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Simultaneous determination of haloperidol and its metabolite, reduced haloperidol, in plasma, blood, urine and tissue homogenates by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic method was developed for the simultaneous determination of haloperidol and reduced haloperidol in human plasma, urine and rat tissue homogenates using bromperidol as an internal standard. The method involved extraction followed by injection of 50–80 μl of the aqueous layer onto a C_{18} reversed-phase column. The mobile phase was 0.5 *M* phosphate buffer–acetonitrile–methanol (58:31:11, v/v/v) and the flow-rate was 0.6 ml/min. The column effluent was monitored by ultraviolet detection at 214 nm. The retention times for reduced haloperidol, haloperidol and bromperidol were 5.4, 7.2 and 8.4 min, respectively. The detection limits for haloperidol and reduced haloperidol in human plasma were both 0.5 ng/ml, and the corresponding values in human urine were both 5 ng/ml. The coefficients of variation of the assay were generally low (below 10.7%) for plasma, urine, blood and tissue homogenates. No interferences from endogenous substances or any drug tested were found.

INTRODUCTION

High-performance liquid chromatographic (HPLC) methods for the determination of haloperidol (HP), a psychotic drug, in plasma have been developed earlier [1–11]. Recently, the reduced metabolite of HP, reduced haloperidol (RH), has been found to possess dopamine blocking activity of *ca.* 20% or more of the potency of HP [12,13], and a high RH-to-HP ratio seemed to be a predictor of a poor response in HP-treated patients [4]. Therefore, the determination of RH in biological samples seems to be essential. Simultaneous determination of HP and RH in human plasma by HPLC with UV [4,6,11] or electrochemical [5,7] detection has been reported. According to Parkinson [9] electrochemical detectors are not, however, in widespread use, and routine higher sensitivity operation at high electrode voltages can be difficult. In the present study, the strong UV absorption of HP and RH at 214 nm offers the possibility of UV detection to assay these compounds in the low nanogram range. None of the HPLC methods describes

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the simultaneous analysis of HP and RH in plasma, blood, urine and tissue homogenates, such as liver, brain, spleen, heart, kidney, adipose, lung, muscle and intestine. The analysis of HP in rat brain was reported [9], and its detection limit was 25 ng/g of brain when the whole extract (1.5 ml) was injected into the HPLC column.

This paper describes a method for the simultaneous analysis of HP and RH in human plasma and urine and rat tissue homogenates with better sensitivity than other HPLC methods with UV detection [1,4–6,11].

EXPERIMENTAL

Chemicals

HP was purchased from Sigma (St. Louis, MO, USA) and hexane and isoamyl alcohol were products of Fisher Scientific (Fairlawn, NJ, USA). RH and an internal standard, bromperidol (BP), were kindly supplied by Janssen Pharmaceutical (Beerse, Belgium). Methanol and acetonitrile were obtained from Merck (Rahway, NJ, USA). The other chemicals were reagent grade and used without further purification.

Sample extraction

Rat tissue was homogenized with four volumes of normal saline solution (adipose was homogenized without addition of any solution) using a Polytron (Brinkman Instruments, Westbury, N.Y. Rexdale, Canada). After centrifugation for 20 min at 2500 *g*, the supernatant was used. Blood was haemolysed in an ultrasonicator (Branson, Shelton, CT, USA) for 20 min. Approximately 0.2–1.0 ml of the biological sample was added to a 15-ml polyethylene tube containing BP dissolved in 50 μl of methanol. The mixture was then made alkaline by the addition of 300–500 μl of 2 *M* NaOH and extracted with 5 ml of hexane–isoamyl alcohol (98:2, v/v). After vigorous shaking for 10 min (40–60 min for tissue homogenates), the organic layer was separated by centrifugation at 2500 *g* for 10 min and transferred to a centrifuge tube containing 120 μl of 0.025 *M* H_2SO_4 (200 μl for tissue homogenates). After vortex-mixing for 0.5 min, the mixture was centrifuged at 2500 *g* for 20 min. The organic layer was aspirated and 50–80 μl of the aqueous layer were injected directly into the HPLC column.

HPLC apparatus

The HPLC system consisted of a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model 1330 pump (Bio-Rad, Japan Servo Company, Japan), a reversed-phase column (Nova-Pak, C_{18}, 15 cm × 2.9 mm I.D., particle size 4 μm, Waters Assoc., Milford, MA, USA), a Model 1306 UV detector (Bio-Rad) and a Model 1200 recorder (Linear, Reno, NV, USA). The mobile phase, 0.5 *M* KH_2PO_4–acetonitrile–methanol (58:31:11, v/v/v) adjusted to pH 3.8–4.0 with phosphoric acid, was run at a flow-rate of 0.6 ml/min and the eluate was detected at 214 nm.

The internal standard peak-height ratio was used to calculate the concentrations of HP and RH.

RESULTS AND DISCUSSION

Fig. 1. shows the UV absorption spectra of HP and RH dissolved in the present HPLC mobile phase. The UV absorption at 214 nm of both HP and RH is strong compared with that at 254 nm. Although there is also a strong UV absorption at 200 nm for both compounds, the background is much higher, so 214 nm was chosen.

Fig. 2 shows typical chromatograms of drug-free human plasma, drug standards in the human plasma, and plasma from a patient receiving HP: the corresponding chromatograms for human urine and rat liver homogenate are shown in Figs. 3 and 4, respectively. No interferences from endogenous sources were observed in any of the biological samples studied. The peaks of RH, HP and BP are symmetrical and eluted at 5.4, 7.2 and 8.4 min, respectively. We also noted no interferences from other commonly used psychoactive drugs, such as desipramine, imipramine, nortriptyline and amitriptyline. However, loxapine did interfere with the present chromatogram and it was separated by changing the buffer composition from 58 to 65% in the mobile phase. It is to be noted that both HP and RH in human plasma were fairly stable when stored at −70°C for one month.

The detection limits for HP and RH in human plasma were both 0.5 ng/ml (Table I), and the corresponding values in human urine were both 5 ng/ml (Table

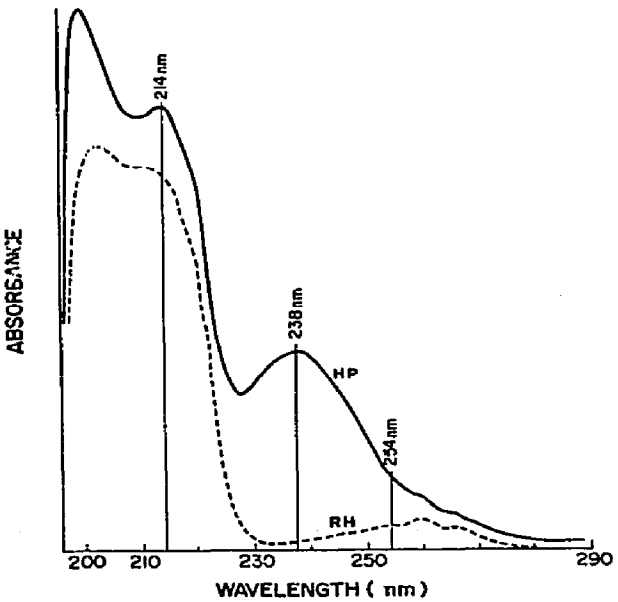

Fig. 1. UV absorption spectra of HP and RH dissolved in the mobile phase 0.05 *M* phosphate buffer–acetonitrile–methanol (58:31:11, v/v/v). The concentrations of HP and RH were both 100 ng/ml.

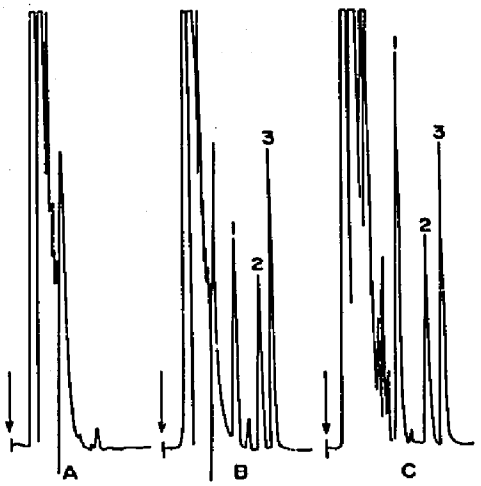

Fig. 2. Chromatograms of extracts from blank human plasma (A), human plasma spiked with 25 ng/ml HP, 22.5 ng/ml RH and 50 ng/ml BP (B), and plasma obtained from a psychotic patient during steady state after oral administration of HP at 50 mg per day (C). Peaks: 1 = RH (5.4 min); 2 = HP (7.2 min); 3 = BP (8.4 min). The arrows mark the points of injection.

II) based on a signal-to-noise ratio of 3.0. The mean within-day coefficients of variation (C.V.) in human plasma were 3.99% (range 1.14–9.31%) and 4.57% (range 0.59–9.54%) for HP and RH (Table I), respectively, and the corresponding values in human urine were 2.10% (range 1.00–4.96%) and 4.67% (range 2.42–8.16%, Table II). The between-day C.V. for the analysis of the same plasma

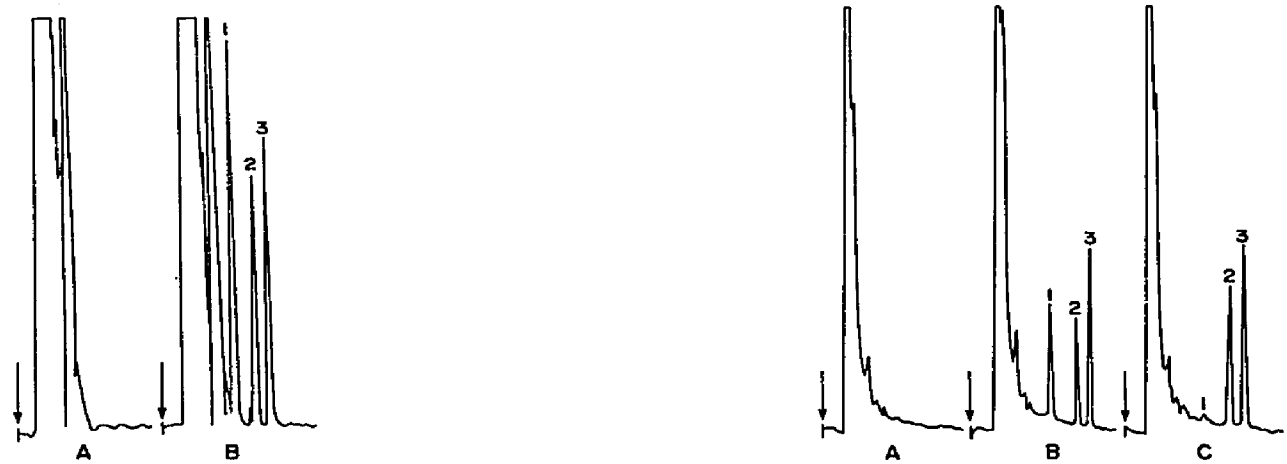

Fig. 3. Chromatograms of extracts from blank human urine (A) and urine spiked with 40 ng/ml HP, 40 ng/ml RH and 50 ng/ml BP (B). Peaks: 1 = RH (5.4 min); 2 = HP (7.2 min); 3 = BP (8.4 min). The arrows mark the points of injection.

Fig. 4. Chromatograms of extracts from blank rat liver homogenate (A), rat liver homogenate spiked with 250 ng/ml HP, 250 ng/ml RH and 500 ng/ml BP (B), and liver homogenate obtained from a rat 120 min after intravenous bolus administration of HP at 1 mg/kg (C). Peaks: 1 = RH (5.4 min); 2 = HP (7.2 min); 3 = BP (8.4 min). The arrows mark the points of injection.

.BLE I

RESPONSE FACTORS AND RECOVERIES OF HALOPERIDOL AND REDUCED HALOPERIDOL AT VARIOUS CONCENTRATIONS IN HUMAN PLASMA

Values in parentheses are C.V. (%); $n = 6$.

Concentration (ng/ml)	Haloperidol		Reduced haloperidol	
	Response factor[a]	Recovery[b] (%)	Response factor[a]	Recovery[b] (%)
100	1.2548 ± 0.0143 (1.14)	99	1.5580 ± 0.0091 (0.59)	75
50	1.2590 ± 0.0146 (1.15)	–	1.6107 ± 0.0251 (1.33)	–
20	1.2156 ± 0.0189 (1.55)	94	1.5236 ± 0.0325 (2.13)	73
10	1.2458 ± 0.0285 (2.29)	–	1.6005 ± 0.0609 (3.08)	–
5	1.2241 ± 0.0335 (2.75)	95	1.5775 ± 0.0725 (4.59)	72
2	1.3275 ± 0.1128 (6.88)	–	1.5754 ± 0.1204 (7.64)	–
1	1.2254 ± 0.0844 (6.88)	93	1.5919 ± 0.1519 (9.54)	73
0.5	1.3097 ± 0.1219 (9.31)	–	1.6157 ± 0.1243 (7.69)	–

[a] (Drug peak height divided by its concentration)/(internal standard peak height divided by its concentration); mean ± S.D.

[b] Relative recovery compared with water.

TABLE II

RESPONSE FACTORS AND RECOVERIES OF HALOPERIDOL AND REDUCED HALOPERIDOL AT VARIOUS CONCENTRATIONS IN HUMAN URINE

Values in parentheses are C.V. (%); $n = 6$.

Concentration (ng/ml)	Haloperidol		Reduced haloperidol	
	Response factor[a]	Recovery[b] (%)	Response factor[a]	Recovery[b] (%)
1000	1.2451 ± 0.0154 (1.24)	99	1.6953 ± 0.0[illegible] (2.45)	81
500	1.2174 ± 0.0147 (1.21)	–	1.6724 ± 0.040[illegible]	–
200	1.3015 ± 0.0151 (1.55)	95	1.7531 ± 0.05[illegible]	86
100	1.2762 ± 0.0127 (1.00)	–	1.7747 ± 0.0883 (4.98)	–
50	1.2832 ± 0.0266 (2.07)	98	1.7674 ± 0.1443 (8.16)	85
20	1.2536 ± 0.0253 (1.55)	–	1.7125 ± 0.1127 (6.58)	–
10	1.2613 ± 0.0452 (3.59)	100	1.7452 ± 0.0834 (4.78)	84
5	1.3121 ± 0.0651 (4.96)	–	1.7915 ± 0.0912 (5.09)	–

[a] (Drug peak height divided by its concentration)/(internal standard peak height divided by its concentration); mean ± S.D.

[b] Relative recovery compared with water.

TABLE III

RESPONSE FACTORS AND RECOVERIES OF HALOPERIDOL AND REDUCED HALOPERIDOL AT VARIOUS CONCENTRATIONS IN RAT BLOOD AND TISSUE HOMOGENATES

Sample[a]	Volume (ml)	Concentration (ng/ml)	Haloperidol		Reduced haloperidol	
			Response factor[b]	Recovery[c] (%)	Response factor[b]	Recovery[c] (%)
Blood	1	1 (4)[d]	1.065 ± 0.109 (10.2)[e]	80	1.783 ± 0.159 (8.91)	81
		20 (3)	1.217 ± 0.055 (4.52)	94	1.875 ± 0.100 (5.32)	89
		100 (3)	1.223 ± 0.041 (3.34)	96	1.868 ± 0.047 (2.54)	89
Liver	0.3	50 (4)	1.174 ± 0.097 (8.26)	90	1.532 ± 0.149 (9.71)	70
		500 (3)	1.193 ± 0.064 (5.35)	93	1.853 ± 0.116 (6.25)	88
		1000 (3)	1.226 ± 0.051 (4.13)	97	1.874 ± 0.089 (4.73)	90
Brain	0.5	20 (4)	1.026 ± 0.054 (5.27)	79	1.482 ± 0.109 (7.36)	74
		100 (3)	1.167 ± 0.063 (5.41)	91	1.562 ± 0.083 (5.34)	74
		500 (3)	1.201 ± 0.033 (2.72)	95	1.762 ± 0.060 (3.42)	88
Spleen	0.5	20 (3)	1.105 ± 0.092 (8.34)	85	1.552 ± 0.159 (10.2)	71
		500 (3)	1.152 ± 0.059 (5.12)	90	1.564 ± 0.083 (5.29)	75
		1000 (3)	1.201 ± 0.042 (3.52)	95	1.821 ± 0.088 (4.81)	87

Heart	1	20 (3)	1.062 ± 0.078 (7.34)	81	1.534 ± 0.129 (8.43)	73
		100 (3)	1.120 ± 0.059 (5.23)	89	1.677 ± 0.106 (6.34)	80
		500 (3)	1.109 ± 0.052 (4.72)	87	1.752 ± 0.079 (4.53)	84
Kidney	0.5	20 (4)	1.101 ± 0.083 (7.54)	84	1.531 ± 0.146 (9.51)	76
		500 (3)	1.226 ± 0.081 (6.63)	95	1.452 ± 0.063 (4.35)	72
		1000 (3)	1.205 ± 0.039 (3.24)	95	1.691 ± 0.064 (3.76)	82
Adipose	0.5	20 (3)	1.083 ± 0.104 (9.56)	83	1.564 ± 0.167 (10.7)	78
		500 (3)	1.156 ± 0.062 (5.34)	90	1.567 ± 0.082 (5.23)	77
		1000 (3)	1.151 ± 0.052 (4.55)	91	1.644 ± 0.068 (4.13)	79
Lung	0.2	100 (3)	1.174 ± 0.062 (5.31)	90	1.573 ± 0.098 (6.22)	78
		500 (3)	1.303 ± 0.042 (3.24)	96	1.699 ± 0.070 (4.12)	81
		5000 (4)	1.312 ± 0.031 (2.33)	97	1.724 ± 0.037 (2.15)	86
Muscle	1	20 (3)	1.124 ± 0.082 (7.31)	86	1.463 ± 0.122 (8.35)	73
		100 (3)	1.212 ± 0.066 (5.42)	94	1.632 ± 0.071 (4.38)	77
		400 (3)	1.198 ± 0.029 (2.43)	94	1.756 ± 0.044 (2.48)	85
Intestine	1	20 (4)	1.151 ± 0.106 (9.25)	88	1.552 ± 0.166 (10.7)	71
		100 (3)	1.203 ± 0.076 (6.34)	94	1.775 ± 0.111 (6.23)	85
		400 (3)	1.215 ± 0.053 (4.37)	96	1.863 ± 0.066 (3.54)	89

[a] Tissue samples were homogenated with four volumes of normal saline except adipose.
[b] (Drug peak height divided by its concentration)/(internal standard peak height divided by its concentration); mean ± S.D.
[c] Relative recovery compared with water.
[d] Sample numbers.
[e] C.V. (%), $n = 3$ or 4.

samples on six days were 3.97 and 3.66% for HP and RH, respectively, and the corresponding values in urine were 4.15 and 4.20%. Mean analytical recoveries of added HP and RH from plasma by this extraction procedure were 95% (range 93–99%) and 73% (range 72–75%) for HP and RH, respectively (n = 6, Table I), and the corresponding values from urine were 98% (range 95–100%) and 84% (range 81–86%, Table II).

The present assay method was also successful for the analysis of HP and RH in rat tissues (Table III). The detection limits for both HP and RH were 1, 25, 20, 20, 10, 20, 20, 50, 10 and 10 ng/g of tissue for blood, liver, brain, spleen, heart, kidney, adipose, lung, muscle and intestine, respectively. The mean within-day C.V. for HP ranged from 3.63% (lung) to 6.65% (intestine) and the corresponding values for RH were from 4.16% (lung) to 6.90% (liver). The mean analytical recoveries of added HP ranged from 85.1% (heart) to 94.3% (lung), and the corresponding values for RH were from 76.7% (kidney) to 86.3% (blood).

The mean plasma profiles of HP and RH after intravenous bolus administration of HP, 10 mg to eight psychotic patients, and RH, 5 mg/kg to five rats, are shown in Figs. 5 and 6, respectively. The mean terminal half-life, total body clearance, volume of distribution at steady state and mean residence time of HP were 676 min, 12.4 ml/min/kg, 11.9 l/kg and 925 min, respectively, in the patients, and the corresponding values of RH were 481 min, 96.1 ml/min/kg, 43.5 l/kg and 500 min in the rats. It is of interest to note that RH was detected in the plasma

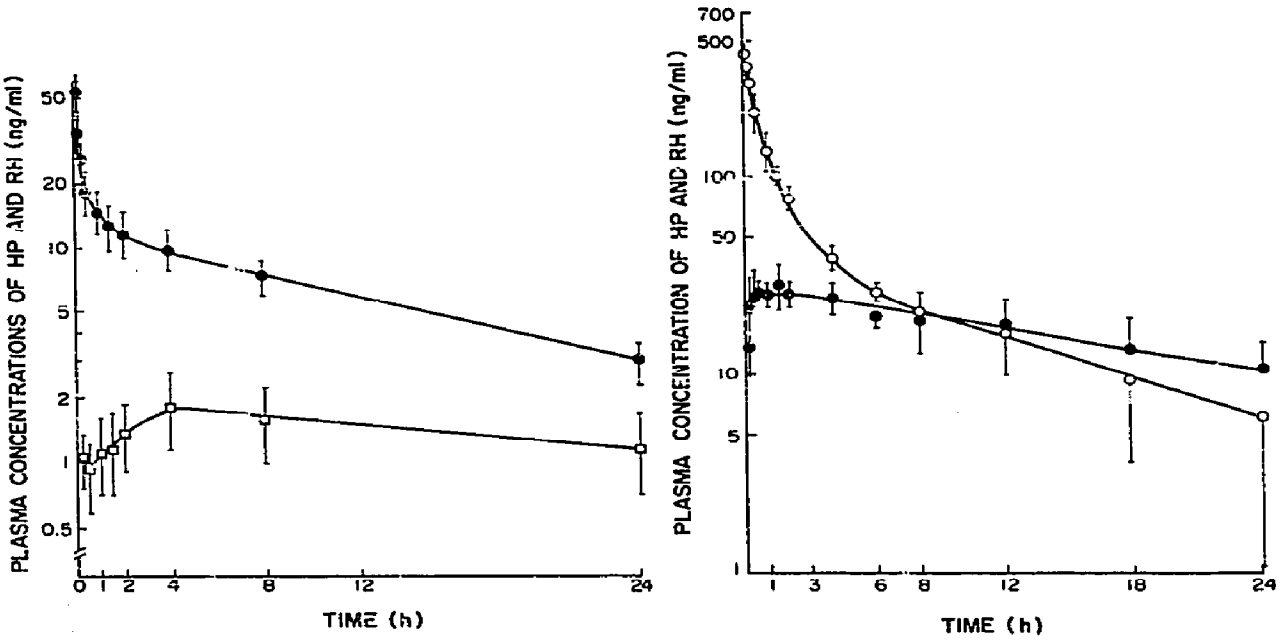

Fig. 5. Mean plasma concentration–time profiles of HP (●) and RH (□) after intravenous bolus administration of HP (10 mg) to eight psychotic patients. Bars represent standard deviations.

Fig. 6. Mean plasma concentration–time profiles of HP (●) and RH (○) after intravenous bolus administration of RH (5 mg/kg) to five rats. Bars represent standard deviations.

when HP was injected into rats, rabbits and humans, and HP was detected in the plasma when RH was injected into rats (Fig. 6). However, HP was not detected in plasma when RH was injected into rabbits [14].

The mean blood partition (blood-to-plasma concentration ratio) values from fifteen psychotic patients were 1.04 and 1.77 for HP and RH, respectively, in the concentration ranges 3–60 ng/ml for HP and 1–100 ng/ml for RH. The tissue concentrations of HP and RH were measured at 120 min after intravenous injection of HP (1 mg/kg) into rats ($n = 5$); the mean values for HP were 20.8 ng/ml and 1230, 899, 6680, 507, 1250, 2520, 352, 326 and 1600 ng/g of tissue for plasma, liver, brain, lung, heart, kidney, spleen, intestine, muscle and adipose, respectively, and the corresponding values for RH were 2.30 ng/ml and 55.2, 96.4, 289, 53.6, 90.5, 137, 95.2, 20.3, and 257 ng/g of tissue.

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