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Assay of acebutolol in pharmaceuticals by analytical capillary isotachophoresis

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Abstract

Acebutolol [*N*-{3-acetyl-4-[(2-hydroxy-3-(isopropylamino)propoxy]phenyl} butanamide] is a cardioselective β-blocker with a potent antihypertensive and antiarrhythmic effect. The optimised operational system of electrolytes for the newly developed ITP separation of acebutolol consisted of 10 mM potassium acetate +10 mM acetic acid (pH 4.65) as the leading electrolyte and 10 mM β-alanine with pH ≈4 (adjusted with acetic acid) as the terminating electrolyte. The driving and detection currents were 75 and 20 μA, respectively and the analysis took ≈13 min. Under these conditions the effective mobility of acebutolol was determined as 20.7×10^{-9} m^2 V^{-1} s^{-1}. The calibration dependence was rectilinear in the range 0.14–1.4 mg ml^{-1} of acebutolol base ($r = 0.9995$); relative standard deviation (RSD) values were 1.1% and 1.2% ($n = 6$) when determining 0.42 and 0.98 mg ml^{-1} of acebutolol in a pure standard solution. The method, with the limit of detection (LOD) of 0.04 mg ml^{-1} and limit of quantification (LOQ) of 0.12 mg ml^{-1}, was applied to the assay of acebutolol in Sectral tablets, Acecor tablets, Apo-acebutol tablets (nominal content 400 mg of acebutolol per tablet) and Acebirex tablets (nominal content 200 mg of acebutolol per tablet) with RSD = 0.7–1.7% ($n = 6$). No interference from any excipients present in the tablets was observed. The recoveries ranged from 98.8% to 102.4% as found by the standard addition technique.

Keywords: Capillary isotachophoresis; Acebutolol; Pharmaceutical analysis

1. Introduction

Acebutolol, ACE (Fig. 1) is a cardioselective, hydrophilic β-adrenoreceptor blocking agent with mild intrinsic sympathomimetic activity for use in treating patients with hypertension and ventricular arrhytmias. It is marketed in tablet form for oral administration [1].

The papers published until now have been concerned with the development of rapid and sensitive methods for the separation, identification or determination of acebutolol and several other basic β-blockers in body fluids using gradient high-performance liquid chromatography (HPLC) with photodiode-array UV detection [2], HPLC-mass spectrometry (MS) [3], RP-HPLC using both isocratic and gradient elution [4] or RP-HPLC using solvent modifiers [5]. Gas chromatography with mass spectrometry (GC–MS) was presented for the sensitive detection of beta-blocking agents in human urine recently [6]. Three β-blockers (propranolol, timolol, acebutolol) were separated by capillary electrophoresis (CE) with electrochemical detection [7].

A strategy for rapid screening for the separation of chiral molecules of pharmaceutical interest by normal-phase LC using cellulose/amylose stationary phases was proposed [8]. Capillary electrochromatography (CEC) was used for enantioseparations of ACE and other active substances [9–12]. The super/subcritical fluid chromatography was similarly applied to the chiral separations of β-blockers [13]. The rapid enantiomeric chiral separation of a set of 12 chiral amino-containing pharmaceutical compounds belonging to various therapeutic categories was achieve by capillary electrophoresis with carboxymethylcyclodextrins as selectors [14].

A nonaqueous capillary electrophoresis (NACE) method coupled with either UV or electrospray mass spectrometry (ESI-MS) was described for the simultaneous analysis of seven β-blockers [15]. The enantiomeric resolution of various kinds of basic pharmaceuticals was investigated in NACE systems using an ion-pairing reagent [16].

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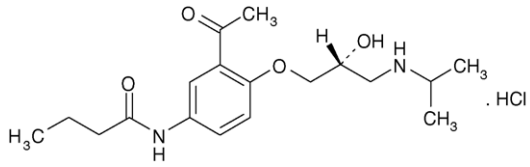

Fig. 1. Chemical structure of acebutolol hydrochloride.

Existing publications concerning the individual determination of acebutolol in pharmaceutical preparations were based on first derivative spectrophotometry, TLC-densitometry and HPLC [17,18], spectrophotometry [19] and GC [20].

To our best knowledge, the ACE has not yet been determined in pharmaceuticals by conventional capillary isotachophoresis. Only the separation of β-blockers oxprenolol, atenolol, timolol, propranolol metoprolol, and acebutolol in human urine by zone electrophoresis with transient isotachophoresis on a poly (methyl methacrylate) chip was investigated recently [21].

The aim of this work was the systematic development and the validation of capillary ITP method with conductimetric detection for the assay of ACE in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

ITP separation performed by using a PC-controlled Villa Labeco EA 100 analyser (VILLA, Spišská Nová Ves, Slovak Republic) operated in a single-column mode. A 160 mm × 0.3 mm (i.d.) analytical capillary made of fluorinated ethylene-propylene copolymer (FEP) and equipped with a conductivity detector was employed. The volume of sampling valve was 30 μl. The data were collected with ITP Win Software, KasComp, Bratislava, Slovakia.

The PHM 220 (Radiometer, France) pH-meter with PHC2401-8 combined glass electrode calibrated with standard Radiometer buffers was employed for pH measurements. The ultrasonic bath Tesson, Tesla Prague, Czech Republic, was used for degassing of solutions.

2.2. Chemicals

Acebutolol hydrochloride was obtained from Sigma–Adrich, Prague, Czech Republic. The dosage forms analysed: Acecor tablets (producer Societa Prodotti Antibiotici Milano, Italy); Apo-acebutol tablets (Toronto, Canada); Sectral tablets (Specia, Nontrouge, France); Acebirex tablets (BTT Erstein, France). The pharmaceuticals were obtained from the pharmacy.

All chemicals (formic, acetic and picolinic acids, tetraethylammonium iodide (TEA), potassium hydrogen carbonate, ε-aminocapronic acid (EACA), β-alanine (BALA), γ-aminobutyric acid (GABA) and methanol were of analytical grade.

A Millipore Milli-Q RG ultra pure water was used for the preparation of the electrolytes and stock solutions.

2.3. Standard and electrolyte solutions

The stock solution (1.4 mg ml^{-1}) of ACE base was prepared by dissolving of ACE·HCl in 1 mM hydrochloric acid. The calibration curve was measured with 0.14–1.4 mg ml^{-1} (0.46–4.6 mM) ACE base solutions (six concentrations, each measured in triplicate).

The leading electrolyte (LE) consisted of 10 mM potassium acetate +10 mM acetic acid (pH 4.65). 10 mM BALA of pH ≈4 (adjusted with acetic acid) was used as the terminating electrolyte (TE). The electrolyte solutions were degassed for 5 min in an ultrasonic bath.

The measurement of the effective mobility of ACE was carried out with 0.5 mM solution of ACE and 0.1 mM TEA as the standard of mobility and calculated from ionic mobilities of K^+, TEA^+ and relative step heights of ACE according to the relevant equation in [22].

2.4. Analysis of tablets

Ten tablets were weighed, crushed and pulverised in an agate mortar, and the weight of the resulting tablet powder equivalent to ≈400 mg of ACE base was transferred into a 100 ml volumetric flask with ≈50 ml of 1 mM HCl and dissolved by applying a 10 min sonication on the ultrasonic bath; thereafter the suspension was diluted to the mark and filtered through a membrane filter (pore size 0.45 mm). The extract subjected to ITP analysis after 10-fold dilution with 1 mM HCl.

2.5. Accuracy test

All the pharmaceutical formulations were initially analysed by ITP as described above; thereafter the samples were treated with known amounts of acebutolol standard at two concentration levels and again subjected to ITP assay. The recoveries of the added amount of ACE were calculated.

3. Results and discussion

3.1. Acid–base properties of the analyte, selection of the electrolyte system and determination of effective mobilities of the drug

The drug under study involves basic amine group which is an important prerequisite for applying any electro-migration method for ITP analysis. According to the earlier literature data [23] the pK_a value of ACE = 9.4 (secondary aliphatic amine group); it means that in aqueous solutions of pH ≤7.4 this compound is practically completely ionised.

Table 1
Mobilities of terminating cations in two electrolyte systems calculated by the RFQ method [21]

L^+ (mmol l^{-1}) R^-; pH[a]	$\bar{u} \times 10^9$(m^2 V^{-1} s^{-1})[b]/pH in the zone T^+[b]			
	BALA	EACA	GABA	ACE[c]
K^+ (10) acetate; 4.65	13.1/3.81	17.6/4.18	15.0/4.03	20.7
K^+ (10) picolinate; 5.25	6.90/4.19	10.77/4.60	8.51/4.43	–

[a] Experimental value.
[b] Calculated by RFQ [21] using $u(L)$, $c(L)$, $pK_a(R)$, $c(R)$, $pK_a(T)$, $u(T)$.
[c] Experimental value (calculated by [22]).

Furthermore, ACE is present in the pharmaceuticals in the form of ionised hydrochloride that is appropriate for its direct ITP assay.

The ITP operational systems were optimised with respect to the quality of separation and the time of analysis. Slightly acidic isotachophoretic operational systems with K^+ or NH_4^+ as the leading ions and acetate ($pK_a = 4.76$) or picolinate ($pK_a = 5.39$) as the counter ions (that are conventionally used in ITP analyses of organic bases) were tested. Three amino acids namely EACA, BALA and GABA were chosen and examined as the terminating ions.

Since all mobilities and pK_a values of the electrolyte system components as well as their concentrations in the leading electrolyte were known the effective mobilities of individual terminating ions in their zones and pH values in the zones could be calculated by using our own computer program based on the RFQ method [24]. For the corresponding calculated data see Table 1 including calculated pH values in the zones of terminating electrolytes.

Considering the reproducibility of the time of analysis (the first zone boundary) K^+ was found to be more convenient leading ion than NH_4^+. The ITP analysis in picolinate system of pH 5.2 as LE took expectedly less time due to the lower mobility of picolinate ion compared to acetate but with the picolinate system all three terminating ions exhibited too low mobility ($\bar{u}(\text{BALA}) = 6.90 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$; $\bar{u}(\text{EACA}) = 10.77 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$, $\bar{u}(\text{GABA}) = 8.51 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$; therefore the ITP analysis of ACE appeared to be less suitable.

Three different concentrations of K^+, 5, 10 and 20 mM were tested. The good quality of separations was achieved in all cases but as expected, the sensitivity and duration of analyses was affected. The use of 5 mM potassium acetate can lead to the breakdown of the analysis due to the lower conductivity of the solution.

On nearer view of the calculated effective mobilities of the terminating ions (T^+) summarized in Table 1 it follows that the most convenient T^+ should be BALA with estimated mobility value of $13.1 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$. In the operational system with BALA the experimental value of effective mobility of ACE is $20.7 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$. With regard to the pH in the ITP zone of ACE the value is in fact equal to ionic mobility. Single isotachophoregram of ACE is shown in Fig. 2.

In the course of the optimisation of the conditions of ITP analysis of ACE the effect of acidity of the leading electrolyte

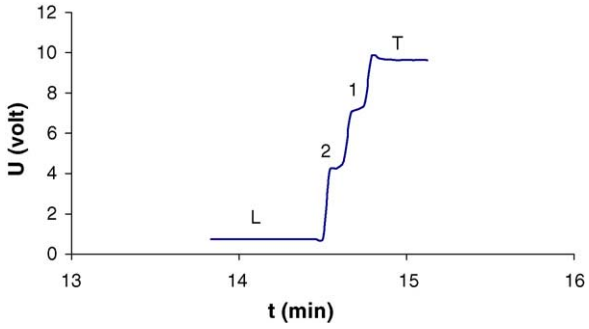

Fig. 2. Isotachophoregram of 0.5 mM solution of acebutolol (1) and 0.1 mM tetraethylammonium (2) as the mobility standard; operational system: 10 mM acetic acid + 10 mM potassium acetate (LE, pH 4.65) and 10 mM BALA (TE).

(acetate buffer) was examined in the range of pH 4.15–5.15. Evaluation of the experimental data indicated unambiguously that with increasing pH the relative step height of ACE (h_{rel}) decreases in relation to the line of the terminating electrolyte; this is accompanied by slight increase of the effective mobility of ACE (see Table 2).

As a fairly strong base the analyte ACE ($pK_a = 9.4$) is completely ionized in the whole pH range 4.15–5.15 tested and tetraethylammonium (TEA^+) as the standard for the evaluation of mobility bears permanent positive charge; nevertheless under these conditions the ionization degree of the terminating ion BALA ($pK_a = 3.55$) and consequently its effective mobility $\bar{u}$ will be affected. It can be assumed that the lesser extent of ionization of BALA as a consequence of using more alkaline leading electrolyte results in its higher step height which is the reason for the observed surprising position of the ACE step in the isotachophoregram (lower h_{rel} of ACE).

Relatively small differences in the calculated $\bar{u}$ values of ACE may be caused by the variations in concentrations of the components of operational solutions (particularly of acetic acid).

Table 2
Effect of pH of LE on position of the ACE step in the isotachophoregram

Acetate buffer (pH)	$\bar{u}_{ACE} \times 10^9$($m^2 V^{-1} s^{-1}$)	$h_{rel\,(ACE)}$[a]
4.15	20.3	0.94
4.65	20.7	0.75
5.15	21.0	0.63

[a] $h_T = 1.0$.

Consequently optimum operational system has composition: LE-10 mM potassium acetate +10 mM acetic acid (pH 4.6) and 10 mM β-alanine of pH ≈4 (adjusted with acetic acid) as TE.

The optimum current and time regime of the ITP analysis is as follows: initially the separation was carried out with the driving current of 75 μA for approximately 600–650 s; the detection current was 20 μA. The overall time of analysis is about 13 min.

3.1.1. Quantitative analysis of ACE

The stability of ACE (0.4 mg l^{-1}) dissolved in 1 M HCl was monitored at ambient temperature (22 to 24 °C) for 8 h by measuring the step length of ACE in time zero (l_0) and in 1 h intervals (l_i) and calculating $S_T(\%) = 100|l_i - l_0|l_0$. No signs if instability were observed since S_T (%) < 1.

The calibration dependence $t = f$ [c(analyte)] (where t stands for the ITP step length in seconds and c(analyte) is the concentration of ACE basis in mg ml^{-1}) was examined with calibration solutions containing 0.14–1.4 mg ml^{-1} of ACE base (six different concentrations 0.14; 0.21; 0.42; 0.70; 0.98 and 1.4 mg ml^{-1}, each calibration solution was measured in triplicate). The linear regression equation $t = a\,c + b$ is described by the following parameters: $a = 45.37 \pm 0.68$; $b = -0.02 \pm 0.53$; correlation coefficient $r = 0.9995$. The low value of the intercept and the high values of the correlation coefficient are positive signs of the analytical stability of the zones and rectilinearity of the calibration curves, respectively. The repeatability of the step length at concentrations of 0.42 and 0.98 mg ml^{-1} of ACE ($n = 6$) showed relative standard deviations (RSD) of 1.1 and 1.2% thus indicating good repeatability of the ITP method. The limit of detection (LOD) value was calculated from the linear regression data as $3.3SD_i$/slope where SD_i stands for the standard deviation of intercept; similarly the limit of quantification (LOQ) value was estimated as $10SD_i$/slope. The LOD and LOQ values are 0.04 and 0.12 mg ml^{-1} of ACE. It can be seen that our LOD value is much higher (over one to three orders) that the LODs attained by HPLC [2,3]. These analytical procedures have been developed for determination of drugs in blood plasma [2] or toxicological screening [3] where the highest sensitivity of the method is needed.

3.2. Determination of ACE in pharmaceuticals

The ITP method was used for determining of ACE in four formulations in the form of tablets and the results are summarised in Table 3 including the repeatability data ($n = 6$). In all instances preparations containing appropriate drug as a single active component have been analysed. The content of ACE in all formulations using ITP method is with good agreement with declared amount. The isotachoforegram of the analysis of Sectral tablets is shown in Fig. 3.

Table 3
Amount of acebutol found in formulations by ITP

Formulation	Declared (mg/tablet)	Content ± RSD (%) (mg/tablet) ($n = 6$)
Acebirex tablet	200	201.0 ± 0.7
Acecor tablet	400	402.8 ± 0.8
Apo-acebutol tablet	400	401.3 ± 1.7
Sectral tablet	400	398.1 ± 1.3

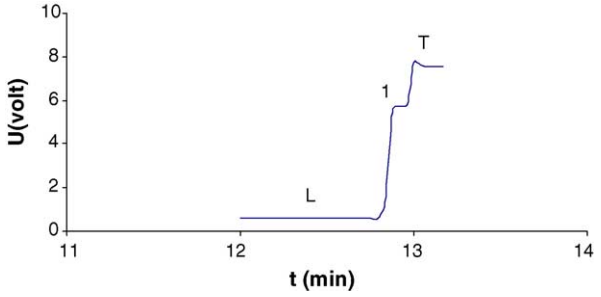

Fig. 3. Isotachophoregram of Sectral tablets, 1 = acebutolol. For operational system see Fig. 2.

The accuracy of the ITP method is the degree of agreement of test results generated by the method to the true value. It must be noted that only the ACE as a bulk substance is official in Pharmacopoeia Bohemica 2002 [25] (and also in European Pharmacopoeia 4 [26]) and its assay is based on the potentiometric titration of the hydrochloride of drug base. The application of such a non-selective method to the determination of the content of the active principle in mass produced pharmaceutical preparations would be obviously unreasonable.

For lack of placebo and pharmacopoeial reference methods for determining the ACE in tablets, we examined the accuracy of the devised ITP assay of ACE in pharmaceuticals by the method of the standard addition. The recoveries of the added amount of ACE at two concentration levels (200 and 400 mg l^{-1}) ranged between 98.8% and 102.4% (see Table 4). Hence it can be concluded that the accuracy of the proposed ITP assay of ACE in various dosage forms is acceptable.

The USP XXVIII article on acebutolol capsules stipulates HPLC assay of the active substance with methanol–acetate

Table 4
ITP recoveries of ACE added to pharmaceutical formulations

Formulation (drug)	Added (mg/l)	Found ± RSD (%) (mg/l) ($n = 6$)	Recovery[a] (%)
Acebirex tablet	200	199.0 ± 0.8	99.5
	400	406.2 ± 0.5	101.6
Acecor tablet	200	204.4 ± 1.6	102.2
	400	408.0 ± 0.8	102.0
Apo-acebutol tablet	200	204.8 ± 1.1	102.4
	400	402.4 ± 1.5	100.6
Sectral tablet	200	197.6 ± 1.2	98.8
	400	396.9 ± 2.1	99.2

[a] Recovery (%) = found × 100/added.

buffer (60:40) of pH 3.5 as the mobile phase and UV detection at 254 nm; the sample pre-treatment takes 30 min and single HPLC separation (sample containing 0.2 mg mL^{-1} of ACE is injected) takes 8 min [18]. In comparison with this HPLC technique, our ITP assay of ACE in tablets including the sample preparation takes also about 30 min and the sensitivity is practically the same.

The separation and monitoring of β-blockers in human urine using isotachophoresis coupled with zone electrophoresis on the microchip system were devised recently. ITP was used as a simple and effective method for preconcentration of ionic sample compounds before the actual CZE separation. Water-soluble hydrophilic matrix components were removed from the urine by solid-phase ecxtraction (SPE) and limits of quantification 5–10 μg ml^{-1} could be achieved [21]. The ITP method developed in our laboratory was performed as an independent separation technique with the sufficient sensitivity for the assay of acebutolol in pharmaceuticals.

For all dosage forms analysed by the ITP method was selective with respect to ACE (no interfering zones appeared in the isotachophoregrams of real samples). The formulations contain either some isotachophoretically inactive species or the ionogenic excipient such as magnesium stearate. This compound does not interfere with the separation of ACE due to its insolubility in water.

Ruggedness. As for the proposed method, no interlaboratory assays were carried out. An indication of acceptable ruggedness of the method could be the result that the RSD values for day-to-day assays of ACE in the analysed pharmaceuticals made in our laboratory by two persons within one week did not exceed 3.5%. Deliberate variations of the leading ion concentration within 5–20 mM and changes of the pH values within range of pH 4.1–5.1 as well as the driving and detection currents between 50 and 80 μA and 10 and 20 μA respectively did not affect considerably the accuracy and repeatability of the results.

It can be noted that the main objective of the proposed method was not the separation of different β-blockers from each other but the development of selective ITP technique aimed to control the quality of monocomponent formulations containing acebutolol as biologically active compound. For this reason, the ITP method was not focused on the separation of mixtures of different β-blockers.

4. Conclusions

In our study, the assay of acebutolol in four pharmaceutical preparations was accomplished by conventional ITP in the acetate operational electrolyte system. The sensitivity, precision and accuracy of this technique were fully sufficient and acceptable. The analyse time was about 13 min, which completes the requirements for routine analysis. The results obtained and low running costs make the ITP method a suitable tool for quality control of various pharmaceutical dosage forms.

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