DETERMINATION OF CAFFEINE AND THEOPHYLLINE IN HUMAN WHOLE BLOOD BY STIR BAR SORPTIVE EXTRACTION (SBSE)-THERMAL DESORPTION-CAPILLARY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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原　　報

DETERMINATION OF CAFFEINE AND THEOPHYLLINE IN HUMAN WHOLE BLOOD BY STIR BAR SORPTIVE EXTRACTION (SBSE)—THERMAL DESORPTION—CAPILLARY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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Stir bar sorptive extraction — 加熱脱着 —キャピラリーガスクロマトグラフィー —マススペクトロメトリーによる全血中カフェインおよびテオフィリンの分析

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Summary

Caffeine and theophylline have been found extractable from human whole blood by stir bar sorptive extraction (SBSE). Their determination was made using capillary gas chromatography with thermal desorption and mass spectrometry. Extraction efficiencies of caffeine and theophylline in whole blood samples were 0.10-0.14 and 0.04 %, respectively. The regression equations for caffeine and theophylline showed excellent linearity in the range of 0.15-2.5 (r=0.9991) and 1.87-15 μg/ml (r=0.9999), respectively. The detection limits (signal-to-noise ratio=3) were 0.06 μg/ml for caffeine and 0.4 μg/ml for theophylline. The data obtained from actual determination of caffeine in a male subject after ingestion of coffee were also presented.

Key words: Stir bar sorptive extraction (SBSE); Caffeine; Theophylline; Thermal desorption; Capillary gas chromatography; Mass spectrometry

Introduction

Stir bar sorptive extraction (SBSE) is a new extraction technique first introduced in 1999 [1]. This procedure employs a stationary phase of polydimethylsiloxane (PDMS) coated on a glass-lined magnetic stir bar to extract a compound from aqueous or headspace samples in sealed vials; the stir bars have been commercialized under the name Twister™. After equilibration between the coated stir bar and liquid or headspace, the stir bar is placed in a thermal desorption (TD) unit coupled on-line to capillary gas chromatography (GC) or GC-mass spectrometry (MS); the analytes are thermally desorbed from the sir bar in an injector of GC. SBSE has been applied to analyses of organochlorine pesticides and chlorobenzenes in strawberries [2], organotin compounds (tributyltin and triphenyltin) in environmental samples [3], off-flavor compounds (2-methylisoborneol, geosmin and 2,4,6-trichloroanisole) in drinking water [4], dicarboximide fungicides in wine [5], aroma and flavor compounds in roasted coffee and coffee brew [6], preservatives in beverages, vinegar, aqueous sauces or quasi-drug drinks [7], polychlorinated biphenyls in human sperm [8], and terpenes, sesquiterpenes, steroides, nicotine, cocaine, fatty acid and phenols in human urine [9]. However, application of SBSE in the field of forensic toxicology has recently started [9, 10]. In this paper, we have established a recommendable procedure for analysing caffeine and theophylline in human whole blood by use of SBSE-TD-GC-MS. To our knowledge, this is the first report to use SBSE for extraction of methylxanthines from human body fluids.

Experimental

Materials

Caffeine anhydrous, theophylline anhydrous and pentifylline were obtained from Sigma (St. Louis, MO, USA). The stir bar coated with a 500 μm (24 μl) of PDMS (Gerstel, Müllheim an der Ruhr, Germany: the magnetic stirring rod incorporated in a glass jacket and coated with PDMS) was donated from Yokogawa Analytical Systems Inc. (Tokyo). Other common chemicals used were of analytical-reagent grade. Whole blood samples were obtained from healthy subjects.

SBSE procedure

To a 1-ml sample of human whole blood containing caffeine, theophylline and pentifylline as an internal standard (IS), was added 1 ml of 1 M perchloric acid solution for deproteinization. After stirring vigorously with a vortex mixer for 3 min, the sample was centrifuged at 3,000 rpm for 5 min. The clear supernatant was decanted into a 10-ml glass vial. The pH of the supernatant was adjusted to about 7.5 with 5 M KOH solution, and the vial was crimped with a Teflon-coated silicone rubber septum. The stir bar was immersed directly into the sample solution in the vial to allow adsorption of the compounds. After immersion with continuous stirring for 60 min at 300 rpm, the stir bar was removed from the sample solution with forceps, dried with a lint-free tissue and transferred to a glass TD tube.

TD-GC-MS conditions

TD-GC-MS analysis was performed by use of a Gerstel TDS-2 thermodesorption system (Gerstel) equipped with a Gerstel CIS-4 programmable temperature vaporization (PTV) injector (Gerstel) and an Agilent 6890 gas chromatograph with a 5973 mass-selective detector (MSD) (Agilent Technologies, Little Falls, DE, USA). The CIS-4 PTV injector was used for cryofocusing of the analytes thermally desorbed from the stir bar. The TD tube containing the stir bar was placed in the TD unit, where the stir bar was thermally desorbed by programming the TDS-2 from 20 (1 min hold) to 250°C (5 min hold) at 60°C/min. The desorbed compounds were cryofocused in the CIS-4 PTV injector at −100°C. The CIS-4 PTV injector was then programmed from −100 (0.5 min hold) to 300°C (10 min hold) at 12°C/sec to inject trapped compounds into a GC capillary column. Injection was performed in the splitless mode and the splitter was opened after 3 min. The GC separation was made with an HP-5 fused silica capillary column (30 m x 0.25 mm i.d., film thickness 1.0 μm, J & W Scientific, Folsom, CA, USA), and the column temperature was programmed at 10°C/min from 60 (3 min hold) to 295°C (2 min hold). Helium was used as carrier gas at a flow rate of 1.2 ml/min.

The MSD was operated in the positive electron impact mode with the ionizing energy of 70 eV,

generating full scan spectra between 29 and 300 amu at 2.73 scans/sec. The ion source temperature was set at 230 °C. The following ions were monitored (quantitation ions shown by bold letter): caffeine **194**, 109, 82, 67 and 55; theophylline **180**, 95 and 68; pentiphylline 264, 193, **180**, 137 and 109.

Samplings after coffee intake

A healthy male subject (body weights: 66 kg) abstained from caffeine-foods and beverages for 10 days before the study. He was given 200 ml of freshly brewed coffee containing 52.6 mg caffeine. Whole blood was collected from the subject 60 min after the ingestion and stored at −20 °C until assays.

Results and discussion

Figure 1 shows mass chromatograms for non-extracted authentic compounds (4 ng each on column) dissolved in methanol directly spiked to the stir bar and for an SBSE extract from a whole blood sample, to which caffeine, theophylline and IS had been added. All compounds were separated from each other and gave sharp peaks under our TD-GC-MS conditions; the blank chromatograms gave no impurity peaks and no interfering peaks appearing around the test peaks (data not shown).

The calibration curves for caffeine and theophylline from whole blood showed good linearity in the ranges of 0.15-2.5 and 1.87-15 μg/ml, respectively; they were drawn against pentifylline as IS. The equations and regression coefficients were: $y=4.70\ x - 0.34$ and $r=0.9991$ for caffeine; and $y=28.1\ x + 0.28$ and $r=0.9999$ for theophylline. The detection limits (signal-to-noise ratio=3) under optimal conditions for caffeine and theophylline were 0.06 and 0.4 μg/ml, respectively, which sufficiently cover therapeutic concentrations of both compounds [11].

Figure 2 shows mass chromatograms for whole blood obtained from a 43-year-old healthy subject 60 min after ingestion of coffee. The IS (80 ng) had been added to the samples at the initial step. Sixty minutes after ingestion of coffee, the blood concentration of caffeine was 0.16 μg/ml.

The extraction efficiencies with the SBSE were calculated by comparing the peak areas obtained from the extracts of the spiked whole blood with those obtained by direct analysis of non-extracted authentic compounds dissolved in methanol, which was spiked to the stir bar placed in the TD tube (Table 1). The efficiencies of caffeine and theophylline were 0.04-0.14 %, and that of IS was 4.57 %. In our previous study, we reported that caffeine and theophylline could be extracted from human whole blood by use of solid-phase microextraction (SPME) with four SPME fiber coatings [12]; the polar carbowax/divinylbenzene (CW/DVB) fiber showed high efficiencies (0.02-0.04 %) for the compounds and the PDMS showed efficiency of 0.001-0.002 %. The fundamental principle of

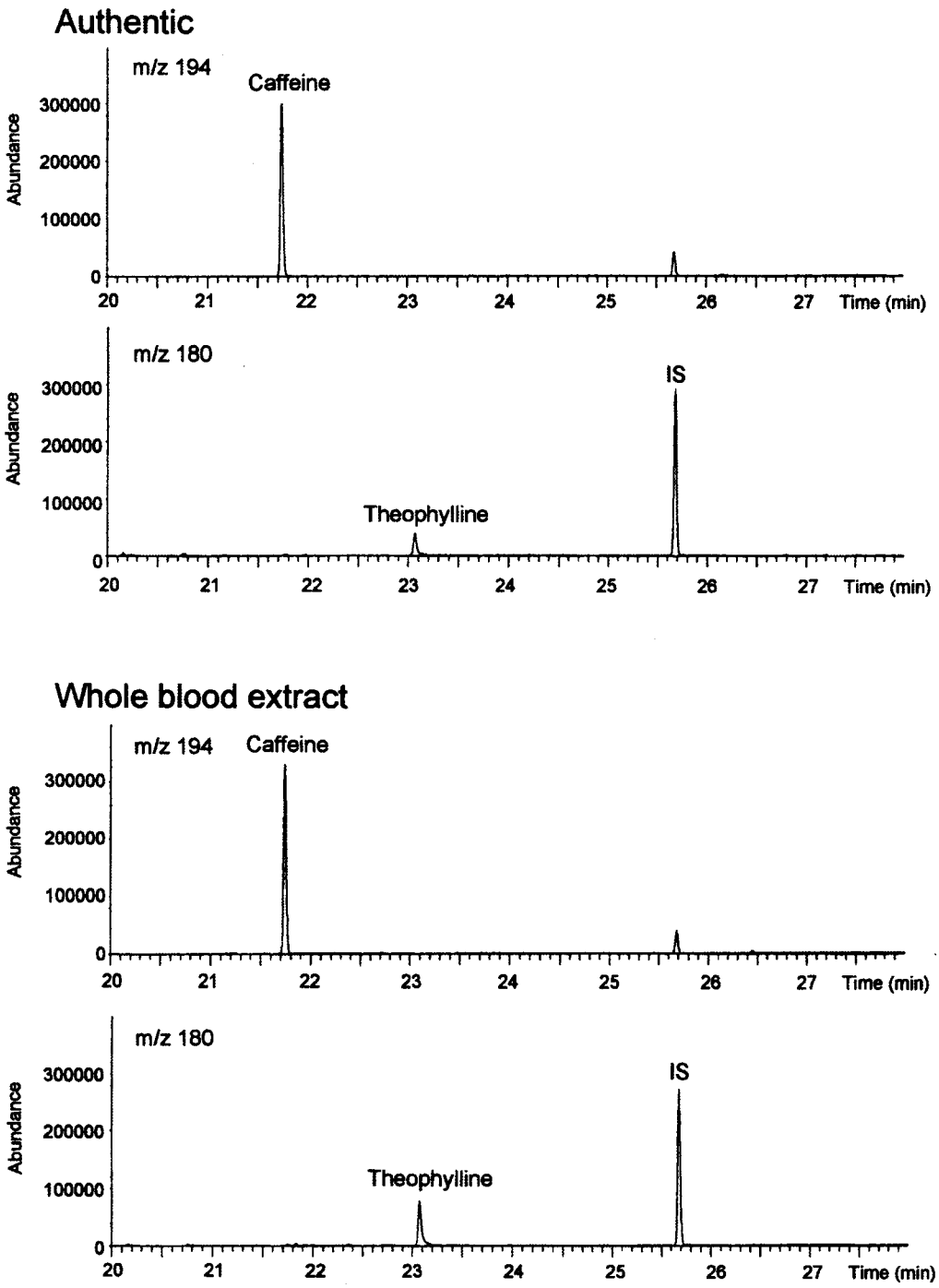

Fig. 1. Mass chromatograms of TD-GC-MS for caffeine, theophylline and IS extracted from human whole blood by SBSE. The amounts of analytes spiked to 1 ml of whole blood were: caffeine, 2.5 μg; theophylline, 15 μg; and IS, 80 ng. The stir bar was immersed into sample solution for 60 min at 50 °C. The amount of the authentic compounds was 4 ng each on column.

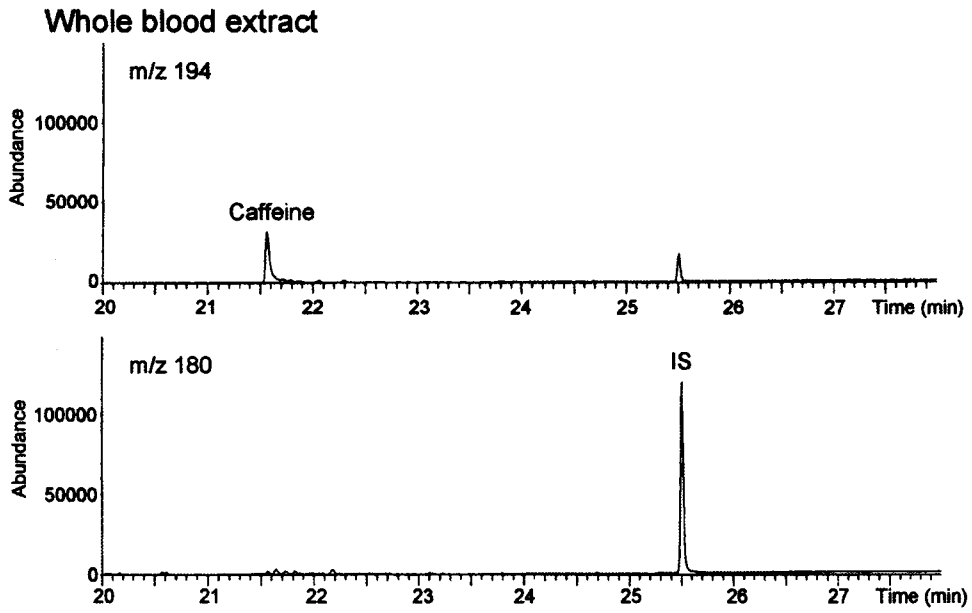

Fig. 2. Mass chromatograms TD-GC-MS for the SBSE extract of whole blood sample obtained 60 min after ingestion of coffee. The amount of pentifylline used as IS spiked into 1 ml of whole blood was 80 ng.

Table 1. Extraction efficiencies for caffeine, theophylline and IS from human whole blood by SBSE

Compound	Amount added (ng/ml)	Amount extracted (ng/ml)	Extraction efficiency, %
Caffeine	2,500	2.49	0.10
	625	0.89	0.14
Theophylline	15,000	6.70	0.04
	3,750	1.54	0.04
Pentifylline	80	3.54	4.57

The values are means of duplicate determinations. The efficiencies were calculated by comparing the peak areas obtained from the extracts of the spiked whole blood with those obtained by direct analysis of non-extracted authentic compounds dissolved in methanol spiked on the stir bar.

the present SBSE is the same as that of SPME [1, 13]. Although SPME is a simple and rapid technique, the small amount of coating of the SPME fiber results in low extraction efficiencies; the volume of PDMS coating in SPME fiber is 0.5 μl or less, whereas in SBSE the stir bar contains 24 μl of PDMS, resulting in much higher extraction efficiencies as compared to those in SPME. Therefore, the extraction efficiencies of the methylxanthines with the present SBSE using PDMS were 40-60 and 2-3 times higher than with the SPME using PDMS and CW/DVB, respectively. If Twister™ coated with 500 μm CW/DVB becomes available, the efficiencies may be far enhanced to give much higher sensitivity.

In conclusion, we have been able to extract and detect caffeine and theophylline in human whole blood by SBSE-TD-GC-MS. The present informations on the analysis of caffeine and theophylline by SBSE-TD-GC-MS suggest its applicability to a number of other drugs and poisons in the field of forensic toxicology.

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