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Liquid chromatographic–mass spectrometric determination of haloperidol and its metabolites in human plasma and urine

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Abstract

Haloperidol and its two metabolites, reduced haloperidol and 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) in human plasma and urine were analyzed by HPLC–MS using a new polymer column (MSpak GF-310), which enabled direct injection of crude biological samples without pretreatment. Recoveries of haloperidol and reduced haloperidol spiked into plasma were 64.4–76.1% and 46.8–50.2%, respectively; those for urine were 87.3–99.4% and 94.2–98.5%, respectively; those of CPHP for both samples were not less than 92.7%. The regression equations for haloperidol, reduced haloperidol and CPHP showed good linearity in the ranges of 10–800, 15–800 and 400–800 ng/ml, respectively, for both plasma and urine. Their detection limits were 5, 10 and 300 ng/ml, respectively, for both samples. Thus, the present method was sensitive enough for detection and determination of high therapeutic and toxic levels for haloperidol and its metabolites present in biological samples.

Keywords: Haloperidol

1. Introduction

Haloperidol is widely used as an antipsychotic drug and frequently encountered in the fields of forensic and clinical toxicology. Analytical methods for haloperidol and its metabolites (Fig. 1) in biological samples so far reported include gas chromatography (GC) [1–3], GC–mass spectrometry (MS) [4], high-performance liquid chromatography (HPLC) [5–10] and HPLC–MS [11–15].

Existing methods for the detection of these molecules usually involve time-consuming multi-step pretreatment including liquid–liquid extraction and/or solid-phase extraction to remove impurities contained in plasma or serum. To avoid such sample pretreatments, HPLC columns such as internal-surface reversed-phase (ISRP) silica support, which enables direct injection of biological samples into HPLC, have been developed [16–20]. These columns are usually used in switching arrangement [17,19,20].

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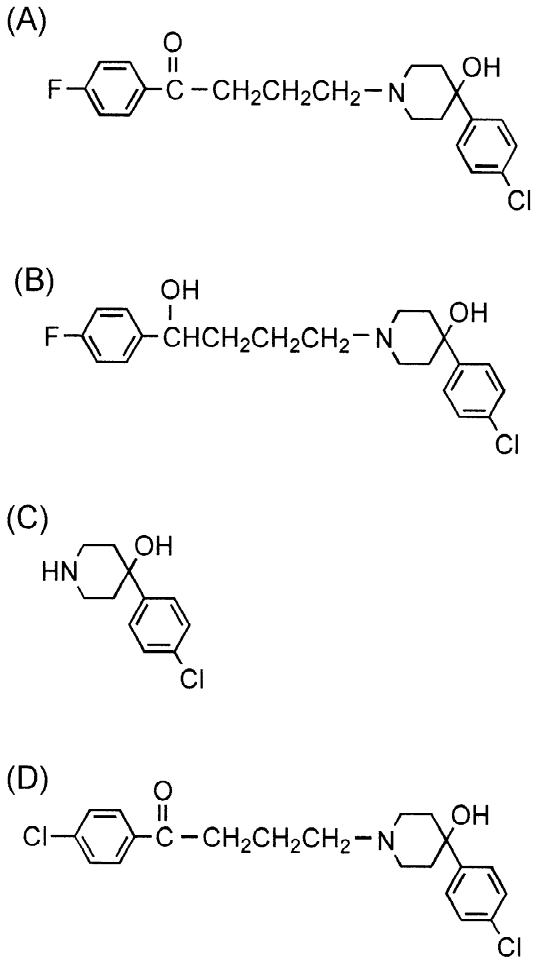

Fig. 1. Molecular structures of the investigated compounds; (A) haloperidol, M_w 375.9; (B) reduced haloperidol, M_w 377.9; (C) 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP), M_w 211.7; (D) haloperidol chlorinated analog, M_w 392.3, used as internal standard (I.S.) or external standard (E.S.).

Recently, a new HPLC polymer stationary phase (MSpak GF-310), which enables direct injection of crude biological samples into the HPLC column, has been developed for use in HPLC–MS in Japan.

In this report, we present a rapid HPLC–MS analysis of haloperidol and its two metabolites, reduced haloperidol and 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) by direct injection of human plasma and urine using the MSpak GF-310 column without sample pretreatment and without the column switching technique.

2. Experimental

2.1. Materials

Haloperidol, reduced haloperidol, CPHP and 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-(4-chlorophenyl)-1-butanone, used as internal standard (I.S.) or external standard (E.S.), were commercially obtained from Research Biochemical International (Natick, MA, USA). Acetonitrile (HPLC grade), high-purity water (HPLC grade), ammonium acetate (reagent grade) and formic acid (reagent grade) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Whole blood and urine were obtained from healthy subjects.

2.2. Sample preparation

To 1 ml plasma or urine in the presence of haloperidol, its two metabolites and I.S. (300 ng), were added 3 ml of the initial HPLC mobile phase (distilled water containing 0.09% formic acid and 20 m*M* ammonium acetate). The mixture was frozen at −20 °C. After thawing, the mixture was centrifuged at 3000 rev./min for 15 min. A 20-μl aliquot of the supernatant fraction was directly injected onto the HPLC column.

2.3. HPLC–MS conditions

Analyses were made on an M-8000 ion trap mass spectrometer (LC-3DQMS, Hitachi, Tokyo, Japan) with a sonic spray ionization (SSI) interface, and connected to a Hitachi L-7100 pump with a Hitachi L-7420 UV–Vis detector set at 280 nm (Hitachi, Tokyo, Japan).

SSI was developed as a new technique to generate molecular ions under a very wide range of solvent systems and liquid flow-rates [21,22], and has become commercially available very recently. Organic compounds are ionized by nebulising sample solutions using a high-speed gas flow only, at room temperature [21,23].

The HPLC column used was an MSpak GF-310 4B, 4.6×50 mm (Showadenko, Tokyo, Japan). The mobile phase consisting of distilled water, containing 0.09% formic acid and 20 m*M* ammonium acetate (solvent A) was set at a flow-rate of 0.3 ml/min for 5

min, and then gradient elution was performed using 100% A to 20% A (80% solvent B; acetonitrile) over 20 min. The mobile phase was discarded for 5 min, and then reintroduced into the mass spectrometer by a switching valve.

The SSI MS conditions were as follows. The mass spectrometric detection, positive ionization mode; the temperatures of the cover plate, aperture-1 and aperture-2, 200, 150 and 120 °C, respectively; the voltages of the drift, focus, SSI chamber and detector, 70, 30, 0 and 500 V, respectively; the ion accumulation time, 350 ms; scan range, 100 to 500 m/z by the centroid mode. Nitrogen gas was generated by AT-10NP-C (Air Tech, Yokohama, Japan) with output pressure of 0.50 MPa and with ion source inlet pressure at 0.39 MPa; buffer gas, helium with output pressure of 0.53 MPa; ion source inlet pressure, 0.25 MPa.

3. Results and discussion

3.1. Comparison of the MSpak GF-310 column with the ISRP column

We compared the GF-310 column with the ISRP column (Pinkerton GFF2, Regis, Morton Grove, IL, USA) for direct injection of biological samples. As a result, the HPLC–MS detector was contaminated within several hours only by solvent (water, methanol or acetonitrile) passed through the ISRP column. The cause of contamination of the MS detector was due to the elution of some kinds of chemical substance constituting the stationary phase, therefore, the column was not suitable in our experiments for HPLC–MS analysis. On the other hand, HPLC–MS detector was not contaminated under the same condition mentioned above by using the GF-310 column; this indicates that this column is chemically and structurally more stable.

The separation by the GF-310 column is based on the size exclusion chromatography (SEC) principle associated with slight action of partition and adsorption, and this column is suited to eliminate proteins, nucleic acids and polysaccharides from biological samples, because their molecular size is too large to enter the pores of the stationary phase, whereas drugs with small molecular masses can enter the pores and be retained on the polyvinyl alcohol phase; the principle has enabled the direct injection of crude biological samples. This polymer support is chemically and structurally stable, and can be used in a wide pH range between 2 and 9, compared to the 2.5–7.5 range of the ISRP silica column.

Another advantage of the present polymer support is that both water and various organic solvents can be used for elution. The expansion or constriction of stationary phase associated with solvent replacement are very small owing to a highly cross-linked hard gel of polyvinyl alcohol, as compared with the conventional polymer columns.

3.2. HPLC–UV detection and HPLC-mass chromatogram

Fig. 2 shows chromatograms obtained by HPLC–UV detection (at 280 nm) for monitoring proteins and nucleic acids, chromatograms of total ion current (TIC) in the scan range of m/z 100 to m/z 500, and reconstructed chromatograms of m/z 212, 378, 376 and 392, showing $[M+H]^+$ ions of CPHP, reduced haloperidol, haloperidol and I.S., respectively, which had been spiked to human plasma and urine samples. They were obtained from 600 ng each of haloperidol and its two metabolites and 300 ng of I.S. spiked to 1 ml of a sample. As shown in the chromatograms of HPLC–UV detection, most of the proteins and/or nucleic acids in plasma and urine were eluted within 5 min. Haloperidol, reduced haloperidol, CPHP and I.S. were well separated and could be determined simultaneously only by the GF-310 column without pretreatment and without a column switching technique.

3.3. Reliability of the method

Recovery rates of haloperidol and its reduced haloperidol from plasma and urine were determined by adding 25, 200 and 600 ng of each compound to 1 ml of plasma or urine; those for CPHP by adding 600 ng to 1 ml of each sample (Table 1). The quantitation was carried out with external calibration. The recoveries of haloperidol from plasma and urine were 64.4 to 76.1% and 87.3 to 99.4%, respectively; those of reduced haloperidol from plasma and urine, 46.8 to 50.2% and 94.2 to 98.3%, respectively; those

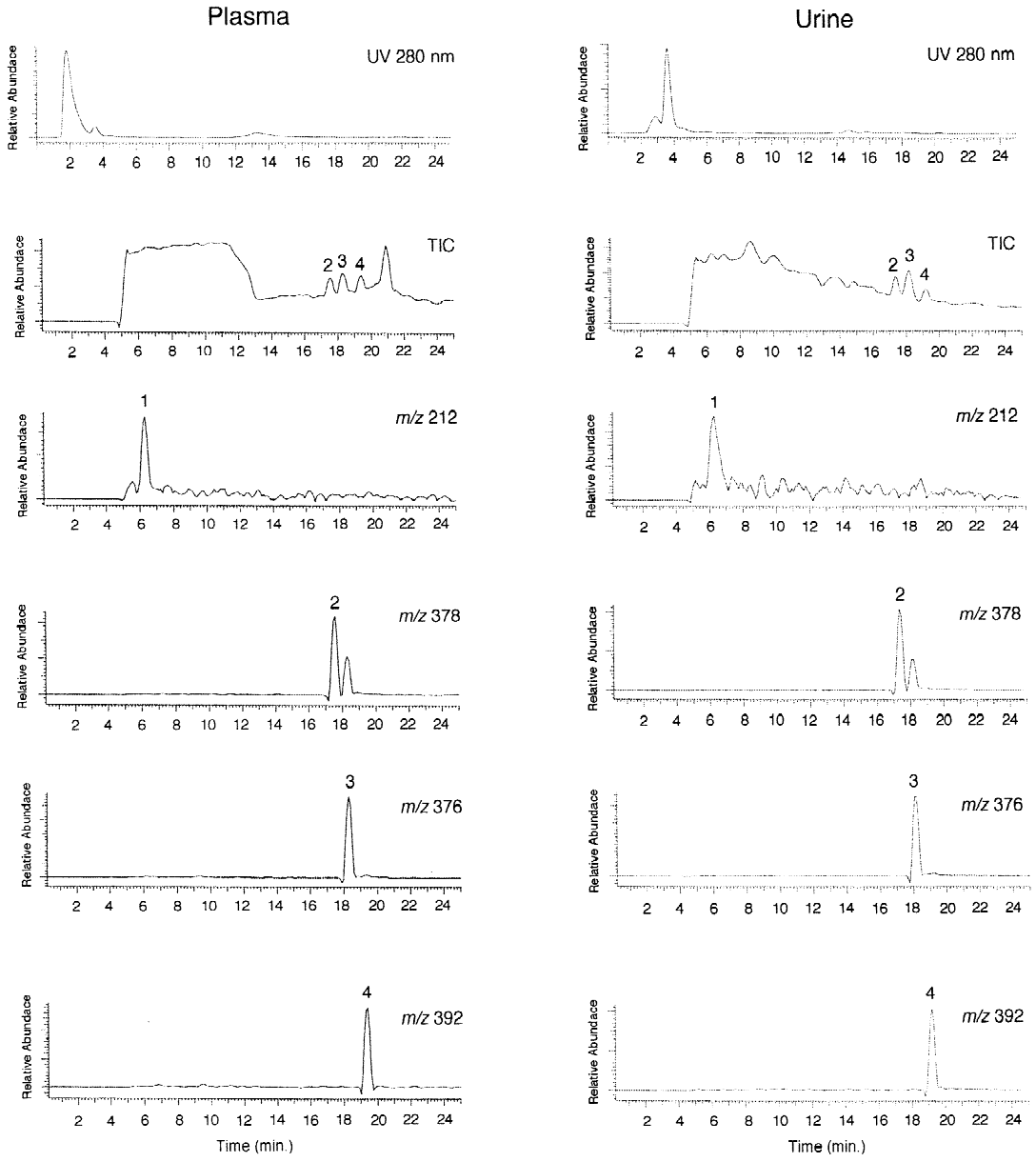

Fig. 2. Typical HPLC–UV chromatograms (at 280 nm), total ion current (TIC) and reconstructed chromatograms at m/z 212, 378, 376 and 392, showing $[M+H]^+$ ions of CPHP, reduced haloperidol, haloperidol and haloperidol chlorinated analog, respectively, in human plasma (left column) and urine (right column) samples. The amount of haloperidol, reduced haloperidol and CPHP spiked to 1 ml plasma or urine was 600 ng, and that of I.S. was 300 ng. Peak 1, CPHP; peak 2, reduced haloperidol; peak 3, haloperidol; peak 4, haloperidol chlorinated analog (I.S.).

of CPHP from plasma and urine, 97.4 and 92.7%, respectively. It is evident that haloperidol and reduced haloperidol give much higher recoveries for urine than for plasma. This is due to the binding of both compounds to proteins, which are eluted very fast from the HPLC column, whereas CPHP does not bind to proteins, though this is not supported by this study.

Table 1
Recoveries of haloperidol and its metabolites from human plasma and urine

Analytes	Samples ($n=10$)							
	Plasma				Urine			
	Added (ng/ml)	Found[a] (ng/ml) (mean±SD)	Recovery (%) (mean±SD)	C.V. (%)	Added (ng/ml)	Found[a] (ng/ml) (mean±SD)	Recovery (%) (mean±SD)	C.V. (%)
Haloperidol	25	16.1±2.4	64.4±9.6	14.8	25	24.8±2.9	99.4±11.6	11.7
	200	135±10.9	67.7±5.4	8.0	200	175±11.1	87.3±5.5	6.3
	600	457±27.1	76.1±4.5	5.9	600	583±24.4	97.1±4.1	4.2
Reduced haloperidol	25	11.7±2.0	46.8±8.2	17.4	25	24.0±2.9	95.8±11.5	12.0
	200	97.0±9.4	48.5±4.7	9.6	200	188±9.1	94.2±4.6	4.9
	600	300±15.9	50.2±2.7	5.3	600	590±24.9	98.3±4.3	4.2
CPHP	600	584±95.2	97.4±15.9	16.3	600	556±87.9	92.7±14.7	15.8

[a] Quantitation of haloperidol and its metabolites was carried out with external calibration.

Linearity and detection limits of the present method for haloperidol and its metabolites were investigated over the concentration range of 1–800 ng/ml (14 points of concentrations) using spiked plasma and urine samples. The results are summarized in Table 2. Haloperidol, reduced haloperidol and CPHP showed good linearity in the ranges of 10–800, 15–800 and 400–800 ng/ml, respectively, for both plasma and urine. The limits of detection (signal-to-noise ratio = 3) for haloperidol, reduced haloperidol and CPHP in plasma and urine were 5, 10 and 300 ng/ml, respectively. The therapeutic serum concentrations of haloperidol were reported to be 0.8–33 ng/ml [24]. The toxic blood levels of haloperidol were reported to be greater than 50 ng/ml [24], and to be 50–500 ng/ml [25]. Therefore, high therapeutic blood levels and any toxic level of haloperidol can be detected by the present method.

Accuracy and precision for quantitation of haloperidol and its two metabolites were determined. Within-day (one person and five persons) and day-to-day (for 5 days of one person) assays were performed using plasma and urine samples spiked with different concentrations (25, 200 and 600 ng/ml) of the analytes. The results are summarized in Table 3. The coefficients of variation (C.V.s) for within-day measurements using one person, within-day measurements using five persons, and day-to-day measurements using one person for haloperidol were not greater than 12.8, 23.3, and 26.8% in plasma, respectively; and not greater than 5.8, 6.0 and 19.8% in urine, respectively; those for reduced haloperidol not greater than 7.0, 34.7 and 26.5% in plasma,

Table 2
Calibration equations, quantitation ranges and detection limits of haloperidol and its metabolites in human plasma and urine samples[a] ($n=10$)

	Calibration equation ($y = ax + b$)		Quantitation range (ng/ml)	Correlation coefficient (r)	Detection limit (ng/ml)
	a	b			
Plasma					
Haloperidol	3.32×10^{-3}	4.28×10^{-2}	10–800	0.995	5
Reduced haloperidol	2.73×10^{-3}	2.10×10^{-3}	15–800	0.995	10
CPHP	2.28×10^{-4}	1.90×10^{-2}	400–800	0.916	300
Urine					
Haloperidol	3.67×10^{-3}	1.01×10^{-1}	10–800	0.997	5
Reduced haloperidol	3.66×10^{-3}	4.69×10^{-2}	15–800	0.997	10
CPHP	2.16×10^{-4}	4.50×10^{-3}	400–800	0.912	300

[a] y is the ratio of peak area of haloperidol or its metabolites to that of I.S., and x is the concentration (ng/ml) of haloperidol or its metabolites.

Table 3
Accuracy and precision (C.V.) data for quantitation of haloperidol and its metabolites in spiked plasma and urine samples

	Added (ng/ml)	Within-day				Day-to-day (for 5 days) Samples from one person ($n=5$)	
		Samples from one person ($n=10$)		Samples from five persons ($n=5$)			
		Found (mean±SD) (ng/ml)	C.V. (%)	Found (mean±SD) (ng/ml)	C.V. (%)	Found (mean±SD) (ng/ml)	C.V. (%)
Plasma							
Haloperidol	25	24.7±3.2	12.8	17.0±4.0	23.3	28.9±7.8	26.8
	200	211±19.5	9.2	187±17.6	9.4	218±29.1	13.3
	600	624±14.8	2.4	526±36.8	7.0	567±46.4	8.2
Reduced haloperidol	25	32.2±2.3	7.0	25.8±9.0	34.7	33.4±8.8	26.5
	200	216±11.2	5.2	171±34.0	19.9	197±10.2	5.2
	600	658±33.0	5.0	468±95.2	20.3	584±57.7	9.9
CPHP	600	581±82.9	14.3	527±118.4	22.5	628±129	20.6
Urine							
Haloperidol	25	19.7±1.1	5.8	13.8±0.6	4.2	22.5±4.4	19.8
	200	223±11.5	5.2	185±11.0	6.0	189±13.2	7.0
	600	610±23.0	3.8	536±26.1	4.9	610±30.3	5.0
Reduced haloperidol	25	19.2±1.5	7.8	18.3±3.4	18.8	19.9±2.1	10.4
	200	226±10.8	4.8	175±18.0	10.3	217±15.8	7.3
	600	621±29.7	4.8	509±44.4	8.7	570±29.6	5.2
CPHP	600	637±58.9	9.3	653±152	23.2	548±72.6	13.4

respectively, and 7.8, 18.8 and 10.4% in urine, respectively; those for CPHP 14.3, 22.5 and 20.6% in plasma, respectively and 9.3, 23.2 and 13.4% in urine, respectively.

From the view points of detection limit, simplicity, and analytical precision, the method presented here seems recommendable in forensic toxicology.

4. Conclusion

In this study, we have established a simple and rapid determination method for haloperidol and its two metabolites in human body fluids without complicated sample pretreatment by using a new polymer HPLC column (MSpak GF-310), which enables direct injection of biological samples without a column switching technique. We can conclude that the present method is useful for identification and determination of haloperidol and its metabolites after high therapeutic and toxic dosing; the new polymer HPLC column should gain popularity for analysis of drugs and poisons in biological samples.

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