Zopiclone impairment

Characterization and measurement in an experimental study

Thesis for the degree of Philosophiae Doctor (PhD)

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Knut Hjelmeland

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Summary

Zopiclone is a hypnosedative drug that was introduced into clinical practice in the late 80s. The drug is frequently prescribed in Norway, especially among elderly women. Zopiclone is related to the benzodiazepines, but is short-acting and is only indicated for short-term treatment of insomnia. Related to the therapeutic effects of zopiclone is impairment of cognitive functioning and psychomotor skills and there is probably an increased risk of falls and fractures, addiction, dementia and involvement in road traffic crashes. Impairment caused by a drug is closely related to its concentration in blood, and this relation is well documented for ethanol. Oral fluid (OF) is an attractive specimen due to the noninvasive nature of sampling procedure.

This thesis is based on a double blind, placebo-controlled, crossover, randomized trial in 16 healthy male subjects. The subjects attended a research unit for four study days, and on each study day they received 5 mg zopiclone, 10 mg zopiclone, 50 mg ethanol or a placebo. During each study day the subjects delivered 10 pairs of blood and OF samples. OF was collected with the Intercept® Oral Specimen Collection Device. At one time point an additional OF sampling with the Statsure Saliva Sampler™ was performed. After intake of the study drug, the subjects performed twice a simplified clinical test of impairment and three times computerized cognitive and psychomotor tests. The computerized tests applied were the Connors Continuous Performance Test, the Stockings of Cambridge Test and the choice reaction test.

We found that a simplified clinical test was less able to detect impairment than more advanced computerized tests. A simplified clinical test should only be used in a population where there is a high prevalence of impairment. We found a dose- and concentration-related impairment of both zopiclone and ethanol for both the simplified clinical test and the computerized tests. The computerized tests consisted of several test components that were categorized in two different ways according to their relation to either behavior level or reaction time, impulsivity and attention/cognition. We found more impairment for 10 mg zopiclone than 50 g ethanol for

automative behavior (behavior level 1) while we found similar impairment for zopiclone 10 mg and ethanol 50 g for controlled behavior (behavior level 2) and executive planning behavior (behavior level 3). Tests that measure reaction time were more likely to be influenced by zopiclone, tests that measure impulsive responses were more likely to be affected by ethanol. Tests of cognition/attention did not demonstrate any clear difference between ethanol and zopiclone. I found acute tolerance for zopiclone, but most clearly expressed for psychomotor tests in behavior level 1 and for psychomotor tests measuring reaction time.

The zopiclone concentration in OF was dependent on the OF sampler device. The OF/blood concentration ratio had a large variation and range, and the intra- and inter individual differences were vast. This OF/blood concentration ratio was dependent on several variables, such as amount of OF delivered and intake of food. We found a prolonged excretion of zopiclone in OF, up to 14 days after intake of 10 mg zopiclone.

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Abbreviations

ACP	2-amino-5-chloropyridine
BAC	Blood Alcohol Concentration
CI	Confidence Interval
CNS	Central Nervous System
CPT	Connors Continuous Performance Test
CPTI	Computerized Psychomotor Tests of Impairment
CRF	Case Report Form
CRT	Choice Reaction Time
CTI	Clinical Test of Impairment
DDD	Defined Daily Dose
DSST	Digit-Symbol Substitution Test
EtOH	Ethanol
GABA	γ-aminobutyric acid
HGN	Horizontal Gaze Nystagmus
Intercept	Intercept® Oral Specimen Collection Device
OF	Oral Fluid
OR	Odds Ratio
POUS	Pharmacy at Oslo University Hospital, Rikshospitalet
RCT	Randomized Controlled Trial
RR	Relative Risk
RT	Reaction Time
RTC	Road Traffic Crashes

RU	Research Unit at Oslo University Hospital, Rikshospitalet
SCTI	Simplified Clinical Test of Impairment
SFST	Standardized Field Sobriety Test
SIR	Standardized Incidence Ratio
SOC	Stockings of Cambridge Test
SPC	Summary of Product Characteristics
Statsure	Statsure Saliva Sampler™
TDM	Therapeutic Drug Monitoring
THC	Tetrahydrocannabinol
TOL	Tower of London (psychomotor test)
ZOBCR	Zopiclone Oral Fluid/Blood Concentration Ratio

List of papers

I. Hjelmeland K, Gustavsen I, Bernard JP, Mørland J. Can a simple clinical test detect impairment of zopiclone and alcohol? – A randomized controlled trial (2015). For Sci Int 248: 129–133.

II. Gustavsen I, Hjelmeland K, Bernard JP, Mørland J. Psychomotor performance after intake of zopiclone compared with intake of ethanol: A randomized, controlled, double-blinded trial (2011). J Clin Psychopharmacol 31(4): 481–488

III. Gjerde H, Øiestad EL, Øiestad ÅM, Langødegård M, Gustavsen I, Hjelmeland K, Bernard JP, Christophersen AS. Comparison of zopiclone concentrations in oral fluid sampled with Intercept® Oral Specimen collection device and Statsure Saliva Sampler™ and concentrations in blood (2010). J of Anal Tox 34:590–593

IV. Hjelmeland K, Gustavsen I, Øiestad EL, Øiestad ÅML, Høiseth G, Mørland J. Zopiclone concentration in oral fluid and blood, after administration of therapeutic doses of zopiclone (2017). For Sci Int 278:177–183

1 Introduction

1.1 The therapeutic role of zopiclone

Insomnia is defined as a nocturnal disturbance of normal sleep patterns that adversely affects daytime functioning (1). Studies of the prevalence of insomnia in the general population indicate that one third of the adults in Western countries experience difficulty with sleep initiation or maintenance of sleep at least once a week. There is a higher incidence of insomnia in women and incidence increase in both men and women as they get older (2). In older adolescents an insomnia prevalence of 23.8 % was reported when insomnia was defined according to DSM-IV criteria (3), and up to 50 % of older adults report insomnia symptoms (4).

The licensed drugs for treatment of insomnia are benzodiazepines, z-hypnotics and melatonin, and these drugs are effective in treatment of insomnia. In Norway two z-hypnotics are registered, zopiclone and zolpidem. Zopiclone is a hypnosedative drug belonging to the family of cyclopyrrolones. The cyclopyrrolones are a family of drugs chemically unrelated to benzodiazepines, but with similar pharmacological profiles to the benzodiazepines. Zopiclone can induce hypnotic, tranquilizing, anticonvulsive and sedative effects (5). Related to the therapeutic effects of zopiclone is impairment of cognitive functioning and psychomotor skills, and these effects increase the risk of misjudgments and accidents.

This thesis deals with the effects of the hypnotic drug zopiclone, in particular with the impairing side effects of this drug, their importance and detection.

1.2 Zopiclone and the z-hypnotics

1.2.1 History

In 1955 Leo Sternbach identified the first benzodiazepine chlordiazepoxide. The benzodiazepines were found to have hypnotic, anxiolytic and muscle relaxant effects and were greeted by medical professionals enthusiastically at first. In the 1960s the benzodiazepines were introduced for broad clinical use as potent anxiolytics. Benzodiazepines were considered a safe alternative to the more toxic formerly used barbiturates. In the 1970s and 1980s benzodiazepines were very frequently prescribed (6). There were few concerns regarding the potential of abuse and dependence (7), although the abuse potentials were recognized as early as 1967. The benzodiazepines became a part of the popular culture. For example the rock band Rolling Stones' hit "Mother's little helper" from 1966 referred to the nickname associated with the widespread use of diazepam by middle-class housewives (8). Several reliable reports in the 1980s established evidence of abuse potential and dependence of the benzodiazepines. The benzodiazepinelike z-hypnotics zopiclone and zolpidem were introduced into clinical practice in 1985 and 1988. These drugs were originally considered by physicians as almost devoid of abuse and dependence potential (9) and with better safety profiles due to their favorable pharmacokinetic profiles (1). Zopiclone and zolpidem were introduced on the market in Norway from 1994 (10). After introduction of zopiclone and zolpidem the prescriptions of these drugs increased substantially both in Norway and worldwide. The very short-acting z-hypnotic zaleplon was introduced on the market in the U.S. in 2000 (11, 12), but with a lack of marketing authorization, zaleplon has never reached the market in Norway.

Zopiclone is available as a racemic mixture of enantiomers (13). The dextrorotatory stereoisomer ((S)-enantiomer), eszopiclone is marketed in the United States as Lunesta®. The S-enantiomer is approximately 50-fold more potent than the R-enantiomer in binding studies and is responsible for the hypnotic effect of the racemate (14). Eszopiclone is sold in the U.S. with recommended therapeutic doses of 1–3 mg (15). In Europe and Asia, the racemate zopiclone is marketed with

therapeutic doses of 5–7.5 mg. Eszopiclone is not marketed in the European Union due to a decision by the European Medicines Agency in 2009. The European Medicines Agency decided that eszopiclone was too similar to zopiclone to be considered a new patentable substance (16). The lack of the new active substance designation implied that the sponsor (Sepracor) would not benefit from 10 years of market exclusivity. As a consequence, the sponsor withdrew the marketing authorization application in 2009 (17).

1.2.2 Prevalence in the population

Zopiclone was marketed in Norway in the 1990s as Imovane® by the pharmaceutical company Rhone-Poulenc Rorer, now a part of Sanofi. In 2020 drugs containing zopiclone in Norway are sold as Imovane®, Zopiklon Mylan®, Zopiclone Actavis® and Zopitin®, and the available doses are 3.5, 5 and 7.5 mg tablets (18). Zopiclone is very frequently prescribed by physicians in Norway for the last two decades. The number of defined daily doses (DDD) of zopiclone sold in Norway was constantly increasing from 1994 to 2004 (19). From 2008 to 2017 the number of individuals prescribed z-hypnotics in Norway has been stable around 360 000, and for the last two years slightly decreasing number of individuals have been prescribed zopiclone. In 2018 around 284 000 Norwegians had at least one prescription of zopiclone (20), among the 5.3 million inhabitants in Norway. Even though hypnotics are indicated for short-time treatment (up to 2–4 weeks) only (21, 22), a study demonstrated that 17 % of new users prescribed z-hypnotics in 2009 were prescribed z-hypnotic for the following four years, and among these long term recurrent users the treatment intensity was high with mean amounts of 199 and 169 DDD per patient per year in men and women respectively (23). Z-hypnotics are sometimes also prescribed in higher doses than recommended (24).

Worldwide zolpidem is the most prescribed drug for insomnia (9) and zolpidem is one of the most commonly used drugs in the United States (8). Zolpidem was initially prescribed in an immediate-release formulation in doses from 5-10 mg (Ambien®). Since zolpidem has a short half-life and is less suitable for patients having difficulties in staying asleep a modified-release formulation of zolpidem

(Ambien CR®) was approved for insomnia in 2005. In 2011 a sublingual, lower-dose (1.75 – 3.5 mg) tablet (Intermezzo®) was approved for difficulty falling back to sleep after middle-of-the-night awakening (25). In Norway only the immediate-release formulation in doses of 5 and 10 mg is marketed.

The z-hypnotics are frequently prescribed among the elderly, some studies quote up to a third of elderly North Americans are prescribed a z-hypnotic or benzodiazepine for sleep disturbance (26). A study from 2013 showed an extensive use of z-hypnotics among elderly women in Norway (27). Recent data from the Norwegian Prescription Database (20) shows that about 51 % of the individuals prescribed zopiclone in Norway in 2018 were over 65 years old. In these data, prescriptions in hospitals and nursing homes are not included. In all age groups the majority (56-66 %) of individuals prescribed z-hypnotics are women. The prevalence of the use of z-hypnotics divided into sex and age in 2018 is shown in Figure 1.

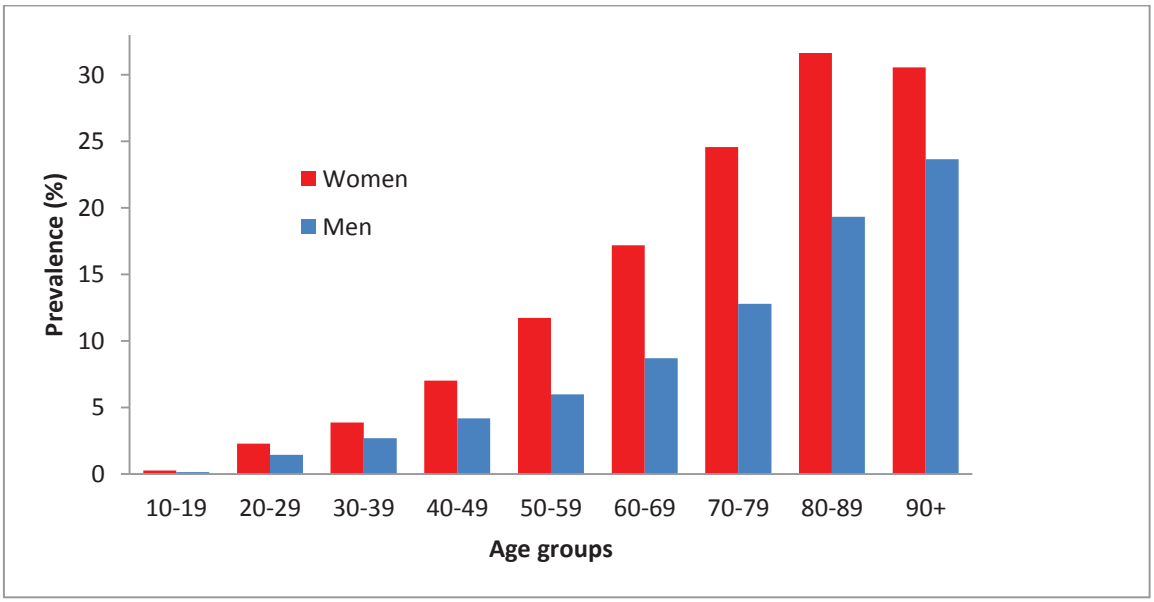

Figure 1. Prevalence of Norwegians prescribed at least one prescription of zopiclone or zolpidem in 2018. Prescriptions in hospitals and nursing homes are not included in the figure. The total amount of individuals prescribed zopiclone and zolpidem were 284 000 and 72 000, respectively (data from the Norwegian Prescription Database (20)).

1.2.3 Zopiclone – Recommended use

Zopiclone (and eszopiclone in the USA) is only indicated for short-term treatment of insomnia. According to the Norwegian Summary of Product Characteristics (21), the treatment should not exceed 2–4 weeks. In the Product monograph from Sanofi-Aventis in Canada it is noted that treatment should not usually exceed 7–10 consecutive days (28). The outer and inner packages containing zopiclone in Norway have a red warning triangle to indicate that zopiclone reduces the ability to drive or operate machinery.

The recommendation is that the lowest effective dose to initiate and maintain sleep should be used. Zopiclone should be taken as a single dose orally before retiring for the night. The initial dose in adults is 5 mg which can be increased to 7.5 mg. In the elderly the recommended initial dose is 3.75 mg, and the dose can be increased to 5 mg and further to 7.5 mg if clinically indicated (21). The dose should be decreased to 3.75 mg in patients suffering from chronic respiratory failure or impaired renal or liver function (5).

1.2.4 Pharmacokinetics of zopiclone

1.2.4.1 Absorption, distribution, metabolism and elimination

Zopiclone is rapidly absorbed and peak plasma concentration is usually reached within 1–2 h (hours) (13, 21). Another study concludes that peak plasma concentration is reached in 0.5 to 4 h (29). An older study observed that 95 % of all absorption occurred within one hour (30). The oral bioavailability of zopiclone averages 80 % (21), implying that the first-pass effect is relatively small. Oral administration of 3.5, 7 and 15 mg zopiclone have shown that the pharmacokinetics are linear (31).

Plasma protein binding is low, approximately 45 % (21, 31), although higher values have been reported (29). The blood/plasma ratio for zopiclone is 1.0 (32, 33). Zopiclone is rapidly distributed from the vascular component to the various body tissues, including the brain (31). The volume of distribution is quite low (1.3-1.6 l/kg) (21, 32).

Zopiclone is metabolized in the liver. Three main biotransformation pathways are identified: oxidation, demethylation and decarboxylation. In humans the two major metabolites are N-oksid-zopiclone and N-desmetylzopiclone. N-oksid-zopiclone is a pharmacological active metabolite in animals, but is less active than the parent compound (5). N-desmetylzopiclone is an inactive metabolite (21, 29). In an in vitro study the cytochrome P-450 isoenzyme CYP3A4 is found to be the major enzyme involved in zopiclone metabolism (both oxidation and demethylation) in vitro, and CYP2C8 contributes significantly to N-desmetylzopiclone formation (34). Co-administration of CYP3A4 inhibitors, such as erythromycin, clarithromycin, ketoconazole may increase the plasma levels of zopiclone and a dose reduction of zopiclone may by required. Co-administration of CYP3A4 inducers, such as rifampicin, carbamazepine and St. John's wort (hypericum perforatum), may require a dose increase (21, 28, 35). CYP2C8 does not significantly metabolize zopiclone in vivo (36). The degradation of zopiclone also leads to the formation of the degradation product 2-amino-5-chloropyridine (ACP) (37). ACP can be detected in urine (38) and whole blood. Several studies (39, 40) have reported instability of zopiclone in blood specimens after sampling, and analysis of ACP might be useful when zopiclone is not detected in blood (41).

In blood approximately 50% of the administered dose is converted to other inactive metabolites via decarboxylation (31), these metabolites are excreted as carbon dioxide via the lungs (42). Zopiclone, N-oksid-zopiclone and N-desmetylzopiclone and other inactive metabolites are eliminated by renal excretion. Less than 7% of the administered dose is excreted as unchanged drug in the urine. In healthy individuals the terminal elimination half-life was found to range from 3.5 to 6.5 h (29), but in patients ≥65 years old the half-life is increased to around 9 h (12). Zopiclone is mainly eliminated in urine (80%) and feces (16%) (21). Zopiclone is the only z-hypnotic where dosage reduction in patients with renal impairment is recommended (13), even though no accumulation of metabolites or zopiclone was found in seven chronic renal failure patients given 7.5 mg zopiclone for seven consecutive nights. The authors concluded that zopiclone is considered to be a safe hypnotic therapy also in patients with renal impairment (43).

1.2.4.2 Plasma concentrations and expected detection times in blood

A summary of several studies observed peak plasma concentration after intake of 7.5 mg zopiclone between 54–86 ng/ml (29). Applying the cut-off levels commonly used in laboratories (in the Department of Forensic Sciences: 10 ng/ml) one can expect to detect zopiclone in blood in about 6–18 h after intake of a hypnotic dose of 7.5 mg zopiclone.

1.2.5 Pharmacodynamics of z-hypnotics

The benzodiazepines and the z-hypnotics mediate their effect by binding to the benzodiazepine receptors at the $GABA_A$ (γ-aminobutyric acid type A) receptor complex. GABA is the primary inhibitory neurotransmitter in the mammalian central nervous system (CNS) and activation of $GABA_A$ receptors by GABA tends to decrease neuronal excitability (44). This may lead to sedating symptoms (i.e. sleepiness, apathy) and deteriorating effects on memory and judgement. Binding to the $GABA_A$-receptor facilitates the opening of GABA-activated chloride channels (13). The $GABA_A$-receptors in the CNS consist of five homologous subunits (pentamer) surrounding the central chloride ion-selective channel. How many isoforms of the receptor exist is far from clear (45). The majority of $GABA_A$-receptors consist of α-, β- and γ-subunit families. The $GABA_A$-receptors are responsive to a wide variety of drugs. The benzodiazepine/z-hypnotic action appears to be determined by presence of particular α-subunits. The benzodiazepines and the z-hypnotics bind predominantly to the interface of the γ_2-subunit with either α_1, α_2, α_3 or α_5-subunit. $GABA_A$-receptors containing α_1-receptors mediate sedation and amnesia, while α_2 and α_3 subunits have been implicated in the anxiolytic effect. Receptors containing α_5-subunits might play a role in memory processes. Drugs are inactive at α_4 or α_6-containing receptors (46, 47).

The main difference between the benzodiazepines and z-hypnotics is in their receptor affinities toward the different $GABA_A$ subunits. Benzodiazepines show similar affinity to the α_1, α_2, α_3 or α_5-receptor subunits. The different z-hypnotics have different affinity to the different α-receptor subtypes. Many researchers claim that zopiclone has no selectivity for the different α subunits on the

$GABA_A$-receptors (12, 48, 49) and therefore zopiclone shares similar pharmacological properties with benzodiazepines, exhibiting anxiolytic, anticonvulsant and myorelaxant effects (1). Other researchers claim that racemic zopiclone shows preferential activity at the α_1 subunit of the $GABA_A$ receptor. The R-antiomer of zopiclone, eszopiclone, differs from its racemic mixture in that it has greater efficacy at the α_2 and α_3 subunits (13). The addition of the R-enantiomer in racemic zopiclone may augment efficacy at the α_1 subunit and potentially lead to increased sedation and residual effects (50). Zolpidem has the highest binding affinity to the α_1-receptor subtype (47, 51), though it has some agonist activity at the α_2 and α_3 subunits. Hence, zolpidem is considered a potent sedative and hypnotic with minimal anxiolytic activity (13). The ultra-short-acting z-hypnotic zaleplon has modestly higher affinity for the α_1 compared to α_2 and α_3 subtypes, although the affinity at the α_1 subtype is about twofold lower than zolpidem. Unlike zolpidem, zaleplon also has affinity for the α_5 subtype (49).

Even though there are some pharmacodynamic differences between the benzodiazepines and the z-hypnotics (especially zolpidem) the main differences are related to specific pharmacokinetic properties (52), and especially the shorter half-lives of the z-hypnotics and accordingly the reduced risk of next-day effects (5). The differences in the selectivity to the different α subtypes between the different z-hypnotics gives a theoretic assumption of how these drugs works as anxiolytics and how memory function is affected, even though the clinical implications of these differences are not clear.

1.2.6 Side effects

There are several studies that have reported adverse cognitive and psychomotor effects following treatment with zopiclone (and other z-hypnotics), including physical consequences, falls, fractures, traffic accidents, daytime fatigue, addiction and increased mortality (53). The effects of the z-hypnotics have been studied in the elderly, but there is an increasing interest in the z-hypnotic effectiveness and residual effects in shift workers, pilots and military personnel (54).

Residual daytime sedation as evidenced by poor coordination, decreased concentration and cognitive deficits are well known “hangover” effects of benzodiazepines, but these effects are less frequent in z-hypnotics due to shorter half-life and duration of action (1, 55). Reduction of cognitive psychomotor activity which may impair activities of daily living are therefore more relevant to benzodiazepines than z-hypnotics. Like benzodiazepines, zopiclone appears to have dose-dependent effect on anterograde amnesia (53) and impair memory and cognitive functioning (56).

The abuse and dependence potential of benzodiazepines is well known (57-59). This abuse and dependence potential is considered to be remarkably lower for the z-hypnotics (9, 60-63) even though dependence of zopiclone is described in several case reports (64-68), and zopiclone is misused among clients attending a methadone maintenance program in Dublin, Ireland (69).

Discontinuation of zopiclone may cause transient insomnia, so-called rebound insomnia (21). Rebound insomnia probably occurs rarely and did not occur after abrupt discontinuation of zopiclone in a RCT of 612 insomniacs ingesting 7.5 mg every night for 28 days (70). Another study of 209 patients treated with 2 or 3 mg eszopiclone for 6 weeks found no evidence of tolerance to sleep-inducing effects of eszopiclone or rebound insomnia and no evidence on next-day psychomotor performance using a memory test (71).

It appears that zopiclone does not considerably affect respiratory function (5, 72, 73). Death caused by overdoses of z-hypnotics is rare (74), even though there are several studies and case reports of overdose deaths caused by zopiclone (33, 75-78).

A common side effect of (es)zopiclone is a bitter, metallic, unpleasant taste reported as frequent as 3.6–34 % (79, 80) and no satisfactory mechanism or explanation has been identified (17). Dry mouth is also frequently reported (21).

Several meta-analyses have concluded that benzodiazepine use is significantly associated with dementia risk (81-84). A similar risk of dementia as was seen with benzodiazepines is suggested for z-

hypnotics (85, 86), even though the evidence is primarily restricted to a few sub-analyses in benzodiazepine studies.

A systematic review, including 14 studies, also found a significant increased risk for fractures among users of z-hypnotics (Odds Ratio [*OR*] = 1.63; 95% Confidence Interval [CI] = 1.42–1.87) (87) and quite similar results were also found for zopiclone in a study from New Zealand (not included in the systematic review above) investigating 74 787 older individuals with first time fractures (88). In a nationwide prospective study from Norway of older people (*n*=906 422) z-hypnotics were associated with higher excess risk of hip fracture at night (Standardized incidence ratio [SIR] 1.3, 95 % CI 1.2–1.4) than during the day (SIR 1.1, 95 % CI 1.1–1.2) (89).

There exist a vast amount of epidemiological studies regarding driving under the influence of non-alcohol drugs (90), and several studies have investigated the risk of being involved or killed in road traffic crashes (RTC) when using zopiclone. Gustavsen et al. (91) used Norwegian databases to study involvement in RTC during the first week after zopiclone was dispensed and a SIR of 2.8 (95% CI 2.0–2.8) was found. In a Belgian part of the DRUID-project (Driving Under the Influence of Drugs, Alcohol and Medicines in Europe) case-control studies found a significant association between the use of z-hypnotics and being injured (crude Odds Ratio [*OR*] 6.45, 95% CI 1.63–25.52) (92). A Norwegian study from 2013 found no significant association for the use of zopiclone and fatal injury among drivers (93). Chang et al. found an *OR* of 1.37 (95% CI 1.06–1.75) for being involved in an RTC after one week's use of z-hypnotics (94). In a case-crossover study from Taiwan, an *OR* for RTCs of 1.55 (95% CI 0.98–2.45) was found. Some other studies have also found no significant associations between RTC and use of z-hypnotics (95). In a study from Sweden, newly initiated treatment with zolpidem or zopiclone showed an increased risk of occurrence of RTC that was highest in the two weeks after the start of the treatment (*OR* 2.66; 95% CI 1.04–6.81) in drivers 50–80 years old (96).

To summarize it can be concluded that zopiclone is a quite safe drug with quite few side-effects and moderate dependence potential. On the other hand, however, the potential increased risk of

dementia and fractures among z-hypnotic users is of particularly concern in the elderly female population, since more than 25 % above the age of 70 are prescribed z-hypnotics (Figure 1). Epidemiological studies have shown mixed results when investigating whether use of zopiclone (or zolpidem) increases the risk of being involved in RTCs or not. Since both sporadic and chronic use of zopiclone can cause detrimental effects on psychomotor function and cognition, further research on impairment of zopiclone is of scientific interest and is further investigated in this thesis.

1.3 Measurement of relevant impairment

1.3.1 Clinical impairment

Measurement of clinical impairment worldwide

Investigation of clinical impairment is relevant in many different settings. For a doctor prescribing a drug a clinical judgement of potential impairing/side effects is valuable. A clinical evaluation may contribute to describe how the drug affects the patients in their daily living or at work. A clinical test to evaluate impairment is of special interest for police officers working roadside. The development of a clinical test in order to reveal impairment of drugs is often designed to reveal traffic-relevant impairment. When a suspected impaired driver is stopped it is essential to have additional tools that can help the police in evaluating whether the apprehended driver is impaired or not. The real-life setting is far from experimental laboratory setting and complex testing which involves laboratory or computerized tools. In some countries, i.e. the USA, the Standardized Field Sobriety Test (SFST) is a clinical tool to investigate impairment. The three test components that make up the SFST are the horizontal gaze nystagmus (HGN), the walk and turn and the one-leg stand test. The SFST was originally designed to assist law enforcement officers in making roadside decisions for alcohol-impaired driving (97) and is found to be an accurate and reliable decision aid for discriminating between blood alcohol concentration (BAC) above and below 0.8 g/l (98). There are some studies

that demonstrate clinical impairment on the SFST after intake of cannabis (99-101), and also to CNS stimulants and depressants and narcotic analgesics (102). The Drug Evaluation and Classification program (DEC) is a systematic and standardized procedure which involves a series of physical and psychomotor tests and concludes with the toxicological examination of a bodily fluid sample. The DEC-program includes a more thorough investigation than the SFST and is performed in intended facilities and by certified drug recognition experts. The use of the DEC-program expands across the United States and into Canada, Europe and Australasia (103).

Measurement of clinical impairment in Norway

The clinical test of impairment (CTI) is a comprehensive clinical test of impairment that is performed by a police physician shortly after apprehension of drivers suspected of driving under the influence of non-alcoholic drugs (104). The CTI has been used for several decades and consists of 25 different subtests, including 7 tests of alertness, cognitive- and vestibular function, 4 observations on eyes, 2 observation of intravenous drug abuse, 4 tests of motor activity/coordination and 8 observations concerning appearance. At the end of the examination the physician concludes based on his/her general impression whether the suspect is impaired or not. The outcome of the performed CTI, especially the conclusion regarding judgement of impairment, has been studied in several observational studies. In these studies, suspected drugged drivers where only a single drug is detected are selected and the relationship between impairment judged by the physician's general impression and the concentration of a certain drug in blood is compared. The concentration range in these studies are wide, which illustrates that supratherapeutic doses of drugs have been ingested. This method of studying observed clinical impairment in relation to drug concentration has been applied for zopiclone/zolpidem (105), GHB (106), codeine (107), heroin/morphine/morphine-6-glucuronide (108), methadone (109), benzodiazepines (104, 110), flunitrazepam (111), carisoprodol (112), amphetamine/methamphetamine (113), cannabis/tetrahydrocannabinol (THC) (114, 115) and THC in combination with ethanol (116). Even though these observational studies are performed by

several different physicians in a selected population of apprehended drivers, they all demonstrate drug-concentration related effects.

In Norway police officers are trained during their education in a simplified test to measure impairment called “Sign and Symptoms” (S&S, Norwegian: Tegn- og symptomer). The test includes: measurement of pupils’ size, the pupils’ reaction to light, HGN, convergence insufficiency and measurement of pulse rate, balance and time control (117). A master’s thesis concluded that the S&S performed by the police was more sensitive in detecting impairment than the CTI (118). To my knowledge the S&S is not validated in other studies.

1.3.2 Measurement of impairment in an experimental setting

There exist recommendations for experimental research on drugs and driving (119). These recommendations involve three core levels of behavior:

1. Automative behavior (well-learned, automatic action patterns)
2. Control behavior or maneuvering level (controlled action patterns)
3. Executive planning behavior or strategic level (general plans for interactions with ongoing traffic)

There are several psychomotor performance tests that measure functions relevant for each core level of behavior. The automative behavior (core level 1) includes functions such as well-learned skills, tracking, steering, alertness, vigilance and sustained attention. Examples of relevant tests are tracking (i.e. on-the-road driving tests that measure the standard deviation of lateral lane position [SDLP] (120)), alertness in continuous performance tests (i.e. The Connors Continuous Performance Test [CPT] (121)), omissions [no response to stimulus] in CPTs and variations in reaction time (RT). The control behavior (core level 2) includes functions such as maintaining distance, speed estimation, response time (too fast/slow), visual search, motor performance, maneuvers, divided attention and perception. Examples of relevant tests are reaction time (RT), too fast responses in choice reaction tests (CRT) and dual attention tests. The executive planning behavior (core level 3) includes functions

such as interactive functions with ongoing traffic, risk taking, impulsivity, cognition, judgement and planning skills. Examples of relevant tests are errors in CRTs, in CPTs and other tests, planning tests (i.e. “Tower of London” (122), “Stockings of Cambridge”), memory and gambling tests.

Studies that investigate impairment by psychoactive drugs should include laboratory tests relevant to the aim of the study. If impaired driving is the issue, a wide range of traffic-relevant tests is applicable, such as measuring sedation, drowsiness, divided attention, continuous perceptual-motor coordination, speed and accuracy of decision making, vigilance and short-time memory. Many of these specific measurements can be evaluated in basic computerized tests and will probably also be relevant for other purposes as impairment of importance for performance in the home or at work.

More comprehensive tests are performed in driving simulators (123, 124) and the on-the-road driving tests (120). The on-the-road driving test measures the vehicle’s lateral position relative to the road delineation (weaving, SDLP) and is considered to be a specific and robust method to examine driving ability (120, 125-127). Measurement of weaving (SDLP) has also been performed in Norway evaluating impairment by alcohol, both in a driving simulator (128) and in real driving on a closed track (129). The on-the-road driving test measures highly automated behaviors, such as road tracking control and it has been argued that the test mainly represents a subtask of driving. These automated behaviors are more affected by a psychoactive drug compared to more complex driving tasks requiring conscious control (130). Psychomotor testing can detect deteriorated performance due to other impairing effects that are not revealed in the on-the road driving test. In a recent study that investigated impairment of buprenorphine, even more impairment was found for the psychomotor testing than measurement of the SDLP in the on-the-road driving test (131).

The current guidelines (119) recommend that pharmacokinetic measurements of substance concentrations (preferably in blood) should be included in experimental studies. A reference drug should also be included in the studies. Since impairment research on alcohol is investigated in many

studies, alcohol can be used as a reference drug (verum) (125). Deteriorating driving performance related to blood alcohol concentration is documented in experimental studies (132).

1.4 Impairment of ethanol and zopiclone – experimental studies and relation to drug concentration in blood

1.4.1 Ethanol

Impairment in relation to drug concentration in blood has become a well-established notion for ethanol. Ethanol intoxication results in diminished psychomotor and cognitive abilities (133) such as decreased coordination, slurred speech, impaired short-time memory, reduced ability to learn, affection of critical sense, decreased error control, increased impulsivity and aggression. This wide span of clinical effects can be explained at a cellular level, since ethanol modulates the activity of a variety of neuroreceptors and ion channels. Several ligand-gated ion channels, such as $GABA_A$, NMDA, glycine, neuronal nicotinic and 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) have been shown to be directly modulated by ethanol. $GABA_A$ receptors appear to occupy a central role in mediating the effects of ethanol in the CNS. The different ligand-gated ion channels are affected by ethanol at different BAC levels. The $GABA_A$ receptors are the most sensitive and can be potentiated at BAC as low as 0.05‰. The function of the GABA receptor is described in Chapter 1.2.5. Through activation of the opioid receptors and $GABA_A$ receptors, ethanol may cause sedation and respiratory depression. The NMDA receptors are inhibited by ethanol and may impair memory and cognitive functioning (134). The NMDA receptors are sensitive, and a considerable inhibitory effect is mediated from BAC as low as 0.25‰. Facilitation of $5\text{-}HT_3$ receptors may increase release of dopamine in the nucleus acumbens and is probably the basis of the arousal effects of ethanol. Effects mediated via opioid- and $GABA_A$- receptors might also be involved in the dopaminergic effects of ethanol.

Ethanol has well-documented impairing effects on cognition and psychomotor functions. A large proportion of the harm linked to alcohol use can be attributed to such impairment. It is further established that there is a rough concentration effect relationship for the level of ethanol in blood and the degree of impairment and risk of harm. Even if such relations have been shown at a group level and not necessarily at the individual level, blood ethanol levels are considered as an acceptable proxy for the degree of cognitive and psychomotor impairment (132). This is, e.g. reflected in road traffic rules/laws.

1.4.2 Zopiclone

Psychomotor effects of zopiclone have been investigated in randomized controlled studies, where healthy volunteers have been given 5, 7.5 or 10 mg zopiclone. Psychomotor tests have been performed both a short time and the morning after ingestion. A short time (i.e. 1–3 h) after ingestion, several studies demonstrated significant impairment in different psychomotor tests, such as CRT, eye-hand coordination test, digit symbol substitution test (DSST), body balance and memory (135-137). When performing psychomotor tests a long time (often the next morning) after ingestion, often no cognitive or psychomotor dysfunction is found in psychomotor tests (such as the DSST, symbol copying time or CRT) (138-141). In a study only the critical flicker fusion test had significant worse results in the early morning after intake of 1 mg eszopiclone. There were no significant differences between eszopiclone and placebo in other objective assessments (142).

Using on-the-road driving tests to investigate performance, impairment is found a long time after intake. These studies are often designed to investigate residual effects of zopiclone in relation to traffic-relevant impairment. In a study using a driving simulator, impairment was found 10 h after intake of 7.5 mg zopiclone (143). The on-the-road driving test is previously described in Chapter 1.3.2, and several studies are performed to evaluate impairment of zopiclone. Evening administration of 7.5 mg zopiclone increased significant next-day SDLP compared with placebo in a driving simulator (144). Another study found that 7.5 mg impaired a highway driving test in healthy

people aged 55–75 years (in the article defined as elderly) at least until 11 h after intake (145). Specific on-the-road driving tests have revealed impairment as long as 10–12 h after intake of 7.5 mg zopiclone (56, 145-154).

Similar relationships between blood zopiclone concentration levels and the degree of cognitive and psychomotor impairment have been indicated (137, 143, 147), although not as convincingly as for ethanol. In a meta-analysis of 21 studies which measured 267 different effects of impairment of zopiclone a concentration-dependent impairment was found (55). Since 2012 Norway has had a traffic act under which e.g. the concentration of un-prescribed zopiclone in blood is linked to the level of punishment, analogously to ethanol. This was based on the assumption that roughly similar relations exist between blood concentrations of ethanol or zopiclone on one hand and risk of accidents on the other (155, 156). A previous thesis from our group (157) was highly related to the introduction of fixed legal limits for blood concentrations in road traffic. The main conclusion of her thesis was overall, that blood zopiclone concentrations seem as suited for traffic-related legal limits as blood ethanol concentrations. That thesis also found a positive relationship between blood zopiclone concentrations and impairment starting at 16 ng/mL and which was sustained throughout supra therapeutic concentrations. It was also concluded that the concentration-effect relationships for zopiclone and ethanol were compatible to each other within a given blood concentration window.

1.5 Oral Fluid

OF includes secretions from salivary glands (saliva), upper gastrointestinal and respiratory tracts and the gingival sulcus (158). The total volume of OF produced by an adult may be in excess of 1000 ml/day (159) and OF is considered to be an attractive specimen due to the noninvasive nature of sampling procedure. In contrast to blood, sampling of OF does not require a suitable environment, sterile equipment or trained personnel. Compared to urine, OF has reduced adulteration potential

and detection in OF often indicates recent intake (160-163). OF also carries a smaller risk of spreading infection (164). Therapeutic drug monitoring (TDM) is defined as the clinical laboratory measurement of a chemical parameter that, with appropriate medical interpretation, will directly influence drug prescribing procedures (165). Traditionally TDM refers to the individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range or window. Due to the simplified sampling, the value of OF as an alternative specimen in TDM is of particular value in drug treatment of psychiatric disorders (166). To establish OF for the purpose of TDM the relationship between concentrations in OF and serum/plasma must be documented. In a study of patients (27 children and 9 adults) with attention-deficit/hyperactivity disorder (ADHD) a significant correlation between concentrations of methylphenidate in serum and saliva was found (167). Some studies have tried to document the potential of measuring different psychoactive substances in OF, such as antipsychotics (168, 169), antidepressants (170) and antiepileptic drugs (171).

OF is also used roadside as a screening devices to indicate drug use in driving populations (172). In a study from Wille et al. blood and OF samples were collected from drivers suspected of driving under the influence of abused drugs (stimulants, morphine, codeine, THC and several benzodiazepines). It was concluded that there was great variability of the OF/blood ratios (173). The relationship between OF (Saliva Sampler™ device) and whole blood concentration for different drugs of abuse (amphetamines, opioid, cocaine and metabolites, THC, benzodiazepines and other psychoactive medicines) was investigated in a former study by Langel et al. A correlation for all substances, except lorazepam and THC, was found even though there was a vast individual variation (174). In another study 4080 paired whole blood/OF (Statsure Saliva Sampler) samples from drivers were analyzed. The authors concluded that analysis of concentrations of different illicit drugs could not be used to accurately identify drivers with drug concentrations above selected cut-offs in blood (175). The OF/plasma ratio of codeine was investigated in one study and it was found that the ratio depends on which OF collection kit is used. The correlation coefficient between OF and plasma codeine

concentrations was statistically significantly (p=0.005) higher for the Saliva Collection System (R^2=0.745) than for Quantisal (R^2=0.403) (176). Quite constant OF/blood (serum) concentrations have been found for theophylline (177) and diazepam (178). In another study tramadol concentrations correlated significantly between plasma and OF (mean R^2=0.77) (179).

There is limited knowledge of the relationship between the concentration of zopiclone in OF and the concentration in blood (serum), this has been previously investigated in two studies on a small number of subjects. In a study by Caille of 10 volunteers the ratio between zopiclone concentration in OF and serum was not the main aim of the study and is briefly described; a ratio AUC (Area Under Curve) saliva/AUC plasma of 2.3 was reported (30). In a population of suspected drugged drivers a median OF/blood zopiclone ratio of 2.4 was calculated for 6 individuals (174); in that study OF was collected with the Saliva Sampler™ device.

In the present work, I have looked further into the relation between blood zopiclone or ethanol concentrations and the outcome of different measures of impairment (such as clinical testing and various cognitive and psychomotor tests). In addition, the suitability of measurement of OF zopiclone concentration as a proxy for blood zopiclone concentrations was investigated.

2 Aims of Thesis

The aims of the present thesis:

2.1 Aim 1

Impairment of zopiclone in relation to blood zopiclone concentration and similarities/differences between zopiclone and ethanol.

a) How useful is a simplified clinical test (SCTI) to measure impairment in comparison with a computerized test (CPTI) after single intakes of zopiclone and ethanol? (Paper I)
b) Do zopiclone and ethanol affect various components of the computerized test (CPTI) differently? (Paper II)
c) How is the acute tolerance to zopiclone in various components of computerized tests (CPTI)? (Paper II)

2.2 Aim 2

Can measurement of OF zopiclone concentration substitute blood zopiclone concentration?

a) Is OF zopiclone concentration dependent on the OF sampler device? (Paper III)
b) Is the relation OF-zopiclone-concentration/blood-zopiclone-concentration constant, and if not, which factors are influencing the ratio? (Paper IV)

3 Material and methods

3.1 Study design

We performed a double blind, placebo-controlled, randomized trial in 16 healthy young male subjects. The study was approved by the Regional Ethical Committee for Medical Research (reference: S-07288a), the Norwegian Medicines Agency (Eudract number 2007-002974-75) and the data protection representative at the Norwegian Institute of Public Health. The Norwegian Directorate of Health gave permission for establishment of a biobank, and also approved that biologic specimen could be sent out of the country. In the early stages in planning of the study collaboration with colleagues from the U.K. was relevant, but this collaboration ceased. No biologic specimen was sent abroad and all analysis was performed at the Norwegian Institute of Public Health. The subjects were students recruited from the University of Oslo (UiO) and the Norwegian Business School (BI). At the end of an ordinary lecture at the UiO a short presentation of the study was given by the authors to students present in the auditorium. Subjects who were interested in participating in the study were encouraged to take contact with the authors in the break for further information. All subjects attended the Research Unit (RU) at the Department of Pharmacology at Oslo University Hospital, Rikshospitalet about one week ahead of the first study day for (further) information, practice of the psychomotor tests and to make sure that inclusion and exclusion criteria were met and an informed consent were signed. Inclusion and exclusion criteria and restrictions are shown in Table 1 and the age and weight of the subjects are shown in Table 2.

Table 1. Inclusion criteria, exclusion criteria and restrictions

Inclusion criteria:	Generally good health Body weight within 70–90 kg Male Age 20–35 Approval of written informed consent
Exclusion criteria:	Intake of zopiclone within 3 months before the study Regular (daily) intake of any prescribed drug. History or presence of drug/alcohol abuse Former abnormal reaction to any hypnotic drug History of severe allergic disease History of significant mental, cardiovascular, renal or hepatic disorder, or other significant disease as judged by the investigator Requirement for a specific diet (e.g. vegetarian) Positive pre-session urine sample of any of the following substances: ethanol, benzodiazepines (confirmation analysis), zolpidem, tetrahydrocannabinol (THC), cocaine, amphetamine, methamphetamine, morphine and codeine
Restrictions:	Intake of more than 3 alcohol-units (45 g ethanol) within 48–96 h ahead of each session Intake of alcohol within 48 h ahead of each session Intake of any medication within one week ahead of each session, except medication which does not affect the study or interact with the investigational products as judged by the investigator (e.g. paracetamol etc.) Non-compliance with the study protocol Incorrect randomization of the volunteer Occurrence of a serious event or any other reason judged by the investigator Positive urine test in baseline samples

Table 2. Study population. *N* = 16 males

	Median	Range
Age	23.5 years	20–28 years
Weight	76.5 kg	69–88 kg

Upon attendance on each study day, baseline urine-, blood- and OF-samples were collected. One of the subjects tested positive for tetrahydrocannabinolic acid (THCA) in the baseline urine sample of one of the study days, proving intake of cannabis. The reason for still including this subject in the study is discussed in Chapter 5.

During the study day the subjects were served two meals. A light lunch was served 2.5 h after intake of study drugs (after sampling of blood and OF). The lunch consisted of several slices of bread, served with ham, cheese or strawberry jam, and apple juice. Dinner (regular meal of the hospital menu) was served after sampling of blood and OF 8 h after intake of study drugs. A study day flow chart of procedures is shown in Figure 2 and a picture from the RU is shown in Figure 3.

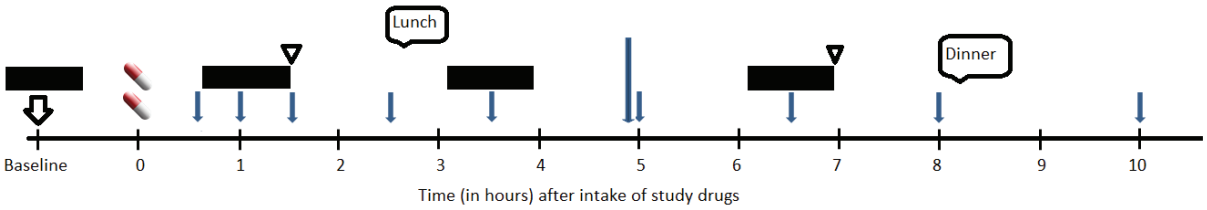

Figure 2. Study day flowchart of procedures. The figure shows the sampling and testing in relation to time (in h) after intake of study drugs (capsules and drink). The big open arrow indicates baseline sampling (blood, OF (Intercept) and urine). The short arrows indicate simultaneously blood and OF (Intercept)-sampling. The long arrow indicates OF (Statsure)-sampling ahead of the OF (Intercept)-sampling 5 h after intake of study drugs. The black boxes indicate the time intervals for the performances measured by the computerized tests (CPTI). The CPT test was performed ahead of sampling and the SOC and CRT were performed right after sampling. The triangles indicate the point of time for the performances on the simplified clinical test of impairment (SCTI). The meals ingested within the study day are shown in the speech balloons.

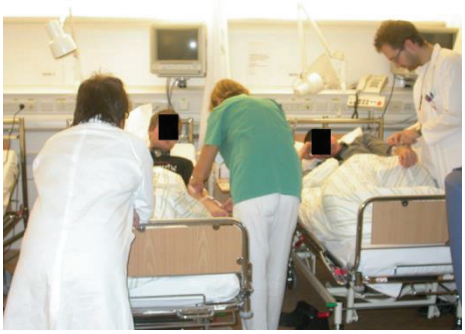

Figure 3. Two of the male study persons, the research nurses, Anne Marie Halstensen and Kristin Villa and the PhD-candidate.

3.2 Study drug

The subjects attended the RU for four study days and received in randomized order one of the following four regimens: 10 mg zopiclone, 5 mg zopiclone, 50 g ethanol or placebo. The washout period between each study day was at least one week. The study medicine package consisted of two capsules and one drink. Each capsule consisted of placebo or 5 mg zopiclone. The randomization of the different investigational products was performed by an external group at the Division of Diagnostics and Intervention, Oslo University Hospital. This group has no relation to the research team. The randomization list was sent directly to the pharmacy at Oslo University Hospital, Rikshospitalet (POUS) without any correspondence with the researchers. POUS is the GMP (Good Manufacturing Practice)-certified pharmacy that prepared the study drinks and capsules for the study. The placebo drinks contained 156 ml water and the ethanol drinks contained 156 ml vodka (40

vol %) (50 g ethanol). To disguise the taste of ethanol, 1 ml Tabasco and 113 ml lime juice were added to both placebo and ethanol (180). The total volume of the study drink (ethanol or placebo) was 270 ml. In further addition a spray of preservative (methylparahydroxybenzoate 8 %, propyl-parahydroxybenzoate 2 % and spirits 90%) was added to both the placebo and ethanol drink in order to camouflage the possible smell of ethanol. The amount of ethanol in the preservative did not contribute to any measurable blood ethanol concentration. The active substance in the gelatin capsules was zopiclone (Zopiklon Merck NM®). Lactose powder was added to the placebo and zopiclone capsules. It was impossible to distinguish between the placebo- and zopiclone capsules based upon appearance. All investigational products were packed identically to maintain blinding. The drink was given in dark-colored bottles and the subjects were instructed to drink all the liquid in the bottle over a maximum of 15 minutes. Median drinking time was 5 minutes (range: 0–15 minutes). They were also instructed to swallow the capsules with the drink. At the end of each study day, the subjects were asked which of the drugs (zopiclone 5/10 mg, placebo or ethanol) they believed they had received.

3.3 Sampling of blood and oral fluid

Blood and OF (Intercept® Oral Specimen Collection Device [Intercept]) samples were collected simultaneously 9 times after intake of study drugs. Samples were collected frequently around expected time to reach maximum concentration (T_{max}) in blood and OF (Figure 2). We did not manage to draw blood samples from one of the volunteers on his zopiclone 5 mg day. Adverse events were registered during the study day. The sampling of the OF Statsure Saliva Sampler™ (Statsure) was performed in relation to the sampling 5 h after intake of study drugs. In addition the subjects were requested to deliver an OF (Intercept) sample 24-81 h after intake of study drug. Both OF sampling devices consist of cotton pads that are placed within the oral cavity for 2 minutes. The OF collection pad used in the Statsure device is made of cellulose and does not contain any chemicals that stimulate the production of OF (181). The OF collection pad used in the Intercept device is made of cotton treated with a solution containing sodium chloride, citric acid, sodium benzoate, potassium

sorbate, gelatin and sodium hydroxide, according to the package insert. The vial contained chlorhexidine digluconate, Flag Blue dye, Tween 20 (nonionic surfactant and deionized water (182). Some of the compounds in the collection pad of the Intercept device stimulate the production of saliva. For this reason, the sampling of OF (Statsure) was performed before the sampling of OF (Intercept), and last (stimulated) sampling of OF (Intercept) was performed 1.5 h ahead of OF (Statsure) sampling (Figure 2).

3.4 Clinical test (SCTI)

After intake of study drugs, a simplified clinical test of performance (SCTI) was performed at 1.5 and 7 h (Figure 2). The Norwegian CTI consists of 25 subtests (104), and five of these subtests were selected. The five selected subtests were: gait-on-line test, turn-on-line test, finger-to-finger test, finger-to-nose test and Romberg's test (standing steady on one leg with eyes closed for at least 5 s [seconds]). The performances of these subtests were scored as either "habitual", "somewhat deviant", or "deviant". In addition, an "overall impression" of impairment was graded as "not impaired", "slightly impaired" or "moderately impaired". The "overall impression" was also referred to and treated like the other subtests. In this thesis the SCTI thus consisted of six subtests.

3.5 Psychomotor test (CPTI)

To establish test components that can be categorized into the three core levels of behavior (119) described in Chapter 1.3.2, the following three computerized cognitive and psychomotor tests were used:

- The Connors Continuous Performance Test (CPT) Version II for Windows is a computerized attention test often used to differentiate patients with attention-deficit/hyperactivity disorder (ADHD) from normal groups and to measure treatment efficacy (121). The test subjects / patients are placed in front of a screen and instructed to press the spacebar on the computer's keyboard in response to any letter excluding "X" appearing on the screen. The test measures how fast the test subject responds to a stimulus and how often the subject

responds incorrectly (i.e. hits the spacebar when the letter “X” appears on the screen). One test session lasted for 14 min.

- The Stockings of Cambridge (SOC) test is a computerized version of the Tower of London (TOL) test. In the TOL test the test subject is presented with two vertical columns of colored balls, one which represents the desired arrangement. The other must be rearranged to match the first, moving one ball at a time. The objective is to use the minimum number of moves in the shortest possible elapsed time (183). The computerized SOC session lasted for 10 minutes (min).
- Choice reaction time (CRT) is a test where a series of stimuli, which may be auditory and/or visual, is presented to the test subject using an electronic apparatus or a computer screen. The subject is instructed to respond appropriately and rapidly through hand movements to pre-selected signals. The subject is graded on the speed and accuracy of the performance. One test session lasted for 7 min.

The three cognitive and psychomotor tests (CPT, SOC and CRT) were performed at baseline (before intake of study drugs) and three times (0.5-1.5 h, 3-4 h and 6-7 h) after intake of study drugs (Figure 2). 20 minutes ahead of each sampling (OF and blood) the Connors Continuous Performance tests version II for Windows (CPT) were performed and right after sampling the Stockings of Cambridge (SOC) and the Choice Reaction Time (CRT) were performed. The psychomotor performances of the three occasions are in this thesis hereafter referred to as 1, 3.5 and 6.5 h after intake of study drug.

Even though there is a general description of what the three psychomotor tests measure, they contain different test components. Twenty-three test components were available from these three psychomotor tests. Fifteen test components that were relevant to measure impairment were selected. These test components constituted the computerized tests (CPTI). They were categorized into three behavior levels (automative, control and executive behaviors) (119). Level 1 (automative behaviors) included 6 test components, 2 and 4 test components from the CRT and CPT test

respectively. Level 2 (control behaviors) included 5 test components, 1 test component from the SOC test and 2 test components from both the CRT and CPT test. Level 3 (executive planning) included 4 test components, 2 test components from the SOC test and 1 test component from the CRT and the CPT test.

In this thesis I present unpublished results where the test components were categorized differently than in three levels of behavior. In the new optional categorization, the test components were categorized into three new groups measuring “impulsivity”, “reaction time” and “attention/cognition”. To measure “impulsivity”, “reaction time” and “attention/cognition”, 4, 7 and 3 test components were categorized, respectively. An explanation of each test component, the categorization into behavior levels and the new optional categorization is presented in Table 3, and the categorization is discussed further in Chapter 4.6. To explain the nomenclature for some of the test components it can be mentioned that test components named “perseverations” measure errors in form of repetition of a response regardless of absence/cessation of a stimulus; “commissions” measure wrong response(s) and “omissions” measure the number of targets when the volunteer did not respond. For one of the test components (CRT omis – measures the number of targets where the volunteer did not respond) only three volunteers tested differently from placebo and the therefore this test component was not included in the unpublished results.

Table 3. Description of the 15 test components and how they are categorized in Paper II (behavior levels) and in the new optional categorization

Test component	Description	Behavior level	Optional Categorization
CRT pers	Perseverations: the percentage of trials the volunteer responded too fast. Measured perception and motor performance.	2	Impulsivity
CPT pers	Perseverations: the number of times the volunteer responded too fast. Measured perception and motor performance.	2	Impulsivity
CRT com	Commissions: the percentage of trials the volunteer pressed the wrong button. Measured information processing and attention.	3	Impulsivity
CPT com	Commissions: the number of times the volunteer responded to a non-target. Measured impulsivity and attention.	3	Impulsivity
CRT rt var	The standard deviation of the reaction time. Measured consistency of reaction time.	1	Reaction time
CPT rt var	The standard error of reaction time. Measured consistency of reaction time.	1	Reaction time
CPT alert	The slope of change in reaction time over the 6 blocks. Measured the ability to stay alert. A vigilance measure.	1	Reaction time
CPT adjust	The slope of change in reaction time related to the inter stimulus intervals. A positive slope indicated a slower reaction time as the inter stimulus interval increased. Measured the ability to adjust to the presented speed.	1	Reaction time
SOC r time	Reaction time: the volunteers' speed of movement from initial move to last move for the 5 moves-problems.	2	Reaction time
CRT r time	Reaction time: the mean latency from stimulus appearance to button press.	2	Reaction time
CPT r time	Reaction time: the mean response time for all 6 blocks.	2	Reaction time
CPT omis	Omissions: the number of targets to which the volunteer did not respond. Measured automative behavior.	1	Attention/ Cognition
SOC plan	The mean time to select the first ball in the 5 moves problems. Measured planning and cognition.	3	Attention/ Cognition
SOC incor	How many times the volunteer did not complete the problems in the minimum possible number of moves. Measured information processing, attention and cognition.	3	Attention/ Cognition
CRT omis	Omissions: the number of targets to which the volunteer did not respond. Measured automative behavior.	1	

The test components and a short description of what they measure. A perseveration is the repetition of a particular response regardless of the absence or cessation of a stimulus. An omission is no response to a stimulus. A commission is a wrong response to a stimulus. The three core levels of behavior: level 1 (Automative behaviors), level 2 (Control Behaviors) and level 3 (Executive Planning).

The specific method applied in Papers I-IV is described in each paper.

4 Methodological considerations

4.1 Study design

Our study was performed as a double-blind, placebo-controlled, randomized trial (RCT). An RCT has several advantages such as minimizing potential biases (i.e. assessment and performance bias) and confounding factors. The study design allows us to compare the performance after intake of an active substance (i.e. zopiclone) to a reference drug (i.e. ethanol) or placebo under controlled conditions. The resource intensive design of an RCT was essential when investigating impairment (Aim 1), but not of critical importance to evaluate pharmacokinetics/concentrations in OF and blood (Aim 2). In our study the volunteers were given 4 different regimens of study drugs, i.e. two different doses of zopiclone, ethanol and placebo on 4 different study days. That means that if a volunteer had suspicion of what kind of study drug he ingested on one study day, for example by experience of alcohol inebriation, he could assume that he will not receive this study drug on a later study day. An alternative design of the study, to optimize the blinding, could have been that the volunteers had attended the RU for five study days receiving the 4 different study drugs in a randomized order. The extra fifth study day, where a random choice of the study drugs was given, would occur in a randomized order before, within or after the other study days. It was considered, however, that the extra effort of implying an extra study day would be larger than the potential gain.

A total of 630 blood samples and 667 OF samples were retrieved in the study. About a quarter of the samples were collected on a study day when the subjects ingested alcohol and about half of the samples were retrieved on the study day when the subjects ingested zopiclone. To reduce unnecessary workload because of analysis of samples that did not contain any active study substance only samples where the subjects received active drugs were analyzed. Therefore, the samples were unblinded for the personnel that performed the analyses. The unblinding was not performed by anyone in the research group. The samples retrieved were only marked with a study number and study day, and the personnel performing the analyses had no possibility of identifying the volunteers.

We considered that breaking the blinding ahead of the analysis would not affect the study or the processing of data. Analysis of ethanol in OF was not performed since the main focus of the study was to compare concentration of zopiclone in blood and OF, the OF/blood ethanol concentration ratio is previously found to be close to one (184-186).

4.2 Study drug

The volunteers were given two capsules and a drink which consisted of one active substance or placebo on each study day. Initially we wanted the volunteers to ingest zopiclone tablets similar to those dispensed at pharmacies. We asked one pharmaceutical company for possible production of placebo tablets looking identically like active zopiclone tablets. The company, however, feared that the result of the study could damage the reputation and cause negative publicity and was therefore not interested in contributing to the study. As an alternative to tablets we administered zopiclone and placebo in capsules.

POUS was willing to produce capsules and drinks for the study. The capsules were produced by packing crushed zopiclone tablets and lactose into capsules. An advantage of capsules compared to tablets was that the potential bitter taste of the zopiclone tablets was omitted. In switching from tablets to capsules the research group was concerned that ingesting capsules might cause delayed absorption and reduced bioavailability compared to ingesting tablets. In order to confirm that the tablets and the capsules of zopiclone had approximately the same pharmacokinetic profiles a small pilot study was performed. A 5 mg tablet or a capsule was given to three of the authors (Paper II) on two separate study days and blood samples were collected several times after ingestion. We found that the time-concentration curves were quite similar for both tablets and capsules. Based on this pilot study we were quite confident that capsules could replace tablets in the study.

4.3 Assumption of intake

At the end of each study day the volunteers were asked which of the drugs they believed they had received. Intake of 10 mg and 5 mg zopiclone was correctly suggested on 8 (50%) and 9 (56%)

occasions, respectively; placebo was correctly suggested 9 times (56%), whereas ethanol intake was correctly suggested on all occasions except for one (93 %) (Paper II). Based on the researchers' subjective experience it seemed that the volunteers had difficulty distinguishing between the taste of ethanol- and the placebo-drink. We wanted to evaluate if we managed to disguise the ethanol taste and the bitter taste of zopiclone, but at the end of each study day the possible experienced subjective pharmacological effects of ethanol and zopiclone were used to differentiate between intakes of the different study drugs. The volunteers' subjective experience of intoxication is also interesting but interferes with the "taste-evaluation" of the study drug/drink. In hindsight, we should have asked the volunteers right after ingestion of the study drink, before the pharmacological effects occurred, and at the end of each study day what kind of study drug they thought they had ingested.

If the volunteers experience side-effects after intake of an active drug in a clinical trial, a systematic difference in expectations emerges between the placebo and the drug arm, and the placebo arm fails to control for the nonspecific factors that play a role in the drug arm (187). As an example, if a volunteer experiences known side effects after intake of zopiclone there is a potential of more pronounced impairment of expected effects, such as increased reaction time if feeling tired.

4.4 Dysgeusia

The most frequently reported side-effect of zopiclone intake, dysgeusia (distortion of taste) is probably partly caused by a direct bitter taste of the tablet. Dysgeusia is also experienced as a side effect after intravenous intake of other drugs (188) and it is likely that dysgeusia may occur after intravenous intake of zopiclone, even though this has not been investigated. A former study found that dysgeusia correlated with drug plasma and saliva levels after intake of eszopiclone. In that study the dysgeusia was reported for several days during a treatment period and was most pronounced for women (189). In the written informed consent, the volunteers were informed that "bitter taste" is a well-known side effect of zopiclone and therefore the occurrence of dysgeusia may have affected the blinding. Dysgeusia was, however, only reported 4 times (Chapter 6.5, Table 6). Even though the

volunteers were informed of the risk of dysgeusia in advance of the study they did not necessarily understand that the dysgeusia was caused by intake of zopiclone. An example (not among the reported 4 occasions of dysgeusia) is that one of the volunteers complained about the terrible taste of the apple juice he got for lunch. This expressed bitter taste was probable due to dysgeusia after intake of zopiclone. Since dysgeusia occurred quite seldom, this side effect did probably not affect the blinding procedure more than the fact that zopiclone (and ethanol) was accompanied by a subjective feeling of intoxication/impairment. In a recent study it was found that beverages containing citric acid suppressed the bitterness intensity of zopiclone tablets (190), so orange juice (containing citric acid) could perhaps been a better drink to serve for lunch. Orange juice could also lower the pH in the oral cavity, which could increase the zopiclone concentration in OF. This is further discussed in Chapter 7.2.2.

4.5 Analytical methods

The analytical method of the blood analyses is described in Paper II and the analytical methods of the OF are described in Paper IV. Zopiclone is well known as a notoriously unstable analyte in biological matrices, and analytical results might therefore depend on pre-analytical factors, such as storage time and temperature (39, 40, 191, 192). A stability study of zopiclone in blood showed that zopiclone was stable less than 1 day at 20 °C, less than 2 weeks at 5 °C but stable for 3 months at -20 °C (193). Due to the known instability of zopiclone (39, 194) the pre-analytical conditions from sampling to analysis were thoroughly controlled. Both the blood and OF samples were put in a refrigerator immediately after the samples were collected. They were stored in a refrigerator (5°C) for maximum 24 h after sampling and then either analyzed or frozen (–20°C) for later analysis. About half of the OF and blood samples were analyzed within two days and all samples were analyzed within nine days. This strict timeline of analysis minimized the risk of pre-analytical degradation of zopiclone.

4.6 Psychomotor tests – expression of drug effects

In this study three laboratory cognitive and psychomotor tests are used. When investigating the impairing effects of a drug there are several benefits of using such psychomotor tests, such as cost-effectiveness and relative ease of administration. The chosen tests were selected because they included thorough measurement of the three core levels of behavior (119).

Which behavior level each test component should be categorized into, was a consequence of a thorough discussion in the research group, also involving neuropsychological specialists Drs Anne-Kristine Schanke and Hans Johansen at Sunnaas Rehabilitation Hospital (195). Even though the different functions relevant for each core level are defined, other researchers could have categorized the test components differently. While working on this thesis, the discussion of how to categorize the cognitive and psychomotor test components in another way was continued in the research group. This led to other possible categorization than the behavior level. This is described in Chapter 3.5 and the unpublished results are presented in Chapter 6.2.2. The new categorization reflected functional similarities between the different test components, and the test components were categorized into three new groups measuring “impulsivity”, “reaction time” and “attention/cognition”. For the test components categorized to measure reaction time, three of the test components measure simplified simple reaction time (SOC r time, CRT r time and CPT r time), while the other four test components measure change of reaction time over time (Table 3).

While working on this thesis I wanted to question the magnitude of deteriorated performance between the different test components, but this turned out to be impossible to explore. Many of the test components measure quite different size parameters, with a mixture of units and continuous variables. Some of them measure time from when a stimulus is given to a certain response from the volunteer. Other test components measure the number of times when a volunteer gives no (omission) or wrong (commission) response to a stimulus. Since the performances for each test component are measured in such different ways, it is difficult to compare the magnitude of altered

performance of a single test component to another test component where performance is measured with another size parameter. For a single test component, a comparison of the magnitude of deteriorated performance between the two doses of zopiclone (and ethanol) is possible to explore, like it is done in Paper II. In the unpublished results I present the results by reporting the frequency of individuals that show deteriorated performance after intake of zopiclone or ethanol compared to their own placebo performance. No statistical analysis is performed on the unpublished results.

In the morning on each study day the volunteers performed a baseline test of the psychomotor tests before they received the study drug. In the research group we had a fruitful discussion on whether the placebo or baseline performance should be used as the "controlled performance". In Papers I and II (published and unpublished results) the (individual or group) psychomotor performance after intake of active substance was compared to the performance after intake of placebo. In another paper (195), we compared individual psychomotor impairment after intake of active drug to the baseline performance. If we compare the performance after intake of an active substance to the placebo performance at the same time of the day, we risk that the placebo-performance is not a good comparison if there is large difference in performance between days. If a volunteer has a "bad day" caused by for example mental stress or sleep deprivation on his placebo-day, the placebo-performance could differ from the habitual performance and the placebo-performance would be worse than expected. On the other hand, if we compare the performance after intake of an active substance to the baseline-performance we have to keep in mind that the performance could differ throughout the study day. We experienced that some of the volunteers were quite sleepy in the morning, while others seemed to be quite refreshed. This morning tiredness/freshness could affect the performance of the baseline performance. To sum up there are pros and cons whether the "control-performance" should be based on placebo or baseline-performance, due to varying intra- and inter-day performance.

4.7 Investigation of acute tolerance to ethanol and zopiclone

We intended to evaluate acute tolerance to both zopiclone and ethanol. To measure acute tolerance, two performances at similar concentrations (increasing and decreasing) of drugs had to be present. The maximum mean concentrations were, however, achieved at rather different times after intake for ethanol 1.0 h (SEM ±0.1 h) and zopiclone 1.7 h (SEM ±0.2 h) (paper II).

At the time of the first psychomotor tests (1 h) the concentration of ethanol for all individuals was close to the maximum concentration (and in the end of the absorption phase), while after intake of zopiclone, the absorption was still ongoing. Therefore the concentration of ethanol was generally much higher around the time of the first psychomotor tests (1 h after ingestion of study drugs) than around the time of comparable psychomotor tests (3.5 h after ingestion of study drugs) and our data was therefore not suitable for evaluation of acute tolerance for ethanol. Actually, it was possible to estimate acute tolerance to ethanol if only test components from the CPT test were included, since the CPT test was the only psychomotor test that was performed before ethanol reached maximum mean concentration at 1 h, but since we wanted to include all psychomotor test components this was not done. For zopiclone, the median (and mean) concentrations around the time of the psychomotor tests 1 h and 3.5 h after intake were quite equal (Chapter 6.2.2, Table 5) and investigation of acute tolerance to zopiclone effects was possible.

4.8 Statistics

In Paper II (Figure 2), it could be discussed if multiple testing should be considered. *P*-values are expressed for six of the computerized tests, and if using the Bonferroni method, the *p*-values required for significance should be reduced to 0.0083 (0.05/6). It could also be discussed whether even lower *p*-values should be required considering that multiple doses are compared for each test component. This would reduce the number of significant differences in Paper II. On the other hand, the Bonferroni correction is considered very conservative and it should be noted that the different test component in this study is derived from only three basic computerized tests.

According to the Altmann nomogram, post hoc power analyses can be expressed. We observed that if the standardized difference between the two groups was above 1.0, a study with 16 participants would have 80% power to detect a significant difference using a *p*-value below 0.05. In Paper II, we calculated mean of all differences between the highest measured concentration and placebo performance for the 16 subjects. Standard deviation was calculated from baseline performances and the standardized differences were obtained by dividing mean differences by the standard deviation. In Paper II (appendix), the estimated differences in 9 of the 15 tests for zopiclone 10 mg were in an area where power was sufficient, while for the lower doses and ethanol, power was insufficient in a larger number of tests. This power calculations would ideally be done before planning the study.

As drug effect sizes varied between individuals and test components, I also found it of value to use the percentage of subjects showing impairment instead of mean values and standard deviation for each test component (unpublished results). In general, I found rather similar results by these two ways of presenting the findings, where the latter was less influenced by outliers.

In Paper IV, the introduction of mixed linear statistical methods was considered. The results consist of 231 pairs of OF/blood ratios from 16 subjects. As we did not include the mixed model statistics, we only compared two values from each participant in each statistical test and used the Wilcoxon paired samples non-parametric test. An exception was the scatterplot, where we included all paired samples.

5 Ethical considerations

The volunteers recruited in the study were all students at the UiO and the BI. At the time of the recruitment of subjects to the study one of the supervisors, Prof. Jørg Mørland, gave lectures at the Faculty of Medicine at the UiO. The recruitment of volunteers did therefore not include students at the Faculty of Medicine, due to the potential bond or connection between the research group and the volunteers enrolled in the study. The two volunteers studying at BI contacted the research group after they heard about the study through common friends studying at the UiO.

In the early stages of planning the study the inclusion age criterion was set to 18–35 years. The alcoholic beverage to be ingested as a part of the study originated from vodka (40 volume percent of alcohol). It can be calculated that diluted with lime juice and Tabasco sauce the alcoholic beverage contained 23 volume percent of alcohol (Paper I). In Norway the legal age for buying strong liquor (alcoholic beverage containing more than 22 volume percent of alcohol) is 20 years (196). Based on this the inclusion criterion was changed to 20–35 years of age. One individual was excluded from the study ahead of the first study day, because he was 18 years old.

One of the volunteers tested positive for tetrahydrocannabinolic acid (THCA) in urine. THCA is an inactive metabolite of THC, the active component of cannabis. THC was not detected in the baseline blood sample and the THC/creatinine ratio in urine was quite low. These analytical findings were in accordance with his explanation of smoking cannabis on three consecutive days 11–13 days ahead of his first study day. A strict interpretation of the exclusion criterion would exclude the subject from the study. A “sporadic” intake of cannabis more than one week ahead of a study day was not considered to interfere with the study by the investigators and the subject was still included.

The study included only young men, even though elderly women are most frequently being prescribed zopiclone (Fig. 1). The elderly are also the group of people that probably experience most side effects of the drugs, like increased risk of falls and fractures (87). However, zopiclone is also highly relevant for traffic impairment. Young males are by far, the most frequent individuals

apprehended by the police under suspicion of impaired driving; 87 % of suspected drugged drivers in Norway in 2018 were men (197). Nevertheless, it would have been preferable to include both sexes in the study. In the study alcohol was given on one of the study days. It was also important that the individuals participating in the study achieved approximately the same maximum BAC and zopiclone concentration. The maximum BAC achieved after intake of alcohol depends largely on the gender and weight of the individual (198). Over the past few years there has been discussion of, especially in the USA, gender differences when dosing the z-hypnotic drug zolpidem. Studies have demonstrated that women have greater functional impairment than men taking the same dose of zolpidem, and the Food and Drug administration (FDA) in the USA reduced the recommended dosage for women down to 50% of the dose for men (199). For zopiclone this gender difference has not been reported. To avoid huge inter-individual pharmacokinetic and potential pharmacodynamics differences for the study drugs (especially ethanol) only the same sex (men) were included in the study. If women had been enrolled into the study, also potential pregnancy had to be investigated in accordance with current procedures, and participating women had to be recommended to use contraception during the study period. This aspect of interference into the private sphere of the participants also spoke against the inclusion of women.

Alcohol is probably the most popular psychoactive substances in the world. It can have powerful effects on mood and mental state. Alcohol is potentially addictive and the toxic effects in both short-term and long-term perspective are well known. The individuals participating in the study were social drinkers and the mean maximum BAC achieved on the day the individuals ingested alcohol was 0.078 %. The alcohol effects at this BAC level leads to moderate impairment of alcohol. The impairment achieved on this study day is probably similar to what the individuals had experienced earlier and was not considered to represent an increased risk for development of alcohol use disorder. The acute risks connected to alcohol intake were considered controlled by all individuals being observed and taken care of during the study day at the RU at the University Hospital.

Zopiclone is a benzodiazepine-like drug that has abuse potential (Chapter 1.2.6). The individuals were given a dose of zopiclone which is slightly higher than the recommended maximal sleeping dose. We aimed to study a concentration/psychomotor effect of zopiclone and therefore it was necessary to administer a slightly supratherapeutic dose of zopiclone. Zopiclone given in these doses is not considered to be toxic, and the expected side-effects are sparse. Two exposures of zopiclone are not considered to be of any potential harm and pose no risk of trigging abuse of this drug. The authors of a recent Cochrane review conclude that there is no or little evidence of harm of eszopiclone if taken as recommended (200). Toxicity of zopiclone is low and deaths caused by intoxication of zopiclone alone are rare, and in the reported deaths in the literature the postmortal zopiclone concentrations were much higher than the mean maximum zopiclone concentrations in our study (77, 201). We observed 20 occasions of individuals falling asleep after ingestion of zopiclone (Chapter 6.5, Table 6), the individuals were observed while they were sleeping and were awakened according to the study day schedule (Fig. 2).

All individuals were paid 4000 NOK for participating in the whole study. It is desirable that all human research is performed by individuals that want to contribute to research on an ideal basis and that participation is not financially motivated. In our study we found that the “workload” and time spent in the study days was substantial and therefore payment was necessary to enroll enough study volunteers. The students participating had to take a break from their studies for 4 whole days, where each study day lasted for about 11 hours. By participating in the study, the volunteers exposed themselves to discomfort in the form of quite moderate intoxication and sleepiness. Since lime and tabasco was added to the study drink, some of the individuals expressed that the drink tasted bad. Initially the research group found the payment of a total of 5000 NOK to be a reasonable compensation for the discomfort, workload and time spent during the study. As a response to the study application to the Regional Ethical Committee for Medical Research (REK) it was argued that the study persons should not be paid at all. The total payment to each volunteer was therefore reduced from 5000 NOK to 4000 NOK, which was accepted by the REK. When it comes to payment of

volunteers in clinical studies, I believe that there has to be a balance between a desired idealistic participation in a research project and a reasonable compensation for discomfort and workload.

6 Summary of Results

6.1 Paper I

In this paper we wanted to compare the findings in a clinical test (SCTI) with a test battery of computerized cognitive and psychomotor tests (CPTI). To be registered as "impaired" after intake of ethanol and zopiclone the performance of a clinical subtest / psychomotor component for an individual had to be deteriorated compared with the performance after intake of placebo at the same time point. At T_{max}, around 1.5 h after intake of both study drugs (zopiclone and ethanol), the SCTI was able to demonstrate impairment in approximately 15–30 % of the subtests, while the CPTI revealed impairment in more than 50 % of the test components. Only 5 % of the SCTI subtests and 32–41 % of test components in the CPTI revealed impairment 6.5 h after intake. At all concentration levels after intake of both study drugs the CPTI detected a significantly higher proportion of impaired observations compared to the SCTI. For the SCTI the fractions of impaired subtests were greater in groups of zopiclone concentrations above 23 ng/ml and BAC above 0.5 g/kg. The sensitivity of a clinical subtest in detecting blood drug concentrations often associated with impairment, due to zopiclone (above 23 ng/ml) and alcohol (above 0.5 g/kg), was low, revealing 27 % and 18 %, respectively. The specificity was higher, 88 % for zopiclone and 96 % for alcohol.

6.2 Paper II

6.2.1 Published results

The performances of the computerized cognitive and psychomotor tests in relation to blood concentrations of zopiclone and ethanol were studied in 15 test components in relation to three core levels of behavior defined as essential to safe driving. Three computerized tests were the same as used for assessing psychomotor performance in Paper I.

As shown in Table 4, intake of 10 mg zopiclone compared with placebo impaired the mean performance of 10 of the single test components 1 hour after intake, whereas 5 mg zopiclone

impaired 4, and 50 g ethanol impaired 6 of the 15 test components. At behavior level 1, 5 out of 6 test components was impaired 1 h after intake of 10 mg zopiclone. Only 3 of the 15 test components were still demonstrating impairment compared with placebo at 3.5 hours after intake. No treatment resulted in any difference from placebo for any test component at 6.5 hours after intake.

Table 4. Deteriorated test components

	Number of test components showing significantly deteriorated performance			
Study drug	1 h after intake			3.5 h after intake
Zopiclone 10 mg		10/15		2/15
	Level 1	Level 2	Level 3	
	5/6	3/5	2/4	
Zopiclone 5 mg		4/15		0/15
	Level 1	Level 2	Level 3	
	2/6	1/5	1/4	
Ethanol 50 g		6/15		1/15
	Level 1	Level 2	Level 3	
	1/6	3/5	2/4	

Number of the 15 test components that showed significantly deteriorated performance 1 and 3.5 h after intake of study drug (Wilcoxon test, $p < 0.05$). The performance of all 16 individuals on group level was compared to the performance of all 16 individuals after intake of placebo. One hour after intake the number of test components that caused impairment within the three core levels of behavior is shown. Although there were differences, no test components showed significant impairment compared with placebo on group level after 6.5 h.

Taking the mean blood drug concentrations 1 hour after intake into account, 39 ng/ml zopiclone was associated with more pronounced impairment than 0.74 g/kg BAC for behavior level 1. For performance at behavior levels 2 and 3, 39 ng/ml zopiclone seemed comparable to BAC level of 0.74 g/kg.

6.2.2 Unpublished results

The different test components responded somewhat differently in subjects influenced by zopiclone compared to subjects influenced by ethanol. For further analysis of this difference between zopiclone and ethanol, I studied the fraction of the 16 subjects impaired for each test component. Since the differences between individuals with respect to the size of an effect for a test component were vast, it was therefore considered interesting to show if performance was deteriorated compared to individual placebo performance or not. The percentages of subjects demonstrating impaired performance according to behavior level are presented in Figure 4a.

The results for each test component expressed as fractions of the 16 individuals that showed deteriorated performance, were often in accordance with the measured psychomotor performance in Paper II. For example, CPT r time showed more deteriorated performance for both doses of zopiclone compared to ethanol measuring size parameters on group level (Paper II, Figure 2) and in Figure 4a.

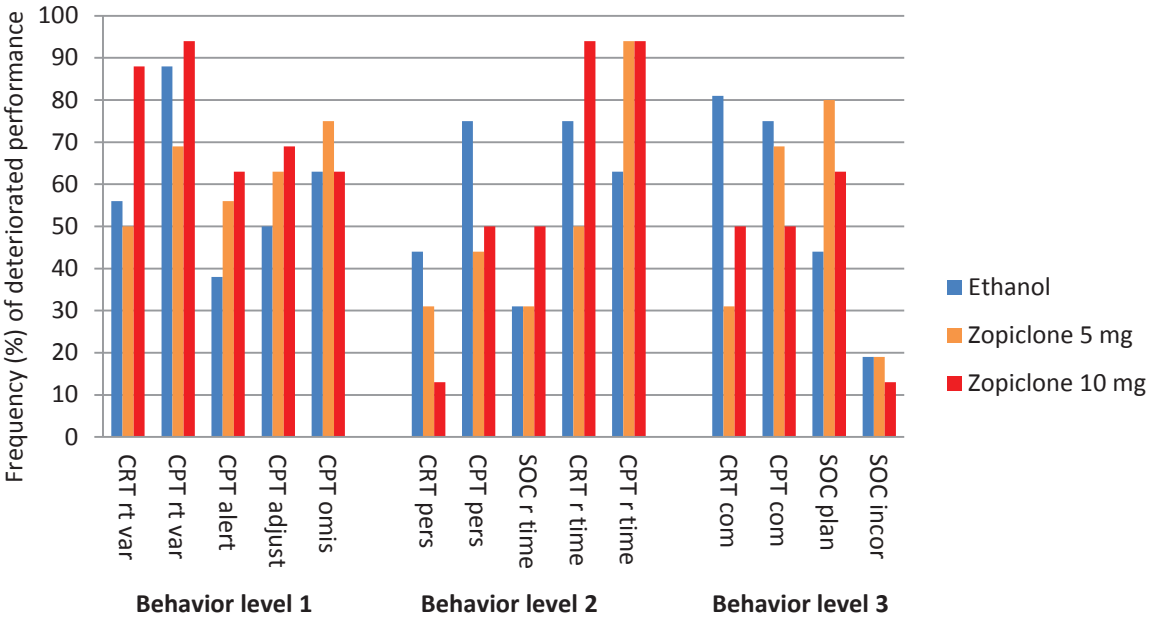

Figure 4a. Fractions (in percent) of the 16 individuals that showed deteriorated performance after intake of active drug compared to placebo performance 1 h after intake. The test components are sorted in order of the three core levels of behavior: 1 (automative behaviors), 2 (control behaviors) or 3 (executive behavior).

Figure 4a shows that the different test components within each level of behavior varied with respect to whether intake of zopiclone or ethanol was impairing the largest fraction of subjects. When I compared the effects of 10 mg zopiclone with ethanol, more subjects were affected from 10 mg zopiclone at level 1, while the differences between zopiclone and ethanol was less pronounced at levels 2 and 3.

A regrouping of test components according to their testing of either impulsivity, reaction time or attention/cognition was also performed (Fig. 4b). Like in Figure 4a, Figure 4b shows the performance of the computerized tests performed after 1 h compared to each individual placebo performance.

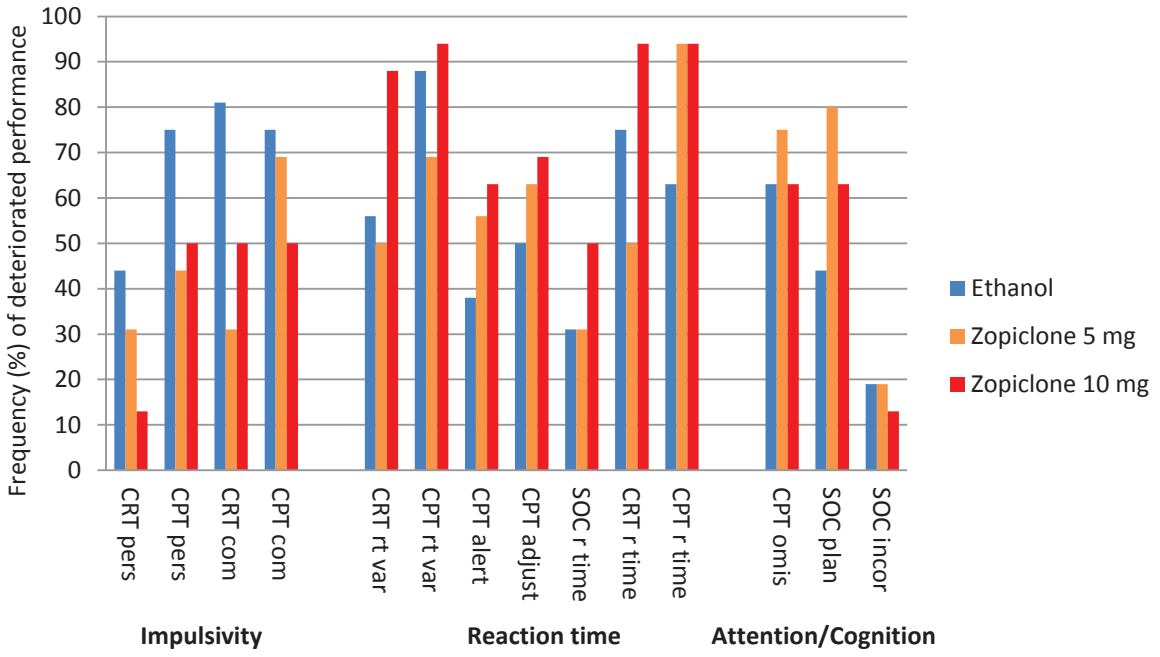

Figure 4b. Proportions (in percent) of the 16 individuals that showed deteriorated performance after intake of active drug compared to placebo performance 1 h after intake. The four test components to the left (CRT pers, CPT pers, CRT com and CPT com) are specific measures of impulsivity. In the middle, seven test components (CRT rt var, CPT rt var, CPT alert, CPT adjust, SOC r time, CRT r time and CPT r time) which measures reaction time are presented. To the right the test components (CPT omis, SOC plan and SOC incor) which measures attention/cognition are shown.

In all 4 test components categorized to indicate impulsivity a higher proportion of individuals show deteriorated performance after intake of ethanol compared to performance after intake of zopiclone (both doses). For all test components that measure reaction time a higher proportion of individuals showed deteriorated performance after intake of 10 mg zopiclone compared to ethanol. For the test components that are categorized to measure “attention/cognition” varying differences in placebo-compared test performance after intake of the different active substances and doses (ethanol and zopiclone) were demonstrated.

Acute tolerance after zopiclone administration

To measure acute tolerance for various test components I chose the following procedure: Blood samples were retrieved in the middle of the psychomotor testing (after CPT and before CRT and SOC) 1.0 h and 3.5 h after intake of zopiclone (Figure 2). The zopiclone-concentrations of the 16 individuals are presented in Table 5.

Table 5. Concentrations of zopiclone in blood 1.0 and 3.5 h after intake. The mean, total and interquartile (IQ) range

	Concentrations (ng/ml) 1.0 h after intake				Concentrations (ng/ml) 3.5 h after intake			
Zopiclone dose	Median	Mean	Range	IQ range	Median	Mean	Range	IQ range
5 mg	17.0	16.4	2–51	8–22	17.0	18.0	13–27	16–20
10 mg	38.0	34.7	3–71	25–45	35.0	35.0	20–50	31–39

The median and mean concentrations of zopiclone for blood samples retrieved 1.0 h and 3.5 h after intake were quite equal for both doses of zopiclone. I could therefore compare the test performances for each test component at 1.0 and 3.5 h in order to investigate whether there was any acute tolerance to zopiclone, expressed as reduced drug effect at 3.5 h compared to 1 h.

Figures 5a and 5b show the change in (placebo-compared) performance 1 h after intake (Figures 4a and 4b) compared to the performance 3.5 h after intake (data not shown) for the two doses of zopiclone. In Figure 5a the test components are sorted according to the three core levels of performance, in Figure 5b according to “impulsivity”, “reaction time” or “attention/cognition”. The change in performance is described by a change in the fraction of individuals that show deteriorated performance after 1 h compared to performance after 3.5 h. An improvement of performance would be an indication of acute tolerance. A positive bar in Figures 5a and 5b shows a lower fraction of impaired subjects at 3.5 h compared to 1 h, and expresses acute tolerance. A negative bar indicates more deteriorated subjects and no acute tolerance.

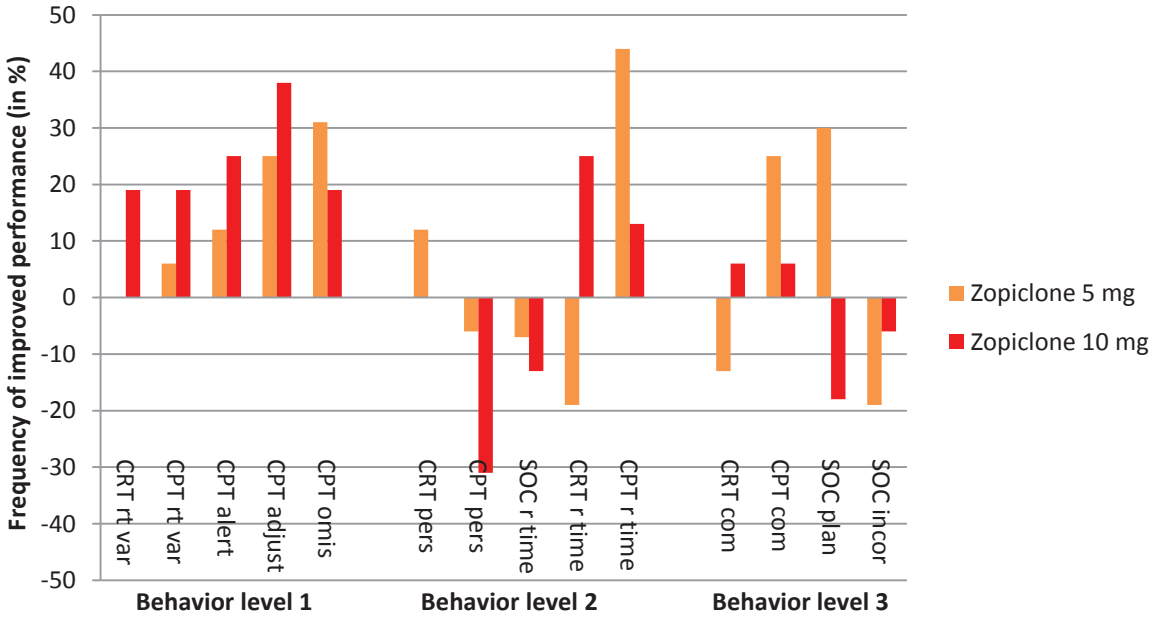

Figure 5a. Change in (placebo-compared) performance (fractions in percent of the 16 individuals) for the 14 test components from 1 h (Figure 4a) to 3.5 h after intake of zopiclone. A positive bar shows lower percentage of deteriorated subjects for performance after 3.5 h compared to performance after 1 h (acute tolerance) and a negative bar shows more deteriorated subjects. The test components are sorted in order of the three core levels of behavior: 1 (automative behaviors), 2 (control behaviors) or 3 (executive behavior).

I found that acute tolerance was most pronounced at behavior level 1 (automative behavior). For behavior levels 2 and 3 the development of acute tolerance was variable. For the three test components that originate from the SOC test acute tolerance was not observed (except for the SOC plan after intake of 5 mg zopiclone).

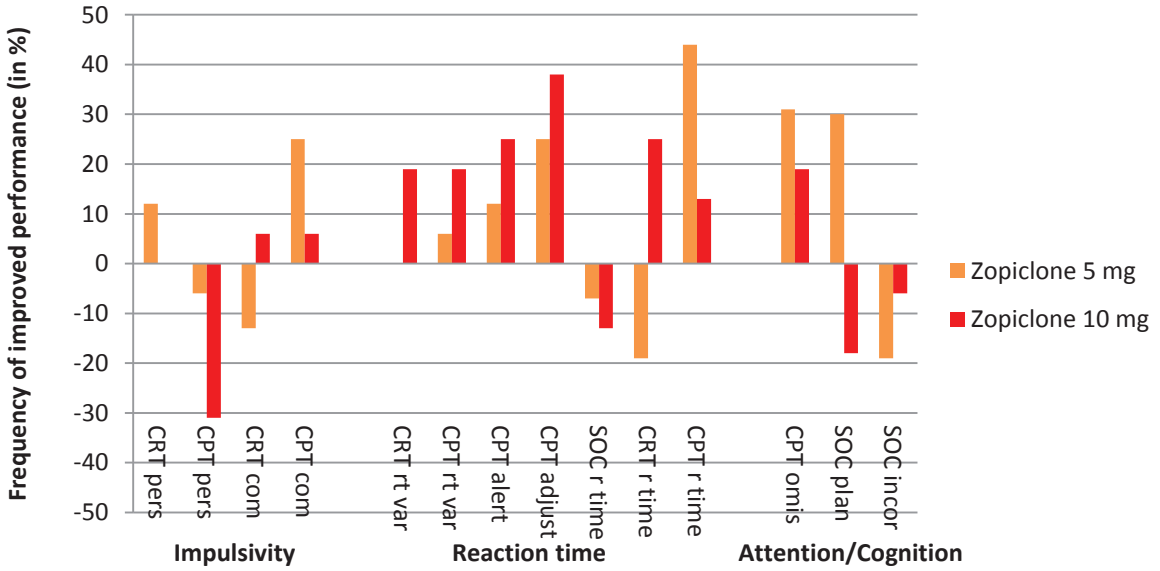

Figure 5b. Change in (placebo-compared) performance (fractions in percent of the 16 individuals) for the 14 test components from 1 h (Figure 4b) to 3.5 h after intake of zopiclone. A positive bar shows lower percentage of deteriorated subjects for performance after 3.5 h compared to performance after 1 h (acute tolerance) and a negative bar shows more deteriorated subjects. The test components are sorted in order as components that measure "impulsivity", "reaction time" or "attention/cognition".

In Figure 5b the test components are sorted as groups that measure "impulsivity", "reaction time" or "attention/cognition". I found that acute tolerance was most pronounced for the test components that measured reaction time. For the test components categorized to measure impulsivity and attention/cognition acute tolerance was more variable.

6.3 Paper III

In this short communication, the zopiclone concentrations in OF collected by two different devices (Intercept® Oral Specimen Collection device and Statsure Saliva Sampler™) and in blood were investigated. Among 16 individuals, 21 paired samples were collected simultaneously and the zopiclone concentrations were compared. The concentrations of zopiclone in OF were generally higher when using the Intercept® Oral Specimen Collection device compared to the Statsure Saliva Sampler™. Wide variations of zopiclone OF/blood concentration ratios (ZOBCR) were found for both devices, the median (range) ZOBCR was 3.8 (1.5-15.9) for the Intercept device and 1.9 (1.2-4.6) for the Statsure device. The correlation between zopiclone concentration in OF collected with the two devices was fairly poor, $r^2 = 0.35$.

6.4 Paper IV

In this paper the zopiclone concentrations in OF and blood after controlled intakes were studied. The concentrations in OF were measured by using the Intercept® Oral Specimen Collection device. All 279 OF samples retrieved 0.5–10 h after intake had zopiclone concentrations above cut-off (0.2 ng/ml). Zopiclone was detected in both OF and blood in 231 OF/blood pairs. A significant, but weak overall correlation between OF and blood concentration was seen (r^2 = 0.30). The median (range) in ZOBCR for all samples was 3.3 (0.8–18). The ZOBCR decreased when the OF volume increased. After 30 of 31 given doses of zopiclone, the ZOBCR was higher in samples collected before lunch than samples collected after lunch. All individuals also delivered an OF sample 23–76 h after intake of zopiclone, and 12 out of 15 of these were positive after intake of 5 mg, and all were positive after intake of 10 mg. For two individuals zopiclone was measurable in low concentrations (0.23 and 0.27 ng/ml) in OF baseline samples obtained 7 and 14 days after intake of 10 mg zopiclone.

6.5 Adverse events (unpublished results)

All reported adverse events and side effects during each study day were registered in a Case Report Form (CRF). The individuals were allowed to take a nap whenever they had the opportunity, and if an individual fell asleep it was reported in the CRF. The adverse events reported after each of the study drugs are shown in Table 6. The individuals were not specifically asked for potential side effects, but spontaneous remarks and the researchers' observations of sleep were registered.

Table 6. Reported adverse events after intake of study drug

	Zopiclone 5 mg	Zopiclone 10 mg	Ethanol	Placebo
Falling asleep*	8	12	4	7
Dysgeusia	2	2	0	0
Visual disturbances / Diplopia	1	4	0	0
Headache	0	0	0	1

* Falling asleep is not really an adverse event of zopiclone since this is the desired effect of the drug

7 Discussion of the main findings

7.1 Aim 1

Impairment of zopiclone in relation to blood zopiclone concentration and similarities/differences between zopiclone and ethanol.

Blood ethanol levels are considered to be an acceptable proxy for the degree of cognitive and psychomotor impairment (132) and this is globally reflected in traffic laws. Similar relationships between blood zopiclone levels and the degree of cognitive and psychomotor impairment has been indicated (137, 143, 147), although not as convincingly as for ethanol. In accordance with former studies (55, 132, 195) we found a positive relationship between the degree of impairment and the blood concentrations of both zopiclone and ethanol.

In Aim 1a, I compared the results of a simplified clinical test of impairment (SCTI) after intake of zopiclone with a standard reference drug of impairment, ethanol. In addition, these results for both drugs were compared to those obtained with the global results of a computerized test battery of impairment. A final goal was to evaluate to what extent a SCTI could yield information comparable to that obtained by methods that are more sophisticated. In Aim 1b, I wanted to find if any test component or combinations of components would be particularly valuable in detecting zopiclone impairment, and if there was a difference between zopiclone and ethanol in this respect. In Aim 1c, it was of interest to find if and how acute tolerance to the effects of zopiclone for various test components developed, as this could indicate changes of importance for zopiclone impairment.

7.1.1 Aim 1a

How useful is a simplified clinical test (SCTI) to measure impairment in comparison with a computerized test (CPTI) after single intakes of zopiclone and ethanol? (Paper I)

How is the SCTI affected by intake of zopiclone and ethanol?

Previous literature on the usefulness of a clinical test in detecting relevant impairment is sparse, especially for zopiclone, other z-hypnotics and benzodiazepines. The only comparable studies where clinical impairment of zopiclone and alcohol is investigated, are two older trials from Finland (202, 203). The study design and the blood drug concentrations detected in the Finnish studies were quite similar to the results in our study. Since the present thesis included both clinical and psychomotor testing in addition to collecting biological samples from the individuals, the time schedule was tight (Figure 2) and therefore, we had to include a selection of subtests from the CTI in the SCTI (Chapter 3.4).

One and a half hours after intake of zopiclone 5 mg we found that about 18 % of the subtests of the SCTI were impaired (compared to the placebo performance), while after intake of 10 mg zopiclone about 29 % of the subtests were impaired. After intake of 50 g ethanol we found that about 17 % of the subtests in the SCTI were impaired. We found that the number of impaired subtests was quite equal for the zopiclone 5 mg dose and the ethanol dose. In contrast to our findings, the Finnish studies demonstrated that alcohol impaired a clinical test, while zopiclone appeared to have minor impairing effects 2 h after intake (202, 203). In the Finnish studies measurement of HGN was also included, a test of a well-known effect of alcohol impairment (97). HGN was not included in the SCTI and this can explain why the Finnish studies found more pronounced impairment of ethanol than zopiclone compared to our study. If other clinical subtests from the CTI had been included in the SCTI the results could have been different. The SCTI failed to detect any major clinical impairment 7 h after intake of zopiclone and ethanol. I am not aware of any studies that have revealed clinical

impairment of zopiclone using the SCTI or a similar test many hours after intake. The possibility of acute tolerance is further discussed under Aim 1c. A study that investigated ethanol showed that 70% of drivers tested with the CTI were judged to be impaired with BAC in the range 0.2-0.5 g/kg, while 83% were concluded as impaired with BAC in the range 0.5-1.2 g/kg (204). This is also a considerably higher occurrence than in the present study and could be explained by different populations.

The sensitivity and the specificity of a clinical subtest's (Table 7) ability to detect impairment according to a threshold zopiclone concentration limit of 23 ng/ml can be calculated. We found a sensitivity of 27 % and a specificity of 88 % (Table 8a). From a previous study of suspected drugged drivers (105), the sensitivity and specificity of the outcome of the CTI can be calculated with a threshold zopiclone concentration of 33 ng/ml. The sensitivity and the specificity among the impaired drivers can be calculated to be 90 % and 29 % respectively. In other words, the sensitivity is considerably higher, and the specificity is considerably lower than we found in the healthy volunteers included in our study. In that study a small number of individuals (*N* = 7) had zopiclone concentrations below 33 ng/ml. The reason for the higher sensitivity can be that the threshold zopiclone concentration is somewhat higher. In the former study only the conclusion of the CTI is evaluated. In our study the conclusion (overall judgment) is included as one of six subtests. The conclusion of the CTI is found to reveal impairment to a higher degree than any of the single subtests (104) and that can explain the higher degree of detected clinical impairment. These results are in accordance with a previous study which found that fewer subjects demonstrate impairment in an experimental setting compared to real-life drug use settings (204). It can also be argued that the physicians performing the CTI might be biased by a subjective expected impairment in a population of suspected drugged drivers, or that the clinical impairment seen in low concentration (low specificity) in the real-life setting is caused by the stress associated with being apprehended by the police and tested by a physician. These explanations are less likely because few (14 %) apprehended

drivers completely negative at toxicology analyses show little impairment in the CTI (108). Another study demonstrated that 26 % of drug naïve users failed the SFST (205).

How is the CPTI affected by intake of zopiclone and ethanol?

Previous studies that have demonstrated more pronounced effects for higher doses of zopiclone, compared to lower doses when applying different psychomotor tests (135, 143, 206-208). One hour after intake of both zopiclone and ethanol we demonstrated significant impairment of more than 50 % of the test components of the CPTI (Figure 2 in Paper I). In accordance with previous studies we found that the fraction of impaired test components increased at the higher concentration of zopiclone. Several large epidemiological studies have shown a BAC-related increasing risk of traffic accident involvement (209, 210) and a deteriorating driving performance in experimental studies (132). In accordance with previous studies we also found that the fraction of impaired test components increased in a BAC-related manner.

Comparison of the SCTI and the CPTI

For both the SCTI and the CPTI we found that the fraction of impaired observations (clinical subtests/test components) increased in a dose- and concentration-related manner after intake of zopiclone; for ethanol we found that the fraction of impaired observations increased in a BAC-related manner. We found a higher proportion of impaired observations applying the computerized tests (CPTI) than the clinical subtests (SCTI). This leads us to conclude that the CPTI was more sensitive than the SCTI. Even though the CPTI demonstrated more impairment than the SCTI the sensitivity of the CPTI is limited. I am not aware of any previous studies that have compared the performance of a clinical test to other more comprehensive “impairment” tests after intake of zopiclone.

Is a clinical test, like the SCTI a suitable tool to evaluate impairment?

Since the SCTI is easy to perform in many settings I wanted to evaluate its usefulness. Our data was processed to calculate the sensitivity and specificity of the SCTI in relation to a certain BAC or

zopiclone concentration level. We wanted to investigate if the SCTI could identify individuals that had BAC above 0.5 g/kg or zopiclone concentrations in blood comparable to this BAC (i.e. 23 ng/ml) (155, 156, 211). A definition of sensitivity, specificity, positive and negative predictive values are given in Table 7 and the given values of these statistical parameters from the present thesis are shown in Table 8.

Table 7. Definition of sensitivity, specificity, positive and negative predictive values

Sensitivity	The probability of a positive clinical subtest/test component if the concentration is above the "threshold concentration"
Specificity	The probability of a negative clinical subtest/test component if the concentration is below the "threshold concentration"
PPV	The probability of a concentration above the "threshold concentration" if a clinical subtest/test component is positive
NPV	The probability of a concentration below the "threshold concentration" if a clinical subtest/test component is negative

A definition of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) is given. The outcome of each single subtest was classified as either "impaired" or "not impaired" compared to placebo performance. The threshold concentrations for ethanol and zopiclone are defined as 0.5 g/kg and 23 ng/ml respectively.

Table 8. Sensitivity, specificity, positive and negative predictive values for the SCTI and the CPTI calculated from the results in the present thesis

a) Zopiclone

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
SCTI	27 %	88 %	51 %	72 %
CPTI	52 %	60 %	47 %	66 %

b) Ethanol

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
SCTI	18 %	96 %	81 %	54 %
CPTI	50 %	65 %	55 %	61 %

The outcome of a subtest/test component was given a performance score after intake of zopiclone and ethanol. A similar score or an improved performance compared with the placebo performance was registered as "not impaired", while a deteriorated performance was registered as an "impaired" performance. Subtest/test component performance were classified according to blood zopiclone concentrations or BACs, above or below/equal to 23 ng/ml or 0.5 g/kg respectively. The sensitivity, specificity, positive and negative predictive values for a clinical subtest (SCTI) or a psychomotor test component (CPTI) according to concentration limits are given for zopiclone (a) and ethanol (b). For the SCTI a total number of 372 and 192 clinical subtests are included for zopiclone and ethanol respectively. For the CPTI a total number of 1395 and 720 test components are included for zopiclone and ethanol respectively.

As seen in Table 8, applying a more sophisticated method to measure impairment, like the CPTI, will increase the sensitivity to detect impairment for blood drug concentrations of zopiclone and alcohol

relevant to impairment of a BAC of 0.5 g/kg. On the other hand, the specificity decreases applying CPTI compared to the SCTI for both zopiclone and ethanol.

In our experimental study of volunteers, we calculated the sensitivity and specificity of a clinical subtest to detect zopiclone concentration above 23 ng/ml (comparable to a BAC of 0.5 g/kg) (Table 8). In this thesis I apply the calculated sensitivity and specificity in a predicted low prevalence population (like a population of random drivers) and in a predicted high prevalence group. These examples demonstrate that both the positive and negative predictive values are directly related to the prevalence of impairment.

Applying the SCTI in a low prevalence group

A former roadside study performed in Norway has estimated (from analyses of OF samples collected roadside from randomly stopped drivers) that the prevalence of driving with a BAC above the legal limit 0.2 g/l was 0.2%, and for zopiclone the prevalence above the legal limit of 12 ng/ml (comparable to BAC of 0.2 g/l) was 0.63 % (212). The prevalence of impaired drivers according to zopiclone concentrations corresponding to a BAC of 0.5 g/l is of course lower. To demonstrate the usefulness of the SCTI in a low prevalence group I wanted to apply the calculated sensitivity and specificity for zopiclone (Table 8a) in a population where 0.63 % is impaired (Table 9). A positive predictive value (PPV) of 1.4 % for zopiclone can be calculated. The NPV in this low prevalence group is 99.5 %. If the calculated sensitivity and specificity for ethanol (Table 8b) are applied in a low prevalence group where 0.2 % is impaired, a very low PPV (0.9 %) and a very high NPV (99.8 %) can be calculated also for ethanol. These calculations show that a positive subtest in this population should not be used as a proof or an indication of impairment by zopiclone or ethanol. This example demonstrates that applying a single subtest in this population has hardly any value. Performing the complete SCTI will add value, but since the PPV is very low and NPV is very high for the single subtests it is not advisable to perform any simplified clinical tests in this low prevalence group as a blind screening procedure.

Table 9. Outcomes of the clinical subtests after intake of zopiclone in a predicted low prevalence group

	Zopiclone conc. > 12 ng/ml	Zopiclone conc. ≤ 12 ng/ml	Sum
Impaired subtests	2	119	121
Not impaired subtests	5	874	879
Summed	6	994	1000

The outcome of each single subtest was classified as either "impaired" or "not impaired" in a low prevalence group in a population of 1000 individuals, applying sensitivity and specificity from Table 8a. A predicted prevalence of 0.63 % is used. Due to rounding of decimals the summation of the subtests for zopiclone concentrations above and ≤ 12 ng/ml is incorrect as it appears in the table. The PPV (positive predictive value) is 1.4 % (1.7 out of 121). The NPV (negative predictive value) is 99.4 % (874 out of 879).

Applying the SCTI in a high prevalence group

Driving related impairment is thoroughly discussed in the literature, but it is also important to pay attention to impairment related to activities of daily living. Elderly women are often prescribed zopiclone (Figure 1). It is well documented that use of z-drugs is associated with significant increased risk of fractures (87). It can be difficult to evaluate the possible impairment effects for the prescribing doctor. If a patient suffering from insomnia attends the doctor´s office for a clinical check some days/weeks after initiating zopiclone, the general practitioner might be in doubt as to whether the patient is impaired by zopiclone or not. Another example of a high prevalence group could be an apprehended driver stopped by the police after observed weaving of a vehicle or other "suspicious driving pattern". The police officers often report that they are in doubt if the driver is impaired by drugs. If we in these examples estimate that there is 50 % probability (prevalence) that the patient/driver is impaired by zopiclone we can calculate the outcome of the clinical tests in this high prevalence population (Table 10). The calculated sensitivity/specificity from our study applied in this high prevalence group gives a PPV of 69 % and a NPV of 55 % for zopiclone. This means that a single positive clinical subtest in this population increases the "probability of impairment" from 50% to 69 %, while a negative result is less helpful. The outcome of a clinical subtest in a predicted high prevalence group of 1000 patients/drivers is shown in Table 10.

Table 10. Outcomes of the clinical subtests after intake of zopiclone in a predicted high prevalence group

	Zopiclone conc. > 23ng/ml	Zopiclone conc. ≤ 23ng/ml	Sum
Impaired subtests	135	60	195
Not impaired subtests	365	440	805
Summed	500	500	1000

The outcome of each single subtest was classified as either "impaired" or "not impaired" in a high prevalence group in a population of 1000 individuals, applying sensitivity and specificity from Table 8a. A predicted prevalence of 50% is used. The PPV (positive predictive value) is 69 % (135 out of 195). The NPV (negative predictive value) is 55 % (440 out of 805).

In conclusion, the usefulness of a simplified clinical test (SCTI) to detect impairment by zopiclone is related to the population where the clinical tests are used. In a low prevalence population, e.g. randomly stopped drivers, the PPV is very low and therefore the risk of a false positive clinical test is too high. In this population the performance of a clinical test to evaluate impairment is not recommended.

By using the SCTI as a tool to evaluate impairment it is important to have in mind, that in our study we investigated young males, and the results would probably be different in another study population. Age-related slowing in cognitive and motor processes includes longer reaction time and movement execution time. Probably an elderly population will fail more clinical subtests than a younger population. On the other hand, this will be corrected for by our study structure since the performance of the clinical subtests was compared to placebo performance.

In our study we have calculated the sensitivity and specificity for the outcome of any single subtest in the SCTI. An alternative to calculating the single subtests per se is to study the combination of more/all subtests. In one study measuring impairment after intake of THC it was found that the best single predictor for driving behavior was overall performance (involving the combination of all three subtests in the SFST) (213).

7.1.2 Aim 1b

Do zopiclone and ethanol affect various components of the computerized test (CPTI) differently? (Paper II)

The three chosen computerized tests and their ability to evaluate impairment

To evaluate impairment we primarily wanted to use cognitive and psychomotor tests that measured impairment according to the defined three levels of behavior related to driving (119). Three different psychomotor tests (CPT, CRT and SOC) were chosen and all contained several test components that evaluated behavior at different levels. The sensitivity of the CPT test assessing driving-related skills after intake of alcohol has been evaluated in a literature review (125). It was concluded that one out of two studies indicated impairment for different psychomotor effects for a high BAC range (0.61–1.0 g/l), but in the medium BAC range (0.31–0.6 g/l), only a few of the studies indicated impairment. The CRT test measures response speed. The same review also evaluated alcohol effects on two-choice reaction time (TCRT) at medium and high BACs. In five out of nine measurements, assessed in eight studies, an increase of reaction time at a medium BAC was found; all five reviewed studies indicated impairment at a high BAC. The Stockings of Cambridge (SOC) test is a computerized version of the TOL test. The TOL is a decision-making task that measures executive function and planning (122). One study found that the TOL was not affected by intake of alcohol at medium and high BACs (214), while another study showed that alcohol could indicate impairment at high BAC on the TOL test (215). These psychomotor tests' ability to detect impairment of ethanol is, as shown above, evaluated in previous studies, the ability to detect impairment induced by other drugs, such as zopiclone, is however, less investigated.

Comparison of impairment of zopiclone and ethanol

In Paper II the results for different test components during the period after intake are investigated. We found more impairment for zopiclone 10 mg than ethanol for test components at behavior level

1, while the number of impaired test components at levels 2 and 3 were similar for zopiclone 10 mg and ethanol (Table 4). Impairment was most pronounced a short time after intake, i.e. 1 h after intake. We found less impairment for the test components after 3.5 h and none of the test components were impaired 6.5 h after intake. In the unpublished results the proportions of the 16 individuals that showed deteriorated placebo-compared performance 1 h after intake were investigated and a new categorization of the test components was introduced. Zopiclone showed most impairment for test components measuring reaction time, while ethanol showed most impairment for test components measuring impulsivity. The demonstrated difference in pharmacodynamics between alcohol and zopiclone is in accordance with the small number of previous studies addressing this question (56, 180, 216).

The dose-dependent deteriorated performance for zopiclone shown for the performance 1 h after intake is demonstrated for 6 of the 7 test components that are categorized to measure reaction time (Figure 4b). This shows that reduced reaction time and probably sedative effects are closely related to the given zopiclone dose. Since the mean zopiclone concentration 1 h after intake of zopiclone 10 mg was about double of the concentration after intake of zopiclone 5 mg, 39 ng/ml and 19 ng/ml respectively, the dose-dependent deteriorated performance is also related to the zopiclone concentration in blood. For all the test components that measure attention/cognition and half of the test components that measure impulsivity this dose-dependent performance was not seen for zopiclone. An explanation could be that the deteriorated performance of these tests is more closely related to the presence of zopiclone and not directly to the concentration of zopiclone, and the most pronounced deteriorated psychomotor performance appears before reaching the maximum zopiclone concentration in blood.

In the new categorization we found that 3 of the 4 test components categorized to measure impulsivity show that more than 70 % of the performances are deteriorated compared to placebo

performance after intake of ethanol (Figure 4b). The inhibitory deficits of ethanol are well known (217-219).

The new categorization presented in this thesis adds awareness of the difference between increased impulsivity for ethanol and reduced reaction time for zopiclone. Since zopiclone acts selectively on the $GABA_A$ receptors, decreased reaction time might be a specific $GABA_A$-mediated effect, while the reduced impulse control (impulsivity) for ethanol is probably mediated by other receptors. For highly complex activities, like driving a vehicle or maneuvering advanced machinery, both impulsivity and reaction time are of importance since these activities involves a wide range of cognitive, perceptual, and motor activities (125).

It can be concluded that zopiclone and ethanol affect various components of the computerized test (CPTI) and impairment by zopiclone and ethanol are expressed differently. For zopiclone, psychomotor tests that measure reaction time will be more likely to detect impairment, while tests that measure impulsive responses are more likely to detect impairment of ethanol.

7.1.3 Aim 1c

How is the acute tolerance to zopiclone in various components of computerized tests (CPTI)? (Paper II)

I documented acute tolerance for zopiclone in psychomotor test components categorized into behavioral level 1 and for test components measuring reaction time. I defined acute tolerance as tolerance to drug effects in the period after a single intake of a drug, i.e. improved performance on the descending concentration compared with the same concentration on the ascending concentration.

Acute tolerance for the effect of ethanol is well known. As early as 1919 Mellanby et al. noted that motor skills were impaired at a lower BAC-level when BACs were rising compared with when BAC were descending, a phenomenon referred to as acute tolerance (220). Even though acute tolerance has been a known phenomenon for decades and ethanol is a thoroughly studied substance, little attention has been given to the pharmacokinetic profile of ethanol and whether the performance tests were made on the absorptive or postabsorptive limbs of the BAC curve (221). Acute tolerance was demonstrated in a study of cognitive and psychomotor performance (222) and acute tolerance for different impairing effects, such as motor coordination, reaction time and subjective ratings of intoxication is demonstrated in several studies (223-225). In a study, information processing and selective attention were shown to be better on the descending limb compared with the same BAC on the ascending limb (226). In another study, 20 adults performed a test battery that measured simulated driving, other psychomotor tests and subjective intoxication ratings after intake of placebo and a moderate dose of alcohol (0.65 g/kg). The authors found that motor coordination and subjective intoxication showed acute tolerance, whereas driving performance and inhibitory control showed no recovery from impairment (227). A large meta-analysis of experimental studies of psychomotor impairment did not find any acute tolerance effect of ethanol when they separated observations into absorption phase and elimination phase (132). A few studies have demonstrated acute tolerance for willingness to drive (228, 229) after intake of alcohol. This can cause drivers to

underestimate their level of intoxication, particularly when their BAC is declining. They may decide to drive even though other essential aspects of their driving and cognitive performance are still impaired. Self-evaluation of the impairment effects of alcohol is in general poor, but this appears to be particularly inaccurate during declining BACs. If a driver is aware of the rapid recovery of some behavior functions when BAC remains high, it may lead them to think they are safe to drive (230). On the other hand, however, several studies have failed to observe development of acute tolerance for measures of inhibitory control (224, 231, 232). The acute tolerance for ethanol effects might vary depending on which ethanol effect is evaluated.

To my knowledge acute tolerance for zopiclone is only sparsely investigated in previous studies (195), but is investigated in a few studies regarding benzodiazepines. Ingum found that acute tolerance develops with respect to some psychomotor skills, most pronounced for simple reaction time after administration of a related hypnotic drug flunitrazepam (1 and 2 mg), and that tolerance is expressed after approximately 4–6 h following intake (233). In that study reaction time was measured, and our findings are in accordance to what Ingum found for flunitrazepam. In the same study no acute tolerance was found for diazepam. Acute tolerance is also found for benzodiazepines like alprazolam, diazepam (234) and clonazepam (235).

The mean zopiclone concentration 1 h after intake of 5 mg zopiclone was similar to the mean zopiclone concentration 6.5 h after intake of 10 mg zopiclone (Paper II, Figure 1). Since impairment was not observed 6.5 h after intake, this could be an indication of acute tolerance. The investigation of acute tolerance presented in the unpublished results was performed based on the fact that the median and mean concentration of zopiclone for the test performed at 1 h after intake were quite equal to the median and mean concentration for the tests performed at 3.5 h after intake (Table 4). These similar concentrations were seen after ingestion of both doses of zopiclone. Frequency (share of the 16 individuals) that showed acute tolerance is indicated by a positive bar in Figures 5a and 5b. The blood samples retrieved after 1 h are in the absorption phase after intake of zopiclone, and

therefore the measured zopiclone concentrations of the individuals have a wide range. The rapid ascending concentration in the absorption phase illustrates the difficulties in investigating acute tolerance in clinical studies. I found acute tolerance for test components categorized to measure reaction time and behavior level 1 (automative behavior). Zopiclone enhanced impulsivity (Fig. 4b), although not as pronounced as ethanol. The degree of acute tolerance to impulsivity was, however, variable. Deteriorating impulse control is not a typical effect of zopiclone and benzodiazepines and acute tolerance for this effect has therefore not been the main focus in previous studies.

Surprisingly, for two of the test components (CRT r time and SOC plan) acute tolerance is found for one of the doses of zopiclone, but not for the other dose. For the test component SOC plan this lack of acute tolerance for the 10 mg zopiclone dose can also be seen in Figure 2 in Paper II. This indicates that these test components do not develop acute tolerance. The instruction ahead of the performance of the SOC on how to plan the performance of the SOC test could perhaps have been better, and the results of the SOC plan test components are perhaps not as reliable as the other test components.

In a previous paper regarding the same clinical study described in this thesis (195), acute tolerance was investigated in a different way. An estimated blood drug concentration of zopiclone and ethanol was grouped into four concentration groups. Thereafter the psychomotor performance was dichotomized to “impaired” or “not impaired” for each test result of the test components, for each individual, based on their baseline performance (on the same study day). The mean Tmax of ethanol was 1 h. When investigating acute tolerance in that study only the calculations from the CPT test were used, because the CPT test was the only test performed before 1 h after intake. The observations were separated into those obtained before and after 1 h after intake. The share of impaired observations for the CPT test components per blood drug concentration group related to whether the blood concentration represented a short (<1 h) or long time (>1 h) after intake were

calculated. It was concluded that acute tolerance was found for both ethanol and zopiclone. Acute tolerance for different effects or behavior levels was not investigated in that paper.

Like other benzodiazepines and z-hypnotics, zopiclone acts selectively on the GABAA-receptors. The GABAA-receptors are allosterically modulated by zopiclone, mediate a depressant effect and slow down the processes of the central nervous system. Zopiclone and other benzodiazepines do not directly affect receptors for other neurotransmitters. Since acute tolerance for zopiclone was mainly found for reaction time and not for impulsivity, it might indicate that primary GABA agonism may lead to different effects, and these effects may have different potential for acute tolerance. Since acute tolerance for zopiclone is found for reaction time, one can speculate that the α1-subunits at the GABAA–receptors, mediating sedation (13), are involved in developing acute tolerance to this effect.

Tolerance to drugs can be divided into acute and chronic (long-term). Chronic tolerance to drugs develops when an individual adapts to constant exposure to a drug over weeks or months. A further discussion of chronic tolerance to benzodiazepines / z-hypnotics is outside the scope of this thesis, but the possible lack of chronic tolerance to different psychomotor effects is described in a meta-analysis from 2004 (236, 237) and a possible partial tolerance to benzodiazepines is described in a recent review (238).

Acute tolerance to different effects of a drug is theoretically important because this can generate knowledge and explain differences and similarities between different drugs. This is also important for practical reasons. Reaction time might be related to the sedative effects. If tolerance is generated to the sleep-inducing effects of zopiclone and the patient awakens in the middle of the night, some patients could ingest another dose of zopiclone even though zopiclone is still present in blood. This additional dose can lead to unfortunate side effects for other effects of zopiclone where tolerance is not developed.

7.2 Aim 2

Can measurement of OF zopiclone concentration substitute blood zopiclone concentration?

For most psychoactive drugs, the concentration in blood is closely related to the amount of drug reaching the CNS and the pharmacologic response. The use of OF to determine the magnitude of impairment would require a rather strong and constant correlation between the concentrations in OF and blood. Drugs are detected in OF either because residual drug is present in the oral cavity after recent "oral" intake or because of drug transfer from blood into saliva/OF. If a drug is ingested orally, sublingually, by snorting or inhalation, a reservoir of drugs can be deposited in the oral cavity. If a drug is ingested recently, very high concentrations can be detected in OF (Crouch 2005). In our laboratory we have experienced very high concentrations of buprenorphine in OF in some cases (131). Actually, the concentrations were so high that the laboratory analyzing the OF sample experienced difficulties. The very high concentration in OF was a result of residual buprenorphine in the oral cavity, since the OF sample was collected simultaneously or shortly after the patient had taken a sublingual buprenorphine tablet.

In our study, zopiclone was given in capsules, which were swallowed whole and dissolved in the stomach. Therefore, local deposition/contamination of the oral cavity was avoided. The zopiclone detected in OF in our study therefore originated entirely through transfer from blood, and not from contamination of the oral cavity. Zopiclone will pass through the capillary wall, the basement membrane, glandular epithelial cells and into the salivary duct (239). Several factors affect the drug transfer from blood into saliva. This may explain the observed huge inter-individual differences in the ratio between the concentration in OF and the concentration in blood (ZOBCR). Based on the vast variance in ZOBCR, measurement of the OF zopiclone concentration cannot substitute determination of the zopiclone concentration in blood.

7.2.1 Aim 2a

Is OF zopiclone concentration dependent on the OF sampler device? (Paper III)

A total of 21 parallel samples of OF using Statsure and Intercept devices were obtained combined with simultaneously taken blood samples. The median ZOBCR for Intercept was 3.8 (range 1.5–15.9) and for Statsure 2.3 (range 1.2–4.6). The concentration of zopiclone in OF thus demonstrated some dependence on the OF collection device used. OF can be collected by a variety of methods and by applying different collection devices. The easy, but very time consuming, draining method is performed by allowing OF to drip from the mouth into a container. Other methods have been developed to stimulate saliva production, by mechanical chewing (240) or by placing citric acid in the mouth (241).

The main difference between the two devices used in our studies is that the OF collection pad of the Intercept device contains saliva-stimulating agents whereas the collection pad of the Statsure device does not. Huge variations in concentrations in OF due to different OF collection methods have been found for several drugs such as codeine (242), cocaine (and metabolites) (243) THC, and methamphetamine (244).

Some of the OF samples were stored in a freezer and analyzed some weeks later according to the analytical capacity in the laboratory. The instability of zopiclone in biological matrices is well known (193, 245) and it can be questioned whether the mean higher ratio for the Intercept device was due to larger instability in the Statsure device. This was investigated by performing stability studies of two separate OF sample pools made by mixing large numbers of OF samples collected from drug users by using the Intercept device or the Statsure device (191). These two sample mixtures were kept 3 months in a freezer at – 20 °C. For the Intercept device, the zopiclone concentration was 83 % of the originally measured concentration, and for the Statsure device the concentration increased to 111 % of the originally measured concentration. We found that the ZOBCR for the Intercept device was

higher than for the Statsure device. The vast difference in ZOBCR can therefore not be explained by a greater instability in the Intercept device. However, the results of the stability study should be interpreted with caution since only one OF sample pool was investigated for each device.

In Paper III, the median (3.8) and range (1.5–15.9) of the ZOBCR for the Intercept device is calculated based on 21 random selected OF/blood pairs. In Paper IV the ZOBCR was calculated based on all 231 OF/blood pairs where zopiclone concentration was measurable in blood (and OF). The median ZOBCR was quite similar (3.3). Since the number of OF/blood pairs was 11 times higher, the wider range (0.8–18) was expected. A large variation of the ZOBCR has also been found for many other drugs (173, 174).

Different commercial OF collection devices show differences in drug absorption (recoveries) (181). It has also been reported that the volume of OF recovered from the devices for use in the laboratory varied between 50 % and 90 % (242). The amount of OF collected was different for the two OF collection devices, but the volume was determined by weighting the samples in order to correct for the different amounts of OF collected when calculating drug concentrations in neat OF.

In our study, we found a fairly poor correlation, r^2 = 0.35, between the OF concentrations in the two devices (Paper III). The correlation is quite similar to the correlation we found between OF and blood, r^2 = 0.30, for the Intercept device (Paper IV). This indicates that the variation in OF concentration between the Intercept and Statsure device is vast and can vary as much as the variation in concentration between OF and blood. In many studies, the authors have neglected to address the effects of the collection procedures and devices on their results (246). In interpretation of the concentration of zopiclone (or another drug) in OF, it is important to be aware that OF volume/drug recovered from the device, and stimulation of OF production varies in different devices. A weakness of many previous studies is that the amount (volume) of OF delivered is not measured. If the OF is mixed with a buffer solution in the collection device, it is not possible to accurately calculate the correct concentration of the neat OF collected without correction for sample volume.

To conclude, the zopiclone concentration in OF is dependent on the OF sampler device. We measured higher zopiclone concentrations in samples collected by the Intercept® Oral Specimen device (with a sampling pad treated with saliva stimulating chemicals) than the Statsure Saliva Sampler™ (not treated with saliva stimulating chemicals). The variation of the ZOBCR was also higher for the Intercept device.

7.2.2 Aim 2b

Is the relation OF-zopiclone-concentration/blood-zopiclone-concentration constant, and if not, which factors are influencing the ratio? (Paper IV)

OF/blood concentration ratio (ZOBCR)

We found a median ZOBCR of 3.3 (range 0.8–18) when using the Intercept sampling device. Vast intra- and inter individual differences in ZOBCR were demonstrated. When the individual ZOBCR was shown, one or more samples from 13 out of the 16 individuals had outliers (defined as ZOBCR greater than 1.5 times the interquartile range) of the ZOBCR (Paper IV, Figure 4). The mean ZOBCR and the interquartile range differed much for different individuals. The correlation between OF and blood concentration ratio for zopiclone was investigated in a former study (174). In that study, the correlation was higher (R^2=0.636) than the correlation in our study (R^2=0.30). The number of OF/blood pairs was higher in our study (*N*=231) compared to the study by Langel (*N*=6). In the former study, OF samples were collected with the Statsure Saliva Sampler™ and the population consisted of drivers suspected of driving under the influence, drivers stopped randomly roadside and injured drivers admitted to hospital after a traffic accident. It is difficult to compare the correlation between the concentration in OF and blood found in our study, to the correlation found in the previous study by Langel et al., since two outliers were removed before calculation of correlation in the previous study. In Paper III we demonstrated that the variation (range) of ZOBCR was greater for the Intercept device (applied in our study), than for the Statsure device (applied in the study by Langel). Another explanation for the higher correlation found in Langel's study, can be the OF collection device that was applied.

In an older study, a mathematical model was developed to predict OF/blood concentration ratios of drugs (247). This theoretical calculation was also modified and used in a study by Caille (30) which presented this equation to predict the theoretical OF/blood concentration ratio:

Theoretical OF/blood ratio = $(1 + 10^{pKa-pH(OF)}) / (1 + 10^{pKa-pH(blood)})$

Zopiclone is a lipophilic substance with a pKa of 6.89 (248). Assuming a pH in OF of 6.5 and a pH in blood of 7.4 a theoretical ZOBCR of 2.6 can be calculated. In Paper IV the calculated theoretical ZOBCR (1.7) is lower because different pKa and pH(OF) are used. As the equation indicates, the ratio is highly dependent on the pH in OF and blood and the pKa of the drug. It must be noted that other important factors affecting drug concentrations in OF, like drug size, protein binding, lipophilicity of the drug and the sampling device/procedure are not variables in the equation. This implies that the mathematical equation to predict the OF/blood concentration ratios only should be considered as a guiding tool.

The 231 OF/blood pairs in our study originated from the 16 volunteers enrolled in the study where concentrations of zopiclone above the cut-off in blood were found. The OF and blood samples originated from only two single intakes of zopiclone. Therefore, it is important to note that the OF/blood pairs were not completely unrelated. A complex mixed model statistical analysis could have been performed to investigate this further.

Transfer of zopiclone into OF

Since the time to reach maximum concentration was quite equal in OF and blood, the process of transfer of zopiclone from blood to OF is not considered to cause any delay. Several factors affect the drug transfer from blood into saliva, such as pKa, physical size, degree of protein binding and lipophilicity of the drug (246, 249).

We found for the first time that the concentration of zopiclone in OF (and the ratio ZOBCR) decreased after intake of a light meal (lunch). Intake of food and chewing stimulates the gland to produce saliva. The salivary flow rate affects the salivary distribution of drugs (249) and an explanation for the decreased zopiclone concentration could be that the diffusion of zopiclone from the circulating plasma could not keep up with the increased saliva secretion rate. This dilution effect

was previously suggested for methamphetamine in a controlled study (250). This is also in accordance with a previous study that showed decreased OF concentrations for codeine when the OF sample was collected after acidic stimulation (242).

Zopiclone is slightly basic, so when entering into saliva, partitioning will occur, since the pH of saliva in general is more acidic than in blood. Previous studies have showed that basic drugs in OF are largely influenced by salivary flow. An increased flow usually increases the pH. For this reason, unstimulated saliva has a low pH and stimulated saliva has a higher pH (251). In our study the served lunch stimulated increased salivary flow, probably causing an increased pH in OF. The raised pH decreased zopiclone's movement from blood to OF by decreasing its degree of ionization. This can be a possible additional explanation for the low zopiclone concentration for the OF sample retrieved shortly after intake of lunch.

Recovery volumes of OF

By the set-up of our study we investigated the relationship between the collected volume of OF and the ZOBCR. In a previous study of about 22 000 OF samples collected in five population studies (mainly drivers) in Norway it was found that the prevalence of alcohol and drugs was found to be higher in OF samples with small volumes than those with large volumes (252). In the population of impaired drivers in Norway, abusers of illegal and legal drugs are overrepresented. Abusers of drugs, e.g. amphetamines and opioids, often suffer from hyposalivation. The increased prevalence of drugs in the small OF volume group could be explained by the fact that drug abuse may lead to small amounts of OF. Since our study was a randomized controlled trial, selection bias was eliminated. The participants of the study were healthy volunteers and no drug (ab)users were included in the study. In accordance with the previous study we found that OF samples with low volume expressed higher ZOBCR, even though the statistical correlation is weak (Pearson correlation R^2=0.17). The zopiclone concentrations in OF samples with low volume tended to demonstrate larger variation in ZOBCR and to include more ZOBCR outliers. The OF volume was measured by weight determination. For the low

volumes it is especially important that the weight of the OF is accurate, as weight in-accuracy may have contributed to a larger variation of the ZOBCR.

We showed that if the OF samples with low volumes (less than 0.4 ml) were excluded, this would lead to a lower zopiclone concentration in OF, and thereby a lower ZOBCR (median reduced from 3.3 to 2.8), and a lower ZOBCR interquartile range (reduced from 2.1–4.9 to 1.9–4.3). The number of extreme outliers (defined as ZOBCR greater than 3x the interquartile range) were reduced from 7 to 2. Many of the next generation OF sampling devices (e.g. Intercept i2) have a color indicator window on the shaft that changes color once 1 ml of OF is sampled. Applying this "volume indicator window" will to some extent ensure that sufficient amount of OF is collected. The concentration of zopiclone in OF and the ZOBCR, however, will be lower.

In our laboratory, several thousand analyses of OF are performed routinely for illegal and legal drugs every year, and the new generation Intercept device with the 1 ml volume indicator is used. Some individuals have to keep the Intercept sampling set in their mouth for a very long time (more than 15 minutes) because the volume indicator does not indicate that enough OF volume is collected. In many cases the Intercept sampling device is thrown in the trash due to "invalid amount of OF collected". Our study shows that even if small amounts/volumes of OF are collected, zopiclone (and probably other drugs) can be detected. Our advice to these customers is therefore that all OF sampling should always be sent in for analysis even though a sufficient volume of OF is not collected within 5 minutes. An OF sample should not be discarded due to "invalid amount of OF collected". Since the amount of OF fluid collected is quite variable (range in our study 0.07-1.02 ml), it is not possible to perform a quantitative interpretation of the zopiclone-concentration without correction for sample volume.

When investigating impairment, OF sampling can be applied for a drug screening test, even if small volumes of OF are collected. Detection of zopiclone in OF should initiate blood sampling. On the

other hand, if the zopiclone concentration in OF is important per se, the OF volume collected is crucial.

Detection time of zopiclone in OF / interpretation of analysis

Our study was not designed to investigate the duration of the detection time of zopiclone in OF. When planning the study, we settled on an interval of at least one week between each study day. We considered that a "washout" time of a week was necessary to eliminate zopiclone from all biologic matrices. When analyzing the data, we were quite surprised to find that we detected zopiclone in baseline samples from two individuals that had ingested 10 mg zopiclone 7 and 14 days earlier. The zopiclone concentrations were measurable just above the cut-off limit (0.23 ng/ml and 0.27 ng/ml). The volunteers were healthy, not treated for insomnia, and we had no indication or suspicion that zopiclone was used by the participants in the interval between the study days. A detection time up to 14 days is very long after intake of a single dose of zopiclone. By using the estimated half-life from blood concentration for every single individual, all individuals were expected to have blood zopiclone concentrations far below the applied cut-off 24 h after intake of zopiclone (Chapter 1.2.4.2). The ratio between the median maximum concentration of zopiclone and the applied cut-off was higher in OF than in blood. This can to some degree explain the prolonged detection-time in OF, but detection times longer than 2–3 days must be explained by other reasons. Accumulation/storage in oral mucosa can be an explanation, even though this is not described in previous literature. The demonstrated prolonged detection time of zopiclone is in contrast to the known short elimination half-life. A pharmacokinetic explanation for this can be that there is a two or multi-phase elimination of zopiclone, which is seen e.g. for THC (253, 254), and buprenorphine (255, 256). When the zopiclone concentration is below measurable concentrations in blood, the elimination half-life might probably be longer than in the initial elimination phase. The prolonged detection time of zopiclone in OF after a single intake may have contributed to the high prevalence of zopiclone in OF collected roadside in Norway (257).

The detection time of zopiclone in OF was investigated in a recent study from our laboratory. Bruun et al. designed a pharmacokinetic study investigating detection time of the benzodiazepine oxazepam and zopiclone in OF and urine (258). In that study, ten healthy volunteers received 7.5 mg zopiclone and urine/OF samples were collected twice daily for the following 6 days. OF samples were collected with the Intercept®i2 Oral Fluid Collection device and the limit of quantification was lower (0.04 ng/ml) compared to the measured concentrations in the two baseline samples (0.23 and 0.27 ng/ml) in our study. One of the 10 volunteers still had measurable zopiclone concentrations in the last OF sample 6 days after ingestion of 7.5 mg zopiclone. In our study, a higher dose was applied, and a higher number of individuals included, which might explain the extremely long detection time detected.

The prolonged excretion of zopiclone in OF can not only be explained by the fact that zopiclone is well suited for passive transfer into saliva. Other transport mechanisms like P-glycoproteins and organic anion and cation transporters could be involved. Other drugs like metformin and lithium are substrates of such transporters and are present in high concentrations in saliva (259, 260). Such transport mechanisms have not been described for zopiclone, but if present, this could partly explain both the high ZOBCR and the long detection time for zopiclone in OF (258). It is described that other drugs can be redistributed and stored in different human tissue, for instance it is demonstrated that THC is deposited in fat tissue (261), resulting in long terminal elimination half-life of THC. To my knowledge, such a reservoir in any human tissue is not described for zopiclone.

Zopiclone is a sedative drug that has been reported involved in drug-facilitated crimes like sexual assaults and robbery (262, 263). One practical important consequence of the long detection time of zopiclone in OF could be in the context of suspected drug-facilitated sexual assaults where the biologic sample of the offended often is retrieved a long time after the incident.

In our study, only the parent drug, zopiclone was analyzed in OF, the metabolites N-desmethylzopiclone and zopiclone N-oxide were not analyzed. These metabolites have been

previously analyzed in urine (38, 264), but studies that have analyzed these metabolites in OF have not been published. Analysis of metabolites in different matrices can give additional information and help in interpretation of a positive analytic result for many drugs. The excretion of drugs into urine requires hydrophilic compounds, which are often facilitated by metabolic conversion of drugs into more water-soluble metabolites. Lipid-soluble molecules are more likely to transfer from blood to OF (246). By a theoretical assumption the metabolites of zopiclone are probably more difficult to detect in OF than the parent drug, zopiclone, and it is doubtful that analysis of the metabolites of zopiclone in OF will be a fruitful contribution for interpreting drug test analysis.

In forensic toxicology testing urine is the most frequently used biologic material for analysis. Along with the analysis of the requested drug, analysis of creatinine is performed. Creatinine serves as a documentation that the material analyzed is urine, and also serves as a marker for detecting diluted (adulterated) urine samples (265). There is not established knowledge of any endogenous substance to ensure that a valid and representative OF-specimen has been collected. It has been suggested that an OF specimen was valid if it contained ≥0.1, 0.5 or 1.0 µg/ml of Immunoglobulin G (IgG) (266). A study of six volunteers found that even if the mouth was rinsed with 50 ml tap water twice right ahead of OF sampling, the IgG concentration was still approximately 2 standard deviations above the 1.0 µg/ml criterion, questioning the utility of the IgG concentration as an indicator of OF specimen validity (159). Obviously, more studies are needed to ensure that IgG concentration is an acceptable marker for specimen validity. I am not aware of any other endogenous substance/marker argued to document specimen validity.

It can be concluded that even though some correlation between concentration in OF and blood was found, we demonstrated that the ZOBCR had a large variation and range, and the intra- and inter individual differences were vast. The ZOBCR was dependent on several variables, such as amount of OF delivered and intake of food. Due to the huge variation of ZOBCR OF is not suited to substitute

blood sampling. On the other hand, due to long detection time, OF sampling is a valuable tool to confirm intake of zopiclone.

It has to be noted that plasma zopiclone is found as both free unbound drug and bound to proteins. Drug concentrations in OF usually reflect unbound drug in plasma (blood) (249). For some drugs the unbound fraction in blood is more representative for the concentration at neuron receptors than the concentration in whole blood (267). It can therefore be speculated that the zopiclone concentration in OF can be closely related to the concentration in the CNS, where the drug has its pharmacodynamic effect. In our study, we have measurement of concentration in OF at the time of performance of the psychomotor tests. Therefore, a check of the performance of the test component CPT r time in our data set was done. The mean performance score of the 5 individuals who had the highest zopiclone concentration in OF 1 h after intake of zopiclone was quite equal to the mean performance score of all 16 individuals. The data set is therefore so far not further investigated regarding psychomotor performance in relation to zopiclone concentration in OF. Further research is needed to test whether this is a sustainable theory. Clinical studies where psychomotor and cognitive human performance is related to the concentration of zopiclone and other drugs in OF are still warranted.

8 Conclusions

At the time when zopiclone and ethanol reached maximum concentrations in blood we found for both the SCTI and the CPTI that the fraction of impaired observations (clinical subtests/test components) increased in a dose- and concentration-related manner. The CPTI were more sensitive than the SCTI. The SCTI were found to have a low sensitivity to detect impairment above BAC 0.5 g/kg and zopiclone concentration comparable to this BAC (23 ng/ml). It is not recommended to apply the SCTI in a population of predicted low prevalence of impairment as a blind screening procedure. Zopiclone and ethanol affect various components of the CPTI and impairment of zopiclone and ethanol are expressed differently. Impairment of psychomotor tests that measure reaction time is more likely to be detected after intake of zopiclone, while tests that measure impulsive responses are more likely to be detected after intake of ethanol. We found acute tolerance to zopiclone in psychomotor test components that were categorized into behavior level 1 (automative behavior) and for test components measuring reaction time.

The zopiclone concentration in OF is dependent on the OF sampler device. We measured higher zopiclone concentrations in samples collected by the Intercept® Oral Specimen device than the Statsure Saliva Sampler™. The variations of OF/blood concentration ratios were higher for the Intercept® Oral Specimen device. There is some correlation between concentration in OF and blood, but we demonstrated that the ZOBCR had a large variation and range, and intra- and inter-individual differences were vast. The ZOBCR was dependent on several variables, such as amount of OF delivered and intake of food. Due to the huge variation of ZOBCR OF is not suited to replace blood sampling. On the other hand, due to the long detection time, OF sampling is a valuable tool to confirm intake of zopiclone.

9 Practical implications

- A clinical test of impairment can be used to evaluate impairment in populations where zopiclone is prevalently used, e.g. if a physician wants to evaluate daytime impairment in a patient being prescribed zopiclone.
- Clinicians should be aware that therapeutic doses of zopiclone lead to impairment comparable to rather high BACs, and that there is a dose- and concentration-related deteriorated performance.
- The majority of impairing effects of zopiclone are markedly diminished 6–7 h after intake.
- Acute tolerance to zopiclone is developed differently to different cognitive and psychomotor effects of zopiclone. This can be important for night awakenings due to waning of hypno-sedative effects, leading to a new intake which possibly is increasing present impulsivity.
- In the context of evaluating impairment measurement of zopiclone concentration in OF cannot substitute measurement of zopiclone concentration in blood. OF zopiclone analysis can, however, be applied as a screening method, ahead of subsequent blood sampling.

10 References

1. Wagner J, Wagner ML. Non-benzodiazepines for the treatment of insomnia. Sleep Medicine Reviews. 2000;4(6):551-81.
2. Everitt H, Baldwin DS, Stuart B, Lipinska G, Mayers A, Malizia AL, et al. Antidepressants for insomnia in adults. The Cochrane Database of Systematic Reviews. 2018;5:CD010753.
3. de Zambotti M, Goldstone A, Colrain IM, Baker FC. Insomnia disorder in adolescence: Diagnosis, impact, and treatment. Sleep Medicine Reviews. 2018;39:12-24.
4. Brewster GS, Riegel B, Gehrman PR. Insomnia in the Older Adult. Sleep Medicine Clinics. 2018;13(1):13-9.
5. Terzano MG, Rossi M, Palomba V, Smerieri A, Parrino L. New drugs for insomnia: comparative tolerability of zopiclone, zolpidem and zaleplon. Drug Safety. 2003;26(4):261-82.
6. Speaker SL. From "happiness pills" to "national nightmare": changing cultural assessment of minor tranquilizers in America, 1955-1980. Journal of the History of Medicine and Allied Sciences. 1997;52(3):338-76.
7. Wick JY. The history of benzodiazepines. The Consultant Pharmacist: The Journal of the American Society of Consultatnt Pharmacists. 2013;28(9):538-48.
8. Licata SC, Rowlett JK. Abuse and dependence liability of benzodiazepine-type drugs: GABA(A) receptor modulation and beyond. Pharmacology, Biochemistry and Behavior. 2008;90(1):74-89.
9. Hajak G, Muller WE, Wittchen HU, Pittrow D, Kirch W. Abuse and dependence potential for the non-benzodiazepine hypnotics zolpidem and zopiclone: a review of case reports and epidemiological data. Addiction. 2003;98(10):1371-8.
10. Berg C, Sakshaug S, Handal M, Skurtveit S. Z-hypnotika - sovemidlene som dominerer markedet i Norge [Norwegian]. Norsk Farmaceutisk Tidsskrift. 2011;4:20-3.
11. Heydorn WE. Zaleplon - a review of a novel sedative hypnotic used in the treatment of insomnia. Expert Opinion on Investigational Drugs. 2000;9(4):841-58.
12. Schroeck JL, Ford J, Conway EL, Kurtzhalts KE, Gee ME, Vollmer KA, et al. Review of Safety and Efficacy of Sleep Medicines in Older Adults. Clinical Therapeutics. 2016;38(11):2340-72.
13. Gunja N. The clinical and forensic toxicology of Z-drugs. Journal of Medical Toxicology. 2013;9(2):155-62.
14. Bacon ER, Chatterjee S, Williams M. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering Comprehensive Medicinal Chemistry II, Chapter 6.06.6.4.4.1 Zopiclone, zolpidem, and eszopiclone. 2007;6:139-67.
15. Brielmaier BD. Eszopiclone (Lunesta): a new nonbenzodiazepine hypnotic agent. Proceedings (Baylor University. Medical Center). 2006;19(1):54-9.
16. EMA. European Medicines Agency. Seprator Pharmaceuticals Ltd withdraws its marketing authorisation application for Lunivia (eszopiclone). http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2009/11/news_detail_000083.jsp&jsenabled=true Press release 15.05.09.
17. Greenblatt DJ, Zammit GK. Pharmacokinetic evaluation of eszopiclone: clinical and therapeutic implications. Expert Opinion on Drug Metabolism & Toxicology. 2012;8(12):1609-18.
18. Norwegian Medicines Agency (Legemiddelverket). www.legemiddelverket.no, accessed 17.01.20.
19. Mellingsaeter TC, Bramness JG, Slordal L. [Are z-hypnotics better and safer sleeping pills than benzodiazepines?]. Tidsskrift for den Norske Legeforening. 2006;126(22):2954-6.
20. Norwegian Prescription Database, www.norpd.no.
21. Summary of Product Characteristics (SPC). Imovane tablets 5/7.5 mg. Norwegian Medicines Agency [Norwegian]. www.legemiddelverket.no. Updated 17.06.19.

22. Summary of Product Characteristics (SPC). Stilnoct tablets 5/10 mg. Norwegian Medicines Agency [Norwegian]. www.legemiddelverket.no. Updated 18.03.19.
23. Sakshaug S, Handal M, Hjellvik V, Berg C, Ripel A, Gustavsen I, et al. Long-term Use of Z-Hypnotics and Co-medication with Benzodiazepines and Opioids. Basic & Clinical Pharmacology & Toxicology. 2017;120(3):292-8.
24. Ineke Neutel C, Skurtveit S, Berg C. Polypharmacy of potentially addictive medication in the older persons--quantifying usage. Pharmacoepidemiology and Drug Safety. 2012;21(2):199-206.
25. Farkas RH, Unger EF, Temple R. Zolpidem and driving impairment--identifying persons at risk. The New England Journal of Medicine. 2013;369(8):689-91.
26. Glass J, Lanctot KL, Herrmann N, Sproule BA, Busto UE. Sedative hypnotics in older people with insomnia: meta-analysis of risks and benefits. BMJ (Clinical research ed.). 2005;331(7526):1169.
27. Bjorner T, Tvete IF, Aursnes I, Skomedal T. [Dispensing of benzodiazepines and Z drugs by Norwegian pharmacies 2004-2011]. Tidsskrift for den Norske Legeforening. 2013;133(20):2149-53.
28. Product monograph. Imovane tablets 5/7.5 mg. Sanofi-aventis Canada Inc. http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjpt9G3tLzbAhXDhqYKHZnnCPkQFggnMAA&url=http%3A%2F%2Fproducts.sanofi.ca%2Fen%2Fimovane.pdf&usg=AOvVaw0G_p2JjZlH4WwCkEfwRRRh. Revision 27.09.18.
29. Fernandez C, Martin C, Gimenez F, Farinotti R. Clinical pharmacokinetics of zopiclone. Clinical Pharmacokinetics. 1995;29(6):431-41.
30. Caille G, du Souich P, Spenard J, Lacasse Y, Vezina M. Pharmacokinetic and clinical parameters of zopiclone and trimipramine when administered simultaneously to volunteers. Biopharmaceutics & Drug Disposition. 1984;5(2):117-25.
31. Gaillot J, Heusse D, Hougton GW, Marc Aurele J, Dreyfus JF. Pharmacokinetics and metabolism of zopiclone. Pharmacology. 1983;27 Suppl 2:76-91.
32. Baselt CR. Disposition of Toxic Drugs and Chemicals in Man. 11th ed. Foster City, CA, USA: Biomedical Publication. 2017.
33. Bramness JG, Arnestad M, Karinen R, Hilberg T. Fatal overdose of zopiclone in an elderly woman with bronchogenic carcinoma. Journal of Forensic Sciences. 2001;46(5):1247-9.
34. Becquemont L, Mouajjah S, Escaffre O, Beaune P, Funck-Brentano C, Jaillon P. Cytochrome P-450 3A4 and 2C8 are involved in zopiclone metabolism. Drug Metabolism and Disposition: The biological fate of chemicals. 1999;27(9):1068-73.
35. Hesse LM, von Moltke LL, Greenblatt DJ. Clinically important drug interactions with zopiclone, zolpidem and zaleplon. CNS Drugs. 2003;17(7):513-32.
36. Tornio A, Neuvonen PJ, Backman JT. The CYP2C8 inhibitor gemfibrozil does not increase the plasma concentrations of zopiclone. European Journal of Clinical Pharmacology. 2006;62(8):645-51.
37. Mannaert E, Tytgat J, Daenens P. Detection of 2-amino-5-chloropyridine in urine as a parameter of zopiclone (Imovane) intake using HPLC with diode array detection. Journal of Analytical Toxicology. 1997;21(3):208-12.
38. Nilsson GH, Kugelberg FC, Ahlner J, Kronstrand R. Quantitative analysis of zopiclone, N-desmethylzopiclone, zopiclone N-oxide and 2-amino-5-chloropyridine in urine using LC-MS-MS. Journal of Analytical Toxicology. 2014;38(6):327-34.
39. Nilsson GH, Kugelberg FC, Kronstrand R, Ahlner J. Stability tests of zopiclone in whole blood. Forensic Science International. 2010;200(1-3):130-5.
40. Gebauer MG, Alderman CP. Validation of a high-performance liquid chromatographic method for the enantiospecific quantitation of zopiclone in plasma. Biomedical Chromatography. 2002;16(4):241-6.

41. Nilsson GH, Kugelberg FC, Ahlner J, Kronstrand R. Validation of an LC-MS/MS method for the determination of zopiclone, N-desmethylzopiclone and 2-amino-5-chloropyridine in whole blood and its application to estimate the original zopiclone concentration in stored specimens. International Journal of Legal Medicine. 2015;129(2):269-77.
42. Goa KL, Heel RC. Zopiclone. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy as an hypnotic. Drugs. 1986;32(1):48-65.
43. Viron B, De Meyer M, Le Liboux A, Frydman A, Maillard F, Mignon F, et al. Steady state pharmacokinetics of zopiclone during multiple oral dosing (7.5 mg nocte) in patients with severe chronic renal failure. International Clinical Psychopharmacology. 1990;5 Suppl 2:95-104.
44. Davies M. The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. Journal of Psychiatry & Neuroscience: JPN. 2003;28(4):263-74.
45. Sigel E, Steinmann ME. Structure, function, and modulation of GABA(A) receptors. Journal of Biological Chemistry. 2012;287(48):40224-31.
46. Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different GABAA receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(3):915-20.
47. Wisden W, Yu X, Franks NP. GABA Receptors and the Pharmacology of Sleep. Handbook of Experimental Pharmacology. 2017;253.
48. Smith AJ, Alder L, Silk J, Adkins C, Fletcher AE, Scales T, et al. Effect of alpha subunit on allosteric modulation of ion channel function in stably expressed human recombinant gamma-aminobutyric acid(A) receptors determined using (36)Cl ion flux. Molecular Pharmacology. 2001;59(5):1108-18.
49. Atack JR. Development of Subtype-Selective GABAA receptor Compounds for the Treatment of Anxiety, Sleep Disorders and Epilepsy.2010.
50. Nutt DJ, Feetam CL. What one hand giveth the other taketh away: some unpredicted effects of enantiomers in psychopharmacology. Journal of Psychopharmacology. 2010;24(8):1137-41.
51. Tan KR, Brown M, Labouebe G, Yvon C, Creton C, Fritschy JM, et al. Neural bases for addictive properties of benzodiazepines. Nature. 2010;463(7282):769-74.
52. Handal M, Skurtveit S, Morland JG. [Co-medication with benzodiazepines]. Tidsskrift for den Norske Legeforening. 2012;132(5):526-30.
53. Gunja N. In the Zzz zone: the effects of Z-drugs on human performance and driving. Journal of Medical Toxicology. 2013;9(2):163-71.
54. Paul MA, Gray G, Sardana TM, Pigeau RA. Melatonin and zopiclone as facilitators of early circadian sleep in operational air transport crews. Aviat Space Environ Med. 2004;75(5):439-43.
55. Berghaus G, Sticht G, Grellner W, Lenz D, Neumann T, Wiesenmüller S. Meta-analysis of empirical studies concerning the effects of medicines and illegal drugs including pharmacokinetics of safe driving. (https://www.bast.de/Druid/EN/deliverales-list/downloads/Deliverable_1_1_2_B.pdf?__blob=publicationFile&v=1). Driving under the influence of drugs, alcohol, and medicines (DRUID), 6 th framework programme 2011.
56. Mets MA, de Vries JM, de Senerpont Domis LM, Volkerts ER, Olivier B, Verster JC. Next-day effects of ramelteon (8 mg), zopiclone (7.5 mg), and placebo on highway driving performance, memory functioning, psychomotor performance, and mood in healthy adult subjects. Sleep. 2011;34(10):1327-34.
57. Krzyzowski J, Michniewicz M. [Case of librium addiction]. [Polish] Neurologia, Neurochirurgia i Psychiatria Polska. 1966;16(2):195-6.
58. Griffiths RR, Johnson MW. Relative abuse liability of hypnotic drugs: a conceptual framework and algorithm for differentiating among compounds. Journal of Clinical Psychiatry. 2005;66 Suppl 9:31-41.

59. Griffiths RR, Weerts EM. Benzodiazepine self-administration in humans and laboratory animals--implications for problems of long-term use and abuse. Psychopharmacology. 1997;134(1):1-37.
60. Soldatos CR, Dikeos DG, Whitehead A. Tolerance and rebound insomnia with rapidly eliminated hypnotics: a meta-analysis of sleep laboratory studies. International Clinical Psychopharmacology. 1999;14(5):287-303.
61. Bianchi M, Musch B. Zopiclone discontinuation: review of 25 studies assessing withdrawal and rebound phenomena. International Clinical Psychopharmacology. 1990;5 Suppl 2:139-45.
62. Inman W, Kubota K, Pearce G, Wilton L. PEM Report Number 10. Zopiclone. Pharmacoepidemiology and Drug Safety. 1993;2:499-521.
63. Zammit G. Comparative tolerability of newer agents for insomnia. Drug Safety. 2009;32(9):735-48.
64. Aranko K, Henriksson M, Hublin C, Seppalainen AM. Misuse of zopiclone and convulsions during withdrawal. Pharmacopsychiatry. 1991;24(4):138-40.
65. Jones IR, Sullivan G. Physical dependence on zopiclone: case reports. BMJ (Clinical research ed.). 1998;316(7125):117.
66. Ayonrinde O, Sampson E. Physical dependence on zopiclone. Risk of dependence may be greater in those with dependent personalities. BMJ (Clinical Research ed.). 1998;317(7151):146.
67. Flynn A, Cox D. Dependence on zopiclone. Addiction. 2006;101(6):898.
68. Thakore J, Dinan T. Physical dependence following zopiclone usage: A case report. Human Psychopharmacology. 1992;7:143-5.
69. Bannan N, Rooney S, O'Connor J. Zopiclone misuse: an update from Dublin. Drug and Alcohol Review. 2007;26(1):83-5.
70. Hajak G, Clarenbach P, Fischer W, Haase W, Ruther E. Zopiclone improves sleep quality and daytime well-being in insomniac patients: comparison with triazolam, flunitrazepam and placebo. International Clinical Psychopharmacology. 1994;9(4):251-61.
71. Zammit GK, McNabb LJ, Caron J, Amato DA, Roth T. Efficacy and safety of eszopiclone across 6-weeks of treatment for primary insomnia. Current Medical Research and Opinion. 2004;20(12):1979-91.
72. Muir JF, DeFouilloy C, Broussier P, Locquet R, Maillard F. Comparative study of the effects of zopiclone and placebo on respiratory function in patients with chronic obstructive respiratory insufficiency. International Clinical Psychopharmacology. 1990;5 Suppl 2:85-94.
73. Lofaso F, Goldenberg F, Thebault C, Janus C, Harf A. Effect of zopiclone on sleep, night-time ventilation, and daytime vigilance in upper airway resistance syndrome. The European Respiratory Journal. 1997;10(11):2573-7.
74. Buckley NA, McManus PR. Changes in fatalities due to overdose of anxiolytic and sedative drugs in the UK (1983-1999). Drug Safety. 2004;27(2):135-41.
75. Druid H, Holmgren P. A compilation of fatal and control concentrations of drugs in postmortem femoral blood. Journal of Forensic Sciences. 1997;42(1):79-87.
76. Pelissier-Alicot A, Kintz P, BDeveux M. A premeditated infanticide by administration of zopiclone. (http://www.soft-tox.org/toxtalk). ToxTalk (Society of Forensic Toxicologists) 2013;37 (3):21-2.
77. Druid H, Holmgren P, Hallander S, Ahlner J. Interpretation of postmortem femoral blood concentrations of newer antidepressants and hypnotics. Presented at the annual meeting of the American Academy of Forensic Sciences, Seattle, WA, USA (February 22). 2001.
78. Jonsson AK, Soderberg C, Espnes KA, Ahlner J, Eriksson A, Reis M, et al. Sedative and hypnotic drugs--fatal and non-fatal reference blood concentrations. Forensic Science International. 2014;236:138-45.
79. Allain H, Delahaye C, Le Coz F, Blin P, Decombe R, Martinet JP. Postmarketing surveillance of zopiclone in insomnia: analysis of 20,513 cases. Sleep. 1991;14(5):408-13.

80. Najib J. Eszopiclone, a nonbenzodiazepine sedative-hypnotic agent for the treatment of transient and chronic insomnia. Clinical Therapeutics. 2006;28(4):491-516.
81. Takada M, Fujimoto M, Hosomi K. Association between Benzodiazepine Use and Dementia: Data Mining of Different Medical Databases. International Journal of Medical Sciences. 2016;13(11):825-34.
82. Islam MM, Iqbal U, Walther B, Atique S, Dubey NK, Nguyen PA, et al. Benzodiazepine Use and Risk of Dementia in the Elderly Population: A Systematic Review and Meta-Analysis. Neuroepidemiology. 2016;47(3-4):181-91.
83. Zhong G, Wang Y, Zhang Y, Zhao Y. Association between Benzodiazepine Use and Dementia: A Meta-Analysis. Plos One. 2015;10(5):e0127836.
84. Lucchetta RC, da Mata BPM, Mastroianni PC. Association between Development of Dementia and Use of Benzodiazepines: A Systematic Review and Meta-Analysis. Pharmacotherapy. 2018;38(10):1010-20.
85. Brandt J, Leong C. Benzodiazepines and Z-Drugs: An Updated Review of Major Adverse Outcomes Reported on in Epidemiologic Research. Drugs R D. 2017;17(4):493-507.
86. Chen PL, Lee WJ, Sun WZ, Oyang YJ, Fuh JL. Risk of dementia in patients with insomnia and long-term use of hypnotics: a population-based retrospective cohort study. Plos One. 2012;7(11):e49113.
87. Treves N, Perlman A, Kolenberg Geron L, Asaly A, Matok I. Z-drugs and risk for falls and fractures in older adults-a systematic review and meta-analysis. Age Ageing. 2018;47(2):201-8.
88. Nishtala PS, Chyou TY. Zopiclone Use and Risk of Fractures in Older People: Population-Based Study. Journal of the American Medical Directors Association. 2017;18(4):368 e1- e8.
89. Bakken MS, Engeland A, Engesaeter LB, Ranhoff AH, Hunskaar S, Ruths S. Risk of hip fracture among older people using anxiolytic and hypnotic drugs: a nationwide prospective cohort study. European Journal of Clinical Pharmacology. 2014;70(7):873-80.
90. Gjerde H, Strand MC, Morland J. Driving Under the Influence of Non-Alcohol Drugs--An Update Part I: Epidemiological Studies. Forensic Science Review. 2015;27(2):89-113.
91. Gustavsen I, Bramness JG, Skurtveit S, Engeland A, Neutel I, Morland J. Road traffic accident risk related to prescriptions of the hypnotics zopiclone, zolpidem, flunitrazepam and nitrazepam. Sleep Medicine. 2008;9(8):818-22.
92. Kuypers KP, Legrand SA, Ramaekers JG, Verstraete AG. A case-control study estimating accident risk for alcohol, medicines and illegal drugs. Plos One. 2012;7(8):e43496.
93. Gjerde H, Christophersen A, Normann P, Mørland J. Associations between substance use and fatal road traffic accidents amiong car and van drivers in Norway: a case-control study. Transportation Research Part F Traffic Psychology and Behavour. 2013;17:134.
94. Chang CM, Wu EC, Chen CY, Wu KY, Liang HY, Chau YL, et al. Psychotropic drugs and risk of motor vehicle accidents: a population-based case-control study. British Journal of Clinical Pharmacology. 2013;75(4):1125-33.
95. Ravera S, van Rein N, de Gier JJ, de Jong-van den Berg LT. Road traffic accidents and psychotropic medication use in The Netherlands: a case-control study. British Journal of Clinical Pharmacology. 2011;72(3):505-13.
96. Nevriana A, Moller J, Laflamme L, Monarrez-Espino J. New, Occasional, and Frequent Use of Zolpidem or Zopiclone (Alone and in Combination) and the Risk of Injurious Road Traffic Crashes in Older Adult Drivers: A Population-Based Case-Control and Case-Crossover Study. CNS Drugs. 2017;31(8):711-22.
97. Burns M, Moskowitz H. Psychophysical Tests for DWI Arrest. Washington, DC: National Highway Traffic Safety Administration. Report No. DOT HS 802 424.; 1977.
98. Stuster J. Validation of the standardized field sobriety test battery at 0.08% blood alcohol concentration. Human factors. 2006;48(3):608-14.

99. Papafotiou K, Carter JD, Stough C. The relationship between performance on the standardised field sobriety tests, driving performance and the level of Delta9-tetrahydrocannabinol (THC) in blood. Forensic Science International. 2005;155(2-3):172-8.
100. Bosker WM, Theunissen EL, Conen S, Kuypers KP, Jeffery WK, Walls HC, et al. A placebo-controlled study to assess Standardized Field Sobriety Tests performance during alcohol and cannabis intoxication in heavy cannabis users and accuracy of point of collection testing devices for detecting THC in oral fluid. Psychopharmacology. 2012;223(4):439-46.
101. Doroudgar S, Mae Chuang H, Bohnert K, Canedo J, Burrowes S, Perry PJ. Effects of chronic marijuana use on driving performance. Traffic Injury Prevention. 2018;19(7):680-6.
102. Porath-Waller AJ, Beirness DJ. An examination of the validity of the standardized field sobriety test in detecting drug impairment using data from the Drug Evaluation and Classification program. Traffic Injury Prevention. 2014;15(2):125-31.
103. Beirness DJ, LeCavalier J, Singhal D. Evaluation of the Drug Evaluation and Classification program: a critical review of the evidence. Traffic Injury Prevention. 2007;8(4):368-76.
104. Bramness JG, Skurtveit S, Morland J. Testing for benzodiazepine inebriation--relationship between benzodiazepine concentration and simple clinical tests for impairment in a sample of drugged drivers. European Journal of Clinical Pharmacology. 2003;59(8-9):593-601.
105. Gustavsen I, Al-Sammurraie M, Morland J, Bramness JG. Impairment related to blood drug oncentrations of zopiclone and zolpidem compared to alcohol in apprehended drivers. Accident; Analysis and Prevention. 2009;41(3):462-6.
106. Al-Samarraie MS, Karinen R, Morland J, Stokke Opdal M. Blood GHB concentrations and results of medical examinations in 25 car drivers in Norway. European Journal of Clinical Pharmacology. 2010;66(10):987-98.
107. Bachs L, Skurtveit S, Morland J. Codeine and clinical impairment in samples in which morphine is not detected. European Journal of Clinical Pharmacology. 2003;58(12):785-9.
108. Bachs L, Hoiseth G, Skurtveit S, Morland J. Heroin-using drivers: importance of morphine and morphine-6-glucuronide on late clinical impairment. European Journal of Clinical Pharmacology. 2006;62(11):905-12.
109. Bernard JP, Morland J, Krogh M, Khiabani HZ. Methadone and impairment in apprehended drivers. Addiction. 2009;104(3):457-64.
110. Bramness JG, Skurtveit S, Morland J. Clinical impairment of benzodiazepines--relation between benzodiazepine concentrations and impairment in apprehended drivers. Drug and Alcohol Dependence. 2002;68(2):131-41.
111. Bramness JG, Skurtveit S, Morland J. Flunitrazepam: psychomotor impairment, agitation and paradoxical reactions. Forensic Science International. 2006;159(2-3):83-91.
112. Bramness JG, Skurtveit S, Morland J. Impairment due to intake of carisoprodol. Drug and Alcohol Dependence. 2004;74(3):311-8.
113. Gustavsen I, Morland J, Bramness JG. Impairment related to blood amphetamine and/or methamphetamine concentrations in suspected drugged drivers. Accident; Analysis and Prevention. 2006;38(3):490-5.
114. Khiabani HZ, Bramness JG, Bjorneboe A, Morland J. Relationship between THC concentration in blood and impairment in apprehended drivers. Traffic Injury Prevention. 2006;7(2):111-6.
115. Khiabani HZ, Morland J, Bramness JG. Frequency and irregularity of heart rate in drivers suspected of driving under the influence of cannabis. European Journal of Internal Medicine. 2008;19(8):608-12.
116. Bramness JG, Khiabani HZ, Morland J. Impairment due to cannabis and ethanol: clinical signs and additive effects. Addiction. 2010;105(6):1080-7.
117. Kjeldsen T, Sundvoll A, Øiseth O. Tegn og symptomer [Norwegian]. 3 utg. Høvik, Forlaget Vett&Viten. 2012.

118. Nesse O. Politiets bruk av tegn- og symptomtesten for å avdekke ruspåvirket kjøring i Norge [Norwegian]. "The Police use of "Sign and Symptoms" to reveal impaired driving in Norway". https://core.ac.uk/download/pdf/30843239.pdf. Master's Thesis. University of Stavanger, Norway. 2014.
119. Walsh JM, Verstraete AG, Huestis MA, Morland J. Guidelines for research on drugged driving. Addiction. 2008;103(8):1258-68.
120. O'Hanlon JF. Driving performance under the influence of drugs: rationale for, and application of, a new test. British Journal of Clinical Pharmacology. 1984;18 Suppl 1:121S-9S.
121. Epstein JN, Erkanli A, Conners CK, Klaric J, Costello JE, Angold A. Relations between Continuous Performance Test performance measures and ADHD behaviors. Journal of Abnormal Child Psychology. 2003;31(5):543-54.
122. Shallice T. Specific impairments of planning. Philosophical Transactions of the Royal Society of London, Biological Sciences. 1982;298(1089):199-209.
123. Kenntner-Mabiala R, Kaussner Y, Jagiellowicz-Kaufmann M, Hoffmann S, Kruger HP. Driving performance under alcohol in simulated representative driving tasks: an alcohol calibration study for impairments related to medicinal drugs. Journal of Clinical Psychopharmacology. 2015;35(2):134-42.
124. Irwin C, Iudakhina E, Desbrow B, McCartney D. Effects of acute alcohol consumption on measures of simulated driving: A systematic review and meta-analysis. Accident; Analysis and Prevention. 2017;102:248-66.
125. Jongen S, Vuurman EF, Ramaekers JG, Vermeeren A. The sensitivity of laboratory tests assessing driving related skills to dose-related impairment of alcohol: A literature review. Accident; Analysis and Prevention. 2016;89:31-48.
126. Jongen S, Vermeeren A, van der Sluiszen NN, Schumacher MB, Theunissen EL, Kuypers KP, et al. A pooled analysis of on-the-road highway driving studies in actual traffic measuring standard deviation of lateral position (i.e., "weaving") while driving at a blood alcohol concentration of 0.5 g/L. Psychopharmacology. 2017;234(5):837-44.
127. Verster JC, Roth T. Standard operation procedures for conducting the on-the-road driving test, and measurement of the standard deviation of lateral position (SDLP). International Journal og General Medicine. 2011;4:359-71.
128. Helland A, Jenssen GD, Lervag LE, Westin AA, Moen T, Sakshaug K, et al. Comparison of driving simulator performance with real driving after alcohol intake: a randomised, single blind, placebo-controlled, cross-over trial. Accident; Analysis and Prevention. 2013;53:9-16.
129. Helland A, Jenssen GD, Lervag LE, Moen T, Engen T, Lydersen S, et al. Evaluation of measures of impairment in real and simulated driving: Results from a randomized, placebo-controlled study. Traffic Injury Prevention. 2016;17(3):245-50.
130. Ramaekers JG, Berghaus G, van Laar M, Drummer OH. Dose related risk of motor vehicle crashes after cannabis use. Drug and Alcohol Dependence. 2004;73(2):109-19.
131. Strand MC, Ramaekers JG, Gjerde H, Morland J, Vindenes V. Pharmacokinetics of Single Doses of Methadone and Buprenorphine in Blood and Oral Fluid in Healthy Volunteers and Correlation With Effects on Psychomotor and Cognitive Functions. Journal of Clinical Psychopharmacology. 2019;39(5):489-93.
132. Schnabel E, Hargutt E, Krüger H. Meta-analysis of empirical studies concerning the effects of alcohol on safe driving: TREN-05-FP6TR-S07.61320-518404-DRUID. (https://www.bast.de/Druid/EN/deliverales-list/downloads/Deliverable_1_1_2.html?nn=613800). University of Wuerzburg, Germany. 2010.
133. Brumback T, Cao D, King A. Effects of alcohol on psychomotor performance and perceived impairment in heavy binge social drinkers. Drug and Alcohol Dependence. 2007;91(1):10-7.
134. Narahashi T, Kuriyama K, Illes P, Wirkner K, Fischer W, Muhlberg K, et al. Neuroreceptors and ion channels as targets of alcohol. Alcohol, Clinical and Experimental Research. 2001;25(5 Suppl ISBRA):182S-8S.

135. Billiard M, Besset A, de Lustrac C, Brissaud L. Dose-response effects of zopiclone on night sleep and on nighttime and daytime functioning. Sleep. 1987;10 Suppl 1:27-34.
136. Billiard M, Besset A, de Lustrac C, Brissaud L, Cadilhac J. [Effects of zopiclone on sleep, daytime somnolence and nocturnal and daytime performance in healthy volunteers]. [French] Neurophysiol Clin. 1989;19(2):131-43.
137. Mattila MJ, Vanakoski J, Kalska H, Seppala T. Effects of alcohol, zolpidem, and some other sedatives and hypnotics on human performance and memory. Pharmacology, Biochemistry, and Behavior. 1998;59(4):917-23.
138. Nicholson AN, Stone BM. Efficacy of zopiclone in middle age. Sleep. 1987;10 Suppl 1:35-9.
139. Ponciano E, Freitas F, Camara J, Faria M, Barreto M, Hindmarch I. A comparison of the efficacy, tolerance and residual effects of zopiclone, flurazepam and placebo in insomniac outpatients. International Clinical Psychopharmacology. 1990;5 Suppl 2:69-77.
140. Tafti M, Besset A, Billiard M. Effects of zopiclone on subjective evaluation of sleep and daytime alertness and on psychomotor and physical performance tests in athletes. Progress in Neuro-psychopharmacology & Biological Psychiatry. 1992;16(1):55-63.
141. Meskali M, Berthelon C, Marie S, Denise P, Bocca ML. Residual effects of hypnotic drugs in aging drivers submitted to simulated accident scenarios: an exploratory study. Psychopharmacology. 2009;207(3):461-7.
142. Takahashi J, Kanbayashi T, Ito Uemura S, Sagawa Y, Tsutsui K, Takahashi Y, et al. Residual effects of eszopiclone and placebo in healthy elderly subjects: a randomized double-blind study. Sleep and Biological Rhythms. 2017;15(3):235-41.
143. Bocca ML, Marie S, Lelong-Boulouard V, Bertran F, Couque C, Desfemmes T, et al. Zolpidem and zopiclone impair similarly monotonous driving performance after a single nighttime intake in aged subjects. Psychopharmacology. 2011;214(3):699-706.
144. Simen AA, Gargano C, Cha JH, Drexel M, Bautmans A, Heirman I, et al. A randomized, crossover, placebo-controlled clinical trial to assess the sensitivity of the CRCDS Mini-Sim to the next-day residual effects of zopiclone. Therapeutic Advances in Drug Safety. 2015;6(3):86-97.
145. Leufkens TR, Vermeeren A. Highway driving in the elderly the morning after bedtime use of hypnotics: a comparison between temazepam 20 mg, zopiclone 7.5 mg, and placebo. Journal of Clinical Psychopharmacology. 2009;29(5):432-8.
146. Leufkens TR, Ramaekers JG, de Weerd AW, Riedel WJ, Vermeeren A. Residual effects of zopiclone 7.5 mg on highway driving performance in insomnia patients and healthy controls: a placebo controlled crossover study. Psychopharmacology. 2014;231(14):2785-98.
147. Vermeeren A, Riedel WJ, van Boxtel MP, Darwish M, Paty I, Patat A. Differential residual effects of zaleplon and zopiclone on actual driving: a comparison with a low dose of alcohol. Sleep. 2002;25(2):224-31.
148. Verster JC, Veldhuijzen DS, Volkerts ER. Residual effects of sleep medication on driving ability. Sleep Medicine Reviews. 2004;8(4):309-25.
149. Vermeeren A, Vets E, Vuurman EF, Van Oers AC, Jongen S, Laethem T, et al. On-the-road driving performance the morning after bedtime use of suvorexant 15 and 30 mg in healthy elderly. Psychopharmacology. 2016;233(18):3341-51.
150. Vermeeren A, Sun H, Vuurman EF, Jongen S, Van Leeuwen CJ, Van Oers AC, et al. On-the-Road Driving Performance the Morning after Bedtime Use of Suvorexant 20 and 40 mg: A Study in Non-Elderly Healthy Volunteers. Sleep. 2015;38(11):1803-13.
151. Vermeeren A, Vuurman EF, Leufkens TR, Van Leeuwen CJ, Van Oers AC, Laska E, et al. Residual effects of low-dose sublingual zolpidem on highway driving performance the morning after middle-of-the-night use. Sleep. 2014;37(3):489-96.
152. Leufkens TR, Vermeeren A. Zopiclone's residual effects on actual driving performance in a standardized test: a pooled analysis of age and sex effects in 4 placebo-controlled studies. Clinical Therapeutics. 2014;36(1):141-50.

153. Ramaekers JG, Conen S, de Kam PJ, Braat S, Peeters P, Theunissen EL, et al. Residual effects of esmirtazapine on actual driving performance: overall findings and an exploratory analysis into the role of CYP2D6 phenotype. Psychopharmacology. 2011;215(2):321-32.
154. Verster JC, Spence DW, Shahid A, Pandi-Perumal SR, Roth T. Zopiclone as positive control in studies examining the residual effects of hypnotic drugs on driving ability. Current Drug Safety. 2011;6(4):209-18.
155. Vindenes V, Jordbru D, Knapskog AB, Kvan E, Mathisrud G, Slordal L, et al. Impairment based legislative limits for driving under the influence of non-alcohol drugs in Norway. Forensic Science International. 2012;219(1-3):1-11.
156. Strand MC, Innerdal C, Mathisrud G, Morland J, Riedel B, Slordal L, et al. [Revision of fixed limits for drugs in traffic.]. Tidsskrift for den Norske Legeforening. 2016;136(19):1619-20.
157. Gustavsen I. Zopiclone and Traffic Safety - Introducing Legalized Blood Zopiclone Concentration Limits - Is it Evidence Based? (PhD thesis). University of Oslo, Faculty of Medicine. 2012.
158. Yeh CK, Christodoulides NJ, Floriano PN, Miller CS, Ebersole JL, Weigum SE, et al. Current development of saliva/oral fluid-based diagnostics. Texas Dental Journal. 2010;127(7):651-61.
159. Crouch DJ. Oral fluid collection: the neglected variable in oral fluid testing. Forensic Science International. 2005;150(2-3):165-73.
160. Bosker WM, Huestis MA. Oral fluid testing for drugs of abuse. Clinical Chemistry. 2009;55(11):1910-31.
161. Huestis MA, Verstraete A, Kwong TC, Morland J, Vincent MJ, de la Torre R. Oral fluid testing: promises and pitfalls. Clinical Chemistry. 2011;57(6):805-10.
162. Caplan YH, Goldberger BA. Alternative specimens for workplace drug testing. Journal of Analytical Toxicology. 2001;25(5):396-9.
163. Toennes SW, Kauert GF, Steinmeyer S, Moeller MR. Driving under the influence of drugs — evaluation of analytical data of drugs in oral fluid, serum and urine, and correlation with impairment symptoms. Forensic Science International. 2005;152(2–3):149-55.
164. Toennes SW, Steinmeyer S, Maurer HJ, Moeller MR, Kauert GF. Screening for drugs of abuse in oral fluid--correlation of analysis results with serum in forensic cases. Journal of Analytical Toxicology. 2005;29(1):22-7.
165. Touw DJ, Neef C, Thomson AH, Vinks AA, Cost-Effectiveness of Therapeutic Drug Monitoring Committee of the International Association for Therapeutic Drug M, Clinical T. Cost-effectiveness of therapeutic drug monitoring: a systematic review. Therapeutic Drug Monitoring. 2005;27(1):10-7.
166. Neumann J, Beck O, Dahmen N, Bottcher M. Potential of Oral Fluid as a Clinical Specimen for Compliance Monitoring of Psychopharmacotherapy. Therapeutic Drug Monitoring. 2018;40(2):245-51.
167. Preiskorn J, Studer S, Rauh R, Lukacin R, Geffert C, Fleischhaker C, et al. Inter- and Intraindividual Variation of Methylphenidate Concentrations in Serum and Saliva of Patients with Attention-Deficit/Hyperactivity Disorder. Therapeutic Drug Monitoring. 2018.
168. Fisher DS, Partridge SJ, Handley SA, Couchman L, Morgan PE, Flanagan RJ. LC-MS/MS of some atypical antipsychotics in human plasma, serum, oral fluid and haemolysed whole blood. Forensic Science International. 2013;229(1-3):145-50.
169. Patteet L, Maudens KE, Morrens M, Sabbe B, Dom G, Neels H. Determination of Common Antipsychotics in Quantisal-Collected Oral Fluid by UHPLC-MS/MS: Method Validation and Applicability for Therapeutic Drug Monitoring. Therapeutic Drug Monitoring. 2016;38(1):87-97.
170. de Castro A, Concheiro M, Quintela O, Cruz A, Lopez-Rivadulla M. LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma. Study of correlation between venlafaxine concentrations in both matrices. Journal of Pharmaceutical and Biomedical Analysis. 2008;48(1):183-93.

171. Maldonado C, Fagiolino P, Vasquez M, Rey A, Olano I, Eiraldi R, et al. Therapeutic Carbamazepine (CBZ) and Valproic acid (VPA) Monitoring in Children Using Saliva as a Biologic Fluid. Journal of Epilepsy and Clinical Neurophysiology. 2008;14(2):55-8.
172. Houwing S HM, Mathijssen R. Prevalence of alcohol and other psychoactive substance in drivers in general traffic. Part I: General results. DRUID Deliverable D 2.2.3 (http://www.druid-project.eu/Druid/EN/deliverales-list/downloads/Deliverable_2_2_3_Part1.pdf?__blob=publicationFile&v=1). SWOV Institute for Road Safety Research, Leidschendam, The Netherlands 2011.
173. Wille SM, Raes E, Lillsunde P, Gunnar T, Laloup M, Samyn N, et al. Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of driving under the influence of drugs. Therapeutic Drug Monitoring. 2009;31(4):511-9.
174. Langel K, Gjerde H, Favretto D, Lillsunde P, Oiestad EL, Ferrara SD, et al. Comparison of drug concentrations between whole blood and oral fluid. Drug Testing and Analysis. 2014;6(5):461-71.
175. Gjerde H, Langel K, Favretto D, Verstraete AG. Detection of illicit drugs in oral fluid from drivers as biomarker for drugs in blood. Forensic Science International. 2015;256:42-5.
176. Coucke LD, De Smet L, Verstraete AG. Influence of Sampling Procedure on Codeine Concentrations in Oral Fluid. Journal of Analytical Toxicology. 2016;40(2):148-52.
177. Gal P, Jusko WJ, Yurchak AM, Franklin BA. Theophylline disposition in obesity. Clinical Pharmacology and Therapeutics. 1978;23(4):438-44.
178. DiGregorio GJ, Piraino AJ, Ruch E. Diazepam concentrations in parotid saliva, mixed saliva, and plasma. Clinical Pharmacology and Therapeutics. 1978;24(6):720-5.
179. Meyer MR, Rosenborg S, Stenberg M, Beck O. First report on the pharmacokinetics of tramadol and O-desmethyltramadol in exhaled breath compared to plasma and oral fluid after a single oral dose. Biochemical Pharmacology. 2015;98(3):502-10.
180. Morland J, Setekleiv J, Haffner JF, Stromsaether CE, Danielsen A, Wethe GH. Combined effects of diazepam and ethanol on mental and psychomotor functions. Acta Pharmacologica et Toxicologica. 1974;34(1):5-15.
181. Langel K, Engblom C, Pehrsson A, Gunnar T, Ariniemi K, Lillsunde P. Drug testing in oral fluid-evaluation of sample collection devices. Journal of Analytical Toxicology. 2008;32(6):393-401.
182. Oiestad EL, Johansen U, Christophersen AS. Drug screening of preserved oral fluid by liquid chromatography-tandem mass spectrometry. Clinical Chemistry. 2007;53(2):300-9.
183. Baselt CR. Drug Effects on Psychomotor Performance: Biomedical publications, Foster City, California, USA; 2001.
184. Jones AW. Inter- and intra-individual variations in the saliva/blood alcohol ratio during ethanol metabolism in man. Clinical Chemistry. 1979;25(8):1394-8.
185. Jones AW. Pharmacokinetics of ethanol in saliva: comparison with blood and breath alcohol profiles, subjective feelings of intoxication, and diminished performance. Clinical Chemistry. 1993;39(9):1837-44.
186. Hoiseth G, Yttredal B, Karinen R, Gjerde H, Morland J, Christophersen A. Ethyl glucuronide concentrations in oral fluid, blood, and urine after volunteers drank 0.5 and 1.0 g/kg doses of ethanol. Journal of Analytical Toxicology. 2010;34(6):319-24.
187. Rief W, Bingel U, Schedlowski M, Enck P. Mechanisms involved in placebo and nocebo responses and implications for drug trials. Clinical Pharmacology and Therapeutics. 2011;90(5):722-6.
188. Peeters PA, van den Heuvel MW, van Heumen E, Passier PC, Smeets JM, van Iersel T, et al. Safety, tolerability and pharmacokinetics of sugammadex using single high doses (up to 96 mg/kg) in healthy adult subjects: a randomized, double-blind, crossover, placebo-controlled, single-centre study. Clinical Drug Investigation. 2010;30(12):867-74.
189. Doty RL, Treem J, Tourbier I, Mirza N. A double-blind study of the influences of eszopiclone on dysgeusia and taste function. Pharmacology, Biochemistry, and Behavior. 2009;94(2):312-8.

190. Yoshida M, Kojima H, Uda A, Haraguchi T, Ozeki M, Kawasaki I, et al. Bitterness-Masking Effects of Different Beverages on Zopiclone and Eszopiclone Tablets. Chemical & Pharmaceutical Bulletin. 2019;67(5):404-9.
191. Lund HM, Oiestad EL, Gjerde H, Christophersen AS. Drugs of abuse in oral fluid collected by two different sample kits--stability testing and validation using ultra performance tandem mass spectrometry analysis. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences. 2011;879(30):3367-77.
192. Jantos R, Vermeeren A, Sabljic D, Ramaekers JG, Skopp G. Degradation of zopiclone during storage of spiked and authentic whole blood and matching dried blood spots. International Journal of Legal Medicine. 2013;127(1):69-76.
193. Nilsson GH, Kugelberg FC, Ahlner J, Kronstrand R. Influence of pre-analytical conditions on the interpretation of zopiclone concentrations in whole blood. Forensic Science International. 2011;207(1-3):35-9.
194. Mata DC. Stability of 26 Sedative Hypnotics in Six Toxicological Matrices at Different Storage Conditions. Journal of Analytical Toxicology. 2016;40(8):663-8.
195. Gustavsen I, Hjelmeland K, Bernard JP, Morland J. Individual psychomotor impairment in relation to zopiclone and ethanol concentrations in blood--a randomized controlled double-blinded trial. Addiction. 2012;107(5):925-32.
196. "Alkoholloven". “Lov om omsetning av alkoholholdig drikk m.v. (alkoholloven)” §1-5 cf. §1-3. [Norwegian].1989 https://lovdata.no/dokument/NL/lov/1989-06-02-27, last update 01.11.18.
197. Årving A, Middelkoop G, Hjelmeland K. Funn i blodprøver hos bilførere mistenkt for ruspåvirket kjøring 2018 [Norwegian]. Toxicological findings in blood samples from suspected impaired drivers 2018. https://oslo-universitetssykehus.no/seksjon/avdeling-for-rettsmedisinske-fag/Documents/Rusmiddelstatistikk%20-%202018.pdf. Oslo University Hospital, Department of Forensic Sciences. 2019.
198. Barbour AD. Simplified estimation of Widmark "r" values by the method of Forrest. Science & Justice : Journal of the Forensic Science Society. 2001;41(1):53-4.
199. Greenblatt DJ, Harmatz JS, Roth T. Zolpidem and Gender: Are Women Really At Risk? Journal of Clinical Psychopharmacology. 2019;39(3):189-99.
200. Rosner S, Englbrecht C, Wehrle R, Hajak G, Soyka M. Eszopiclone for insomnia. The Cochrane Database of Systematic Reviews. 2018;10:CD010703.
201. Jones AW, Holmgren A, Ahlner J. Post-mortem concentrations of drugs determined in femoral blood in single-drug fatalities compared with multi-drug poisoning deaths. Forensic Science International. 2016;267:96-103.
202. Kuitunen T, Mattila MJ, Seppala T. Actions and interactions of hypnotics on human performance: single doses of zopiclone, triazolam and alcohol. International Clinical Psychopharmacology. 1990;5 Suppl 2:115-30.
203. Kuitunen T, Mattila MJ, Seppala T, Aranko K, Mattila ME. Actions of zopiclone and carbamazepine, alone and in combination, on human skilled performance in laboratory and clinical tests. British Journal of Clinical Pharmacology. 1990;30(3):453-61.
204. Hoiseth G, Berg-Hansen GO, Oiestad AM, Bachs L, Morland J. Impairment due to alcohol, tetrahydrocannabinol, and benzodiazepines in impaired drivers compared to experimental studies. Traffic Injury Prevention. 2017;18(3):244-50.
205. Yoshizuka KP, PJ. Upton, G. Lopes, I. Eric, J. standardized Field Sobriety Test: False Positive Test Rate among Sober Subjects. Journal of Forensic Toxicology & Pharmacology. 2014;3:2.
206. Broadhurst A, Cushnaghan RC. Residual effects of zopiclone (Imovane). Sleep. 1987;10 Suppl 1:48-53.
207. Lader M, Denney SC. A double-blind study to establish the residual effects of zopiclone on performance in healthy volunteers. Pharmacology. 1983;27 Suppl 2:98-108.
208. Nicholson AN, Stone BM. Zopiclone: sleep and performance studies in healthy man. Pharmacology. 1983;27 Suppl 2:92-7.

209. Blomberg RD, Peck RC, Moskowitz H, Burns M, Fiorentino D. The Long Beach/Fort Lauderdale relative risk study. Journal of Safety Research. 2009;40(4):285-92.
210. Moskowitz H, Fiorentino D. A review of the literature on the effects of low doses of alcohol on driving-related skills: National Highway Traffic Safety Administration. Washington DC, US. 2000. Report No.: DOT HS 809 028.
211. Strand MC, Morland J, Slordal L, Riedel B, Innerdal C, Aamo T, et al. Conversion factors for assessment of driving impairment after exposure to multiple benzodiazepines/z-hypnotics or opioids. Forensic Science International. 2017;281:29-36.
212. Gjerde H, Christophersen AS, Normann PT, Assum T, Oiestad EL, Morland J. Norwegian roadside survey of alcohol and drug use by drivers (2008-2009). Traffic Injury Prevention. 2013;14(5):443-52.
213. Papafotiou K, Carter JD, Stough C. An evaluation of the sensitivity of the Standardised Field Sobriety Tests (SFSTs) to detect impairment due to marijuana intoxication. Psychopharmacology. 2005;180(1):107-14.
214. Ramaekers JG, Theunissen EL, de Brouwer M, Toennes SW, Moeller MR, Kauert G. Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. Psychopharmacology. 2011;214(2):391-401.
215. Weissenborn R, Duka T. Acute alcohol effects on cognitive function in social drinkers: their relationship to drinking habits. Psychopharmacology. 2003;165(3):306-12.
216. Haffner JF, Morland J, Setekleiv J, Stromsaether CE, Danielsen A, Frivik PT, et al. Mental and psychomotor effects of diazepam and ethanol. Acta Pharmacologica et Toxicologica. 1973;32(3):161-78.
217. Mulvihill LE, Skilling TA, Vogel-Sprott M. Alcohol and the ability to inhibit behavior in men and women. Journal of Studies on Alcohol. 1997;58(6):600-5.
218. Marczinski CA, Fillmore MT. Preresponse cues reduce the impairing effects of alcohol on the execution and suppression of responses. Experimental and Clinical Psychopharmacology. 2003;11(1):110-7.
219. Fillmore MT, Vogel-Sprott M. An alcohol model of impaired inhibitory control and its treatment in humans. Experimental and Clinical Psychopharmacology. 1999;7(1):49-55.
220. Mellanby E. Alcohol and alcohol intoxication. British Journal of Inebriety. 1920;17:157-78.
221. Amlung MT, Morris DH, McCarthy DM. Effects of acute alcohol tolerance on perceptions of danger and willingness to drive after drinking. Psychopharmacology. 2014;231(22):4271-9.
222. Hiltunen AJ. Acute alcohol tolerance in cognitive and psychomotor performance: influence of the alcohol dose and prior alcohol experience. Alcohol. 1997;14(2):125-30.
223. Beirness D, Vogel-Sprott M. The development of alcohol tolerance: acute recovery as a predictor. Psychopharmacology. 1984;84(3):398-401.
224. Fillmore MT, Marczinski CA, Bowman AM. Acute tolerance to alcohol effects on inhibitory and activational mechanisms of behavioral control. Journal of Studies on Alcohol. 2005;66(5):663-72.
225. Marczinski CA, Fillmore MT. Acute alcohol tolerance on subjective intoxication and simulated driving performance in binge drinkers. Psychology of Addictive Behaviors : Journal of the Society of Psychologists in Addictive Behaviors. 2009;23(2):238-47.
226. Schweizer TA, Vogel-Sprott M. Alcohol-impaired speed and accuracy of cognitive functions: a review of acute tolerance and recovery of cognitive performance. Experimental and Clinical Psychopharmacology. 2008;16(3):240-50.
227. Weafer J, Fillmore MT. Acute tolerance to alcohol impairment of behavioral and cognitive mechanisms related to driving: drinking and driving on the descending limb. Psychopharmacology. 2012;220(4):697-706.
228. Morris DH, Treloar HR, Niculete ME, McCarthy DM. Perceived danger while intoxicated uniquely contributes to driving after drinking. Alcohol, Clinical and Experimental Research. 2014;38(2):521-8.

229. Cromer JR, Cromer JA, Maruff P, Snyder PJ. Perception of alcohol intoxication shows acute tolerance while executive functions remain impaired. Experimental and Clinical Psychopharmacology. 2010;18(4):329-39.
230. Starkey NJ, Charlton SG. The effects of moderate alcohol concentrations on driving and cognitive performance during ascending and descending blood alcohol concentrations. Hum Psychopharmacol. 2014;29(4):370-83.
231. Miller MA, Fillmore MT. Protracted impairment of impulse control under an acute dose of alcohol: a time-course analysis. Addictive Behaviors. 2014;39(11):1589-96.
232. Ostling EW, Fillmore MT. Tolerance to the impairing effects of alcohol on the inhibition and activation of behavior. Psychopharmacology. 2010;212(4):465-73.
233. Ingum J, Bjorklund R, Volden R, Morland J. Development of acute tolerance after oral doses of diazepam and flunitrazepam. Psychopharmacology. 1994;113(3-4):304-10.
234. Ellinwood EH, Jr., Nikaido AM, Heatherly DG, Bjornsson TD. Benzodiazepine pharmacodynamics: evidence for biophase rate limiting mechanisms. Psychopharmacology. 1987;91(2):168-74.
235. dos Santos FM, Goncalves JC, Caminha R, da Silveira GE, Neves CS, Gram KR, et al. Pharmacokinetic/pharmacodynamic modeling of psychomotor impairment induced by oral clonazepam in healthy volunteers. Therapeutic Drug Monitoring. 2009;31(5):566-74.
236. Barker MJ, Greenwood KM, Jackson M, Crowe SF. Cognitive effects of long-term benzodiazepine use: a meta-analysis. CNS Drugs. 2004;18(1):37-48.
237. Barker MJ, Greenwood KM, Jackson M, Crowe SF. Persistence of cognitive effects after withdrawal from long-term benzodiazepine use: a meta-analysis. Archives of Clinical Neuropsychology. 2004;19(3):437-54.
238. van der Sluiszen N, Vermeeren A, Jongen S, Vinckenbosch F, Ramaekers JG. Influence of Long-Term Benzodiazepine use on Neurocognitive Skills Related to Driving Performance in Patient Populations: A Review. Pharmacopsychiatry. 2017;50(5):189-96.
239. Aps JK, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. Forensic Science International. 2005;150(2-3):119-31.
240. Hold KM, de Boer D, Bos KL, van Ooijen RD, Zuidema J, Maes RA. Enantioselective quantitation of (R)- and (S)-alprenolol by gas chromatography-mass spectrometry in human saliva an plasma. Journal of Chromatographic Science. 1996;34(1):13-9.
241. Navazesh M. Methods for collecting saliva. Annals of the New York Academy of Sciences. 1993;694:72-7.
242. O'Neal CL, Crouch DJ, Rollins DE, Fatah AA. The effects of collection methods on oral fluid codeine concentrations. Journal of Analytical Toxicology. 2000;24(7):536-42.
243. Kato K, Hillsgrove M, Weinhold L, Gorelick DA, Darwin WD, Cone EJ. Cocaine and metabolite excretion in saliva under stimulated and nonstimulated conditions. Journal of Analytical Toxicology. 1993;17(6):338-41.
244. Drummer OH. Drug testing in oral fluid. The Clinical Biochemist. Reviews. 2006;27(3):147-59.
245. Nilsson GH, Kugelberg FC, Kronstrand R, Ahlner J. Stability tests of zopiclone in whole blood. Forensic Science International. 2010;200(1-3):130-5.
246. Crouch DJ, Walsh JM, Flegel R, Cangianelli L, Baudys J, Atkins R. An evaluation of selected oral fluid point-of-collection drug-testing devices. Journal of Analytical Toxicology. 2005;29(4):244-8.
247. Matin SB, Wan SH, Karam JH. Pharmacokinetics of tolbutamide: prediction by concentration in saliva. Clinical Pharmacology and Therapeutics. 1974;16(6):1052-8.
248. Drugbank. https://www.drugbank.ca/drugs/DB01198 (accessed 04.01.20).
249. Jusko WJ, Milsap RL. Pharmacokinetic principles of drug distribution in saliva. Annals of the New York Academy of Sciences. 1993;694:36-47.

250. Schepers RJ, Oyler JM, Joseph RE, Jr., Cone EJ, Moolchan ET, Huestis MA. Methamphetamine and amphetamine pharmacokinetics in oral fluid and plasma after controlled oral methamphetamine administration to human volunteers. Clinical Chemistry. 2003;49(1):121-32.
251. Moffat ACO, M.D. Widdop, B. Watts, J. Clarke's Analysis of Drugs and Poisons. 4th edition. 2011.
252. Gjerde H, Normann PT, Christophersen AS. The prevalence of alcohol and drugs in sampled oral fluid is related to sample volume. Journal of Analytical Toxicology. 2010;34(7):416-9.
253. Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clinical Pharmacokinetics. 2003;42(4):327-60.
254. Heuberger JA, Guan Z, Oyetayo OO, Klumpers L, Morrison PD, Beumer TL, et al. Population pharmacokinetic model of THC integrates oral, intravenous, and pulmonary dosing and characterizes short- and long-term pharmacokinetics. Clinical Pharmacokinetics. 2015;54(2):209-19.
255. Bullingham RE, McQuay HJ, Porter EJ, Allen MC, Moore RA. Sublingual buprenorphine used postoperatively: ten hour plasma drug concentration analysis. British Journal of Clinical Pharmacology. 1982;13(5):665-73.
256. Kuhlman JJ, Jr., Lalani S, Magluilo J, Jr., Levine B, Darwin WD. Human pharmacokinetics of intravenous, sublingual, and buccal buprenorphine. Journal of Analytical Toxicology. 1996;20(6):369-78.
257. Furuhaugen H, Jamt REG, Nilsson G, Vindenes V, Gjerde H. Roadside survey of alcohol and drug use among Norwegian drivers in 2016-2017: A follow-up of the 2008-2009 survey. Traffic Injury Prevention. 2018;19(6):555-62.
258. Bruun LD, Kjeldstadli K, Temte V, Birdal M, Bachs L, Langodegard M, et al. Detection Time of Oxazepam and Zopiclone in Urine and Oral Fluid after Experimental Oral Dosing. Journal of Analytical Toxicology. 2019;43(5):369-77.
259. Lee N, Duan H, Hebert MF, Liang CJ, Rice KM, Wang J. Taste of a pill: organic cation transporter-3 (OCT3) mediates metformin accumulation and secretion in salivary glands. Journal of Biological Chemistry. 2014;289(39):27055-64.
260. Shetty SJ, Desai PB, Patil NM, Nayak RB. Relationship between serum lithium, salivary lithium, and urinary lithium in patients on lithium therapy. Biological Trace Element Research. 2012;147(1-3):59-62.
261. Kreuz DS, Axelrod J. Delta-9-tetrahydrocannabinol: localization in body fat. Science. 1973;179(4071):391-3.
262. Kintz P, Villain M, Dumestre-Toulet V, Ludes B. Drug-facilitated sexual assault and analytical toxicology: the role of LC-MS/MS A case involving zolpidem. Journal of Clinical Forensic Medicine. 2005;12(1):36-41.
263. Stockham TL, Rohrig TP. The Use of Z-Drugs to Facilitate Sexual Assault. Forensic Science Review. 2010;22(1):61-73.
264. Mannaert E, Daenens P. Development of a fluorescence polarization immunoassay for the routine detection of N-desmethylzopiclone in urine samples. Analyst. 1996;121(6):857-61.
265. Arndt T. Urine-creatinine concentration as a marker of urine dilution: reflections using a cohort of 45,000 samples. Forensic Science International. 2009;186(1-3):48-51.
266. Guidelines Workplace Testing Programs. Substance Abuse and Mental Health Services. Notice of Proposed Revisions to the Mandatory Guidelines for Federal Workplace Testing Programs, (69 FR 16973). April 13, 2004.
267. Rowland M, Tozer TN. Clinical Pharmacokinetics - Concepts and Applications. 3rd edition. 1995.

11. Errata

- Different units for BACs have been applied in the different papers. In Paper I g/l is applied, even though the promille value or g/kg is correct. We should have corrected for the specific weight of blood (1.055).
- In paper I, Fig. 3 (and Fig. 2) the numbers in (or above) each bar represent the total number of clinical subtests/test components and not the number of «impaired» subtests/test components. In Fig. 3b the bar for the clinical subtests (SCTI) in the BAC range 0.51-0.93 should have been 18 % and not 10 %. The bars for the SCTI in Fig. 3a should have been up to 3 % higher.
- In Paper II under results and "Psychomotor Performance" in the first sentence should be: "Figure 2 shows the effect of the different drugs on **6** of the test components related to time after intake."
- In Paper IV the title should be written without a comma.
- In Paper IV, page 180, the last sentence under Chapter 3.1 should be: … the median was reduced from 3.3 to 2.8, …
- In Paper IV page 182, in the equation of the theoretical ZOBCR, pH(OF) and pH(blood) should be noted in superscript. The calculated value is however correct. In this thesis the correct use of superscript is given in Chapter 7.2.2.

11 Papers I-IV

I

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Can a simple clinical test detect impairment of zopiclone and alcohol? – A randomized controlled trial

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ABSTRACT

Purpose: The risk of traffic accident involvement is increased among patients prescribed the z-hypnotic drug zopiclone. Clinical test observations able to indicate drug impairment are therefore essential. This study compared the findings of a simplified clinical test of impairment (SCTI) with those of a battery of computerized psychomotor tests of impairment (CPTI).
Methods: 16 healthy young male volunteers attended a research unit on four different study days, receiving in randomized order either placebo, zopiclone 5 mg, zopiclone 10 mg, or alcohol 50 g. The SCTI was performed twice and the CPTI was performed three times on each study day, with blood samples being collected for drug analysis.
Results: The SCTI (and the CPTI) was able to demonstrate impairment at 1.5 h, but no major impairment was found at 7 h with the SCTI, after intake of both zopiclone and ethanol. The CPTI detected a significantly higher proportion of impaired observations than the SCTI, both for zopiclone and for ethanol, at all concentration levels. The sensitivity of the clinical tests in detecting blood drug concentrations often associated with impairment, due to zopiclone (above 23 ng/ml) and alcohol (above 0.5 g/l), was low, revealing 27 per cent and 18 per cent, respectively. The specificity, however, was higher, both for zopiclone (88 per cent) and for alcohol (96 per cent).
Discussion: The SCTI may be a useful tool, especially during roadside investigation, when the police are in doubt as to whether the apprehended driver is impaired or not. A subject, who has consumed zopiclone or alcohol, tested with the SCTI, with one or more subtests diverging from a habitual result, is likely to have a blood zopiclone concentration above 23 ng/ml or a BAC above 0.5 g/l. A negative result, however, is less helpful.

1. Introduction

The negative effects of alcohol on driving performance are well-documented. Several epidemiological studies have shown a blood alcohol concentration (BAC)-related increasing risk of traffic accident involvement [1,2], and a deteriorating driving performance, in experimental studies [3]. The detection of drunk driving, in the field, is often performed by the combined use of various simple clinical tests for impairment (e.g. the standardized field sobriety tests (SFST)) and breath alcohol screening.

Throughout the past few decades, driving under the influence of non-alcoholic drugs has received considerable attention and become of increasing concern [4,5]. As rapid roadside screening tests (e.g. qualitative saliva tests), for all non-alcoholic drugs of interest, are not yet available to detect cases of suspected drugged driving, other means of observation indicating impairment are still required. These observations are of importance, both in detecting cases which should be subjected to blood sampling and further examinations, and in documenting impairment for the possible subsequent handling of such cases in court. For these purposes, different approaches are currently being used in different countries, such as the SFST [6] and various clinical tests of impairment (CTIs) [7].

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The SFST consists of a specific set of tests, aiming to assess different aspects of cognitive functioning and psychomotor performance related to driving. It was originally designed to assist law enforcement officers in making roadside decisions on suspected alcohol-impaired driving, and has been found to be an accurate and reliable decision-aiding device for discriminating between blood alcohol concentrations (BAC) above or below 0.8 g/l [8]. Regarding the usefulness of clinical testing to other drugs than alcohol, a Finnish CTI was found to be more sensitive in detecting the intake of alcohol 0.8 g/kg than the intake of zopiclone 7.5 mg [9,10].

Previous studies have suggested that driving performance can be assessed testing three different aspects of driving-related behavior: the level of control; the ability to maneuver; and the ability to strategize [11]. Guidelines, established by The International Council on Alcohol, Drugs and Traffic Safety (ICADTS), suggest that three levels of behavior are tested to investigate traffic relevant psychomotor performance: automotive behavior, control behavior and executive planning behavior [12]. However, we are not aware of any systematic comparison between the SFSTs or the CTIs and such comprehensive testing of cognitive functioning and psychomotor performance. A systematic evaluation of cognitive functioning and psychomotor performance cannot be performed roadside, as it requires a laboratory with special equipment, while SFSTs and CTIs can easily be performed in almost any traffic-related setting.

One of the most frequently used non-alcoholic drugs among Norwegian drivers is zopiclone [13], and the risk of being involved in a traffic accident, causing personal injury, is increased among patients prescribed this drug [14]. Zopiclone can also be representative of the drug class acting through the GABA-receptor, including drugs like benzodiazepines, which are likewise commonly detected among drugged drivers involved in motor vehicle accidents [15].

The aim of the present study was to compare the findings of a simplified clinical test of impairment (SCTI) with a "gold standard" i.e. a battery of computerized cognitive and psychomotor tests of impairment (CPTI) covering all three aspects mentioned, in relation to detecting impairment. The aim was also to study if the findings of the SCTI could indicate blood drug concentrations of zopiclone or alcohol over a level often associated with impairment. This was done by applying a double-blind randomized trial, comparing the proportion of impaired subtests retrieved from the SCTI with the proportion of impaired test components retrieved from the CPTI, in subjects tested on four different occasions, after the administration of one or two capsules of 5 mg zopiclone, 50 g of alcohol, or placebo.

2. Materials and methods

2.1. Experimental design

A double-blind, placebo-controlled, randomized trial was performed on 16 healthy young male volunteers. The trial was approved by the Regional Ethical Committee for Medical Research and the Norwegian Medicines Agency. The study has been described in detail elsewhere [16,17]. The volunteers attended the research unit on four different study days, separated by at least a week, receiving in randomized order either placebo, zopiclone 5 mg, zopiclone 10 mg, or alcohol (ethanol) 50 g. Alcohol was given in an alcoholic drink containing 23 vol% [18]. Blood samples were collected during the study day, and analyzed for zopiclone and alcohol. The blood drug concentrations of zopiclone and alcohol have previously been reported on a group level [16] and on an individual level [17]. Clinical impairment was judged based on the performance on a simplified version of the Norwegian CTI, the SCTI, and on three licensed computerized tests.

2.2. Simplified clinical test of impairment (SCTI)

Five subtests from the Norwegian CTI were selected: gait-on-line test, turn-on-line test, finger-to-finger test, finger-to-nose test, and Romberg's test (standing steady on one leg for at least 5 s with arms stretched out and eyes closed). For each of the five subtests, the performance was measured and scored as either "habitual", "somewhat deviant", or "deviant". An overall impression of the subject, termed the "global impression", being the sixth subtest, was graded as either "not impaired", "slightly impaired" or "moderately impaired". The volunteers were assessed twice on each study day, at 1.5 and 7 h after drug intake (Fig. 1). The tests were performed around the points of time where zopiclone and alcohol reached their highest blood drug concentrations, as well as at the end of the study day, where any previously detected impairment was expected to be gone. Zopiclone and alcohol reached their highest drug concentrations in blood at 1.7 (±0.2 SEM) and 1.0 (±0.1 SEM) hours after intake, respectively [16]. The 768 (4 study days × 16 volunteers × 2 clinical tests on each study day × 6 subtests) individual clinical subtests were performed on the volunteers by three of the authors (A1, A2, A3). There were no significant differences between the authors with regard to the performance scores (Fisher's exact test).

2.3. Computerized psychomotor test of impairment (CPTI)

Three computerized tests were used in the study: stockings of Cambridge (SOC) and Choice Reaction Time (CRT), both from CANTABeclipse™ (version 3, ©2006, Cambridge Cognition Ltd.), and Connors Continuous Performance Test version II for Windows (CPT), from Multi-Health Systems Inc (©2000, 2004). Together, these three tests included 15 different test components with 4–6 components for each of the three behavior aspects [16]. The tests were each repeated three times (Fig. 1) after intake. In summation, the total number of computerized test component observations, after intake of the study drugs, were 2880 (4 study days × 16 volunteers × 3 sets of computerized tests on each study day × 15 test components in each set).

2.4. Defining impairment

For each subtest of the SCTI and each test component of the CPTI, each individual's placebo performance was defined as "not impaired". The placebo performances were consequently compared with every individual's performance at the same point of time after intake of an active drug. A similar score or an improved performance was registered as "not impaired", while any deteriorated performance, after active drug intake, was registered as "impaired".

The outcome of each subtest of the SCTI and each test component of the CPTI were arranged into groups according to blood drug concentrations. To define the drug concentration

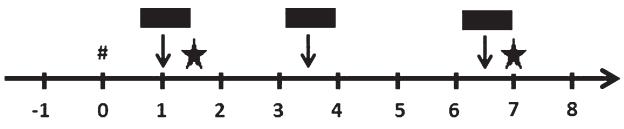

Fig. 1. Study day flowchart, showing the variables included in the study, in relation to time (in hours) after intake (#) of the study drugs. Arrows indicate the points of time for blood sampling. Black boxes indicate the time intervals for the performances measured by the computerized tests (CPTI). Stars indicate the points of time for the performances on the simplified clinical test of impairment (SCTI).

windows, the limits for impairment and graded sanctions, in Norway, were applied; with blood zopiclone concentration limits at 12 ng/ml, 23 ng/ml, and 58 ng/ml [19], and BAC limits at 0.2 g/l, 0.5 g/l, and 1.2 g/l, respectively. The highest blood zopiclone concentration measured was 77 ng/ml, allowing the following four concentration intervals: 0–12 ng/ml, 13–23 ng/ml, 24–58 ng/ml, and 59–77 ng/ml. For the BACs, the highest measured concentration was 0.93 g/l, allowing three concentration intervals: 0–0.20 g/l, 0.21–0.50 g/l, and 0.51–0.93 g/l. Hence, there were three concentration groups for alcohol and four concentration groups for zopiclone. Significant differences between the clinical subtests and the computerized test components were calculated by Pearson's Chi-squared test. All statistical analyses were performed using Microsoft Excel 2010 and SPSS Statistics 20.0.

2.5. Sensitivity and specificity of the SCTI

The outcomes of the subtests after intake of zopiclone were categorized into four different groups, depending on whether the concentrations were above or below 23 ng/ml, which in a previous study was shown to accompany impairment roughly corresponding to a BAC of 0.5 g/l [16,19], and on whether the outcome of the subtests were "impaired" or "not impaired" (Table 1a). For the subtests after intake of alcohol, the BACs were likewise categorized into four different groups, depending on whether the BACs were above or below 0.5 g/l, and on if the outcome of the subtests were "impaired" or "not impaired" (Table 1b). Based on this classification, it became possible to calculate the sensitivity and the specificity of the subtests.

3. Results

At the points of time where zopiclone and alcohol reached their highest blood drug concentrations, around 1.5 h after intake of the study drugs, the SCTI was able to demonstrate impairment in approximately 15–30 per cent of the subtests (Fig. 2), while only 5 per cent of the subtests revealed impaired results at 7 h after intake (data not shown).

At 1.5 h after study drug intake, the CPTI was able to reveal impaired results in more than 50 per cent of the test components (Fig. 2). Six to seven hours after intake, this percentage was down to 41 per cent for zopiclone 10 mg, and 32 per cent for zopiclone 5 mg and alcohol 50 g (data not shown). At 1–1.5 h after drug intake, a significantly lower proportion of the subtests (of the SCTI) were categorized as "impaired" compared with test components (of the CPTI), after all drug treatments (Fig. 2).

Table 1
Outcomes of the clinical subtests after intake of zopiclone **(a)** and alcohol **(b)** are presented. The outcome of each single subtest was classified as either "impaired" or "not impaired". A similar score or an improved performance compared with the placebo performance was registered as "not impaired", while any deteriorated performance after active drug intake was registered as "impaired". Subtest performances were consequently classified according to the blood zopiclone concentration or BACs, above or below/equal to 23 ng/ml or 0.5 g/l, respectively.

(a)

	Zopiclone conc. >23 ng/ml	Zopiclone conc. ≤23 ng/ml	Sum
Impaired subtests	32	31	63
Not impaired subtests	88	221	309
Summed	120	252	372

(b)

	BAC > 0.5 g/l	BAC ≤ 0.5 g/l	Sum
Impaired subtests	17	4	21
Not impaired subtests	79	92	171
Summed	96	96	192

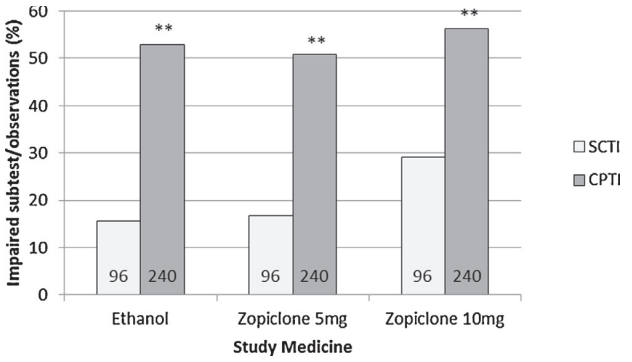

Fig. 2. Performances on the clinical subtests (SCTI) compared with the performances on the test components (CPTI), at 1–1½ hours after intake of the study drugs. An "impaired" subtest or test component was defined as any deteriorated performance after intake of a study drug, compared with the performance after intake of placebo. Significant differences between the SCTI and the CPTI were calculated using Pearson's Chi-squared test and are marked by asterisks in the figure: $^{**}p < 0.001$.

The fractions of impaired observations at the different blood zopiclone concentration levels are presented in Fig. 3a. For the SCTI, the fractions of impaired subtests were greater in the groups of zopiclone concentrations above 23 ng/ml. For the CPTI, the fractions of impaired test components increased in a concentration-related manner. The CPTI was able to detect a significantly

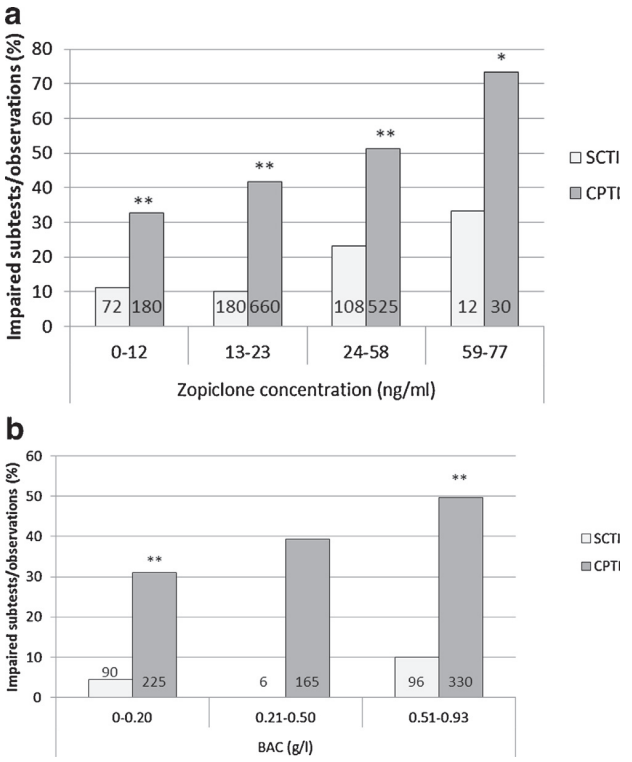

Fig. 3. (a) Performances on each subtest (SCTI) and test component (CPTI), compared with placebo performances, for the different zopiclone concentration levels. The total number of "impaired" subtests and test components at each concentration level is shown in each bar. Significant differences between the SCTI and the CPTI were calculated using Pearson's Chi-squared test and are marked by asterisks in the figure: $^{*}p < 0.05$; $^{**}p < 0.001$. (b) Performances on each subtest (SCTI) and test components (CPTI), compared with placebo performances, for the different BAC levels. The total number of "impaired" subtest and test components at each concentration level is shown in (or above) each bar. Significant differences between the SCTI and the CPTI were calculated using Pearson's Chi-squared test and are marked by asterisks in the figure: $^{*}p < 0.05$; $^{**}p < 0.001$.

higher fraction of impaired observations compared with the SCTI, at all zopiclone concentration levels.

The fractions of impaired observations at the different BAC levels are shown in Fig. 3b. There were comparatively more impaired subtests with the SCTI at BACs above 0.5 g/l than below this concentration level. For the test components of the CPTI, the fractions of impaired test components increased with increasing BAC levels. The CPTI was able to detect a significantly higher fraction of impaired observations compared with the SCTI, across all BAC levels.

In Tables 1a and b the outcomes of all the clinical subtests after intake of zopiclone and alcohol are presented. In Table 1a the subtest performances are classified according to the zopiclone concentration levels, above or below/equal to 23 ng/ml (comparable to a BAC of 0.5 g/l), and in Table 1b the subtest performances are classified according to the BAC, above or below/equal to 0.5 g/l. With reference to these limits, the sensitivity and the specificity for zopiclone was calculated to 27 per cent and 88 per cent, respectively. For alcohol, the sensitivity was 18 per cent and the specificity 96 per cent.

4. Discussion

In this study, the effects due to intake of zopiclone and alcohol in test subjects, as detected by the SCTI and the CPTI, were studied, as well as the usefulness of the SCTI in detecting drug concentrations often accompanying impairment. The study showed a higher proportion of impaired observations applying the CPTI, compared to the SCTI, when the subjects were studied under the influence of zopiclone or alcohol. For the CPTI and the SCTI the fractions of impaired observations increased in a concentration-related manner, after the administration of active drugs. When used for the purpose of detecting impairment due to zopiclone concentrations above 23 mg/ml and BACs above 0.5 g/l the SCTI was found to have a low sensitivity, but the specificity was quite high.

The CPTI was able to reveal *more impairment* than the SCTI at 1.5 h after intake of both zopiclone and alcohol. This difference in the impairment detecting ability, between the CPTI and the SCTI, was significant at all concentration levels, except for the BACs in the middle of the range (0.21–0.50 g/l). The number of clinical observations at this BAC level was low since the SCTI was performed either when the BAC was high (1.5 h after intake) or low (7 h after intake), which may explain the lack of a significant difference. The results indicate that the chosen computerized psychometric tests are more suitable in detecting impairment than the selected clinical subtests. The SCTI failed to detect major impairment 7 h after drug intake, while the CPTI was able to reveal impairment at this time, both for zopiclone and for alcohol. This result is in accordance with previous studies, revealing impairment due to similar dosages of zopiclone, at 7.5 h after intake [20], and at more than 10 h after intake [21–23]. A recent study compared the outcomes of various psychometric tests with measurements of the standard deviation of the lateral position (SDLP), while driving a car in an experimental situation [24]. The study found a higher sensitivity with the SDLP compared to certain psychometric tests. On the other hand, the SDLP measures only one aspect of driving behavior critical to safe driving [11,25].

This study found a positive concentration-effect relationship, both for zopiclone and for alcohol, with the CPTI, and also to some extent with the SCTI. This result is in accordance with former randomized studies demonstrating more pronounced effects due to higher dosages of zopiclone, compared with that of lower dosages [16,21,26–29]. A recent study supports a hypothesis that the SFST is also able to identify impairment associated with the use of CNS stimulants, CNS depressants, cannabis, and narcotic analgetics [30].

The results show a deteriorated performance over quite a wide concentration range, both for zopiclone and for alcohol. A former Finnish study, by Kuitunen et al., concluded that alcohol impaired both motor – (walking with eyes open and closed, gait-in-turning, finger-to-finger, collecting small objects) and vestibular (Romberg with eyes open and closed, nystagmus) functions on a clinical test, while zopiclone appeared to have minor impairing effects [9,10], at 2 h after intake of the study drugs. The study design and the blood drug concentrations detected in the Finnish study were quite similar to the results in the present study. The clinical test in the Finnish study also included additional clinical observations, such as for nystagmus, which is a well-established effect of alcohol impairment [6]. Nystagmus was, however, not among the chosen subtests in this study. Deteriorated performance on tests not included in this study (e.g. nystagmus), after the intake of alcohol, may to some degree explain the diverging results. Zopiclone reached its maximum concentration at a later point of time than that of alcohol, respectively at 1.7 versus 1.0 h after intake, leaving a declining BAC-concentration when the clinical tests were performed, in contrast to a rising zopiclone concentration. The volunteers could thereby have developed some acute tolerance to the effects of alcohol, but not to the effects of zopiclone. Acute tolerance has previously been found for both drugs [17].

Presently, we have no knowledge of any other randomized trial investigating the usefulness of clinical testing in detecting zopiclone and alcohol impairment. For the SCTI to be useful in practice, knowledge of whether an impairing concentration of a drug, like zopiclone or alcohol, will be revealed with some certainty, e.g. among suspected drugged drivers, is crucial. In a country where legal limits for driving under the influence of non-alcoholic drugs have been introduced, like Norway [19], this becomes particularly important. An additional tool, like the SCTI, could be helpful in evaluating if an apprehended driver might have a blood drug concentration above the legal limits. The results show that the SCTI detected impairment, due to zopiclone and alcohol, with almost equal sensitivity, but the sensitivities were low, at 27 and 18 per cent, respectively. The specificity for alcohol was 96 per cent, indicating that the risk of being judged as "impaired" with a BAC below 0.5 g/l is small. This was similarly the case for the blood zopiclone concentrations below 23 ng/ml, with the specificity being calculated to 88 per cent. The SCTI does not require any special equipment or skills from the investigator, and can easily be performed roadside, shortly after apprehension, by a police officer or a physisian. The SCTI is especially useful during roadside investigation, when the police are in doubt as to whether the apprehended driver is impaired or not. A subject who has consumed zopiclone or alcohol, and who is tested by the SCTI, with one or more of the six subtests diverging from a habitual performance, is likely to have a blood zopiclone concentration above 23 ng/ml or a BAC above 0.5 g/l. A negative result, however, would be less helpful. This study was performed on young male volunteers given only one drug. Investigating other study populations, such as the elderly or regular drug users, might have yielded different results.

The results from this study, with respect to the SCTI, would probably also be of relevance when investigating impairment due to benzodiazepines, which have quite similar pharmacodynamic properties to zopiclone (all acting through the GABA-receptor). Previous studies on reported outcomes of CTIs, performed in real life [7], support this assumption.

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References

[1] R.D. Blomberg, R.C. Peck, H. Moskowitz, M. Burns, D. Fiorentino, The Long Beach/Fort Lauderdale relative risk study, J. Saf. Res. 40 (2009) 285–292.
[2] H. Moskowitz, D. Fiorentino, A Review of the Literature on the Effects of Low Doses of Alcohol on Driving-Related Skills, National Highway Traffic Safety Administration, Washington, DC, USA, 2000.
[3] E. Schnabel, E. Hargutt, H. Krüger, Meta-analysis of Empirical Studies Concerning the Effects of Alcohol on Safe Driving: TREN-05-FP6TR-S07.61320-518404-DRUID, University of Wuerzburg, Germany, 2010.
[4] H. Gjerde, P.T. Normann, A.S. Christophersen, S.O. Samuelsen, J. Morland, Alcohol, psychoactive drugs and fatal road traffic accidents in Norway: a case–control study, Accid. Anal. Prev. 43 (2011) 1197–1203.
[5] H. Schulze, M. Schumacher, R. Urmeew, K. Auerbach, The DRUID project, in: Final Report: Work performed, Main Results and Recommendations; 6th Framework Programme, 2012, http://www.druid-project.eu/.
[6] M. Burns, H. Moskowitz, Psychophysical Tests for DWI Arrest, National Highway Traffic Safety Administration, Washington, DC, 1977,Report No. DOT HS 802 424.
[7] J.G. Bramness, S. Skurtveit, J. Morland, Testing for benzodiazepine inebriation – relationship between benzodiazepine concentration and simple clinical tests for impairment in a sample of drugged drivers, Eur. J. Clin. Pharmacol. 59 (2003) 593–601.
[8] J. Stuster, Validation of the standardized field sobriety test battery at 0.08% blood alcohol concentration, Hum. Factors 48 (2006) 608–614.
[9] T. Kuitunen, M.J. Mattila, T. Seppala, Actions and interactions of hypnotics on human performance: single doses of zopiclone, triazolam and alcohol, Int. Clin. Psychopharmacol. 5 (Suppl. 2) (1990) 115–130.
[10] T. Kuitunen, M.J. Mattila, T. Seppala, K. Aranko, M.E. Mattila, Actions of zopiclone and carbamazepine, alone and in combination, on human skilled performance in laboratory and clinical tests, Br. J. Clin. Pharmacol. 30 (1990) 453–461.
[11] J. Michon, A Critical View of Driver Behaviour Models: What Do We Know, What Should We Do? Plenum Press, New York, 1985.
[12] J.M. Walsh, A.G. Verstraete, M.A. Huestis, J. Morland, Guidelines for research on drugged driving, Addiction 103 (2008) 1258–1268.
[13] H. Gjerde, P.T. Normann, B.S. Pettersen, T. Assum, M. Aldrin, U. Johansen, et al., Prevalence of alcohol and drugs among Norwegian motor vehicle drivers: a roadside survey, Accid. Anal. Prev. 40 (2008) 1765–1772.
[14] I. Gustavsen, J.G. Bramness, S. Skurtveit, A. Engeland, I. Neutel, J. Morland, Road traffic accident risk related to prescriptions of the hypnotics zopiclone, zolpidem, flunitrazepam and nitrazepam, Sleep Med. 9 (2008) 818–822.
[15] L. Orriols, L.R. Salmi, P. Philip, N. Moore, B. Delorme, A. Castot, et al., The impact of medicinal drugs on traffic safety: a systematic review of epidemiological studies, Pharmacoepidemiol. Drug Saf. 18 (2009) 647–658.
[16] I. Gustavsen, K. Hjelmeland, J.P. Bernard, J. Morland, Psychomotor performance after intake of zopiclone compared with intake of ethanol: a randomized, controlled, double-blinded trial, J. Clin. Psychopharmacol. 31 (2011) 481–488.
[17] I. Gustavsen, K. Hjelmeland, J.P. Bernard, J. Morland, Individual psychomotor impairment in relation to zopiclone and ethanol concentrations in blood – a randomized controlled double-blinded trial, Addiction 107 (2012) 925–932.
[18] J. Morland, J. Setekleiv, J.F. Haffner, C.E. Stromsaether, A. Danielsen, G.H. Wethe, Combined effects of diazepam and ethanol on mental and psychomotor functions, Acta Pharmacol. Toxicol. 34 (1974) 5–15.
[19] V. Vindenes, D. Jordbru, A.B. Knapskog, E. Kvan, G. Mathisrud, L. Slordal, et al., Impairment based legislative limits for driving under the influence of non-alcohol drugs in Norway, Forensic Sci. Int. 219 (2012) 1–11.
[20] J. Boyle, J.A. Groeger, W. Paska, J.A. Cooper, C. Rockett, S. Jones, et al., A method to assess the dissipation of the [corrected] residual effects of [corrected] hypnotics: eszopiclone versus zopiclone, J. Clin. Psychopharmacol. 32 (2012) 704–709.
[21] M.L. Bocca, S. Marie, V. Lelong-Boulouard, F. Bertran, C. Couque, T. Desfemmes, et al., Zolpidem and zopiclone impair similarly monotonous driving performance after a single nighttime intake in aged subjects, Psychopharmacology 214 (2011) 699–706.
[22] T.R. Leufkens, A. Vermeeren, Highway driving in the elderly the morning after bedtime use of hypnotics: a comparison between temazepam 20 mg, zopiclone 7.5 mg, and placebo, J. Clin. Psychopharmacol. 29 (2009) 432–438.
[23] A. Vermeeren, W.J. Riedel, M.P. van Boxtel, M. Darwish, I. Paty, A. Patat, Differential residual effects of zaleplon and zopiclone on actual driving: a comparison with a low dose of alcohol, Sleep 25 (2002) 224–231.
[24] J.C. Verster, T. Roth, Predicting psychopharmacological drug effects on actual driving performance (SDLP) from psychometric tests measuring driving-related skills, Psychopharmacology 220 (2012) 293–301.
[25] T. Rothengatter, Psychological aspects of road user behaviour, Appl. Psychol. Int. Rev. 46 (1997) 223–234.
[26] M. Billiard, A. Besset, C. de Lustrac, L. Brissaud, Dose–response effects of zopiclone on night sleep and on nighttime and daytime functioning, Sleep 10 (Suppl. 1) (1987) 27–34.
[27] A. Broadhurst, R.C. Cushnaghan, Residual effects of zopiclone (Imovane), Sleep 10 (Suppl. 1) (1987) 48–53.
[28] M. Lader, S.C. Denney, A double-blind study to establish the residual effects of zopiclone on performance in healthy volunteers, Pharmacology 27 (Suppl. 2) (1983) 98–108.
[29] A.N. Nicholson, B.M. Stone, Zopiclone: sleep and performance studies in healthy man, Pharmacology 27 (Suppl. 2) (1983) 92–97.
[30] A.J. Porath-Waller, D.J. Beirness, An examination of the validity of the standardized field sobriety test in detecting drug impairment using data from the Drug Evaluation and Classification program, Traffic Inj. Prev. 15 (2014) 125–131.

IV

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Zopiclone concentrations in oral fluid and blood after, administration of therapeutic doses of zopiclone

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ABSTRACT

Purpose: Little is known about the relationship between concentrations in oral fluid (OF) and blood for the widely prescribed hypnotic drug zopiclone. The purpose of this study was to investigate the usefulness of OF zopiclone concentrations to predict blood zopiclone concentrations in order to introduce OF testing as an alternative to more cumbersome blood testing.
Methods: 16 healthy young male volunteers received capsules of either 5 or 10 mg zopiclone on two different study days separated by at least one week. Blood and OF were collected simultaneously at baseline and 9 times after intake of zopiclone on each study day. In addition an OF sample was collected 24–81 h after intake. Lunch was served between samples taken 2.5 and 3.5 h after intake. All samples were analysed for zopiclone, and the cut-off was 10 ng/ml in blood and 0.2 ng/ml in OF–buffer mixture.
Results: Zopiclone was detected in all OF samples during the study day. After 24–81 h, all subjects were also positive for zopiclone in OF, except from three subjects ingesting the 5 mg dose. In a single case zopiclone was detected in a baseline OF sample 14 days after intake on an earlier study day. Zopiclone was detected in both OF and blood in 231 OF/blood pairs, and a significant but weak correlation between OF and blood concentration was seen (R^2 of 0.30). The median (range) zopiclone OF/blood concentration ratio (ZOBCR) for all samples were 3.3 (0.8–18). The ZOBCR decreased when the OF volume increased. After 30 of 31 given doses of zopiclone, the ZOBCR was higher in samples collected before lunch than samples collected after lunch.
Discussion: Vast intra- and interindividual differences in ZOBCR were found, and the correlation between OF and blood concentration was less pronounced than reported in former studies. In accordance with earlier studies we found a negative correlation between ZOBCR and OF volume. The ZOBCR decreases in relation to recent intake of a meal, probably because stimulated saliva production causes "dilution" of saliva. OF zopiclone concentration appeared unsuitable for estimation of blood zopiclone concentration. Due to long detection time, analysis of zopiclone in OF might be useful to detect non-recent, previous intake.

1. Introduction

The magnitude of cognitive and psychomotor (side) effects of many drugs are often related to the blood drug concentration [1]. This is reflected in a correlation between the degree of driving impairment and the level of the particular drug in blood [2]. Blood sampling with determination of drug concentration is therefore considered important in the handling of suspected driving under the influence (DUI) cases. However, collection of blood samples would require suitable environment, sterile equipment and trained personnel. Of alternative matrices oral fluid (OF) has several advantages such as easy sample collection which can be performed roadside by most people. Compared to urine OF has reduced adulteration potential and often indicates recent drug intake [3–7]. In the DRUID project OF screening devices were used to indicate drug use in driving populations [8]. OF is also used widely roadside

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as a routine matrix in law enforcement of suspected impaired driving, and positive confirmation analysis of drugs in OF may be followed by judicial punishment e.g. in Queensland, Australia [9,10] and Belgium [11].

The application of the use of OF to determine the magnitude of impairment would require a rather strong and constant correlation between drug concentrations in OF and blood. The measurement of drugs in saliva/OF is also of interest for purpose of therapeutic drug monitoring for some therapeutic drugs [12].

For several potential drugs of abuse it has been attempted to establish conversion factors for drug concentrations in OF to concentrations in whole blood e.g. for amphetamines, opioids, cocaine and benzodiazepines [2,13]. Cross sectional population studies have found variable ratios between drug concentrations in OF and blood for some drugs, including benzodiazepines [2,14,15]. In a controlled experimental study a variable correlation between OF and blood concentrations of oxazepam was demonstrated [16].

Zopiclone is a benzodiazepine-like hypnotic drug widely prescribed in many countries [17,18]. In previous studies we have found a high prevalence of zopiclone use among Norwegian car drivers [8,19] and an increased risk of being involved in a car accident among those being prescribed this medication [20]. It has further been demonstrated that blood zopiclone concentrations are related to the degree of impairment as measured by psychomotor and cognitive tests [21,22]. Little is, however, known about the relationship between concentrations in OF and concentrations in blood (serum) [15,23].

In the present study we investigated the usefulness of OF zopiclone concentrations to predict blood zopiclone concentrations in order to possibly introduce OF testing as an alternative to more cumbersome blood testing. We performed a controlled experiment on 16 volunteers who were given zopiclone in randomized order in two different doses, at two occasions, followed by repeated measurements of zopiclone concentration in OF and blood during a period of at least ten hours subsequent to drug administration.

2. Methods

2.1. Study procedure

16 healthy young males were enrolled in a double blind, placebo-controlled randomized trial. The individuals were given alcohol, placebo or two different doses of zopiclone on four separate study days. The individuals had to fulfil certain inclusion criteria, such as age 20–30, body weight within 70–85 kg and approval of written informed consent. Individuals with a history or presence of mental or significant somatic disease, drug/alcohol abuse, any regular (daily) intake of any prescribed drug or intake of zopiclone within three months ahead of the study were excluded. The study has been described in detail elsewhere [21,22,24]. For the present study only the study days with zopiclone administration were relevant. The subjects were in a randomized order given either zopiclone 5 mg or zopiclone 10 mg. To prevent contamination of the oral cavity zopiclone tablets (Zopiklon "Merck NM") were crushed, mixed with lactose and packed into capsules. This procedure was performed at the GMP certified Pharmacy at Oslo University Hospital (Rikshospitalet), Norway. The capsules were rapidly swallowed by the individuals. The washout period between each study day was at least one week. Before intake of study medication, baseline samples of OF, blood and urine were retrieved. Blood and OF samples were collected simultaneously 9 times (0.5, 1.0, 1.5, 2.5, 3.5, 5, 6.5, 8 and 10 h) after drug intake. We did not manage to draw any blood samples from one individual on his zopiclone 5 mg study day. Out of a total of 279 OF/blood pairs, zopiclone was detected in both blood and OF in 231 pairs. A light meal (lunch) was served between 2.5 and 3 h after drug intake. Dinner was served between 8 and 8.5 h after drug intake. The individuals were also requested to deliver an OF sample 24–81 h after intake of study medication. At this time no simultaneous blood samples were taken. The OF was collected by using the Intercept Oral Specimen Collection Device (OraSure Technologies, Bethlehem, PA, USA). The Intercept collection pad is treated with preservatives and compounds that stimulate the production of OF (sodium chloride, citric acid, sodium benzoate, potassium sorbate, gelatine and sodium hydroxide), while the preservative buffer contains an antibacterial agent, non-ionic surfactant and colour (chlorhexidine digluconate, Tween 20, Flag Blue Dye) in addition to water. A collection device recovery of 80% has previously been reported for zopiclone with this collection device [25].

2.2. Analysis and calculation of zopiclone concentration in OF

Zopiclone can hydrolyze to 2-amino-5-chloropyridine [26,27], and instability has been reported for both blood and OF [28–30]. Fast analysis or storage at −20 °C is therefore recommended [29,30]. Both OF and blood samples were therefore only stored in a refrigerator (5 °C) for maximum 24 h after sampling and then either analysed or frozen (−20 °C) for later analysis. About half of the OF and blood samples were analysed within two days and all samples were analysed within nine days.

Whole blood samples were protein precipitated and analysed with liquid chromatography mass spectrometry (LC–MS), while the OF samples underwent liquid–liquid extraction before analysis with liquid chromatography tandem mass spectrometry (LC–MS/MS) [25,31]. Blood calibration samples were prepared by spiking whole blood. Oral fluid calibration samples were made by spiking Negative Calibrator Oral Fluid, purchased from Orasure Technologies Inc., with the same contents as the collection devices. In addition the blue dye used for visibility in the collection devices was added. In the present study a cut-off for zopiclone in blood of 10 ng/ml and in OF–buffer mixture of 0.2 ng/ml were used. No analysis of any metabolites of zopiclone was performed. The Intercept device contained 0.8 ml buffer. The collected OF was therefore diluted with this buffer volume after sampling. Drug concentrations in undiluted OF (C) were calculated as follows: $C = C_0 \times V_{total}/V_{OF}$, where C = drug concentration in OF, C_0 = drug concentration in OF–buffer mixture, V_{total} = volume of buffer and OF and V_{OF} = volume of OF [31]. To calculate the volume of OF, we weighed the OF collection device. The volume of the OF delivered by each subject (V_{OF}) was calculated by subtraction of the standard weight of the OF Collection device (with buffer) from the total weight of each OF sample (including device, buffer and added OF).

2.3. Statistics

The concentrations of zopiclone in OF and blood were compared, and the zopiclone OF/blood concentration ratios (ZOBCR) for all 16 individuals were calculated for each time point of simultaneous OF/blood sampling. All statistical analyses were performed using Microsoft Excel 2010 and SPSS Statistics 23.0. The results were not normally distributed. Median and 25th–75th percentile values are reported for continuous variables and frequency distributions are reported for categorical variables. Wilcoxon rank test were used to analyse the difference in time to reach maximum concentration (T_{max}) in blood and OF and to analyse the difference in ZOBCR between different time points of sampling. The Pearson correlation was used to analyse the correlations between the zopiclone concentration in OF and blood, between ZOBCR and OF volume and between ZOBCR and blood zopiclone concentration.

2.4. Approvals

The trial was approved by the Regional Ethical Committee for Medical Research and the Norwegian Medicines Agency.

3. Results

The study population was male college students within the age 20–28 (median 23.5) with a body weight of 70–85 (median 76.5) kg. The time–concentration profiles for all individuals after intake of 5 and 10 mg zopiclone in blood are shown in Fig. 1a and b. After intake of 5 and 10 mg zopiclone the maximum blood drug concentration was reached from 0.5 to 2.5 h after drug intake, with a median T_{max} of 1.5 h for both doses. For the blood sample taken 10 h after intake of 5 or 10 mg zopiclone, 1 out of 15 and 11 out of 16, respectively had zopiclone concentrations above the cut-off limit in blood.

The individual time–concentration profile for all 16 individuals after intake of 5 and 10 mg zopiclone in OF are shown in Fig. 2a and b. The maximum OF drug concentration was reached between 1 and 2.5 h with a median T_{max} at 1.5 h for the 5 mg doses and at 2.5 h for the 10 mg doses. All OF samples retrieved after intake of zopiclone on each study day had zopiclone concentrations above cut-off in OF. T_{max} was not significantly longer in OF than in blood for either of the doses ($p > 0.05$, Wilcoxon signed rank test).

23–81 h after intake of 5 mg zopiclone 15 individuals delivered an OF sample, and 23–76 h after intake of 10 mg zopiclone 12 individuals delivered an OF sample. After intake of 5 mg zopiclone, OF samples were positive for zopiclone up to 50 h after intake, which included 12 of the 15 cases. For the intake of 10 mg zopiclone the drug was found in OF in all samples from 12 individuals, i.e. up to 76 h after intake.

For two of the individuals zopiclone was measurable above the cut-off limit (0.23 and 0.27 ng/ml) in OF baseline samples. These two individuals had received 10 mg zopiclone in a previous session 7 and 14 days, respectively, prior to the study day. Zopiclone was analysed in OF baseline samples on the study days the individuals received zopiclone. After intake of 5 mg OF baseline samples from 6 individuals were analysed on the following study day, while after intake of 10 mg OF baseline samples from 5 individuals were analysed on the following study day. For the other OF baseline samples analysed, zopiclone was not measurable.

In Fig. 3 a scatterplot of the zopiclone concentration in OF vs. blood with regression line is shown. Only the 231 time points where zopiclone concentrations were measurable in blood were included in the scatterplot. The linear regression analysis for the relationship of OF and blood concentration showed a correlation with a coefficient of determination R^2 of 0.30 ($p < 0.01$, Pearson correlation).

The overall median (range) of the ZOBCR (individual zopiclone OF/blood concentration ratios) for all samples were 3.3 (0.8–18),

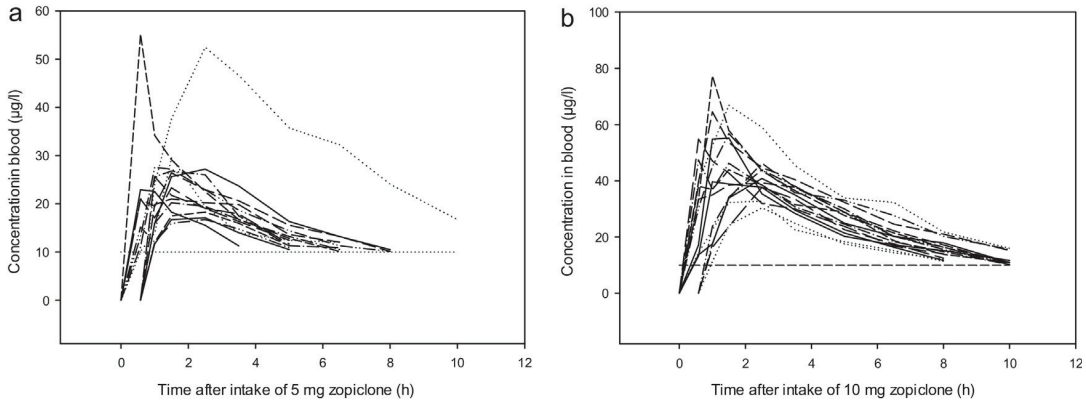

Fig. 1. Concentration of zopiclone in blood vs. time after intake of 5 mg zopiclone (a) (N = 15) and 10 mg zopiclone (b) (N = 16). Each line represents one individual. The horizontal line in the figures shows the LOQ (10 µg/l).

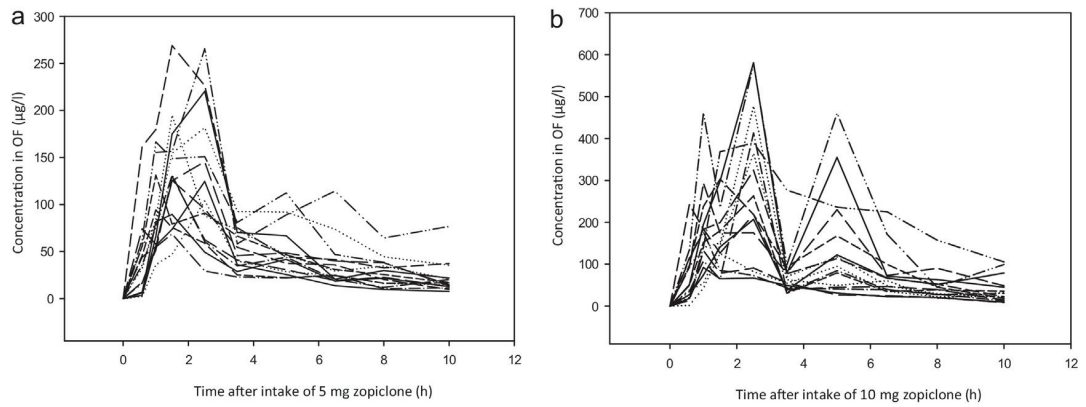

Fig. 2. Concentration of zopiclone in OF vs. time after intake of 5 mg zopiclone (a) and 10 mg zopiclone (b) for all 16 individuals up to 10 h after intake. Each line represents one individual. All OF samples retrieved after intake of zopiclone on each study day had zopiclone concentrations above the applied cut-off in OF.

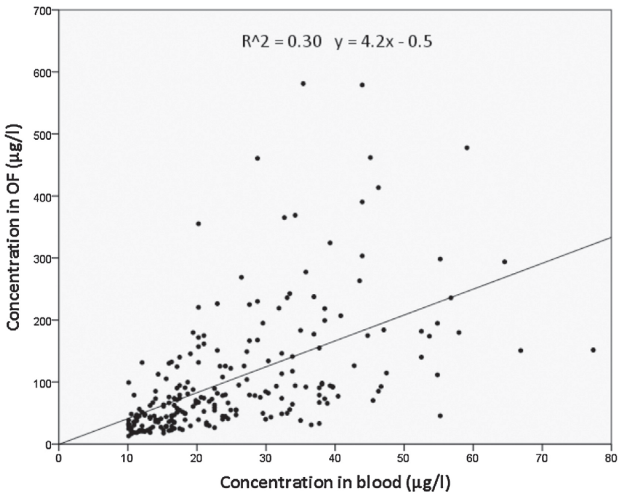

Fig. 3. The zopiclone OF vs. blood zopiclone concentration with regression line. Only OF/blood pairs where zopiclone were measurable in blood were included. N = 231 blood/OF pairs from 16 subjects.

and the 25th and 75th percentiles were respectively 2.1 and 4.9. For the 16 individuals the number of OF–blood paired samples available for both doses (max 18) varied from 8 to 17 and the individual median ratios (ZOBCR) varied from 1.8 to 7.9. A boxplot of ZOBCR for each individual is shown in Fig. 4.

Due to the large variations in ZOBCR the importance of factors like OF volume and time after intake of zopiclone in blood were further investigated.

3.1. OF volume

The volume of the OF (V_{OF}) had a wide range (0.07–1.02 ml). A scatter plot of ZOBCR vs. volume of the OF is shown in Fig. 5. The figure indicates that the ZOBCR decreased when the OF volume increased, and the samples with low OF volume (less than 0.4 ml) tended to have high ZOBCRs. Result of linear regression analysis for the relationship of ZOBCR and OF weight showed a coefficient of determination R^2 of 0.17 ($p < 0.01$, Pearson correlation). When including only the OF samples with volume $\geq$0.4 ml, the number of extreme outliers (defined as ZOBCR greater than 3× the interquartile range) were decreased from 7 to 2, the median range was reduced from 3.3 to 2.8, and the 25- and 75-percentiles from 2.1 and 4.9 to 1.9 and 4.3 respectively.

3.2. Time after intake

The ZOBCR for all time points after intake are shown in Fig. 6. For the samples collected 0.5, 3.5, 6.5, 8 and 10 h after intake, the median ZOBCR for each time point was lower than the overall median. The highest median (and also the highest 25th and 75th percentile) ZOBCR (5.1) were found in samples collected 2.5 h after drug intake, and the lowest median ZOBCR (2.1) was observed after 3.5 h. For all individuals and both doses, except for one (after a 5 mg dose), ZOBCR was decreasing between these two time-points of sampling. Within this time interval (between 2.5 h and 3.5 h after intake) a light lunch meal was served. OF and blood samples were retrieved immediately before and 0.5 h after intake of lunch. The median volume of all samples was 0.59 ml, and the volume of the samples collected 2.5 h and 3.5 h after intake was 0.48 ml and 0.66 ml respectively. For samples collected before and after intake

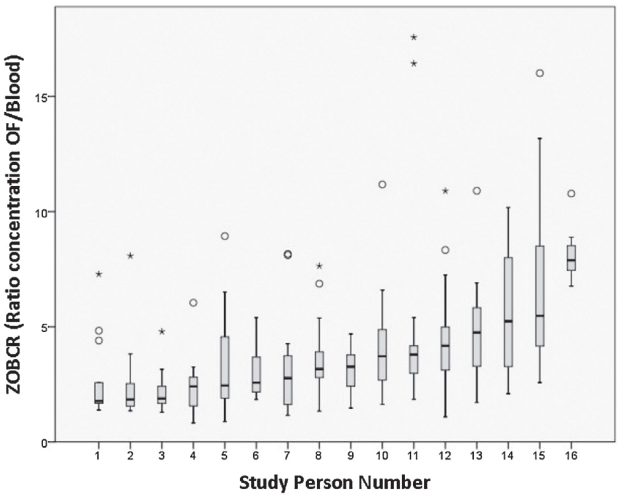

Fig. 4. ZOBCR for all individuals. The boxes represent the interquartile range (lower limit — 25th percentile; upper limit 75th percentile), with the middle (horizontal) line of the box representing the median ZOBCR for each study person for all time points at both doses. The whiskers represents the range of concentrations for each group, not including outliers (O — ZOBCR greater than 1.5× the interquartile range; * — concentrations greater than 3× the interquartile range). The individuals are sorted according to ascending median concentration ratios. For subject number 16 ZOBCR was obtained only after intake of 10 mg.

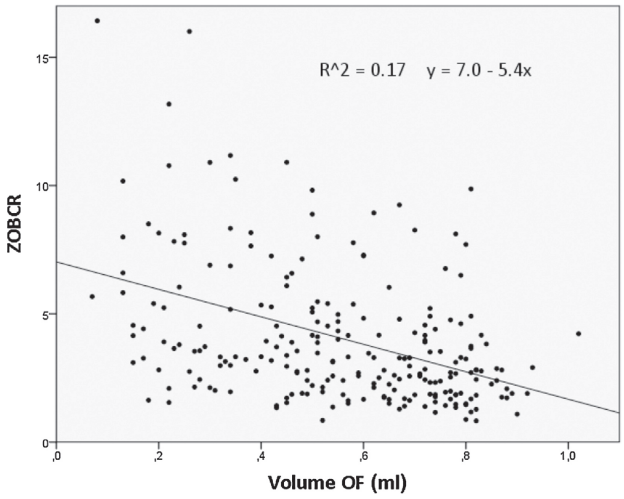

Fig. 5. Scatter plot of ZOBCR vs. OF volume with regression line. N = 231 blood/OF pairs from 16 subjects.

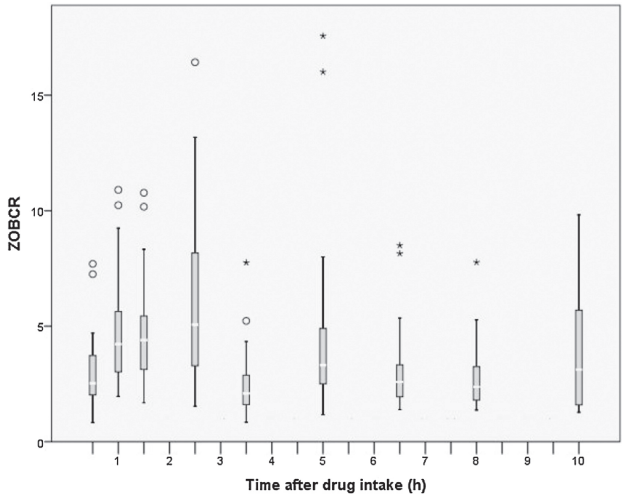

Fig. 6. ZOBCR vs. time after drug intake for all samples (both doses). The boxes represent the interquartile range (lower limit — 25th percentile; upper limit 75th percentile), with the middle (transparent horizontal) line of the box representing the median ZOBCR for each time after intake. The whiskers represents the range of concentrations for time after intake, not including outliers (O — ZOBCR greater than 1.5× the interquartile range; * — concentrations greater than 3× the interquartile range).

of dinner (8 and 10 h after intake) we found no significant differences in ZOBCR.

4. Discussion

In this study we compared the concentrations of zopiclone in OF and blood in samples collected simultaneously, repeatedly over a time period as long as zopiclone could be detected in blood after a single intake. There was some correlation between the concentration in OF and in blood, although vast intra- and interindividual differences in ZOBCR were found. These differences could partly be due to differences in OF sample volume as we found a significant negative correlation between ZOBCR and volume. The negative correlation persisted, although less pronounced, even if only OF-volumes above 0.4 ml were studied. Another factor found to influence ZOBCR was the relation of sampling to food intake. We also observed that zopiclone could be detected in OF at least up to 14 days after a single intake of 10 mg zopiclone for some individuals.

In a former study Langel et al. found a correlation between the OF and whole blood zopiclone concentration ($R^2 = 0.636$) [15] which was higher than our findings ($R^2 = 0.30$). The former study contained only eight OF/blood pairs (from eight individuals), while our study included 231 OF/Blood pairs (from 16 individuals). With the present study design and the high number of OF/blood pairs, our study indicates that the individual variation in ZOBCR ratio is higher than previously reported. The 231 OF/blood pairs were, however, not completely unrelated since all pairs originated from only 16 individuals.

Our results show that the OF samples with a low volume of OF expressed higher ZOBCR than those with high volume. This is in accordance with a former study [32] that found higher prevalence of alcohol and drugs in OF samples with small volumes than in those with larger volumes. The authors recommended that samples with smaller volumes than required by the analytical methods should not be discarded, but instead be analysed using the smaller sample volume, if necessary, after dilution. In the former study the average volume collected roadside in cases containing zopiclone was smaller (0.31 ml, N = 163) than the median volume (0.59 ml) collected in the present study. We also found that zopiclone concentration in OF samples with low volume tended to demonstrate larger variation in ZOBCR and to include

more ZOBCR outliers. The OF volume was measured by weight determination. For the low volumes it is of great importance that the weight of the OF is accurate, as weight inaccuracy may have contributed to the larger ZOBCR. If only the OF samples with large volumes (weight higher than 0.4 g) were included the number of extreme outliers and the individual variance in ZOBCR was reduced. In the former study [32] OF samples were collected from roadside surveys, and positive findings may indicate samples from impaired drivers and drug abusers. The population of suspected impaired drivers include individuals that use large doses of drugs that are known to cause hyposalivation. Since our study was a controlled study of healthy volunteers (no drug abusers) there must be other explanations to the increased concentration of zopiclone in low volume OF samples.

There was no significant difference in time to reach maximum concentration in blood and OF for either of the doses of zopiclone. Since zopiclone was administered in coated capsules and swallowed such, the measured zopiclone in OF most likely origins as a consequence of transport from blood and not from local contamination during intake. Absorbed zopiclone will then pass from the circulating plasma, through the capillary wall, the basement membrane, the glandular epithelial cells and into the salivary duct by simple passive diffusion [12]. Due to "weak" protein binding (45–80% [33]) and a pK_a value close to physiologic pH (6.7) [15,34] zopiclone is suitable for detection in OF, and the transport from plasma to OF is not likely time-limiting. The high levels of zopiclone concentration in OF depend on the physiochemical properties of the drug and the pH in blood and OF. Assuming that average pH in OF is 6.7 (pH(OF)) [35] and the average pH in blood is 7.4 (pH(blood)) it is possible to predict the theoretical ZOBCR by using the following equation [23]:

$$\text{ZOBCR(theoretical)} = (1 + 10^{pK_a} - \text{pH(OF)})/(1 + 10^{pK_a} - \text{pH(blood)}) = 1.7$$

indicating higher concentrations in OF than in blood.

After intake of a light meal (lunch), the ZOBCR was significantly reduced for almost all individuals at both zopiclone doses. The OF and blood samples were collected within short time (less than 30 min) after intake of lunch. Intake of food and chewing stimulates the salivary glands to produce saliva. The secretion rate can vary from 0 ml/min to 6 ml/min. Before zopiclone circulating in plasma can be discharged into the salivary duct it must pass through the capillary wall, which is a rate-determining step [12]. A possible explanation for the lower ZOBCR after the light meal is that the speed of diffusion of zopiclone from the circulating plasma and into the salivary duct could not keep up with the increased saliva secretion rate. After stimulation of the flow rate the concentration of drugs in OF decrease [36]. This "dilution" by extra saliva production may explain the sudden reduction of ZOBCR after intake of a light meal. Vice versa if the production of saliva was low, the dilution would be low and could explain the high zopiclone concentration in low volume samples. This dilution effect is also suggested for methamphetamine in a controlled study [37]. After intake of dinner we did not observe any significant reduction in ZOBCR, possibly due to a long time period (1.5 h) before next sampling. Dinner was served in the end of the study day (between 8 and 8.5 h after intake of zopiclone) and at this time the concentrations in OF was low and in some individuals not detectable in blood.

Our results show that zopiclone could be measured in OF for more than 24 h after intake of 5 or 10 mg zopiclone and up to at least 14 days after a single intake of 10 mg zopiclone in a single case. The information collected on drug use by the participants gave no evidence for zopiclone use outside the study days by anyone. Before intake of study drug on every study day a urine sample was delivered and analysed for several common drug of abuse, they were all negative for zopiclone and benzodiazepines. Zopiclone was not detected in baseline samples if alcohol or placebo was ingested on the previous study day. Using the estimated half-life from blood zopiclone concentration for every single individual, all individuals were expected to have zopiclone concentrations in blood far below our applied cut-off 24 h after intake of zopiclone. The ratio between the median maximum concentration of all individuals and the applied cut-off was far higher in OF than in blood. This can to some degree explain the prolonged detection-time in OF, but detection times longer than 2–3 days would more likely be explained by accumulation/storage of zopiclone in the oral mucosa, even though this is not described in previous literature. Former road-side studies have shown that zopiclone is the most frequently detected drug in OF in Norway, where about 1–2% of the driving population had measurable zopiclone in OF [8,19]. The LOQ in OF in these studies were 10 ng/ml [38], which is 17 times higher than the applied cut-off in this study (corrected for an average dilution factor of three in the Intercept sampling set [25,32]). According to the Norwegian prescription database (http://www.norpd.no) about 6% of the population were prescribed zopiclone at least once in 2016. Zopiclone has a short half-life and the detection time in blood is limited, but the long detection time in OF may have contributed to the high prevalence in roadside OF samples, even though the applied cut-off in the present study was lower than in the former road side studies.

To our knowledge simultaneous measurements of zopiclone concentrations in OF and blood (plasma) have previously been performed only once in a controlled experimental setting [23]. In this previous study the concentration ratio between OF and blood concentrations was not the main focus, but to study the pharmacokinetic and clinical parameters of zopiclone and the interaction between zopiclone and trimipramine. By the study procedure in the present study allowing many samples to be taken from the same group of individuals, inter- and intraindividual variability can be studied in a more informative manner than by collecting OF and blood samples at random in a general population or in drivers. Although less variation could be expected under controlled conditions, we observed marked variations in inter- and intraindividual ZOBCRs. Even if cut-offs with respect to sample volumes were introduced, OF zopiclone concentrations appeared unsuitable for estimation of blood zopiclone concentration. On the other hand, due to the long detection time, analysis of zopiclone in OF may be suitable for documentation of former, non-recent drug intakes. Urine is often the preferred matrix to detect non-recent drug intakes. Former studies have revealed detection times in urine at least up to two days after a single intake of 5 mg [39] and up to six days after a single intake of 10 mg zopiclone [40]. Due to the longer detection time and the simplicity of sample collection, obviating the need for special restroom facilities and same sex collectors and making adulteration more difficult [41], OF could be preferred above urine. In the context of suspected drug-facilitated sexual assaults, the biological sample of the offended is often retrieved a long time after the presumed assault and analysis of OF could also be tried.

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References

[1] C. Hiemke, P. Baumann, N. Bergemann, A. Conca, O. Dietmaier, K. Egberts, et al., AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011, Pharmacopsychiatry 44 (2011) 195–235.
[2] S.M. Wille, E. Raes, P. Lillsunde, T. Gunnar, M. Laloup, N. Samyn, et al., Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of driving under the influence of drugs, Ther. Drug Monit. 31 (2009) 511–519.
[3] D. Lee, M.A. Huestis, Current knowledge on cannabinoids in oral fluid, Drug Test Anal. 6 (2014) 88–111.
[4] W.M. Bosker, M.A. Huestis, Oral fluid testing for drugs of abuse, Clin. Chem. 55 (2009) 1910–1931.
[5] M.A. Huestis, A. Verstraete, T.C. Kwong, J. Morland, M.J. Vincent, R. de la Torre, Oral fluid testing: promises and pitfalls, Clin. Chem. 57 (2011) 805–810.
[6] Y.H. Caplan, B.A. Goldberger, Alternative specimens for workplace drug testing, J. Anal. Toxicol. 25 (2001) 396–399.
[7] S.W. Toennes, G.F. Kauert, S. Steinmeyer, M.R. Moeller, Driving under the influence of drugs—evaluation of analytical data of drugs in oral fluid, serum and urine, and correlation with impairment symptoms, Forensic Sci. Int. 152 (2005) 149–155.
[8] S. Houwing, M. Hagenzieker, R. Mathijssen, Prevalence of alcohol and other psychoactive substance in drivers in general traffic, Part I: General Results. DRUID Deliverable D 2.2.3, SWOV Institute for Road Safety Research, Leidschendam, The Netherlands, 2011 http://www.druid-project.eu/Druid/EN/deliverales-list/downloads/Deliverable_2_2_3_Part1.pdf?__blob=publicationFile&v=1.
[9] J. Davey, K. Armstrong, P. Martin, Results of the Queensland 2007–2012 roadside drug testing program: the prevalence of three illicit drugs, Accid. Anal. Prev. 65 (2014) 11–17.
[10] M. Chu, D. Gerostamoulos, J. Beyer, L. Rodda, M. Boorman, O.H. Drummer, The incidence of drugs of impairment in oral fluid from random roadside testing, Forensic Sci. Int. 215 (2012) 28–31.
[11] T. Van der Linden, S.M. Wille, M. Ramirez-Fernandez, A.G. Verstraete, N. Samyn, Roadside drug testing: comparison of two legal approaches in Belgium, Forensic Sci. Int. 249 (2015) 148–155.
[12] J.K. Aps, L.C. Martens, Review: the physiology of saliva and transfer of drugs into saliva, Forensic Sci. Int. 150 (2005) 119–131.
[13] V. Vindenes, H.M. Lund, W. Andresen, H. Gjerde, S.E. Ikdahl, A.S. Christophersen, et al., Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers, Forensic Sci. Int. 219 (2012) 165–171.
[14] H. Gjerde, K. Langel, D. Favretto, A.G. Verstraete, Detection of 4 benzodiazepines in oral fluid as biomarker for presence in blood, Ther. Drug Monit. 36 (2014) 252–256.
[15] K. Langel, H. Gjerde, D. Favretto, P. Lillsunde, E.L. Oiestad, S.D. Ferrara, et al., Comparison of drug concentrations between whole blood and oral fluid, Drug Test Anal. 6 (2014) 461–471.
[16] B.E. Smink, B.J. Hofman, A. Dijkhuizen, K.J. Lusthof, J.J. de Gier, A.C. Egberts, et al., The concentration of oxazepam and oxazepam glucuronide in oral fluid, blood and serum after controlled administration of 15 and 30 mg oxazepam, Br. J. Clin. Pharmacol. 66 (2008) 556–560.
[17] A.M. Hausken, K. Furu, S. Skurtveit, A. Engeland, J.G. Bramness, Starting insomnia treatment: the use of benzodiazepines versus z-hypnotics. A prescription database study of predictors, Eur. J. Clin. Pharmacol. 65 (2009) 295–301.
[18] N. Gunja, The clinical and forensic toxicology of Z-drugs, J. Med. Toxicol. 9 (2013) 155–162.
[19] H. Gjerde, P.T. Normann, B.S. Pettersen, T. Assum, M. Aldrin, U. Johansen, et al., Prevalence of alcohol and drugs among Norwegian motor vehicle drivers: a roadside survey, Accid. Anal. Prev. 40 (2008) 1765–1772.
[20] I. Gustavsen, J.G. Bramness, S. Skurtveit, A. Engeland, I. Neutel, J. Morland, Road traffic accident risk related to prescriptions of the hypnotics zopiclone, zolpidem, flunitrazepam and nitrazepam, Sleep Med. 9 (2008) 818–822.
[21] I. Gustavsen, K. Hjelmeland, J.P. Bernard, J. Morland, Psychomotor performance after intake of zopiclone compared with intake of ethanol: a randomized, controlled, double-blinded trial, J. Clin. Psychopharmacol. 31 (2011) 481–488.
[22] I. Gustavsen, K. Hjelmeland, J.P. Bernard, J. Morland, Individual psychomotor impairment in relation to zopiclone and ethanol concentrations in blood—a randomized controlled double-blinded trial, Addiction 107 (2012) 925–932.
[23] G. Caille, P. du Souich, J. Spenard, Y. Lacasse, M. Vezina, Pharmacokinetic and clinical parameters of zopiclone and trimipramine when administered simultaneously to volunteers, Biopharm. Drug Dispos. 5 (1984) 117–125.
[24] K. Hjelmeland, I. Gustavsen, J.P. Bernard, J. Morland, Can a simple clinical test detect impairment of zopiclone and alcohol? A randomized controlled trial, Forensic Sci. Int. 248 (2015) 129–133.
[25] E.L. Oiestad, U. Johansen, A.S. Christophersen, Drug screening of preserved oral fluid by liquid chromatography–tandem mass spectrometry, Clin. Chem. 53 (2007) 300–309.
[26] C. Fernandez, F. Gimenez, J. Mayrargue, A. Thuillier, R. Farinotti, Degradation and racemization of zopiclone enatiomers in plasma and partially aqueous solutions, Chirality 7 (1995) 267–271.
[27] E. Mannaert, J. Tytgat, P. Daenens, Detection of 2-amino-5-chloropyridine in urine as a parameter of zopiclone (Imovane) intake using HPLC with diode array detection, J. Anal. Toxicol. 21 (1997) 208–212.
[28] K. Pil, F.M. Esposito, A. Verstraete, External quality assessment of multi-analyte chromatographic methods in oral fluid, Clin. Chim. Acta 411 (2010) 1041–1045.
[29] G.H. Nilsson, F.C. Kugelberg, R. Kronstrand, J. Ahlner, Stability tests of zopiclone in whole blood, Forensic Sci. Int. 200 (2010) 130–135.
[30] H.M. Lund, E.L. Oiestad, H. Gjerde, A.S. Christophersen, Drugs of abuse in oral fluid collected by two different sample kits—stability testing and validation using ultra performance tandem mass spectrometry analysis, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 879 (2011) 3367–3377.
[31] H. Gjerde, E.L. Oiestad, A.M. Oiestad, M. Langodegard, I. Gustavsen, K. Hjelmeland, et al., Comparison of zopiclone concentrations in oral fluid sampled with Intercept® oral specimen collection device and Statsure Saliva™ Sampler and concentrations in blood, J. Anal. Toxicol. 34 (2010) 590–593.
[32] H. Gjerde, P.T. Normann, A.S. Christophersen, The prevalence of alcohol and drugs in sampled oral fluid is related to sample volume, J. Anal. Toxicol. 34 (2010) 416–419.
[33] C. Fernandez, C. Martin, F. Gimenez, R. Farinotti, Clinical pharmacokinetics of zopiclone, Clin. Pharmacokinet. 29 (1995) 431–441.
[34] C.R. Baselt, Disposition of Toxic Drugs and Chemicals in Man, 10th ed., Biomedical Publication, Foster City, CA, USA, 2014.
[35] S. Baliga, S. Muglikar, R. Kale, Salivary pH: a diagnostic biomarker, J. Indian Soc. Periodontol. 17 (2013) 461–465.
[36] R. Haeckel, Factors influencing the saliva/plasma ratio of drugs, Ann. N. Y. Acad. Sci. 694 (1993) 128–142.
[37] R.J. Schepers, J.M. Oyler, R.E. Joseph Jr., E.J. Cone, E.T. Moolchan, M.A. Huestis, Methamphetamine and amphetamine pharmacokinetics in oral fluid and plasma after controlled oral methamphetamine administration to human volunteers, Clin. Chem. 49 (2003) 121–132.
[38] K. Pil, E. Raes, A. Verstraete, The toxicological challenges in the European project DRUID, Forensic Sci. Int. Suppl. Ser. 1 (2009) 29–32.
[39] H.K. Nordgren, K. Bodin, O. Beck, Chromatographic screening for zopiclone and metabolites in urine using liquid chromatography and liquid chromatography–mass spectrometry techniques, Ther. Drug Monit. 24 (2002) 410–416.
[40] M. Deveaux, M. Cheze, G. Pepin, The role of liquid chromatography–tandem mass spectrometry (LC–MS/MS) to test blood and urine samples for the toxicological investigation of drug-facilitated crimes, Ther. Drug Monit. 30 (2008) 225–228.
[41] E. Gallardo, J.A. Queiroz, The role of alternative specimens in toxicological analysis, Biomed. Chromatogr. 22 (2008) 795–821.