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Rapid isolation of some antiepileptic hydantoins and their analogues with Sep-Pak C_{18} cartridges and capillary gas chromatography with splitless injection

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Summary. A simple and rapid method for isolation of seven antiepileptics (2 hydantoin, 2 oxazolidin, and 3 suximide derivatives) from urine and plasma is presented. Urine and plasma (1 ml) samples containing seven antiepileptics were mixed with distilled water (4 ml), and the sample solution was poured into a pretreated Sep-Pak C_{18} cartridge; this was washed with water and chloroform/methanol was passed through it to elute the antiepileptics. The eluate was mixed with isoamyl acetate and evaporated under a stream of N_2. The drugs were detected by gas chromatography with fused silica capillary columns, splitless injection and flame ionization detection. Separation of the seven antiepileptics from each other and from impurities was satisfactory with the use of an SPB-1 capillary column. The detection limit for the seven antiepileptics with the present method was 0.1–1.0 μg/ml urine or plasma. The recovery of the drugs from urine and plasma was more than 70% and 50%, respectively.

Key words: Toxicology, hydantoins – Antiepileptics, capillary gas chromatography – Sep-Pak C_{18} cartridges

Zusammenfassung. Es wird eine einfache und schnelle Methode zur Extraktion und zum gaschromatographischen Nachweis von sieben Antiepileptika (2 Hydantoin-, 2 Oxazolidin- und 3 Succinimid-Derivaten) in Harn und Plasma beschrieben. Harn und Plasma (1 ml) werden mit dest. Wasser (4 ml) versetzt und auf eine vorbehandelte Sep-Pak C_{18} Kartusche gegeben. Nach Waschen mit dest. Wasser werden die Verbindungen mit Chloroform/Methanol eluiert. Das Eluat wird mit Isoamylacetat versetzt und unter N_2 eingeengt.

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Dann erfolgt die gaschromatographische Trennung: Quarzkapillarsäule, splitlose Injektionstechnik und FID. Mit der SPB-1 Kapillarsäule gelang eine gute Trennung der sieben Antiepileptika voneinander und von weiteren Substanzen aus der Matrix. Unter den angegebenen Analysebedingungen betrug die Nachweisgrenze 0.1–1.0 µg/ml Harn oder Plasma. Die Wiederfindungsraten lagen bei den Harnanalysen über 70%, im Plasma über 50%; bei einigen wurden Wiederfindungsraten von mehr als 100% ermittelt.

Schlüsselwörter: Toxikologie, Hydantoin – Antiepileptika, Kapillar-Gaschromatographie – Sep-Pak C_{18} Kartusche

Introduction

Antiepileptic hydantoins and their analogues are highly toxic and are occasionally encountered in forensic cases of suicide or accidental ingestion. In this paper, we present a simple and rapid isolation method for antiepileptic hydantoins and their analogues using Sep-Pak C_{18} cartridges and their detection by capillary gas chromatography (GC) with splitless injection in their underivatized forms.

Materials and methods

Materials

Phensuximide and methsuximide were obtained from Warner-Lambert (Ann Arbor, Mich., USA); ethotoin, phenytoin and trimethadione from Dainippon Pharmaceutical Co. (Osaka); ethosuximide from Eisai (Tokyo); and paramethadione from U.S.P.C (Rockville, Md., USA). Other chemicals used were of analytical grade. Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, Mass., USA): fused silica narrow-bore capillary columns (SPB-1, 15 m × 0.35 mm i.d., film thickness 1.0 µm; and OV-17, 15 m × 0.32 mm i.d., film thickness 0.1 µm) from Supelco (Bellefonte, Pa., USA) and Quadrex (New Haven, Conn., USA), respectively. Urine and plasma samples were obtained from healthy Japanese subjects.

Methods

GC conditions. GC was carried out on a Shimadzu GC-4CM instrument with two different fused silica narrow-bore capillary columns, SPB-1 and OV-17, flame ionization detection and a splitless/split injector. The GC conditions were: column temperature 80–300°C (10°C/min) for the SPB-1 column and 80–280°C (10°C/min) for the OV-17 column; injection temperature 300°C, nitrogen (carrier gas) flow rate 2 ml/min and make-up gas flow rate 30 ml/min for both columns. The samples were injected splitless at a column temperature of 80°C and the splitter was opened after 30 s.

Isolation of antiepileptics. Sep-Pak C_{18} cartridges were pretreated by passing through 10 ml of chloroform/methanol (9:1), 10 ml acetonitrile and 20 ml distilled water. For new cartridges, this procedure was repeated more than twice to reduce background noise. Cartridges could be re-used more than ten times when this procedure was carried out once after each usage.

Urine or plasma samples (1 ml) containing a mixture of seven antiepileptic hydantoins and their analogues (5 µg each) were mixed with 4 ml distilled water and then poured into the pretreated cartridge. This was then washed with 10 ml distilled water followed by 3 ml chloroform/methanol (9:1) to elute the antiepileptics from the cartridge. After addition of 100 µl isoamyl acetate [1, 2], the organic layer was evaporated to 100 µl under a stream of nitrogen in less than 30 min. A 1 µl aliquot of the concentrated solution was used for GC analysis.

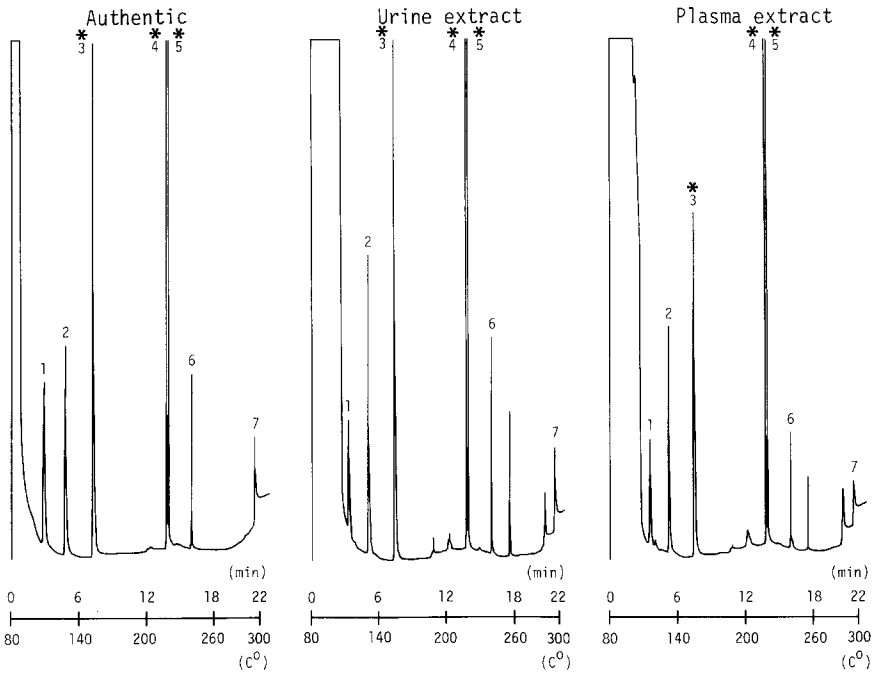

Fig. 1. Narrow-bore capillary GC (SPB-1 column) for antiepileptics isolated from human urine and plasma by means of Sep-Pak C_{18} cartridges. *1,* trimethadione; *2,* paramethadione; *3,* ethosuximide; *4,* methsuximide; *5,* phensuximide; *6,* ethotoin; *7,* phenytoin. GC was carried out with a fused silica narrow-bore capillary column (SPB-1, 15 m × 0.35 mm i.d., film thickness 1.0 μm). The mixture of seven antiepileptics (5 μg each) was added to 1 ml of urine and plasma. * Peaks 3, 4 and 5 have been scaled up to magnify other small peaks. The real ratios of heights for peaks 3, 4 and 5 were 100%, 359% and 353% for authentic, 153%, 400% and 394% for urine extract and 60%, 388% and 388% for plasma extract

Results

Figure 1 shows gas chromatograms obtained with a non-polar SPB-1 capillary column for seven drugs that had been added to urine and plasma samples. Most drugs could be satisfactorily distinguished from each other and from impurities on the gas chromatograms. The retention times and recoveries of the seven antiepileptics are shown in Table 1. Recovery of the drugs from the urine and plasma samples was more than 70% and 50%, respectively. The recovery of paramethadione (peak 2), ethosuximide (peak 3), methsuximide (peak 4), phensuximide (peak 5) and ethotoin (peak 6) from the urine extract and paramethadione (peak 2), methsuximide (peak 4) and phensuximide (peak 5) from the plasma extract was more than 100%.

To check background reactions, urine and plasma (1 ml each) were treated as above in the absence of antiepileptics and subjected to GC with a SPB-1 column (Fig. 2). The urine extract gave impurity peaks at column temperatures of approximately 255 and 285°C. The plasma extract also gave impurity peaks at 255, 270 and 285°C. Except for these peaks, the background noise was generally low, especially below 200°C.

Table 1. Retention times and recoveries of hydantoins and their analogues from urine or plasma[a]

Drug	Retention time (min)	Recovery (%)	
		Urine	Plasma
Trimethadione	3.2	70	53
Paramethadione	5.0	143	109
Ethosuximide	7.4	153	60
Methsuximide	13.6	111	108
Phensuximide	13.9	112	110
Ethotoin	15.9	124	67
Phenytoin	21.4	98	53

[a] Five micrograms of each drug was added to 1 ml urine or plasma. The SPB-1 narrow-bore capillary column (15 m × 0.35 mm i.d., film thickness 1.0 μm) was used. Column temperature was 80–300°C (10°C/min); nitrogen flow rate was 2 ml/min. The experiments were repeated 4–6 times and typical data are presented in this table. Variation of each recovery value was less than 20%

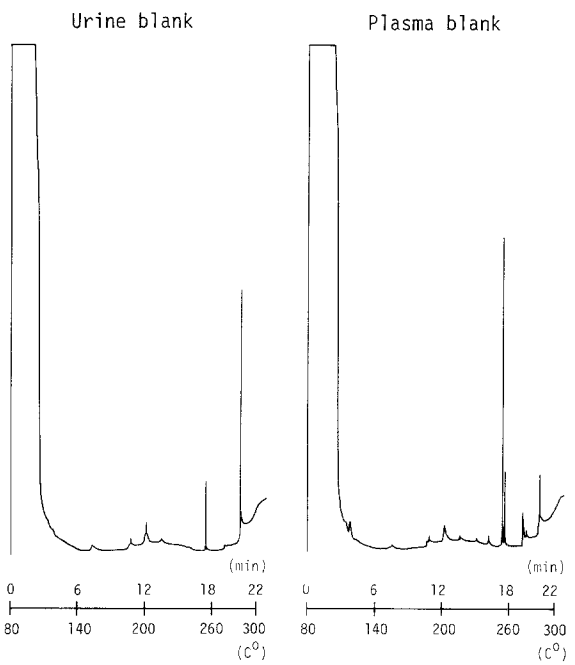

Fig. 2. Narrow-bore capillary GC (SPB-1 column) showing background levels obtained from human urine and plasma (1 ml each) in the absence of antiepileptics by means of Sep-Pak C_{18} cartridges

An intermediately polar OV-17 capillary column was also tested as shown in Fig. 3, and a greater separation of methsuximide (peak 4) and phensuximide (peak 5) was found than with the SPB-1 column (Fig. 1). Impurity peaks also appeared in background levels of urine and plasma extracts (data not shown) at column temperatures of approximately 200, 220–230 and 275°C. These impurity peaks overlapped the peaks for phensuximide (peak 5) and ethotoin (peak 6).

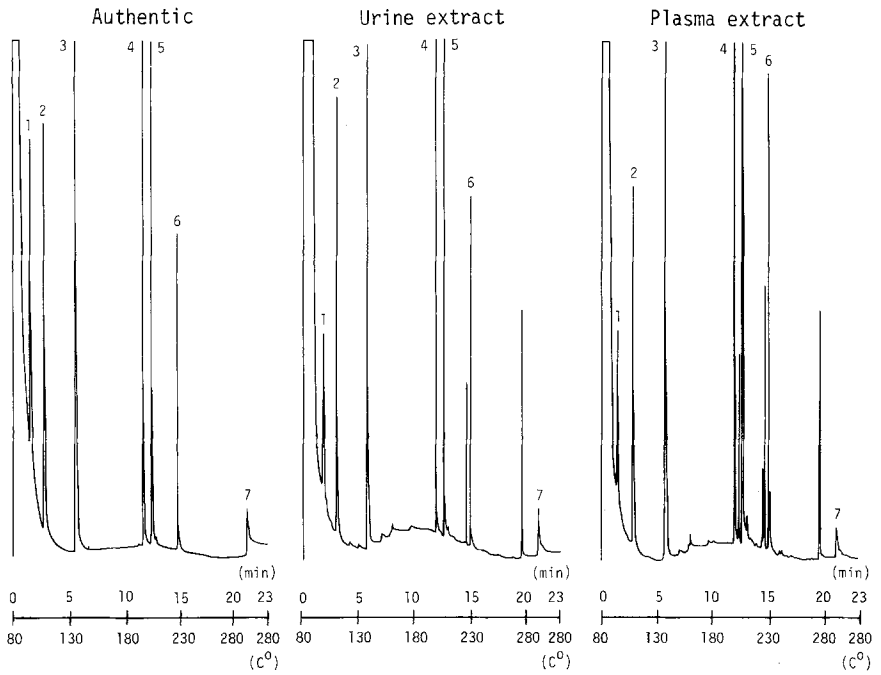

Fig. 3. Narrow-bore capillary GC (OV-17 column) for antiepileptics isolated from human urine and plasma by means of Sep-Pak C_{18} cartridges. The key numbers are the same as specified in Fig. 1. GC was carried out with a fused silica narrow-bore capillary column (OV-17, 15 m × 0.32 mm i.d., film thickness 0.1 μm). The mixture of seven antiepileptics (5 μg each) was added to 1 ml of urine and plasma

The response of the detector differed markedly with different compounds (Figs. 1 and 3). The highest sensitivity was obtained with methsuximide (peak 4) and phensuximide (peak 5) and the lowest sensitivity with phenytoin (peak 7). The detection limits for the suximides and phenytoin were 1 and 10 ng, respectively, in the injected volume (0.1 and 1.0 μg/ml urine or plasma).

Discussion

In the present report, a method for the isolation of antiepileptic hydantoins and their analogues by means of Sep-Pak C_{18} cartridges is presented. The advantages of the cartridges are that the analytical procedure is much simpler and faster than when organic solvents are used and much cleaner extracts are obtained [3]. To our knowledge, only one report dealing with the isolation of antiepileptics with Sep-Pak C_{18} cartridges has been published [3]. However, only phenytoin and ethosuximide were examined using high-performance liquid chromatography and the drugs were eluted from the cartridge with acetonitrile. In the present investigation chloroform/methanol (9:1) was used as the elution solvent. Evaporation of the 3 ml chloroform/methanol eluate to 0.1 ml required less than 30 min, while evaporation of acetonitrile or methanol eluates [4] requires more than 1.5 h.

Antiepileptics contain many functional groups in their structures, and in the underivatized forms, are generally not suitable for GC analysis with packed columns. Even using capillary GC, hydantoins must be methylated or alkylated before analysis [5, 6]. However, derivatization is usually undesirable for analysis of unknown toxic substances, because the analysis then becomes much more complicated. In the present experiments, seven compounds of hydantoins and their analogues could be detected by narrow-bore capillary GC without any derivatization (Figs. 1 and 3), probably because of their low adsorption to the capillary columns.

Benzodiazepines [7] and butyrophenones [8] could be detected without any decomposition by means of wide-bore capillary GC, because they were more stable during passage through the wide-bore column due to faster flow (20 ml/min) and correspondingly shorter exposure to heat. Benzodiazepines and butyrophenones are easily decomposed during slow flow through narrow-bore capillary columns [9]. However, hydantoins and their analogues have been found to be relatively resistant to heat decomposition during GC analysis and thus narrow-bore capillary GC was preferred to wide-bore capillary GC because of its higher resolution.

The split injection mode has been used exclusively for narrow-bore capillary GC analysis of hydantoins in earlier work reported [5, 6]; however, in the present experiments, a splitless/split injection system was used for analysis of the antiepileptics. All samples were injected splitless at low column temperatures and thus accumulated inside the column. The splitter was then opened to supply a slow carrier gas flow appropriate for the narrow-bore column before elevation of the column temperature. This system thus causes no loss of samples when they are injected into a GC port. The present splitless/split system of narrow-bore capillary GC possesses both high sensitivity and high resolution.

The recovery of some drugs in urine and plasma extracts exceeded 100% with the SPB-1 column (Fig. 1, Table 1). This phenomenon was not due to contamination by impurities; the gas chromatograms for the extracts without any addition of drugs did not show any impurity peaks where the drugs would be expected to appear (Fig. 2). This phenomenon can be due to certain impurities contained in the extracts, which may act to stabilize the drugs or to prevent them from adsorbing onto the column.

The detection limit for seven antiepileptics by the present method was 1–10 ng in an injected volume (0.1–1.0 μg/ml urine or plasma). The drugs are clinically effective at high doses (0.2–3 g p.o.), and therapeutic plasma concentrations have been reported to be in the range of 3.9–100 μg/ml [10]. Thus, our analytical method for seven antiepileptics is sufficiently sensitive even for therapeutic monitoring of low plasma levels in clinical pharmacology, in addition to the detection of high levels in forensic and clinical toxicology.

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