ELSEVIER

Journal of Chromatography B, 712 (1998) 237–241

JOURNAL OF CHROMATOGRAPHY B

High-performance liquid chromatographic assay for a new fasciolicide agent, αBIOF10, in biological fluids

Lauro Misael del Rivero, Helgi Jung*, Rafael Castillo, Alicia Hernández-Campos

Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., Mexico

Received 29 September 1997; received in revised form 9 March 1998; accepted 17 March 1998

Abstract

This paper describes a high-performance liquid chromatographic method with ultraviolet absorbance detection at 304 nm for the determination of 6-chloro-5-(1-naphthyloxy)-2-methylthio benzimidazole (αBIOF10) — a new fasciolicide agent — and its sulphoxide (SOαBIOF10), in plasma and urine. It requires 2 ml of biological fluid, an extraction using Sep-Pak cartridges, and methanol for drug elution. Analysis is performed on a μBondapak C_{18} (10 μm) column, using methanol–acetonitrile–water (40:30:30, v/v) as the mobile phase. Results showed that the assay is sensitive: 7.2 ng/ml for αBIOF10 and SOαBIOF10 in plasma and 3.6 ng/ml for both compounds in urine. The response was linear between 0.195 and 12.5 μg/ml. Maximum intra-day coefficient of variation was 5.3%. Recovery obtained was 97.8% for both αBIOF10 and SOαBIOF10. In urine, recovery was 99.6% and 93.1% for αBIOF10 and SOαBIOF10 respectively. The method was used to perform a preliminary pharmacokinetic study in two sheep and was found to be satisfactory.

Keywords: 6-Chloro-5-(1-naphthyloxy)-2-methylthio benzimidazole (αBIOF10)

1. Introduction

Searching for new fasciolicide agents, a new benzimidazole derivative, 6-chloro-5-(1-naphthyloxy)-2-methylthio benzimidazole (αBIOF10), was recently synthesized by our group at the National University of Mexico. This compound has shown a potent fasciolicide activity in vitro and in vivo in sheep [1].

In order to determine the pharmacokinetic parameters of αBIOF10, a sensitive and specific method of assay was needed in order to measure the drug levels in plasma and urine. This paper describes a rapid, specific, reliable, and sensitive analytical method based on reversed-phase liquid chromatography for the quantitation of αBIOF10 and its metabolite in plasma and urine of sheep. The method was validated according to procedures and acceptance criteria based on USP XXIII guidelines [2] and recommendations of Shah et al. [3], Bresolle et al. [4] and Braggio et al. [5].

2. Experimental

2.1. Materials and reagents

αBIOF10 and αBIOF10 sulphoxide (SOαBIOF10) were synthesised at the Department of Pharmacy, National University of Mexico

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00145-5

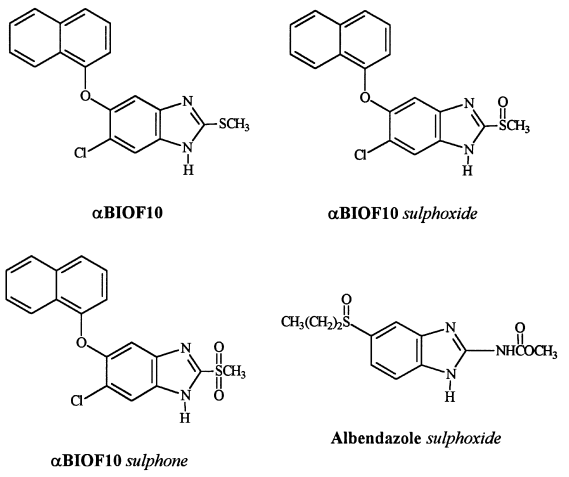

Fig. 1. Structural formulae of αBIOF10, SOαBIOF10, SO_2αBIOF10 and internal standard (albendazole sulphoxide) used for the analytical method.

(México). Fig. 1 shows the chemical structures of these compounds. The internal standard, albendazole sulphoxide, was obtained from SmithKline Beecham (Philadelphia, PA, USA). Methanol and acetonitrile were of chromatographic grade (Mallinckrodt Specialty Chemicals, KY, USA). All other reagents were analytical grade. Pooled plasma and urine samples from sheep were used for the validation of the method.

2.2. Chromatographic conditions

The instrument used was a high-performance liquid chromatograph equipped with a Shimadzu pump Model LC10AS (Kyoto, Japan), a Shimadzu variable-wavelength UV absorbance detector (Model SPD10AV), a Rheodyne loading valve (Model 7125) fitted with a 20-μl sample loop (Cotati, CA, USA), a Shimadzu integrator Model C-R501 (chart speed, 2 mm/min.), a stainless steel analytical column of 300×3.9 mm I.D. (Waters Associates, Milford, MA, USA) packed with μBondapak C_{18} (particle size, 10 μm) with methanol–acetonitrile–water (40:30:30, v/v) as the mobile phase. The column was kept at room temperature (20–24°C), and the flow-rate was kept constant at 1 ml/min. The absorbance at 304 nm was recorded at a sensitivity of 0.1 a.u.f.s.

2.3. Sample preparation

To 2 ml of plasma or urine samples, 150 μl of methanolic solution of the internal standard, albendazole sulphoxide (7.5 μg/ml) were added, spiked with αBIOF10 and SOαBIOF10 in the range of 0.19 to 12.5 μg/ml, and extracted by passing through a Sep-Pak C_{18} cartridge. The cartridge was previously conditioned by flushing with 5 ml of methanol followed by 4 ml of water. After spiked samples had been passed through the cartridge it was washed with 20 ml of water. The compounds were then eluted with 3 ml of methanol. The methanolic fractions were evaporated to dryness in a water bath at 40°C under nitrogen and the residues were redissolved in 0.5 ml of methanol. Aliquots of 20 μl were injected into the HPLC system.

3. Results and discussion

Chromatograms of plasma and urine samples are shown in Fig. 2. Retention times for internal standard, SOαBIOF10 and αBIOF10 were 4.9, 6.8 and 8.8 min respectively, in plasma and in urine. The capacity factors in plasma and urine were 2.3, 3.3 and 6.1 for internal standard, SOαBIOF10 and αBIOF10. No interfering peaks at the retention times of internal standard, SOαBIOF10 or αBIOF10 were detected (Fig. 2). A linear relationship ($r=0.999$) was found when the ratio of the peak-height of SOαBIOF10 and αBIOF10 in plasma to the peak height of the internal standard were plotted against various concentrations ranging from 0.195 to 12.5 μg/ml. The same relationship ($r=0.999$) was obtained for urine.

In plasma samples, the intra-assay reproducibility was determined from calibration curves prepared on the same day in triplicate using the different stock solutions. The corresponding mean±S.D. coefficient of the linear regression analysis was $0.999\pm7.353\cdot10^{-3}$ (C.V.=0.9%). For the SOαBIOF10 the corresponding mean±S.D. coefficient of the linear regression analysis was $0.999\pm7.353\cdot10^{-3}$ (C.V.=0.6%). The results from the urine analysis showed that the corresponding mean±S.D. coefficient of the linear regression analysis was $0.999\pm4.125\cdot10^{-3}$ (C.V.=0.8%), whereas for SOαBIOF10 the corresponding

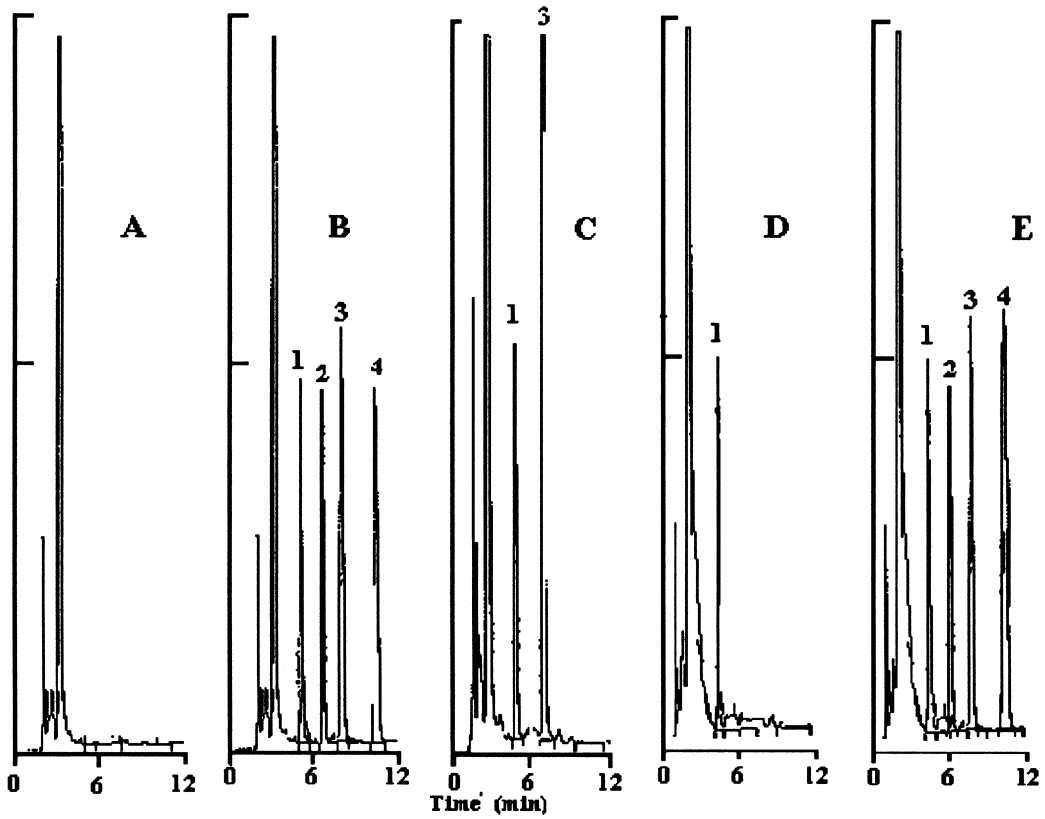

Fig. 2. Chromatograms of (A) blank plasma, (B) plasma spiked with 1.56 μg/ml of αBIOF10, SOαBIOF10 and SO_2αBIOF10, (C) plasma sample from a sheep receiving 12 mg/kg of αBIOF10, (D) blank urine and (E) urine spiked with 1.56 μg/ml of αBIOF10, SOαBIOF10 and SO_2αBIOF10. The peaks are (1) internal standard (2) SO_2αBIOF10 (3) SOαBIOF10 (4) αBIOF12.

mean±S.D. coefficient of the linear regression analysis was $0.999 \pm 2.346 \cdot 10^{-3}$ (C.V.=0.7%).

Intra-day and inter-day (2 days) precision and accuracy of the method, assessed by analyzing quality control samples, are given in Table 1. It can be seen that the maximum within-day coefficient of variation was 5.3% at 0.195 μg/ml for plasma and 4.9% at 1.56 μg/ml for urine samples.

The recoveries of αBIOF10 and SOαBIOF10 were determined by comparing peak heights from plasma or urine, spiked with known amounts of the analyte (0.19, 0.39, 0.78, 1.56, 3.12, 6.25 and 12.5 μg/ml) using the extraction procedure versus peak height of the same concentrations prepared in methanol and injected directly onto the analytical column. Each sample was determined in triplicate. The mean recovery of αBIOF10 in plasma and urine averaged 97.2±2.4% (n=7) and 93.1±2.3% (n=7), and for SOαBIOF10 values were 97.8±3.7% (n=7) and 99.6 0±2.3% (n=7), respectively.

The limit of quantitation (LOQ) was determined from the peak and the standard deviation of the noise level, S/N. The LOQ was defined as the sample concentration resulting in a peak height of 10 times S/N, Value obtained for αBIOF10 and SOαBIOF10 in plasma was 24 ng/ml. When urine was analyzed, limit of quantitation was 12 ng/ml for both compounds.

The limit of detection (LOD) was defined as the sample concentration resulting in a peak height of 3 times S/N. Results in plasma showed a value of 7.2 ng/ml for αBIOF10 and SOαBIOF10, in urine the LOD value was 3.6 ng/ml for both compounds.

Stock solutions of αBIOF10 and SOαBIOF10 (500 μg/ml), and internal standard in methanol (150 μg/ml) were stable for at least 3 and 6 months,

Table 1
Accuracy and precision of the HPLC method in plasma and urine samples

Theoretical concentration (μg/ml)	Experimental concentration (mean)(μg/ml)		C.V. (%)		Recovery (%)	
	SOαBIOF10	αBIOF10	SOαBIOF10	αBIOF10	SOαBIOF10	αBIOF10
Plasma						
Intra-day (n=3)						
0.19	0.178	0.188	5.3	4.3	91.2	96.1
0.39	0.377	0.389	2.4	2.7	96.5	99.7
0.78	0.773	0.738	1.7	0.8	99.0	94.5
1.56	1.534	1.497	3.3	1.9	98.2	95.8
3.12	3.222	2.987	1.4	2.3	103.1	95.6
6.25	6.05	6.30	1.0	2.6	96.8	100.8
12.5	12.45	12.23	2.9	1.8	99.6	97.8
Inter-day (n=6) in 2 days						
0.19	0.187	0.189	4.5	4.7	95.6	96.9
0.39	0.381	0.360	4.5	4.1	97.5	92.3
0.78	0.773	0.725	2.2	4.8	99.0	92.8
1.56	1.533	1.503	2.6	3.2	98.1	96.2
3.12	2.925	2.862	1.8	2.5	93.6	91.6
6.25	6.050	6.043	3.1	2.8	96.8	96.7
12.5	12.51	12.33	2.7	2.2	100.1	98.6
Urine						
Intra-day (n=3)						
0.19	0.1937	0.188	3.1	3.5	99.2	96.4
0.39	0.3949	0.355	3.4	1.5	101.1	90.9
0.78	0.7617	0.740	2.6	3.9	97.5	94.7
1.56	1.5141	1.469	2.9	3.6	96.9	94.0
3.12	3.063	2.819	1.4	1.7	98.0	90.2
6.25	6.356	5.816	1.6	1.2	101.7	93.1
12.5	12.85	11.54	1.7	1.6	102.8	92.3
Inter-day (n=6) in 2 days						
0.19	0.175	0.172	3.7	4.4	89.8	88.1
0.39	0.338	0.355	2.8	1.7	86.6	91.0
0.78	0.699	0.737	5.3	3.0	89.5	94.4
1.56	1.361	1.492	4.9	3.5	87.1	95.5
3.12	2.919	3.122	1.6	2.8	93.4	99.9
6.25	6.344	6.444	1.3	1.5	101.5	103.1
12.5	12.625	11.95	1.5	2.7	101.0	95.6

respectively. For the stability study, control sheep plasma and urine, and plasma and urine standards were spiked with 0.195, 1.562 and 12.5 μg/ml of αBIOF10 and SOαBIOF10. Each concentration determination was performed in triplicate.

A short term stability test performed at room temperature showed that the samples spiked with αBIOF10 and SOαBIOF10 at concentrations of 1.562 and 12.5 μg/ml of αBIOF10 and SOαBIOF10 were stable for 6 h (recoveries 92–105%, 81.5–88.6% respectively). Daylight did not modify the stability of either compound.

The long-term stability of the analytes in plasma and urine from sheep stored at −20°C was determined by periodic analysis over a span of 3 months. Samples were analyzed immediately after preparation and after storage. The results indicated that αBIOF10 and SOαBIOF10 were stable for 1.5 months, with an average recovery between 95 and 98%, respectively (Table 2).

The freeze–thaw stability was also determined. Spiked biological fluids were analyzed immediately after preparation, and on a daily basis, after repeated freezing–thawing cycles at −20°C on 3 consecutive

days. At least three freeze–thaw cycles can be tolerated without a loss higher than 10%.

Although the method was not validated for αBIOF10 sulphone, we found that our method is able to detect this compound. Fig. 2B and Fig. 2E show typical chromatograms of plasma and urine spiked with albendazole sulphoxide, αBIOF10 and αBIOF10 sulphoxide and sulphone. It can be seen that the four analytes were well separated eluting at 4.9, 5.3, 6.8 and 8.8 min.

Following the optimization of the chromatographic conditions and validation of the assay, the method was used in a preliminary pharmacokinetic study in two sheep that received a single oral dose of 12 mg/kg. Blood samples were taken into heparinized tubes from 0 to 168 h, plasma was separated by centrifugation and stored at −20°C until assayed. Fig. 2C shows a typical chromatogram obtained. No chromatographic interferences from any endogenous compounds were found. In this study αBIOF10 could not be detected at any of the sampling times, only the SOαBIOF10 could be determined. These results are in accordance with other benzimidazoles, which are extensively metabolized in different species and in humans [6–8]. In our study also αBIOF10 sulphone could not be detected. Fig. 3 shows the plasma concentration–time profile of αBIOF10 sulphoxide after the administration 12 mg/kg αBIOF10. Maximum plasma concentration in plasma was 8.32 μg/ml at 10 h. The half life obtained was 20.5 h. When comparing our results with those obtained with triclabendazole sulphoxide [9] the C_{max} for this drug 16 μg/ml with a halflife of 30 h. Although this study was performed only in two sheep, the long halflife obtained with our compound combined with its efficacy suggests that αBIOF10 could be a new promising fasciolicide agent.

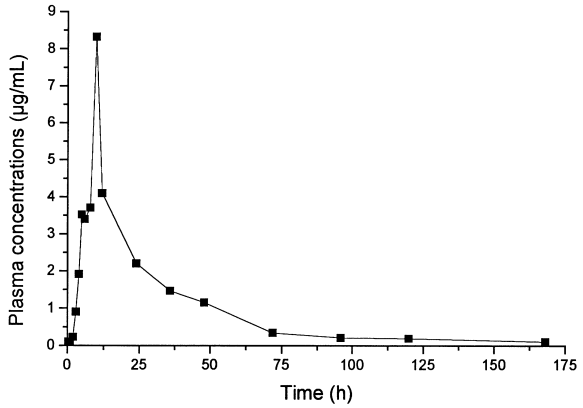

Fig. 3. Mean plasma concentration–time curve following oral administration of αBIOF10 (12 mg/kg) in two sheep.

4. Conclusions

The developed method proved to be useful and reliable for the determination of αBIOF10 and SOαBIOF10 in biological fluids. The sample pretreatment procedure, involving a direct extraction with Sep-Pak cartridge, is simple and rapid. The method, validated for concentrations ranging from 0.195 to 12.5 μg/ml, had good repeatability and accuracy and low limits of quantitation and detection. This method is sufficiently sensitive to perform pharmacokinetic studies.

The HPLC developed method is simple, sensitive, and fast (36 samples can be analyzed in 1 day) so it is suitable for the study of the biopharmaceutics and pharmacokinetics of αBIOF10.

Acknowledgements

This project was supported by DGAPA (UNAM) number IN214496.

References

[1] O.F. Ibarra, R.Y. Vera, E. García, A. Hernández, R. Castillo, Eficacia Fasciolicida de un Compuesto Experimental en Ovinos, XII Congreso Latinoamericano de Parasitología, Santiago de Chile, October 1995, p. 42.
[2] US Pharmacopoeia XXIII, The United States Pharmacopeial Convention, Rockville, MD, 1994, p. 1929.
[3] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[4] F. Bresolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[5] S. Braggio, R.J. Barnaby, P. Grossi, M. Cugola, J. Pharm. Biomed. Anal. 14 (1996) 375.
[6] L.D.B. Kinabo, J.A. Bogan, J. Vet. Pharmacol. Therap. 11 (1988) 254.
[7] S.E. Mariner, J.A. Bogan, Am. J. Vet. Res. 42 (1981) 1143.
[8] S.E. Mariner, D.L. Morris, B. Dickson, J.A. Bogan, Eur. J. Clin. Pharmacol. 30 (1986) 705.
[9] M. Alvinerie, P. Galtier, J. Chromatogr. 374 (1986) 409–414.