

## Method validation for simultaneous determination methamphetamine and its metabolite amphetamine in rat liver by using GC-FID

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### Abstract

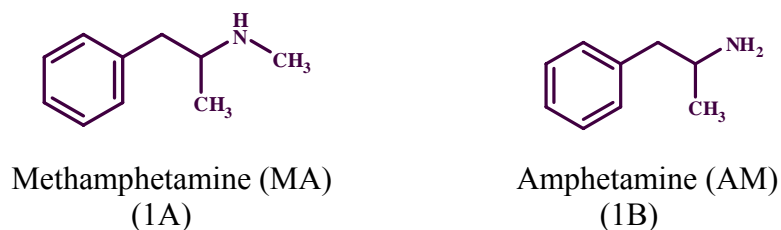
The purpose of these studies was to develop method for simultaneous determination of methamphetamine (MA) and its metabolite amphetamine (AM) in rat liver. Quantification of MA and AM in rat liver by using gas chromatography with a flame ionization detector (GC-FID) was validated. The chromatography used a VertiBond<sup>TM</sup> 5 capillary column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Sample of rat liver was extracted by liquid-liquid extraction. MA and AM were well separated within 12.8 min. The calibration curves were linear with good correlation coefficient. The intra- and inter-day precisions of the method were 8.35-10.97%RSD and 4.79-8.58%RSD for AM, 7.94-9.58%RSD and 8.51-12.21%RSD for MA. The accuracy ranged from (-) 14.77 to (+) 5.41%DEV for AM and (-) 6.98 to (+) 4.11%DEV for MA. The results were shown good recovery both AM and MA. The limit of detection and limit of quantification of AM and MA were 6.25 µg/g and 9.375 µg/g, respectively. It was shown to be useful for the study of pharmacokinetic and forensic toxicology.

**Keywords:** methamphetamine, amphetamine, GC-FID, liver.

### Introduction

Methamphetamine (MA; Fig.1A) and its metabolite amphetamine (AM; Fig.1B) are powerful stimulants of central nervous system and are abuse in many countries including Thailand. MA is a dangerous narcotic drug which causes health problems. MA produces many toxic effects such as, wakefulness, hyperthermia, hypertension and euphoria (1). The death is a hallmark of toxic effect from MA. Therefore, it is important to indicate level of MA and its metabolite AM in metabolic organ, liver. Gas chromatography (GC) (2,3,4) and liquid chromatography (LC) (5) are successful technique to detect MA and its metabolite AM in tissues after derivatization to minimized tailing peak or unsuccessful baseline separation. However, derivatization has several inherent disadvantages over direct analysis. In addition, MA and AM have been determined by using GC-MS which is a powerful technique. However, this technique has been limited since this running cost is very expensive. The gas chromatography-flame ionization detector (GC-FID) is considered to be more possible for determining the MA and its metabolite AM in liver.

Therefore, the aim of this study was to develop suitable condition of GC-FID for determination of MA and its metabolite AM in rat liver without derivatization.



**Figure 1** Chemical structure of MA and AM

## Methods

### Chemicals and reagents

Reference standard methamphetamine hydrochloride was obtained from the Regional Forensic Science Division 4, Office of the Police Forensic Science, Thailand. Amphetamine was provided by Department of Pharmacology, Faculty of Science, Prince of Songkla University.

### Preparation of standard solutions

The stock solution of 1,000 µg/mL of MA and AM standards were prepared in pure methanol. Working standard solutions were prepared by diluting the stock solutions to a concentration range of 2.5-50 µg/mL as a mixture of the standard MA and AM.

### Chromatographic instrument and condition

A gas chromatography model GC 6890 (HP, U.S.A.) equipped with a flame ionization detector, capillary column VertiBond<sup>TM</sup> 5 (5% diphenyl and 95% dimethylpolysiloxane, 30 m x 0.32 mm i.d., 0.25 µm film thickness) (Vertical<sup>®</sup>, Thailand). Determination of MA and its metabolite AM in liver were modified (6). Temperature of the column was programmed initially at 70 °C (2 min) and increased with a rate of 30 °C/min to 120 °C and then increased to 150 °C at a rate of 5 °C/min. Finally, the temperature was increased at a rate of 70 °C/min to 300 °C where it was held for 1 min. 1 µL of sample was injected in a splitless injection mode (splitless injection with inlet insert purge time of 0.3 min and split vent flow rate of 80 mL/min). Helium gas was used as the carrier gas with a flow rate of 2.5 mL/min. Fuel gas (H<sub>2</sub>) and make-up gas (N<sub>2</sub>) flow rate were 30 mL/min. Oxidant gas (O<sub>2</sub>) flow rate was 300 mL/min. Injector and detector temperature were 200 °C and 300 °C, respectively.

### Liver preparation and extraction procedure

Rat liver was quickly removed after euthanized with diethyl ether and perfused with 0.9% normal saline. Prior to extraction procedure, MA and AM free-liver was homogenized using a homogenizer (Kinematica CH-6010 Kriens-LU, Polytron<sup>®</sup>, Switzerland) and sonicated with ultrasonic processor (Branson Sonifier 450, U.S.A.) in an ice-chilled tube containing an ice. Homogenized tissue was divided into 2 portions. One was used as blank and the others were used for mixture standard MA and AM spiked tissue. The extraction procedure was modified by method of Moriya and Hashimoto (7). The final MA and AM concentration in the liver were in a range of 6.25-125 µg/g. Finally, 1 µL of the reconstituted volume was injected into the GC-FID.

### Method validation

The method of analysis was validated in accordance with the Guidance for Industry: Bioanalytical Method Validation (8).

Linearity was evaluated by preparing eight standard concentrations (6.25-125 µg/g; 5 replicates each concentration) of MA and AM in liver. The calibration curve was constructed by plotting the peak area of the analyte versus its concentrations. Regression analysis for each calibration curve was performed to obtain the calibration equation and correlation coefficient (*r*).

Precision was evaluated from intra- and inter-day precision by using 3 quality control samples (5 replicates for each concentration) into liver. Precision is frequently expressed as the percentage of the relative standard deviation (%RSD). The level of acceptance for precision is 15% RSD value.

The accuracy was also determined using QC with five replicates for each concentration. Accuracy was expressed as the deviation (DEV) and is acceptable when the DEV is within  $\pm 15\%$ .

Recovery was determined in three concentration (15.625, 31.25, 125  $\mu\text{g/g}$ ; 5 replicates of each concentration).

The limit of detection (LOD), signal to noise ratio equal to or greater than 3, and the limit of quantification (LOQ), signal to noise ratio equal to or greater than 10, were using concentrations of MA and AM (6.25, 9.375, 12.5  $\mu\text{g/g}$ ; 5 replicates of each concentration).

## Results

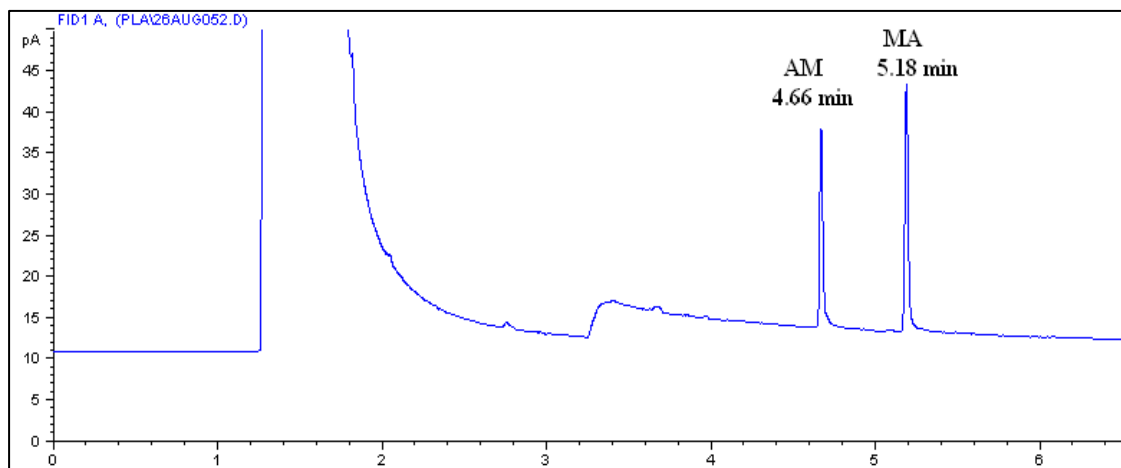
The chromatographic separation of MA and AM in rat liver is shown in Fig. 2. MA and AM were well separated with no interference. The retention times of MA and AM were 4.66, and 5.18 min, respectively within a run time of 12.8 min. Regression analysis results showed that the calibration curves were linear over the concentration ranges of 9.375-125  $\mu\text{g/g}$  for MA and AM. The regression equation was  $y = (8.23 \pm 0.69)x - (2.03 \pm 0.95)$ ,  $r = 0.9996$  for AM and  $y = (10.25 \pm 0.80)x - (2.30 \pm 0.79)$ ,  $r = 0.9997$  for MA. The accuracy (%DEV) for determination of all analytes ranged between  $\pm 15\%$ . Intra- and inter-day precisions for determining AM and MA are shown in Table 1. Both values for all analytes were found to be within the acceptable value (15%RSD). The LOD and LOQ of AM and MA were 6.25  $\mu\text{g/g}$  and 9.375  $\mu\text{g/g}$ , respectively. The mean percentages of recovery were 86-114% for AM and 88-110 % for MA

**Table 1** Precision and accuracy of the method for determination of MA and AM in rat liver (n=5)

Analyte	Concentration ( $\mu\text{g/g}$ )	Precision (%RSD)		Accuracy (%DEV)	
		Intra-day	Inter-day	Intra-day	Inter-day
AM	125	8.35	8.55	+5.41	-6.23
	31.25	8.47	8.58	-2.88	-10.51
	18.75	10.97	4.79	-13.80	-14.77
MA	125	7.94	8.51	+4.11	+1.26
	31.25	9.58	8.51	-2.46	-3.40
	18.75	9.39	12.21	-6.98	+0.37

## Discussion

The present liquid-liquid extraction method which was modified is more available. This study is present a good recovery and cost saving apparatus. In addition, without derivitization the chromatogram are symmetry and shorter run time than previous reported (6). Therefore, this study may be useful for study of pharmacokinetic and forensic toxicology.



**Figure 2** Representative chromatograms of AM and MA in rat liver (62.5 µg/g)

### Conclusion

GC-FID technique is universal, laboratories can afford, cost saving technique, simple, sensitive, precise and accurate to determine MA and AM in tissue.

### Acknowledgements

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