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Determination of bopindolol in pharmaceuticals by capillary isotachophoresis

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Abstract

Capillary isotachophoresis (ITP) with conductimetric detection has been used for separating and determining bopindolol (**I**) in commercial mass-produced pharmaceutical preparations. The optimised operational electrolyte system consisted of 5 mM potassium picolinate and 5 mM picolinic acid (leading electrolyte, LE; pH 5.37) and 10 mM formic acid as the terminating electrolyte (TE). The driving and detection currents were 50 μA (for 350 s) and 10 μA, respectively. The single analysis took about 12 min. Under such conditions the effective mobility of **I** was determined as 16.73 10^{-9}m^2 V^{-1} s^{-1} (with tetraethylammonium as the mobility standard). The calibration graph relating the ITP zone length to the concentration of **I** was rectilinear ($r = 0.99990$) in the range 10–100 mg l^{-1}. The relative standard deviation (R.S.D.) was 0.90% ($n = 6$) when determining 50 mg l^{-1} of **I** in pure test solution. Sample pre-treatment of the tablets involved ice-cooled extraction of **I** with methanol. The method was suitable for determining **I** in Sandonorm tablets with R.S.D. value 1.45% ($n = 6$). According to the validation procedure based on the standard addition method the recovery was 97.3%.

Keywords: Bopindolol; Isotachophoresis; Sandonorm; Validation; Pharmaceutical preparation

1. Introduction

Bopindolol, 4-(2-benzoyloxy-3-tert-butylamino-propyl)-2-methylindole hydrogen malonate (**I**), is a potent and specific β-adrenoreceptor antagonist with more prolonged action compared with other β-blockers. The drug **I** undergoes very rapid hydrolysis catalysed by endogenous esterases to form 4-(2-hydroxy-3-tert-butylamino-propyl)-2-methylindole (**II**) as the metabolite showing the major pharmacological effect (cf. Fig. 1).

The communications published until now have been concerned with the development of sensitive and selective methods for the separation of bopindolol or its metabolite from blood plasma. The high performance liquid chromatography (HPLC) methods using fluorescence [1], electrochemical [2], or coulometric [3] detection were described. The enantiomeric HPLC separation of bopindolol using a chiral immobilised protein stationary

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phase with ultra violet (UV) detection was reported for the first time in 1986 [4]. Recently the ion-pair supercritical fluid chromatography was applied to the assay of ionic drug substances including bopindolol [5].

To our best knowledge, no attempts have yet been made to determine **I** by any electro-migration separation method. The basic character of **I** should facilitate the formation of its cationic species in a slightly acidic medium and hence to make the determination of **I** by capillary isotachophoresis (ITP) or capillary zone electrophoresis (CZE) possible.

The aim of the present study was to develop and validate an ITP method suitable for selective assay of the content of **I** in mass-produced pharmaceutical preparations. The preliminary steps were: (i) finding optimum system of operational electrolytes to ensure correct ITP migration of **I** and calculation of its effective mobility value; (ii) finding out quantitative ITP calibration characteristics for the drug standard; and (iii) devising optimum procedure for sample pre-treatment before the ITP separation.

2. Experimental

2.1. Instrumentation

ITP separations were performed using a computer-controlled Villa Labeco EA 100 analyser (Spišská Nová Ves, Slovak Republic) operated in a 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 copolymer (FEP) and a conductivity detector. Quantitative data (length of the ITP zones) were obtained by off-line processing of the stored isotachophoregrams by using the appropriate ITP software package supplied by the Villa Company.

Bandelin SONOREX RK 100 ultrasonic bath (Berlin, Germany) was employed for the sonication of solid dosage form to facilitate the extraction of **I**.

2.2. Materials

Bopindolol hydrogen malonate and Sandonorm, tablets (nominal content 1 mg of **I** per tablet) were obtained from Léčiva a.s., Czech Republic. All chemicals (formic acid, picolinic acid, tetraethylammonium iodide (TEAI), potassium hydrogen carbonate and methanol purchased from Sigma-Aldrich) 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. Isotachophoretic conditions

The optimum leading electrolyte (LE) was a buffer solution containing 5 mM picolinic acid and 5 mM potassium picolinate (pH 5.37). The terminating electrolyte (TE) of pH $\approx$ 2.6 was 10 mM formic acid (terminating ion H^+). All buffers contained 20% of methanol. The electrolytes were degassed ultrasonically before use.

The driving current for the analytical capillary was 50 μA for about 350 s, the detection current was 10 μA. The samples were injected with a 30 μl sampling valve. The separation capillary was maintained at 25 ± 0.2 °C by using a lab-made unit based on Peltier element.

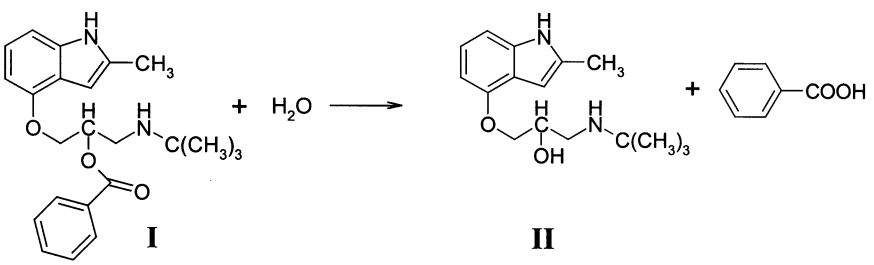

Fig. 1. Chemical structure of bopindolol (**I**) and its hydrolytic product (**II**).

The ultrasonic bath was used for degassing the LE. All solutions were filtered through a 0.45 μm filter (Synpor, Prague, Czech Republic) before ITP measurements.

2.4. Procedures

2.4.1. The effective mobility measurement

The measurements of the effective mobilities of **I** and **II** were carried out with 0.4 mM analyte solution and 0.2 mM TEAI as the mobility standard. The numerical values were calculated from the relative step heights taking into account the tabulated values of ionic mobility of K^+ and $TEAI^+$ of 76.1×10^{-9} and 33.8×10^{-9} m^2 V^{-1} s^{-1}, respectively, [6].

2.4.2. Calibration

The calibration curve was measured with 10–100 mg l^{-1} of **I** in aqueous 20% methanol (five concentrations, each measured in triplicate). The time (t, in s) of the passage of the zone of **I** through the detector was read as the quantitative parameter; the dependence of t on the concentration of **I** (mg l^{-1}) was evaluated by linear regression.

2.4.3. Analysis of Sandonorm tablets

To extract bopindolol from the dosage form ten tablets were weighed, crushed and pulverised, and the weight of the resulting tablet powder equivalent to ≈ 5 mg of **I** was transferred into a beaker cooled on an ice bath and treated with 20 ml of cold methanol. The suspension was agitated by using a magnetic stirrer for 15 min and thereafter it was transferred into a 100 ml volumetric flask where it was diluted to the mark with water. This suspension was filtered through a membrane filter (Synpor, pore size 0.45 μm) and then analysed directly by ITP. Alternatively, appropriate amount of pulverised tablets was sonicated in 100-ml volumetric flask with 20 ml of methanol for 15 min but this procedure had to be rejected since the **I** was partially hydrolysed to **II**.

To isolate the hydrolytic product (**II**) of bopindolol, 0.5 g sample of **I** was dissolved in 20 ml of aqueous methanol, the solution was treated with 1 M hydrochloric acid and the mixture was heated on a boiling water-bath under a reflux condenser for 1 h. Thereafter, most of the liquid was evaporated, the residue was neutralised with sodium hydrogen carbonate and the non-ionised base **II** was separated from the reaction mixture (containing benzoate) by extraction into chloroform. The extract was evaporated to dryness, the residue was dissolved in aqueous 20% methanol and the solution containing **II** was subjected to ITP analysis.

2.4.4. Extraction efficiency

The pharmaceutical preparation was initially analysed by ITP as described above, thereafter the sample was treated with known amounts of bopindolol standard and again subjected to ITP assay. The recovery of the added amount of **I** was calculated.

2.5. Validation

The validation of the ITP method was carried out according to recommendations given in [7,8].

- Precision, the overall reproducibility of the ITP method was determined by assaying six individual samples of commercial preparation Sandonorm tablets and it has been expressed as the relative standard deviation (R.S.D.).
- Linearity, the calibration function $t = f\,[c(\mathbf{I})]$ (where t stands for the ITP zone length in s and $c(\mathbf{I})$ is the concentration of **I** in mg l^{-1}) was examined with five solutions covering the concentration range 10–100 mg l^{-1} of **I** and the parameters of the calibration line $t = ac(\mathbf{I}) + b$ were evaluated by linear regression; each of five calibration solution was measured in triplicate).

  ITP system suitability test was performed by analysing six standard solutions containing 50 mg l^{-1} of **I** and the result was expressed in terms of R.S.D.
- Sensitivity of the method, the limit of detection (LOD) and limit of quantitation (LOQ) were determined as the concentrations of **I** giving the response as zone lengths of 1 or 10 s, respectively.
- Accuracy, the accuracy of the ITP method has been checked by the standard addition technique by analysing six real samples spiked with

Table 1
Composition of electrolytes for ITP analysis of **I** and characteristic features of the separation including mobilities ($\bar{u}$) of **I**

$c(K^+)/c(R^-)$ (mol l^{-1})	I (μA)	t_1/t_2 (s)	pH_{LE}	$\bar{u} \times 10^9 \pm$ R.S.D. ($m^2\ V^{-1}\ s^{-1}$)	Shape of the step	Duration of analysis (s)
Counter ion (R^-): picolinate; TE: 10 mM formic acid						
0.01/0.02	50/10	600/500	5.30	13.02 ± 0.32	Regular, rectangular	1050
0.005/0.01	50/10	350/400	5.37	16.73 ± 0.24	Regular, rectangular	750-800
Counter ion (R^-): acetate; TE: 10 mM acetic acid						
0.01/0.02	75/25	400/300	4.80	15.35 ± 0.13	distinctly descending	620
0.005/0.01	75/25	200/300	4.83	16.68 ± 0.35	regular, descending	430
0.005/0.01	75/10	200/600	4.83	N/A	regular, rectangular	770

K^+, leading ion; I, driving/detection current; c (**I**), 0.4 mmol l^{-1}; t_1/t_2, timing programme in driving/detection current; R.S.D., relative standard deviation in percent ($n = 3$).

a known amount of analyte and six replicates of original non-spiked samples. The mean recovery of the added **I** was calculated.

- Ruggedness, (i) stability: stock solution of bopindolol (100 mg l^{-1}) in aqueous 20% methanol was stored at 4 °C for 7 days. The **I** was determined in this solution in 1 day intervals. (ii) The effect of repeated preparation of the LE (pH 5.5) on the reproducibility of the ITP zone length values of bopindolol was tested ($n = 3$). (iii) To examine the effect of pH of LE three different LEs (pH 5.0, 5.4, and 5.8) were prepared and five replicate analyses were carried out with a standard solution of bopindolol and real samples. (iv) Another two separation capillaries of the same size (160×0.3 mm) and a shorter one (90×0.3 mm) purchased from the same supplier were also used to generate validation data (v) The changes of the driving (± 20 μA) and detection ($+10$ μA) currents on the quality of ITP separation of **I** and **II** were examined.

3. Results and discussion

3.1. Method development and determination of the effective mobility

Generally, analytes of basic character are completely ionised in acid aqueous medium if $(pH)_{medium} \leq (pK_a)_{base} - 2$. We could not find the pK_a value of **I** in appropriate literature but it can be supposed that its pK_a is close to that of structurally similar compound pindolol (pK_a 8.8) [9]. It means that **I** will be practically completely ionised bellow pH 7. In fact, bopindolol occurs in the form of ionised salt (hydrogen malonate) in formulations and hence it is ready for the assay by cationic ITP with no need of additional chemical pre-treatment.

The ITP operational system of electrolytes was optimised with respect to the quality of separation, sensitivity of the ITP determination and duration of the analyses. Different weakly acid operational systems with K^+ as the leading ion and acetate and/or picolinate as the counter ion have been tested. Acetic acid and/or formic acid (10 mmol l^{-1}) were used as the TEs. All the operational systems contained 20% of methanol to enhance solubility and separation of **I**. The optimisation of the electrolyte system involved critical selection of the kind and concentration of the counter-ion and, consequently, the pH value of the LE. The parameters of the operational electrolyte systems including brief commentary of the results are summarised in Table 1. The picolinate system of pH 5.37 containing 5 mmol l^{-1} K^+ gave optimum ITP results considering the

shape of the ITP step, duration of analysis and sensitivity (the zone length of **I**). The calculated effective mobility of **I** = 16.73×10^{-9} m^2 V^{-1} s^{-1} (cf. Table 1) is in fact its ionic mobility value. The optimal time and driving current regime was 50 μA for 350 s followed by and 10 μA detection current. A single analysis took about 12 min.

The precision of the ITP system determined by making six consecutive injections of the bopindolol standard solution (see ITP system suitability test in Section 2) gave satisfactory results (R.S.D., 0.90%). Inter-day precision (reproducibility) was not examined.

3.2. Application of the method to real pharmaceutical samples

3.2.1. Extraction procedure

Two extraction procedures employed are described in detail in Section 2. Initially the **I** was extracted from pulverised tablets with methanol by 15 min sonication at ambient temperature. If this extract was subjected to the ITP two zones appeared in the isotachophoregram, one corresponding to **I** and another one suggesting the presence of some cationic species of higher mobility. Considering the fact that the ester group of **I** is readily hydrolysed (see Fig. 1) the compound giving rise to the additional zone is presumably 4-(2-hydroxy-3-*tert*-butylaminopropyl)-2-methylindole (**II**) that should possess higher effective mobility compared with **I** due to its smaller molecule. Theoretical estimate of effective mobility of this hydrolytic product using empirical Jokl equation [10] gave the value of 19.5×10^{-9} m^2 V^{-1}s^{-1} which is in excellent agreement with 19.2×10^{-9} m^2 V^{-1}s^{-1} calculated from the height of the appropriate ITP step. The identity of **II** in the extract was confirmed by comparing the isotachophoregram of the extract (see Fig. 2(B)) with that of isolated **II** prepared by acid hydrolysis of bopindolol standard (Fig. 2(A)). It can be clearly seen that the zone of species with higher effective mobility in the isotachophoregram of the extract from tablets compares well with the zone of pure hydrolytic product **II**.

The problem of hydrolysis of **I** during the sample pre-treatment was overcome by carrying out the extraction on an ice bath at 0 °C with conventional magnetic agitation instead of sonication. In this instance the isotachophoregram of the extract showed only a single zone of **I**.

3.3. Method validation

3.3.1. Linearity

The calibration line $t = ac(\mathbf{I}) + b$ (where t stands for the ITP zone length in s and $c(\mathbf{I})$ is the concentration of **I** in mg l^{-1}) is described by the

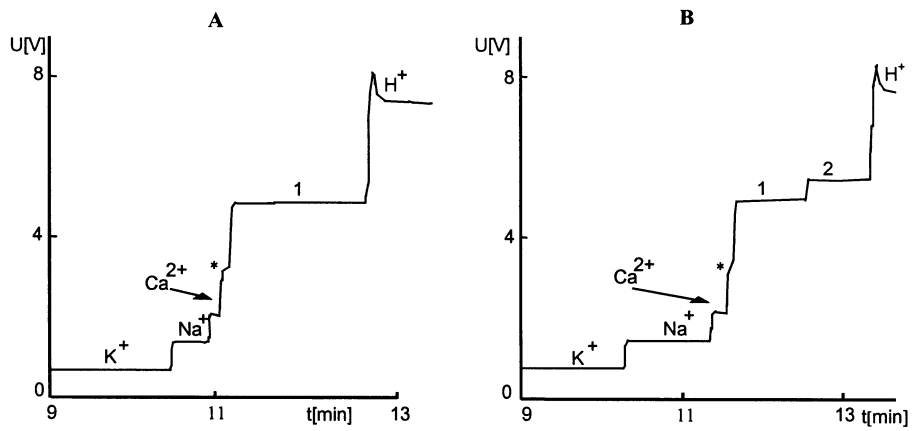

Fig. 2. Isotachophoregrams of (A), hydrolysed bopindolol (**II**) (zone 1); (B) sonicated extract from Sandonorm tablets, bopindolol (zone 2), **II** (zone 1);*, unidentified impurity in the electrolyte system.

Table 2
ITP recovery of **I** added to pharmaceutical formulation

Formulation	Added (mg l^{-1})	Found (mg l^{-1})	R.S.D. (%)[a]	Mean recovery (%)
Sandonorm	40	38.92	2.05	97.31

[a] Six replicate results.

following linear regression parameters, $a = 1.212 \pm 0.014$; $b = 2.503 \pm 0.889$; the correlation coefficient $r = 0.9999$. Relatively low value of the intercept b is positive sign of correctly passed migration and analytical stability of the ITP zone and the high value of the correlation coefficient confirms good rectilinearity of the calibration graph.

3.3.2. Sensitivity

With an injection volume of 30 μl the LOD is approximately 0.7 μg ml^{-1} of **I** and the LOQ amounts to 6 μg ml^{-1} of **I.** The sensitivity of the method proposed is fully sufficient for the assay of **I** in solid dosage forms.

3.3.3. Accuracy

To our best knowledge no official reference method for the assay of **I** is mentioned in internationally recognised pharmacopoeias. For lack of reference methods and unavailability of appropriate placebo the accuracy of the ITP method had to be checked by the standard addition technique in accordance with the recommendation of Pharmaceutical Authorities of the Czech Republic [8]. The mean recovery of the added **I** was 97.3% (see Table 2).

3.3.4. Selectivity

The proposed ITP method with given operational electrolytes is selective regarding the assay of **I** in commercial formulation Sandonorm tablets. Sandonorm is a simple pharmaceutical preparation containing just a single active principle. Other constituents present in the tablet matrix are either isotachophoretically inactive or they are cations such as Na^+ and Fe^{3+}. The zones of these cations are distinctly separated from the zone of **I** due to their high mobilities and so they cannot interfere with the ITP assay of bopindolol. The presence of such excipients can just prolong the duration of the ITP analysis by about 2 min. On the other hand the ITP method is apt to indicate partial hydrolytic decomposition of **I** to **II** and to quantify the extent of hydrolysis if this eventually happened in an improperly stored formulation.

3.3.5. Ruggedness

(i) Stability, stock solution of bopindolol (100 mg l^{-1}) in aqueous 20% methanol stored at 4 °C was stable for at least 7 days (the decrease of the concentration of I as found by the ITP was $\leq 0.8\%$. (ii) Repeated preparation of the LE (pH 5.5) had negligible effect on the reproducibility of the ITP zone length values of bopindolol (R.S.D. $\leq 1\%$; $n = 3$). (iii) The effect of pH of the LE. Within the tested pH interval (pH 5.0–5.8) the lengths of the ITP zone of **I** measured for standard solution of bopindolol and of real samples showed reproducibility characterised by R.S.D. $\leq 1\%$. These results indicate that the method is sufficiently rugged with respect to changes in electrolyte composition in the tested pH interval. (iv) For all the capillaries employed (160×0.3 and 90×0.3 mm) purchased from the same supplier the ITP results were consistent with those obtained with the original ITP set-up. (v) Also the examined changes of the driving (± 20 μA) and detection ($+10$ μA) currents gave regular results.

The results of ITP assay of bopindolol in Sandonorm, tablets using proposed ITP method are summarised in Table 3. The intra-assay precision of this method expressed as the R.S.D., was 1.45%. The inter-day precision (reproducibility) results were not investigated.

Table 3
ITP determination of bopindolol in Sandonorm tablets

Formulation	Nominal content	Found by ITP	R.S.D. (%)[a]
Sandonorm	1 mg per tablet	0.99 mg per tablet	1.45

[a] Six replicate results.

4. Conclusion

- The feasibility of validating ITP method for the determination of bopindolol in pharmaceutical preparation Sandonorm is shown.
- The proposed ITP method is suitable for determining 10–100 mg l^{-1} of bopindolol; it is characterised by good selectivity, reproducibility and accuracy.
- The method is acceptably time efficient; a single analysis takes about 12 min.
- Compared with earlier published LC methods predominantly utilised for the determination of bopindolol in body fluids [1–3], the proposed assay shows somewhat lower sensitivity but it is still fully sufficient for the analysis of pharmaceutical preparations containing **I** as the active principle. The possibility of determining milligram amounts of **I** in pharmaceutical preparations and low running costs make the ITP method a good alternative to existing methods currently used in quality control of various pharmaceutical dosage forms.
- In the proposed ITP operational system the separation of bopindolol and **II** as its active metabolite or decomposition product is possible.

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References

[1] C.J. Oddie, G.P. Jackman, A. Bobik, J. Chromatogr. Biomed. Appl. 273 (1983) 469–474.
[2] H. Humbert, J. Denouel, H.P. Keller, J. Chromatogr. Biomed. Appl. 422 (1987) 205–215.
[3] S.L. Perkins, B. Tattrie, P.M. Johnson, E.Z. Rabin, Ther. Drug Monit. 10 (1988) 480–485.
[4] E. Kuesters, D. Giron, J. High Resolut. Chromatogr. Chromatogr. Commun. 9 (1986) 531–533.
[5] W. Steuer, J. Baumann, F. Erni, J. Chromatogr. 500 (1990) 469–479.
[6] V. Jokl, J. Pospíchalová, M. Polášek, Chem. Listy 80 (1986) 1305–1309.
[7] R. Causon, J. Chromatogr. B 689 (1997) 175–180.
[8] Information Bulletin of State Institute of Drug Control 11, Skarnitzl Foundation, Prague, 1991.
[9] B. Silverman, M. Rawlings, C.R. Hitchings, Martindale—The Extra Pharmacopoeia, 29th ed., The Pharmaceutical Press, London, 1989, pp. 24–28.
[10] V. Jokl, J. Chromatogr. 13 (1964) 451–455.