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Determination of cimetidine in human plasma by free capillary zone electrophoresis☆

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Abstract

The separation of cimetidine from the metabolites cimetidine amide and cimetidine sulfoxide, endogenous creatinine and the internal standard ranitidine was achieved by capillary electrophoresis in less than 5 min. All compounds were well separated from cimetidine, including possible plasma ingredients, as the UV spectra of cimetidine standard and cimetidine from the plasma extract match. Plasma levels of cimetidine were determined in the range 250–3000 ng/ml in plasma and higher concentrations were determined by dilution of the sample with blank plasma.

1. Introduction

The determination of drug levels in biological matrices is currently performed by chromatographic methods, mostly HPLC. Chromatographic columns are usually adversely affected by these matrices, especially plasma, resulting in a relatively rapid decrease in column performance. Solid-phase extraction is the most promising solution for obtaining cleaner sample extracts. However, even in clean sample extracts some late-eluting components occur. Hence capillary electrophoresis (CE) is the method of choice for separation. The elution order in CE is different from that in HPLC and plasma proteins that still remain in the extract usually have longer migration times because of their high molecular mass compared with relatively small drug molecules. Components with migration times longer than those of the molecules of interest are simply washed out of the capillary during the purge after each run.

Cimetidine, N - cyano - N - methyl - N′ - [2 - (5 - methyl - 1H - imidazol - 4 - yl)methylthio - ethyl]-guanidine (Fig. 1) is a histamine H_2 receptor antagonist and as such a potent inhibitor of gastric acid secretion [1]. Cimetidine and ranitidine have already been determined by HPLC with the usual liquid–liquid extraction [2–7], by HPLC with solid-phase extraction [8] and also by micellar electrokinetic chromatography (MEKC) with electrochromatographic solid-phase extraction for sample clean-up [9].

The method reported in this paper permits

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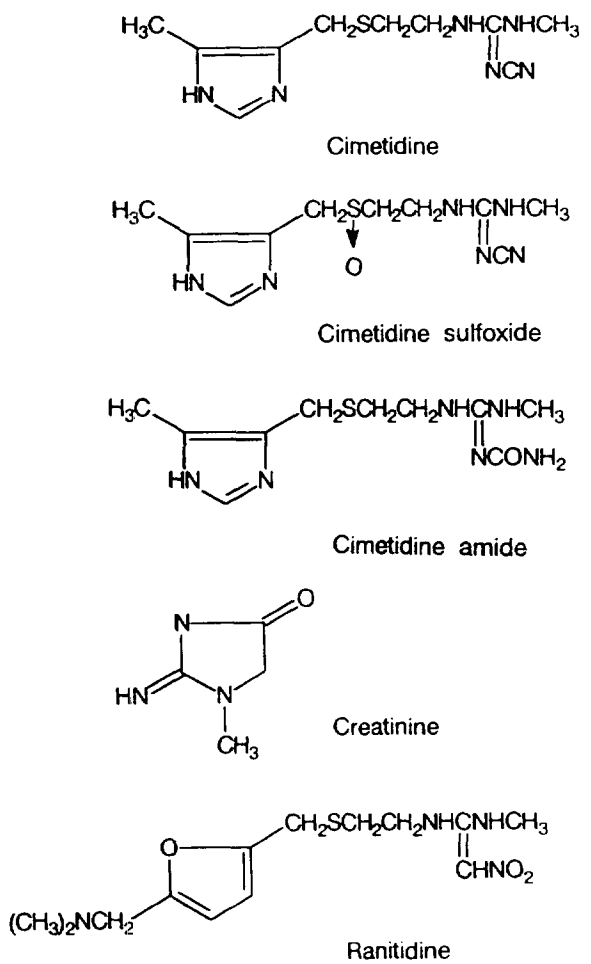

Fig. 1. Structures of cimetidine, its metabolites, cimetidine sulfoxide and cimetidine amide, the possible endogenous compound creatinine and the internal standard, ranitidine.

separation in the free solution mode of capillary electrophoresis technique [capillary zone electrophoresis (CZE)]. The HPLC determination of cimetidine is performed according to Ziemniak et al. [7]. The analysis time is about 30 min because of some very late-eluting peaks, as can be seen from the HPLC trace (see Fig. 2). For large numbers of samples, as in bioavailability and bioequivalence studies, this method is time consuming and large amounts of organic solvents are needed and discarded.

2. Experimental

2.1. Chemicals

Cimetidine, cimetidine sulfoxide and cimetidine amide were produced and characterized at Lek (Ljubljana, Slovenia). Ranitidine was obtained from Amipharma (Barcelona, Spain). K_2HPO_4, NaH_2PO_4 and H_3PO_4 were of analytical-reagent grade from Kemika (Zagreb, Croatia). A solution of 50 m*M* K_2HPO_4 (pH 8.4, no adjustment needed) was prepared for solid-phase extraction column pretreatment and a solution of 100 m*M* NaH_2PO_4 (pH 2.0, adjusted with concentrated H_3PO_4) as electrophoretic running buffer. Methanol of LiChrosolv grade (Merck, Darmstadt, Germany) was used for sample treatment.

2.2. Equipment

Solid-phase extraction was carried out with Supelclean LC-18 columns, with 1 ml on a Visiprep manifold with a manometer (Supelco, Gland, Switzerland) and the extracts were evaporated in a vacuum centrifuge (Univapo UVC 150H; Martinsried, Germany). Electrophoretic separations were performed using a BioFocus 3000 capillary electrophoresis instrument with a coated capillary (24 cm × 25 μm I.D.) (Bio-Rad, Vienna, Austria).

2.3. Solid-phase extraction (SPE)

Solid-phase extraction was carried out on Supelclean LC-18 columns on a Visi-prep manifold which provided about 100 mm Hg pressure applied at the outlets of the SPE columns. Series of columns were washed and activated with 1 ml of water, 1 ml of phosphate buffer (50 m*M*, pH 8.4), 3 ml of methanol and 5 ml of water. To 1.0 ml of plasma sample or standard solution in plasma the internal standard ranitidine was added and the mixture was applied to a Supelclean LC-18 column and washed with 1 ml of phosphate buffer. Elution was carried out with three 1-ml volumes of methanol and each of the three portions of the eluate were collected separ-

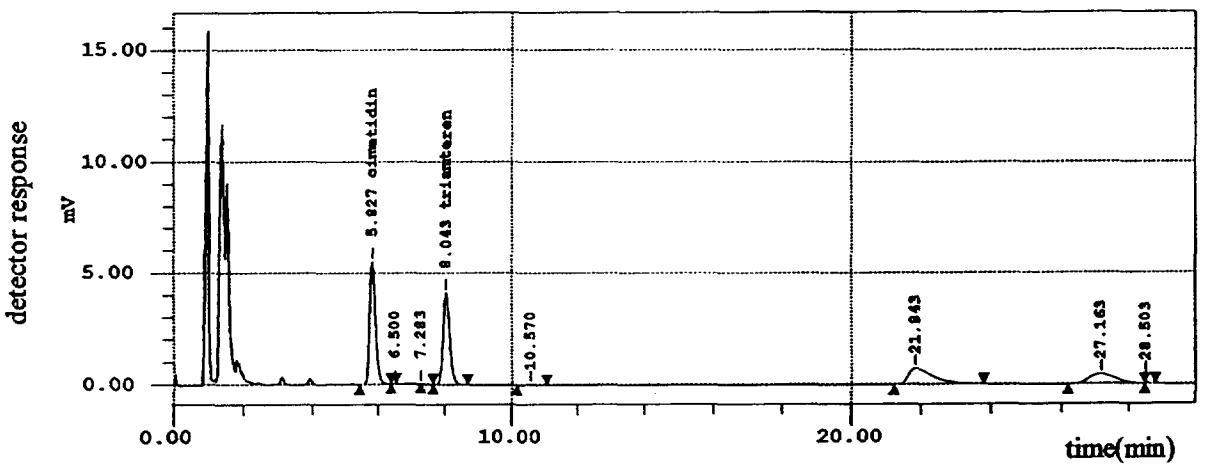

Fig. 2. Chromatogram of cimetidine in plasma from a healthy volunteer who had taken 400 mg of cimetidine as a single dose. The HPLC conditions were the same as those of Ziemniak et al. [7] and the internal standard was triamteren.

ately. The methanolic fractions were dried in a vacuum centrifuge and the dry residues were reconstituted in 50 μl of sample buffer.

2.4. Free capillary zone electrophoresis

Capillary electrophoretic separation was achieved in a coated capillary (24 cm × 25 μm I.D.) in free solution under the following instrumental conditions. The electrophoretic running buffer was 100 m*M* NaH_2PO_4 (pH 2.0). The sample buffer was 10 m*M* NaH_2PO_4 (tenfold dilution of running buffer) as the electrokinetic mode of sample injection was used (8 kV, 8 s, positive to negative polarity). Separation was achieved by free capillary zone electrophoresis at an applied voltage 15 kV, positive to negative, and components were detected at 208 nm. The temperature of the sample carousel and capillary cartridge was maintained at 15°C. Quantification of the electrophoretic responses was carried out using least-squares linear regression for calibration and the areas were normalized by migration times.

3. Results and discussion

Sample preparation using solid-phase extraction is very efficient with respect to the removal of plasma proteins, as shown in the electropherogram in Fig. 3. Cimetidine and ranitidine are sufficiently retained on the solid-phase ex-

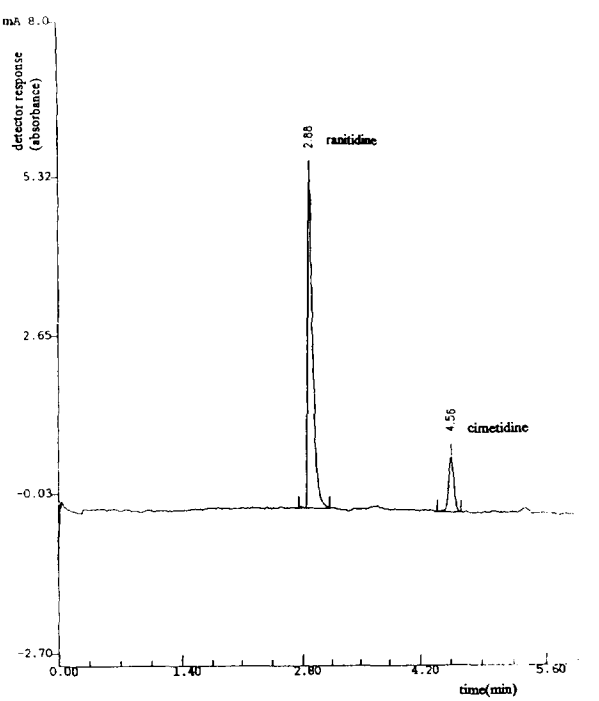

Fig. 3. Electropherogram of a plasma sample from a healthy volunteer who had taken 400 mg of cimetidine as single dose. Conditions as described under Experimental.

traction column and after washing the plasma proteins with buffer and the first two portions (1.0 ml each) of methanol, the third 1.0-ml fraction of methanol elutes cimetidine and ranitidine in a relatively clean solution. After drying in a vacuum centrifuge, the residue was reconstituted in sample buffer and poor recoveries were found (10–30%), but we were still able to measure the signal because of the very good and stable baseline, as demonstrated in the electropherograms (Figs. 3–7). The sample was injected into the capillary in the electrokinetic mode so that electrophoretically slowly migrating species were excluded in the selective process of electrokinetic sample loading.

Chromatographic problems of usually broad peaks (Fig. 2) for cimetidine due to unwanted matrix interactions were successfully avoided in free capillary zone electrophoresis. The electrophoretic peaks are sharp owing to the exclusion

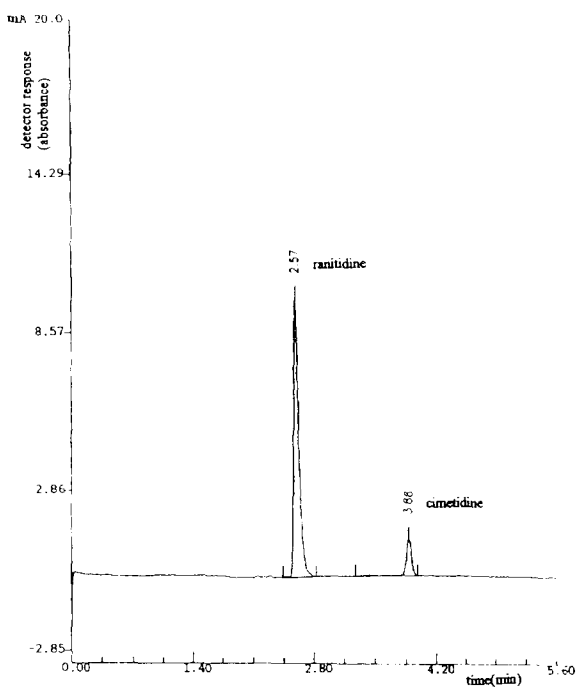

Fig. 4. Electropherogram of cimetidine and ranitidine in human plasma after solid-phase extraction. Concentration point 3000 ng from calibration graph. Conditions as described under Experimental.

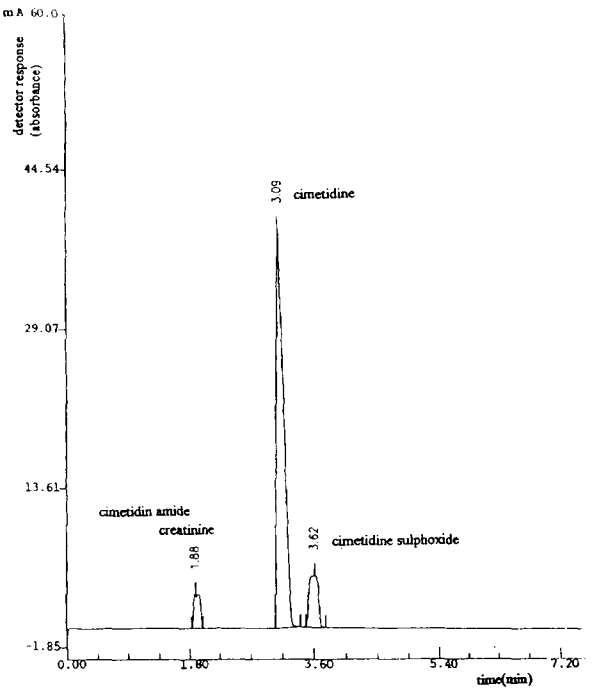

Fig. 5. Electropherogram of cimetidine together with creatinine and metabolites (cimetidine amide and cimetidine sulfoxide). Conditions as described under Experimental.

of any matrix interactions in the coated capillary. Electropherograms of a plasma sample from a volunteer who had taken a single dose of 400 mg of cimetidine and a plasma standard from the calibration graph are shown in Figs. 3 and 4. Cimetidine and related compounds shown in Fig. 1 were successfully separated in a free solution of phosphate buffer at pH 2.0. Relative migration times with respect to cimetidine were about 0.7 for ranitidine, about 0.6 for cimetidine amide and creatinine and about 1.2 for cimetidine sulfoxide (Fig. 5). Cimetidine amide and creatinine co-migrate, but they are not quantified, so no further separation was needed. Components of interest were resolved from cimetidine in 5 min while 30 min were needed for the HPLC analysis, hence a considerably greater throughput was achieved. All other possible endogenous compounds from plasma were washed out from the capillary, so the analysis

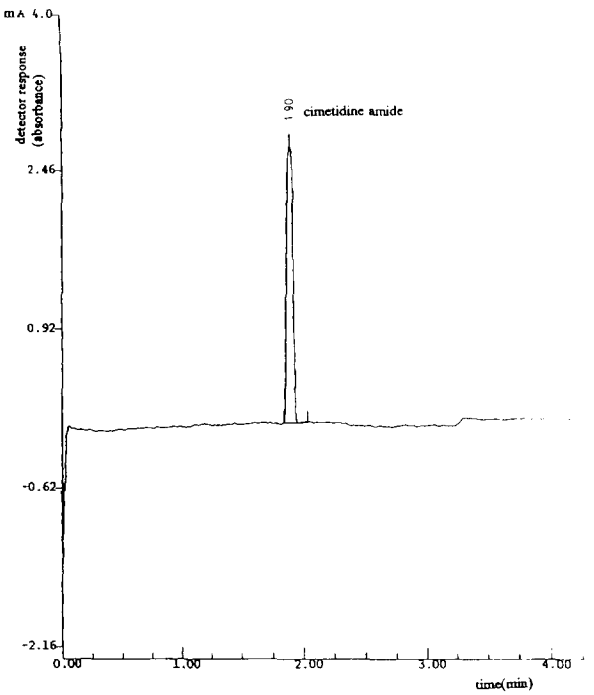

Fig. 6. Electropherogram of metabolite cimetidine amide. Conditions as described under Experimental.

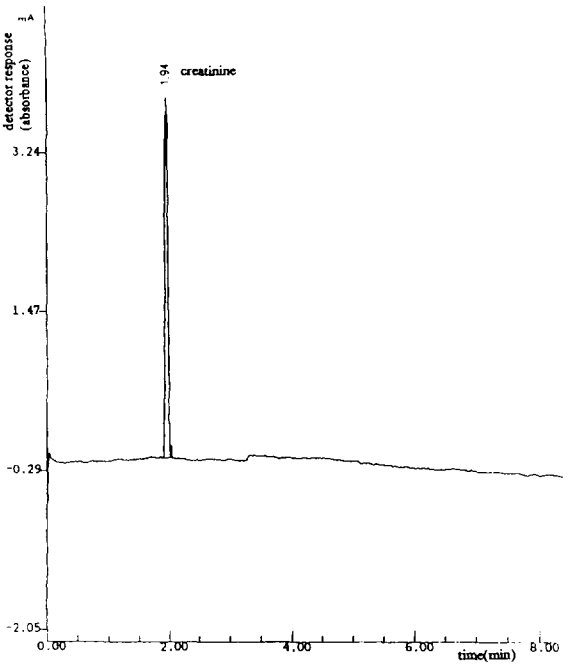

Fig. 7. Electropherogram of the endogenous compound creatinine, which might occur in plasma samples. Conditions as described under Experimental.

time is only that needed to separate the components of interest, i.e., 5 min plus a few minutes to purge the capillary. The precision of the method was estimated using spiked samples; the R.S.D. ($n = 8$) was 8.3%. The CZE method has not yet been fully validated, but the drug levels obtained by CZE compare with those of HPLC to within 20%.

A calibration graph in the concentration range 250–3000 ng/ml of human plasma was used for the analysis of plasma samples from healthy volunteers. The correlation coefficient of the least-squares linear regression curve was 0.9983, the slope was 3.064 and the intercept was 0.166. This range is suitable for routine monitoring of cimetidine plasma levels. The upper limit is exceeded only in a few cases. This is overcome by diluting the samples with blank plasma to obtain concentrations corresponding to the calibration graph. The limit of quantification (LOQ) was found to be 250 ng/ml of plasma (signal-to-noise ratio = 5:1) and the limit of detection (LOD) was 100 ng/ml of plasma at a signal-to-noise ratio of 3:1. The sensitivity in CZE is slightly lower than that in HPLC, where the LOQ is 100 mg/ml and the LOD is 50 ng/ml, mostly owing to low recoveries of solid-phase extraction.

Two metabolites, cimetidine amide and cimetidine sulfoxide, and three possible exogenous compounds (acetylsalicylic acid, paracetamol and caffeine) and the possible endogenous compound creatinine were added to human plasma. The metabolites and creatinine migrate at the applied voltage, but are well separated from cimetidine (Fig. 5); acetylsalicylic acid, paracetamol and caffeine are not extracted by solid-phase extraction. The cimetidine peak is homogeneous according to the UV spectrum scanned during the electrophoretic run (Fig. 8).

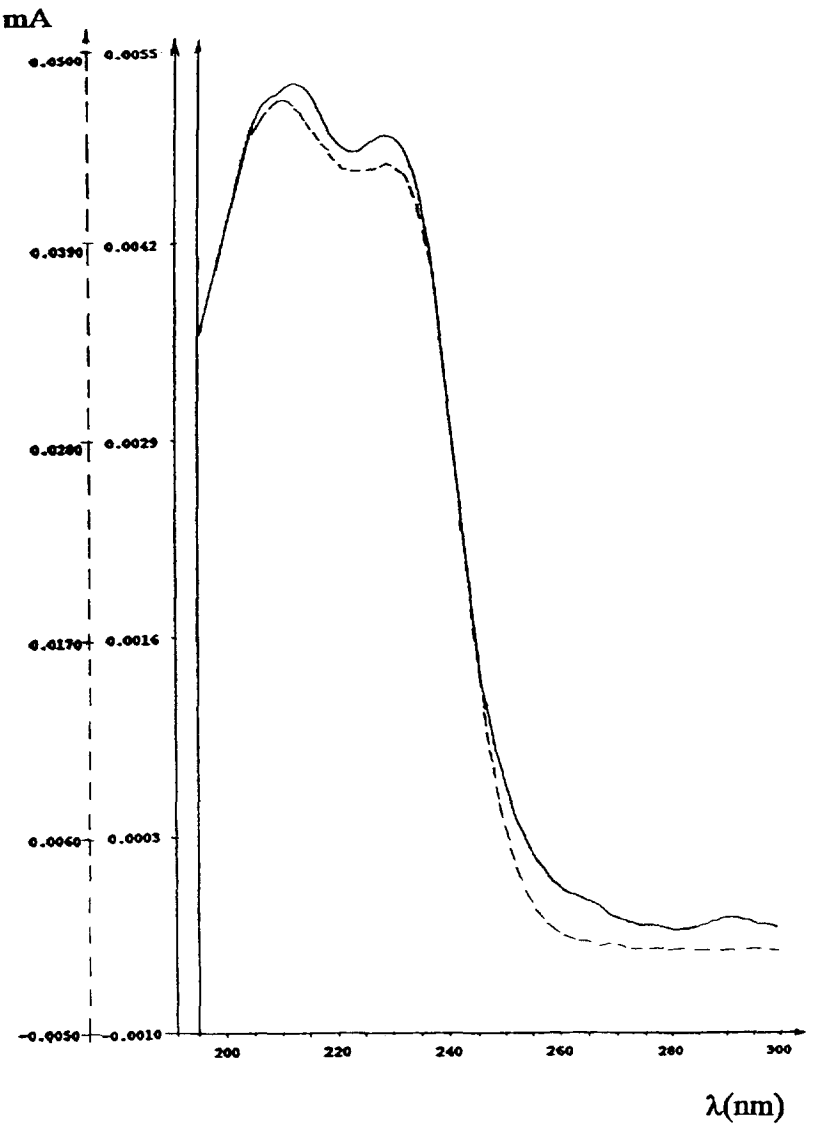

Fig. 8. UV spectra of cimetidine standard (dashed line) and cimetidine from plasma extract (solid line). Conditions as described under Experimental.

4. Conclusions

The determination of cimetidine using free capillary zone electrophoresis in a coated capillary is much faster than using HPLC analysis: the run time is reduced from 30 to about 8 min (5 min for separation and about 2–3 min for capillary purge steps). The solvent consumption is reduced from several tens of litres of organic solvents to less than 1 l for the whole pharmacokinetic study; this is important with respect to both costs and environmental protection.

This method can be used for rapid pilot pharmacokinetic studies and after full validation

it can be applied to ordinary bioequivalence studies of different cimetidine pharmaceutical finished products.

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