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Technical Report

Automatic Identification and Semi-quantitative Analysis of Psychotropic Drugs in Serum Using "GC/MS Forensic Toxicological Database"

Hitoshi Tsuchihashi[1]

Abstract:

A sample consisting of serum extract from a patient administered with psychotropic drugs was analyzed by GC/MS; identification and semi-quantitation of the substances were conducted using the "GC/MS Forensic Toxicological Database". All three administered substances were identified, and their semi-quantitative values were calculated. The results indicated that it is possible to qualitatively and semi-quantitatively determine drugs using this database.

Keywords: GC/MS, psychotropic drug, phenobarbital, semi-quantitation, forensic medicine, toxicology

Introduction

Accidental poisoning due to the abuse and excessive intake of stimulants and other illegal drugs or psychotropic drugs continues to be a troublesome social issue. In particular, when death occurs due to acute drug poisoning, identifying the specific drug responsible and determining its concentration in the blood are essential subjects in university forensic classrooms and critical activities in prefectural police department forensic laboratories.

Identification and quantitative analysis of a drug substance in the blood can be very time-consuming, requiring a calibration curve for verification of retention times using a standard drug sample, and determination of the appropriate preparation and pretreatment of the actual sample. In addition to the automatic search algorithm built into the GC/MS Forensic Toxicological Database, a semi-quantitative analysis feature is also included which uses relative response factors for drugs that often lead to poisoning. These features allow identification of drugs for which standard samples are difficult to obtain, and evaluation based on estimated quantitation values (via semi-quantitation) for these substances.

In this Technical Report, we utilized the substance identification and semi-quantitation features included in the GC/MS Forensic Toxicological Database to identify 3 substances, including the barbiturate, Phenobarbital (used to treat epilepsy), the antipsychotic, chlorpromazine, and the antihistamine antiemetic, promethazine (used to treat Parkinson's disease), in a spiked sample of actual serum. In addition, we verified the results using a serum sample from an actual patient administered these 3 substances.

Experiment

Reagents

The phenobarbital sodium, chlorpromazine hydrochloride, and promethazine hydrochloride were obtained from Wako Chemicals, and each was adjusted to a free-state concentration of 10 mg/mL (by using methanol for phenobarbital sodium and distilled water for chlorpromazine hydrochloride and promethazine hydrochloride). After preparing a mixed solution of these, each at a concentration of 100 µg/mL, the mixture was added to blank serum, and the concentration was adjusted to 10 µg/mL. This was then used as the analytical sample (spiked serum). In addition, after receiving informed consent from a psychiatric patient who had been administered these 3 substances, we used the received blood serum as the actual sample. In addition, two custom standard solutions were obtained from Shimadzu GLC, one an n-alkane C7 – C33 sample (Custom Retention Time Index Standard, Restek Corp.) for retention time correction, and the other an internal standard (Custom Internal Standard, Restek Corp.) sample necessary for the semi-quantitation.

1 Department of Legal Medicine, Osaka Medical College

Pretreatment Procedure

Into 500 µL of each serum sample (spiked serum and actual sample), 20 µL of 10% hydrochloric acid was titrated to acidify, 1000 µL of a chloroform-isopropanol mixture (3:1, v/v) was added, and after vigorously mixing, centrifuging was conducted for 15 minutes, and the organic layer (acidic fraction) was collected. After conducting this operation twice in succession, 20 µL of 28% aqueous ammonia was added to the aqueous layer to make the mixture basic. Then, 1000 µL of a chloroform-isopropanol mixture (3:1, v/v) was added, and after conducting the mixing / centrifuging process described above, the organic layer (basic fraction) was collected.

After combining the acidic and basic fractions, dewatering of the mixture was conducted using anhydrous sodium sulfate, and evaporative drying was performed at 40°C under nitrogen. The obtained residue was dissolved in 250 µL ethyl acetate, and this was used as the sample for GC/MS analysis.

Equipment

For the GC/MS analysis, the GCMS-QP2010 Ultra was used, and GC-MSsolution software was used for data processing. Table 1 shows the analytical conditions that were used for the analyses. For retention time adjustment, the AART (Automatic Adjustment of Retention Time) feature included in GCMSsolution Postrun Analysis was used to calculate the retention times of the 162 substances (included in the free-state substance analytical method for psychiatric drugs) from the retention indices, and these were used as the standard retention times for identification. The retention time windows were set to ±0.2 minutes, and the substances included in the samples were identified using the automatic identification feature. In addition, the internal standard used for quantitation was introduced automatically into the GC injection port simultaneously with the sample using the internal standard automatic addition feature of the AOC-20i+s.

Table 1 Analytical Conditions

Instruments	
GC-MS	: GCMS-QP2010 Ultra
Auto-injector	: AOC-20i + s
Column	: Rxi®-5Sil MS (30 m × 0.25 mm I.D. df=0.25 µm, Restek Corporation)
GC condition	
Column Temp.	: 60°C (1 min)-10°C/min-320°C (10 min)
Carrier Gas	: He (Constant Linear Velocity Mode)
Carrier Gas Velocity	: 45.6 cm/sec
Injection Mode	: Splitless
Sample injection volume	: 1 µL
IS injection volume	: 1 µL
MS condition	
Interface Temp.	: 280°C
Ion Source Temp.	: 200°C
Scan Interval	: 0.3 sec
Monitor ion for semi-quantitation	: *m/z* 204 for phenobarbital
	: *m/z* 318 for chrolpromazine
	: *m/z* 72 for promethazine

Results and Discussion

Semi-Quantitation Results of Spiked Serum

The total ion current chromatogram (TIC) obtained from analysis of the spiked serum using the abovementioned procedure is shown in Fig. 1. the 3 added substances were detected within ±0.03 minutes of the expected calculated retention time, and accurate identification was achieved based on the retention times (Fig. 2). Since each substance is detected based on the extracted ion chromatogram (EIC) of multiple *m/z* values set beforehand, the EIC chromatogram can be detected even at trace concentrations or when a discrete chromatographic peak in the TIC cannot be detected due to matrix interferences. The detected chromatographic peak can be accurately identified automatically through confirmation of the degree of similarity with the standard mass spectrum (Fig. 3). In addition, the quantitation values were calculated from the obtained peak intensity ratio of each target substance and internal standard substance, and the relative response factor.

Thus, utilizing the automatic search feature of the GC/MS Forensic Toxicological Database, phenobarbital, chlorpromazine, and promethazine were all detected automatically, and semi-quantitation values were obtained for these 3 substances. The semi-quantitation values obtained using the semi-quantitation feature of the GC/MS Forensic Toxicological Database and the relative response factors (obtained from analysis of the standard solution) are shown in Table 2.

As shown in Table 2, fairly good quantitative results were obtained for chlorpromazine and promethazine, but the quantitative results for phenobarbital indicate a value 1.8 times that of the added amount. The semi-quantitation feature of the GC/MS Forensic Toxicological Database generates a semi-quantitation value which is a rough estimate of "the drug concentration in the final sample" based on the response factor obtained from the previously-analyzed standard sample. Therefore, the semi-quantitation value can vary considerably depending on the drug recovery ratio and sample concentration during sample pretreatment, as well as the matrix effect. Especially, in the case of a serum sample, the recovery of a drug with high lipid solubility will decrease greatly, increasing the likelihood that the difference between the obtained semi-quantitation value and the true value will widen considerably. Thus, since the calculated quantitation value can be expected to vary depending on the pretreatment procedure, the GC injection port, and the column condition, it is necessary to regard it only as an approximate estimated value. For quantitative analysis requiring great accuracy, standard samples must be used.

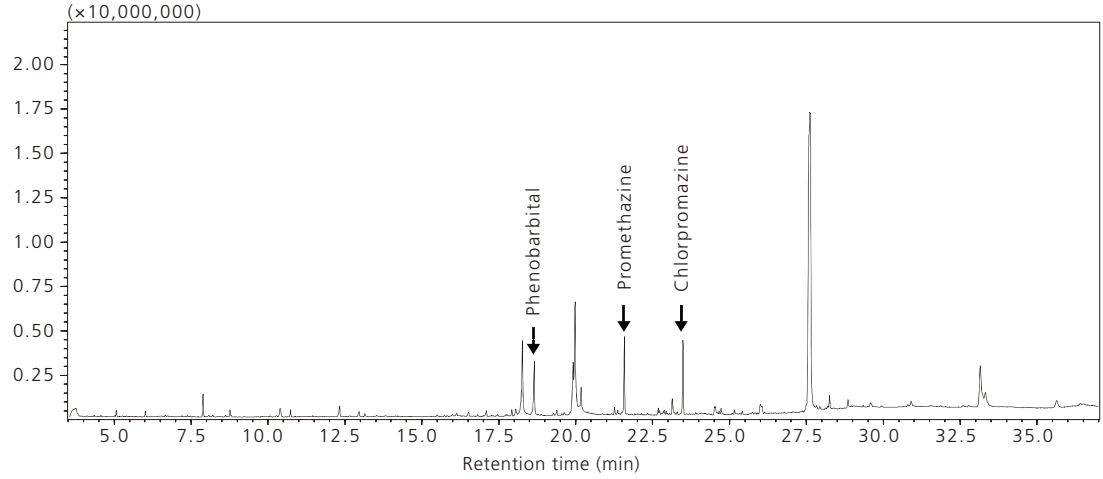


Fig. 1 Total Ion Current Chromatogram Obtained from Spiked Serum

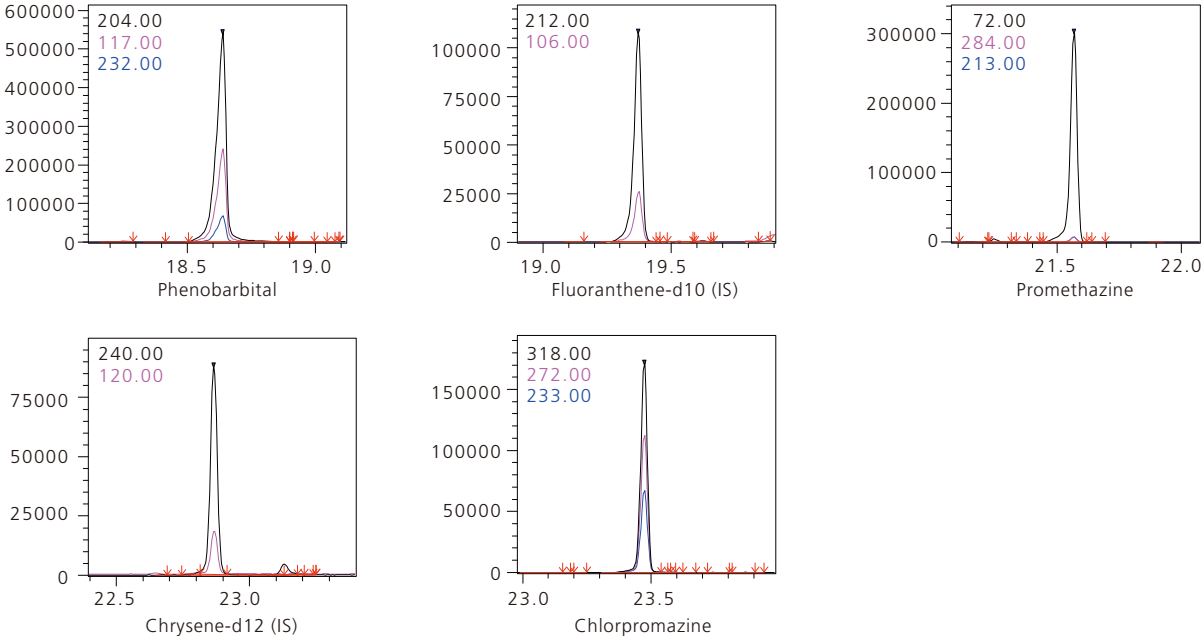


Fig. 2 Mass Chromatograms of 3 Added Substances and Principal Internal Standard Samples

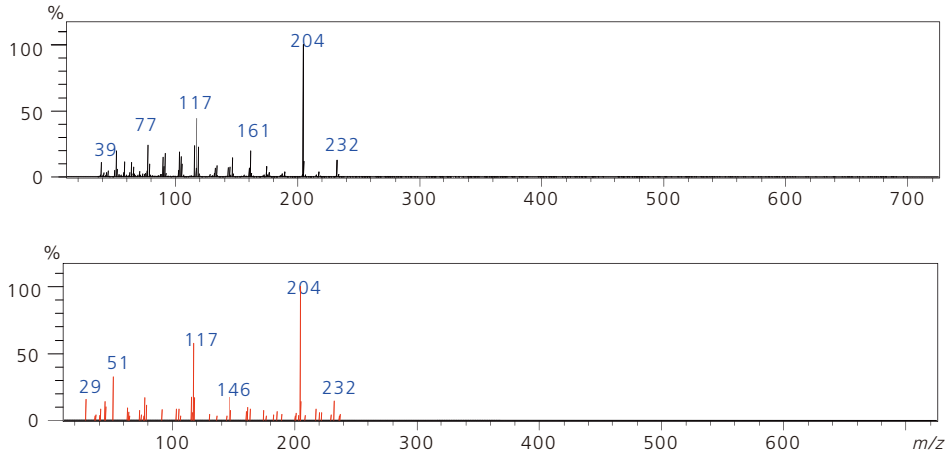


Fig. 3 Comparison of Measured Mass Spectrum of Phenobarbital (above) and Standard Mass Spectrum (below)

Table 2 Semi-Quantitated Results of Spiked Plasma

Compounds	Additive Amount (μg/mL)	Semi-quantitated (μg/mL)	Response Factor
Phenobarbital	10.0	17.6	0.175
Chlorpromazine	10.0	11.3	0.144
Promethazine	10.0	11.4	1.782

Semi-Quantitation Results for Actual Sample

In the case of the actual sample, just as with the spiked serum, the 3 substances were automatically detected by the automatic search algorithm, and the semi-quantitative results were obtained. The chromatogram obtained from analysis of the actual sample is shown in Fig. 4. Also, with respect to the actual sample, the quantitation values obtained using the internal standard method were compared with the semi-quantitation values calculated from the GC/MS Forensic Toxicological Database. Those data are shown in Table 3.

As with the spiked serum, the semi-quantitation values obtained for chlorpromazine and promethazine were relatively close to the values obtained from the calibration curve, but the semi-quantitation value for phenobarbital indicated a value that was about 3 times higher.

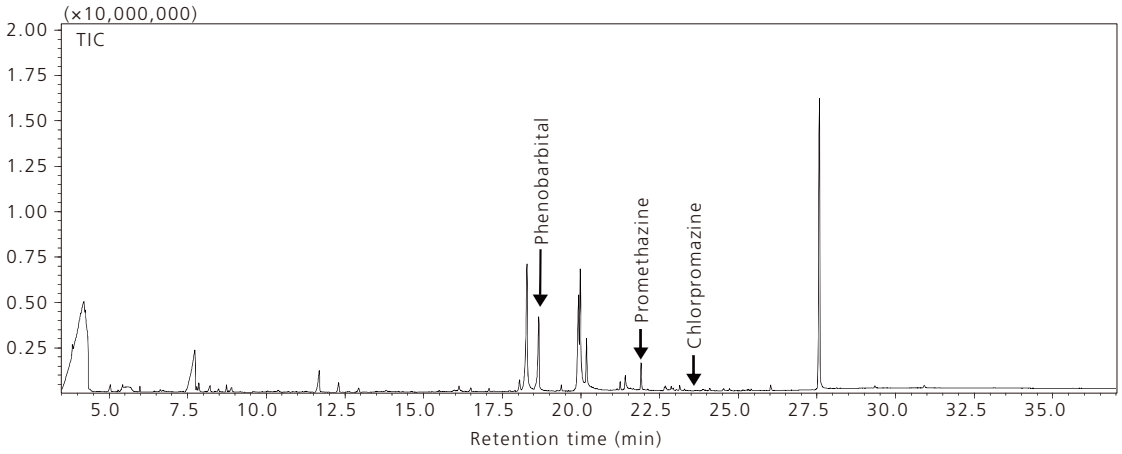


Fig. 4 Total Ion Current Chromatogram Obtained Using Actual Sample

Table 3 Comparison of Quantitated Results and Semi-Quantitated of Real Specimen

Compounds	Quantitation* (µg/mL)	Semi-Quantitation (µg/mL)
Phenobarbital	11.6	33.2
Chlorpromazine	0.03	0.02
Promethazine	0.13	0.15

* Determined from calibration curve generated using spiked serum.

Conclusion

Automatic qualitative and semi-quantitative analyses were conducted for 3 psychotropic drug substances (phenobarbital, chlorpromazine, and promethazine) in serum using the GC/MS Forensic Toxicological Database. Using a method which incorporated information on 162 psychiatric drugs, automatic identification of phenobarbital, chlorpromazine, and promethazine was possible using retention time correction via the AART feature of the GCMSsolution software. Relatively accurate quantitative results were obtained for chlorpromazine and promethazine, but the results obtained for phenobarbital tended to be 2 to 3 times higher than the spiked levels. Since the semi-quantitative values obtained using this feature of the GC/MS Forensic Toxicological Database are just quantitative estimates of the concentrations in the final sample, they should be considered as values subject to great variation, depending on such factors as the error in the drug recovery ratio or sample concentration in pretreatment, matrix effects, and instrument condition. However, since automatic quantitative analysis using this database can be conducted simultaneously with an automatic database search, it can certainly be useful for obtaining a rough estimate of a drug's concentration while conducting qualitative analysis, or for quickly estimating concentration of a specific drug when there is no time for preparing calibration standards.

Gas Chromatograph Mass Spectrometer

GCMS-QP2010 Ultra

Features

1. High sensitivity
2. Easy maintenance
3. Identification of compounds using retention indices

The Shimadzu GCMS-QP2010 series has optimum functions and performance for forensic toxicology.

1. The Shimadzu GCMS-QP2010 series features an extremely high sensitivity and capability to measure forensic toxicology-related compounds down to low concentrations.
2. Urine and blood samples contain lots of contaminants. When samples such as these are measured by GC/MS, contamination of the ion source becomes problematic.The GCMS-QP2010 series is less likely to become dirty, and, moreover, can be easily cleaned even if the ion source is contaminated.
3. It is difficult to obtain standard samples for forensic toxicology-related compounds. However, in the GC/MS Forensic Toxicological Database for the GCMS-QP2010 series, the information of more than 500 medicinal toxicants is registered to method files together with optimum analysis conditions.

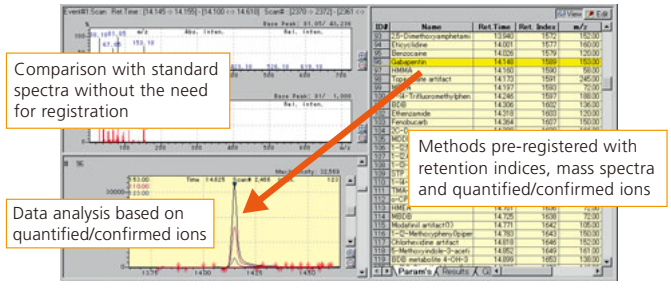
GC/MS Forensic Toxicological Database (Drugs of Abuse / Medicines / Pesticides)

The "GC/MS Forensic Toxicological Database" is exclusively for the GCMSsolution workstation software for GCMS-QP2010 series gas chromatograph mass spectrometers. It is pre-registered with 1011 mass spectra including free-, TMS- and TFA- body types for 502 compounds that are required in forensic toxicological analysis of drugs of abuse, drugs for psychiatric and neurological disease, and other medicines and pesticides.

This database comprises the following: method files pre-registered with analytical conditions, mass spectra, retention indices, etc., compound information including CAS numbers, etc., libraries containing mass spectra and retention indices, and a handbook (printed version of library information).

Spectra for 591 drugs of abuse, 274 drugs for psychiatric and neurological disease, 110 medicines, and 36 pesticides are registered to the methods and libraries.

Use of this database enables high-precision identification of compounds based on the AART (Automatic Adjustment of Retention Time) that uses retention indices, and based on mass chromatograms compared with standard mass spectra and quantified/confirmed ions.



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