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# Electrochromatographic solid-phase extraction for determination of cimetidine in serum by micellar electrokinetic capillary chromatography

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## ABSTRACT

A highly effective electrochromatographic solid-phase extraction and preconcentration method is reported for the determination of cimetidine in serum in the concentration range 0.233–11.4 μg/ml. Preconcentrated samples were determined by micellar electrokinetic capillary chromatography while ranitidine was used as an internal standard. Sample preparation included retention of the analyte on a $C_{18}$ solid-phase cartridge, followed by elution assisted by an applied voltage of 150 V. From 0.5-ml serum samples, 20–50-μl aliquots were collected for electrophoretic analysis. Within the studied concentration range, the method was linear and provided adequate precision.

## INTRODUCTION

Cimetidine, N-cyano-N-methyl-N′-[2(5-methyl-1H-imidazoyl-4-yl)methylthioethyl]guanidine, is a histamine $H_2$ receptor antagonist which is used to reduce acid secretion in treatment of gastrointestinal ulcers [1]. This pharmaceutical has traditionally been determined in serum by high-performance liquid chromatography (HPLC) [2—11]. Unfortunately, some analytical problems are encountered with this chromatographic approach. Cimetidine exhibits a high retention with most HPLC systems and, in addition, asymmetric and broad peaks are often experienced owing to undesirable interaction with the packing materials. Typical column efficiencies are seldom sufficient to separate cimetidine adequately from various interfering serum or plasma constituents.

Isolation of cimetidine as a pure component from serum is difficult owing to its neutral character ($pK_a$ = 6.8) [12] and high solubility in water. In some instances, laborious, multiple extractions with methylene chloride have been reported [2] to be necessary. An alternative approach has been solid-phase extraction with Extrelut [6]

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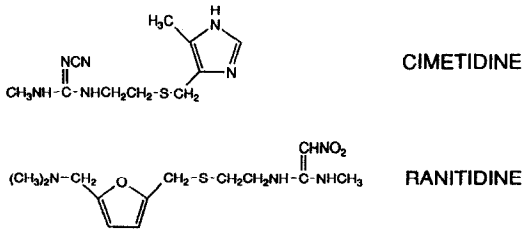


Fig. 1. Structures of cimetidine and ranitidine.

or a reversed-phased material [5,7–11]. In this general procedure, large volumes of solvent can be avoided, while the occurrence of coextracted blood constituents is also suppressed. However, relatively large volumes of either acetonitrile or methanol (1–5 ml) must be reduced to the permissible injection volumes (40–100 $\mu$l) for HPLC.

Capillary electromigration techniques, with their relatively high efficiencies and various selective mechanisms, now offer interesting alternatives in drug analysis. As such techniques are relatively new, only few quantitative methods for determining drug substances in serum or plasma have been reported [13–18]. At this stage of development, small sample volumes (of the order of nanoliters) are essential in capillary zone electrophoresis (CZE) or micellar electrokinetic capillary chromatography (MECC), so that very low levels of therapeutic drugs can be reached only through highly sensitive detection methods, such as laser-induced fluorescence measurements of derivatized samples [17].

This investigation concerns the use of cimetidine as a model compound in exploring certain analytical merits of MECC in serum analysis. Sample purification and preconcentration are accomplished through electrochromatography, which combines certain advantages of electrophoresis and sorptive interactions [19]. In its more modern version, the separation modes of electrochromatography have been explored with slurry-packed reversed-phase microcapillaries, where the model compounds were separated under various conditions of pressurized flow and applied electrical field [20–23]. A potential role of electrochromatography in sample pretreatment has also been predicted previously [23].

During our analyses of cimetidine, ranitidine (Fig. 1) has been used as an internal standard. In order to determine the drug levels in the range 0.2–10 $\mu$g/ml, sample preconcentration proved essential. We therefore combined a traditional solid-phase extraction with electrically driven elution to provide a sample treatment compatible with electrophoretic analysis. We have termed this method "electrochromatographic solid-phase extraction". Various experimental variables were further explored to ensure quantitative results, while the capabilities of providing pharmacologically relevant measurements were also considered.

## EXPERIMENTAL

### *Chemicals*

Cimetidine and ranitidine were a gift from Orion Pharmaceutica (Espoo, Finland). Hexadecyltrimethylammonium bromide (HTAB) and tris(hydroxymethyl)

aminomethane (TRIZMA base) were purchased from Sigma (St. Louis, MO, USA), glacial acetic acid, rosaniline hydrochloride (Magenta) and tetrahydrofuran (THF) from Fisher Scientific (Fair Lawn, NJ, USA), trifluoroacetic acid from Mallinkrodt (Paris, KY, USA), sodium hydrogenphosphate from Aldrich (Milwaukee, WI, USA) and acetonitrile (OmniSolv) from EM Science (Cherry Hill, NJ, USA). VWR 50-$\mu$l micropipets (Drummond Scientific, Broomall, PA, USA), and Kimble 20-$\mu$l microcapillary pipets (Toledo, OH, USA) were used for sample collection. Supelclean LC-18 1.0-ml SPE tubes ($C_{18}$, 100 mg, 40-$\mu$m particle size, 20-$\mu$m porous polyethylene frits) were obtained from Supelco (Bellefonte, PA, USA). Tuberculine 1-ml disposable syringes (Becton Dickinson, Rutherford, NJ, USA) were used for pressing liquids through sample cartridges. Nylon 66 membranes (0.2 $\mu$m) (Alltech, Deerfield, IL, USA) were used for filtering all buffer solutions. Water was purified by distillation and ion exchange.

*Micellar electrokinetic capillary chromatography*

A laboratory-made capillary electrophoresis apparatus described previously [24] was used. A high-voltage power supply (0–60 kV) was a product of Spellman High Voltage Electronics (Plainview, NY, USA). Negative voltages, $-18$ to $-23$ kV, were used (positive ground). The uncoated fused-silica capillary was 60 cm $\times$ 50 $\mu$m I.D. $\times$ 180 $\mu$m O.D. from Polymicro Technologies (Phoenix, AZ, USA). The polyimide coating was removed in a small area about 15 cm from the capillary end to form an on-column flow cell for UV detection. The detector was a Jasco UVIDEC-100-IV (Japan Spectroscopic, Tokyo, Japan) adjusted to 228 nm. Sample injection was performed hydrodynamically by dipping the capillary end into the sample solution for 10–15 s. The height difference between the injection point and buffer level was 13.5 cm to produce the necessary pressure gradient. The buffer system included the cationic detergent HTAB (9.8 m*M*), TRIZMA base (3.3 m*M*) and sodium hydrogenphosphate (9.4 m*M*) at pH 6.4.

A new capillary was rinsed with water for 30 min and, overnight, with 0.1 *M* sodium hydroxide solution. After a short wash with water, the buffer was stabilized for several hours, followed by injections of cimetidine standard solutions. The same buffer solution was used for at least 2 days continuously. When changing the buffer, rinsing for 15 min was performed. A short rinse with the buffer between injections was found to be necessary. The capillary remained in the buffer solution overnight.

When the separation system lost its efficiency, a short rinse with water (10 min), 45% trifluoroacetic acid (2 h) and water (10 min) restored its original characteristics. When using an acid wash, equilibration with the analytical buffer containing cationic detergent was easily established within 2 h. However, an alkaline wash was necessary with overnight equilibration.

*Spiked serum samples*

Stock cimetidine and ranitidine standard solutions of 5 mg/ml in methanol were prepared. Additional working standard solutions were prepared by appropriate dilutions. Cimetidine standard solutions (50 $\mu$l) were added to 0.5 ml of serum. In all samples, 5 $\mu$g per 5 $\mu$l of ranitidine were added as an internal standard (concentration 10 $\mu$g/ml in serum).

*Electrochromatographic solid-phase extraction*

Samples were concentrated on the reversed-phase $C_{18}$ (commercially packed cartridges, 6 mm I.D.). Solvents and samples were moved by means of a disposable 1-ml syringe in the voltage "off" mode (Fig. 2). The general procedure for the solid-phase extraction included the steps of conditioning, retention, washing and elution, as described previously [25,26].

The cartridge was subsequently activated with methanol (1 ml) by a syringe suction (downflow) (Fig. 2). The remaining methanol was further removed with 1.0 ml of water, while the cartridge was conditioned to pH 7.7 with 1.0 ml of buffer (10 m*M* TRIZMA–3.2 m*M* acetic acid, buffer TAC-2). Serum (0.5 ml) was diluted (1:1) with a buffer (40 m*M* TRIZMA–13 m*M* acetic acid, buffer TAC-8). Sample was dispensed by means of a 1.0-ml syringe, from the bottom of the cartridge to the upflow direction, followed by washing with 1.0 ml of water in the same direction. Finally, water in the cartridge was compensated for in the suction mode with 160 $\mu$l of the elution solvent [tetrahydrofuran–buffer, 5 m*M* TRIZMA–1.7 m*M* acetic acid (pH 7.7) (50:50)].

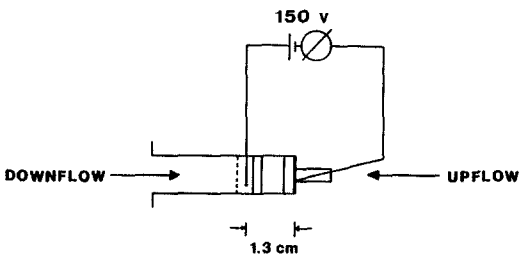


Fig. 2. Electrochromatographic solid-phase extraction set-up. Retention of the drug: voltage off/upflow direction with pressure. Elution: voltage on/flow towards negative electrode (downflow).

During elution of the sample, an electrical field was applied as a driving force. The cartridge was set in a horizontal position between the platinum electrodes, as shown in Fig. 2 (a hole was drilled in the cartridge for the positive electrode, which was in contact with the elution solvent). The negative electrode was sharpened and attached to the front part of the polyethylene frit.

## RESULTS AND DISCUSSION

While developing the solid-phase electrochromatographic technique, several situations had to be recognized during retention and elution of the analytes of interest. These were different from the previously used elution mode using a pressure gradient.

Preconcentration of cimetidine on a hydrophobic octadecyl phase was previously found to be effective [5,7–11]. The interaction between cimetidine and the $C_{18}$ phase is strong at pH 7.7 where cimetidine appears in its neutral, secondary amine form. However, the known disadvantages of the $C_{18}$ phase are its limited selectivity and the ionic activity of the free silanol groups [27]. Consequently, the roles of TRIZMA buffer during the cartridge conditioning and as a diluting buffer for the serum

sample were assessed as necessary to both adjusting pH and covering the active silanol sites, in order to prevent strong interactions with cimetidine molecules [25].

When introducing the serum samples into the cartridge at the same side (upflow, Fig. 2) as that where the analytes were driven out (downflow, Fig. 2) with the eluting solvent, the elution volume and time could be effectively decreased. This is analogous to the coupled-column backflush mode in HPLC [28]. Water served for washing out the salts, while leaving part of the serum proteins on the cartridge.

During the electrically driven elution, solvents of medium polarity were necessary to overcome the interactions between the solute of interest and the octadecyl moieties [25,26]. While selecting the percentage and type of eluting solvent, rosaniline (aromatic primary amine) was used as a marker due in spiked cimetidine aqueous samples or the serum samples. Eluting colored zones were observed visually and the cimetidine concentrations were measured in collected (colored) sample fractions. Cimetidine and the dye eluted together. According to these results, the solvent-to-buffer ratio was selected. Tetrahydrofuran, acetonitrile and methanol were all tested in a 50:50 solvent–50% buffer [3.7 m*M* TRIZMA–1.8 m*M* acetic acid (pH 7.7)] ratio. Table I summarizes certain important characteristics of these studied solvents.

For the three different solvents, fractions were evaluated for their content of cimetidine (contained originally at 1.85 $\mu$g per 0.5 ml of serum). For the elution procedure driven by 150 V, the retention and elution steps were repeated 3–6 times with each solvent. Fig. 3 shows the corresponding elution profiles. Tetrahydrofuran with the lowest dielectric constant and the largest elution strength on ODS (Table I) resulted in the most effective medium.

The electrically driven elution (150 V) was further compared with a pressurized elution (syringe action). While the cimetidine elution trends were similar (Fig. 4), there was a substantial difference in precision (5.9 *vs.* 18.9% in favor of the electrically driven elution). In addition, it was considerably easier to collect precisely the relatively small volumes in the electrical mode. Table II shows the calculated concentration factors together with standard deviations (S.D.s) acquired when cimetidine peak heights were compared with those in the standard solutions and serum samples.

Elution flow stability was found to be influenced by the applied voltage and percentage and type of solvent, and also the mobility of ions in the buffer solution. At

TABLE I

PROPERTIES OF SOLVENTS USED IN ELECTROCHROMATOGRAPHIC MEASUREMENTS

| Solvents[a] | Elution strength on ODS[b] | Viscosity, $\eta$(cP) (25°C)[c] | Dielectric constant, $\varepsilon$ (20°C)[c] |
|---|---|---|---|
| THF | 3.7 | 0.55[d] | 7.6 |
| ACN | 3.1 | 0.34 | 37.5 |
| $CH_3OH$ | 1.0 | 0.55 | 32.6[e] |

[a] THF = tetrahydrofuran; ACN = acetonitrile; $CH_3OH$ = methanol.
[b] Ref. 29.
[c] Ref. 30.
[d] 20°C.
[e] 25°C.

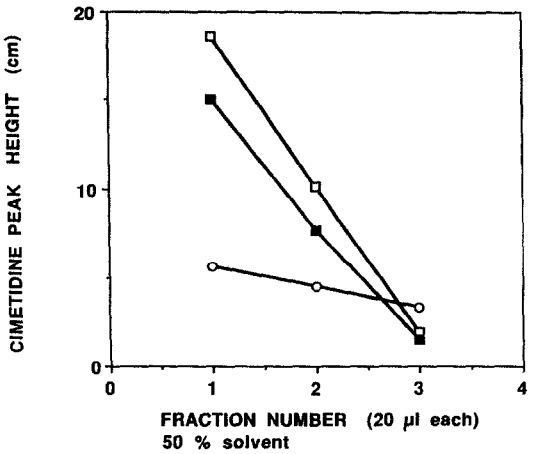


Fig. 3. Elution profiles of cimetidine with different solvent–buffer (50:50) compositions on a 100-mg $C_{18}$ cartridge. Cimetidine peak heights were measured in three successively collected 20-µl sample fractions ($n$ = 3–6). □ = THF; ■ = acetonitrile; ○ = methanol.

150 V, flow-rates were stable and Joule heating was insignificant. With a voltage increase to 200 V, bubbles were generated with all the solvents studied. The tetrahydrofuran–buffer mixture used was found to be the most stable and effective. The flow-rates experienced with the methanol–buffer medium were highly variable, while the elution strength was also insufficient.

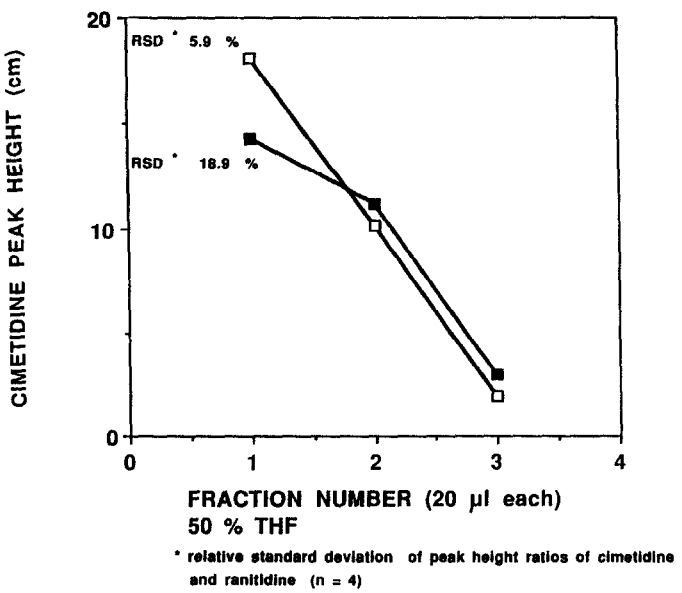


Fig. 4. Elution profiles of cimetidine with (□) the electrical elution mode, R.S.D. = 5.9% ($n$ = 4), and (■) pressure mode, R.S.D. = 18.9% ($n$ = 4). Cimetidine peak heights were measured in the fractions obtained. For calculation of precision data, the cimetidine–ranitidine peak-height ratio was evaluated.

TABLE II

CONCENTRATION FACTORS FOR CIMETIDINE IN SERUM SAMPLE: ENRICHMENT BY ELECTRICAL (E) AND PRESSURE (P) ELUTION MODE

| Solvent (50%) | Elution mode | Concentration factor | S.D. | *n* | Flow-rate (μl/min) |
|---|---|---|---|---|---|
| THF | E | 13 | 2 | 6 | 40 |
| THF | P | 8 | 2 | 5 | –[a] |
| ACN | E | 10 | 3 | 3 | 60 |
| MeOH | E | 4 | 2 | 3 | 30–60 |

[a] Not measured.

Tsuda [22] reported the use of a pressurized system to avoid bubbles in electrochromatography. In our experience, a non-pressurized system is effective provided that the high-mobility ions (*e.g.*, buffers containing sodium or potassium) are avoided. Otherwise, inappropriately high flow-rates, cartridge overheating and formation of bubbles typically occur.

For precise sample introduction, it is desirable to match the composition of the solution eluting from the cartridge with that of a buffer used in the analytical MECC. Table III summarizes the types and concentrations of the respective buffers. Hexadecyltrimethylammonium bromide (HTAB) was used here at concentrations above the critical micelle concentration. The flow direction was oriented toward the positive electrode, as reported earlier by Liu *et al.* [31]. Under stable MECC conditions, the precision [relative standard deviation (R.S.D.)] of the cimetidine retention was 2.5% ($n = 6$), and for the cimetidine–ranitidine peak-height ratio, the R.S.D. was 1.0% ($n = 6$) (concentration 3.7 μg/ml).

The method was found to be linear ($r > 0.999$) using both 20- and 50-μl collected fraction volumes. The linear regression calibration data for the peak-height ratios of cimetidine and the internal standard ranitidine from electrochromatographically extracted serum samples are shown in Table IV.

TABLE III

BUFFERS, SOLVENTS AND CONDITIONS USED

| Parameter | Eluting solvent | MECC buffer |
|---|---|---|
| THF | 50% | – |
| TRIZMA | 3.7 m*M* | 3.3 m*M* |
| Acetic acid | 1.8 m*M* | – |
| HTAB | – | 9.8 m*M* |
| $NaH_2PO_4$ | – | 9.4 m*M* |
| pH | 7.7 | 6.4 |
| Flow direction | To cathode | To anode |
| Voltage, current | +150 V, 0.5 mA | –20 kV, 11–13 μA |
| Tube radius | 6 mm | 50 μm |

TABLE IV

LINEAR REGRESSION CALIBRATION DATA FOR SPIKED CIMETIDINE SERUM SAMPLES

| Range (μg/ml) | No. of points, *n* | Correlation coefficient, *r* | *x*-Axis intercept | *y*-Axis intercept | Collected volume (μl) |
|---|---|---|---|---|---|
| 0.233–11.4 | 6 | 0.9991 | 0.126 | −0.016 | 50 |
| 0.233–11.4 | 5 | 0.9993 | 0.047 | −0.004 | 20 |

Precision was studied with repeated analyses of 0.5-ml serum samples at concentrations of 0.47 and 3.7 μg/ml. The R.S.D. values were 9.2% and 4.5% ($n = 4$), respectively. The peak-height ratios for cimetidine and the internal standard ranitidine were also measured. Accuracy (relative error) was calculated by using the linear regression calibration graph for the measurement data. The results are shown in Tables V and VI. Means ($x$), standard deviation (S.D.) values and R.S.D. values were calculated ($n = 4$).

Drug recovery was estimated by comparing the peak heights of cimetidine in the first eluted 20- or 50-μl fractions with a standard solution sample, which was

TABLE V

PRECISION AND ACCURACY FOR SPIKED SERUM SAMPLES (0.47 μg/ml OF CIMETIDINE)

| | Cimetidine–ranitidine peak-height ratio | Added (μg) | Found (μg) | Relative error (%) |
|---|---|---|---|---|
| | 0.076 | 0.233 | 0.262 | +12.4 |
| | 0.079 | 0.233 | 0.276 | +18.5 |
| | 0.082 | 0.233 | 0.289 | +24.0 |
| | 0.066 | 0.233 | 0.219 | − 6.0 |
| *x* | 0.076 | | 0.261 | +12.2 |
| S.D. | 0.007 | | 0.03 | 13 |
| R.S.D. (%) | 9.2 | | 11.6 | |
| *n* | 4 | | 4 | 4 |

TABLE VI

PRECISION AND ACCURACY FOR SPIKED SERUM SAMPLES (3.7 μg/ml OF CIMETIDINE)

| | Cimetidine–ranitidine peak-height ratio | Added (μg) | Found (μg) | Relative error (%) |
|---|---|---|---|---|
| | 0.402 | 1.85 | 1.71 | −7.6 |
| | 0.428 | 1.85 | 1.83 | −1.1 |
| | 0.446 | 1.85 | 1.91 | +3.2 |
| | 0.414 | 1.85 | 1.77 | −4.3 |
| *x* | 0.423 | | 1.81 | −2.5 |
| S.D. | 0.019 | | 0.085 | 4.6 |
| R.S.D. (%) | 4.5 | | 4.7 | |
| *n* | 4 | | 4 | 4 |

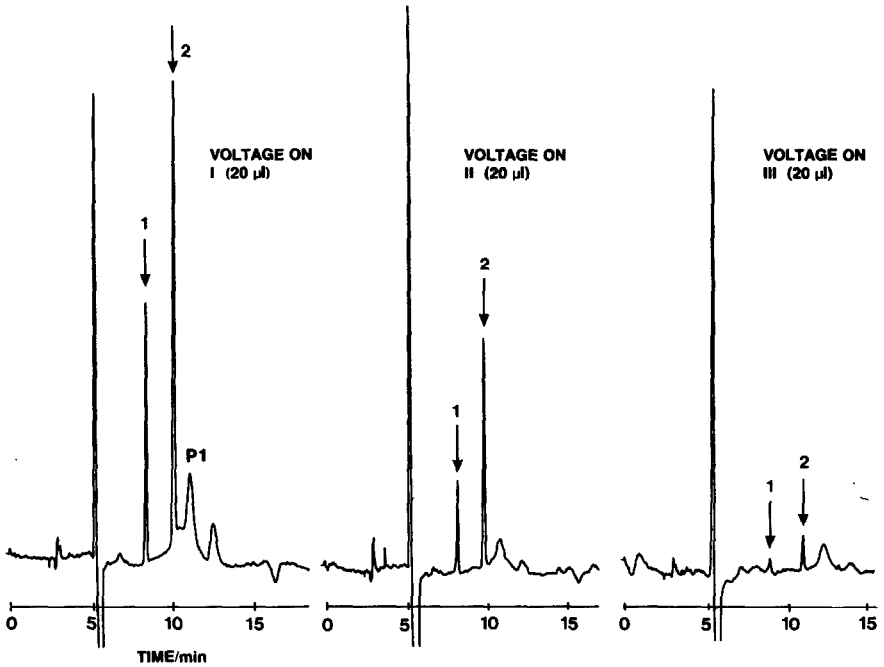


Fig. 5. Series of electropherograms for successively collected 20-µl fractions (I–III) after electrical elution (3.7 µg/ml of cimetidine in serum). 1 = Cimetidine; 2 = ranitidine; P1 = unknown serum background component.

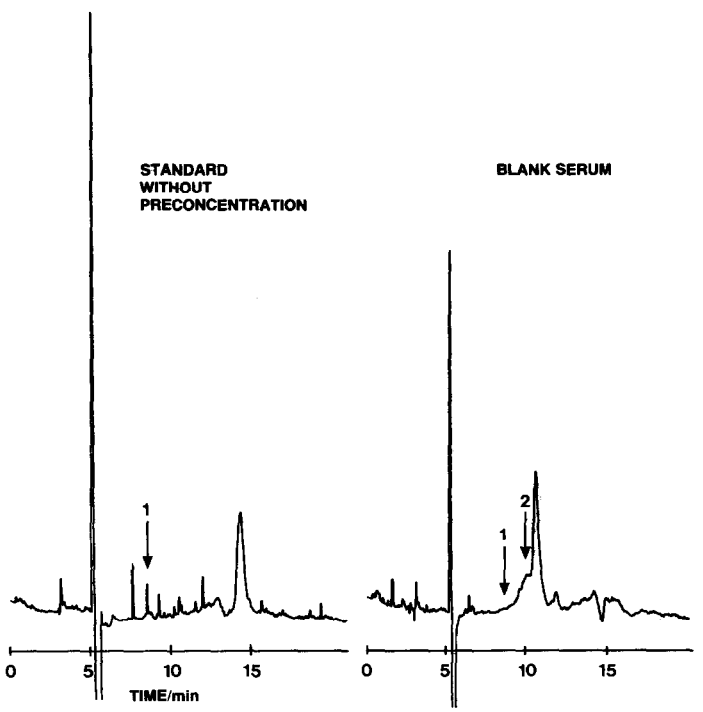


Fig. 6. Cimetidine standard (11.4 µg/ml) in THF–buffer (50:50) without preconcentration and a blank serum extract after electrochromatographic preconcentration. 1 = Cimetidine; 2 = ranitidine.

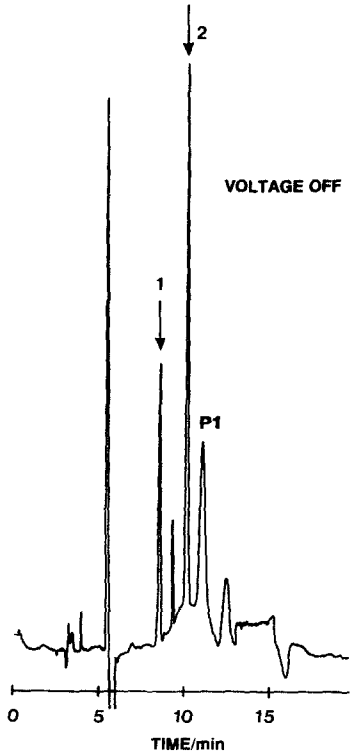


Fig. 7. Electropherogram of a cimetidine serum sample enriched by pressure elution. 1 = Cimetidine; 2 = ranitidine; P1 = unknown serum background compound.

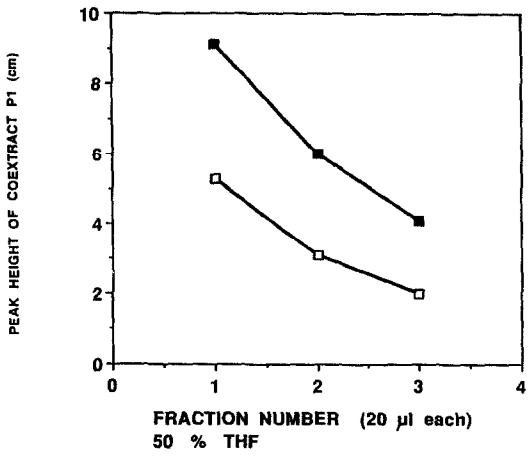


Fig. 8. Elution profiles of unknown serum background component P1 in the (□) electrochromatographic elution (150 V) and (■) pressure elution modes.

prepared in the eluting solvent [THF–buffer (50:50)]. Recoveries were 74% (S.D. = 15%, $n$ = 4) and 93% (S.D. = 2%, $n$ = 4) for 20- and 50-$\mu$l samples, respectively. Fig. 5 shows electropherograms for three successive electrically eluted 20-$\mu$l sample fractions. Fig. 6 compares the cimetidine standard (11.4 $\mu$g/ml) prepared in an elution solvent mixture without preconcentration with a blank serum extract after electrical preconcentration.

The electrochromatographic mode of elution appeared to have a beneficial effect on sample purification. Fig. 7 shows the appearance of an unidentified serum component (labeled P1) in the electropherogram of the cimetidine serum sample eluted by pressure. Electrochromatographic elution decreased the amount of this unwanted component by *ca.* 40% [Fig. 5, I (20 $\mu$l)]. Fig. 8 shows the elution profiles of compound P1 in both elution modes for three successively eluted 20-$\mu$l fractions.

Overall, the proposed electrochromatographic solid-phase extraction procedure serves to achieve a *ca.* 10–15-fold increase in sample concentration for cimetidine, while decreasing the occurrence of interfering peaks. Through the use of internal standards, satisfactory analytical results could be obtained. Further, such results were achieved with just a laboratory-built apparatus, and considerably better accuracy and reproducibility are to be expected from the rapidly emerging commercial instruments.

## ACKNOWLEDGEMENTS

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