UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

Academiejaar 2010 - 2011

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Karen MEERT

Promotor: Prof. Dr. Alain Verstraete

Scriptie voorgedragen in de 2de Master in het kader van de opleiding tot

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I would like to thank my promotor, Prof. Dr. A. Verstraete, for his remarks and notions. I appreciated also his fast corrections.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

6-AM	6-acetylmorphine
BZE	Benzoylecgonine
COC	Cocaine
EME	Ecgonine methylester
EMIT	Enzyme Multiplied Immunoassay Technique
GC-MS	Gas Chromatography-Mass Spectrometry
HMA	4-hydroxy-3-methoxyamphetamine
HMMA	4-hydroxy-3-methoxymethamphetamine
IA	Immunoassay
IN	Intranasal
IV	Intravenous
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDA	3, 4-methylenedioxyamphetamine
MDMA	3, 4-methylenedioxymethyl-amphetamine
SAMHSA	The Substance Abuse and Mental Health Services Administration
THC	Δ^9-tetrahydrocannabinol
THCCOOH	11-nor-Δ^9-carboxy-tetrahydrocannabinol-9-carboxylic acid

ABSTRACT

This review summarizes how soon different drugs (methamphetamine, MDMA, cannabis, cocaine and heroin) or their metabolites can be detected in urine after a typical dose. However, in the literature no study was found that was specifically designed to answer this question. The information of this review comes from the limited urinary excretion data from controlled clinical studies. Furthermore the four drugs are discussed.

With a 500 ng/ml cut-off concentration, the first positive methamphetamine occurred at a mean time of 5.5 h (1.4-11.3 h) after a 10 mg dose and at 4.3 h (1.2-8.8 h) after a 20 mg dose. Lowering the cut-off value from 500 ng/ml to 250 ng/ml didn't change the time to first positive. The median time to first positive of MDMA and its main metabolite HMMA occurred at 1.3 h (0.8-3.3 h) after 1.0 mg/kg body weight and at 1.2 h (0.4-1.4 h) after 1.6 mg/kg body-weight with a cut-off value of 25 ng/ml.

After smoking a marihuana cigarette of 15.8 mg and 33.8 mg THC, the mean time to first positive of THCCOOH was 4.9 $\pm$ 1.8 h (3.2-8 h) and 3.4 $\pm$ 0.6 h (2.3-4 h), respectively. Very large differences in the first urine concentration of THCCOOH were reported. After hydrolysis with bacterial β-glucuronidase, the presence of significant quantities of THC and 11-OH-THC can be demonstrated in urine. Five minutes after smoking a low dose marijuana cigarette (17.7 mg THC), THC was already measurable in two of the six cases after a low dose and in five of the seven cases after a high dose (35.8 mg THC). Five minutes after use, 11-OH-THC could also be measured in four of the six subjects after 17.7 mg THC and in six of the seven subjects after 35.8 mg THC. In enzyme-hydrolyzed urine, one study found a peak concentration of 8, 11-diOH-THC in the earliest urine samples in nine of the ten cases, but no other study has confirmed the presence of 8, 11-diOH-THC.

The time to first positive of benzoylecgonine was 2.6 $\pm$ 1.3 h (0.4-4.3 h) after 25 mg IV cocaine, 1.6 $\pm$ 0.5 h (0.8-2.3 h) after 32 mg intranasal cocaine and 1.9 $\pm$ 0.8 h (1.0-3.3 h) after 42 mg smoked cocaine. Measuring cocaine as unchanged drug could be a valuable alternative to get rapid positive values. After administration of 1.5 mg/kg intranasal cocaine, one subject was found who excreted free cocaine (1400 ng/ml at 1 h post-dose) in the absence of benzoylecgonine.

After different doses (3 mg, 6 mg and 12 mg) of intravenous heroin, 6-AM was measurable in all the first urines above the cut-off value of 10 ng/ml. In most cases (23 out of 24), the peak concentration of 6-AM was observed in the first urine. After smoked heroin, 6-AM was also measurable in all the first urines, however there were two cases with a first 6-AM value below the cut-off value, while the concentrations of total and free morphine were clearly positive.

One can conclude that, after a relatively low dose of drugs, methamphetamine appears in detectable concentrations in the urine after 1-11 hours, MDMA after 1- 4 hours, THCCOOH after 2-8 hours, BZE after 0.5- 4 hours and 6-AM after 1 – 3 hours. For most drugs, the appearance of metabolites in urine is quite rapid, and the risk of having a negative result in the first hours after intake of the drug is rather low.

NEDERLANDSE SAMENVATTING

Deze literatuurstudie bespreekt de snelheid waarmee verschillende drugs (methamfetamine, MDMA, cannabis, cocaine en heroine) of hun metabolieten verschijnen in de urine, na gebruik van een typische dosis. Er werd echter geen enkele studie gevonden die speciaal gericht was op het beantwoorden van deze vraag. Wel werd er informatie verkregen uit studies die onderzoek verrichten naar de detectie tijden van urine. Uit deze artikels werden dan waarden van drugs of metabolieten gevonden kort na gebruik.

In de literatuur zijn er weinig gecontroleerde studies gekend na gebruik van (meth)amfetamine of MDMA ("ecstasy"). Na 10 mg en 20 mg oraal methamfetamine, werd het eerste positieve staal voor methamfetamine gedetecteerd na een gemiddelde tijd van 5,5 h (1,4-11,3 h) en 4,3 h (1,2-8,8 h), respectievelijk. De SAMHSA richtlijnen die gelden voor arbeidsomstandigheden, spreken pas van een positief staal, als methamfetamine (cut-off-waarde: 500 ng/ml) samen gaat met een amfetamine-concentratie van meer dan 200 ng/ml. Wanneer deze richtlijnen worden gevolgd, dan is de tijd tot het eerste positieve staal veel langer. Waarden van zelfs 14 h werden dan opgemeten. Na gebruik van 1,0 mg MDMA/kg, was de mediaan van de tijd tot het eerste positieve staal voor MDMA en HMMA 1,3 h (0,8-3,3 h) en na gebruik van 1,6 mg/kg was deze mediaan 1,2 h (0,4-1,4 h). MDMA en zijn metaboliet HMMA werden gedetecteerd in alle eerst urinestalen na 1,0 mg MDMA/kg en in 14 van de 16 gevallen na een dosis van 1,6 mg/kg. De vroegste urineafname vond plaats na 0,4 h en zelfs die waarden van MDMA en HMMA waren al positief. Gezien HMMA concentraties bijna altijd hoger waren dan MDMA, kan HMMA een snellere manier zijn om een positief staal te detecteren. Op dit moment echter wordt de bepaling van HMMA niet gebruikt bij routine procedures.

Na een cannabis dosis van 15,8 mg en 33,8 mg THC, was de gemiddelde tijd tot het eerst positieve staal voor THCCOOH 4,9 $\pm$ 1,8 h (3,2-8 h) en 3,4 $\pm$ 0,6 h (2,3-4 h), respectievelijk. Zeer grote verschillen van THCCOOH concentratie in de eerste urine werden gerapporteerd. Na een dosis van 15,8 mg THC, varieerden de THCCOOH waarden in de eerste urine van 5,5 ng/ml tot 138,4 ng/ml, wat een 25-voudige variatie inhoudt. Na een dosis van 33,8 mg THC, varieerden de waarden van 9,1 ng/ml tot 318,0 ng/ml, wat zelfs een 35 voudige variatie inhoudt. De piek van THCCOOH werd gemiddeld gevonden na 4 uur en dit met een gemiddelde concentratie van 179,4 $\pm$ 146,9 ng/ml na gebruik van 35,8 mg THC. Gezien de enorme verschillen van THCCOOH tussen de verschillende vrijwilligers, en gezien de eerder late piek, werd er op zoek gegaan naar andere metabolieten die eventueel kunnen gebruikt worden als marker voor recent cannabis gebruik. 8, 11-diOH-THC werd naar voor geschoven als mogelijke marker, omdat in negen van de tien gevallen de hoogste concentratie van deze metaboliet werd gevonden in de eerste urine. (gemiddelde tijd van de eerste urine was 2,6 h). Een andere studie probeerde de aanwezigheid van deze metaboliet te bevestigen, maar vond slechts in één geval van de acht deze metaboliet terug. Na hydrolyse met bacterieel β-

glucuronidase, kunnen THC en 11-OH-THC in significante hoeveelheden worden gedetecteerd in de urine. Vijf minuten na het roken van een lage dosis sigaret (17,7 mg THC), was THC reeds meetbaar in twee van de zes gevallen en na een hoge dosis sigaret (35,8 mg THC) was dit in vijf van de zes gevallen. Ook 11-OH-THC kan gemeten worden 5 min na gebruik. Deze metaboliet was al meetbaar in vier van de zes gevallen na 17,7 mg THC en in zes van de zeven gevallen na 35,8 mg THC.

Na gebruik van 25 mg intraveneus cocaine, 32 mg intranasaal cocaine en 42 mg gerookte cocaine, kwam het eerste positieve staal van benzoylecgonine gemiddeld na 2,6 $\pm$ 1,3 h (0,4-4,3 h), na 1,6 $\pm$ 0,5 h (0,8-2,3 h) en na 1,9 $\pm$ 0,8 h (1,0-3,3 h), respectievelijk. Ongeveer 40 % van de cocaine dosis wordt geëxcreteerd als benzoylecgonine (BZE) en daarmee kan benzoylecgonine beschouwd worden als de belangrijkste metaboliet van cocaine. Nochtans komt de piek van BZE na de piek van cocaine en ecgonine methylester. Gezien cocaine als drug zelf, het eerst verschijnt in urine, kan het nuttig zijn om cocaïne op te sporen kort na druggebruik. Een vrijwilliger met 1400 ng/ml cocaine 1 uur na gebruik, maar in afwezigheid van BZE, is beschreven in de literatuur. Echter, aangezien deze studie maar 6 proefpersonen bevatte, is de vraag of deze vrijwilliger inderdaad uniek is of niet, moeilijk te beantwoorden. Anderzijds, werden er ook twee personen beschreven met excretie van BZE in afwezigheid van cocaine. Vals negatieve waarden bekomen, zijn dus in ieder geval mogelijk wanneer er maar naar één specifiek metaboliet wordt gekeken.

Na gebruik van verschillende dosissen intraveneus heroïne (3 mg, 6 mg en 12 mg), was 6-acetylmorphine al meetbaar in alle eerste urinestalen en dit boven de cut-off waarde van 10 ng/ml. In de meeste gevallen (23 van de 24), bevatte de eerste urine de piekconcentratie van 6-AM. Voor elke dosis waren alle eerste urinestalen (gemiddelde afname na 2 h) positief voor totaal morfine (cut-off: 300 ng/ml) en dit in concentraties die ruimschoots boven deze cut-off waarde lagen, zelfs ook bij de lage dosis. Dit doet vermoeden dat vroegere stalen ook nog positief zouden geweest zijn. De snelst positieve eerste urines werden gecollecteerd na 1,2 u en na 1,4 u. De respectievelijke concentraties van totaal morphine hierbij, waren 1855 ng/ml en 6950 ng/ml, veruit boven de cut-off waarden.

Na roken van heroïne, was 6-AM ook meetbaar in alle eerste urinestalen, hoewel er twee gevallen werden beschreven met een waarde onder de cut-off waarde, terwijl de concentraties van totaal en vrij morfine duidelijk positief waren. De cut-off van 6-AM verlagen kan een manier zijn om sneller positieve waarden van heroïne te bekomen.

Uit deze thesis kunnen we concluderen dat, na een eerder lage dosis, metamfetamine in de urine verschijnt na 1- 11 uur, MDMA na 1-4 uur, THCCOOH na 2-8 uur, BZE na 0,5-4 uur and 6-AM na 1-3 uur. Voor de meeste drugs geldt dat er een maar een kleine kans is dat er een negatief resultaat wordt bekomen in de eerste uren na gebruik.

INTRODUCTION AND OBJECTIVES

Drug use is a worldwide problem. To get an idea of the situation in Belgium, some data are given. In the "Belgian National Report on drugs 2009" (Lamkaddem et al.(1)), data from 2007 are available, in comparison with these from 2003. In 2007, there was a lifetime prevalence, last year and last month of cannabis use of 23.9 %, 18.8 % and 12.4 %, respectively in the category 15-16 years old. This was a decrease with 7.1 %, 7.3 %, 1.6 % in comparison with 2003. This decrease was steeper among boys compared to girls. Unlike cannabis, the prevalence rates for amphetamines, "ecstasy" and cocaine increased: the lifetime prevalence of cocaine increased from 2.4 % to 4.8 %, amphetamines from 2.8% to 4.8% and "ecstasy" from 4.4 % to 5.3 %. The use of heroin remained the same. The report "Analyses of illegal drugs in Belgium, 2008" (Deprez et al. (2)) gives a national overview of the results of all drug analyses that were conducted in Belgium. Although not all laboratories could provide all data, estimations could be made concerning the drugs that are used. The substances most often found were from the group of cannabinoids (31 %) and opiates (29 %), followed by cocaine (12 %) en amphetamines (10 %). Taken into account that reporting the results of the analysis of cannabis is no longer required, the percentage of cannabis is probably even larger than reported here.

Drug testing involves the analysis of biological material to detect these substances or their metabolites in the body. Drugs can be detected in blood, urine, saliva, sweat , hair and breath samples. This document is focussing on the urine detection of drugs. Four drugs will be discussed: amphetamines, cannabis, cocaine and heroin.

Initial screening of drugs in urine can be performed by immunoassay, which detects the presence of opiates, amphetamines, benzodiazepines, cannabinoids, cocaine,…. The result is usually reported as positive (substance is found) or negative (no substance is found). Immunoassays are sensitive, but not specific. When the test result is positive, second line testing, using gas chromatography-mass spectrometry (GC-MS) as recommended analytical technique, is used to confirm and to identify the drug or metabolite.

A frequently asked question is how long drugs can be detected after they were used. That is a difficult question because the detection time is influenced by many factors: the dose, the route of administration (oral, smoked, intravenous,…), acute versus chronic use, the choice of the matrix (urine, hair,…), the cutoff value of the analytical technique, the nature of the molecule, the pH, the concentration of the urine and the interindividual variation in metabolism (Vandevenne et al., (3)). Only a few studies have focused on the detection time (Verstraete (4)).

Another question is how soon drugs can be detected after they were used. It is a less common question, but however interesting: Is the first urine after smoking a marijuana cigarette already positive or do we have to wait longer? How long do we have to wait? When will the peak concentration be observed in

the urine? Can we test too early so that the result is falsely negative because the drug is not yet metabolized or excreted?

There are some situations where it can be important to know how soon drugs can be detected in urine, like all the acute intoxications, toxicological investigations of drug-related cases, sport doping-cases… In the literature we didn't find any study that was specifically designed to give an answer to this question. But there are urinary excretion profiles of drugs where one can find information about the first urine specimens after single doses of drugs.

METHODOLOGY

The first objective of this literature study was to obtain a general overview of the subject. I started by reading chapters from the book 'Disposition of Toxic Drugs and Chemicals in Man, Fifth Edition' (Baselt (5)). Thereafter I mostly used digital archives such as *Pubmed* and *Web of Science*. Key words in my research were urine, detection, cocaine, heroin, marijuana, amphetamine, detection time, false negatives, GC-MS, IA.

In the next stage I refined my search for the specific clinical question for which an answer must be sought and again I used *Pubmed* and *Web of Science*. Furthermore it was very useful to use the references of the selected articles to find more interesting articles. Important in such research is to realize that the quality of the reviews and articles can vary.

In many occasions an article looked interesting when reading the abstract, but once the article was found, it didn't seem relevant at all for our study-question. Interesting articles were often hidden behind titles that were irrelevant at first sight.

RESULTS

1. INTRODUCTION

1.1. General information

Urine, blood, hair, saliva, sweat and nails are some biological specimens used to perform laboratory drug testing, and they provide different levels of specificity, sensitivity, and accuracy. Urine is most often the preferred matrix because of ease of collection. Concentrations of drugs and metabolites also tend to be high in the urine, allowing longer detection times than concentrations in serum (Schwartz (6)).

Two types of urine drug analytical methods are typically used, immunoassay and gas chromatography-mass spectrometry (GC-MS). Immunoassays, that use uses antibodies to detect the presence of specific drugs or metabolites, are the most common method for the initial screening process. Gas chromatography-mass spectrometry is considered as the standard for confirmatory testing. The method is able to detect small quantities of a substance and confirm the presence of a specific drug (Karasek et al. (7)).

1.2. Immunoassay screening

Urine testing for drugs of abuse generally consists of an initial screening test followed by confirmation by a more specific technology like gas chromatography/mass spectrometry (GC-MS). SAMHSA requires the use of an immunoassay as the initial test method (Aziz et al. (8)).

Several different types of immunoassay are routinely performed in the laboratory. Although they differ in the types of reagents and instrumentation used, they are all based on the same scientific principle, namely the binding of drugs to antibodies. The four types of immunoassay that are commonly used for drug testing are enzyme multiplied immunoassay (EMIT), fluorescence polarization immunoassay (FPIA), kinetic interaction of micro particles in solution (KIMS) and cloned enzyme donor immunoassay (CEDIA). Immunoassay screens are fast, inexpensive and the preferred initial test for urine drug screening. They are sensitive but relative nonspecific. That means that when they give a negative result, no further consideration is needed, but when they give a positive result, it can be false-positive due to cross-reactivity with related chemical compounds. Unexpected positive test results should be confirmed with gas chromatography/mass spectrometry or high-performance liquid chromatography.

1.3. GC-MS as reference method

A GC/MS instrument is the serial combination of a gas chromatograph and a mass spectrometer. It has been widely recognized as the standard instrumentation for the separation and detection of (mixtures of) different organic compounds, because of the possibility to both identify and quantify unknown compounds rapidly. The separation of the chemicals with a gas chromatograph is based on the

difference in their partition coefficient. This coefficient is the ratio of the concentration of the compound in the stationary (liquid or solid) phase and the mobile gas phase. How a compound behaves in the environment of these phases depends on its chemical properties: mass, boiling point, polarity… The partition coefficient determines the retention of the compounds and therefore the elution pattern. After elution, the pure compounds are identified by mass spectroscopy. His technique is based on the fragmentation of the molecule in recognizable fragments (Karasek et al. (7)). For the detection of drugs in urine, GC-MS can be considered as the reference method. It is the most accurate, sensitive and reliable method of testing. However, the test is time-consuming, requires a high level of expertise to perform, and is costly. For these reasons, GC-MS is usually performed after a positive result obtained from immunoassay.

1.4. Cutoff values

Drug testing cutoff levels are the minimum concentrations of drugs or metabolites that must be present in specimens, before labs will report the drug testing results as positive. Substance Abuse and Mental Health Services Administration (SAMHSA) (9) has published his new guidelines for the cutoff values in 2010. Earlier studies described in this thesis, will be interpreted with earlier cutoff values.

1.5. Drugs and their metabolites

1.5.1. Ampethamine, methamphetamine and MDMA

Amphetamine and methamphetamine are derivatives of phenylethylamine. Those are central nervous system stimulant drugs that have limited legitimate pharmacological use (narcolepsy, attention-deficit disorder) (Wigal et al. (10)). 3, 4-methylenedioxymethyl-amphetamine (MDMA or “ecstasy”) is an illegally synthesized derivative of amphetamine.
Methamphetamine is rapidly absorbed by all routes, but time to onset of effects is fastest by intravenous and smoked routes. Acidic urine enhances methamphetamine excretion and shortens its half-life whereas basic urine slows excretion (Beckett et al. (11)). The SAMHSA screening and confirmation cut-offs are 500 ng/ml and 250 ng/ml, respectively (SAMHSA(9)).

Reviews of the pharmacology of MDMA have appeared in the literature (Green et al. (12)). Two metabolic pathways for MDMA in humans have been described (Figure 1). In the major pathway, MDMA is demethylenated to form 3, 4-dihydroxymethamphetamine (HHMA). HHMA, an unstable intermediate, is subsequently converted to 4-hydroxy-3-methoxymethamphetamine (HMMA) and further metabolized to 4-hydroxy-3-methoxyamphetamine (HMA). In the minor pathway, MDMA is *N*-demethylated to 3, 4-methylenedioxyamphetamine (MDA) with further demethylenation to from 3, 4-dihydroxyamphetamine (HHA). Furthermore, HHA forms HMA. These four metabolites, particularly HMMA and HMA, are known to be excreted in the urine as conjugated glucuronide and sulfate metabolites (de la Torre et al. (13)).

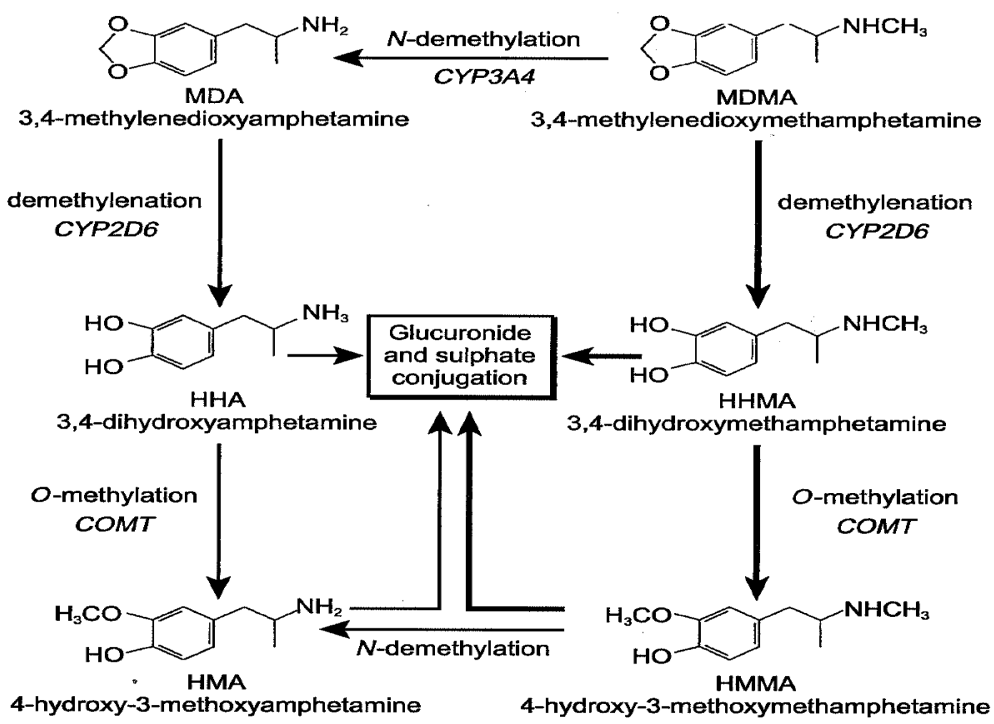

Figure 1: Metabolism of 3.4- methylenedioxymethamphetamine (source: de la Torre et al.(13))

1.5.2. Cannabis

Cannabis, also known as marijuana, refers to any number of preparations of the Cannabis plant, intended for use as a psychoactive drug of for medicinal purposes (Farnsworth (14)). Smoking is the preferred route of administration because of the rapid absorption and distribution of the primary psychoactive component, Δ^9-tetrahydrocannabinol (THC), to the brain (Huestis et al. (15)). The metabolism of THC in humans has been extensively reviewed (Agurell et al. (16)). Initially THC is hydroxylated primarily at the C-11 position, forming the active metabolite, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) (see Figure 2). Oxidation of 11-OH-THC results in the formation of the most abundant, but not psychoactive THC metabolite found in urine, 11-nor-Δ^9-carboxy-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) (Foltz et al. (17), Fraser et al. (18)). Without prior glucuronide hydrolysis, THC and 11-OH-THC concentrations had been shown to be insignificant in urine after smoking (Wall et al. (19)). But the addition of an enzymatic hydrolysis step in the extraction protocol, using bacterial β-glucuronidase (from *E. coli*) demonstrated the presence of significant quantities of THC and 11-OH-THC in urine. (Kemp et al. (20)). Hydroxylation of 11-OH-THC can also lead to the minor metabolite 8, 11-diOH-THC.

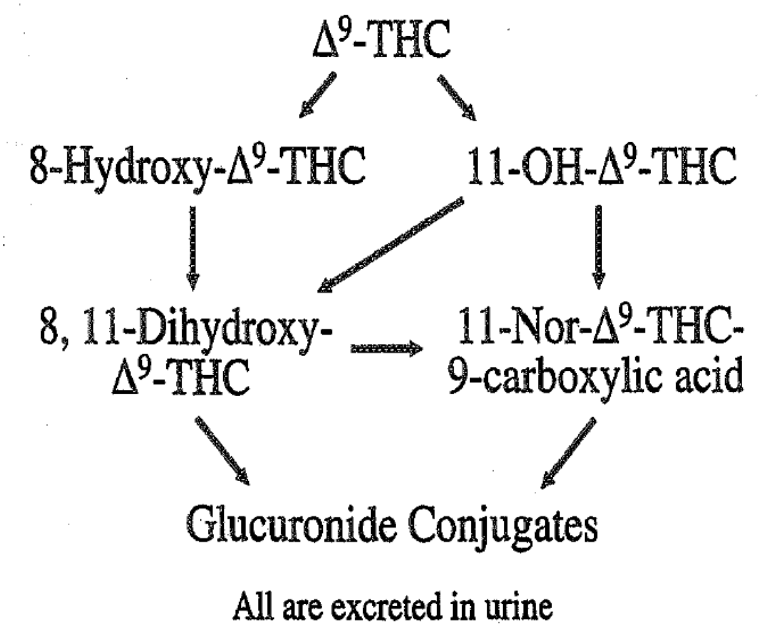

Figure 2: Scheme for biotransformation of THC (Source: Manno et al. (21)).

Urine concentrations of 50 ng/ml THCCOOH or more are considered as positive for screening and a cutoff value of 15 ng/ml is used for confirmation. This cutoff value of 15 ng/ml should avoid positive results due to passive inhalation.

1.5.3. Cocaine

Cocaine is one of the most commonly self-administered illicit drugs of abuse and is ranked as one of 20 most misused substances causing significant physical and social harm to humans (Nutt et al. (22)) It is commonly taken as the hydrochloride by nasal insufflation, intravenous injection or as the free base by smoking, in doses of 10-120 mg (Baselt (5)). The major metabolic routes for cocaine are well-documented (see Figure 3) (Cone et al. (23)). Cocaine is extensively metabolized, primarily in the liver, with only about 1 % excreted unchanged in the urine. The metabolism is dominated by hydrolytic ester cleavage, so the eliminated metabolites consist mostly of benzoylecgonine (BE), the major metabolite, and other significant metabolites in lesser amounts such as ecgonine methylester (EME) and norcocaine (Kolbrich et al. (24)).

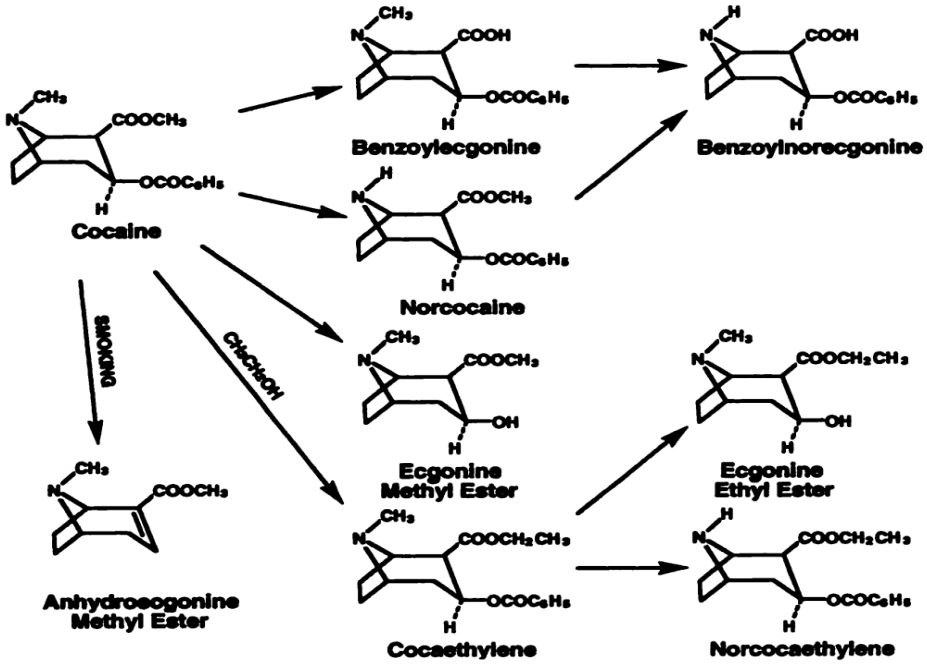

Figure 3: Metabolism of cocaine (source: Cone et al. (23)).

Most immunoassays are targeted against benzoylecgonine that is the most stable of the urinary metabolite of cocaine. An antibody selective for benzoylecgonine, having little cross-reactivity with cocaine and other metabolites, is used. The SAMHSA require 150 ng/ml for screening and 100 ng/ml for confirmation with GC-MS (SAMHSA (9)).

1.5.4. Heroin

It is known that heroin (diacetylmorphine) is rapidly deacetylated to 6-acetylmorphine (6-AM), and that 6-AM is further hydrolyzed to morphine at a slower rate (see Figure 4). Heroin is not pharmacologically active itself but is rather a pro-drug which must be metabolized to morphine for activity (Vandevenne et al. (3)). The typical dose of a beginning user is 10-15 mg, but tolerant users can use up to 2 grams per day. It is commonly taken by intravenous injection or by smoking.

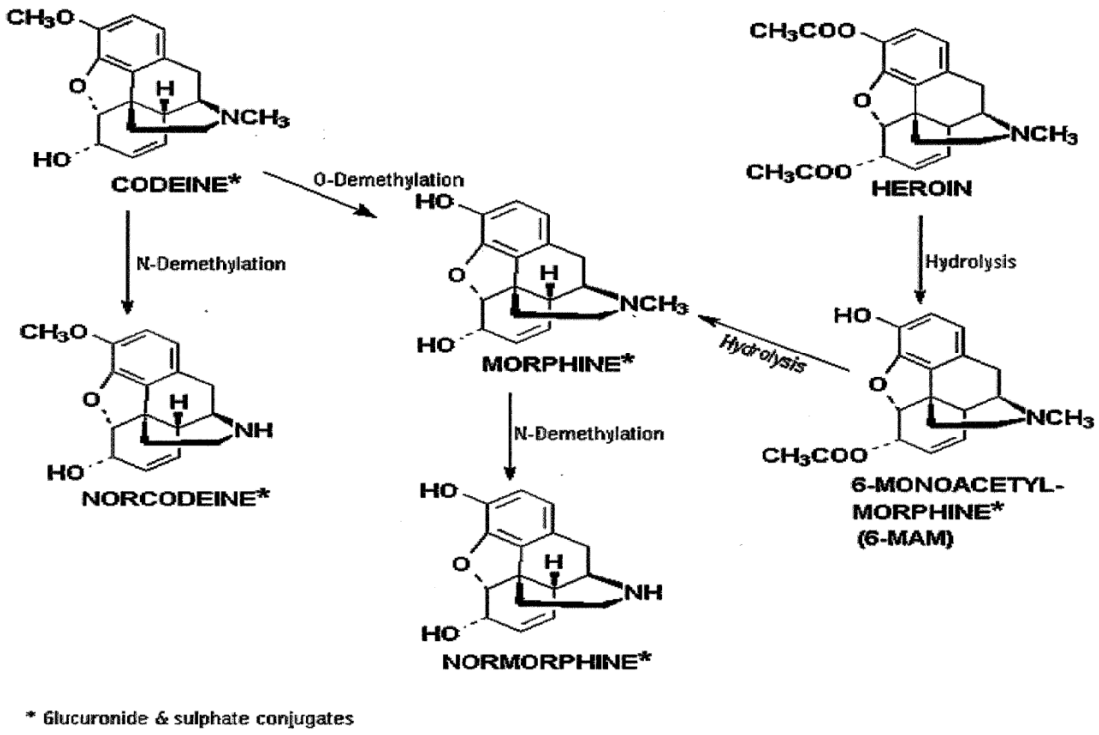

Figure 4: Metabolism of heroin (Source: (25))

Detection of heroin use is one of the major tasks in urine drug testing for opiates. The established way of doing this is by using the predominant urinary metabolite, morphine, as the principal analytical target, both in the screening and confirmation. This may sometimes be complicated, as the presence of morphine in urine is not a unique indicator of heroin intake (Musshoff et al. (26)). The immunoassays – calibrated with morphine - have important cross-reactivity with 6-acetylmorphine and codeine. (Beck et al. (27)). Screening for opiates occurs with a cut-off value of 300 ng/ml, but there is discussion about this value (Vandevenne et al. (3)). The confirmation of 6-AM occurs with a cutoff value of 10 ng/ml.

2. RESULTS AND DISCUSSION

2.1. AMPHETAMINES, METAMPHETAMINES AND MDMA

Few controlled clinical studies exist in which subjects ingest MDMA or (met)ampethamine. Some clinicians (Vollenweider et al. (28)) consider even a single dose as controversial. The few studies that exist, give dextro-methamphetamine, dextro-amphetamine or MDMA ("ecstasy"). Although methamphetamine is not widely used in Europe, yet it is discussed because of a number of interesting articles.

2.1.1. Methamphetamine

Valentine et al. (29) studied the urine excretion profiles after intake of oral dextro-methamphetamine, in a dose of 30 mg/70 kg, what is a relatively large dose. The subjects were ten male volunteers who were divided into two groups of five. One group was given the initial dose of d-methamphetamine at 09.30h, the other group at 21.30h. One week later, each subject was given the same dose but at the other time. Urine specimens were collected prior to the experiment and at libitum during a 12-h period after dosing. Urinary concentrations for methamphetamine and amphetamine were averaged for each subject during successive 2-h intervals. Figure 5 shows the mean methamphetamine concentration for subjects 1-5. This group started with the night dose. The mean peak concentration occurs in the 2-4 h interval. Because of the design of the study (the authors collected all the urine specimens in the first 0-2 interval and averaged these values to one mean value for the interval), we don't know the exact values of the first specimens. The only conclusion we can make is that the mean of the specimens in the 0-2h interval (1300 ng/ml) is apparently above the SAMHSA cut-off value of 500 ng/ml for immunoassay. The value is more than two times the cut-off value. In the 0-2 h interval, two subjects did not micturate. A week later subjects 1-5 received the day dose at 9.30 h. Figure 5 shows that the mean methamphetamine concentrations in the specimens prior to the experiment were not zero, but reached already the 500 ng/ml–value. Therefore for our study, these values are not applicable.

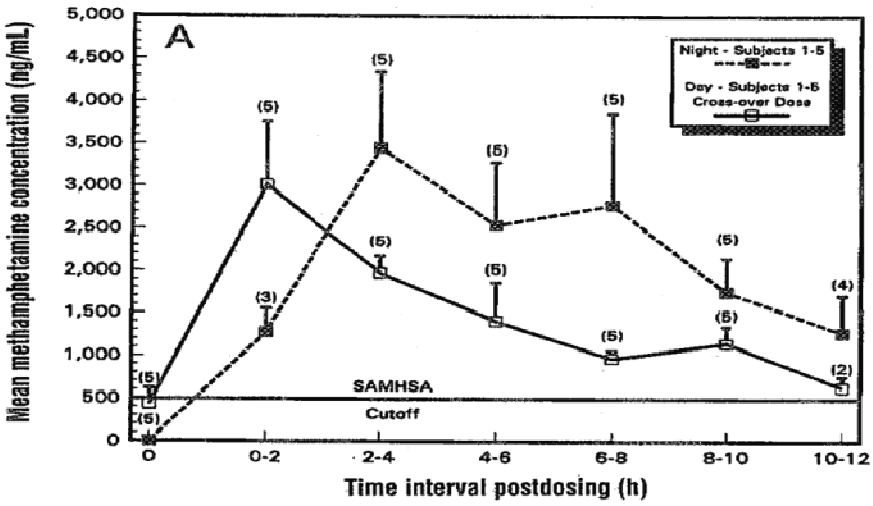

Figure 5: Mean plus or minus standard error of the mean for methamphetamine concentration in 2-h intervals for subjects 1-5 during the 12-h period following administration of 30 mg/70 kg d-methamphetamine (source: Valentine et al.(29)).

Subjects 6-10 received first the day-dose. The mean value of the specimens in the 0-2 h interval is 1500 ng/ml (3 times the cut-off value). A week later, after the night dose, the mean of the specimens prior to the experiment were negative. The mean value of the specimens in the 0-2h interval was 1600 ng/ml after the night dose, again far above the cut-off value of 500 ng/ml. Metamphetamine concentrations were consistently greater than the 500 ng/ml cutoff in all post-dosing specimens, whereas amphetamine concentrations generally did not achieve the 200-ng/ml cutoff specified by SAMHSA. The mean value of the specimens in the 0-2 h interval was about 80 ng/ml amphetamine for both night and day doses. In the following intervals (2-4 h, 4-6 h and 6-8 h), the concentration of amphetamine was higher (mean about 160 ng/ml) but did not reach the cut-off value of 200 ng/ml.

Huestis et al. (30) published a controlled dosing study involving 5 drug-free volunteers. Subjects received 10 mg and 20 mg oral doses of d-methamphetamine. All urine specimens were collected ad libitum. The results are summarized in table 1.

Table 1: Time to detection of the first positive specimen of methamphetamine and amphetamine in urine at different cut-off concentrations following a single dose of 10 and 20 mg of methamphetamine administered to five subjects (Meth = methamphetamine; Amp = amphetamine)

Meth dose (mg)	Analyte	N	cut-off (ng/ml)	Time to first positive (range) (h)
10	Meth	5	500	5.5 (1.4-11.3)
	Amp	5	200	14.5 (9.3-19.6)
20	Meth	5	500	4.3 (1.2-8.8)
	Amp	5	200	14.2 (7.9-19.6)
10	Meth	5	250	5.5 (1.4-11.3)
	Amp	5	100	9.9 (4.2-17.2)
20	Meth	5	250	3.9 (1.2-8.8)
	Amp	5	100	9.1 (3.3-13.1)

Immunoassay screening usually detects methamphetamine at a cut-off value of 500 ng/ml. The time to the first positive for amphetamine is much longer than for methamphetamine. Methamphetamine was first detected in urine at the cutoff value at mean times of 5.5 h and 4.3 h, respectively for the 10 mg and the 20 mg doses. The great range should be noticed. Since the urine specimens were collected ad libitum, the mean values were strongly influenced. Persons who drink little, will deliver a specimen later, that might be highly positive, while an early mandatory specimen may also have been positive. In the workplace, the SAMHSA guidelines require that methamphetamine be accompanied by amphetamine at 200 ng/ml for a "positive" methamphetamine specimen. If these guidelines are to be followed, the time to first positive is much longer. After both the 10 mg dose and the 20 mg dose at the cut-off value of 200 ng/ml, amphetamine is positive after about 14 h. So, according to these guidelines, more urine specimens will be falsely negative. This is illustrated in figure 6.

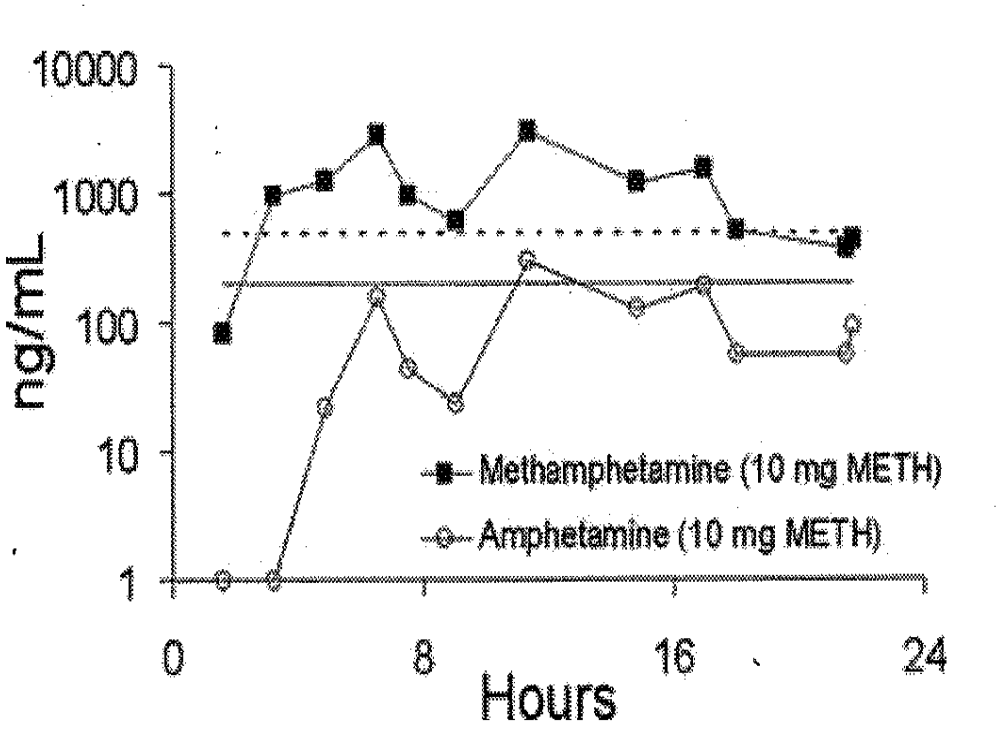

Figure 6: Methamphetamine (METH) and amphetamine excretion in urine of one subject following administration of a single dose (first day) of 10 mg methamphetamine. The dotted line illustrates the 500 ng/ml confirmation cutoff concentration for methamphetamine and the solid line illustrates the 200 ng/ml confirmation cutoff concentration for amphetamine (Source: Huestis et al. (30)).

In this figure the methamphetamine and amphetamine excretion in urine of one subject is shown. All the specimens in the first hours (0-10 h) would be negative with the SAMHSA guidelines. A critique is that the dose (10 mg) was very low and not representative for the current doses in practice. Valentine et al. (29) used already a higher dose (30 mg/70 kg d-methamphetamine), but they also found only values of 160 ng/ml amphetamine in the first specimens (0-6 h). They also had only a few values around the cut-off value of 200 ng/ml and this in the 4-12 h interval.
Striking is that when lowering the cut-off concentration of 500 ng/ml to 250 ng/ml (Table 1), the time

to first positive didn't change at all with the 10 mg dose and changed only a little with the 20 mg dose. Regardless of the dose we can say that there is a real risk that sampling too early will lead to false-negatives. The fact that the time to first positive changed little when lowering the cut-off value for methamphetamine (MAMP), was also found by Oyler et al (31). They studied the urinary excretion of methamphetamine and amphetamine after single doses of oral 10 mg MAMP.HCl. Their values are summarized in Table 2.

Table 2: Initial MAMP detection times and initial detection voids at different cutoff concentrations after 10 mg MAMP.HCl (n = 5)

	Initial detection time (h)		Initial detection void	
Cut-off value	Range	Mean ± SD	Range	Mean
500/200	4.2-12.0	8.3 ± 3.3	1-7	4.8
500	1.4-4.3	3.0 ± 1.3	1-2	1.8
250/100	0.5-4.3	3.0 ± 1.7	1-4	1.8
250	0.5-4.2	2.0 ± 1.4	1-2	1.2
LOQ	0.7-4.2	2.8 ± 1.6	1-2	1.4

With the 2.5 ng/ml LOQ, initial detection of MAMP generally occurred in the first urine void. Two of the five participants had an initial detection of MAMP in the second void, but their first voids were collected < 0.5 h after drug administration. These voids were too soon to get a positive specimen, even with a cut-off value/LOQ of 2.5 ng/ml. Based on a 500/200 ng/ml cut-off value, the initial detection time was much longer (mean 8.3 ± 3.3 ng/ml). Lack of AMP detection was responsible for most negative results early in the excretion of MAMP. Reducing the cutoff from 500/200 to 250/100 shifts initial confirmation by up to three voids, which corresponds with 5 hours. Lowering the AMP cutoff value was responsible for earlier MAMP detection in all the cases. That means that MAMP reached very quickly a level above 500 ng/ml. Elimination of the 100 ng/ml AMP cutoff value shifts initial MAMP detection to the left by a mean of 1 hour or about a half void.
This study (Table 2) confirms are our previous propositions that the mean time of initial detection of metamphetamine is not really influenced by the cut-off value of methamphetamine. With a cut-off value of 500 ng/ml, the mean time to the first positive is about 3 hours, while it is 2 hours with a 250 ng/ml cut-off value. Lowering the cut-off value to 250 ng/ml changed the initial detection time very little.

Cook et al. (32) studied the urinary excretion kinetics of MAMP after single smoked and intravenous doses in six male volunteers who were regular users. All contracted to avoid use of prescription drugs while participating. The volunteers inhaled an average dose of 21.8 ± 0.3 mg of S-(+)-methamphetamine hydrochloride, and were given an intravenous injection of 15.5 mg of S-(+)-methamphetamine hydrochloride. The authors concluded an equivalent renal clearance for the two

routes. They propose a hypothesis that the fraction of methamphetamine excreted in urine decreases with increasing dose and the amount of methamphetamine in urine is not proportional to the dose of the absorbed drugs.

2.1.2. Ampethamine

Poklis et al.(33) studied the urinary excretion in 7 volunteers following administration of 5-, 10-, and 20 mg oral doses of dextro-amphetamine. Each volunteer received one of four treatments: an oral dose of 5 mg, 10mg, 20 mg of d-amphetamine or a placebo, in a double-blind manner. The study was divided into four periods, so each subject received each dose once. Urine specimens were taken prior to dosing, at 2 h post-dose and at 4 h post-dose. Unfortunately, the urine concentrations of all the subjects were not provided. After the 5 mg dose, three of the seven subjects had their peak concentration of d-amphetamine (620 ng/ml, 3120 ng/ml and 3160 ng/ml) in the first urine (2 h). With a cut-off value of 500 ng/ml for immunoassay and 200 ng/ml for GC-MS, all these specimens were positive. The four other subjects had their peak concentration at 4 h or 8 h post dose (mean peak concentration of 1137 ng/ml). Unfortunately, no data of the first urine was provided. After the 10 mg and the 20 mg dose, two subjects had their peak concentration in the 2h urine with values of 2390 ng/ml and 2980 ng/ml (after the 10 mg dose) and values of 1510 ng/ml and 3240 ng/ml (after the 20 mg dose), far above the cut-off values.

Poklis et al.(33) mention another important aspect of amphetamine detection. The urinary pH is the single most important factor in amphetamine excretion. Amphetamine excretion increases with increasing urine flow and decreasing urine pH. When urine is acidified the excretion of unchanged amphetamine is approximately four times that of the metabolites (Beckett et al.(11)). Since we are interested in how soon we can detect drugs in urine, we are focused on the parent drug. Thus we can say that the more acidic the urine, the faster the drug can be detected. Foods ingested can change the acidity or alkalinity of the urine. The highest acid loads originated in cheese, following by meat, fish and cereal products, whereas more vegetarian food is more neutral of alkaline (Remer et al. (34)).

2.1.3. MDMA

Very few controlled MDMA studies have been performed with doses commonly ingested by young adults. Helmlin et al. (35) collected urine samples from two patients in a controlled clinical study. After administration of a single oral dose of 1.5 mg MDMA/kg body weight, urine samples were collected for up to 20 h. The first urine samples from subject A were collected at 0, 1.5, 3.5 and 5.5 h after the administration of MDMA and the first urine samples from subject B were collected at 0, 1, 1.25, 1.5, 3.75, 4.2 and 5 h after administration of MDMA. Figure 7 represents the urine excretion profile of subjects A and B.

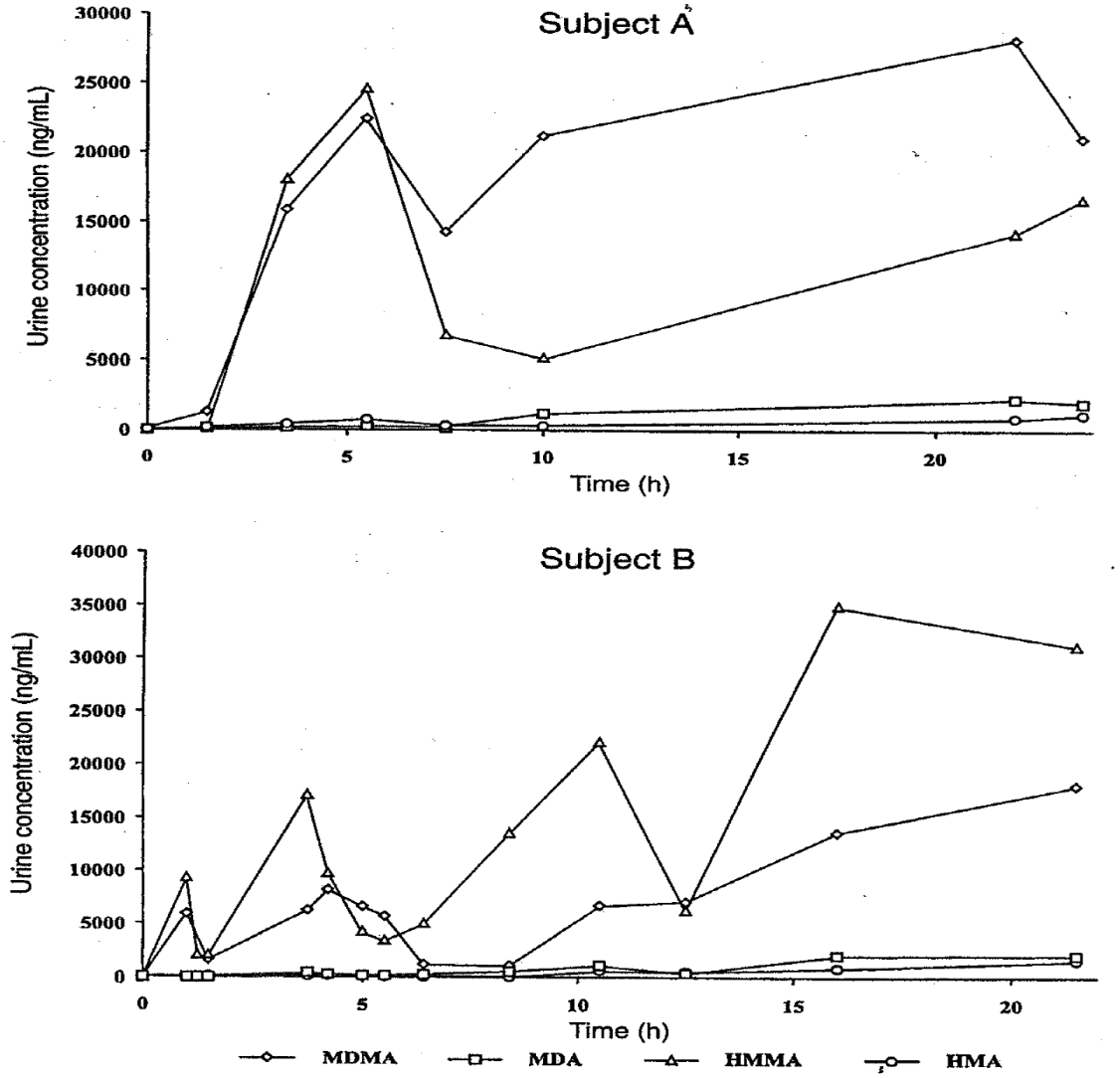

Figure 7: Urine profiles of subjects A and B after an oral dose of 1.5 mg/kg 3.4-methylenedioxymethamphetamine (MDMA) (Source: Helmlin et al. (35))

These urine data reflect the interindividual differences in the excretion pattern. The urine specimen at 1.5 h of subject A had a positive value of MDMA (> 1000 ng/ml), but the other metabolites (HMMA, MDA and HMA) were still negative. Since the concentration of MDMA is above 500 ng/ml, the urine specimen will be positive, both with immunoassay and GC-MS as confirmation. The next urine specimen at 3.5 h was highly positive for MDMA and HMMA, the major urinary metabolite of MDMA. The HMMA peak concentration may even exceed that of the parent compound MDMA (Subject B). The urine specimen at 1 h of subject B was already positive for both MDMA (6000 ng/ml) and HMMA (9000 ng/ml), clearly positive with GC-MS. The specimens at 1.25 and 1.5 h showed a relapse, but the concentrations of MDMA and HMMA stayed above the cut-off value of 200 ng/ml for confirmation and 500 ng/ml for immunoassay. Considering the high values at 1.0 h with subject B, we can predict that earlier voids would be also positive.

Abraham et al. (36) studied 16 healthy adults after administration of placebo, 1.0 mg/kg and 1.6 mg/kg oral MDMA doses in a double-blind, randomized and controlled manner. All urine was collected and quantified by GC-MS. Active drug was prepared as the hydrochloride salt; placebo contained only lactose. Each urine specimen was collected ad libitum. Concentration maxima (Cmax) and times of maximum concentration (Tmax) were determined for each specimen after the low and the high doses. The time to first positive is with a cut-off value of 25 ng/ml (LOQ). The results are summarized in Table 3.

Table 3: Median of Urine concentration maximum, Time of Urine Concentration and Time to first positive of MDMA, HMMA, MDA, and HMA after Low (1.0 mg/kg) and High (1.6 mg/kg) oral administration of MDMA.

	1 mg/kg (n=16)	1.6 mg/kg (n=16)
Cmax (MDMA) (ng/ml)	12376 (5438-31777)	21470 (12292-48947)
Cmax (HMMA) (ng/ml)	13633 (5698-46876)	20793 (7399-36492)
Cmax (MDA) (ng/ml)	1116 (454-3256)	2229 (1064-5135)
Cmax (HMA) (ng/ml)	784 (270-1716)	876 (465-3217)
Tmax (MDMA) (h)	12.3 (5.5-22.9)	13.9 (3.3-30.4)
Tmax (HMMA) (h)	9.9 (2.1-23.3)	9.2 (3.3-30.4)
Tmax (MDA) (h)	13.5 (7.0-26.8)	23 (3.3-30.4)
Tmax (HMA) (h)	19.8 (8.3-23.8)	23.3 (11.3-30.4)
Time to first positive (MDMA) (h)	1.34 (0.83-3.33)	1.17 (0.42-1.65)
Time to first positive (HMMA) (h)	1.34 (0.83-3.33)	1.17 (0.42-1.65)
Time to first positive (MDA) (h)	2.17 (0.83-3.33)	1.63 (0.92-4.50)
Time to first positive (HMA) (h)	2.17 (0.97-3.33)	3.08 (1.12-5.32)

Urinary excretion patterns over time showed substantial intersubject variability. Generally, HMMA exceeded MDMA concentrations. There appeared to be a direct correlation between dosing and median MDMA Cmax, with high dose Cmax increasing 1.7-fold over low dose Cmax. This is comparable to the 1.6-fold increase in MDMA dose between the low and the high doses. Intersubject Tmax are highly variable for all analytes with ranges as wide as 3.3-30.4 h after a single MDMA dose. Median time to first positive of MDMA occurred first at approximately 1.3 h with similar median times for the low and the high doses. A lower range is found with the high dose.

2.2. CANNABIS

Limited urinary excretion data from controlled studies with marijuana use are available. Huestis et al. (37) studied six healthy men, all with a history of earlier cannabis-use. The subjects all started smoking cannabis when they were 13 to 16 years old and that with a mean quantity of 2.3 cigarettes a week. Before starting the experiment, negative urine was needed at least for 5 consecutive days (immunoassay Syva EMIT, 100 ng/ml). The experiment was performed in a double blind manner during three weeks. Each week the subjects smoked one cigarette. Each cigarette had a different dose: a placebo, 15.8 mg THC (normal dose of a joint) and 33.8 mg THC (high dose of a joint). To reduce the variability between the different subjects, the number of cigarettes-puffs, the inhalation time and the time between the puffs, was controlled. The depth of inhalation wasn't checked in this study. The urine was collected ad libitum. The first three hours, the subjects were encouraged to keep up the urine. Finally, all urine specimens were analyzed for THCCOOH by GC-MS (cut-off 15 ng/ml). The results are summarized in Table 4.

Table 4: Mean THCCOOH concentration in the first urine, mean time to peak and mean peak THCCOOH concentration after low (15.8 mg THC) and high (33.8 mg THC) smoking dose of cannabis

Dose	THCCOOH conc (first urine) (ng/ml)	Tmax (THCCOOH) (h)	Cmax (THCCOOH) (ng/ml)
15.8 mg THC	47 ±22.3 (5.5-138.4)	7.7 ± 0.8 (6.0-11.3)	89.8 ± 31.9 (20.6-234.2)
33.8 mg THC	75.3 ±48.9 (9.1-318.0)	13.9 ± 3.5 (5.6-28.0)	153.4 ± 49.2 (29.9-355.2)

With the low dose cigarette (15.8 mg THC), three of the six subjects had a first positive urine (GC-MS > 15 ng/ml). With the high dose (33.8 mg), five of the six subjects had a positive first urine (GC-MS > 15 ng/ml). The time of the first urine specimen was 3.7 ± 0.6 h (low dose) and 3.0 ± 0.5 h (high dose), which is similar. As a consequence, the THCCOOH-concentration in the first urine depended on the dose of the marihuana-cigarette. The higher the dose, the higher the concentration of THCCOOH and the chance that the first urine will be positive. After the low dose (15.8 mg THC) and the high dose (33.8 mg THC), the mean time to first positive was 4.9 ± 1.8 (3.2-8) h and 3.4 ± 0.6 (2.3-4) h, respectively.

Except for one subject, the THCCOOH concentration was dose-dependent: the mean THCCOOH concentration in the first urine was 47 ± 22.3 ng/ml and 75.3 ± 48.9 ng/ml for the low and the high dose, respectively. None of the first urine specimens (low dose and high dose) contained the peak concentration of THCCOOH. The maximum concentration came later: after 7.7 +0.8 h (low dose) and after 13.9 ± 3.5 h (high dose) and it was also dose-dependent. Although the mean peak concentration seemed to be dose dependent (89.8 ± 31.9 ng/ml (low dose) and 153.4 ± 49.2 ng/ml (high dose)), there was a 12-fold variation between the subjects: peak concentrations ranged from 20.6 to 234.2 ng/ml for the low dose and from 29.9 to 355.2 ng/ml for the high dose. The same big variation could be seen in the THCCOOH concentration in the first urine specimen: from 5.5 to 138.4 ng/ml for the low dose and from 9.1 to 318.0 ng/ml for the high dose, implying a 25-fold variation for the low dose and a 35-fold

variation for the high dose. Given the very high inter-individual variation, differences in drug metabolism and excretion appeared to play a role.

Surely, in this study, the differences in the way of smoking were kept to a minimum. There were three first urines that were negative with GC-MS after the low dose and one negative urine after the high dose. In Table 5 the results of the negative urine specimens are given.

Table 5: Negative first urine specimens with the GC-MS value (ng/ml) and time of the first urine (h) after the low (15.8 mg THC) and high (33.8 mg THC) dose.

Low dose		High dose	
Time (first urine) (h)	THCCOOH (first urine) (ng/ml)	Time (first urine) (h)	THCCOOH (first urine) (ng/ml)
2.2	5.5	1	9.1
3.5	11.3		
3.3	10		

From this table it appears that sampling too early can lead to false negative results. After both the doses, the second urines were all positive with GC-MS. The mean time of the second urine was 6.7 $\pm$ 2.3 h for the low dose and 5.6 $\pm$ 2.0 h for the high dose. The same large differences in peak concentration and in first urine concentration of THCCOOH were also found by Mc Burney et al. (38) who performed a similar study. Ten young men with a history of occasional marihuana use stopped smoking a week before the study. During the experiment they smoked 2 cigarettes in 30 minutes, with a balanced time distribution between the two cigarettes. These two cigarettes together contained 150 µg THC/kg body weight. If calculated with an average weight of 75 kilo for a young man, this corresponds to 11 mg THC, which is a normal but low dose for a marihuana cigarette. The urine specimens were taken ad libitum, whereupon the urine was evaluated with GC-MS (cutoff: 15ng/ml). The urine of one subject contained 9.9 ng/ml THCCOOH before the experiment started and therefore these results were cancelled. The mean time of the first urine was 2.6 h and the mean THCCOOH-concentration was 17.2 ng/ml (range: 0.5-53.8 ng/ml). The mean peak concentration was 38.6 ng/ml (range: 5.8-128 ng/ml), and came after a mean time of 6.8h (2.6-13.9h). A 20-fold variation between the THCCOOH peak concentrations was observed. This variation is higher than what Huestis et al. (37) observed and this could be the result of the fact that the study of McBurney et al. (38) had no indications on the way the cigarette was smoked. The THCCOOH-concentration of the first urine sample had a 100-fold variation compared to the 25- and 35-fold variation found by Huestis et al. (37). Only three of the nine subjects tested positive (>15 ng/ml) with their first urine sample, and only one of the nine subjects had a peak concentration of THCCOOH in the first urine sample. Considering the high variation between the subjects regarding the THCCOOH concentration and regarding the high variation between the peak concentrations of THCCOOH, sampling too soon can lead to concentrations below the cut-off-value. Therefore it would be desirable to find one or more early-

occurring metabolites whose presence in urine would indicate very recent drug use. Different studies looked into other metabolites that might serve as evidence for recent cannabis use.
McBurney et al. (38) studied 8, 11-diOH-THC as metabolite. Figure 8 shows the mean urinary concentrations of THCCOOH and 8, 11-diOH-THC by GC-MS and by immunoassay (EMIT) after smoking marihuana in a dose of 150 µg THC/kg body mass.

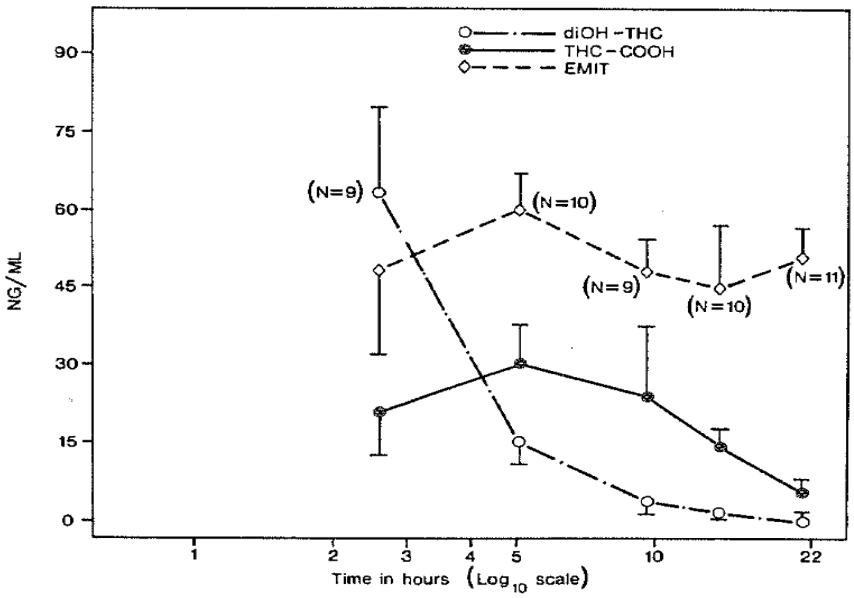

Figure 8: Mean urinary levels of THCCOOH and 8, 11-diOH-THC by GC-MS and EMIT, after smoking 150 µg THC/kg body mass (Source: McBurney et al. (38))

In the first hours, the metabolite 8, 11-diOH-THC has a higher concentration in comparison with THCCOOH. In the next urine specimens there is a fast decrease of 8,11-diOH-THC. After 22 hours, the concentration of this metabolite for all subjects-except one-is below the detection limit. Nine of the ten subjects had the highest concentration of 8, 11-diOH-THC in their first urine sample (mean time of first urine is 2.6 h). After- estimated- 4 hours, THCCOOH has the highest concentration. In one of the ten cases the urine contained no 8,11-diOH-THC, nor in the first urine, nor later. Given the low creatinine value (< 33 mg/dl) and the low THCCOOH-value (the peak-concentration was 5.8 ng/ml), this value was the most dilute. The mean time to first urine came after 2.6 h and have a mean 8,11-diOH-THC-concentration of 62.8 ng/ml (range: 19.8-102.7 ng/ml). Although this was a 5-fold range, the range was much smaller than the THCCOOH-concentrations in the first urine-range, found by McBurney et al. (38) and Huestis et al. (37). The mean peak concentration of 8.11-diOH-THC was 51.6 ng/ml (range: 16.1-102.7 ng/ml) and had a mean time of 3.3 h (nine times at 2.6 h and one time at 9.4 h). Mc Burney et al. (38) suggested 8.11 –diOH-THC as possible indicator for recent marihuana use and suggested 15 to 20 ng/ml as cutoff value. With 20 ng/ml as cutoff, all values -except two- were negative after 4-6 h. McBurney et al. (38) state that, if this metabolite is found, this is an indication for marihuana use in the last 4-6 hours.

Manno et al. (21) also studied 8, 11-diOH-THC as a marker for recent cannabis use. The authors studied eight young subjects, four men and four women, all casual users (1-3 cigarettes a week or less). The subjects smoked one cigarette a week, in two doses: 17.7 mg THC (normal dose) and 35.8 mg THC (high dose). A urine specimen was analyzed before the experiment started, five minutes after use, 60 minutes after use and then every hour for up to eight hours after use. After smoking the 17.7 mg THC-cigarette, no subject tested positive for 8,11-diOH-THC, neither shortly after use nor after hours (detection limit GC-MS: 0.9 ng/ml). After smoking the 35.8 mg THC-cigarette, only one person tested positive for 8,11 –diOH-THC. Later, this person admitted to be a frequent user. Like in the study of McBurney et al. (38) the peak concentration of 8,11-diOH-THC in this subject was found in the first urine (2h after use). Since 8,11-diOH-THC wasn't found in the urine (except for one frequent user), Manno et al. (21) concluded that 8, 11-diOH-THC can't be used as an indicator for recent cannabis use, which is the opposite of McBurney et al. (38).

Looking to 11-OH-THC and THC, two studies were performed (Manno et al. (21) , Brenneisen et al. (39)). Both authors used bacterial β-glucuronidase (from *E. coli*) in the sample preparation protocol. Manno et al. (21) studied the urine concentration of THC and 11-OH-THC. Their subjects smoked 17.7 mg THC and 35.8 mg THC. One of the eight subjects had already positive results prior to smoking and his results were cancelled. In figure 9, the urinary concentrations of THC, THCCOOH and 11-OH-THC are illustrated. For both the low dose and the high dose, the mean time to peak of THC was observed after 120 min. This mean peak THC concentration was 9.1 ng/ml (range: 1.5-21.5 ng/ml) for the low dose and 21.5 ng/ml (range: 3.2-53.3 ng/ml) for the high dose. The first urine specimen was taken after five minutes. In three of the seven cases this first specimen was already positive for THC (LOD 1.5 ng/ml) after using the low dose and in six of the eight cases after using the high dose. After the peak concentration, there was a fast decline. Urinary THC concentrations were above the LOD (1.5 ng/ml) after five hours for the low dose, and after seven hours for the high dose.

The experiment of Manno et al. (21) took only eight hours and in the last hours one can see clearly that values go to zero. With an LOD of 2 ng/ml, the values dropped below the threshold value after five hours, both for the low dose and the high dose. Therefore, when concentrations of THC above 2 ng/ml were found, one can estimate that marihuana was used less than five hours before sampling. This high interindividual variation was due to the big differences in the way of smoking and the differences in metabolism of marihuana. For 11-OH-THC, mean peak concentrations were achieved at 180 minutes, for both the low and the high dose. Peak concentrations ranged from 14.0 to 79.3 ng/ml with a mean of 48.7 ± 11.5 ng/ml for the low dose. For the high dose, the peak concentrations ranged from 27.4 to 169 ng/ml with a mean of 77.3 ± 29.7 ng/ml. Since the study took only eight hours it is impossible to say when this metabolite drops under the LOD. The data after 8 hours proved that the values drop slowly, but don't tend to approach zero. After the low dose the first urine (five minutes post use) was positive in five of the eight cases for 11-OH-THC en in seven of the eight cases for the high dose. THCCOOH had the highest intersubject variability (see figure 9). Peak THCCOOH-concentrations of 94.0 ± 62.7 ng/ml and 179.4 ± 146.9 ng/ml were detected 240 min after the low and the high dose, respectively. Thus, Manno et al. (21) obtained for the high dose cigarette a THC peak of 21.5 (range 3.2-53.3) ng/ml two hours after smoking, a 11-OH-THC peak of 77.3 ± 29.7 ng/ml after three hours and a THCCOOH peak of 179.4 ± 146.9 ng/ml after four hours. For our question, these data suggest that THC may be a biologic marker for the identification of recent marihuana use.

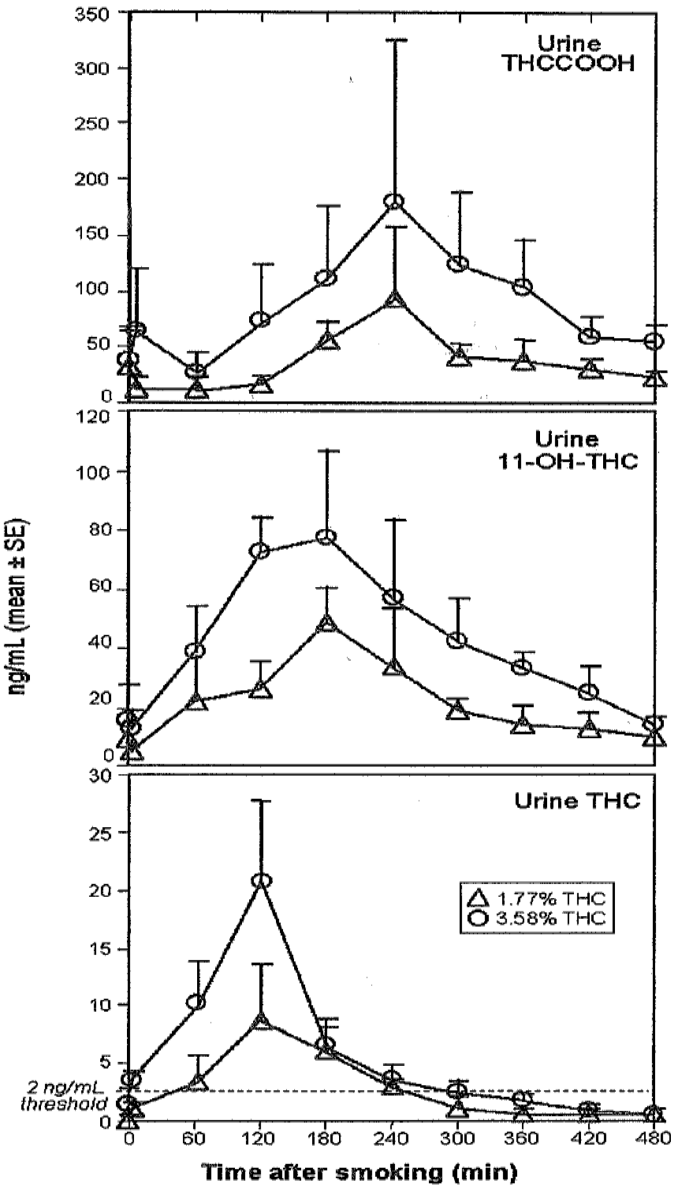

Figure 9: Urine concentrations of THC, 11-OH-THC and THCCOOH (mean ± SEM) after smoking marihuana (Source: Manno et al. (21))

Brenneisen et al.(39) studied twelve men who occasionally smoke cannabis (less than once a month). A negative urine test for cannabis was required before starting. They all smoked a standard cannabis cigarette of 70 mg THC. They used a standardized smoke-and inhalation procedure. After smoking, the residual THC content was measured, therefore the mean THC-content became 45 ± 7 mg and the estimated lung dose was 25 mg. Urine was sampled 2, 4, 6 and 8 h after administration. THC, 11-OH-THC and THCCOOH were measured with GC-MS. The results are summarized in table 6.

Table 6: Urine levels of THC, 11-OH-THC and THCCOOH (Mean + SEM) after smoking a standardized 70 mg THC cigarette

Metabolite/Time	2 h	4 h	6 h	8 h	12 h
THC	0.7 ± 0.3 ng/ml	0.3 ± 0.2 ng/ml	0.1 ± 0.2 ng/ml	0	0
11-OH-THC	6.7 ± 4.2 ng/ml	4.2 ± 2.5 ng/ml	4.2 ± 3.3 ng/ml	2.5 ± 1.6 ng/ml	1.4 ± 1.6 ng/ml
THCCOOH	2.5 ± 1.4 ng/ml	6.6 ± 3.9 ng/ml	13.4 ± 9.2 ng/ml	10.6 ± 9.8 ng/ml	8.1 ± 5.8 ng/ml

The peak THC concentration came in the urine at 2 h in eleven of the twelve cases, which was also found by Manno et al. (21). In one subject, the peak concentration was measured in the 4 h urine. The mean concentration in the 2-h urine void was 0.7 ± 0.3 ng/ml (range: 0.2-1.3 ng/ml). Except for one, the THC-value was under the LOD after eight hours.

For 11-OH-THC, the peak concentration was observed in the first urine (2 h) in nine of the twelve cases, in the second urine (4h) in one case and in the third urine (6h) in two cases. The mean concentration in the 2-h urine void was 6.7 ± 4.2 ng/ml (range: 2.2-14.4 ng/ml). Except in one case, the 11-OH-THC dropped below the LOD after 12 h to 72 h.

The peak concentration of THCCOOH came usually after 6-8 h. No first urine sample contained a peak concentration of THCCOOH. The mean concentration of THCCOOH in the first urine (2 h) was 2.5 ± 1.4 ng/ml, far below the cut-off value of 15 ng/ml. In the second urine specimens (4h), the mean concentration was 6.6 ± 3.9 ng/ml, also far below the cut-off value. Every first urine sample (2h) and every second urine sample (4h) was negative for THCCOOH. At the third urine sample (6h) four of twelve subjects tested positive for THCCOOH.

2.3. COCAINE

2.3.1. Intravenous cocaine

Cone et al. (40) studied the urinary excretion profiles of 6 healthy men, after use of IV 25 mg cocaine HCl, that can be considered as a low starting dose. The urine specimens were measured by GC-MS and were collected ad libitum. In figure 11, the excretion profiles of cocaine (COC), benzoylecgonine (BZE) and ecgonine methylester (EME) are illustrated. A summary of these graphs can be found in table 7. Mean maximum concentrations generally followed the following order: BZE > EME > COC. Logically, the peak of COC came first, followed by the peak of EME and finally the peak of BZE.

Table 7: Time to peak (h), maximum concentration (ng/ml), time to first positive (h) and concentration of first positive (ng/ml) of COC, BZE and EME after 25 mg iv cocaine HCl.

	COC (range)	BZE (range)	EME (range)
Time to peak (h)	3.9 ± 0.9 (1.2-8.0)	5.6 ± 1.4 (1.8-11.2)	4.1 ± 0.6 (2.8-8.0)
Maximum conc. (ng/ml)	775 ± 297 (100-2078)	15611 ± 3095 (7693-25127)	4968 ± 1118 (2786-8663)
Time to first positive (h)	2.6 ± 1.3 (0.4-4.3)	2.6 ± 1.3 (0.4-4.3)	2.6 ± 1.3 (0.4-4.3)
Conc. of first positive (ng/ml)	750 ± 678 (97-2078)	10473 ± 7637 (1741-23061)	3939 ± 3283 (828-8663)

In two of the six subjects the first urine was voided after 1.2 h. At that time BZE (GC-MS) concentrations of 7520 ng/ml and 1741 ng/ml were found, 50 and 11 times the cut-off value of 150 ng/ml. On the other hand, two subjects had their first urine at 0.5 h and at 0.4 h, and in both cases no metabolites were found with GC-MS. BZE appeared clearly very fast after administration of intravenous cocaine. In figure 10, a detail of the urinary concentration of the first three hours is seen.

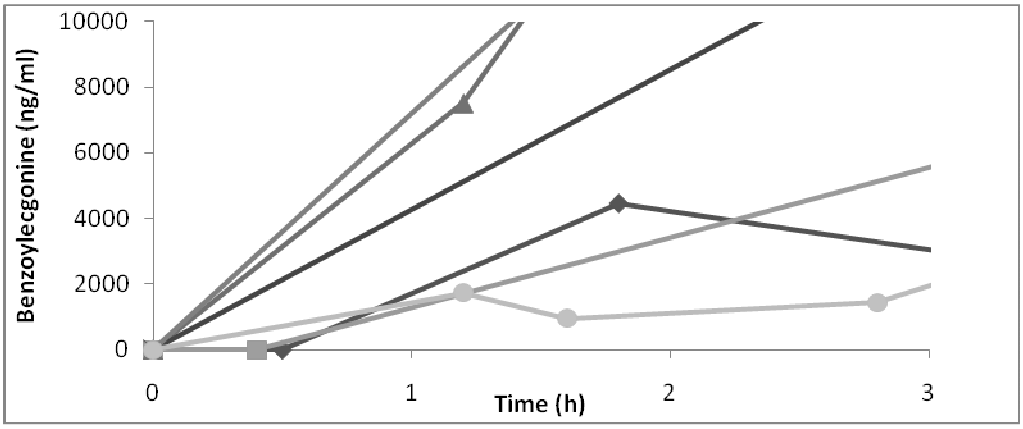

Figure 10: Urinary excretion of benzoylecgonine in the first three hours after intake of 25 mg IV cocaine HCl (Based on data from:Cone et al. (40)).

In this figure, the rate at which the values were getting positive, is clearly seen. Cone et al. (40) found false-negative values when the urine sampling happened 30 min after use or earlier.

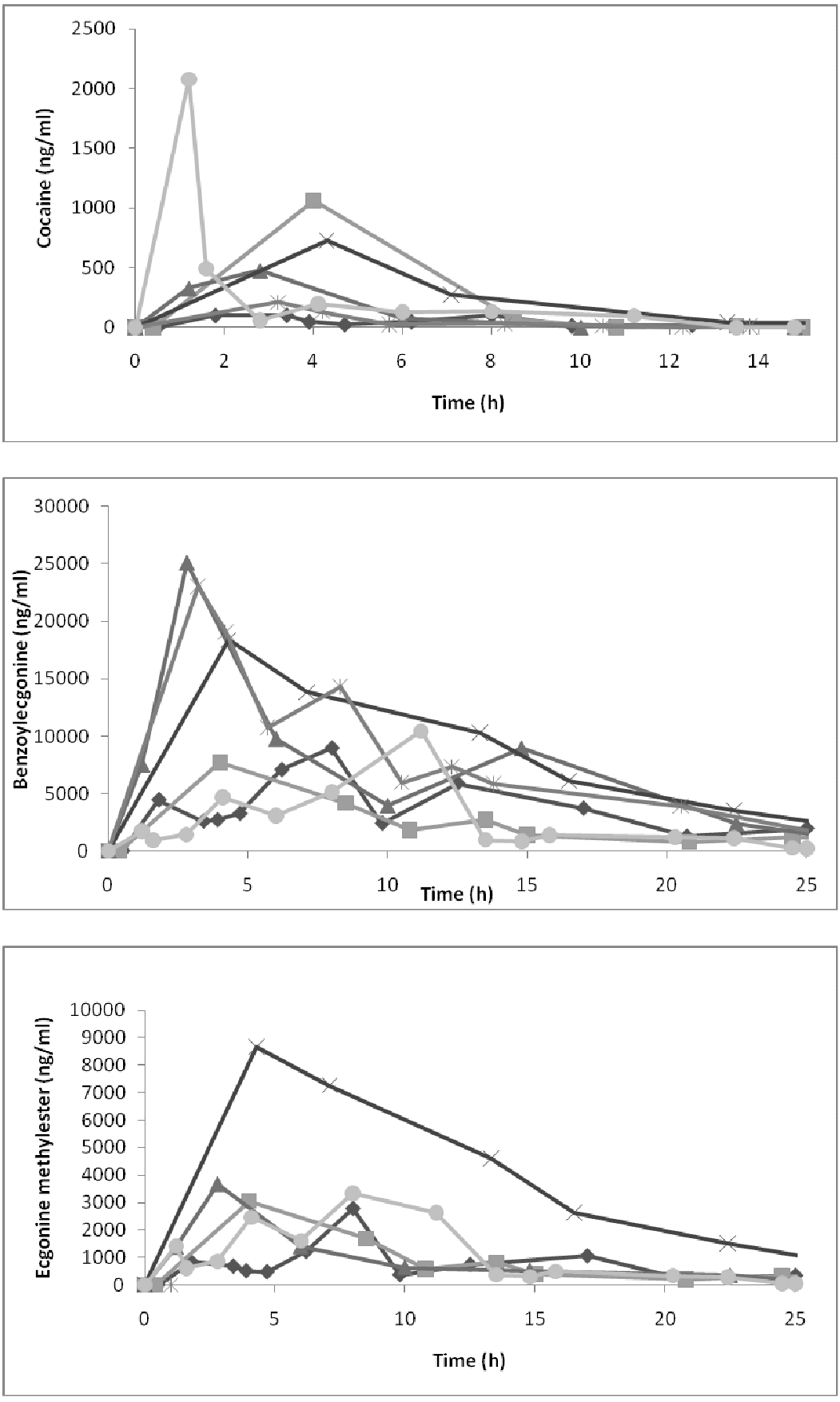

Figure 11: Urinary excretion profile of cocaine (ng/ml), benzoylecgonine (ng/ml) and ecgonine methylester (ng/ml) in six subjects after 25 mg IV cocaine HCl (Based on data from: Cone et al. (40))

After different doses of iv cocaine (11.2 mg, 22.4 mg, 25 mg and 44.8 mg), Smith et al. (41) initially detected all metabolites in the first or second urine voids for most subjects. More details of the percentage of the subjects who had a positive value at their first specimen were not given. The time to peak of cocaine occurred at the time of the first urine void. Unfortunately, the authors gave no time-indications.

Kogan et al. (42) reported data from two subjects given 100 mg of cocaine HCl intravenously. COC and BZE were determined in urine, collected from 0 to 2 h. High positive values of 43500 ng/ml BZE and 9300 ng/ml COC were measured.

2.3.2. Intranasal cocaine

Cone et al. (40) studied the urinary excretion profiles of 6 men after use of 32 mg IN cocaine HCl, an approximately equipotent dose with 25 mg IV cocaine HCl. The urine specimens were measured by GS-MS and collected ad libitum. In table 8, the results are summarized.

Table 8: Time to peak (h), maximum concentration (ng/ml), time to first positive (h) and concentration of first positive (ng/ml) of COC, BZE and EME after intake of 32 mg IN cocaine HCl.

	COC (range)	BZE (range)	EME (range)
Time to peak (h)	5.1 ± 1.0 (2.5-8.1)	7.8 ± 1.2 (3.1-12.2)	5.0 ± 1.1 (2.3-8.1)
Maximum conc.(ng/ml)	412 ± 126 (62-903)	13681 ± 3719 (4418-29838)	5831 ± 2103 (1980-12963)
Time to first positive (h)	2.6 ± 1.8 (1.3-6.5)	1.6 ± 0.5 (0.8-2.3)	1.6 ± 0.5 (0.8-2.3)
Conc. of first positive (ng/ml)	134 ± 75 (59-237)	2430 ± 1314 (485-4003)	922 ± 664 (240-1496)

As with the intravenous administration, the mean maximum concentrations generally followed the following order: BZE > EME >COC. The peaks of COC and EME coincided at a mean time of 5 hours, followed by the peak of BZE. The earliest sampling happened at 0.8 h post dose: with a value of 1040 ng/ml BZE -seven times the cut-off value- it is highly positive. Also other early samples (1.3 h, 1.5 h) were already highly positive: values of 3221 ng/ml and 4003 ng/ml BZE were measured. A detail of the urinary excretion in the first three hours is seen in figure 12.

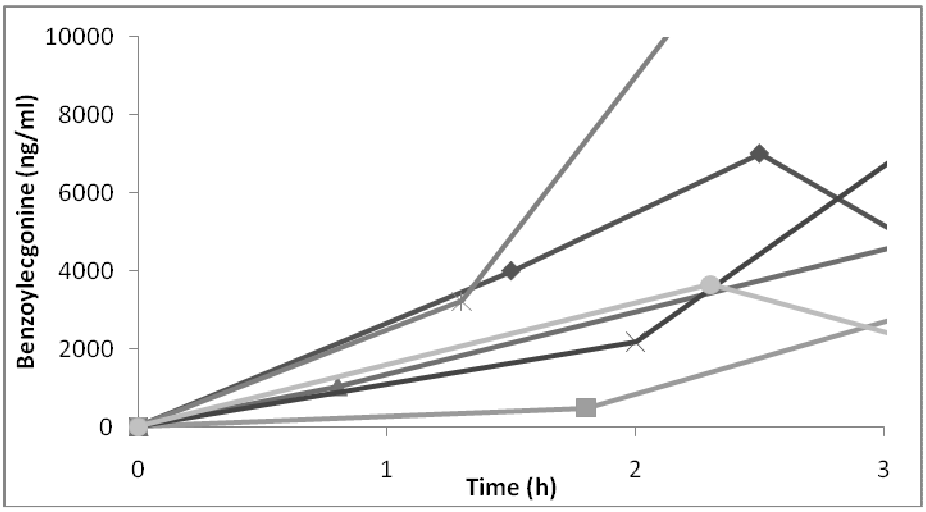

Figure 12: Urinary excretion of benzoylecgonine in the first three hours after intake of 32 mg IN cocaine HCl in 6 subjects (Based on data from: Cone et al. (40))

Urinary excretions patterns were also examined in an old study by Hamilton et al. (43). The subjects were six adults who received intranasal cocaine hydrochloride in a dose of 1.5 mg/kg body weight. Pools were obtained 1 h, 2 h and 4 h post-dose. The specimens were measured by gas-liquid chromatographic (GLC). At this moment, GLC is no longer used in the detection of drugs in urine, but the dose used in this study is a street-dose and therefore, the study is interesting. The excretion of cocaine peaked early and diminished rapidly. Three of the six subjects had maximum concentrations of cocaine in the 0 to 1 h pool, and the other subjects showed their peak concentration of cocaine in the 1 to 2-h urine specimens. Benzoylecgonine excretion was maximal after 1 to 12 h. The mean peak concentration occurred during the 4 to 8 h interval. In the 0-1 h pool, the mean concentration of benzoylecgonine was 13400 ± 15100 ng/ml (range: 1200-37400), which implies a huge variability. After already one hour, they were all -except one- positive for benzoylecgonine (>150 ng/ml). One subject had 0 ng/ml BZE in his 0-1 h pool, but had a normal value of COC (1400 ng/ml) in that pool. When this urine was measured by immunoassay, it would have been false-negative.

2.3.3. Smoked cocaine

The urinary excretion profile of 6 men after use of 42 mg smoked cocaine (base) was studied by Cone et al. (40). Urine specimens were measured by GC-MS and collected ad libitum. The results are summarized in table 9.

Table 9: Time to peak (h), maximum concentration (ng/ml), time to first positive (h) and concentration of first positive (ng/ml) of COC, BZE and EME after intake of 42 mg smoked cocaine (base).

	COC (range)	BZE (range)	EME (range)
Time to peak (h)	2.6 ± 0.4 (1.4-3.9)	4.1 ± 0.6 (2.8-6.0)	4.1 ± 0.6 (2.8-6.0)
Maximum conc. (ng/ml)	707 ± 413 (48-2289)	9395 ± 3284 (1095-20873)	3193 ± 874 (469-5802)
Time to first positive (h)	2.3 ± 1.0 (1.0-3.9)	1.9 ± 0.8 (1.0-3.3)	1.9 ± 0.8 (1.0-3.3)
Conc. of first positive (ng/ml)	586 ± 791 (48-2289)	4581 ± 5739 (115-17180)	1572 ± 1530 (22-4690)

Five of the six subjects were positive for BZE. One subject had his first specimen at 1.4 h with a value of 115 ng/ml BZE (<150 ng/ml), 22 ng/ml EME and 0 ng/ml COC. The next specimen at 3.3 h was positive with a value of 1095 ng/ml BZE. On the other hand, one subject had a specimen at 1.0 h that was positive for BZE (2187 ng/ml). Figure 13 illustrates the excretion pattern of benzoylecgonine during the first three hours.

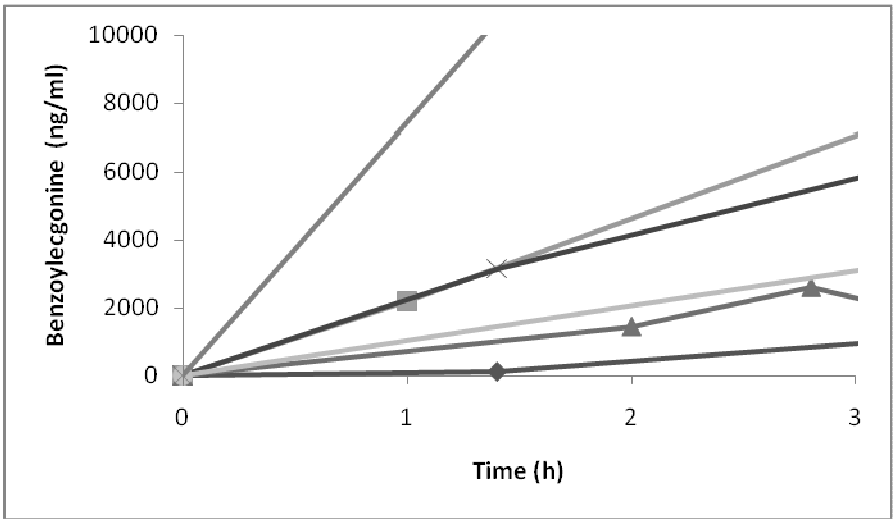

Figure 13: Urinary excretion of benzoylecgonine in the first three hours after intake of 42 mg smoked cocaine (base) (Based on data from: Cone et al. (40))

A few years later, Huestis et al. (44) also studied the excretion profile after smoked cocaine in three different doses (10 mg, 20 mg and 40 mg). Unfortunately, all information needed for our study was not given. Mean maximum concentrations decreased as follows: BZE > EME > COC in accordance to the results of Cone et al. (40). Cocaine was measurable in all first urine voids regardless of dose, unlike Cone et al. (40) who found one person in whom cocaine was not measurable in the first (1.4 h) and in the second (3.3 h) void. Only from the third void (3.9 h), cocaine was measurable (48 ng/ml).

2.3.4. Oral cocaine

A 25 mg oral dose of cocaine HCl was given to a single volunteer by Baselt et al. (45) what resulted in a peak urinary COC concentration of 269 ng/ml at 1 h and 3740 ng/ml BZE at 1 h. The peak of BZE (7940 ng/ml) came later, at 12 h post dose. Earlier voids were not taken. The immunoassay value at 1 h had a value > 1000 ng/ml.

2.3.5. Comparison of the different routes of administration

Six males were given approximately equipotent doses of intravenous, smoked and intranasal cocaine (25 mg IV cocaine HCl, 42 mg smoked cocaine, 32 mg IN cocaine HCl) by Cone et al. (40). The metabolic excretion patterns were generally similar, but with some important differences. The maximum concentrations of COC, BZE and EME tended to be higher –but not significantly- following IV and IN route, in comparison with the smoked route (Table 7, 8 and 9). Whether this means that

faster positive samples will be measured by the IV and the IN route, is difficult to say. More important for our question, is the fact that the recovery (% dose) of COC and its metabolites seemed to be higher following IV and IN routes compared to the smoked route (Table 10). The bio-availability, which is much higher with the IV route, plays an important role, illustrated by the different doses of Cone et al. (40). Especially for BZE, there is a remarkable difference between the different routes. Since BZE is the target metabolite with IA and GC-MS, theoretically it may have an effect on the time to the first positive, where it takes longer to the smoked route to get a first positive specimen.

Table 10: Mean % dose (SEM) for COC, BZE and EME in urine after single dose administration by the iv, in and smoked routes (% dose was determined over three days).

	% dose (SEM)		
	BZE	EME	COC
intravenous, 25 mg cocaine HCl	39.2 (3.7)	15.3 (2.3)	1.0 (0.4)
intranasal, 32 mg cocaine HCl	29.9 (3.4)	12.9 (2.2)	0.5 (0.2)
smoked, 42 mg cocaine (base)	16.4 (5.1)	7.2 (2.1)	0.5 (0.2)

A few years later, Smith et al. (41) concluded that the route of cocaine administration did not have an obvious impact on the peak concentration for cocaine metabolites. They reported that peak concentration increased with dose and time to peak was independent of dose. Also Huestis et al. (44) - who followed urine excretion profile after smoking- found a time to peak who was not dose-dependent.

2.3.6. Failure to detect an intoxication

Baker et al. (46) reported a case of an individual who died of COC intoxication but whose immunoassay screen (EMIT) for COC metabolite was negative. GC-MS of the urine detected 75 ng/ml BZE and COC at 55 ng/ml. These concentrations explained the negative screening considering that the cut-off concentration of the immunoassay was 300 ng/ml for BZE. The time when the sample was taken was not known; however it is an interesting fact for our question. When sampling happens too soon, little COC has been metabolized, so a low concentration of BZE is present, too low to give a positive immunoassay screening result. After intranasal administration of 1.5 mg cocaine/kg body weight, Hamilton et al. (43) found one individual where no BZE was detected in the 0 to 1 h and the 1 to 2 h specimens, although cocaine was detectable (1400 ng/ml).

2.4. HEROIN

In this chapter, four different routes of administration of heroin are described, which the intravenous route and the smoked route are used the most.

2.4.1. Intranasal heroin

The intranasal route of heroin achieves similar pharmacologic effects to the intravenous route, but allows the user not to use needles and reduces the associated diseases (47). When compared with the intramuscular route, the relative potency of the intranasal route is about 50 %. The intramuscular route releases the majority of the drug into the bloodstream as heroin, 6-acetylmorphine and morphine. Intranasal administration produces lower blood concentrations. This phenomenon is a result of loss of drug by swallowing a part of the dose (47).

Cone et al. (48) studied the urinary excretion profiles of 6 men after use of intranasal heroin under double-blind, double dummy conditions. Each person experienced the following conditions: 6 mg heroin hydrochloride (intranasal), 12 mg heroin hydrochloride (intranasal) and placebo at weekly intervals. Prior to the experiment, all subjects were required to test negative for three consecutive days by immunoassay (EMIT, cut-off value: 300 ng/ml). The urine specimens were taken ad libitum until a week after use. All specimens were tested by immunoassay (EMIT assay for opiates) and by GC-MS for heroin metabolites (total morphine, free morphine and 6-acetylmorphine). Figure 14 shows the excretion profile of total morphine after intake of 6 mg intranasal heroin (cut-off value: 300 ng/ml). This dose can be considered as half of a normal starting dose.

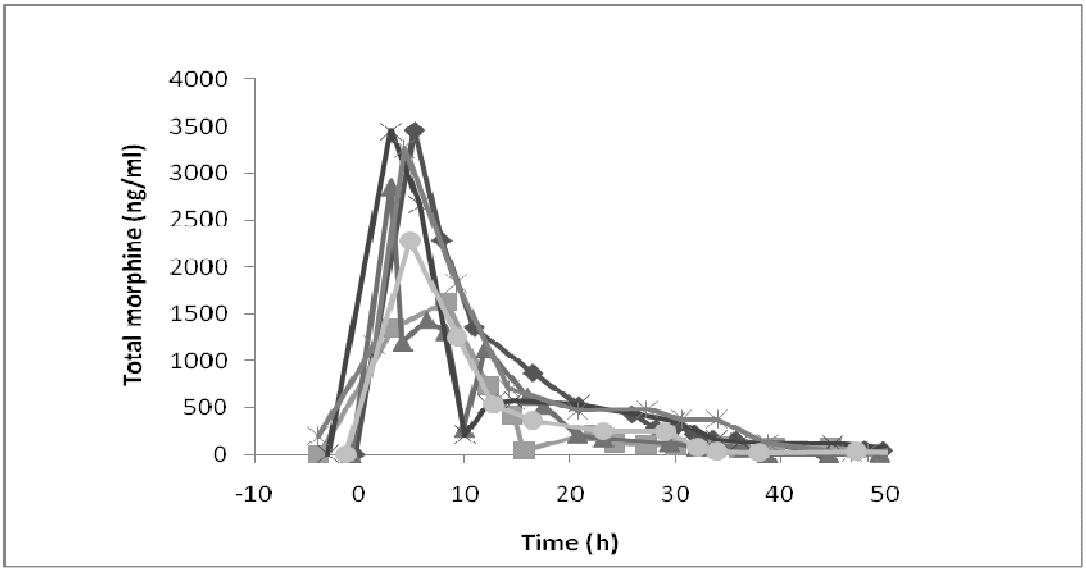

Figure 14: Urinary excretion profile of total morphine (ng/ml) after 6 mg intranasal heroin (n=6). (Based on data from Cone et al. (48))

The mean peak concentration of total morphine came after 4.8 h (range: 3.0-8.2 h). The peak concentrations ranged from 1628 to 3455 ng/ml. The mean time of the first urine was 3.5 h (range:

1.4-5.3 h) and the mean concentration 2426 ng/ml (range: 1174-3455 ng/ml). All the first urine specimens were positive for total morphine (cut-off value: 300 ng/ml). The fastest first urine was taken at 1.4 h and had a concentration of 1174 ng/ml, far above the cut-off value. This sample should be considered as unreliable, because the values of total morphine, free morphine and 6-AM were already positive prior to dosing. After the peak concentrations, concentrations of total morphine declined rapidly. After a mean time of 24 h, the concentration dropped below the 300 ng/ml cutoff concentration. After intake of 12 mg intranasal heroin, which is a typical starting dose, similar trends were seen, but something more variable (figure 15). The mean peak concentration came later (after 6.1 h; range: 2.5-8.0 h) and the peak concentrations were higher, approximately double in comparison with the 6 mg dose (range: 3085-10425 ng/ml). All the first urine specimens were already positive for total morphine. The mean concentration of the first urine was 5975 ng/ml (range: 1362-10425 ng/ml) with a mean time of 4.3 h (range: 1.3 h-8.0 h). The fastest first urine was taken at 1.3 h and had a concentration of 1362 ng/ml, more than four times the cut-off value.

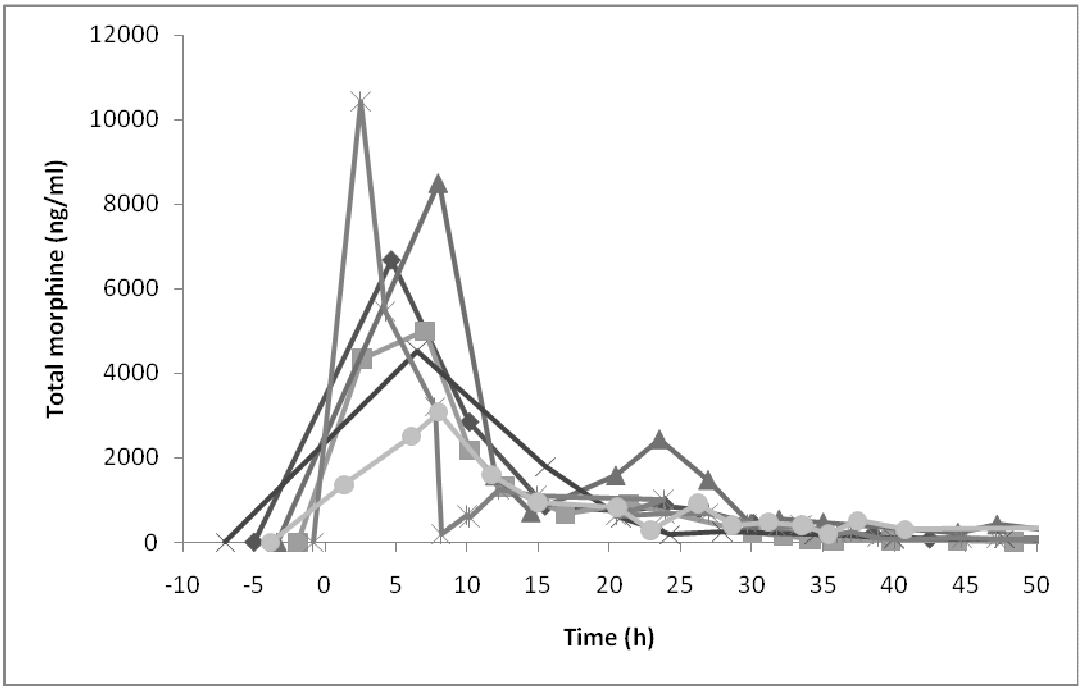

Figure 15: Urinary excretion profile of total morphine (ng/ml) after 12 mg intranasal heroin (n=6). (Based on data from Cone et al. (48))

For both the low and the high dose, all the first urine specimens were positive for total morphine. As the mean concentrations of the first urine specimens were 2426 ng/ml and 5975 ng/ml for the low and the high dose, respectively, in comparison with the cut-off value of 300 ng/ml, it can be predicted that if the samples were taken earlier they would also have been positive.

The excretion of free morphine (cut-off: 25 ng/ml) in the study of Cone et al. (48) showed a similar trend. The mean peak concentration came fast after a mean time of 4.0 h (range: 3.0-5.3 h) and 4.8 h (range: 2.5-8.0), for the low and the high dose, respectively. For both the doses, the first urine was

positive for free morphine (> 25 ng/ml). For one person, the concentration of free morphine had a value of 465 ng/ml after 1.3 h, far above the cut-off value, which suggests that earlier urine specimens would be positive as well.

The peak of 6-acetylmorphine (cut-off: 10 ng/ml) appeared almost always in the first urine, followed by a rapid decline. In a mean time of 1.8 h (range: 0-3.3 h) and 2.9 h (range: 0-6.5 h), the concentration had fallen under the cut-off value, after the low and the high dose, respectively. The mean concentration of the first urine, which coincided with the peak concentration, had for the low dose a value of 34 ng/ml (range: 0-81 ng/ml) and for the high dose a value of 73 ng/ml (range: 0-183 ng/ml). While the ratio of the mean concentrations of total morphine and free morphine (of the first urine) compared to the respective cut-off values were 8 and 14, this was only three for 6-acetylmorphine after the low dose. With the high dose, the ratios became 20 and 35 for total morphine and free morphine compared to seven with 6-acetylmorphine. For occasional users it is possible that their dose is too low to get positive values of 6-AM, considering the low ratio of the mean peak concentration compared to the cut-off value. In two cases in the study of Cone et al. (48), the first urine contained a concentration of 6-AM below the cut-off value (<10 ng/ml), while the concentrations of total and free morphine were clearly positive. 6-AM is a marker of recent heroin intake, but considering these two cases of Cone et al. (48), for our study-question, lowering the cut-off value of 6-AM could be a manner to get rapid and positive values. Nevertheless, finding of 6-AM gives certainty about heroin-intake, which is not the case with total and free morphine.

All specimens were also tested by immunoassay (EMIT for opiates). Both after low and high dose, all the first urine samples were positive (>300 ng/ml). In five of the six cases, the first urine coincided with the peak concentration of the assay, for both the low and high dose. The mean concentrations of the first urine specimens (363.5 ng/ml and 375 ng/ml, low and high dose respectively) suggest that earlier urine specimens would probably be positive as well.

2.4.2. Intramuscular heroin

Cone et al. (1991) (49) studied the urine excretion profiles of six men after intake of 3 mg and 6 mg intramuscular heroin. Prior to the experiment, a negative urine specimen was needed during three consecutive days (Abuscreen immunoassay, cut-off: 300 ng/ml). All specimens were taken ad libitum and were tested by GC-MS for total morphine, free morphine and 6-AM. In figure 16 one can see the urine excretion profile of 6 persons of total morphine after intake of 3 mg intramuscular heroin.

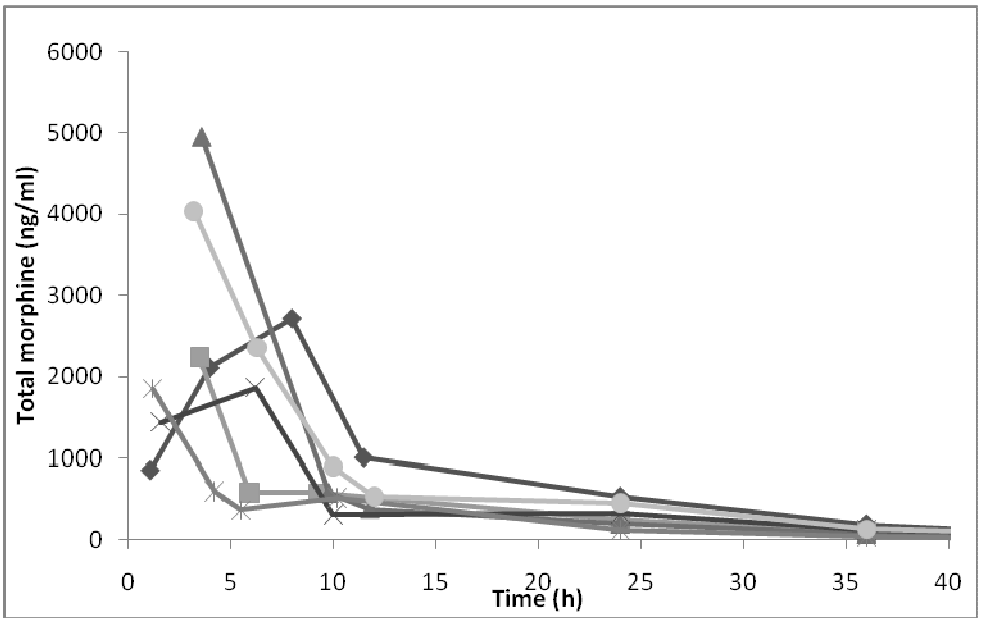

Figure 16: Urinary excretion profile of total morphine (ng/ml) after 3 mg intramuscular heroin (n=6). (Based on data from Cone et al. (49))

The peak of total morphine came fast, after a mean time of 4.3 h (range: 1.2-8.0 h). The peak concentration ranged from 1852 to 4953 ng/ml and had a mean concentration of 2944 ng/ml. The mean time of the first specimen was 2.4 h (range: 1.1-3.6 h) and it had a mean concentration of 2563 ng/ml (range: 1442-4953 ng/ml). All the first urine specimens were positive for total morphine (cut-off: 300 ng/ml). The fastest first urine was already produced after 1.1 h and had a concentration of 850 ng/ml total morphine. After the peak concentration, the concentrations of total morphine declined rapidly below the cut-off value, after a mean time of approximately 24 h. After intake of 6 mg intramuscular heroin (see figure 17), the mean peak concentration came after 3.8 h (range: 2.8-4.5 h) and the peak concentrations were higher, namely an average of 7535 ng/ml (range: 3316-12022 ng/ml). Also here, all the first urine specimens were positive for total morphine. The mean concentration of the first urine was 6022 ng/ml (range: 1342-12022 ng/ml) and the samples were collected after a mean time of 2.9 h (range: 1.1-4.3 h). Two specimens were already taken after 1.1 h with 1621 ng/ml and 1342 ng/ml as value, four to five times higher than the normal cut-off value. Cone et al. (48) also studied the urine excretion profile after use of 6 mg intramuscular heroin with 6 healthy volunteers. Also in this study, the first urine was positive for total morphine in all the cases: the mean concentration in the first urine was 3892 ng/ml (range: 1869-6117), far above the cut-off value. The first urine came after a mean time of 3.9 h (range: 1.7-8.3). Compared to Cone et al. (49), the mean concentration of the first urine had a value of 3892 ng/ml, which is about the half of 6022 ng/ml. In the study of Cone et al. (48), the peak concentration of total morphine was also much lower, namely 3892 ng/ml (range: 1869-6117 ng/ml) compared to 7535 ng/ml of Cone et al. (49).

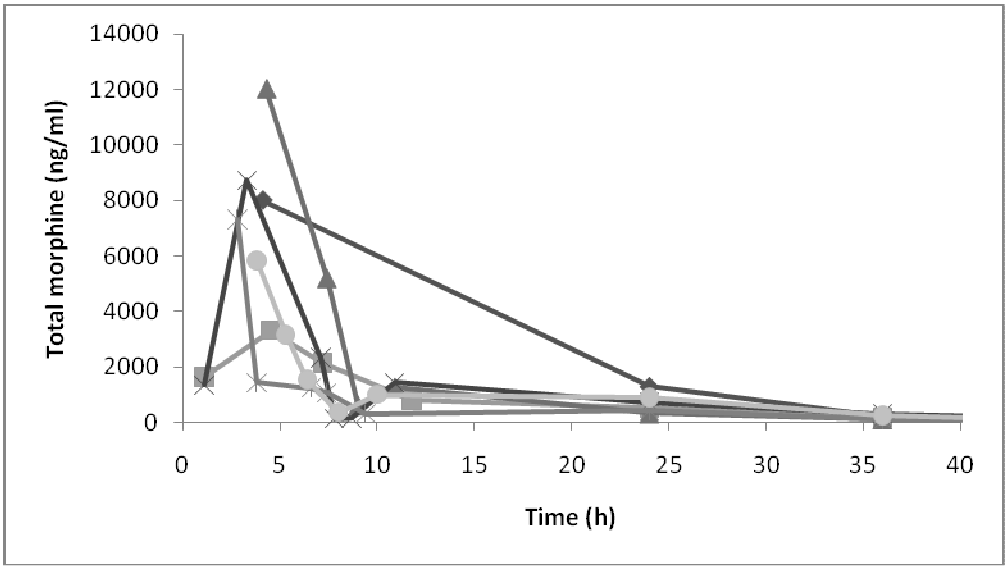

Figure 17: Urinary excretion profile of total morphine (ng/ml) after 6 mg intramuscular heroin (n=6) (Based on data from Cone et al. (49)).

The peak of free morphine (cutoff: 25 ng/ml) in the study of Cone et al. (49) came after a mean time of 2.9 h (range:1.2-4.0) and after 3.8 h (range: 2.8-4.5 h), for the low and the high dose, respectively. The mean peak concentration was 341 ng/ml (range: 144-724 ng/ml) and 916 ng/ml (range: 296-1280 ng/ml), for the low and the high dose. The mean concentration of the first urine was 333 ng/ml (range: 144-724 ng/ml) for the low dose and 810 ng/ml (range: 244-1280 ng/ml) for the high dose. The first urine came after a mean time of 2.4 h (range: 1.1-3.6 h) for the low dose and after 2.9 h (range: 1.1-4.3 h) for the high dose.

Regarding 6-AM, one can make the same observation as with the intranasal administration: the peak concentration of 6-AM coincided with the first urine specimen, and this both after 3 mg IM and after 6 mg IM heroin. Afterwards, a fast decline followed. The mean concentration of the first urine specimen for 6-AM was 73 ng/ml (range: 42-148) for the low dose and 138 ng/ml (range: 50-236) for the high dose. Compared with the intranasal route, these values are about double.

2.4.3. Intravenous heroin

Smith et al. (50) studied the urine-excretion profiles after intravenous heroin use, and determined total morphine, free morphine and 6-AM with GC-MS. Eight subjects used intravenous heroin hydrochloride in three different doses (3mg, 6 mg and 12 mg), with three days intervals. Each subject had to be tested negative during three consecutive days (EMIT immunoassay, 300 ng/ml) prior to dosing. The samples were collected ad libitum. In table 11, the results are summarized.

Table 11: Time to peak (h), maximum concentration (ng/ml), time of first specimen (h) and concentration of first specimen (ng/ml) of total morphine and free morphine after different doses of iv heroin.

dose		total morphine (range)	free morphine (range)
3 mg	time to peak (h) maximum concentration (ng/ml) time of first specimen (h) concentration of first specimen (ng/ml)	2.3 (1.2-4.5) 3495 (2207-5100) 2.0 (1.2-2.8) 3405 (2207-5100)	2.0 (1.2-2.8) 367 (153-680) 2.0 (1.2-2.8) 367 (153-680)
6 mg	time to peak (h) maximum concentration (ng/ml) time of first specimen (h) concentration of first specimen (ng/ml)	2.9 (1.2-6.2) 6832 (1855-9250) 2.4 (1.2-5.1) 6598 (1855-9250)	2.4 (1.2-5.1) 780 (390-1160) 2.4 (1.2-5.1) 780 (390-1160)
12 mg	time to peak (h) maximum concentration (ng/ml) time of first specimen (h) concentration of first specimen (ng/ml)	5.6 (4.1-9.3) 16651 (11150-29030) 2.8 (1.4-4.0) 10774 (6040-16300)	3.0 (2.1-4.6) 1530 (1091-2300) 2.8 (1.4-4.6) 1302 (478-2259)

For each of the three doses, all the first urine specimens were positive for total morphine (cut-off: 300 ng/ml). The higher the dose, the higher the first urine concentration. Even at the low dose (3 mg), the mean concentration of the first urine (3405 ng/ml) was already ten times higher than the cut-off value, which suggests that earlier urine specimens would probably be positive as well. The fastest first urines were after 1.2 h for the 3 mg and the 6 mg dose and after 1.4 h for the 12 mg dose. The respective concentrations were 3730 ng/ml, 1855 ng/ml and 6950 ng/ml, all above the cut-off value of total morphine. In seven of the eight cases, the peak concentration coincided with the first urine concentration (for 3 mg and 6 mg). This was the case in only two of eight cases for the 12 mg dose. For free morphine one observes a similar trend: all the first urine specimens were positive for each dose. They had concentrations far above the cut-off value of 25 ng/ml. For the two low doses one can see in table 11 that the concentration of the first urine coincided with the peak concentration. For the 12 mg dose this occurred in seven of the eight cases.

6-AM concentrations showed a similar trend, like Cone et al.(48) and Cone et al. (49) already described: most cases (23 of the 24) had their peak concentration of 6-AM in their first urine specimen at a mean time of 2.0 h (range: 1.2-2.8 h) for the 3 mg dose, at 2.4 h (range: 1.2-5.1 h) for the 6 mg dose and at 2.8 h (range: 1.4-4.6 h) for the 12 mg dose. Afterwards there was a fast decrease of 6-AM.

2.4.4. Smoked heroin

Smith et al. (50) studied the urine-excretion profile after smoking heroin and determined total morphine, free morphine and 6-AM by GC-MS. Depending of the dose of the cigarette, the subjects were divided into two groups (low dose and high dose). A part of the results is summarized in table 12.

Table 12: Time to peak (h), maximum concentration (ng/ml), time of first specimen (h) and concentration of first specimen (ng/ml) of total morphine after different dose of smoked heroin.

smoked heroin dose		total morphine (range)
low dose (3.5-5.2 mg) (n=5)	time to peak (h) maximum concentration (ng/ml) time of first specimen (h) concentration of first specimen (ng/ml)	3.3 (2.3-4.9) 2758 (1392-4620) 2.9 (2.3-4.9) 2690 (1050-4620)
high dose (7-10.5 mg) (n=5)	time to peak (h) maximum concentration (ng/ml) time of first specimen (h) concentration of first specimen (ng/ml)	2.5 (2.2-3.1) 8157 (2065-17721) 2.5 (2.2-3.1) 8157 (2065-17721)

These results are in agreement with the results of the other routes of administration of heroin. In all cases for both the low and the high dose, the first urine was positive for total morphine with values far above the cut-off value of 300 ng/ml. With the high dose, the first urine coincided with the peak concentration in all cases, with the low dose in four of the five cases. The 6-AM concentrations showed a similar trend as the other routes of administrations. In all the ten cases, the first urine concentration coincided with the peak concentration. The mean concentration of the first urine was 56 ng/ml (range: 7.2-133 ng/ml) for the low dose and 239 ng/ml (range: 6.1-568 ng/ml) for the high dose. For both the low and the high dose, there was one case with a peak concentration (= first urine concentration) lower than the cut-off value for 6-AM (10 ng/ml), while the concentrations of total morphine and free morphine were clearly positive. This is in agreement with Cone et al. (48), who also found some cases who were negative for 6-AM, but positive for total morphine and free morphine.

DISCUSSION AND CONCLUSION

Prior to the discussion of the individual drugs, some generalities and limitations of this study were given. It is difficult to get approval for studies with drugs because they have to be given to healthy volunteers. Some clinicians (Vollenweider et al.(28)) consider even a single dose as controversial. In a consequence, the administered doses are lower than the normal "street" ones, so the excretion patterns and the corresponding concentrations of the drugs in the studies are not always realistic. For example, Huestis et al. (30) worked with volunteers who received 10 mg or 20 mg oral doses of methamphetamine, while the normal street dose of methamphetamine is about 3 to 5 times higher (Baselt (5)). As a consequence, the concentrations of methamphetamine and amphetamine were lower and the time to first positive can be influenced by that.

Another result of the difficult approval of the study is the very low number of subjects (often no more than ten volunteers) and as a consequence the study is much less powerful than a study working with a large population. And when an individual is found who is unique in his data, the question is raised whether this individual is indeed unique. It is always possible that there would have been more subjects with this data if the population had been larger.

It also happens that the route of administration is different from the normal one and this manner of use determines the amount of drug that is absorbed and the kinetics. Indeed, the route of drug administration directly influences pharmacologic outcome (Cone et al. (48)). For example, Cone et al. ((48)) studied 6 men after intake of intranasal heroin in double-blind, double dummy conditions, what makes it a valuable study, but for the fact that heroin is quite rarely used intranasally compared to the intravenous and smoked routes.

Another problem with this type of studies, is that unlike blood specimens, urine cannot be collected every ten minutes. In most studies, the urine specimens were collected ad libitum, with the result that the first sample was often produced after about 1-2 hours or even later. Since we are interested in the time just after taking, the ad libitum-method is less interesting than the mandatory-method.

Another drawback of urine use is that the concentrations of the drugs are dependent on the fluid intake and the diurnal fluctuations. Persons whose fluid intake is low, will deliver a specimen later, that might be have a high positive value, while an earlier mandatory specimen might also have been positive. Most of the authors didn't control the fluid intake of their volunteers. One study (Huestis et al.((37)) even encouraged the volunteers to keep up their urine for the first three hours.

In literature, no studies had done any research about the time and rate in which the metabolites appear in urine just after intake. In contrast, there are more publications about detection time of drugs (Verstraete (4)). These publications had another research question, so time indications were only sporadically given. For example, when first urine collection was indicated, the specific time of the collection was not often specified (Smith et al. (41)). Many authors didn't publish many details about the first urine specimen. For this reason these publications didn't deliver the necessary information for

our study. The low number of relevant studies, the low number of study subjects, the low doses of drugs together with the disability to continuous monitoring, makes it difficult to answer our study-question.
Furthermore the different drugs of this study are discussed.

With a 500 ng/ml cut-off concentration, the first positive methamphetamine occurred at a mean time of 5.5 h (1.4-11.3 h) after a 10 mg dose and at 4.3 h (1.2-8.8 h) after a 20 mg dose (Huestis et al. (30)). With the same cut-off value, Oyler et al.(31) found a mean initial detection time of 3.0 h (1.4-4.3 h) after a 10 mg dose. Both authors didn't find a change in time of first positive when lowering the cut-off value from 500 ng/ml to 250 ng/ml (Table 1 and Table 2). Two explanations are possible. One explanation could be that methamphetamine concentrations rise rapidly post-dose. When the curve is very steep after drug use, the cut-off value will be of less importance and will not have a significant influence of the time of first positive.
Another explanation could be that the number of people in this study was too low. Huestis et al. (30) worked only with five people, which means that when there is only one outlier, it has a great influence on the mean value, but not on the median value. Considering the great range of the values of the time to first positive (1.4-11.3 h for the low dose and 1.2-8.8 h for the high dose), it is hard to compare the mean values. This could be an explanation for the small difference in initial detection time between the low cut-off value and the high cut-off value.
After administration of different doses (5 mg, 10 mg and 20 mg) of oral amphetamine, all the specimens were positive at 2 h post-dose (Poklis et al. (33)). No data on the time the first urine was provided and no other controlled study after oral amphetamine with data of the first urine, was found in the literature.
Helmlin et al. (35) and Abraham et al. (36) studied the urinary excretion profile after 1.5 mg MDMA/kg body weight and after 1.0 mg MDMA/kg body weight, respectively. In real life, MDMA is usually taken in oral doses of 60 – 70 mg (range: 20-110 mg) as the hydrochloride form (Cole et al. (51)), which makes the doses used by Helmlin et al. (35) and Abraham et al.(36) very realistic. The median time to first positive of MDMA and its main metabolite HMMA occurred at 1.3 h (0.8-3.3 h) after 1.0 mg/kg body weight and 1.2 h (0.4-1.4 h) after 1.6 mg/kg with a cut-off value of 25 ng/ml (Abraham et al. (36), n=16). MDMA and HMMA were detected in all first voids after the high dose and in 14 of 16 first specimens after the low dose. After the high dose, the earliest void came at 0.42 h with positive values for MDMA and HMMA. These results were similar to those of Helmlin et al.(35) who also found positive values of MDMA for two volunteers at the first voids (1 h and 1.5 h). Considering that HMMA concentrations were consistently greater than those of MDMA in most urine specimens (Figure 4, subject B), HMMA could be a faster manner to get a positive value, but measurement of urinary HMMA is not included in routine monitoring procedures.
Another critique on the studies with (meth)amphetamine is the fact that urine pH is not taken into

account. It is known that the excretion of (meth)amphetamine is pH dependent. Under normal conditions, up to 43 % of a methamphetamine dose is excreted as unchanged drug with 4 %-7 % as amphetamine in 24 hours. This can be changed by adjusting the urine pH. In acidic urine, up to 76 % of the methamphetamine dose appears as parent drug, and only 7 % as amphetamine in the first 24 hours (Kim et al. (52)). This large difference between % dose excreted as unchanged drug can have a influence on the time to first positive.

Enormous differences in first urine concentration of THCCOOH were reported by Huestis et al.(37) and McBurney et al. (38) (Table 4). The authors reported a 25-fold variation and even a 100-fold variation between the subjects. Such wide variation in levels of metabolite excretion is probably a reflection both of differences in smoking efficiency and of inter-individual differences in metabolism. After the low dose (15.8 mg THC) and the high dose (33.8 mg THC), the mean time to first positive was 4.9 $\pm$ 1.8 (3.2-8) h and 3.4 $\pm$ 0.6 (2.3-4) h, respectively.
Considering the high variation in THCCOOH concentration between the subjects, it would be desirable to find one or more analytes with shorter urinary excretion times, whose presence in urine would indicate very recent drug use.
In enzyme-hydrolyzed urine, McBurney et al. (38) found a peak concentration of 8,11-diOH-THC in the earliest urine samples in nine of the ten cases. They concluded that this metabolite is the best indicator of recent use in urine. In contrast -with the exception of one heavy cannabis user- 8,11-diOH-THC was not detected in urine by Manno et al. (21) . It is difficult to explain this contradiction. The authors didn't give an explanation. It could be that Manno et al.(21) worked with healthy users who reported a history of light marijuana use (1-3 cigarettes per week or less), while the volunteers in the study of McBurney et al. (38) were not well defined. The authors described them only as "experienced occasional users". In reality, maybe they were more like heavy cannabis users. Analysis of pre-smoking urines confirmed the presence of THCCOOH for one subject at a concentration of 9.9 ng/ml, so at least there was one heavy user.
After addition of bacterial β- glucuronidase, the presence of significant quantities of THC and 11-OH-THC, can be demonstrated in urine (Kemp et al. (20)). The data of Manno et al. (21) suggested that THC and 11-OH-THC may be two biologic markers for the identification of recent marijuana use. Five minutes after smoking a low dose marijuana cigarette (17.7 mg THC), THC was already measurable (LOD: 1.5 ng/ml) in two of the six cases and in five of the seven cases after a high dose marijuana cigarette (35.8 mg THC). Five minutes after use, 11-OH-THC could also be measured in four of the six subjects after the low dose and in six of the seven subjects after the high dose. Unfortunately, in the study of Brenneisen et al. (39) –who also studied THC and 11-OH-THC as a possible marker – the first urine was collected at 2 h post-dose and not earlier. In these 2 h-urines, THC and 11-OH-THC were measurable in all cases. Other studies with larger experimental population are needed that confirm the rapid presence of THC and 11-OH-THC in urine after use.

Another critique on the cannabis studies is that the mean amount of THC released by smoking from cigarettes was not measured. It would be better to measure it because with this information, the average inhaled dose can be estimated, which would make the studies more valuable to compare.

That sampling too soon can lead to negative results, was observed by Cone et al. (40) after use of intravenous cocaine. They observed two volunteers whose first urine samples were taken at 0.5 h and 0.4 h post dose. At these times, no cocaine nor metabolites (EME and BZE) were found. In contrast, the next specimens at 1.8 h and 4.0 h, had huge values of BZE, 4447 ng/ml and 7693 ng/ml, respectively. Between 0.5 h and 1.8 h, the BZE value went from 0 ng/ml to 4447 ng/ml, which illustrates the disadvantage of urine that it cannot be taken off every moment. One can only conclude that for this person BZE appears in the urine between 0.5 h and 1.8 h.
Previous studies have indicated that cocaine administered to humans is excreted into urine almost entirely in the form of metabolites (Fish et al. (53), Inaba et al. (54)). BZE is known to be a major urinary metabolite and to account for approximately 40 % of the cocaine dose. However, the peak of BZE usually comes after COC and EME. Since the parent drug appears first in urine, maybe it is useful for our question to focus on unchanged cocaine. Hamilton et al.(43) found one subject with excretion of free cocaine (1400 ng/ml at 1 h post-dose) in the absence of benzoylecgonine, after administration of 1.5 mg/kg body weight intranasal cocaine. With a study with only six subjects, the question is raised whether this individual is indeed unique or not. On the other hand, Cone et al. (40) found two subjects with excretion of benzoylecgonine in the absence of cocaine soon after use. After 42 mg smoked cocaine, one subject had no measurable cocaine in the first three hours, while benzoylecgonine was 115 ng/ml at 1.4 h and 1095 ng/ml at 3.3 h. After 32 mg intranasal cocaine, one subject was found with 1040 ng/ml benzoylecgonine at 0.8 h, but no measurable cocaine. The potential for false negatives when using techniques sensitive only to one metabolite, is thus demonstrated.

The urinary excretion of metabolites after intranasal, intramuscular, intravenous and smoked heroin, was described in this thesis. Only the two latter are frequently used. After different doses (3 mg, 6 mg and 12 mg) intravenous heroin, 6-AM was measurable in all the first urines above the cut-off value of 10 ng/ml (Smith et al.(50)). In most cases (23 of the 24), the peak concentration 6-AM was observed in the first urine. Since 6-AM is the first metabolite of heroin, detection of this molecule is useful for our study-question. After smoked heroin, 6-AM was also measurable in all the first urines.However there were two cases with a first 6-AM value below the cut-off value, while the concentrations of total and free morphine were clearly positive (Smith et al. (50)). If only 6-AM is analysed, this can lead to false negatives. Lowering the cut-off value of 6-AM could be a manner to get rapid and positive values of heroin.

From this review we can conclude that, after a relatively low dose of drugs, methamphetamine appears in detectable concentrations in the urine after 1-11 hours, MDMA after 1- 4 hours, THCCOOH after 2-8 hours, BZE after 0.5 -4 hours , and 6-AM after 1 – 3 hours. For most drugs, the appearance of metabolites in urine is quite rapid, and the risk of having a negative result in the first hours after intake of the drug, is rather low.

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