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Rapid isolation with Sep-Pak C_{18} cartridges and wide-bore capillary gas chromatography of some butyrophenones

H. Seno, O. Suzuki, T. Kumazawa, and M. Asano

Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan

Summary. A simple and rapid method for isolation of five butyrophenones with Sep-Pak C_{18} cartridges from human samples, and their wide-bore capillary gas chromatography (GC), are presented. The GC was made by both flame ionization and electron capture detections. The drugs contained in alkaline samples were directly applied to the cartridges and eluted with chloroform/isopropanol (9:1). The recoveries with use of the cartridges were excellent for most drugs in both urine and plasma samples. We can recommend the Sep-Pak C_{18} cartridges for isolation of butyrophenones because of simplicity and rapidity, and also wide-bore capillary GC because of high sensitivity and low decomposition of drugs during passage through the column.

Key words: Butyrophenones, Sep-Pak C_{18} cartridges – Wide-bore capillary gas chromatography, butyrophenones

Zusammenfassung. Es wird eine einfache und rasche Methode zur Isolierung von fünf Butyrophenonderivaten mittels Sep-Pak C_{18} Säulen aus Humanproben sowie deren gaschromatographische Bestimmung mit wide-bore Kapillaren beschrieben. Bei der Gaschromatographie kamen die FID- und ECD-Detektion zum Einsatz. Die im alkalisierten Untersuchungsmaterial enthaltenen Substanzen wurden direkt auf die Säulen gegeben und mit Chloroform/iso-Propanol (9:1) eluiert. Die mit den Säulen erzielten Wiederfindungsraten waren für die meisten Arzneistoffe, sowohl beim Einsatz von Urin als auch bei Plasmaproben, hervorragend. Die Sep-Pak C_{18} Säulen können wegen ihrer einfachen und schnellen Anwendung für die Isolierung von Butyrophenonderivaten empfohlen werden, ebenso wie die wide-bore Kapillar-Gaschromatographie mit ihrer hohen Empfindlichkeit

Offprint requests to: H. Seno

und geringen Gefahr der Zersetzung der Proben während des Trennvorganges.

Schlüsselwörter: Butyrophenone, Sep-Pak C_{18}-Säulen – Wide-bore Kapillar-Gaschromatographie, Butyrophenone

Introduction

Butyrophenones are one of the major tranquilizers and are occasionally encountered in forensic science practice. Their analysis in blood of psychiatric patients is also required to provide the best effective doses [1–5].

In this paper, we demonstrate a simple and rapid isolation method using Sep-Pak C_{18} cartridges for butyrophenones and their wide-bore capillary gas chromatography (GC).

Materials and methods

Materials

Haloperidol-HCl was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; moperone-HCl from Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan; bromperidol from Yoshitomi Pharmaceutical Ind. Co., Ltd., Osaka, Japan; spiperone and pipamperone-2HCl from Eisai Co., Ltd., Tokyo, Japan. Sep-Pak C_{18} cartridges were purchased from Waters Associates, Milford, MA, USA; and fused silica wide-bore capillary column (SPB-1, 10 m × 0.53 mm i.d., film thickness 1.5 μm) from Supelco, Inc., Bellefonte, PA, USA. Other common chemicals used were of the highest purity commercially available.

Urine and plasma obtained from healthy subjects were also used for the extraction experiments.

Isolation of butyrophenones

Isolation of butyrophenones from biologic impurities with use of Sep-Pak C_{18} cartridges was made as follows. To 1 ml urine or plasma containing butyrophenones (10 μg or 1 μg each) were added 1 ml carbonate buffer solution (pH 9.8, 5 g Na_2CO_3 and 5 g $NaHCO_3$ dissolved in 100 ml water), 3 ml water, and 1 g NaCl. After activating a Sep-Pak C_{18} cartridge by passing 10 ml chloroform/isopropanol (9:1), 20 ml acetonitrile, and then 20 ml water, the sample solution was poured into the cartridge at a flow rate not greater than 5 ml/min. The sample tube was rinsed with 2 ml water, which was also poured into the same cartridge. The cartridge was washed with 10 ml water; finally, 3 ml chloroform/isopropanol (9:1) was passed through it to elute butyrophenones and collected in a vial. The eluate consisted of a major amount of an organic layer (lower phase) and a small amount of an aqueous layer (upper phase); the latter was discarded by aspiration with a Pasteur pipette. The organic layer was evaporated to dryness under a stream of nitrogen and the residue dissolved in 100 μl methanol. The 1-μl aliquot of it was subjected to the GC analysis.

The Sep-Pak cartridge could be re-utilized several times by repeating washings with 10 ml chloroform/isopropanol (9:1), 20 ml acetonitrile and 20 ml water.

GC Conditions

GC was carried out on a Shimadzu GC-4CM instrument with a fused silica wide-bore capillary column (SPB-1, 10 m × 0.53 mm i.d., film thickness 1.5 μm), to which a glass-made direct injection conversion kit (Supelco, Inc., Bellefonte, PA, USA) had been attached. GC was made

by flame ionization detection (FID) when 10 μg each of the drugs was added to the 1 ml samples, and by electron capture detection (ECD) when 1 μg each of the drugs was added to them. The GC conditions were: injection temperature 280°C and column temperature 170°–290°C (10°C/min) for FID; injection temperature 280°C, and column temperature 190–290°C (10°C/min) for ECD; and nitrogen flow rate 20 ml/min.

Results

Figure 1 shows SPB-1 wide-bore capillary gas chromatograms with use of FID for the mixture of five butyrophenones, 10 μg of each, which had been added to 1 ml urine or plasma, and directly extracted with Sep-Pak C_{18} cartridges. Separation of the drugs from biologic impurities was satisfactory for both urine and

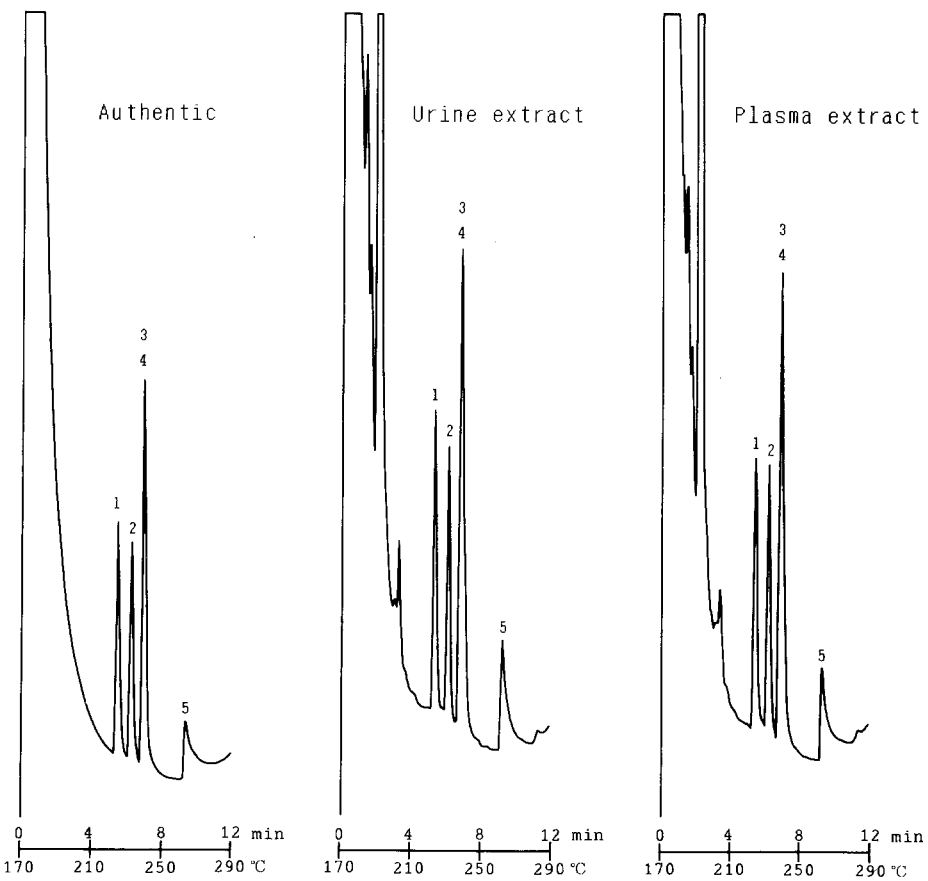

Fig. 1. Wide-bore capillary GC with FID for butyrophenones isolated from human urine and plasma with use of Sep-Pak C_{18} cartridges. *Key:* 1, moperone; 2, haloperidol; 3, bromperidol; 4, pipamperone; and 5, spiperone. GC was carried out with a fused silica wide-bore capillary column (SPB-1, 10 m × 0.53 mm i.d., film thickness 1.5 μm). Its conditions were: column temperature 170°–290°C (10°C/min) and nitrogen flow rate 20 ml/min. The mixture of five butyrophenones (10 μg each) was added to 1 ml urine or plasma

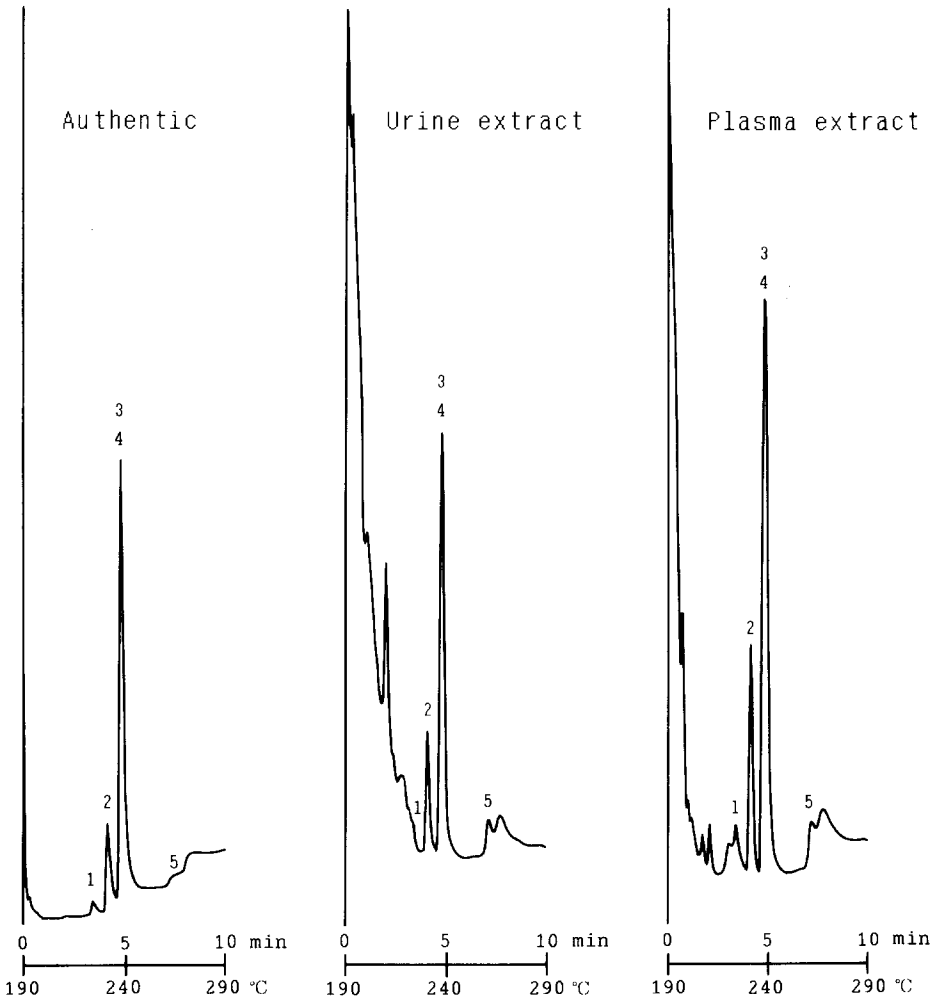

Fig. 2. Wide-bore capillary GC with ECD for butyrophenones isolated from human urine and plasma with use of Sep-Pak C_{18} cartridges. *Key:* 1, moperone; 2, haloperidol; 3, bromperidol; 4, pipamperone; and 5, spiperone. GC was carried out with a fused silica wide-bore capillary column (SPB-1, 10 m × 0.53 mm i.d., film thickness 1.5 μm). Its conditions were: column temperature 190°–290°C (10°C/min) and nitrogen flow rate 20 ml/min. The mixture of five butyrophenoses (1 μg each) was added to 1 ml urine or plasma

plasma. Bromperidol and pipamperone (Peaks 3, 4) could not be separated under the present GC conditions. The retention times for moperone, haloperidol, bromperidol, pipamperone, and spiperone were 5.5, 6.2, 6.9, 6.9, and 9.4 min, respectively, with a column temperature of 170–290°C (10°C/min) and a nitrogen flow rate of 20 ml/min. The recoveries were very excellent for all drugs added to urine or plasma and exceeded 100%; more than 120% for moperone, haloperidol, bromperidol, and pipamperone, and more than 150% for spiperone. These phenomena were not due to contamination by impurities

because the gas chromatograms for the extracts without any addition of drugs did not show any impurity peak at each site where drugs were expected to appear.

Figure 2 shows the gas chromatograms with use of ECD for the mixture of the drugs, 1 μg of each, which was also added to 1 ml urine or plasma. The peak heights remarkably differed according to different compounds. Bromoperidol (Peak 3) gave a very strong peak. The recoveries were generally excellent also for the 1 μg drugs and most of them exceeded 100%.

Discussion

In this paper we have presented a simple and rapid isolation procedure for butyrophenones with use of Sep-Pak C_{18} cartridges. Butyrophenones have been isolated by extraction and washings with organic solvents [1, 4–6], and with Extrelut columns [7]. These extraction methods are much more complicated and time-consuming than the present Sep-Pak method.

During the preparation of this manuscript, Hayakari et al. [8] reported a similar technique prior to high-performance liquid chromatography (HPLC). However, they dealt with only haloperidol; they used Bond Elut C_{18}, which is probably made of the same material. Under their conditions, the elution of haloperidol from the cartridge was made with 1.6 ml acidified methanol. Thus, we have carefully compared the time required for evaporation of the present chloroform/isopropanol eluate with that of the acidified methanol eluate recommended by Hayakari et al. [8], under the stream of nitrogen. By their method, it took more than 2 h, while it completed within 30 min by our method (unpublished observation).

Packed GC columns were used in many reports [1, 2, 4–7] for analyses of butyrophenones. In 1979, Moulin et al. [3] used a narrow-bore capillary column (0.25 mm × 15 m, SE-30) for analysis of haloperidol. To our knowledge, our report is the first one to present wide-bore capillary GC for butyrophenones. This technique is recommendable because the drugs are relatively stable during passage through a wide-bore capillary column due to much faster flow inside the column and thus much shorter exposure to heat [9]. In addition, it is splitless, in contrast to the narrow-bore capillary GC, which makes the analysis much more sensitive.

In the present experiments, we have used a glass-made direct injection conversion kit for capillary GC. We also tested an injection kit made of metal (Shimadzu Seisakusho Ltd., Kyoto, Japan), which gave much smaller peaks especially for spiperone (unpublished observation). This result shows that butyrophenones are easily decomposed on contact with metals at high temperatures.

The peak of each drug added to urine or plasma was higher than that of the corresponding authentic compound (Figs. 1, 2), showing that the recoveries apparently exceeded 100%. This was not due to overlapping impurity peaks. This phenomenon can be explained by the possibility that certain impurities contained in the urine or plasma extracts act to stabilize and protect

butyrophenones from their decomposition during the passage through the column at high temperatures, or act to prevent the drugs from their adsorbing to the column.

The present isolation method for butyrophenones with use of Sep-Pak C_{18} cartridges, together with the wide-bore capillary GC, seems very useful in the fileds of forensic chemistry, clinical toxicology, and clinical pharmacology.

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