Journal of Chromatography, 593 (1992) 73–78
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2432

Determination of Zy 17617B in plasma by solid-phase extraction and liquid chromatography with automated pre-column exchange

D. Chollet* and M. Salanon

Zyma S.A., Department of Toxicology and Pharmacokinetics, Nyon (Switzerland)

ABSTRACT

An automated chromatographic system, combining solid-phase extraction and automated pre-column exchange, is described for the routine determination of Zy 17617B at the pmol/ml level in human plasma. The sample extraction and elution onto the analytical column were performed automatically and concomitantly using a conventional liquid chromatographic apparatus equipped with a Merck OSP-2 on-line sample preparator. Validation data demonstrate the reliability of the method.

INTRODUCTION

Zy 17617B, 1,3-dihydro-1-{1-[(4-methyl-4H,6H-pyrrolo[1,2*a*][4,1]benzoxazepin-4-yl)methyl]-4-piperidinyl}-2H-benzimidazol-2-one maleate (Fig. 1), is a pharmaceutical product with antidiarrheal properties [1]. A sensitive assay is required for the determination of pharmacokinetic data after oral intake of this drug.

Solid-phase extraction of drugs from plasma, via disposable extraction pre-columns or by column switching, is being increasingly used in combination with liquid chromatography (LC) as an alternative to time-consuming liquid–liquid extraction. Semi-automated or fully automated systems have been introduced for off-line analysis [2–4]; however, very few of them allow complete on-line analysis with automated pre-column exchange [5].

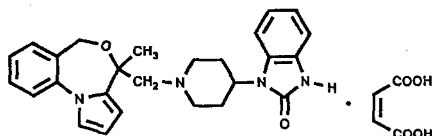

Fig. 1. Molecular structure of Zy 17617B; mol.wt. 544.61.

In this study, a system is described for the routine quantification of Zy 17617B in human plasma. Both sample extraction, carried out using a new pre-column, and elution onto the analytical column were performed automatically and concomitantly using a conventional LC apparatus equipped with an OSP-2 on-line sample preparator from Merck.

EXPERIMENTAL

Materials

Anhydrous sodium dihydrogenphosphate of Suprapur grade was from Merck (Darmstadt, Germany). Orthophosphoric acid (85%, w/v), sodium hydroxide and tetrahydrofuran (THF) were of analytical grade and were also from Merck. THF was freshly distilled. Methanol and acetonitrile (high-performance liquid chromatography grade) were purchase from Mächler (Basle, Switzerland). Water was bidistilled.

Zy 17617B was from Ciba-Geigy (Summit, NJ, USA). A typical reference stock solution was prepared by dissolving 1.14 mg of Zy 17617B in 5 ml of acetonitrile and diluting to 100 ml with water. Di-

0021-9673/92/$05.00 © 1992 Elsevier Science Publishers B.V. All rights reserved

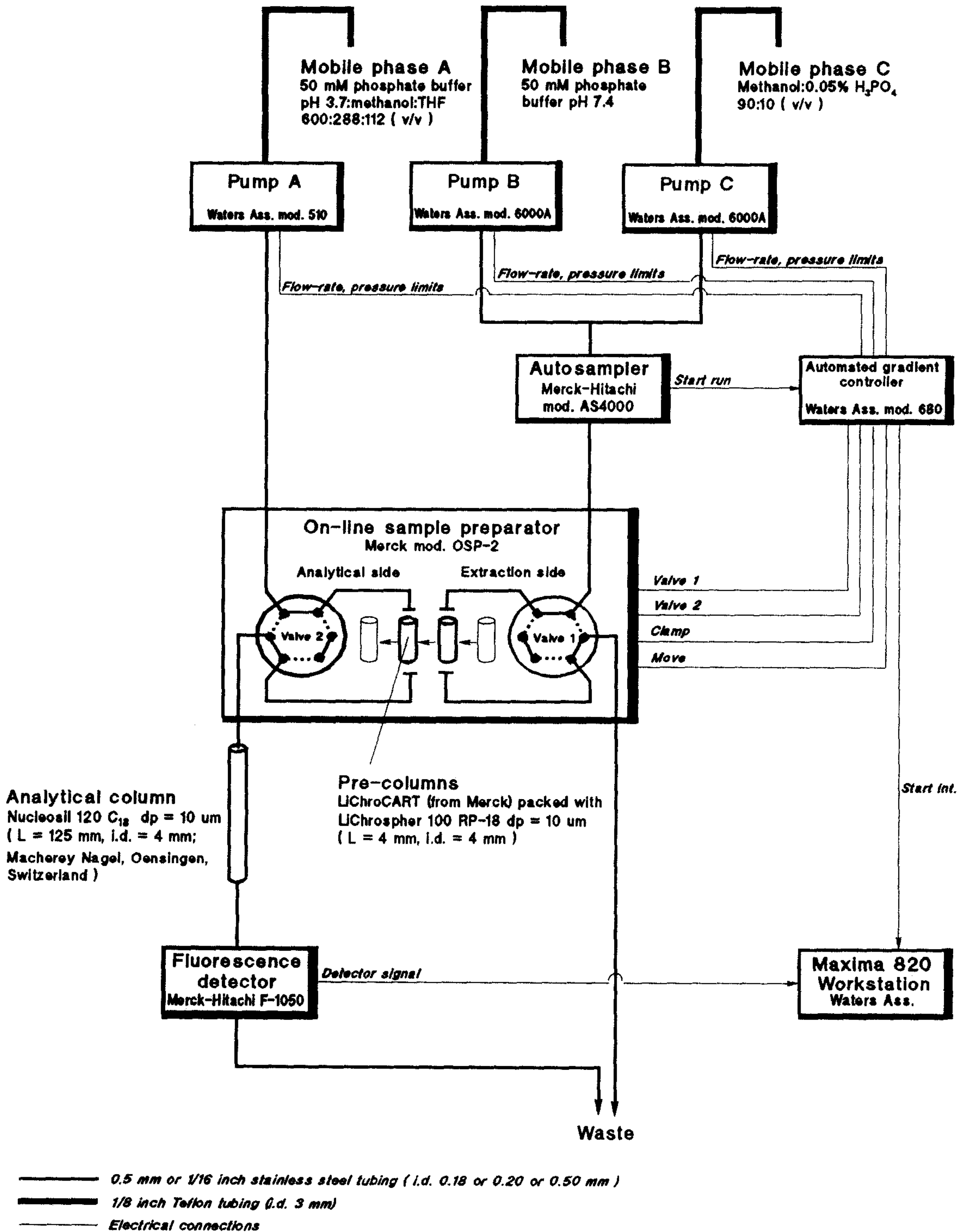

Fig. 2. Scheme of the chromatographic system.

luted solutions were obtained by successive dilutions with water. The reference solutions were stored at 4°C.

Apparatus

The chromatographic system is described in Fig. 2. The autosampler was equipped with a 400-μl fixed loop and a temperature-controlled rack set to 10°C. The mobile phases are also described in Fig. 2.

Method

Sample extraction was performed on LiChroCART pre-columns (4 mm × 4.0 mm I.D.; from

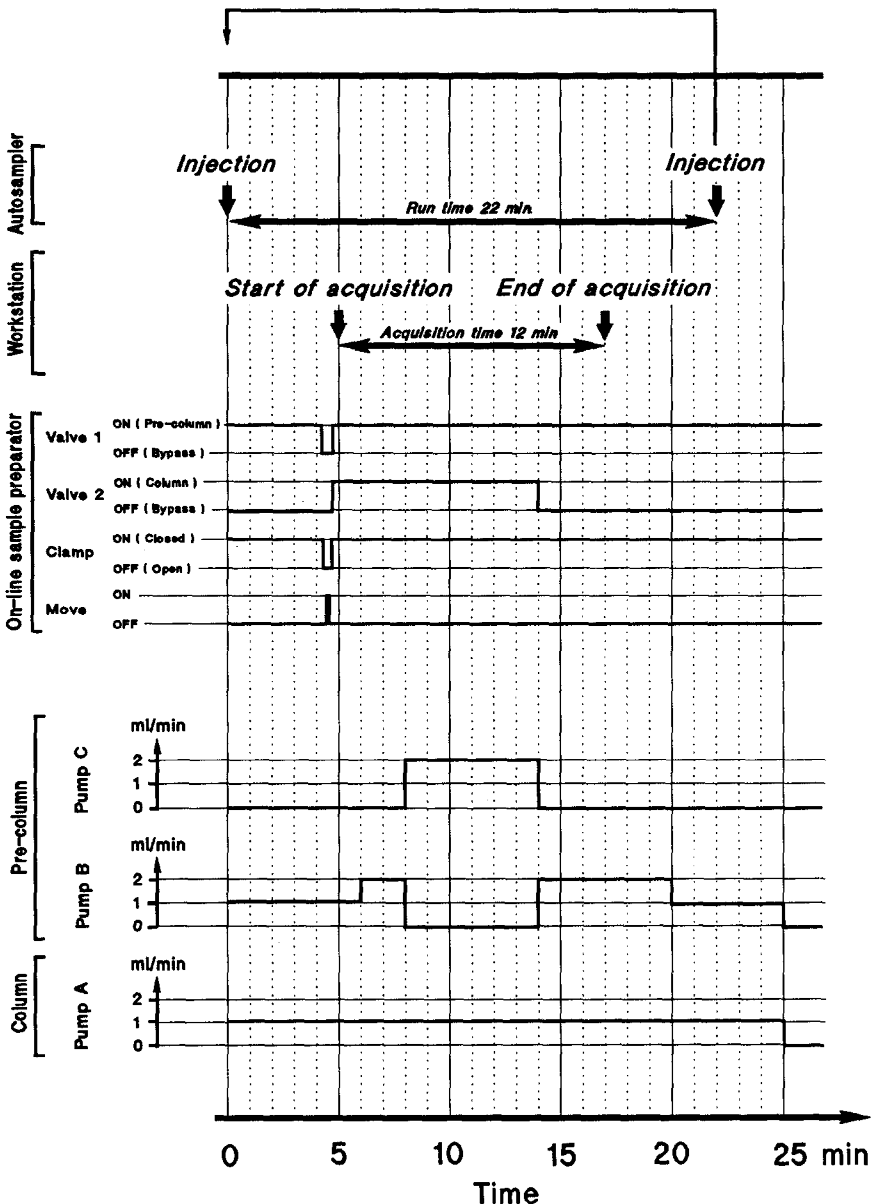

Fig. 3. Experimental conditions.

Merck), which were packed with LiChrospher 100 RP-18 (particle diameter, d_p = 10 μm). These were conditioned following the instructions in Figs. 2 and 3. Concomitantly, the analytical column (Nucleosil 120 C_{18}, d_p = 5 μm, 125 mm × 4.0 mm I.D.) was eluted isocratically using mobile phase A (Fig. 2). The flow-rates were set as shown in Fig. 3. Detection was performed by spectrofluorimetry at an excitation wavelength of 285 nm and an emission wavelength of 315 nm.

Sample preparation

An aliquot of 1.0 ml of thawed plasma was diluted with 1.0 ml of mobile phase B (Fig. 2) in a glass tube, vortexed for 1 min and filtered on a single-use 0.22-μm filter unit (Model Millex-GS, Millipore). The resulting solution was then placed in the autosampler rack for analysis (injection of 400 μl).

Standard preparation

Standard plasma samples were prepared by diluting drug-free plasma with reference solutions followed by further dilution with mobile phase B.

Calibration and quantification

Calibration was performed by linear regression analysis of the detector response over the concentration range 10–84 pmol/ml. The concentration of Zy 17617B in each sample was quantified using the calibration curves.

RESULTS AND DISCUSSION

Zy 17617B was extracted from the plasma by solid-phase extraction by means of an OSP-2 on line sample preparator. This device allowed the on-line extraction of a sample whilst another was being eluted onto the analytical column in a continuous automatic fashion (Fig. 2). A new or reconditioned pre-column was used for each analysis. Typical chromatograms are given in Fig. 4.

Solid-phase extraction

Solid-phase extraction of Zy 17617B was performed on short C_{18} reversed-phase pre-columns (4 mm × 4 mm I.D., d_p = 10 μm) using phosphate buffer pH 7.4 (50 mM) as the mobile phase.

The total recovery of the analysis was found to be 81.5 ± 1.4% (n = 3) for a plasma standard of 41.9 pmol/ml. Zy 17617B was strongly adsorbed onto plastic or glassware. In a set of experiments, the volume fraction of plasma in a standard sample was increased from 0.00 to 1.00. As shown in Fig. 5, the increase in the level of plasma proteins reduced this

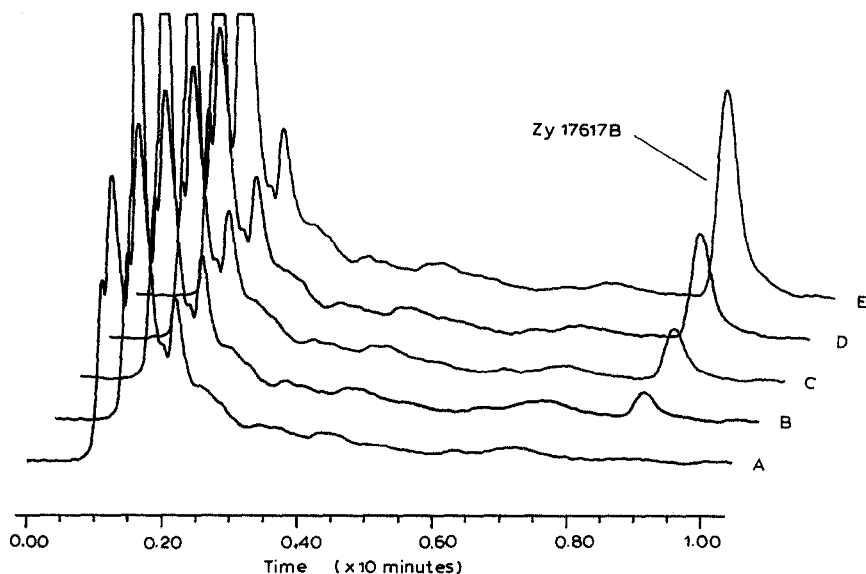

Fig. 4. Typical chromatograms of plasma standards: (A) 0.0 pmol/ml; (B) 10.5 pmol/ml; (C) 21.0 pmol/ml; (D) 41.9 pmol/ml; and (E) 83.7 pmol/ml. For experimental conditions see Fig. 3.

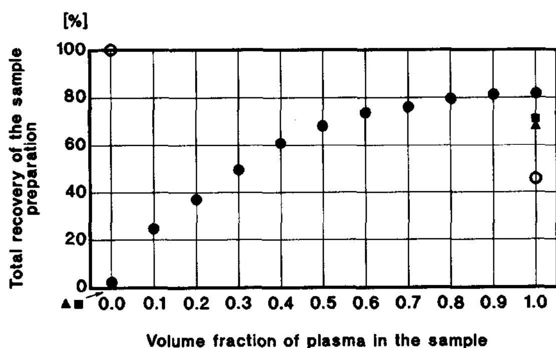

Fig. 5. Recovery of the sample preparation. Sample 1.00 ml + 41.9 pmol of Zy 17617B in 200 μl of water + 0.80 ml of mobile phase B (pH 7.4, closed symbols) or 0.80 ml of diluted acetic acid (pH 3.0, open symbols). ● = glass tubes; ▲ = polyethylene tubes; ■ = polystyrene tubes.

adsorption at physiological pH. This is probably the result of protein binding. This effect was less pronounced in acidic medium.

The recovery of the extraction step was found to be approximately 99%. Liquid–liquid extraction in basic medium with diisopropyl ether, ethyl acetate and *n*-hexane gave values of 87, 47 and 7% respectively.

The capacity of the pre-columns was found to be more than 400 pmol of Zy 17617B. The pre-columns may be used up to eighteen times. Pre-column to pre-column variations as well as peak broadening were found to be negligible.

Chromatography

Zy 17617B was desorbed from the pre-column and eluted onto a C_{18} reversed-phase analytical column by isocratic elution with a mobile phase containing phosphate buffer pH 3.7 (50 m*M*–methanol–tetrahydrofuran (600:288:112, v/v). The flow-rate was set to 1.0 ml/min. The data acquisition was started when the pre-column was switched to the analytical column. The retention time of Zy 17617B was about 9 min. Spectrofluorimetric detection was selected for its enhanced selectivity and sensitivity compared with UV detection.

Method validation

The precision and the accuracy were determined over the range 10.5–83.7 pmol/ml. The results are given in Table I. The intra-assay and inter-assay precisions ranged from 1.5 to 4.8% and from 3.0 to 8.3% (n = 6), respectively. The intra-assay and inter-assay accuracies ranged from −5.7 to +0.3% and from −5.5 to +4.9% (n = 6), respectively.

The calibration graphs relating the Zy 17617B peak height to its concentration in prepared standards were linear in the range 10.5–83.7 pmol/ml in

TABLE I

PRECISION AND ACCURACY OF Zy 17617B ASSAY

Analyses carried out using the experimental conditions of Fig. 3.

Nominal concentration (pmol/ml)	Concentration found (mean ± S.D., n = 6) (pmol/ml)	Relative standard deviation (%)	Confidence interval of the mean value (P = 95%) (pmol/ml)	Accuracy[a] (%)
Intra-assay variability				
10.47	10.01 ± 0.48	4.8	10.01 ± 0.50	−4.4
20.93	19.74 ± 0.35	1.8	19.74 ± 0.37	−5.7
41.86	41.99 ± 0.62	1.5	41.99 ± 0.66	0.3
83.73	84.01 ± 1.82	2.2	84.01 ± 1.91	0.3
Inter-assay variability				
10.47	10.98 ± 0.39	3.5	10.98 ± 0.40	4.9
20.93	20.60 ± 0.62	3.0	20.60 ± 0.66	−1.6
41.86	39.55 ± 1.47	3.7	39.55 ± 1.54	−5.5
83.73	85.11 ± 7.03	8.3	85.11 ± 7.38	1.6

[a] Defined as the percentage deviation between the mean concentration found and the theoretical concentration.

plasma. The regression characteristics were typical: slope = 0.000949, intercept = 0.06, with a correlation coefficient (r) of 0.999.

The limit of quantification for the assay was of the order of 4 pmol/ml.

Zy 17617B was found to be stable under the experimental conditions for up to 12 h.

CONCLUSION

The developed method is suitable for the determination of Zy 17617B in plasma at the pmol/ml level. It allows automated extraction and analysis of plasma samples to be carried out. Full validation data demonstrate its reliability.

REFERENCES

1 K. H. Antonin, B. Ensslin-Haasis, R. Schulz and P. R. Bieck, *Eur. J. Gastroenterol. Hepatol.*, 2(Suppl. 1) (1990) 54.
2 M. W. F. Nielen, R. W. Frei and U. A. Th. Brinkman, in R. W. Frei and K. Zech (Editors), *Selective Sample Handling and Detection in HPLC, Part A* (*Journal of Chromatography Library*, Vol. 39A), Elsevier, Amsterdam, 1988, Ch. 1, pp. 1–80.
3 R. Huber and K. Zech, in R. W. Frei and K. Zech (Editors), *Selective Sample Handling and Detection in HPLC, Part A* (*Journal of Chromatography Library*, Vol. 39A), Elsevier, Amsterdam, 1988, Ch. 2, pp. 81–143.
4 M. C. Rouan, J. Campestrini, J. B. Lecaillon, J. P. Dubois, M. Lamontagne and B. Pichon, *J. Chromatogr.*, 456 (1988) 45.
5 M. W. F. Nielen, A. J. Valk, R. W. Frei, U. A. Th. Brinkman, Ph. Mussche, R. de Nijs, B. Ooms and W. Smink, *J. Chromatogr.*, 393 (1987) 69.