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Determination of Pentazocine in Urine by Gas Chromatography-Mass Spectrometry

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ABSTRACT

A sensitive and specific gas chromatography-mass spectrometry (GC-MS) method using selected ion monitoring (SIM) has been developed for quantification of pentazocine in urine. This method uses solid-solid extraction (SPE) and *N*, *O*-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane derivatization, followed by GC-MS analysis using dextromethorphan as the internal standard. The method was linear between 125 and 1500 ng/mL (r^2 = 0.997). The limit of quantification and limit of detection were 125 and 62.5 ng/mL, respectively. The intraday accuracy and precision were -6.3~4.7% and 3.6~11.2%, respectively. The interday accuracy and precision were -7.5~7.0% and 6.2~10.0%, respectively. These data showed that the GC-MS method was suitable for the determination of pentazocine in urine. This method was further applied to an interlaboratory study. The collaborative study was given by the Association of Official Analytical Chemists International (AOAC International). Nine drug abuse urine testing laboratories in Taiwan participated to analyze 3 samples in duplicate. The repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R) values were 3.0~5.2% and 6.8~10.3%, respectively. The method showed acceptable reproducibility, as evidenced by HORRAT values of 1.1 to 1.5. The method recommended for use by the drug abuse urine testing laboratories in Taiwan.

Key words: pentazocine, GC-MS, urine, collaborative study

INTRODUCTION

Pentazocine, classified in Schedule 2 under the Controlled Drugs Act in Taiwan, is a widely abused narcotic analgesic drug that frequently combines with morphine or tripelenamine to produce herion-like effects. Pentazocine metabolizes and becomes inactive conjugate forms mainly in liver. The conjugate forms of pentazocine can be easily hydrolysed enzymetically by using β-glucuronidase. The daily dosage of pentazocine abused case could go from 300 to 2000 mg by intravenous injection, and thus detection of the unchanged forms should be adequate to determine its presence within the body[1-3]. The analysis of pentazocine in urine has been accomplished with thin-layer[4], gas chromatography[5], gas chromatography-mass spectrometry (GC-MS)[1,6], and gas chromatography/surface ionization organic mass spectrometry (GC-SIOMS)[1]. Currently, protocols for forensic urine drug testing generally involve immunoassay screening or thin-layer chromatography (i.e., the commercially marketed "Toxi-Lab" system) followed by a confirmatory GC-MS of the positive samples.

Reid & Gerbeck[6] presented a GC-MS method to detect pentazocine in urine. However, this method lacks an internal standard and uses a toxic reagent, chloroform, as the extraction solvent. The GC-MS apparatus for this method was a Finnigan 9610/4000 (Finnigan Corp., Sunnyvale, CA 94068). Seno *et al.*[1] presented a GC-SIOMS and a GC-MS method to determinate pentazocine. In this method, sample was carried out by a Sep-Pac C18 cartridge and then performed on a GC-17A gas chromatography (Shimadzu, Kyoto, Japan). For the equipment reason, none of the current methods[1,6] is suitable for the drug abuse urine testing laboratories in Taiwan. A sensitive and specific GC-MS method with selected ion monitoring (SIM) data analysis is required to establish a reference among all testing laboratories in Taiwan.

The purposes of this paper are to establish a GC-MS method and evaluate a collaborative study to validate the determination method of pentazocine in urine.

MATERIALS AND METHODS

I. *Reagents*

Pentazocine-HCl (1 mg/mL in methanol, Lot No. P-039) was purchased from Cerilliant (USA). Dextro-

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methorphan, the internal standard, was purchased from U.S.P.C. (Rockville, MD, USA). N, O-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA-TMCS) was purchased from Sigma. Methanol, H_3PO_4, K_2HPO_4, KH_2PO_4, NaOH, CH_2Cl_2, and 2-propanol were purchased from Merck (Darmstadt, Germany). Ammonia hydroxide was purchased from J.T. Baker.

The acetate solution (100 mM, pH = 4.5) was prepared by adding 2.93 g of $CH_3COONa•3H_2O$ and 1.62 mL of glacial acetic acid to double deionized water to make 1 L. K_2HPO_4 (1.70 g) and KH_2PO_4 (12.14 g) were dissolved in double deionized water to make 1 L of phosphate buffer solution (100 mM, pH = 6.0). Sodium hydroxide (10 N) was used to adjust the pH value to 6.0 ± 0.1. The elution solvent was prepared daily by mixing with 20 mL of 2-propanol and 2 mL of NH_4OH (14.8 M), then adding CH_2Cl_2 to 100 mL.

Individual stock solutions containing 10 μg/mL of pentazocine-HCl and 20 μg/mL of dextromethorphan in double deionized water were prepared. Working solution of 2 μg/mL of pentazocine-HCl was subsequently prepared.

Blank urine specimen was collected from a non-drug user and no drug was detected by GC-MS. The blank human urine was spiked with pentazocine-HCl at the concentrations of 125, 250, 500, 1000, and 1500 ng/mL for quantitative comparisons. Another quality-control samples (250, 500 and 750 ng/mL) were prepared in the same way for precision evaluation. All pentazocine solutions were kept in the dark at 4 °C until analysis.

II. *Instrumentation*

GC-MS analysis was performed with an Agilent 6890 gas chromatography equipped with a J&W DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperature was set at 250 °C and 280 °C, respectively. The column temperatures was initially held at 150 °C for 2 min, increased to 280 °C at a rate of 20 °C/min, and held at 280 °C for 2 min.

Agilent 5973N mass selective detector in selected ion monitoring (SIM) mode coupled to GC was used for quantitative analysis. The electron impact of 70 eV was used for the ionization of the compounds. The following ions were monitored at dwell 25 ms: pentazocine: m/z 289, 274, 244 and dextromethorphan: m/z 271, 214 (quantification ions are underlined).

III. *Extraction*

Solid-phase extraction was carried out. Fifty microliter of dextromethorphan solution (20 μg/mL) and 1 mL of phosphate buffer solution (100 mM) were added to 1 mL of the urine samples, calibrators and controls. Ammonium hydroxide solution (14.8 M) or phosphoric acid (3 M) was used to adjust the pH value to 6.0 ± 0.5. After activating a Clean-Screen CSDAU203 (United Chemical Technologies, Bristol, UK) cartridge with 2 mL of methanol, 2 mL of double deionized water, and 1 mL of phosphate buffer at a flow rate of 30 mL/min, the sample solution was loaded on the cartridge at a flow rate below 2 mL/min. The solution was washed subsequently again with 2 mL of double deionized water, 2 mL of acetate buffer solution, and 2 mL of methanol at a flow rate 18 mL/min. Finally, 3 mL of elution solvent at a flow rate of 1-2 mL/min passed through the cartridge and the eluate was collected in a vial. The eluate was evaporated to dryness under a gentle stream of nitrogen at room temperature.

IV. *Derivatization*

Fifty microliter of BSTFA-TMCS was added to the dried extract as the derivatization reagent, and allowed to derivatize for 30 min at 80 °C. The resultant derivatives were transferred to an autosampler vial and 2 μL was injected into the GC-MS.

V. *Method Validation*

Pentazocine was identified by comparing the retention time and relative abundance of three ions monitored with standards analyzed at the same time. Quantification of pentazocine was determined using the internal standard method. A five-point standard curve was prepared by linear least-square regression analysis of the ratio of the peak area of pentazocine to the peak area of the internal standard, dextromethorphan.

To determine the analytical recoveries, one set of drug-free urine was spiked with known amounts of pentazocine-HCl (250, 500, and 750 ng/mL) without the internal standard. Another set of standards containing the same corresponding amount of pentazocine-HCl in methanol was also prepared. After solid-phase extraction, elutes in both sets were evaporated to dryness at room temperature under a stream of nitrogen. Fifty microliter of the internal standard (20 μg/mL) was added to each extract. The analytes and the internal standard in the residue were then derivatized and analyzed by GC-MS. The average recovery of pentazocine was determined by comparing the ratio of the peak area of pentazocine to the peak area of the internal standard from extracted urine specimens spiked with pentazocine and extracted methanol solution.

The limits of quantification (LOQ) for pentazocine in urine was established as the lowest concentration that the method can detect with a consistent response to the actual solution concentration[7]. The signal-to-noise ratio for limits of detection (LOD) was greater than 3 for the specified quantification ion, when present with the required confirming ions. Accuracy and precision of this analytical method were evaluated by preparing samples with known concentrations of each analyte. Intraday and interday precision were assessed and expressed in terms of RSD (relative standard deviation), where as accuracy was expressed in terms of DFA (difference from the actual value)[8]. The within- and between-day precision

of the GC-MS analytical method was studied with five replicates within one day and once daily for five days.

VI. *Application of Analytical Method to Collaborative Study*

Nine drug-abuse urine testing laboratories in Taiwan participated in the collaborative study. All of the testing laboratories passed their own quality control and were accredited by the National Bureau of Controlled Drugs, Department of Health, Excutive Yuan, Taiwan. Each collaborator received a reference standard of pentazocine, an internal standard of dextromethorphan, and three duplicate samples (300, 500, 1250 ng/mL) of pentazocine-HCl spiked urine. The collaborators also received a set of instructions regarding the amount of sample to take for analysis, a copy of the method and a report form for recording results. They were also asked to describe specific operational parameters of the instrument system used and to submit their report forms along with their chromatograms. Each laboratory was asked to use routine GC-MS equipment. In order to obtain a wide diversity of systems, analysts were encouraged to use their own columns.

The statistical terms used are those given by the Association of Official Analytical Chemists International (AOAC International)[9], including (a) repeatability (intra-laboratory) standard deviation (S_r), (b) repeatability relative standard deviation (RSD_r), (c) repeatability value (r, 2.8 × S_r), (d) reproducibility (inter-laboratory) standard deviation (S_R), (e) reproducibility relative standard deviation (RSD_R), (f) reproducibility value (R, 2.8 × S_R), and (g) HORRAT values. The acceptability of reproducibility of the method was assessed on the basis of HORRAT values. Moreover, the Cochran and Grubbs tests were used for outliers.

Cochran test is used to remove the extreme individual values from a set of laboratory values (1-tail test at a probability value of 2.5%). Similarly, Grubbs tests are used to eliminate extreme averages using single value test (2-tail; P = 2.5%) and pair value test (2 values at the highest end, 2 values at the lowest end, and 2 values, one at each end, at an overall P = 2.5%). HORRAT value is the ratio of observed RSD_R to predicted RSD_R (predicted $RSD_R = 2C^{-0.1505}$, C is the estimated mean concentration). HORRAT value between 0.5 and 1.5 indicates good performance of the method. The limits for the performance acceptability are 0.5 to 2[9].

RESULTS AND DISCUSSION

This paper reported a validated GC-MS method for the determination of pentazocine in urine. This method was further applied to a collaborative study in Taiwan.

I. *Mass Spectra Profile and Reliability of the Method*

Dextromethorphan was used as an internal standard due to lack of deuterated pentazocine compound in the commercial market. Figure 1 shows the full-scan mass

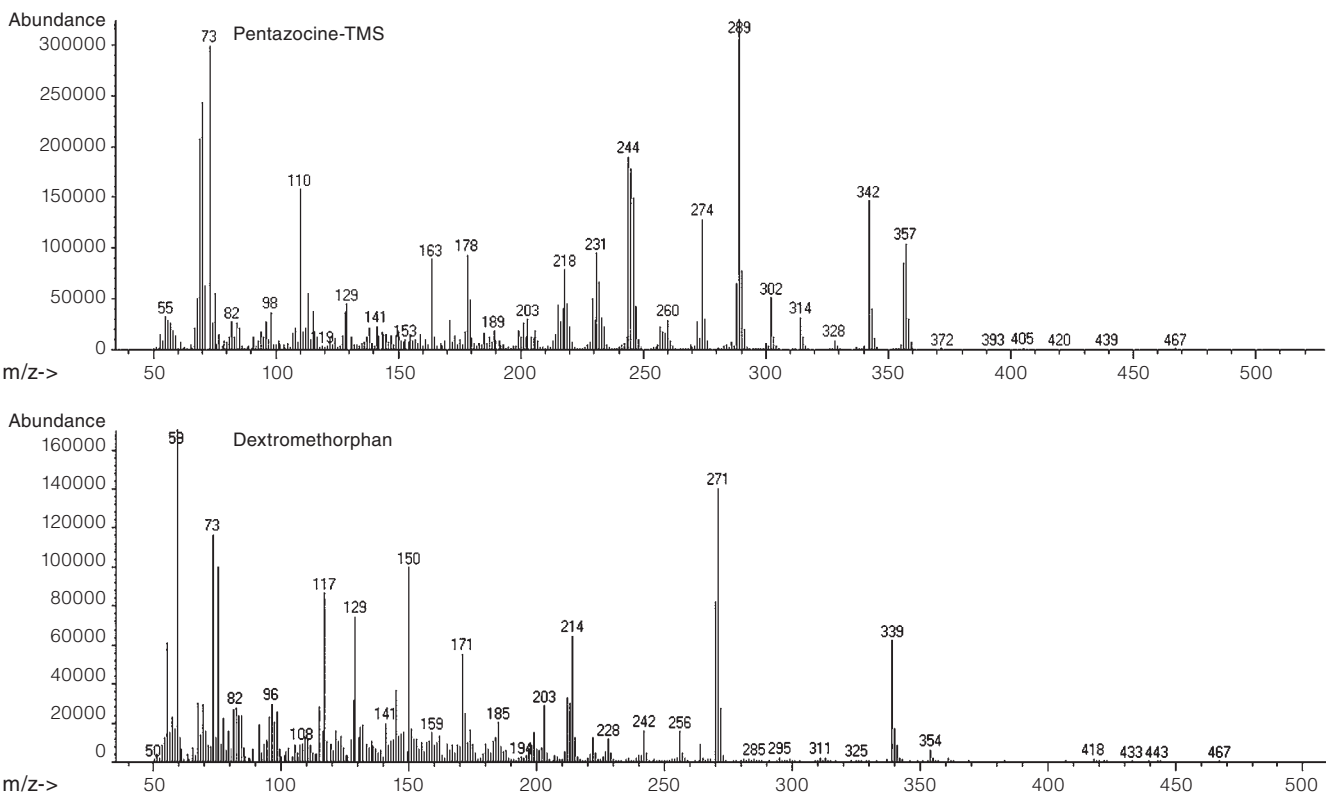

Figure 1. Mass spectra of pentazocine-TMS and dextromethorphan.

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spectra of pentazocine-TMS and dextromethorphan. A typical chromatogram GC-MS profile of derivative urine extract is shown in Figure 2. The retention times of pentazocine and the dextromethorphan were 7.8 and 7.2 min, respectively. Standard curve for pentazocine was linear across the range of urine assayed (125~1500 ng/mL). The correlation coefficient of the standard curve was 0.997. Limit of quantification and detection was 125 ng/mL and 62.5 ng/mL, respectively. The average extraction recoveries (n = 3) were greater than 72.4% (Table 1).

Table 1 also shows the accuracy and precision of the analytical method for the quantification of pentazocine.

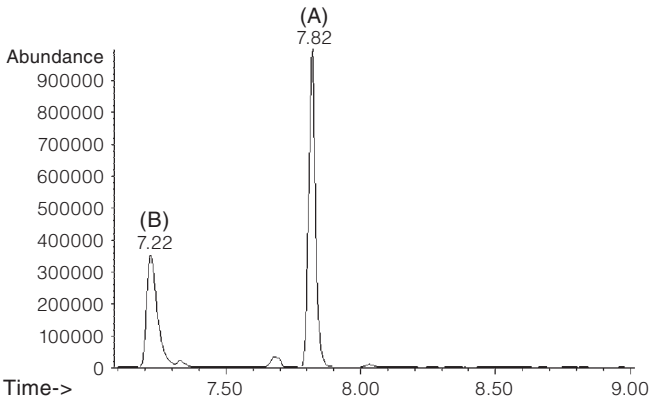

Figure 2. GC-MS (selected ion monitoring) profile of a derivatized urine extract containing (A) 500 ng/mL of pentazocine-HCl and (B) 1000 ng/mL of dextromethorphan.

The intraday variability was determined by analyzing 5 replicate controls prepared in blank urine spiked at 250, 500, and 750 ng/mL on a single day. The intraday accuracy and precision was -6.3~4.7% and 3.6~11.2%, respectively. The interday variability was determined in 5 different days using the same concentrations. The interday accuracy and precision was -7.5~7.0% and 6.2~10.0%, respectively. These results demonstrated that this method was suitable for quantification of pentazocine in urine with satisfactory accuracy and precision.

II. *Collaborative Study*

Table 2 shows the diversity of instrument systems used by the collaborators. Adoption of suitability tests can obviate many problems arising from deficient analytical instrument systems because they demonstrate whether a particular system yields satisfactory performance. All collaborators were able to meet the system suitability requirements of the method. The results were reported by the collaborators varied from one to two months.

No outlier was observed when the Cochran and Grubbs tests were applied for each sample. The analysis of variance by the AOAC International method is shown in Table 3. The RSDr values were 3.0~5.2% and the RSD_R values were 6.8~10.3%. HORRAT values ranged from 1.1 to 1.5. These data further indicated that the proposed GC-MS method for the determination of pentazocine in urine showed good reproducibility between laboratories. Most collaborators commented favorably on the method.

Table 1. Precision, accuracy and recovery of pentazocine in urine

Spiked conc. (ng/mL)	Intraday[a]			Interday[a]			Recovery[b] (%)
	Mean ± S.D.	RSD (%)	DFA (%)	Mean ± S.D.	RSD (%)	DFA (%)	
250	253.6 ± 28.3	11.2	1.4	267.6 ± 16.6	6.2	7.0	82.1 ± 13.1
500	468.4 ± 25.8	5.5	-6.3	462.6 ± 33.3	7.2	-7.5	94.6 ± 17.6
750	785.4 ± 28.0	3.6	4.7	741.4 ± 73.9	10.0	-1.2	72.4 ± 2.9

[a]n = 5, [b]n = 3.

Table 2. Analytical condition of the GC-MS system used by the collaborators

Lab.	Instrument	Column			
		Model	Length (m)	Diameter (mm)	Film (μm)
1	Agilent 6890/5973N	J&W DB-5	30	0.25	0.25
2	Agilent 6890N/5973N	HP-5MS	15	0.25	0.25
3	Agilent 6890/5973	HP-5MS	30	0.25	0.25
4	Agilent 6890/5973	HP-5MS	12	0.20	0.33
5	Agilent 6890/5973	Quadrex UAC-1	15	0.25	0.50
6	Agilent 6890N/5973N	J&W DB-5	15	0.25	0.25
7	Finnigan GC/Polaris Q	CP-Sil 8CB-MS	30	0.25	0.25
8	Finnigan GC 8000 top/Voyager	Rtx-5MS	15	0.25	0.25
9	Agilent 6890N/5973N	HP-1	15	0.25	0.25

Table 3. Collaborative study results for determination of pentazocine in urine by GC-MS

Added (ng/mL)	Found (ng/mL)	No. of labs	S_r	r	RSD_r (%)	S_R	R	RSD_R (%)	HORRAT
300	288.3	9	11.6	32.4	4.0	29.7	83.2	10.3	1.5
500	517.0	9	15.3	42.9	3.0	35.2	98.6	6.8	1.1
1250	1171.5	9	61.3	171.7	5.2	81.6	228.6	7.0	1.3

S_r: repeatability standard deviation; r: repeatability value; RSD_r: repeatability relative standard deviation; S_R: reproducibility standard deviation; R: reproducibility value; RSD_R: reproducibility relative standard deviation.

CONCLUSIONS

The analytical method described for the determination of pentazocine was sensitive, reproducible and accurate. This method was successful applied to a collaborative study and showed good reproducibility between laboratories. The method is recommended for drug abuse urine testing laboratories in Taiwan.

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