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Application Data Sheet

No.124

GC-MS

Gas Chromatograph Mass Spectrometer

Semi-Quantitation of Toxicological Substances in Whole Blood Using the Quick-DB Forensic.

The sample preparation is an indispensable process in GC-MS analysis. The recovery rate of sample preparation must be considered when quantifying target compounds in samples. However, the experience and time needed to determine the optimal sample preparation method and recovery rate increase with the number of compounds targeted for measurement. In addition, it is costly and difficult to acquire standard samples for quantitation and create calibration curves.

The Quick-DB GC/MS/MS Forensic Toxicology Database (Quick-DB Forensic) contains the GC/MS/MS analytical conditions for 68 toxicological substances often involved in poisonings, as well as calibration curve information for multiple points throughout the sample preparation. This enables standard-less semi-quantitation of toxicological substances in whole blood samples using pretreatment in accordance with the optimized QuEChERS protocol.

This application data sheet introduces an evaluation of the semi-quantitative accuracy by calculating quantitative results for a pretreated sample of whole blood with drugs added, using calibration curves registered in Quick-DB Forensic.

Quick-DB Forensic

This database is intended to provide a total solution for everything from sample preparation to GC/MS/MS measurement and data processing for 68 toxicological substances often involved in poisonings. QuEChERS, which is known as a simple sample preparation method, has been adopted so pretreatment can easily be performed even by first-time users. The calibration curve information contained in the database is multipoint data obtained by first adding targets compounds to whole blood samples and then performing pretreatment. This reduces quantitative errors due to interference from the matrix and pretreatment recovery rates, which have a significant effect on quantitative accuracy. As a result, high-accuracy semi-quantitative values can be obtained without standard samples.
In addition, the performance control function, which automatically assesses the system status, quickly determines problematic areas such as contamination of columns or glass inserts. As a result, toxicological substances can be analyzed while consistently maintaining the highest status level.

Analysis Protocol of Quick-DB Forensic

Figure 1 shows an overview of the protocol for analysis using Quick-DB. Dilute a 200 µL whole blood sample with distilled water and add the internal standard. Then, perform a cleanup via QuEChERS extraction and dispersive solid phase extraction (dSPE) in accordance with the protocol. Perform concentration and evaporation to dryness, and dilute it with ethylacetate. The analysis method file is created automatically by Smart MRM, which creates a method file from the database. The calibration curve information is also registered simultaneously. Semi-quantitative values can be obtained for the toxicological substances detected just by measuring the test sample with the method file created. Phenethylamine drugs can be measured by on-column TFA derivatization via an autoinjector, so the derivatization process is automated.

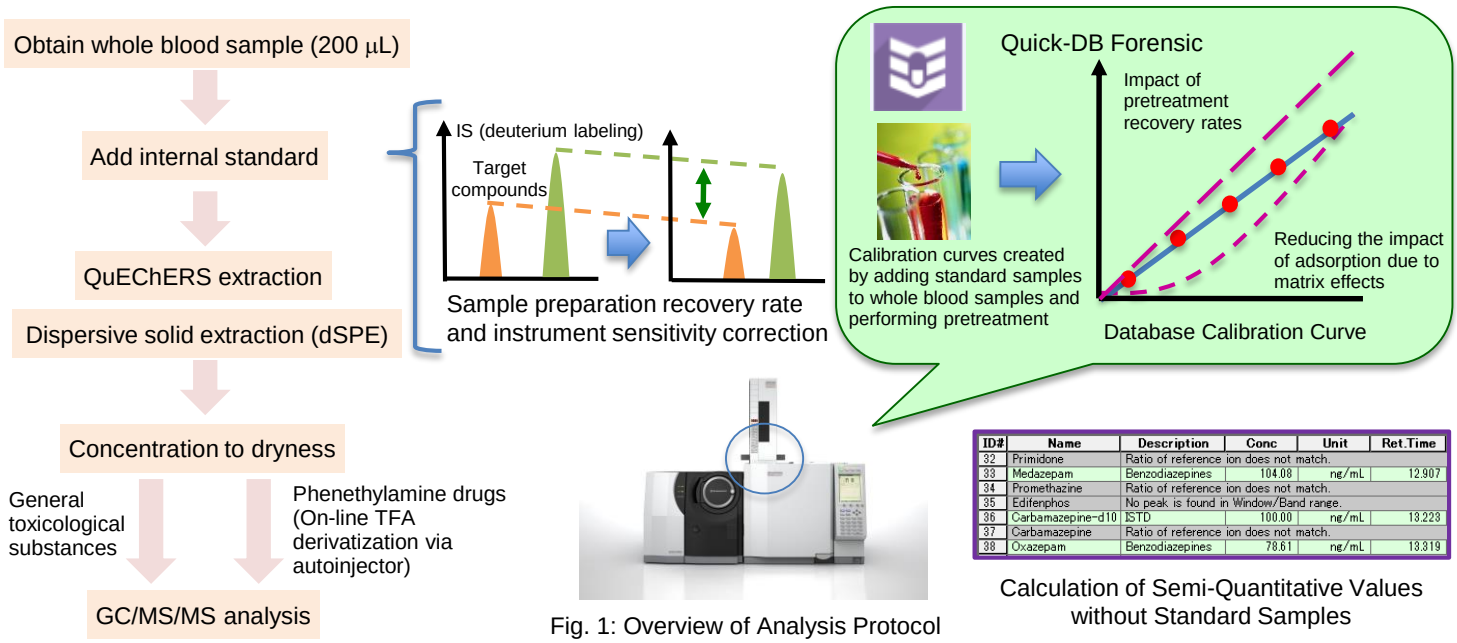

Fig. 1: Overview of Analysis Protocol

ID#	Name	Description	Conc	Unit	Ret.Time
32	Primidone	Ratio of reference ion does not match.			
33	Medazepam	Benzodiazepines	104.08	ng/mL	12.907
34	Promethazine	Ratio of reference ion does not match.			
35	Edifenphos	No peak is found in Window/Band range.			
36	Carbamazepine-d10	ISTD	100.00	ng/mL	13.223
37	Carbamazepine	Ratio of reference ion does not match.			
38	Oxazepam	Benzodiazepines	78.61	ng/mL	13.319

Calculation of Semi-Quantitative Values without Standard Samples

Analysis Results

In order to evaluate the accuracy of the semi-quantitation by the database, benzodiazepine drugs and phenethylamine drugs were added at a concentration of 100 ng/mL, respectively, to a whole blood sample. After pretreatment, the extracted sample was measured. The semi-quantitative results for benzodiazepines are shown in Table 1, and typical chromatograms are shown in Fig. 2. In addition, the semi-quantitative results for phenethylamine drugs are shown in Table 2, and typical chromatograms are shown in Fig. 3. A low-interference chromatogram was obtained due to the high selectivity of MS/MS. In terms of quantitation, by applying calibration curves with consideration to the impact of the matrix, favorable quantitative results were obtained at 78 to 117% accuracy, even with a semi-quantitative method not using a standard sample.

Table 1: Semi-Quantitative Values and Accuracy for Benzodiazepine Drugs

	Semi-Quantitative Values (ng/mL)	Accuracy (%)
Medazepam	104.08	104.08
Oxazepam	78.61	78.61
Fludiazepam	97.78	97.78
Diazepam	97.89	97.89
Clotiazepam	107.74	107.74
Clobazam	109.29	109.29
Flunitrazepam	111.46	111.46
Bromazepam	117.66	117.66
Prazepam	105.92	105.92
Nimetazepam	111.86	111.86
Flurazepam	117.83	117.83
Alprazolam	92.9	92.90
Tofisopam	80.19	80.19
Etizolam	84.13	84.13
Triazolam	81.86	81.86

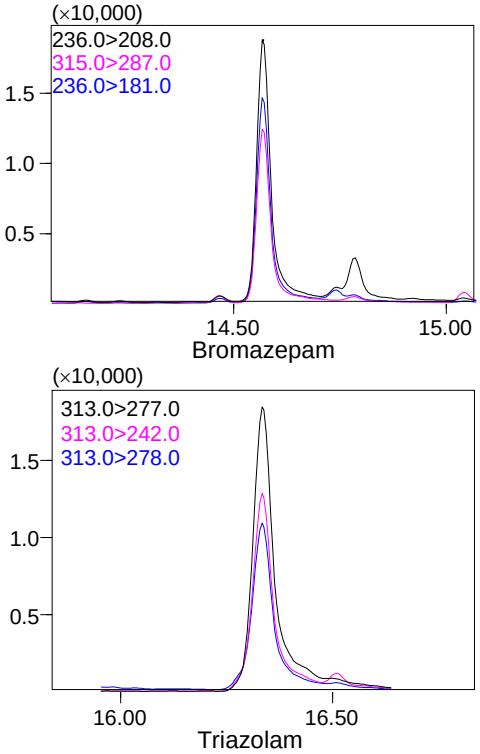

Fig. 2: Typical Chromatograms for Benzodiazepine Drugs

Table 2: Semi-Quantitative Values and Accuracy for Phenethylamine Drugs

	Semi-Quantitative Values (ng/mL)	Accuracy (%)
Amphetamine-TFA	96.76	96.76
Methamphetamine-TFA	88.37	88.37
MDA-TFA	87.63	87.63
MDMA-TFA	80.01	80.01

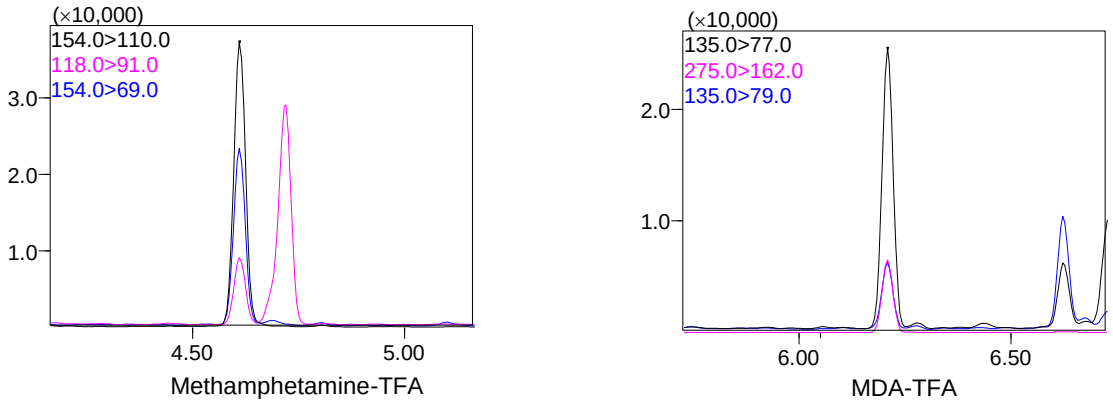

Fig. 3: Typical Chromatograms for Phenethylamine Drugs-TFA Derivatives

The quantitative information obtained with this system consists only of quantitative values obtained without using standard products. Be sure to implement tests using standard samples to calculate accurate quantitative values for use in expert written opinions or reports.

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