ELSEVIER

Journal of Chromatography A, 706 (1995) 451–457

JOURNAL OF
CHROMATOGRAPHY A

Plasma level determination of 1,4-butanedisulphonate by ion chromatography and conductimetric detection

E. Moro*, M. De Angelis, B. Fugazza

Department of Pharmacokinetics, Research and Development, BioResearch SpA, BASF Pharma, 20060 Liscate (MI), Italy

Abstract

A rapid, specific and reproducible liquid chromatographic method was developed for the determination of 1,4-butanedisulphonate in plasma. The method involves protein precipitation with perchloric acid, precipitation of perchlorate ions by addition of potassium carbonate followed by ion chromatography on an ion-exchange column connected with a conductimetric detector. Calibration graphs were linear over the concentration range 2.5–25 μg/ml; the intra-assay precision was $\leq$3.6% and the inter-assay precision was $\leq$5.8%. The analyte was stable in plasma and in perchloric acid at 37°C for 24 h. The assay procedure was applied to monitoring plasma levels in animals receiving chronic intravenous and oral administration of the analyte.

1. Introduction

1,4-Butanedisulphonic acid is the counter ion utilized for the preparation of a stable salt of S-adenosylmethionine, a drug with anticholestatic activity. As this acid is not commonly used for salt preparation, data on its pharmacokinetic properties in different animal species were required. For this purpose we had to develop a suitable assay method for this acid in biological fluids.

Although analytical procedures for determining inorganic anions such as sulphate in biological fluids [1] and in tissue [2] have been described, to date there is no method available for assaying butanedisulphonate and other linear alkylsulphonates in this kind of matrix. The main problems in developing such a method are related to the high polarity of the analyte, which makes both extraction with solvents and the subsequent chromatography and detection difficult.

In this paper, we describe a liquid chromatographic procedure that utilizes recent advances in separation and detection technology such as ion chromatography and conductimetric detection and allows the determination of concentrations of 1,4-butanedisulphonic acid in plasma of 2.5 μg/ml with no interference from endogenous components present in biological samples and good performance in terms of precision, accuracy and specificity.

2. Experimental

2.1. Chemicals and reagents

Standard 1,4-butanedisulphonic acid disodium salt [$NaO_3S(CH_2)_4SO_3Na$] was obtained from BioResearch (BASF-Pharma) with chemical purity $\geq$98%. 1,2-Ethanedisulphonic acid disodium salt, [$NaO_3S(CH_2)_2SO_3Na$], used as an

* Corresponding author.

0021-9673/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(94)01288-1

internal standard, was purchased from Fluka (Buchs, Switzerland).

Standard solutions of 1,4-butanedisulphonic acid disodium salt and of the internal standard in distilled water were stored at 4°C and used for 2 months; their detector response was checked daily. A 0.67 *M* perchloric acid solution was prepared by dilution with distilled water of 70% perchloric acid (Sp.gr. 1.67) of analytical-reagent grade (Merck, Darmstadt, Germany). Solutions of 5 m*M* sodium carbonate, 1.8 m*M* sodium hydrogencarbonate (used for mobile phase preparation), 3 *M* potassium carbonate and 0.014 *M* sulphuric acid were prepared diluting with distilled water of the Baker Analyzed Reagents (J.T. Baker, Deventer, Netherlands).

Water used for preparing all solutions was demineralized, distilled and filtered through a Type GTTP 0.2-μm filter (Millipore, Bedford, MA, USA).

2.2. Liquid chromatography

A DX-100 ion chromatograph (Dionex, Sunnyvale, CA, USA) equipped with a conductivity detector and an AMMS-II anion micro membrane suppressor (Dionex) was used.

The analytical column was a 25 cm × 4 mm I.D. Ion Pac AS9-SC (Dionex) packed with a 13-μm polyethylvinylbenzene–divinylbenzene substrate and an anion-exchange stationary phase protected by an AG9-SC guard column (Dionex).

The mobile phase contained 5 m*M* Na_2CO_3 and 1.8 m*M* $NaHCO_3$ pumped isocratically at room temperature at a flow-rate of 2.0 ml/min. The 0.014 *M* sulphuric acid for the suppression system was used at a flow-rate of 4.0 ml/min.

Samples were injected manually using a Model 7125 injector with a 100-μl loop (Rheodyne, Cotati, CA, USA) or a Model 231 XL automatic sample injector (Gilson, Villiers le Bel, France).

On-line data acquisition and subsequent calculations were performed with a Data Jet Integrator (Thermo Separation Products, Riviera Beach, FL, USA) and a Spectra 386 computer using Winner/386 Autolab software (Thermo Separation Products).

2.3. Sample preparation

Aliquots of 0.2–0.5 ml of plasma samples were mixed with suitable volumes (8–20 μl) of a 1 mg/ml solution of 1,2-ethanedisulphonic acid (internal standard) in water in order to obtain concentrations of 40 μg/ml plasma and with two volumes of 0.67 *M* perchloric acid. The mixture was vortex mixed and centrifuged at 6000 *g* at 0°C for 15 min.

A 0.5-ml volume of the clear, upper phase were measured into a test-tube and mixed with 0.1 ml of 3 *M* K_2CO_3 solution at 0°C in order to precipitate the main amount of ClO_4^- ions as the potassium salt. After centrifugation at 6000 *g* at 0–4°C for 15 min, 0.1 ml of the clear supernatant phase was transferred into another test-tube and diluted to 2.0 ml by addition of distilled water. Aliquots of 0.1 ml of this solution were injected into the chromatographic column.

2.4. Calibration graphs

Calibration graphs were constructed by transferring aliquots of the standard solutions of 1,4-butanedisulphonic acid (SD4) and internal standard into blank plasma to give final concentrations of 2.5, 5, 7.5, 12.5, 17.5 and 25 μg/ml of SD4 and a constant concentration of internal standard (40 μg/ml). These calibration standards were extracted as described above. The calibration graphs were obtained by plotting the peak-area ratio of SD4 to the internal standard versus the concentration of SD4 by least-squares analysis. For each calibration graph the correlation coefficient, *r*, the intercept and the slope were calculated. This procedure was repeated six times.

3. Results and discussion

3.1. Sample preparation and chromatographic system.

Owing to the high polarity characteristics of 1,4-butanedisulphonate, its extraction from biological matrices using organic solvents was not

practicable. We obtained good results by applying a method based on protein precipitation followed by direct injection into the chromatographic column of the clear supernatant obtained after centrifugation.

Addition of perchloric acid to plasma was optimum for protein precipitation and gave the clearest supernatant with the advantage of avoiding column contamination by plasma constituents and subsequent decrease in column efficiency. The amount and concentration of perchloric acid had to be optimized to minimize the dilution factor and the interference of the broad peak of perchlorate with a retention time of about 15 min that could overlap the internal standard peak.

The addition of K_2CO_3 solution to the clear supernatant phase precipitates the main amount of perchlorate but the aliquot remaining in solution (ca. 10%) represents a limiting factor of the analytical procedure in terms of sensitivity. By applying the method without addition of an internal standard it was possible to increase the sensitivity limit up to 1 μg/ml with minor consequences in terms of accuracy. The validation of this procedure is in progress.

By replacing the precolumn every 500 injections, the analytical column can be used for up to about 1500 analyses of biological samples. During routine analysis, the mobile phase could be slightly modified in relation to column efficiency: normally the concentration of $NaHCO_3$ solution was maintained constant with minor changes in Na_2CO_3 solution concentrations (between 5 and 7.5 m*M*).

In order to maintain good performance for a long time, the analytical column was washed with 200 ml of a CH_3CN–1 *M* NaCl (80:20, v/v) mixture after every 2 months of continuous use.

The retention times under the above chromatographic conditions for 1,4-butanedisulphonic acid and the internal standard were 5.0 and 10.0 min, respectively.

3.2. Limit of quantification

The limit of quantification of 1,4-butanedisulphonic acid in plasma samples was 2.5 μg/ml using a 0.2-ml specimen; this concentration represents the lowest level of the calibration graphs. The inter-assay relative standard deviation (R.S.D.) at this concentration was 5.8% ($n = 6$).

3.3. Linearity

The calibration graphs were linear over the concentration range 2.5–25 μg/ml with correlation coefficients $r > 0.998$ and minimal intercepts (0.0057; R.S.D. = 54.5%, $n = 6$). The mean value of the slope was 0.021 with a day-to-day R.S.D. of 2% ($n = 6$) (Table 1).

3.4. Reproducibility and accuracy

The precision (defined as the R.S.D. of replicate analyses) and the accuracy (defined as the deviation between the found and added concentrations) of the analytical procedure were evaluated on spiked samples at concentrations of 3.75 and 20 μg/ml. The intra-assay reproducibility was determined by analysing five specimens for each concentration of spiked plasma samples on the same day. The inter-assay reproducibility was obtained by analysing two specimens of spiked plasma samples for the two concentrations on six different days. The concentrations of SD4 in these spiked samples were determined by using the linear regression line of peak-area ratios versus concentration of calibration graphs constructed as described above. The results obtained are reported in Tables 2 and 3.

The R.S.D.s were 3.6% (3.75 μg/ml) and 2.9% (20 μg/ml) for the intra-day reproducibility ($n = 5$) and 2.28% (3.75 μg/ml) and 2.31% (20 μg/ml) for the inter-day reproducibility ($n = 12$). The accuracy, calculated for the same samples, was 99.9% and 102.3% for intra-day and 99.9% and 100.3% for inter-day assay, respectively.

3.5. Specificity

Fig. 1 shows typical chromatograms of (a) an extract of blank rat plasma, (b) a plasma standard spiked with SD4 at 5 μg/ml and internal standard and (c) a plasma sample of rat receiving

Table 1
Reproducibility of calibration graphs

Standard concentration (μg/ml)	Peak-area ratio (SD4 to internal standard)		
	Mean ($n = 6$)	Range	R.S.D. (%)
2.5	0.046	0.044–0.050	5.8
5.0	0.101	0.096–0.108	4.5
7.5	0.154	0.143–0.161	4.0
12.5	0.255	0.242–0.261	2.9
17.5	0.362	0.333–0.375	4.2
25.0	0.522	0.515–0.526	0.7
Calibration graph Parameter	Mean ($n = 6$)	Range	R.S.D. (%)
Slope	0.021	0.020–0.021	2.0
y-Intercept	−0.00574	−0.00085 to −0.0028	54.5
Correlation coefficient	0.9988	0.998–0.999	0.041

oral SD4 chronically at a dose of 225 mg/kg per day during a long-term toxicity study.

Blank plasma samples from humans, mouse and dog analysed by this method were found to be, as for rat plasma, free from endogenous contaminants at the retention times corresponding to SD4 and the internal standard. The chromatographic behaviour of the drug and possible impurities and metabolites was also evaluated to determine their potential for interference in the assay. The possible interference due to excipients administered with the drug were checked by analysing plasma samples from humans and animals treated with placebo preparations. The resulting chromatograms from these tests revealed no interfering peaks.

The high specificity of the method is enhanced through the use of a conductimetric detector, which is very sensitive for ionic substances, giving no signal for other constituents in the biological matrices.

3.6. Chromatographic system suitability test

Column efficiency

This was evaluated as the number of theoretical plates of the column calculated by using Labnet software (Thermo Separation Products)

Table 2
Intra-assay precision and accuracy

Nominal concentration (μg/ml)	Measured concentrations (μg/ml) and accuracy (%)						
	1	2	3	4	5	Mean	R.S.D. (%)
3.75	3.58	3.63	3.88	3.87	3.76	3.74	3.6
Accuracy (%)[a]	95.5	96.8	103.5	103.2	100.3	99.86	—
20.0	20.6	19.9	21.4	20.0	20.4	20.5	2.9
Accuracy (%)[a]	103.0	99.5	107.0	100.0	102.0	102.3	—

Rat plasma samples were prepared to contain SD4 at two concentrations and five replicates of each sample were analysed on the same day.

[a] Accuracy is expressed as (measured concentration/nominal concentration) · 100.

Table 3
Inter-assay precision and accuracy

Day	Nominal concentration (μg/ml)					
	3.75			20.0		
	Measured concentration (μg/ml)	Accuracy (%)[a]	R.S.D. (%)	Measured concentration (μg/ml)	Accuracy (%)[a]	R.S.D. (%)
1	3.72, 3.79	99.2, 101.1	1.3	21.2, 19.8	106.0, 99.0	4.8
2	3.55, 3.80	94.7, 101.3	4.8	20.2, 20.7	101.0, 103.5	1.7
3	3.75, 3.79	100.0, 101.1	0.7	20.0, 19.9	100.0, 99.5	0.3
4	3.76, 3.73	100.3, 99.5	0.6	20.1, 19.6	100.5, 98.0	1.8
5	3.64, 3.85	97.1, 102.7	4.0	19.8, 19.7	99.0, 98.5	0.3
6	3.72, 3.84	99.2, 102.7	2.2	19.7, 20.1	98.5, 100.5	1.4
Overall mean (n = 12)	3.74	99.9	2.28	20.1	100.3	2.31

Rat plasma samples were prepared to contain SD4 at two concentrations. Two aliquots of each concentration were analysed on six different days using a separate calibration graph for each day.
[a] Accuracy is expressed as (measured concentration/nominal concentration) · 100.

by the equation $N = 5.54\ (t_R/W)^2$, where t_R is the retention time of the SD4 peak and W its width at half-height. The calculated value of N was 2400.

Peak symmetry

Using the same software, the tailing factor of the SD4 peak was found to be 0.95. A similar value was obtained for the internal standard peak (0.96).

3.7. Stability

Rat plasma samples were spiked with two concentrations of 1,4-butanedisulphonic acid (2.5 and 8 μg/ml). Two samples for each concentration were immediately analysed as described above and used as reference samples ($t = 0$).

Two samples for each concentration were stored in a water-bath at 37°C for 24 h before analysis for testing the stability in plasma. To two samples for each concentration were added twice their volume of 0.67 M perchloric acid, then the mixture was centrifuged and 0.5 ml of the supernatant was stored in a water-bath at 37°C for 24 h before analysis for testing the stability in deproteinized samples by comparison with results obtained from two samples for each concentration analysed immediately after centrifugation.

The results obtained are reported in Table 4 and indicate that the analyte is stable for 24 h at 37°C both in rat plasma and in the dilute solution of perchloric acid used for the deproteinization procedure.

3.8. Application to biological samples

The method was successfully applied to the analysis of several plasma samples from humans and animals from pharmacokinetic and toxicology studies following oral and intravenous administration of SD4 alone and as the counter ion of S-adenosyl-L-methionine.

4. Conclusions

The described method is suitable for the determination of 1,4-butanedisulphonate ion in plasma samples deproteinized with perchloric acid. The results of the validation tests indicated that with this method it is possible to measure with good precision, accuracy and specificity up to 2.5 μg/ml of this analyte in plasma samples.

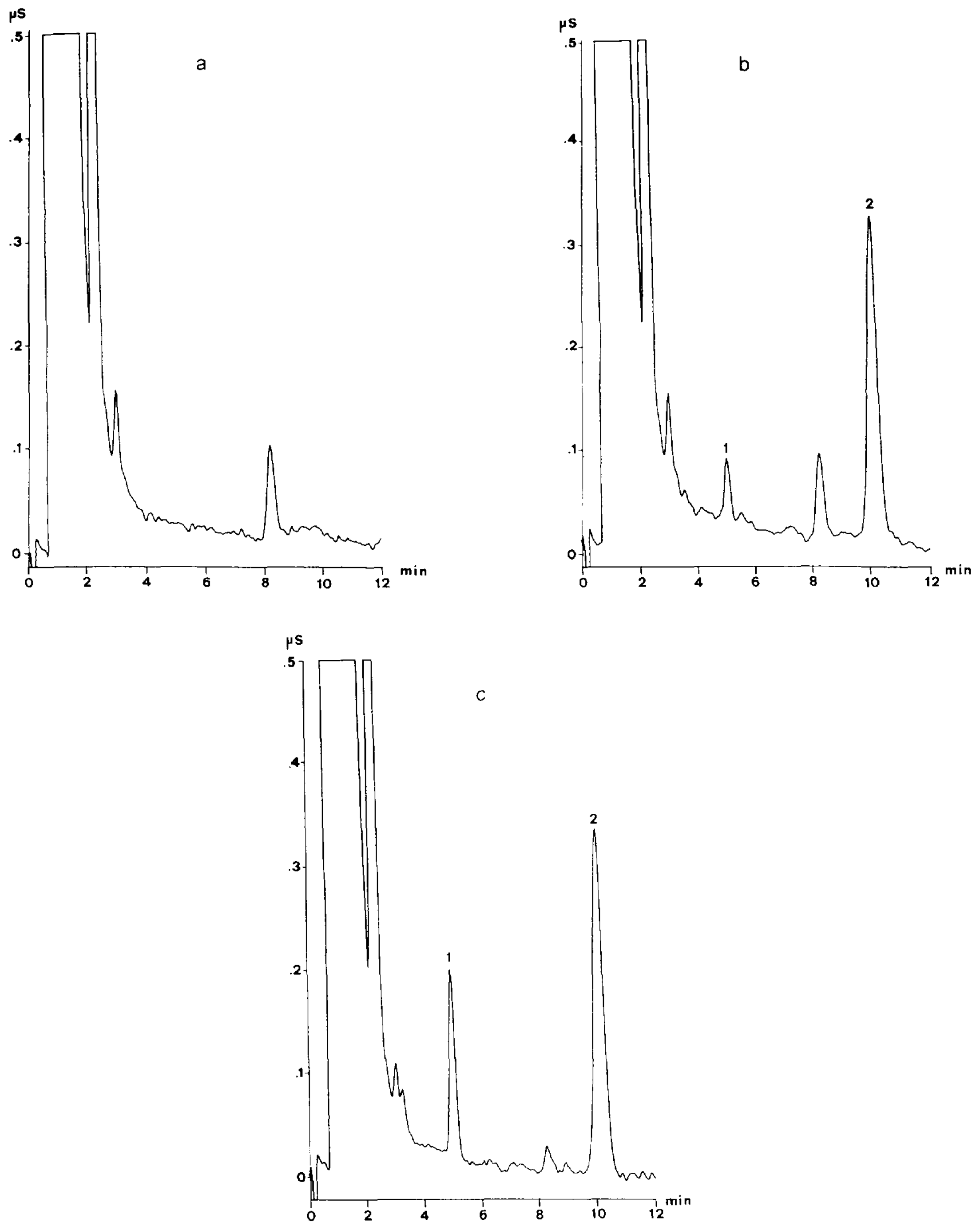

Fig. 1. Chromatograms of extracts from (a) blank rat plasma, (b) plasma spiked with 5μg/ml of 1,4-butanedisulphonic acid (SD4) and (c) plasma sample from a rat receiving oral SD4 chronically. Peaks: 1 = SD4; 2 = internal standard.

Table 4
Stability of SD4 in plasma and deproteinized samples

Concentration (μg/ml)	Sample and conditions	Mean recovery ($n=2$) (%)	Sample and conditions	Mean recovery ($n=2$) (%)
2.5	Rat plasma,	94.7	Rat plasma,	103.0
8.0	freshly prepared	95.1	24 h at 37°C	101.3
2.5	Rat plasma deproteinized with $HClO_4$,	100.4	Rat plasma deproteinized with $HClO_4$,	102.6
8.0	freshly prepared	105.0	24 h at 37°C	109.4

This procedure can be applied to the analysis of deproteinized plasma samples from rat, mouse, dog and man and in urine after the appropriate validation tests for each of the different biological matrices.

References

[1] D.E.C. Cole and C.R. Scriver, *J. Chromatogr.*, 225 (1981) 359–367.

[2] P. Rozman, H.J. Kim, C. Madhu and C.D. Klaassen, *J. Chromatogr.*, 574 (1992) 146–149.