Drug and Alcohol Dependence 198 (2019) 136–139

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Full length article

Detection of heroin intake in patients in substitution treatment using oral fluid as specimen for drug testing

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ARTICLE INFO

Keywords:
Substitution
Treatment
Oral fluid
Drug testing
Heroin
6-Acetylmorphine

ABSTRACT

Background: Detection of heroin use is among the major tasks for drug testing and can be best performed by using 6-acetylmorphine as the target analyte. This study was performed to document analytical findings in oral fluid after OF heroin intake.

Methods: The samples were from routine drug testing of patients in substitution treatment. The analytical investigation was made with a forensic accredited liquid chromatography-tandem mass spectrometry method.

Results: Out of 2814 samples, from 1875 patients, sent for routine drug testing, 406 contained one or more opiate in the drug screening when applying a cutoff limit of 1 ng/mL neat OF. Out of these 406, 314 had a measured 6-AM concentration in neat OF ≥ 1 ng/mL. The study demonstrated that 6-AM is a viable parameter in oral fluid drug testing with an about 80% sensitivity compared to using morphine and codeine as biomarkers. An additional value of using 6-AM is the confidence in concluding a heroin intake. The 6-AM concentrations varied between 1 and > 1000 ng/mL, with a median value of 18.6 ng/mL. Heroin was measured in 35 samples with a median value of 0.72 ng/mL. The positive rate for opiates in urine and OF drug testing was the same, 13.5%, in similar populations of patients.

Conclusions: 6-AM is a preferred parameter in OF drug testing for monitoring heroin use and makes OF drug testing for detecting heroin use more effective than urine drug testing when using highly sensitive mass spectrometry methods.

1. Introduction

Drug testing is essential when monitoring compliance in patients treated for opiate addiction with substitution therapy. The established and most common specimen for drug testing is urine, that is used to screen for possible drug content using commercial immunochemical assays. However, there is an increasing interest for using alternative specimens to urine, e.g., oral fluid, mainly to minimize risk for adulteration and intrusion on privacy (Palmer and Krasowski, 2019).

Detection of relapse into heroin use is one of the major tasks for doing drug testing and is usually done by screening urine for morphine content. To distinguish a heroin intake from other sources of morphine the relative proportion of morphine and codeine, as measured in the confirmation assay, has been used as basis for interpretation (Tenore, 2010; Stefanidou et al., 2010). An alternative way is to focus on the specific heroin metabolite 6-acetylmorphine (6-AM) (Cone et al., 1991), which provides evidential confidence in heroin intake. For many years the general conception in urine drug testing was that 6-AM is short-lived and therefore not a suitable parameter. However, it has since been demonstrated that focusing on 6-AM in drug testing with a sensitive method is a viable approach (Andersson et al., 2014). The sensitivity of modern liquid chromatography-mass spectrometry instruments has made it possible to include 6-AM as a parameter in routine confirmation methods (Andersson et al., 2014; Coles et al., 2007). Use of 6-AM as a parameter even detects samples that are negative in established opiate testing focusing on morphine (Beck and Böttcher, 2006).

In recent time 6-AM has become a target analyte already in the screening assay (Holler et al., 2004; Borriello et al., 2015; Wang et al., 2015). The reason for focusing on 6-AM as target already in drug screening is that morphine can originate from other common sources as ingestion of morphine, codeine and poppy seeds (Tenore, 2010; Cone et al., 1991). In addition, morphine is becoming established as a medication in substitution treatment (Beck et al., 2014).

It is known that the detectability of 6-AM is good in OF after heroin intake (Fierro et al., 2017) and that it provides a better detection rate than urine (Dams et al., 2007). However, even though immunochemical assays exist for OF these do not include 6-AM at present. Additional complications with immunoassay screening in general are that reagents

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https://doi.org/10.1016/j.drugalcdep.2019.02.011
Received 5 January 2019; Received in revised form 3 February 2019; Accepted 5 February 2019
Available online 23 March 2019
0376-8716/

for OF are developed for use together with a specific sample collection system (i.e., extraction buffer solution), and that the applied cutoff limits in these assays result in a shorter detection time as compared to urine drug testing (Verstraete, 2004; Bosker and Huestis, 2009). To overcome these obstacles the screening of oral fluid samples can alternatively be done with more sensitive and universal methods, such as mass spectrometry (Grabenauer et al., 2018). Such a method is established in the authors laboratory since more than five years and is applied for clinical drug testing under ISO 15189 accreditation (Reinstadler et al., 2018; Wagner et al., 2018).

The present study was aimed to collect more data about detected opiate analytes in OF by investigating samples for opiates from drug users in substitution treatment with methadone or buprenorphine.

2. Materials and methods

2.1. Standards and controls

Reference compounds of heroin, heroin-d9, 6-AM, 6-AM-d3, morphine, morphine-d6, codeine, codeine-d6, norcodeine, 6-acetylcodeine, dihydrocodeine and hydromorphone-d3 were obtained as ampoulled methanol solutions from LGC Standards GmbH (Wesel, Germany). Working multi-component solutions were prepared by taking aliquots of these solutions, evaporating the solvent and dissolving the residues in methanol. The working solution of the analytes was used to prepare standards and controls, by mixing with a 1 + 1 solution of blank saliva and SES-buffer (Greiner Bio-One GmbH, Kremsmünster, Austria). A pool of blank saliva was collected from laboratory personnel. Standards for calibration were made in multiple concentrations ranging from 0.025 to 20 ng/mL for all analytes, and up to 1000 ng/mL for 6-AM. Quality controls were made in concentrations 0.5 ng/mL and 1.5 ng/mL and at 80 ng/mL for 6-AM.

2.2. Clinical samples

OF samples were collected with the Greiner Bio-One saliva collection device (Greiner Bio-One GmbH) (GBO, 2018) containing SES-buffer (Raggam et al., 2008; Coucke et al., 2016). Observed sampling was conducted by the medical staff at the clinics. Routine measurements were made at the day of arrival and additional analyses with the modified method after storage at −24 °C. OF and urine samples were from the routine toxicology service of patients in substitution therapy at several outpatient clinics using methadone or buprenorphine. In general, the patients were well established in the programs and relapse into illicit drug use was tolerated. Additional analytical investigations were made on anonymized surplus aliquots, and results from the routine measurements were anonymized.

For comparison in detection rate between OF and urine samples, samples were randomly collected during a 3-month period from the same outpatient clinic. The clinic had a patient population of about 200 patients, and the samples were non-paired.

2.3. Analytical methods

The OF samples were analyzed with an UPLC-MS/MS multi-target method with a cutoff limit at 1 ng/mL in neat OF and with an upper quantification limit of 20 ng/mL. The sample preparation procedure was based on salted out liquid/liquid extraction (Reinstadler et al., 2018; Yanes and Lovett, 2012). The method was run under accreditation (ISO15189 standard). In addition, a slightly modified method with less sample volume and wider measuring range were used to measure heroin and 6-AM with measuring ranges from 0.1 to 10 and 1–1000 ng/mL, respectively.

The method validation of intra- and inter-day variability in quantification demonstrated measuring uncertainties within the measuring range of < 12%. Stability of the analytes in the OF/SES-buffer mixture was demonstrated for 4 weeks at +4 °C.

Urine screening was performed using DRI opiate reagents (Thermo Fisher Scientific, Passau, Germany) applied on an Olympus AU680 instrument (Beckman-Coulter GmbH, Krefeld, Germany) with an applied cutoff at 100 ng/mL. The 100 ng/mL calibrator was prepared by diluting the commercial 300 ng/mL calibrator with the negative calibrator. The method was run under accreditation (ISO15189 standard). Morphine is the calibrator substance, and cross-reactivities for other related opiates are; morphine-3-glucuronide 88%, morphine-6-glucuronide 111%, codeine 200%, 6-AM 107%, heroin 79%.

Table 1
Results from the analyses of heroin and metabolites in 2814 oral fluid specimens from patients in substitution treatment.

Substance	N	Positive rate %	Concentration range ng/mL	Statistics
Heroin	35	8.6		
6-AM in all samples	314	77.3	1- > 1000	Mean ± SD 222 ± 344 Median 18.6 13% > 1000[a]
6-AM in heroin positive samples	35	100	50- > 1000	54% > 1000
Morphine	405	99.8	1- > 20	54% > 20
Codeine	280	69.0	1- > 20	51% > 20
6-Acetylcodeine	156	38.4	1- > 20	38% > 20
			Ratio range	
Morphine/codeine ratio in 6-AM positive samples	40		0.95-10.8	Mean ± SD 3.8 ± 2.6 Median 3.1
6-AM/6-acetylcodeine ratio	96		9.2-108	Mean ± SD 23.6 ± 11.8 Median 21.3

[a] Used as 1001 in above calculation.

3. Results

3.1. Evaluation of 6-AM as key analyte

Of the total 2814 samples from 1875 patients, 406 contained one or more opiate in the drug screening when applying a cutoff limit of 1 ng/mL neat OF (Table 1). This represents 14.4% of the samples and 16.7% of the patients. Out of the 406 opiate positives, 314 had a measured 6-AM concentration in neat OF ≥ 1 ng/mL. 6-AM was detected in an additional 25 samples, but the concentration was < 1 ng/mL in neat OF when correcting for the OF dilution resulting from the sampling process. In 57 of the samples with no 6-AM detected the dominance of morphine indicated a possible heroin intake. In one sample, only dihydrocodeine was present at 13.1 ng/mL concentration. In another 11 samples with no 6-AM detected there was a dominance of codeine over morphine (ratio > 1) indicating a codeine intake. Thus, in 12 samples no indication of a heroin intake was evident from the analytical results. The sensitivity for detecting a heroin intake based on 6-AM being measured was 79.4% when using 6-AM results above the 1 ng/mL cutoff limit as indication a heroin intake, and 85.6% when using the additional 25 samples with 6-AM detected.

The 6-AM concentrations were measured between 1 and 1000 ng/mL. The distribution of 6-AM concentrations in the 314 samples is shown in Fig. 1. In 42 samples (13%) the 6-AM concentration was > 1000 ng/mL. In the remaining 271 samples, the mean concentration was 104 ng/mL, while the median concentration value was 13.4 ng/mL. When using all samples and a 1001 value for those 42 > 1000 the calculated mean value was 222 ng/mL and the median value was 18.6. Heroin was present (> 0.1 ng/mL) in 35 samples (11.2%), with a mean value of 1.83 ± 2.49 (SD) ng/mL and with a median value of 0.72 ng/mL. When heroin was detected the 6-AM concentration was always > 50 ng/mL and in most cases (54%) > 1000 ng/mL.

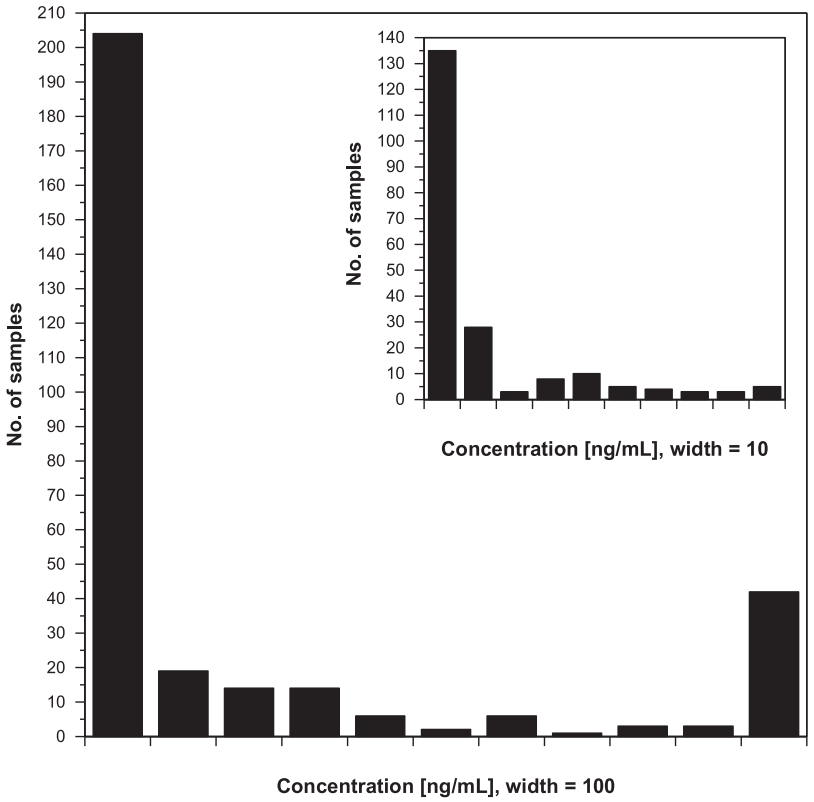

Fig. 1. The distribution of 6-AM concentrations in neat oral fluid.

In the samples with 6-AM ≥1 ng/mL, 40 samples had morphine and codeine within the measuring range. The mean morphine/codeine ratio was 3.81 ± 2.65 (SD), with a range between 0.9 and 10.8. In 6 of the 25 samples with 6-AM detected but below 1 ng/mL, the mean morphine/codeine ratio was 3.17 ± 0.48.

6-Acetylcodeine was present in 156 of the 314 6-AM positive samples (50%). In 98 samples the 6-acetylcodeine concentration was within the measuring range (1–20 ng/mL) and in 58 cases > 20 ng/mL. The ratio between 6-AM and 6-acetylcodeine was possible to calculate in all 98 cases when the 6-AM concentration was within the measuring range, and the mean value was 23.6 ± 11.8 (SD), with a range between 9.2 and 108. Most cases (58%) with 6-acetylcodeine > 20 ng/mL had 6-AM concentrations > 1000 ng/mL.

3.2. Comparison of OF with urine screening from the same patient population

The positive rate for opiates in this setting was 13.5% (n = 902) in OF with LC–MS/MS and 13.5% for urine (n = 968) with immunoassay screening. 6-AM was detected in OF in 76.2% of all opiate positive samples.

4. Discussion

This study successfully measured heroin and related opiates and their metabolites in OF from patients in substitution therapy as part of routine drug testing. The primary aim was to document how well 6-AM performs as a key analytical parameter to accurately reveal relapse into heroin use in this population. This is important since OF is a specimen that is already widely applied for drug testing and there is a need to learn how analytical results should be interpreted. One advantage of OF is that the collection procedure is less intrusive and easy to perform as compared with urine, especially when supervision is required to avoid adulteration or substitution of the sample. One drawback with replacing urine with OF for drug testing has been the shorter detection time (Verstraete, 2004; Bosker and Huestis, 2009). However, this study found that by lowering the reporting limits by improved methodology, the detection rate for opiates was the same in urine and OF in the population of patients in substitution treatment. It should be noted that the applied urine screening cutoff level of 100 ng/mL is lower than the 300 ng/mL that is usually applied. A similar observation was reported by Kunkel and co-workers who were using a mass spectrometry method with low cutoff level found the OF testing was superior to urine regarding the positive rate, which was assumed to be due to adulteration or substitution of the urine specimen as they were unobserved (Kunkel et al., 2015). One reason for high detectability of 6-AM in oral fluid is probably the high ratio between saliva and blood (Kauert, 2000).

Our study found that about 80% of the cases with a probable heroin intake were discovered using 6-AM as the detection parameter, which is higher than in urine (Andersson et al., 2014; Beck and Böttcher, 2006). A similar detection rate of 6-AM has been seen in another investigation (Di Rago et al., 2016; Wagner et al., 2018), and even a higher detection rate than for morphine in patients in opiate substitution treatment has been reported in OF (Kunkel et al., 2015; Vindenes et al., 2011). A similar observation was also made in a study using OF samples collected from a more general drug addiction population (Flood et al., 2016), and in samples from workplace testing (Presley et al., 2003).

The classification of the other cases than 6-AM positives as probable heroin intake was based on the morphine/codeine ratio being ≥1. This classification follows the criterion used for urine (Stefanidou et al., 2010). The similar mean values of the morphine/codeine ratio in cases with 6-AM at low concentrations, quantified or only detected, supported this classification. However, an intake of other sources (e.g., poppy seed) of morphine and codeine at these low concentrations cannot be ruled out. Therefore, basing the analytical result as heroin intake on the morphine to codeine ratio might be risky. The advantage of having the interpretation based upon presence of 6-AM is that the heroin intake can be regarded as proven regardless of morphine/

codeine ratio.

The ratio between 6-AM and 6-acetylcodeine was much higher than between morphine and codeine, and more in agreement with the fact that morphine dominates over codeine in raw opium. The observation that the morphine/codeine ratio is lower is most likely related to the fact that the measured values were later in the dosing interval when the 6-AM concentrations were low. However, from studies on codeine pharmacokinetics, it is known that codeine is eliminated slightly more rapid from plasma than morphine (Lafolie et al., 1996), which is contrary to our observation.

Our study also successfully measured heroin concentrations in 35 cases. The concentrations were always much lower than for 6-AM, indicating a low sensitivity in detecting heroin intake and that it is a less useful parameter for clinical drug testing.

It is important to be aware of the difference between sampling techniques used for collecting an OF specimen. It is most common to collect 1 mL of liquid from the oral cavity and put it in an extraction buffer, which is then used as a specimen. Problems with this technique will be cases of dry mouth and that the volume of sample is inexact and the time required can be variable. The GBO device used in this study collects a sample of oral fluid by using an oral extraction buffer with slightly acid pH and therefore offers a more standardized procedure. Despite this, the final performance for drug testing seems to be similar (Langel et al., 2008).

In conclusion, this study demonstrates that 6-AM is a preferred parameter in OF drug testing for monitoring heroin use and that OF drug testing is as effective as urine drug testing when using highly sensitive mass spectrometry methods.

Role of funding

None.

Contributors

MB initiated and designed the study, collected the study results, participated in the data analysis and writing of the manuscript.

SL and AP were involved in the planning of the study, did the analytical work, put the results in data base, and participated in the manuscript work.

OB was involved in the initiation of the study, data analysis and did the main part of writing the manuscript.

All authors have approved the final version.

Conflict of interest

No conflict declared.

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