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Comparative study of postmortem blood, urine and vitreous humor methamphetamine

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Abstract

Methamphetamine (MA) is a highly addictive and toxic central nervous system stimulant. Urine samples are used as evidences of MA abuse under the Narcotics Laws, whereas blood samples are specimens for investigation of MA poisoning. However, both specimens are occasionally not available or contaminated. Vitreous humor is a specimen which is less contaminated and easy to work with analytically. The aim of this study is to examine the relationships between MA concentrations in blood, urine and vitreous humor. Those three specimens were collected from 40 deceased and their MA concentrations were quantitatively analyzed by headspace-solid phase microextraction/gas chromatography-mass spectrometry technique. The results showed that the relationships between MA concentrations in urine vs blood, urine vs vitreous humor, and vitreous humor vs blood were linearly correlated with a correlation coefficient (r) of 0.89, 0.99, and 0.88, respectively. Linear regression equations of those relationships between urine vs blood, urine vs vitreous humor, and vitreous humor vs blood were y = 0.001x + 8.08, y = 0.056x - 262.86, and y = 0.027x + 16.20, respectively. This study suggests vitreous humor as an alternative specimen for MA investigation.

Key Words: Methamphetamine, Blood, Urine, Vitreous humor

Introduction

Methamphetamine (MA) is an amphetamine-type central nervous system stimulant. Even though MA is the active compound of medication prescribed for narcolepsy, attention deficit hyperactivity disorder and appetite suppression, it is commonly used as illicit drug. In the United States, MA is classified as a Schedule II controlled substance under the Controlled Substances Act of 1970 [1].

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Its widespread and addictive uses are currently a worldwide issue needed to be urgently solved. Drug testing in biological samples is used as a deterrent to illicit drug uses as well as information for forensic or clinical purposes. Most drug testing activities are based on urine even though alternative specimens are gaining in popularity. The cutoff concentrations of MA in urines mandated by the Department of Health and Human Services, Substance Abuse and Mental Health Services Administration (SAMSHA) are 1000 ng/ml of amphetamines using immunoassay preliminary screening and 500 ng/ml of amphetamine or MA using gas chromatography-mass spectrometry (GC-MS) confirmatory assay [2].

While MA concentrations in urine samples are used as an evidence of MA abuser under the law, its concentrations in urines are not related to its effects. MA concentrations in

blood samples represent MA physiological effects. Thus, blood samples are also collected for forensic investigation so as to address whether drugs or illicit substances are suspected to be a cause of death. MA use or toxicity is implicated as either a direct/an antecedent cause of death or only a significant contributing factor. Therapeutic, toxic and fatal levels of MA in blood are reported to be used as a guideline for interpretation of MA effects in human [3, 4]. Our previous preliminary study on the relationship between urine and blood MA concentrations demonstrated a closely linear relationship between these 2 parameters [5]. Even though drug concentrations in urine cannot be extrapolated to blood or vice versa, this relationship is advantageous for approximate estimation of MA concentration in urine from MA concentration in blood sample while urine sample is not available or vice versa.

Vitreous humor is one of the specimens used in forensic toxicology. Its value for postmortem analysis has been reported for many compounds such as alcohol [6, 7]; opiates: morphine, heroin, methadone [8-12]; cocaine and its metabolites [6, 8, 13-16]; cannabinoids [17]; as well as amphetamines or hallucinogenic amines [18-24]. Amphetamine-type stimulants that had been determined in vitreous humor in previous studies were mostly 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy), 3,4-methylenedioxyamphetamine (MDA; love pill) using GC-MS [18], high performance liquid chromatography (HPLC) [19, 21-23] and liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) [20]. Regarding MA, there is a study investigating MA in blood and vitreous humor of 18 deceased using GC-MS following liquid-liquid extraction. They found that the ratio of MA concentrations in vitreous humor to peripheral blood was shown by the mean ± S.D. of 1.63 ± 0.75 [24].

Vitreous humor possesses a number of advantages for analysis of chemicals/drugs for forensic purpose. It is easily collected even though autopsy is not completely performed. Vitreous humor is clear and contains 99 % of water. Besides collagen, hyaluronic acid and other non-collagenous proteins, vitreous humor comprises several substances comparable to the serum such as sodium ion, chloride ion, calcium ion, glucose, urea and creatinine, etc. This specimen is easy to be used in the analytical procedure without complicated sample preparation. The advantageous property of vitreous humor over blood, urine and other tissue specimens is its anatomically isolated location resulting in more protection from putrefaction, charring and trauma [25]. These advantages of vitreous humor as well as the previous report of distribution of MA into vitreous humor [24], indicate that vitreous humor may be a useful alternative specimen, in the situation that blood, urine or other specimens are not satisfactory or not available. Thus, this study aimed to investigate the relationship between MA concentrations in vitreous humor and blood or urine samples of MA abusers and the correlation equations were constructed for the prediction of MA concentrations in blood or urine from vitreous humor. The findings of this study may contribute beneficial information of alternative specimens for postmortem MA investigation in the cases that either urine or blood samples is not suitable or not available.

Materials and Methods

Chemicals and Reagents. Methamphetamine hydrochloride was from Lipomed (U.S.A.). Diphenhydramine hydrochloride was purchased from Sigma Chemical Ltd. (U.S.A.). All other chemicals (methanol, sodium chloride, and sodium hydroxide) were analytical grade (Merck, Darmstadt, Germany).

Blood, urine and vitreous humor samples. Blood, urine and vitreous humor samples were collected from corpses whose bodies were sent to be performed autopsies at the Institute of Forensic Medicine, Police General Hospital, Royal Thai Police Headquarter. Firstly, all urine samples in the bladders were collected and tested with Orange Test Methamphetamine Strip® (True line Med. Co. Ltd., Switzerland) to exclude the deceased whose urine samples were negative. The deceased whose urine samples were positive, their blood and vitreous humor samples were further collected. Blood samples were collected from basilar artery whereas vitreous humor was collected from the vitreous chamber of both eyes. All samples were stored at 4 ºC until analysis.

Sample preparations. Blood samples: Preparation of blood samples was modified from the method of Namera et al [26]. Briefly, 4 ml of blood sample was transferred to an extraction tube and then 2 ml of 5 M sodium hydroxide was added. The mixture was vortex-mixed before centrifugation at 14,000 rpm for 20 min. Two milliliter of the supernatant was transferred to a 20 ml SPME glass vial and 200 µl of diphenhydramine in methanol (20 µg/ml) as well as 0.5 g of sodium chloride were added. The glass vial was capped with aluminum cap before analysis.

Urine and vitreous humor samples: Preparation of urine and vitreous humor samples was modified from the method of Myung et al [27]. Briefly, 1 ml of urine or vitreous humor sample was transferred to a 20 ml SPME glass vial. Then, 200 µl of diphenhydramine in 1 M sodium hydroxide (1 µg/ml) was added. Then, 0.3 g of sodium chloride was added into the glass vial which was capped with aluminum cap before analysis.

Instrumentation. Headspace-solid phase microextraction/gas chromatrography-mass spectrometry (HS-SPME/GC-MS) consisted of GC/MS (QP-2010 plus Shimadzu, Kyoto, Japan) equipped with an AOC-5000 Auto injector. Samples were separated on a crossband 100 % dimethyl polysiloxane 0.25 mm i.d. x 0.5 µm thickness x 30 m length Rtx-1MS column (Restex, U.S.A.). SPME assembly consisted of a

replaceable extraction fiber coated with 100 µm polydimethylsiloxane fused silica/SS 57341-U (Supelco, Bellefonte, U.S.A.). Initial oven temperature of 100 ºC was held for 5 min, then increased at the rate of 15 ºC/min to 150 ºC for 1 min, and finally increased to 250 ºC at 15 ºC/min for 3 min. The injection port and interface temperature were set at 240 ºC and 220 ºC, respectively. The split injection mode and helium carrier gas was used. MS detection was operated in selective ion monitoring mode and characteristic ion for MA quantification was m/z = 58.

Calibration samples. Calibration curves were prepared using MA standard solutions prepared in the corresponding blank matrix. MA standard solutions in blank urine or vitreous humor (250, 500, 1000, 1250, 2000, 2500 and 4000 ng/ml) as well as MA standard solutions in blank blood (100, 200, 400, 800 and 1000 ng/ml) were prepared by performing dilution from the stock MA standard solution of 5000 ng/ml. MA concentrations in all MA standard solutions were analyzed using the procedure and detected by HS-SPME/GC-MS as mentioned above. Each concentration of MA standard solutions was analyzed in triplicate.

Method validation. Linearity: Separated calibration curves were prepared in blank blood, urine and vitreous humor in the same manner as preparing the MA calibration curves mentioned above. Each sample was analyzed for MA concentrations using HS-SPME/GC-MS for 5 times. Relationship between actual and measured MA concentrations was assessed by Pearson's correlation and simple linear regression. Linearity should be achieved with coefficient of determination (R^2) of 0.99 [28-30].

Limits of detection and quantification: Limit of detection (LOD) is the lowest concentration of a compound that produces a response three times the background noise. Limit of quantification (LOQ) is the lowest concentration of a compound in a matrix that can be determined with accep precision and accuracy. Signal-to-noise ratio of ten is generally used for estimating LOQ [28, 30].

Precision: Precision of the assays was evaluated both within- and between-day by assessing from the percentage of coefficient of variation (% CV). To evaluate within-day precision, urine/vitreous humor samples containing 1000, 2000, and 4000 ng/ml of MA in blank urine/vitreous humor samples were analyzed according to the sample preparations mentioned above followed by HS-SPME/GC-MS technique.

Table 1 Accuracy, within- and between- day precision of the method using for determination of MA concentrations in urine, blood, and vitreous humor samples

Specimens	MA Concentrations (ng/ml)	Accuracy (% Recovery)[a]	Precision (% CV)	
			Within-day[b]	Between-day[c]
Blood	200	90.04 ± 1.98	2.19	2.30
	400	90.68 ± 1.95	2.15	-
	800	91.13 ± 1.43	1.57	-
Urine	1000	99.63 ± 2.57	2.58	2.98
	2000	98.68 ± 2.72	2.75	-
	4000	98.56 ± 1.89	1.91	-
Vitreous humor	1000	100.39 ± 8.00	7.96	6.25
	2000	98.86 ± 3.26	3.29	-
	4000	98.43 ± 3.64	3.70	-

[a] The data shown were mean ± S.D. of n = 5.
[b] The data shown were calculated from mean and S.D. of n = 5 within one day.
[c] The data shown were calculated from mean and S.D. of n = 4 (4 days). The experiments were performed in triplicate in each day.

Each concentration was performed 5 times. Blank blood samples containing 200, 400, 800 ng/ml of MA were also performed in the same manner 5 times for each concentration within 24 hours. Between-day precision was evaluated by analyzing blank urine/vitreous humor samples containing 1000 ng/ml of MA with 3 replicate analyses as well as blank blood sample containing 400 ng/ml of MA with 3 replicate analyses. The experiments were performed for 4 consecutive days. The % CV should not exceed 15 % [29].

Accuracy: To evaluate accuracy, urine/vitreous humor samples containing 1000, 2000, and 4000 ng/ml of MA in blank urine/vitreous humor samples were analyzed according to the sample preparations mentioned above followed by HS-SPME/GC-MS technique. Each concentration was performed 5 times. Blank blood samples containing 200, 400, 800 ng/ml of MA were also performed in the same manner 5 times for each concentration. Accuracy of the assay was evaluated by the percentage of recovery. The mean value of % recovery should be within 15 % [29].

Statistical analyses. Results were expressed as mean ± standard error of the mean (S.E.) or standard deviation (S.D.). Relationship of MA concentrations between the specimens was tested by Pearson's correlation and simple linear regression using SPSS for Windows, version 16.0. A *p*-value of less than 0.05 was considered statistically significant.

Results

Method validation. The standard curves plotting from peak area ratio of MA to internal standard (diphenhydramine) and MA concentrations in blank blood, urine and vitreous humor samples were constructed with the R^2 of 0.9993, 0.9994 and 0.9998 respectively. These standard curves were used for determinations of MA concentrations in the corresponding specimens of the subjects as well as in the method validation process.

The method validation of this study was reported by linearity, LOD, LOQ, precision, and accuracy. Linearity was shown by the closely linear relationship between measured MA concentrations and actual MA concentrations in blood samples (R^2 = 0.9998, p = 0.000), urine samples (R^2 = 0.9996, p = 0.000) and vitreous humor samples (R^2 = 0.9998, p = 0.000). LOD of the method was shown to be 2.5, 25 and 25 ng/ml for blood, urine and vitreous humor samples, respectively. LOQ of the method for determining of MA in all three matrixes was shown to be 100 ng/ml. Accuracy as well as within-day and between-day precision of the method for determination of MA concentrations in blood, urine and vitreous humor samples were shown in Table 1. It was shown that % CV of both within- and between-day precision of all concentrations of MA in all matrixes were not exceeded 15 %. Likewise, the mean values of % recovery were all within 15 %. Representative chromatogram of the MA standard in blank blood, urine and vitreous humor were shown in Figure 1.

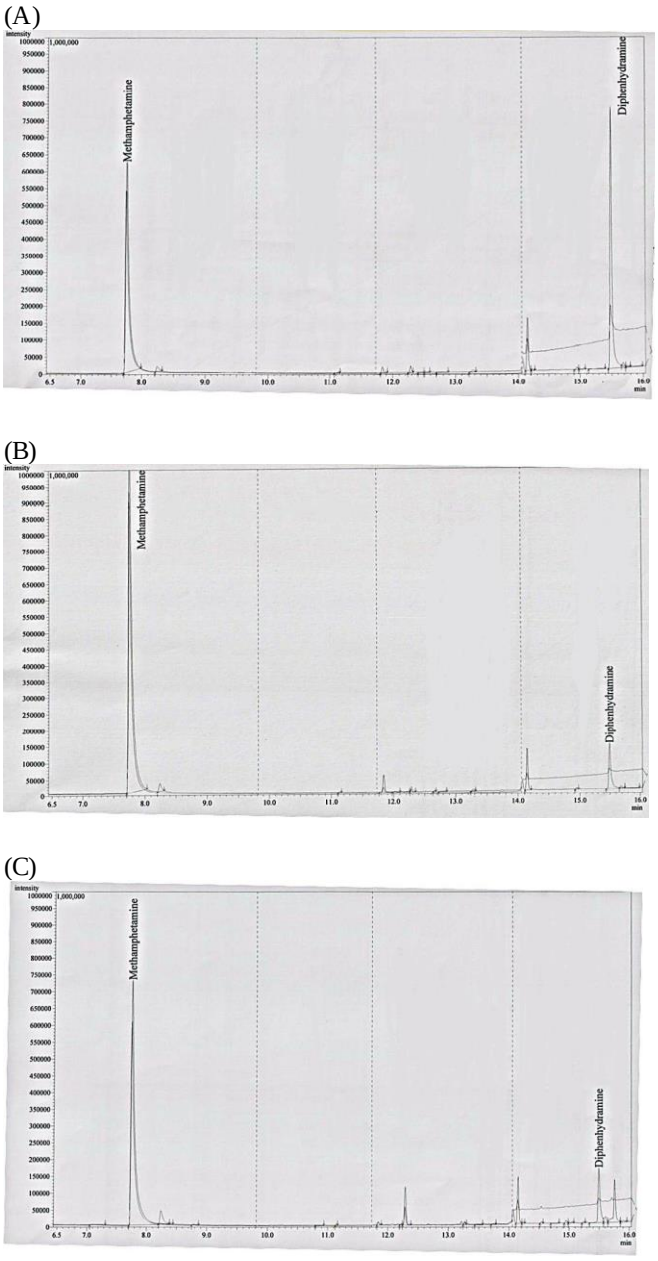

Figure 1 Representative chromatogram of the MA standard in blank blood (A), urine (B), and vitreous humor (C)

MA concentrations in blood, urine and vitreous humor samples of the deceased. Forty male and female deceased were recruited into the study. Most of them were male (n = 38, 95 %) whereas the remaining (n = 2, 5 %) were female.

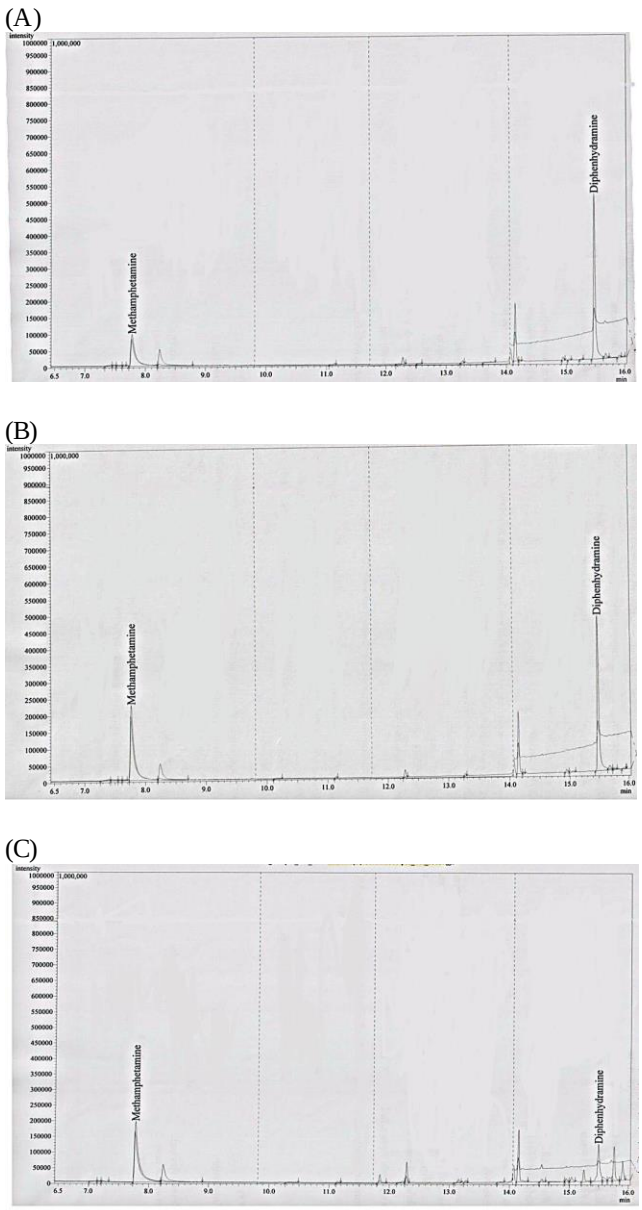

Figure 2 Representative chromatogram of MA in the blood (A), urine (B), and vitreous humor (C) samples of the deceased

Mean ± S.E. of their ages was 30.84 ± 1.54 years (range of 16 - 60 years). Majority of causes of death was unknown (35 %).

Urine MA concentrations of all deceased were higher than 1000 ng/ml. MA concentrations detected in urine samples were far higher than those in blood and vitreous humor. Also, MA concentrations detected in vitreous humor were higher than those in blood samples. Mean ± S.E. of MA concentrations in urine, blood and vitreous humor samples were 19914.22 ± 4627.70 ng/ml (range = 1132.02 - 144715.99 ng/ml), 44.70 ± 9.31 ng /ml (range = 2.52 - 316.42 ng/ml), and 1068.76 ± 306.32 ng/ml (range = 42.62 - 7691.95 ng/ml), respectively. Mean ratios (S.E.) of MA concentrations from blood/urine, vitreous humor/urine and blood/vitreous humor were 0.0025 (0.0002), 0.0336 (0.0022) and 0.0855 (0.0008), respectively. Blood and vitreous humor samples of 7 deceased were MA negative while their urine samples contained MA with the concentrations in the range of approximately 1000 - 3000 ng/ml. Representative chromatogram of MA in blood, urine and vitreous humor samples of the deceased were shown in Figure 2.

Relationships between MA concentrations in blood, urine and vitreous humor samples. To determine the relationships between MA concentrations in these three biological samples, the data of 33 deceased (82.5 %) from the total of 40 deceased were used because MA concentrations of 7 cases (17.5 %) could not be detected in blood and vitreous humor samples. The results showed that MA concentrations in blood, urine and vitreous humor samples were linearly correlated with a correlation coefficient (r) of 0.89 (urine *vs* blood, p-value < 0.05), 0.99 (urine *vs* vitreous humor, p-value < 0.05) and 0.88 (vitreous humor *vs* blood, p-value < 0.05). The corresponding linear regression equations were $y = 0.001x + 8.08$, $y = 0.056x - 262.86$, and $y = 0.027x + 16.20$, respectively (Figure 3-5, respectively).

Discussion and Conclusion

Regarding method validation, the results showed that linearity of the procedure (as shown by the correlation coefficient of more than 0.99 for all specimens) is acceptable according to the criteria suggested by the guidelines [28-30]. LODs of the procedure for determination of MA in urine and vitreous humor were 25 ng/ml while that for blood sample was 2.5 ng/ml. This could be explained by the higher volume of blood sample used in the method. Four milliliters of blood sample was added with sodium hydroxide and centrifuged to obtain clear supernatant. Then 2 ml of the supernatant was used in each SPME glass vial while only 1 ml of urine or vitreous humor was used in each SPME glass vial without alkali precipitation. Even though total volume of vitreous humor in both eyes of each person is approximately 4 ml [25], in this study, only 2-3 ml of vitreous humor could be practically collected. Thus, vitreous humor could be used at the maximum volume of 1 ml for each of the experiment which was needed to perform in duplicate. As mentioned in the Methods, the procedure used to determine MA concentrations in urine and vitreous humor samples in this study was modified from the method of Myung et al [27]. In that study, they demonstrated that LOD of the method was 10 ng/ml whereas LOD of the method shown in this study was 25 ng/ml. The difference of LOD between studies could

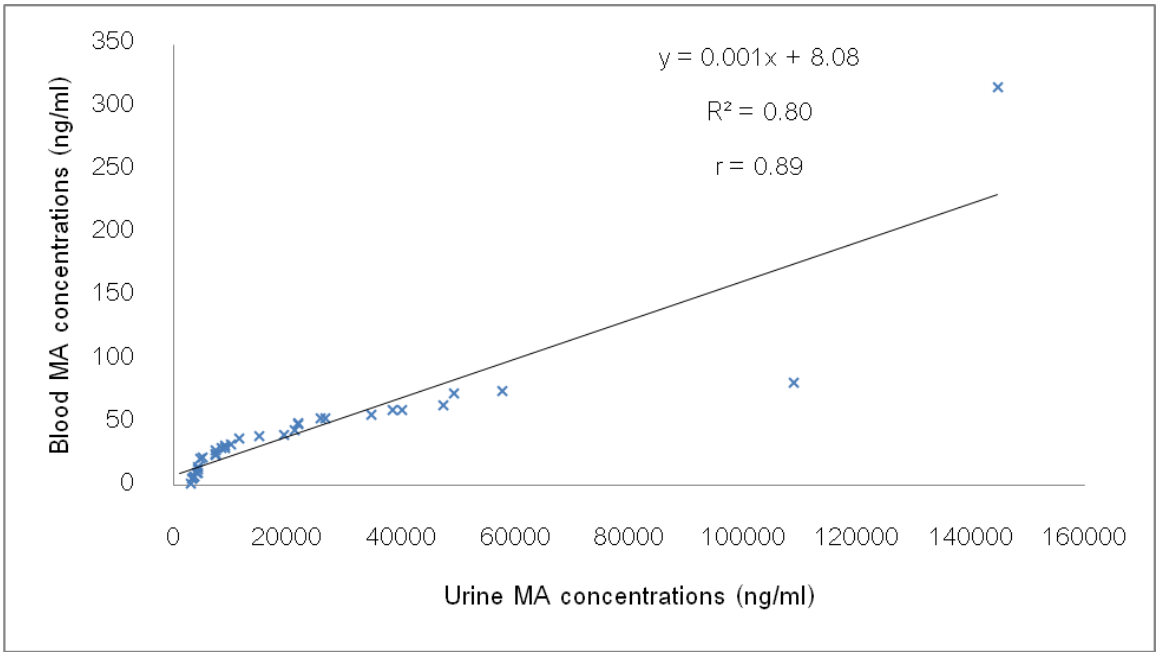

Figure 3 Relationship between MA concentrations in urine and blood samples (n = 33)

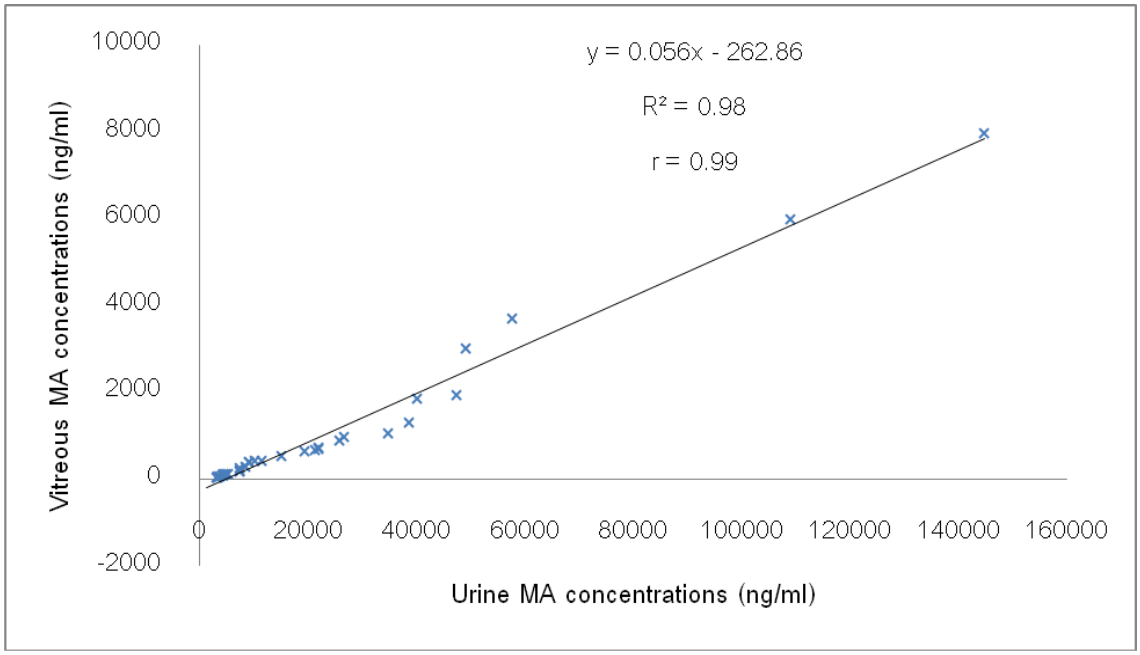

Figure 4 Relationship between MA concentrations in urine and vitreous humor Samples (n = 33)

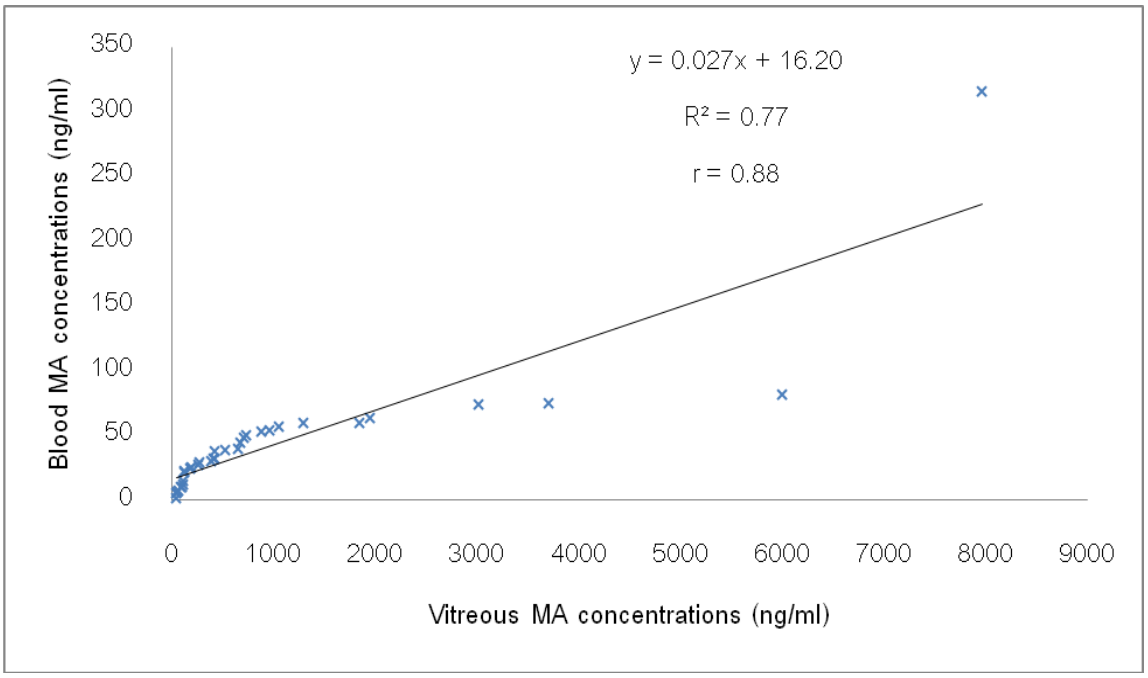

Figure 5 Relationship between MA concentrations in vitreous humor and blood samples (n = 33)

be contributed from any of these contributing factors: volumes of the specimens (3 ml *vs* 1 ml); differences of the extraction process by SPME (direct immersion-SPME *vs* head space-SPME); GC-MS conditions (e.g. length of column, oven temperature, etc); interlaboratory variation, etc. Those two extraction processes provided different advantages and disadvantages. Regarding the direct immersion-SPME technique, the fiber directly contacted with the sample in sample phase and all kinds of molecule dissolved in the sample phase were adsorbed. Thus, more concentrated molecules were likely to be adsorbed and injected to the column resulting in lower LOD. However, undesired molecules which could damage the column could also be adsorbed. For HS-SPME technique, only small vaporized molecules could be adsorbed, fewer molecules could be injected to the column resulting in higher LOD. On the other hand, this technique more prolonged the column lifetime because of reducing chance of undesired molecules exposure to damage the column. Determination of MA concentrations in blood samples was modified from the method of Namera et al [26]. In that study, LOD of the method was shown to be 5 ng/g. Because an average density of whole blood is approximately 1.060 g/ml [31], thus, LOD of the method reported by Namera et al [26] could be approximately as 5 ng/ml. LOD of the method demonstrated in this study was 2.5 ng/ml which was small different to the value reported by Namera et al [26]. Somewhat difference of the LOD between studies could be explained by some differences between these 2 studies such as volume of the whole blood used (0.5 g or 0.5 ml *vs* 4 ml); utilization of derivatizing agent *vs* non-derivatizing agent method; as well as interlaboratory variation, etc. Regarding the accuracy and precision test, the results showed that accuracy of the procedure as presented by the % recovery as well as the % C.V. of both within- and between-day precision were within 15 % which are suggested by the guidelines [28-30].

The results showed that MA concentrations in urine were far higher than the corresponding MA concentrations in blood. This is consistent to a previous study of Lebish et al [32]. Actually, peak plasma MA concentration was shown to occur after 4 hours and 2.5 hours via intranasal administration and smoking, respectively [33, 34] and approximately 70 % of MA dose was excreted in urine within 24 hours [35]. In addition, MA could be detected in urine several days (7 days) after repeated MA doses [36]. The reports regarding toxic/fatal MA concentrations in blood vary among studies. Toxic concentrations of MA in blood were ranged from 200-5000 ng/ml and fatal MA concentrations in blood samples were reported > 10000 ng/ml [3, 4]. These variations could be due to differences of route of administration, amount and purity of the substance, co-administrated drugs/substances, individual variation, etc. [37-40]. However, blood MA concentrations of all the subjects in this study (Mean ± S.E. of blood MA concentrations = 44.70 ± 9.31 ng /ml) were much lower than the reported toxic/fatal concentrations. MA exposures of the subjects in this study seem to be possibly a contributing factor not the direct cause of death.

The closely linear relationships between MA concentrations in vitreous humor and other specimens using

for different purposes (urine for forensic purpose and blood samples for physiological effect interpretation) suggest that vitreous humor can be used as an alternative to urine or blood samples in the situation that both samples are not available or contaminated. Further study to verify the linear regression equations obtained from this study is suggested. This could be simply performed by using the specimens (urine, blood and vitreous humor samples) collected from other unrelated deceased. All specimens are analyzed for MA concentrations by the same procedure as in this study. Calculated MA concentrations of each sample can be obtained by calculation using the linear regression equations. Then, the calculated MA concentrations are statistically analyzed compared to the actual MA concentrations.

Vitreous humor possesses several advantages. Collection of this specimen is easy even if an autopsy is not completely performed. Because of its clear characteristic and mainly consists of water (99 %), vitreous humor is easy to analyze with reduced time and less requirement of sample preparation. Analytical method which is developed for urine or blood can be adapted to vitreous humor. Normally, putrefaction, charring, and trauma may affect sample quality. For example, tyramine, phenethylamine as well as other decomposition products may interfere blood and tissues extraction and analysis. These situations were less occurred with vitreous humor due to its anatomically isolated location. Also, as severe major organ damage occurs, an available specimen such as blood sample is potentially contaminated from tissues or stomach contents, in this situation, vitreous humor may be useful as a promising specimen [25].

In conclusion, MA concentrations in blood, urine and vitreous humor samples collected from 33 deceased were linear correlated with a correlation coefficient (r) of 0.89 (urine *vs* blood), 0.99 (urine *vs* vitreous humor) and 0.88 (vitreous humor *vs* blood). The corresponding linear regression equations were $y = 0.001x + 8.08$, $y = 0.056x - 262.86$, and $y = 0.027x + 16.20$, respectively. This relationship is preliminarily advantageous for prediction of MA concentrations in urine from MA concentration in blood sample while urine sample is not available or vice versa. Also, vitreous humor can be used as an alternative to blood and urine samples for determination of MA concentrations in case that both samples are not available or contaminated.

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References

[1] C. Franco. Methamphetamine: Legistration and issues in the 109th Congress, In L.V. Barton (ed.), *Illegal Drugs and Governmental Policy*, Nova Science, New York, 2007, pp. 137-142.
[2] T.C. Kwong. Introduction to drugs of abuse testing. In: A. Dasgupta (ed.), *Handbook of Drug Monitoring Methods*, Humana Press, New Jersey, 2008, pp. 297-315.
[3] C.L. Winek, W.W. Wahba, C.L. Winek Jr., and T.W. Balzer. Drug and chemical blood-level data 2001. *Forensic Sci. Int.* 122: 107–123 (2001).
[4] M. Schulz, A. Schmoldt, Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* 58: 447–474 (2003).
[5] R. Narapanyakul, W. Tungtananuwat, P. Yongpanich, T. Sinchai, N. Thong-ra-ar, S. Lawanprasert. Relationship between methamphetamine concentrations in urine and blood of Thai methamphetamine abusers, *Thai J. Pharmacol.* 34: 126-132 (2012).
[6] S. Mackey-Bojak, J. Kloss, and F. Apple. Cocaine, cocaine metabolite, and ethanol concentrations in postmortem blood and vitreous humor. *J. Anal. Toxicol.* 24: 59-65 (2000).
[7] W. Tungtananuwat, S. Lawanprasert, and N. Suwongthrom. Comparative study of blood, vitreous humor and urine alcohol concentrations in Thai postmortem traffic accidental victims. *Thai J. Pharm. Sci.* 23: 165-172 (2000).
[8] W.Q. Sturner, and J.C. Garriott. Comparative toxicology in vitreous humor and blood. *Forensic Sci.* 6 (1975) 31-39.
[9] K.R. Ziminski, C.T. Wemyss, J.H. Bidanset, T.J.Manning, and L. Lukash. Comparative study of postmortem barbiturates, methadone, and morphine in vitreous humor, blood and tissue. *J. Forensic Sci.* 29: 903-909 (1984).
[10] A.M. Bermejo, I. Ramos, P. Fernández, M. López-Rivadulla, A. Cruz, M. Chiarotti, N. Fucci, and R. Marsilli. Morphine determination by gas chromatography/mass spectroscopy in human vitreous humor and comparison with radioimmunoassay. *J. Anal. Toxicol.* 16: 372-374 (1992).
[11] M.J. Bogusz, R.D. Maier, and S. Driessen. Morphine, morphine-3-glucuronide, morphine-6-glucuronide and 6-monoacetylmorphine determined by means of atmospheric pressure chemical ionization-mass spectrometry-liquid chromatography in body fluids of heroin victims. *J. Anal. Toxicol.* 21: 346–355 (1997).
[12] J. Wyman, S. Bultman. Postmortem distribution of heroin metabolites in femoral blood, liver, cerebrospinal fluid, and vitreous humor. *J. Anal. Toxicol.* 28: 260-263 (2004).
[13] A. Poklis, M.A. Mackell, and M. Graham. Disposition of cocaine in fatal poisoning in man. *J. Anal. Toxicol.* 9: 227–229 (1985).
[14] B.K. Logan, and D.T. Stafford. High-performance liquid chromatography with column switching for the determination of cocaine and benzoylecgonine concentrations in vitreous humor. *J. Forensic Sci.* 35: 1303–1309 (1990).
[15] C.W. Chronister, J.C. Walrath, and B.A. Goldberger. Rapid detection of benzoylecgonine in vitreous humor by enzyme immunoassay. *J. Anal. Toxicol.* 25: 621–624 (2001).
[16] C. Furnari, V. Ottaviano, G. Sacchetti, and M. Mancini. A fatal case of cocaine poisoning in a body packer. *J. Forensic Sci.* 47: 208–210 (2002).
[17] D.L. Lin, and R.L. Lin. Distribution of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in traffic fatality cases. *J. Anal. Toxicol.* 29: 58-61 (2005).
[18] J. Crifasi, and C. Long. Traffic fatality related to the use of methylenedioxy- methamphetamine. *J. Forensic Sci.* 41: 1082–1084 (1996).
[19] K.M. Clauwaert, J.F. Van Bocxlaer , E.A. De Letter , S. Van Calenbergh , W.E. Lambert, and A.P. De Leenheer. Determination of the designer drugs 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine and 3,4-methylenedioxyamphetamine with HPLC and fluorescence detection in whole blood, serum, vitreous humor, and urine. *Clin. Chem.* 46: 1968-1977 (2000).
[20] T. Decaestecker, E. De Letter, K. Clauwaert, M.P. Bouche, W. Lambert, J. Van Bocxlaer, M. Piette, E. Van den Eeckhout, C. Van Peteghem, and A.P. De Leenheer, Fatal 4-MTA intoxication: development

of a liquid chromatographic—tandem mass spectrometric assay for multiple matrices. *J. Anal. Toxicol.* 25: 705-710 (2001).
[21] E.A. De Letter, P. De Paepe, K.M. Clauwaert, F.M. Belpaire, W.E. Lambert, J.F. Van Bocxlaer, and M.H. Piette. Is vitreous humour useful for the interpretation of 3,4-methylenedioxymethamphetamine (MDMA) blood levels?: Experimental approach with rabbits. *Int. J. Legal Med.* 114: 29–35 (2000).
[22] E.A. De Letter, K.M. Clauwaert, W.E. Lambert, J.F. Van Bocxlaer, A.P. De Leenheer, and M.H. Piette. Distribution study of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in a fatal overdose. *J. Anal. Toxicol.* 26: 113-118 (2002).
[23] E.A. De Letter, M.P. Bouche, J.F. Van Bocxlaer, W.E. Lambert, and M.H. Piette. Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: discussion by means of a distribution study in two fatalities. *Forensic Sci. Int.* 141: 85–90 (2004).
[24] I.M. Mclntyre, C. Hamm, and E. Bader. Postmortem methamphetamine distribution. *J. Forensic Res.* 2: 122 (2011).
[25] B.S. Levine, and R.A. Jufer. Drugs-of-Abuse Testing in Vitreous Humor. In: A. J. Jenkins, Y.H. Caplan (eds.), *Forensic Science and Medicine: Drug Testing in Alternate Biological Specimens*, Humana Press, New Jersy, 2008, pp. 117-130.
[26] A. Namera, M. Yashiki, J. Liu, K. Okajima, K. Hara, T. Imamura, and T. Kojima. Simple and simultaneous analysis of fenfluramine, amphetamine and methamphetamine in whole blood by gas chromatography-mass spectrometry after headspace-solid phase microextraction and derivatization. *Forensic Sci. Int.* 109: 215-223 (2000).
[27] S.W. Myung, H.K. Min, S. Kim, M. Kim, J.B. Cho, and T.J. Kim. Determination of amphetamine, methamphetamine and dimethamphetamine in human urine by solid-phase microextraction (SPME)-gas chromatography/mass spectrometry. *J. Chromatogr. B* 716: 359-365 (1998).
[28] The International Committee Harmonization. ICH-Q2B, Guidance for Industry: Validation of Analytical Procedures: Methodology. [online]. 1996. Available from http://www.fda.gov/cder/guidance/index.htm [2011, May 21].
[29] The United States Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. Guidance for industry: Bioanalytical Method Validation. [online]. 2001. Available from http://www.fda.gov/CDER/GUIDANCE/4352fnl.htm [2011, May 21]
[30] The Society of Forensic Toxicologists/ the Society of Forensic Toxicologists. Forensic Laboratory Guidelines 2006 version. [online]. 2006. Available from http://www.softtox.org/docs/Guidelines%202006%20Final.pdf [2011, May 21]
[31] J.D. Cutnell, and K.W. Johnson. *Physics*, 4th ed. John Wiley & Sons, New York, 1998,pp. 308.
[32] P. Lebish, B.S. Finkle, and J.W. Brackett Jr. Determination of amphetamine, methamphetamine, and related amines in blood and urine by gas chromatography with hydrogen- flame ionization detector. *Clin. Chem.* 16: 195-200 (1970).
[33] C.L. Hart, E.W. Gunderson, A . Perez, M.G. Kirkpatrick, A. Thurmond, S.D. Comer, and R.W. Foltin. Acute physiological and behavioral effects of intranasal methamphetamine in humans. *Neuropsychopharmacol.* 33: 1847–1855 (2008).
[34] M. Perez-Reyes, W.R. White, S.A. McDonald, R.E. Hicks, A.R. Jeffcoat, J.M. Hill, and C.E. Clinical effects of daily methamphetamine administration. *Clin. Neuropharmacol.* 14: 352–358 (1991).
[35] C.C. Cruickshank, and K.R. Dye. A review of the clinical pharmacology of methamphetamine. *Addiction* 104: 1085-1099 (2009).
[36] J.M. Oyler, E.J. Cone, R.E. Joseph Jr., E.T. Moolchan, and M.A. Huestis. Duration of detectable methamphetamine and amphetamine excretion in urine after controlled oral administration of methamphetamine to humans. *Clin. Chem*. 48: 1703–1714 (2002).
[37] B.K. Logan, C.L. Fligner, and T. Haddix. Cause and manner of death in fatalities involving methamphetamine. *J. Forensic Sci.* 43: 28–34 (1998).
[38] R. Matoba. Cardiac lesions in methamphetamine abusers. *Jpn J. Legal Med*. 55: 321–330 (2001).
[39] M. Ago, K. Ago, and M. Ogata. Determination of methamphetamine in sudden death of a traffic accident inpatient by blood and hair analyses. *Legal Med*. 11: S568-S569 (2009).
[40] H. Inoue, N. Ikeda, K. Kudo, T. Ishida, M.Terada, and R. Matoba. Methamphetamine-related sudden death with a concentration which was of a 'toxic level'. *Legal Med*. 8: 150-155 (2006).