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Journal of Chromatography B, 820 (2005) 237–242
JOURNAL OF CHROMATOGRAPHY B
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Sensitive method for the quantitative determination of bromocriptine in human plasma by liquid chromatography–tandem mass spectrometry

Arnaud Salvador[a,c,*], Didier Dubreuil[a], Jannick Denouel[b], L. Millerioux[a]

[a] *CEPHAC Europe, 90 Avenue des Hauts de la Chaume, BP 28, 86281 Saint-Benoit, France*
[b] *Novartis Pharma S.A.S, 2-4 rue Lionel-Terray, BP 308, 92506 Rueil-Malmaison, France*
[c] *Université Claude Bernard Lyon 1, UMR 5180, 43 Boulevard du 11 novembre 1918, 69622 Villeurbanne Cedex, France*

Received 26 May 2004; accepted 29 March 2005

Abstract

A sensitive LC–MS–MS assay for the quantitative determination of bromocriptine has been developed and validated and is described in this work. The assay involved the extraction of the analyte from 1 ml of human plasma using a solid phase extraction on Oasis MCX cartridges. Chromatography was performed on a Symmetry C_{18} (2.1 mm × 100 mm, 3.5 μm) column using a mobile phase consisting of 25:75:01 acetonitrile–water–formic acid with a flow rate of 250 μl/min. The linearity was within the concentration range of 2–500 pg/ml. The lower limit of quantification was 2 pg/ml. This method has been demonstrated to be an improvement over existing methods due to its greater sensitivity and specificity.

Keywords: Bromocriptine; Mass spectrometry; Liquid chromatography

1. Introduction

Bromocriptine (2-bromo-α-ergocriptine) (BC) is an ergot derivative that has been marketed for over 20 years, and has a potent dopamine receptor agonist activity, which activates post-synaptic dopamine receptors. It is used in the treatment of hyperprolactinaemic disorders, acromegaly and Parkinson's disease [1,2]. The total daily doses typically employed in these indications are 2.5–15 mg, 20–30 mg and 10–40 mg, respectively [3]. In each case, the daily dose is given in two- to fourfold smaller doses to decrease potential side effects and to achieve a longer lasting therapeutic action. The most common adverse events observed in healthy volunteers and in patients are the following: nausea, dizziness, hypertension, orthostatic reactions, vomiting, nasal congestion, and confusion. The first three are relatively common, especially during initial use. Although BC is sometimes used in doses up to 100 mg/day daily in Parkinson's disease, doses higher than 5 mg have rarely been given to healthy volunteers. Even at 5 mg, a small proportion of healthy volunteers drop out of clinical studies due to severe nausea, vomiting and dizziness.

Fortunately, available evidence indicates that a dose of 5 mg is sufficient to detect the known pharmacokinetic interaction of bromocriptine with co-administrated compound [4,5]. For this daily dose, evaluation of plasma pharmacokinetics of BC requires a very sensitive analytical technique.

Several analytical procedures for quantifying BC levels have been published. Radioimmunoassay provided good sensitivity, but was not sufficiently selective, due to extensive metabolism of BC [6–8]. High performance liquid chromatography (HPLC) methods showed low sensitivity and required extended chromatography run times in order to be specific against other co-extracted plasma compounds [9,10]. Gas chromatography revealed a higher sensitivity but required a complex extraction procedure [11]. To our knowledge [12], mass spectrometry coupled to liquid chromatography (LC–MS) has not been extensively used for BC determination plasma in the pg/ml range. Therefore, a single,

* Corresponding author. Tel.: +33 4 72 43 11 52; fax: +33 4 72 44 62 02.
E-mail address: arnaud.salvador@univ-lyon1.fr (A. Salvador).

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doi:10.1016/j.jchromb.2005.03.029

specific and sensitive LC–MS–MS assay method for BC determination in the pg/ml range was developed.

2. Experimental

2.1. Chemicals

Bromocryptine (purity = 98%) and α-ergocryptine (α-EC) (purity = 99%) were obtained from Sigma (St. Louis, MO, USA). The structure of BC and the internal standard (α-ergocryptine) used in the assay are shown in Fig. 1. HPLC-grade methanol, acetonitrile and ammonium hydroxide 25% were purchased from Carlo Erba (Val de Reuil, France). Guaranteed grade 90% formic acid was purchased from Fisher Bioblock (Illkirch, France). Deionized water was purified using a Milli-Q filter system (Millipore, Milford, MA, USA).

2.2. Instrumentation

The HPLC system consisted of an HTS Pal autosampler from CTC analytics AG (Zwingen, Switzerland) with the sample cooler set at 10 °C and a Perkin-Elmer series 200 pump (Norwalk, USA). A Labpro switching valve from rheodyne (California, USA) and a LC-6A Pump from shimadzu (Duisburg, Germany) were used to split the mobile phase eluted during the first minute to the waste. A model API 4000, triple stage quadrupole mass spectrometer from Applied Biosystem/MDS Sciex (Concord, Canada) was connected to the HPLC system as an MS–MS detector. A Symmetry C_{18} (2.1 mm × 100 mm, 3.5 μm) column from Waters (Milford, MA, USA) was used for HPLC separation.

2.3. LC–MS–MS conditions

The mobile phase consisted of 250 ml of acetonitrile, 750 ml of water and 1 ml of formic acid. The two pumps, connected to the same isocratic mobile phase, were set to a flow-rate of 250 μl/min. Thirty micro-litres of the extracted samples were separated by the analytical column left at room temperature. BC and the internal standard (α-EC) were introduced into the mass spectrometer via the electrospray source under atmospheric pressure. Detection was performed by monitoring the positive ions of the combined parent and product compounds in multiple selected reaction monitoring mode (MRM). The theoretical *m*/*z* values of the parent and daughter ions $[M + H]^+$(Fig. 1) were set at 656.7 and 348.2 for BC, 576.7 and 268.3 for the internal standard. The 81 Br isotope of BC rather 79 Br isotope was selected because we found less noise in the corresponding MRM trace. The heater probe was set at 550 °C. N_2 gas was used as the nebulizer and collision gas. The collision energies for the analysis of BC and α-EC were set to 35 eV. Analysis time was 6.5 min.

2.4. Standards and quality control samples

Stock solutions of BC and α-EC were prepared by dissolving accurately weighed standard compounds in methanol to yield a concentration of 50 μg/ml. All subsequent dilutions were made with methanol. Standard working solutions of BC at concentrations of 0.04, 0.1, 0.2, 0.61.6, 4 and 10 ng/ml were prepared by serial dilutions. QC working solutions at concentrations of 0.1,1, 10 and 200 ng/ml were also prepared by successively diluting the 50 μg/ml QC stock solution. The internal standard stock solution was diluted to a working concentration of 200 ng/ml. These working solutions were stored at +4 °C until use.

Plasma standards of BC were prepared fresh daily by spiking 50 μl of the appropriate working solutions into 1 ml of heparinized human plasma to yield calibration concentrations of 2, 5, 10, 30, 80, 200 and 500 pg/ml. Fifty micro-litres of methanol was added to control plasma to make up plasma double blank and blank samples, respectively.

Plasma QC samples were prepared by adding appropriate QC working solutions and plasma volumes to yield concentrations of 2, 5, 250 and 400 pg/ml. After preparation, the bulk QC samples were aliquoted into 2.5 ml volumes and stored at −25 °C until used.

2.5. Plasma extraction

A 1 ml aliquot of plasma was spiked with 0.2 ml of formic acid solution (5% in water), followed by 20 μl of internal

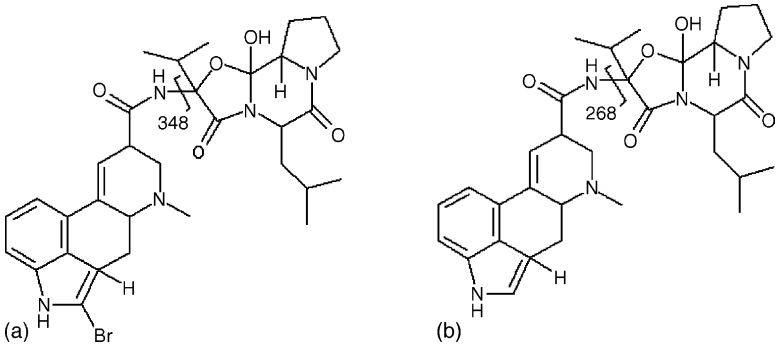

Fig. 1. Structures of bromocriptine (a) and the internal standard (α-ergocryptine) (b).

standard working solution. The sample was then loaded onto an Oasis MCX (3 ml, 60 mg) extraction cartridge (Waters), pre-conditioned with 1 ml of methanol and 1 ml of water. Following rinsing with 1 ml of 5% formic acid aqueous solution and 1 ml of methanol, analytes were eluted with a mixture of 1 ml of methanol ammonium hydroxide (97.5/2.5, v/v). The eluate was then evaporated to dryness under a gentle stream of nitrogen at approximately +40 °C. The residue was dissolved in 100 μl of mobile phase, and an aliquot (30 μl) was injected into the LC–MS–MS system.

Concentrations of BC were back-calculated from the weighted ($1/x^2$) linear least squares fitted line of peak area ratio of BC to the internal standard versus standard concentrations.

3. Results and discussion

3.1. Sample preparation and LC–MS–MS conditions

Since BC and it internal standard (α-EC) are weak bases (the pk_a values of BC are approximatively 6.6 and 15), they mainly exist in ionic form in solution at low pH (<4). In this assay, the sample applied onto the SPE cartridge was acidified to less than pH 3 with a formic acid solution (5%). In their ionic form, the strong binding of the analytes to the SPE cartridge allows for sufficient sample clean up (with acidic water and methanol). However, the analytes were easily eluted with 500 μl of an ammonia/methanol solution. The evaporated samples were dissolved in 100 μl of mobile phase.

Due to the presence of basic nitrogen in the molecule, BC exhibited favourable sensitivity in positive ion mode detection because of the efficiency of ionisation of the analyte. The electrospray ionisation (ESI) method was to be found to be more sensitive than atmospheric pressure chemical ionisation (APCI).

Furthermore, preventing the introduction of plasma components into the mass spectrometry by utilizing a switching-valve, may have decreased ion source contamination and allowed for an increase in the number of assay samples per batch.

3.2. Recovery

The extraction recovery for BC at three QC concentration levels was determined by comparing the area ratios of the analyte to internal standard obtained from plasma samples spiked before extraction to the area ratios obtained from plasma samples spiked after extraction. The internal standard was spiked after extraction in each case. The extraction recovery for the internal standard was assessed in a similar manner using the high QC as a reference considering BC as the internal standard. The mean extraction recovery for BC, calculated as the average recoveries from the three QC levels, was 79.6%. The extraction recovery for the internal standard was 86% (Table 1).

Table 1
Extraction recovery of bromocriptine and its internal standard ergocryptine in human plasma by SPE

	Mean peak area ratio[a]		Mean recovery[b]
	Pre-spiked	Post-spiked	
Bromocriptine concentration of QC sample (pg/ml)			
5.0	0.043	0.050	84.9
250	1.859	2.378	78.2
400	2.934	3.887	75.6
Overall mean recovery			79.6
α-Ergocryptine concentration of IS in sample (pg/ml)			
80	0.221	0.257	86.0

[a] $n = 6$, calculated as (mean peak area of BC)/(mean peak area of I.S.) for BC and vice versa for α-ergocryptine.
[b] Calculated as (pre-spiked mean peak area ratio/post-spiked mean peak area ratio) × 100%.

3.3. Selectivity

Assay selectivity was assessed by analysing drug-free pooled plasma and plasma from six individual humans and examining for peaks that interfered with BC and the internal standard. Representative chromatograms obtained from blank human plasma and plasma spiked with BC and the internal standard are shown in Fig. 2. There were no chromatographically interfering peaks observed at the retention times of either analyte or internal standard in the samples. Under the chromatographic conditions described, the retention times of BC and the internal standard were 5.1 and 3.2 min, respectively (Fig. 2).

3.4. Sensitivity

The lower limit of quantification (LLOQ) of the assay was determined by measuring the peak-area ratios of known and decreasing concentrations of BC. An LLOQ of 2 pg/ml was determined to be the lowest concentration of BC that could be measured with a precision better than ±20% and an accuracy of at least ±20% of nominal.

3.5. Linearity

A weighted ($1/x^2$) least-squares linear regression of response versus concentration was used for the calibration. Good linearity was obtained in the range of 2–500 pg/ml with typical correlation coefficients higher than 0.99, and an average slope of 0.010253 ± 0.0021227 (mean ± S.D., $n = 8$).

3.6. Precision and accuracy

The intra-assay precision and accuracy were assessed by extracting and analysing six replicates of the plasma standards at four concentrations levels. The intra-assay precision (expressed as percent relative standard deviation, %R.S.D.) ranged from 4.94 to 18.97% (at LLOQ level) and the intra-assay accuracy (expressed as percent of nominal values)

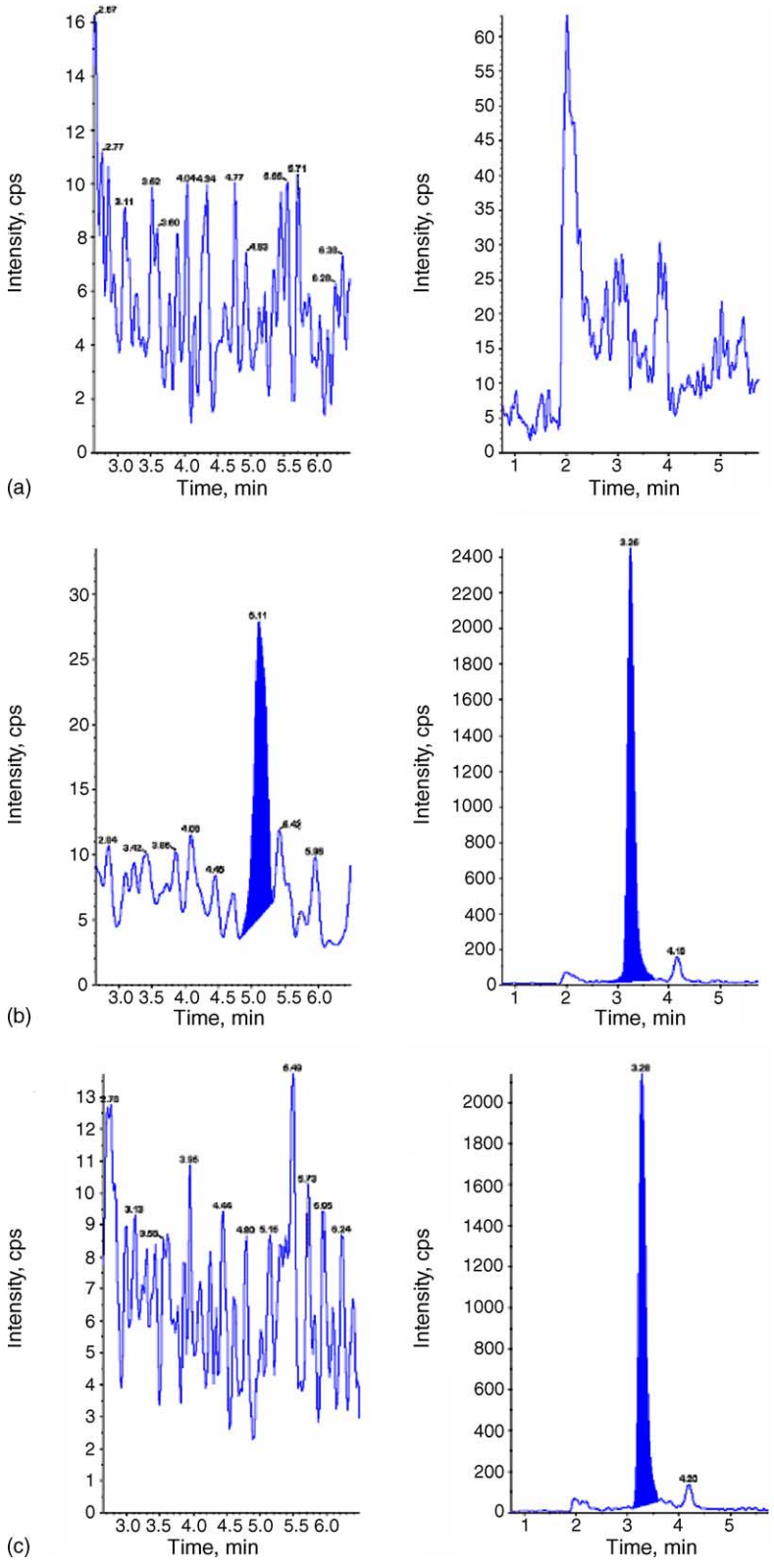

Fig. 2. Chromatograms of (a) a blank plasma; (b) spiked plasma sample at LLOQ (2 pg/ml BC and 80 pg/ml IS), (c) a control blank plasma (80 pg/ml IS).

Table 2
Intra-day assay precision and accuracy for BC

Nominal concentration (pg/ml)	Determined concentration[a] (mean ± S.D. (pg/ml))	Precision[b] (%)	Accuracy[c] (%)
2	2.39 ± 0.453	18.97	119.5
5	5.02 ± 0.252	5.02	100.4
250	261 ± 12.9	4.94	104.4
400	403 ± 27.4	6.80	100.7

[a] $n = 6$.
[b] Expressed as %R.S.D.: (S.D./mean) × 100.
[c] Calculated as (mean determined concentration/nominal concentration) × 100%.

Table 3
Inter-day assay precision and accuracy for BC

Nominal concentration (pg/ml)	Determined concentration[a] (mean ± S.D. (pg/ml))	Precision[b] (%)	Accuracy[c] (%)
2	2.34 ± 0.395	16.88	117.0
5	5.17 ± 0.410	7.93	103.4
250	259 ± 37.1	14.32	103.6
400	400 ± 30.6	7.65	100.0

[a] $n = 3$ days with two replicates per day.
[b] Expressed as %R.S.D.: (S.D./mean) × 100.
[c] Calculated as (mean determined concentration/nominal concentration) × 100%.

ranged from 100.4 to 119.5% (at LLOQ level) (Table 2). The initial inter-assay precision and accuracy were determined by analysing two replicates of the quality control samples through three assay runs. The method showed reproducibility with an inter-assay precision ranging from 7.65 to 16.88% (at LLOQ level). The inter-assay accuracy ranged from 100.0 to 117.0% (at LLOQ level) (Table 3). The %R.S.D. of the second concentration level (200 pg/ml) is 14.3% which is nearly as high as the %R.S.D. at the LLOQ level. This anomaly is likely due to an error during the experimental procedure. During routine analysis of bromocriptine samples, we found a %R.S.D. of 6.0% ($n = 34$) at this concentration level (data not shown).

3.7. Short-term and long-term stability

Table 4 displays the short-term and long-term stability of BC. As per FDA guidelines, the compound is deemed "stable" if the concentration of the compound through the stability experiment does not deviate by more than 15% compared to a control (time 0) concentration. Stability for BC under the following conditions has been shown; (1) stability of BC in human plasma for at least 4 h on the benchtop at room temperature, (2) stability of BC in human plasma through at least three freeze–thaw cycles, (3) stability of BC for at least 10 weeks frozen at −25 °C.

Table 4
Stability of BC under a variety of conditions

Statistical variable	Theoretical concentration (pg ml)	
	5	400
Freeze–thaw stability		
Mean[a]	5.08	432
S.D.	0.470	15.3
%R.S.D.	9.25	3.54
%Accuracy[b]	101.6	108.0
Short-term stability (4 h at room temperature)		
Mean[a]	5.37	426
S.D.	0.401	21.9
%R.S.D.	7.47	5.14
%Accuracy[b]	107.4	106.5
Long-term stability (−25 °C for 10 weeks)		
Mean[a]	5.01	428
S.D.	0.604	17.9
%R.S.D.	12.06	4.18
%Accuracy[b]	100.2	107.0

[a] $n = 6$.
[b] Calculated as (mean calculated concentration/nominal concentration) × 100%.

3.8. Matrix effects

The reliability of quantitative LC–MS–MS data obtained from the determination of drugs in biological matrices can be adversely affected by endogenous components in the biological fluids. These matrix effects generally take the form of either ion suppression or ion enhancement and may vary between different sources of plasma. Several methods have been reported for matrix effect evaluation. Our approach to determining the effect of different lots of plasma on the performance of the analytical method was to determine to what extent, if any, suppression or enhancement affected the method. This was determined based on the differences between the area of neat standards and that of post-extraction spiked control plasma at two concentration levels. Based on the result Table 5, the amount of ion suppression (expressed as [(mean peak area of post-extraction spiked plasma samples/mean peak area of neat samples) × 100) − 100)] is in the range of −12.34–3.13%. The inter lot of plasma variation on peak area (expressed as percent relative standard deviation, %R.S.D.), was found to be in the range 3.08–4.23%. Based on these results any variation between different

Table 5
Matrix effect evaluation

	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Neat standard	Inter-lot
5 pg ml^{-1} of BC								
Mean area[a]	4707	4283	4644	4756	4718	4540	4858	4608
S.D.	211	623	99	454	457	508	433	176
%R.S.D.	4.48	14.55	2.13	9.55	9.69	11.19	8.91	3.83
%Matrix effect	−3.11	−11.84	−4.41	−2.10	−2.88	−6.55	–	−5.15
400 pg ml^{-1} of BC								
Mean area[a]	391091	389198	428823	427649	416367	419006	443995	412022
S.D.	52685	24282	5519	12028	36440	9379	11698	17625
%R.S.D.	1347	624	129	281	875	224	11.70	4.28
%Matrix effect	−11.92	−12.34	−3.42	−3.68	−6.22	−5.63	–	−7.20
80 pg ml^{-1} of IS								
Mean area[a]	73608	68611	73246	74028	70988	70000	71779	71747
S.D.	5789	4317	1428	3278	4097	2341	4.049	2208
%R.S.D.	7.86	6.29	1.95	4.43	5.77	3.34	5.64	3.08
%Matrix effect	2.55	−4.41	2.04	3.13	−1.10	−2.48	–	−0.04

[a] $n = 6$.

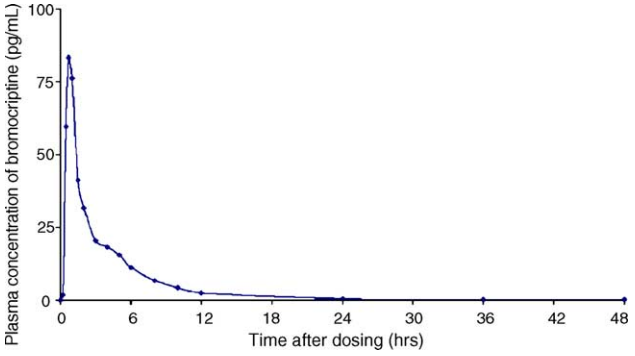

Fig. 3. Representative data showing mean plasma concentration-time profiles of healthy volunteers after the administration of an oral single dose of 5 mg tablet of bromocriptine.

sources of plasma should have effect on the quantitated results.

3.9. Method application

The described analytical procedure was applied to plasma samples obtained from healthy volunteers after the administration of a single dose of a 5 mg bromocriptine tablet. The plasma concentration-time profile of one volunteer is represented in Fig. 3.

4. Conclusion

A sensitive and reliable LC–MS–MS method for the analysis of bromocriptine in human plasma has been successfully developed and validated. To extract bromocriptime from plasma, SPE using cationic cartridges was used. This method has an LLOQ of 2 pg/ml using 1 ml of plasma and has a linear calibration range of 2–500 pg/ml. Compared to previous methods, this method provided a very simple procedure and much better sensitivity for the determination of bromocriptine in human plasma

Acknowledgement

The authors thank M. Di Ioia for the review of the manuscript.

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