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ORIGINAL ARTICLE

Determination of Some Butyrophenones in Body Fluids by Gas Chromatography with Surface Ionization Detection

Hiroshi SENO[1], Takeshi KUMAZAWA[2], Akira ISHII[1], Keizo SATO[2] and Osamu SUZUKI[1]
[1]*Department of Legal Medicine, Hamamatsu University School of Medicine, Shizuoka 431-31, Japan*
[2]*Department of Legal Medicine, Showa University School of Medicine, Tokyo 142, Japan*

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Abstract. Haloperidol, moperone, pipamperone and bromperidol were found measurable with high sensitivity by gas chromatography (GC) with surface ionization detection (SID). The calibration curves using moperone as internal standard were linear in the range of 0.4–4 pmol on column for haloperidol, pipamperone, and bromperidol. The detection limit of the drugs was about 0.1 pmol on column. For their actual determination with forensic samples, a detailed procedure for their extraction from human whole blood and urine was established with Sep-Pak C_{18} cartridges. The recovery of the 4 drugs, which had been added to whole blood and urine, was more than 90%. Haloperidol in whole blood and urine of a schizophrenic patient, who had been administered with this drug (3 mg/day p.o.), could be quantitated by the present GC-SID, and the levels were 7.18 and 43.2 pmol/ml, respectively.

Key words: Toxicology, Butyrophenones, Haloperidol, Moperone, Pipamperone, Bromperidol, Gas chromatography, Surface ionization detection, Sep-Pak C_{18} cartridges

Introduction

Butyrophenones are widely used for schizophrenia and are frequently encountered in forensic science practice. Their analyses are being made by gas chromatography (GC)[1)2)], GC/mass spectrometry[3)~5)], high-performance liquid chromatography[6)] and radioimmunoassay[7)]. In 1985, surface ionization detection (SID) for GC was first introduced by Fujii and Arimoto[8)], and was suggested to be very sensitive and specific especially to tertiary amines. Application of GC-SID has recently started for some drug groups[9)~16)]. In this study, we report that haloperidol, moperone, bromperidol and pipamperone in biological samples can also be detected with high sensitivity by GC-SID.

Materials and Methods

Materials

Chemical structures of butyrophenones used in this study are shown in Fig. 1. Haloperidol-HCl was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka; moperone-HCl from Yamanouchi Pharmaceutical Co., Ltd., Tokyo; bromperidol from Yoshitomi Pharmaceutical Ind. Co., Ltd., Osaka; pipamperone-2HCl from Eisai Co., Ltd., Tokyo. Sep-Pak C_{18} cartridges were purchased from Waters Associates, Milford, MA, U.S.A.; and a DB-1 fused silica capillary column (15 m × 0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific, Folsom, CA, U.S.A. Other common chemicals were of the highest purity commercially available.

Whole blood and urine were obtained from healthy subjects and from a 76-year-old male schizophrenic patient to whom haloperidol (3 mg/day p.o.) had been administered for years; informed consent was obtained from the latter.

Isolation with Sep-Pak C_{18} cartridges

Procedures for pretreatment of Sep-Pak C_{18} cartridges and for extraction of drugs from whole blood and urine samples were the same as described in our previous paper[14)].

For the addition tests, the mixture of the 4 standard drugs (50 pmol each) dissolved in 100 μl methanol was added to 1 ml of whole blood or urine at the initial step of the experiments.

GC conditions

GC was carried out on a Shimadzu GC-14B gas

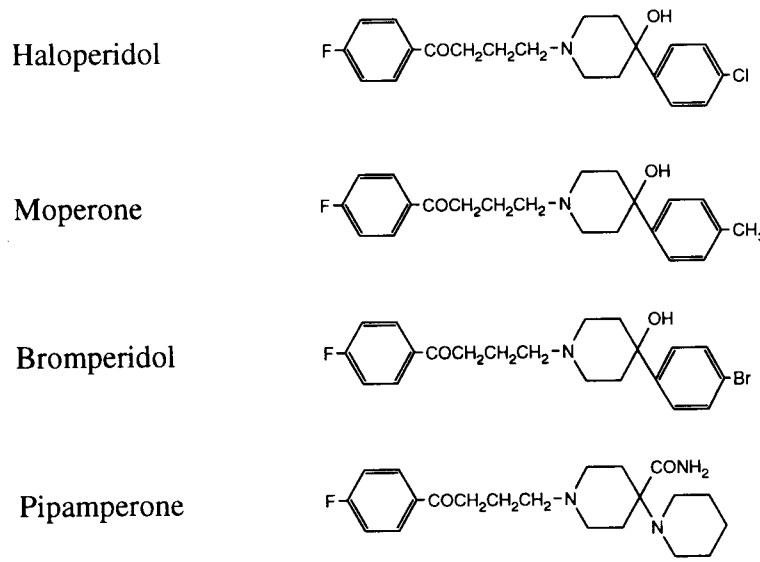

Fig. 1. Chemical structures of haloperidol, moperone, bromperidol and pipamperone.

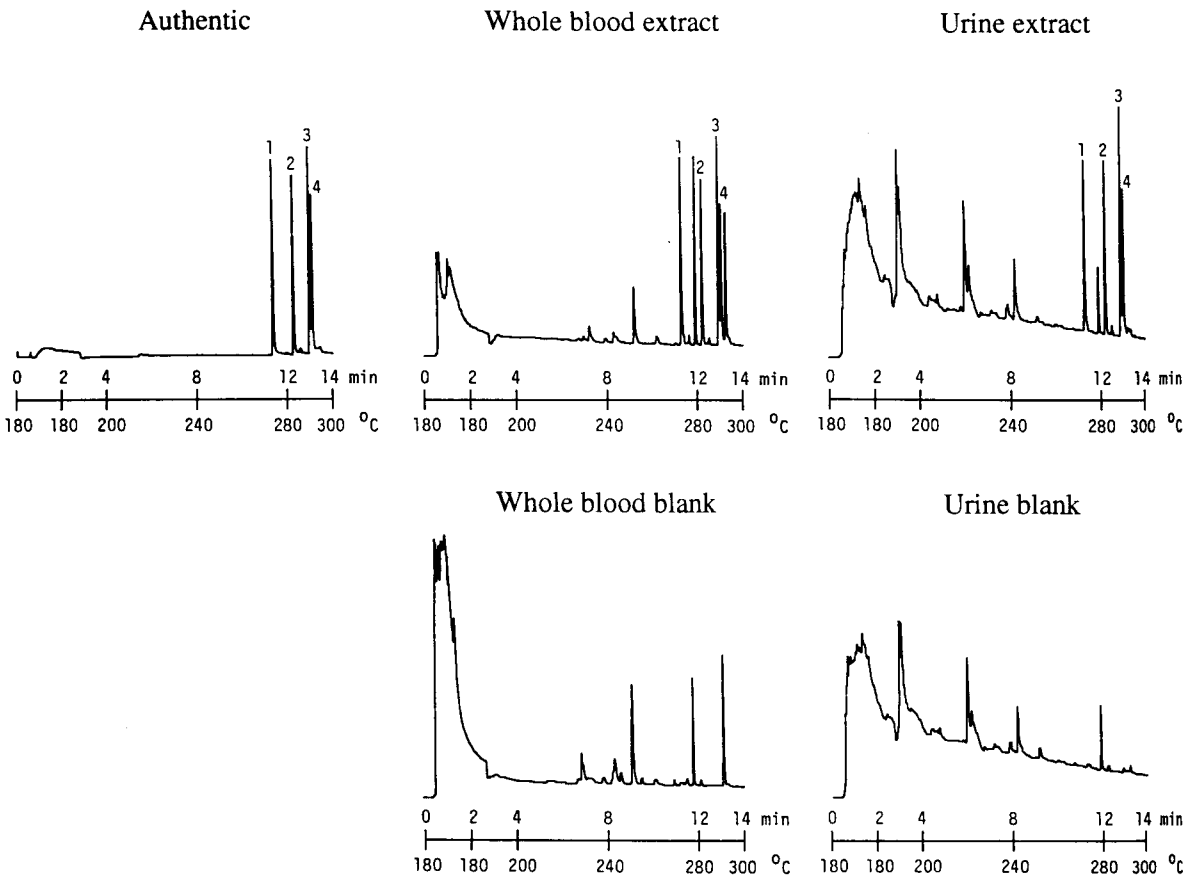

Fig. 2. Capillary GC-SID for moperone (Peak 1), haloperidol (Peak 2), pipamperone (Peak 3) and bromperidol (Peak 4) extracted from whole blood and urine, and for their backgrounds. The mixture of 50 pmol each of drugs was added to 1 ml of samples. GC was carried out with a fused silica DB-1 capillary column (15 m × 0.32 mm, i.d., film thickness 0.25 μm). GC conditions were: column temperature, 180–300°C (10°C/min); injection and detector temperature, 235°C; helium flow rate, 3 ml/min.

chromatograph equipped with SID. A DB-1 fused silica capillary column and a split-splitless injector were used. The GC conditions were: column temperature, 180–300°C (2 min hold at 180°C and 10°C/min); injection and detector temperature, 235°C; helium flow rate, 3 ml/min. The SID conditions were: heating current through the platinum emitter, 2.2 A; emitter temperature, *ca.* 600°C; ring electrode bias voltage, +200 V with respect to the collector electrode. The samples were injected in the splitless mode at 180°C of the column temperature and the splitter was opened after 2 min.

Results

Figure 2 shows gas chromatograms by GC-SID for the extracts of whole blood and urine samples obtained from healthy subjects with and without addition of the drugs (50 pmol each). Very small impurity peaks overlapped peaks 1 and 2 in whole blood sample and peaks 1-4 in urine sample, but their contamination gave almost no problems. The retention times were 11.2, 12.1, 12.9 and 13.0 min for moperone, haloperidol, pipamperone and bromperidol, respectively. The recovery of all drugs was more than 90% for whole blood and urine.

The calibration curves for haloperidol, pipamperone and bromperidol are shown in Fig. 3; moperone (50 pmol on column) was used as an internal standard (IS). Those for haloperidol and pipamperone showed linearity in the range of 0.2–4 pmol on column, and that for bromperidol in the range of 0.4–4 pmol. The equation and r values for the curves were: $y=0.967x + 0.00448$, $r=$

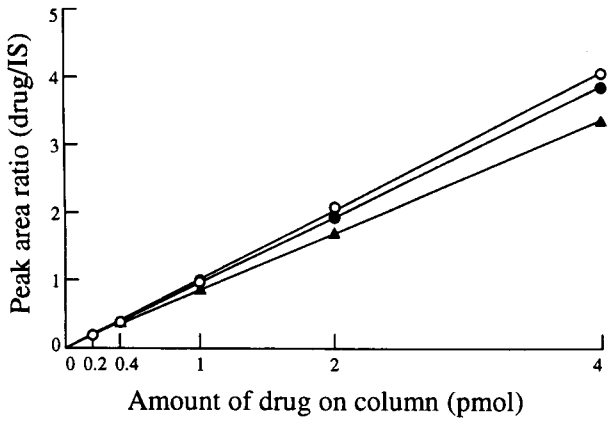

Fig. 3. Calibration curves by GC-SID for haloperidol (●), pipamperone (○), and bromperidol (▲). The vertical axis shows the peak area ratio of drug to IS (moperone, 1 pmol on column). GC conditions were as specified in Fig. 2.

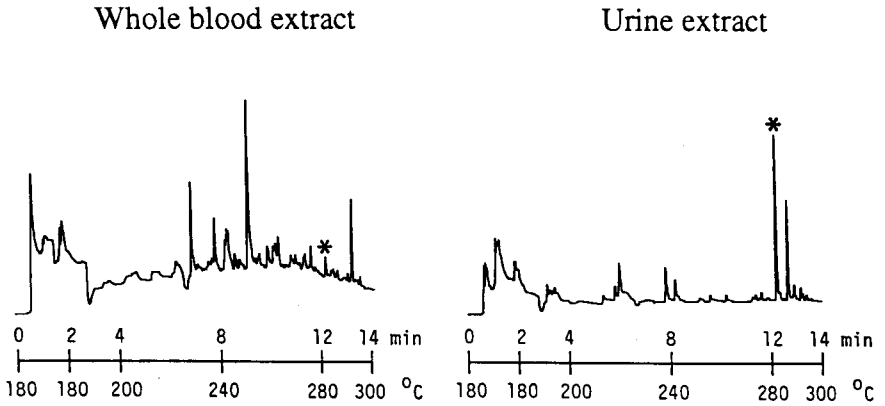

Fig. 4. Capillary GC-SID for the extracts of whole blood and urine obtained from a 76-year-old male schizophrenic patient, who received oral administration of 3 mg of haloperidol daily. The asterisks show the peaks of haloperidol.

0.99998 for haloperidol; y=1.02x − 0.0136, r= 0.99959 for pipamperone; y=0.833x + 0.00316, r=0.99978 for bromperidol. The detection limit of the drugs was about 5 pmol per ml of a sample (about 0.1 pmol on column).

Figure 4 shows gas chromatograms by GC-SID for the extracts of whole blood and urine obtained from a 76-year-old male schizophrenic patient, who had received 3 mg haloperidol daily. The 50-pmol aliquot of moperone was added to the same samples at the initial step of extraction to quantitate haloperidol; the levels were 7.18 and 43.2 pmol/ml for the whole blood and urine, respectively.

Discussion

In this paper, we have shown that butyrophenones can be detected by GC-SID with high sensitivity; the detection limit of haloperidol, moperone, pipamperone and bromperidol was about 0.1 pmol on column. Since these drugs have halogen groups in their structures, the sensitivity should be compared with that by GC-electron capture detection (ECD). Tyndale and Inaba reported that the detection limit of non-extracted haloperidol was 1 pmol on column by GC-ECD with a megabore fused silica capillary column[1]. In comparison with that by GC-nitrogen phosphorus detection in the literature[2], in which haloperidol was extracted from plasma and a packed column was used, the sensitivity of the present GC-SID is also about ten times higher than theirs.

In this study, we have used moperone as internal standard to measure other butyrophenones (Fig. 3). Moperone can also be measured against either of other butyrophenones as internal standard, conversely.

In the original paper on GC-SID by Fujii and Arimoto[8], tertiary amino compounds, such a tributylamine and triethylamine, were reported to give very high sensitivity, suggesting that a tertiary amino group with straight side chain structures gives the highest response by this method. However, during the progress of our studies on GC-SID, various tertiary amino compounds with ring structures, such as mepivacaine[10], dextromethorphan[14], dimemorphan[14] and pethidine[15], also gave relatively high response to SID. The high sensitivity obtained with the present butyrophenones is probably due to the piperidinyl group contained in common in this drug group (Fig. 1).

Therapeutic concentration of haloperidol in serum was reported to be below 0.05 μg/ml (about 133 pmol/ml) and toxic effects may occur with blood concentrations greater than about 0.05 mg/ml[17]. The detection limit obtained by GC-SID is below the therapeutic and toxic levels; actual measurement could be made by GC-SID for blood and urine samples obtained from a schizophrenic patient as shown in Fig. 4. Therefore, the present method seems very useful in all fields of forensic toxicology, clinical toxicology and clinical pharmacology.

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表面電離ガスクロマトグラフィーによる体液中ブチロフェノン類の測定

妹尾 洋[1]・熊沢 武志[2]・石井 晃[1]
佐藤 啓造[2]・鈴木 修[1]

[1]浜松医科大学法医学教室，[2]昭和大学医学部法医学教室

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摘要 ハロペリドール，モペロン，ピパムペロン，ブロムペリドールの4種のブチロフェノン系薬物につき表面電離検出ガスクロマトグラフィーによる微量分析を行つた．モペロンを内部標準として作成した検量線は，ハロペリドール，ピパムペロンならびにブロムペリドールでは0.4～4pmolで直線性を示し，検出感度は，注入量で約0.1pmolであつた．Sep-Pak C_{18} カートリッジを用いてこれらの薬剤をヒト全血及び尿から分離したところ回収率は良好で，問題となるような不純ピークとの重なりもなかつた．ハロペリドール（3 mg/day）を経口投与されている76歳の男性精神分裂病患者より採取した全血及び尿中ハロペリドールを本法によつて測定する事ができ，その濃度はそれぞれ7.18ならびに43.2pmol/mlであつた．