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Analyses of butyrophenones and their analogues in whole blood by high-performance liquid chromatography–electrospray tandem mass spectrometry

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Abstract

Five butyrophenones and two analogues contained in human whole blood have been analyzed by high-performance liquid chromatography (HPLC)–electrospray (ES)-tandem mass spectrometry (MS). All compounds gave the base peaks due to $[M+1]^+$ by HPLC–ES-single MS. The product ions formed from each quasi-molecular ion by HPLC–ES-tandem MS showed the base peaks at m/z 165 for four compounds. The mass chromatography of HPLC–ES-tandem MS showed much higher sensitivity than that of HPLC–ES-single MS for all drugs spiked to whole blood. Therefore, regression equations, detection limits, recovery rates and precision were studied for haloperidol, bromperidol and floropipamide spiked to human whole blood by means of mass chromatography of HPLC–ES-tandem MS. The three compounds showed good linearity in the range of 0.2–0.8 ng/ml with a detection limit of about 0.1 ng/ml. Recoveries of the three compounds spiked to whole blood (0.2 and 0.8 ng added to 1 ml whole blood) were 23.6–81.2%; the coefficients of intra- and inter-day variations were 8.4–10.4 and 14.5–17.5%, respectively. The three compounds in whole blood could be actually determined 3 and 6 h after oral administration of 1 mg each of haloperidol and bromperidol, and 10 mg of floropipamide in a volunteer.

Keywords: Butyrophenones

1. Introduction

Butyrophenones are widely used drugs for treatment of psychoses and are frequently encountered in forensic chemistry and clinical toxicology. Many analytical methods for these drugs have been reported using gas chromatography (GC) [1–3], GC–mass spectrometry (MS) [1] or high-performance liquid chromatography (HPLC) [4–13] and recently, two reports have appeared on HPLC–electrospray (ES)-single MS for haloperidol and its metabolite in plasma [14] and on HPLC–thermospray-tandem MS for floropipamide (pimozide), benperidol, droperidol, haloperidol and pipamperone in reference solutions (not biosamples) [15]. In the present report, five butyrophenones and two analogues, most of which do not overlap those of the latter report [15], have

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been detected from human whole blood by HPLC–ES-tandem MS with high sensitivity and high selectivity; validation data have been presented for quantification of three representative butyrophenones in whole blood.

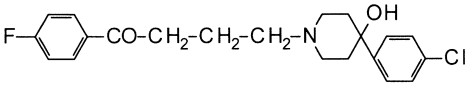
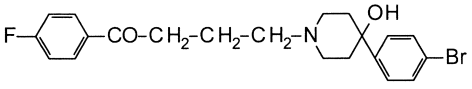
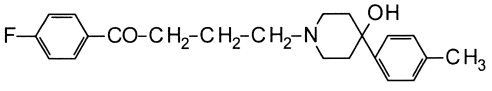
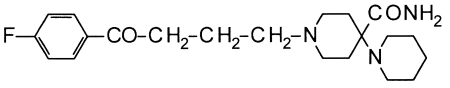
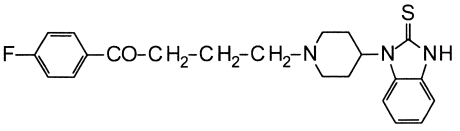
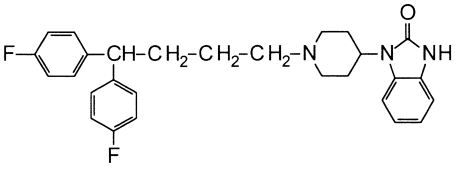
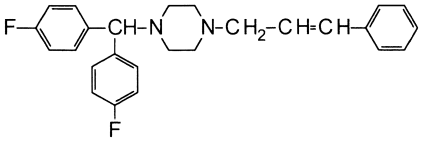
2. Experimental

2.1. Materials

Chemical structures of five butyrophenones and two analogues used in the present study are listed in Table 1. Haloperidol was obtained from Dainippon Pharmaceutical (Osaka, Japan); bromperidol from Yoshitomi Pharmaceutical (Osaka, Japan); moperone from Yamanouchi Pharmaceutical (Tokyo, Japan); floropipamide from Eisai (Tokyo, Japan); timiperone from Daiichi Pharmaceutical (Tokyo, Japan); pimozide from Fujisawa Pharmaceutical (Osaka, Japan); and flunarizine from Kyowa Hakko Kogyo (Tokyo, Japan). Oasis HLB 3-cc Extraction Cartridges were purchased from Waters (Milford, MA, USA). Other common chemicals used were of the highest purity commercially available.

Whole blood was obtained from healthy subjects.

Table 1
Chemical structures of butyrophenones and their analogues used in the present study, and their mass spectra obtained by HPLC–ES-MS and by HPLC–ES-tandem MS

Compound		MW	MS (% intensity)	MS–MS (% intensity)
Haloperidol		375	376 (100), 378 (34), 219 (28), 377 (23)	165 (100), 358 (53), 123 (19), 359 (18)
Bromperidol		419	420 (100), 422 (98), 423 (20)	165 (100), 402 (50)
Moperone		355	356 (100), 357 (24)	165 (100), 338 (58), 153 (22), 123 (17)
Floropipamide		375	376 (100), 377 (25)	291 (100), 165 (33), 331 (32), 262 (15)
Timiperone		397	398 (100), 399 (26)	165 (100), 217 (90), 234 (26), 380 (12)
Pimozide		461	462 (100), 463 (30)	328 (100), 201 (47), 326 (22), 173 (17), 147 (15)
Flunarizine		404	405 (100), 406 (26), 203 (21)	203 (100)

2.2. Clean-up procedure

To 1 ml whole blood (in the presence and absence of the drugs including internal standard (I.S.)), were added 2 ml of distilled water and 0.5 ml of 1 *M* sodium bicarbonate. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant fraction was applied onto an Oasis HLB 3-cc Extraction Cartridge, which had been conditioned with 2 ml of methanol, followed by 2 ml of distilled water. The mixture in the cartridge was aspirated by use of a vacuum manifold with a flow-rate not greater than 2 ml/min. It was washed with 2 ml of distilled water and the drugs were eluted with 2 ml of acetonitrile. The eluate was evaporated to dryness under nitrogen stream. The residue was dissolved in 50 μl of methanol, followed by addition of 100 μl of distilled water. A 100-μl aliquot of the solution was subjected to HPLC–MS(–MS) analyses.

2.3. HPLC–MS(–MS)

The HPLC used in connection with MS(–MS) was an HP 1100 Series instrument (Hewlett-Packard, Palo Alto, CA, USA). The HPLC column used for chromatographic separation was Capcell Pak C_{18} UG80, S-5 μm, 250×1.0 mm (Shiseido, Tokyo, Japan). The mobile phase consisting of 15% B (85% A) was set at a flow-rate of 50 μl/min for 5 min and then gradient elution was performed using 15% B (85% A) to 90% B (10% A) over 25 min; solvent A was distilled water containing 0.1% formic acid and 10 m*M* ammonium acetate, and solvent B 100% acetonitrile.

ES ionization-MS(–MS) was performed on a Finnigan (San Jose, CA, USA) MAT LCQ ion trap mass spectrometer with an LCQ Navigator (version 1.2) in the positive-ion mode. The ES ionization conditions were: capillary temperature, 230°C; spray needle voltage, +5.5 kV; sheath gas pressure, 80 units; auxiliary gas flow, 15 units. The tandem MS conditions were: collision energy, 30%; maximum injection time, 200 ms; isolation width, 2.0 amu. Full-scan mode was used in both HPLC-single MS and HPLC-tandem MS for mass spectral measurements and for quantification by mass chromatography.

3. Results and discussion

3.1. Mass spectra

The mass spectra of the ES-single MS and ES-tandem MS are shown in Table 1. The ES-single MS gave quasi-molecular ions of $[M+H]^+$ constituting the base peaks for the seven compounds without exception.

Therefore, these quasi-molecular ions were subjected to product ion formation by tandem MS. Four compounds gave the base peaks at m/z 165 due to $[F–C_6H_4–CO–C_3H_6]^+$. The base peaks of the product ions were used for quantification by mass chromatography.

3.2. Comparison between mass chromatograms by HPLC–ES-single MS and by HPLC–ES-tandem MS

Fig. 1 shows mass chromatograms of HPLC–ES-single MS obtained from 0.5 ng each of the seven drugs spiked to 1 ml of human whole blood (upper panel) and for non-spiked blood extracts (lower panel). Most of the drug peaks overlapped impurity peaks.

Fig. 2 shows mass chromatograms of HPLC–ES-tandem MS for the same amounts (0.5 ng each) of the drugs spiked to whole blood. Distinct peaks appeared for all compounds with almost no impurity peaks. When the chromatograms in Fig. 2 were compared with those in Fig. 1, it was obvious that HPLC–ES-tandem MS gave much higher sensitivity and selectivity than HPLC–ES-single MS. Therefore, the following experiments were aimed at quantification of a butyrophenone in whole blood by mass chromatography of HPLC–ES-tandem MS.

3.3. Quantification of haloperidol, bromperidol and floropipamide in whole blood

Quantification of haloperidol, bromperidol and floropipamide in human whole blood was made by mass chromatography using each base peak of product ions of HPLC–ES-tandem MS. Selected-reaction monitoring (SRM) can be also used for sensitive quantification, but the sensitivity obtained by SRM was similar to that obtained by mass

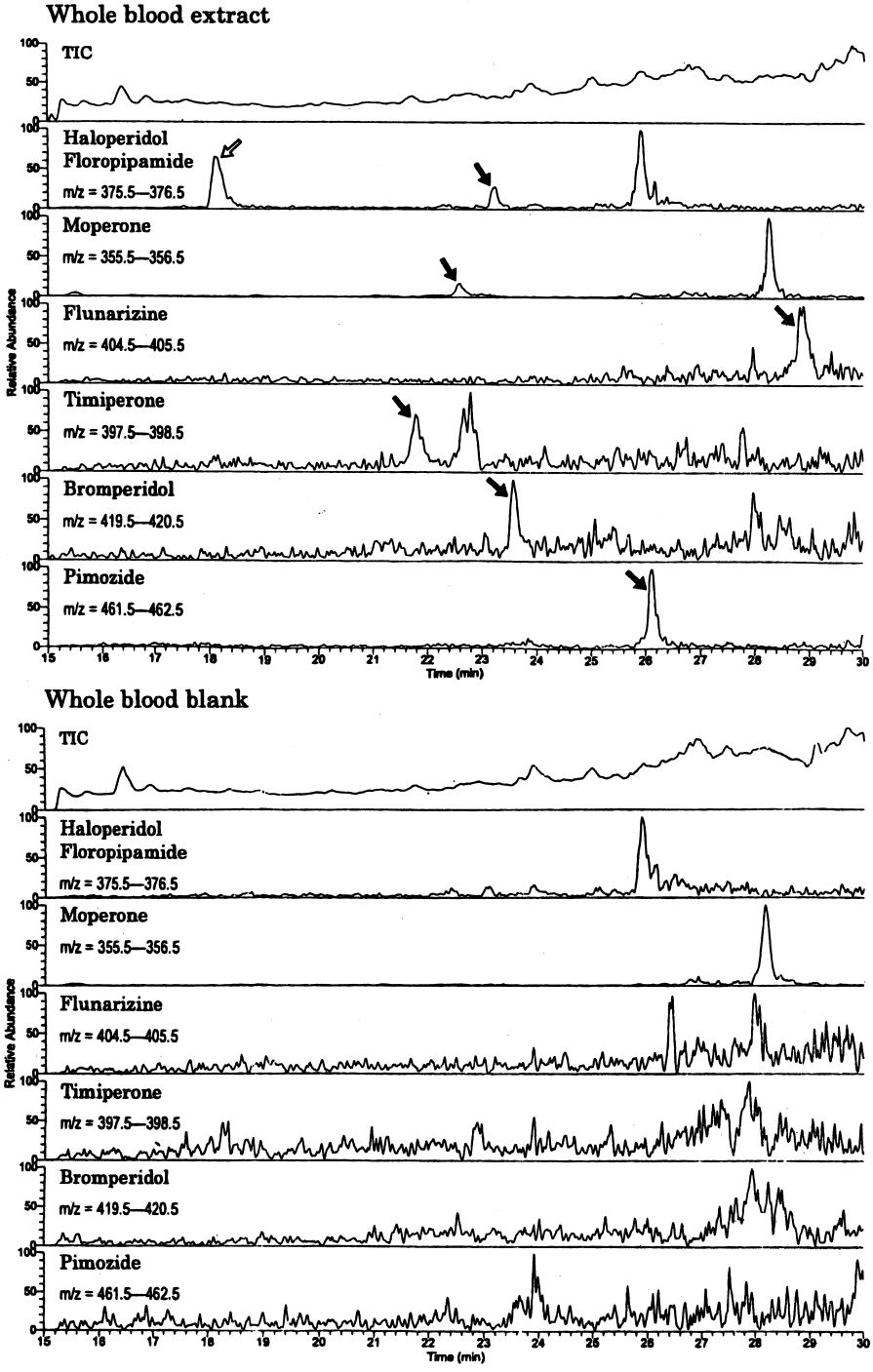


Fig. 1. Mass chromatograms of HPLC–ES-single MS for butyrophenones and their analogues extracted from human whole blood. The upper panel shows chromatograms obtained from whole blood spiked with each drug (0.5 ng/ml); the lower panel those not spiked with the drugs. The arrows show the peaks of the drugs. In the mass chromatogram for haloperidol and floropipamide, the open and filled arrows show the peaks of floropipamide and haloperidol, respectively.

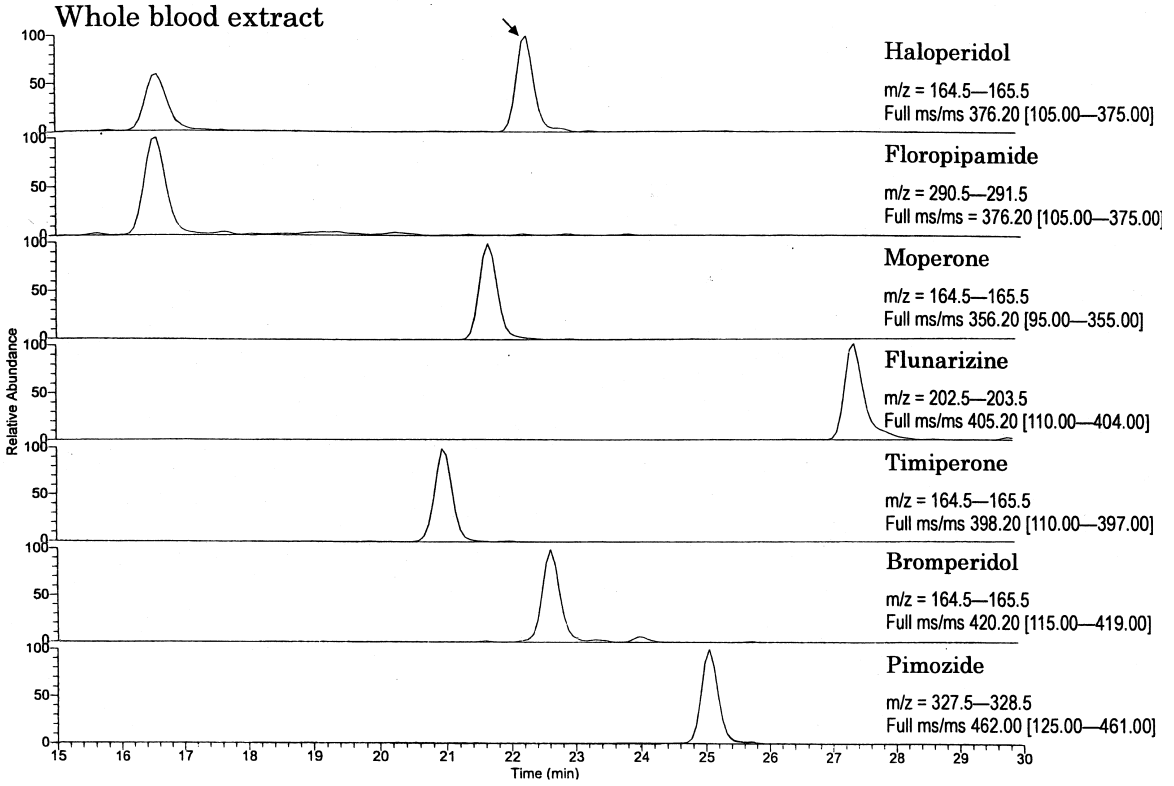


Fig. 2. Mass chromatograms of HPLC–ES-tandem MS for butyrophenones and their analogues extracted from human whole blood. The amount of each drug spiked to 1 ml whole blood was 0.5 ng. The arrow shows the peak of haloperidol.

chromatography of the product ions, because of the intrinsic character of the ion trap MS. Thus, we adopted the latter in view of capability of both quantification and identification using full-scan of product ions.

The calibration curves were constructed for haloperidol, bromperidol and floropipamide at 0.2, 0.4, 0.6 and 0.8 ng/ml of whole blood using 0.4 ng of pimozide as I.S., and they were linear in the above range. The equations and r values for the curves were: $y=0.287x+0.066$ ($r=0.998$) for haloperidol; $y=0.845x+0.013$ ($r=0.999$) for bromperidol; and $y=0.258x+0.031$ ($r=0.999$) for floropipamide. The detection limit was about 0.1 ng/ml for the three compounds.

Recovery rates from human whole blood were also tested for the three compounds by adding 0.2 or 0.8 ng each to 1 ml of whole blood. They were 47.6±2.8 (mean±SD, $n=4$) and 60.5±4.5% at 0.2 and 0.8 ng/ml of whole blood, respectively, for haloperidol; 33.9±2.0 and 23.6±1.3%, respectively, for bromperidol; 81.2±7.1 and 76.8±10.8%, respectively, for floropipamide. The low recovery rates of bromperidol may be due to easy liberation of the bromine from the molecule, although this is not evidenced in this study.

Table 2
Precision data for three butyrophenones spiked to human whole blood

Compound	Concentration spiked (ng/ml)	Coefficient of variation (%)	
		Intra-day ($n=5$)	Inter-day ($n=5$)
Haloperidol	0.2	9.7	14.8
	0.8	8.6	14.5
Bromperidol	0.2	8.9	16.2
	0.8	8.4	15.7
Floropipamide	0.2	10.4	17.5
	0.8	9.7	16.9

The precision data are shown in Table 2. The intra-day C.V.s were less than 10.4%, and inter-day C.V.s 14.5–17.5%.

3.4. Determination of haloperidol, bromperidol and floropipamide in whole blood of a volunteer after oral ingestion

The present HPLC–ES-tandem MS method was applied to real whole blood samples to confirm its utility. One milligram of haloperidol, 1 mg of bromperidol or 10 mg of floropipamide were administered orally to a male volunteer, 50 years of age, and blood was obtained 3 and 6 h after each administration. The typical mass chromatograms of the samples are shown in Fig. 3.

The blood concentrations calculated by I.S. calibration were 0.18 and 0.25 ng/ml at 3 and 6 h after administration, respectively, for haloperidol; 0.21 and 0.17 ng/ml, respectively, for bromperidol; and 2.00 and 1.85 ng/ml, respectively, for floropipamide.

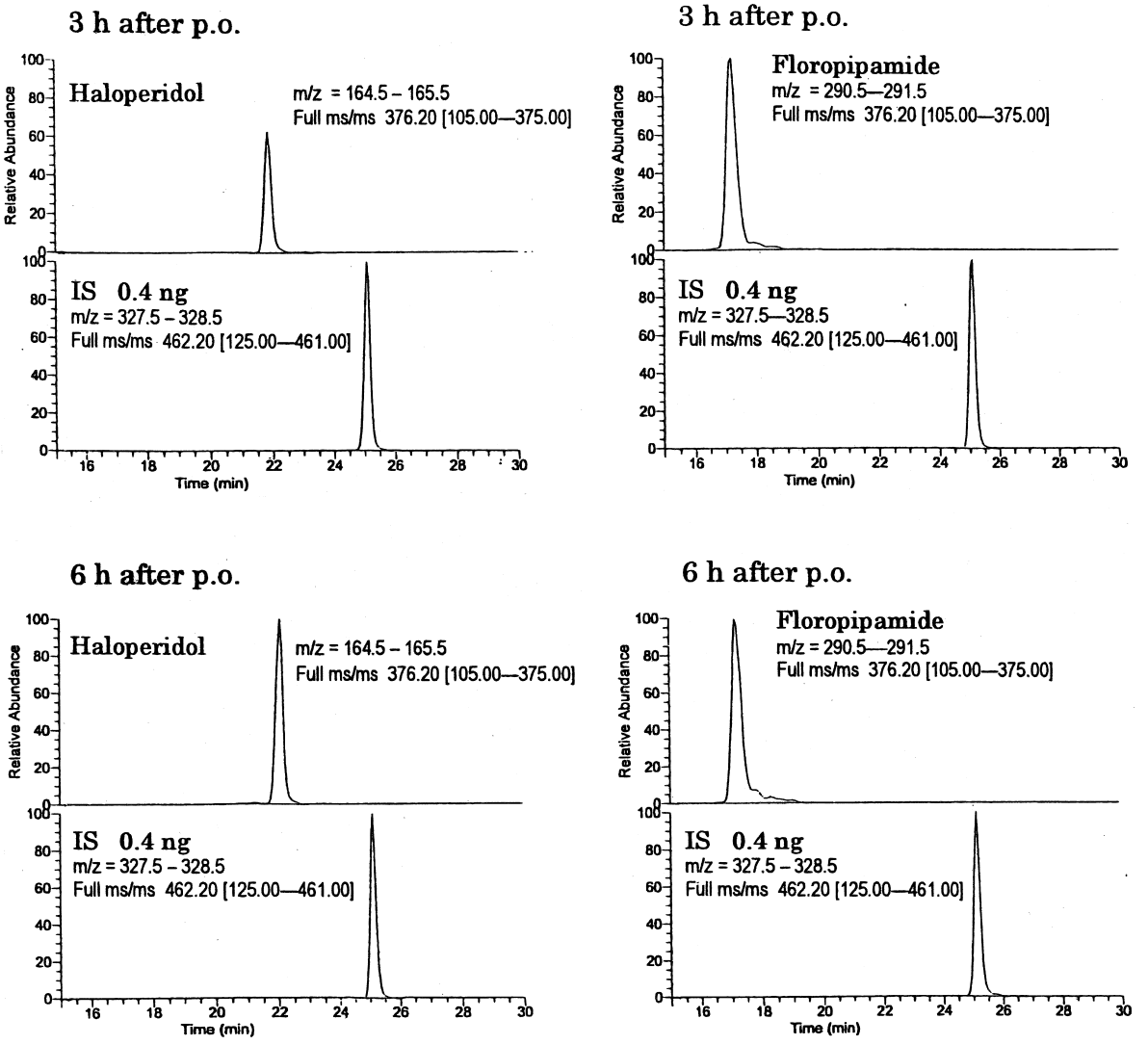


Fig. 3. Mass chromatograms of HPLC–ES-tandem MS for haloperidol and floropipamide after oral administration of 1 and 10 mg of the drugs, respectively, in a 50-year-old male.

4. Conclusion

In the present study, we have established a detailed procedure for sensitive analysis of butyrophenones and their analogues in whole blood by HPLC–ES-tandem MS. To our knowledge, this is the first report dealing with HPLC–ES-tandem MS for butyrophenones in biological samples. For ion trap MS, mass chromatography gives a sensitivity comparable to that of selected ion monitoring or SRM; we thus adopted mass chromatography with full scan for detection of each product ion. This is advantageous because both quantification and identification with product ion spectra can be made simultaneously. The present method is recommendable for use in therapeutic monitoring, clinical toxicology and forensic toxicology for butyrophenones.

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