Electrochemical detection of illicit drugs in oral fluid : potential for forensic drug testing

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Abstract

Illicit drugs continue to pose a serious threat to society and public health. Drug (ab)use is linked to organised crime and violence. Therefore, to fight the so-called war on drugs, police and law enforcement agencies need to be equipped with accurate and efficient sensors for the detection of illicit drugs and drug use. Even though colour tests (for powders) and lateral flow immunoassays (for biological samples) lack accuracy, they are relied upon for fast and easy on-site detection. Alternatively, in recent years, there has been an increasing interest in electrochemical sensors as a promising technique for the rapid and accurate on-site detection of illicit drugs. While a myriad of literature exists on the use of electrochemical sensors for drug powder analysis, literature on their use for the detection of drug use in biological samples is scarce. To this end, this review presents an overview of strategies for the electrochemical detection of illicit drugs in oral fluid. First, pharmacokinetics of drugs in oral fluid and the legal limit dilemma regarding the analytical cut-offs for roadside drug detection tests are elaborated to present the reader with the background knowledge required to develop such a test. Subsequently, an overview of electrochemical strategies developed for the detection of illicit drugs in oral fluid is given. Importantly, key challenges to address in the development of roadside tests are highlighted to improve the design of the next electrochemical devices and to bring them to the field. Overall, electrochemical sensors for illicit drugs detection in oral fluid show promise to disrupt current strategies for roadside testing.

Keywords Electrochemistry; Oral fluid testing; Illicit drugs; Forensic analysis

1. Introduction

An illicit drug may be defined as a psychoactive substance whose purpose for production, sale or use is non-medical and prohibited.[1] The use and abuse of illicit drugs continue to be a problem for society. Over the last decade, the number of drug users worldwide has increased by almost 30% to reach 269 million in 2018.[2,3] One growin concern regarding drug use is that of driving under the influence of drugs (DUID).[4,5] Depending on the drug used, the risk of traffic accidents and fatal crashes is increased to dramatic degrees.[6] The World Health Organization (WHO) has estimated that over 39,600 traffic deaths were caused by DUID in 2013.[4] Over half of these cases were attributed to the use of amphetamines, while cannabis was estimated to cause approximately one fifth of the reported drug-driving fatalities. These numbers demonstrate that it is of paramount importance to tackle the DUID

issue and consequently improve road safety. With this aim, a potential solution to this problem is to perform more roadside tests to identify DUID.

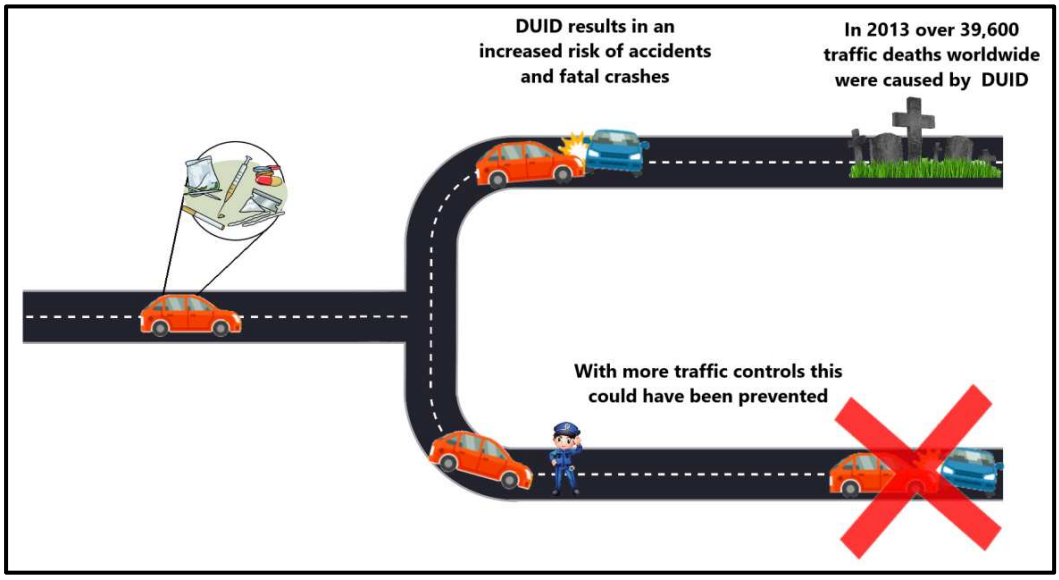

Figure 1. *DUID results in an increased risk of accidents and fatal car crashes.*

In recent years, oral fluid has become the preferred matrix of choice for roadside drug testing over urine.[7] Oral fluid provides a better indication of impairment as this matrix might reflect recent drug use. Moreover, oral fluid collection can easily be performed on-site under the supervision of a police officer without privacy concerns, contrary to the collection of urine. The use of oral fluid as a matrix makes the testing procedure easier for law enforcement, which drastically increases the number of performed roadside controls. Ultimately, this has a deterring effect on road users and importantly, it leads to increased knowledge of drug use in drivers. The Euorpean Monitorin Centre for Drugs and Drug Addiction (EMCDDA) proposed analytical cut-off values for roadside drug detection in oral fluid (Table 1).[8]

Table 1 Cut-off values for whole blood and oral fluid proposed by the European DRUID project.[8]

	Whole blood analytical cut-off		Oral fluid analytical cut-off	
	µg/L	nM	µg/L	nM
6-acetylmorphine	10	31	5	15
Amphetamine	20	150	25	18
Benzoylecgonine	50	170	10	35
Cocaine	10	33	10	33
MDA	20	110	25	140
MDMA	20	100	25	130
Methamphetamine	20	130	25	170
Morphine	10	35	20	70
THC	1	3.2	1	3.2
THCCOOH	5	14.5	N/A	N/A

The standard method of illicit drug use detection in the frame of driving under the influence of drugs consists of two steps.[9] First, a presumptive test is performed on-site. If this test generates a positive result, a biological sample is collected and transported to a laboratory for a confirmation analysis using techniques such as gas chromatography or liquid chromatography coupled to mass spectrometry (GC-

or LC-MS). For presumptive tests, lateral flow immunoassays (LFAs) are currently the gold standard even though they might exhibit some drawbacks: (i) lack of specificity from cross-reactivity with similar chemicals; (ii) time consuming (> 5 min); (iii) expensive (ca €25); and (iv) short shelf lives due to the use of bioreceptors.[10,11] Importantly, a study has shown that the false positive rates of LFAs can range between 9-53%, depending on the device used and the target drug.[12] As part of the EU-DRUID project, eight commercial LFAs were assessed based on sensitivity, specificity, and accuracy.[13] The target values were set at 80% for all three parameters. It was found that none of the testing devices reached these target values for the separate drug tests they entailed.

Due to the high selectivity and sensitivity, versatility, portability and simplicity of use, electrochemical sensors have gained more interest in the forensic field.[14] Forensic applications for which the use of electrochemical methods is being explored include the detection of gunshot residue,[15] explosives,[16] and illicit drugs.[17] Multiple illicit drugs have successfully been detected in powders by means of electrochemical sensing. Several studies report the electrochemical detection of cocaine,[18] cannabis,[19] opiates,[20] and amphetamine-type stimulants (ATS).[21] However, roadside drug testing and the use of oral fluid as matrix come with new challenges, *i.e.*, (i) lower limit of detection (LOD) required (low µg/L range), (ii) detection of metabolites, and (iii) the influence of matrix effects of oral fluid on the electrochemical signal. While several reviews exist on the electrochemical sensing of illicit drugs in street samples,[22–24] a review focused on the electrochemical detection of illicit drug consumption in oral fluid is not yet reported. First, the pharmacokinetic features and metabolism pathways of illicit drugs will be described. Second, the cut-off values for roadside illicit drug tests will be assessed. Subsequently, the state-of-the-art of electrochemical tests will be evaluated. Therefore, this review will supply the reader with a descriptive perspective of current electrochemical sensors for illicit drug consumption in oral fluid, remaining challenges, and advances to assist in the future development of DUID tests based on electrochemical technology.

2. Drug pharmacokinetics

The concentrations of illicit drugs (structures shown in supporting material) in oral fluid show inter-individual variations and depend on the administration route and time after consumption. Therefore, it is important to understand the pharmacokinetics of illicit drugs to know the expected concentrations in a biological matrix. This is paramount when designing an analytical tool aiming to be used in the field.

2.1 Amphetamine-type stimulants (ATS)

2.1.1 MDMA

The recreational drug 3,4-methylenedioxymethamphetamine (MDMA) is an entactogen that is often used at dance parties or raves.[25] Its effects include increased energy, pleasure, and altered sensation of space and time.[26] While MDMA is the most abundant species in plasma and urine, its main metabolites in these matrices are 3,4-dihydroxymethamphetamine (HHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA). These metabolites are mostly present as their glucuronide or sulphate conjugates. Similarly, MDMA in oral fluid is present as parent compound in higher concentrations than its metabolites.[27,28] In a study by Navarro *et al.* (2001), eight volunteers were given a single dose of 100 mg oral MDMA.[27] Oral fluid samples were obtained without any stimulation. In both oral fluid and plasma, MDMA peak concentrations were achieved 1.5 hours after administration. Oral fluid concentrations (range 1,728.9 – 6,510.6 µg/L) were higher than plasma concentrations (134.9 – 223.0 µg/L). After that, mean concentrations decreased to 126.2 and 13.5 µg/L at 24 hours in oral fluid and plasma, respectively. MDMA disappeared more rapidly from oral fluid than from plasma. However, oral fluid offered a longer window of detection due to higher concentrations. While HMMA was only detected in trace amounts in oral fluid, 3,4-methylenedioxyamphetamine (MDA) concentrations were found to be approximately 4-5% of the

MDMA concentration in this matrix. When there was no MDMA detected, MDA was also not found.[28,29]

2.1.2 Methamphetamine and amphetamine

Few studies have been performed on the pharmacokinetics of methamphetamine and its metabolite amphetamine in oral fluid. Studies on the pharmacokinetics of amphetamine as a parent drug in oral fluid were not found. Cook *et al*. (1993) evaluated the pharmacokinetics of methamphetamine after self-administration by smoking.[30] Urinary excretion of methamphetamine represented large portions of the administered dose (37-45%). Others have also reported that methamphetamine is almost entirely (90%) eliminated in urine.[31] Immediately after smoking, methamphetamine concentrations in oral fluid were very large compared with plasma concentrations.[30] After that, oral fluid/plasma (OF/P) ratios declined over time and averaged 5.1 from 4-24 hours after smoking. Amphetamine oral fluid concentrations were generally too low to measure. Research conducted by Schepers *et al*. (2003) has shown that amphetamine concentrations were always lower than methamphetamine concentrations in both plasma and oral fluid after controlled oral methamphetamine administration.[31] Eight volunteers were administered four daily 10 mg oral doses of methamphetamine within a week. After three weeks, five out of eight volunteers received four additional daily doses of 20 mg. Oral fluid and plasma samples were collected to determine the pharmacokinetic parameters, as shown in Table 2. The elimination half-lives in oral fluid were comparable to those in plasma. Poor correlation was found between oral fluid and plasma concentrations for methamphetamine (R^2 = 0.222). Methamphetamine and amphetamine concentrations in oral fluid were below the Substance Abuse and Mental Health Services Administration (SAMHSA) cut-offs 48 hours after the last administration of 10 or 20 mg methamphetamine.[32]

2.2 THC

After marijuana consumption, the active ingredient Δ^9-tetrahydrocannabinol (THC or Δ^9-THC) is rapidly distributed in the human body and is eventually eliminated in faeces and urine as metabolites.[33] THC is first metabolised to 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) after which it can be conjugated with glucuronic acid and sulphate.[34] During smoking, THC is stored in the oral mucosa.[35] This is thought to be the main source of THC that is measured during drug testing. Lee *et al.* (2012) monitored THC, THCCOOH, cannabidiol (CBD) and cannabinol (CBN) concentrations in the oral fluid of cannabis smokers after controlled administration.[34] Prior to smoking, four out of ten participants tested positive for THC and nine for THCCOOH. This indicated that these individuals had already consumed marijuana before participating in the tests. THC peak concentrations occurred before or at the first collection moment (0.25 h) and were in the range of 68 – 10,284 µg/L. Interestingly, THC concentrations at 0.25 h postdose were significantly correlated with the lifetime years of cannabis smoking, with participants who had smoked cannabis for less than ten years showing lower maximum concentrations. Three hours after administration, oral fluid THC concentrations were reduced by more than 95% from the concentrations at 0.25 h for all participants. Detection times were generally longer for THCCOOH than for THC as its concentrations decreased more slowly. The time courses of CBD and CBN showed similar to that of THC, but their concentrations were mostly one order of magnitude lower.

2.3 Cocaine

Cocaine is metabolised by three major pathways: i) hydrolysis to benzoylecgonine (BZE), ii) hepatic N-demethylation to norcocaine, and iii) reaction with plasma cholinesterases to form ecgonine methyl ester (EME).[36] The disposition of cocaine and its metabolites in oral fluid is dependent on the manner of cocaine administration.[37] For the intranasal and smoked routes, the cocaine concentration was found to be highest immediately after administration as a result of oral contamination. After two

hours, the OF/P ratios obtained for these routes appeared normal, with comparable values as achieved by the intravenous route (Table 3). The metabolite anhydroecgonine methyl ester (AEME) was only detected in oral fluid from the smoked route. No significant differences were found in pharmacokinetic parameters for cocaine and BZE between smoked and intravenous administration.[38] Peak cocaine concentrations in plasma were lower for the intranasal route than for the intravenous and smoked routes. Cone *et al*. (1988) observed significant correlations between oral fluid and plasma cocaine levels after intravenous cocaine administration in five healthy male participants.[39] Additionally, almost identical correlations were found with behavioural and psychological effects. This provides the opportunity for oral fluid drug testing as a non-invasive method for recent drug use tests. Greater variability in the estimation of pharmacokinetic parameters in oral fluid was found as compared to plasma.

2.4 Heroin

The opiate heroin is rapidly hydrolysed to 6-monoacetylmorphine (6-MAM) in human plasma.[40] 6-MAM is subsequently metabolised to morphine, which can be conjugated with glucuronic acid at its 3- and 6-positions. In a study by Jenkins *et al.* (1995), heroin was already detected in oral fluid after only two minutes.[41] Peak concentrations were observed two minutes after smoking and two to five minutes after intravenous administration. After that, the amount of heroin decreased swiftly. One hour after administration, heroin levels were equal to or below 15 µg/L. Due to this rapid metabolism, the detection of heroin use relies upon the detection of 6-MAM and morphine.[42] While 6-MAM is specific to heroin consumption, the presence of morphine could have other sources, including medication.[42,43] This makes 6-MAM a more reliable marker for the detection of heroin use, although its very short half-life can limit its usefulness.[42] After smoked administration, 6-MAM could be detected in oral fluid for 0.5 – 8 hours.[41] The detection window ranged up to 1 – 4 hours after intravenous administration, with peak concentrations between 18 – 141 µg/L in the first ten minutes.

Table 2 Pharmacokinetic parameters of methamphetamine and amphetamine after different dosages of methamphetamine.[31]

		Methamphetamine (10 mg dose)	Methamphetamine (20 mg dose)	Amphetamine (10 mg dose)	Amphetamine (20 mg dose)
Plasma	T_{first} (h)	1.5 ± 0.8	1.1 ± 0.5	6.6 ± 5.3	7.4 ± 4.3
	T_{max} (h)	5.4 ± 2.5	7.5 ± 3.4	11.9 ± 6.9	14.3 ± 5.5
	C_{max} (µg/L)	20.2 ± 6.4	32.4 ± 7.7	4.7 ± 2.5	5.6 ± 3.2
	$T_{1/2}$ (h)	9.3 ± 3.7	11.1 ± 7.2		
Oral fluid	T_{first} (h)	0.6 ± 0.6	0.4 ± 0.1	3.1 ± 1.5	4.1 ± 4.3
	T_{max} (h)	5.0 ± 1.9	4.7 ± 3.9	9.1 ± 3.0	8.2 ± 3.5
	C_{max} (µg/L)	106.1 ± 100.8	192.2 ± 120.8	8.6 ± 6.5	14.3 ± 6.1
	$T_{1/2}$ (h)	7.1 ± 2.3	8.1 ± 0.9		

Abbreviations: T_{first} = time of first detection; T_{max} = time of highest detected concentration; C_{max} = highest detected concentration; $T_{1/2}$ = half-life.

Table 3 Pharmacokinetic parameters of cocaine and its metabolites after different administration routes.[37,38]

Dose (mg)	Route	Analyte	Oral fluid T_{first} (h)	Oral fluid T_{last} (h)	$T_{1/2}$ (h)		C_{max} (µg/L)		T_{max} (h)		Oral fluid/ Plasma
					Oral fluid	Plasma	Oral fluid	Plasma	Oral fluid	Plasma	
25	IV	COC	0.08	4 – 12	2.45	5.05	258 – 1303	230.4 (97.7 – 349.4)	0.08	0.04 (0.02 – 0.05)	0.1 – 10.1
		BZE	0.08	6 – 12	3.50	4.72	2 – 43	110.6 (60.6 – 165.4)	0.75 – 6	2.02 (1.10 – 2.64)	0.1 – 1.3
		EME	0.08 – 1.50	1.50 – 12	2.56		2 – 50		1.50 – 4		
32	IN	COC	0 – 0.17	6 – 12	1.61	5.90	75 – 147,436	62.7 (40.1 – 88.5)	0.08 – 0.75	0.62 (0.39 – 0.85)	0.1 – 17,781.3
		BZE	0.08 – 1.50	6 – 12	4.68	5.25	28 – 1,931	128.9 (94.0 – 158.1)	0.17 – 2	3.44 (2.94 – 3.81)	0.1 – 63.7
		EME	0.08 – 1.50	4 – 12	4.60		15 – 154		0.33 – 4		
42	SM	COC	0.08 – 0.17	1 – 12	2.63	8.64	94 – 12,582	227.1 (56.3 – 345.1)	0.08 – 0.17	0.04 (0.02 – 0.05)	0.1 – 63.5
		BZE	0.25 – 0.50	3 – 12	3.50	4.59	0 – 51	86.7 (46.7 – 168.7)	1 – 6	1.84 (1.27 – 2.52)	0.1 – 0.6
		EME	0.08 – 0.33	3 – 6	3.63		28 – 110		0.33 – 3		
		AEME	0.08	0.17 – 1	0.19		5 – 775		0.08		

Abbreviations: T_{first} = time of first detection; T_{last} = time of last detection; $T_{1/2}$ = half-life; C_{max} = highest detected concentration; T_{max} = time of highest detected concentration; IV = intravenous administration; IN = intranasal administration; SM = administration by smoking; COC = cocaine; BZE = benzoylecgonine; EME = ecgonine methyl ester; AEME = anhydroecgonine methyl ester.

3. Electrochemical strategies for the detection of illicit drug use in oral fluid

3.1 Amphetamine type stimulants (ATS)

3.1.1 MDMA

To overcome the problem of cross-reactivity associated with immunoassays targeting amine groups, the group of Guilbalt developed a disposable amperometric immunosensor for the detection of MDA and its analogues MDMA and MDEA in oral fluid and urine using methylenedioxy-specific antibodies.[44] High specificity was achieved by employing an antibody targeting the methylenedioxy moiety of an MDA-bovine serum albumin (BSA) conjugate. To increase the sensitivity of the assay, horseradish peroxide (HRP) enzyme was conjugated to the analytes and added simultaneously during the analysis to realise a competitive assay. This highly sensitive and specific method for ecstasy detection outperformed ELISA methods at a lower cost (€ 2-3). However, it was regrettably limited in its feasibility for roadside testing by a long incubation time of 45 minutes.

More recently, Oiye *et al.* (2020) reported the voltammetric detection of MDMA in oral fluid at carbon SPE without any chemical modifications.[45] Blank oral fluid samples were collected by non-stimulated expectoration and mixed with buffer solution. Cyclic voltammetry (CV) was performed with an anodic pretreatment applied at +0.7 V for 30 seconds. Importantly, the MDMA peak potential was not influenced by the viscosity of the oral fluid when it was mixed in buffer solution. The peak current for the oxidation of MDMA was not proportional to the MDMA concentration in both synthetic oral fluid and authentic oral fluid, thus precluding the determination of the LOD. Even though the method was adequate for the identification of MDMA use in the first two hours after consumption, the LOD was not sufficiently low for roadside drug testing according to the recommendations from the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).

Finally, our group has reported the enhanced detection of MDMA in oral fluid at bare carbon SPE by the use of a surfactant-mediated solution.[46] Surfactants, which can easily be added to the buffer solution used in electrochemical measurements, have the ability to modify and enhance electrode surfaces.[47] We exploited the adsorption of the surfactant sodium dodecyl sulphate (SDS) at the electrode surface to improve the affinity of our target analytes towards the sensor surface. Oral fluid samples were collected by expectoration and spiked with the drug. The samples were diluted 10-fold in a buffer solution containing the SDS before analysis. In oral fluid, a slight shift in peak potential was observed. This change was ascribed to a potential change in pH due to the oxidation of compounds in the oral fluid matrix sample. Even though this research had promising results for future applications due to the simple modification of the electrode, the LOD and analysis time needs to be decreased before the sensing strategy can be used in real scenarios.

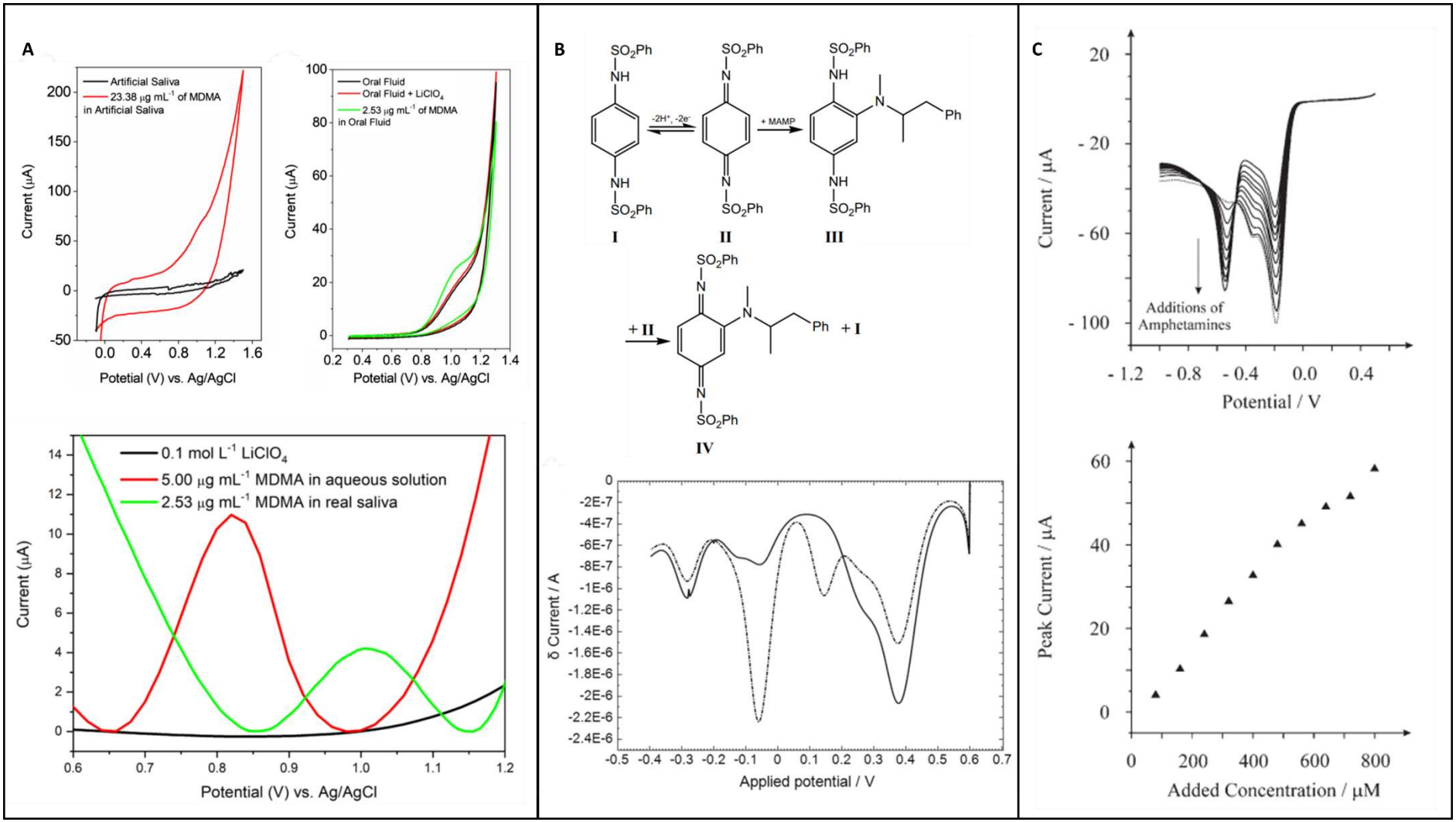

Figure 2 *Electrochemical strategies for the detection of ATS in oral fluid. A) SWV detection of MDMA at carbon paste electrode and SPCE.*[45] *Copyright 2020, Elsevier B.V. B) N,N'-(1,4-phenylene)-dibenzenesulfonamide mediated SWV detection of methamphetamine at SPCE.*[48] *Copyright 2016, Springer. Adapted. C) NQS-mediated detection of D-amphetamine sulphate.*[49] *Copyright 2006, WILEY-VCH Verlag GmbH & Co. KGaA.*

3.1.2 Methamphetamine and amphetamine

Methamphetamine has been detected in synthetic oral fluid using a bioelectrochemical sensor with polypeptides with electroactive end-groups as sensing platforms.[50] The fluorescent-labelled polypeptide EDOT-BTDA-Pala was used as the matrix for the immobilisation of methamphetamine antibodies on the electrode. Detection of methamphetamine was achieved by measuring the decrease in differential pulse voltammetry (DPV) signal as a result of restricted electron transfer through GCE/EDOT-BTDA-Pala/Antibody by the selective binding of the drug. To test the system in synthetic oral fluid, standard addition of known amounts of methamphetamine was performed. The methamphetamine recovery was between 90 – 93%. The validity of the method in synthetic oral fluid was confirmed via LC-MS analysis.

Another methodology for identifying methamphetamine use in oral fluid involved indirect detection of the drug via *N,N'*-(1,4-phenylene)-dibenzenesulfonamide mediated buffer.[48] The oxidised mediator can react with the secondary amine group of methamphetamine, leading to the formation of an adduct which can be detected through its reduction. It is important to note that this mediator might also react with other secondary amines present in oral fluid, which can contribute to false positives. In authentic oral fluid collected by expectoration, the reduction peaks were significantly reduced as compared to oral fluid buffer (85 – 95%). This decrease could be explained by the biofouling issue from proteins, which were absent in the oral fluid buffer. Interestingly, the effect of donor variation on the methamphetamine signal was evaluated. Oral fluid samples from two individuals were centrifugally filtered with different filters (3, 10, 30, and 100 kDa). The methamphetamine signal increased with decreasing molecular weight cut-off of the filter. This signified the negative impact of high molecular weight compounds such as proteins on the electrochemical response. However, when the 3 kDa filter was used, the variation between the responses of the two donors was still considerable.

Besides voltammetry, electrochemiluminescence (ECL) can be used for methamphetamine detection. Dokuzparmak *et al.* (2021) recently reported an ECL sensor using $[Ru(bpy)_3]^{2+}$ modified SPE for the detection of methamphetamine and its metabolites amphetamine and *para* hydroxy-methamphetamine.[51] Commercially available oral fluid was used in the experiments. This was diluted five times to overcome problems regarding its high viscosity. While methamphetamine showed maximum ECL intensity around 1.10 V vs Ag/AgCl, the peaks for both metabolites were found to be around 1.15 V. These small differences in peak potential hindered the distinction between the three compounds. However, this screening method would still be fit for the determination of methamphetamine use. More importantly, the blank artificial oral fluid showed a background peak in the same region as the ATS. While the authors noted that this does not interfere with ATS detection as it still allows for a LOD of 10 µM, this background peak may interfere with the detection of lower concentrations found in intoxicated drivers. Therefore, an ECL intensity threshold may need to be selected for ATS identification. However, this may be difficult in real oral fluid samples which can show inter-individual differences.

The illicit drug amphetamine is harder to directly detect using electrochemical sensors.[52] The direct oxidation of primary amines in an aqueous solution occurs at high potential at carbon SPEs. This limits the oxidation of amphetamine at carbon SPE. To overcome this problem, Goodwin *et al.* (2006) exploited the mediator sodium 1,2-naphthoquinone-4-sulfonate (NQS), which is commonly used in colorimetric tests for amines.[49] In this proof-of-concept study, NQS was used to indirectly detect the model amines D-amphetamine sulphate and pseudoephedrine. For detection in oral fluid, a synthetic oral fluid solution was used that reflected the mineral and mucin content of human oral fluid. The sensitivity of the system was considerably lower in artificial oral fluid compared to the buffer solution, likely as a result of a change in viscosity and the presence of mucins. Finally, the developed system was tested for the determination of amphetamine in authentic oral fluid provided by one of the authors. The results showed that a signal could be obtained in under two minutes, demonstrating the possible use for roadside testing. However, the LOD should be lowered for this method to be suitable for roadside testing.

3.2 THC

The electrochemical recognition of cannabis use in oral fluid has recently received a lot of attention. The first to report the voltammetric detection of THC in artificial oral fluid were Goodwin *et al.* (2006), who showed the indirect sensing of the cannabinoid in their proof-of-concept study.[53] They used 2,6-dichloro-p-amino-phenol as a mediator, which produced quinoneimine (QI) when oxidised. This oxidation process could be followed using CV, together with the reverse reaction in which QI was reduced back to the aminophenol. Upon the addition of THC to the solution, the reduction signal for the reverse reaction decreased, as THC reacted with the QI. For the detection of THC in artificial oral fluid, graphite micropowder was modified with 4-amino-2,6-diphenylphenol, which was immobilised onto a basal plane pyrolytic graphite electrode. Here, square wave voltammetry (SWV) was used as detection method. Even though the obtained LOD (low micromolar range) would be sufficient to detect THC in oral fluid in the first hours after cannabis consumption, the LOD did not meet the recommended cut-off values of the EMCDDA.[8,34]

Another method for the indirect detection of THC in oral fluid was developed by Wanklyn *et al.* (2016).[54] In this study, the mediator *N*-(4-amino-3-methoxyphenyl)-methanesulfonamide (OX0245) was used for the detection of THC in undiluted oral fluid at SPCE. The determination of THC was based on the electrochemical reduction of an adduct formed between THC in the sample and the oxidised form of the mediator. Oral fluid specimens were collected immediately prior to analysis by expectoration. An electrochemical procedure using chronoamperometry was developed that made it possible to correct for donor variation. Detection of 25 – 50 µg/L THC was reached with an analysis time of 30 seconds. The developed sensor was tested and validated using LC-MS. Interestingly, real samples from four cannabis smoking donors and a control group of non-smokers were used. Oral fluid samples were classified as negative (0 µg/L) or positive (> 0 µg/L), based on the LC-MS results. Ultimately, the sensitivity, selectivity, and accuracy of the sensor were determined to be 28, 99, and 52%, respectively. It is important to note that these values cannot be compared with the values necessitated by the EMCDDA, due to a different definition used for false negatives and false positives. However, from the high LOD it can be concluded that the sensor does not fulfil the requirements.

A more straightforward approach to the recognition of cannabis use is the direct measurement of THC at the electrode surface. However, the direct oxidation of hydroxyl groups has the disadvantage that radicals and radical cations are formed, which leads to electrode passivation.[55,56] To overcome this problem, Nissim and Compton (2014) exploited the use of adsorptive stripping voltammetry at a carbon paste electrode.[19,57] Carbon past electrodes (CPEs) were immersed in deoxygenated THC for three minutes, after which they were removed and placed in blank buffer for SWV analysis. To decrease the LOD, the preconcentration time was increased to five minutes and the amount of carbon paste (graphite/mineral oil) was doubled. Additionally, the effect of stirring the solution was evaluated on low levels of THC. Here, the results suggested that the detection was restricted by the amount of THC that can be accumulated at the carbon paste. It could also be concluded that stirring the solution decreased the practical LOD for THC. While the authors claimed that the obtained LOD was useful for roadside detection, it is still two orders of magnitude higher than the analytical cut-off value recommended by the EMCDDA.[58]

Another method for the direct detection of THC detection was achieved by Oiye *et al*. (2020) who developed and patented a disposable 3D-printed electrode for the detection of THC and its metabolite THCCOOH.[59] The electrochemical behaviour of THC and THCCOOH at the sensor was analysed in aqueous solution containing 0.1 M KNO_3 using CV. Here, the LOD for THC was 4.7 mg/L (15 µM). When THC was analysed in a spiked oral fluid sample, which was 1:1 diluted with aqueous solution, no oxidation peak was observed. The metabolite THCCOOH on the other hand, did show an oxidation peak in spiked oral fluid when the same experimental conditions were used. As THC and THCCOOH only differ in a carboxylic group, this oxidation peak was also ascribed to the phenolic group. The LOD of THCCOOH was found to be 15 mg/L (45 µM). While this research showed that it is possible to build a disposable and even biodegradable device at low cost (75% reduction in price compared to

commercial SPE), the developed sensor should be developed further to reach lower LODs and to successfully detect THC in oral fluid.

An innovative and novel approach to the direct, ultra-low level detection of THC was achieved by Ortega *et al.* (2022).[60] For the first time, the electrode was modified with the same analyte as the target analyte. Here, the presence of THC on the electrode surface improved the affinity of THC for the sensor via "peer interaction". SWV was used to successfully measure THC in simulated oral fluid, reaching a limit of 1.6 µg/L at pristine electrodes. When real oral fluid was used, it was found that the higher viscosity of this matrix affected the revsibility of THC oxidation at the modified electrodes. Additionally, the increase in viscosity lowered the adsorption-oxidation of THC. Importantly, unwanted adsorption of different components of the simulated oral fluid on the electrode was observed. Uric acid was identified as the main interference in simulated oral fluid, due to its oxidation near +0.4 V. In real oral fluid, the oxidation peak of uric acid shifted towards a more positive value, making it less interferent with THC detection. As the developed method had a higher sensitivity than commercially available tests for cannabis use, it showed promise for future use.

An important consideration in roadside testing is the concurrent use of multiple drugs and/or drugs and alcohol. Recently, the first step towards simultaneous detection of alcohol and THC use has been made by the group of Wang.[61] This group realised a wearable electrochemical ring sensor which allowed for direct SWV sensing together with amperometric detection of alcohol. For simultaneous detection, a four electrode system with two working electrodes was employed. THC was detected via SWV at an electrode modified with multi-walled carbon nanotubes. Ethanol biosensing was performed at a Prussian blue/Alchohol oxidase modified carbon-electrode via amperometric measurements to monitor the reduction of enzymatically generated hydrogen peroxide. For detection in oral fluid, samples were collected by expectoration immediately before analysis and centrifuged for two minutes. It was found that the simultaneous detection of THC and ethanol was possible without cross-reactivity. SWV analysis of THC in oral fluid showed an oxidation peak at a more positive potential than in buffer solution. No interferences from the oral fluid matrix were observed. The ring-based sensor had a total analysis time of approximately six minutes, including three minutes for oral fluid collection and three minutes for performing the measurements. While it showed that the miniaturization of electrochemical sensors for the detection of drug use is possible, it is important to consider whether a ring is the most viable option to ensure hygienic analysis.

While most research has been focused on voltammetric detection of THC, Stevenson *et al.* (2019) reported the use of non-faradaic electrochemical impedance spectroscopy (EIS).[62] They constructed a biosensor that could recognise THC based on the presence of a THC-BSA hapten. After EIS analysis, the zeta potential and dose-dependent response were analysed. Subsequently, binary classification was employed to predict THC presence with a true positive rate of 94.3% and a false positive rate of only 5%. Finally, the limit of detection was found to be 100 ng/L.

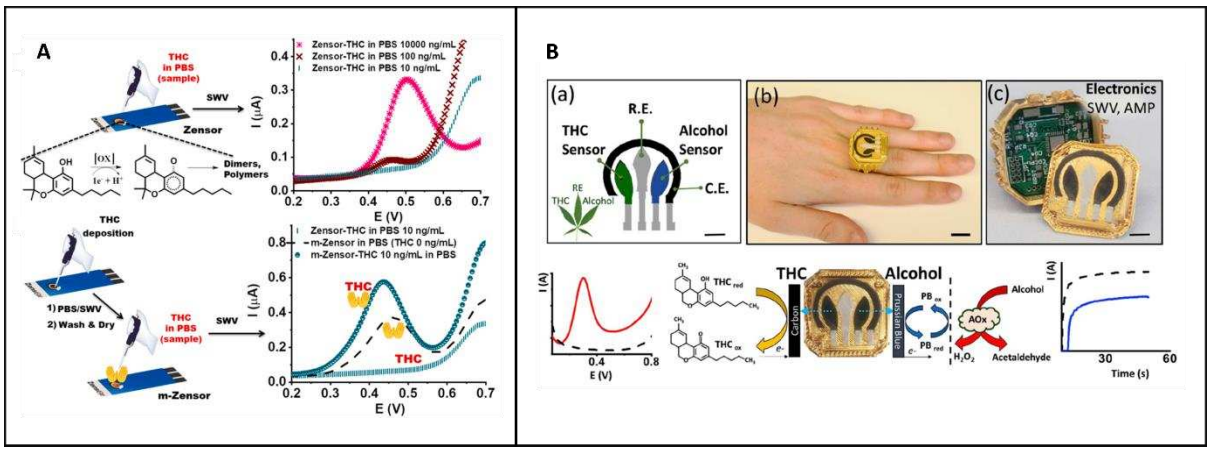

Figure 3 *Electrochemical strategies for the detection of THC in oral fluid. A) Detection of ultra-low concentration of THC by using THC as sensor analyte.*[60] *Copyright 2021, Elsevier B.V. B) Wearable sensor for the simultaneous detection of THC and ethanol.*[61] *Copyright 2020, Elsevier B.V.*

3.3 Cocaine

Several groups have reported the electrochemical detection of cocaine in oral fluid. Multiple groups have employed antibodies for highly specific detection.[63–65] Sengel *et al.* (2017) proposed an electro-immunosensor for the detection of both BZE and cocaine.[63] An innovative sensor surface was developed using a combination of a BZE antibody and poly-L-phenylalanine bearing electroactive monomer (EDOT-BTDA-PPhe) on a glassy carbon electrode. Indirect detection was exploited via the decrease in DPV signal upon binding of the antibody with cocaine or BZE. To determine the applicability of the sensor for recent drug use testing, the performance of the sensor was evaluated using synthetic oral fluid samples with known BZE concentrations. The sensor signals were compared to those in buffer solution to calculate the recovery, which ranged between 92 – 95%.

Abdelshafi *et al.* (2018) developed a microfluidic electrochemical immunosensor based on magnetic beads coated with anti-cocaine antibodies.[64] Microfluidic chip systems, just as other embedded systems, are promising in the field of chemical sensing as they allow for the easy adaption of recognition, thus enabling possibilities for multiplexing. The assay was specific to cocaine, exhibiting medium affinity for BZE and norcocaine and low affinity for ecgonine and EME. To establish whether the sensor was suitable for cocaine detection in oral fluid, samples collected from a random group member were spiked with 1 and 100 µg/L cocaine. Assays were performed without any further pretreatment of the oral fluid specimens. At low concentrations, the recovery of cocaine was 106%, while at higher concentrations this decreased to 88%.

A different method of lowering the LOD of electrochemical immunosensors was adopted by Sanli *et al.* (2020).[65] They exploited the ability of cobalt oxide nanoparticles to improve the sensor sensitivity by enhancing the surface area and promoting electron transfer. A biosensor based on single-chain antibody fragments (scFvs) was developed for the rapid detection of cocaine at functionalised SPEs using DPV. As the presence of cocaine demoted electron transfer, a decrease in peak current could be observed allowing the difference in signal (Δ µA) to be used for cocaine determination. The sensor had a LOD of 3.6 µg/L cocaine (11.9 nM) in buffer solution. The addition of 100 µg/L BZE, JWH-073, codeine, and methamphetamine at the electrode surface did not show any significant positive change compared with cocaine. Ultimately, the applicability of the sensor for several synthetic body fluids including synthetic oral fluid was assessed. The specimen was spiked with 100 µg/L cocaine and the results were compared to those in buffer solution. Recovery of 107% was found, which confirmed the potential of the developed sensor for testing in oral fluid.

Another interesting approach to cocaine detection in oral fluid is the use of aptamers. Aptamers offer high specificity and selectivity, can be produced with high reproducibility, and have a lower cost than antibodies.[66,67] A solid-state probe-based electrochemical aptasensor for cocaine determination was developed by Du *et al*. (2010).[68] In the presence of cocaine, a decrease in the DPV signal occurred, allowing for the determination of cocaine. The sensor was also applied for the detection of 1.2 mg/L (3.8 µM) cocaine in 25% human oral fluid. Before analysis, the oral fluid was centrifuged for 15 minutes to remove precipitates. The recovery of cocaine was found to be 96.1%. Even though the LOD (30 µg/L (0.1 µM)) was lower than had been reported for other available cocaine aptasensors at the time of publication, it was not sufficient for roadside drug testing. Moreover, long incubation times of 40 minutes and the centrifugation of the oral fluid samples limited the applicability of the sensor for on-site detection.

More recently, Su *et al.* (2017) investigated the use of two-dimensional zirconium-based metal-organic framework nanosheet composites embedded with Au nanoclusters (2D AuNCs@521-MOF) for the detection of cocaine.[69] A large amount of cocaine aptamer strands could be immobilised on the substrate. The aptasensor was submerged in cocaine solutions of varying concentrations for two hours during DPV analysis. The performance of the developed aptasensor in oral fluid was evaluated by spiking oral fluid samples provided by healthy volunteers with 0.001 – 0.1 µg/L cocaine. All samples were diluted 1000 times with PBS solution before measurement. While dilution of the sample is beneficial for minimizing matrix effects from the oral fluid, it has the disadvantage that it also requires a more sensitive method. Correcting for the dilution factor, the LOD of the developed method would be 673 ng/L (2.22 nM) in oral fluid. However, the actual LOD in oral fluid might be higher due to interferences or matrix effects.

Since aptamers have some issues regarding their stability depending on the conditions used, our group employed the more robust molecularly imprinted polymers (MIPs) for cocaine detection in real samples.[70] These biomimetic materials can be tailored towards the analyte of interest to offer highly specific detection. Here, a sensor based on electrodeposited MIPs and palladium nanoparticles on graphene-functionalised SPEs was designed. While the MIPs were employed for specificity, the nanoparticles and graphene were used to increase the sensitivity of the sensor. Oral fluid specimens were collected using a Quantisal collection device and subsequently diluted 1:10 in PBS (pH 7.0). After that, the samples were spiked with known amounts of cocaine for electrochemical analysis. Before starting the measurement, cocaine was allowed to accumulate at the electrode surface for five minutes. The recovery of cocaine from the oral fluid samples was 99.4% (RSD 13.2%) and the LOD was 15 mg/L (50 µM). Even though the LOD was not low enough for roadside testing, the short analysis time and high cocaine recoveries showed that the use of a MIP-based sensor is promising for recent drug use testing.

Recently, a more straightforward and simple approach to cocaine detection in artificial oral fluid by direct SWV at unmodified carbon paste electrodes was reported.[71] A pretreatment at +1.0 V was applied for 30 seconds before taking the voltammogram. Under optimised conditions, the LOD and LOQ were 0.80 mg/L (2.64 µM) and 2.4 mg/L (7.95 µM), respectively. An interference study was performed using caffeine and lidocaine. While the presence of caffeine did not interfere with the cocaine signal, lidocaine was found to hinder cocaine detection. In artificial oral fluid, the oxidation peak appeared at the same potential but had a lower current value than that obtained with the freebase cocaine standard. The LOD of the developed method in artificial oral fluid was not evaluated, but would be too high for roadside testing applications.

The use of surfactant-mediated buffer solution was shown to be another simple strategy to enhance the electrochemical oxidation signal for cocaine by Parrilla *et al.*[46] Upon addition of SDS, the cocaine signal increased 5.2-fold. Here, the oxidation process of the deprotonated tertiary amine was found to be adsorption controlled, contrary to the diffusion-controlled process in solution without SDS.[72] As a result of this, the signal could be enhanced further by increasing the adsorption time. This method proved useful to detect cocaine concentrations down to 3.6 mg/L (12 µM) in real oral fluid samples.

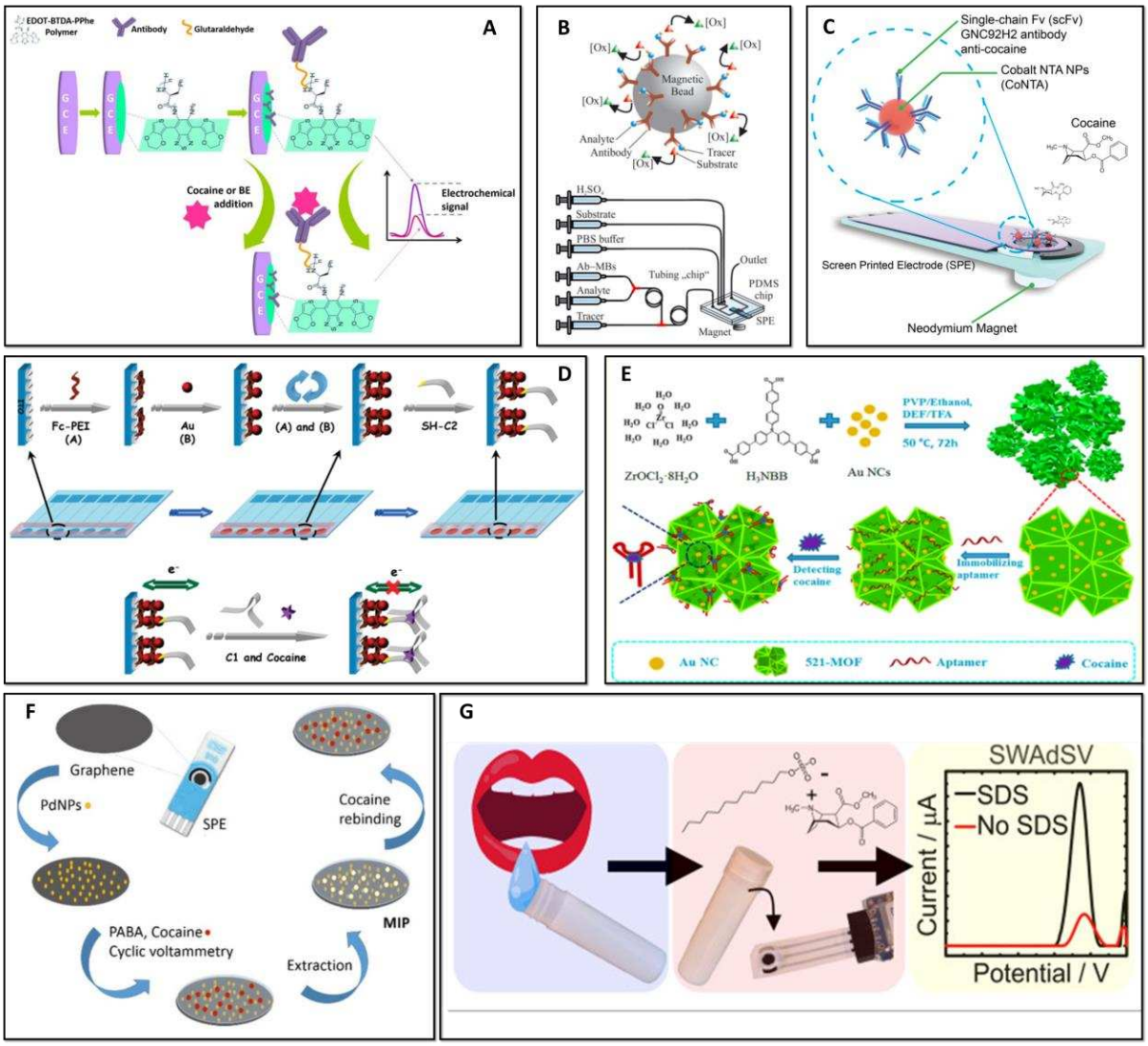

Figure 4 *Electrochemical strategies for the detection of cocaine in oral fluid. A) Immunoelectrochemical platform for biosensing of cocaine use.* [63] *Copyright 2017, Elsevier B.V. B) Microfluidic electrochemical immunosensor for trace analysis of cocaine.*[64] *Copyright 2018, John Wiley & Sons, Ltd. C) SPE-based biosensor functionalised with magnetic cobalt/single-chaint antibody fragments.*[65] *Copyright 2020, Elsevier B.V. D) Solid-state probe based electrochemical aptasensor.*[68] *Copyright 2010, American Chemical Society. E) Aptasensor with two-dimensional zirconium-based metal-organic framework nanosheet composites embedded with Au nanoclusters.*[69] *Copyright 2017, American Chemical Society. F) Electrochemical sensing based on electrodeposited biomimetic affinity ligands.*[70] *Copyright 2019, The Royal Society of Chemistry. G) Surfactant-mediated SWV detection at SPE.*[46] *Copyright 2021, Elsevier B.V.*

3.4 Heroin

Literature on the electrochemical detection of heroin in oral fluid is scarce. Shang *et al.* (2014) reported an electrochemiluminescence (ESL) sensor.[73] Heroin detection was achieved at a Tris(2,2'-bipyridyl) ruthenium ($Ru(bpy)_3^{2+}$) immobilised glassy carbon electrode modified with MIPs. Reproducibility and stability tests indicated that the sensor had good long-term stability, with the ECL intensity stable and reproducible for at least 50 times of measuring 3.7×10^{-6} µg/L (1×10^{-14} M) heroin. To assess whether the developed sensor could be applied to forensic testing, the sensor performance in human oral fluid and urine was evaluated. The oral fluid and urine samples were directly diluted 100-fold and 200-fold, respectively, after the addition of the standard heroin. For oral fluid, recoveries

ranged between 97.4% and 99.1%. It is important to note that no ECL responses for heroin detection in the human matrices are shown.

Parrilla *et al.* (2021) investigated the use of surfactant-mediated buffer solution for the detection of heroin in authentic oral fluid.[46] In comparison to heroin in buffer solution without SDS, the electrochemical signal was increased 2.5-fold for heroin upon SDS addition. The performance of the sensor in oral fluid was assessed using oral fluid spiked with heroin and 10-fold. Prior to analysis, 15 minutes of waiting time was used to allow heroin to accumulate at the electrode surface. For the method to be usable in real settings, this analysis time should be decreased. Additionally, the LOD of 8.9 mg/L (24 µM) was too high for roadside applications.

3.5 Other drugs

3.5.1 Novel psychotic substances

An important trend in the illicit drug markets is the rise of novel psychotic substances (NPS).[74] These drugs are often synthesised due to their similar structures compared to the traditional illicit drugs or because they mimic the effects of traditional illicit drugs. Importantly, NPS are not regulated when they reach the market. Therefore, it is important that these substances can be distinguished from their illegal counterparts. This poses a new challenge for forensic practitioners, as the detection techniques in the forensic laboratory might be unable to do so. Additionally, little information is known about the pharmacokinetics of NPS and often there are no reference standards available. This has caused a knowledge gap in the current research. Hence, it is important that these compounds are correctly identified to avoid false accusations and to put a warning system in place for the emergence of NPS, as they can pose a threat to public health.

Besides the use of marijuana, the usage of new synthetic cannabinoids (SCs) has gained more popularity. Recently, these drugs have led to increasing health concerns due to their potential for abuse and unpredictable toxicity.[75] To tackle this issue, Dronova *et al*. studied the electrochemical detection of six indole- and five indazole-containing SCs which were predominant in the drug market using CV.[76] In artificial oral fluid, indole-based SCs showed a signal around +1.2 V, whereas the peaks for indazole-based SCs appeared around +1.5 V. SCs with naphthalene or quinoline moieties exhibited a second oxidation peak. While the distinction between indole- and indazole-based SCs could be made using this approach, it was not possible to differentiate between the different compounds within the same group. As new SCs will keep emerging, this might not be a problem for law enforcement who ultimately have the goal to detect impairment caused by drug usage in drivers instead of differentiation between a drug used.

Brown *et al.* (2020) reported the detection of the NPS scopolamine, a naturally occurring hallucinogen that is also used recreationally.[77] The drug was detected in undiluted artificial oral fluid (Bioténe® oral balance gel) using ECL at $[Ru(bpy)_3]^{2+}$-modified electrode. In this oral fluid matrix, a LOD of approximately 1 µM was achieved. This LOD was higher than that obtained in serum, due to the higher viscosity of the oral fluid sample which resulted in poorer electron transfer kinetics. Importantly, in this work concerns were raised about selectivity of the ECL sensor and the possibility of interreference from species with similar molecular structures. This is an important issue regarding the detection of NPS, which often are structurally similar to their licit analogues. Therefore, the authors of this work reported that they continue to work on this problem using alternative metal luminophores. Additionally, their group has shown that the distinction between species can be made using pH-controlled ECL.[78] This distinction between structurally similar drugs is pivotal for tackling the NPS challenge and shows the advantage of electrochemical detection methods.

3.5.2 Methadone

Methadone is an opioid that has been used for the treatment of heroin or morphine addicts over the past decades.[79] As methadone also has the potential for abuse, Amiri-Aref *et al.* (2013) reported an

electrochemical sensor for its detection in biological fluids and pharmaceutical samples to tackle this problem.[80,81] They explored the simultaneous sensing of methadone and acetaminophen at glassy carbon electrodes modified with functionalised MWCNTs. These modifications facilitated the kinetics of the electrochemical processes for the target analytes, resulting in lower peak potentials and increased sharpness of the peaks. An interference study showed no shifts in peak currents for methadone and acetaminophen in presence of ascorbic acid, L-cysteine, and uric acid. For the detection in real samples, oral fluid was collected using Salivette (Sarstedt) cotton swabs. The obtained samples were centrifuged and diluted in 10 mL PBS (pH 7). In this matrix, the simultaneous detection of both target analytes at concentrations as low as 3.0 – 12 mg/L (20-40 µM) was still possible using DPV. The recovery of methadone was approximately 98%. However, no voltammograms were shown to corroborate these results.

Another electrochemical solution to the issue of methadone use has been proposed by Afkhami *et al.* (2014), who described the sensing of the opioid in biological fluids at CPE modified with gold nanofilm.[82] Adsorptive SWV was successfully employed for the detection of methadone in both buffer solution and oral fluid. At the gold nanofilm modified CPE, the LOD for methadone in buffer solution was found to be as low as 1.5 µg/L (5 nM), which would be suitable for DUID testing. The analytical procedure would however not be applicable to roadside settings. The oral fluid samples were first centrifuged and diluted, after which they were purged with nitrogen. While centrifugation of the samples would be possible with portable centrifuges, purging of the samples cannot be performed outside the laboratory. Additionally, the number of steps involved in the procedure would be too high for simple analysis.

3.5.3 Ketamine

While the drug ketamine has legal use as anaesthetic in hospitals, it is also recreationally used for its hallucinogenic effects.[83] In many countries worldwide, the drug is listed as a controlled substance.[84] Its electrochemical detection in oral fluid has first been reported by Fu *et al.* (2019) who developed a sensor based on MIPs at modified electrodes.[85] Graphene and metal-organic frameworks (MOFs) were used as substrates to enhance sensor sensitivity. The detection of ketamine was explored using DPV, where an incubation time of five minutes was required to obtain the adsorption equilibrium. In buffer solution, the sensor exhibited high sensitivity and selectivity, and had a LOD of 9.5 ng/L (4.0×10^{-11} M). Only norketamine, a metabolite of ketamine, was found to produce signals comparable to those of ketamine due to the similar molecular structure. To test the applicability of the sensor for ketamine detection in biological samples, oral fluid was collected from healthy volunteers. The samples were refrigerated overnight and diluted 10-fold in ionised water immediately prior to analysis. The recovery of ketamine from the spiked oral fluid samples was found to range between 100% and 104% with RSD between 2.0-2.5%.

More recently, Parrilla *et al.* (2021) reported the use of SDS for the electrochemical detection of several illicit drugs including ketamine.[46] In buffer solution with 0.1 mg/mL SDS, a 4.9-fold enhancement in peak current was found compared to SDS-free buffer. Interestingly, the electrochemical signal for the oxidation of ketamine's secondary amine was located at ca. +0.95 V in buffer, but shifted slightly towards lower potential in 10-fold diluted oral fluid to +0.93 V. Under optimised conditions the LOD for ketamine in oral fluid samples was found to be 0.62 mg/L (2.6 µM), which would correspond to ketamine concentrations of 6.2 mg/L (26 µM) in the oral fluid from the donor.

3.5.4 Fentanyl

The group of Arroyo-Mora has recently reported the electrochemical detection of the opioid fentanyl for forensic samples, including oral fluid, using adsorptive stripping SWV.[86] For fentanyl determination in oral fluid, samples were first treated by "protein crashing" which involves denaturising the proteins, followed by spiking of the samples. In diluted oral fluid, the peak potentials of fentanyl were found to have shifted slightly as compared to fentanyl in buffer solution. Interestingly, several compounds were

found to interfere with the detection of fentanyl. Most importantly, the presence of cocaine and quinine obstructed the detection of the second oxidation peak of fentanyl. When quinine was present in high concentrations compared to fentanyl, it also hindered the detection of the first fentanyl peak, rendering the detection of the opioid not possible. Overall, the developed method showed good potential for both qualitative and quantitative analysis.

Table 4 Electrochemical strategies for the detection of illicit drugs in oral fluid.

Method	Electrode	E_p (V)	pH	LOD (µg/L)	LOD (µM)	Analysis time	Ref
			MDMA				
Amperometry	Ab-MDMA-HRP/SPE	-	9.5	1.02	5.28×10^{-3}	45 min	44
SWV	Carbon paste SPE	+1.2	4.5	1.93×10^3	10		45
		+1.03*					
SWV	SDS/SPE	+1.00	5.0	1.74×10^2	0.9	10-15 min adsorption	46
		+1.03*		1.93×10^2	1.0*		
			Methamphetamine				
DPV	GCE/EDOT-BTDA-Pala/Antibody/METH	~ +0.2	7.0	13.07×10^3	87.6	30 min preincubation	50
Split SWV	*N,N'*-(1,4-phenylene)-dibenzenesulfonamide/SPE	-0.04	10.8	400	2.68	10 min centrifugation	48
		-0.046				55 s analysis	
		+ 0.15					
		+0.38**					
ECL	$[Ru(bpy)_3]^{2+}$/GCE	+1.10	9	1.49×10^{-3}	10		51
			Amphetamine				
LSV	1,2-naphthoquinone-4-sulfonate/edge plane pyrolytic graphite	–0.17**	9.1	2.6×10^3	19 (peak 1)	< 2 min	49
		-0.52		8.5×10^3	63 (peak 2)		
		–0.17**	6.8*	3.0×10^3	22 (peak 1)*		
		-0.53*		10×10^3	74 (peak 2)*		
			THC				
SWV	2,6-dichloro-p-amino-phenol/basal plane pyrolytic graphite	~ 0.0	9	3×10^2	1		53
	4-amino-2,6-diphenylphenol/basal plane pyrolytic graphite	~ –0.05	9	5.7×10^3	18		
			6.8*	0.47×10^3	1.5*		
CV/ chrono-amperometry	*N*-(4-amino-3-methoxyphenyl)-methanesulfonamide/carbon SPE	+0.059	9.5		-	30 s	54
		–0.005					
AbSWV	Carbon paste	+0.35	10	0.15	0.48×10^{-3} (stationary)	5 min preconcentration	19
				0.13	0.41×10^{-3} (stirred)		
CV	3D-printed SPE	~ +0.1	7	4.7×10^3	15		59
SWV	THC/SPE	+0.4 – +0.6	7.4	1.1	3.5×10^{-3}	30 s preconcentration	60
				1.6	5.1×10^{-3} *		
SWV	MWCNT/carbon	+0.35	7.4	0.16×10^3	0.5	3 min	61

EIS	Gold	-	4 & 6	0.10	3.2×10^{-4}	15 min incubation	[62]
			Cocaine				
DPV	EDOT-BTDA-PPhe/Antibody/GCE	+0.1 – +0.4	7.0	0.12×10^{3}	0.4 (BZE)		[63]
LSV	SWCNT-COOH/carbon SPE	+0.44 – +0.66	-	0.15	4.9×10^{-4}	25 min	[64]
				1	3.3×10^{-3} *		
DPV	CoNTA-Ab/SPE	+0.1**	8	3.6	11.9×10^{-3}		[65]
DPV	$(Fc\text{-}PEI/AuNPs)_2/ITO$	~ +0.35	6.5	30	0.1	15 min centrifugation 40 min analysis	[68]
DPV	2D AuNCs@521-MOF/AE	~ +0.2	7.4	6.73×10^{-4}	2.22×10^{-6}	2 hours	[69]
EIS	2D AuNCs@521-MOF/AE	-	7.4	3.91×10^{-4}	1.29×10^{-6}		[69]
SWV	MIP-PdNPS/GPH-SPE	+0.88	11.0	15×10^{3}	50	5 min incubation	[70]
SWV	Carbon paste	+0.90	9.5	900	2.97	30 s preconcentration	[71]
SWV	SDS/SPE	+0.83	9	0.2×10^{3}	0.7	10-15 min adsorption	[46]
		(+0.89*)		0.36×10^{3}	1.2*		
			Heroin				
ECL	Nafion/MWCNT GCE	~ +1.1	7.0	1.5×10^{-6}	4.0×10^{-9}	5 min preconcentration	[73]
SWV	SDS/SPE	+0.92	6	0.66×10^{3}	1.8	10-15 min adsorption	[46]
		+0.96*		0.89×10^{3}	2.4*		
			Indole-based SCs				
DPV	BDD	~ +1.5	5	0.28-0.84		4 min pretreatment	[76]
		~ +1.2*					
			Indazole-based SCs				
DPV	BDD	~ +1.7	5	0.23-0.56		4 min pretreatment	[76]
		~ +1.5*					
			Scopolamine				
ECL	$[Ru(bpy)_3]^{2+}/GCE$	~ +1.05*		1.49×10^{3}	1*		[77]
			Methadone				
DPV	fMWCNT/MGCE	+0.91	7.0	87	0.28	5 min centrifugation*	[81]
AdSWV	GNPs/MWCPE	1.00	9.0	1.5	5×10^{-3}	5 min purging + 100s preconcentration	[82]
			Ketamine				
DPV	KT-MIM/MOF/MOFs@G/SPE	+0.1**	6.0	9.5×10^{-3}	4.0×10^{-5}	5 min incubation	[85]
SWV	SDS/SPE	+0.87	8	0.26×10^{3}	1.1	10-15 min adsorption	[46]
		(+0.93*)		0.62×10^{3}	2.6*		
			Fentanyl				
SWAdSV	Carbon SPE	+0.75	8.5	37	0.11	~ 6 min	[86]

											+0.88

All peak potentials and LODs are related to detection in buffer solution, unless specified otherwise. * For (artificial) oral fluid ** Change in peak current observed upon addition of target analyte in comparison to peak present in blank. Abbreviations: CV = cyclic voltammetry; DPV = differential pulse voltammetry; ECL = electrochemiluminescence; EIS = electrochemical impedance spectroscopy; LSV = linear sweep voltammetry; SPE = screen printed electrode SWCNT = single-walled carbon nanotube; SWV = square wave voltammetry.

4. Prevailing challenges

Over the last two decades, the detection of illicit drugs in oral fluid has received more attention from the scientific community. Electrochemical sensing methods have been shown promising in this regard, but still, no electrochemical drug tests are commercially available. Prior to these techniques reaching the market, it is crucial to address the key challenges regarding electrochemical sensors for illicit drug tests in oral fluid: (i) understanding of matrix effects, (ii) interferences from common electroactive cutting agents, and (iii) feasibility in roadside use. **Figure 6** depicts the three main challenges identified by the authors of this review. In this section, these challenges will be elaborated upon, assuming that the required limits of detection have been reached.

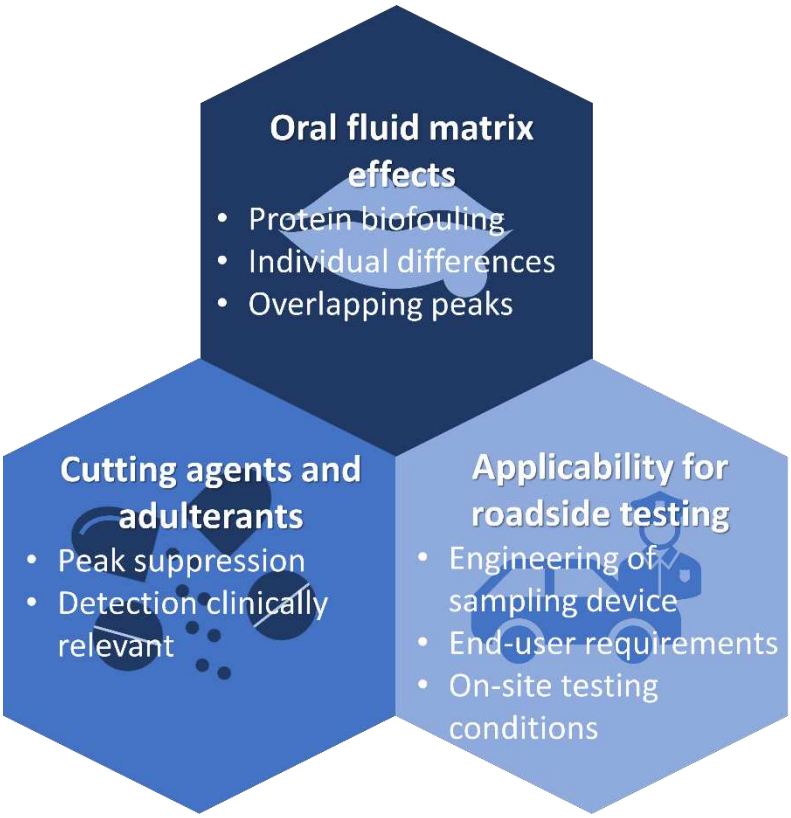

Figure 5 *Prevailing challenges in the development of electrochemical sensors for illicit drug detection in oral fluid: understanding the oral fluid matrix effects; understanding the influence of cutting agents and adulterants on the electrochemical signals of illicit drugs; tailoring the sensors towards applicability for roadside testing.*

4.1 Oral fluid matrix effects

Oral fluid is a complex heterogeneous mixture containing proteins, electrolytes and small organic compounds, and is rich in antioxidants.[87,88] The electrochemical behaviour of analytes in this matrix can differ greatly as compared to their behaviour in standard aqueous solutions.[89] Electroactive species in oral fluid can overlap with the redox signals of illicit drugs, hindering their detection. An example of this is the protein albumin, which shows an oxidation peak around +0.5 V (depending on the electrode's materials), hindering the detection of the drug 4-chloro-alpha-pyrrolidinovalerophenone (Cl-PVP).[46] Currently, the oral fluid matrix effects on electrochemical detection of illicit drugs remain not understood as no study on this topic exists. It is of great importance that the electrochemical species in oral fluid are identified. Since the oral fluid composition between individuals can differ, dilution of the sample is necessary to better control the chemical composition and to minimise these individual differences. Moreover, it is paramount to determine the suppressing effects of oral fluid constituents. Proteins are known to cause biofouling of electrodes due to their non-specific adsorption on the sensor surfaces.[90] This can result in a

decrease of the performance of the biosensor and loss in sensitivity and specificity for the target analyte. As oral fluid contains over 1000 different proteins, antifouling strategies need to be developed to mitigate these biofouling effects.[91] A review on antifouling strategies including (i) nanoengineered surfaces, (ii) antifouling coatings, (iii) nanoporous membranes, and (iv) hydrogels has been published by Russo *et al.* (2021).[90] Furthermore, the effect of exogenous compounds present in oral fluid – *e.g.*, food, beverages, or therapeutic drugs – should be examined as some of these compounds might be electroactive and can interfere/overlap with the electrochemical profile of the illicit drug, thus causing a false positive. An interesting effect of drinks can be that the pH of oral fluid can be lowered after drinking acidic beverages such as sodas.[92] This should be kept in mind when choosing the buffer and dilution factor. Finally, the developed electrochemical methods should not be tested in artificial oral fluid, as this can be a poor model medium, but in authentic oral fluid.[89] No restrictions on eating, drinking or smoking should be imposed on the sample donors, to keep the oral fluid samples as representative as possible to those obtained in real drug testing scenarios.

Additionally, there are other strategies that one may consider in mitigating the effects of the oral fluid matrix. First of all, the rational design and synthesis of multi-functional nanostructured electrocatalysts can be used as nano-electrocatalysts have the inherent ability to enhance the signal/current respons of the analytes in appropriate pH media. To achieve rational design of such nano-electrocatalysts, the use of computational (DFT) calculations cannot be over-emphasised. Secondly, stable electrochemical immunosensors are known for their high selectivity and specificity. Immunosensors are already being used from illicit drugs, e.g., THC where electrode-immobilised anti-THC can be used to detect THC.[93] Biocompatiple electrode materials can be designed and synthesised to encapsulate these antibodies without negatively impacting on their stable electrochemical responses at room temperature. Thirdly, the specificity of MIPs and aptamers for analytes calls for increased efforts in designing and producing them for enhanced electrochemical sensing of illicit drugs.

4.2 Interference of cutting agents and adulterants

Besides interferents from the oral fluid matrix, the illicit drugs consumed can also contain components that might hinder the electrochemical signals. Additional compounds, called adulterants or cutting agents, can be added to illicit drugs by manufacturers to increase the amount, enhance or mimic the effects of the drug, or assist with drug delivery.[94] Commonly used adulterants include caffeine, paracetamol, procaine and sugars. However, little is known about adulteration patterns or when they are added in the drug supply chain.[95] Research has shown that many of the adulterants supplemented with cocaine and heroin in the United States are toxic when ingested alone or in combination with drugs.[96] Detection of the presence of adulterants in the oral fluid of drug users could provide valuable information to health providers. For example, levamisole is frequently used for cocaine adulteration and can lead to anxiety, rash, necrotic tissue, nausea, and vomiting among other things.[97] Research has shown that this compound was found in approximately 75% of oral fluid samples from Australian cocaine users.[98] Importantly, several adulterants are known to be electrochemically active.[99] Interestingly, levamisole was found to hinder the electrochemical detection of cocaine and heroin in powder form in PBS pH 7.[72,100] At this pH, heroin detection was also obstructed in presence of procaine due to overlap of the oxidation peaks.[100] To overcome this issue, the pH was adjusted from 7 to 12 (dual pH screening) or a preconditioning step was added.[72,100] Currently, no extensive study of the effect of adulterants and cutting agents on the electrochemical behaviour of illicit drugs in oral fluid matrix exist. Moreover, no information is available on how the redox signals for drugs are affected in presence of metabolites of adulterants. Hence, it is imperative that a thorough study on this issue is performed. Several nanostructured electocatalysts have proven themselves as viable electrode materials for simultaneous detection of target analytes and their potential interfering species. The rationality in materials design is giving due consideration to the introduction of appropriate functionalities to electrocatalysts that will allow for their specific and selective interaction with the

analyte of interest. For example, it is possible to design an electrocatalyst that can detect the illicit drug as well as the licit ones (such as paracetamol) at the same time.

4.3 Applicability for roadside testing

Finally, before an electrochemical sensor for drug abuse can reach the market, the applicability of the developed methods to roadside testing scenarios should be considered. All of the developed methods described above have only been tested in laboratory settings, but have not been subjected to the conditions that police officers have to work in. Roadside drug testing devices are required to work under varying weather and lighting conditions. Therefore, temperature studies of the electrochemical behaviour of the drugs in oral fluid are required to ensure that the potential intervals of the target analytes are stable over the working temperature range. Additionally, the sensing methods should be combined with oral fluid collection and sampling steps for straightforward application at the roadside by a police officer. To do this, the collection and sampling steps should be integrated into a testing device that can be coupled to a miniaturised sensing device.

In the development of such a device for the application in real scenarios, the demands of end-users should be taken into account. The European Traffic Police Network (TISPOL) has outlined several requirements for roadside testing devices. These requirements have been summarised in Table 5.[101] An important aspect is the simplicity of the devices. A short training of only 0.5 – 1 hour should be adequate for police officers, after which the officers can learn from experience and use the devices. Importantly, the police officers who will be handling the roadside testing devices do not have expertise in electrochemistry. In this regard, software to convert the electrochemical signal into user-friendly output is invaluable. Moreover, pretreatment steps such as purging and centrifugation of the samples might not be feasible at the roadside. These steps are laborious and require additional equipment and increase analysis time. In the ROSITA project, it was suggested that an overall analysis time of 15 minutes would be realistic to accept for roadside drug testing.[101] This time includes an explanation to the tested person, collection of the sample, and sample analysis. While police officers seem to accept collection times of less than five minutes, a collection time of three minutes was considered acceptable by only 57% of tested persons in this project.[101] Last but not least, the sensing device needs to be disposable to avoid contamination between samples as well as between the police officer and the suspected intoxicated driver. Therefore, to allow decentralisation of the test and massive testing, the devices need to be affordable.

Table 5. Requirements of roadside drug testing devices set out by the European Traffic Police Network (TISPOL)[101]

Police user requirements for roadside testing devices	
Training	Operational testing
- Police officers trained by police instructors (0.5 – 1 hr)	- 75% of tests qualified as simple to operate
- Police instructors trained by manufacturer (1 – 2 hrs)	- Hygienic use of device
- Learning by demonstrating	- Sufficient amount of collected oral fluid
- Learning by doing	- Detectable substances at least cannabis, cocaine, opiates, amphetamines (analogues)
- Information about do's and don'ts	- At least 75% of the tests should be correct for at least one of the substances
- Clear hygienic and safety measures	- Indication lines should remain visible for at least 3 minutes
- Instruction card for each officer during training	Documentation
- Material available through police intranet	- Device user manual in native language
- All materials in native language	- Device instruction card for each trained officer
	- Digital material (guidelines) available for each force/unit
	- User manual for electronic reader

5. Conclusions and future perspectives

In this review, the electrochemical strategies for illicit drugs detection in oral fluid have been discussed. Compared to the detection of powdered drugs, the main challenge remains achieving low LOD (µg/L) without increasing electroanalysis times or adding laborious (electrode) pretreatment steps. A limited number of articles included centrifugation, purging of the samples, or incubation times, while most of the strategies involve modifications to the electrode surface in order to enhance the LOD. However, most detection strategies do not meet the required analytical cut-off values for roadside drug testing. In this regard, nanomaterials may be used to enhance the electrochemical signals. Additionally, the prevention of biofouling phenomena by proteins in the oral fluid matrix could prove essential to enhance the sensor performance and further improve the LOD for drug detection in biological fluids. Importantly, the effects of the oral fluid matrix on the electrochemical signals of illicit drugs are as of yet non-understood. Studies to elucidate the electroactive species in oral fluid should be conducted, as well as studies to determine the effect of compounds present in the matrix on the electrochemical signal of illicit drugs. Besides compounds from the biological matrix, cutting agents and adulterants could also affect the signals for drugs. Therefore, it is crucial to understand which additional drug-related substances might be present in oral fluid and what effects these have on drug detection. Another prevailing challenge that needs to be addressed in future work is the applicability of the detection strategies at the roadside. It is essential to make the shift from laboratory settings to working conditions in the field where law enforcement activities occur. Ultimately, interdisciplinary collaborations with end-users will be vital to achieve electrochemical sensors that allow illicit drug detection in oral fluid at the roadside.

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Supplementary material

Electrochemical detection of illicit drugs in oral fluid: potential for forensic drug testing

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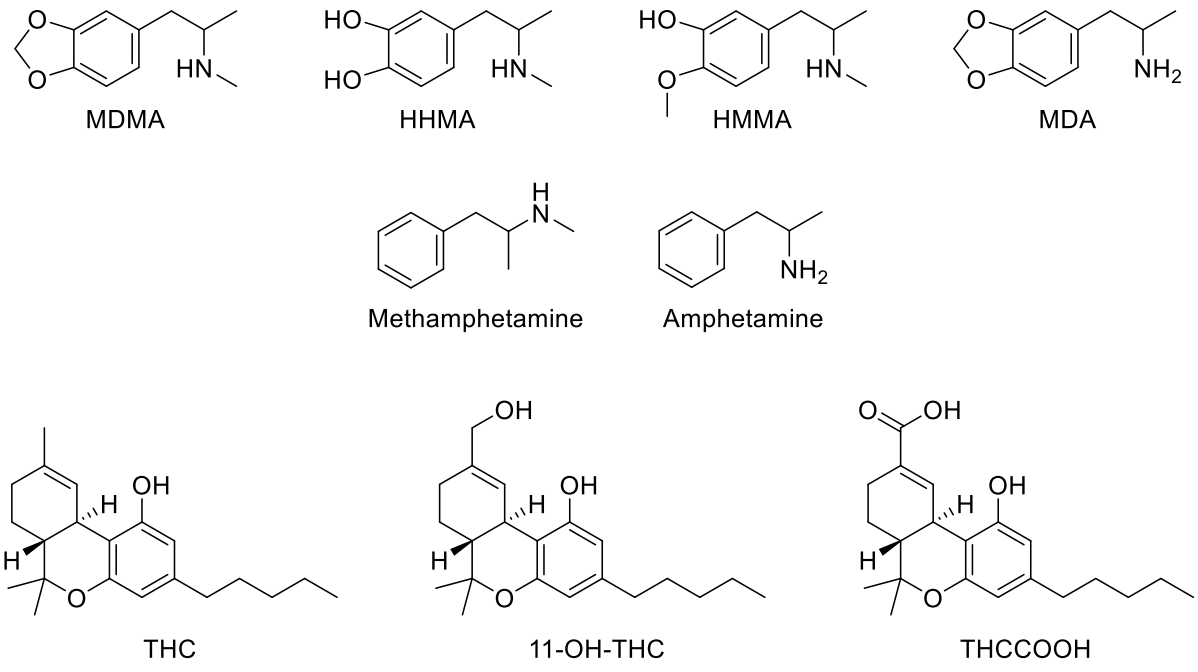
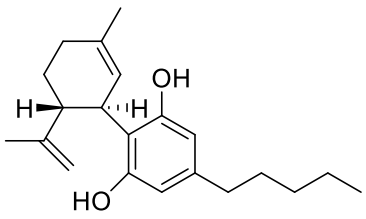
Structures of the illicit drugs and their metabolites





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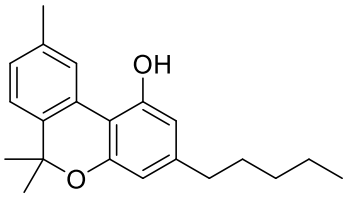

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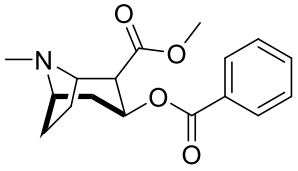

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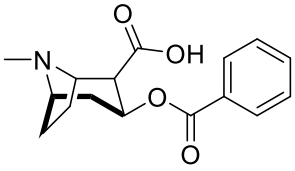

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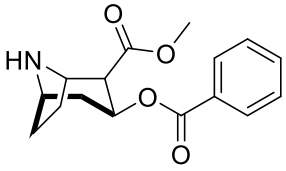

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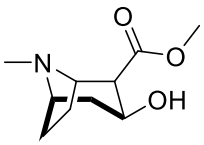

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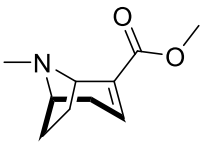

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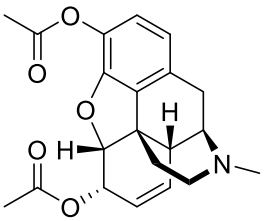

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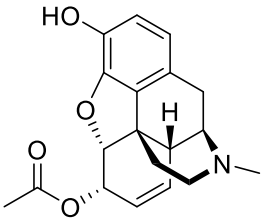
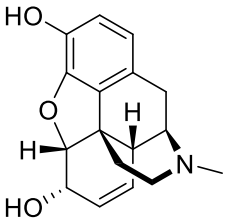


6-MAM



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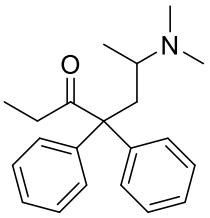

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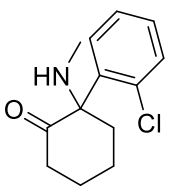

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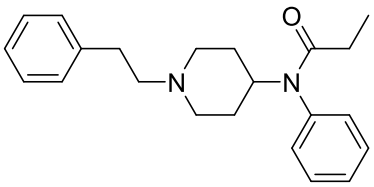

Fentanyl