Comparing the Concentrations of Drugs and Medicines in Whole Blood, Plasma and Saliva Samples of Drivers Suspected of Driving Under the Influence

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ABSTRACT

In my thesis I discuss analysing drugs and medicines of abuse from whole blood, plasma and saliva. I go through different methods to analyse these matrices and previous studies where these matrices have been compared. I also deal with sample collection and different qualities of saliva for example drug transfer from blood to saliva.

The objective of my thesis was to compare drug and medicine concentrations in whole blood, plasma and saliva in samples taken from people suspected of driving under the influence. The aim was to determine what kind of differences there are and do the results correlate between the matrices. Samples were collected from voluntary DUI (driving under the influence) drivers at the Helsinki University Department of Forensic Medicine with the cooperation of a forensic pathologist and the police. Samples were analysed in the Drug Research Unit of the National Public Heath Institute in Finland.

I got samples from 28 voluntary suspected DUI driver and from these the comparable results there were for amphetamine, cannabinoids and benzodiazepines. Due to the small number of samples definite conclusions could not be made from the results but indicative results for amphetamines and benzodiazepines could be found. In whole blood and plasma there were only a few positive samples for cannabinoids so no comparison could be made with saliva. There were all together 14 amphetamine positive samples and according to these results the correlation between whole blood and plasma was very strong. Amphetamine concentrations in saliva were much higher than in whole blood and plasma but there was still some correlation between all the matrices. In benzodiazepines comparable results were found in six substances; diazepam, alprazolam, nordiazepam, oxazepam, clonazepam and temazepam. Whole blood and plasma had strong correlations for each of these substances. In comparison with saliva there was correlation with whole blood and plasma only in oxazepam, nordiazepam and temazepam.

These results indicate that any of these matrices could be used to detect amphetamines from suspected DUI drivers. For other substances this study cannot give proper conclusions. Of the benzodiazepines analysed in this study the results are too narrow for saliva to be used reliably. Whole blood and plasma are both usable matrices for detecting benzodiazepines according to this study.

Keywords

whole blood, plasma, saliva, drugs and medicines of abuse

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Huumaus- ja lääkeaineiden pitoisuuksien vertailu kokoveressä, plasmassa ja syljessä rattijuopumusesta epäillyillä henkilöillä

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TIIVISTELMÄ

Työssäni käsittelen huumaus- ja lääkeaineiden analysointia veri-, plasma- ja sylkinäytteistä. Käyn läpi erilaisia menetelmiä näiden matriisien analysoimiseksi sekä aiempia tutkimuksia, joissa eri matriiseja verrataan. Käsittelen myös näytteiden keräystä ja syljen ominaisuuksia kuten huumausaineiden siirtymistä verestä sylkeen.

Työn tarkoituksena oli verrata huumaus- ja lääkeaineiden pitoisuuksia veri-, plasma- ja sylkinäytteissä rattijuopumuksesta epäillyiltä henkilöiltä otetuista näytteissä. Tavoitteena oli selvittää minkälaisia eroja eri matriisien välillä on ja kuinka ne korreloivat keskenään. Näytteet kerättiin vapaaehtoisilta rattijuopumuksesta epäillyiltä henkilöiltä oikeuslääkäriasemalla yhteistyössä oikeuslääkärin ja poliisin kanssa. Näytteet analysoitiin Kansanterveyslaitoksen huumetutkimusyksikön laboratoriossa.

Näytteitä saatiin 28 vapaaehtoiselta ja niistä saatiin keskenään verrattavia tuloksia amfetamiinista, kannabiksesta ja bentsodiatsepiineista. Näytteiden vähäisen määrän vuoksi vahvoja päätelmiä tuloksista ei voinut tehdä, mutta suuntaa antavia tuloksia sain amfetamiinille ja bentsodiatsepiineille. Kokoveressä ja plasmassa oli niin vähän kannabispositiivisia näytteitä, että vertailua syljen kanssa ei voinut tehdä. Amfetamiinipositiivisia näytteitä oli kaiken kaikkiaan 14 ja näiden tulosten perusteella saatiin vahva korrelaatio kokoveren ja plasman välille. Amfetamiinipitoisuudet olivat syljessä huomattavasti korkeampia kuin kokoveressä ja plasmassa. Kokoveri ja plasma myös korreloivat jonkin verran syljen kanssa. Bentsodiatsepiinien ryhmään kuuluvista aineista verrattavia tuloksia oli kuudelle aineelle; diatsepamille, alpratsolamille, nordiatsepamille, klonatsepamille, oksatsepamille ja tematsepamille. Kokoverellä ja plasmalla oli vahva korrelaatio näiden kaikkien aineiden kohdalla. Sylki sen sijaan korreloi kokoveren ja plasman kanssa vain oksatsepamissa, nordiatsepamissa ja tematsepamissa.

Nämä tulokset antavat viitteitä siitä, että mitä tahansa näistä matriiseista voitaisiin käyttää amfetamiinin analysoimiseen päihteiden alaisena ajamisesta epäillyiltä henkilöiltä. Muista aineista ei tämän tutkimuksen perusteella voi juuri tehdä johtopäätöksiä. Tutkituista bentsodiatsepiineista syljen osalta tulos on liian suppea, jotta sitä voitaisiin käyttää luotettavasti. Kokoveri ja plasma taas soveltuvat molemmat käytettäväksi bentsodiatsepiinien analysointiin tämän tutkimuksen valossa.

Avainsanat

kokoveri, plasma, sylki, huumaus- ja lääkeaineet

APPENDICES

Appendix 1 List of substances analysed in DRUID-project and short description of each substance.

Appendix 2 Sample collection form

1 INTRODUCTION

Drugs and medicines of abuse are known to impair the ability to drive a car and therefore deteriorating the road safety. Police may not see the state of the driver and ask for a drug test if alcohol cannot be shown in the breath test. Also drugs and medicines of abuse are often used together with alcohol and therefore are not detected. (Lillsunde, Pirjo - Luntiala, Pertti - Seppä, Heikki - Gunnar, Teemu – Hokkanen, Arto – Penttilä, Antti 2003, 3.)

Internationally Finland belongs to the high road safety countries. It has been shown that generally deaths in traffic accidents have decreased in previous decades in Finland even though the traffic volume has increased. Alcohol's share of serious accidents has been increasing and sadly with other intoxicants the statistics look even worse. From 1989 to the year 2002 the amount of traffic accidents involving at least one party under the influence of drugs or medicines increased five times. Still alcohol is the greatest risk in traffic but other substances must be taken in to account also. (Lillsunde et al. 2003, 7-9.)

The objective of the EU road safety programme is to bisect the amount of the victims of traffic accidents in the EU-area by the year 2010. It has been shown that traffic safety has increased in the last decade and deaths in traffic have decreased in the EU-area. (Lillsunde et al. 2003, 9.)

The title of my thesis is comparing the concentrations of drugs and medicines in whole blood, plasma and oral fluid samples of drivers suspected of driving under the influence. My thesis is part of the sub study of the EU-project DRUID (Driving Under the Influence of Drugs, Alcohol and Medicines). The sub study is about comparing the levels of drugs and medicines in different matrices.

This EU-project is conducted in Finland by the National Public Health Institute (KTL). It is done in cooperation with the Police and Helsinki University Department of Forensic Medicine. All the samples are analysed in the National Public Health Institute Drug Research Unit. The blood and plasma samples are analysed in the routine drug laboratory. The National Public Health Institute is in charge of all the sampling and analyses and they provide all the materials and supplies for this study.

The research problem investigates what kind of differences there are in drug levels when comparing whole blood, plasma and saliva samples that are taken at the same time from drivers suspected of driving under the influence of drugs, medicines or alcohol. These matrices are chosen because of their accessibility and possibilities. If the correlations between the matrices are good for example saliva could be used to determine drugs of abuse from drivers instead of blood. Blood and plasma are more traditional and already widely used matrices for detecting drugs of abuse. Collection and handling are well documented and the analysing method is similar for both. Saliva on the other hand is more an alternative matrix and it has several potential qualities. Ease of collection and handling makes it interesting matrix to take in to wider use in detecting abuse of drugs and medicines.

Whole blood contains all the components of blood whereas plasma is the liquid portion of blood without blood cells. Plasma still contains some of the blood proteins.

1.1 Aim of the study

The objective of my thesis is to get information on the whole blood-plasma-oral fluid concentrations of different drugs and medicines. The samples are collected at the Helsinki University Department of Forensic Medicine from people suspected of driving under the influence of drugs or medicines and alcohol (DUI). The aim for the whole study is to get the samples from 300 DUI cases. In my thesis the amount of cases will be about 30 so the amount of samples will be about 90.

This study will give information about the efficiency of traffic control of drugs and medicines. It can also give answers to the questions about the more reliable methods for monitoring driver's impairment and whether the current method is the best. It may help in changing the opinions toward alternative matrices in DUI investigation.

1.2 Implementation of the study

The beginning of the study was to start taking samples at the Department of Forensic Medicine. As my development study for Stadia I organised a place for taking samples at the Department of Forensic Medicine and we started taking the samples in cooperation with MD Philippe Lunetta. The samples will be analysed with immunological methods

and GC–MS (gas chromatography-mass spectrometry). The whole blood samples are analysed in the routine lab since they are real police DUI samples. I will get the results when the analyses are ready and then according to the results I will decide what to analyse from the plasma. Whole blood samples will be analysed again together with the plasma samples according to the routine results. Whole blood is therefore analysed two times so that the results are from samples with similar storage conditions. The saliva samples will be analysed with a semi-quantitative method for all the substances that are included in the list of analyzed substances in DRUID-project. The list of these substances is in table 1.

2 BACKGROUND

The background of this study is very tightly tied on the DRUID-project since it belongs to the sub-study of the project. The methodology and ethics and purpose of this study are all defined by the DRUID-project. This study belongs to the area of road-safety and methodology of detecting the abuse of substances harmful in traffic.

2.1 DRUID-project

DRUID (Driving Under the Influence of Alcohol, Drugs and Medicines) is an EU-Project involving 19 European countries: 17 EU countries and Norway and Switzerland. From these countries there are 37 research institutes taking part in the project. The DRUID-project consists of seven work packages. In several of these work packages biological samples are collected. These biological samples are for example whole blood, oral fluid and plasma. In the project the aim is to determine the prevalence of drugs of abuse and medicines in traffic and in accident victims, the accident risk caused by psychoactive substances, the medicines actions in healthy persons and patients, and the effect of drugs on driving capabilities. The biological samples collected are not the same in every country. Legislation and practises in every country are taken into account and samples are collected accordingly. The aim of this project is also to produce new information about ratios of drugs of abuse and medicines in whole blood, plasma and oral fluid, and levels in urine. The analyses will be done with immunological methods and GC–MS. In addition the aim is to find out whether the blood spot sample is suitable

for DUI sampling since it has been established in researches that cocaine can hydrolyse in whole blood samples. The whole project started 15.10.2006 and will finish 14.10.2010. (Druid-tutkimussuunnitelma 2006.)

2.2 DRUID Sub Study: Comparison of the Whole Blood-Plasma-Oral Fluid Ratios of Different Drugs and Medicines

This particular sub study of the project is carried out in close cooperation with the police and Helsinki University Department of Forensic Medicine. When stopping a suspected DUI driver the police fill out an observation form in which they make an evaluation of weather, roadway and driving manner. They also mark up their observations made during the stopping. With the help of the observation form the police make decisions about the driver's ability to drive the vehicle and decide whether the driver should be taken to the blood test.

Police brings the suspected DUI drivers to the Department of Forensic Medicine for a blood test. The doctor taking the samples at the Forensic Medicine Department makes a clinical evaluation about the level of functional disorder of the driver and fills out the intoxication investigation form. The police's observation form and doctor's intoxication evaluation form are a part of normal preliminary investigation material and therefore normal practices when driving under the influence is suspected. The results of these forms have not been systematically compared with the findings of the blood samples so proper information of correspondence between these does not exist. In this study one of the interests is to compare the police's and doctor's evaluation form results to the actual level of drugs of abuse in blood.

The aim in this study is to get in total of 300 suspected DUI drivers to participate in the research. Drivers are asked to sign a written consent for taking part in the research. Participation is completely voluntary and does not affect the drivers DUI detection or his position in court. The driver can refuse his participation in the study at any time. There is an identification sticker in the information form the driver receives with which he can retract his participation in the study. Personal identification information is removed from the samples so that the samples that have already been taken cannot be found with the personal data.

2.3 Drugs and Medicines Analysed In the Study

There are 28 different substances that are analysed in DRUID. Here is a chart of those drugs and medicines and their cut-off limits. These are the cut-off limits defined by DRUID, the lowest concentrations of positive samples. Any concentration above these limits will be interpreted as a positive result.

TABLE 1. Substances and their cut-off limits in DRUID (ng/ml)

Substance	Whole blood cut-off	Plasma cut-off	Saliva cut-off
Ethanol	0,1 g/l	-	0,1 g/l
Morphine	10	10	20
Amphetamine	20	20	25
MDMA	20	20	25
MDA	20	20	25
Cocaine	10	10	10
THC	1	1	1
THC-COOH	5	5	-
Diazepam	20	20	5
Alprazolam	10	10	1
Clonazepam	10	10	1
Benzoylecgonine	50	50	10
Codeine	10	10	20
6-acetylmorphine	10	10	5
Methamphetamine	20	20	25
Methadone	10	10	20
Oxazepam	50	50	5
Nordazepam	20	20	1
Zopiclone	10	10	10
MDEA	20	20	25
Lorazepam	10	10	1
Flunitrazepam	2	2	1
Zolpidem	20	20	10
Temazepam	50	50	5
Midazolam	10	10	1
Phenazepam	20	20	1
Nitrazepam	10	10	1
Zaleplon	5	5	5

3 ANALYSING THE SUBSTANCES

There are several methods for determining the drugs of abuse in biological matrices. Here are outlined some of the methods and how research has evaluated the suitability of

certain methods for each respective matrix. Here are also some results that different researchers have found for the analysing methods.

3.1 Determining Drugs of Abuse and Medicinal Drugs in Blood and Plasma

Determination of drugs in blood is of great importance in clinical and forensic toxicology. Analyses are done for the actual substance and for some of its metabolites and degradation products. For screening drugs of abuse there are sensitive immunological methods available for many of the analytes. Mostly the screening and confirmation is done with gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS). (Kraemer - Paul 2007, 1415.)

When comparing different methods for analysing certain drugs of abuse, Kraemer and Paul used papers written in English between 2002 and 2007. Their main perspective was to describe methods suitable for forensic toxicological determination. The methods they were interested in had to meet the expectations of being reliable and providing quantitative results. In the review they compared the determination methods for cannabinoids, opiates, amphetamines and amphetamine-derived designer drugs, cocaine and their metabolites. (Kraemer - Paul 2007, 1415-1435.)

According to the Kraemer and Paul review there are several different immunological screening methods available. Positive immunoassay results must always be confirmed by a second independent method. This other method must be as sensitive as the screening method and provide the highest level of confidence in the results. Usually the confirmation is done by GC-MS or LC-MS. When the prevalence of positive samples is high, the screening should also be done by chromatographic procedures. (Kraemer - Paul 2007, 1416-1426.)

3.1.1 Cannabinoids

In the determination of cannabinoids there are several things to take into account. The primary psychoactive component of cannabis is THC. It is rapidly metabolised in to OH-THC and further to inactive THC-COOH. It is recommended to determine all of these from a toxicological point of view. THC is almost completely bound to plasma

proteins and its distribution into red blood cells is very poor and that's why the concentrations in plasma and blood are not completely comparable. It should always be mentioned whether plasma or blood has been used in quantitative analysis. (Kraemer - Paul 2007, 1426-1427.)

According to Kraemer and Paul GC-MS method is the most commonly used method for quantitative determination and confirmation of cannabinoids in blood and plasma. Most often methylation and silylation were chosen as derivatization method for GC-MS. Derivatization is used to increase thermal stability, sensitivity and to get more specific mass fragments of the target analytes in mass spectra. In addition to GC-MS, also an increasing number of methods based on LC-MS has been used for quantitation of THC and THC-COOH. One advantage of LC-MS is that no derivatization of the extracts is required. (Kraemer - Paul 2007, 1426-1427.)

3.1.2 Opiates

In many European countries opioids, mainly heroin, accounted for about 60% of all recorded illegal drug treatment requests in 2004 according to Kraemer and Paul. In their review they concentrated in the analysis of morphine, codeine and heroin and their principal metabolites. (Kraemer - Paul 2007, 1428.)

Heroin is quickly hydrolyzed to 6-MAM (6-monoacetylmorphine) and next to morphine. Morphine is also rapidly metabolized and its major metabolite is M3G (morphine-3-glucuronide). There have been several techniques to quantify morphine and its metabolites. Immunoassays are simple to handle but they fail to specify opiates from their glucuronides and should only be used as screening methods. (Kraemer - Paul 2007, 1428.)

According to Kraemer and Paul to overcome matrix interference in the determination of opiates procedures were applied for purification of bio matrices. In the whole blood analyses, protein precipitation with acetonitrile or methanol was often conducted. Several GC-MS methods for the analysis of opiates have bees described since 2002. LC-MS/MS technique has become popular for simultaneous determination of low concentrations of morphine, M3G and M6G. It provides better sensitivity than EI-GC-

MS (electron impact-gas chromatography-mass spectrometry) which is also used as a method for the analysis of the opiates. (Kraemer - Paul 2007, 1429.)

3.1.3 Amphetamines

Amphetamine and metamphetamine are strong stimulants of the central nervous system. They are drugs of abuse and also doping agents. When determining amphetamine and metamphetamine from blood the target analytes are the parent compounds, not metabolites. (Kraemer - Paul 2007, 1430.)

Amphetamine-derived designer drugs have gained popularity and they are often used as "rave drugs". They cause feelings of euphoria and energy and aspiration to socialize. These designer drugs are MDA, MDMA and MDEA. When determining the designer drugs in blood the target analytes are the parent compounds and for MDMA and MDEA also the metabolite MDA. (Kraemer - Paul 2007, 1430.)

According to Kraemer and Paul amphetamines can be analysed from blood with GC-MS. It is also getting more popular to analyse amphetamines with LC-MS procedures. For routine analysis there are traditional alkaline LLE (liquid-liquid extraction) procedures and acylation using perfluoroacylated reagents followed by GC-MS detection in use. Peters et al. have presented a new method for simultaneous screening and quantitation of amphetamines and designer drugs in plasma. (Kraemer - Paul 2007, 1430.)

3.1.4 Cocaine

In many parts of the world cocaine has become one of the most frequently used drugs of abuse. The most common routes for cocaine administration are intranasal, intravenous and through smoking. (Kraemer - Paul 2007, 1431.)

Cocaine metabolizes quickly into many metabolites. The major metabolite benzoylecgonine (BZE) should be included in screening and quantitation from plasma according to Kraemer and Paul. Cocaine demands special care during analysis since it seems to have a tendency for hydrolysis and pyrolysis, extensive metabolism and

formation of thermo labile products. For determining cocaine, inhibition of plasma esterases by e.g. sodium fluoride is necessary. For a routine forensic laboratory the determination of cocaine and BZE may be adequate. (Kraemer - Paul 2007, 1432.)

In testing for cocaine and its metabolites there are methods for GC-MS and LC-MS procedures. GC-MS may cause some problems when analysing cocaine after smoking crack. AEME (anhydroecgonine methyl ester) is formed during smoking but also in the GC injection port. This may cause false positive results for AEME. Thus LC-MS has proven to be a valuable tool for clarifying oxidative metabolism of cocaine. (Kraemer - Paul 2007, 1433.)

3.1.5 Benzodiazepines

Benzodiazepines are often prescribed drugs for treating many medical and psychiatric disorders. They are anticonvulsive, centrally muscle relaxing, sedative hypnotics and anxiolytic agents. Different benzodiazepines have different duration of action and potencies in these categories. There are several methods for analysing single benzodiazepine or a selective group of these analytes and a few for non-benzodiazepine hypnotic agents. These procedures that have been published lack the possibility for determination of both groups simultaneously. (Gunnar - Ariniemi - Lillsunde 2005, 175-176.)

A method has been presented for simultaneous screening of benzodiazepines in whole blood using GC with MS and electron capture detection (ECD). Most of the analytes were analyzed quantitatively and others semi-quantitatively. Gunnar et al. note that Kratzsch et al. have recently presented a method for quantitative analysis for these analytes from plasma using atmospheric pressure chemical ionization (APCI) liquid chromatography-mass spectrometry (LC-MS). Quantitative methods for analysing benzodiazepines from whole blood, plasma or serum have recently included dual-column GC, GC-MS, GC-tandem mass spectrometry (GC-MS/MS), liquid chromatography (LC), liquid chromatography-mass spectrometry, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). According to Gunnar et al GC-MS has remained the method of choice in many routine laboratories because of the separation efficiency, versatility, and ease of operation and maintenance. This method

offers also lower costs of the analysis and investment expenses compared to LC-MS/MS. (Gunnar et al. 2005, 176.)

According to Gunnar et al, when using GC based methods for analysing benzodiazepines the derivatization of polar functional groups containing reactive hydrogen agents atoms is very important. Silylating reagents are often used for derivatization since they are versatile, easy to prepare and can be injected directly into the GC-MS system. With use of derivatization increased thermal stability, sensitivity and more specific mass fragments of the target analytes in mass spectra are commonly achieved. (Gunnar et al. 2005, 176.)

3.2 Determining Drugs of Abuse and Medicinal Drugs in Saliva

Saliva is an alternative matrix for determining drug abuse. There has been a lot of interest toward salivary drug testing for several years and its possibilities are still unclear. Particularly saliva has been of interest for road-side testing for potentially intoxicated drivers and now it is in use for that. For forensic purposes the use of saliva is still limited. (Schramm – Smith – Craig - Kidwell 1992, 1; Samyn – Verstraete - van Haeren - Kintz 1999,40)

For drug testing saliva is easy to collect non-invasively and collection can be done under supervision. Problems with saliva testing have been found to be variability in collection protocols and therefore in results. For example in road-side testing the sample volume can be very small. Also it has been known that the salivary concentrations are smaller than urine concentrations. The problem earlier was lack of commercialized immunoassay tests but now they are available. (Samyn et al. 1999, 40.)

According to Samyn et al salivary drug concentration is defined by the route of administration, the salivary pH, the degree of plasma protein binding, and the physicochemical properties of the abused drug. They find that saliva might be a better tool than blood for testing potentially intoxicated drivers since the saliva/plasma ratio is so good. (Samyn et al. 1999,40) Schramm et al thinks that saliva is potentially better in indicating a state of intoxication than blood since it contains the free component of drug that is physiologically active. Many drugs are bound to proteins in blood and they are

not physiologically active but saliva is an ultra-filtrate of interstitial fluid and contains the free components of drugs. (Schramm et al. 1992, 1.)

3.2.1 Formation of the Saliva

Samyn et al recite three main functions of saliva to be: (1) the moistening of the mucous membranes of the upper aero-digestive area in order to ease speech and solubilise food to help swallowing, (2) to control the bacterial flora of the mouth and generate defence mechanisms, (3) to supply enzymes for food digestion and hormones, and other pharmacologically active compositions. The saliva is mostly produced by the major salivary glands. Saliva contains ordinary body fluid electrolytes. Primary saliva formation is situated in the end-pieces of the salivary glands. Formation of saliva is dependent on the active transport of one or more of the principal ions from the interstitial fluid to the acinar cells and the lumen. Water enters the lumen by osmosis. Saliva is mostly water (99%) and mineral salts. It also contains proteins and enzymes. Almost all of the organic compounds of plasma may be detected in saliva even though the total protein concentration in saliva is less than 1% of that of plasma. An adult produces saliva 500-1500 ml per day. (Samyn et al. 1999, 40-41.)

3.2.2 Drug Transport In To Saliva

Samyn et al describes the drug transport in to saliva. Before any drug can be released from plasma to saliva it must pass the capillary wall, the basal membrane, and the membrane of the glandular epithelial cells. They find that the following mechanisms occur since saliva is not a simple ultra filtrate of plasma: passive diffusion through the membrane; active processes against a concentration gradient; filtration through pores in the membrane; and pinocytosis. Most of the drugs seem to enter saliva by a simple passive diffusion process. This is dependent of the drug's physicochemical characteristics which are pKa (indicates the strength of the acid; the higher the pKa value, the weaker the acid), liposolubility, molecular weight, and spatial composition. Also diffusion defining factors are the degree of plasma protein binding and the pH of both media. The lipophilic drugs with a low degree of ionization can cross the barrier between plasma and saliva easily so that the concentration of saliva is a reflection of the non-protein bound plasma concentration. Nonetheless, for weakly basic drugs, the pH

of the saliva is the main importance for the concentrations discovered in saliva. (Samyn et al. 1999, 42.)

3.2.3 Saliva Collection

There are several methods for collecting saliva. For example the subjects can be asked to spit in to the vial or saliva can be collected by tilting the head forward allowing the saliva to flow freely into a container. There are also some special devices for saliva collection for example the Omni-Sal device with a sterile collection pad, transport tube and buffer solution. Different stimuli can be used to cause salivation for example chewing Teflon or chemical stimuli can be used with citric acid crystals or sour candy. However it might be better to use un-stimulated saliva since drug concentrations can decrease when salivary flow is increased. There are some limitations for this: social barrier to spitting may cause dry mouth, some drugs have been observed to cause diminished salivary flow, and there is often more froth than actual liquid so that sufficient sample is not obtained. (Samyn et al. 1999, 42.)

3.2.4 Testing Saliva for Cocaine

It has been established that cocaine can be detected from saliva also after intravenous administration so it is evident that cocaine enters the salivary glands from the blood circulation. Thus, the positive result is not just residue from nasal or oral administration, but still contamination of the buccal cavity after smoking or sniffing can have a significant effect on the salivary cocaine levels. Immunoassay can detect cocaine from saliva as long as 10 days after intake. According to Schramm et al the cocaine concentration in saliva is usually higher than in plasma. (Schramm et al. 1992, 4.)

Samyn et al. noted that Thompson et al. had observed a good correlation between the salivary cocaine concentration and its plasma concentration. Cocaine half-life in saliva is known to be about 35 minutes which also correlates well with plasma half-life of about 40 minutes. Confirmation is usually done with GC-MS method although the detection time is shorter. Cocaine can generally be detected from saliva for several hours after administration by different routes. Analytical methods with detection limits

of 5 to 10 ng/ml are sufficiently sensitive and can detect also the metabolites that appear little later into the saliva.(Samyn et al. 1999, 43-44.)

3.2.5 Testing Saliva for Cannabinoids

There have been attempts to analyse THC from saliva since the early seventies. Analytical methods for cannabis detection from saliva have been first thin layer chromatography and later on different methods with for example HPLC, GC/MS, GC/ECD and immunoassay. It has been shown that salivary THC reflects positive results in blood reliably even though the concentrations do not correlate specifically. (Schramm et al. 1992, 2; Samyn et al. 1999, 45.)

With radio-labelled THC intravenous administration it has been shown that it is not likely that THC or its metabolites pass into the saliva or lungs from the blood. THC and its active metabolites are substantially plasma protein-bound. THC detected from saliva is therefore mostly from contamination of the buccal cavity during smoking. THC concentrations between 5 and 330 ng/ml have been detected from saliva after 8 hours since smoking. Analytical methods used for detecting THC are immunoassays and GC-MS. According to Samyn et al, most immunoassays cross-react with THC and it's other metabolites even though they are selective for the THC metabolite THC-COOH. (Schramm et al. 1992, 3; Samyn et al. 1999, 45.)

Samyn et al note that cannabinoid detection in saliva is a better indication of recent cannabis use than detection in urine. Even though the transport of cannabinoids from blood to saliva is minor they are easily detectable from saliva for several hours after administration via the usual routes of smoking and ingestion. The only problem with saliva testing is possible mouth washing if drinking beverages or other liquids after administration. In the review of Samyn et al they found one study (by Maseda, Hama, Fukui, Matsubara, Takahashi & Akane 1986) which shows that drinking beer lowers the salivary THC levels. In contrast other studies demonstrate that consumption of food or drink has no significant effect on THC levels. (Samyn et al. 1999, 45.)

3.2.6 Testing Saliva for Opioids

In the early studies chemical and metabolic instability of heroin limited the measurement of morphine in saliva and other body fluids. First the detections were made with immunoassays and in those studies it was noted that saliva correlated badly with urine and that morphine could be detected longer in plasma than in saliva. In the review of Samyn et al they note that recently Goldberger et al have developed a new more sensitive method to quantify heroin and its metabolites MAM and morphine in body fluids. This method is based on solid phase extraction and GC/MS and it enables heroin to be detected after sniffing, smoking, and intravenous administration (Goldberger, Darwin, Grant, Alen, Caplan & Cone 1993). (Samyn et al. 1999, 46.)

According to Schramm et al's review methadone has been detected in saliva and the correlation between saliva and plasma is very good. In determination of codeine a higher concentration was found in saliva than in plasma after oral administration. There was much variation in codeine levels between individuals (Sharp, Wallace, Hindmarsh & Peel 1983). In another study it was found that there are similar concentrations of codeine in saliva and plasma (Cone 1990). Detection methods were different in these codeine studies. In the first study the analyses were conducted with GC and the other with RIA. Also differences can be caused by salivary pH which would affect the saliva/plasma ratio. (Schramm et al. 1992, 5.)

3.2.7 Detection of Amphetamines in Saliva

As Schramm et al states, saliva has been recommended to be the matrix for diagnostic evaluation because of the high amphetamine levels found in saliva and the strong dependency of urinary pH on the excretion of the drug. The amphetamine concentration in saliva is found to be three times higher than in plasma. (Schramm et al. 1992, 6.)

Samyn et al has similar findings concerning amphetamine analysis. Amphetamine is bound to plasma proteins at about 16% and therefore is a mostly free component in blood. With GC/MS three times higher amphetamine concentrations in saliva than in plasma have been detected. They note that care must be taken when using immunoassays for amphetamine detection due to the risk of cross-reaction. Vapaatalo et

al had shown that TLC (Thin Layer Chromatography) is unsuitable for amphetamine detection because of the low sensitivity of the method. (Samyn et al. 1999, 47.)

3.2.8 Detection of Benzodiazepines in Saliva

Saliva can be used for detecting the use of benzodiazepines in on-site tests for roadside drug screening with a benzodiazepine quick tester even though the concentrations are generally low in saliva. Benzodiazepines are highly bound to proteins in blood and therefore they are not easily transferred to saliva. Also the problem with quick testers is the cross-reactivity of the benzodiazepine antibodies with other medicinal drugs. According to Pehrsson et al the need for a specific and sensitive tester is evident for roadside drug testing. (Pehrsson, Anna - Gunnar, Teemu - Engblom, Charlotta - Seppä, Heikki – Jama, Ahlam – Lillsunde, Pirjo 2007, 140-146.)

In the study performed by Pehrsson et al they made an evaluation of saliva quick testers and compared the results to saliva and whole blood samples analysed with GC-MS. The result for saliva was that the sensitivity for benzodiazepines was 74.4%. Specificity was 84.2% and accuracy 79.2%. They found the use of saliva for testing benzodiazepines promising and concluded that improvement can be made regarding saliva testing for benzodiazepines. (Pehrsson et al. 2007, 146-147.)

4 PREVIOUS STUDIES OF COMPARING LEVELS OF DRUGS AND MEDICINES IN DIFFERENT MATRICES

A number of different studies have been conducted comparing different drugs in different biological matrices. Mostly the matrices have been saliva and whole blood or plasma and saliva. Blood is a traditional and probably the most commonly used specimen when suspecting driving under the influence of drugs. Saliva on the other hand is widely used in quick-testers that police use when suspecting the abuse of drugs. Saliva is easy to collect in a non-invasive manner and it is easily processed for testing whereas blood sample requires more demanding specimen collection and handling. A blood sample must be taken by a medical professional and needs suitable surroundings.

In most of the studies I found the comparison was done with only a few substances that were determined beforehand. The voluntary subjects in the studies had to test negative in drug tests before the study. The amount of drugs given to them was strictly monitored. This differs quite a lot from my study since I don't know what, when and how much the subjects have used drugs and in what manner. Here are two different studies performed to compare different matrices for analysing drugs. The studies have been executed in quite a different manner to each other.

4.1 Comparison of Heroin and Cocaine Concentrations in Saliva with Concentrations in Blood and Plasma

Amanda J. Jenkins, Jonathan M. Oyler, and Edward J. Cone from Addiction Research Center, National Institutes of Health in Baltimore conducted a study where they compared heroin and cocaine concentration in saliva to those in blood and plasma. In this study they also compared the results of administration of drugs via different routes. The subjects were administered three intravenous and three smoked doses of heroin. They were also administered one dose of cocaine smoked and one dose of cocaine intravenously. (Jenkins, Amanda – Oyler, Jonathan – Cone, Edward 1995, 359.)

The subjects were healthy male volunteers with a history of drug use and they were required to test negative in drug tests before the study and pass physical and psychiatric evaluation. The study took place in a closed research unit of the Addiction Research Center. (Jenkins et al. 1995, 360.)

Two of the subjects were administered heroin and seven of them cocaine. The administration of drug doses was scheduled so that the risk of carryover from previous doses was minimal and the safety of the subject was ensured. Samples were collected 30 minutes before the drug administration and 0.03, 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after the administration of drugs. Saliva and blood samples were collected at the same time. The whole blood samples were collected in tubes containing sodium fluoride. Plasma samples were collected in tubes containing saturated sodium fluoride and acetic acid. Collection of blood samples was made with a syringe. For saliva samples the saliva flow was stimulated with sour candy containing citric acid. Saliva was collected by expectorating into a tube containing sodium fluoride. The samples were frozen at -30°C until the analysis took place. (Jenkins et al. 1995, 360-361.)

In heroin analysis blood and saliva were analysed for heroin and metabolites 6-asetylmorphine and morphine by solid-phase extraction (SPE) and (gas chromatography-mass spectrometry) GC-MS. In cocaine analysis saliva and plasma samples were analysed for cocaine and metabolites AEME, benzoylecgonine (BZE), norbenzoylecgonine, norcocaine, cocaethylene, and norcocaethylene by SPE and GC-MS. (Jenkins et al. 1995, 361.)

As a result they found that heroin was detected in saliva at 2 minutes after the administration of drug by both routes. It seems that the concentration of heroin was higher and was detectible for longer in saliva after a smoked dose than in blood. The same usually happened with the metabolites. Only morphine was sometimes lower in saliva and was detected later than in blood. Peak heroin concentrations in saliva were different between routes due to the contamination of the oral cavity. After reaching their peak heroin concentrations in saliva decreased rapidly. All together heroin was detectable in saliva for 2-24 hours after smoking. (Jenkins et al. 1995, 361-364.)

Peak heroin saliva concentrations declined rapidly after intravenous administration and reached the sensitivity limit of the assay between 5 and 30 minutes for most intravenous doses. For metabolites the peak concentrations were substantially lower and were achieved more slowly after intravenous administration. Concentrations of morphine in saliva were generally higher after smoking than after intravenous administration. (Jenkins et al. 1995, 364-365.)

Heroin appeared rapidly in blood through both routes and peak concentrations were reached in 5 minutes. Generally higher heroin concentrations were found in blood after intravenous administration. Heroin concentrations declined rapidly in blood by both routes of administration and reached the sensitivity limit of the assay by 30 minutes. (Jenkins et al. 1995, 365.)

According to their calculations the half-life of heroin in saliva after smoking was approximately 14-60 times longer than in blood. After intravenous administration the half-life of heroin is 2-208 times longer in saliva than in blood. (Jenkins et al. 1995, 365-366.)

The cocaine and metabolites were analysed in saliva and plasma. After smoking the highest concentrations were detected after 2 minutes. After intravenous administration the peak concentration in saliva was reached in 5 to 30 minutes. Peak concentrations in saliva were lower after intravenous administration than smoking but cocaine was detected for longer in saliva after an intravenous dose. In plasma the peak cocaine concentrations were generally higher after intravenous administration than after smoking. Also in cocaine samples smoking resulted in high concentrations in saliva in the early collecting period due to the contamination of oral cavity. All together the half-life of cocaine was shorter in saliva compared with plasma after smoking but longer after intravenous administration. (Jenkins et al. 1995, 366-371.)

4.2 Amphetamine Concentrations in Saliva and in Whole Blood

In another study, done by Charlotta Engblom, Teemu Gunnar, Anna Rantanen and Pirjo Lillsunde from the National Public Health Institute of Finland amphetamine concentrations in saliva and whole blood samples were compared. The study investigated amphetamine concentrations in oral fluid and whole blood samples from persons suspected of driving under the influence of drugs. The oral fluid samples were collected from persons that police suspected of drugged driving and they voluntarily took part in the study. Blood samples were collected by routine procedures. (Engblom, Charlotta – Gunnar, Teemu – Rantanen, Anna – Lillsunde, Pirjo 2007, 1.)

The saliva samples were collected using an Intercept device. The collection pad was kept in the oral cavity for 3 minutes and then placed in the cup containing buffer solution. The samples were stored in -20 °C until they were analyzed. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride. The samples were stored at 4 °C until analysed. Analyses were done with GC-MS. (Engblom et al. 2007, 1-2.)

They found that cases positive for amphetamine in whole blood were also positive in saliva. The concentrations of amphetamines were much higher in saliva than in whole blood. Also the detection time window in which the amphetamine was found was longer for saliva than whole blood. They concluded that saliva sample would be a suitable matrix for amphetamine when suspecting driving under the influence.
(Engblom et al. 2007, 1-4.)

5 CONDUCTING THE STUDY

My thesis will be answering the following questions:

- What kind of differences there are between the concentrations of drugs and medicines of abuse in whole blood and plasma?
- What kind of differences there are between the concentrations of drugs of abuse in whole blood and saliva?
- What kind of differences there are between the concentrations of drugs of abuse in plasma and saliva?

Handling of the samples has been as similar as possible so that the results would be comparable. Blood and plasma samples of each voluntary DUI driver have been first held in the fridge in +4 ºC and then put to freezer in -20 ºC at the same time. This way all the whole blood-plasma pairs should be well comparable.

As written earlier in the thesis, the samples were collected at the Helsinki University Department of Forensic Medicine with forensic pathologist. Sampling took place in facilities provided specially for this project. Here are more detailed directions of how the samples are collected and handled.

5.1 Sampling and Processing the Blood Samples

According to National Public Health Institute sampling and sample processing guidelines, venous blood samples must primarily be taken from the elbow crook. Principally the vacuum technique is to be used. The open technique can also be used when necessary. The subject is normally in a sitting position when the samples are taken but the samples can also be taken from the subject when they are lying down. When taking a venous sample, the subject's arm must be well supported, so that they can hold their arm as relaxed as possible. A tourniquet can be used for finding the vein if needed. The vein is found by looking and exploring with a finger. The puncture area must be cleaned with a dampened cloth, but the puncture area must be dry before piercing the skin. (Näytteenotto- ja näytteidenkäsittelyohje VTL-MO.K001 s.18) When taking intoxication samples, it is forbidden to use any solvents for cleaning the skin.

When using the vacuum technique to take the blood sample, the person taking the sample attaches a clean needle to the adapter and releases the needle cover. The needle is taken to the vein and a sample tube is connected to the other end of the adapter. The tourniquet must be loosened as soon as the blood starts to flow to the tube. Vacuum-tubes fill themselves automatically to the needed volume. A new tube will be changed to the adapter after the previous is filled. After the last tube is released from the adapter, a cotton patch is placed on top of the puncture area and the needle is taken out of the vein. To stop the bleeding, pressure must be applied to the puncture area. The needle is released from the adapter and dropped to a needle container. (Näytteenotto- ja näytteidenkäsittelyohje VTL-MO.K001 s.18) When using a safety needle with wings, the needle cover is put on the needle after it has been taken out of the vein. The needle is released from the adapter and dropped to the container for used needles.

According to National Public Health Institute drug laboratory intoxication investigation package instructions, you should always handle only one patient's samples at the time. Each sample tube and clinical intoxication investigation form must be marked with an identifier label. The intoxication investigation form is filled with the required information and signed. Blood samples are taken to two gray-topped 10ml Venoject-Glycaemia-VT-100SFX07 sample-tubes, so that there is at least 4ml blood in both of them. The tubes are turned upside down 7-8 times to ensure dissolving of the anticoagulant. (Päihdetutkimuspakkauksen käyttöohje versio 1.0)

For the DRUID-project two tubes of whole blood are first taken to Venoject-Glycaemia-VT-100SFX07-tubes with 100mg of sodium fluoride and 22,50mg of potassium oxalate as additives. These are the routine sample tubes for a intoxication investigation. In addition one 10ml green-topped sodium heparin plasma tube (Venoject-Plasma-VT-100SH) is taken. For ethical reasons the plasma tube is taken after the routine samples even though this is against the manufacturers instructions. The tube is turned upside down at least 5 times. The plasma tube is allowed to cool down to room temperature for 15 minutes before centrifugation. Centrifugation happens with a speed of 3500 rpm for 10 minutes. After the centrifugation the plasma is separated to a gray-topped Venoject-Glycaemia-VT-100SFX07-tube with a Pasteur pipette. At least 3ml of plasma is preferably taken.

The plasma samples are stored in the +4 ˚C until the whole blood sample results are ready from the routine analysis. When the whole blood sample is released from the routine lab I can take part of the sample for our purposes. At this point the whole blood and the plasma sample are frozen and stored at -20 ˚C until they are analysed. This way the storage environment is similar for both samples and it makes them more comparable. There were two whole blood samples for which I couldn't take any for reanalysis because there were so little left from the routine analysis. These samples were numbers 93193 and 93197. In these cases the plasma sample has not been frozen but kept at fridge temperature the whole time.

5.2 Collection and Handling of the Saliva Samples

Saliva samples were collected with a Statsure saliva collection device. Saliva collection packages include a collection device with saliva collection pad and plastic transportation tube with 1ml of buffer solution. On the handle of the collection device there is an indicator window, which turns blue when the collector has enough saliva (1ml). (DRUID-tutkimussuunnitelma)

According to the manufacturers instructions the collection package should not be used after the expiration date on the package. Subject's identifier label is attached to the plastic tube. The tube is checked so that there is enough buffer solution, if not, the tube can not be used. (DRUID-tutkimussuunnitelma)

At the beginning of the collection the collection device is taken out of the package. The subject collects saliva to the mouth as much as possible and the collection pad is placed under the tongue. The mouth is kept closed and timing begins. The collection device is not allowed to be moved inside the mouth during the collection and the collection pad is not allowed to be sucked or bitten. The collection device is kept in the mouth until the indicator window turns completely blue. Collection time varies between 2 and 5 minutes. If the collection lasts over 5 minutes and the indicator does not turn blue, the collection should be put to a halt and a note of insufficient sample made. At the end the collection device is taken out of the mouth and put in to the plastic transportation tube. The collection device must not be put back in to the mouth after it has been in the buffer solution. The cap is pressed on to the transportation tube until you hear the click-sound.

Gently shake the tube so that the whole collection pad is saturated with the buffer solution. (DRUID-tutkimussuunnitelma)

Samples should be transported to the laboratory within a day and be analysed within 3 days. The samples must be stored at +4 ˚C. (DRUID-tutkimussuunnitelma)

Since we are not able to analyse the samples within 3 days, the samples are stored in the freezer in -20 ˚ C until the analysing takes place. This way the samples were preserved as well as possible during the storage time.

6 RESULTS

The whole blood and plasma samples were de-frozen at fridge temperature +4 ˚C. De-freezing did not happen without problems. Most of the whole blood tubes broke during the melting process. They were kept in the cover tube so that the sample was not lost but it made the processing of the sample more difficult for the laboratory analysts. Fortunately the analysis was successful and I got positive results for amphetamines, benzodiazepines or cannabinoids from 21 cases out of my original 28 cases. I chose to reanalyze cases in which there were positive results in amphetamines, benzodiazepines and cannabinoids in routine analysis. Only two whole blood samples 93190 and 93197 were not analyzed again since there was not enough sample left. Plasma samples for these cases were analyzed according to the original routine results in the same manner as the other plasma samples also. Amphetamines, benzodiazepines and cannabinoids were chosen for reanalyzing because there were several positive results in the routine analysis so that there would be some comparison to be made.

6.1 Amphetamine Results for Whole Blood and Plasma Samples

The whole blood and plasma samples were analyzed with the quantitative amphetamine method using GC-MS at the National Public Health Institute routine laboratory. For all the samples I got two results from which I calculated the mean which then was used as a final result. There were 14 positive amphetamine results.

TABLE 2. Amphetamine Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Whole Blood-Plasma Ratio Amphetamines	14	,75	1,13	1,0020	,10818
Plasma Amphetamines	14	23,44	1777,33	371,5396	519,84161
Whole Blood Amphetamines	14	26,50	1841,24	385,5871	555,33304
Valid N (listwise)	14				

As chart 2 shows there are no big differences between whole blood and plasma results for the amphetamines in general. Minimums and maximums are close to each other and the mean whole blood-plasma ratio is close to one.

TABLE 3. Concentrations of Amphetamines and Blood-Plasma Ratio

				Whole blood Amphetamine	Plasma Amphetamine	Whole Blood-Plasma Ratio
Case number						
	93160	1		199,70	199,07	1,00
		Total	N	1	1	1
	93161	1		214,68	287,10	,75
		Total	N	1	1	1
	93163	1		39,85	49,04	,81
		Total	N	1	1	1
	93164	1		148,86	138,51	1,07
		Total	N	1	1	1
	93182	1		29,83	30,81	,97
		Total	N	1	1	1
	93184	1		156,17	155,12	1,01
		Total	N	1	1	1
	93185	1		1841,24	1777,33	1,04
		Total	N	1	1	1
	93188	1		214,85	226,57	,95
		Total	N	1	1	1
	93190	1		26,50	23,44	1,13
		Total	N	1	1	1
	93194	1		1434,26	1293,50	1,11
		Total	N	1	1	1
	93189	1		33,75	33,13	1,02
		Total	N	1	1	1
	93208	1		590,80	538,15	1,10
		Total	N	1	1	1
	93209	1		202,91	200,26	1,01
		Total	N	1	1	1
	93210	1		264,85	249,56	1,06
		Total	N	1	1	1
	Total	N		14	14	14

In chart 3 the individual paired samples with amphetamine results in whole blood and plasma can be seen. This shows the actual differences between whole blood and plasma samples in amphetamines. For the blood sample in case 93190 the result is from the original routine analyse and that sample has not been analysed again. Therefore there is some time gap between the whole blood and the plasma analysis. Apparently this has not had a significant effect on the results. In this chart the blood-plasma ratio which can also be seen indicates that the results are quite similar since the ratio is close to one.

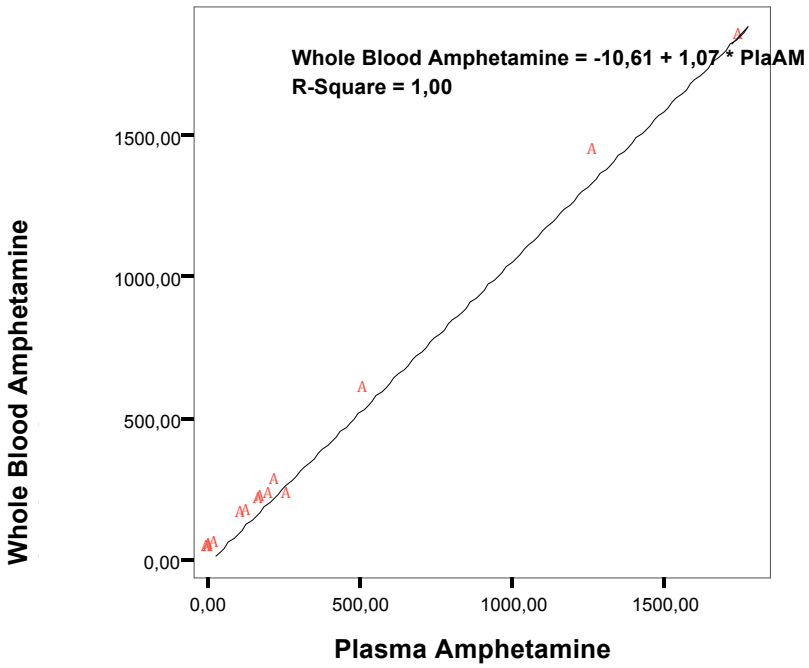

CHART 1: Linearity of Amphetamine in Comparison of Whole Blood and Plasma

Chart 1 shows the correlation of plasma and whole blood results. This linear curve shows that these two matrices correlate well for amphetamines. As a conclusion I can say that similar results are to be expected when comparing amphetamine concentrations in whole blood and plasma.

6.2 Benzodiazepine Concentrations in Whole Blood and Plasma

Benzodiazepines were analysed with a quantitative benzodiazepine method using GC-MS. There were always two results from which I calculated the mean as a final result. All the analyses were conducted in the National Public Health Institute drug laboratory.

In benzodiazepine-positive whole blood samples there were two samples that could not be analyzed again since there was too little sample left. In these cases (93197 and 93190) plasma sample has been stored in the fridge temperature +4 ºC and was not frozen. Plasma results in these cases are compared with the original routine results. The time gap between the analyses is longer than in the other cases but the storage conditions are the same for whole blood and plasma.

Positive benzodiazepine results were detected for nordiazepam, oxazepam, temazepam, nitrazepam, clonazepam, diazepam, zopiclone, midazolam, and alprazolam. More than one positive result was found for diazepam, alprazolam, clonazepam, oxazepam, nordiazepam, and temazepam. These are therefore the substances I have concentrated on when comparing the matrices.

TABLE 4. Whole Blood-Plasma Ratios for Benzodiazepines

	N	Minimum	Maximum	Mean	Std. Deviation
Whole Blood-Plasma Ratio Diazepam	12	,46	,71	,5844	,07124
Whole Blood-Plasma Ratio Alprazolam	7	,63	,84	,7403	,07115
Whole Blood-Plasma Ratio Clonazepam	6	,49	,83	,6484	,13348
Whole Blood-Plasma Ratio Oxazepam	10	,47	,87	,6390	,11608
Whole Blood-Plasma Ratio Nordazepam	12	,47	,69	,5784	,06646
Whole Blood-Plasma Ratio Temazepam	8	,49	,80	,6201	,09734
Valid N (listwise)					

Table 4 demonstrates the blood-plasma ratios for benzodiazepines. The minimum is 0.46 for diazepam and the maximum is 0.87 for oxazepam. The mean ratio varies from 0.5784 to 0.7403. In further detail the mean whole blood concentration for diazepam is 58.4% of plasma concentration. The mean whole blood concentration for alprazolam is 74% of plasma concentration. On average the whole blood concentration for the substances handled here is 63.5% of plasma concentration.

TABLE 5. Correlations of Blood and Plasma in Benzodiazepines

		N	Correlation	Sig.
Pair 1	Blood Diazepam & Plasma Diazepam	12	,999	,000
Pair 2	Blood Alprazolam & Plasma Alprazolam	7	,998	,000
Pair 3	Blood Clonazepam & Plasma Clonazepam	6	,894	,016
Pair 4	Blood Oxazepam & Plasma Oxazepam	10	,998	,000
Pair 5	Blood Nordazepam & Plasma Nordazepam	12	,986	,000
Pair 6	Blood Temazepam & Plasma Temazepam	8	,998	,000

In table 5 we can compare the correlations of different substances. Correlation is close to one so it is very strong in each of the substances. The difference is statistically significant since the significance number is small (sig> 0.01 and sig>0.05 for Clonazepam). This shows that the differences between the whole blood and plasma concentrations are not caused by chance but rather are consistent.

As a conclusion I can say that the whole blood concentration for benzodiazepines is about 60% of that in plasma. The difference is quite consistent between different substances within the benzodiazepine group.

6.3 Cannabinoid Concentrations in Whole Blood and Plasma

Cannabinoids were also analysed in the National Public Health Institute drug laboratory with a quantitative GC-MS cannabis method. From two analysis results I again calculated the mean as the final result.

Within the cases there were four samples with positive results for cannabinoids. The statistical analysis and comparison of the concentrations between whole blood and plasma have been done for THC and THC-COOH because these are the cannabinoids analysed for DRUID. For one case there was not enough sample left so whole blood results are from the original routine analysis. This sample is number 93190. As

mentioned before the plasma sample for that case has been stored at fridge temperature so that the storage conditions have been the same for whole blood and plasma.

TABLE 6. THC and THC-COOH Concentrations in Whole Blood and Plasma

				Whole Blood THC	Plasma THC	Whole Blood-Plasma Ratio THC	Whole Blood THC-COOH	Plasma THC-COOH	Whole Blood-Plasma ratio THC-COOH
Case number									
	93161	1		,00	1,86	,00	9,94	22,85	,44
		Total	N	1	1	1	1	1	1
	93189	1		1,97	3,22	,61	31,68	62,22	,51
		Total	N	1	1	1	1	1	1
	93190	1		3,05	3,36	,91	178,70	248,18	,72
		Total	N	1	1	1	1	1	1
	93212	1		,00	,00	.	6,51	14,47	,45
		Total	N	1	1		1	1	1
	Total	N		4	4	3	4	4	4

In table 6 we can see all the cannabinoid results for the studied cases. There are also the whole blood-plasma ratios in the table. There were only two cases with positive results in whole blood and in plasma for THC. So the blood-plasma ratio established for THC cannot really tell anything about THC blood-plasma ratios in general.

For THC-COOH there were four cases with positive results. These four cases are still way too small a number of results to make any real interpretations about blood-plasma ratios and differences in concentrations but it can give some kind of hint about the reality. On average the blood-plasma ratio is 0.53 so the whole blood concentration is about 53% of the plasma concentration for THC-COOH.

I didn’t see any point in making more statistical analyses for cannabinoids since there are not enough results. With a study group this size the results are not reliable and no interpretations can be made of the results.

6.4 Amphetamine Concentrations in Saliva Compared to Whole Blood and Plasma Concentrations

The saliva samples were defrosted at room temperature. Samples were weighed to measure the actual amount of saliva in the collection tube. Analyses were made with a GC/MS semi quantitative method. For saliva samples only one analysis was made for each sample in comparison to blood and plasma for which there were always two parallel analyses done.

I decided to go through amphetamine results for saliva as a whole and compare the outcomes together for plasma and whole blood since the results are so similar. I had 14 amphetamine positive saliva samples.

Amphetamine concentrations are significantly higher in saliva than in plasma or whole blood as seen in table 7. The mean whole blood amphetamine concentration in whole blood is about 3.8 % of the saliva concentration. Similarly the mean plasma amphetamine concentration is about 3.9 % of saliva concentration. With these results one has to keep in mind that the really high amphetamine concentrations in saliva are not as quantitative as other results since they are so much over the highest standard.

TABLE 7. Descriptive Statistics of Amphetamines

	N	Minimum	Maximum	Mean	Std. Deviation
Whole Blood Amphetamine	14	26,5	1841,2	385,6	555,3
Plasma Amphetamine	14	23,4	1777,3	371,5	519,8
Saliva Amphetamine	14	754,5	44363,1	10616,1	13509,1
Whole Blood-Saliva ratio Amphetamine	14	,01	,06	,038	,02
Plasma-Saliva ratio Amphetamine	14	,01	,06	,039	,02
Valid N (listwise)	14				

Correlations are shown in table 8. Correlations between saliva and plasma and also between saliva and whole blood are significant but not very strong (plasma-saliva 0,730 is relatively close to one and whole blood saliva 0,739 is relatively close to one).

TABLE 8. Amphetamine Correlations of Different Matrices

		Whole Blood Amphetamine	Plasma Amphetamine	Saliva Amphetamine
Whole Blood Amphetamine	Pearson Correlation	1,000	,998**	,739**
	Sig. (2-tailed)		,000	,003
	N	14	14	14
Plasma Amphetamine	Pearson Correlation	,998**	1,000	,730**
	Sig. (2-tailed)	,000		,003
	N	14	14	14
Saliva Amphetamine	Pearson Correlation	,739**	,730**	1,000
	Sig. (2-tailed)	,003	,003	
	N	14	14	14

**. Correlation is significant at the 0.01 level (2-tailed).

In table 9 there are individual amphetamine concentrations for each sample in whole blood, plasma and saliva. There are also ratios for whole blood and saliva as well as plasma and saliva.

TABLE 9. Concentrations and Ratios of Amphetamine in Different Matrices

				Whole Blood Amphetamine	Plasma Amphetamine	Saliva Amphetamine	Whole Blood-Saliva ratio Amphetamine	Plasma-Saliva ratio Amphetamine
Case number								
	93160	1		199,7	199,1	5176,8	,04	,04
		Total	N	1	1	1	1	1
	93161	1		214,7	287,1	11930,5	,02	,02
		Total	N	1	1	1	1	1
	93163	1		39,9	49,0	754,5	,05	,06
		Total	N	1	1	1	1	1

	93164	1	148,9	138,5	7069,9	,02	,02
		Total N	1	1	1	1	1
	93182	1	29,8	30,8	796,2	,04	,04
		Total N	1	1	1	1	1
	93184	1	156,2	155,1	3193,5	,05	,05
		Total N	1	1	1	1	1
	93185	1	1841,2	1777,3	28558,5	,06	,06
		Total N	1	1	1	1	1
	93188	1	214,9	226,6	40167,0	,05	,06
		Total N	1	1	1	1	1
	93189	1	33,8	33,1	1216,9	,03	,03
		Total N	1	1	1	1	1
	93190	1	26,5	23,4	1467,4	,02	,02
		Total N	1	1	1	1	1
	93194	1	1434,3	1293,5	29140,8	,05	,04
		Total N	1	1	1	1	1
	93208	1	590,8	538,2	44363,1	,01	,01
		Total N	1	1	1	1	1
	93209	1	202,9	200,3	3564,8	,06	,06
		Total N	1	1	1	1	1
	93210	1	264,9	249,6	7375,9	,04	,03
		Total N	1	1	1	1	1
	Total	N	14	14	14	14	14

6.5 Cannabinoid Concentrations in Saliva Compared to Whole Blood and Plasma

I had 9 THC positive saliva cases. Table 10 shows the descriptive measures for cannabioids. It is obvious that there are more cannabinoid positive cases in saliva than in whole blood or plasma. Also, THC concentrations are higher in saliva than in whole blood or plasma. Since there are not more comparable cases in cannabinoids I will not do any deeper analyses for these results.

TABLE 10. THC Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Saliva THC	9	,88	32,52	10,95	10,93
Whole Blood THC	4	,00	3,05	1,26	1,51
Plasma THC	4	,00	3,36	2,11	1,56
Valid N (listwise)	3				

In table 11 there are all the THC concentrations and whole blood-saliva and plasma-saliva ratios.

TABLE 11. THC Concentrations and Whole Blood-Saliva and Plasma-Saliva ratios

				Whole Blood THC	Plasma THC	Saliva THC	THC Whole Blood-Saliva Ratio	THC Plasma-Saliva Ratio
Case number								
	93161	1		,00	1,86	23,91	,00	,08
		Total	N	1	1	1	1	1
	93163	1		.	.	11,56	.	.
		Total	N			1		
	93164	1		.	.	13,02	.	.
		Total	N			1		
	93166	1		.	.	,88	.	.
		Total	N			1		
	93189	1		1,97	3,22	32,52	,06	,10
		Total	N	1	1	1	1	1
	93190	1		3,05	3,36	.	.	.
		Total	N	1	1			
	93196	1		.	.	3,58	.	.
		Total	N			1		
	93208	1		.	.	9,04	.	.
		Total	N			1		
	93210	1		.	.	1,96	.	.
		Total	N			1		
	93212	1		,00	,00	2,11	,00	,00
		Total	N	1	1	1	1	1
	Total	N		4	4	9	3	3

6.6 Benzodiazepine Concentrations in Saliva Compared to Whole Blood and Plasma

Comparable positive benzodiazepine results were detected for diazepam, alprazolam, clonazepam, oxazepam, nordiazepam, and temazepam. There were some individual positive results detected in saliva also for other substances in the Benzodiazepine group but I will be concentrating only on the ones from which can be done some comparison with plasma and whole blood results.

Table 12 shows the whole blood-saliva ratios and table 13 plasma-saliva ratios from the benzodiazepines in question.

TABLE 12. Whole Blood-Saliva Ratios for Benzodiazepines

				Whole Blood-Saliva Ratio Diazepam	Whole Blood-Saliva Ratio Alprazolam	Whole Blood-Saliva Ratio Clonazepam	Whole Blood-Saliva Ratio Oxazepam	Whole Blood-Saliva Ratio Nordiazepam	Whole Blood-Saliva Ratio Temazepam
Case number									
	93160	1		.	,17	.	10,89	45,05	5,48
		Total	N		1		1	1	1
	93161	1		39,66	.	.	7,16	29,44	5,52
		Total	N	1			1	1	1
	93163	1		.	,67	,21	.	35,50	.
		Total	N		1	1		1	
	93164	1		46,98	.	.	13,69	46,11	
		Total	N	1			1	1	
	93165	1		.	11,47	,15	.	.	.
		Total	N		1	1			
	93166	1		58,38	4,35	.	.	47,63	.
		Total	N	1	1			1	
	93182	1		18,54	2,97	.	4,03	35,13	10,27
		Total	N	1	1		1	1	1
	93184	1		.	.	5,88	.	.	.
		Total	N			1			
	93185	1		.	4,23	.	.	39,15	.
		Total	N		1			1	
	93188	1		62,83	.	1,36	21,21	68,55	6,68
		Total	N	1		1	1	1	1
	93189	1		.	.	.	7,08	.	.
		Total	N				1		
	93190	1		1,28	.	6,66	4,66	24,73	1,42
		Total	N	1		1	1	1	1
	93191	1		77,54	.	.	13,56	66,27	
		Total	N	1			1	1	
	93195	1		54,84	.	.	.	33,27	.
		Total	N	1				1	
	93197	1		23,06	5,15	.	.	69,41	.
		Total	N	1	1			1	
	Total	N		9	7	5	8	12	5

Variation in the whole blood – saliva ratios seems to be quite large for each of the studied substances in the Benzodiazepines. In general the concentrations are mostly much higher in whole blood than in saliva for each substance with a few exceptions in alprazolam, clonazepam and temazepam.

TABLE 13. Plasma-Saliva Ratios for Benzodiazepines

				Plasma-saliva ratio Diazepam	Plasma-saliva ratio Alprazolam	Plasma-saliva ratio Clonazepam	Plasma-saliva ratio Oxazepam	Plasma-saliva ratio Nordiazepam	Plasma-saliva ratio Temazepam
Case number									
	93160	1		.	,21	.	14,49	73,27	9,12
		Total	N		1		1	1	1
	93161	1		78,28	.	.	15,30	59,28	11,37
		Total	N	1			1	1	1
	93163	1		.	,98	,40	.	75,01	.
		Total	N		1	1		1	
	93164	1		65,74	.	.	15,72	66,41	
		Total	N	1			1	1	
	93165	1		.	13,58	,18	.	.	.
		Total	N		1	1			
	93166	1		105,87	5,87	.	.	90,45	.
		Total	N	1	1			1	
	93182	1		33,54	4,69	.	5,94	60,24	17,04
		Total	N	1	1		1	1	1
	93184	1		.	.	12,12	.	.	.
		Total	N			1			
	93185	1		.	5,48	.	.	69,73	.
		Total	N		1			1	
	93188	1		110,65	.	1,83	29,98	116,78	10,61
		Total	N	1		1	1	1	1
	93189	1		.	.	.	11,91	.	.
		Total	N				1		
	93190	1		2,30	.	9,32	8,17	41,23	2,46
		Total	N	1		1	1	1	1
	93191	1		134,89	.	.	25,24	115,75	
		Total	N	1			1	1	
	93195	1		82,31	.	.	.	60,96	.
		Total	N	1				1	
	93197	1		35,46	7,19	.	.	101,25	,37
		Total	N	1	1			1	1
	Total	N		9	7	5	8	12	6

Similarly as in table 12 for whole blood - saliva we can see in table 13 that there is also quite a lot of variation in plasma – saliva ratios. As in blood also in plasma the concentrations are generally much higher than in saliva with a few exceptions in alprazolam, clonazepam and temazepam.

Table 14 and 15 shows the correlations between plasma and saliva and whole blood and saliva.

TABLE 14. Plasma-Saliva Correlations for Benzodiazepines

		N	Correlation	Sig.
Pair 1	Plasma & Saliva Diazepam	9	,309	,418
Pair 2	Plasma & Saliva Alprazolam	7	,232	,616
Pair 3	Plasma & Saliva Clonazepam	5	,157	,801
Pair 4	Plasma & Saliva Oxazepam	8	,934	,001
Pair 5	Plasma & Saliva Nordiazepam	12	,892	,000
Pair 6	Plasma & Saliva Temazepam	6	,920	,009

Plasma – saliva correlation is very good for oxazepam and it is statistically significant because the correlation is very close to one (0,934) and significance is very small, close to zero. Also nordiazepam and temazepam correlate well since their correlations are close to one also (0,892 and 0,920) and significances are close to zero for both. Contrastingly correlations for diazepam, alprazolam and clonazepam have no correlation what so ever.

TABLE 15. Whole Blood-Saliva Correlations for Benzodiazepines

		N	Correlation	Sig.
Pair 1	Whole Blood & Saliva Diazepam	9	,296	,439
Pair 2	Whole Blood & Saliva Alprazolam	7	,211	,650
Pair 3	Whole Blood & Saliva Clonazepam	5	,074	,906
Pair 4	Whole Blood & Saliva Oxazepam	8	,944	,000
Pair 5	Whole Blood & Saliva Nordiazepam	12	,888	,000
Pair 6	Whole Blood & Saliva Temazepam	5	,953	,012

It seems the correlations are strong and statistically significant for oxazepam and nordiazepam also in whole blood – saliva comparison. For temazepam the correlation between whole blood and saliva appears to be strong too but it is not statistically that significant. Similarly as in plasma – saliva comparison diazepam, alprazolam and clonazepam have no correlation between whole blood and saliva. This would indicate that saliva is a more usable matrix for detecting oxazepam, nordiazepam or temazepam than diazepam, alprazolam or clonazepam. According to these results saliva gives no proper indication of the level of drugs in blood or plasma for these particular Benzodiazepines.

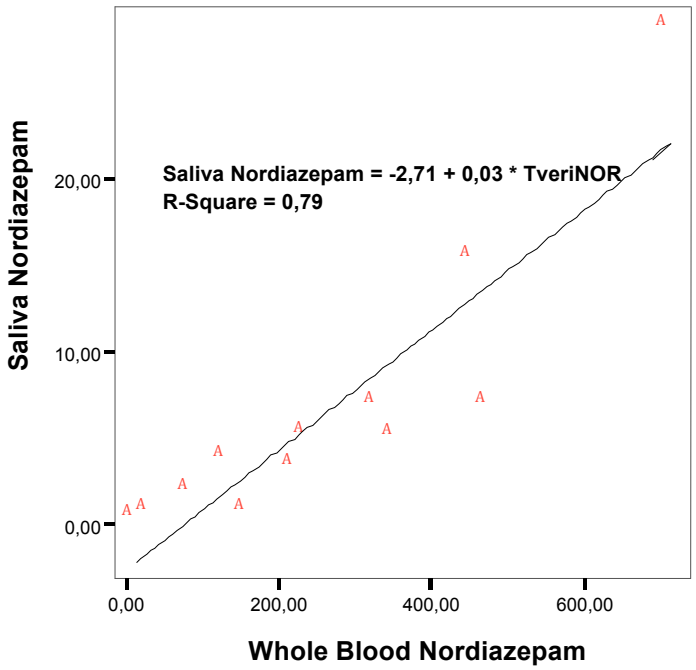

CHART 2. Linearity of Nordiazepam in Saliva Compared to Whole Blood

In chart 2 can be seen that saliva nordiazepam results are linear with whole blood nordiazepam results. This gives more indication of the correlation of these results.

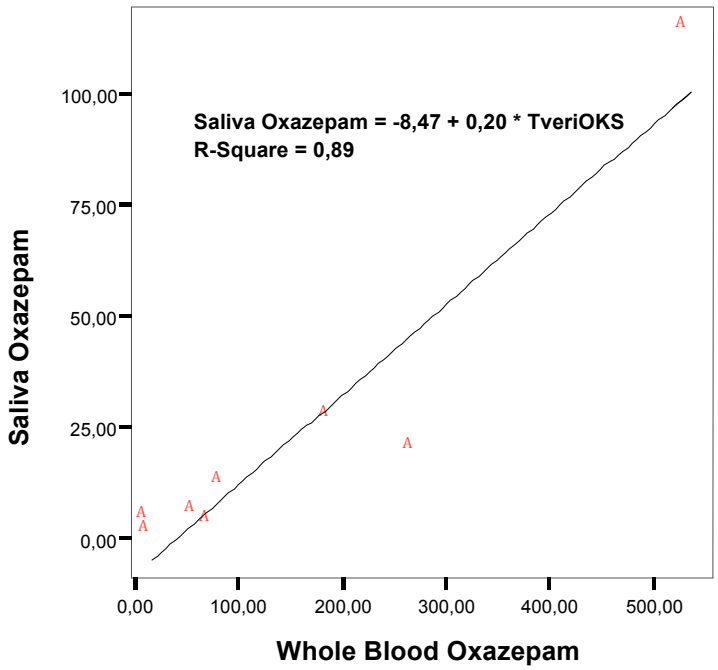

CHART 3. Linearity of Oxazepam in Saliva Compared to Whole Blood

Oxazepam also gives more indication of the correlation between saliva and whole blood with its linear regression.

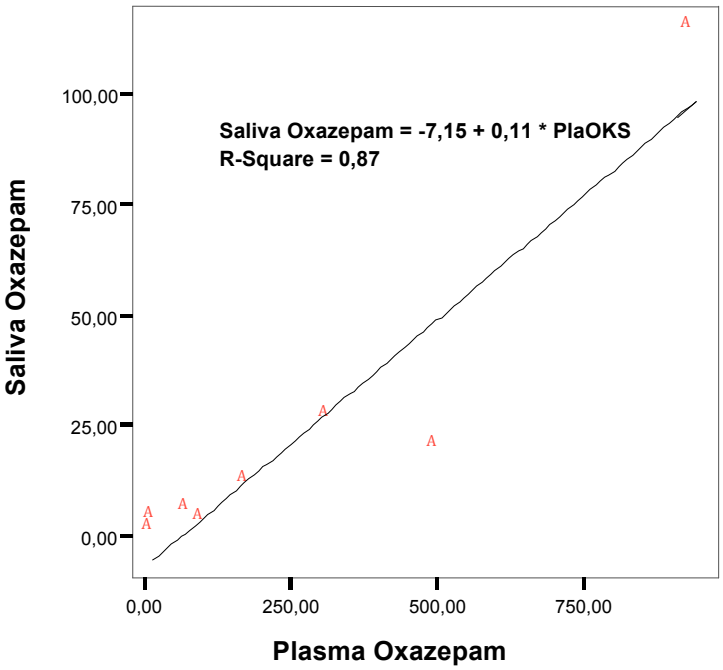

CHART 4: Linearity of Oxazepam in Saliva Compared to Plasma

As in whole blood – saliva comparison linearity of saliva – plasma also backs up the correlation results for oxazepam. This is visible in chart 4.

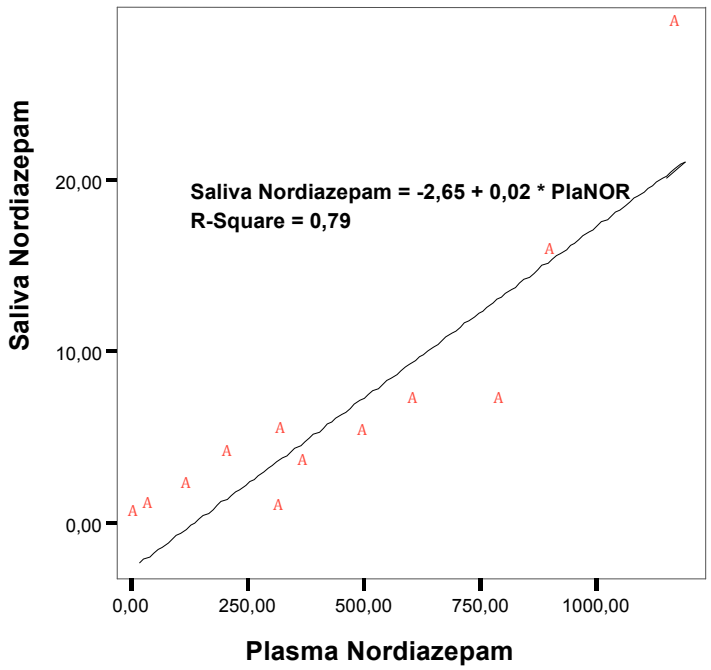

CHART 5. Linearity of Nordiazepam in Saliva Compared to Plasma

Chart 5 shows very well the linearity of nordiazepam in the comparison of saliva and plasma as was also well established for saliva – whole blood comparison.

7 CONCLUSIONS

7.1 Analysis of the Results

The purpose of the research was to offer my commissioner, the National Public Health Institute, Druid project, information concerning the differences between the concentrations of drugs of abuse in different matrices. The matrices in question were whole blood, plasma and saliva. The goal was to collect the samples from more than 30 voluntary suspected DUI drivers. Unfortunately I got samples from only 28 suspected DUI drivers.

Within the samples there were all together 14 positive cases for amphetamine. Amphetamine results were very promising as the results seemed to correlate very well. Even though the saliva concentrations were significantly higher the correlations to whole blood and plasma were good. Whole blood and plasma concentrations were very similar and whole blood-plasma ratios varied from 0.75 to 1.13 and the mean was 1.002. Whole blood-saliva ratios varied from 0.01 to 0.33 with a mean of 0.07 and the plasma-saliva ratio varied from 0.01 to 0.41 with a mean of 0.08.

As a result I can say that according to this research any of the matrices can be used to detect amphetamine from DUI drivers. The results are only suggestive since my sample is so small. But my results are in agreement with a previous study by Engblom et al. They concluded that saliva is a suitable matrix for detection of amphetamine from suspected DUI drivers for a comparison to whole blood.

With larger sample number the results could be more generalized and proper rates could be calculated for the matrices. The results could indicate that amphetamine concentration could be analysed from any of the matrix in question and to calculate the concentration in others.

Cannabinoids were detected only in four whole blood and plasma samples. Within the saliva samples there were nine cannabinoid positive samples. The amount of comparable samples was so small that no proper conclusions could be made. The only obvious result is that cannabinoids are more visible in saliva than in whole blood or plasma. This could be due to the fact that cannabinoids are usually consumed by smoking which may result in contamination of the oral cavity. According to my study I can not say how well saliva is suited for detection of cannabinoids from suspected DUI drivers. No rates between the matrices can be calculated with these results. A much bigger sample number would be needed to give any conclusions about the concentrations between different matrices.

In benzodiazepines I got comparable positive results in six different substances. These were diazepam, alprazolam, clonazepam, nordiazepam, oxazepam and temazepam. Whole blood and plasma correlate well in each of the substances. This indicates that these matrices can both be used for detecting the benzodiazepines in question from suspected DUI drivers.

Saliva samples gave more conflicting results. From the same substances good correlations between whole blood and saliva and plasma and saliva were seen in nordiazepam, oxazepam and temazepam. Diazepam, alprazolam and clonazepam did not correlate at all. Again the sample was too small to make any definite conclusions about the ratios between different matrices. The concentrations varied quite lot between the matrices and substances studied. Variation in concentrations may be due to the fact that we don't know when the substances have been consumed and in what dosages. According to these results I would conclude that saliva can not be used to measure the concentrations of benzodiazepines from suspected DUI drivers. A much bigger sample numbers would be needed to establish any proper ratios between the matrices. Even for the substances that seemed to correlate well between the matrices it would need a larger sample group to verify the results. It would also require specific knowledge about the substance that was suspected to have been abused to use saliva for measuring the concentration.

7.2 Evaluation of the Research

Validity

The term validity is used to determine whether the research measures what it was supposed to measure (Uusitalo 1996, 84.). To increase validity I planned the research in close cooperation with my commissioner. The basis of my thesis is being done according to the sub study of larger entity. As my study is part of the bigger sub study, it is giving suggestive results for the main study and is therefore valid for the purpose it was planned for. Originally I attempted to get more results for a higher variety of substances. Since the final sample number was so small the validity also suffered. On the other hand the research measured what it was supposed to measure but on a smaller scale. All in all, I came to the conclusion that the validity in my research was not as high as I wanted it to be. This is largely due to the fact that it was more difficult to get samples that I had expected.

Reliability

The reliability of the research measures how dependable the results are, i.e. whether the same results would be achieved if the research was repeated (Uusitalo 1996, 84). The sample collection procedures and sample handling, storage and analysing procedures effect the reliability of my study. All the stages were done according to well determined

procedures. The study could be repeated and the results would probably be same or at least very similar. I would say that the reliability of the study is high even though some problems occurred in the thawing process of the whole blood and plasma samples and collecting sufficient amounts of some samples.

7.3 My Own Reflections on the Thesis

The whole thesis project started with the development assignment for school. As my development project I organised the sample collection facilities at the Helsinki University Department of Forensic Medicine and started the sample collection with the cooperation of a forensic pathologist in April 2008. This gave me a good starting point for my thesis since I was already familiar with the topic and the whole project that my thesis was based on.

Difficulties arose with the sample collection. We were not able to get as many samples as we had hoped for. It was difficult to arrange sample collection times that were suitable for both the forensic pathologist and me. Also when we managed to arrange a time there weren't as many suspected DUI drivers as we had hoped for. This was quite frustrating.

I started to collect material for my thesis in June 2008 and at the same time I wrote the theoretical framework for the thesis. The theoretical part was ready in August 2008. Finding suitable articles was not too difficult since there were materials readily available at the National Public Health Institute, where I also worked.

Sample collection took place during the whole summer. Originally I was supposed to get the samples to analysis during the summer and fall. Unfortunately there become problems with that and there were some delays with the analysis. Finally I got all the analyses results by mid December 2008. Statistical analyses were made as I got results. All statistical data was ready in the end of December 2008.

The thesis project has been very educational since I had to familiarize myself with a topic with which I was not particularly well acquainted. I had not been in contact with drugs of abuse and driving under the influence of drugs and medicines. Also, saliva was not a familiar analytical matrix for me.

On the whole I found the project quite straight forward. It went on systematically and according to plan. The only thing that did not really work as planned was the time table. I was not able to get as many samples as I had hoped for. Also, the analyses were not finished in the time frame originally planned.

During this project I was able to work in cooperation with different kinds of people. I think that the cooperation with the National Public Health Institute, Department of Forensic Medicine, and police worked quite well. Also, the suspected DUI drivers were positive about the study and most of them were happy to take part. All in all I think that this was a successful project which gave me lot of new skills and connections in working life.

My thesis gives indicative results for the continuing project for the researchers at the National Public Health Institute. This topic needs further investigation and there is lot further to do with this subject. One further research objective is obviously continuing the study with more samples. Since The National Public Health Institute continues this study there will be many interesting results to get in the future.

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Appendix 1 (1/2)

List of substances analysed in DRUID-project and short description of each substance.

1. Ethanol	Ethanol, also called ethyl alcohol, is a flammable, colourless, chemical compound. It is best known as the type of alcohol found in alcoholic beverages and is also used as a solvent and antiseptic substance. In common use it is often referred to as alcohol. (Encyclopedia Britannica Online, http://search.eb.com/eb/article-9033143, 8.8.2008.)
2. Morphine	Morphine is one of the alkaloids that opium contains. Like other opioids, e.g., diacetylmorphine (heroin), morphine acts directly on the central nervous system to relieve pain. Generally, the term opioid has been used to refer to compounds with morphine-like activity. Effects on morphine use are for example analgesia, drowsiness, reduced gastrointestinal motility, miosis, nausea, and respiratory depression. Strong physical dependence may result in frequent use. (Samyn,Verstraete, van Haeren & Kintz 1999, 45.)
3. Amphetamine	Amphetamine is a strong stimulant of the central nervous system. Amphetamine acts as indirect sympathomimetic drug. Amphetamine is used as drug of abuse and also as doping agent (Kraemer & Paul 2007, 1430). Amphetamine causes euphoria, increased energy, and alertness (Samyn et al 1999, 47).
4. MDMA	MDMA is also known as ecstasy. It is an amphetamine-derived designer drug. It produces a feeling of euphoria, energy and urges to socialize. As with other designer drugs it is used often as a "rave drug". (Kraemer & Paul 2007, 1430.)
5. MDA	MDA is known as "love pills". As for MDMA, MDA is also an amphetamine-derived designer drug and produces similar feelings. It is also used as a party drug. These drugs may lead to intoxication and impairment of ability to drive a car. (Kraemer & Paul 2007, 1430.)
6. Cocaine	Cocaine is derived from the leaves of the coca plant that grows in South America. For illegal abuse cocaine is sold as cocaine hydrochloride for oral, intravenous and intranasal use and also cocaine base ("crack") for smoking. Cocaine produces a feeling of well-being and euphoria. (Samyn et al 1999, 43-44.)
7. THC	THC is the primary psychoactive component in cannabis. Cannabis products are for example marijuana, hashish and hashish oil. Cannabis is the most widely produces plant-based illegal drug in the world. It is reported to be the most frequently used illegal drug in Europe and the USA. (Kraemer & Paul 2007,1426). Cannabis administration can

	cause sedation, euphoria, hallucinations, and temporal distortion (Samyn et al 1999, 44.)
8. THCCOOH	THCCOOH is the inactive metabolite of THC. (Kraemer & Paul 2007, 1426.)
9. Diazepam	Diazepam is the most popular among the benzodiazepines and Valium is most widely known trademark for it. Benzodiazepines are tranquilizers. They are prescribed as sedative-hypnotics, muscle relaxants, anticonvulsants, and for relief of anxiety and psychiatric disorders. (Schramm, Smith, Craig & Kidwell 1992, 6.)
10. Alprazolam	Alprazolam belongs to benzodiazepines. It is used as medication for panic disorders and anxiety disorders. (Moffat, Osselton & Widdop 2004, 606.)
11. Clonazepam	Clonazepam belongs to benzodiazepines. It can be used for epilepsy, anxiety disorders, and panic disorders. (Moffat et al 2004, 830.)
12.Benzoylecgonine	Benzoylecgonine is the primary metabolite of cocaine. It is further metabolized in to ecgonine. (Moffat et al 2004, 688.)
13. Codeine	Codeine is also an alkaloid that opium contains and it belongs to the group of opiates. Codeine is used commonly as an analgesic and antitussive. (Samyn, et al 1999, 45-46.)
14.6-acetylmorphine	6-acetylmorhine known as MAM is active metabolite of heroin which is further hydrolyzed to morphine. (Samyn et al 1999, 45.)
15. Methamphetamine	As with amphetamine, metamphetamine is also a strong stimulant of the central nervous system. Metamphetamine acts as an indirect sympathomimetic drug. Metamphetamine is used as drug of abuse and also as a doping agent. (Kraemer & Paul 2007, 1430.)
16. Methadone	Methadone is a synthetic opioid analgesic. It is used to administer opioid dependence. Methadone is usually taken orally or by intramuscular injection. To suppress the withdrawal symptoms initial doses are adequate. Use of methadone may cause dependence when the use lasts a long time. (Samyn et al 1999, 46.)
17. Oxazepam	Oxazepam is a metabolite of several benzodiazepines. It is used to treat for example anxiety disorders. (Moffat et al 2004, 1376.)
18. Nordiazepam	Nordiazepam is a metabolite of many benzodiazepines and it is metabolized to oxazepam. (Moffat et al 2004, 1353.)
19. Zopiclone	Zopiclone is a benzodiazepine-like substance. It is used to treat insomnia. (Pharmaca Fennica 2005, 2969.)
20. MDEA	Similarly to MDA and MDMA, MDEA is also an

	amphetamine-derived designer drug. It is known as "Eve" and it is used similarly to other amphetamine-derived designer drugs. (Kraemer & Paul 2007, 1430.)
21. Lorazepam	Lorazepam belongs to the benzodiazepines and it is used to treat for example anxiety disorders. (http://www.drugs.com/lorazepam.html, 5.8.2008.)
22. Flunitrazepam	Flunitrazepam belongs to the group of benzodiazepines. It is used as a hypnotic to treat insomnia. (Moffat et al 2004, 1045.)
23. Zolpidem	Zolpidem is a benzodiazepine-like substance. It is used to treat severe insomnia. (Pharmaca Fennica 2005, 2955.)
24. Temazepam	Temazepam is a metabolite of many benzodiazepines. It is used as a hypnotic to treat insomnia. (Moffat et al 2004, 1603.)
25. Midazolam	Midazolam is a benzodiazepine derivative. It is used as a sedative and as premedication before anaesthesia. (Pharmaca Fennica 2005, 1693.)
26. Phenazepam	Phenazepam belongs to the group of benzodiazepines. It is used to treat for example epilepsy, alcohol withdrawal and insomnia. (http://en.wikipedia.org/wiki/Phenazepam, 6.8.2008.)
27. Nitrazepam	Nitrazepam belongs to the group of benzodiazepines. It is mostly used to treat insomnia. (Moffat et al 2004, 1344.)
28. Zaleplon	Zaleplon is a benzodiazepine-like substance that is used to treat insomnia. (http://en.wikipedia.org/wiki/Zaleplon, 6.8.2008.)

Appendix 2 (2/2)

Sample collection form

TAPAUSLOMAKE
OIKEUSLÄÄKETIETEEN LAITOS

veri-plasma -tutkimus

Versio 1.0 25.3.2008

Kansanterveyslaitos
Folkhälsoinstitutet
National Public
Health Institute

Tapaustunnus
(T-alkuinen) tähän

DRUID

Huumaus- ja lääkeaineiden pitoisuudet eri matriiseissa rattijuopumuksesta epäillyillä henkilöillä

Tapaukseen liittyvä HULAVA-numero tähän
(R-alkuinen)

Virtsanäyte otettu rattijuopumustutkintaa varten ☐ Kyllä ☐ Ei

Tutkimusta varten otetut näytteet

☐ Plasma ____/____/20____ Näyte otettu klo____:____
☐ Poikkeava näytteenotto, kommentit:

☐ Sylki ____/____/20____ Näyte otettu klo____:____
☐ Normaali näytteenotto, kesto alle 5 min
☐ Poikkeava näytteenotto, kommentit:

☐ Veritäplä ____/____/20____ Näyte otettu klo____:____
☐ Poikkeava näytteenotto, kommentit:

☐ Virtsa ____/____/20____ Näyte otettu klo____:____
☐ Poikkeava näytteenotto, kommentit:

Hematokriittitulos

Muut kommentit ja huomiot: