ELSEVIER

Journal of Chromatography B, 698 (1997) 123–132

JOURNAL OF CHROMATOGRAPHY B

Quantitative determination of BMS-186716, a thiol compound, in rat plasma by high-performance liquid chromatography–positive ion electrospray mass spectrometry after hydrolysis of the methyl acrylate adduct by the native esterases

Mohammed Jemal*, Dara J. Hawthorne

Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 191, New Brunswick, NJ 08903-0191, USA

Received 14 January 1997; received in revised form 22 April 1997; accepted 12 May 1997

Abstract

During method development in support of non-clinical studies in animal models, BMS-186716 was found to be extremely unstable in blood and plasma. Stabilization of the compound was achieved by reacting the compound with methyl acrylate (MA) in blood, from which the plasma was then prepared. While the resulting BMS-186716–MA adduct was found to be stable in dog plasma, and hence it was used as the basis for the method developed for analysis of dog plasma samples, the BMS-186716–MA adduct was found to be unstable in rat plasma as it was readily hydrolyzed to BMS-186716–acrylic acid (AA) by native esterases found in rat plasma. Although the finding of the instability of BMS-186716–MA in rat plasma was not the result of prospective planning, we were able to successfully develop a quantitative bioanalytical method using BMS-186716–AA as the analyte instead of the originally planned BMS-186716–MA analyte. The standard and quality-control (QC) samples were prepared by spiking blank plasma with BMS-186716–MA, and then allowing them to stand at room temperature for 1 h to convert BMS-186716–MA to BMS-186716–AA. After adding the internal standard BMS-188035–AA, each sample was acidified with HCl and then extracted with methyl *tert.*-butyl ether. The reconstituted extract was injected into a HPLC–electrospray ionization mass spectrometric system for detection by positive ion electrospray ionization. A lower limit of quantitation (LLQ) of 5 ng/ml was achieved, using 0.1 ml plasma and a standard curve range of 5–5000 ng/ml. © 1997 Elsevier Science B.V.

Keywords: BMS-186716; Methyl acrylate

1. Introduction

BMS-186716 (Fig. 1) is a novel dual metalloprotease inhibitor which is being developed for the treatment of hypertension and congestive heart failure. A quantitative bioanalytical method based on HPLC–electrospray ionization mass spectrometry has been developed for the quantification of BMS-186716 in rat plasma to support pharmacokinetic, bioavailability and toxicokinetic studies in rat.

Because of extreme instability of the sulfhydryl compound BMS-186716 in blood and plasma, it was found necessary to stabilize the compound in the biological matrices by adding methyl acrylate (MA),

*Corresponding author.

PII S0378-4347(97)00292-2

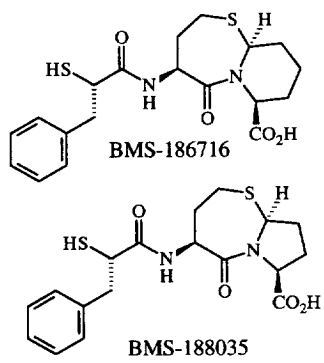

Fig. 1. Structures of BMS-186716 and BMS-188035, the analytical internal standard.

which forms the BMS-186716–MA adduct [1,2]. A quantitative bioanalytical method based on HPLC–electrospray ionization mass spectrometry for the quantification of BMS-186716 in dog plasma was previously developed and validated [2]. The method involved: (a) stabilization of the BMS-186716 in blood via the formation of BMS-186716–MA, from which the plasma was prepared by centrifugation; (b) utilization of standards and quality-control (QC) samples obtained by spiking drug-free plasma with BMS-186716–MA; (c) extraction of BMS-186716–MA from plasma; (d) HPLC–MS, with single-ion-monitoring (SIM) of $[M+NH_4]^+$. However, during the process of applying this method to rat plasma, we found that BMS-186716–MA was unstable in rat plasma due to the hydrolysis of BMS-186716–MA to BMS-186716–acrylic acid (BMS-186716–AA), presumably due to native esterases. This finding prompted us to develop a method in rat plasma based on utilizing BMS-186716–AA as the analyte instead of the originally planned BMS-186716–MA. Stabilization of BMS-186716 in blood samples obtained from rats dosed with BMS-186716 was achieved by mixing the blood sample with MA immediately after drawing the blood. After a 10-min reaction of the blood with MA in ice, the plasma was prepared by centrifuging the blood in a refrigerated centrifuge. The study samples were stored at −70°C until time of analysis. On the day of analysis, the samples were kept at room temperature for 1 h to hydrolyze any remaining BMS–186716-MA to BMS-186716–AA. Plasma QC and standard curve samples were prepared by spiking blank plasma with BMS-186716–MA and then allowing them to stand at room temperature for 1 h to hydrolyze BMS-186716–MA to BMS-186716–AA. The BMS-186716–AA in the plasma samples was extracted by liquid–liquid extraction and was analyzed by high-performance liquid chromatography–positive ion electrospray mass spectrometry. HPLC coupled with electrospray mass spectrometry, both in the positive and negative ion mode, is a proven technique for rapid and sensitive quantitative determination of drugs and metabolites in biological matrices [3–10].

2. Experimental

2.1. Reagents and chemicals

BMS-186716 and BMS-188035 (the internal standard) are characterized products of Bristol-Myers Squibb Pharmaceutical Research Institute. Methyl *tert.*-butyl ether (HPLC grade) and 0.1 *M* HCl (Mallinckrodt brand) were purchased from Baxter Scientific Products (McGaw Park, IL, USA). House deionized water, further purified with a Milli-Q water purifying system (Millipore, Bedford, MA, USA), was used. Ammonium acetate, ACS grade, was from Sigma (St. Louis, MO, USA). Methanol (HPLC grade), acetonitrile (HPLC grade), formic Acid (88%) and disodium hydrogen phosphate were from Fisher (Fair Lawn, NJ, USA). Methyl acrylate was purchased from Aldrich (Milwaukee, WI, USA). Drug-free rat plasma was purchased from Pel Freez Biologicals (Rogers, AK, USA).

A 0.01 *M* phosphate buffer was prepared by dissolving 1.42 g of disodium hydrogen phosphate in 100 ml of Milli-Q water and then diluting ten-fold with Milli-Q water. A 0.02 *M* HCl solution was prepared by diluting 50 ml of 0.1 *M* HCl to 250 ml with Milli-Q water. Mobile phase A was prepared by dissolving 770 mg (10 m*M*) of ammonium acetate in a mixture of 750 ml of Milli-Q water and 250 ml of methanol and then adjusting the pH to 3.0 by the addition of formic acid. Mobile phase B was prepared by dissolving 770 mg of ammonium acetate (10 m*M*) in 1000 ml of methanol. Reconstitution

solution was prepared by mixing 55 ml of mobile phase A with 45 ml of mobile phase B.

2.2. Equipment

The HPLC–MS analysis was performed using a Sciex (Concord, Ont., Canada) API I mass spectrometer equipped with an articulated ionspray interface and a Macintosh data system. The API I was coupled to a Hewlett–Packard (Palo Alto, CA, USA) 1090L HPLC system equipped with a ternary pumping unit and a variable volume automatic sampler. The HPLC column, BDS Hypersil C_{18}, column plus frit (CPF), 3 μm, 100×2.0 mm, was from Keystone (Bellefonte, PA, USA). A Turbovap LV evaporator from Zymark (Hopkinton, MA, USA) was used. The biofuge centrifuge, 15R, (drum rotor with 20×0.5 or 0.4 ml racks) was from Baxter. The IEC centrifuge, model PR-7000M (IEC, Needham Hts., MA, USA) was also used. The shaker used was an Eberbach two-speed shaker, purchased from Baxter. Disposable polyethylene transfer pipettes with extended fine tips (1-ml draw, 10.4 cm and 3-ml draw, 15.3 cm) and microcentrifuge tubes with snap caps (0.25 ml) were purchased from Fisher. Polypropylene conical microvials (0.25 ml) were obtained from Sun Brokers (Wilmington, NC, USA). Cryule vials with color-coded caps (Wheaton-2 ml) and a Nalgene polyethylene pan for the dry ice acetone bath were purchased from Baxter.

2.3. Chromatographic and mass spectrometric conditions

For the HPLC conditions, an isocratic system was employed using a combination of 55% of mobile phase A and 45% of mobile phase B (Section 2.1). The flow-rate through the HPLC column was 0.3 ml/min and the effluent was split so that only one-sixth of the effluent was directed to the mass spectrometer. The injection volume was 15 μl. Ultrahigh purity nitrogen was used as the nebulizing gas (60 p.s.i.g.), and as the curtain gas (1.2 l/min). The interface temperature was maintained at 60°C. The sprayer voltage was set at +5000 V and the orifice was set at +45 V. The instrument was tuned with mass peak widths of 0.8 a.m.u. at half height. The multiplier voltage was optimized daily.

2.4. Preparation of the MA-adduct stock solutions

Two separate stock solutions of BMS-186716–MA, one to be used for the standard curve set (stock solution A) and the second for the QC samples (stock solution B), were prepared during validation of the method. For each stock solution, accurately weighed (12.62 mg for the standard curve set and 12.59 mg for the QCs) BMS-186716 was added to a 25-ml volumetric flask containing 12.5 ml of 0.01 *M* phosphate buffer and 50 μl of MA which had been mixed with the buffer. The solution was sonicated for 10 min with intermittent vortexing and inversion, after which the flask was partially filled with acetonitrile. After the flask content was allowed to come to room temperature, the flask was filled to volume with acetonitrile. The stock solution of the internal standard (BMS-188035–MA) was prepared by adding 4.96 mg of BMS-188035 into a 10-ml volumetric flask containing 5 ml of 0.01 *M* phosphate buffer and 50 μl of MA which had been mixed with the buffer. Following a 10-min sonication with intermittent mixing, the flask was filled to volume with acetonitrile. All solutions were kept refrigerated (4°C) when not in use.

2.5. Standard and QC preparations

The calibration set consisted of twelve concentrations, each in duplicate, prepared in drug-free rat plasma. The curve range was 5 to 5000 ng/ml of rat plasma. The highest concentration standard was prepared by diluting 50 μl of stock solution A (described in Section 2.4) to 5 ml with drug-free rat plasma in a volumetric flask and then letting it stand at room temperature for 1 h to allow the completion of the hydrolysis reaction of BMS-186716–MA to BMS-186716–AA. The rest of the standards were prepared by further dilutions of the highest standard with drug-free rat plasma. Five levels of QCs, prepared in drug-free plasma and stored at −70°C until analysis, were used for each set of QC samples used for method validation. Four QCs were within the standard curve range; the fifth QC, known as the dilution QC, had a concentration about five times higher than the highest concentration of the standard curve. For the QC preparations, a portion of stock solution B was diluted with acetonitrile to obtain

three diluted stock solutions: a 2-fold dilution, a 10-fold dilution and 33.33-fold dilution, respectively. The QCs were prepared by diluting the appropriate volume of the stock solution, or one of the diluted stock solutions, with 10 or 25 ml of drug-free rat plasma. The QC solutions were allowed to stand at room temperature for 1 h for the completion of the hydrolysis reaction. After the 1-h reaction time period, the QC samples were aliquotted into 2-ml cryule vials and stored at −70°C. The working internal standard solution (12,400 ng/ml) was prepared by diluting 125 μl of the internal standard stock solution (described in Section 2.4) to 5.0 ml with drug-free rat plasma in a volumetric flask and then letting it stand at room temperature for 1 h to allow the completion of the hydrolysis reaction of BMS-188035–MA to BMS-188035–AA.

2.6. Extraction and reconstitution

To a 0.1-ml portion of each rat plasma standard, QC, or study sample, 50 μl of the working internal standard solution (to obtain 6200 ng/ml of plasma) was added followed by 0.5 ml of 0.02 *M* hydrochloric acid solution. Samples were then vortexed. For the dilution QC, a total volume of 0.1 ml plasma was obtained by combining 0.01 ml of the dilution QC and 0.09 ml of drug-free rat plasma. To each acidified plasma sample, methyl *tert.*-butyl ether (3.0 ml) was added, and the sample then shaken for 10 min using the high setting of the Eberbach shaker. The aqueous and the methyl *tert*-butyl ether layers were separated by centrifugation at 2000 *g* in the IEC centrifuge for 10 min. After freezing the aqueous layer, the organic layer was removed by pouring into 16×100 mm test tubes and was then evaporated using a Turbovap LV evaporator, at 40°C for 10 min.

The reconstitution solution, 60 μl, (Section 2.1) was added to each tube containing dried extract. The test tubes were vortexed on a multitube vortexer for 1 min and each sample solution transferred using transfer pipettes with extended fine tips (15.3 cm) to microcentrifuge tubes (0.25 ml). The microcentrifuge tubes were capped and the samples centrifuged for 5 min at 4000 *g* using the Biofuge 15R centrifuge (20×0.5 or 0.4 ml racks) for removal of any particulates. The microcentrifuge tubes were uncapped and, leaving the bottom contents of the tube, each sample solution was then transferred into polypropylene conical microvials (0.25 ml) using transfer pipettes with extended fine tips (10.4 cm) and capped for injection.

2.7. Analysis

The samples were analyzed via selected ion monitoring (SIM), using the $[M+NH_4]^+$ ion of BMS-186716–AA (*m/z* 498) and the $[M+NH_4]^+$ ion of the internal standard BMS-188035–AA (*m/z* 484).

3. Results and discussion

The formation of the adduct BMS-186716–MA and the subsequent hydrolysis of BMS-186716–MA to BMS-186716–AA is depicted in Fig. 2. The thiol of BMS-186716 undergoes the Michael addition reaction across the conjugated carbon–carbon double bond of MA to give BMS-186716–MA [1]. While BMS-186716–MA was found to be stable in dog plasma, it was found to readily hydrolyze to BMS-

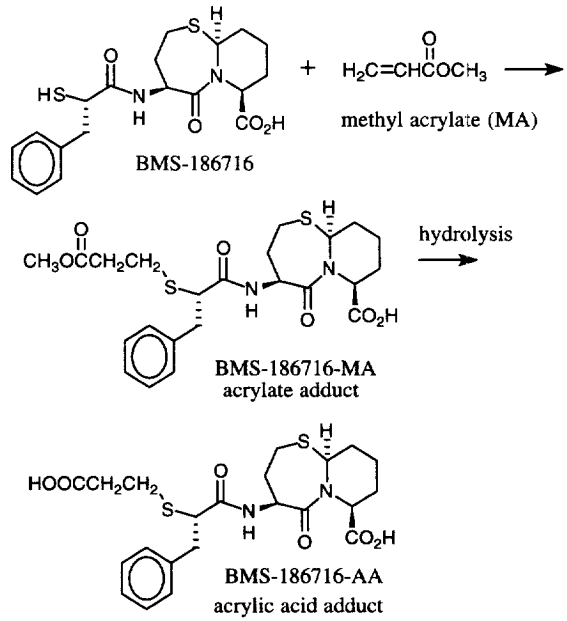

Fig. 2. Reaction of BMS-186716 with methyl acrylate (MA) to form the adduct BMS-186716–MA and hydrolysis of the adduct to form BMS-186716–AA.

186716–AA in rat plasma. The hydrolysis reaction is believed to be catalyzed by the presence of endogenous esterases in rat plasma. The internal standard MA adduct, BMS-188035–MA, also undergoes the same hydrolysis reaction in rat plasma, producing BMS-188035–AA.

Initially, the reaction of BMS-186716 with acrylic acid (AA), both in the free acid and sodium salt forms, was investigated. BMS-186716–AA could not be formed when BMS-186716 was reacted in the sodium phosphate buffer with AA under the conditions used for the MA reagent, as described in Section 2.4. This was not surprising since the nucleophilic addition reaction across the carbon–carbon double bond of AA, which possesses a carboxylate anion in the alkaline reaction medium used, is expected to be much slower when compared to that of MA, which does not possess the carboxylate anion. Thus, the direct formation of BMS-186716–AA for the preparation of standards and QCs was not possible. It was then decided to obtain BMS-186716–AA by spiking rat plasma with BMS-186716–MA, and then allowing time for the rat plasma native esterases to hydrolyze BMS-186716–MA to BMS-186716–AA.

During method development, the conversion of BMS-186716–MA to BMS-186716–AA in rat plasma was investigated by spiking rat plasma with BMS-186716–MA and allowing the samples to stand at room temperature for 0, 10, 20, 30, 45, 60, 90 and 120 min. After allowing hydrolysis for the specified length of time, the plasma samples were extracted (as described in Section 2.6) and chromatographed to monitor the decrease in the BMS-186716–MA peak and the increase in the BMS-186716–AA peak. The conversion of BMS-188035–MA to BMS-188035–AA was monitored similarly. Fig. 3a–c and Fig. 4a–c depict the conversion of BMS-186716–MA to BMS-186716–AA and of BMS-188035–MA to BMS-188035–AA, respectively, in rat plasma after incubation periods of 0, 10 and 45 min. The results of the hydrolysis are summarized in Table 1, which gives the estimated degree of hydrolysis at different times. The conversion was nearly complete (≥99%) after 20 min at room temperature for both the analyte and internal standard at the concentrations tested: (4970 ng/ml for BMS-186716–MA and 12 400 ng/ml for BMS-188035–MA). It was found that the lower the concentration of BMS-186716–MA or BMS-188035–MA in plasma, the faster was the hydrolysis reaction. During the preparation of the AA adduct of the internal standard (Section 2.5), the concentration of internal standard in the plasma was 12 400 ng/ml. Since the standard curve range of the method is 5–5000 ng/ml, the BMS-186716–MA concentrations of all standard points and QC samples will be at ≤5000 ng/ml, the concentration at which the hydrolysis of BMS-186716–MA was studied. All standard, QC and study samples were kept at room temperature for 1 h for the completion of the hydrolysis reaction. Although a period of 60 min is more than needed for complete hydrolysis of the MA adduct, a 60-min reaction time was still followed to provide for differences in the ambient temperature prevalent during the time of the work performed.

The HPLC conditions described here do not chromatographically separate BMS-186716–AA from BMS-188035–MA. This is relevant because the two compounds have a common analytical ion, m/z 498. Since complete hydrolysis of BMS-186716–MA and BMS-188035–MA occurs in rat plasma during the 60-min reaction time period, it is not necessary to monitor BMS-186716–MA or BMS-188035–MA during routine execution of the method.

The results of the quadratic regression analysis (weighted to $1/X$, where X is the concentration in ng/ml plasma) of a typical standard curve are shown in Table 2. The accuracy of each standard point and the precision of the duplicate points were very good, as the deviation (%) and C.V. (%) values were less than 15% at all concentrations of the standard curve. Table 3 presents the summary of the results of the QC samples analyzed during the method validation on three different days. The deviation of the grand mean was less than 13% for all QC levels. The inter-day and intra-day C.V. values were 8.0% or less at all levels.

For the verification of the lower limit of quantitation (LLQ) of the method, six different lots of blank rat plasma were spiked with BMS-186716–MA at the lowest level of the standard curve (5.01 ng/ml) and the internal standard. The samples were then analyzed against the standard curve. The six different lots of blank rat plasma were also analyzed after spiking with the internal standard only (QC0), and with no spiking at all, in order to determine if any

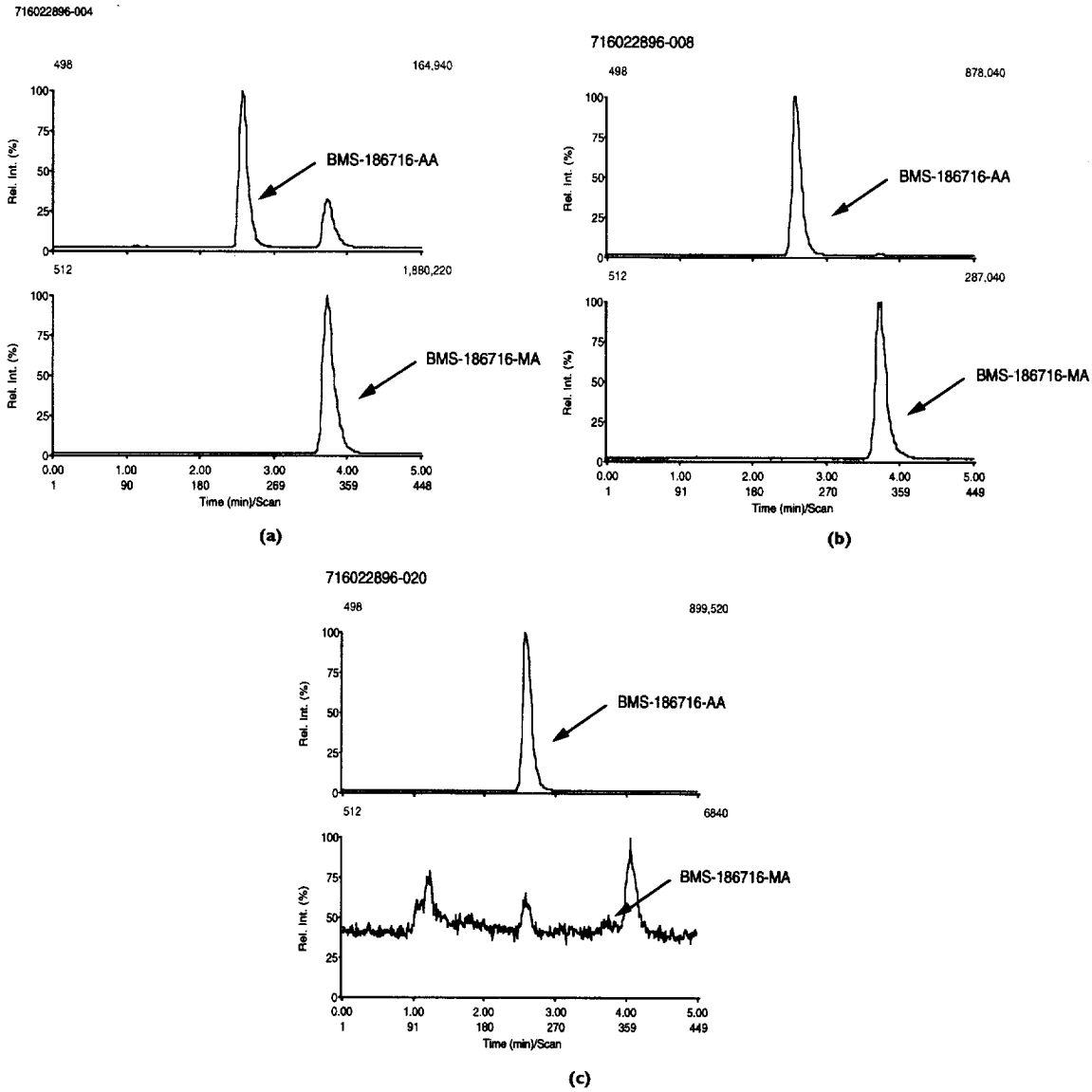

Fig. 3. (a) Conversion of BMS-186716–MA to BMS-186716–AA in rat plasma after 0 min at room temperature at 4970 ng/ml. (b) Conversion of BMS-186716–MA to BMS-186716–AA in rat plasma after 10 min at room temperature at 4970 ng/ml. (c) Conversion of BMS-186716–MA to BMS-186716–AA in rat plasma after 45 min at room temperature at 4970 ng/ml.

endogenous plasma constituents coeluted with the analyte or the internal standard. The values obtained for the LLQ verification are listed in Table 4. The deviation from the nominal value was ±17% or less. The precision at this level was excellent, with the C.V. being 7.5%.

Figs. 5 and 6, which compare the chromatogram of a QC0 against that of an LLQ sample (5.01 ng/ml) demonstrate the specificity of the method. In the six lots of blank plasma used, little to no response was detected in the analyte or internal standard channels. An acceptable peak was obtained for BMS-186716–AA at the LLQ level. The BMS-186716–AA response in the analyte channel of the

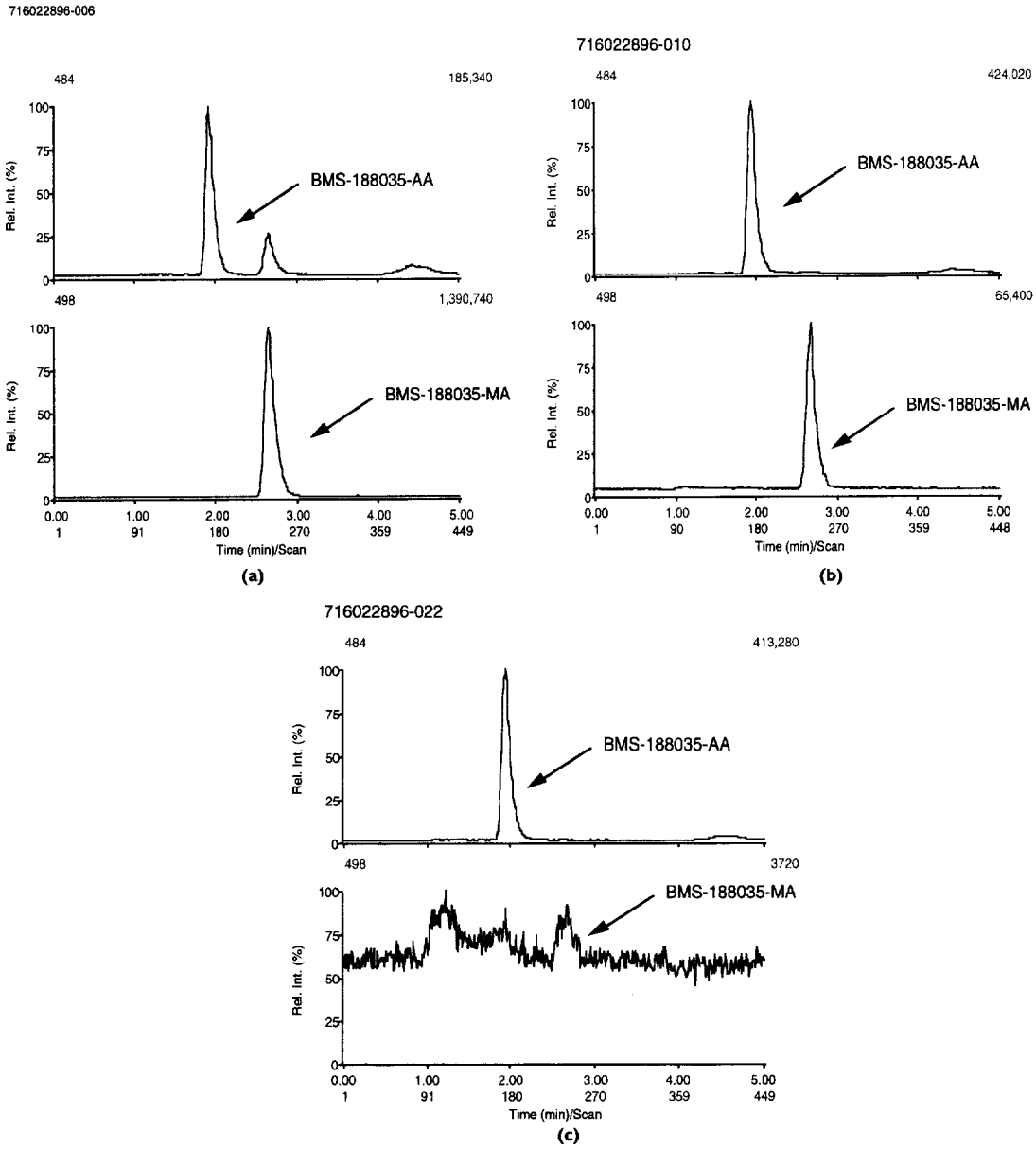

Fig. 4. (a) Conversion of BMS-188035–MA to BMS-188035–AA in rat plasma after 0 min at room temperature at 12 400 ng/ml (only 40 μl of the incubated sample was extracted, which was equivalent to extracting 0.1 ml of a 4960 ng/ml solution). (b) Conversion of BMS-188035–MA to BMS-188035–AA in rat plasma after 10 min at room temperature at 12 400 ng/ml (only 40 μl of the incubated sample was extracted, which was equivalent to extracting 0.1 ml of a 4960 ng/ml solution). (c) Conversion of BMS-188035–MA to BMS-188035–AA in rat plasma after 45 min at room temperature at 12 400 ng/ml (only 40 μl of the incubated sample was extracted, which was equivalent to extracting 0.1 ml of a 4960 ng/ml solution).

QC0 samples, when present, was significantly lower than that in the LLQ sample. Fig. 7 shows a typical chromatogram for a high-level QC sample.

BMS-186716–AA QC samples in rat plasma were found to be stable for at least 7 months at −70°C at all concentrations evaluated. BMS-186716–AA QC

Table 1
Hydrolysis of BMS-186716–MA and BMS-188035–MA in rat plasma

Incubation time (min)	BMS-186716–MA hydrolyzed (%)	BMS-188035–MA hydrolyzed (%)
10	85	95
20	99	99.7
30	99.9	100
45	100	100

Percentage hydrolysis was estimated by dividing the response of the MA adduct after the specified incubation time by the response of the MA adduct at 0 h and multiplying by 100.

Table 2
Quadratic regression analysis results of a typical standard curve in rat plasma

Nominal concentration (ng/ml)	Deviation (%)	C.V. (%)
5.010	+4.4, +12.5	1.5
10.02	−8.6, +1.8	3.1
25.04	+9.1, −2.6	5.2
50.08	−13.1, +11.9	13.8
150.2	−12.7, +5.4	12.1
250.4	−10.8, +4.9	10.8
500.8	+0.1, −4.5	3.2
751.5	−6.0, +4.0	7.0
1503	−3.0, +4.5	5.2
2003	−2.1, +4.0	4.1
3005	−4.9, +6.6	7.8
5008	−2.7, +1.8	3.1

The concentrations are for BMS-186716–AA, expressed in terms of BMS-186716. Deviation (%) is calculated by subtracting the back-calculated concentration from the nominal concentration and then dividing the difference by the nominal concentration and multiplying by 100. The two values given under the heading deviation (%) are for the duplicate standards at each level; the precision of the duplicate values are given under the heading C.V. (%). The quadratic regression parameters: intercept=0.004219; linear slope=0.0002983; quadratic slope=−0.000000005; R^2:0.998.

Table 4
Lower limit of quantitation (LLQ) for BMS-186716–AA in rat plasma

Blank rat plasma No.	Deviation (%)
1	−9.1
2	−17.4
3	−7.4
4	−2.4
5	−14.4
6	+0.76
Mean	4.592
Std. deviation	0.3454
C.V. (%)	7.5

Deviation (%) is calculated by subtracting the calculated concentration from the nominal concentration and then dividing the difference by the nominal concentration and multiplying by 100.

samples in rat plasma were found to be stable for at least 2 months at −20°C. Rat plasma QC samples were stable after they were subjected to a 3-cycle freeze–thaw process. At room temperature and at 4°C, BMS-186716–AA in rat plasma was found to be stable for at least 24 h.

Table 3
Summary of the results of rat plasma QC samples analyzed on three different days

Nominal concentration (ng/ml)	Grand mean (ng/ml)	Deviation (%)	Inter-day precision C.V. (%)	Intra-day precision C.V. (%)
20.98	21.17	+0.9	2.9	5.4
99.92	100.6	+0.7	0.6	3.2
999.2	1014	+1.4	3.6	7.0
3497	3641	+4.1	8.0	3.8
24 980	28 102	+12.5	0.0[a]	7.3

[a] No significant additional variation was observed as a result of performing the assay on different days. The concentrations are for BMS-186716–AA, expressed in terms of BMS-186716.

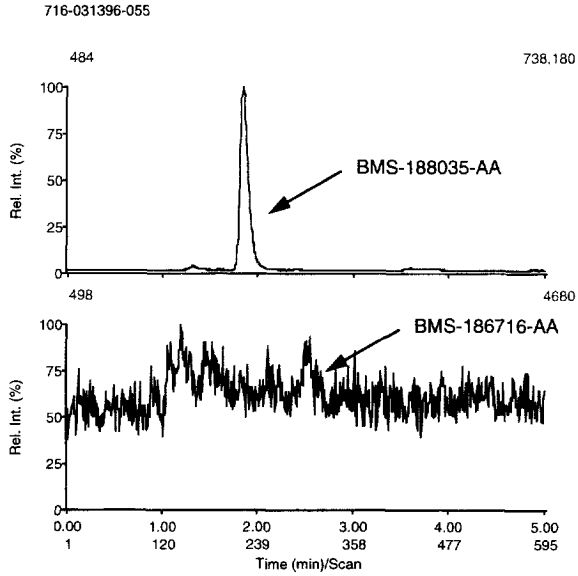

Fig. 5. Chromatogram of BMS-186716–AA at 0 ng/ml and the internal standard BMS-188035–AA at 6200 ng/ml in rat plasma.

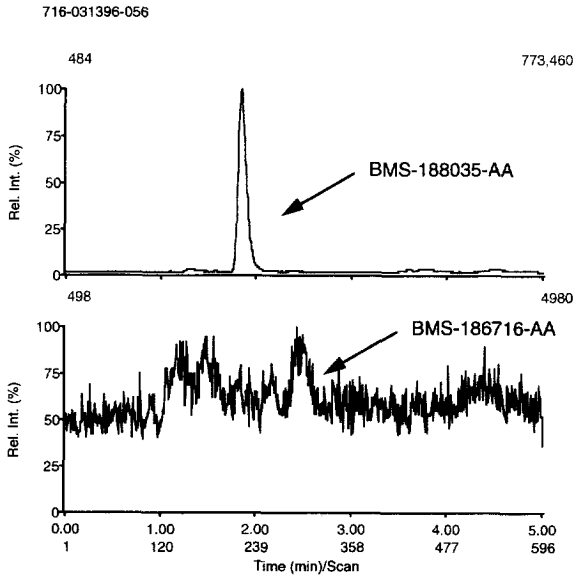

Fig. 6. Chromatogram of BMS-186716–AA at 5 ng/ml (lower limit of quantitation) and the internal standard BMS-188035–AA at 6200 ng/ml in rat plasma.

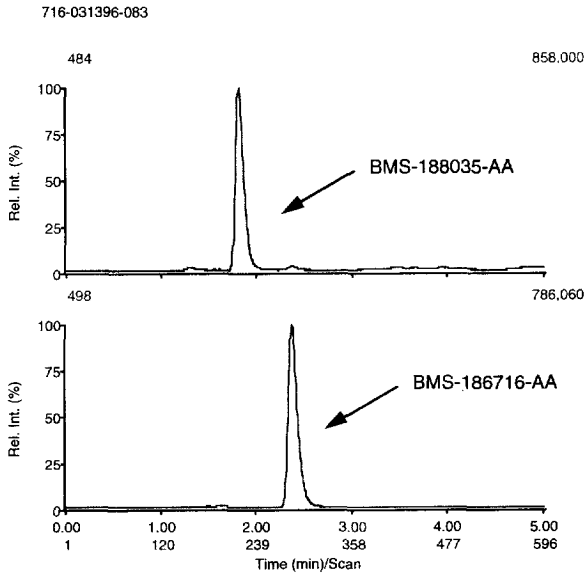

Fig. 7. Chromatogram of a BMS-186716–AA QC sample at 3497 ng/ml and the internal standard BMS-188035–AA at 6200 ng/ml in rat plasma.

4. Conclusion

We have shown that the MA-adduct of a sulfhydryl compound BMS-186716 (BMS-186716–MA) is unstable in rat plasma. This is in contrast to the marked stability of BMS-186716–MA in dog plasma [2]. In rat plasma, the methyl ester of the MA portion of BMS-186716–MA was found to be unstable due to hydrolysis to the acrylic acid (AA), resulting in a new adduct, BMS-186716–AA. Although this finding of instability of BMS-186716–MA was not the result of prospective planning, we were able to successfully develop a quantitative bioanalytical method using BMS-186716–AA as the analyte instead of the originally planned BMS-186716–MA. An LLQ of 5 ng/ml plasma was achieved.

References

[1] M. Jemal, D. Hawthorne, Rapid Commun. Mass Spectrom. 8 (1994) 854.
[2] M. Jemal, D. Hawthorne, J. Chromatogr. B 693 (1997) 109.
[3] A.T. Murphy, P.L. Bonate, S.C. Kasper, T.A. Gillespie, A.F. DeLong, Biol. Mass Spectrom. 23 (1994) 621.

[4] F.J. Hayes, T.R. Baker, R.L.M. Dobson, M.S. Tsueda, J. Chromatogr. A 692 (1995) 73.
[5] P. Michelsen, B. Jergil, G. Odham, Rapid Commun. Mass Spectrom. 9 (1995) 1109.
[6] A. Roda, A.M. Gioacchini, C. Cerrè, M. Baraldini, J. Chromatogr. B 665 (1995) 281.
[7] A.E. Mutlib, J.T. Strupczewski, J. Chromatogr. B 669 (1995) 237.
[8] M. Jemal, D. Hawthorne, J. Pharma. Biomed. Anal. 14 (1995) 43.
[9] Y. Oda, N. Mano, N. Asakawa, J. Mass Spectrom. 30 (1995) 1671.
[10] J.D. Mooree, J.P. Sommadossi and the ACTG 215 Study Team, J. Mass Spectrom, 30 (1995) 1707.