High-Throughput SPE/GC-MS Method for Quantification of Methamphetamine and Amphetamine In Urine Samples of Drug Users

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Introduction

Methamphetamine is the most common drug of abuse in Thailand. Bureau of Drug and Narcotic, Department of Medical Sciences, as the national drug testing laboratory, has analyzed more than hundred thousand urine specimens collected from drug users each year. It is necessary to develop a high-throughput method capable of simultaneously determining methamphetamine and its meta-bolite, amphetamine, in urine specimens.

Method

A simple and rapid GC-MS method was developed using automated solid-phase extraction for sample clean up. Phentermine was used as internal standard. Derivatization was performed with pentafluo-ropropionic acid at 65ºC for 25 minutes. Chromatography was conducted on a fused silica capillary column and analytes were deter-mined in selected-ion-monitoring (SIM) mode. Mass spectra of penta-fluoropropionyl derivatives showed peak at m/z 190, 118 and 91 for amphetamine, at m/z 204, 160 and 118 for methamphetamine and at m/z 204, 132 and 91 for phentermine. The method was fully validated according to the current recom-mendations of the USFDA bioanaly-tical method validation guidance.

Table 1 Precision, accuracy and recovery data

Analyte	Concentration (ng/mL)	Intraday %CV	Intraday %RD	Inter-day %CV	Inter-day %RD	%Recovery
Methamphetamine	100	0.87	8.53	12.98	3.78	-
	250	3.92	4.04	9.12	3.37	91.80
	2,500	1.54	2.39	5.68	-0.56	76.89
	4,000	6.30	8.54	3.08	0.13	90.86
Amphetamine	100	4.15	16.76	3.84	17.23	-
	250	10.17	-0.54	9.52	3.52	99.46
	2,500	8.42	5.98	10.04	-0.34	75.58
	4,000	6.14	8.33	5.23	9.08	75.40

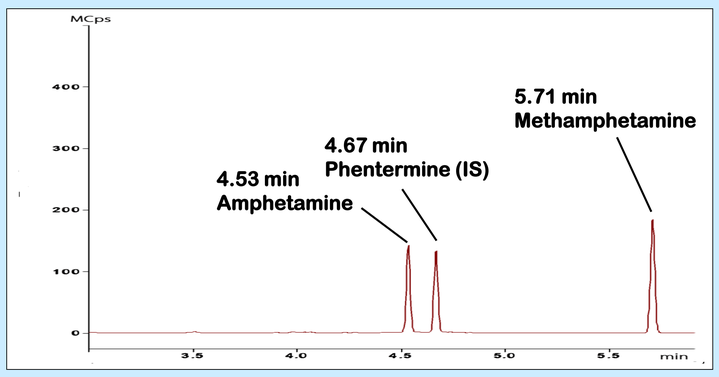

Fig. 1 Chromatogram of a spiked standard mixture containing methamphetamine (2,500 ng/mL), amphetamine (2,500 ng/mL), and phentermine (1,000 ng/mL)

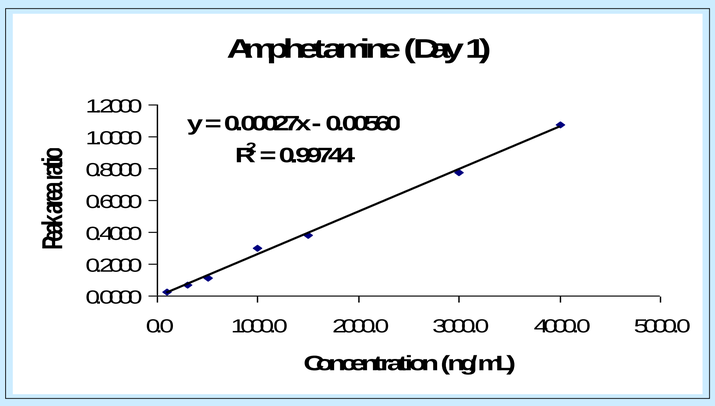

Fig. 2 Calibration curve of amphetamine spiked standard in range of 100 -4,000 ng/mL

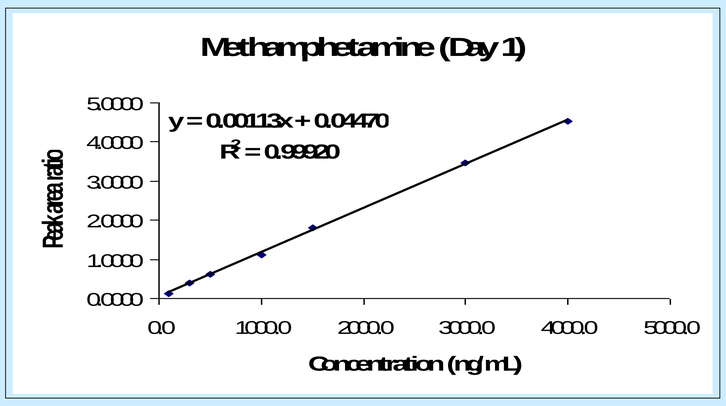

Fig. 3 Calibration curve of methamphetamine spiked standard in range of 100 -4,000 ng/mL

Results

There were no interfering peaks from endogenous components in blank urine chromatograms. Calibration curves were linear cover 100-4,000 ng/mL with correlation coefficients greater than 0.992 (Fig.2-3). Recovery was 75.4-99.5% for amphetamine and 76.9-91.8% for methamphetamine (Table 1). Accuracy and precision were performed at 4 different concen-trations cover the calibration range. Accuracy, expressed as relative deviation (%RD) was less than 16.8% for intra-day and less than 17.2% for inter-day (Table 1). Precision, expressed as coefficient of variation (%CV) was less than 10.2% for intra-day and less than 13.0% for inter-day (Table 1). Stability of amphetamine and methamphetamine in various storage conditions was also determined.

Conclusion

The method was proved to be high-throughput and successfully used in our laboratory to quantify amphetamine and methamphetamine in urine samples of drug users.

Acknowledgment

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