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High-performance liquid chromatographic–electrochemical assay for the quantitation of BMS-181885 in monkey plasma

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Abstract

A high-performance liquid chromatographic–electrochemical assay was developed and validated for the quantitation of BMS-181885 (**I**), an anti-migraine agent, in monkey plasma. The assay involved a solid-phase extraction of **I** and BMY-46317 (internal standard; I.S.) on a 1-ml cyano cartridge using the automatic solid-phase extraction cartridge (ASPEC) system. Immediately following the conditioning of the cyano column (3 ml of methanol and 2 ml of 1% glacial acetic acid), plasma (0.25 ml) was loaded on to the column. The column was then washed with a 3 ml of 0.1 *M* ammonium acetate buffer (pH 6). The final elution of the analytes was performed using 2 ml of methanol. The eluate was then evaporated to dryness (gentle stream of nitrogen at 40°C) and the residue was dissolved in the mobile phase and injected on to a YMC basic column (15 cm×4.6 mm; 5 μm particle size) at a flow-rate of 1 ml/min. A mixture of 0.1 *M* ammonium acetate at pH 6–acetonitrile–methanol (70:20:10, v/v) was used as the mobile phase. Standard curves, with a lower limit of quantitation of 2 ng/ml of **I** were linear ($r^2 \geq 0.998$; range: 2–50 ng/ml). Based on the analysis of the quality control (QC) samples, the assay was both accurate and precise. The stability of **I** was established following freeze–thaw cycles and storage at or below −20°C. The extraction recovery of **I** from monkey plasma was about 82%. The validated assay method was applied to determine the pharmacokinetics of I in monkeys following a single 1 mg/kg intravenous dose.

Keywords: BMS-181885

1. Introduction

Migraine is an episodic, throbbing headache pain, often associated with symptoms that may include photophobia, gastrointestinal distress and nausea. The severity of the attack may vary in migraine patients and the duration vary from 4 to 72 h. Serotonin (5-hydroxytryptamine; 5-HT) is believed to play an important role in the pathogenesis of migraine [1]. Since 5-HT alleviates both spontaneous and reserpine-induced migraine [2,3] and causes constriction of cranial blood vessels [4,5], a sound rationale appears to be to develop a 5-HT agonist to treat episodes of migraine.

BMS-181885, 3-[[3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-1H-indol-5-yl]-4-methyl-3-cyclobuten-1,2,-dione free base (**I**; Fig. 1), is under development for the treatment of migraine.

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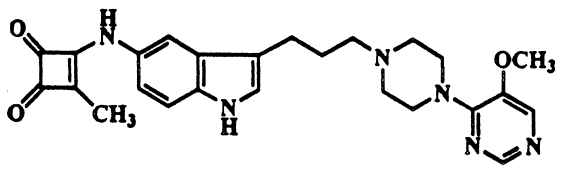

Fig. 1. Structural representation of BMS-181885 (**I**).

Similar to sumatriptan, another 5-HT agonist, the pharmacological activity of **I** is elicited by binding to 5-$HT_{1\text{-like}}$ (specifically 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$) receptors. In order to support toxicokinetic and pharmacokinetic studies of **I** in monkeys, a rapid and sensitive assay for the measurement of intact **I** in monkey plasma was developed and validated.

2. Experimental

2.1. Reagents

Compound **I** (95.6% purity) and the internal standard (I.S.; BMY-46317) were obtained from Bristol-Myers Squibb (New Brunswick, NJ, USA). Ammonium acetate was obtained from Fisher Scientific (Fair Lawn, NJ, USA). HPLC grade acetonitrile and methanol were purchased from Burdick and Jackson (Muskegon, MI, USA). Glacial acetic acid (ACS reagent) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Ethanol was obtained from Aaper Alcohol and Chemical (Shelbyville, KY, USA). Water was double-distilled and filtered using a Milli-Q system from Millipore (Milford, MA, USA). Control monkey plasma (EDTA as anticoagulant) was obtained from Buckshire (Peskasie, PA, USA). All other reagents were of analytical grade and were used without further purification.

2.2. Apparatus and high-performance liquid chromatographic conditions

The high-performance liquid chromatography (HPLC) system consisted of a Waters Model 590 pump, a WISP 717 automatic sample injector (Waters, Milford, MA, USA), an ESA Model 5020 electrochemical detector equipped with a Model 5010 analytical cell (electrodes 1 and 2 set at +150 mV and +600 mV, respectively) and a Model 5020 guard cell (ESA, Chemsford, MA, USA). The analytical column was a YMC basic, 15 cm×4.6 mm, 5 μm particle size (YMC, Wilmington, NC, USA) which was a preceded by a YMC basic guard column, 23×4.0 mm, 5 μm particle size. The isocratic mobile phase consisted of 0.1 *M* ammonium acetate (pH 6)–acetonitrile–methanol (70:20:10) and the flow-rate was maintained at 1 ml/min. Chromatograms were electronically acquired on a Multichrom 2 VG Data System (Fisons Instruments, Danvers, MA, USA).

2.3. Standard solutions

A stock solution of **I** (250 μg/ml; A) was prepared in an ethanol–water (8:2) mixture. After which, a 500 ng/ml concentration of **I** was prepared in monkey plasma using 1 part of A and 49 parts of control monkey plasma. The 500 ng/ml plasma stock was subsequently used to prepare working standard concentrations of 2, 5, 10, 25, 40 and 50 ng/ml in monkey plasma by employing a serial dilution technique. A working I.S. solution (100 ng/ml) was prepared in Milli-Q water. The stock solutions were stored at −20°C and were stable for at least one month.

2.4. Sample preparation

An automated solid-phase extraction controller (ASPEC) system (Gilson Medical Electronic, Middleton, WI, USA) was used to perform solid-phase extraction. 1-ml volumes of 0.1 *M* ammonium acetate buffer (pH 6 adjusted by glacial acetic acid) and 100 μl of I.S. solution (100 ng/ml) were mixed with 250 μl of monkey plasma standards or samples. The mixture was slowly placed on 1 ml Isolute cyano cartridge (International Sorbent Tech., Mid Glamorgan, UK) which had been activated (i.e., conditioned) by sequential washing with 3 ml of methanol and 2 ml of 1% glacial acetic acid. A single step washing was then performed using 3 ml of 0.1 *M* ammonium acetate buffer. Finally, the elution was performed with 2 ml of methanol, after which the eluent was evaporated to dryness at 40°C using a gentle stream of nitrogen. The residue was reconstituted in 500 μl of the mobile phase and 75 μl was injected onto the analytical column.

2.5. Standards and quality control samples

Standard calibration curves were constructed using six non-zero concentrations of **I** over the range of 2–50 ng/ml. These samples were processed as described in Section 2.4. The intra-assay accuracy and precision of the assay were estimated by analyzing at least seven replicates of the quality control (QC) samples containing **I** at four different concentrations (10, 20, 35 and 202 ng/ml). The inter-assay accuracy and precision were determined for six different analytical runs. The criteria for acceptability of the data included an accuracy of within (±) 15% deviation (DEV) from the nominal values and a precision of within 15% relative standard deviation (R.S.D.).

2.6. Stability experiments

The stability of **I** and I.S. in the injection solvent was determined periodically injecting replicate preparations of processed samples for up to 48 h after the initial injection; the peak area obtained at the 0 h for each analyte was used as the reference. The stability of **I** after freeze–thaw was investigated for four cycles. Freezer stability of **I** was assessed by analyzing monkey plasma samples kept frozen at −20°C for at least three months.

2.7. Extraction recovery

Two sets of standards containing **I** in the concentration range of 2–50 ng/ml, were prepared. One

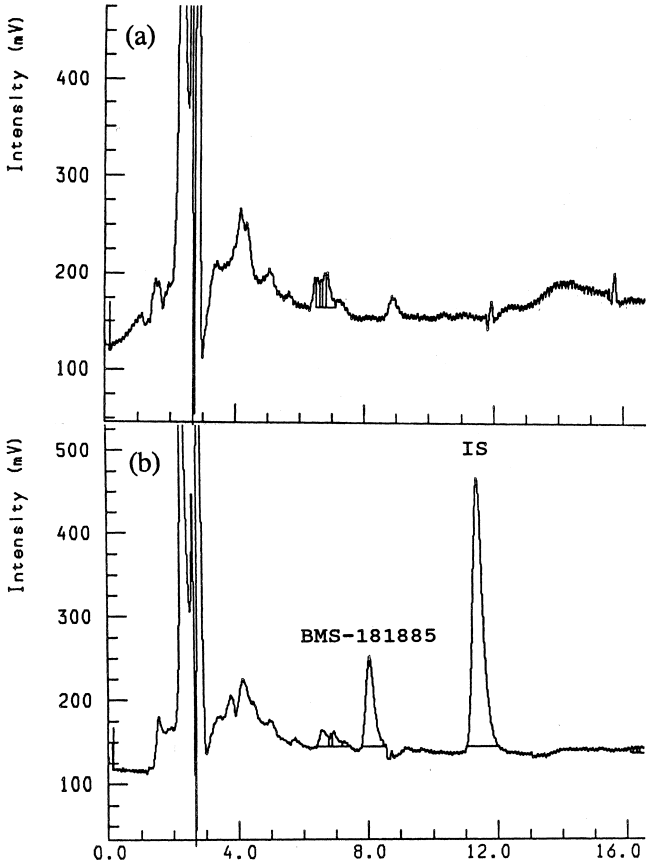

Fig. 2. Chromatograms of processed samples: (a) monkey blank plasma with internal standard; (b) monkey plasma standard. Peaks: 1=**I**, 8 min; 2=I.S., 11 min.

Table 1
Lower limit of quantitation for **I** added to seven sets of monkey plasma

Animal No.	Nominal concentration (ng/ml)	Measured concentration (ng/ml)	Deviation (%)
1	2	2.0	0.0
2	2	2.1	5.0
3	2	2.1	5.0
4	2	2.2	10.0
5	2	2.1	5.0
6	2	2.0	0.0
7	2	2.2	10.0
Overall mean		2.1	
% R.S.D.		3.9	
% DEV		5.0	

set was prepared in monkey plasma and the other set was prepared in the mobile phase. Plasma standards were processed and chromatographed as described in Sections 2.2 and 2.4; while standards prepared in mobile phase were injected onto the column directly. The percent recovery of each analyte extracted from plasma was calculated using the following equation:

$$\% \text{ Recovery} = 100 \times \frac{\text{Slope of standard curve prepared in plasma}}{\text{Slope of standard curve prepared in mobile phase}}$$

3. Results and discussion

3.1. Specificity

Chromatograms were obtained and compared between the blank and spiked monkey plasma matrices (Fig. 2). The nominal retention times were 8 and 11 min for **I** and I.S., respectively. No significant interfering peaks were detected at the retention times of the peaks of interest.

3.2. Range of reliable response

A weighted linear regression of the peak area ratios versus standard concentrations was performed for **I** using a weight of 1/concentration. The observed peak area ratios were linear over the concentration range of 2–50 ng/ml in monkey plasma. The values of r^2 ($\geq$0.998) and the consistency in slope values (0.02143–0.02616; $n=3$), demonstrated that the standard curve had a reliable response over the studied concentration range.

3.3. Lower limit of quantitation (LLQ), accuracy and precision

The results of the accuracy and precision for the LLQ determination of **I** in monkey plasma are provided in Table 1. The predicted mean concentration was 2.1 ng/ml for **I** with a precision of within 3.9% R.S.D. and an accuracy of 5.0% DEV from the nominal value. The intra- and inter-assay accuracy and precision values for QCs are provided in Table 2. The intra-assay precision values (% R.S.D.) for the 10, 20, 35 and 202 ng/ml QC concentrations were 5.6%, 3.5%, 5.3% and 3.9%, respectively. The inter-assay precision (% R.S.D.) for all four concentrations were 0.5%, 2.5%, 2.2% and 0.5%, respectively.

Table 2
Accuracy and precision results for **I** in monkey plasma

Nominal concentration (ng/ml)	Mean measured concentration (ng/ml)	R.S.D. inter-assay (%)	R.S.D. intra-assay (%)	Deviation (%)
10	9.9	0.5	5.6	−1.1
20	19.6	2.5	3.5	−1.9
35	34.1	2.2	5.3	−2.7
202[a]	198.9	0.5	3.9	−1.6

[a] Diluted 10-fold prior to analysis.

Table 3
Freeze–thaw stability of **I** in monkey plasma

Freeze–thaw cycles	10 ng/ml		30 ng/ml	
	Measured concentration (ng/ml)	Mean±R.S.D.	Measured concentration (ng/ml)	Mean±R.S.D.
0	9.5	9.6±3.3	30.7	28.8±6.9
	9.4		29.1	
	10.0		26.7	
1	11.6	11.1±15.3	29.0	28.7±0.9
	12.5		28.7	
	9.2		28.5	
2	11.9	10.6±10.4	30.3	29.3±9.6
	9.9		31.5	
	10.0		26.1	
3	11.0	11.1±1.5	28.2	28.1±5.7
	11.0		29.7	
	11.3		26.5	
4	10.0	10.8±7.0	30.5	30.5±6.6
	11.5		30.3	
	10.9		28.6	

3.4. Stability

Both **I** and the I.S. showed stability in the injection media at room temperature for at least 48 h. The % DEV during the course of this experiment for the analytes was generally found to be within 10–15%. Based on the results in Table 3, the monkey plasma samples containing **I** could be frozen and thawed up to at least four cycles. In addition, **I** was found to be stable when stored at −20°C for at least

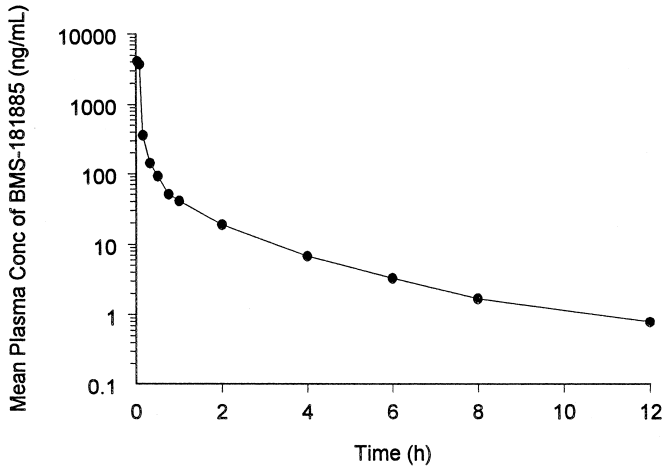

Fig. 3. Mean plasma concentration–time profile of BMS-181885 (**I**) in monkeys ($n=3$) receiving an intravenous dose of 1 mg/kg of **I**.

Table 4
Mean (±standard deviation) pharmacokinetic parameters of **I** in monkeys ($n=3$) receiving 1 mg/kg intravenous dose of **I**

C_{max} (ng/ml)	AUC_{inf} (ng h/ml)	MRT (h)	$t_{1/2}$ (h)	Cl_{tot} (ml/min/kg)	V_{ss} (l/kg)
3765±955	500±94	0.7±0.2	2.9±1.1	34±5.8	1.5±0.5

C_{max}: Concentration at the end of intravenous infusion.
AUC_{inf}: Area under the plasma concentration–time curve extrapolated to infinity.
MRT: Mean residence time.
$t_{1/2}$: Terminal elimination half-life.
Cl_{tot}: Total body clearance.
V_{ss}: Volume of distribution at steady-state.

three months. The percent deviations of the predicted concentrations were within ±10% of the nominal concentrations.

3.5. Extraction recovery

The results of the comparison of neat standards versus plasma extracted standards in the concentration range 2–50 ng/ml indicated that the extraction recovery of **I** in monkey plasma was 82%.

3.6. Application of the method

The method was applied to determine the levels of **I** in monkeys ($n=3$) that received a 1 mg/kg intravenous dose of **I**. The mean plasma concentration versus time profile for **I** is depicted in Fig. 3 and the mean pharmacokinetic parameters are tabulated in Table 4.

4. Conclusion

The assay described herein is specific, accurate, precise and reproducible for the analysis of **I** in monkey plasma.

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