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Collaborative Study on the Determination of Phencyclidine in Urine by Gas Chromatography-Mass Spectrometry

KUEI HUI CHAN[1], MEI-CHICH HSU[2*], WEI-LAN CHU[3], WEN-ING TSAY[4] AND CHIAREIY LIU[4]

[1.] *Graduate Institute of Coaching Science, National Taiwan Sport University, Taoyuan, Taiwan (R.O.C.)*
[2.] *Graduate Institute of Sports Science, National Taiwan Sport University, Taoyuan, Taiwan (R.O.C.)*
[3.] *Division of Clinical Toxicology, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan (R.O.C.)*
[4.] *National Bureau of Controlled Drugs, Department of Health, Executive Yuan, Taipei, Taiwan (R.O.C.)*

ABSTRACT

A gas chromatography-mass spectrometry method for the analysis of phencyclidine (PCP) in urine was subjected to an inter-laboratory study. The collaborative study followed the guidelines provided by the Association of Official Analytical Chemists International. Ten laboratories participated, analyzing 3 samples of PCP-spiked urine as blind duplicate. The repeatability relative standard deviation (RSD_r) and the reproducibility relative standard deviation (RSD_R) were between 2.1%-3.6% and 4.2%-7.3%, respectively. HORRAT values for the reproducibility showed 0.4-0.7, indicating acceptable precision between laboratories. The method was thus proposed to be used by the drug-abuse urine testing laboratories in Taiwan.

Key words: phencyclidine, GC-MS, urine, inter-laboratory study

INTRODUCTION

Phencyclidine (PCP) is a synthetic drug which possesses of anesthetic properties and reportedly used as a treatment of psychiatric patients in England in the early 1960's[1]. Because of its high psychological dependence, low to moderate physical dependence, and hallucinogenic effect, PCP was placed in Schedule II under the Controlled Substances Act by the Drug Enforcement Administration (DEA) in United States and also in Taiwan. Despite retaining its popularity in the United States, PCP is apparently not a common drug of abuse in other countries[1]. Two cases of PCP abuse were observed from emergency visiting in the Veterans General hospital, Taipei, Taiwan in 2007. PCP is not considered as a routine screening drug in most of the drug-abuse urine testing laboratories in Taiwan, therefore, the popularity of abuse is not determined. A method with good reproducibility for determination the PCP in urine for drug-abuse urine testing laboratories of Taiwan seems to be necessary.

The analysis of PCP in urine has been accomplished with gas chromatography[2,3], gas liquid chromatogra-phy[4], enzyme-linked immunosorbent assay (ELISA)[5], gas chromatography-mass spectrometry (GC-MS)[6,7], immunoassays and gas chromatography-mass spectro-metry[8] and gas chromatography/surface ionization organic mass spectrom-etry (GC-SIOMS)[9]. Currently, using GC-MS to confirm the screening of positive urine sample is becoming inevitable[10-13]. None of the present methods[6-9] is suitable for most of the drug abuse urine testing laboratories in Taiwan concerning the analytical equipment system. A sensitive and specific GC-MS method with selected ion monitoring (SIM) data analysis is required for establishing the reference among the testing laboratories in Taiwan. This paper reports the result of a collaborative study designed to validate a GC-MS method for the determination of the PCP in urine. The analytical protocol was introduced by the United Chemical Technologies (Bristol, PA, U.S.A.), with minor modification for this study.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

PCP (1 mg/mL in methanol) and PCP-d_5 (100 μg/mL in methanol) were purchased from Cerilliant (Austin, TX, USA). Methanol, ethyl acetate, 2-propanol (IPA), glacial acetic acid, H_3PO_4, NaOH, CH_2Cl_2, K_2HPO_4, and KH_2PO_4 were purchased from Merck (Darmstadt, Germany). Sodium acetate trihydrate and NH_4OH (14.8 M) were purchased variously from Riedel-deHaen (Seelze, Germany) and J. T. Baker (New Jersey, USA).

The acetate solution (100 mM, pH = 4.5) was pre-

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* Author for correspondence. Tel: +866-3-3283201 ext. 2421;
　Fax: +866-3-3280592; E-mail: meichich1224@mail2000.com.tw

pared by adding 2.93 g of sodium acetate trihydrate and 1.62 mL of glacial acetic acid in double deionized water to reach a final volume of 1 L. Phosphate buffer solution (100 mM, pH = 6.0) was prepared by adding 1.70 g of K_2HPO_4 and 12.14 g of KH_2PO_4 in double deionized water to reach a final volume of 1 L and adjusting the pH value to 6.0 ± 0.1 by NaOH (10 N). Phosphoric acid solution (3 M) was prepared by adding 10.2 mL of H_3PO_4 (14.7 M) in double deionized water to reach a final volume of 50 mL.

Individual stock solutions containing 4 μg/mL of PCP and 1 μg/mL of PCP-d_5 in double deionized water were prepared. Working solution of 400 ng/mL of PCP was subsequently prepared. A blank urine specimen was collected from a non-drug user and none of the drug was detected by GC-MS. The blank human urine was spiked with PCP at the concentrations of 3.125, 6.25, 12.5, 25, 100, and 200 ng/mL for quantitative comparisons. Other quality-control samples (12.5, 25, and 50 ng/mL) were prepared in the same way for precision and recovery evaluation. All of the PCP solutions were kept in the dark at 4°C until analysis.

Ⅱ. *Extraction*

The analytical protocol was performed on a GC-MS method with solid-phase extraction as described by United Chemical Technologies (Bristol, PA, U.S.A.)[14] with slight modification. The extraction columns were Clean Screen® CSDAU203 containing 200 mg of sorbent in a 3 mL column (United Chemical Technologies, Bristol, PA, U.S.A).

Fifty microliters of PCP-d_5 (1 μg/mL) and 1 mL of phosphate buffer solution were added to 1 mL of the urine samples, calibrators or controls. Phosphoric acid solution or NH_4OH was used to adjust the pH to 6.0 ± 0.5. After activating the column with 2 mL of methanol, 2 mL of double deionized water and 1 mL of phosphate buffer solution at a flow rate of 30 mL/min, the sample solution was loaded over the cartridge at a flow rate of 1-2 mL/min. The cartridge was washed subsequently with 2 mL of double deionized water, 2 mL of acetate solution and 2 mL of methanol at a flow rate of 18 mL/min. The cartridge was then dried under a stream of nitrogen gas for 2 min. Finally, 3 mL of eluent (CH_2Cl_2/IPA/NH_4OH, 78/20/2, v/v/v) at a flow rate of 1-2 mL/min passed through and the eluent was collected in a vial. The extract was evaporated to dryness under a gentle stream of nitrogen gas at room temperature and reconstituted in 50 μL of ethyl acetate. One microliter aliquot of the solution was injected into the GC-MS analysis in SIM mode.

Ⅲ. *GC-MS Procedures*

An Agilent 6890 GC/5973N mass selective detector system was used to acquire the full-scan and selected ion monitoring (SIM) mass spectrometric data. A Chrompack DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used under the following conditions: split-less injection; helium flow rate, 0.8-1.2 mL/min; injection port, 200°C; interface, 280°C; column oven programming: starting at 70°C and holding for 1 min, increasing to 180°C at 20°C/min, then increasing to 230°C at 35°C/min, holding for 3 min. Ions selected for PCP were *m/z* 200, 91, 242; the corresponding ions for PCP-d_5 were *m/z* 205, 96, 246 (quantification ions are underlined).

The total analysis time was 11 min per sample with a solvent delay of 6.5 min. The transfer line temperature and MS source temperature were 280°C and 150°C, respectively. The nominal electron energy was set at 70 eV. Full-scan mass spectra of derivatized analytes and internal standard were collected in the range of *m/z* 45-550 at a scan rate of 2.94 scan/s.

Ⅳ. *Collaborative Study*

The procedures for the preparation of solutions and analyze were written as a form of standard operation procedure (SOP). Ten 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, Executive Yuan, Taiwan. Each collaborator received a reference standard of PCP, an internal standard of PCP-d_5, and test samples (15, 25, and 75 ng/mL in duplicates) of PCP-spiked urine. Concentration of the test samples was unknown to the collaborators. The collaborators also received a set of instructions regarding the SOP and a report form for recording results. They were asked to follow the SOP to analyze the samples, to describe specific operational parameters of the instrument system used, and to submit the report forms along with the chromatograms. Each laboratory was encouraged to use one's routine analytical system (e.g. instrument, injector, and column) and to make individual judgment in adjusting the operating conditions.

V. *Statistical Analysis*[15]

The statistical terms used are those given by the Association of Official Analytical Chemists (AOAC international), including (a) repeatability (intra-labor-atory) standard deviation (S_r), (b) repeatability relative standard deviation (RSD_r), (c) reproducibility (inter-laboratory) standard deviation (S_R), (d) reproducibility relative standard deviation (RSD_R), and (e) 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.

The Cochran test is used to remove the extreme individual values from a set of laboratory values. Grubbs test is used to remove the laboratories with extreme average. The maximum outlier rate is 2/9 and a study should maintain valid data from a minimum of 8 laboratories. HORRAT value is the ratio of observed RSD_R to predicted RSD_R ($PRSD_R = 2C^{-0.1505}$, C is the mean concentration found).

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Table 1. GC-MS systems used by the collaborators

| Lab. | Instrument | Column | | | |
		model	length (m)	diameter (mm)	film (μm)
1	Agilent 6890/5973N	Agilent, J&W DB-5	30	0.25	0.25
2	Agilent 6890N/5973N	Agilent, HP-5MS	15	0.25	0.25
3	Agilent 6890/5973	Agilent, HP-5MS	30	0.25	0.25
4	Agilent 6890/5973	Supeleo, Equity-5	12	0.20	0.33
5	Agilent 6890/5973	Quadrex, UAC-1	15	0.25	0.50
6	Agilent 6890N/5973N	Agilent, J&W DB-5	15	0.25	0.25
7	Finnigan GC/Polaris Q	Chrompack, CP-Sil 8CB-MS	30	0.25	0.25
8	Finnigan GC 8000 top/Voyager	Restek, Rtx-5MS	15	0.25	0.25
9	Agilent 6890N/5973N	Agilent, HP-1	15	0.25	0.25
10	Agilent 6890/5973	Agilent, J&W DB-5 MS	29	0.25	0.25

HORRAT value between 0.5 and 1.5 may be taken to indicate that the performance value for the method corresponds to good performance. Consistent deviations from the ratio on the low side (values < 0.5) may indicate unreported averaging or excellent training and experience[16].

RESULTS AND DISCUSSION

I. *Reliability of the Method*

The retention time of PCP and the PCP-d_5 were 9.89 and 9.87 min, respectively. Standard curve for PCP was linear over the range of urine assayed (3.125-200 ng/mL). The correlation coefficient of the standard curve was 1.000. Limit of detection and limit of quantification were 3.125 ng/mL and 6.25 ng/mL, respectively. The recovery obtained at three triplicate concentration levels was 86.6%.

The intraday and interday variability were determined by analyzing 5 replicate controls prepared in blank urine spiked at 12.5, 25, and 50 ng/mL on a single day and once daily for five days for each concentration. The precision was assessed as both intraday and interday and expressed in terms of RSD (relative standard deviation). The accuracy was expressed in terms of DFA (difference from the actual value). The intraday precision obtained for PCP was 2.9%, 2.2%, and 3.2%, intraday accuracy was 3.9%, 2.7%, and 10.5%; interday precision was 2.2%, 4.7%, and 6.0%, and the interday accuracy was 0.9%, 0.8%, and 6.9%. These results demonstrated that this method was suitable for the quantification of PCP in urine with satisfactory accuracy and precision.

II. *Analytical Apparatus of the Collaborators*

Table 1 shows the diversity of instrument systems used

Table 2. Laboratory analysis results for the determination of phencyclidine in urine by GC-MS

| | phencyclidine (ng/mL) | | | | | |
	Sample 1		Sample 2		Sample 3	
1	16.21	16.73	25.60	26.35	74.23	74.65
2	15.54	15.87	26.18	26.93	77.21	77.05
3	16.71	17.15	25.39[a]	32.48[a]	70.69	78.67
4	15.40	16.08	26.24	26.69	74.79	80.61
5	14.67[a]	8.33[a]	25.28	25.33	70.76	74.02
6	16.28	17.77	26.57	26.99	75.27	69.75
7	15.29	14.79	24.99	24.99	68.99	71.21
8	17.85	18.04	27.98	29.24	82.37	81.57
9	15.60	16.23	26.57	26.30	79.17	80.93
10	14.15	14.24	26.25	27.84	79.23	81.28

[a] Cochran outlier. Six samples (3 pairs of blind duplicates) were sent to each laboratory.

by the collaborators. Other analytical conditions of the GC-MS system (eg. injection mode, column pressure, gas flow rate, ionization mode, interface temperature, and electron energy etc.) were similar among the collaborators. All of the collaborators were able to meet the system suitability requirements of the method. The results were reported by the collaborators varied from one to three weeks.

III. *Outlier Treatment of the Collaborative Study*

Table 2 illustrates the results of a collaborative study. The Cochran and Grubbs tests for outliers were conducted

Table 3. Inter-laboratory study results for the determination of phencyclidine in urine by GC-MS

Sample No.	Added (ng/mL)	Found (ng/mL)	No. of labs[a]	S_r	S_R	RSD_r, %	RSD_R, %	HORRAT
1	15	16.11 ± 1.13	9 (1)	0.47	1.17	2.9	7.3	0.7
2	25	26.46 ± 1.05	9 (1)	0.56	1.12	2.1	4.2	0.4
3	75	76.12 ± 3.92	10	2.75	4.38	3.6	5.8	0.7

[a] Each value is the number of laboratories retained after elimination of outlier; each value in parenthesis is the number of laboratories removed as outliers. S_r: repeatability standard deviation. RSD_r: repeatability relative standard deviation. S_R: reproducibility standard deviation. RSD_R: reproducibility relative standard deviation.

on the data of 10 laboratories for the 3 duplicate samples. One Cochran outlier was identified for sample 1 and sample 2. None Grubbs outlier was found. The outliers were below 2/9 for all the laboratories and within acceptable limits of the protocol. Although there are two laboratories shown deviant results among the others in low concentration and middle concentration of blind samples, no questions or any further improvement concerning the analytical method has been requested from collaborators.

Ⅳ. *Repeatability and Inter-laboratories Reproducibility*

Table 3 presents S_r, S_R, RSD_r, RSD_R, and HORRAT, which were calculated based on the result of the collaborative study, excluding the statistical outliers in accordance with the precision criteria. The RSD_r values (2.1%-3.6%) were less than the RSD_R values (4.2%-7.3%). HORRAT was 0.4-0.7, less than 2, indicating an acceptable precision of method and good performance.

CONCLUSIONS

The collaborative study of the GC-MS method for the determination of PCP in urine has demonstrated good inter-laboratory reproducibility. The method was proposed to be used by the drug-abuse urine testing laboratories in Taiwan.

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REFERENCES

1. Mozayani, A. 2002. Phencyclidine-Effects on human performance and behavior. Forensic Sci. Rev. 15: 62-72.
2. Ishii, A., Seno, H., Kumazawa, T., Nishikawa, M., Watanabe, K. and Suzuki, O. 1996. Simple and sensitive detection of phencyclidine in body fluids by gas chromatography with surface ionization detection. Int. J. Legal Med. 108: 244-247.
3. Casari, C. and Andrews, A. R. 2001. Application of solvent microextraction to the analysis of amphetamines and phencyclidine in urine. Forensic Sci. Int. 120: 165-171.
4. Clark, C. C. 1979. Gas-liquid chromatographic quantitation of phencyclidine HCl in powders: collaborative study. J. Assoc. Off. Anal. Chem. 62: 560-563.
5. Kerrigan, S. and Phillips Jr., W. H. 2001. Comparison of ELISAs for opiates, methamphetamine, cocaine metabolite, benzodiazepines, phencyclidine, and cannabinoids in whole blood and urine. Clin. Chem. 47: 540-547.
6. Vorce, S. P., Sklerov, J. H. and Kalasinsky, K. S. 2000. Assessment of the ion-trap mass spectrometer for routine qualitative and quantitative analysis of drugs of abuse extracted from urine. J. Anal. Toxicol. 24: 595-601.
7. Elsohly, M. A., Little, T. L., Mitchell, J. M., Paul, B. D., Mell, L. D. and Irving, J. 1988. GC/MS analysis of phencyclidine acid metabolite in human urine. J. Anal. Toxicol. 12: 180-182.
8. Tsai, S. C., ElSohly, M. A., Dubrovsky, T., Twarowska, B., Towt, J. and Salamone, S. J. 1998. Determination of five abused drugs in nitrite-adulterated urine by immunoassays and gas chromatography-mass spectrometry. J. Anal. Toxicol. 22: 474-480.
9. Ishii, A., Seno, H., Watanabe-Suzuki, K., Kumazawa, T., Matsushima, H., Suzuki, O. and Katsumata, Y. 2000. Ultrasensitive determination of phencyclidine in body fluids by surface ionization organic mass spectrometry. Anal. Chem. 72: 404-405.
10. Cone, E., Vaupel, D. and Buchwald, W. 1980. Phencyclidine detection and measurement of toxic precursors and analogs in illicit samples. J. Anal. Toxicol. 4: 119-123.

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11. Gibb, R. P., Cockerham, H., Goldfogel, G. A., Lawson, G. M. and Raisys, V. A. 1993. Substance abuse testing of urine by GC/MS in scanning mode evaluate by proficiency studies, TLC/GC, and EMIT. J. Forensic Sci. 38: 124-133.

12. Goldberger, B. A. and Cone, E. J. 1994. Confirmatory tests for drugs in workplace by gas chromatography-mass spectrometry. J. Chromatogr. A 674: 73-86.

13. Mauurer, H. H. 1992. Systematic toxicological analysis of drugs and their metabolites by gas chromatography-mass spectrometry. J. Chromatogr. 580: 3-41.

14. Phencyclidine for GC or GC/MS confirmations using: 200 mg Clean Screen® extraction column, Procedure code: PCU200DAU050191, United Chemical Technologies, Inc.

15. AOAC International. 2002. Guidelines for collaborative study procedures to validate characteristics of a method of analysis. http://www.aoac.org/ vmeth/Manual_Part_6.pdf.

16. Horwitz, W. and Albert, R. 2006. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J. AOAC Int. 89: 1095-1099.