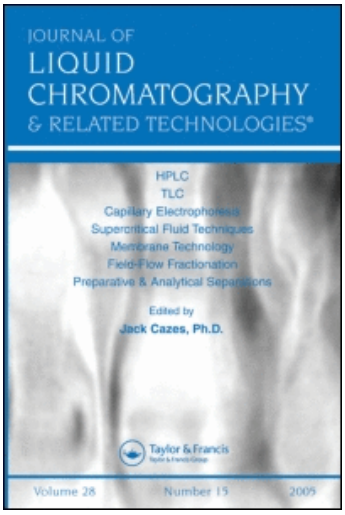

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Cimetidine in Biological Fluids by High Performance Liquid Chromatography

J. A. Apffel[a]; U. A. Th. Brinkman[a]; R. W. Frei[a]
[a] Department of Analytical Chemistry, Free University, Amsterdam, HV, The Netherlands

To cite this Article Apffel, J. A. , Brinkman, U. A. Th. and Frei, R. W.(1982) 'Analysis of Cimetidine in Biological Fluids by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 5: 12, 2413 — 2422
To link to this Article: DOI: 10.1080/01483918208067643
URL: http://dx.doi.org/10.1080/01483918208067643

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(12), 2413-2422 (1982)

ANALYSIS OF CIMETIDINE IN BIOLOGICAL FLUIDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

J.A. Apffel, U.A.Th. Brinkman
and R.W. Frei
Department of Analytical Chemistry,
Free University,
De Boelelaan 1083,
1081 HV Amsterdam, The Netherlands

ABSTRACT

The clean-up and analysis of cimetidine in human urine and blood is described. Samples were prepared by adsorption on Waters Sep-pak C-18 disposable pre-columns in basic solution followed by elution in 1 ml methanol. For blood samples, the eluate was concentrated under a stream of nitrogen; urine samples required no further concentration. The separation was performed on a reversed phase column using a mixture of methanol-1 mM sodium dodecyl sulphate in a 10 mM phosphate buffer of pH 3.0 (35:65) as mobile phase. Procaine was used as internal standard. Detection was by UV at 228 nm yielding a minimum detectable quantity of 20 ng with linearity over three decades of concentration.

INTRODUCTION

Cimetidine (Tagamet^{R}, SKF) (N"-cyano-N-methyl-N'-[2(5-methyl-1H--imidazol-4-yl)methyl-thioethyl]guanidine), a histamine H_2 receptor antagonist, inhibits the secretion of gastric acids and has been used very effectively in the treatment and maintenance of patients with duodenal and peptic ulcers and in the treatment of Zollinger-Ellison syndrome (1-3).

Although adverse side effects are generally minor and gastrointestinal in nature (4,5), there are reports of more serious reactions including CNS adverse effects (6,7). Furthermore, the action of cimetidine has been shown to be dose dependent (8). For these reasons, the analysis of cimetidine in biological fluids, particularly blood and plasma is of clinical interest.

Several high performance liquid chromatography (HPLC) analyses of cimetidine have appeared in the literature (9-15) utilizing both normal phase and reversed phase separations. Reversed phase separations have been performed with a variety of mobile phases, including highly basic solutions with high percentages of organic modifier (9) and acidic solutions with low modifier concentrations (14). While the use of mobile phase of pH 8 or above easily leads to column degradation via silica dissolution, the acidic mobile phases used offer limited range of capacity factors due to the low retention of cimetidine under acidic conditions. Sample preparation procedures reported have required double or triple extractions (9,10) or at least single extraction steps followed by concentration steps (13-15).

This paper describes a reversed phase separation using a mobile phase containing ion pair reagent offering improved control over capacity factors. The clean-up procedure utilizes Waters Sep-pak C-18 cartridges for a rapid clean-up and concentration.

EXPERIMENTAL

Reagents and Samples

Analytical grade methanol was obtained from E. Merck (Darmstadt, GFR). HPLC-grade water was prepared using a Milli Q water system (Waters Millipore, Millford, MA, USA). Potassium phosphate and sodium dodecyl sulphate (SDS) were obtained from Baker (Deventer, The Netherlands). Cimetidine (CMT) was obtained from Smith, Kline and French Labs (Philadelphia, PA, USA) and procaine hydrochloride from Sigma (St. Louis, MO, USA).

Blood and urine samples were taken from a duodenal ulcer patient receiving cimetidine in 400 mg doses at 12 hour intervals. Blood samples were prevented from coagulating by the addition of NaEDTA. All samples were stored in a refrigerator at 5°C.

HPLC Apparatus

The HPLC system consisted of a Gilson (Villers le Bel, France) Model 302 single piston pump with a Kontron (Zürich, Switzerland) Model 812 pulse dampener. A Valco Instruments (Houston, TX, USA) high pressure six port model CV UH Pa valve with a 20 µl loop was used for sample introduction. A Pye Unicam (Cambridge, UK) Model LC 3 variable wavelength UV detector was used as a detector at 228 nm. A Kipp and Zonen (Delft, The Netherlands) BD-8 multi range recorder was used to record detector out-put. The HPLC column consisted of a 10 cm x 4.6 cartridge column system packed with 10 µm C-18 material from Brownlee Labs (Clara, CA, USA) Sep-pak^{R} C-18 cartridges were obtained from Waters.

METHODS

The HPLC mobile phases consisted of mixtures of methanol and 1 mM sodium dodecyl sulphate in a 10 mM phosphate buffer of pH 3.0. Routine analyses were performed using 35% methanol. The use of mixtures of methanol and 0.1% solution of ammonium hydroxide was also investigated. The mobile phases were filtered using a 0.5 µm Waters Millipore solvent filtration system and degassed under vacuum in an ultrasonic bath.

Samples were prepared as follows. To 1-5 ml of blood or urine was added one drop of ammonium hydroxide giving the sample a pH of approximately 10. An appropriate amount of the internal standard procaine (1 mg/ml for urine and 20 µg/ml for blood) was also added. The sample was passed through a Sep-pak C-18 cartridge which had previously been washed with a dilute ammonium hydroxide solution. The cartridge was then washed with 10 ml of a dilute solution of ammonium hydrox-

ide (pH 10) and 10 ml of the 1 mM sodium dodecyl sulphate in 10 mM phosphate buffer at pH 3.0. Following the washes, the cimetidine was eluted from the cartridges with 1 ml methanol. For the analysis of blood, this eluent was concentrated to 50 µl under a stream of nitrogen. In the case of urine samples this concentration step was not required.

RESULTS AND DISCUSSION

Initial tests were run using a mobile phase consisting of mixtures of methanol and 0.1% aqueous solution of ammonium hydroxide. Figure 1 shows a plot of log k' vs % methanol. This system provided adequate retention, but rapid degradation of the analytical column resulted due to silica dissolution. Although this effect could be circumvented via the use of silica presaturation of mobile phases, we preferred to develop a simpler system using ion pair chromatography. Using sodium dodecyl sulphate to form ion pairs with the protonated amino groups in cimetidine, retention behaviour similar

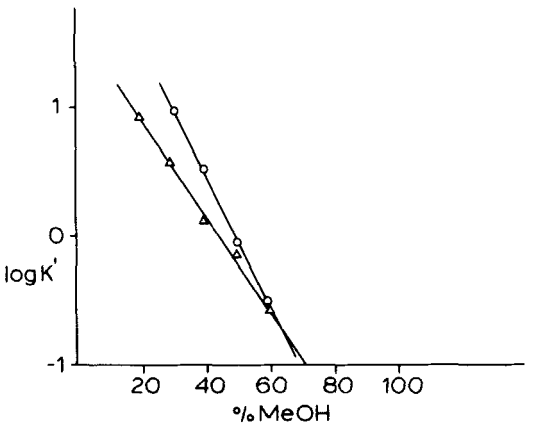

Figure 1. Retention behaviour of Cimetidine on reversed phase (C-18) material. Δ; CH_3OH: 1% NH_3 in H_2O, pH 10, O; CH_3OH: 20 mM KH_2PO_4, 1 mM SDS in H_2O, pH 3.0.

to the ammonium hydroxide mobile phase was obtained. This is illustrated also in Figure 1. The amount of methanol added to the sodium dodecyl sulphate solution was dictated in both blood and urine samples by the resolution of cimetidine from the internal standard, procaine. At 35% methanol, the resolution of these two compounds was found to be 1.3 with cimetidine eluting in 9 ml at a flow-rate of 2 ml/min.

The clean-up procedure was found to be adequate for both blood and urine samples. Figure 2 shows a plot of the concentration of ci-

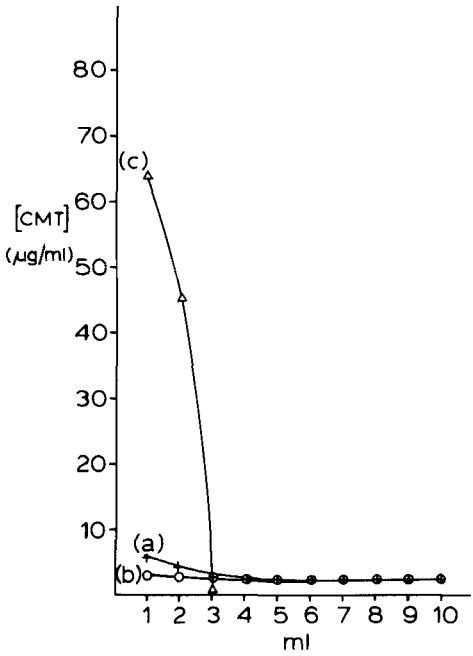

Figure 2. Concentration and elution of cimetidine on Sep-pak C-18 cartridges. (a) loading sample with equilibration if Sep-pak with 0.1% NH_3 in water, (b) loading sample without equilibration with 0.1% NH_3 in water, (c) elution of sample (10 ml loaded) by methanol. Original sample concentration: 20 μg/ml.

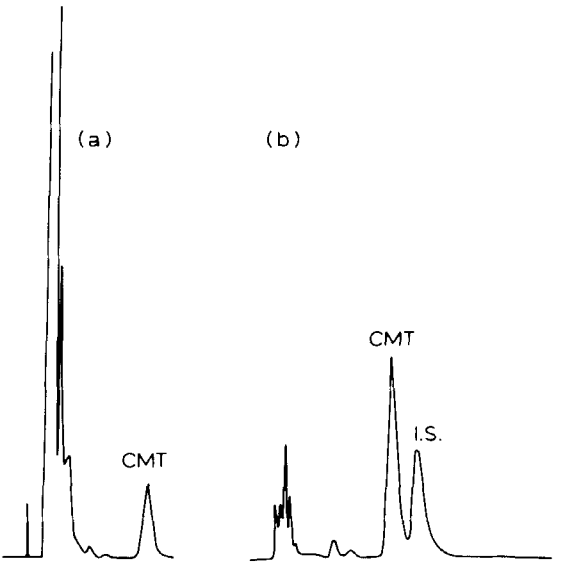

Figure 3. Analysis of cimetidine (CMT) in urine: Column: 10 cm x 4.6 mm C-18. Mobile phase: 35:65 CH_3OH: 20 mM KH_2PO_4 1 mM SDS in H_2O, pH 3.0; Flow-rate 2 ml/min; Detection UV at 228 nm; 20 µl loop injection. a) Raw urine, without Sep-pak clean--up: No internal standard added b) Urine with Sep-pak clean--up. Original sample volume: 50 ml with 1 mg/ml procaine internal standard (1.5.) added.

metidine eluting from the Sep-pak as a function of the volume of sample (curve A) or eluent (curve C) passed through the Sep-pak. Curve A represents the cimetidine eluting from the Sep-pak during sample loading. A relatively constant low level of cimetidine elutes throughout the loading step. The mechanism of this phenomenon is not known; however, the use of the internal standard which behaves similarly negates any adverse effect on the quantitation. Curve B represents the elution of cimetidine during sample loading when the Sep-pak is not initially washed with a basic solution. A larger concentration of cimetidine elutes in the first 2 ml of sample due to a lack of equilibration. Curve C shows the elution of the analyte after loading 10 ml of sample from the Sep-pak with methanol.

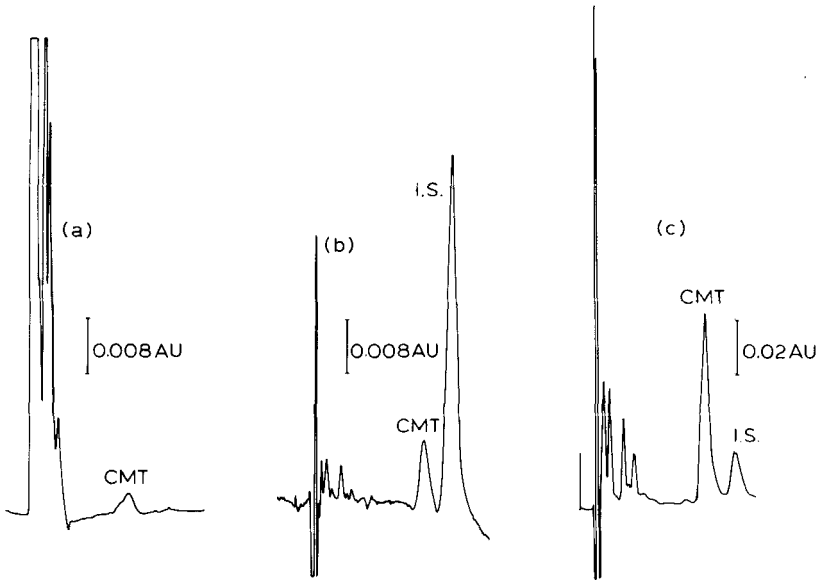

Figure 4. Analysis of cimetidine in blood. Conditions as in figure 3. a) Untreated whole blood. No internal standard added. b) whole blood after Sep-pak clean-up. Original sample: 1 ml blood + 20 µg/ml procaine internal standard. c) Whole blood after Sep-pak clean-up and concentration of eluent to 50 µl. Original sample as in (b).

Figure 3 shows the analysis of cimetidine in urine with and without the clean-up procedure. No procaine is present in the untreated samples. As can be seen, in the case of urine, the clean-up is not absolutely necessary since the analyte and internal standard are well resolved from the rest of the matrix. Figures 4 a, b and c shows the analysis of blood; without clean-up, with clean-up and with clean-up and concentration, respectively. The amount of internal standard added was less in the concentrated sample to keep the procaine peak on scale. Again it can be seen that, in general, the major components in the sample are well resolved from the analyte, but the concentration in the blood is approximately 1000 times less than in urine. Even with the clean-up on a Sep-pak cartridge which involves a concentration of supernatant from full blood

present at a low level (Fig. 4b). In the routine method further concentration by evaporation of the solvent was used and the cimetidine was easily detected at therapeutic levels (Fig. 4c). Lower detection limits could also be achieved by using larger injection volumes with little loss in resolution.

Using ultraviolet absorbance detection at 228 nm, a minimum detectable quantity of 20 ng was obtained. Data from a calibration plot yielded a correlation coefficient of 0.9988 over three decades of concentration.

The concentration of cimetidine in blood following administration of the drug was examined as a function of time. This is shown graphically in Figure 5. The samples were taken at 2 hour intervals for 10 hours following a 400 mg dose of cimetidine. Although the blood concentration reaches a maximum of approximately 3.3 µg/ml 6 hours after dosage, there appears to be a basal concentration of at least 1.5 µg /ml maintained by the dosage schedule. These analyses were preformed in duplicate and the resulting values were within 2.1% of each other.

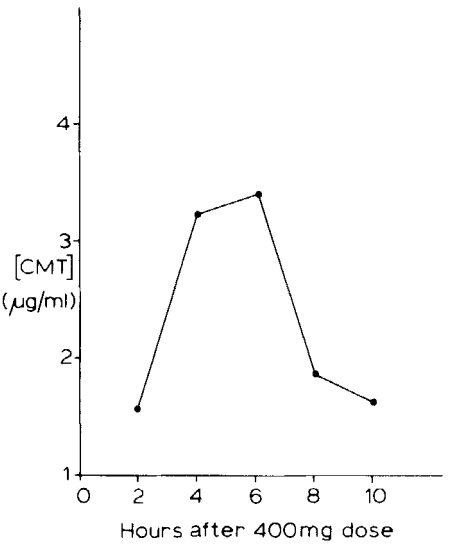

Figure 5. Blood concentration of cimetidine following 400 mg dose.

CONCLUSION

The method described offers a rapid, easy and sensitive technique for the analysis of cimetidine in biological fluids. The use of ion pair chromatography has significant advantages over previously reported approaches to this analysis, in that it avoids the use of highly basic mobile phases which lead to short column lifetimes and offers greater control over the retention than the use of acidic buffered mobile phase. The clean-up technique described has the advantages of speed and simplicity. It has been shown to be useful for both blood and urine samples. According to Larsen et al. (9) cimetidine undergoes metabolic conversion in blood (but not in plasma) to the sulphoxide, and it may be advantageous to assay cimetidine in plasma rather than whole blood. The technique described here should be applicable for such samples as well.

Subsequent work is under way to place the analysis on-line in an automated column switching system which should supply rapid automated clean-up and analysis of cimetidine in plasma and urine.

RÉFERENCES

(1) R.N Brogan, R.C. Heel, T.M. Speight and G.S. Avery, Drugs 15 (1978) 93.

(2) D.M. McCartny, Gastroenterology, 74 (1978) 453.

(3) J.W. Freston, Gastroenterology, 74 (1978) 426.

(4) J.M. McGuigan, Gastroenterology, 80 (1981) 181.

(5) L.M. Gifford, M.E. Aeugle, R.M. Myerson and P.J. Tennenbaum, J. Amer. Med. Assoc., 243 (1980) 1532.

(6) J.J. Schentag. G. Caller, J.Q. Rose, F.B. Cerra, E. De Glopper and H. Bernhard, Lancet, 1 (1979) 177.

(7) B.J. Kimelblatt, F.B. Cerra, G. Caller, M.J. Berg, M.A. McMillen and J.J. Schentag, Gastroenterology, 78 (1980) 791.

(8) E. Aadland, A. Berstad and L.S. Semb in W.L. Burland and M.A. Simkines (Editors), Cimetidine, Excerpta Medica, Amsterdam 1979, p. 87.

(9) N.E. Larsen, P. Hesselfieldt, S.J. Rune and E.F. Bleidbeurg, J. Chromatogr., 163 (1979) 57.

(10) W.C. Randolph, V.L. Osborne, S.S. Walkenstein and A.P. Intoccia, J. Pharma. Sci., 66 (1977) 11, 48.

(11) R.M. Lee and P.M. Osborne, J. Chromatogr., 146 (1978) 354.

(12) J.A. Ziemniak, D.A. Chiarmonte and J.J. Scheutag, Clin. Chem., 27 (1981) 272.

(13) S.J. Saldin, D.R. Fingold, P.C. Feuge and W.A. Mahon, Ther. Drug Monitoring, 1 (1979) 371.

(14) D.R.P. Guay, H.N. Bockbrader, G.R. Matzke, J. Chromatogr., 228 (1982) 389.

(15) J. Fleitman, G. Torosian and J.H. Perrin, J. Chromatogr., 229 (1982) 255.