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Comparison of Centrifugation and Solid Phase Extraction (SPE) Methods of Sample Preparations in Determination of Residues of Drugs of Abuse in Urine

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Abstract

Detection of drugs of abuse in urine samples depends on their composition, absorption, distribution, physical and chemical properties, concentrations, elimination properties, and the techniques employed. This study compared the performance of centrifugation and solid phase extraction (SPE) methods in the determination of levels of drugs of abuse and their metabolites in urine samples collected from drug abusers. Analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS-MS). Calibration was done using both internal and external standards. The recovery values were generally greater in centrifugation method than in SPE method, but the recoveries in both methods were within an acceptable range. Linearity values (R^2) ranged from 0.9789 to 0.9944 with an average of 0.99. The LOD and LOQ values were satisfactory. The concentrations of 6-monoacetylmorphine (6-MAM), morphine and cocaine were up to 3.37, 4.21 and 0.03 µg/mL, respectively in samples prepared by centrifugation method, while in samples prepared by SPE method were up to 6.67, 6.66 and 0.13 µg/mL, respectively. The mean concentrations and detection frequencies of 6-MAM and morphine were higher in samples prepared by centrifugation method than those of SPE method. Cocaine had the same mean concentrations and detection frequencies in samples prepared by both methods. There were no significant differences in the concentrations of the drugs of abuse between the samples prepared by centrifugation and SPE methods, although the levels of the drugs in the samples prepared by centrifugation method were slightly greater than those prepared by SPE method.

Keywords: Centrifugation; Solid Phase Extraction; Cocaine; Heroin; Urine; LC-MS-MS

Introduction

Suitable sample preparation is a key aspect of quantitative chemical analysis and it is usually the most time consuming part of the analyses. Interfering matrix compounds such as proteins, lipids, salts and other endogenous and background compounds should be removed during sample pretreatments, not only to avoid column clogging and instrument soiling, but also to improve the sensitivity and reliability of the analyses. Selection of an appropriate preparation procedure depends upon analytes characteristics, their expected concentrations, the sample size and matrix, and the availability of suitable analytical techniques for analytes quantification (González-Mariño et al. 2012).

Insufficiently treated samples may cause interfering peaks when using spectroscopic detection techniques such as UV-absorbance or fluorescence. Analyses by LC-MS-MS are less prone to sample matrix effects and therefore usually require less conceited sample clean-up. Commonly and widely applied sample preparation techniques include protein

precipitation, liquid-liquid extraction (LLE) and solid-phase extraction (SPE). Manual operations associated with sample treatments may be very labour intensive and time consuming, and that could be avoided with direct sample injection followed by online extraction methods (Roškar and Lušin 2012).

The goal of sample preparation is to provide the target analyte(s) in solution, provide the analyte(s) at concentrations appropriate for detection or measurements. Concentration of the analyte helps to increase sensitivity and achieve lower limits of detection and removes interfering matrix elements (e.g., phospholipids, salts, proteins, nucleic acids, and sugar) that alter the instrument responses or co-elute with the target analytes. Matrix effects result in ion suppression (loss of signal) or enhancement (gain signal). Matrix effects have negative impacts on the accuracy, precision and robustness of the methods, and add to the method variability (Pedrouzo et al. 2011).

The importance of clean samples include better separation, lower limits of detection, decreased analysis variability, more robust analysis, reduced matrix effects and fewer reanalysis. Also less chances of false positive results and false negative results, longer column lifetime, less instrument downtime, minimizes costs in manpower and equipment maintenance (Robandt et al. 2008). The bottlenecks of sample preparation include the fact that the processes are difficult and time consuming; about 75% of the activities and operating costs in an analytical laboratory are spent in processing and preparing samples for analysis (Danaceau 2013).

Centrifugation method is simple and straightforward method widely used in analysis of certain samples such as plasma samples. It is accomplished by using organic solvents (typically acetonitrile or methanol) and is commonly used to separate proteins from liquid supernatant. The supernatant is sometimes diluted with chromatographically compatible solvents. Moreover, the supernatant can be directly injected or pre-concentrated after evaporation and reconstitution. The method has been extended to quantification of drugs and metabolites from whole blood and urine. The absence of interfering compounds such as proteins in these matrices allows direct injection without sample pretreatments (Danaceau 2013).

Solid phase extraction (SPE) is frequently used for urine sample extraction and clean-up methods in recent techniques of drug testing in urine (Cao et al. 2015). It has become very popular and is considered as a basic technique in many laboratories for sample preparation for determination of drugs and their metabolites in biological matrices. SPE is considered to be a very versatile sample preparation technique for various analytes in complex matrices, e.g., blood, serum, plasma, oral fluid, tears, nasal fluid, urine, faeces, postmortem samples and many others (Robandt et al. 2008). It is the best technique for minimizing matrix interferences including proteins, phospholipids, salts and other compounds. This is the best clean-up option, fast, easy to automate, achieves the highest recovery and reproducibility, can be manipulated for optimum recovery and clean-up and uses a variety of device formats and sorbent chemistries. However, the method may require method development to optimize the protocol and it is difficult and costly (Danaceau 2013).

The objective of this study was to compare the performance of centrifugation and SPE methods in the determination of the types and levels of drugs of abuse and their metabolites in urine samples.

Materials and Methods

Sampling, sample storage and ethical considerations

Urine samples for this study were collected from Ubungo darajani site in Dar es Salaam Region. Samples were collected directly from drug abusers who volunteered to participate in the study. The sampling exercise was performed on 21st May 2018. A total of 30 samples were randomly collected from drug users who volunteered to give urine. 100 mL

plastic containers were used for collection of the samples. Immediately after sample collection, each sample container was tightly closed, labelled and packed in a sampling container for easy transportation and avoiding contamination of samples. The samples were transported to the laboratories of the Government Chemist Laboratory Authority (GCLA) for storage where they were kept in a refrigerator at 8 °C prior to laboratory analysis.

Ethical approval was granted from the National Institute of Medical Research in Tanzania (Ref. No. NIMR/HQ/R.8a/Vol. IX/2943). The consent of each participant was sought before collection of urine sample. The participants were assured on the confidentiality of the information and urine samples provided. The information obtained from the participant was not intended to be used for any other purpose except for research study only.

Chemicals and materials

The chemicals used included methanol, acetonitrile, Milli-Q water, distilled water. The drugs of abuse standards were cocaine, heroin, benzoylecgonine, 6-monoacetylmorphine and morphine. The chemicals (solvents, reagents and standards) were of high purity and of analytical grade. The solvents and reagents were obtained from Sigma-Aldrich, whereas the standards were obtained from the United Nations Office on Drugs and Crime (UNODC).

Sample preparation and processing

Centrifugation method

This procedure was done according to Dams et al. (2003), whereby 1 mL of urine sample was drawn into an Eppendorf tube, then centrifuged for 5 min at 13000 rpm. After centrifugation, a 0.2 mL volume of the sample was drawn and combined with 0.8 mL mixture of acetonitrile and Mill-Q water (50:50) (mobile phase) to form 1 mL followed by vortex-mixing. A 10 µL volume of the supernatant was drawn and injected into the LC-MS-MS system.

Solid phase extraction (SPE) method

The solid phase extraction (SPE) system arrangement was composed of a tank, rack, test tube holder, lids (covers), needle pump (inlet and outlet), pressure regulator and pressure gauge (setting pressure to allow flow of the sample drop-wise). The cartridge was preconditioned with 1 mL of 100% methanol and 1 mL of water. Then, the sample (2 mL) was loaded into the cartridge and washed with 1 mL water followed by 1 mL of 5% methanol, then dried for approximately 2 minutes. Retained drugs were eluted with 2 mL of 100% methanol and evaporated almost to dryness using nitrogen at about 45 °C. The extraction process was done through a vacuum master. After drying, the extract was reconstituted with 1 mL of the mobile phase into an autosampler vial. The vial was vortexed for 2 min before transferring to the instrument where a volume of 10 µL was injected into the LC-MS-MS.

Gas chromatographic analysis, identification and quantification

Sample analysis was done at the Government Chemist Laboratory Authority (GCLA) Dar es Salaam using LC-MS-MS. The analyses were carried out by Thermo Scientific TM Q- Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific) coupled with an ultra-high performance liquid chromatography (UHPLC) system. A thermo Scientific Hypersil GOLD Aq analytical column with length x ID x particle size of 100 mm x 2.1 mm x 1.9 µm, respectively from Agilent was used to accomplish the chromatographic separation of analytes. Water containing 0.1% formic acid (phase A) and acetonitrile containing 0.1% of formic acid (phase B) were both used as mobile phase components. The gradient flow was 95% A to 95% B in about 15 minutes with a flow rate of 0.3 mL/min. The LC flow rate was set at 0.3 mL/min and the elution gradient was as follows: 0% of phase B was kept as initial mobile phase from 0 to 0.5 min, the percentage of phase B was increased to 50 from 1 to 2 min and 90 from 3.5 to 5.5 min. Finally, the

percentage of phase B decreased again to 0 from 6 to 8 min also establishing a post-time of 10 min to return to the initial conditions. Analytes were detected by mass spectrometry using single reaction monitoring (SRM) in either positive or negative electrospray ionization (ESI) modes by infusing 1 µg/mL of analytes in 0.1% of formic acid in Milli-Q water. The parameters of the electrospray ionization (ESI) were as follows: the nitrogen flow was set to 10 mL/min and it was kept at 300 °C. The nebulizer pressure was 18 psi and the capillary voltage was 45 eV in positive ionization mode. Trace Finder Software was used to process all data analysis. Single Reaction Monitoring (SRM) was used to detect standards by infusing 1 µg/mL of analytes in 0.1% of formic acid in Milli-Q water. The precursor ions corresponding with their $[M + H]^+$ adducts were m/z 304.15 for cocaine; 290.14 for benzoylecgonine; 370.16 for heroin; 328.1 for 6-monoacetylmorphine (6-MAM) and 286.1 for morphine. Product ions, collision energies, fragmentor voltage and retention times are summarized in Table 1.

Table 1: Mass spectrometric parameters for the analytes

Analyte name	Precursor ions (m/z)	Product ions (m/z)		Fragmentor (V)	Collision energy (eV)	RT (min)
		Primary ion (*m/z*) (Q)	Secondary ion (*m/z*) (I)			
Cocaine	304.15	182.1	82.1	125	20; 32	13.26
Benzoylecgonine	290.14	168.1	105.1	125	26; 30	8.98
Heroin	370.16	171.1	105.1	130	20; 30	12.87
6-MAM	328.15	165.1	201	155	40; 30	11.32
Morphine	286.14	152	201.1	150	70; 30	6.29

(Q) = quantification transition, (I) = identification transition and RT = retention time.

Identification of analytes (drugs of abuse ad metabolites) was done using the retention times of the analytes in the chromatograms. This was done by comparing the retention times of the analytes in the samples to those of reference standards run in parallel and at the same conditions with the samples. A specific analyte was identified if it had the same retention time to that of the standard. All analytes were monitored within a ± 0.5 min retention time window. The mass spectra of the analytes in the standards and samples were also compared with the mass spectra in the Trace Finder library for the LC-MS-MS. Quantification was performed using calibration curves. Since the initial volume of the sample was 2 mL and the final volume of the extract was 1 mL, the concentrations of the analytes obtained from the calibration curves were divided by 2.

Analytical quality assurance and control parameters

Method validation parameters were assessed using spiked drug-free urine samples which were obtained from volunteers who were non-drug users as described in other previous studies (Dams et al. 2003, Shin et al. 2014, Jeanville et al. 2000, Lee et al. 2016). The following parameters were evaluated: linearity, recovery, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy. The stock solutions of target analytes were cocaine, benzoylecgonine, heroin, 6-monoacetylmorphine and morphine; they contained 5 mg/mL in methanol and stored at – 20 °C in the dark until use. Working standard solutions were prepared by diluting with methanol.

Calibration curves prepared using standards were used to obtain the linearity range. The calibration graphs were derived by plotting the peak heights of the analytes against the

concentrations of the standard analytes. The data were fitted to a linear regression curve. Calibration was performed by linear regression analysis over a concentration range of 10–30 µg/mL (10, 15, 20, 25 and 30 µg/mL). Solutions of matrix-matched calibrators were prepared by fortifying drug-free urine samples with high and low working solutions (10 and 30 µg/mL). The recovery was determined by measuring the spiked analytes in drug-free urine sample after the extraction procedure as for the sample. The percentage recovery of each drug of abuse analyte was calculated. Procedural blank tests involved checking the reagents and solvents.

Limit of detection (LOD) is defined as the lowest concentration of an analyte in a given sample that can be detected but not necessarily quantified, under the stated conditions. The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of the tests. Common methods used for the determinations of LOD and LOQ include visual definition, and calculation from the signal-to noise ratio which corresponds to 3 and 10 times the noise level, respectively (Kocourek 2012). The LOD was calculated based on the standard deviation of the mean concentration of the analyte detected from the small amount of the standard, and then the standard deviation was multiplied by 3. The LOQ was calculated by multiplying the standard deviation by 3–10. The linearity was checked by preparing series of concentrations of each standard analyte, then plotting curves of concentrations versus signals (peak heights) obtained.

The precision was tested by analyzing drug-free urine samples spiked with low and high concentrations of analytes (Shin et al. 2014). Relative standard deviations (RSD) of replicate measurements were calculated and suitable precision was obtained as the relative standard deviations were < 20%.

Data analysis

Paired t-test was used to test whether there were significant differences in the concentrations of the analytes obtained in the samples prepared using centrifugation and SPE methods.

Results and Discussion

Blanks, recoveries, linearity, precision, detection limits and limits of quantification

Analytes of drugs of abuse were not detected in the matrix and procedural blank samples. The percentage recovery of each analyte in each recovery test was calculated and the results are presented in Table 2. The percentage recoveries for cocaine, heroin, 6-monoacetylmorphine and morphine ranged from 82.8% to 104.2% and 82.2% to 115.3% in samples prepared by centrifugation and SPE, respectively. The mean percentage recoveries for the compounds in samples prepared by centrifugation and SPE varied from 89.7 to 101.8% and 91.7% and 104.1%, respectively. The recovery values were generally comparable, although slightly greater in SPE method than in centrifugation method. The recovery values in both methods were within the acceptable range of 70% to 120% (Kocourek 2012). The results were therefore not corrected to recoveries.

Table 2: Recoveries of drugs of abuse and metabolites in samples

Analyte	Centrifugation (n = 3)		SPE (n = 5)	
	Range (%)	Mean (%)	Range (%)	Mean (%)
Cocaine	82.80–96.60	89.70	95.5–111.2	104.1
Heroin	101.1–101.3	101.2	85.8–106.0	99.20
6-monoacetylmorphine	99.00–104.2	101.8	82.2–104.7	91.70
Morphine	93.40–99.30	96.30	86.6–115.3	97.90

The results of precision and linearity are summarized in Table 3. The precisions (RSD) ranged from 7% to 19% and were suitable. The peak height ratios of the calibration standards were proportional to the analyte concentrations in each analysis to the nominal concentration range of 10 to 30 µg/mL for cocaine, benzoylecgonine, heroin, 6-monoacetylmorphine, and morphine. Linear fits were employed to describe the calibration curves. Correlation coefficients (R^2) ranging from 0.980 to 0.994 for standards were obtained for the relationships between the single reaction monitoring ion abundances of the analytes and the corresponding calibration concentrations. The LODs and LOQs ranged from 0.01 to 0.03 µg/mL and 0.03 to 0.09 µg/mL, respectively (Table 3). The LODs and LOQs determined in this study were comparable to those reported by other studies. For example, Jagerdeo and Abdel-Rehim (2009) reported LODs for ecgonine methyl ester, benzoylecgonine, cocaine, and cocaethylene of 0.0229, 0.0237, 0.004, and 0.0098 µg/mL, respectively. The LOQs for ecgonine methyl ester, benzoylecgonine, cocaine, and cocaethylene in that study were 0.065, 0.075, 0.095, and 0.075 µg/mL, respectively.

Table 3: Linearity, precision, limits of detection and limits of quantification of analytes

Analyte	LOD, µg/mL	LOQ, µg/mL	Linearity/R^2, µg/mL	Precision (RSD), %
Cocaine	0.01	0.03	0.9789	19
Benzoylecgonine	0.01	0.03	0.9825	7
Heroin	0.01	0.03	0.9885	18
6-monoacetylmorphine	0.02	0.06	0.9944	18
Morphine	0.03	0.09	0.9931	13

Levels of heroin and metabolites in urine

Heroin was not detected in any of the urine samples, but its metabolites were detected in some samples. The metabolites of heroin detected in urine samples were 6-monoacetylmorphine (6-MAM) and morphine (Table 4). The presence of these metabolites showed the use of heroin by the people who provided the urine samples. The compound 6-MAM was detected in 13 (43.3%) of the samples prepared by centrifugation method and 9 (30%) of the samples prepared by solid phase extraction method. The concentrations of 6-MAM in the urine samples from the drug abusers at Ubungo darajani ranged from not detected/below detection limit (ND) to 3.37 µg/mL and ND to 6.67 µg/mL for the samples prepared by centrifugation and solid phase extraction methods, respectively (Table 4). Morphine was detected in 12 (40%) and 10 (33.3%) of the samples prepared by centrifugation method and solid phase extraction method, respectively. The concentrations of morphine in the urine samples from the drug abusers at Ubungo darajani ranged from ND to 4.21 µg/mL and ND to 6.66 µg/mL for the samples prepared by centrifugation and solid phase extraction, respectively.

Table 4: Concentrations of heroin and metabolites in urine samples prepared by centrifugation and solid phase extraction (SPE) methods (µg/mL)

Sample codes	Heroin		6-MAM		Morphine	
	Centrifugation	SPE	Centrifugation	SPE	Centrifugation	SPE
WU01	nd	nd	0.74	nd	nd	3.28
WU02	nd	nd	nd	nd	nd	nd
WU03	nd	nd	3.37	nd	nd	nd
WU04	nd	nd	nd	nd	nd	nd
WU05	nd	nd	nd	nd	nd	nd
WU06	nd	nd	0.54	0.29	nd	nd
WU07	nd	nd	nd	nd	nd	nd
WU08	nd	nd	1.53	nd	nd	3.22
WU09	nd	nd	nd	nd	nd	3.22
WU10	nd	nd	2.18	6.67	3.77	nd
WU11	nd	nd	nd	nd	3.25	3.21
WU12	nd	nd	nd	nd	nd	nd
WU13	nd	nd	nd	nd	nd	nd
WU14	nd	nd	nd	nd	nd	nd
WU15	nd	nd	nd	nd	nd	nd
WU16	nd	nd	nd	nd	nd	nd
WU17	nd	nd	1.24	0.71	4.09	4.47
WU18	nd	nd	0.39	0.35	3.43	nd
WU19	nd	nd	0.91	1.05	4.21	6.66
WU20	nd	nd	nd	nd	3.76	4.49
WU21	nd	nd	1.01	0.66	3.58	4.45
WU22	nd	nd	0.54	0.6	3.71	4.39
WU23	nd	nd	nd	nd	3.24	nd
WU24	nd	nd	nd	nd	nd	nd
WU25	nd	nd	0.34	1.02	3.44	4.33
WU26	nd	nd	0.18	nd	3.59	nd
WU27	nd	nd	nd	nd	nd	nd
WU28	nd	nd	nd	nd	nd	nd
WU29	nd	nd	nd	nd	nd	nd
WU30	nd	nd	0.22	0.08	3.56	nd

SPE = solid phase extraction; nd = not detected (below detection limit).

The absence of heroin in all the urine samples revealed that heroin was rapidly metabolized to 6-MAM after administration and then to morphine (Hanisch and Meyer 1993). Additionally, the heroin detection time length shows that it stays in the body for 1 to 2 days after being consumed (Moeller et al. 2008, Gourlay et al. 2010, Marlowe and Meyer 2011). The rapid metabolism can be the reason that caused the heroin not to be detected in the urine samples. The 6-MAM is a product (metabolite) of heroin which makes the confirmatory testing of heroin. Nevertheless, 6-MAM, the metabolite of heroin, has a short half-life of 36 minutes and is only detected in urine samples up to 8 hours after heroin use (Moeller et al. 2008).

Levels of cocaine and metabolite in urine

Cocaine and one of its metabolites (benzoylecgonine) were detected in some of the urine samples collected from the drug abusers at the study site. Table 5 shows that, 2 (6.7%) of the urine samples in centrifugation

method and 2 (6.7%) of the urine samples in the SPE method contained cocaine. The concentrations of cocaine in the urine samples were up to 0.03 and 0.13 µg/mL in the samples prepared by centrifugation and solid phase extraction methods, respectively. Benzoylecgonine, the metabolite of cocaine, was not detected in any of the samples prepared by both methods and analysed by LC-MS-MS.

The observed concentrations of cocaine in the urine samples were very low compared to the concentrations of other analytes. The results obtained in the samples prepared by both methods suggested that the number of people using cocaine were very few among the persons who participated in providing the urine samples for the present study.

Table 5: Concentrations of cocaine and metabolites in urine samples (µg/mL)

Sample codes	n	Cocaine		Benzoylecgonine	
		Centrifugation	SPE	Centrifugation	SPE
WU01	1	0.01	0.05	nd	nd
WU02– WU21	20	nd	nd	nd	nd
WU22	1	0.03	0.13	nd	nd
WU23 –WU30	8	nd	nd	nd	nd

Comparison of the levels of drugs of abuse in all samples and findings of other researchers

Morphine was the leading compound in terms of concentrations with mean values of 1.45 and 1.39 µg/mL in centrifugation and SPE methods, respectively. This was followed by 6-MAM with mean concentrations of 0.44 and 0.38 µg/mL in centrifugation and SPE methods, respectively. The least detected compound was cocaine which had mean concentrations of 0.001 and 0.006 µg/mL, respectively. Heroin and benzoylecgonine were not detected in the samples in both methods. The findings indicated that heroin was the more commonly used drug than cocaine. Statistical analysis using one way ANOVA with Tukey Kramer Multiple Comparisons Test showed that there were significant differences among the concentrations of the analytes detected in the urine samples (centrifugation $F_{(2,\ 87)} = 12.755$, $p < 0.0001$ and SPE $F_{(2,\ 87)} = 7.879$, $p < 0.0007$).

Table 6 summarizes the concentrations of 6-MAM, morphine, cocaine and benzoylecgonine detected in urine samples in this study and as observed by other researchers using similar methods. The concentrations of morphine in the urine samples found in this study were comparable to the concentrations of morphine observed by other researchers such as Cao et al. (2015) in USA and Cao et al. (2019) in China. The concentrations of morphine observed in urine samples by Smith et al. (2014) in USA were higher than the concentrations of morphine observed in urine in this study. The concentrations of 6-MAM detected in the urine samples in this study were higher than the concentrations of 6-MAM in urine samples observed in Norwegian drug drivers by Vindenes *et al*. (2012) and in USA by Cao et al. (2015). Benzoylecgonine (the metabolite of cocaine) was not detected in urine samples in this study, while benzoylecgonine was reported in the studies conducted in Spain, Norway and USA (Fernandez et al. 1996, Vindenes *et al*. 2012, Cao et al. 2015) as shown in Table 6. The concentrations of cocaine detected in this study were lower than the levels reported by Williams et al. (2000) in urine samples from patients in an urban emergency medicine setting. The observation of cocaine in very few urine samples from the drug abusers at the study site implied that it was not commonly used; the results suggest that the majority were using heroin.

Table 6: Concentrations of 6-MAM, morphine, cocaine and benzoylecgonine observed in urine samples in various studies

Analyte	Sample preparation and analysis method	Concentration, µg/mL	Reference
Morphine	Roche Opiates II immunoassay; SPE, GC-MS	<300–7,522,000	Smith et al. (2014)
	Dilute-and-shoot; LC–MS-MS	0.086–54.191	Cao et al. (2015)
	Competitive fluorescence immunoassay; GC-MS/MS	4.392–13.80; 4.060–12.95	Cao et al. (2019)
	Centrifugation	nd–4.21	This study
	SPE; LC-MS-MS	nd–6.66	
6-MAM	Liquid-liquid extraction; LC-MS-MS	0.02	Vindenes *et al*. (2012)
	Dilute-and-shoot; LC–MS-MS	0.304–0.380	Cao et al. (2015)
	Centrifugation	nd–3.37	This study
	SPE; LC-MS-MS	nd–6.67	
Cocaine	Solid-liquid extraction; HPLC	nd–4.14	Fernandez et al. (1996)
	SPE; GC-MS	0.004 to 40.13	Williams et al. (2000)
	Centrifugation	nd–0.03	This study
	SPE; LC-MS-MS	nd–0.13	
Benzoylecgonine	Solid-liquid extraction; HPLC	nd–4.14	Fernandez et al. (1996)
	Liquid-liquid extraction; LC-MS-MS	0.03	Vindenes et al. (2012)
	Dilute-and-shoot; LC–MS-MS	0.626–0.653	Cao et al. (2015)
	Centrifugation	nd	This study
	SPE; LC-MS-MS		

Comparison of centrifugation and solid phase extraction methods

The concentrations of analytes detected in the urine samples prepared by centrifugation and solid phase extraction methods are summarized in Table 7. The concentrations of the drugs of abuse in the urine samples prepared by the centrifugation and SPE methods were also compared in order to check the variations. The paired t-test revealed that there were no significant differences in the concentrations of the analytes detected in the urine samples between the two methods of sample preparations (6-MAM: $t = 0.2912$, $df = 29$, and $p = 0.7730$; morphine: $t = 0.1858$, $df = 29$ and $p = 0.8539$). This indicated similarity in the performance of the two methods (centrifugation and SPE methods) as shown in Figure 1. However, the concentrations and detection frequencies of 6-MAM and morphine in the samples prepared by centrifugation method were generally greater than the concentrations in the samples prepared by the SPE method. This could be due to minor

losses in the SPE preparations, may be because of adsorption effects or challenges in elution of the analytes. The samples prepared by the SPE method were clean, while some of the samples prepared by the centrifugation method were dirty.

Table 7: Summary of levels of drugs of abuse in human urine samples prepared by centrifugation and solid phase extraction (SPE) methods

Analytes	Centrifugation method (n = 30)			SPE method (n = 30)		
	Mean, µg/mL	Range, µg/mL	Detection frequency, %	Mean, µg/mL	Range, µg/mL	Detection frequency, %
6- MAM	0.44	nd–3.37	43.3	0.38	nd–6.67	30.0
Morphine	1.45	nd–4.21	40.0	1.39	nd–6.66	33.3
Cocaine	0.001	nd–0.03	6.7	0.01	nd–0.13	6.7
Heroin	nd	nd	0	nd	nd	0
Benzoylecgonine	nd	nd	0	nd	nd	0

nd = not detected (below detection limit).

The concentrations of cocaine in the samples prepared by the SPE method were slightly greater than the concentrations in the samples prepared by centrifugation method. Heroin and benzoylecgonine (the major metabolite) of cocaine were not detected in the samples prepared by both methods.

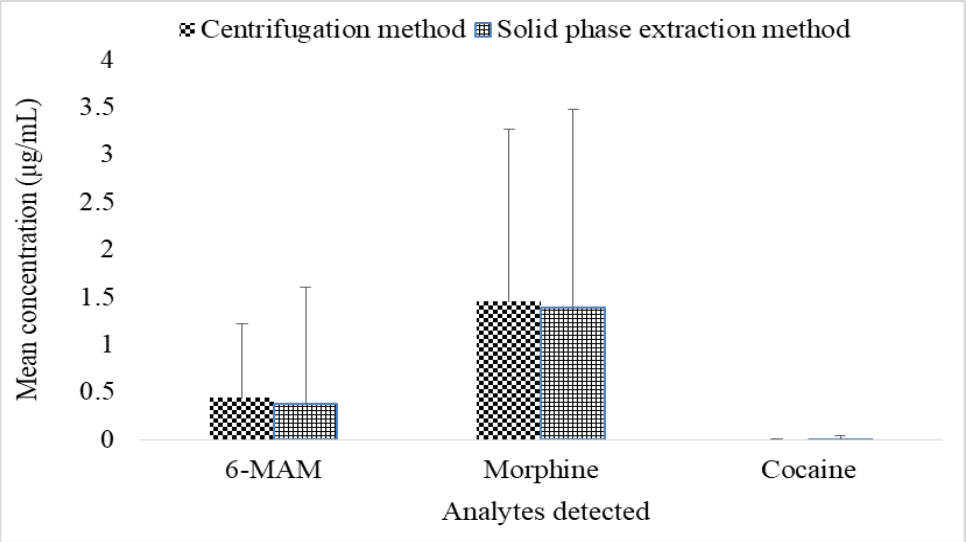

Figure 1: Comparison of concentrations of drugs of abuse in urine samples prepared by centrifugation and solid phase extraction methods. Error bars indicate standard deviations.

Conclusions

The results obtained indicated that there were no significant differences in the concentrations of drugs of abuse between the samples prepared by centrifugation and solid phase methods, although the levels of the drugs in the samples prepared by centrifugation method were slightly greater than those prepared by solid phase extraction method. Therefore, both the centrifugation and SPE preparation methods are suitable, except that the analyses of the samples prepared by centrifugation method could be complicated by the presence of impurities in the samples.

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