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Technical Note

Effects of biological matrices and sample collection conditions on the reliability of methamphetamine analysis

Chayanant Hongfa [1,*] and Rasda Boonprasert [2]

[1] Science Division, Mahidol University International College, Mahidol University, 999 Salaya, Phutthamonthon, Nakhon Pathom, Thailand
[2] Division of Medical Toxicology, Department of Preventive and Social Medicine, Faculty of Medicine at Siriraj Hospital, Mahidol University, Bangkok, Thailand
* Corresponding author, email: chayanant.hon@mahidol.ac.th

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Abstract: Breast milk and urine samples from thirty-nine mother-infant pairs were tested for the methamphetamine-level measurement reliability under four separate conditions: freeze-thaw cycle, short-term storage, long-term storage and on-instrument/extract storage. The four different conditions yielded methamphetamine levels within ±10% variation of that obtained from the freshly prepared samples. For most consistent results, milk samples should be frozen immediately after collection, while urine samples can be kept at room temperature for up to six hours before the longer-term storage. Finally, the use of an autosampler only has minimal effects on the measurement reliability.

Keywords: methamphetamine, breast milk, urine, biological matrices

INTRODUCTION

The methamphetamine epidemic is an especially worrisome trend in South-east Asia [1]. The widespread of abuse is especially severe in Thailand [1-4]. Unfortunately, the epidemic of the addiction has also spread to many breastfeeding mothers. The investigation by Bartu et al. [5] has confirmed the transfer of methamphetamine from the breast milk of drug-addict mothers to their infants.

There have been several investigations on fast and reliable chromatographic methods for determining both the level of methamphetamine in the milk of drug-addict mothers and that which can be passed on to their infants. The detection method usually involves the modification of methamphetamine into a more detectable derivative, followed by such quantification methods as high-performance liquid chromatography, gas chromatography, and gas chromatography - mass spectrometry [6-8]. Many of the newer methods utilising similar techniques, however, can

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quantitatively detect methamphetamine, along with other illicit drugs, without any chemical modifications. These methods typically require the use of either solid-phase extraction or tandem chromatographic techniques [9-17].

Although these previous investigations have addressed the issue of detection and quantification of methamphetamine, the effects of biological matrices and sample collection conditions on the analysis results have not been well established. The data reliability of analytes in biological samples collected in the field is a critical component to any clinical studies. The purpose of this study is to assess the reliability of methamphetamine analysis of biological samples (breast milk and urine) based on the consensus recommendation by the American Association of Pharmaceutical Scientists [18]. The sample collection conditions studied are multiple freeze-thaw cycle, short-term storage, long-term storage and on-instrument/extract storage.

MATERIALS AND METHODS

Chemicals and Biological Samples

Methamphetamine and phentermine (internal standard) were obtained from Sigma-Aldrich (USA). HPLC-grade acetonitrile and methanol were purchased from Merck (Germany) and *n*-hexane was obtained from Tedia (USA). Deionised water was purified by a water purification system from Millipore (USA). Drug-free urine and breast milk samples were obtained from healthy volunteers from the project "A pilot study on the extraction, analysis and excretion of methamphetamine in urine and breast milk of mother and baby exposed to methamphetamine during pregnancy" and stored at -20^{o}C until use. The study was approved by the ethics committee of the Faculty of Medicine, Siriraj Hospital, Mahidol University. Written consents were obtained from all patients. Breast milk and urine samples of their infants were collected every 6-8 hours. The samples were immediately stored after collection at −20^{o}C until analysis. Other common chemicals used were of the highest purity and commercially available.

Preparation of Standard Solutions and Calibration Curve

The stock standard solution of methamphetamine (100,000 ng/mL) and internal standard solution of phentermine (50,000 ng/mL) were prepared separately by dissolving appropriate amounts of each compound in 50% aqueous methanol. All working solutions were prepared using the same solvent to desired concentrations. The calibration curve was constructed by a linear regression model ($y = mx + b$) and weighted by 1/x, where y is the ratio of peak area of methamphetamine to that of internal standard (2000 ng/mL), x is the concentration at different levels, i.e. 100, 250, 500, 1,000, 2,000 and 3,000 ng/mL. All calibration ranges yielded a linear relationship with the coefficient of determination (r^2) and its value exceeded 0.995.

Sample Extraction

An aliquot of 500 uL of urine or breast milk sample containing a known amount of methamphetamine was spiked with 20 uL of internal standard solution. The sample was then made alkaline by 0.1M NaOH (100 uL) and extracted with *n*-hexane (1.2 mL). The organic layer was centrifuged at 3000 rpm (4^{o}C) for 10 min. After centrifugation, it was extracted with 0.05% trifluroacetic acid (500 uL) and the aqueous phase was used for further analysis.

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Chromatography

Chromatographic separations were performed with an Acquity™ ultra-performance liquid chromatograph (UPLC) (Waters, USA) with an Acquity UPLC® photodiode-array detector (Waters, USA), which was set at 215 nm. The separation was carried out on a reverse-phase column (Acquity UPLC™ BEH Shield RP, 100x2.1 mm i.d., 1.7 μm) (Waters, USA) at 40°C, with a flow rate of 0.45 mL/min. The elution gradient of the mobile phase was programmed as follows: 0.05% trifluoroacetic acid: acetonitrile (10:90 v/v) for 2 min., then changing to 12:88 v/v in 2 min., then back to 10:90 v/v in 4 min. The Empower 2 software was used for data management.

Reliability Study

The data reliability of methamphetamine assay in urine and breast milk was determined by the replicated analysis of three sets of samples spiked with three different concentrations of methamphetamine under four different conditions. The first condition was the freeze-thaw cycle, in which the urine and breast milk samples were frozen at −20°C for at least 24 hr and then thawed to room temperature (25°C). The process was repeated three times before analysis. In the second condition, i.e. the short-term storage reliability test, urine and breast milk samples were stored at room temperature for 6 hr before analysis. For the long-term storage reliability test (third condition), urine and breast milk samples were frozen at −20°C for 3 months before analysis. The on-instrument/extract storage reliability test (fourth condition) was done by placing vials of urine and breast milk samples in an auto-sampler at 8°C for 10 hr before analysis.

RESULTS AND DISCUSSION

The chromatographic separation provided a good separation for methamphetamine and its internal standard in both urine and breast milk samples, which was achieved within 3 min. for each run. No interference from endogenous peaks was observed in the selectivity testing in both urine and breast milk samples, as shown in Figures 1a-b. The retention times were approximately 1.9 min. for methamphetamine and 2.3 min for the internal standard, as shown in Figures 1c-d.

Linear calibration curves were obtained using the concentration range of 100–3,000 ng/mL. This gave a linear calibration curve with the coefficient of determination (r^2) of 0.9988, 0.9970 and 0.9994 for urine samples and 0.9992, 0.9981 and 0.9972 for breast milk samples at day 1, day 2 and day 3 for all three concentrations of methamphetamine respectively. The limit of quantification was demonstrated at 100 ng/mL using 500 μL of urine and breast milk samples. The limit of detection was 50 ng/mL for both types of samples.

For urine samples, the absolute recoveries of methamphetamine at 300, 1200 and 2500 ng/mL were 42.4, 54.3 and 51.2 % respectively. The recovery of the internal standard (phentermine) at 2000 ng/mL was 45.4%. For breast milk samples, the absolute recoveries for methamphetamine at 300, 1200 and 2500 ng/mL were 78.7, 89.6 and 89.2 % respectively. The absolute recovery of phentermine at 2000 ng/mL was 74.8%. The extraction method was much more efficient for breast milk samples than urine samples for both methamphetamine and phentermine.

Under the four conditions as stated in the reliability study section, the percentage of variation in each condition was within an acceptable range as illustrated in Table 1. The urine and breast milk samples containing methamphetamine were stable in all tested conditions.

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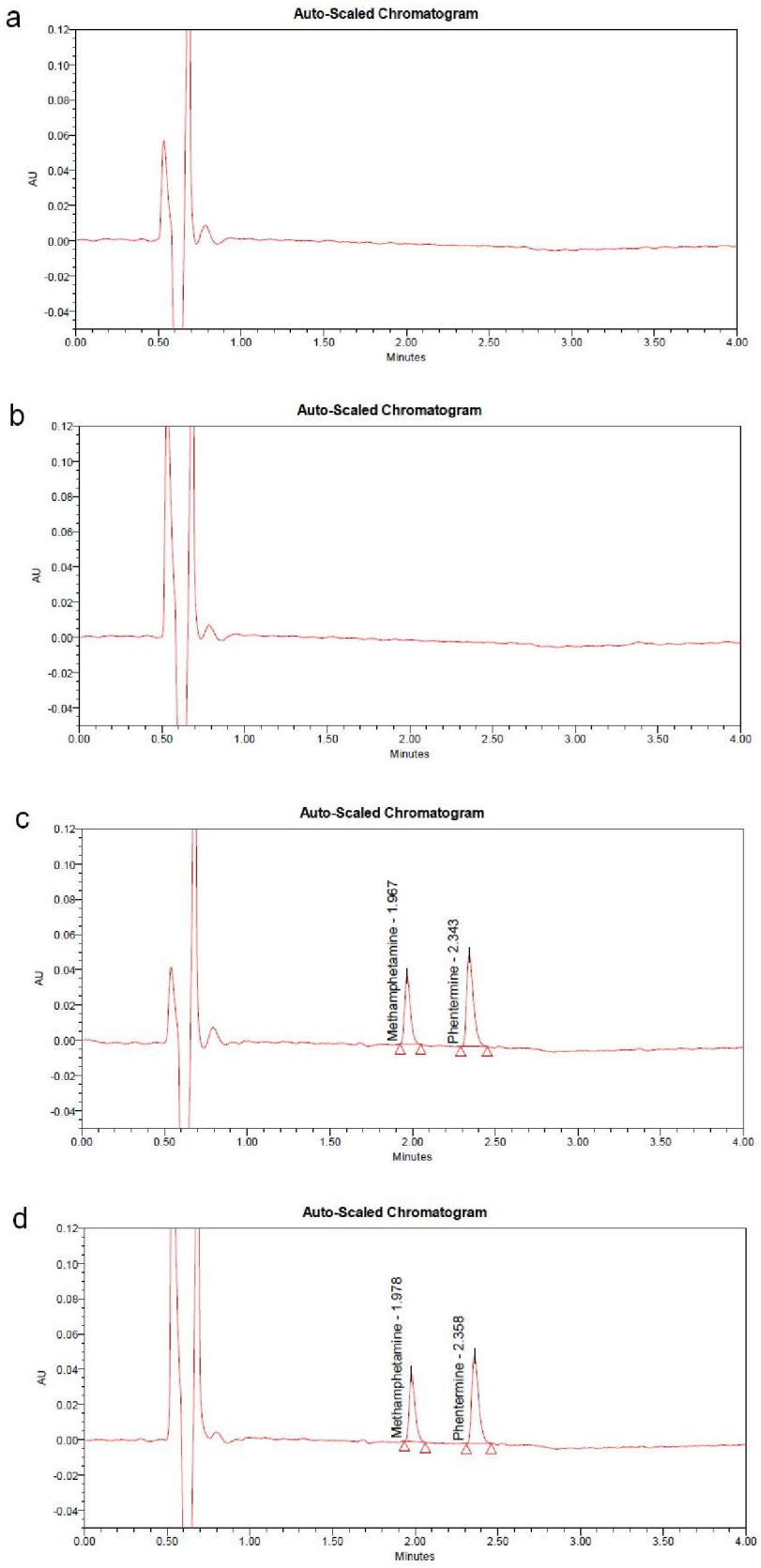

Figure 1. Chromatograms of (a) blank urine sample; (b) blank breast milk sample; (c) urine sample spiked with 1,000ng/mL of methamphetamine and 2,000 ng/mL of phentermine (internal standard); (d) breast milk sample spiked with 1,000ng/mL of methamphetamine and 2,000 ng/mL of phentermine

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Table 1. Variation of methamphetamine level in spiked urine and breast milk samples under four different conditions

Concentration (ng/mL) (n=3)	Variation (%)	
	Urine	Breast milk
Freeze-thaw for 3 cycles		
300	6.2	-0.5
1200	-6.5	0.6
2500	-3.5	2.1
Short-term storage for 6 hr at room temperature		
300	-3.2	1.6
1200	-2.8	1.0
2500	-3.7	5.5
Long-term storage for 3 months		
300	8.8	-4.0
1200	0.5	0.7
2500	0.6	4.4
On-instrument/extract storage for 10 hr in auto-sampler (8 ℃)		
300	-1.4	0.6
1200	-4.3	-0.8
2500	-3.2	-4.9

Methamphetamine-addict mothers are often socially deprived and financially unstable, and as high as 55% of them continue using the drug during their pregnancy and nursing period [19]. As a result, breast milk and urine collection from these mothers and their infants typically takes place in situations that may require these samples to be kept in less-than-ideal storage conditions for many hours. Our present study involves an evaluation of the measurement reliability of methamphetamine in both breast milk and urine samples in these less-than-ideal conditions based on the guideline from the American Association of Pharmaceutical Scientists [18]. In order to minimise the fluctuation of the analytes, our data suggest that the best way to handle breast milk samples is to freeze them immediately, according to Table 1. If they are kept at room temperature, the variation of methamphetamine level can be as high as 5.5%. In contrast, for urine samples, the low variation of 3.7% suggests that it is better to keep them at room temperature if the samples cannot be refrigerated within six hours after collection time. If they are frozen and thawed out during the transportation process, the analysis fluctuation can be as high as -3.5 to 6.2 %. From Table 1, the long-term storage seems to affect both biological samples to the same extent, but it is still within an acceptable range, viz. ±10% of the freshly prepared samples. As for the on-instrument/extract storage, the deviations are less than 5% of the freshly prepared samples in both cases. The use of an autosampler for a large number of samples is therefore acceptable, with minimal effects on the accuracy of the analytes.

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CONCLUSIONS

This study proposes a general guideline that may assist in the improvement of the procedure for collection of urine and breast milk samples. The findings of this investigation have demonstrated the effects of biological matrices and some field and lab conditions on the reliability of methamphetamine analysis results. A new bioanalytical method for methamphetamine determination has also been developed and validated.

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