Department of Biopharmaceutical Sciences, University of Illinois at Chicago, USA

Determination of endothelin antagonist BMS182874 in plasma by high-performance liquid chromatography

M. D. CHAVANPATIL, N. V. RAJESHKUMAR, A. GULATI

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Dr. Mahesh D. Chavan Patil, Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, 259, Mack Avenue, Room No: 3250, Detroit, Michigan, 48201, USA

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A simple and rapid high performance liquid chromatography (HPLC) method was developed for the determination of BMS182874 (BMS) in mouse plasma. The drug was extracted from plasma by a liquid-liquid extraction process. The method consists of reversed-phase chromatography using a Thermo Hypersil-Keystone RP-18 5 μm, 250 × 2.1 mm column and UV spectrophotometer detection at 255 nm. The mobile phase consists of 45% (v/v) acetonitrile: 55% (v/v) trifluoroacetic acid (0.015% v/v; pH 3.0) at a flow rate of 0.6 ml/min. Validity of the method was studied and the method was precise and accurate with a linearity range from 100 ng/ml to 1000 ng/ml. The extraction efficiency was found to be 81, 84 and 87% for 100, 500 and 1000 ng/ml, respectively for spiked drug in plasma. The limit of quantification and limit of detection were found to be 50 and 10 ng/ml, respectively in plasma. Within-day and between-day precision expressed by relative standard deviation was less than 4% and inaccuracy did not exceed 4%. The assay was also used to analyze samples collected during animal studies. The suitability and robustness of the method for *in vivo* samples were confirmed by analysis of BMS from mouse plasma and tissues dosed with BMS.

1. Introduction

Endothelin-1 (ET-1) is a potent and long-lasting vasoconstrictor (Yanagisawa et al. 1988). Furthermore, ET-1 is involved in bronchoconstriction and also plays a role in neurotransmitter release, inflammation, cell proliferation and fibrosis. It exerts its actions via two characterized ET receptors, ET_A and ET_B. These receptors are very similar in structure and belong to the superfamily of G protein-coupled receptors (Kedzierski and Yanagisawa 2001).

The first generation of ET receptor antagonists included cyclic pentapeptides isolated from *Streptomyces misakiensis* (Ihara et al. 1991) and synthetic peptide analogs of ET1, such as BQ788 (Ishikawa et al. 1994), TAK-044 (Watanabe and Fujino 1996), and FR139317 (Sogabe et al. 1993). The second generation has included orally active nonpeptide compounds derived from benzene sulfonamide, such as bosentan (Clozel et al. 1993), from naphthalenesulfonamide (Stein et al. 1994), such as BMS-182874 (Webb et al. 1995), from indane-carboxylic acid (SB209670) (Nambi P. et al. 1994), or from butenolide (Doherty et al. 1995). Quantitating these drugs in plasma, urine, or in other body fluids is essential to the elaboration of appropriate dosing regimens and the study of their *in vivo* toxicity, pharmacokinetics, and pharmacodynamics.

HPLC has been reported for the determination of BMS from plasma (Griffith et al. 1998). The authors describe gradient reversed-phase HPLC and a multiple step extraction procedure for the determination of BMS from plasma. The resultant process is time consuming in routine analysis. The purpose of the present study was to develop a rapid, reproducible, reliable and selective chromatographic method for the determination of BMS in mouse plasma. In this study, a simple single step liquid-liquid extraction process and sensitive assay for routine analysis of BMS in plasma is presented.

2. Investigations, results and discussion

Typical chromatograms of blank and plasma sample spiked with BMS are shown in Fig. 1. Under the chromatographic conditions described, plasma protein peaks and BMS were well resolved in plasma sample and eluted at 9.1 min, following injection into HPLC. Optimization was achieved by monitoring varying reversed-phase columns, mobile systems, flow rate and using a simple one-step extraction method.

BMS is insoluble in water and could be extracted from plasma into organic solvents. In this method, we used methanol as an extraction solvent and a C18 column as analytical stationary phase. In another experiment, we also tried extraction solvents such as acetonitrile and chloroform but the extraction efficiency was found to be 50–52%. A 45% of acetonitrile – 55% of (0.015%) trifluroacetic acid was optimum to achieve the best resolution between BMS and plasma protein peaks. The increased percentage of acetonitrile reduced the retention time of the BMS peak resulting in interference of the latter and endogenous plasma protein peaks. Using acetonitrile below 45% gave tailing for the BMS peak. The similar interference was observed when the flow rate was adjusted below 0.6 ml/min.

The mean extraction efficiency of BMS at three different concentrations (100, 500, 1000 ng/ml) shown in Table 1

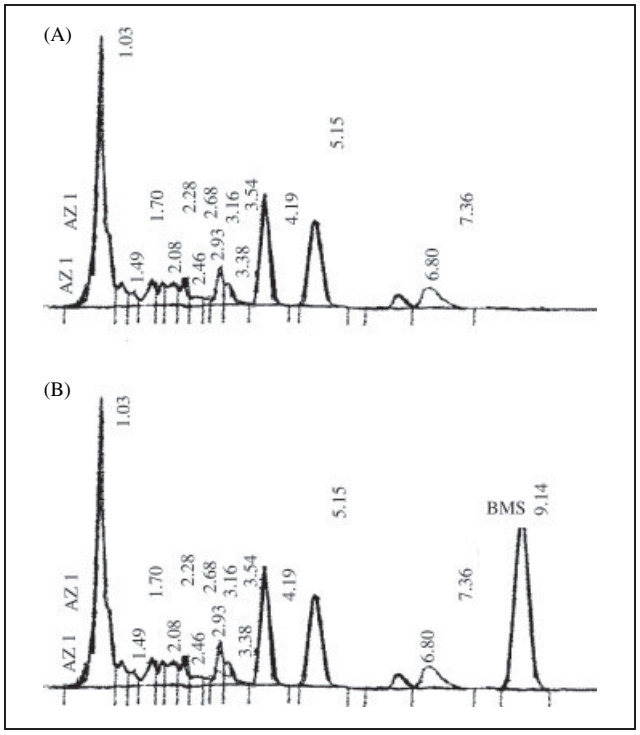

Fig. 1: HPLC chromatograms of BMS (A) Drug free plasma; (B) plasma spiked with BMS (400 ng/ml); retention time for BMS was found to be 9.14 mins

was determined by comparing peak areas from extracted standard samples with standard solution (100, 500, 1000 ng/ml) in methanol. Extraction efficiency was found to be 81, 84 and 87% for 100, 500 and 1000 ng/ml, respectively, for spiked drug in plasma.

The standard curve for BMS for the concentrations 100, 200, 400, 600, 800 and 1000 ng/ml spiked in plasma was prepared. The calibration curve displayed excellent linearity ($r^2 > 0.999$) over the concentration range investigated. It is described by $Y = 555.04X + 103483$, where Y is the peak-area of BMS and X is BMS concentration (ng/ml). Three calibration curves have been examined and the mean values of slopes, intercepts and correlation coefficients are illustrated in Table 2.

The accuracy and precision were determined by preparing four replicate samples of BMS at concentrations of 100, 200, 600, and 1000 ng/ml of plasma on each and three separate days. Concentrations were determined using a calibration curve for each day. Fifteen blank samples were

Table 1: Extraction efficacy of BMS from plasma at various concentrations, mean ± standard deviation (n = 3)

Concentration (ng/ml)	Extraction recovery ± S.D. (%)
100	81.23 ± 3.7
500	84.63 ± 2.8
1000	87.08 ± 3.1

Table 2: Mean ± standard deviation of slope, intercept, and correlation coefficient of calibration curve equation (n = 3)

Slope ± S.D	555.04 ± 16.31
Intercept ± S.D.	103483 ± 8475.06
Correlation coefficient ± S.D.	0.999 ± 0.00015

Table 3: Accuracy and precision of BMS in spiked plasma (n = 5; four sets for 3 days)

Concentration	Found concentration	CV (%)
Intraday (n = 5)		
100	100.14 ± 1.18	1.18
200	199.10 ± 4.03	2.02
600	596.11 ± 8.46	1.42
1000	1017.91 ± 36.24	3.56
Inter-day (n = 5)		
100	100.05 ± 2.09	2.09
200	199.90 ± 3.19	1.59
600	603.31 ± 11.63	1.92
1000	1012.89 ± 39.17	3.86

determined for method validation. According to the intra-day (within-run) and inter-day (between-run) data good accuracy and precision were observed over the entire concentration range. The results are presented in Table 3. The within-run and between-run variability showed CV values less than 3.9 in all four selected concentrations. The limit of quantification of the method, defined as the minimum concentration that could be measured with a CV <5% was found to be 50 ng/ml in 250 µl of plasma sample. The limit of detection with a S : N ratio of 3 : 1 was 10 ng/ml in plasma.

Stability of standards and plasma samples was evaluated at −20 °C for 1 month and at a room temperature for 24 h. Under these conditions, samples preserved their potency (>95%).

The present method was used to determine the body distribution of BMS in mice. The animals were sacrificed 1 h after i.v. injection of BMS. Fig. 2 shows the mean ± standard error of the mean (S.E.M). Analysis of BMS concentration (ng/100 mg of tissue) of 0.3 mg BMS solution in 10% DMSO provided the following body distribution in different organs (mean ± S.D): Brain = 236 ± 58; liver = 320 ± 132; heart = 323 ± 150; lung = 206 ± 98; kidney = 364 ± 200; spleen = 115 ± 50; Plasma = 594 ± 284 ng/100 mg of tissue.

3. Experimental

3.1. Chemicals and reagents

BMS182874 was purchased from Tocris Pharmaceuticals Inc; Ellisville, MO. Trifloroacetic acid was purchased from Sigma Aldrich, MO. HPLC grade acetonitrile and methanol were purchased from Fishers Scientific, IL. All other chemicals and solvents were of analytical grade and used without any further purification.

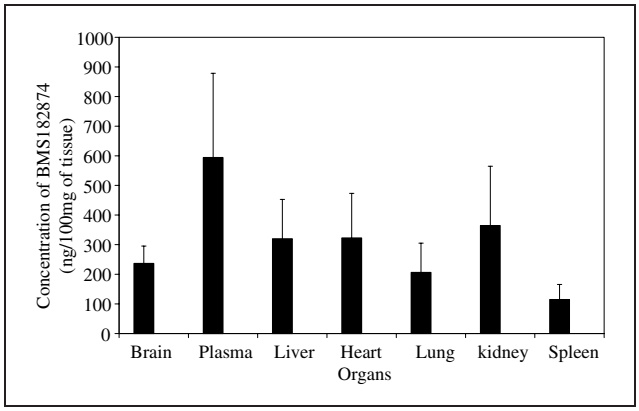

Fig. 2: Concentration of drug (ng/100 mg of tissue) in mice after i.v administration of 0.3 mg of BMS182874

3.2. Solutions

Stock solution of BMS was prepared by dissolving 5 mg BMS in methanol to give a final concentration of 1 mg/ml. Standard solutions were obtained by diluting this solution with methanol to give concentrations over the range of 100–1000 ng/ml.

3.3. Chromatographic conditions

The HPLC system consisted of a 717 plus Auto sampler, variable 2487 UV detector, model 600 pump, 746 data module from Waters, MA. The analytical column was Thermo hypersil-keystone RP-18, 5 μm, 250 × 2.1 mm. The mobile phase was composed of 45% (v/v) acetonitrile (spectroscopic grade) – 55% (v/v) (0.015%) trifluoroacetic acid (pH 3.0), adjusted with 1N sodium hydroxide solution prepared daily and degassed by passing through a 0.45 μm filter. All chromatographic separations were performed at room temperature. The flow rate was set to 0.6 ml/min. The UV detection was performed at 255 nm.

3.4. Sample preparation

Frozen plasma samples were obtained from mouse blood thawed and allowed to reach room temperature. A 250 μl aliquot of plasma was placed into a test tube. 50 μl of BMS standard solutions was spiked in the blank plasma. Plasma was diluted with 5 ml methanol and vortexed for 5 min. The precipitated proteins were separated by centrifugation at 10,000 rpm for 10 min. An aliquot of 10 μl clear supernatant was injected into the HPLC system.

3.5. Quantification

Calibration standards of BMS were prepared by spiking 50 μl of BMS standard solutions into 250 μl of blank human plasma to give final concentrations over the range of 100–1000 ng/ml. The sample extraction and HPLC analysis was performed as described in section 3.4. Calibration curves were constructed by plotting the measured peak area of BMS Vs concentration. The intra-day (within-run) and inter-day (between-run) accuracy and precision of the method was determined by measuring four replicate samples of BMS standard solutions (100, 200, 600 and 1000 ng/ml) on three separate days.

3.6. Extraction yield

Aliquots of 50 μl of BMS standard solutions were added into 250 μl of plasma. Samples were extracted according to the sample preparation method. An aliquot of 10 μl was injected into the HPLC system. The peak area of these samples was compared with the peak area of the standard solution and % extraction efficiency was calculated. The experiment was repeated in triplicate.

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