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Detection of accompanying substances, impurities and combinations of active ingredients in the context of narcotics abuse

Master Thesis

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Declaration of oath

I hereby certify that I have written this Thesis independently without outside help and have used only the sources and aids indicated. Passages based on other works, verbatim or in spirit, are marked as such.

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Abstract

Consumption of illicit substances is accompanied with severe health risks. Even more so if they are adulterated with others. With the development of new pharmacological active compounds suitable for mimicking or enhancing the drug's effects on the body, new patterns emerged in recent years. Identifying those and including them in analytical methods applied on human urine samples to find most commonly used substances was the goal of this thesis. The main focus was chosen to be on heroin and cocaine in particular as both substances make up a large amount of Europe's total drug market.
As possible candidates, levamisole, aminorex, lidocaine and procaine were found during literature research, included in routine analytics and added to LC-MS/MS methods for identification. Limits of this method were found to be 0.56 ng/mL for detection of levamisole and 1.47 ng/mL for its quantitation, a LOD of 0.99 ng/mL and LOQ of 2.96 ng/mL for aminorex, while lidocaine could be detected at 0.66 ng/mL and quantified at 2.14 ng/mL and lastly procaine only being applied for quantitative analysis above its LOD of 0.96 ng/mL and LOQ of 3.07 ng/mL. Patient samples were measured and screened for the presence of any of the new substances, resulting in a total of 244 levamisole positive samples, five of these containing aminorex as well. 66 samples contained lidocaine and 105 procaine, however both were found in both cocaine positive and negative samples. In parallel, an evaluation of past samples measured in the lab from January 2024 to date was performed to verify whether some of these or other substances named as possible adulterants had previously been detected.
The amount of cocaine positive samples containing levamisole with the augmented method, confirmed recently published trends. Only one case of a levamisole positive sample was found in past samples, highlighting the potential analytical gain of its addition. Heroin was expected to contain synthetic opioids like fentanyl and nitazene as these gained popularity. The majority of samples tied to illicit opioid consumption contained 4-ANPP, a precurser of fentanyl known to be used in its illicit synthesis pathways.

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Nomenclature

Notation	Description	Unit
4-ANPP	N-Phenyl-1-(2-phenylethyl)piperidin-4-amine	
6-MAM	6-monoacetylmorphine	
ACN	Acetonitrile	
AIDS	Acquired immunodeficiency syndrome	
ALL	Seeming signal yield	
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	
Amph	Amphetamines	
ATP	Adenosine triphosphate	
BE	Benzoylecgonine	
cAMP	Cyclic adenosine monophosphate	
CE	Cocaethylene	
CE	Collision energy	eV
CNS	Central nervous system	
CXP	Cell exit potential	eV
CYP2D6	Cytochrome P450 2D6 enzyme	
CYP3A4	Cytochrome P450 3A4 enzyme	
CYP450	Cytochromes P450 superfamily of enzymes	
D1, D2, D3	Dopamine receptors	
DA	Dopamine	
DAT	Dopamine transporters	
DCT	Drug confirmation test in serum QC sample	
DOP	δ-opioid receptor	
DP	Declustering potential	eV
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine	
EMCDDA	European monitoring centre for drugs and drug addiction	
EME	Ecgonine methyl ester	
EU	European Union	
GC-MS	Gas chromatography–mass spectrometry	
GIRK	G protein-coupled inwardly rectifying potassium channel	
GTFCh	Society of Toxicological and Forensic Chemistry	
KOP	κ-opioid receptor	
LC-MS/MS	Liquid chromatography tandem mass spectrometry	
LOD	Limit of detection	ng/mL
LOQ	Limit of quantitation	ng/mL
Max	Maximum	
MDMA	3,4-Methylenedioxymethamphetamine (Ecstasy/ Molly)	

will be continued on next page...

Notation	Description	Unit
ME	Relative signal intensity	
MeOH	Methanol	
Min	Minimum	
MOP	μ-opioid receptor	
MOR	μ-opioid receptor (alternative abbreviation)	
MRM	Multiple reaction monitoring	
MS/MS	Tandem mass spectrometry	
MV	Mean value	
NE	Norepinephrine	
NET	Norepinephrine transporters	
NK1	Tachykinin	
NMDA	N-methyl-D-aspartate	
p53	Tumor-suppressor gene p53	
PChE	Pseudocholinesterase	
QC	Quality control	
RC	Recovery	
SD	Standard deviation	
SERT	Serotonin transporter	
THC	Tetrahydrocannabinol	
TLC	Thin-layer chromatography	
VMAT	Vesicular monoamine transporter	

1. Introduction

Throughout human history, the discovery and use of substances with intoxicating, stimulant or narcotic effects has been a reoccurring topic. Cocaine was reportedly consumed in form of plant leaves as soon as 5000 years ago in south america.[1] Today, the illicit consumption of drugs of abuse is still a generally well known health risk as well as a key factor in criminal networks. Therefore, the European monitoring centre for drugs and drug addiction (EMCDDA) as well as the European Union (EU) law enforcement agency Europol strictly monitor current developments. Regarding their efforts, especially economic but also societal findings are of interest. In total, it is estimated that the EU drug market had a retail value of at least €31 billion as of 2021. Apart from Cannabis, cocaine and heroin were identified as the highest contributing substances, as shown in Figure 1. Together, they account for more than half of the assumed total value.[2] As a result, both are of particular interest.

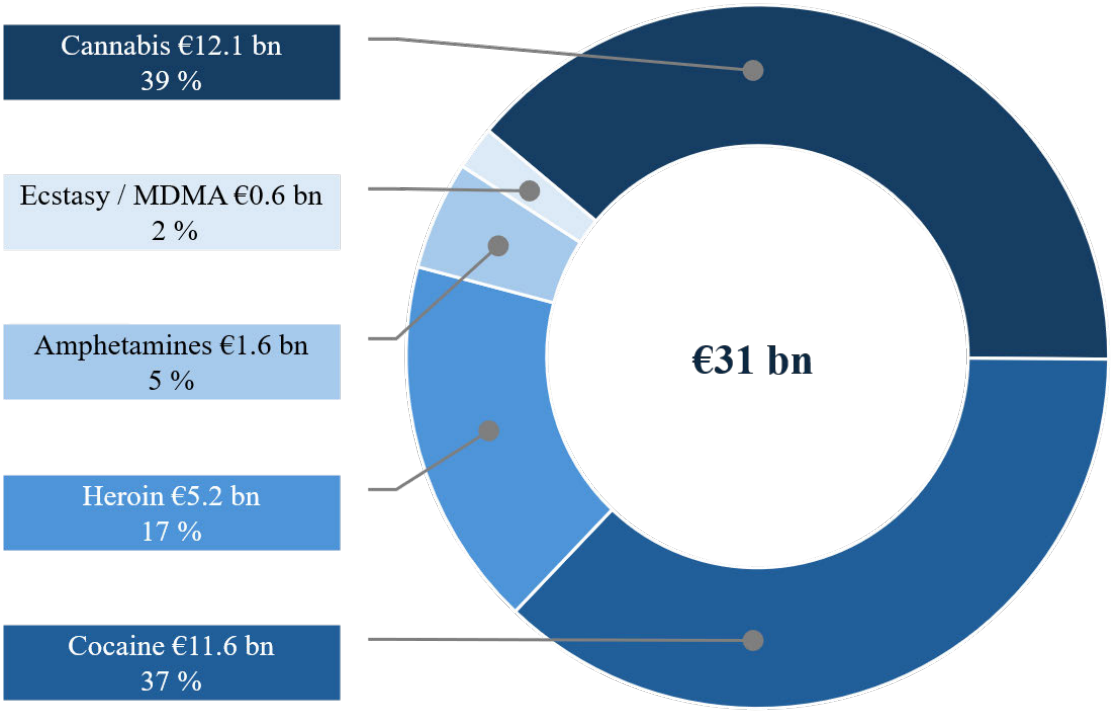

Figure 1: Absolute value and percentage share of most common illicit drugs in the European drug market. (Source: cf. EMCDDA and Europol 2024[2])

Apart from monetary concerns positively influencing other areas like money laundering or even trafficking of firearms,[2] severe health concerns raised by consumption of illicit drugs are to be noted as well. When consumed in their pure form, their effects on the body are severe but generally well studied. However, this is rarely the case. Most drugs bought from street sellers are cut with inert or active substances for various reasons, for example to maximise profit while minimising investment. While inactive compounds

are unlikely to cause additional harm, active ones can greatly increase the risk for severe adverse reactions, overdoses or even death. Especially if shortages of a substance, complications in synthesizing them or stricter regulations decrease the amount of available illicit substances, the addition of other compounds or "cutting" increases. As new, especially synthetic compounds, are being discovered continuously, composition of these adulterated street drugs is subject to change. Biologically active substances can be added for various reasons, mostly altering or enhancing the effects of the drug itself.
According to surveys, the general public expects the presence of toxic substances like cleaning agents, brick dust or even glass shards and strychnine.[3,4] In practice, chemical profiling of seized samples reveal a different reality. Consequentially, it is to be expected that most consumers are unaware of the exact composition of the substances they consume. From a medical perspective, this can have severe consequences even for experienced or routine consumers.[5] Synthetic compounds are often more potent, which can cause severe adverse effects, both short and long term, and even overdose at much lover dosages than expected. As a result, detecting accompanying biologically active compound can be crucial in finding the appropriate therapy approach and eventually saving lives. New developments in the adulteration pattern, especially of cocaine and heroin, were discovered recently. Namely the use of highly potent synthetic opioids in heroin[2] and of anthelmintic substances in cocaine are of interest, as they are known to cause severe health risks. It is therefore highly important to establish new analytical methods to detect not only the known drugs of abuse but to also include potential adulterants.

2. Research Question

In this thesis, it is to be examined, which adulterants can be found in illicit drugs of abuse with a focus on heroin and cocaine. It is to be verified whether the identified adulterants can be found during routine testing of urine samples. The addition of Substances that are not yet present in routine analytical methods is to be performed if possible. Additionally, a validation of the added substances in the applied analytics is to be performed. Considering substances that can be found during routine analytics of samples send in for heroin or cocaine testing, their relative frequency and importance in the context of adulteration is to be evaluated. A special focus is set on pharmacologically active substances and their effects in combination to the drugs of abuse they are potentially used to adulterate.

3. Theoretical Background

Over time, many substances, that were initially intended to be used for their pharmacological effects but were eventually taken off the market due to their addictive nature, were afterwards illicitly synthesized and used in an abusive manner. Just like active pharmaceutical ingredients are continuously improved to achieve better effects, a similar process is happening for illicit substances. However, substances like heroin and cocaine that have been used as drugs of abuse for a long time continue to be frequently consumed. In their respective cases, adulteration is used as a form of improving or modifying the effects of the drug itself.[6] To identify these adulterations in human samples, highly sensitive and selective detection methods like LC-MS/MS are necessary.

3.1. Heroin

3,6-diacetylmorphine, mainly known as Heroin, can be derived from morphine contained in poppy plants and is classified as a semi synthetic opioid.[7,8] Its chemical structure can be found in Figure 2.

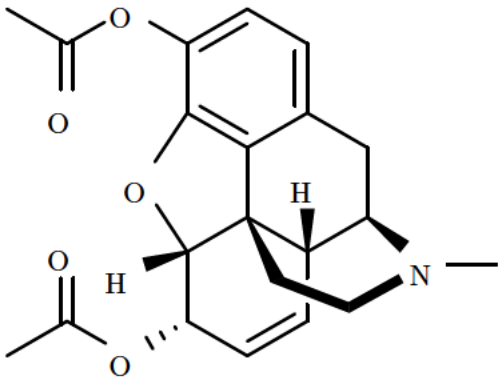

Figure 2: Chemical structure of the opioid heroin (Source: cf. Milella et al. 2023[8])

Heroin as it is known today was initially developed in 1874 by C.R. Alder Wright as a more potent and less addictive alternative for morphine. From 1898 it was introduced as medication mainly as a treatment for asthma and tuberculosis. First concerns regarding addictive potential were raised in 1912. Due to heroin being readily available while also highly potent, cases of it being used in a similar way as cocaine started to surface.[9]
A large amount of heroin is produced in Afghanistan. In recent years however, a decline of produced heroin from this region has led to the use of other opioids, especially in the form of adulteration.[10]

3.1.1. Pharmacokinetics

The distribution of heroin inside the body depends greatly on the method of administration. These include intravenous as well as intramuscular and subcutaneous injection, inhalation, insufflation and oral administration. A key factor concerning popularity of each route is the availability of the different forms of heroin, but the desired duration of the drugs effects play an equally important role. Intravenous injection shows fastest onset as well as shortest half-life, while subcutaneous injection leads to a slow release of heroin into the bloodstream and significantly prolonged half-life. This is due to little to no metabolisation taking place in muscle tissue. After oral administration, which is mainly applied in a medical context rather than abuse, hepatic first-pass effect as well as hydrolysis in the duodenum and colon lead to no detectable heroin or 6-monoacetylmorphine (6-MAM) concentration in the bloodstream. However, high morphine levels can be achieved this way.[8]

Following intravenous injection, peak plasma concentrations can be detected after 30 seconds to 2 minutes. The rapid decline with half-life set around 3-4 minutes leads to concentrations of heroin below common detection limits within 10-45 minutes. Despite fast formation of metabolites, heroin is the dominant substance in blood in the first 8 minutes following administration. In the brain, peak heroin concentrations can be found after 1.5-2 minutes followed by rapid metabolisation with a half-life of 1-2 minutes before becoming undetectable after 10-30 minutes, as found in research performed in rat brain.[8]

For inhalation, heroin is either evaporated and inhaled or mixed into tobacco. The chosen route depends mostly on the available form of the substance, as the hydrochloride, which is generally considered the prevailing form, cannot withstand the temperatures needed for evaporation. In contrast to this, freebase heroin can be consumed this way. Both subtypes of inhalation lead to rapid absorption due to heroins lipophilic properties. However, bioavailability is estimated to not exceed 38-53 % if taken via evaporation and as low as 14 % if consumed alongside tobacco. Maximum plasma concentrations can be found after 5 minutes and half-lives of 3-4 minutes were reported.[8]

Insufflation of heroin is mainly used in a replacement treatment context in form of nasal sprays. Compared to injection, absorption is low, resulting in low peak concentration and longer time frames to reach maximum concentration of 4-5 minutes. Additionally, half-life is increased to 5-6 minutes.[8]

Intramuscular and subcutaneous injection results in about double as high peak plasma concentrations compared to insufflation. Additionally, half-life can be increased up to 7.8 minutes using this form of administration. Reason for both these changes being the negligible metabolism inside muscle tissue. As a result, it is mostly used if a longer and less immediate effect is desired. It is however seen as equally likely that most cases of this type of drug admission are due to poor injection practice.[8]

Little data is available for the oral administration route. Maximum morphine concentrations can be achieved faster than after administration of morphine itself.[8]
Once heroin reaches the bloodstream, further distribution and metabolisation takes place. Due to its lipophilic properties derived from the two acetyl groups, heroin is able to cross the blood brain barrier faster than morphine. As a result, onset of effects happen sooner. Relatively low affinity to opioid receptors has led to theories of heroin being used as a prodrug for its metabolites rather than for its own activity. The metabolisation process into 6-MAM and from this into morphine takes place soon after administration and is performed by esterase enzymes. From morphine, multiple pathways can be taken for further metabolisation.[9] Metabolisation into 6-MAM, a substance with an especially high affinity to mu opioid receptors, is taking place in the brain as well.[11]
Heroin itself has a very short half-life of only five minutes in plasma, leading to detection windows of only up to 45 minutes. Analysis of saliva can expand the detection window to up to 24 hours. The excretion of intact heroin via urine is considered highly unlikely due to fast metabolisation in the liver as well as erythrocytes and plasma, while stability in aqueous media is very low.[9] Due to this, heroin itself is not routinely analysed for the identification of possible heroin consumption. Instead, its metabolite 6-MAM is used for this purpose. However, as it is also a degradation product that can be formed outside of the body, only contact with heroin but not its consumption can be verified in this way.

3.1.2. Pharmacodynamics

In general, the substances classified as opioids can be described as drugs acting on opioid receptors. As they all share the amino acid sequence known as the canonical opioid motif, both natural and synthetic opioids can bind to all three classical opioid receptors mu, delta and kappa with varying affinity. These binding sites, responsible for the analgesic action of agonist morphine and its derivatives, can be found on the three main types of endogenous opioid receptors μ-, δ- and κ-opioid receptors (MOP, DOP and KOP).[8,12] Naloxone has been identified as a common antagonist for all binding sites. The biological effect is based on G protein interactions and identical second messenger systems. Those result in an inhibition of the accumulation of cyclic adenosine monophosphate (cAMP),[8] the opening of G protein-coupled inwardly rectifying potassium channel (GIRK) potassium channels[13] as well as the inhibition of voltage gated Ca^{2+} channels and downstream kinases stimulation.[12] As a result, the neuronal excitability through hyperpolarisation is reduced. It can be said that the analgesic effect is based on the inhibition of nociceptive pathways. This general method of action for opioids can be found visualised in Figure 3. Depending on the type of endogenous receptor, biological effects and abuse potential of the respective agonists differ greatly.[11]

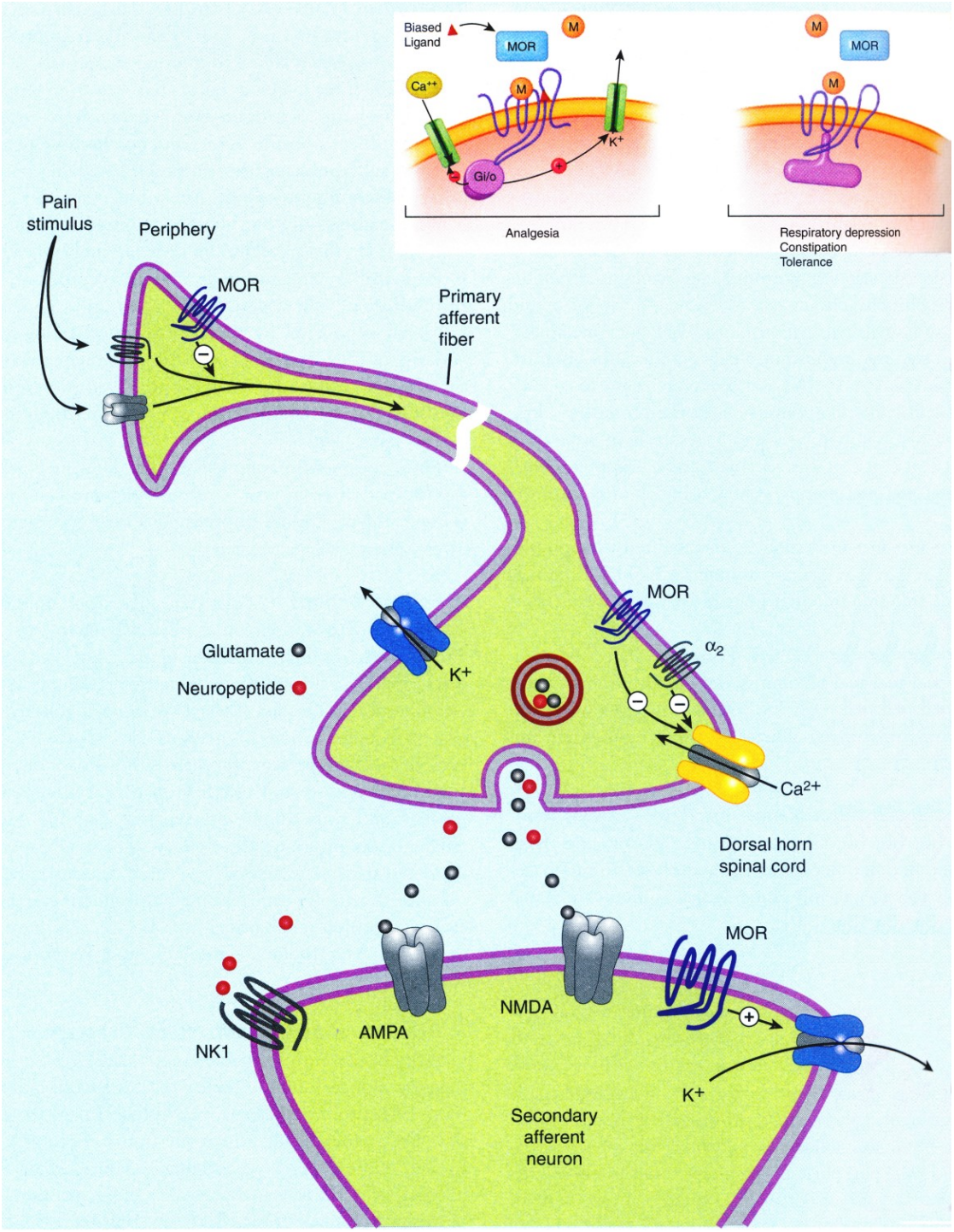

Figure 3: Working mechanism of pain transduction and effects of analgesic drugs. Opioids acting on the μ-opioid receptor (MOR) attenuate the pain stimulus and can inhibit the postsynaptic neuron. Other substances like α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and its receptor or N-methyl-D-aspartate (NMDA) and tachykinin (NK1) receptors can have an influence on this postsynaptic pain response as well. The Inset figures highlight the effects of morphine as an opioid acting on MOR. The analgesic effect is based on coupling of MOR to $G_{i/o}$-mediated inhibition of calcium channels and activation of of potassium channels (left). If instead a coupling to β-arrestin is favoured, side effects like respiratory depression increase (right).
(Source: Vanderah 2024[12])

The affinity of heroin itself to MOP receptors was found to be low in comparison to its metabolites 6-MAM and morphine, due to a lack of free phenolic hydroxyl groups in heroins molecular structure. This can be seen as further indication of heroin use as a prodrug for 6-MAM and morphine, facilitating their transport into the brain amongst other body regions. A comparison of heroin and its metabolites affinity to all opioid receptors shows, that heroin has the lowest affinity to any type. Morphine has the highest affinity of the three for MOP. Regarding MOP, data concerning not only affinity but also efficacy and potency was found. Heroin and 6-MAM showing equal efficacy, surpassing morphine. In context of potency, 6-MAM and morphine share equal levels, while those of heroin are deemed to be considerably higher.[8]

Due to its acetyl functional groups, heroin can easily pass the blood brain barrier. This allows for it to directly affect multiple different brain regions, negatively influencing their functional connectivity. Numerous adverse consequences of heroin abuse have been identified in the past. Some were linked to heroin consumption itself and its toxicity, others are expected to be due to oxygen deprivation as a result of heroins biological action on the respiratory centre. Lesions, oedemas as well as other defects and a general reduction in matter volume were reported alongside other brain abnormalities in the majority of brain regions. An increase in marker proteins for inflammation and local injury was detected in brain tissue of heroin users, likely as a response to the severe brain damage. Another indicator of brain damage is the activation of cell types involved in the immune response typical for neuronal damage. This was shows in immunohistopathological studies focusing on leukoencephalopathie and hypoxic-ischaemic encephalopathy. Unlike other adverse effects on the brain, the reduction of gray matter is reportedly non permanent and can be recovered after just one month of abstinence.[11]

The development of heroin addiction is a highly researched topic. Several biological alterations following consumption have been observed, some being linked to development of strong withdrawal symptoms. Additionally, biochemical changes in the abundance of mu opioid receptors were observed. This is assumed to be involved in the development of heroin addictions, especially as genetic mutations affecting those receptors were observed to increase susceptibility.[11]

For opioids or substances acting on the opioid receptors, especially the *mu*-opioid receptor, a common antagonist was found to reverse effects and ultimately prevent overdoses. The competitive antagonist naloxone binds to unbound receptors and prevents the agonists, opioids, to bind themselves.[10] When administered before or shortly after heroin, naloxone is able to suppress the drugs effect completely. This is further evidence, that an effective treatment is possible and overdoses can be successfully prevented this way.[14]

3.2. Cocaine

Cocaine is a central nervous system (CNS) stimulant drug with local anaesthetic and sympathomimetic properties. It has been widely used for its pharmacological effects but also as a drug of abuse for over 5000 years.[15] The chemical structure of coocaine can be seen in Figure 4.

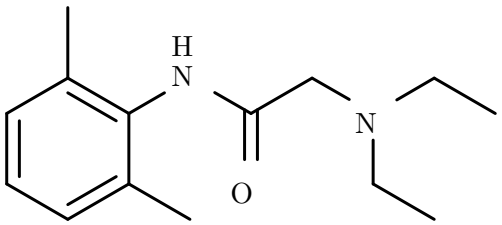

Figure 4: Chemical structure of the stimulant cocaine (Source: cf. Vanderah 2024[12])

First isolation of cocaine was performed by Albert Niemann in 1860.[1,15] However, it has already been used for religious occasions and medical purposes by indigenous populations like the Inca in the Andean region, mostly by chewing the leaves of plants of the *Erythroxylum* genus. Mainly, *E. coca* and *E. novogranatense*, two of the 250 known species are used as a basis for cocaine extraction. Apart from cocaine, the leaves of *E. coca* var. *coca*, which is used for the majority of extraction processes, contain various other alkaloids. These are in general less toxic and less euphoric than cocaine itself, however some can still be found as product related impurities in the finished cocaine product.[1] Apart from extraction processes, cocaine can be obtained via synthesis. The first pathway starting from tropinone was discovered in 1901 by Richard Martin Willstätter. Over time, many other procedures for cocaine synthesis were introduced.[16]
Throughout the late 1800s and early 1900s, cocaine was widely used as a local anaesthetic and pain medication, even with a rising number of addictions and even fatal cases. The medical use for cocaine was limited in 1914 in the US, however, it is still relatively easily available and at low cost, resulting in increased abuse cases.[15] In most countries, it is illegal to transport, sell, or possess cocaine as well as cultivating coca plants. However, there are some exceptions for possession of amounts deemed as personal use, mostly around 2 g or less.[1]

3.2.1. Pharmacokinetics

The majority of consumers prefers the powdered form of cocaine hydrochloride via intranasal administration, especially in a more social or nightlife context. It can also be consumed orally and intravenously, as it is soluble in water. However, the inhalation of the free base, known as crack cocaine, can more often be observed in a frequent abuse as well as poly-drug abuse patterns. Admission through the respiratory system lead to an onset of effects after 6 to 8 seconds. Smoking or inhalation showing faster drug uptake as well as higher peak plasma concentrations, with a bioavailability of 90 %. For the intranasal route, vasoconstrictive properties of cocaine result in a slower uptake leading to a delay in reaching peak plasma concentration of up to 60 minutes and reduced bioavailability of 80 %. Both routes are more commonly used over other forms of administration like intravenous injection due to their ability to reach brain circulation faster. Oral administration or application of cocaine hydrochloride to mucous membranes is also possible. However, especially oral administration results in low cocaine concentrations compared to the respiratory pathways. Reason for this being gastric breakdown and metabolism. Onset of drug effects via oral administration and the longest duration between 1 and 2 hours are also observed in this context. Similarly, chewing powdered leaves of the coca plant results in peak plasma concentrations after 0.4 to 2 h. Finally, preparation of a tea made from coca leaves can be used as a route of administration resulting in the lowest amounts of consumed cocaine.[1]

Once administered, cocaine distribution inside the body takes place rapidly. Highest concentrations can be found in the brain as well as kidney, spleen and lungs, followed by blood, heart and muscle tissue. As average half-life of cocaine 40 to 90 minutes have been reported.[1]

There are several metabolic pathways for cocaine inside the human body. pseudocholinesterase (PChE) in plasma and carboxylesterase type 2 in the liver are responsible for the formation of ecgonine methyl ester (EME). The formation of benzoylecgonine (BE) by carboxylesterase type 1 takes place spontaneously under physiological pH or in the liver. Both main metabolites BE and EME, undergo further metabolisation into ecgonine and are pharmacologically inactive.[1] The N-demethylation of cocaine by CYP450 and CYP3A4 leads to formation of norcocaine, which is able to cross the blood brain barrier and is considered hepatotoxic. Additionally, norcocaine is inhibiting noradrenaline uptake by brain synaptosomes more effectively than cocaine itself. Several other minor metabolic pathways are possible, however they make up a minor part of cocaines metabolisation process. In case of co-administration of other substances, specific metabolites of both can be found. This will be further discussed in the co-administration and drug interaction section found below.[1]

Cocaine as well as the main metabolites described above are mainly excreted through urine. Longest detection times after consumption were found for EME at up to 164 h after a dose of 40 mg.[1]
In analytics, metabolites are often used as markers for consumption in addition to the drug itself to verify a passage through the body took place rather than only brief contact. For cocaine, the main metabolites are routinely used for this purpose, while minor metabolisation products are mostly not referred to due to low concentrations being present in blood urine or hair. Of the considered analytes, highest concentrations can generally be found in urine, leading to this being the preferred type of sample used.[1]

3.2.2. Pharmacodynamics

Cocaine has an influence on many biological processes inside the human body, leading to a multitude of perceived effects. While some occur due to a chronic abuse pattern, others have been found to arise after acute abuse or even a single dose of cocaine.[1]
The local anaesthetic properties are the result of cocaine mediated stabilization of voltage gated sodium channels in their inactive state, which stops the sodium transport into the cell, effectively blocking the polarization process.[1] As cardiac sodium channels are also affected, this is one of the main mechanism causing cocaines cardiotoxic properties.[16] Intracardial signal conduction is negatively affected, ultimately causing dysrythmia.[1] Another aspect is the vasoconstrictive nature, often leading to severe damage on blood vessels. Most fatal overdoses are due to these symptoms.[16]
For the psychostimulant and sympathomimetic properties, setting it apart from other local anaesthetics, multiple involved transport processes were identified. Due to cocaine consumption, the presynaptic transporters for serotonin, dopamine and noradrenaline reuptake are blocked, resulting in an increased concentration.[1,12,15] This process can be found visualised for norepinephrine in Figure 5, representing the process for all three discussed biogenic amines.[12]

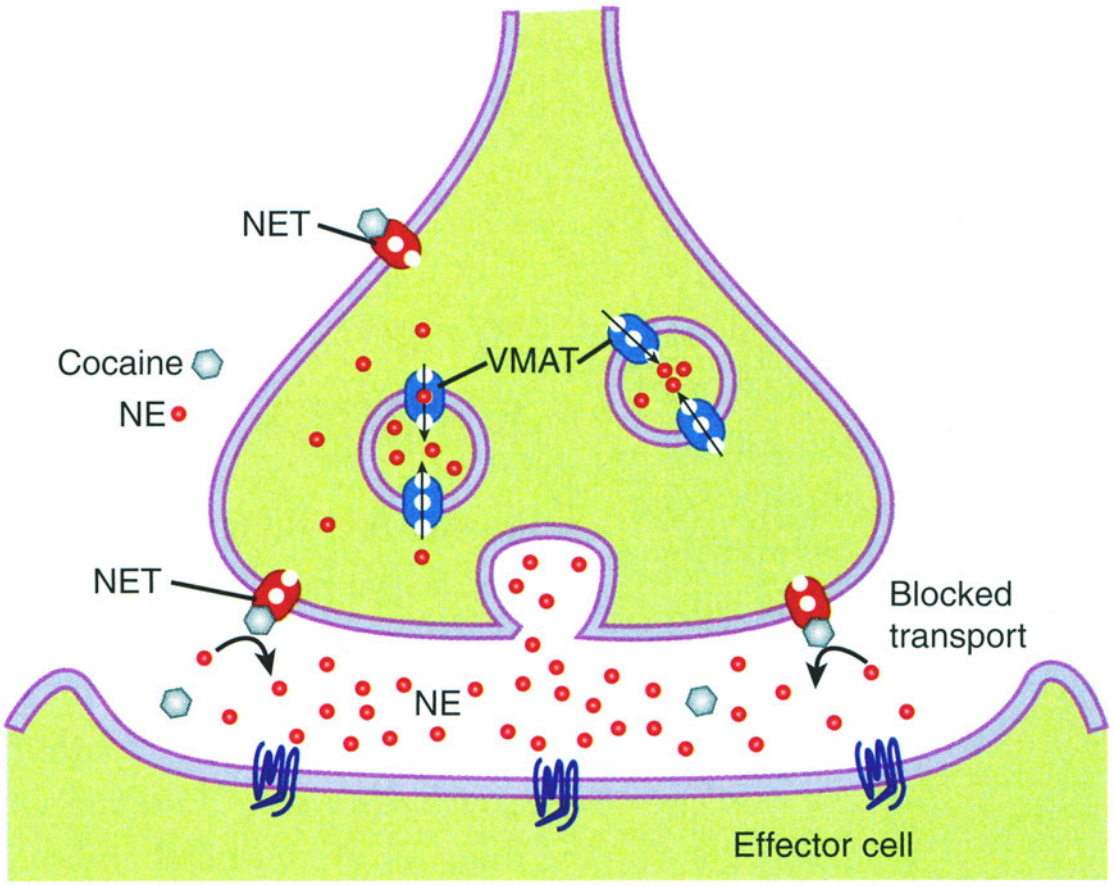

Figure 5: Cocaines effect on monoamine transporters, exemplary illustrated for norepinephrine (NE) and norepinephrine transporters (NET) as well as vesicular monoamine transporter (VMAT). (Source: Vanderah 2024[12])

In the case of dopamine, this results in an overstimulation of dopaminergic postsynaptic receptors causing an euphoric rush. Over time, a decrease in the number of dopamine receptors D2 and D3 can be found as well as an increase in the presynaptic dopamine transporter DAT. Therefore, tachyphylaxis occurs and a higher dosage of cocaine will be needed in the future to achieve the same effect as it initially had.[1,15] Similarly, the reuptake of serotonin is blocked. The resulting increase in serotoninergic activity may induce seizures and significantly enhances the addictive potential of cocaine. Lastly, noradrenaline reuptake is blocked in a comparable manner. The adrenergic response is heightened due to the increased stimulation of α and β andrenergic receptors. This effect has been suggested to be the reason for cardiotoxic and vasoconstrictive properties of the drug. The induced increase in the vasoconstrictor endothelin 1 while simultaneous reduction of the vasodilator nitric oxide is a key factor in vasoconstriction of the coronary arteries. As a result, blood pressure and heart rate increase significantly while oxygen supply to tissues decreases.[1]

The activation of dopaminergic and serotoninergic receptors has been suggested as the underlying mechanism for the hyperthermia that can occur as a consequence of cocaine abuse. Increased levels of dopamine, serotonin and noradrenaline in hypothalamic thermoregulatory centre were found to result in an imbalanced heat production. Further aggravated by cocaine induced hyperactivity and impaired heat distribution due to vasoconstriction limiting dermal blood flow, body temperature of users can reach high enough levels to cause a heat infarct.[1] Of special interest are the effects of cocaine on the brain. The influence on dopaminergic and serotoninergic pathways as well as noradrenalin lowers seizure threshold even after first consumption. Chronic low intensity stimulation of the limbic system, also known as kindling, is involved in this process. It was found to be caused by heightened expression of the tumor-suppressor gene p53 in brain tissue.[1]

While effects on the cardiovascular system and brain tissue occur independently from the form of administration, any pathway involving the inhalation of cocaine specifically influence the respiratory system. Local irritant effects of cocaine can induce bronchospasms. Moreover, during evaporation, toxic pyrolysis and combustion products of cocaine are formed. Adulterants added before consumption also increase toxicity. Potentially fatal symptoms like pneumothorax or pulmonary haemorrhage, among others are associated with cocaine inhalation and smoking.[1] General effects of cocaine on the lung include those of inhalation, but also secondary pulmonary complications like a predisposition to infections or pulmonary infarction. Moreover, many users of cocaine experience the production of black sputum containing blood and carbonaceous flecks. Burns throughout the airways frequently occur in cocaine smocking individuals as well. All of these mentioned symptoms worsen with chronic consumption.[15]

Most of cocaines toxic effects on other organ structures is generally attributed to its vasoconstrictive properties reducing blood flow to the respective tissue leading to necrosis and formation of ulcers, among others.[15] As cocaine passes through the body, metabolisation is taking place in the liver, leading to hepatotoxic effects. Inflammation and hepatocellular necrosis are the most prevalent results of cocaine consumption in this context. Bioactivation of cocaine by cytochrome P450 (CYP450) and a resulting inhibition of hepatic metabolism were found to be strongly related to hepatotoxic properties. The metabolites that are formed in the liver are highly reactive, irreversibly binding cellular proteins and subsequently inducing cell death. Another aspect of this metabolisation is the formation of free radicals, which significantly increase oxidative stress. Mitochondrial respiration processes can be blocked by cocaines metabolites as well, affecting intercellular adenosine triphosphate (ATP) levels. Both of these effects eventually lead to cell death.[1] Additionally, cocaine is able to modulate the subunit expression of NMDA, altering their distribution and communication pathways with D1 dopaminergic receptors. An affinity to σ-opioid receptors, type 1 more prevalent than type 2, heightens the risk of seizures.[1]

In general, cocaine as well as some of its metabolites are able to cross the blood brain barrier, leading to the brain being especially susceptible to the drugs toxic effects. It has furthermore been suggested that there could be an additional functionality as DAT inverse agonist. This would result in cocaine reversing the transport direction of DAT. However, this needs further research and is based on similarities in symptoms to other substance acting this specific way on the receptor.[1]

3.3. Co-Administration and Drug Interactions

The pharmacodynamics of any drug can be influenced greatly by the presence of other pharmacologically active substances. These can either be administered shortly before or after the drug itself or mixed into the substance before consumption. In the first case, this is refered to as co-administration, co-consumption or poly-drug use, while the latter is reffered to as adulteration. The interaction of these substances can lead to various effects, incuding changes in the working meachanisms related to affected metabolic pathways. In some cases, unique metabolites of the substances can be formed, which have the potential to increase toxicity in different ways than the drug itself.[1]
Heroin is often combined with substances acting in a similar way, especially other opioids. In general, the consumption of multiple opioids is known to cause severe respiratory depression, which has been found to be the most common reason for overdose induced deaths.[17]
Additionally, other substance classes are also known to be taken frequently alongside heroin. The combination of opioids with psychostimulants leads to the symptoms of the stimulant like myocardial infarction being masked and less likely to be recognised in time. Specifically the combination with cocaine can result in an increased risk of neurotoxicity and behavioural change.[17]
Cocaine is also often consumed alongside other substances. Although unlikely as an adulterant, co-administration of methylenedioxymethamphetamine (MDMA) leads to a significant increase in MDMA plasma concentration as the respective metabolism is slowed down. Reason for this is cocaine inhibiting the related hepatic CYP450 isoenzyme CYP2D6. Additionally, MDMA is known to induce a serotonergic effect, which is enhanced due to cocaine acting as a serotonin reuptake inhibitor.[17]
The simultaneous consumption of cocaine and amphetamines has the potential to cause significant increases of norepinephrine but most importantly dopamine concentrations in the synaptic cleft.[17] While mechanisms of action of the substances differ, one substances effect is hightened in the presence of the other. As stated above, cocaine is a reuptake inhibitor for dopamine, among others. Amphetamines however strongly increase nonvesicular dopamine release by acting as a substrate to dopamine transporters.[12] This process is shown in Figure 6.

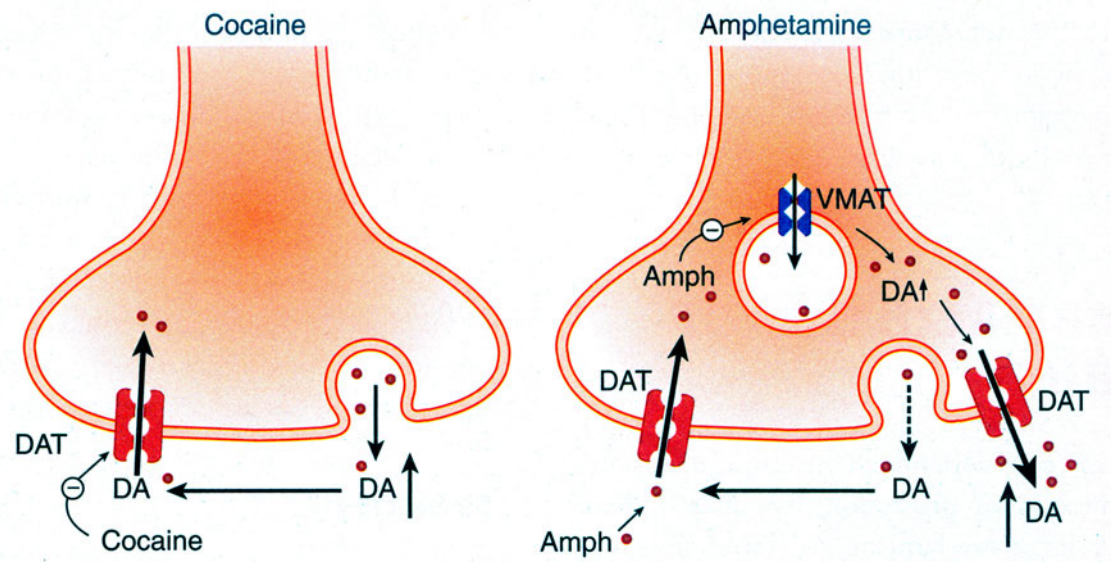

Figure 6: Cocaines effect on dopamine transporters (DAT) and resulting dopamine (DA) concentration in the synaptic cleft (left). Amphetamines (Amph) acting as substrate on DAT, inhibiting transport of DA. Inside the cell, amphetamiines then impedes the filling process of synaptic vesicles by interfering with vesicular monoamine transporters (VMAT). This ultimately results in a reversal of transport direction for DAT and an increased release of DA. (Source: Vanderah 2024[12])

If taken alongside CNS depressants, synergistic effects suppress the activity of the respiratory centre.[17]

Substances that counteract cocaines stimulant effects are commonly combined with it, aiming to prolong the duration of the effects. Mainly alcohol and heroin are used in this context.[1] If alcohol is consumed at the same time as cocaine, a transesterification product of both, cocaethylene (CE), is formed in vivo. Contrary to the others, this substance was found to be pharmacologically active. It can lead to graver acute toxic reactions as well as being generally considered more lethal than cocaine itself. Reason for this is the higher selectivity of CE for DAT compared to cocaine. It leads to enhanced effects on the CNS and an increase in heartrate and blood pressure. Longer duration of these effects is due to a longer half-life. When CE is present, the formation of the otherwise main metabolite BE is inhibited.[1]

The combined consumption of cocaine and heroin is generally referred to as speedball.[1] The duration of biological activity is increased as both drugs bind to the same enzymes during their metabolic pathways, causing competitive inhibition.[1] If combined with opioids, the number of cocaine related fatal overdose increases significantly. This is most likely due to the masking of cocaine induced symptoms of myocardial infarction. The risk of neurotoxicity and behavioural change is increased as well.[17]

3.4. Adulterants in Drugs of Abuse

In contrast to co-administration, there are other ways for multiple substances to take effect inside the body at the same time. As mentioned above, other substances than the illicit drug itself can be found mixed into the finished product prior to consumption. Compounds that are present due to production related reasons are mostly referred to as (product related-) impurities. These include precursor molecules, metabolisation products or specific chemicals needed during synthesis. Mostly, their presence is rather considered tolerated than desired. However, other substances that are deliberately added to drugs of abuse can serve various purposes. While bulking agents like sugars are predominantly used to increase weight and improve handling of sold drugs, others are added for their biological activity. These adulterants are as numerous as their effects, ranging from mimicking properties of the substance they are added to in order to mask poor quality to a pharmacological effect either enhancing or changing those of the drug. Depending on the form of administration, some can even be added in order to facilitate evaporation for smoking or lower the melting point if injection is preferred. In general, it can be said that adulteration is performed following a thoughtful process instead of the pure bulking that is widely assumed to be the reason for cutting of illicit substances. Additionally, the investigation of adulteration in seized drugs from various countries and comparison of their composition indicate, that adulteration is performed at the production site or early in the distribution chain already. At street level, almost no adulteration or cutting could be detected.[3] Depending on the specific drug and its working mechanism, different adulterants are used. While it is mostly established to enhance the drugs effects by adulteration, in some cases other substances can be used to mask a substances adverse side effects.[6]

Apart from identifying the specific drugs exact composition in an attempt to better understand and investigate trading routes, it is also crucial in a different context. The same effects described above as drug interactions are occurring in adulterated drugs, as mentioned before. While this is desired in some cases, it can lead to serious health concerns if taking place unbeknownst to the consumer.[5] Especially if a drug was adulterated to enhance perceived quality of a product containing less of the proclaimed substance than expected this can be the case. Regarding the consumption of pharmacologically active substances, being unaware of the presence of others that modify the body's response significantly increases the risk for fatal consequences. These can either occur immediately or delayed and as a result of chronic consumption or abuse.[17] In both cases, but especially the latter, knowing the exact composition of the consumed final drug product can help understanding occurring symptoms as well as enabling the application of a more precise medical therapy.

3.5. LC-MS/MS

The abbreviation LC-MS/MS is used to describe liquid chromatography tandem mass spectrometry analytics. A separation using a liquid chromatography column is performed and resulting fractions are injected into a triple quadrupole mass spectrometer. In the following, the basic procedures will be explained while implementing the abbreviations as utilised by the used software

For the liquid chromatography, a column has to be chosen to retain all selected analytes. As in this case a general analysis of numerous substances is to be performed, a less specific column is to be preferred to make sure that no relevant substances are washed out due to a lack of binding capacity of the column. This also plays a significant role in the choice of mobile phases used. During modification of the existing chromatographic methods used in routine analysis, it is crucial to achieve a clear separation from the other substances. If necessary, this can be done by modification of the implemented gradient of mobile phases or their composition.[18,19]

In the first phase of the chromatographic run, the analytes are bound to or retained by the column. Depending on chemical structure and size, the retention time of each substance differs. Ideally, as many interfering substances as possible are washed out of the column at this time. As a next step, the composition of the mobile phase is changed gradually. This results in the molecules of interest being removed from the column again after their respective retention times.[18,19]

After separation by chromatography, the sample fractions of interest are injected into a mass spectrometer. In this case, tandem mass spectrometry is used for higher selectivity and specificity. This version of mass spectrometry is performed using a triple quadrupole spectrometer. It consists of three separate quadrupoles. Using a declustering potential (DP) chosen to purify the analyte ions as best as possible from solvents, high enough to prevent adducts of sodium or other components to form clusters with the analyte substances, but not high enough to cause fractions of the molecules to break off prematurely. After electrospray ionisation, the ions of the analytes are transported into the first quadrupole, where an electric field is used to separate the target ions from the others. Only those are then further processed in the second quadrupole. In this step, an inert gas is used as collision gas to break apart the selected substances into their characteristic fragments. It is crucial to choose the right collision energy (CE) to make sure the fragmentation pattern is not disturbed. With the cell exit potential (CXP), those chosen characteristic fragments are transported into the third and last quadrupole. Here, the final selection step of target fragments is performed before they are finally led to the detector and the mass spectrum of the substance is created. This process can be performed for multiple substances at once, which is then referred to as multiple reaction monitoring (MRM).[18]

To identify a substance, several of the visible peaks are to be taken into consideration. First, the molecular ion or parent peak, represents the mass of the complete compound. For compounds with a single charge, the mass to charge ratio (m/z) of this peak corresponds to the molecular mass plus or minus the mass of one hydrogen atom $[M+H]^+$ or $[M-H]^-$, a pseudo-molecular ion.[18] Peaks of fragments, also referred to as daughter peaks, can be found at lower m/z values. The specific patterns formed by these peaks are characteristic for each substance and can be used for identification.[18] When looking at a substances' mass spectrum, the two peaks of high intensity are chosen as quantifier or target and qualifier ion transition for routine analysis. The target is then used to quantify the concentration of the molecule in the sample while the qualifier is used to verify the found compound to be the substance of interest. In routine evaluation, both signals are expected to occur in a specific ratio often referred to as ion ratio to ensure none of the peak areas is enhanced or reduced by interfering factors. In routine analytics, peaks of higher m/z, which represent larger fragments of the substance, are preferred for identification. As fragments of a molecule are analysed, they are often referred to as ion transitions from the parent to the fragment peak. In order to be able to detect multiple substances at the same time, the mass spectrometer is used in multi reaction monitoring mode.

In the case of cocaine, fragment peaks with an m/z of 182.20 Da and 105.10 Da are chosen for quantification and identification, respectively. Parameters for identifying metabolites can be found in the appendix. Heroin can be identified using LC-MS/MS as well. However, due to its short detection window in the chosen sample matrices, the metabolite 6-MAM is more frequently used to verify contact with heroin. 6-MAM is quantified and identified using fragment peaks with an m/z of 152.00 Da and 165.20 Da.

3.6. Statistic Evaluation

All analysed samples were evaluated regarding detected substances. Due to a very large amount of data being present for past samples, a limited evaluation was performed. Among all analytical methods that showed a positive result, their total number of occurrence as well as their relative frequency in combination with either heroin or cocaine was calculated. It was then evaluated which of the found substances could be deemed a potential adulterant rather than co-consumption. In this context, results of the literature research were used to provide substances or substance classes of interest.
For substances that were added to the analytical methods, not only their relative frequency was calculated but additional evaluation of the found concentrations was performed. To determine trends within the found concentrations, parameters such as mean[18] and median values were calculated.

$$\overline{x} = \frac{\sum x_{\mathrm{i}}}{N} \tag{3.1}$$

with $\overline{x}$ = arithmetic mean,
x_{i} = individual measurement values
and
N = number of measured values.
The mean and median values were calculated using the respective function in Microsoft Excel. The results could then be used to evaluate the concentration distribution of the measured samples.

3.7. Validation of Added Analytes

In order to reliably evaluate the measured results, a validation of important parameters was performed regarding the added substances. The procedures were based on the regulations of the GTFCh as described by Peters et al.[20,21]

3.7.1. LOD and LOQ

To determine the lower limits of detectable concentrations, the following calculations needed to be performed based on measurement results of samples around the expected concentration range[21].

$$X_{\mathrm{NG}} = s_{\mathrm{x_0}} \cdot t_{\mathrm{f},\alpha} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\overline{x}^2}{Q_{\mathrm{x}}}} \tag{3.2}$$

$$X_{\mathrm{BG}} = k \cdot s_{\mathrm{x_0}} \cdot t_{\mathrm{f},\alpha} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(X_{\mathrm{LOQ}} - \overline{x})^2}{Q_{\mathrm{x}}}} \tag{3.3}$$

with
$k = 3$ = relative uncertainty of measurements;
$s_{\mathrm{x_0}}$ = standard deviation of the method;
$t_{\mathrm{f},\alpha}$ = quantile of the t-distribution;
$\alpha = 0.01$ = significance level;
m = number of analyses per sample;
n = number of measurements;
$\overline{x}$ = mean value of concentrations
and
$Q_{\mathrm{x}} = \sum(x - \overline{x})^2$

The limit of detection is the lowest concentration that can be found using the applied analytical method, while the limit of quantitation is the lowest concentration that can reliably be quantified. In case the limit of quantification is calculated to be lower than the limit of detection, it has to be assumed at the same, higher value.[20,21]

3.7.2. Accuracy

Accuracy is an important parameter of analytical methods. It is composed of the deviation of individual measurement results due to systematic and random errors. A systematic error is generally determined through calculating the bias as a reference for the measurements trueness. For random errors, precision is determined by calculating both intermediate precision ($RSD_{(\mathrm{T})}$) and repeatability (RSD_{r}). In order to do so, the parameters of repeatability variance (S_{r}^2) and variance between days (S_{t}^2) need to be calculated. Lastly, the standard deviation could be utilised to express the methods imprecision.[21]

To obtain the measurement data needed to perform these calculations, samples of a predetermined concentration needed to be prepared and analysed at least six different times in duplicates. It was crucial among these measurements, that one or more factors differ in between samples. For this thesis, sample preparation was performed on different days as well as by different people. The following equations based on the guidelines published by the GTFCh[21,22] were then used to determine the required values.

$$Bias[\%] = \frac{\overline{x}_{\text{Total}} - \mu}{\mu} \cdot 100\% \tag{3.4}$$

$$S_{\text{r}}^2 = \frac{\sum(x - \overline{x}_{\text{Day}})^2}{N_{\text{Days}} \cdot (N_{\text{Measurements/Day}} - 1)} \tag{3.5}$$

$$S_{\text{t}}^2 = \frac{\sum(\overline{x}_{\text{Day}} - \overline{x}_{\text{Total}})^2}{N_{\text{Days}} - 1} - \frac{S_{\text{r}}^2}{N_{\text{Measurements/Day}}} \tag{3.6}$$

$$RSD_{(\text{T})}[\%] = \frac{\sqrt{S_{\text{t}}^2 + S_{\text{r}}^2}}{\overline{x}_{\text{Total}}} \cdot 100\% \tag{3.7}$$

$$RSD_{\text{r}}[\%] = \frac{\sqrt{S_{\text{r}}^2}}{\overline{x}_{\text{Total}}} \cdot 100\% \tag{3.8}$$

$$U_{\text{extended}} = 2 \cdot \sqrt{\frac{Bias^2 + RSD_{(\text{T})}^2}{RSD_{\text{r}}}} \tag{3.9}$$

with
x = measured value;
$\overline{x}_{\text{Day}}$ = mean value of measurement day;
$\overline{x}_{\text{Total}}$ = mean value of all measurement days
and
μ = target value.

Resulting percentages should range from -20 to 20 % in proximity to the LOQ while -15 to 15 % should not be exceeded above that concentration range. For the extended measurement uncertainty, a value below 30 % is to be achieved.[21]

3.7.3. Matrix Effects, Recovery and Ion Suppression

As the analytes was to be measured within human urine samples, the effects this matrix has on the measurement results needed to be determined. To do this, analytes were measured six times following three ways of preparation. First, they were spiked into the mobile phase used in the LC-MS/MS method with no further preparation, which will be referred to as external (A) during calculations. Secondly, blank samples were prepared using the regular sample preparation steps and spiked with the analytes afterward, referred to as external + Matrix (B) during calculations. Lastly, samples were spiked prior

to preparation and measured, which was named as internal (C) for calculations.[21] After analysis, the areas of resulting peaks were evaluated using the following equations. Each analytes recovery (RC), relative signal intensity (ME) and seeming signal yield (ALL) are calculated separately[21].

$$RC = \frac{internal(C)}{external + Matrix(B)} \tag{3.10}$$

$$ME = \frac{external + Matrix(B)}{external(A)} \tag{3.11}$$

$$ALL = \frac{internal(C)}{external(A)} \tag{3.12}$$

Ideally, low matrix effects and high recovery were to be achieved. Values should be as close to 100 % as possible. For RC and ALL values, a range from 50 to 150 % should not be exceeded, while ME values should not fall below 75 % or above 127 %. A standard deviation of 25 % is not to be exceeded.[21]
Low RC values indicate a low yield through sample preparation, which should be optimised as a result. Ion suppression or enhancement can be seen in the ME value. Ion suppression describes an effect in the detection process when interferences at the elution time of the analyte that reduces the perceived signal intensity. Similarly, an ion enhancement is a perceived boost of the analyte signal.[21] While low amount can be tolerated, high derivations should be investigated and the analytical procedure altered to reduce these effects.

3.7.4. Linearity

In quantitative analysis, linear regression is often used to calculate concentrations based on a calibration. Therefore, during method validation it is important to be made sure that the peak area increase can be described by a linear function[20]. Prior to measurements, a realistic range was estimated and samples equally distributed within were prepared. The resulting peak areas were then evaluated using the following equations.

$$\alpha = \frac{\sum(x - \overline{x})(y - \overline{y})}{\sum(x - \overline{x})^2} \tag{3.13}$$

$$b = \overline{y} - a \cdot \overline{x} \tag{3.14}$$

From which the linear equation of the form $y = a \cdot x + b$ was derived.

The individual measured points were then compared to the resulting linear plot and their fit evaluated. The linear range of the analytical method for each individual substance was determined accordingly so that a linear correlation between peak area and concentration can be assumed.

4. Material and Methods

4.1. Literature Research

A literature research was performed using PubMed, Google scholar and the HAW library catalogue HIBS. The keywords searched for were mainly illicit drugs adulterants, drug impurities, impurity profiling, cocaine impurities, heroin impurities, adulterants in cocaine/heroin, determination of heroin and adulterants, drug interactions heroin fentanyl nitazene, cutting of cocaine and heroin, fentanyl in heroin, drug interactions heroin, working mechanism heroin, impurities in heroin, impurities in cocaine, pharmacological effect of impurities in cocaine, among others.
The focus chosen for the used sources were the identification of adulterants of interest in cocaine and heroin as well as possible synergistic effects in combination with the illicit drugs. As a result, pharmacologically active substances are of interest rather than biologically inert ones.
Considering the analytical method was to be applicable for the use on human urine samples, substances of interest were to be measurable in these as well as unlikely to be present if no consumption of the illicit drugs of interest took place.
It was also to be considered whether alternative sample preparation and LC-MS/MS methods could be found. However, the addition of adulterants to existing methods was to be preferred to minimize the increase in workload.

4.2. LC-MS/MS Analytics

Most sources for analytical methods relied on gas chromatography-mass spectrometry (GC-MS)[7,23,24] analysis. However, in this thesis, LC-MS/MS was chosen due to its higher sensitivity and specificity. As methods for this kind of analytics were already implemented in the routine, those were used as a basis for modification. Adulterants of interest, that were identified during literature research, were to be implemented with as little increase in workload as possible. Hence, first approaches were aimed at detecting these substances using existing methods.
After the identification of relevant substances, their respective mass spectra were recorded with the used MS system. Then, suitable peaks were chosen to be used as quantifier and qualifier during later evaluation.

4.2.1. Materials

In the process of method development as well as sample preparation, the following Material shown in Table 1 were used.

Table 1: Chemicals and substances used for experimental aspects of this thesis.

Substance	Concentration or Quantity	expiry date	Supplier
Levamisole	100ng/mL in ACN	18.08.2029	DR. Ehrenstorfer via LGC
Aminorex	10mg neat, dissolved to 1 mg/mL in ACN	08.2026	TRC via LGC
Procaine	10mg neat, dissolved to 1 mg/mL in MeOH	21.04.2030	Caesar & Lorentz GmbH
Morphine-d3	1 mg/mL	31.10.2027	LGC
Benzoylecgonine-d3		31.12.2025	LGC
Methadone-d9		01.06.2021	LGC
Buprenorphine-d4	100 μg/mL	30.01.2029	LGC
Norbuprenorphine-d3	100 μg/mL	30.10.2028	LGC
Fentanyl-d5		31.03.2025	LGC
QC samples			
DCT-A	10.5 ng/mL Lidocaine	03.2027	ACQ Science
DCT-B	102.0 ng/mL Lidocaine	03.2027	ACQ Science
DCT-C	1134.0 ng/mL Lidocaine	03.2027	ACQ Science
Chemicals			
Acetonitrile ULC/MS grade			Biosolve
Acetic acid 99-100 %			J. T. Baker
BG Turbo - High efficiency recombinant β-Glucuronidase			Finden Kura
Disodium hydrogen phosphate			Merck
Fumeric acid 98-100 %			Supelco
Methanol			Biosolve
Potassium dihydrogen phosphate			Merck
Water			Biosolve
2-Propanole			Supelco Lichrosolv

Every solution used was made from these components. It was made sure to work with LC-MS grade material only. In addition to bought pure water, the filtration system mentioned below in Table 3 was used to produce pure water. For sample preparation, an internal standard solution is prepared containing 2 μg/mL morphine-d3, benzoylecgonine-d3 and methadone-d9 as well as 1 μg/mL buprenorphine-d4 and norbuprenorphine-d3 and lastly 0.2 μg/mL fentanyl-d5 dissolved in methanole. 10 mL of this solution is diluted in 200 mL of water to create the IS-water mix used in sample preparation. A phosphate buffer with a pH of 7 was prepared using potassium dihydrogen phosphate and disodium hydrogen phosphate.. To 30 mL of this, 2.5 mL of the BG Turbo enzyme solution was added to create the buffer-enzyme mixture. The mobile phase added to the samples consists of 15 % methanole and 85 % of 0.5 % acetic acid, while the gradient on the measurement systems was performed using methanol as organic phase and 0.1 % fumeric acid as aqueous phase.
While the internal standard solutiuons contained several deuterated substances, only benzoylecgonine-d3 was used as reference for the added analytes during evaluation.
As there was no pure lidocaine standard substance available at the time of measurements, the bought serum QC solutions "drug confirmation tests in Serum" (DCT) were used instead. Prior to any quantification of measured samples, a calibration was measured containing all analytes of interest. The corresponding stock solution was prepared using the pure substances stated above as well as the bought control solution used for lidocaine. Samples of the concentrations shown in Table 2 were produced.

Table 2: Concentrations of new analytes in prepared calibration and quality control (QC) samples.

Name	Concentration of analyte [ng/mL]			
	Levamisole	Aminorex	Lidocaine	Procaine
Calibration 1	5.00	0.50	0.11	0.50
Calibration 2	20.00	1.00	0.51	1.00
Calibration 3	50.00	5.00	1.28	5.00
Calibration 4	100.00	10.00	5.10	10.00
Calibration 5	250.00	20.00	28.35	20.00
Calibration 6	500.00	50.00	56.70	50.00
Quality control (QC) low	10.00	5.00	5.10	5.00
Quality control (QC) high	200.00	20.00	51.03	50.00

4.2.2. Instrumentation and Software

For the analytical aspects of this thesis, the equipment and software shown in Table 3 was used.

Table 3: Laboratory equipment and software used for experimental aspects of this thesis and evaluation of results.

Laboratory equipment	Supplier
Centrifuge "Mikro 220"	Hettich
Centrifuge "Rotana 460 R"	Hettich
Multipettes "E3"	Eppendorf
Pipettes "Reference 2" 10-100 μL and 100-1000 μL	Eppendorf
Pipette "Reference" 0.5-10 μL	Eppendorf
Liquid handling platform "Freedom evo"	Tecan
Thermomixer "MHR 23" & "MKR 23"	HLC Analytics
Variable temperature sealer	Vitl
Water filter system "Milli-Q Q-POD"	Merck
Water filter "Millipak 0.22 μm"	Merck
LC-MS/MS Systems	
Autosampler "RSI 850"	CTC Analytics
Autosampler "Chronect robotic RSI"	Axel Semrau; CTC Analytics
Chromatography column "Kinetex Biphenyl 100x3 mm 2.6 μm"	Phenomenex
Chromatography column "Kinetex Biphenyl 50x2 mm 1.7 μm"	Phenomenex
Column oven "CTO-20AC"	Shimadzu
Degassing unit "DGU-20A5R"	Shimadzu
LC system "1260 Infinity 2"	Agilent
LC pump "LC-20AD XR"	Shimadzu
Mass spectrometer "Triple Quad 6500+"	Sciex
Mass spectrometer "QTRAP 5500"	AB Sciex
Mass spectrometer "Triple Quad 5500+ QTRAP ready"	Sciex
Software	
Mass spectrometry data aquisition software "Analyst" Versions 1.7.1 and 1.7.2	AB Sciex
LC-MS/MS control software "Chronos"	Axel Semrau
LC-Pump control software "Clarity"	Axel Semrau
Data processing software "Sciex OS" Version 3.0.0.3339	AB Sciex
Calculation software "Excel" Version 2508 Build 19127.20240	Microsoft

For this thesis, the two measurement systems used will be refered to as system one and two. System one consists of autosampler RSI 850 and MS device QTRAP 5500 coupled with the individual LC components by Shimadzu listed above. System two however uses the Triple Quad 5500+ QTRAP ready mass spectrometer coupled with the LC-system by agilent and the chronect robotics RSI autosampler listed in Table 3. For chromatography columns, Kinetex Biphenyl 100x3 mm 2.6 μm is used for system one and Kinetex Biphenyl 50x2 mm 1.7 μm for system two. During the tuning process to determine the potential fragment masses of levamisole and aminorex, a triple quad 6500+ device was used.

4.2.3. Sample Preparation

For the analytical aspects of this thesis, the methods currently used for routine analysis for opiates, opioids and cocaine was chosen to be the most suitable starting point, as most of the substances of interest were already included. Both heroin and cocaine as well as identifying metabolites and some of the common adulterants could already be detected simultaneously this way. Details concerning preparative an analytical steps shown in Figure 7 will be further described in the following.

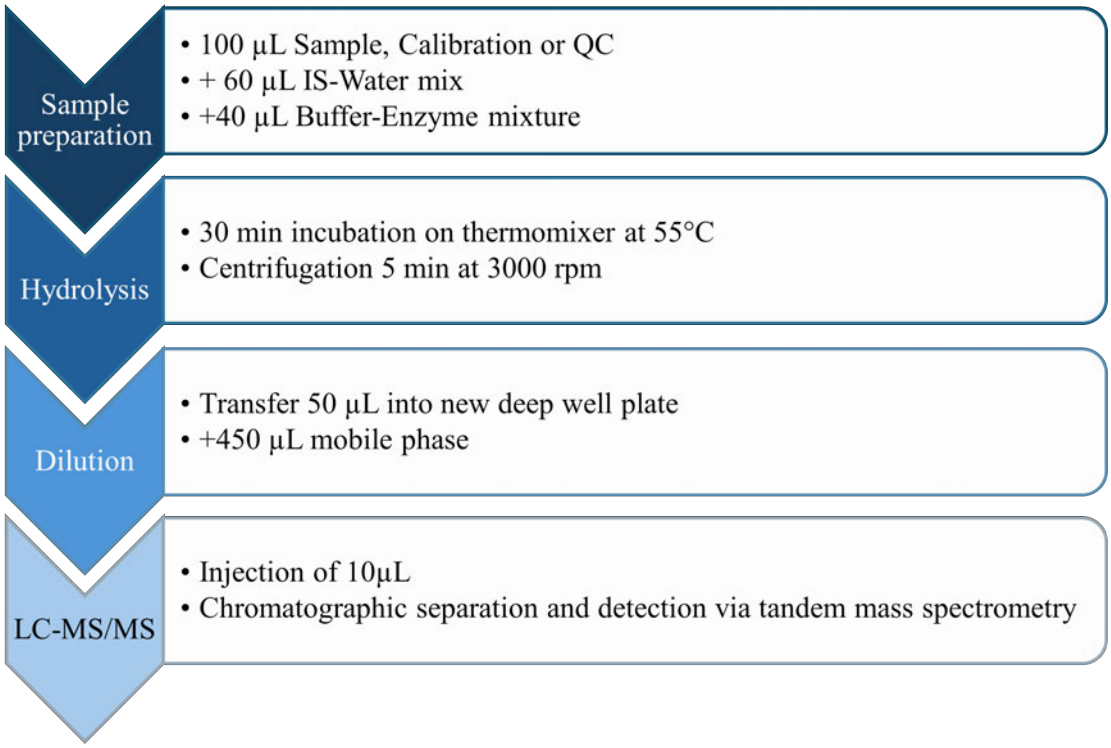

Figure 7: Performed steps during the sample preparation process.

Regarding the preparation of urine samples, it was found that a dilute and shoot approach was sufficient for identification of the added substances. For this purpose, 100 µL of a sample are used and diluted with a buffer-enzyme mixture and the IS-Water mix solution. After 30 minutes of incubation on a thermomixer at 55°C for hydrolysation to take place, samples are briefly centrifuged. 50 µL of this solution are taken out and added to 450 µL of mobile phase before another centrifugation step and injection of 10 µL into the LC-MS/MS system.

4.2.4. Liquid Chromatography

For the separation by liquid chromatography, the gradients of solvents shown in Table 4 were applied for system one and two.

Table 4: Gradient of aqueous phase B applied for liquid chromatography on measurement system one (left) and two (right).

Measurement system one			Measurement system two		
Process time t [min]	% of solvent B	Flow rate [mL/min]	Process time t [min]	% of solvent B	Flow rate [mL/min]
0.00	80.00	0.70	0.00	85.00	0.5
5.00	1.00	0.70	2.50	1.00	0.5
5.10	1.00	0.70	3.20	1.00	0.5
8.00	1.00	0.70	3.25	85.00	0.5
8.11	80.00	0.70	4.00	85.00	0.5
10.00	80.00	0.70	end of gradient		

For system one, the gradient of solvents pictured in Figure 8was applied at a flow rate of 0.7 mL/min.

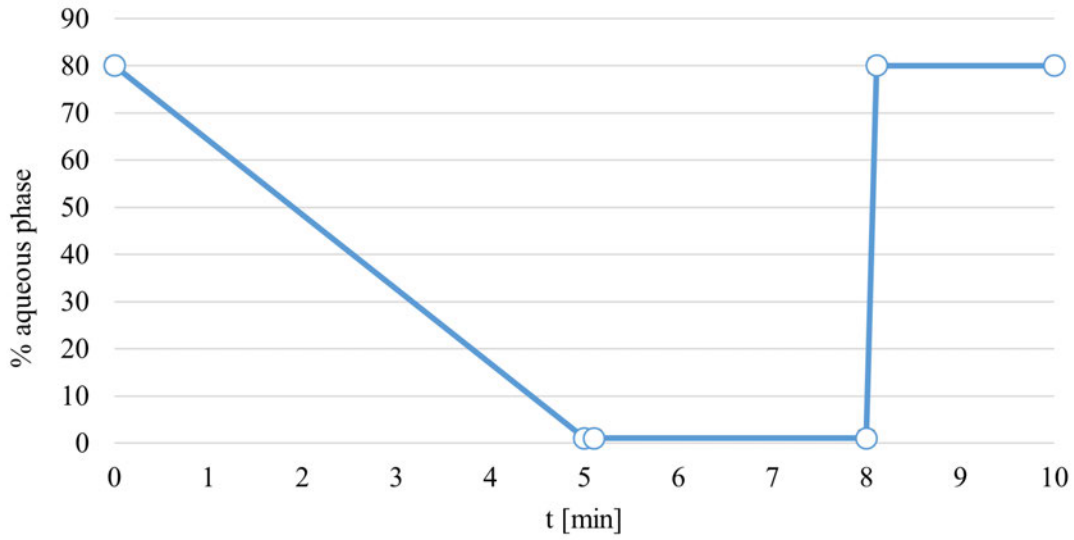

Figure 8: Gradient of aqueous mobile phase applied on measurement system one.

System two was run on a slightly different gradient with shorter run times and a reduced flow of 0.5 mL/min due to deviating column length amd diameter. The gradient applied here can be seen in Figure 9.

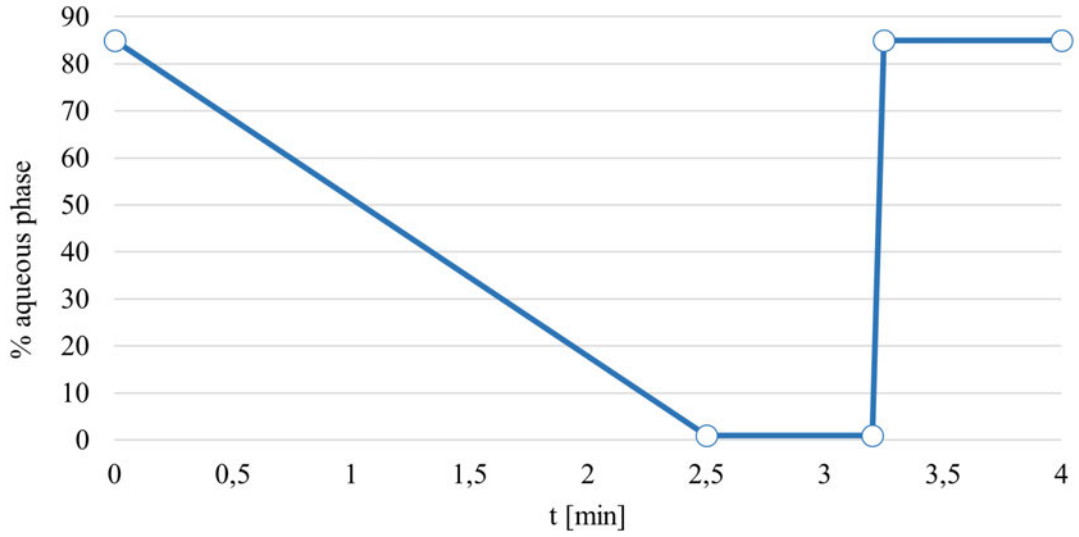

Figure 9: Gradient of aqueous mobile phase applied on measurement system two.

For both systems, this results in a linear gradient with a re-equilibration phase after each sample was measured. The column oven temperature is set to 40°C and of each sample 10 μL are injected into the columns. It can be said that both gradients were similar with only slight changes applied to account for different column dimensions.

4.3. Statistic Evaluation

First, samples that were analysed in the past were evaluate for cocaine and heroin positive results. Among these samples, results were screened for further positive analytes to find possible correlations as well as likely adulteration patterns. All routine analytical methods were considered, regardless of biological material. As a result, human serum, capillary blood, saliva and urine were included. Samples from January 2024 until early September of 2025 were considered. Due to the large amount of samples processed in this time frame, it was decided to only perform detailed evaluation on urine samples to provide best comparability with measurement results of added analytes. In this process, there was no initial differentiation between substances that can be linked directly to consumptions of the illicit drugs and those that could be present for other reasons. In addition to the list of analytes established based on literature research, amphetamines, benzodiazepines, opiates and opioids as well as cannabis were determined to be of particular interest.
Due to some adulterants being added to the analytical methods in the process, these were only to be detected in samples measured after the modification has been made. Exceptions to this occur if additional analytical methods were performed in addition to the urine analysis. Based on this data, the number of total samples that were considered positive for any of the analytes of interest was used to calculate arelative frequency of occurrence in any cocaine or heroin positive sample. It was then evaluated which adulterants were the most common to be detected.

5. Results and Discussion

During literature research, a multitude of substances that are being used as adulterants in the illicit drugs of interest were revealed. The most common ones among those were chosen to be included in the routine analytical methods as well as the statistical evaluation of measured samples.

5.1. Common Adulterants in Illicit Drugs

Of the adulterants identified for both heroin and cocaine, those that cannot be unambiguously linked to the consumption of either drug in blood samples of suspected consumers were excluded from the developed analytical method as well as the statistical evaluation. Additionally, pharmacologically active substances used to adulterate illicit drugs were prioritised over inactive ones.

5.1.1. Heroin

The most common adulterants in heroin at the current time, as found in the reviewed literature, can be seen in Table 5.

Table 5: Most common substances found in adulterated heroin according to literature, grouped by substance class.

Substance class	Substance	Function and Effect	Source(s)
Analgesic	Paracetamol	Similar taste and melting point, masking of poor quality	[6]
	Phenacetin	toxic effects	[12,25]
Barbiturate	Phenobarbital	facilitates smoking, potentially fatal health risks	[6,25]
CNS Depressant	Diazepam	Respiratory depression	[12,17]
Local Anaesthetic	Procaine	lowers heroins vaporizing temperature	[6]
	Lidocaine	numbing sensation	[26]
Stimulant	Caffeine	Lowers evaporation temperature, addictive, induces health issues	[6]
	Cocaine	incresed neurotoxicity, masking of symptoms, increased fatality	[17]
	Methamphetamine	increased neurotoxicity	[17]
Synthetic opioid	Fentanyl	mimicking heroin, enhanced effect	[17]
	Dextromethorphan		
	Methorphan		[26]
	Nitazene		[27]

Recently, a decline in heroin production in Afghanistan has led to less availability of the substance, which led to a significant increase in adulteration and is expected to result in more frequent use of nitazenes as an alternative drug of abuse.[10] Predominantly synthetic opioids are used, as they have similar effects and can be easily synthesized at illicit production sites, if they are not readily available.[6] Due to their similar working mechanisms, there are strong synergistic effects. As most synthetic opioids are more potent than heroin itself, the combination, especially if taking place unbeknownst to the consumer, can have severe consequences.[5,17] Mostly, heroin was found to be cut with different opioid substances like dextromethorphan or various morphines. Reason for this being the increase of heroins effect on the body as well as mimicking it with similar acting but better available or easier and cheaper acquired drugs.[17]

Among the most common adulterants, especially the synthetic opioids fentanyl and nitazene-type substances are of interest. Both are several folds more potent than heroin itself. As a result, both groups significantly enhance the effect of the drug.[14] As both fentanyl and nitazene type substances are highly lipophilic, they are able to cross the blood brain barrier, while concentrations in the brain can be influenced by P-glycoprotein-mediated extrusion from the brain.[10]
Additionally, they pose significant health risks and increase overdose related fatality, as they are not as susceptible to the effects of the opioid antidote naloxone. This leads to acute overdoses that cannot be treated as effectively. Often times, several folds higher dosages would be needed as well as an admission of the antidote within minutes of the initial drug.[14] Especially fentanyl- and nitazene-type substances with a slow dissociation from mu-opioid receptors require high naloxone dosages for reversal of their effects.[10] When injected prior to fentanyl, naloxone was able to suppress the drugs effects in a rodent model experiment. However, in a context of drug abuse, naloxone is more likely to be administered after the consumed drug, once overdose is suspected. After only a 10 minute delay in administration, no reduction of fentanyls effects could be observed, however that of following injections was blocked entirely. Further adulteration with different substances is expected to influence naloxones potential to reverse adverse opioid effects.[14]
Fentanyl is used in medial care for patients that are opioid tolerant as an alternative pain medication. Symptoms of fentanyl consumption include mainly sedation, nausea and respiratory depression. The latter being especially of interest as it is the main reason for overdose induced deaths.[12]
Other side effects like visual hallucinations, insomnia and anxiety are linked to neurotoxic abilities. High dosages of fentanyl have been linked to developing cerebral hemorrage and edema as well as hyperintensities in the hippocampus.[11]

5.1.2. Cocaine

For cocaine, the most commonly mentioned adulterants according to the literature can be found in Table 6. While the available amount of pure cocaine has been increasing in the past years,[2] a number of relatively frequently used adulterants could be identified, with some raising severe health concerns for their users.

Table 6: Most common substances found in adulterated cocaine according to literature, grouped by substance class.

Substance class	Substance	Function and Effect	Source(s)
Analgesic	Acetylsalicylic-acid	Similar bitter taste, anti-inflammatory	[23]
	Ibuprofen		
	Paracetamol		
	Metamizole		[28]
	Phenacetine	Intensified effect, hallucinations, cardiotoxic effects	[17,23]
Anthelmintic	Levamisole	Prolonged and intensified effects, pulmonary lymphocystic vasculitis, agranulocytosis, skin necrosis	[17,23,29]
Antihistamine	Diphenhydramine	Anxiolytic and sedative	[25]
	Hydroxyzine		[23,28]
Calcium channel blocker	Diltiazem	cardiovascular effects	[23,28]
Cannabinoid	THC	potential analgesia, can synergise with μ-opioid receptor agonists	[12,17]
Empathogen	MDMA	agonized serotonergic effect	[17]
Local Anaesthetic	Benzocaine	Mimic cocaines numbing of gums and tongue, synergistically enhance toxic effects	[17,23]
	Lidocaine		
	Procaine		
	Tetracaine		
	Lignocaine		[17]
Opioid	Heroin	incresed neurotoxicity, masking of symptoms, increased fatality	[17]
Stimulant	Amphetamine	Compliments effects of drug	[17,23]
	Ephedrine		[23]
	Caffeine		
	Methamphetamine	sympathomimetic properties, cardiovascular complications	[17,23]

Concerning all substances that are often used as illicit drugs of abuse themselves, it was stated that their presence in samples of seized drugs in literature could be a result of cross-contamination rather than adulteration.[17] As a result, compounds that are less likely to be consumed on their own were favoured during the analytical approach.

Local anaesthetic compounds such as lidocaine, procaine, benzocaine and tetracaine were found to usually be added as a way of masking poor quality, as cocaine itself has local anaesthetic properties. If tested against gum or tongue, this can be mimicked.[23]
As especially lidocaine and procaine were found to be mentioned the most among them, those two, which were previously only analysed in additional measurements like GC-MS or thin-layer-chromatography (TLC) if needed, were also implemented into the same routine measurement as cocaine and heroin. The chemical structure of both lidocaine and procaine can be found in Figure 10.

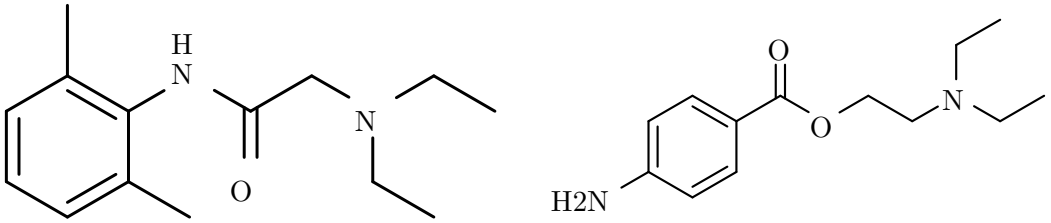

Figure 10: Chemical structure of the local anaesthetics Lidocaine (left) and Procaine (right) (Source: cf. Vanderah 2024[12])

Procaine was introduced as a local anaesthetic shortly after cocaine. It is referred to as the first useful injectable anaesthetic. However, the duration of action is limited and instability is believed to be a contributing factor to induce allergic reactions, as free aromatic acid is released during hydrolysis.[12]
Lidocaine was introduced in 1948 with a higher stability and potency compared to procaine. Both act through a blockage of sodium channels, slowing and ultimately inhibiting action potential propagation. CNS excitation as well as local neurotoxicity are known effects. As of today, lidocaine is still used for anaesthesia of intermediate duration, while procaine is very rarely used for short procedures only.[12]
Some of the commonly found adulterants in seized samples are used as medication themselves, in biological samples those cannot be fully attributed as adulterants in cocaine but might also be a co consumption. This is the case for hydroxyzine or piracetam,[23] which have very specific fields of application. Especially widely used analgesics like ibuprofen, paracetamol or acetylsalicylic acid have to be excluded from consideration as an analyte for that reason. Similarly, substances like THC or amphetamines are most likely to be present in human samples due to consumption of that drug itself rather than adulteration.

A new development, however, is the addition of the anthelmintic levamisole. Levamisole is a drug that was initially developed to treat various diseases such as helminthiasis and colorectal cancer, among others.[30] Being introduced as anthelmintic in 1966 and registered as medicine in 1969, it was withdrawn from the market in 1999 due to severe side effects and alternate medication being readily available.[31] These side effects, namely agranulocytosis and skin necrosis, are the reason that application in humans is still only rarely considered an option. A more frequent application is the use in horses as anthelmintic drug.[30]

While it is considered a pharmacologically active adulterant of cocaine, which will be further addressed in the following, physiological properties are also ideal for use in cocaine. With a melting point below the one of cocaine free base, it can be inhaled alongside it without being noticed by users, maintaining a perception of high purity and quality, while reducing the amount of actual cocaine and adding a cheaper substance that is readily available due to its routine use in veterinary medicine.[31]

Chemically, levamisole can be classified as an imidazothiazole and the S-enantiomer of tetrahydroimidazothiazole. The anthelmintic properties are a result of levamisoles function as a nicotinic acetylcholine receptor agonist, which causes paralysis and spasms in parasitic worms. An influence on the metabolism of monoamine neurotransmitters was found in rats, inducing dopamine release in certain brain regions. Activation of human neuronal nicotinic receptor in combination with monoamine oxidase inhibition is suggested to play a roll in this effect in human. This effect is suspected to be the pharmacological reason for use as adulterant in cocaine, as synergistic effects occur due to this. Additionally, levamisole has an influence on the immune system. Inflammatory responses are induced by migration of monocytes through levamisole, while function of several immune cell types is enhanced. This process is the reason for levamisoles application in some cancer treatments. In combination with dexamisole, it was additionally applied in the treatment of arthritis and AIDS under the name of tetramisole.[31]

In plasma, it was found to have a half life of 2.0 h and a detection window of 36 h.[32] After oral administration, detection of levamisole in urine was found to be possible for up to 39 h, while the metabolite aminorex shows a detection window of up to 54 h.[31]

While the exact method of action in humans is still not fully researched, it is known to enhance the duration of cocaine induced euphoria as well as hallucinogenic effects. This might be due to it's metabolisation by the liver into aminorex (5-phenyl-4,5-dihydro-1,3-oxazol-2-amine), a substance that is known to have amphetamine like effects on humans.[29] Some even speculate the formation of aminorex is the reason for the use of levamisole, which would in this case be added as a prodrug. While both substances show an influence on dopamine and norepinephrine transporters, the affinity of aminorex towards them was found to be significantly higher, further supporting this hypothesis. As shown by the longer detection times, aminorex has a longer half-life than cocaine, which results in an extension of the overall drug effects.[29,31]

The underlying metabolic pathway was first discovered after cases of aminorex positive race horses surfaced, indicating illegal doping. It was then found that all alleged cases concerned horses that have been treated with levamisole beforehand. Since then, the formation of aminorex after levamisole administration has been confirmed to take place in both horses and humans.[29,30] Chemical structures of levamisole as well as aminorex are shown in Figure 11.

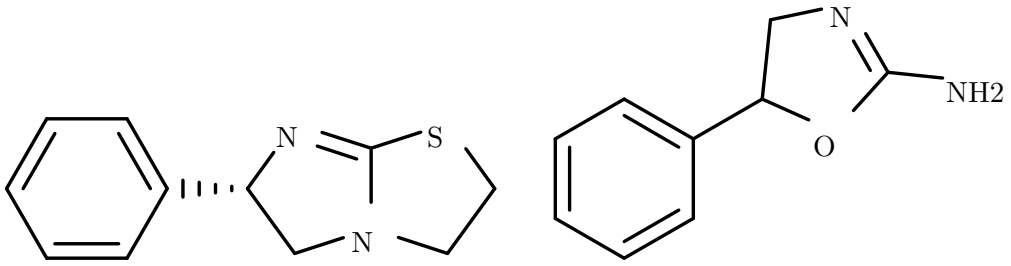

Figure 11: Chemical structure of Levamisole (left) and it's metabolite Aminorex (right) (Source: cf. LGC[33,34])

Amphetamine and amphetamine-like substances act as a dopamine releasing agent,[35] which can be considered the reason for the strong increase in effect if coupled with the reuptake inhibiting cocaine. This pharmacological interaction was taken as a reason to not only analyse the samples for levamisole, but also for aminorex.
In plasma, aminorex can be detected for up to 30 h.[32]
As a pharmacological substance, aminorex was initially developed in 1963 by Poos for McNeil Laboratories as an alternative anorectic drug to suppress the users appetite to be used instead of amphetamines. However, strong adverse side effects, mainly a significant increase in pulmonary hypertension cases, were the reason for removal from the market in 1968 after just three years of application.[30] As of today, it is instead classified as a strictly controlled narcotic substance.
Studies show that aminorex inhibits K^+ channels in the lung and causes pulmonary artery pressure. The main biological activity is linked to the serotonergic system. Due to aminorex, an increased amount of serotonin is released, while its reuptake is inhibited. Reason for this being the accumulation of aminorex inside the cells, its binding to the serotonin transporter (SERT) and the resulting effect on intracellular messaging pathways. As a genetic component is involved as well, it has been speculated that there is a genetic predisposition for the resulting susceptibility for pulmonary arterial hypertension.[30]

5.2. LC-MS/MS Parameters

After the sample preparation process, liquid chromatography was performed to separate the substances of interest from each other as well as possibly remaining interfering substances. Analysis via tandem mass spectrometry followed. For the liquid chromatographie, two seperate systems with slightly different analytical methods were used. Detail on the applied gradients can be found in the liquid chromatography section of the materials and methods chapter.
For each new analyte that had to be added to routine analytical procedures, a tuning process was performed to determine significant fragmentation patterns for identification. Reference values found in literature were used as a starting point for expected fragments.[32] These could be reproduced. The found fragment peaks found for levamisole can be found in Figure 12 and those for aminorex in Figure 13.

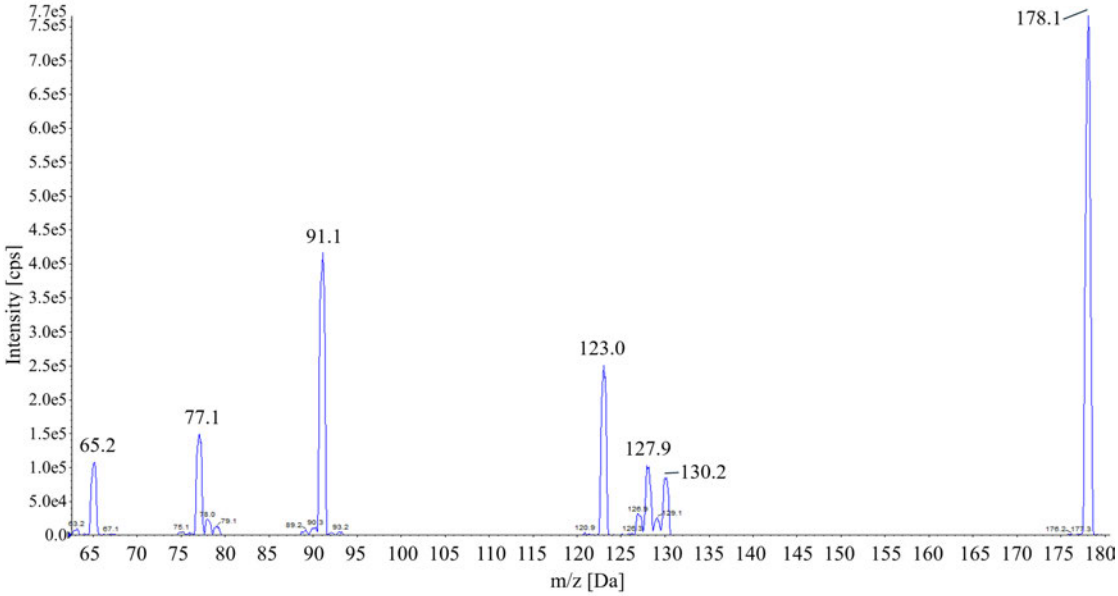

Figure 12: Mass spectrum of levamisoles fragmentation pattern, only daughter peaks are shown. Recorded during tuning process. As base peak, 205.29 m/z was found (not pictured).

The two highest signals were found for the fragments at 178.10 m/z and 91.10 m/z for levamisole. However, as larger fragments are preferred due to higher specificity for the substance, instead of 91.10 m/z, the peak at 123.00 m/z was chosen as qualifier.

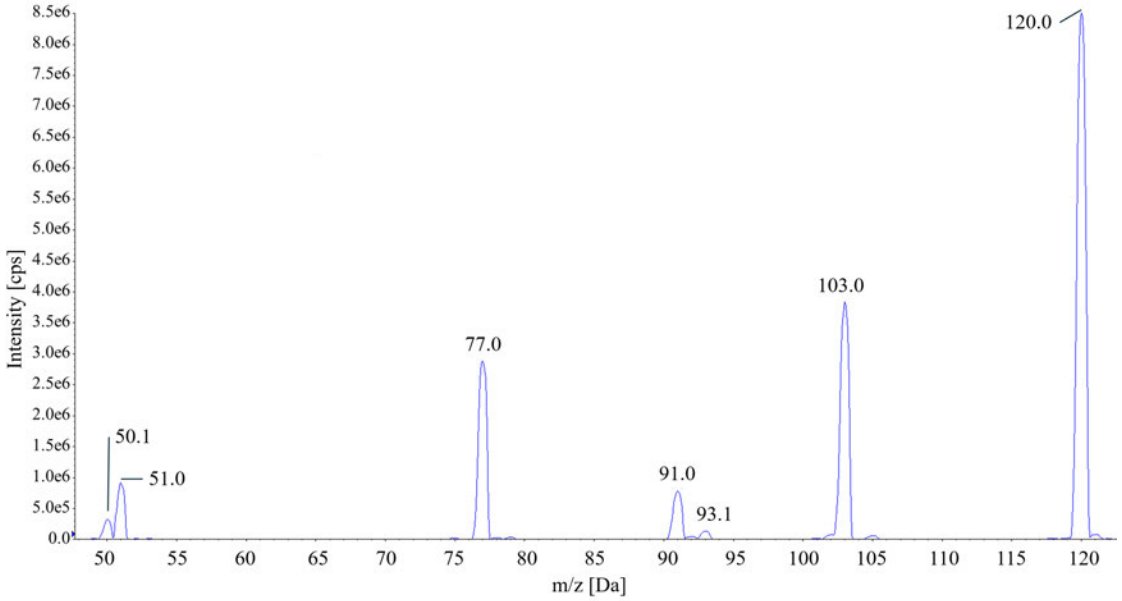

Figure 13: Mass spectrum of aminorex fragmentation pattern, only daughter peaks are shown. Recorded during tuning process. As base peak, 163.05 m/z was found (not pictured).

Six ion transitions for each substance were monitored by the software during the tuning process. Among these, two were chosen for routine use as quantifier and qualifier and MS/MS parameters were optimised for ideal detection. These can be found in Table 7 alongside the parameters for lidocaine and procaine. Their respective fragmentation pattern were not individually determined but adapted from other routine analytical methods where both were already included. The full list of all analysed substances, including those used for cocaine, heroine and identifying markers, can be found in Table 15 in the appendix.

Table 7: Ion transitions and MRM parameters of analytes newly implemented into routine analytics.

Name	Ion transition m/z [Da]	Declustering potential [eV]	Collision energy [eV]	Collision cell exit potential [eV]
Levamisole 1	205.29-178.10	96.00	29.00	14.00
Levamisole 2	205.29-123.00	96.00	39.00	8.00
Aminorex 1	163.05-120.00	6.00	19.00	12.00
Aminorex 2	163.05-103.00	6.00	35.00	10.00
Lidocaine 1	235.10-86.20	48.00	23.00	8.00
Lidocaine 2	235.10-58.20	48.00	35.00	8.00
Procaine 1	237.10-120.10	56.00	21.00	12.00
Procaine 2	237.10-100.00	56.00	37.00	8.00

These parameters were applied to an MRM method to ensure that simultaneous measurement of all analytes of interest can be detected.
Resulting chromatograms were then evaluated using Sciex OS software. For visualisation, Chromatogramms of the four added analytes at the highest calibration point are shown below. Results from system one can be found in Figure 14, Figure 15, Figure 16 and Figure 17, while those of system two can be seen in Figure 18, Figure 19, Figure 20 and Figure 21.

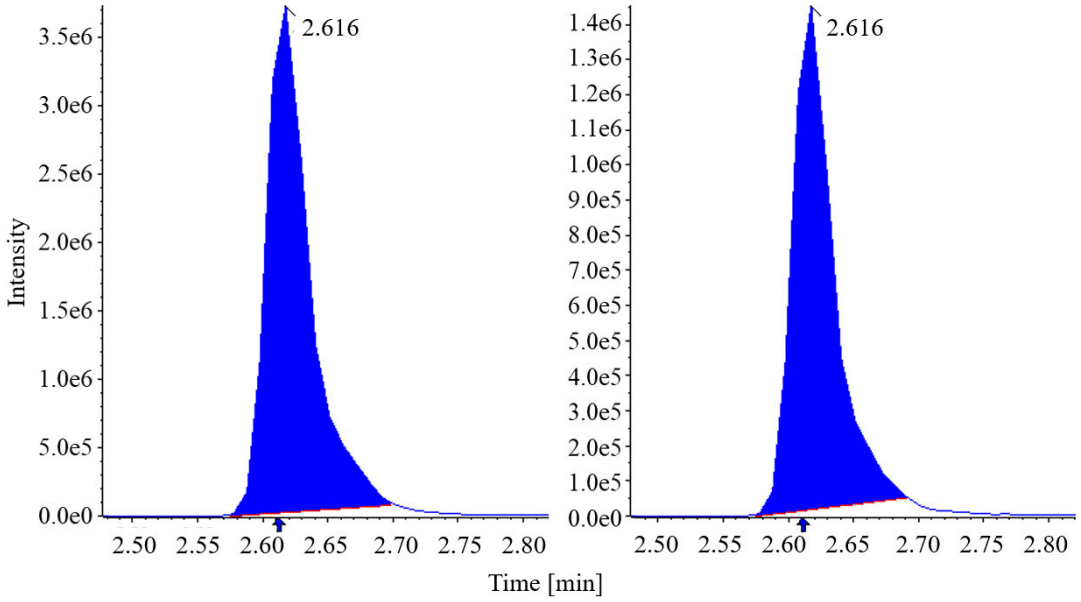

Figure 14: Chromatogram of 500.00 ng/mL levamisole measured on system one. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 2.616 min in this sample.

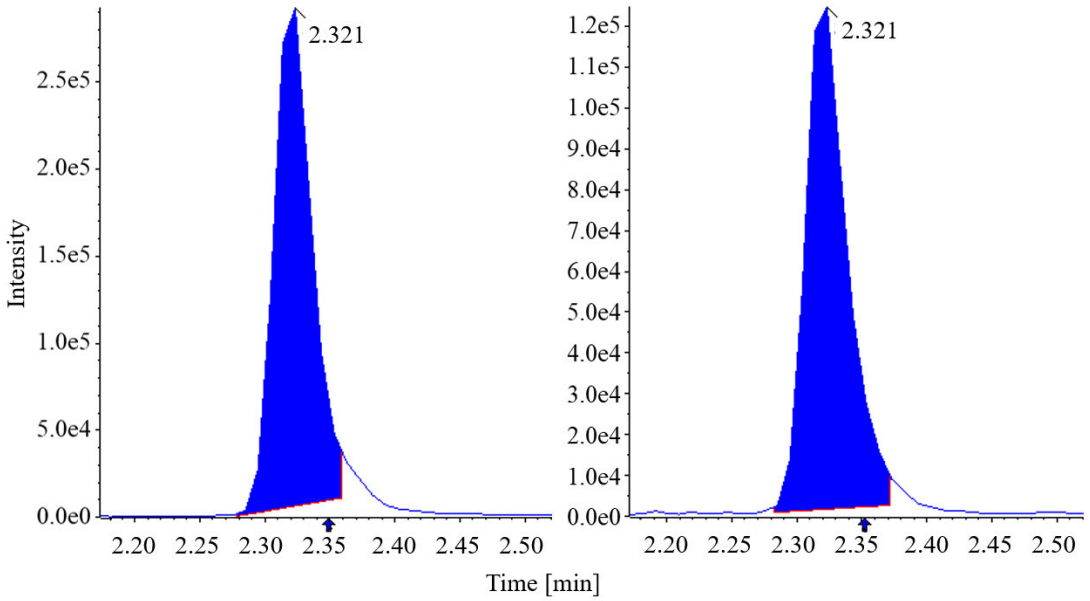

Figure 15: Chromatogram of 50.00 ng/mL aminorex measured on system one. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 2.321 min in this sample.

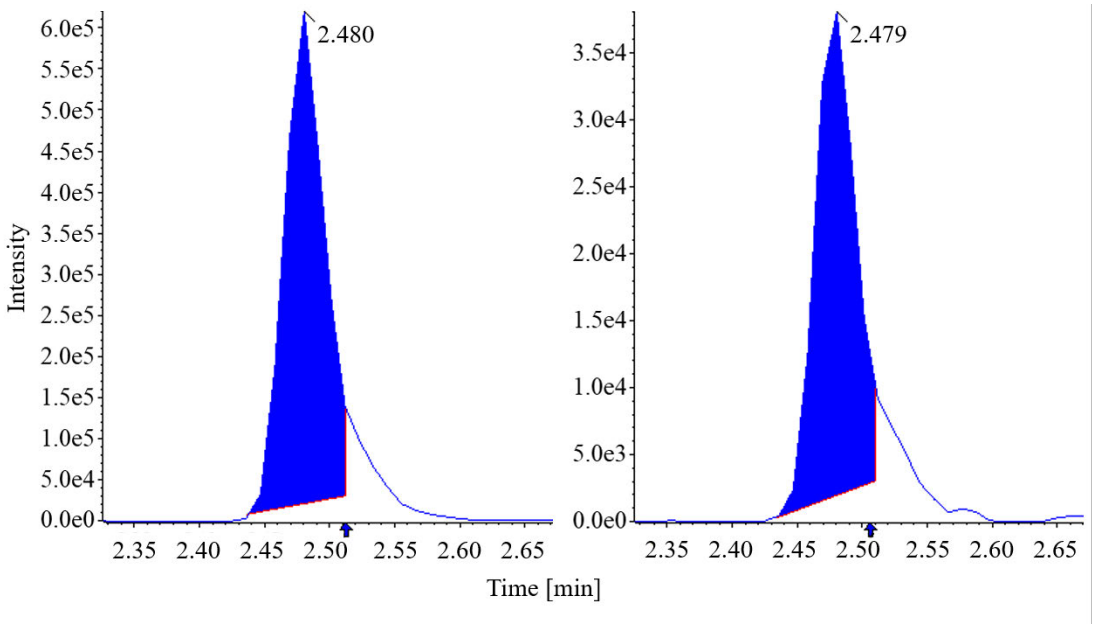

Figure 16: Chromatogram of 56.70 ng/mL lidocaine on system one. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 2.480 min in this sample.

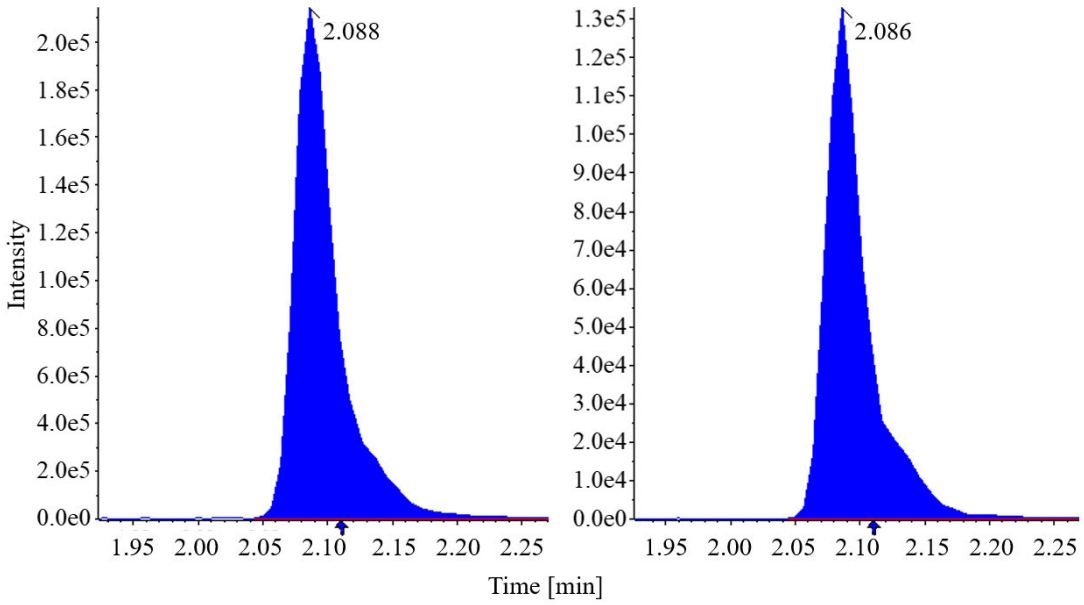

Figure 17: Chromatogram of 50.00 ng/mL procaine on system one. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 2.088 min in this sample.

For all four analytes, peaks with a high intensity were found. Slight tailing could be observed that was removed from the evaluated peak area. For Procaine, this area correction was not performed in the shown example as a qualitative approach was taken.

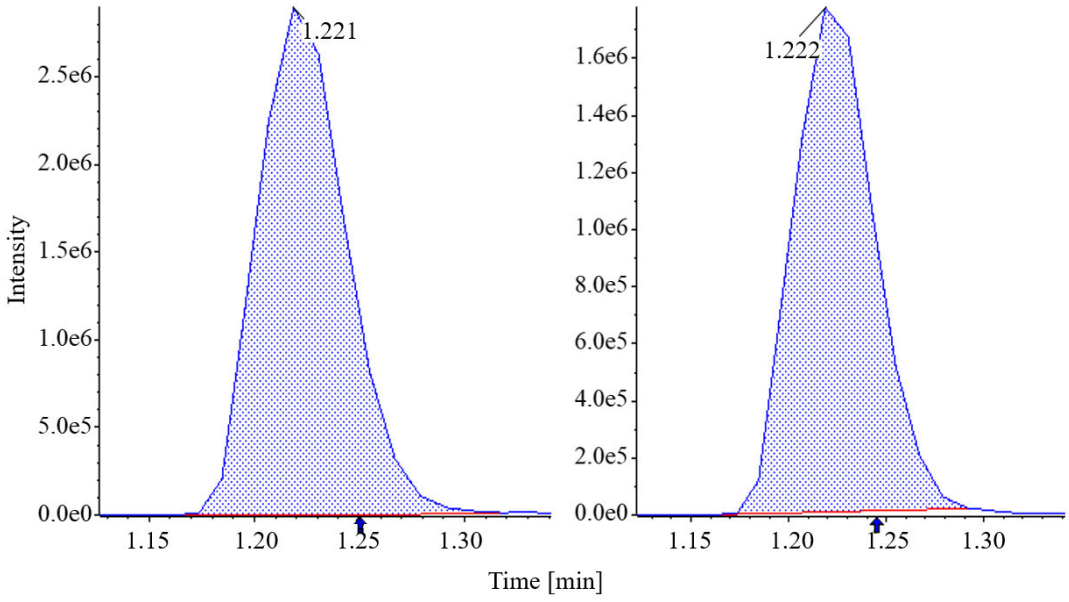

Figure 18: Chromatogram of 500.00 ng/mL levamisole on system two. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 1.221 min in this sample.

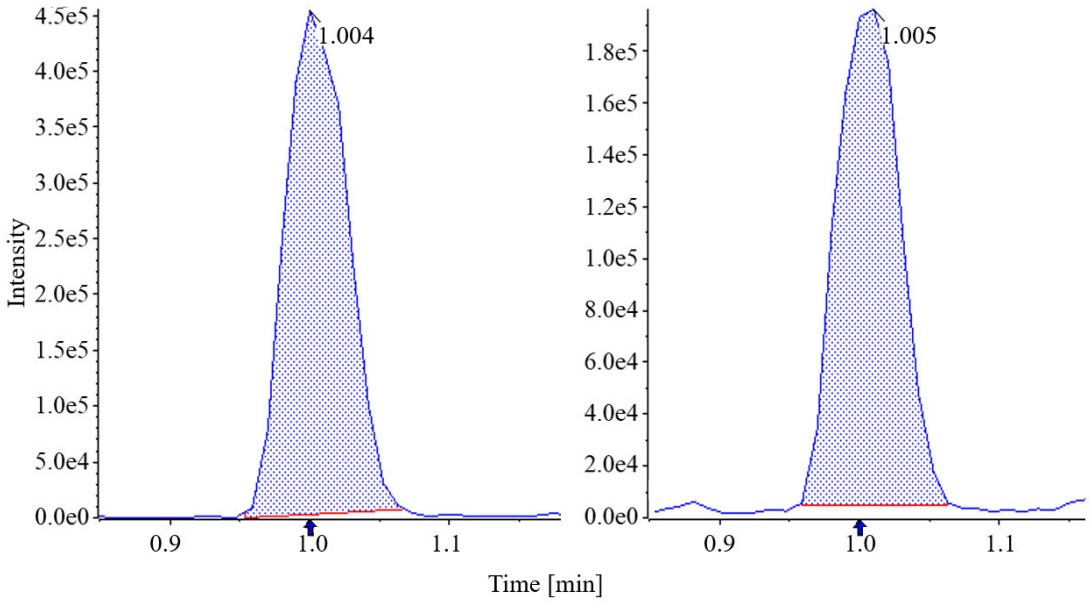

Figure 19: Chromatogram of 50.00 ng/mL aminorex on system two. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 1.004 min in this sample.

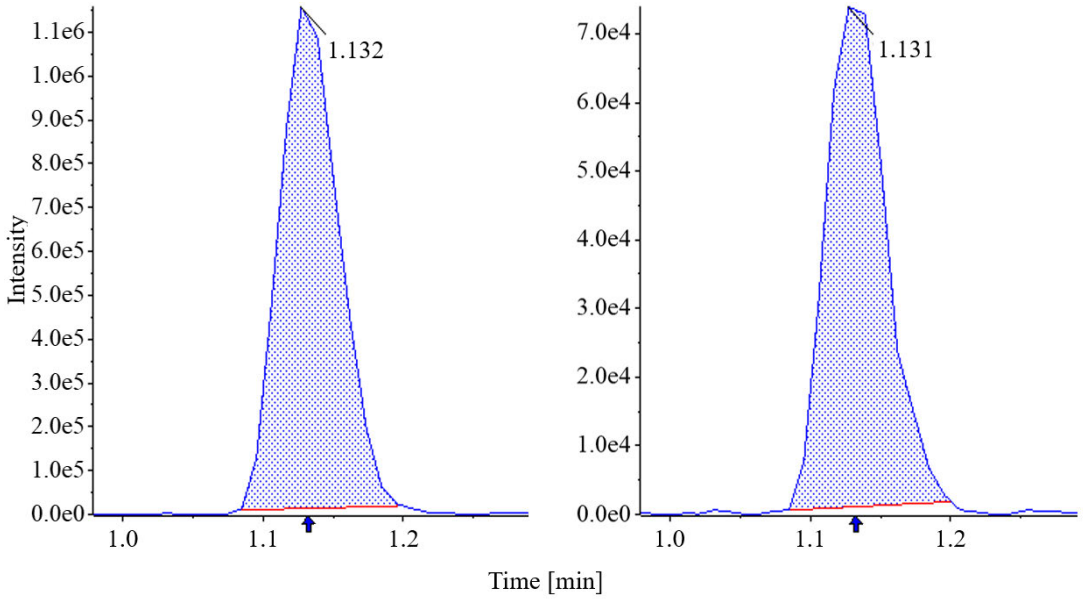

Figure 20: Chromatogram of 56.70 ng/mL lidocaine on system two. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 1.132 min in this sample.

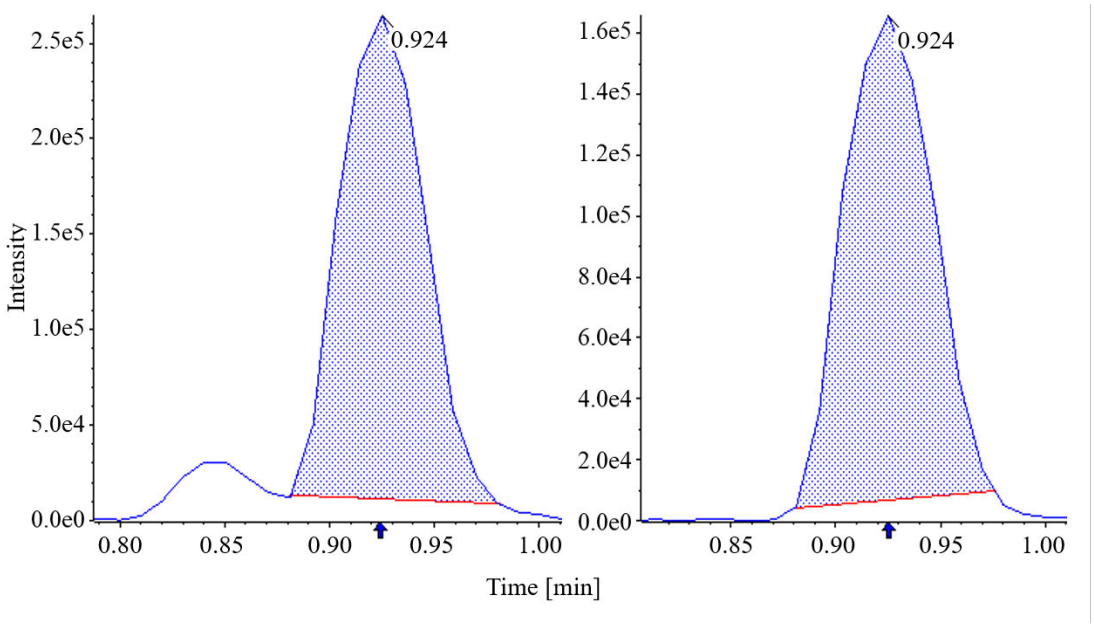

Figure 21: Chromatogram of 50.00 ng/mL procaine on system two. The arrow on the time axis indicates the expected retention time while the number attached to the peak shows the actual retention time of 0.924 min in this sample.

The peaks recorded on system two show similar, for some substances slightly higher signal intensities compared to system one. It is to be noted that at the shown concentrations, less tailing can be seen than on the other measurement system.
For all shown peaks, variations in the third decimal place of the actual retention time can be due to the integration process of the software as well as each individual peaks shape. During evaluation, it was made sure that all peaks that were accounted for as a positive sample exhibit a constant relative retention time to the used internal standard. This value was specific for each measurement system as resulting chromatograms differed due to varying column dimensions, flow rates and the applied gradient. In doing so, differences in individual analytical processes can be accounted for and an identification of the correct peaks is ensured.

5.3. Method Validation

The measurement procedures specified in the theoretical background section were performed and resulted in the data found below. Here, the significant results will be discussed while the full measurement data can be found in the appendix.

5.3.1. LOD and LOQ

The limits of detection and quantitation can be found in Table 8 and Table 9. Per analyte and measurement system, five individual samples were evaluated. However, an exception was made for aminorex and lidocaine on system one. In the case of aminorex, seven samples were included as a lower limit was to be achieved based on literature research results for typical concentration ranges. Lidocaine was measured in a different range due to easier handling of used volumes of the QC serum samples. No significant improvement could be observed for either in doing so. As a result, this was not repeated during validation of system two.

Table 8: Calculated LOD and LOQ of added analytes on measurement system one.

Substance	Target		Qualifier		Total results		Measured range		Unit
	LOD	LOQ	LOD	LOQ	LOD	LOQ	Min	Max	
Levamisole	0.39	1.27	0.56	1.74	0.56	1.27	1.00	5.00	ng/mL
Aminorex	0.92	2.96	0.76	2.48	0.92	2.96	1.00	10.00	ng/mL
Lidocaine	0.60	2.14	0.62	2.23	0.62	2.14	1.02	10.20	ng/mL
Procaine	0.91	3.07	0.96	3.27	0.96	3.07	0.50	10.00	ng/mL

For the measurement system referred to as system one, no differentiation could be made between a peak resulting from present procaine and one of unknown origin and varying peak area found in the target ion transition in all samples including blanks. Therefore, no quantification could be performed. However, as this peak could not be fount in the qualifier ion transition, an evaluation of procaines presence in samples was still considered possible. For this purpose, the LOD and LOQ values were determined despite the interfering signal to make sure that adequately low concentrations can still be distinguished from negative samples.

Table 9: Calculated LOD and LOQ of added analytes on measurement system two.

Substance	Target		Qualifier		Total results		Measured range		Unit
	LOD	LOQ	LOD	LOQ	LOD	LOQ	Min	Max	
Levamisole	0.46	1.47	0.52	1.64	0.52	1.47	1.00	5.00	ng/mL
Aminorex	0.99	2.88	0.58	1.80	0.99	2.88	1.00	5.00	ng/mL
Lidocaine	0.36	1.20	0.66	2.01	0.66	1.20	1.02	5.10	ng/mL
Procaine	0.49	1.56	0.90	2.65	0.90	1.56	1.00	5.00	ng/mL

Samples with concentrations of one of these analytes below the LOQ but above the LOD can be considered positive but concentrations have to be reported as below LOQ. In regards to procaine, it was found that the target peak was sufficiently separated from the interfering peak for system two. A quantification according to the limitations stated above was therefore considered possible. Considering the application in routine analytics, the higher value of both measurement systems should be used to increase comparability and reduce workload during evaluation. As a result, the LOD of levamisole should be set at 0.56 ng/mL and the LOQ at 1.47 ng/mL. For Aminorex, detection can be assumed at 0.99 ng/mL and quntitation above 2.96 ng/mL can be performed. Concerning lidocaine, an LOD of 0.66 ng/mL and an LOQ of 2.14 ng/mL can be set for both systems. Procaine concentrations starting from 0.96 ng/mL could be detected and quantified above 3.07 ng/mL. For this thesis however, the individual values for each system were applied during evaluation.

5.3.2. Accuracy

Concerning accuracy of the used methods, both measurement systems were included in the same evaluation process as both are used simultaneously in routine analytics and should therefore produce comparable results. For significant parameters, the values shown in Table 10 were found. A total of nine samples was included for this evaluation. However, due to the fact that procaine cannot be evaluated quantitatively on system one, only seven samples could be included for this analyte.

Table 10: Measurement results for accuracy determining parameters of added analytes across both measurement systems.

Substance	Bias [%]	Intermediate precision [%]	Repeatability [%]	extended measurement uncertainty [%]
Levamisole	3.4	4.6	3.2	13.1
Aminorex	5.5	7.4	3.3	19.6
Lidocaine	3.3	4.1	3.7	12.9
Procaine	2.2	7.8	3.5	17.7

As stated in the theoretical background section, percentages should fall below 15 % or 20 % close to LOQ concentrations, while the extended measurement uncertainty should not exceed 30 %. These criteria were fulfilled for each of the parameters. The results of this part of the validation process indicates that the chosen analytical method resulted in accurate results for all analytes.

5.3.3. Matrix Effects, Recovery and Ion Suppression

The parameters of matrix effects, recovery and ion suppression were investigated on both measurement systems as well. The results are shown in Table 11 and Table 12.

Table 11: Results for matrix effects, recovery and ion supression of added analytes on measurement system one.

Substance	RC [%]		ME [%]		ALL [%]		Measured concentrations	
	MV	SD	MV	SD	MV	SD	low [ng/mL]	high [ng/mL]
Levamisole	96	21	91	21	87	19	10	200
Aminorex	90	24	84	19	76	20	5	20
Lidocaine	103	22	79	16	81	18	5.1	51.03
Procaine	no evaluation						5	50

For procaine, the evaluation of matrix effects, recovery and ion suppression was not performed as the interfering substance disrupting the target ion had a significant influence on the signal and no samples could be measured with an acceptable ion ratio in relation to the qualifier ion.

Table 12: Results for matrix effects, recovery and ion supression of added analytes on measurement system two.

Substance	RC [%]		ME [%]		ALL [%]		Measured concentrations	
	MV	SD	MV	SD	MV	SD	low [ng/mL]	high [ng/mL]
Levamisole	106	3	93	4	99	3	10	200
Aminorex	103	3	68	5	71	3	5	20
Lidocaine	109	6	83	5	90	4	5.1	51.03
Procaine	110	3	77	4	84	2	5	50

When taking the dilute and shoot approach as well as the transfer step using a quarter of the original volume into account during spiking of the prepared samples, all parameters fall into the desired range on system one. For system two however, aminorex showed a relative signal intensity below the desired range. Only 68 % instead of the required 75 % were achieved here.

5.3.4. Linearity

During method validation, all four added analytes were investigated for their linear range. The underlying data can be found in Table 13 and Table 14.

Table 13: Linearity evaluation and applied concentration range of added analytes on measurement system one.

Substance	Target [ng/mL]		Qualifier [ng/mL]		Total [ng/mL]		Range [ng/mL]	
	up to	result	up to	result	up to	result	Min	Max
Levamisole	2000	linear	2000	linear	2000	linear	1.00	2000
Aminorex	150	linear	150	linear	150	linear	2.00	150.00
Lidocaine	215.46	linear	215.46	linear	215.46	linear	5.10	215.46
Procaine	not applicable, qualitative analytics only							

The visualisation of this data obtained on system one, exemplary included for levamisole, is shown in Figure 22. The remaining graphs can be found in the appendix.

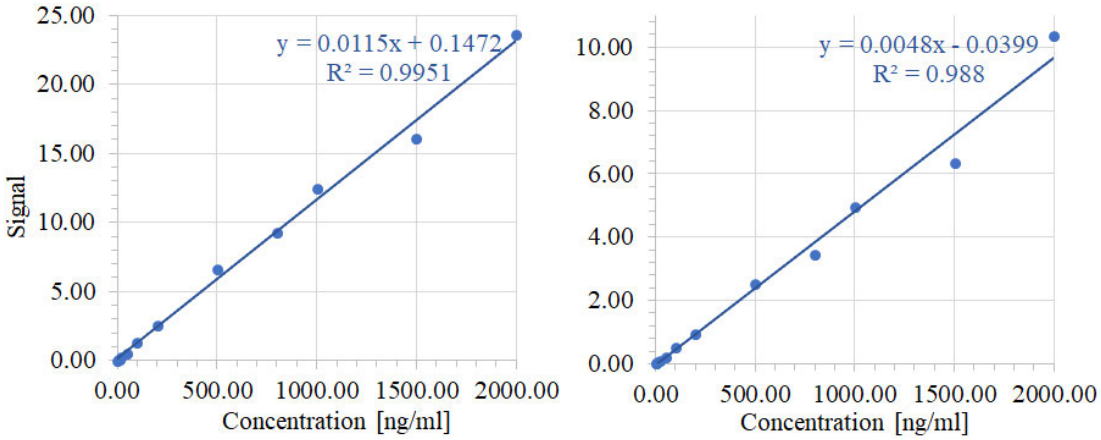

Figure 22: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of levamisole on system one. The calculated equation for the linear model can be found attached to the respective plot.

A clear linear correlation between concentration of the substance and the resulting signal, represented by the peak area, was found. In regards to procaine, linearity data was recorded regardless of the disturbance signal mentioned above. However, the main focus for this substance was set on the qualifier ion transition as a result.

Table 14: Linearity evaluation and considered range of added analytes on measurement system two.

Substance	Target [ng/mL]		Qualifier [ng/mL]		Total [ng/mL]		Range [ng/mL]	
	up to	result	up to	result	up to	result	Min	Max
Levamisole	800	linear	800	linear	800	linear	1.00	800
Aminorex	150	linear	150	linear	150	linear	1.00	150
Lidocaine	215.46	linear	215.46	linear	215.46	linear	1.02	215.46
Procaine	200	linear	200	linear	200	linear	2.00	200

While aminorex, lidocaine and procaine were linear for the whole chosen range on both measurement systems, that was not the case for levamisole. Here, the highest point was chosen as 2000 ng/mL, which led to a non linear result for system two. However, if reduced to a maximum of 800 ng/mL, levamisole concentrations showed a linear correlation to their respective peak areas. For routine analytics, samples exceeding 800 ng/mL that were measured using system two, the concentration would have to be resulted as >800 ng/mL. The largest diversion from the applied linear model can be seen with procaine on both systems. As shown in Figure 23 for system two, the data point corresponding to 120.00 ng/mL had a significantly lower signal than expected. As the others do match the applied regression model, this can be treated as an outlier and does not significantly influence the overall linear result.

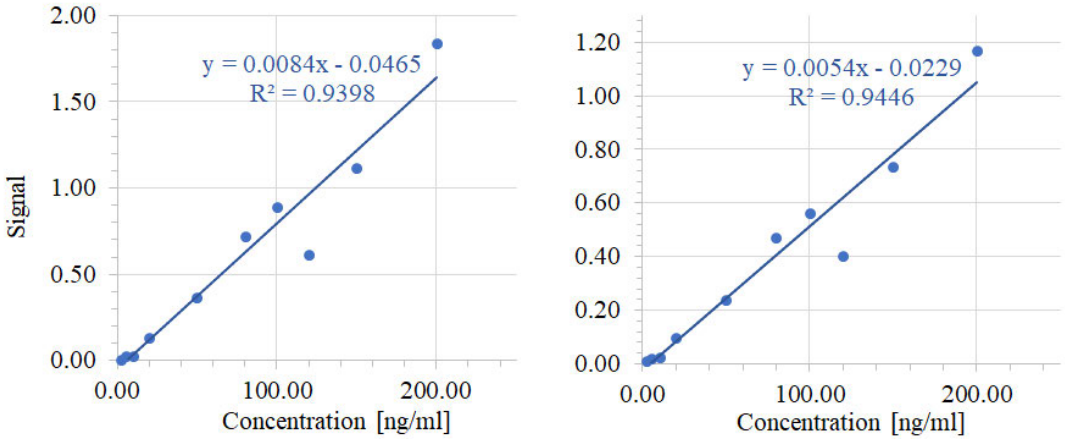

Figure 23: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of procaine on system two. The calculated equation for the linear model can be found attached to the respective plot.

In general, it can be said that similar results were achieved with both measurement systems. This indicates that each can be used for routine analysis of the added analytes. However, in regards to procaine, system two is to be preferred if a qualitative analysis is desired. In case a high concentration of levamisole is expected, system one should be used for analysis.

5.4. Statistic Evaluation

Some of the identified adulterants could be detected in the analysed samples. Due to the matrix being human samples taken after consumption, many frequently used bulking agents, impurities, and adulterants cannot be unambiguously linked to the consumption of illicit drugs. Especially substances like sugars, caffeine, or frequently used readily available pain medications like paracetamol are the most likely to potentially be present due to co-consumption rather than them being present in the consumed heroin or cocaine. Some substances however are not authorised for use in humans and therefore most likely to be consumed in the context of drug abuse. This pitfall was known at the beginning of sample evaluation and was therefore considered in the process.

5.4.1. Evaluation of Past Samples

Concerning past samples, adulterants in heroin could be identified as the most common ones were already being measured alongside the substance. For cocaine however, most adulterants of interest were not routinely tested for in humans. As a result, only other substances of abuse could be identified. Whether these were present due to co-consumption or because of adulterated cocaine can neither be denied nor confirmed. For

samples analysed after the addition of suspected adulterants, more precise evaluation can be performed. These were manually assessed and a separate statistic was set up, due to them not being included in the results entered into the data base. The corresponding results can be found below in the section results of LC-MS/MS analytics.
Evaluation of past samples analysed from January of 2024 until the first week of September 2025 showed a total of around 330 found substances including identifying metabolites. Among these, their likeliness to be used as an adulterant was estimated and the frequency of the respective substances was evaluated. However, the most frequently found substances for both heroin and cocaine were determined regardless of this factor. Furthermore, a list of potential analytes of interest was set up in addition to the results of literature research. Here, a focus was set on amphetamines, benzodiazepines, opiates and opioids as well as cannabis. The full data regarding the found number of samples containing one or more of these substances while being tested positive for either heroin, cocaine or both can be found in Table 23 in the appendix.
The performed analysis of patient samples revealed the substances described and visualised in Figure 24 to be present in confirmed cases of heroin use. As the highest amount of substances and analytical procedures were found in urine samples, these were chosen for the overall evaluation of most common substances. A total of 7718 samples were found to be positive for monoacetylmorphine and therefore considered heroin positive including a metabolic pathway used for identification of consumption.

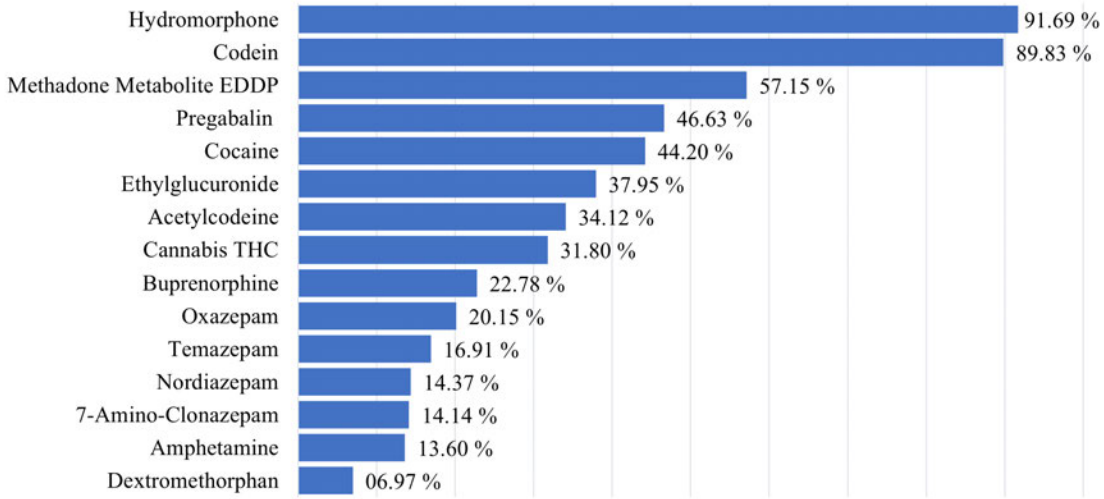

Figure 24: The 15 most commonly found substances alongside heroin from January 2024 until September 2025.

It is to be noted that most of the fifteen most commonly found substances are classified as opioids. Hydromorphone, codeine and acetylcodeine as well as methadone, represented by its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP),[36] but also dextromethorphan and buprenorphine fall into this category. Methadone is a synthetic

opioid that is generally used as replacement therapy for opioid use disorder to lessen withdrawal symptoms.[11] While synthetic opioids were found to be among the substances that are the most likely to be used as an adulterant of heroin, their clinical application prevents them from being classified as an adulterant. Especially codeine and methadone, but also dextromethorphan are most likely linked to independent consumption.[12] Among the 7718 heroin positive samples, hydromorphone was found 7077 times, codeine 6933 times, EDDP 4411 times, acetylcodein 2633 times, buprenorphine 1758 times and dextromethorphan 538 times total.

Pregabalin, which is often used in treatment of seizures but also has analgesic properties[12] was found in 3599 heroin positive samples. It is not commonly listed as a possible adulterant and its various medical applications indicate other reasons for its frequent occurrence.

The combination of heroin and cocaine has been addressed in the co-administration and drug interactions section of the chapter theoretical background. While an adulteration of one with the other can be the case in some of the 3411 samples where both were identified, a deliberate co-administration cannot be ruled out.

2929 samples were found to contain both ethylglucuronide and heroin. Ethylglucuronide is used in routine analytics as a short term marker for alcohol consumption,[37] indicating that heroin is often consumed alongside or in close proximity to alcohol rather than adulteration.

In 2454 samples within the evaluated timespan, the cannabinoid THC could be detected. Simmilarly to most substances discussed above, cannabinoids are often consumed in a medical context, especially for the treatment of excitatory disorders like Parkinson, epilepsy or Tourette syndrome.[12] Additionally, it is often used as a drug itself outside of the medical applications.[17] Consequentially, a classification of THC as an adulterant cannot be made and co-consumption is to be considered the most likely reason for its presence in the urine samples.

The most commonly found members of the group of benzodiazepines, namely oxazepam and temazepam in this case, were accounted for separately in order to give an impression on which substances from that group were found in particular. Benzodiazepines in general are applied as treatment for anxiety and panic attacks, insomnia and seizure disorders. Their effects range from CNS depressant properties to anaesthesia and eventually respiratory depression. Additionally, they are known to have significant addictive potential.[12] In combination with heroin, these effects could be amplified, however, due to their numerous applications as medications, their use as adulterants cannot be confirmed.

In addition, it was specifically searched for analytes mentioned in literature. From that list as found in Table 5, the substances shown in Figure 25 were found in urine samples.

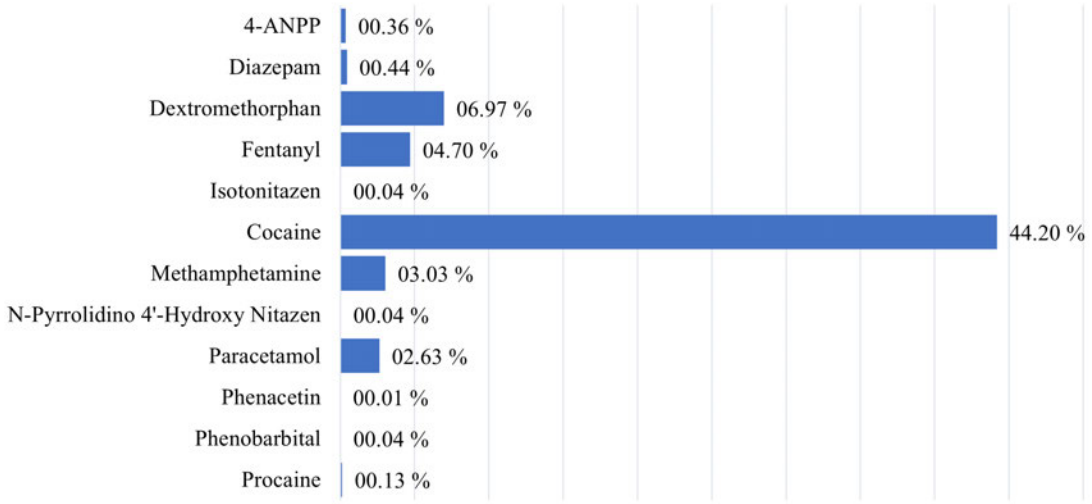

Figure 25: Substances identified during literature research found alongside heroin from January 2024 until September 2025.

Among these substances, it is to be noted that cocaine, dextromethorphan and fentanyl were found most frequently. In routine analytics, the presence of N-Phenyl-1-(2-phenylethyl)piperidin-4-amine (4-ANPP), which is used as a base substance during synthesis of fentanyl, indicates an illicit production pathway.[38] As a result, samples containing this substance are more likely to be linked to illicit consumption of fentanyl which also increases the likeliness for its application as an adulterant. 4-ANPP could be identified in 28 samples also containing heroin or identifying metabolites. A co-consumption can neither be confirmed nor denied, however among all samples containing fentanyl or corresponding markers, these can be considered the most likely to be due to adulterated heroin. In order to determine whether the numbers of cases involving both heroin and fentanyl increase as expected, a more in depth analysis in smaller increments, for example monthly, would be useful and should be considered in the future.

Nitazene type substances, which were named as a new synthetic opioid used to adulterate heroin,[5] could only be identified in three samples each. This marks the second lowest number of all considered samples before phenacetine, which was found in one sample that was also tested positive for cocaine.

Considering the remaining substance groups deemed relevant, most were already described within the most common substances. However, amphetamines were detected as well. In 1050 or 13.60 % of the heroin positive samples, a positive result was found. All three opioid analgesics tilidine, tramadole and oxycodone[12,39] could be detected alongside heroin. This was the case in 31 samples for tilidine, 81 for tramadole and 145 for oxycodone. Despite their potential to increase the effects of heroin, independant consumption cannot be eliminated as a reason for their presence.

The total number of heroin positive samples that contained substances likely used in

adulteration was as low as 29, containing samples that tested positive for 4-ANPP and phenacetin. The latter however, was found in a cocaine positive sample and is therefore more likely linked to cocaine adulteration, which will be further discussed below. while 4-ANPP is tied to illicit opioid consumption, it could also be due to fentanyl abuse. The additional presence of heroin however, indicates a possible adulteration.
For cocaine positive samples, the substances visualised in Figure 26 were identified in past samples. In total, 22129 urine samples were considered cocaine positive and included in this evaluation.

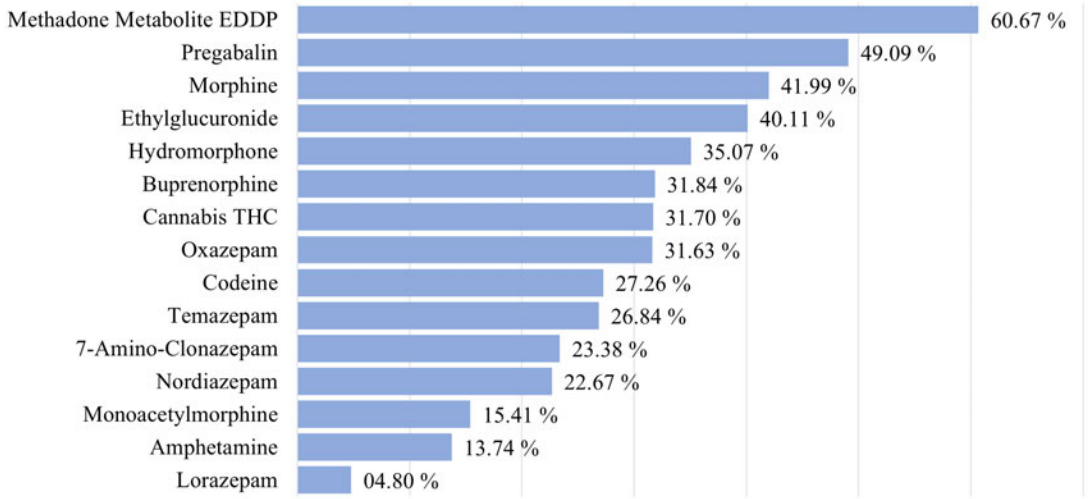

Figure 26: The 15 most commonly found substances alongside cocaine from January 2024 until September 2025.

In the past, the most commonly found substance alongside cocaine was the methadone metabolite EDDP. Other opioids, such as morphine, hydromorphone, buprenorphine, codein and heroin, identified by its metabolite 6-MAM, were also among the most frequently found substances. Potential reasons for the co-administration of opioids with cocaine were discussed above. Their presence as an adulterant cannot be confirmed.
Similarly to the results found for heroin, pregabalin was often found in the context of cocaine as well. However, it was not identified as a frequent adulterant during literature research. Pregabalins application as an anti seizure medication[12] could potentially influence the seizure inducing effects of cocaine. However, no indicators of its use as an adulterant in cocaine were found.
THC was found to also be among the most frequent substances to be found alongside cocaine. While the adulteration of cocaine with THC was found in literature[17] and possible analgesic effects[12] could imply a similar application to opioids in the context of cocaine abuse, co-consumption was deemed the more likely reason for its presence.
Nordiazepam, a metabolite of diazepam[40], was detected more often than its precursor, which was found 224 times total. Both are part of the benzodiazepines group, similarly

to lorazepam, temazepam[12] and 7-amino-clonazepam, a metabolite of clonazepam[41]. The secondary metabolite of diazepam, oxazepam,[40] was even more frequently detected. However, oxazepam is also used as a therapeutic itself.[12] The general therapeutic application as discussed above is the main reason why they cannot be clearly categorised as adulterants in cocaine. However, some of their pharmaceutical effects like respiratory depression[12] indicate that co-administration with cocaine could result in an increase of these symptoms severity.
Lastly, amphetamines were identified in 3041 of the cocaine positive samples. Similarly to most substances discussed above, co-consumption cannot be ruled out as the underlying reason for this. However, due to the enhancing properties amphetamines can have on cocaines effects, based on the biological processes described in the theoretical background section, an intentional simultaneous consumption was considered possible.
Among those substances that were identified during literature research, those listed in Figure 27 were also found in urine samples.

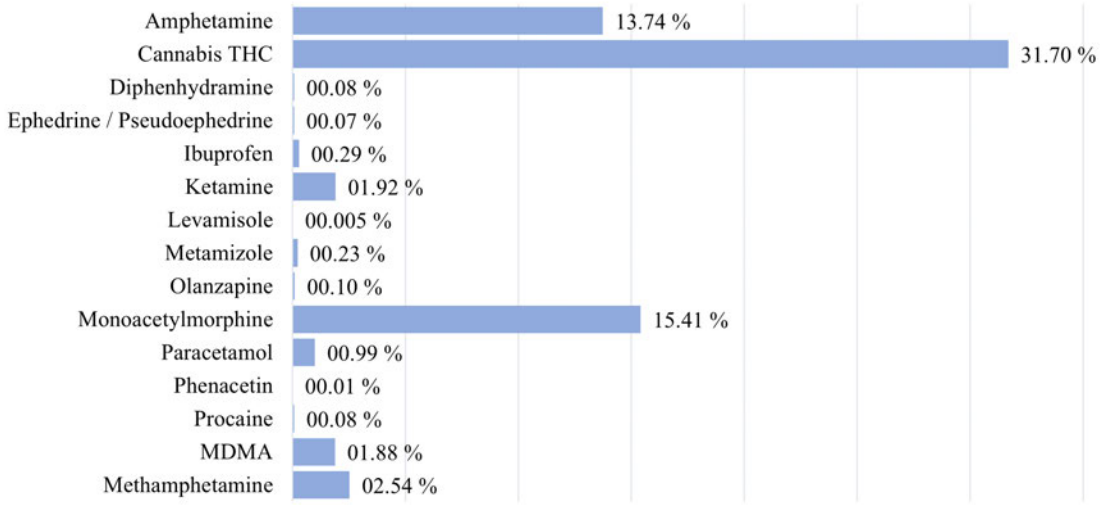

Figure 27: Substances identified during literature research found alongside cocaine from January 2024 until September 2025.

Among the substances identified as a potential adulterant, the most frequently found ones were THC, heroin as identified by its metabolite 6-MAM, and amphetamines. Methamphetamine, ketamine and MDMA were also found in some cases. Ketamine is a known drug of abuse itself. It increases dopamine levels[12] and can therefore increase cocaines effects. Similarly, MDMA strongly increases the extracellular serotonin concentration by reversing its transporters action.[12] If taken alongside cocaine, their respective effects can be heightened. However, MDMA is often consumed as a drug of abuse itself and can as a result not be unequivocally categorised as an adulterant.
Ephedrine and its enantiomer pseudoephedrine were found in 16 samples. their mild stimulant properties[12] can enhance cocaines effect. Pseudoephedrine in particular is

used as a precursor in the illicit production of methamphetamine but is still available in small dosages in readily available medication.[12] Consequentially, it is more likely to be present due to simultaneous methamphetamine consumption rather than cocaine adulteration.

In 220 samples, traces of paracetamol were found. Due to its properties stated above, it is often considered a popularly chosen adulterant. However, it is a very frequently applied drug for pain management.[12] Its presence in urine can therefore not be linked to adulteration of cocaine. A prodrug of acetaminophen, paracetamols active pharmaceutical ingredient, phenacetin was detected in three cases. Due to the metabolisation into acetaminophen, phenacetins effects are similar, however, in its original form, higher toxicity was recorded. As a result, its application is limited.[12] This indicates, that phenacetin was in fact likely to be present due to adulterated cocaine instead of medical reasons.

Similar to paracetamol, ibuprofen is a commonly used pain mediacation and as such readily available.[12] The 64 cases of positive samples containing cocaine can therefore not be linked to adulteration. Metamizole is a non-narcotic analgesic and anti pyretic drug. Its use is strictly regulated due to the possibility for severe side effects. In many countries, it was taken off the market entirely. In Germany however, it is still applied for treatment of severe pain.[42] While metamizole is less commonly used than paracetamol and ibuprofen, it can still be linked to medical reasons rather than adulteration.

Olanzapine is an antipsychotic drug with medium sedative potency.[12] It is commonly used as medication and can therefore not be directly linked to adulteration of the affected cocaine positive samples.

The local anaesthetic procaine was found in 17 cases alongside cocaine. It is used in cocaine to mask poor quality by imitating its immediate local anaesthetic effects.[23] While being among the most likely substances to be used as an adulterant, it can also be present as a result of medical application. Lidocaine, which is similar to procaine in its use as an adulterant for cocaine, was not found in urine samples.

Concerning all substances found alongside cocaine, it is to be noted, that especially levamisole was very rarely detected at all in urine samples using a GC-MS analytical methods. Only one sample contained detectable concentrations.

For the substances detected alongside cocaine, most of these deemed potentially of interest are already included either in the list of most commonly detected substances or those mentioned in literature. However, tilidine, tramadol and oxycodone were not included yet. These substances were found in 176, 351 and 390 samples, respectively. Tramadol is an analgesic drug with the ability to block serotonin and norepinephrine reuptake.[12] Especially if taken alongside other reuptake inhibitors like cocaine, the risk for consumers to develop serotonin syndrome is significantly heightened. Both tilidine and oxycodone are opioid analgesics.[12,39] All three are applied in a medical but also an illicit abuse context. As a result, adulterated cocaine cannot be assumed as the most

likely reason for their presence, however, the combination would have the same effects described in the theoretical background section for opioids in general.
In total, four samples can be linked to possible adulteration of cocaine. Three contained phenacetin and one levamisole, both substances not commonly used in human for other purposes.

5.4.2. Results of LC-MS/MS Analytics

Concerning the analytes that were newly added to the detection method in human urine, the following results visualised in Figure 28 were found. After implementation, presence of levamisole and aminorex in cocaine positive samples could be determined. Samples showing low concentrations of cocaine, benzoylecgonine or any newly added analytes, around the LOD or concentration cut-offs as applied in routine analytics, were considered positive for this thesis if a clear distinction between peak and noise signal could still be made.
In total 5169 samples were evaluated in regards to one or more of the newly added analytes being present. This was found to be the case in 355 or 6.87 % of them, while 4814 or 93.13 % did not show traces of either substance. However, only 308 of the evaluated samples were both positive for one or more newly added analytes and cocaine simultaneously, which amounts to 5.96 %. These are the cases relevant for possible adulteration patterns, while the remaining samples that did not test positive for cocaine imply other reasons for the substances to be consumed apart from cocaine consumption.

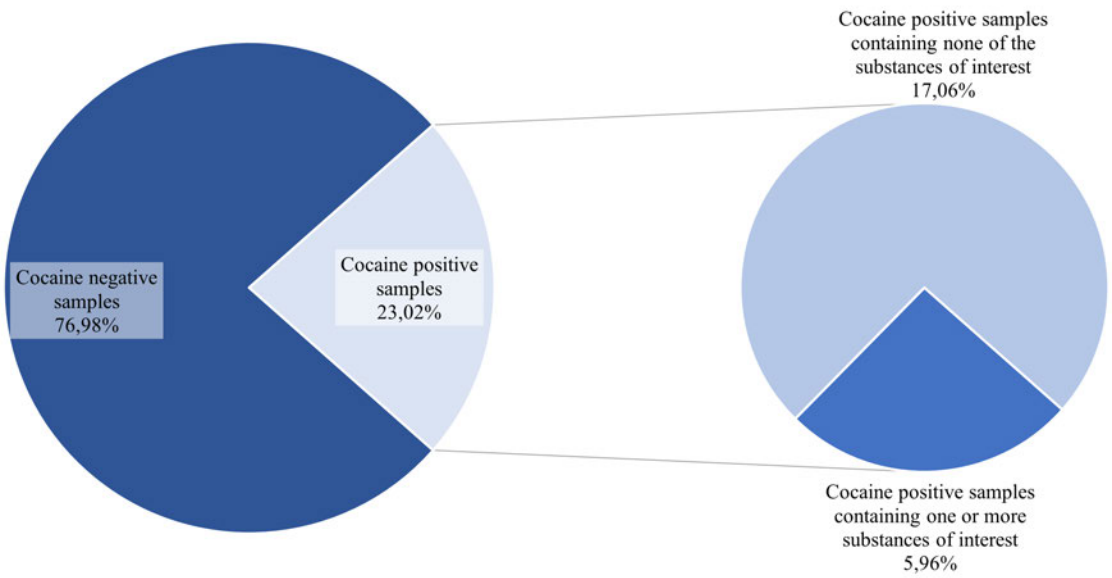

Figure 28: Distribution of samples manually evaluated for the presence of one or more of the newly added analytes. Percentages in reference to total number of samples.

For the 355 samples in which either of the added substances could be found, the frequency of each was investigated, as well as their simultaneous appearance in individual samples. The results of this process were visualised and are shown in Figure 29. In general, it can be said that levamisole was identified in the highest amount of samples of the four, followed by procaine and lidocaine before aminorex.

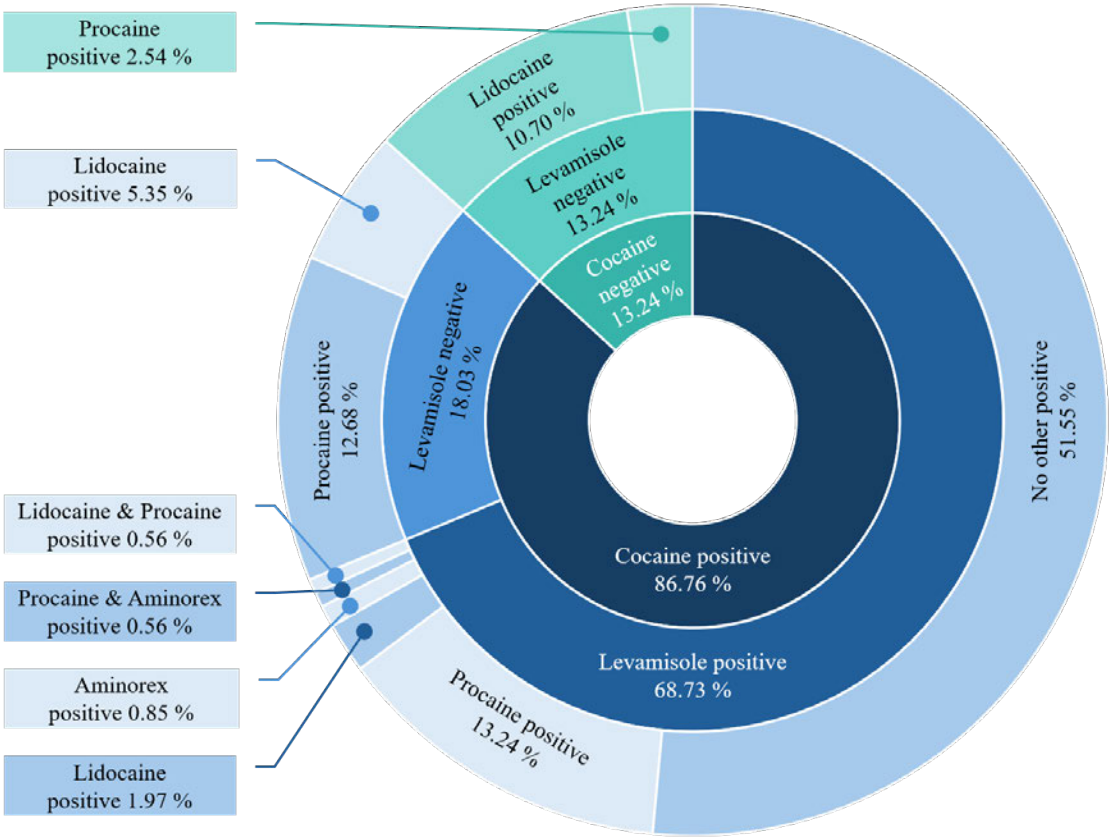

Figure 29: Relative frequency of levamisole, aminorex, lidocaine and procaine in the 355 samples tested positive for one or more of these substances.

A total of 244 samples, which corresponds to a percentage of 68.73 % of all samples tested positive for one or more newly added analyte, contained detectable levels of levamisole. Due to cocaine seizings indicating more pure substance being available,[2] this high numbers of potential adulteration exceeded expectations. A consumption of levamisole on its own cannot generally be traced back to a medical reason, despite its limited applications in some cancer treatments and the arthritis and AIDS medication tetramisole.[31] As a consequence, this pathway of administration can be deemed unlikely. Found concentrations ranged from the detection limits of both systems to as high as 2335.99 ng/mL or >800 ng/mL, depending on the used measurement system. The highest found concentration of levamisole greatly exceeded the linear range of the detection method of 800 ng/mL on system two. Such high results, however, were not routinely found. The mean value of found concentrations was calculated to be 67.27 ng/mL. However, most samples contained only small amounts of the substance, leading to the median value to be calculated as 4.81 ng/mL. A significant amount of samples contained concentrations lower than the LOQ but above LOD. This was the case for 43 samples total or 17.62 % of all levamisole positive samples. When only those samples that were found to contain concentrations above LOQ and therefore can be used in a quantitative evaluation are

considered, the mean concentration was 70.20 ng/mL and the median was found to equal 6.44 ng/mL. This development was expected as a significant amount of samples at low concentrations was excluded. All samples that tested positive for levamisole also tested positive for cocaine and identifying markers. This indicates, that levamisole could indeed be linked to adulteration of cocaine rather than its individual consumption. As a result, it could potentially be used as another factor to identify cocaine consumptions in human urine samples. It is to be noted, that in some levamisole positive samples, the found concentration of cocaine was below the routinely applied cut-off concentration for recent consumption. Some even showed small concentrations of the marker benzoylecgonine only, in rare cases below cut-off limits as well. However, as a clear peak could still be distinguished, these were still counted towards being cocaine positive. Considering the longer half-life of levamisole compared to cocaine,[32] this could be utilised to identify consumption of adulterated cocaine even after cocaine concentrations and those of identifying metabolites in urine have fallen below cut-off limits in the future.
In comparison to the results of past samples, the significant difference in total levamisole positive samples is to be noted. This can likely be attributed to the lack of detection in the same analytical method. In many cases, the GC-MS analytics needed up until this point to identify the substance is not performed in addition to the other routine cocaine analytics. The 244 levamisole positive samples found during the month of august for this evaluation, would otherwise be represented to some extend in the number of levamisole positive samples retrospectively evaluated for adulterants. This further solidifies the hypothesis that including levamisole in the main cocaine detection method would represent an improvement for the identification of cocaine consumption as well as adulteration.
Samples tested positive for the presence of aminorex were also found to contain high concentrations of levamisole. However Aminorex was very rarely detected with only five positive samples total and only 1.41 % of all samples containing at least one new analyte testing positive for it. This indicates, that while aminorex is considered a metabolisation product of levamisole[31], high concentrations have to be present for sufficient concentrations of aminorex being produced to be detectable by the used analytical methods. The lowest concentration of levamisole with a corresponding aminorex signal was found to be 231.23 ng/mL levamisole and 1.69 ng/mL aminorex. Simultaneously, samples showing higher concentrations of levamisole tested negative for aminorex. A sample that was found to contain 462.87 ng/mL levamisole did not show any signal for aminorex. This indicates no proportionality between the consumed amount of levamisole and resulting aminorex production. The found concentrations of aminorex ranged from 0.97 ng/mL to 17.54 ng/mL with a mean value of 4.80 ng/mL and a median of 1.69 ng/mL. In total, four out of the five or 80.00 % of found positive samples were below the respective LOQ of the measurement systems. The highest and only concentration above LOQ was found in a sample that exceeded the linear range for levamisole of system two significantly. This leads to the assumption, that the use of aminorex for routine analytics is limited

at this time. A significant decrease in LOD and LOQ through optimisation of the used analytical methods allowing for lower concentrations to be detected would be needed. If this would be achieved in the future, the even longer half life compared to levamisole[32] would potentially allow for a further increased timeframe for detection of adulterated cocaine consumption. However, under the given conditions, this is not yet possible.
Lidocaine and procaine were also added to the method and positive results could be found in some of the analysed samples. For these substances however, presence was not exclusively tied to the presence of cocaine or identifying markers. In total, 66 samples or 18.59 % of all samples containing one or more of the newly added analytes were found to contain lidocaine, while 105 tested positive for procaine, which corresponds to 29.58 %. However, only 28 total or 42.42 % of the lidocaine and 96 total or 91.43 % of the procaine positive samples were found to contain cocaine or identifying markers as well. This suggests that both local anaesthetics are not necessarily indicative for cocaine consumption but can also be tied to other factors. However, for each of them there are some samples where cocaine alone or cocaine and levamisole both showed a positive result. For these samples, it is likely that the consumed cocaine did in fact contain one or more of the substances as an adulterant. Simillar to levamisole, the numbers of positive results for lidocaine and procaine was higher than those found when evaluating past samples. The addition of both to the analytical method can therefore be expected to result in more samples being identified as positive for either of the two subsances.
Concerning lidocaine, concentrations ranged from the respective detection limits to 894.29 ng/mL. While the calculated mean value was determined to be 80.03 ng/mL, the median of 11.40 ng/mL suggests that most samples contained lower amounts of the local anaesthetic. In total, 9 samples or 13.64 % of all lidocaine positive results were found to be below the limit of quantification. If only those above the LOQ and within the tested linear range are considered, the mean concentration was found to be 38.14 ng/mL, while a median of 11.56 ng/mL was calculated. Reason for this significantly lower mean value is the exclusion of samples with concentrations above 200 ng/mL. In the future, it could therefore be useful to investigate the linearity range of lidocaine to ensure these samples exceeding the current range can be included.
For procaine, only one of the applied analytical methods could be used for quantification. A peak of an interfering substance that was mainly separated here showed a complete overlap with the other method. However, through evaluation of the second ion transition, qualitative results could still be found. Among all considered samples containing procaine, 80.00 % were only evaluated quantitatively while 20.00 % could also be quantified. Within these, one or 0.95 % fell below the quantification limit. The remaining samples ranged between 1.69 ng/mL and 3158.25 ng/mL. As a result, a mean concentration of 265.00 ng/mL and a median of 14.75 ng/mL could be calculated. This indicates that samples generally contain lower concentrations. However, multiple samples were found to contain higher concentrations as well. Among all new analytes, procaine was

the one with the most widespread concentration range.

6. Conclusion and Future Prospects

A large variety of possible adulterants in the drugs of abuse heroin and cocaine could be identified. Among these, most could not be clearly associated with adulteration of heroin or cocaine. In heroin positive samples, only 29 contained substances tied to adulteration patterns, namely 4-ANPP and phenacetin. Even less results were found considering cocaine adulteration with only four samples were strongly suspected to contain adulterated cocaine, as levamisole and phenacetin were identified.
Four substances resulting from literature research were chosen based on recent developments in adulteration patterns and added to the existing analytical method for identification of opioids, cocaine and identifying metabolites, among others. It could be proven that both applied measurement systems can be used to analyse the identified adulterants that were added to the methods. Results were proven to be highly accurate with a bias, intermediate precision and repeatability values below 8 % for all analytes. Low detection and quantitation limits could be established as well. For levamisole, concentrations as low as 0.56 ng/mL can be detected on both measurement systems and quantitation was calculated to be possible from 1.47 ng/mL considering both systems. Aminorex was found to be detectable from 0.99 ng/mL and quntitation above 2.96 ng/mL can be performed. However, due to the low number of positive samples found, its use in routine analytics was deemed low within the stated limitations of detectable concentration. As a result, it will currently not be included in routine sample evaluation processes until adjustments in the analytical method allow for more sensitive detection and the potential use for evaluation of cocaine consumption can be reevaluated. Concerning lidocaine, an LOD of 0.66 ng/mL and an LOQ of 2.14 ng/mL can be assumed for both systems. Procaine could be detected above 0.96 ng/mL and quantified above 3.07 ng/mL. Due to interfering signals, quantitation was only deemed possible on system two, while system one can be applied for qualitative evaluation only. All analytes were proven to be linear within the tested concentration range with that of levamisole being reduced on system two in comparison to system one. Aminoerx as levamisoles pharmacologically active metabolite could be found in a small number of levamisole positive samples. However, high concentrations of levamisole had to be present for detectable concentrations of aminorex to be found. As most levamisole positive samples did not contain sufficient concentrations of aminorex, its use in routine analytics can be considered limited.
Following the successful validation of the new substances, levamisole, lidocaine and procaine were deemed useful for routine analytics and added to the evaluation process. They will be included in future reporting for found substances.
Levamisole could be identified in 244 samples containing cocaine or identifying markers.

Some were found within samples containing cocaine and benzoylecgonine concentrations that would be considered negative using current cut-off limits but still showed a significant signal in the corresponding chromatogram. This suggests that in the future, levamisole concentrations in urine could be evaluated to determine consumption of adulterated cocaine even after the concentration of cocaine itself has fallen below cut-off values. Additionally, the discrepancy in the number of levamisole positive samples found during evaluation of the augmented analytical method and those within the time span evaluated retrospectively shows high potential for future evaluation. This indicator for significantly higher consumption of adulterated cocaine than represented before offers great potential for the better understanding of the drug markets development as well as improvements in the medical approach to affected patients. Simultaneously, it implies that adulteration is an important aspect concerning the illicit consumption of substances, not only in the context of levamisole but highly potent synthetic opioids as well. The possibility to safely consume drugs is limited while side effect inducing components are unknown to the consumer, raising the question if improved drug checking opportunities could lead to a decrease in fatal overdoses.

References

1. Roque Bravo, R. *et al.* Cocaine: an updated overview on chemistry, detection, biokinetics, and pharmacotoxicological aspects including abuse pattern. *Toxins* **14,** 278. doi:10.3390/toxins14040278 (2022).

2. European Monitoring Centre for Drugs and Drug Addiction and Europol. *EU Drug Markets Analysis: Key insights for policy and practice* via https://www.emcdda.europa.eu/publications/eu-drug-markets/analysis-key-insights-policy-and-practice_en. ISBN: 978-92-9497-965-0, doi:10.2810/567901, Accessed: 22.07.2025. 2024.

3. Broséus, J., Gentile, N. & Esseiva, P. The cutting of cocaine and heroin: a critical review. *Forensic science international* **262,** 73–83. doi:10.1016/j.forsciint.2016.02.033 (2016).

4. Coomber, R. Reconceptualising drug markets and drug dealers—the need for change. *Drugs and alcohol today* **10,** 10–13. doi:10.5042/daat.2010.0122 (2010).

5. European Monitoring Centre for Drugs and Drug Addiction. *European Drug Report 2024: Trends and Developments* via https://www.emcdda.europa.eu/publications/european-drug-report/2024_en. ISBN: 978-92-9497-975-9, doi:10.2810/91693, Accessed: 22.07.2025. 2024.

6. Cole, C. *et al.* Adulterants in illicit drugs: a review of empirical evidence. *Drug testing and analysis* **3,** 89–96. doi:10.1002/dta.220 (2011).

7. Chandra, S., Kumar, A., Yadav, S. & Anjana, S. N. Forensic examination of heroin and its cutting agents. *Journal of Forensic Science & Criminology* **10,** 1–14. ISSN: 2348-9804 (2022).

8. Milella, M. S. *et al.* Heroin and its metabolites: relevance to heroin use disorder. *Translational psychiatry* **13,** 120. doi:10.1038/s41398-023-02406-5 (2023).

9. Maas, A., Madea, B. & Hess, C. Confirmation of recent heroin abuse: Accepting the challenge. *Drug testing and analysis* **10,** 54–71. doi:10.1002/dta.2244 (2018).

10. Alhosan, N., Cavallo, D., Santiago, M., Kelly, E. & Henderson, G. Slow dissociation kinetics of fentanyls and nitazenes correlates with reduced sensitivity to naloxone reversal at the μ-opioid receptor. *British Journal of Pharmacology* **182,** 969–987. doi:10.1111/bph.17376 (2025).

11. Blackwood, C. A. & Cadet, J. L. The molecular neurobiology and neuropathology of opioid use disorder. *Current research in neurobiology* **2,** 100023. doi:10.1016/j.crneur.2021.100023 (2021).

12. Vanderah, T. W. *Katzung's basic & clinical pharmacology* 16th Edition. ISBN: 978-1-260-46330-9 (McGraw Hill New York, 2024).

13. Wei, C. *et al.* Response dynamics of midbrain dopamine neurons and serotonin neurons to heroin, nicotine, cocaine, and MDMA. *Cell discovery* **4,** 60. doi:10.1038/s41421-018-0060-z (2018).

14. Kiyatkin, E. A. & Choi, S. Brain oxygen responses induced by opioids: focus on heroin, fentanyl, and their adulterants. *Frontiers in Psychiatry* **15,** 1354722. doi:10.3389/fpsyt.2024.1354722 (2024).

15. Kaye, A. D. & Weinkauf, J. L. in *Perioperative Addiction: Clinical Management of the Addicted Patient* 95–109 (Springer, 2011). ISBN: 978-1-4614-0169-8. doi:10.1007/978-1-4614-0170-4_5.

16. Drake, L. R. & Scott, P. J. DARK classics in chemical neuroscience: cocaine. *ACS chemical neuroscience* **9,** 2358–2372. doi:10.1021/acschemneuro.8b00117 (2018).

17. Peck, Y., Clough, A. R., Culshaw, P. N. & Liddell, M. J. Multi-drug cocktails: Impurities in commonly used illicit drugs seized by police in Queensland, Australia. *Drug and alcohol dependence* **201,** 49–57. doi:10.1016/j.drugalcdep.2019.03.019 (2019).

18. Cammann, K. *Instrumentelle Analytische Chemie* 1st Edition, Reprint 2010. ISBN: 978-3-8274-2739-7 (Spektrum-Verlag Heidelberg, 2001).

19. Thomas, S. N., French, D., Jannetto, P. J., Rappold, B. A. & Clarke, W. A. Liquid chromatography–tandem mass spectrometry for clinical diagnostics. *Nature Reviews Methods Primers* **2,** 96. doi:10.1038/s43586-022-00175-x (2022).

20. Peters, F. T., Drummer, O. H. & Musshoff, F. Validation of new methods. *Forensic science international* **165,** 216–224 (2007).

21. Peters, F. T. *et al.* Requirements for the validation of analytical methods. *APPENDIX B To the GTFCh Guidelines for quality assurance in forensic-toxicological analyses.* **Version 01** (2009).

22. Paul, L. *et al.* Richtlinie der GTFCh zur Qualitätssicherung bei forensisch- toxikologischen Untersuchungen. *Toxichem Krimtech* **76,** 142–176 (2009).

23. Laposchan, S., Kranenburg, R. F. & van Asten, A. C. Impurities, adulterants and cutting agents in cocaine as potential candidates for retrospective mining of GC-MS data. *Science & Justice* **62,** 60–75. doi:10.1016/j.scijus.2021.11.004 (2022).

24. Sharma, S., Rathor, M., Wadhvani, G., Goyal, M. & Sharma, S. K. ANALYSIS AND SEPARATION OF COMMON ADULTERANTS AND DILUENTS FROM THE ILLICIT DRUG (HEROIN) SAMPLES. *Rasayan Journal of Chemistry* **16.** doi:10.31788/RJC.2023.1638268 (2023).

25. Shesser, R., Jotte, R. & Olshaker, J. The contribution of impurities to the acute morbidity of illegal drug use. *The American journal of emergency medicine* **9,** 336–342 (1991).

26. Lurie, I. S., Driscoll, S. E., Cathapermal, S. S. & Panicker, S. Determination of heroin and basic impurities for drug profiling by ultra-high-pressure liquid chromatography. *Forensic science international* **231,** 300–305. doi:10.1016/j.forsciint.2013.06.008 (2013).

27. EUDA. *Signals of the adulteration of heroin with nitazene opioids - France and Germany* RCS ID: EU-EWS-RCS-AD-2025-0001. EU Early Warning System Advisory. Transmitted by: Early warning, Alert, Laboratory network Sector, EUDA. Accessed: 05.03.2025. 2025.

28. Fiorentin, T. R., Fogarty, M., Limberger, R. P. & Logan, B. K. Determination of cutting agents in seized cocaine samples using GC–MS, GC–TMS and LC–MS/MS. *Forensic science international* **295,** 199–206. doi:10.1016/j.forsciint.2018.12.016 (2019).

29. Hofmaier, T. *et al.* Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. *Neurochemistry international* **73,** 32–41. doi:10.1016/j.neuint.2013.11.010 (2014).

30. Maier, J., Mayer, F. P., Brandt, S. D. & Sitte, H. H. DARK classics in chemical neuroscience: aminorex analogues. *ACS chemical neuroscience* **9,** 2484–2502. doi:10.1021/acschemneuro.8b00415 (2018).

31. Brunt, T. M., van den Berg, J., Pennings, E. & Venhuis, B. Adverse effects of levamisole in cocaine users: a review and risk assessment. *Archives of Toxicology* **91,** 2303–2313. doi:10.1007/s00204-017-1947-4 (2017).

32. Hess, C. *et al.* Determination of levamisole, aminorex, and pemoline in plasma by means of liquid chromatography-mass spectrometry and application to a pharmacokinetic study of levamisole. *Drug Testing and Analysis* **6,** 1049–1054. doi:10.1002/dta.1619 (2014).

33. LGC Standards. *Levamisol 100 µg/mL in Acetonitrile* via https://www.lgcstandards.com/DE/de/Levamisol-100-g-mL-in-Acetonitrile/p/DRE-A14629690AL-100. Accessed: 22.04.2025.

34. LGC Standards. *Aminorex* via https://www.lgcstandards.com/DE/de/Aminorex/p/TRC-A629200. Accessed: 22.04.2025.

35. Offermanns, S. & Rosenthal, W. *Encyclopedia of molecular pharmacology* ISBN: 978-3-030-57401-7. doi:10.1007/978-3-030-57401-7 (Springer, 2021).

36. Huq, F. & Al-Shuneigat, H. Molecular modelling analysis of the metabolism of methadone. *Asian J Biochem* **2,** 19–30. doi:10.3923/ajb.2007.19.30 (2007).

37. Heier, C., Xie, H. & Zimmermann, R. Nonoxidative ethanol metabolism in humans—from biomarkers to bioactive lipids. *Iubmb Life* **68,** 916–923. doi:10.1002/iub.1569 (2016).

38. Vandeputte, M. M., Krotulski, A. J., Hulpia, F., Van Calenbergh, S. & Stove, C. P. Phenethyl-4-ANPP: a marginally active byproduct suggesting a switch in illicit fentanyl synthesis routes. *Journal of Analytical Toxicology* **46,** 350–357. doi:10.1093/jat/bkab032 (2022).

39. Radbruch, L. *et al.* Topical review on the abuse and misuse potential of tramadol and tilidine in Germany. *Substance abuse* **34,** 313–320. doi:10.1080/08897077.2012.735216 (2013).

40. St-Pierre, M. V. & Pang, K. S. Kinetics of sequential metabolism. II. Formation and metabolism of nordiazepam and oxazepam from diazepam in the perfused murine liver. *The Journal of pharmacology and experimental therapeutics* **265,** 1437–1445. doi:10.1016/S0022-3565(25)38281-9 (1993).

41. Negrusz, A. *et al.* Quantitation of clonazepam and its major metabolite 7-aminoclonazepam in hair. *Journal of analytical toxicology* **24,** 614–620. doi:10.1093/jat/24.7.614 (2000).

42. Andrade, S., Bartels, D. B., Lange, R., Sandford, L. & Gurwitz, J. Safety of metamizole: a systematic review of the literature. *Journal of clinical pharmacy and therapeutics* **41,** 459–477. doi:10.1111/jcpt.12422 (2016).

Appendix

A. LC-MS/MS Parameters

Table 15: Ion transitions and MRM parameters of all analytes used for routine analytics. Separate transitions for the same analytes are numbered for better differentiation.

Name	Ion transition m/z [Da]	DP [eV]	CE [eV]	CXP [eV]	On System
4-ANPP 1	281.10 - 188.20	41	25	10	1, 2
4-ANPP 2	281.10 - 105.10	41	43	12	1, 2
4-Chloroisobutyrylfentanyl 1	385.20 - 188.10	81	31	12	1, 2
4-Chloroisobutyrylfentanyl 2	385.20 - 105.20	81	53	6	1, 2
4-Flourotropacain 1	264.275 - 124.10	71	29	6	1, 2
4-Flourotropacain 2	264.275 - 67.00	57	57	8	1, 2
4-hydroxy Nitazene 1	369.20 - 100.00	61	27	14	1, 2
4-hydroxy Nitazene 4	369.20 - 72.00	61	65	10	1, 2
4-methoxy-Butyryl Fentanyl 1	381.20 - 188.10	81	31	12	1, 2
4-methoxy-Butyryl Fentanyl 2	381.20 - 105.20	81	53	6	1, 2
6-beta-Naltrexol 1	344.174 - 326.10	80	30	8	1, 2
6-beta-Naltrexol 2	344.174 - 55.00	80	40	8	1, 2
Acetyl Fentanyl 1	323.20 - 188.10	81	31	12	1, 2
Acetyl Fentanyl 2	323.20 - 105.20	81	53	6	1, 2
Acetyl Norfentanyl 1	219.10 - 84.10	81	30	8	1, 2
Acetyl Norfentanyl 2	219.10 - 55.00	81	50	8	1, 2
Acetylcodein 1	342.20 - 225.00	96	37	18	1, 2
Acetylcodein 2	342.20 - 165.20	96	65	10	1, 2
Acryl Fentanyl 1	335.20 - 188.10	81	31	12	1, 2
Acryl Fentanyl 2	335.20 - 105.20	81	53	6	1, 2
Anhydroecgonine methyl ester 1	182.10 - 91.10	81	37	16	1, 2
Anhydroecgonine methyl ester 2	182.10 - 118.10	81	33	20	1, 2
Anhydroecgonine methyl ester 25	182.10 - 122.10	81	31	22	1, 2
Anhydroecgonine methyl ester 26	182.10 - 151.10	81	23	8	1, 2
AH-7921 (Opioid) 1	329.01 - 284.00	81	25	6	1, 2
AH-7921 (Opioid) 2	329.01 - 173.10	81	43	12	1, 2
Aminorex 1	163.054 - 120.00	6	19	12	1, 2
Aminorex 2	163.054 - 103.00	6	35	10	1, 2
ANF 3	219.10 - 56.10	86	40	10	1
ANF 4	219.10 - 136.10	86	25	8	1
ATM4 1	354.122 - 281.10	121	21	10	1, 2

will be continued on next page...

Name	Ion transition m/z [Da]	DP [eV]	CE [eV]	CXP [eV]	On System
ATM4 2	354.122 - 221.10	121	37	10	1, 2
ATM-4-Glucuronide 1	529.981 - 354.10	1	21	14	1, 2
ATM-4-Glucuronide 2	529.981 - 281.10	51	37	12	1, 2
Benzoylecgonine 1	290.20 - 168.20	101	27	30	1, 2
Benzoylecgonine 2	290.20 - 105.10	101	43	22	1, 2
Benzoylecgonine-C13 5	291.187 - 78.00	106	73	8	1
Benzoylecgonine-C13 6	291.187 - 81.90	106	39	12	1
Benzoylecgonine-d3	293.20 - 171.10	101	29	6	1, 2
Buprenorphine 1	468.50 - 55.30	130	93	2	1, 2
Buprenorphine 2	468.50 - 468.50	130	30	2	1, 2
Buprenorphine 3	468.50 - 396.10	130	55	8	1, 2
Buprenorphine 5	468.30 - 396.10	170	55	8	1
Buprenorphine 6	468.30 - 414.30	170	47	16	1
Buprenorphine-C13 1	469.50 - 56.30	130	93	10	1
Buprenorphine-C13 2	469.50 - 469.50	130	30	10	1
Buprenorphine-d4 1	472.30 - 400.20	121	55	10	1
Buprenorphine-d4 2	472.50 - 472.50	121	20	2	1, 2
Butyryl Fentanyl 1	351.20 - 188.10	81	31	12	1, 2
Butyryl Fentanyl 2	351.20 - 105.20	81	53	6	1, 2
Cocaethylene 1	318.00 - 196.20	71	29	12	1, 2
Cocaethylene 2	318.00 - 82.10	71	47	14	1, 2
Cocaine 1	304.20 - 182.20	96	29	14	1, 2
Cocaine 2	304.20 - 105.10	96	39	8	1, 2
Cocaine-C13 1	305.20 - 105.10	96	29	14	1
Cocaine-C13 2	305.20 - 150.10	96	39	8	1
Cocaine-C13 3	305.20 - 150.10	96	39	8	1
Codeine 1	300.20 - 152.20	85	90	10	1, 2
Codeine 2	300.20 - 115.20	85	99	8	1, 2
Codeine-Glucuronide 1	476.20 - 300.10	60	40	8	2
Codeine-Glucuronide 2	476.20 - 152.10	60	90	10	2
Desmethylloperamide 1	463.20 - 252.10	60	53	8	1, 2
Desmethylloperamide 2	463.20 - 196.10	60	53	8	1, 2
Despropionyl p-Fluoro Fentanyl 1	299.20 - 188.10	81	31	12	1, 2
Despropionyl p-Fluoro Fentanyl 2	299.20 - 105.20	81	53	6	1, 2
Dextromethorphan 1	272.10 - 213.10	120	47	21	1, 2
Dextromethorphan 2	272.10 - 215.10	120	47	21	1, 2

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Name	Ion transition m/z [Da]	DP [eV]	CE [eV]	CXP [eV]	On System
Dihydrocodeine 1	302.20 - 199.20	91	30	14	1, 2
Dihydrocodeine 2	302.20 - 128.10	91	89	10	1, 2
Dihydrocodeine-C13 1	303.20 - 199.20	91	30	14	1
Dihydrocodeine-C13 2	303.20 - 128.10	91	89	10	1
Dimethocaine 1	279.06 - 120.10	126	31	6	1, 2
Dimethocaine 2	279.06 - 142.20	126	25	10	1, 2
EDDP 1	278.20 - 249.00	51	37	18	1
EDDP 2	278.20 - 234.10	51	47	20	1
EDDP-C13 4	279.008 - 157.10	126	67	14	1, 2
EDDP-C13 6	279.008 - 116.10	126	87	6	1, 2
Ethylmorphine 1	314.00 - 165.00	80	60	10	1, 2
Ethylmorphine 2	314.00 - 153.00	80	60	10	1, 2
Fentanyl 1	337.20 - 77.30	51	107	12	1, 2
Fentanyl 2	337.20 - 105.30	51	77	5	1, 2
Fentanyl-d5 2	342.20 - 105.30	51	70	5	1, 2
Furanyl Fentanyl 1	375.20 - 188.10	81	31	12	1, 2
Furanyl Fentanyl 2	375.20 - 105.20	81	53	6	1, 2
Hydrocodone 1	300.20 - 199.10	40	35	4	1, 2
Hydrocodone 2	300.20 - 171.10	40	50	4	1, 2
Hydromorphone 1	286.10 - 185.10	40	35	4	1, 2
Hydromorphone 2	286.10 - 157.10	40	50	4	1, 2
Isotodesnitaze 1	366.20 - 100.10	61	27	14	1, 2
Isotodesnitaze 3	366.20 - 107.00	61	79	14	1, 2
Isotonitaze 1	411.188 - 100.00	61	27	14	1, 2
Isotonitaze 2	411.188 - 72.00	61	65	10	1, 2
Isotonitaze 3	411.188 - 130.00	61	33	6	1
Isotonitaze 4	411.188 - 106.90	61	79	14	1
Isotonitaze 5	411.188 - 77.00	61	125	10	1
Isotonitaze 6	411.188 - 102.20	61	39	18	1
Ketamine 1	238.10 - 125.00	40	35	4	1, 2
Ketamine 2	238.10 - 220.20	40	20	4	1, 2
Levamisole 1	205.294 - 178.10	96	29	14	1, 2
Levamisole 2	205.294 - 123.00	96	39	8	1, 2
Lidocaine 1	235.10 - 86.20	48	23	8	1, 2
Lidocaine 2	235.10 - 58.20	48	35	8	1, 2
Loperamide 1	477.20 - 266.30	120	53	12	1, 2

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Name	Ion transition m/z [Da]	DP [eV]	CE [eV]	CXP [eV]	On System
Loperamide 2	477.20 - 210.20	120	53	12	1, 2
Meperidine 1	248.20 - 220.30	40	20	4	1, 2
Meperidine 2	248.20 - 174.20	40	20	4	1, 2
Methadone 1	310.30 - 265.20	76	23	8	1
Methadone 2	310.30 - 105.10	76	43	8	1
Methadone-C13 4	311.095 - 105.60	96	39	12	1, 2
Methadone-C13 6	311.095 - 90.80	96	49	16	1, 2
Methadone-d9	319.00 - 268.20	96	39	12	1, 2
Methaqualone 1	251.10 - 91.10	40	50	4	1, 2
Methaqualone 2	251.10 - 132.10	40	35	4	1, 2
Methiodon 1	346.10 - 72.00	41	55	10	1, 2
Methiodon 2	346.10 - 207.10	41	24	6	1, 2
Methylecgonine 1	200.10 - 182.20	66	23	14	1, 2
Methylecgonine 2	200.10 - 82.20	66	37	2	1, 2
Mitragynine 1	399.222 - 174.00	76	43	10	1, 2
Mitragynine 2	399.222 - 225.90	76	35	14	1, 2
Monoacetylmorphine 1	328.20 - 152.00	101	93	8	1, 2
Monoacetylmorphine 2	328.20 - 165.20	101	57	8	1, 2
Morphine 1	286.20 - 152.10	80	81	10	1, 2
Morphine 2	286.20 - 165.10	80	59	12	1, 2
Morphine-C13 1	287.20 - 153.10	80	81	10	1, 2
Morphine-C13 2	287.20 - 166.10	80	59	12	1, 2
Morphine-d3	289.20 - 152.20	126	85	10	1, 2
MT-45 (Opioid) 1	349.256 - 181.10	106	29	22	1, 2
MT-45 (Opioid) 2	349.256 - 169.20	106	25	12	1, 2
Naloxone 1	328.10 - 310.30	76	29	8	1, 2
Naloxone 2	328.10 - 253.30	76	35	20	1, 2
Naltrexone 1	342.10 - 324.10	81	39	18	1, 2
Naltrexone 2	342.10 - 270.10	81	53	12	1, 2
N-desethyl Isotonitazene 1	383.20 - 72.00	61	65	10	1, 2
N-desethyl Isotonitazene 2	383.20 - 312.10	61	35	10	1, 2
N-Desmethyl Dextromethorphan 2	258.10 - 213.10	120	47	21	1, 2
N-Desmethyl Dextromethorphan 3	258.10 - 215.10	120	47	21	1, 2
Norbuprenorphine 1	414.40 - 55.20	121	99	8	1, 2
Norbuprenorphine 2	414.40 - 414.40	121	20	16	1, 2
Norbuprenorphine 3	414.40 - 165.10	121	125	8	1, 2

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Name	Ion transition m/z [Da]	DP [eV]	CE [eV]	CXP [eV]	On System
Norbuprenorphine 4	414.30 - 152.10	121	125	8	1
Norbuprenorphine 5	414.30 - 187.10	166	49	22	1
Norbuprenorphine 6	414.30 - 340.20	166	41	12	1
Norbuprenorphine-C13 1	415.40 - 56.20	121	99	8	1
Norbuprenorphine-C13 2	415.40 - 415.40	121	20	16	1
Norbuprenorphine-d4	417.30 - 165.10	121	115	10	1
Norbuprenorphine-d3	417.40 - 417.40	121	22	16	1, 2
Norfentanyl 1	233.081 - 84.00	51	25	14	1, 2
Norfentanyl 2	233.081 - 54.90	51	59	8	1, 2
Norketamine 1	224.00 - 207.00	40	20	4	1, 2
Norketamine 2	224.00 - 179.00	40	20	4	1, 2
Noroxycodone 1	302.10 - 284.20	71	25	20	1, 2
Noroxycodone 2	302.10 - 227.20	71	41	16	1, 2
Nortilidin 1	260.20 - 155.20	40	20	4	1, 2
Nortilidin 2	260.20 - 77.20	40	40	4	1, 2
N-Piperidinyl 4'-hydroxy Nitazen 1	381.016 - 112.10	146	35	12	1, 2
N-Piperidinyl 4'-hydroxy Nitazen 2	381.016 - 107.10	146	73	12	1, 2
N-Pyrrolidino 4'-hydroxy Nitazen 1	366.985 - 98.10	141	31	12	1, 2
N-Pyrrolidino 4'-hydroxy Nitazen 3	366.985 - 107.10	141	75	12	1, 2
Ocfentanil 1	371.20 - 188.10	81	31	12	1, 2
Ocfentanil 2	371.20 - 105.20	81	53	6	1, 1
OH-Brorphine 1	416.10 - 234.10	10	30	6	1
OH-Brorphine 2	416.10 - 183.00	10	40	6	1
OH-Brorphine 4	418.10 - 234.10	10	30	6	1
OH-Mitragynine 1	415.435 - 190.00	116	41	12	1
OH-Mitragynine 3	415.435 - 175.00	116	63	10	1
OH-Xylazine 1	237.00 - 89.90	41	31	10	1
OH-Xylazine 2	237.00 - 137.20	41	41	6	1
Ortho-Fluorofentanyl 1	355.20 - 188.10	81	31	12	1, 2
Ortho-Fluorofentanyl 2	355.20 - 105.20	81	53	6	1, 2
Oxymorphone 1	302.10 - 284.10	40	20	4	1, 2
Oxymorphone 2	302.10 - 227.10	40	35	4	1, 2
Oxycodone 1	316.10 - 298.20	86	27	8	1, 2
Oxycodone 2	316.10 - 241.20	86	41	18	1, 2
Phencyclidine 1	244.20 - 159.20	36	21	10	1, 2
Phencyclidine 2	244.20 - 117.10	36	41	18	1, 2

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Name	Ion transition m/z [Da]	DP [eV]	CE [eV]	CXP [eV]	On System
Piritramide 1	431.171 - 98.00	151	35	8	1, 2
Piritramide 2	431.171 - 346.10	151	27	18	1, 2
Procaine 1	237.10 - 120.10	56	21	12	1, 2
Procaine 2	237.10 - 100.00	56	37	8	1, 2
Propoxyphene 1	340.20 - 58.10	36	35	8	1, 2
Propoxyphene 2	340.20 - 266.20	36	15	8	1, 2
Sufentanil 1	387.258 - 238.00	46	27	16	1, 2
Sufentanil 2	387.258 - 111.00	46	53	6	1, 2
Tapentadol 1	222.10 - 107.00	61	33	10	1, 2
Tapentadol 2	222.10 - 76.90	61	63	8	1, 2
Tilidine 1	274.20 - 155.20	40	20	4	1, 2
Tilidine 2	274.20 - 91.10	40	50	4	1, 2
Tramadol 1	264.20 - 58.10	31	39	8	1, 2
Tramadol 2	264.20 - 246.00	31	17	22	1, 2
Tramadol-M1-C13 1	251.10 - 58.30	56	41	6	1, 2
Tramadol-M1-C13 2	251.10 - 42.10	56	117	6	1, 2
Tramadol-M1 1	250.10 - 58.30	56	41	6	1, 2
Tramadol-M1 2	250.10 - 42.10	56	117	6	1, 2
U-47700 (Opioid) 1	331.281 - 286.00	66	25	6	1, 2
U-47700 (Opioid) 2	331.281 - 174.90	66	45	12	1, 2
U47700 a (Opioid) 1	317.10 - 145.10	120	47	10	1, 2
U47700 a (Opioid) 2	317.10 - 173.10	120	47	10	1, 2
U47700 M331 (Opioid) 1	331.30 - 286.00	66	25	10	1
U47700 M331 (Opioid) 2	331.30 - 174.90	66	45	6	1
Valeryl Fentanyl 1	365.20 - 188.10	81	31	12	1, 2
Valeryl Fentanyl 2	365.20 - 105.20	81	53	6	1, 2
W-15 (designer drug) 1	377.10 - 273.10	81	30	8	1, 2
W-15 (designer drug) 2	377.10 - 175.00	81	30	8	1, 2
W-18 (designer drug) 1	422.10 - 105.00	81	30	8	1, 2
W-18 (designer drug) 2	422.10 - 175.00	81	30	8	1, 2
WT18b (designer drug) 1	422.10 - 175.00	151	37	14	1, 2
WT18b (designer drug) 2	422.10 - 111.00	151	63	12	1
WT18b (designer drug) 3	422.10 - 273.00	151	31	8	1, 2
Xylazine 1	221.15 - 90.20	71	37	12	1, 2
Xylazine 2	221.15 - 164.20	71	33	6	1, 2

B. Validation Data

B.1. Accuracy

Table 16: Validation data for the Accuracy evaluation of the added analytes on both measurement systems.

measurement values [ng/mL]								
Levamisole Low 10.00 ng/mL								
10.65	10.96	11.36	10.53	10.01	10.80	11.11	10.31	9.64
10.35	10.91	10.43	9.95	11.19	10.46	10.52	10.56	9.86
				9.57	10.45	10.08	10.13	
Levamisole Low 200.00 ng/mL								
194.56	204.45	203.71	197.45	201.29	207.95	204.77	202.35	225.45
210.31	202.08	203.35	208.48	196.04	196.28	210.73	190.62	228.18
				211.00	202.14	200.90	199.67	
Aminorex Low 5.00 ng/mL								
5.64	5.64	5.45	5.71	5.07	5.05	4.87	4.36	5.06
5.65	5.74	5.43	5.63	4.27	5.01	4.50	4.75	4.77
					4.86	4.86	4.29	
Aminorex High 20.00 ng/mL								
21.99	22.88	21.06	18.83	21.44	21.67	22.57	21.57	21.73
22.86	22.99	21.12	20.78	22.65	22.32	21.75	20.35	22.37
				22.53	22.97	22.27	22.74	
Lidocaine Low 5.10 ng/mL								
5.20	5.09	5.30	5.59	4.85	5.62	5.55	5.09	5.23
5.42	5.67	5.24	5.07	5.47	5.32	5.15	5.67	5.15
				4.81	5.41	5.10	5.15	
Lidocaine High 51.03 ng/mL								
50.58	55.52	52.20	49.77	50.71	53.60	53.98	51.04	52.41
54.61	52.48	52.38	50.51	48.71	51.42	55.67	50.05	55.94
				54.89	53.96	55.19	52.14	
Procaine Low 5.00 ng/mL								
4.50	4.59	5.01	5.28	5.62	5.58	5.47		
4.51	4.43	4.90	5.72	5.37	5.56	5.43		
			4.95	5.71	5.45	5.45		
Procaine High 50.00 ng/mL								
49.99	52.43	44.64	47.60	50.55	50.30	49.30		
56.82	52.31	46.28	47.58	50.19	53.46	47.00		
			52.57	50.82	52.45	49.97		

B.2. System One

B.2.1. LOD and LOQ

Table 17: Validation Data used for the determination of the limit of detection and quantitation on system one.

Name	concentration	Peak area		
	[ng/mL]	Internal Standard	Target Ion	Qualifier Ion
Levamisole	1.00	768094	11691	4711
	2.00	1027290	36023	12235
	3.00	755065	38349	12876
	4.00	959461	67334	23163
	5.00	708920	61848	21006
Aminorex	1.00	892365	7956	3210
	2.00	847457	14735	6879
	2.50	1173696	26886	11144
	3.75	1083504	37878	16688
	5.00	914721	38660	16780
	7.50	920240	56394	25296
	10.00	868252	75167	32405
Lidocaine	1.02	1173696	24381	1596
	2.04	914721	30602	1991
	2.55	1083504	48658	3084
	5.10	1134071	94209	5811
	10.20	868252	138969	8313
Procaine	0.50	1251047	29927	2796
	1.00	1173696	30293	3661
	2.00	914721	25992	5659
	2.50	1083504	33522	9374
	5.00	1134071	46437	17344

B.2.2. Matrix Effects, Recovery and Ion Suppression

Table 18: Validation Data used for the determination of matrix effects, recovery and ion suppression on system one.

	Sample name	Target peak area	
		Low	High
Levamisole low: 10.00 ng/mL high: 200.00 ng/mL	(A) external 1	155410	2667032
	(A) external 2	201926	4348366
	(A) external 3	123739	2948789
	(A) external 4	199063	4243569
	(A) external 5	139935	2656599
	(A) external 6	192480	4400842
	(B) external + Matrix 1	179677	3456868
	(B) external + Matrix 2	139473	2242274
	(B) external + Matrix 3	200063	3673052
	(B) external + Matrix 4	134031	2442370
	(B) external + Matrix 5	196050	4160007
	(B) external + Matrix 6	113681	2466510
	(C) internal 1	129999	2385814
	(C) internal 2	195278	3679087
	(C) internal 3	112824	2396280
	(C) internal 4	171121	3552677
	(C) internal 5	110389	2457508
	(C) internal 6	171698	3728976
Aminorex low: 5.00 ng/mL high: 20.00 ng/mL	(A) external 1	56166	217542
	(A) external 2	66928	344689
	(A) external 3	47830	227758
	(A) external 4	66995	323105
	(A) external 5	53171	219149
	(A) external 6	68866	370555
	(B) external + Matrix 1	63097	240418
	(B) external + Matrix 2	43734	176693
	(B) external + Matrix 3	64343	299683
	(B) external + Matrix 4	41596	195863
	(B) external + Matrix 5	63413	281717
	(B) external + Matrix 6	40019	167751
	(C) internal 1	39240	182579
	(C) internal 2	64828	301690

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	Sample name	Target peak area	
		Low	High
Aminorex low: 5.00 ng/mL high: 20.00 ng/mL	(C) internal 3	34448	166530
	(C) internal 4	54993	213651
	(C) internal 5	35418	146776
	(C) internal 6	46067	265745
Lidocaine low: 5.10 ng/mL high: 51.03 ng/mL	(A) external 1	124226	858592
	(A) external 2	139102	1384424
	(A) external 3	91899	996201
	(A) external 4	148440	1544938
	(A) external 5	118449	844094
	(A) external 6	157171	1366628
	(B) external + Matrix 1	129062	921380
	(B) external + Matrix 2	90431	673209
	(B) external + Matrix 3	128626	1126508
	(B) external + Matrix 4	94891	737758
	(B) external + Matrix 5	112359	1113780
	(B) external + Matrix 6	85012	714219
	(C) internal 1	87216	760522
	(C) internal 2	139216	1121528
	(C) internal 3	80833	709275
	(C) internal 4	135651	1082089
	(C) internal 5	93834	685320
	(C) internal 6	124427	1026003

B.2.3. Linearity

Table 19: Validation Data used for the determination of the linearity on system one.

Name	concentration [ng/mL]	Peak area		
		Internal Standard	Target Ion	Qualifier Ion
Levamisole	1.00	1029113	11633	4524
	5.00	1338361	90690	32637
	10.00	1068498	115042	41941
	20.00	1306686	367053	141152
	50.00	996795	548830	204652
	100.00	1425193	1891247	731157
	200.00	1026735	2636764	983450
	500.00	1325460	8762457	3354244
	800.00	1035374	9624222	3591030
	1000.00	1350460	16873752	6695847
	1500.00	975713	15696515	6198423
	2000.00	1308923	30909431	13529953
Aminorex	2.00	1338361	24692	11121
	5.00	1068498	38198	19196
	7.50	1306686	92320	43821
	10.00	996795	82895	38407
	15.00	1425193	182199	89509
	20.00	1026735	165017	78880
	50.00	1325460	654961	309894
	80.00	1035374	687175	292790
	100.00	1350460	1309568	610913
	120.00	975713	998536	435736
	150.00	1308923	1912863	902243
Lidocaine	5.10	1306686	124677	7535
	10.20	996795	141530	9344
	22.68	1425193	560518	33867
	45.36	1026735	667175	41725
	79.38	1325460	1849809	114355
	102.06	1035374	1477040	99254
	124.74	1350460	3074048	180318
	158.76	975713	2192945	134521
	215.46	1308923	4788736	299594
Procaine	1.00	1338361	30913	4755

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Name	concentration [ng/mL]	Peak area		
		Internal Standard	Target Ion	Qualifier Ion
Procaine	2.00	1068498	25974	4575
	5.00	1306686	54359	19247
	10.00	996795	29558	9993
	20.00	1425193	144812	77741
	50.00	1026735	191338	111173
	80.00	1325460	615655	422121
	100.00	1035374	444772	280724
	120.00	1350460	594352	309779
	150.00	975713	605517	368261
	200.00	1308923	1583858	1008894

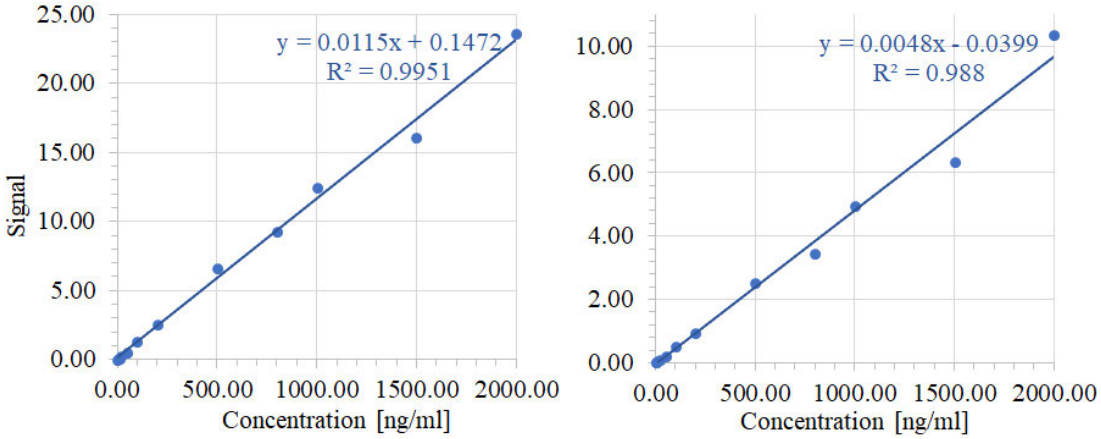

Figure 30: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of levamisole on system one. The calculated equation for the linear model can be found attached to the respective plot.

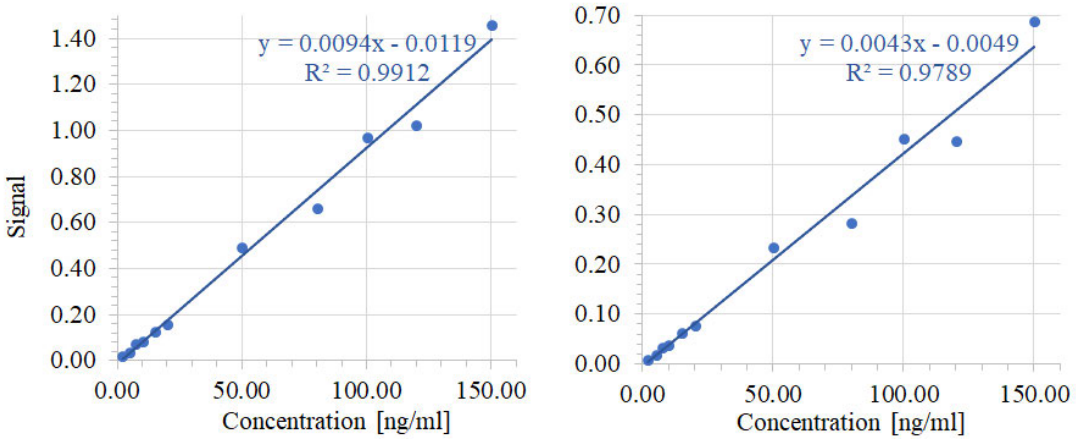

Figure 31: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of aminorex on system one. The calculated equation for the linear model can be found attached to the respective plot.

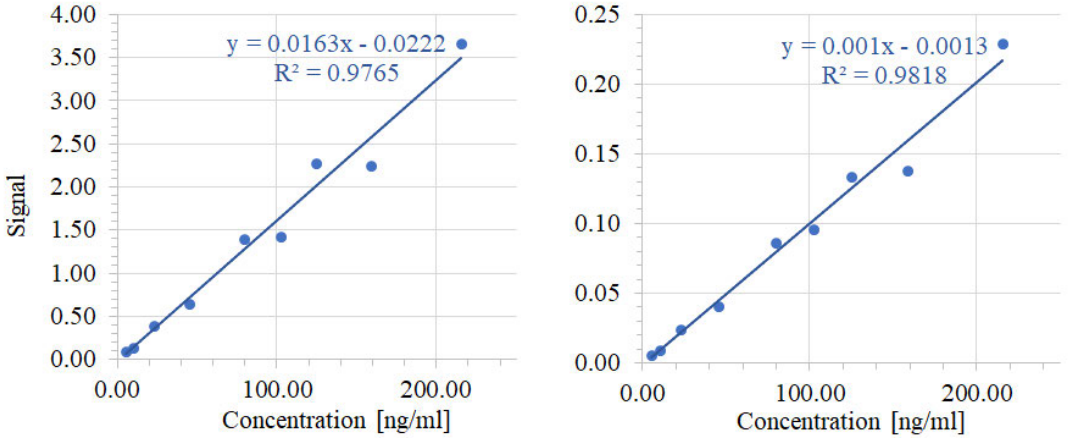

Figure 32: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of lidocaine on system one. The calculated equation for the linear model can be found attached to the respective plot.

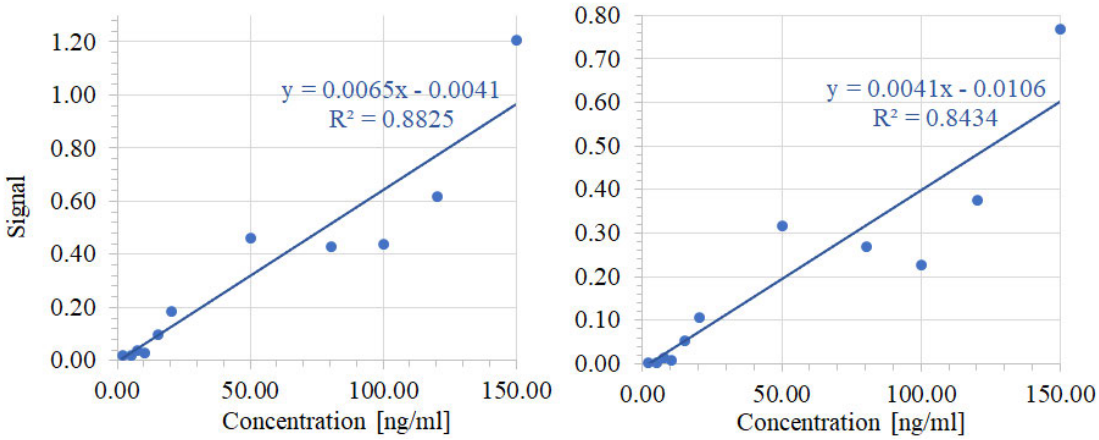

Figure 33: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of procainee on system one. The calculated equation for the linear model can be found attached to the respective plot.

B.3. System Two

B.3.1. LOD and LOQ

Table 20: Validation Data used for the determination of the limit of detection and quantitation on system two.

Name	concentration	Peak area		
	[ng/mL]	Internal Standard	Target Ion	Qualifier Ion
Levamisole	1.00	2438000	40760	15550
	2.00	2569000	81600	33040
	3.00	2305000	112400	42890
	4.00	2429000	149600	57880
	5.00	2611000	201000	80330
Aminorex	1.00	2900000	55110	28320
	2.00	3151000	122000	57620
	3.00	2958000	149500	74250
	4.00	3123000	200400	100100
	5.00	3261000	257700	126200
Lidocaine	1.02	2438000	117800	4601
	2.04	2569000	214700	9839
	3.06	2305000	264500	13500
	4.08	2429000	354500	18550
	5.10	2611000	476500	23900
Procaine	1.00	2438000	18200	20210
	2.00	2569000	33220	34970
	3.00	2305000	39090	38450
	4.00	2429000	53310	52290
	5.00	2611000	69060	69220

B.3.2. Matrix Effects, Recovery and Ion Suppression

Table 21: Validation Data used for the determination of matrix effects, recovery and ion suppression on system two.

	Sample name	Target peak area	
		Low	High
Levamisole low: 10.00 ng/mL high: 200.00 ng/mL	(A) external 1	899100	12050000
	(A) external 2	714800	12760000
	(A) external 3	698500	12100000
	(A) external 4	677500	11410000
	(A) external 5	812500	11790000
	(A) external 6	694900	13030000
	(B) external + Matrix 1	699300	10940000
	(B) external + Matrix 2	653100	10330000
	(B) external + Matrix 3	701000	12200000
	(B) external + Matrix 4	735900	11530000
	(B) external + Matrix 5	675500	11600000
	(B) external + Matrix 6	715500	11470000
	(C) internal 1	716700	12090000
	(C) internal 2	734000	12430000
	(C) internal 3	733300	11820000
	(C) internal 4	756900	12090000
	(C) internal 5	787300	12360000
	(C) internal 6	725600	11660000
Aminorex low: 5.00 ng/mL high: 20.00 ng/mL	(A) external 1	756000	2739000
	(A) external 2	543500	2930000
	(A) external 3	564400	2790000
	(A) external 4	568600	2632000
	(A) external 5	630200	2722000
	(A) external 6	595000	2809000
	(B) external + Matrix 1	402200	1725000
	(B) external + Matrix 2	394200	1676000
	(B) external + Matrix 3	376100	2184000
	(B) external + Matrix 4	435500	1989000
	(B) external + Matrix 5	437200	1950000
	(B) external + Matrix 6	419100	1979000
	(C) internal 1	410600	2090000
	(C) internal 2	414300	1945000

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	Sample name	Target peak area	
		Low	High
Aminorex low: 5.00 ng/mL high: 20.00 ng/mL	(C) internal 3	427000	2028000
	(C) internal 4	409200	2066000
	(C) internal 5	420400	2002000
	(C) internal 6	413000	2030000
Lidocaine low: 5.10 ng/mL high: 51.03 ng/mL	(A) external 1	1099000	7471000
	(A) external 2	977500	7856000
	(A) external 3	930500	7133000
	(A) external 4	927400	6970000
	(A) external 5	1008000	7131000
	(A) external 6	885700	7581000
	(B) external + Matrix 1	764200	5865000
	(B) external + Matrix 2	806100	5487000
	(B) external + Matrix 3	793900	6716000
	(B) external + Matrix 4	847600	5899000
	(B) external + Matrix 5	801000	6044000
	(B) external + Matrix 6	864900	6171000
	(C) internal 1	795000	6846000
	(C) internal 2	806500	6931000
	(C) internal 3	923600	6700000
	(C) internal 4	857100	6753000
	(C) internal 5	830000	6804000
	(C) internal 6	874200	6827000
Procaine low: 5.00 ng/mL high: 50.00 ng/mL	(A) external 1	428900	3605000
	(A) external 2	355800	3610000
	(A) external 3	346000	3446000
	(A) external 4	347000	3473000
	(A) external 5	386500	3613000
	(A) external 6	364800	3819000
	(B) external + Matrix 1	286900	2614000
	(B) external + Matrix 2	275600	2496000
	(B) external + Matrix 3	271000	2973000
	(B) external + Matrix 4	303700	2791000
	(B) external + Matrix 5	273800	2880000
	(B) external + Matrix 6	293600	2823000
	(C) internal 1	307600	3050000
	(C) internal 2	289400	3098000

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	Sample name	Target peak area	
		Low	High
Procaine low: 5.00 ng/mL high: 50.00 ng/mL	(C) internal 3	327400	3073000
	(C) internal 4	312300	3068000
	(C) internal 5	310700	3061000
	(C) internal 6	318000	3011000

B.3.3. Linearity

Table 22: Validation Data used for the determination of the linearity on system two.

Name	concentration [ng/mL]	Peak area		
		Internal Standard	Target Ion	Qualifier Ion
Levamisole	1.00	2093000	25450	9644
	5.00	2057000	113700	54760
	10.00	2093000	241000	115100
	20.00	2250000	511200	255300
	50.00	2133000	1176000	564100
	100.00	2085000	2424000	1146000
	200.00	2173000	5045000	2406000
	500.00	2293000	11750000	5380000
	800.00	2310000	18450000	8414000
Aminorex	1.00	2093000	22550	10330
	2.00	2057000	41740	15900
	5.00	2093000	104700	49230
	7.50	2250000	171700	75590
	10.00	2133000	240100	103700
	15.00	2085000	347000	145100
	20.00	2173000	473500	212400
	50.00	2293000	1220000	570800
	80.00	2310000	2036000	914700
	100.00	2333000	2515000	1148000
	120.00	2447000	2990000	1370000
	150.00	2357000	3937000	1738000
Lidocaine	1.02	2057000	65190	4445
	2.04	2093000	134300	6271
	5.10	2250000	336300	23080
	10.20	2133000	601000	38250
	22.68	2085000	1273000	77220

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Name	concentration [ng/mL]	Peak area		
		Internal Standard	Target Ion	Qualifier Ion
Lidocaine	45.36	2173000	2574000	159700
	79.38	2293000	4749000	299300
	102.06	2310000	6149000	386800
	124.74	2333000	7206000	437300
	158.76	2447000	9280000	600200
	215.46	2357000	12320000	809800
Procaine	2.00	2093000	14020	19160
	5.00	2250000	55310	44190
	10.00	2133000	56860	49050
	20.00	2085000	283200	202900
	50.00	2173000	794100	523700
	80.00	2293000	1657000	1084000
	100.00	2310000	2061000	1295000
	120.00	2333000	1437000	941300
	150.00	2447000	2739000	1801000
	200.00	2357000	4328000	2756000

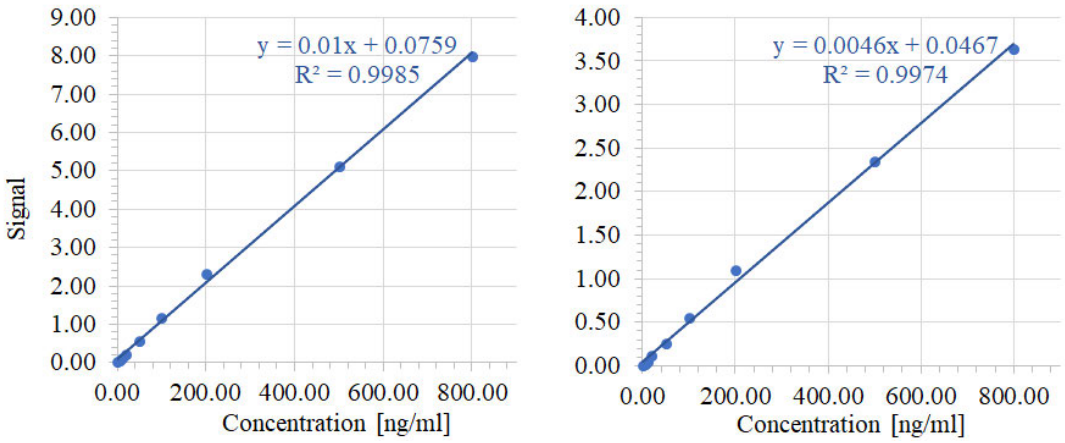

Figure 34: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of levamisole on system two. The calculated equation for the linear model can be found attached to the respective plot.

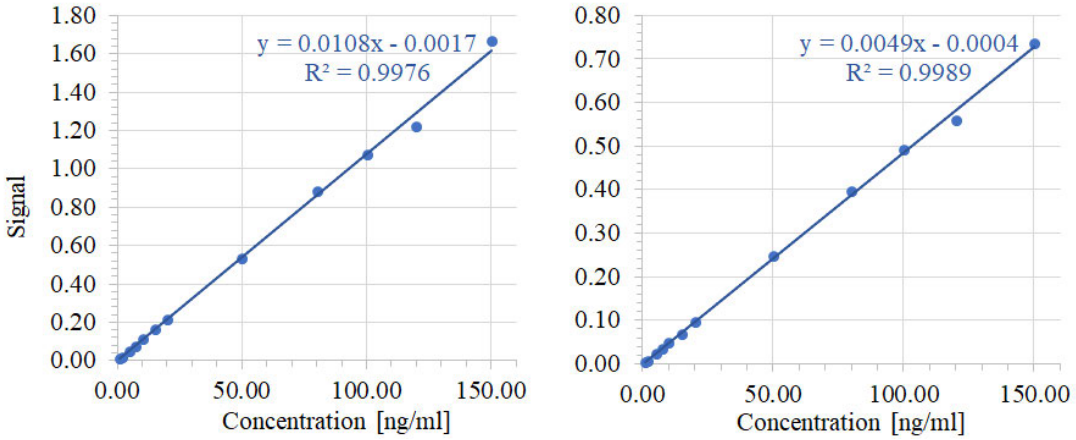

Figure 35: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of aminorex on system two. The calculated equation for the linear model can be found attached to the respective plot.

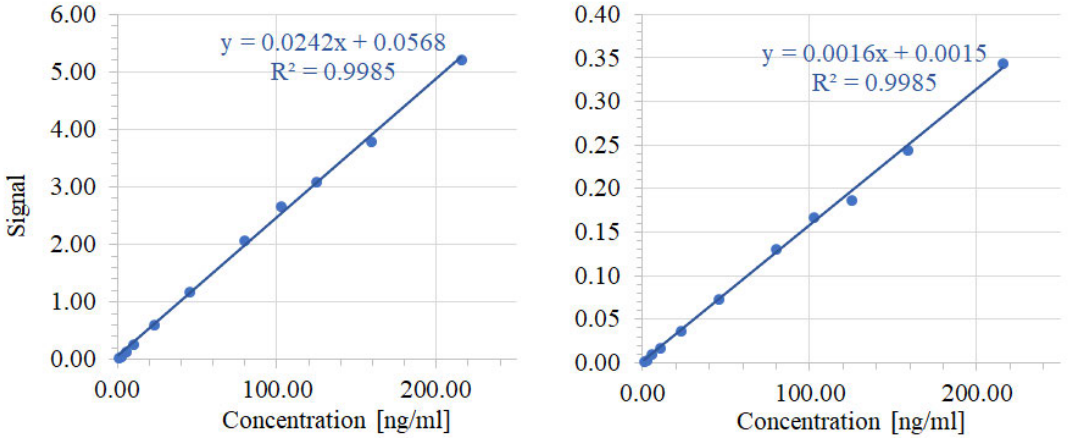

Figure 36: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of lidocaine on system two. The calculated equation for the linear model can be found attached to the respective plot.

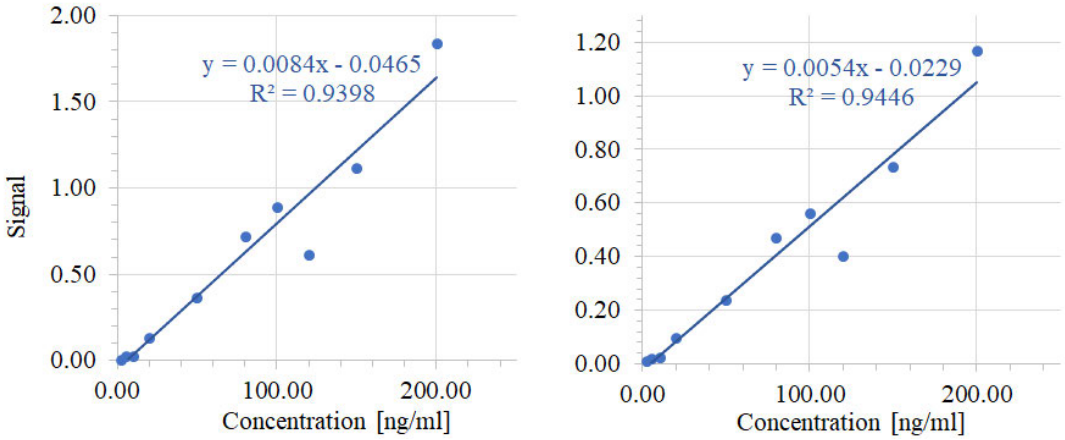

Figure 37: Results of linearity evaluation for the target (left) and qualifier ion transition (right) of procainee on system two. The calculated equation for the linear model can be found attached to the respective plot.

C. Statistic Evaluation of Past Samples

Table 23: Selected substances and metabolites found in heroin and cocaine positive urine samples from January 2024 until September 2025.

Substance	Number substance and cocaine positive	Number substance and heroin positive	Total positive samples
4-ANPP	146	28	155
7-Amino-Clonazepam	5174	1091	5639
Acetylcodeine	1042	2633	2636
Amphetamine	3041	1050	3577
Buprenorphine	7046	1758	8008
Cannabis THC	7014	2454	8349
Cocaine	22129	3411	22129
Codeine	6033	6933	9819
Dextromethorphan	402	538	639
Diazepam	224	34	249
Diphenhydramine	17	13	28
Ephedrine / Pseudoephedrine	16	10	25
Ethylglucuronide	8876	2929	10467
Fentanyl	1047	363	1185
Hydromorphone	7760	7077	11727

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Substance	Number substance and cocaine positive	Number substance and heroin positive	Total positive samples
Ibuprofen	64	15	73
Isotonitazen	15	3	17
Ketamine	424	86	452
Levamisole	1	0	1
Lorazepam	1063	215	1184
MDMA	417	95	438
Metamizole	51	10	55
Methadone	11389	4024	13545
Methadone Metabolite EDDP	13425	4411	15777
Methamphetamine	561	234	689
Monoacetylmorphine	3411	7718	7723
Morphine	9292	7733	13613
Nordiazepam	5017	1109	5567
N-Pyrrolidino 4'-Hydroxy Nitazen	18	3	19
Olanzapine	23	17	32
Oxazepam	7000	1555	7759
Oxycodone	390	145	470
Paracetamol	220	203	300
Phenacetin	3	1	3
Phenobarbital	5	3	8
Piracetam	1	1	1
Pregabalin	10864	3599	12723
Procaine	17	10	17
Temazepam	5940	1305	6587
Tilidine	176	31	195
Tramadol	351	81	387