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Determination of ambroxol or bromhexine in pharmaceuticals by capillary isotachophoresis

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Abstract

Expectorant drugs ambroxol (AX) and bromhexine (BX) were determined by capillary isotachophoresis (ITP) with conductimetric detection. The leading electrolyte (LE) was a buffer solution that contained 5 mM picolinic acid and 5 mM potassium picolinate (pH 5.2). The terminating electrolyte (TE) was 10 mM formic acid. The driving current was 80 μA (for ≈ 200 s) or 50 μA (for ≈ 350 s) and the detection current was 20 μA (a single analysis took about 8 min). The effective mobilities of AX and BX (evaluated with tetraethylammonium as the mobility standard) were 18.8×10^{-9} m^2 V^{-1} s^{-1} and 14.3×10^{-9} m^2 V^{-1} s^{-1} respectively. The calibration graphs relating the ITP zone length to the concentration of the analytes were rectilinear ($r = 0.9993–0.9999$) in the range 10 mg l^{-1} (20 mg l^{-1} for BX) to 200 mg l^{-1} of the drug standard. The relative standard deviations (RSD) were 1.2–1.6% ($n = 6$) when determining 100 mg l^{-1} of the analytes in pure test solutions. The method has been applied to the assay of AX or BX in seven commercial mass-produced pharmaceutical preparations. According to the validation procedure based on the standard addition technique the recoveries were 97.5–102.7% of the drug and the RSD values were 0.11–2.20% ($n = 6$). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ambroxol; Bromhexine; Isotachophoresis; Pharmaceutical analysis

1. Introduction

Bromhexine (BX) and its major metabolite ambroxol (AX) are important mucolytic and expectorant drugs (Fig. 1). The BX or AX hydrochlorides occur as main active principles in a number of commercially available dosage forms such as solutions, drops, capsules, injections and tablets. They are used in the treatment of chronic bronchitis and pneumonia.

Different analytical methods have been used for the individual determination of AX and BX in pharmaceutical preparations as well as in biological materials, including either bulk and separation methods. Derivatisation with subsequent spectrophotometry in visual region [1], or UV spectrophotometry [2] were employed for determining these drugs. Automated flow-injection extrac-

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tion–spectrophotometric determinations of BX or AX in blood serum and in pharmaceutical preparations containing other active ingredients (antibiotics) were devised; these methods are based on the formation of the ion pairs with $Co(SCN)_4^{2-}$ [3], orange IV [4] or bromothymol blue [5]. Another extraction-flow injection method using atomic absorption spectrometric detection was developed for the determination of BX in pharmaceuticals [6]. The automated determination of BX using potentiometric titration involving diazotisation of BX with sodium nitrite has been applied in the content uniformity testing [7]. The presence of a primary aromatic amine group in the BX molecule allowed the development of a direct kinetic method that utilises azo-coupling of the diazotized BX derivative with *N*-(1-naphtyl)-ethylenediamine (NED) for the photometric determination of BX; such a method is used for the assay of BX in pharmaceutical samples containing also antibiotics as e.g. penicillins and cephalosporins [8]. Simultaneous quantitative TLC determination of BX and salbutamol in formulations was published recently [9]. Gas liquid chromatography (GLC) with FID has been used for the assay of AX or BX in pharmaceutical formulations [2,10]; another GLC method with electron capture detection was employed for the determination of AX in body fluids after sample derivatisation with trifluoroacetic anhydride [11]. Particularly the HPLC methods with UV [2,12–17], amperometric [18] or indirect conductimetric detection [19] are frequently used for the quantitation of AX [2,13–15,18] or BX [12,16,17,19] in human body fluids [13,15,18] or in pharmaceuticals [2,12,14,16,17,19].

The use of the highly efficient and fast technique of capillary zone electrophoresis with diode-array detection for the identification of BX an AX in various samples such as pharmaceuticals, urine and serum has been reported in recent years [20].

To our best knowledge, the AX and BX have not yet been determined by capillary ITP. These drugs exist in the form of stable cationic species; hence their determination by cationic ITP should be feasible. The aim of this work was the development of capillary ITP method with conductimetric detection for the assay of AX or BX in pharmaceutical preparations.

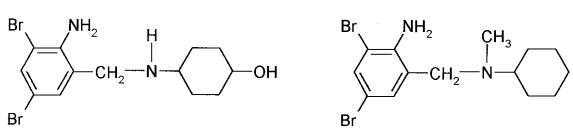

Fig. 1. Chemical structures of ambroxol and bromhexine.

2. Materials and methods

2.1. Reagents

The standards of ambroxol hydrochloride and bromhexine hydrochloride were obtained from the State Institute for Drug Control, Prague, Czech Republic. Other chemicals (picolinic acid, formic acid, potassium hydrogen carbonate and tetraethylammonium iodide purchased from Sigma-Aldrich, Prague, Czech Republic) were of analytical grade. A Millipore Milli-Q RG ultrapure water was used throughout. All solutions were filtered through a 0.85 μm filter (Synpor, Prague, Czech Republic).

2.1.1. Dosage forms analysed

Ambrosan tablets (PRO.MED.CS, Prague, Czech Republic); Ambrobene injection solution (Merckle, Germany); Mucosolvan solution (Boehringer Ingelheim, Germany); Solvolan tablets (KRKA, Slovenia); Bromhexin 12 BC solution (Berlin-Chemie AG, Germany); Bromhexin 8 coated tablets (Berlin-Chemie AG, Germany); Paxirasol solution (EGIS Pharmaceuticals, Hungary).

2.2. Apparatus

ITP analyses were carried out with use of a computer-controlled EA 100 ITP analyser (VILLA s.r.o., Spišská Nová Ves, Slovak Republic) operated in the single-column mode. The analyser was equipped with a 30-μl sampling valve, a 160×0.3 mm (i.d.) analytical capillary made of fluorinated ethylene-propylene (FEP) co-

polymer and a conductivity detector. Quantitative data were obtained by off-line processing of the stored isotachophoregrams providing the length of the isotachophoretic zones by using the ITP software package supplied by the VILLA company. The ultrasonic bath Tesson, Tesla Prague, Czech Republic was used for degassing of solutions.

2.3. Procedure

2.3.1. Determination of the effective mobilities $\bar{u}$

The measurement of the effective mobilities was carried out with 0.2 mM solution of AX or BX and 0.2 mM tetraethylammonium iodide as the standard of mobility. The effective mobility of the drugs was calculated from the waveheights [21] obtained from triplicate ITP measurements taking into account the tabulated values of ionic mobilities of K^+ and tetraethylammonium 76.2×10^{-9} and 33.8×10^{-9} $m^2.V^{-1}.s^{-1}$, respectively.

2.3.2. Examination of isotachophoretic separability of AX and BX

Equimolar mixture of AX and BX (containing 0.2 mmol l^{-1} of either component) and another four mixtures 0.2 mM in one component and 0.5 or 0.8 mM in the other component were subjected to ITP separation.

2.3.3. The ITP conditions, choice of electrolyte systems

The driving currents were 80 μA (for ≈ 200 s) or 50 μA (for ≈ 350 s) and the detection current was 20 μA. The leading electrolyte (LE) was a buffer solution containing 5 mM picolinic acid and 5 mM potassium picolinate (pH 5.20). The terminating electrolyte (TE) of pH 2.50 was 0.01 M formic acid (terminating ion H^+).

2.3.4. The calibration curves

These were measured within the range 10–200 mg l^{-1} of AX.HCl or 20–200 mg l^{-1} of BX.HCl (six concentrations for each analyte, each measured in triplicate). The time (t, in s) of the passage of the zones of AX or BX through the detector was read as the quantitative parameter (obtained by off-line computer-aided processing of the stored isotachophoregrams) and the $t = f[c(\text{analyte})]$ curves were evaluated by linear regression.

2.3.5. Analysis of dosage forms

All dosage forms examined were processed in such a way that the concentration of BX or AX in the final test solutions or extracts fell within the calibration range.

2.3.5.1. Solutions, injections:. Since the active drugs are present in the form of hydrochloride salts, the samples were just diluted with picolinate buffer to achieve the appropriate concentration (Mucosolvan solution and Ambrobene injections 100-fold dilution; Bromhexine 12 BC solution 125-fold dilution and Paxirasol solution 20-fold dilution).

2.3.5.2. Tablets, coated tablets:. The mean weight of a single piece of the dosage form was determined and 10 pieces were homogenised in an agate mortar. The appropriate amount of the powdered mass corresponding to ≈ 10 mg of analyte was weighed and dissolved in ≈ 80 ml of LE in a100-ml graduated flask by applying a 10-min (20-min in the case of Bromhexin preparation) sonication on the ultrasonic bath; thereafter the suspension was diluted to the mark and filtered through Schleicher and Schuell SPARTAN 30/B membrane filter (pore size 0.45 μm) before the ITP analysis.

2.3.5.3. Accuracy test:. In the first step the test solutions of the individual dosage forms (processed in the way mentioned above) were analysed by the proposed ITP method giving the value of the drug content A (mg/l). In the second step the samples were processed in the same way but the known amount of AX or BX was added before diluting the test solutions to the mark. The added amounts corresponded to 50% of the initial analyte contents, e.g. 40 or 50 mg/l. These resulting solutions were analysed by the ITP and the content of AX (or BX) was determined giving the overall drug content B in mg/l; hence added amount found (mg/l) = overall content B − content A (see Table 4).

Table 1
Parameters of aqueous operational system used for ITP of AX (BX) and fundamental ITP characteristics; TE: 10 mM formic acid (pH 2.5)

L	R c(mol l^{-1})	pH_{LE}	$\bar{u} \times 10^9$ (m ^{2} V^{-1} s^{-1})	
			AX	BX
K^+	Picolinate			
0.005	0.01	5.2	18.8 ± 0.1	14.3 ± 0.2

3. Results and discussion

3.1. Acid–base properties of the analytes and choice of the electrolyte system

The drugs under study involve basic aliphatic amine group which is an important prerequisite for applying any electro-migration method for their analysis including quality control of mass produced pharmaceutical preparations. According to the earlier literature data [22] the pK_a value of BX = 8.5 (benzylamino group); it means that in aqueous solutions of pH ≤ 6.5 this compound is completely ionised. The AX is a metabolite formed from BX by *N*-demethylation and hydroxylation of the cyclohexane ring. Such a change of structure results in better solubility of AX in water compared to rather lipophilic BX (even the BX.HCl is sparingly soluble in water and aqueous ethanol). Though we could not find any data on the pK_a of AX it can be presumed, considering the structure of AX, that its pK_a value will be very similar to that of BX. In fact, since the AX and BX are present in the pharmaceutical preparations in the form of ionised hydrochlorides, the drugs are ready for assay by cationic capillary ITP.

To find convenient electrolyte system for the ITP analysis, two different weakly acid buffer solutions with K^+ as the leading ion and acetate or picolinate as the counter ion were tested. In either case systems of different ionic strength were examined ($c_L = 10$ or 5 mmol l^{-1}). Considering the quality of separation and sensitivity and duration of analysis the picolinate buffer was unambiguously selected as the optimum operational system. As the concentration of an analyte in the ITP zone is directly proportional to the concentration of the leading ion while being a function of the mobilities of the migrating ions present, the lower concentration of L^+ ($c_L = 5$ mmol l^{-1}) appears to be convenient especially for the analysis of BX in the context with its low solubility in water (here the lower c_L serves as a tool for decreasing the concentration of BX in its own zone thus avoiding possible accompanying solubility problems). The parameters of the optimised operational system are shown in Table 1.

The optimum current and time regime of the ITP analysis is as follows: initially the separation was carried out with the driving current of 80 μA for approx. 200 s (another alternative was 50 μA for about 350 s), the detection current was 20 μA. The overall time of analysis is about 8 min.

3.2. Determination of effective mobilities of the drugs

As already mentioned in the preceding section, the experimental effective mobilities were calculated from the ITP data with the use of tetraethylammonium (TEA^+) as the standard of mobility. The resulting $\bar{u}$ values were 18.8×10^{-9} m^2 V^{-1} s^{-1} for AX and 14.3×10^{-9} m^2 V^{-1} s^{-1} for BX (Table 1). With regard to the pH in the ITP zones of AX or BX these values are in fact equal to ionic mobilities. Single isotachophoregrams of AX or BX are shown in Fig. 2.

3.3. Investigation of ITP separation of AX from BX

In the given operational electrolyte system the $\bar{u}$ values of AX and BX cited above differ by about 4 units and therefore a mixture of these compounds should be in fact completely separated but the reality is different. If the concentration of AX in the mixture is fixed at c(AX) = 0.2 mmol l^{-1} and the c(BX) is gradually increased from 0.2 to 0.5 and 0.8 mmol l^{-1} (so that the concentration ratios of c(BX)/c(AX) amount to 1.0, 2.5 and 4.0 respectively) in all three instances the separation of pure zone of BX, migrating as the second one, takes place. Regression analysis of the BX zone length l_{BX} as a function of c(BX) (cf. Fig. 3)

confirmed strictly rectilinear correlation ($r = 0.9998$). However, according to the equation of the regression line $l_{BX} = 31.1c(BX) - 54.6$ obtained under the above conditions, the separation of discrete zone of BX occurs only at the concentration ratios $c(BX)/c(AX)$ exceeding 0.9 in the injected mixture. It means that the first zone is a mixed zone of AX + BX of fixed composition.

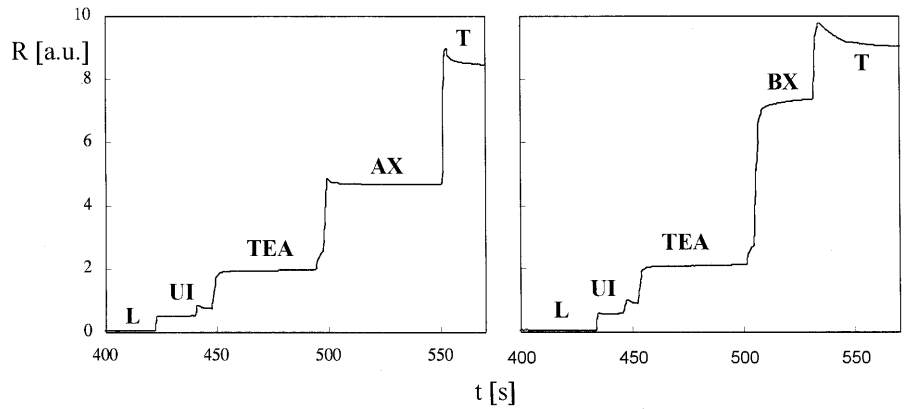

Fig. 2. Isotachophoregrams of 0.2 mM ambroxol (AX) or 0.2 mM bromhexine (BX) and 0.2 mM tetraethylammonium (TEA) as the mobility standard. Operational system: 5 mM picolinic acid + 5 mM potassium picolinate (LE) and 10 mM formic acid (TE); UI = unidentified impurities; R = resistance (in arbitrary units).

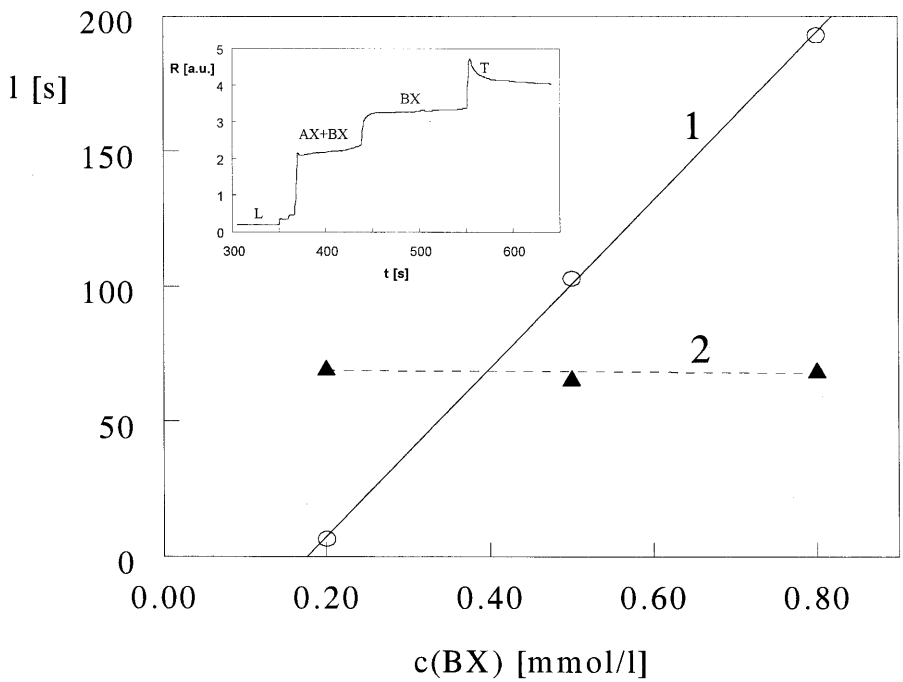

Fig. 3. Dependence of the ITP zone lengths l on the concentration of BX in AX–BX mixtures. Curve 1: zone of BX; curve 2: mixed zone of AX + BX; $c(AX) = 0.2$ mmol l^{-1} in all instances for curve 1; $c(AX) = c(BX)$ in all instances for curve 2; injected sample volume 30 μl. Isotachophoregram of the mixture of 0.2 mM AX + 0.5 mM BX is shown in the insertion; For operational system see Fig. 2.

Table 2
Linear regression calibration parameters[a] for drugs analysed

Analyte	*a*	*b*	*r*	*n*
Ambroxol.HCl	0.6798 ± 0.0050	-0.04 ± 0.56	0.99989	6
Bromhexine.HCl	0.2930 ± 0.0054	0.49 ± 0.63	0.99933	6

[a] $t = ac + b$, where t = ITP zone length (s); c = analyte concentration (mg l^{-1}); a = slope; b = intercept; r = correlation coefficient and n = number of calibration points (each of the six calibration solutions was measured in triplicate).

The $\bar{u}$ value of the mixed zone is close to that of AX and the isotachophoregrams do not show any sign of the formation of discrete zone of pure AX. We observed that the two compounds form such a mixed zone even after 10-fold dilution of the injected mixture or after 10-fold reduction of the injected sample volume. Hence it follows that in the given electrolyte system perfect separation of AX from BX is impossible (this could be probably achieved only by selecting qualitatively different electrolyte system). We mention here the AX – BX separation problem just as theoretically interesting fact concerning migration of these two drugs in the picolinate electrolyte system and it does not bring any contradiction between the aim and outcome of this paper. It is evident that from the pharmaceutical standpoint it would be purposeless to combine both AX and BX as active principles of the same pharmacological effect in a single preparation (in fact there is no such a pharmaceutical preparation available) and in that sense the complete separation of AX and BX is not of practical importance.

3.4. Calibration graph of AX or BX

The calibration dependence $t = f[c(\text{analyte})]$ (where t stands for the ITP zone length in seconds and c(analyte) is the concentration of AX.HCl or BX.HCl in mg l^{-1}) was examined for either analyte as cited in the preceding section and the ITP data were evaluated by linear regression. The linear regression parameters of the calibration line $t = ac(\text{analyte}) + b$ are presented in Table 2. 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 results of replicate analyses of prepared samples containing 100 mg l^{-1} of AX or BX ($n = 6$) showed relative standard deviations (RSD) of 1.2–1.6% thus indicating good repeatability of the ITP method. Day-to-day reproducibility of the calibration curves is characterised by the RSD of the slope and intercept data not exceeding 2.5%. The LOD values are 4 and 8 μg ml^{-1} of AX.HCl and BX.HCl.

3.5. Determination of AX or BX in pharmaceuticals

The ITP method was used for determining AX.HCl or BX.HCl in seven formulations (tablets, coated tablets, injections, solutions for

Table 3
ITP determination of ambroxol and bromhexine in pharmaceutical preparations

Formulation [drug]	Nominal content	ITP method, content ± RSD[a] (%)
Ambrosan (ambroxol) tabl.	30 mg/1 tabl.	102.02 ± 0.11
Ambrobene (ambroxol) inj.	7.5 mg/1 ml inj.	101.33 ± 2.20
Mucosolvan (ambroxol) sol.	7.5 mg/1 ml sol.	101.10 ± 0.33
Solvolan (ambroxol) tabl.	30 mg/1 tabl.	99.05 ± 1.32
Bromhexin 8 (bromhexin) coat. tabl.	8 mg/1 coat. tabl.	101.25 ± 1.71
Bromhexin 12 BC (bromhexin) sol.	12 mg/1ml sol.	100.83 ± 0.72
Paxirasol (bromhexin) sol.	2 mg/1 ml sol.	101.04 ± 0.74

[a] $n = 6$.

Table 4
ITP recoveries of AX (BX) added to pharmaceutical formulations

Formulation (drug)	Added (mg/l)	Found ± RSD[a] (%) (mg/l)	Recovery[b] (%)
Ambrosan (AX) tabl.	50	48.8 ± 0.45	97.5
Ambrobene (AX) inj.	40	40.1 ± 2.57	100.2
Mucosolvan (AX) sol.	40	40.3 ± 1.28	100.8
Solvolan (AX) tabl.	50	48.9 ± 2.19	97.8
Bromhexin 8 (BX) coat. tabl.	50	51.4 ± 2.53	102.8
Bromhexin 12 BC (BX) sol.	50	51.0 ± 0.78	102.0
Paxirasol (BX) sol.	50	50.9 ± 1.45	101.8

[a] $n = 6$.
[b] Recovery (%) = found × 100/added.

internal use) 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 accuracy of the ITP method is the degree of agreement of test results generated by the method to the true value. The true value for accuracy assessment can be usually obtained in three ways — (i) by a different well established reference method, (ii) by the analysis of the model sample, e.g. placebo, with the standard addition of the analyte, or (iii) if the placebo is not available, by the analysis of the sample of interest treated with a known additional standard amount of the analyte. It must be noted that only the BX.HCl as a bulk substance is official in Pharmacopoeia Bohemica 1997 [23] (and also in European Pharmacopoeia 3) [24]) 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 AX or BX in pharmaceutical formulations, we examined the accuracy of the devised ITP assay of AX and BX in pharmaceuticals by the method of the standard addition (cf. way iii) in accordance with the recommendations of Pharmaceutical Authorities of the Czech Republic [25]. The recoveries of the added AX.HCl or BX.HCl ranged between 97.5 and 102.8% (see Table 4). Hence it can be concluded that the accuracy of the proposed ITP assay of AX or BX in various dosage forms is acceptable.

For all dosage forms analysed the ITP method was selective with respect to AX or BX (no interfering zones appeared in the isotachophoregrams of real samples). If a pharmaceutical preparation contains some other isotachophoretically active species (i.e. cations) their zones are distinctly separated from the zone of the drug; the foreign cations exhibit always higher mobility and their zones are located in the lower part of the isotachophoregram. Excipients such as magnesium stearate (for ambrosan and bromhexin tablets), benzalkonium chloride (for mucosolvan solution) or sodium citrate (for paxirasol solution) can be named as typical examples. The ionogenic excipients do not affect reliability of the proposed ITP method at all but they merely prolong duration of the analysis (usually by several minutes).

3.6. Ruggedness/robustness of the ITP method

As for the ruggedness of the proposed ITP method, no real inter-laboratory assays were carried out. An indication of acceptable ruggedness of the ITP method might be the fact that the RSD values for day-to-day assays of AX or BX in the cited formulations performed in the same laboratory by two analysts within one week did not exceed 3%. Deliberate changes of the pH of the leading electrolyte within the range of pH 4.9–5.9 and those of the driving and detection currents between 20 and 80 and 10 and 20 μA respectively

did not exhibit any significant effects on the accuracy and repeatability of the results of ITP assay of AX or BX. The same was true when the original separation capillary (160×0.3mm) was replaced by a shorter one (90×0.3 mm).

4. Conclusions

This study has demonstrated that the cationic capillary ITP is suitable for separating and determining milligram amounts of basic mucolytic agents ambroxol hydrochloride (AX) and bromhexine hydrochloride(BX) in various dosage forms (tablets, coated tablets, injections, solutions). The overall running time of the ITP procedure is short; a single analysis takes about 7–12 min. The sample preparation is easy and electrolyte and sample consumption are low. Moreover, the advantage of the ITP is the fact that the migration medium employed is purely aqueous, free from any organic solvents (compare with the published HPLC assays of AX or BX in various pharmaceutical preparations that were performed with use of mobile phases containing 40–80% of acetonitrile [2,13,14,17,19] or 75% of methanol [12]).

The lower sensitivity of the proposed ITP method compared to the HPLC methods published earlier is fully sufficient for the analysis of pharmaceutical preparations. On the other hand the proposed ITP method cannot be utilised for determining low levels of these drugs in body fluids and for the simultaneous determination of AX and BX when both are present together. Considering its acceptable reproducibility and accuracy the ITP method devised is a promising one for relatively rapid and reliable analytical evaluation of pharmaceutical formulations containing AX or BX as the active ingredient.

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