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Validated HPLC procedures for the analysis of BMY-28090 in human plasma and urine

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Summary. The compound BMY-28090 (elsamicin A) is a new fermentation product with antitumor properties, which has the same aglycone as chartreusin but contains two novel sugars. To define the disposition of BMY-28090 during phase I trials, HPLC procedures were developed and validated for the quantitation of the drug in human plasma and urine. To 1.0 ml plasma were added 0.5 ml 0.2 *M* phosphate buffer (pH 8.0), 125 ng 1-naphthol (internal standard) in 25 µl MeOH and 5 ml ethyl acetate. After mixing and centrifugation, 4 ml ethyl acetate layer was removed, evaporated to dryness, and the residue was dissolved in 250 µl mobile phase and injected (200 µl). To 1.0 ml urine were added 100 µl MeOH and 1.0 ml 0.5 *M* succinate buffer (pH 4.0). After mixing (30 s) and sonication (1 min), the solution was filtered in an Amicon Centrifree micropartition unit and injected (30 µl). An IBM C-8 column 5-µm and fluorescence detection (excitation at 254 mm, 418 nm emission filter) were used for both analyses. The mobile phases for plasma (2 ml/min) and urine (1.3 ml/min) were H_2O/CH_3CN (7:3 v/v) and H_2O/CH_3CN/MeOH (6:3:1 (v/v), respectively, with 1.5 ml 85% H_3PO_4 and 1.5 ml triethylamine/l. BMY-28090 eluted at 8–10 min and 1-naphthol, at 10–11 min. The standard curves were linear from 1 to 50 ng/ml plasma and from 10 to 1000 ng/ml urine. The within- and between-day precision was <3% for plasma and <5% for urine. Accuracies were within 6% of the nominal value and recoveries were 75% and 90% for plasma and urine, respectively. At 37° C, BMY-28090 was stable in plasma for at least 8 h but had a half-life of 36 h in urine. The drug was stable in plasma and urine for 30 days at −20° C.

Introduction

The compound BMY-28090 (elsamicin A) in a new and novel fermentation product produced by an actinomycete strain and has the structure shown in Fig. 1. Its chemical name is benzo[h][i]benzopyrano[5,4,3-cde][i]-benzopyran-5,12-dione,10-[[2-0-(2-amino-2,6-dideoxy-3-0-methyl-α-D-galactopyrano-syl)-6-deoxy-3-c-methyl-β-D-galactopyrano-syl]oxy]-6-hydroxy-1-methyl. It has the same aglycone (chartarin) as chartreusin but contains two different and novel sugars called elsarose and elsaminose [2–4, 7]. The latter amino sugar accounts for the increased water solubility of BMY-28090 relative to chartreusin. The compound is 10–15 times more potent than chartreusin in a number of murine tumor models, including leukemia P388 and L1210 and melanoma B16 [2, 3], is active in subrenal capsule assays and a human colon tumor xenograft [5], and is cytotoxic to murine and human tumor cell lines in vitro [6]. BMY-28090 appears to be cross-resistant in tumor cell lines having the multiple drug resistance phenotype and produces extensive DNA breakage in intact cells but not in pure DNA in vitro [1, 6]. Based on these preclinical activities, the compound was selected for phase I trials. To define the disposition of BMY-28090 during these initial clinical studies, sensitive, accurate, and precise analytical procedures were required for the analysis of the compound in human plasma and urine. This report describes the validation of these methods.

Materials and methods

Chemicals. BMY-28090 (elsamicin A) and chartreusin (BMY-25684) were supplied internally and chartarin was obtained from the Bristol-Myers Research Institute (Tokyo, Japan). Triethylamine and 1-naphthol were obtained from Aldrich Chemical Co. (Milwaukee, Wis). HPLC-grade methanol and acetonitrile, 85% phosphoric acid, succinic acid, sodium hydroxide, and sodium phosphate were purchased from Fisher Scientific Co. (Fairlawn, NJ); HPLC-grade ethyl acetate was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). All water was filtered and deionized with a Milli-Q system from Millipore Corp. (Bedford, Mass). Human urine was collected from laboratory personnel; human plasma, collected with ethylenediaminetetracetate (EDTA) as the anticoagulant, was obtained from the Interstate Blood Bank (Memphis, Tenn)

Equipment. The following equipment was purchased from the sources indicated: Centrifree micropartition system, Amicon Corp. (Danvers, Mass); model 27 electronic balance, Cahn Instruments, Inc. (Cerritos, Calif); Sartoris model 1212 MP balance, Brinkman Instrument Co. (Westbury, NY); model HN-SII centrifuge, IEC/Damon Division, International Equipment Co. (Westbury, NY); model B-52 ultrasonic cleaner, Branson Instruments (Sheton, Calif); model 2600 multi-tube vortex mixer,

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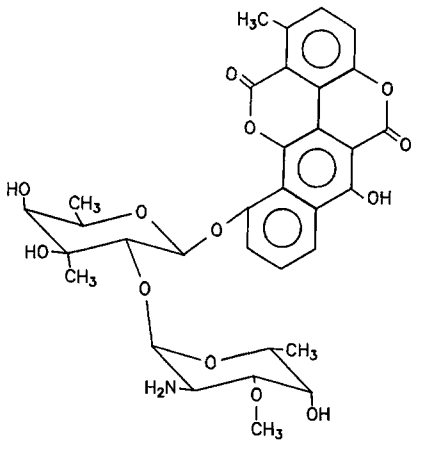

Fig. 1. Structure of BMY-28090

Scientific Manufacturing Industries (Emeryville, Calif); VMR2 vortexer, Scientific Industries, Inc. (Bohemia, NY); Accumet model 815MP pH meter, Fisher Scientific Co. (Fairlawn, NJ); Gilson Pipetman models P1000, P200, P100, and P20, Rainin Instrument Co., Inc. (Woburn, Mass); 25-, 50-, and 100-µl syringes, Hamilton Co. (Reno, Nev); solvent filtration apparatus and 0.22-µm Durapore filters, Millipore (Milford, Mass); N-Evap evaporator, Organomation Associates Inc. (Northborough, Mass); microcentrifuge tubes (330 µl) and 13 × 100-mm polypropylene tubes with caps. Denville Scientific, Inc. (Denville, NJ); 6- and 12-ml polypropylene screw-capped tubes, Sarstedt Inc. (Princeton, NJ); 10-ml repipet dispenser, Rainin Instrument Co. (Woburn, Mass); model 346 hematology/chemistry mixer, Fisher Scientific Co (Fairlawn, NJ).

The HPLC system for urinalysis consisted of a model 590 programmable solvent-delivery module and a model 710B automatic injector from Waters Associates (Milford, Mass) combined with a Spectroflow model 980 fluorescence detector from Kratos Analytical (Ramsey, NJ), a model 1200 recorder from Linear Instrument Corp. (Reno, Nev), and a solvent selector from Autochrome, Inc. (Milford, Mass). The HPLC system for plasma consisted of the same injector and fluorescence detector as were used for urine, attached to a model LC/9560 ternary liquid chromatograph from IBM Instrument Co. (Danbury, Conn), and a model SE-120 recorder from BBC-Metrawatt/Goerz (Bromfield, Colo). The 5-µm, 4.5 × 150-mm C-8 columns were obtained from IBM Instrument Co. (Danbury, Conn).

Solutions. Succinic acid buffer (0.5 *M*, pH 4.0) was prepared by adding 14.75 g succinic acid to 225 ml water and 10 ml 5 *N* NaOH and stirring under low heat until a clear solution was obtained. After it had cooled to room temperature, the pH was adjusted to pH 4.0 with 5 *N* NaOH and the volume was brought to 250 ml with water. The buffer was prepared fresh weekly and was stored at 3° – 7° C. A solution of the internal standard for the plasma assay was prepared by adding 1-naphthol to methanol in a glass vial to give a final concentration of 5 µg/ml. This solution was prepared fresh weekly and was stored in a dark environment at 3° – 7° C. The stock of powdered 1-naphthol was stored in a dark environment under nitrogen in a desiccator. Phosphate buffer (0.2 *M*, pH 8.0) was prepared by adding 14.2 g dibasic sodium phosphate to 400 ml water, bringing the volume to 500 ml, and adjusting the pH to 8.0 with 85% phosphoric acid. The solution was stored at 3° – 7° C. Stock solutions of BMY-28090 were prepared by adding methanol to the compound (0.3 – 1.0 mg) to give a final concentration of 100 µg/ml. After sonication for 2 – 3 min, dilutions were made to give final concentrations of 10.0 and 1.0 µg/ml methanol. The solutions were prepared fresh weekly and were stored in a dark environment at 3° – 7° C.

Mobile Phases. The mobile phase for the plasma assay was prepared by adding 1.5 ml 85% phosphoric acid and 0.6 – 1.5 ml triethylamine to 300 ml acetonitrile in a graduated cylinder and diluting to 1 l with water. Peak separations were optimized by varying the amounts of triethylamine. The mobile phase for the urine assay was prepared by adding 1.5 ml 85% phosphoric acid and 1.5 ml triethylamine to 300 ml acetonitrile plus 300 ml methanol in a graduated cylinder and diluting to 1 l with water. The mobile phases were prepared fresh daily and were filtered through 0.22 µm filters.

Standards of BMY-28090. Samples (1, 2, or 5 µl) of the appropriate stock solutions of BMY-28090 in methanol (1 or 10 mg/ml) were added in duplicate with a 10-ml glass syringe to 1.0 ml plasma in 13 × 100-mm polypropylene tubes to give final concentrations of 1, 2, 5, 10, 20, and 50 ng/ml. Similarly, samples of the stock solutions were added in duplicate with a 100-µl glass syringe to 1.0 ml urine to give 10, 20, 40, 80, 100, 400, 800, 1,000 ng/tube. Additional methanol was added to give a total volume of 100 µl/ml urine. Tubes of plasma and urine containing no BMY-28090 (blanks) were also prepared.

Processing of plasma. To each plasma sample (1.0 ml) and standard was added 125 ng (25 µl) internal standard, 1-naphthol, followed by 0.5 ml 0.2 *M* phosphate buffer (pH 8.0). After mixing on a multi-tube vortex mixer for 30 s, 5 ml ethyl acetate was added and the capped tubes were placed on a rotary mixer for 10 min. Following centrifugation for 7 min at 200 *g*, 4 ml ethyl acetate layer was transferred to separate tubes and evaporated to dryness at 30° C under a stream of nitrogen. The sides of the tubes were then rinsed with 0.75 ml methanol and the solvent was evaporated. The residues were reconstituted in 250 µl mobile phase by mixing on a vortex mixer for 30 s, sonicating for 5 min, and mixing on a vortex mixer for 30 s. The samples were then transferred to autosampler vials for analysis.

Processing of urine. Methanol (100 µl) and 1.0 ml 0.5 *M* succinate buffer (pH 4.0) were added to each urine sample and standard. The capped tubes were mixed on a vortex mixer for 30 s, sonicated for 1 min, and then mixed again. A 1.0-ml sample was transferred to a Centrifree micropartition unit and centrifuged for 8 min at 500 *g*. A 200 µl sample of the ultrafiltrate was transferred to a microcentrifuge tube in an autosampler vial for analysis.

HPLC of plasma extracts. Samples (200 µl) of the reconstituted plasma extracts were injected onto a 5-µm C-

8 column with a mobile phase of H_2O/CH_3CN (7:3 v/v) containing 1.5 ml 85% phosphoric acid and 0.6 – 1.5 ml triethylamine/l at a flow rate of 2 ml/min. Detection was carried out by fluorescence with excitation at 254 nm and emission with a 418 nm cutoff filter. Rise time was 5 s, high voltage was 890 V, and the range was 0.1 AUFS. BMY-28090 and 1-naphthol eluted at about 9.5 and 10.5 min, respectively.

HPLC of urine samples. Samples (30 µl) of ultrafiltrates of the 1 : 1 mixture of urine and 0.5 *M* succinate buffer were injected onto a 5-µm C-8 column with a mobile phase of H_2O/CH_3CN/MeOH (6:3:1 v/v) containing 1.5 ml 85% phosphoric acid and 1.5 ml triethylamine/l at a flow rate of 1.3 ml/min. Detection was done by fluorescence with excitation at 254 nm and emission with a 418-nm cutoff filter. Rise time was 2 s, the PMT signal was set at 0.1, and the range was 0.1 AUFS. BMY-28090 eluted at 7.5 – 8.5 min.

Data collection and calculations. The HPLC detector output was recorded with a Hewlett-Packard model 3357 Laboratory Automation System computer and chromatograms were evaluated with the system software. Least-squares regression of the peak height ratios of BMY-28090/1-naphthol (plasma) or the peak heights of BMY-28090 (urine) from the standards vs the concentrations of BMY-28090 were calculated. The slope, intercept (95% confidence limits), and correlation coefficient were calculated. Sample concentrations were estimated by inverse prediction.

Specificity. Urine and plasma samples from ten different donors were processed and chromatographed, and the detector output at the retention times for BMY-28090 and 1-naphthol (plasma only) was recorded. The retention times of chartarin, the aglycone of BMY-28090 and a potential degradation product, an chartreusin, an analogue of BMY-28090, were determined in each HPLC system.

Detection limit. BMY-28090 was added to plasma (0.05 ng/ml) and urine (5 and 10 ng/ml) samples from ten different donors. These samples, as well as drug-free, blank samples from the same donors, were processed and analyzed. The peak heights at the retention time of BMY-28090 in each blank and test sample were recorded and subjected to a paired Student's *t*-test.

Lower limit of quantitation. BMY-28090 was added to plasma (1 ng/ml) and urine (5 and 10 ng/ml) samples from ten different donors. These samples, as well as corresponding plasma and urine specimens containing no drug, were processed and analyzed and the concentrations of the drug were determined based on a standard curve. Precision was expressed as the percentage of relative standard deviation (%RSD) of the observed concentration. Accuracy was expressed as the percentage of deviation of the observed from the nominal concentration.

Range of reliable response. Standard curves consisting of at least six (plasma) or eight (urine) standards in duplicate, and covering a range of 1 – 50 ng/ml plasma and 10 – 1,000 ng/ml urine, were analyzed on several separate occasions. The slopes, correlation coefficients, and intercepts were calculated and compared for linearity and reproducibility.

Accuracy and precision. Bulk plasma (3.0 and 45 ng/ml) and urine (36 and 850 ng/ml) samples were prepared by someone other than the analyst. Ten replicate samples of each matrix were analyzed on the day of preparation and on 2 subsequent days. Samples of urine (40 and 700 ng/ml) were also prepared, diluted with an equal volume of 0.5 *M* succinate buffer, and similarly analyzed. Accuracy was expressed as the percentage of deviation of the mean observed from the nominal concentration. Precision was expressed as the mean percentage of relative standard deviation (%RSD) of the observed concentration. Between-day accuracy was calculated using the deviation of the grand mean for each concentration from the nominal concentrations. Between-day precision was calculated from the observed mean of all samples at each concentration.

Recovery. Standard curves were prepared in plasma and urine and the samples were processed and analyzed. The slopes of these standard curves were calculated as a percentage of the slopes of standard curves prepared in mobile phase (plasma) or methanol (urine) and injected directly. The concentrations in the mobible phase were adjusted to 80% of the nominal concentrations in plasma, since only 4 ml of the 5-ml ethyl acetate extract was taken for analysis.

Stability in plasma. Stability was initially evaluated at 37° C. A bulk plasma sample containing 20 ng BMY-28090/ml was prepared and 1.0-ml samples were transferred to separate tubes. After capping of the tubes, one was placed directly in dry ice and the others were placed in a constant-temperature water bath at 37° C. Tubes were removed from the water bath at 15, 30, and 45 min and at 1, 2, 4, 6, and 8 h and were placed immediately in dry ice. The samples were then stored at −20° C and were analyzed the following day. Storage stability was evaluated by preparing bulk plasma samples of BMY-28090 (3.0 and 45.0 ng/ml), transferring 1.2-ml, samples to separate tubes, and storing these for various periods of time at −20° C. Several replicate samples of each concentration were analyzed on the day of preparation (day 1) and on days 9, 30, 65, and 91.

Stability in urine. BMY-28090 was added to a bulk urine sample to give a final concentration of 100 ng/ml. A portion of the sample was diluted with an equal volume of 0.5 *M* succinate buffer (pH 4.0), and the latter and the urine were placed in a water bath at 37° C. Samples were removed for analysis at 0, 0.25, 0.50, 1, 2, 4, 8, 24, 48, and 72 h, frozen in dry ice, and stored at −70° C until analyzed. Storage stability at −20° C was evaluated by preparing bulk samples of urine containing 36 and 850 ng BMY-28090/ml, transferring portions to separate tubes, and storing these at −20° C for 2, 3, 9, 18, 30, and 58 days. A sample was also analyzed on the day of preparation. Urine samples containing 40 and 700 ng BMY-28090/ml were prepared, diluted 1:1 (v/v) with 0.5 *M* succinate buffer (pH 4.0), and stored and analyzed with the undiluted urine samples.

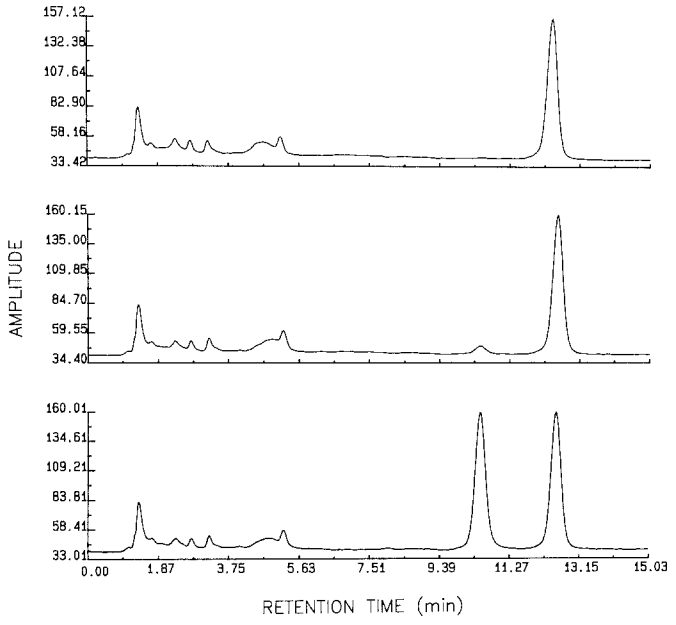

Fig. 2. Representative chromatograms of BMY-28090 in human plasma. *Top to bottom:* 0, 1, and 20 ng/ml

Stability during HPLC analysis. A bulk plasma sample containing 40 ng BMY-28090/ml was prepared. Samples (1.0 ml) were allocated to each of 19 separate tubes and were processed. After reconstitution, the samples were pooled and mixed and portions (225 µl) of the pooled solution were placed in 18 autosampler vials. Triplicate injections were made at the following times: 0, 6, 12, 24, 36, and 48 h. The peak heights and peak height ratios were determined for each injection. BMY-28090 was added to urine to give final concentrations of 40 and 800 ng/ml. The samples were processed and placed in autosampler vials. One set of vials at each concentration was left at room temperature and was injected at 0, 6, 12, 24, 36, and 48 h. Another set was stored at 3° – 7° C and injected at 24 and 48 h. The peak heights were compared to the zero-time peak height.

Results and discussion

Plasma analysis

A simple and rapid procedure using a single extraction with ethyl acetate was developed for the analysis of BMY-28090 in human plasma. An internal standard, 1-naphthol, and 0.5 ml 0.2 *M* sodium phosphate buffer (pH 8.0) were added to 1.0 ml plasma containing BMY-28090. The mixture was extracted with 5 ml ethyl acetate, and 4 ml organic phase was removed and evaporated to dryness. After reconstitution in mobile phase, the sample was chromatographed on a C-8 column with a mobile phase of H_2O/CH_3CN (7:3 v/v) containing 1.5 ml 85% phosphoric acid and 0.6–1.5 ml triethylamine/l. Quantitation was based on the peak height ratio (BMY-28090/1-naphthol) of fluorescence.

Representative chromatograms of BMY-28090 from human plasma are shown in Fig. 2. Fluorescent plasma constituents eluted before 5 min, whereas the drug and 1-naphthol eluted at about 9.5 and 10.5 min, respectively. Endogenous constituents from the plasma of ten different donors did not elute at the retention times of either BMY-28090 or the internal standard. Chartarin, the aglycone of BMY-28090, eluted at 79 min in this system, whereas an analogue, chartreusin, eluted at 8.0 min and was completely separated from BMY-28090. Therefore, the HPLC system appeared to have good specificity, but interference from possible metabolites cannot be evaluated until clinical samples are available and the metabolism of the compound has been defined.

Table 1. Stability of BMY-28090 in human plasma (20 ng/ml) in vitro at 37° C

Time (h)	Observed concentration (ng/ml)
0	19.7
0.25	20.2
0.50	18.7
0.75	21.6
1	20.0
2	19.6
4	22.2
6	19.6
8	18.2

Although the lower limit of detection was 0.05 ng/ml, the lower limit of quantitation was 1.0 ng/ml. Significant detector responses were seen at concentrations below 1.0 ng/ml, but good linearity was observed only between 1.0 and 50.0 ng/ml plasma. Standard curves ($n = 16$) prepared from at least six standards in duplicate and covering a range of 1.0–50.0 ng/ml showed good linearity with correlation coefficients of $\geqslant 0.996$ and a mean ($\pm$ SD) slope of 0.0646 ± 0.00617.

The overall recovery of BMY-28090 from human plasma was 75%, based on a comparison of the slope of a processed standard curve to the slope of a standard curve prepared in mobile phase and assayed without processing. This value takes into account a 20% loss due to removal of only 80% of the ethyl acetate extract for analysis. Recovery was consistent over the entire concentration range, with individual values ranging from 71.4% to 79.9%. The method was accurate and precise. Within-day accuracy was 100% and 105% ($n = 10$) at concentrations of 3.0 and 45 ng/ml, respectively, and the corresponding precision (%RSD) was 1.0% and 2.9%. The between-day accuracy ($n = 10$) at the same concentrations was 103% and 106% and the between-day precision (%RSD), 1.2% and 0.7%, respectively.

BMY-28090 (20 ng/ml) was stable in human plasma in vitro for at least 8 h at 37° C (Table 1). Extracts of plasma samples containing 40 ng/ml were stable for at least 48 h at room temperature following reconstitution in the mobile phase, with a precision (%RSD) of $\leqslant 0.8\%$ ($n = 3$). Therefore, samples could be stored and analyzed for up to 48 h after reconstitution in the mobile phase. At concentrations of 3 and 45 ng/ml, BMY-28090 was stable for 30 days in human plasma stored at −20° C. However, only about 90% and 75% of the original concentrations remained after 65 and 91 days of storage, respectively (Table 2). These results indicate that plasma samples from clinical studies should be stored at −20° C and analyzed within 30 days of sampling.

Table 2. Stability of BMY-28090 in human plasma stored at −20° C

Time (days)	Nominal concentration (ng/ml)	Samples (*n*)	Mean observed concentration (ng/ml)	%RSD	Percentage of deviation from nominal
1	3	10	3.01	4.1	0.3
9	3	10	3.09	3.8	3.0
30	3	5	3.04	4.6	1.3
65	3	5	2.69	4.5	−10.3
91	3	5	2.28	2.6	−24.0
1	45	10	47.3	2.1	5.0
9	45	10	47.5	3.5	5.5
30	45	5	46.2	3.4	2.8
65	45	5	40.1	8.4	−11.0
91	45	5	34.3	2.2	−23.8

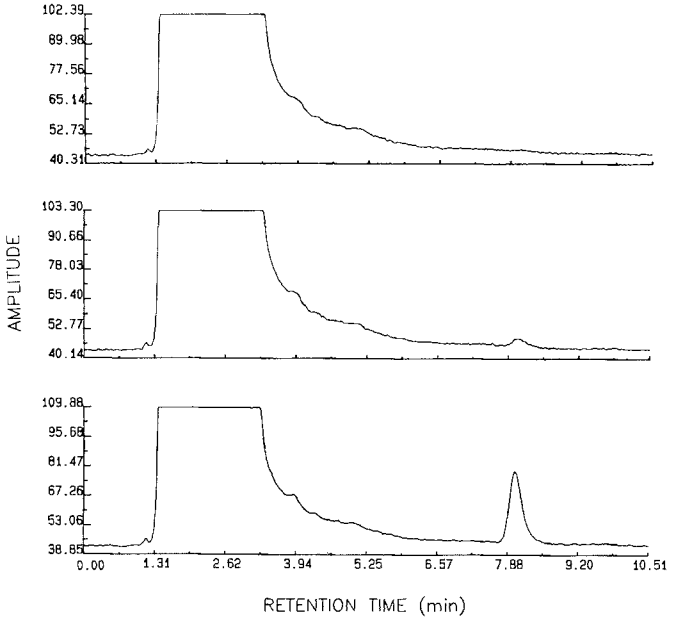

Fig. 3. Representative chromatograms of BMY-28090 in human urine. *Top to bottom:* 0, 10, and 100 ng/ml

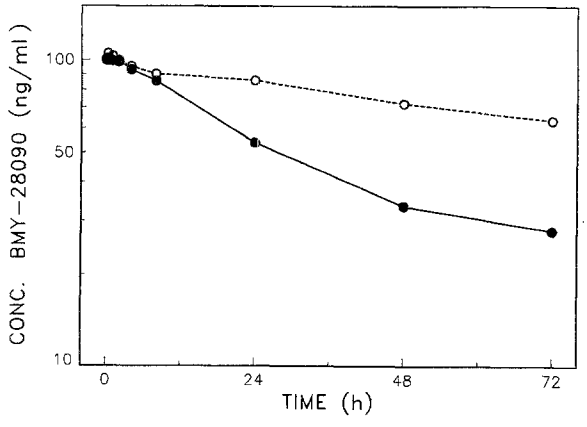

Fig. 4. Concentrations of BMY-28090 in human urine (●) and in human urine plus an equal volume of 0.5 *M* succinate buffer (pH 4.0) (○) as a function of time at 37° C. The half-life was 36 h in urine and 115 h in urine/0.5 *M* succinate buffer

Urinanalysis

Urine samples of BMY-28090 were analyzed by HPLC without extraction. Methanol (100 µl) and 1.0 ml 0.5 *M* succinate buffer (pH 4.0) were added to a 1.0-ml sample of urine; after mixing, the solution was filtered through an Amicon Centrifree unit. A 30-µl sample of the filtrate was chromatographed on a C-8 column with a mobile phase of $H_2O/CH_3CN/MeOH$ (6:3:1) containing 1.5 ml 85% phosphoric acid and 1.5 ml triethylamine/l. Quantitation was based on peak height.

Representative chromatograms of BMY-28090 in human urine are shown in Fig. 3. Fluorescent materials in urine eluted by 6 min and the drug eluted at between 8 and 9 min. No peaks from endogenous materials in the urine from ten different donors were found at the retention time of BMY-28090. As with the HPLC system for plasma, the analogue, chartreusin, eluted before BMY-28090 and the aglycone eluted at a much later time (36 min). The lower limit of detection for the analysis of BMY-28090 in urine was 5 ng/ml; however, the lower limit of quantitation was set at 10 ng/ml. The linear range was 10–1,000 ng/ml urine. Standard curves (n = 11) prepared from at least eight standards in duplicate and covering the range of 10–1,000 ng/ml had correlation coefficients of 0.999 and a mean (±SD) slope of 299±29. The overall recovery was 90%, based on the slope of a processed standard curve relative to the slope of a standard curve prepared in methanol and injected directly.

The analytical method for urine was accurate and precise. At the lower limit of quantitation (10 ng/ml), the mean percentage of deviation from nominal was 2.0% and the mean RSD was 7.8%. The mean within-day error (%RSD) was 3.0% and 3.7% at concentrations of 36 and 850 ng/ml, respectively, and the corresponding accuracy was 98% and 97%. The between-day precision (%RSD) was 2.2% and 4.8% at concentrations of 36 and 850 ng/ml urine, respectively, and the corresponding accuracy was 97% and 98%. The stability of BMY-28090 (100 ng/ml) in human urine and in a 1:1 mixture of urine and 0.5 *M* succinate buffer (pH 4.0) was initially evaluated at 37° C (Fig. 4). The compound had a half-life of 36 h (r = 0.997) in urine and 115 h (r = 0.980) in a 1:1 mixture of a urine/succinate buffer.

Based on the results at 37° C and on the fact that succinate buffer was added to urine as part of the analytical

Table 3. Stability of BMY-28090 in a 1:1 (v/v) mixture of human urine and 0.5 *M* succinate buffer (pH 4.0) at room temperature and at 3° – 7° C

	Room temperature (n = 4):			
	Low Concentration (40 ng/ml)		High concentration (800 ng/ml)	
Time (h)	Mean (± SD) peak height	% of 0 time	Mean (± SD) peak height	% of 0 time
0	13,278 ± 165	–	238,406 ± 1,689	–
6	13,133 ± 236	98.9	242,646 ± 1,196	102
12	12,655 ± 134	95.3	237,238 ± 1,577	99.5
24	12,176 ± 112	91.7	225,566 ± 1,041	94.6
36	11,133 ± 327	83.8	210,590 ± 656	88.3
48	11,072 ± 221	83.4	205,462 ± 1,291	86.2
	3° – 7° C (n = 4):			
0	13,278 ± 165	–	238,406 ± 1,689	–
24	13,684 ± 98	103	256,998 ± 2,012	108
48	13,550 ± 321	102	241,813 ± 3,051	101

Table 4. Storage stability of BMY-28090 in human urine and in a 1:1 (v/v) mixture of urine and 0.5 *M* succinate buffer (pH 4.0) at – 20° C

		Urine:			
		Low concentration (36 ng/ml)		High concentration (850 ng/ml)	
Time (days)	Samples (n)	Concentration[a] (ng/ml)	% of theory	Concentration[a] (ng/ml)	% of theory
1	10	35.4 ± 0.58	98.3	876 ± 29.7	110
2	10	35.6 ± 1.54	98.9	795 ± 34.3	93.5
3	10	34.0 ± 1.05	94.4	829 ± 97.6	97.6
9	6	35.0 ± 0.74	97.2	796 ± 12.1	93.6
18	6	36.8 ± 0.54	102	857 ± 30.2	101
30	6	35.5 ± 0.81	98.6	834 ± 17.4	98.1
58	6	31.0 ± 1.32	86.1	808 ± 13.7	95.0
		Urine + 0.5 *M* succinate buffer (1:1, v/v):			
		Low concentration (40 ng/ml)		High concentration (700 ng/ml)	
1	10	37.6 ± 1.70	94.0	691 ± 17.3	98.3
2	10	35.6 ± 1.57	89.0	634 ± 21.2	90.5
3	10	37.2 ± 1.59	93.0	656 ± 21.7	93.7
9	6	35.7 ± 1.07	89.2	628 ± 21.0	89.7
18	6	38.9 ± 0.87	97.2	676 ± 16.6	96.6
30	6	38.0 ± 1.54	95.0	666 ± 8.10	95.2
58	6	36.1 ± 1.19	90.2	654 ± 0.54	93.4

[a] Mean ± SD

methodology, subsequent stability studies at room temperature and at 3° – 7° C were conducted in the 1:1 mixture of urine/succinate buffer. At concentrations of 40 and 800 ng/ml, BMY-28090 was stable in the urine-succinate buffer mixture for about 12 h at room temperature, and over 90% of the original concentration was still present at 24 h (Table 3). The same mixtures were stable for at least 48 h at 3° – 7° C (Table 3).

Storage stability at – 20° C was evaluated in both urine and a urine/succinate buffer (1:1) mixture. At – 20° C and concentrations of 36 and 850 ng/ml urine, BMY-28090 was stable for at least 30 days, with 86% and 95% of the original concentrations, respectively, remaining at 58 days (Table 4). Similar stability was seen with the same concentrations in the urine/succinate buffer mixture (Table 4). A mean of 95% of the original concentrations were present after 30 days at – 20° C, with 90% and 93% remaining at 58 days. Therefore, human urine samples of BMY-28090 should be stored at – 20° C and analyzed within 30 days of collection to obtain valid results.

In conclusion, sensitive, specific, reproducible, and accurate HPLC procedures were developed and validated for the analysis of BMY-28090 in human plasma and urine. These procedures will be applied in determination of the pharmacokinetics of the drug in patients during phase I trials.

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References

1. Catino JJ, Francher DM, Long BH, Casazza AM (1987) Cellular uptake of elsamicin and DNA breakage in P388 murine leukemia cells. Proc Am Assoc Cancer Res 28: 310
2. Konishi M, Sugawara K, Tsunakawa M, Nishiyama Y, Miyaki T, Kawaguchi H (1985) BMY-28090 (elsamicin), a new antitumor antibiotic related to chartreusin: isolation, chemistry and activity. In: Ishigami J (ed) Recent advances in chemotherapy. Proceedings of the International Congress on Chemotherapy., Anticancer section 1. Tokyo Press, Tokyo, pp 333–334
3. Konishi M, Sugawara K, Kofu F, Nishiyama Y, Tomita K, Miyaki T, Kawaguchi H (1986) Elsamicins, new antitumor antibiotics related to chartreusin: 1. Production, isolation, characterization and antitumor activity. J Antibiot 39(6): 784–791
4. Konishi M, Oki T (1987) Elsamicin A. Drugs Future 12 (12): 1104–1105
5. Schurig JE, Basler GA, Faulkner HC, Duncan GF, Rose WC, Bradner WT (1986) Experimental antitumor activity of elsamicin, a new antitumor antibiotic. Proc Am Assoc Cancer Res 27: 275
6. Schurig JE, Forenza S, Long BH, Rose WC, Catino JJ, Kamei H, Nishiyama Y, Bradner WH, Casazza AM, Stringfellow DA, Doyle TW (1988) Elsamicin and esperamicin: novel fermentation-derived cytotoxic antitumor agents with unique mechanisms of action. Proc Am Assoc Cancer Res 29: 538–539
7. Sugawara K, Tsunakawa M, Konishi M, Kawaguchi H, Krishnan B, Cunheng H, Clardy J (1987) Elsamicins A and B, new antitumor antibiotics related to chartreusin: 2. Structures of elsamicins A and B. J Org Chem 52: 996–1001

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