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Determination of some cardiovascular drugs in serum and urine by capillary isotachophoresis

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Abstract

The separation and determination of amiloride, metoprolol, deacetylmetipranolol, labetalol and furosemide in human serum and urine by capillary isotachophoresis were investigated. Amiloride and β-blockers were separated by cationic isotachophoresis in the electrolyte system sodium morpholinoethanesulfonate buffer (pH 5.5) (c_L = 10 m*M*)–glutamic acid. Furosemide was separated using the anionic electrolyte system histidine hydrochloride buffer (pH 6.2) (c_L = 10 m*M*)–morpholinopropanesulfonic acid. Endogenous and the possible exogenous compounds were almost totally removed from serum and urine by solid-phase extraction using a Separon SGX C_{18} cartridge. The recovery of compounds varied from 98.2 to 103.2%. The linearity range for the compounds was 50–1000 ng/ml. The relative standard deviations varied from 0.1 to 5.6%. The overall limits of determination ranged from 32 to 46 ng/ml of urine and from 39 to 46 ng/ml of serum, depending of the type of drugs.

Keywords: Isotachophoresis; Drugs; β-Blockers

1. Introduction

Hypertension is a common and usually progressive disorder, which, if not effectively treated, has a high mortality resulting from a greatly increased probability of coronary thrombosis, strokes and renal failure.

The basic antihypertensive drugs are diuretics and β-blocking agents that can be prescribed in initial therapy of mild hypertension alone. For patients who have failed to respond to diuretics or β-blockers, β-blockers in combination with diuretics are indicated. Thiazide diuretics or furosemide increase urinary potassium excretion and can cause hypokaliaemia in subjects treated with these medications during term-maintenance therapy. Therefore, thiazides are often prescribed in association with potassium-sparing diurectics (e.g., amiloride) in order to maintain an appropriate body content of electrolytes [1].

The separation of β-blockers has been investigated by many techniques, such as HPLC [2-6], GC–MS [7], capillary zone electrophoresis (CZE) [8–10] and MEKC micellar electrokinetic chromatography [11–14]. A number of screening methods have been published for diuretics based on urine samples and HPLC [15–17] and GC–MS [18,19]. With the CZE method, urine and serum can be effectively screened for diuretics [20–22].

In this paper, we present a capillary isotachophoretic (ITP) method for the determina-

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tion of β-blockers (metoprolol, deacetylmetipranolol and labetalol) and diurectics (amiloride and furosemide) in serum and urine. The linear calibration range was studied for eventual application of the method to real samples obtained by oral administration of the drug.

2. Experimental

2.1. Apparatus

Isotachopherograms were obtained by the use of a Villa Labeco ZKI 02 isotachophoretic analyser equipped with a conductivity detector. The analytical column (160 mm × 0.3 mm I.D.) was connected with a preseparation column (90 mm × 0.80 mm I.D.). The voltage varied between 1 and 15 kV. The measurement data were acquired with a PC/AT with a 12-bit A/D–D/A converter at 100 Hz sampling frequency. For solution pH measurement, a model OP-211 pH meter (Radelkis, Hungary) with a combined glass electrode was employed.

2.2. Materials

All chemicals were of the highest quality commercially available. Deionized, redistilled water was used in the preparation of the electrolyte systems and of the solutions of the model mixtures.

Labetalol hydrochloride, metoprolol tartrate, deacetylmetipranolol (DAmetipranolol), amiloride hydrochloride and furosemide was obtained from the State Institute for the Control of Drugs (Bratislava, Slovak Republic). Control serum was obtained from Imuna Šarišské Michal'any.

Solid-phase extractions of urine and blood serum were carried out with Separon SGX C_{18} tubes (Laboratorní přístroje, Prague, Czech Republic).

2.3. Standard solutions and samples

A stock standard solution of each drug was prepared by dissolving 10.0 mg of the compound in 100.0 ml of methanol (deacetylmetipranolol and furosemide) or water (amiloride, labetalol and metoprolol). These solutions were stored in the dark at 4°C. Working standard solutions were prepared by appropriate dilution. Samples were prepared by adding various amounts of the standard mixture of the drugs to serum and urine.

2.4. ITP operating system

2.4.1. Cationic system

The leading electrolyte was 10 m*M* sodium morpholinoethanesulfonate (pH 5.5)–0.1% methylhydroxyethylcellulose and the terminating electrolyte was 5 m*M* glutamic acid. The driving current for the preseparation column was 150 μA and for the analytical column was 20 μA (separation) and 10 μA (detection).

2.4.2. Anionic system

The leading electrolyte was 10 m*M* histidine–HCl (pH 6.2)–0.1% methylhydroxyethylcellulose and the terminating electrolyte was 5 m*M* morpholinopropanesulfonic acid. The driving current for the preseparation column was 250 μA and for the analytical column 40 μA (separation) and 10 μA (detection).

2.5. Preparation of the serum and urine samples

A 1-ml volume of control serum or urine was spiked with a solution containing accurately known amounts of drugs. Endogenous and the possible exogenous compounds were almost totally removed from serum and urine by solid-phase extraction. A Separon SGX C_{18} cartridge was activated and conditioned by successive washing with 2 ml of methanol and 1 ml of distilled water. A mixture of 1.0 ml of serum or urine spiked with a mixture of standards was loaded on the Separon SGX C_{18} cartridge. The serum and urine samples passed through the cartridge at low pressure in 2–4 min. The cartridge was washed with 2 ml of water and the substances were eluted with 1 ml of acetronitrile–dichloromethane (75:25) (amiloride,

furosemide) and 1 ml of acetronitrile–dichloromethane (25:75) (metoprolol, deacetylmetipranolol and labetalol). These eluents were found to be more efficient than methanol, acetonitrile and other mixtures tested.

The total eluate was evaporated to dryness with a stream of nitrogen, the residue was dissolved in 0.5 ml of ethanol and an aliquot (50–100 μl) was injected.

3. Results and discussion

β-blockers and amiloride, containing an amino group, were separated at acidic pH as cations; furosemide, containing a carboxylic group, was separated as the anion.

Although ITP is also an excellent separation technique, the separation of β-blockers and amiloride by ITP is difficult because of the similarity of their structures and characteristics. To find suitable separation conditions, the effect of the pH of the leading electrolyte on the separation was studied. Na^+ was used as the leading ion in all instances and the pH was adjusted to 3.4–5.0 with acetic acid or to 5.5–6.5 with morpholinoethanesulfonic acid. In order to suppress the electroosmotic flow, 0.1% of methylhydroxyethylcellulose was added.

The terminating electrolyte was always selected at a given pH such that it was well separated from the other compounds in the sample and permitted the use of the highest possible driving current and thus also the attainment the shortest possible separation time. In all systems tested, the differences between the relative step height (R.S.H.) values are acceptable, but metoprolol and deactylmetipranolol can be distinguished in mixtures only using the cationic system mentioned above. Under the other electrolyte conditions used, the effective mobilities of metoprolol and deacetylmetipranolol in the mixed zone were almost identical, so the compounds could not be separated.

Fig. 1 illustrates the isotachopherograms obtained from extracts of (a) serum and (b) urine samples containing a mixture of five drugs each at a concentration of 200 ng/ml using the cationic system. The complete separation of

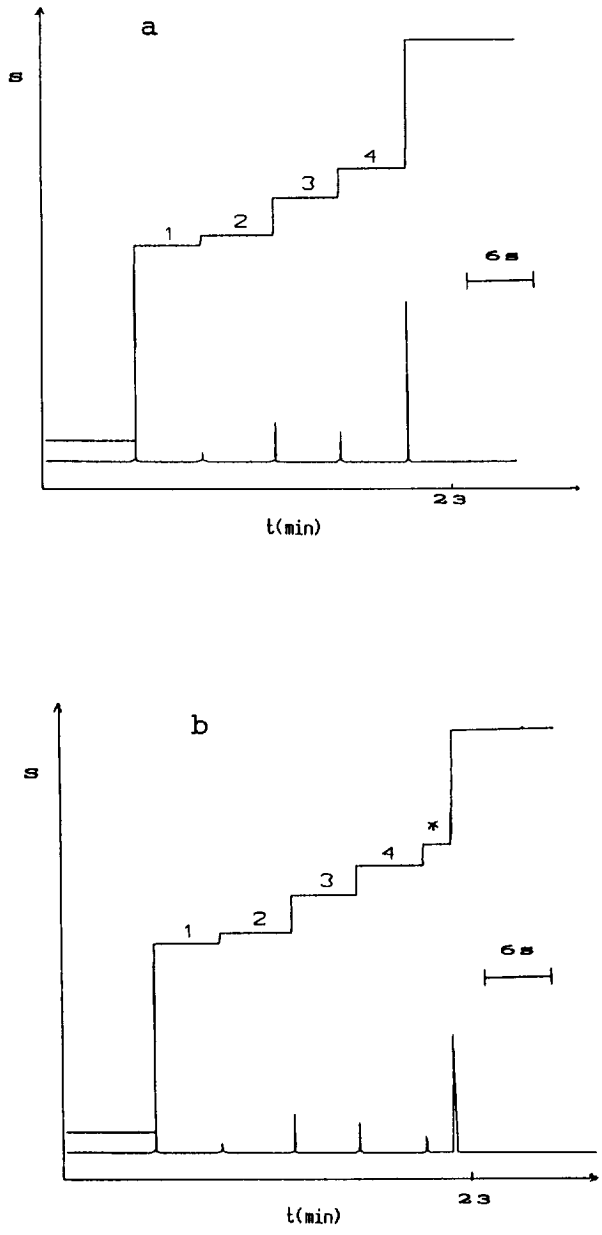

Fig. 1. Isotachopherograms obtained from extracts of (a) serum and (b) urine samples containing a mixture of five drugs (amiloride, metoprolol, deacetylmetipranolol, labetalol and furosemide) at concentrations of 200 ng/ml each using the cationic system. Leading electrolyte, 10 m*M* sodium morpholinoethanesulfonic acid (pH 5.5)–(0.1% methylhydroxyethylcellulose; terminating electrolyte; 5 m*M* glutamic acid; driving current, preseparation column 150 μA, analytical column 20 μA (separation) and 10 μA (detection); conductivity detection. 1 = Amiloride; 2 = metoprolol; 3 = deacetylmetipranolol; 4 = labetalol; * = unidentified zone from urine.

amiloride, metoprolol, deacetylmetipranolol and labetalol was achieved. No compounds present in serum and urine interfered with the analytes.

Fig. 2 shows the same type of isotachopherograms obtained with the anionic system.

3.1. Calibration

Calibration was performed by analysing 1.0-ml aliquots of blank serum to which 50–1000 ng of each drug had been added or 1.0-ml aliquots of blank urine to which 50–5000 ng of each drug had been added. Five replicate analyses were carried out at each concentration and seven concentrations were used for each calibration graph. The linearity range for the compounds was 50–1000 ng. At amounts higher than 1000 ng, the calibration graphs were parabolic. The correlation coefficients of the straight lines ranged from 0.997 to 0.999 (Table 1).

3.2. Recovery and repeatability

Aliquots (1 ml) of serum or urine were spiked with 100, 500 and 750 ng (serum) or 100, 1000 and 2500 ng (urine) of the drugs. After the samples had been extracted and analysed as described, the zone length of each drug was compared with that obtained when the same amount of each drug in the absence of serum or urine which had not undergone extraction was analysed. Five replicate analyses were carried out at each concentration. The recovery of the compounds was studied by individual spiking, but all five drugs were always included in the solution. The recovery and repeatability results for each drug are given in Tables 2 and 3. The recoveries of the compounds varied from 98.2 to 103.2% and the relative standard deviations varied from 0.1 to 5.6%.

3.3. Determination limits

Table 4 shows the therapeutic level and the sensitivity of the procedure using the cationic and anionic systems. The overall limits of determination range from 32 to 46 ng/ml for urine and from 39 to 46 ng/ml for serum, depending on the type of drug. As the therapeutic blood level of amiloride is generally 0.2–10 ng/ml, the method is not sensitive enough for routine analysis.

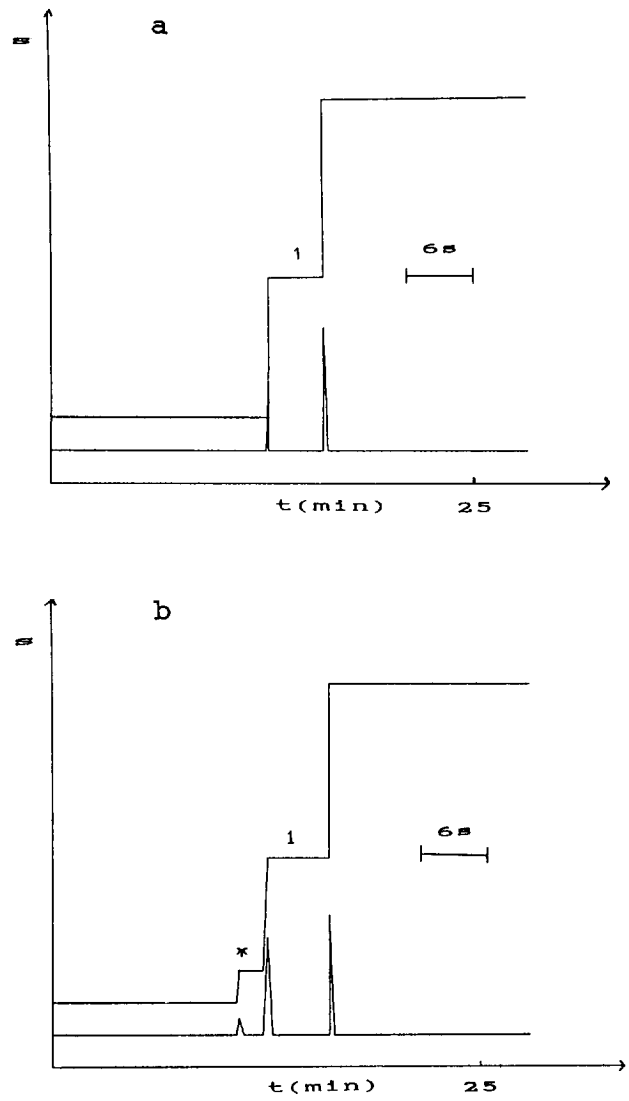

Fig. 2. Isotachopherograms obtained from extracts of (a) serum and (b) urine samples containing a mixture of five drugs (amiloride, metoprolol, deacetylmetipranolol, labetalol and furosemide) at concentrations of 200 ng/ml each using the anionic system. Leading electrolyte, 10 m*M* histidine–HCl (pH 6.2)–0.1% methylhydroxyethylcellulose; terminating electrolyte, 5 m*M* morpholinopropanesulfonic acid; driving current, preseparation column 250 μA, analytical column 40 μA (separation) and 10 μA (detection); conductivity detection. 1 = furosemide; * = unidentified zone from urine.

Table 1
Linearity of the method in the range 50–1000 ng/ml

Compound	Serum			Urine		
	a	*b*	*r*	*a*	*b*	*r*
Amiloride	0.06	69.81	0.998	0.03	70.69	0.999
Metoprolol	0.04	76.40	0.998	0.05	77.88	0.999
DAmetipranolol	0.02	68.65	0.999	0.01	68.87	0.999
Labetalol	0.08	70.81	0.999	0.06	71.47	0.999
Furosemide	0.09	65.05	0.998	0.07	65.16	0.999

Concentrations of the drugs of 50, 100, 200, 300, 500, 750 and 1000 ng/ml were studied. The equation for the straight line is $y = a + bx$, where y is the zone length (mm), a is the intercept on the ordinate, b is the slope (absolute amount injected, μg) and r is the correlation coefficient.

Table 2
Recovery and repeatability of the method at levels of 100, 500 and 750 ng/ml in serum

Compound	Concentration (ng/ml)					
	100		500		750	
	Recovery[a] (%)	R.S.D. (%)	Recovery[a] (%)	R.S.D. (%)	Recovery[a] (%)	R.S.D. (%)
Amiloride	99.7	2.9	98.6	1.6	98.2	0.9
Metoprolol	100.2	3.8	99.5	2.1	99.3	0.9
DAmetipranolol	98.9	5.1	98.7	5.6	98.5	1.4
Labetalol	99.0	3.5	98.9	2.7	98.4	1.1
Furosemide	99.4	1.8	99.0	1.1	98.9	0.7

[a] Mean of five determinations.

Table 3
Recovery and repeatability of the method at levels of 100 and 1000 and 2500 ng/ml in urine

Compound	Concentration (ng/ml)					
	100		1000		2500	
	Recovery[a] (%)	R.S.D. (%)	Recovery[a] (%)	R.S.D. (%)	Recovery[a] (%)	R.S.D. (%)
Amiloride	101.6	1.7	99.8	1.3	99.6	0.2
Metoprolol	103.2	2.2	101.1	0.7	100.5	0.2
DAmetipranolol	99.1	3.4	99.2	2.1	98.6	0.9
Labetalol	99.8	3.8	99.7	2.6	98.7	0.2
Furosemide	100.3	1.3	99.8	1.2	99.3	0.1

[a] Mean of five determinations.

Table 4
Determination limits (LD) and therapeutic levels (TL)

Compound	Serum		Urine	
	LD (ng/ml)	TL (ng/ml)	LD (ng/ml)	TL (ng/ml)
Amiloride	43	0.2–15	42	2000–400 000
Metoprolol	39	12–220	32	200–20 000
DAmetipranolol	44	3000–12 000	36	–
Labetalol	42	2–800	35	20–4000
Furosemide	46	–	46	2000–100 000

3.4. Real samples

Furosemide and deacetylmetipranolol were determined in serum and urine samples, taken 4 h after oral application of 40 mg of furosemide (SPOFA) and 10 mg of trimepranol tablets.

4. Conclusion

The results presented suggest that ITP can easily be used for the determination of metoprolol, deacetylmetipranolol, labetalol and furosemide in human serum or urine and amiloride in urine. Solid-phase extraction of the tested drugs using a Separon SGX C_{18} cartridge has been shown to provide extracts that are sufficiently clean and concentrated for ITP analysis.

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