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Original

ANALYSIS OF BUTYROPHENONES IN WHOLE BLOOD SPECIMENS BY LC/MS/MS USING A NEW POLYMER COLUMN

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ポリマーカラムを用いた全血中ブチロフェノン系薬物の LC/MS/MS 分析

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Summary

Seven butyrophenones in human whole blood were analyzed by LC/MS/MS using a new polymer column (MSpak GF-310), which enabled direct injection of crude biological samples without complicated pretreatments. The quantitation was made by mass chromatography for each product ion. The recoveries were more than 50% for haloperidol, bromperidol, moperone and spiroperidol at 80 ng/ml in whole blood, but less than 10% for pimozide. The regression equations showed good linearity for haloperidol, bromperidol, moperone and spiroperidol in the range of 10–80 ng/ml, for timiperone in the range of 20–80 ng/ml and for pimozide in the range of 50–200 ng/ml using the chlorinated analog of haloperidol as internal standard. Their detection limits were about 5 ng/ml for haloperidol, bromperidol, moperone and spiroperidol, about 10 ng/ml for timiperone and about 25 ng/ml for pimozide. Thus, the present method seems useful for sensitive and specific detection and determination of their high therapeutic and toxic levels present in biological specimens including whole blood.

Key words : Butyrophenones ; Haloperidol ; Bromperidol ; Moperone ; Spiroperidol ; LC/MS/MS ; New polymer column (MSpak GF-310) ; Whole blood

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Introduction

Butyrophenons have a long history as antipsychotic drugs, but are still being used widely; they are frequently encountered in the fields of forensic and clinical toxicology. They were analyzed by gas chromatography (GC) [1-3], GC/mass spectrometry (MS) [4], high-performance (HP) liquid chromatography (LC) [5-10], LC/MS [11,12] and LC/MS/MS [13-17]. In this study, we have combined an LC/MS/MS technique with a new polymer separation column, which enables direct injection of crude biological samples without complicated pretreatments and without a column switching system.

Experimental

Materials

Chemical structures of six butyrophenons and an internal standard (IS) are depicted in Fig. 1. Haloperidol-HCl was obtained from Dainippon Pharmaceutical (Osaka); bromperidol from Yoshitomi Pharmaceutical (now Mitsubishi-Well-Pharma,

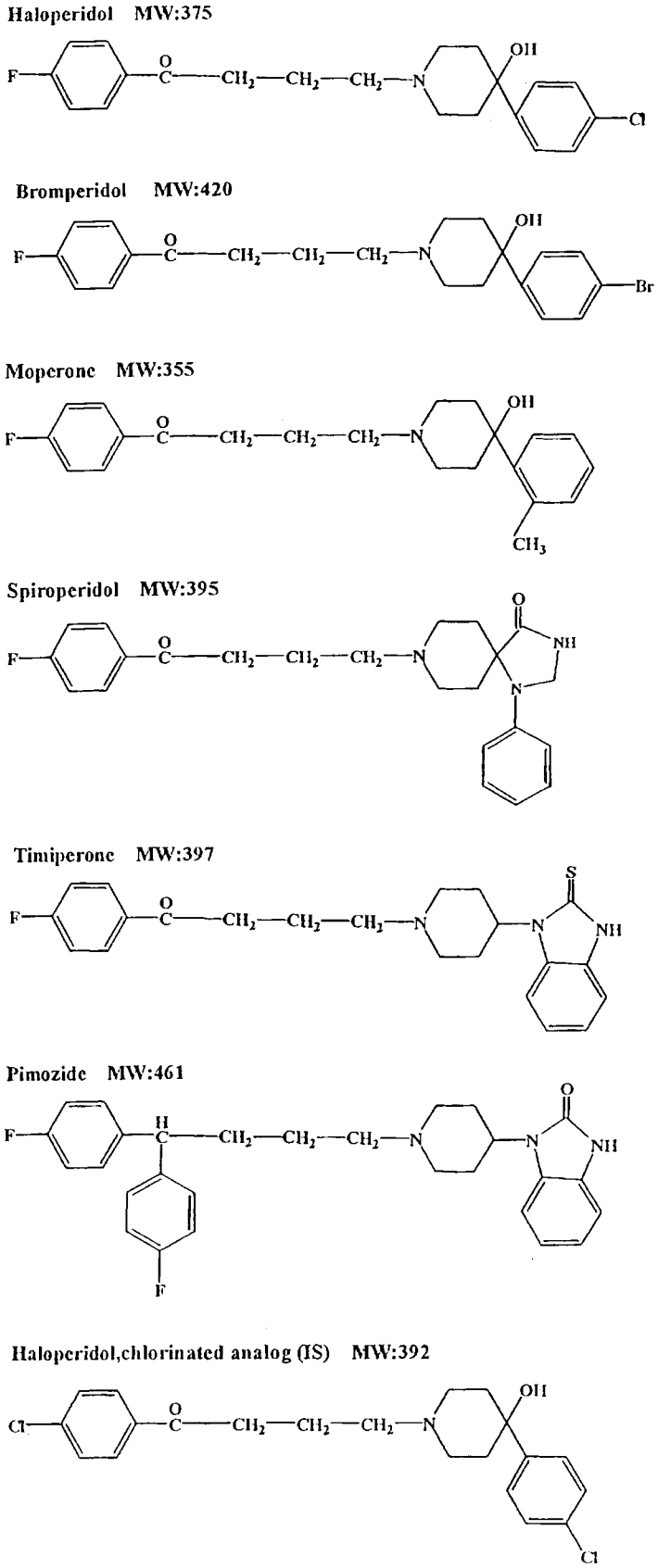

Fig. 1. Structures of butyrophenones dealt with in the present study.

Osaka); moperone-HCl from Yamanouchi Pharmaceutical (Tokyo); spiroperidol from Eisai Co., Ltd. (Tokyo); timiperone from Daiichi Pharmaceutical (Tokyo); pimozide from Fujisawa Pharmaceutical (Osaka); chlorinated analog of haloperidol from Research Biochemical International (Natick, MA, USA). Other common chemicals used were of the highest purity commercially available. Amicon® Ultra-4 centrifuge tubes were purchased from Millipore (Bedford, MA, USA). Whole blood was obtained from healthy volunteers.

Procedure

One milliliter of whole blood was well mixed with 50 ng IS, 1 ml distilled water and 1ml 15% formic acid solution. The solution was poured into the filter unit compartment of an Amicon Ultra-4 centrifuge tube. After centrifugation at 4,000 rpm for 20 min, clear filtrate could be obtained in the outer compartment of the tube. A 0.1-ml aliquot of the filtrate was injected into the LC/MS/MS instrument.

LC/MS/MS conditions

The LC, used in connection with MS/MS, was made on an HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA). The LC column used for chromatographic separation was MSpak GF-310 4B (50 x 4 mm i. d., Showa Denko, Tokyo). This column is made of a new polymer, which enables direct injection of crude biological samples, utilizing by size-exclusion chromatographic separation, and actions of partition and ion-exchange. For the gradient elution with the mobile phase, the following solutions were prepared; solvent A: 7.5 mM ammonium acetate aqueous solution; solvent B: 0.05% formic acid solution in acetonitrile. The mobile phase consisting of 100% A (0% B) was set at a flow rate of 0.2 ml/min for 5 min and then gradient solution was performed using 100% A to 20% A (80% B) over 15 min at the same flow rate.

Electrospray ionization (ESI)-MS/MS was performed on a Thermo Electron Corporation (San Jose, CA, USA) LCQ ion trap mass spectrometer with an LCQ Navigator in the positive-ion mode. The ESI conditions were: capillary temperature, 270 °C; spray needle voltage, + 5.8 kV; sheath gas pressure, 100 units; auxiliary gas flow, 30 units. The tandem MS conditions were: collision energy, 40%; maximum injection time, 200 ms; isolation width, 1.0 amu. Full scan mode was used in both LC/single MS and LC/MS/MS for mass spectral measurements; for quantitation, mass chromatography using product ions of a test compound and IS was used. Each base peak obtained from LC/MS was used for product ion formation by LC/MS/MS; the base or an intense peak of the product ions was used for quantitation. The precursor ions and their corresponding product ions used were: *m/z* 376 → 165 for haloperidol; *m/z* 420 → 165 for bromperidol; *m/z* 356 → 165 for moperone; *m/z* 396 → 165 for spiroperidol; *m/z* 398 → 217 for timiperone; *m/z* 462 → 328 for pimozide; *m/z* 392 → 181 for chlorinated analog of haloperidol (IS).

Results

Mass chromatograms of selected product ions

Figure 2 shows mass chromatograms of each ion selected from product ions for butyrophenons spiked into whole blood obtained by LC/MS/MS; they were arranged according to retention times. Peaks were almost not interfered with by impurity peaks at the concentrations of 10 ng/ml for most compounds, 20 ng/ml for timiperone and 50 ng/ml for pimozide.

Recoveries

Table 1 shows the recoveries of each compound at different concentrations spiked into whole blood specimens. They were

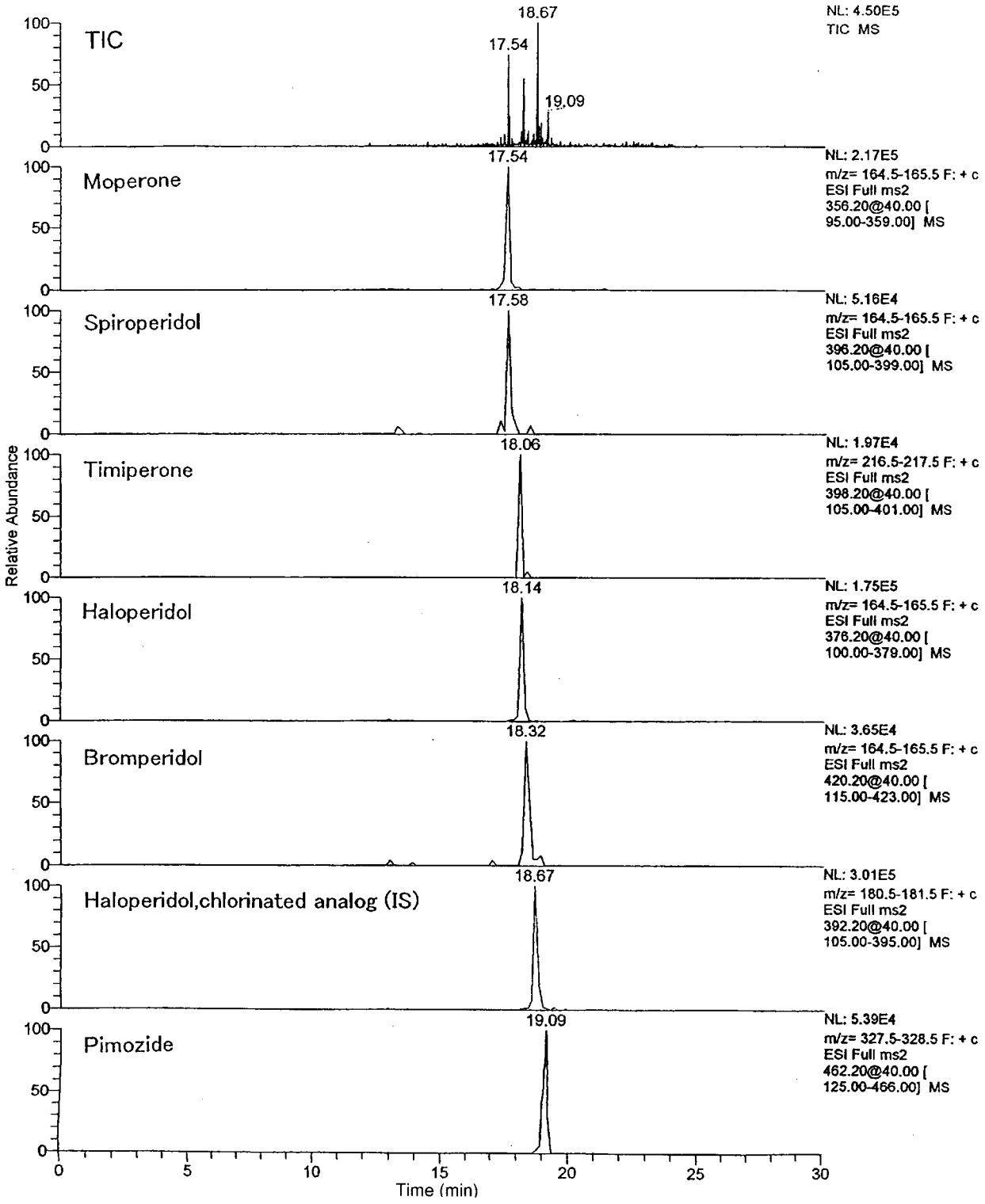

Fig. 2. TIC and mass chromatograms obtained by LC/MS/MS for butyrophenones in human whole blood. The amount of each drug spiked into 1 ml whole blood was 10 ng except for timiperone and pimozide; their amounts were 20 and 50 ng, respectively.

Table 1. Recovery rates of butyrophenones spiked into whole blood specimens

Compound	Concentration spiked (ng/ml)	Recovery (%)[a]
Haloperidol	10	58 ± 6
	80	68 ± 4
Bromperidol	10	41 ± 7
	80	53 ± 6
Moperone	10	51 ± 12
	80	71 ± 4
Spiroperidol	10	40 ± 10
	80	56 ± 3
Timiperone	20	19 ± 7
	80	49 ± 8
Pimozide	50	3 ± 1
	200	8 ± 1
Chlorinated analog of haloperidol (IS)	50	35 ± 3

[a] Each value represents the mean ± SD ($n = 8$).

more than 50% for haloperidol, bromperidol, moperone and spiroperone at 80 ng/ml in whole blood, and only for haloperidol and moperone at 10 ng/ml. For pimozide, they were only less than 10 %. As expected, when the concentration of each compound was higher, the higher recovery could be obtained for every compound.

Linearities

Calibration curves were prepared using various concentrations of each compound spiked into whole blood as shown in Table 2. In spite of their poor recoveries (Table 1), good linearity could be obtained for all compounds. Detection limits were about 5 ng/ml for four typical butyrophenones, about 10 ng/ml for timiperone and about 25 ng/ml for pimozide.

Precision

Intra-day precision data are presented in Table 3. The C.V. values ranged from 1.3 to 20.3%; they tended to be lower at higher concentrations in whole blood.

Discussion

In the present study, we have established a detailed procedure for LC/MS/MS analysis of six butyrophenones in whole blood using a new polymer separation column. In this connection, our previous report dealing with a similar technique [16] should be mentioned; we had used the same instrument for LC/MS/MS analysis for seven butyrophenones and their analogues. In our previous method, the drugs had been purified by solid-phase extraction with Oasis HLB cartridges; the pretreatment had been relatively time-consuming. In the present method, the pretreatment is consisted of only ultrafiltration for whole blood specimens, which is much simpler and faster than the previous procedure. If serum or plasma is dealt with, even ultrafiltration becomes unnecessary; it can be directly injected into the LC/MS/MS system. Such simplicity of the pretreatment is due to the use of a unique polymer separation column MSpak GF-310, which has three properties, such as exclusion of large-molecular proteins and nucleic acids, hydrophobic adsorption and ion-exchanging ability. When a crude sample solution is applied to the column,

Table 3. Precision data as a function of intra-day variation for butyrophenones spiked into whole blood

Compound	Concentration spiked (ng/ml)	C.V. (%)[a]
Haloperidol	10	20.3
	80	6.0
Bromperidol	10	3.4
	80	5.9
Moperone	10	7.3
	80	1.3
Spiroperidol	10	11.1
	80	6.0
Timiperone	20	18.8
	80	11.1
Pimozide	50	12.9
	200	6.7

[a] Each value was obtained from 5 determinations.

Table 2. Calibration equations, quantitation ranges and detection limits of butyrophenones in human whole blood using the chlorinated analog of haloperidol as IS ($n = 5$)

Compound	Calibration equation ($y = ax \pm b$)		Quantitation range	Correlation coefficient	Detection limit
	a	b	(ng/ml)	(r^2)	(ng/ml)
Haloperidol	3.28×10^{-2}	$+2.16 \times 10^{-1}$	10 – 80	0.995	5
Bromperidol	1.40×10^{-2}	-4.60×10^{-3}	10 – 80	0.999	5
Moperone	8.14×10^{-2}	$+3.00 \times 10^{-1}$	10 – 80	0.996	5
Spiroperidol	1.11×10^{-2}	$+3.78 \times 10^{-2}$	10 – 80	0.997	5
Timiperone	3.20×10^{-3}	$+6.50 \times 10^{-3}$	20 – 80	0.997	10
Pimozide	1.80×10^{-3}	-1.05×10^{-2}	50 – 200	0.997	25

large molecules including proteins are eliminated from the column by passing through it very fast; small-molecular drugs to be targeted are separated by the column as shown in Fig. 2.

Because of the absence of condensation process for target compounds, the sensitivity of the present method (Table 2) is lower than that of the previous one [16]. However, the detection limits for haloperidol and bromperidol obtained in the present study are within the range of high therapeutic levels [18]; but for pimozide it is in the toxic range [19]. Therefore, the present method seems to sufficiently meet the forensic toxicological demand in view of sensitivity and simplicity.

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