ELSEVIER

Journal of Chromatography B, 688 (1997) 275–280

JOURNAL OF CHROMATOGRAPHY B

Determination of haloperidol and its reduced metabolite in human plasma by liquid chromatography–mass spectrometry with electrospray ionization

H. Hoja[a], P. Marquet[a,*], B. Verneuil[a], H. Lotfi[a,b], J.L. Dupuy[a], B. Pénicaut[a], G. Lachâtre[a,b]

[a]*Service de Pharmacologie et Toxicologie, Chru Dupuytren, 2 Avenue du Pasteur Martin-Luther-King, 87042 Limoges Cedex, France*
[b]*Laboratoire de Toxicologie, Faculté de Pharmacie, 2 Rue du Docteur Marcland, 87025 Limoges Cedex, France*

Received 18 April 1996; revised 25 June 1996; accepted 25 June 1996

Abstract

A sensitive and accurate liquid chromatographic–electrospray mass spectrometric (LC–ES-MS) method for the determination of haloperidol (H) and reduced haloperidol (RH) in human plasma is presented, using chlorohaloperidol as the internal standard. A 2-ml volume of plasma was subjected to basic (NaOH) extraction, acid (HCl) back-extraction, acid wash and basic (NaOH) re-extraction. The extraction solvent was hexane–isoamyl alcohol (99:1, v/v) for the whole procedure. A Nucleosil C_{18} column (150×1 mm) was used for high-performance liquid chromatography, together with 2 m*M* $HCOONH_4$–acetonitrile (55:45, v/v; pH 3.0) as the mobile phase. For each drug, four characteristic ions were monitored. Linearity was assessed in the ranges 0.1–50 and 0.25–50 ng/ml for H and RH, respectively. Recoveries were 58 and 70% and detection limits were 0.075 and 0.100 ng/ml for H and RH, respectively. Correlation coefficients were better than 0.999 for both compounds. R.S.D.s for repeatability and reproducibility at 0.25 ng/ml were 11.1 and 8.5% for H and 9.4 and 11.2% for RH, respectively. One of the main advantages of (LC–ES-MS) over other detection systems is the increase in selectivity obtained by monitoring three ions of confirmation for each of the drugs.

Keywords: Haloperidol; Reduced haloperidol

1. Introduction

Haloperidol (H) is a potent neuroleptic drug widely used in the treatment of schizophrenia. One of its metabolites, reduced haloperidol (RH), also possesses significant pharmacological activity [1]; therefore, its plasma level is commonly determined together with that of the parent drug. Numerous methods for the determination of H and RH in plasma have been reported, using HPLC with UV- [2–10] or electrochemical detection [11–14], HPLC coupled to thermospray mass spectrometry (TS-MS) [15], GC with thermoionic detection (NPD) [16,17] or coupled to chemical ionization MS [18,19]. Limits of quantification (LOQ) at the low nanogram level and even, for some of them, at medium picogram levels were attained. Apart from methods using MS detection, which gain in specificity on monitoring selected ions, the other detectors may sometimes suffer from interfering compounds when samples

*Corresponding author.

PII S0378-4347(96)00294-0

from patients with multiple medical treatments are determined. A radioimmunoassay (RIA) for H only, showing little cross-reactivity with its metabolites, has also been reported with a limit of detection of the same order of magnitude as that obtained by the most sensitive chromatographic methods [20].

The aim of this study is to enhance selectivity and sensitivity of H and RH determination in human plasma by using reversed-phase HPLC coupled to electrospray mass spectrometry (LC–ES-MS) with selected-ion monitoring (SIM) detection, a method which, to our knowledge, has never been reported.

2. Experimental

2.1. Materials and chemicals

H, RH and chlorohaloperidol (the internal standard, I.S.) were all gifts from Janssen Pharmaceutica (Beerse, Belgium). Stock solutions of drugs were prepared at 1 g/l by dissolution in methanol and were kept at 4°C in the dark. They proved to be stable for several months. Working solutions were freshly prepared by appropriate dilution in deionized water. NaOH, HCl and *n*-hexane Pestinorm were purchased from Prolabo (Paris, France), isoamyl alcohol from Merck (Darmstadt, Germany), high purity formic acid and ammonium formate from Sigma (St. Louis, MO, USA) and HPLC-grade acetonitrile from Carlo Erba (Milan, Italy). Water was purified with a Milli-Q-system (Millipore, Bedford, MA, USA). Samples were extracted with a test-tube rotator (Breda Scientific, Breda, Netherlands) or with a reciprocating shaker (J. Toulemonde, Paris, France). HPLC was performed on a Nucleosil C_{18} 5 μm 100 Å 150×1 mm I.D. column (LC-Packings, Touzart-Matignon, Paris, France). The solvent was dispensed by a Brownlee double-piston syringe pump (Brownlee, CA, USA). Samples were injected with a Rheodyne 7410 injection valve equipped with a 1-μl internal loop (Touzart-Matignon) An API 100 mass spectrometer (SCIEX, Foster City, Canada) with its electrospray-type ionspray source was used. Infusion experiments were carried out with a Harvard Model 11 syringe pump (Harvard Apparatus, South Natick, MA, USA).

2.2. Extraction

Blood samples (approx. 5 ml), received in heparinized Becton-Dickinson tubes, were centrifuged for 15 min at 2500 *g* and plasma was frozen at −20°C until analysis. A mixture of *n*-hexane–isoamyl alcohol (99:1, v/v) was used as the organic solvent for the whole extraction procedure. After each extraction or wash step, the samples were centrifuged for 5 min at 2000 *g*.

To 2 ml of thawed plasma, 50 μl of the internal standard (500 ng/ml), 500 μl of 2 *M* NaOH and 8 ml of the organic solvent mixture were added in 15-ml round-bottomed glass tubes with PTFE-lined screw caps. To prevent the formation of an emulsion, extraction was performed gently for 20 min on a test-tube rotator, keeping the rotating plate in an almost horizontal position. After centrifugation, the organic phase was transferred to a clean set of 15-ml glass tubes with PTFE-lined screw-caps and was back-extracted for 15 min with 1 ml of 0.2 *M* HCl by vigourous shaking on a reciprocating shaker. The organic layer was aspirated and an acid wash step (5 min) with 5 ml of the organic solvent mixture was performed. The solvent was discarded again and samples were re-extracted (15 min) after addition of 500 μl of 2 *M* NaOH and 7 ml of the organic solvent mixture. The organic layer was transferred to 10-ml conical glass tubes and evaporated to dryness under a gentle stream of nitrogen at 37°C. The dry extract was reconstituted in 50 μl of mobile phase, of which 1 μl was injected onto the chromatographic system.

2.3. Liquid chromatography

All chromatographic solvents were degassed (He) and filtered prior to use. Tubing with an I.D. of 100 μm or less and low dead-volume unions were used downstream of the injection valve. Chromatography was carried out at ambient temperature at a flow-rate of 50 μl/min with 2 m*M* ammonium formate–acetonitrile (55:45, v/v), adjusted to pH 3 with formic acid, as the mobile phase. The column outlet was connected to a 100-μm I.D. fused-silica capillary which transferred the whole eluent into the ion source.

2.4. Mass spectrometry

Mass calibration from 30 to 2250 u was done by infusing a standard mixture of poly(propylene glycol)s (PPGs, Applied Biosystems, Paris, France) at a flow-rate of 5 μl/min and by monitoring eight m/z ratios in this range. Resolution was adjusted to give a peak width of 0.7±0.1 u at 50% of the peak height. Stability of mass calibration was checked frequently and found to be excellent (variations of less than 0.1 u in two months), so the mass spectrometer was not recalibrated during this period.

Electrospray ionization was performed with nitrogen as the nebulizing (1.23 l/min) and curtain (1.25 l/min) gas. Instrument response for H, RH and I.S. was optimized by infusion experiments of the pure drugs, dissolved in the mobile phase at a flow-rate of 50 μl/min. Firstly, mass spectra in the range of 30–500 u for solutions of the pure drugs in the mobile phase were acquired at three different orifice voltages (ORV), 20, 60 and 120 V, respectively. This parameter controls the acceleration of the ions in the intermediate pressure region between atmospheric and vacuum and thus the energy of ion–molecule collisions, which, in turn, is responsible for fragmentation. With a low voltage, molecular ions are preserved whereas a higher voltage favors fragmentation. This feature is particularly valuable as the ionizing energy imparted to the ions in atmospheric pressure ionization techniques is often too low to yield fragment ions in addition to the protonated molecular ions. Instrumental responses for the four most abundant ions for each drug identified in the first experiment were further optimized by ramping the ionspray voltage (ISV) and ORV from 3000 to 6000 V with a step size of 250 V and from 20 to 150 V with a step size of 5 V, respectively. Optimum response with regard to signal intensity and stability was obtained for the following voltages: 4750 V for ISV, ORV variable (see Section 3), 400 V for ring (RNG) and −10 V for quadrupole 0 (Q_0). These settings were maintained for further operation and the electron multiplier (CEM) voltage was checked from time to time in order to ensure that analyses were performed in the linear range of the electron multiplier. A dwell time of 120 ms for each ion was used.

2.5. Validation procedure

Recoveries were determined in triplicate at concentrations of 1 and 25 ng/ml, by extracting drug-free plasma samples spiked with H and RH. The I.S. was prepared in the solvent used for sample reconstitution and thus was added after the extraction procedure. Recoveries were calculated by comparison of the analyte/I.S. peak-area ratios of the extracted samples with those of unextracted standard mixtures representing 100% recovery. Repeatability was determined at 0.25, 5 and 25 ng/ml by extraction and analysis, on the same day, of six aliquots of spiked plasma for each concentration.

For the assessment of linearity and reproducibility, blank plasma samples (19 ml) were spiked with 1 ml of aqueous solutions of H and RH in order to obtain respective concentrations of 0, 0.1, 0.25, 0.5, 1, 5, 10, 25 and 50 ng/ml, of which, 2-ml aliquots were stored at −20°C until analysis. One aliquot of each concentration was thawed and analyzed each day for six days. Calibration graphs were constructed using a $1/x$-weighted least-squares linear regression of the drug-to-internal standard peak-area ratios of the most abundant ions, 376.2 and 378.2 for H and RH, respectively, versus theoretical drug concentrations.

3. Results and discussion

Fig. 1 shows mass spectra of H and RH acquired with an ORV voltage of 60 V. Several prominent ions can be identified for H. The protonated molecular ions $[M+H]^+$ at m/z 376.2 and 378.2, with an isotope ratio of 3:1, due to the presence of one chlorine atom in the molecule, while m/z 358.2 and 360.2 ions with the same isotope ratio result from a dehydration process. Other major ions are found at m/z 165.0 and 123.1. The first one results, as previously reported [21], from the charge-initiated cleavage of the alkyl carbon–nitrogen bond, with expulsion of the nitrogen-containing moiety as a neutral species; the second can be described by the formula $[F–C_6H_4–C=O]^+$. Further characteristic ions of lower abundance are found at m/z 194.2 and 206.2.

The molecular mass of RH, which is formed by

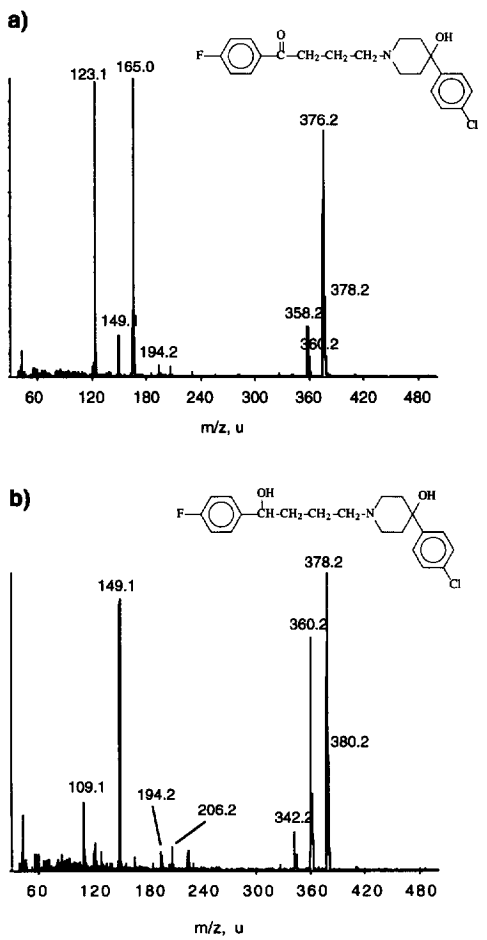

Fig. 1. Mass spectra of (a) H and (b) RH obtained by infusion experiments of the pure drugs dissolved in the mobile phase using an orifice voltage (ORV) of 60 V.

hydroxylation of the carbonyl group, differs from that of the parent compound only by 2 u with $[M+H]^+$ at m/z 378.2/380.2. It undergoes dehydration in two steps, yielding, in the first step, abundant ions at m/z 360.2/362.2, implicating the benzylic hydroxyl group whereas the second step, with ions at m/z 342.3/344.3, is less important. Dominant low mass ions are m/z 109.1 and 149.1 which can be described by the formulae $[F-C_6H_4-CH_2]^+$ and $[F-C_6H_4-C_4H_6]^+$, respectively. They represent a major fragmentation pathway of RH and their high abundance can be explained by their stabilization by resonance. Other less abundant ions are found at m/z 194.2/196.2 and 206.2/208.2 for which the respective formulae $[H-NC_5H_7-C_6H_4-Cl]^+$ and $[CH_2=NC_5H_7-C_6H_4-Cl+H]^+$ have been proposed [21].

ISV had little incidence on ion abundances, therefore, a common value was chosen. The ORV parameter, on the other hand, was found to significantly modify the abundances; thus, optimum settings for each ion were used for data acquisition (i.e. throughout data acquisition, each recorded m/z ratio was monitored with its optimum ORV). Table 1 shows the m/z ratios monitored, their relative intensities and the respective ORV applied in SIM analysis.

Four ions were chosen for each drug, m/z 123.1, 165.0, 376.2 and 378.2 for H, m/z 109.1, 360.2, 378.2 and 380.2 for RH, while I.S. was monitored at m/z 392.2. It should be noted that the ion at m/z 149.1 for RH was not chosen because phthalate esters, yielding fragment ions at this m/z ratio, are ubiquitous in modern laboratories. Quantification was performed on the respective protonated molecular ions, 376.2 and 378.2 for H and RH, while the relative abundances of the three others were used for confirmation, thus increasing the specificity of determination. Typical retention times were 5.0 min for RH, 6.6 min for H and 8.7 min for I.S. Fig. 2 depicts chromatograms obtained for a blank plasma, plasma spiked at 0.1 ng/ml and an extract of a patient plasma, where 9.54 and 13.96 ng/ml concentrations were found for H and RH, respectively. A slight tailing of the peaks was observed, which was due to the basic character of the drugs. Addition of suppressing agents like triethylamine, which might have provided more symmetric peaks was unfortunately

Table 1
Orifice voltages (ORV) applied and relative abundances of molecular and fragment ions from haloperidol and reduced haloperidol during selected ion monitoring acquisition

m/z	ORV (V)	Relative intensity (%)	
		H	RH
109.1	100	<2	28.2
123.1	95	95.5	2.4
165.0	65	43.0	2.3
360.2	50	4.5	49.8
376.2	35	100.0	–
378.2	35	36.6	100.0
380.2	35	–	35.1
392.2	35	–	–

Quantification ions are underlined.

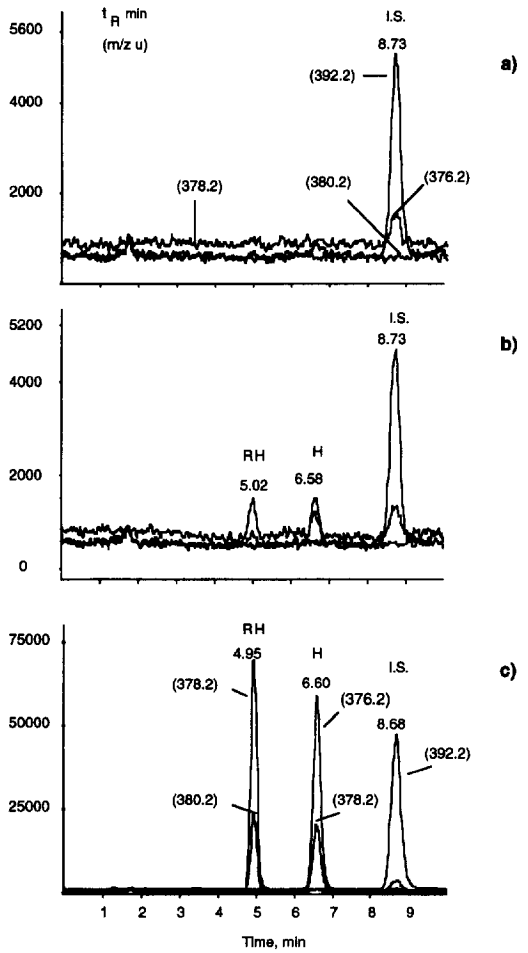

Fig. 2. Extracted ion chromatograms of extracts from (a) a blank plasma, (b) a plasma sample spiked at 0.1 ng/ml and (c) a patient plasma. [Column: Nucleosil C_{18}, 5 μm, 150×1 mm; eluent: 2 m*M* $HCOONH_4$–CH_3CN (55:45, v/v) adjusted to pH 3.0; flow-rate: 50 μl/min].

incompatible with the technique because of its high gas phase basicity, which would lead to a concomitant loss of ionization efficiency of the analytes.

Mean recovery for H was 58% at both concentration levels, while for the metabolite it was 71 and 66% at 1 and 25 ng/ml, respectively. As care was taken to obtain sample extracts that were as clean as possible using a rather time-consuming sample work-up, sample losses were accumulated by multiple extraction steps. These values are of the same order as those reported previously by other workers using similar extraction procedures [16], but are lower than those attained with solid-phase or one-step extraction procedures [7,8,11,12]. Limits of detection were 0.075 and 0.100 ng/ml for H and RH, respectively.

For repeatability, R.S.D. values are reported in Table 2. They are all satisfactory, between 5 and 12%.

Linearity was assessed with nine-point calibration curves in the range of 0.1–50 ng/ml, including a blank. Using $1/x$-weighted least-squares linear regression analysis, typical equations were $A_{(H)}/A_{(I.S.)}=0.10061X+0.00429$ for H and $A_{(RH)}/A_{(I.S.)}=0.06188X+0.00445$ for RH, where $A_{(H)}/A_{(I.S.)}$ and $A_{(RH)}/A_{(I.S.)}$ are the peak-area ratios and X is the theoretical concentration. Correlation coefficients were higher than 0.999 for both compounds. Analysis of variance, performed with the calculated concentrations versus added concentrations, verified an insignificant non-linearity ($n=54$). Table 3 shows the values calculated for the assessment of the reproducibility and the accuracy of the method. Applying the common requirement of the R.S.D. being less than 20%, the limit of quantification (LOQ) for H

Table 2
Repeatability of haloperidol and reduced haloperidol determinations in human plasma

Added concentration (ng/ml)	Haloperidol		Reduced metabolite	
	Mean concentration found ($n=6$) (ng/ml)	R.S.D. (%)	Mean concentration found ($n=6$) (ng/ml)	R.S.D. (%)
0.25	0.24	11.1	0.25	9.4
5	5.63	9.1	5.75	9.2
25	23.82	5.8	23.53	7.4

Table 3
Reproducibility and accuracy of haloperidol and reduced metabolite determinations in human plasma

Added concentration (ng/ml)	Haloperidol (n=6)			Reduced metabolite (n=6)		
	Mean concentration found (ng/ml)	R.S.D. (%)	Accuracy (%)	Mean concentration found (ng/ml)	R.S.D. (%)	Accuracy (%)
0.1	0.087	14.8	86.9	0.079	30.5	79.0
0.25	0.253	8.5	101.2	0.253	11.2	101.1
0.5	0.556	6.2	111.2	0.559	12.3	111.8
1	1.038	8.2	103.8	1.049	9.0	104.9
5	4.953	5.0	99.1	5.240	9.0	104.8
10	9.851	4.3	98.5	10.021	5.6	100.2
25	24.519	2.2	98.1	24.497	4.2	98.0
50	50.592	1.2	101.2	50.152	2.7	100.3

was 0.1 ng/ml, for which R.S.D. was 14.8% and S/N=5. LOQ for RH was 0.25 ng/ml, with an R.S.D. of 11.2% and S/N=9. The higher LOQ value determined for RH might be due to coeluting endogeneous compounds, which could compete for ionization. This phenomenon would be more pronounced for the metabolite, as it eluted first. These lower LOQs were far below the concentrations normally encountered in patients with steady-state treatments. They compared favorably with those of the most sensitive methods previously published, which gave 0.1 and 0.5 ng/ml for HPLC with electrochemical detection (ED) [11,12] and with UV detection [7,9], 0.1 ng/ml for GC–MS [19] and 0.3 ng/ml for RIA [20].

The upper LOQs were 50 ng/ml for both drugs. This wide range of linearity was useful for both drug-monitoring and toxicology uses, where high concentrations are occasionally found. The selectivity and specificity of the determination were enhanced through simultaneous monitoring of four characteristic ions for each drug, which represents another improvement compared to the HPLC–ED method of Korpi et al. [22], which was previously used in the laboratory. As a matter of fact, ED was sometimes affected by interferences of unknown nature, with retention times close to those of H, RH or I.S. This problem has never occurred during the last few months since MS detection was applied.

References

[1] R.T. Rubin and R.R. Poland, in S. Dahl and E. Usdin (Editors), Clinical Pharmacology in Psychiatry, Macmillan, London, 1981, pp. 217–225.
[2] G.T. Vatassery, L.A. Holden and M.W. Dysken, J. Anal. Toxicol., 17 (1993) 304.
[3] T. Ohkubo, R. Shimomaya and K. Suguwara, J. Pharm. Sci., 81 (1992) 947.
[4] K.H. Park, M.H. Lee and M.G. Lee, J. Chromatogr., 572 (1991) 259.
[5] S. Cahard, P.P. Rop, T. Conquy and A. Viala, J. Chromatogr., 532 (1990) 193.
[6] S.T. Tan and P.J. Boniface, J. Chromatogr., 532 (1990) 181.
[7] D. Wilhelm and A. Kemper, J. Chromatogr., 525 (1990) 218.
[8] L.B. Nilsson, J. Chromatogr., 431 (1988) 113.
[9] G.T. Vatassery, L.A. Herzan and M.W. Dysken, J. Chromatogr., 432 (1988) 312.
[10] R.L. Miller and C.L. Devane, J. Chromatogr., 374 (1986) 405.
[11] M. Aravagiri, S.R. Marder, T. van Putten and B.D. Marshall, J. Chromatogr. B, 656 (1994) 373.
[12] N.D. Eddington and D. Young, J. Pharm. Sci., 77 (1988) 541.
[13] D.W. Hoffman and R.D. Edkins, Therap. Drug Monit., 16 (1994) 504.
[14] M. Hariharan, E.K. Kindt, T. van Noord and R. Tandon, Ther. Drug Monit., 11 (1989) 701.
[15] A.M.A. Verweij, M.L. Hordijk and P.J.L. Lipman, J. Anal. Toxicol., 19 (1995) 65.
[16] S. Ulrich, F.P. Meyer, S. Neuhof and W. Knorr, J. Chromatogr. B, 663 (1995) 289.
[17] E.S. Burstein, H. Friedman and D.J. Greenblatt, J. Chromatogr., 423 (1987) 380.
[18] P.A. Szczepanik-van Leeuwen, J. Chromatogr., 339 (1985) 321.
[19] N. Häring, Z. Salama, L. Todesko and H. Jaeger, Arzneim. Forsch., 37 (1987) 1402.
[20] Y. Terauchi, S. Ishikawa, S. Oida, M. Nakao, A. Kagemoto, T. Oida, Y. Utsui and Y. Sekine, J. Pharm. Sci., 798 (1990) 432.
[21] A.J. Tomlinson, L.M. Benson, K.L. Johnson and S. Naylor, J. Chromatogr., 621 (1993) 239.
[22] E.R. Korpi, B.H. Phlebs, H. Granger, W.H. Chang, M. Linnoila, J.L. Meek and R.J. Wyatt, Clin. Chem., 29 (1983) 624.