DETECTION OF SOME PHENOTHIAZINES BY HEADSPACE SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY

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原　　報

DETECTION OF SOME PHENOTHIAZINES BY HEADSPACE SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY

Hiroshi SENO*[a], Takeshi KUMAZAWA[b], Akira ISHII[a], Masanobu NISHIKAWA[a], Kanako WATANABE[a], Hideki HATTORI[c] and Osamu SUZUKI[a]

[a] *Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431−31, Japan*
[b] *Department of Legal Medicine, Showa University School of Medicine, 1−5−8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan*
[c] *Department of Legal Medicine, Aichi Medical University, Nagakute-cho, Aichi 480−11, Japan*

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ヘッドスペース固相ミクロ抽出/ガスクロマトグラフィーによるフェノチアジン系薬物の検出

妹尾　洋[a]，熊沢武志[b]，石井　晃[a]，西川正信[a]，渡部加奈子[a]，服部秀樹[c]，鈴木　修[a]

[a]浜松医科大学法医学教室　〒431−31 静岡県浜松市半田町3600番地
[b]昭和大学医学部法医学教室　〒142 東京都品川区旗の台1−5−8
[c]愛知医科大学法医学教室　〒480−11 愛知県愛知郡長久手町大字岩作字雁又21番地

Summary

Phenothiazine drugs, such as promazine, chlorpromazine, triflupromazine, trimeprazine and methotrimeprazine, have been found extractable from whole blood and urine by headspace solid phase microextraction (SPME). Sample solutions were heated at 140 ℃ in the presence of NaOH, and then an SPME fiber was exposed in the headspace of a vial. Immediately after the fiber was pulled out, they were analyzed by gas chromatography with a flame ionization detector. Recoveries were 0.90−1.67 and 13.1−22.2 % for whole blood and urine samples, respectively. The calibration curves for the drugs extracted by SPME from whole blood was linear in the range of 0.5−5 μg/ml. The detection limits were 0.1−0.2 μg/ml for whole blood and 0.01−0.02 μg/ml for urine.

Key words: Solid phase microextraction (SPME); Headspace method; Gas chromatography; Phenothiazines; Chlorpromazine; Promazine; Triflupromazine; Trimeprazine; Methotrimeprazine

*Correspondence should be addressed to Hiroshi Seno.
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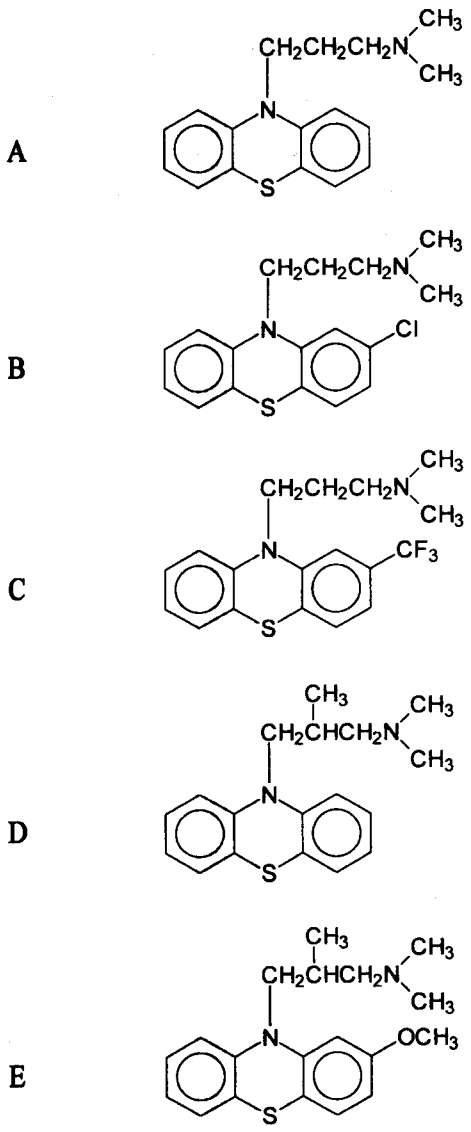

Fig. 1. Chemical structures of promazine (A), chlorpromazine (B), triflupromazine (C), trimeprazine (D) and methotrimeprazine (E).

Introduction

Phenothiazines are widely used as antipsychotic drugs, and their intoxication cases are not rare [1,2]. They are usually extracted from biological samples by liquid-liquid or solid-phase extraction [1,3,4]. Solid phase microextraction (SPME) was first introduced by Arthur and Pawliszyn in 1990 [5], and recently, some reports have appeared for extraction of compounds of forensic interest by SPME [6−8]. In this report, we present that some phenothiazines are extractable from whole blood and urine by headspace SPME.

Experimental

Materials

Chemical structures of five phenothiazines used in this study are presented in Fig. 1. Chlorpromazine hydrochloride was purchased from Sigama Chemical Co., St. Louis, MO, USA. Promazine hydrochloride was a gift from Banyu Pharmaceutical Co., Ltd., Tokyo; triflupromazine hydrochloride from E. R. Squibb & Sons, Princeton, NJ, USA; trimeprazine tartrate from Daiichi Seiyaku Co., Ltd., Tokyo; methotrimeprazine maleate from Yoshitomi Pharmaceutical Ind. Co., Ltd., Osaka. SPME devices and their 100 μl bonded polydimethylsiloxane fiber assemblies were purchased from Supelco Inc., Bellefonte, PA, USA; DB-1 fused silica capillary column (30 m × 0.32 mm ID, film thickness 0.25 μm) from J & W Scientific, Folsom, CA, USA. Other common chemicals were of the highest purity commercially available. Whole blood and urine were obtained from healthy subjects.

Extraction

One milliliter whole blood, 1 ml distilled water and 100 μl 10 M sodium hydrochloride were placed in a 7.5-ml vial. The vial was sealed with a silicone septum cap and shaken for 10 s. In the case of urine, 1 ml of a sample was mixed with 100 μl of 10 M sodium hydrochloride without adding distilled water. For addition tests, 5 μg and 0.5 μg each of the drugs were added to 1 ml of whole blood and urine, respectively. After heating the vial at 140 ℃ for 10 min on an aluminum block heater, the septum piercing needle of the SPME fiber holder was passed through the septum. The fiber was exposed in the headspace of the vial at 140 ℃ for 40 min. The fiber was retracted into the needle, pulled out from the vial, and then immediately inserted into the gas chromatography (GC) port.

GC conditions

GC was carried out on an HP 5890 Series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Palo Alto, CA, USA) and the DB-1 fused silica capillary column. Column temperature was set at 100 ℃ for 1 min for splitless injection, and then programmed from 100 ℃ to 270 ℃ at 10 ℃/min. Injection and detector temperatures were 280 ℃, and helium flow rate 3 ml/min.

Results and discussion

Figure 2 shows gas chromatograms for the authentic compounds (0.1 μg of each drug on column) dissolved in methanol, headspace SPME extracts and their backgrounds. To 1 ml of whole blood or urine, 5 μg or 0.5 μg of each drug was added, respectively. The retention times were 14.0, 14.7, 14.9, 16.4 and 16.6 min for triflupromazine, trimeprazine, promazine, chlorpromazine and methotrimeprazine, respectively. The peak of triflupromazine overlapped a small impurity peak for urine sample, but it gave almost no problems. The recoveries are listed in Table 1; they were calculated by measuring the peak areas for the SPME extracts against those for the authentic compounds.

It was reported that blood concentrations of chlorpromazine in the range of 0.5−2 μg/ml were associated with toxic effects and concentrations of 2 μg/ml or greater may be lethal [9]. The detection limits of the phenothiazines used in this study were about 0.1−0.2 and 0.01−0.02 μg/ml for whole blood and urine, respectively. The calibration curve for the drugs extracted by SPME from whole blood was linear in the range of 0.5−5 μg/ml. Thus the present method is applicable to the measurement of the drugs at toxic levels.

This is the first report to measure phenothiazines from biological fluids with use of SPME. SPME is a simple extraction method and gives cleaner extracts than liquid-liquid or solid-phase extractions; it seems useful in forensic and clinical toxicology.

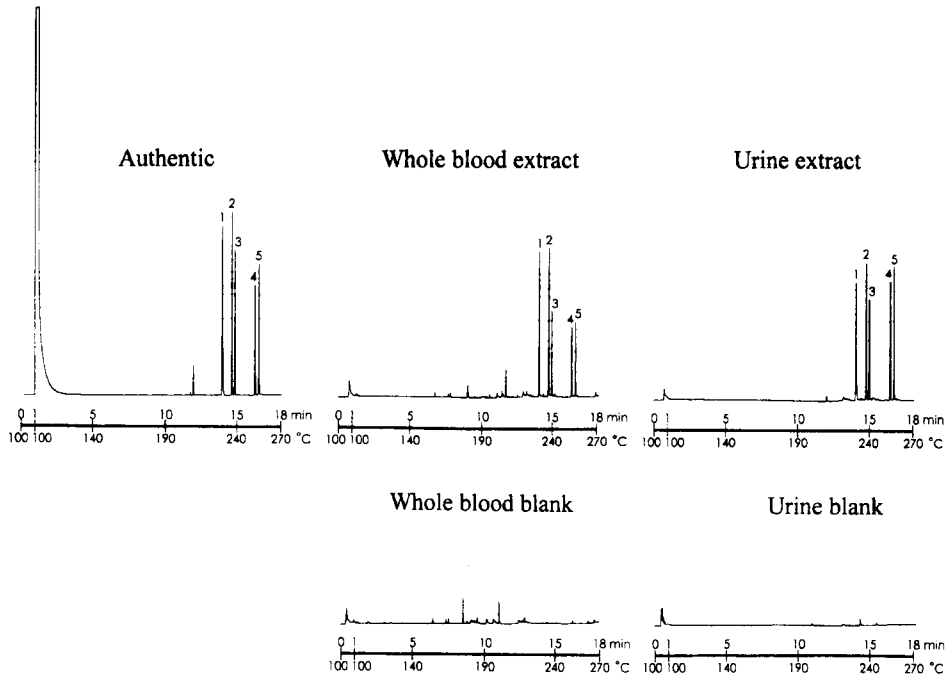

Fig. 2. Gas chromatograms for non-extracted authentic drugs dissolved in methanol (100 ng each on column), extracts from human whole blood (5 μg/ml for each drug) and urine (0.5 μg/ml for each drug) using headspace SPME, and their backgrounds. 1: triflupromazine; 2: trimeprazine; 3: promazine; 4: chlorpromazine; 5: methotrimeprazine.

Table 1. Recoveries of five phenothiazines extracted from human body fluids by headspace SPME

Compound	Recovery (%)	
	Whole blood	Urine
Promazine	1.01	13.1
Chlorpromazine	1.07	22.2
Triflupromazine	1.67	15.1
Trimeprazine	1.42	13.8
Methotrimeprazine	0.90	19.4

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