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Direct determination of procainamide and N-acetylprocainamide by capillary zone electrophoresis in pharmaceutical formulations and urine

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Abstract

In this work a new sensitive capillary zone electrophoresis method for the direct determination of procainamide (PA) and N-acetylprocainamide (NAPA) in pharmaceutical formulations and urine samples without any extraction and/or preconcentration steps has been developed. The determination was carried out in a fused-silica capillary of 43.5 cm (35.9 cm length to the detector)×0.75 μm I.D. Phosphate 0.05 *M* buffer was used as the background electrolyte and 10 kV separation voltage was applied. The separation of PA and NAPA is possible in a wide range of pH from 1.7 to 9.7. However, in order to avoid the effect of the urine matrix, it is optimal to work at pH 7.7. The determination of PA and NAPA takes less than 5 min while high resolution is achieved. The detection limits obtained, 1.235 μg/ml and 0.359 μg/ml for PA and NAPA respectively, are lower than those for GC method normally reported.

Keywords: Pharmaceutical analysis; Procainamide; Acetylprocainamide

1. Introduction

Procainamide (PA) is a widely used drug applied against atrial and ventricular arrhythmias. Its principal metabolic pathway in the liver is its acetylation under the formation of N-acetylprocainamide (NAPA). The decrease of the hepatic capacity for N-acetylation of PA may indicate a genetic defect of a liver enzyme, polymorphic N-acetyltransferase (NAT). There are about 55–60% of individuals with a genetic defect of NAT (slow acetylators, SAs) and 40–45% of individuals with a normal rate of acetylation (rapid acetylators, RAs) within the European and white American population. SAs are generally more prone to the adverse and even toxic effects of polymorphically acetylated drugs due to their low metabolic capacity [1–4]. However, RAs rapidly acetylate PA and therefore develop high levels of NAPA. Accumulation of this active metabolite in the organism may also cause adverse effects. Phenotyping test, which can be performed with PA or another test drug, is very important to classify RAs or SAs, and is used for establishing optimal dose schedules in patients treated with polymorphically acetylated drugs [5].

At present, PA and NAPA are analyzed by gas chromatography [6], spectrophotometry [7], fluorescence polarization immunoassay [8] and ion selective electrode method [9]. The level of PA has officially been determined by titrimetry with sodium nitrite [10]. However, pre-treatment of the sample is necessary in most of the cases. Capillary zone electrophoresis (CZE) has been established as an alternative

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method to GC and HPLC for studies of the metabolism and pharmacokinetics of drugs [11–13]. The method is very useful for therapeutic drug monitoring in hospitals because of the ability of automation and short analysis time. In this work we have studied the possibility of CZE to develop a new sensitive method for the direct determination of PA and NAPA in pharmaceutical formulations and urine samples without any pre-treatment and/or preconcentration steps.

2. Experimental

2.1. Reagents

Procainamide hydrochloride and N-acetylprocainamide hydrochloride were supplied by Sigma (St. Louis, MO, USA). Lyophilized urine was from Chemie Biotrol (Paris, France). The phosphoric acid and sodium hydroxide were of analytical grade from Lachema Chemapol (Brno, Czech Republic). Mesityl oxide (MSO) used as a marker for electroosmotic flow (EOF) determination was from Fluka (Buchs, Switzerland). The standard pH buffers were from the Institute of Serum and Vaccines (Prague, Czech Republic). Deionized water was used to prepare all the solutions and it was double-distilled from a quartz still Heraeus Quarzschmelze (Hanau, Germany.

2.2. Apparatus

Beckman CZE apparatus, model P/ACE System 5500 (Palo Alto, CA, USA) equipped with a diode array detection (DAD) system, automatic injector, a fluid-cooled column cartridge and a System Gold Data station was utilized for all experiments. A fused-silica capillary with 75 μm of I.D. and 43.5 cm total length (35.9 cm length to the detector) was used. Radelkis OP-208 Precision Digital pH meter (Budapest, Hungary) and a Radelkis pH sensitive, combination glass electrode (Budapest, Hungary) were applied for the pH measurements.

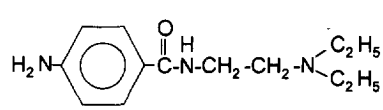
2.3. Procedure

All experiments were carried out at 30°C. The 50 m*M* phosphate buffer, used throughout this study, was prepared daily, passed through 0.2 μm nylon filters and submitted to Ultrasonic Cleaner (Branson, USA). The pH was adjusted with diluted solutions of NaOH and/or HCl. The capillary inlet and outlet vials were replenished after every ten injections. For each urine sample to be analyzed 100 μl were diluted to 1 ml (1:10, v/v) with deionized water. The capillary was every day washed for 10 min 0.1 *M* NaOH, 10 min with deionized water and 10 min background electrolyte (BGE) buffer solution. Each analysis consisted of 2 min of pre-wash with BGE, and sample hydrodynamic (pressure) injection 5 s, 3447.38 Pa (0.5 p.s.i.). The separation potential was 10 kV and the absorbance was monitored at 200 nm. The electroosmotic flow (EOF) was determined using 0.1% MSO (v/v) under the same conditions as for the separation of the samples.

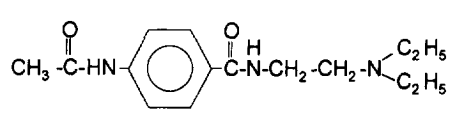
Rat urine was collected for a given time interval after the intravenous injection of PA (50 mg/kg). Human urine was collected for 7 h after the oral administration of PA (APO-Procainamide, one capsule 250 mg) into the glass vial. The total urine volumes were measured and samples (10 ml) were kept at 6–20°C until assayed.

3. Results and discussion

The molecular structures of PA and NAPA are presented in Fig. 1. They can give information about the possible ways how to separate them and about their acidobasic properties. In both cases there are three nitrogen atoms able to be protonized, with pK_a



PROCAINAMIDE



N-ACETYLPROCAINAMIDE

Fig. 1. Chemical structure of PA and NAPA.

values starting from about 9–10 for ternary nitrogen down to 4–5 for the amino-group. The absorption spectra of the compounds (in aqueous solution) show two maxima, at 200 and 280 nm for PA, and at 200 and 268 nm for NAPA (Fig. 2). The detection during CZE measurements was then made at 200 mm where the response was the most sensitive.

3.1. Separation of PA and NAPA in pure solutions

The compounds are present in the form of cations (pH range 1.7 to 9.5) in agreement with reported pK_a (9.23) value for PA [14]. Their effective mobilities were determined in the pH range from 1.7 to 10.6, using 0.1% MSO as a neutral marker (Fig. 3). Due to the sufficiently large difference in the mobility values, the separation of PA and NAPA was possible in a wide range of pH from 1.7 to 9.7. The acetylated amine group in NAPA provides the sufficient change in the molecule with respect to PA to make the difference in the mobilities. Fig. 4 shows the values of resolution in the whole pH range, which can be acceptable ($R_s>1$). Observed mobility changes could be due to the acid–basic behavior of the molecules.

The optimal separation voltage value was determined from the graph of observed current versus applied voltage (Fig. 5), sometimes called Ohm's law plot, $E=I\,R$, where R is the resistance and I the current. Voltage, V, can be plotted instead of the electric field, E, because the capillary length is constant. The maximum voltage that should be used is indicated by the point at which non-linearity

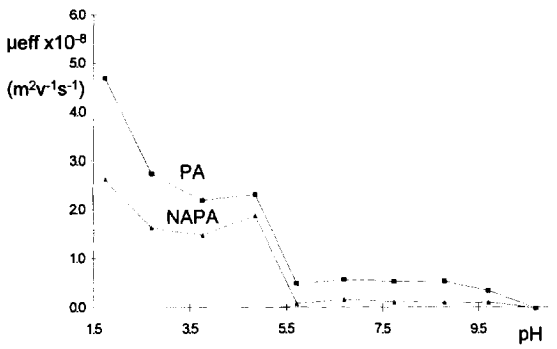


Fig. 3. Effective mobilities as a function of pH. Concentrations: PA $1.18\cdot10^{-4}$ M and NAPA $1.13\cdot10^{-4}$ M, using 0.1% MSO as a neutral marker for the electroosmotic flow. The capillary effective length was 35.9 cm, total length 43.5 cm, applied voltage 10 kV, pressure injection 5 s, 0.5 p.s.i. (1 p.s.i.=6894.76 Pa). Phosphate 0.1 M buffer was used as the background electrolyte. Detection at 200 nm.

occurs, i.e., in this case it was at 10 kV (1.016 W of electric power). The separation efficiency and resolution depend on the concentration of the phosphate buffer, cf. Fig. 6. The increase of the phosphate buffer concentration from 0.02 to 0.1 M caused the increment in the resolution the resolution by the factor of 1.9. This was due to the reduction in the ionic mobilities by the effect of ionic strength [15]. The relative difference in the effective mobilities of analytes was then larger and it was reflected in improving the resolution. Concentration of BGE 0.05 M was chosen as optimal because at higher concentration of the electrolyte an increase of the

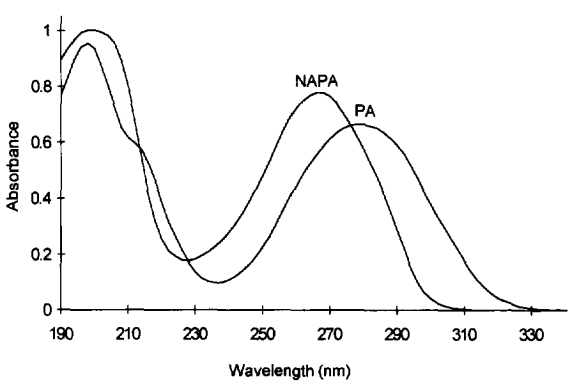


Fig. 2. Absorption spectra of PA and NAPA in aqueous solutions: PA $4.26\cdot10^{-5}$ M; NAPA $4.06\cdot10^{-5}$ M.

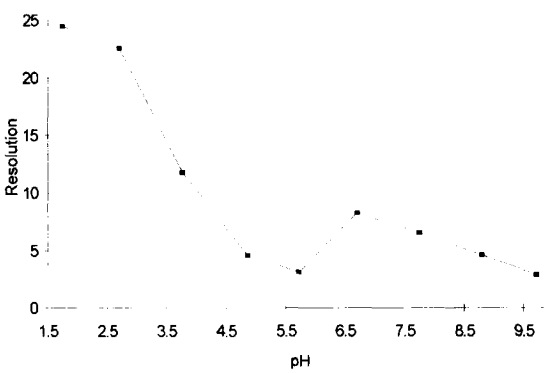


Fig. 4. Resolution of PA and NAPA as a function of pH. Concentrations: PA $1.18\cdot10^{-4}$ M and NAPA $1.13\cdot10^{-4}$ M. The other conditions as in Fig. 3.

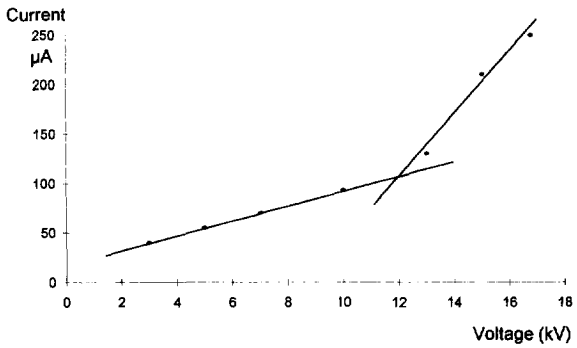


Fig. 5. Ohm's law plot. Current as a function of applied voltage. Concentrations: PA $1.18 \cdot 10^{-4}$ *M* and NAPA $1.13 \cdot 10^{-4}$ *M*. Maximum applied voltage was found to be 10 kV. The other conditions as in Fig. 3, except that the phosphate 0.5 *M* buffer was used.

analysis time was observed and, furthermore, the increased value of the current caused overheating.

3.2. Separation and determination of PA and NAPA in urine

Analysis of PA and NAPA in urine samples is a delicate problem. In our experience, if urine is directly injected into the capillary, proteins and the other biomolecules in the urine matrix are adsorbed to the wall of the capillary and thus quickly deteriorate the column performance. In addition, in order to be able to monitor the samples of urine, the conditions must be found where no other components comigrate with the analytes. The experiments with

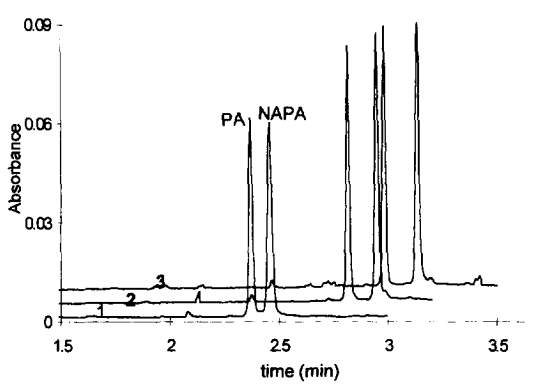


Fig. 6. Effect of different BGE concentrations at pH 7.74. Concentrations: PA $1.18 \cdot 10^{-4}$ *M*. The phosphate buffer concentration was: 1, 0.02 *M*; 2, 0.05 *M*; 3, 0.1 *M*. The other conditions as in Fig. 3.

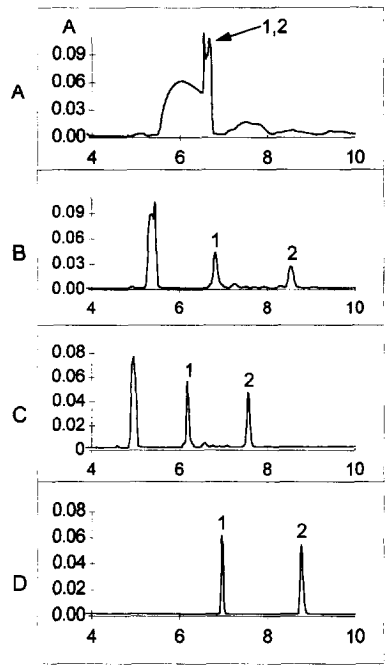


Fig. 7. Electropherograms of PA and NAPA for different urine dilution. The other conditions as in Fig. 3. 1, PA; 2, NAPA. A, Urine without dilution+PA and NAPA; B, urine 1:5 dilution (v/v); C, urine 1:10 dilution (v/v); D, PA and NAPA in water.

different urine dilution ratios (Fig. 7) have shown that a ten-fold dilution of urine and pH 7.7 (Fig. 8) were optimal for the analysis while in the same time the determination of PA and NAPA was kept free of adverse matrix effects.

Known amounts of PA and NAPA (1, 5, 10, 20, 30, 40, 50, 100, 150 and 200 μg/ml) were spiked in ten times diluted urine to establish the calibration curves. In the concentration range studied, the calibration curves were linear. The equations $y = 0.0408x - 0.0327$, with correlation coefficient $r^2 = 0.9995$ for PA, and $y = 0.0406x + 0.0059$, with correlation coefficient $r^2 = 0.9990$ for NAPA were obtained. The detection limits (defined at $S/N = 3$) obtained were 1.235 μg/ml for PA and 0.359 μg/ml for NAPA. The reproducibility of migration times was 0.57% of the relative standard deviation (R.S.D.) for PA and 0.64% of R.S.D. for NAPA ($n = 30$), where n is the number of experiments.

Several samples of rat's urine and one human sample were analyzed with a standard spectrophoto-

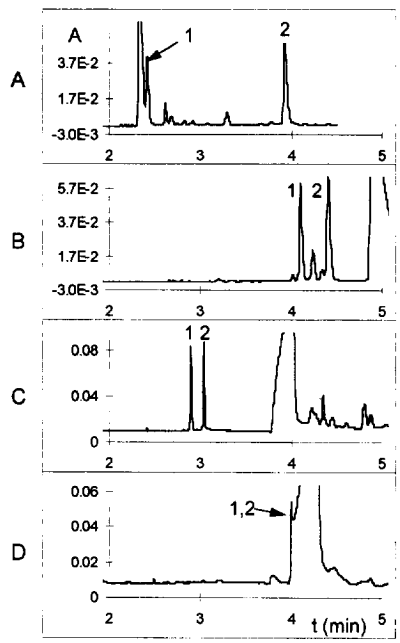


Fig. 8. Electropherograms of PA and NAPA. Effect of pH in urine medium (diluted ten times). The other conditions as in Fig. 3, except that 0.05 *M* phosphate buffer was used as the background electrolyte. 1, PA; 2, NAPA. A, pH 1.74; B, pH 4.86; C, pH 7.74; D, pH 10.69.

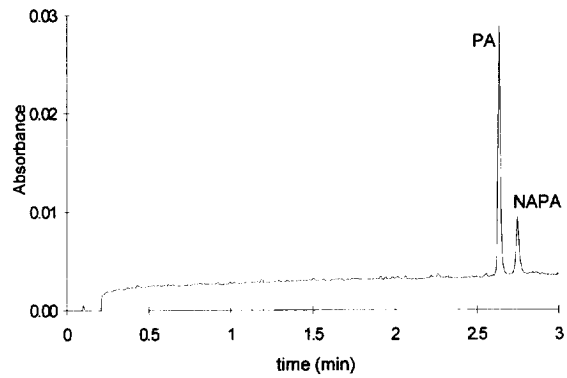


Fig. 9. Typical electropherogram for the determination of PA and NAPA in human urine under the best separation conditions. Phosphate 0.05 *M* buffer of pH 7.7 was used, applied voltage 10 kV, pressure injection 5 s, 0.5 p.s.i., detection at 200 nm. Fused-silica capillary 75 μm I.D. (effective length 35.9 cm, total length 43.5 cm).

metrical method [16] in order to make a comparison with the CZE method developed in this work. The results are given in Table 1. The values obtained by spectrophotometry and CZE agree satisfactorily. Fig. 9 shows the separation of PA and NAPA under the best conditions for a human urine sample.

The method developed has also been used to quantify some medicaments. APO-Procainamide USP Capsules 250 mg were analyzed and the results obtained can be seen in Table 2. The average purity (%) reached was 99.85%±1.35 (σ). It is important to say that PA is normally administered as oral dose 2–5 times per day, and such fluctuations in the dosage can be tolerated.

Table 1
Comparison of the analytical results of PA and NAPA by spectrophotometric and CZE method proposed

Sample	Interval of collecting (h)	Method	PA±σ (μg/ml)	NAPA±σ (μg/ml)
1	1–20	a	936±42	300±26
		b	965.1±14.6	496.1±8.8
2	1–20	a	1737±60	473±25
		b	1846.6±8.3	765.6±7.6
3	0–24	a	88.3±3.2	137±7
		b	96.8±2.2	243.7±3.5
4	0–24	a	96.7±7.6	84±9
		b	86.7±2.2	163.6±12.2
5	0–24	a	94.7±5.5	189±11
		b	123.2±1.8	225.3±2.6
Human urine	0–7	a	168±8	46.6±5.7
		b	139.6±1.4	45.8±2.3

[a] Spectrophotometric method.
[b] CZE method proposed.
All results are average of three injections.

Table 2
Analysis results of APO–Procainamide capsules 250 mg

Sample	Theoretical amount (mg)	Experimental amount (mg)±(σ)	Purity %
1	250	254.61±5.48	101.84
2	250	249.12±4.97	99.65
3	250	245.14±3.33	98.06

All the results are average of three injections.

4. Conclusion

One of the major challenges of capillary electrophoresis is the analysis of drugs at low concentrations in complex matrices, like biological fluids. Optimized conditions for the separation and quantification of PA and NAPA in pharmaceutical formulations and urine samples without extraction and/or preconcentration steps were found and the CZE method developed. The analysis of pharmaceutical formulations can be made directly, while with urine samples it was necessary to dilute ten times, in order to avoid adsorption on the capillary wall. The CZE method proposed makes possible an effective therapeutic drug monitoring of PA and NAPA in patients with arrhythmias, as well as measuring the urine concentrations of PA and NAPA in the acetylator phenotyping test.

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