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Determination of some non-steroid anti-inflammatory drugs by capillary isotachophoresis

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Abstract

The isotachophoretic (ITP) behaviour and separation of the anti-inflammatory drugs kebuzone (KB), tribuzone (TB) and phenylbutazone (PB) was studied in the operational system of HCl/His (leading electrolyte, LE) and 4-nitrophenol (terminating electrolyte, TE). The effective mobilities were 19.4×10^{-9} m^2 V^{-1} s^{-1} for KB, 18.1×10^{-9} m^2 V^{-1} s^{-1} for TB and 18.9×10^{-9} m^2 V^{-1} s^{-1} for PB when using an optimised system with 10 mM HCl + 40 mM His (pH 6.63) as LE and 10 mM 4-nitrophenol as TE. The calibration graphs were rectilinear ($r = 0.9982$–0.9996) in the range 20 to 600 μmol l^{-1} of KB, TB or PB. The ITP method was used for determining the content of KB, TB or PB in mass-produced pharmaceuticals as tablets, coated tablets, injections, and ointments. The results of the ITP determination were in good agreement with those of standard pharmacopoeial methods.

Keywords: Anti-inflammatory drugs; Isotachophoresis; Kebuzone; Pharmaceuticals; Phenylbutazone; Tribuzone

1. Introduction

The 4-substituted-1,2-diphenyl-3,5-pyrazolidinediones (4-butyl, phenylbutazone, PB; 4-(3-oxybutyl), kebuzone, KB; 4-(4,4-dimethyl-3-oxopentyl), tribuzone, TB) are widely used as anti-inflammatory agents. They are officially determined by titration methods [1]. Many instrumental methods, including spectrophotometry [2–5], fluorimetry [6], liquid chromatography [7], HPLC [8–12], gas chromatography [13], ^{15}N NMR spectroscopy [14] and coulometry [15] have been used for the determination of these compounds.

The present paper focuses on the development of a simple, rapid and accurate method for isotachophoretic (ITP) separation and determination of the cited drugs and its application to the analysis of some pharmaceuticals.

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2. Experimental

2.1. Apparatus

Isotachophoregrams were obtained on a ZKI 01 ITP analyser (Spišská Nová Ves, Slovak Republic) equipped with a 120 mm × 0.3 mm (i.d.) analytical capillary and a conductivity detector, and linked to a TZ 4600 chart recorder. Samples were dosed by a 30 μl sampling valve.

2.2. Reagents

The standards of KB, TB, and PB (obtained from Léčiva) were of quality complying with PhBs 4 [1]. Distilled and demineralised water was used throughout. Other chemicals and solvents were of analytical grade.

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2.3. Procedure

(a) Effective mobilities (ū)

The measurement of the effective mobilities was carried out with 0.2 mM solutions of sodium salts of KB, TB and PB, potassium iodate being used as the internal standard of mobility. The calibration curves were measured with 20–600 μM solutions of the sodium salts prepared by appropriate dilution of standard stock solutions (2 mmol l^{-1}). The time (in seconds) of the passage of the zone through the detector was read as the quantitative parameter. The driving and detection currents were 50 μA and 25 μA respectively. The leading electrolyte (LE) containing 0.05% of poly(vinyl alcohol) as an additive was a buffer solution of His.HCl + His, $c(Cl^-)$ = 10 mmol l^{-1}, pH = 6.10. The terminating electrolyte (TE) that was 10 mM in 4-nitrophenolate had a pH of 6.7. The effective mobilities of the drugs were calculated from the values of relative weveheights [16]. Theoretical values of effective mobilities were calculated according to the literature [17].

(b) Analysis of dosage forms

Tablets, coated tablets, injections or ointments were processed in such a way that the concentration of analyte in the final test solution or extract fell within the calibration range.

Injections Since the drugs are present in the form of sodium salts, the samples were just diluted with water to achieve the appropriate concentration.

Tablets or coated tablets The mean weight of a single piece (m_t) was determined and the tablets were homogenised as usual. From a weighed amount of the powdered material (m_S) the analyte was liberated by treating the sample with 0.1 M NaOH (a slight stoichiometric excess of OH^- relative to the expected amount of the analyte) and stirring the mixture for 15 or 30 min. Thereafter, the mixture was filtered on a sintered glass filter and the filtrate was made up to a certain volume (V) in a graduated flask; this solution was either subjected to ITP analysis directly or further diluted with water before the analysis.

Ointments The procedure was the same as for the tablets except that the weighed amount of the ointment was melted in a water bath before stirring for 5–30 min with the NaOH solution.

The content of a drug in a dosage form was calculatedaccordingtotheformula:$m = VcF_rZm_t/m_S$, where m stands for the amount (mg) of the drug in a single tablet (or in 1 g of an ointment or 1 ml of an injection solution); V is the volume of the test solution (see above); c is the concentration (mol l^{-1}) of the analyte in the test solution determined by ITP; F_r stands for the relative formula weight of the analyte; Z stands for the dilution factor; m_t is the mean weight of a single tablet; and m_S is the sample weight (in mg). For injections m_t and m_S are omitted, and for ointments m_t is omitted from the formula.

Control alkalimetric or bromatometric determinations were carried out following pharmacopoeial methods [18].

3. Results and discussion

3.1. Acidity of analytes and selection of the electrolyte system

The pyrazolidinedione derivatives under study are of inogenic character since they exhibit keto–enol tautomerism giving rise to enol forms that can be relatively readily ionised. This makes it possible to use anionic capillary ITP for their analysis. To facilitate the selection of the electrolyte system, initially the pK_a values of the drugs were determined spectrophotometrically at the authors' laboratory; the pK_a values were 4.48 ± 0.01 (PB), 3.79 ± 0.01 (KB) and 3.99 ± 0.01 (TB). Five operational systems with Cl^- as the leading ion were tested. The systems differed by the critical selection of the counter-ion and, consequently, by the pH of the LE. These systems were (counter-ion(pK_a)–terminator): (I) ε-aminocaproic acid(4.3)–picolinate; (II) 1,10-phenanthroline(4.96) – morpholinoethansulfonate; (III) histidine(6.1)–4-nitrophenolate; (IV) TRIS (8.1)–glycinate; (V) dimethylaminoethanol(9.25)–OH^-. From the standpoint of the sensitivity of the ITP determination and the quality of separation, the histidine operational system III with the original, newly introduced 4-nitrophenol as terminator was found to be optimal.

Table 1
Effect of concentration of the leading ion Cl^- on ITP separation of KB, PB and TB

c_L (mmol l^{-1})	Column hold-up (mC)	pH_{LE}[a] exper.	$\bar{u} \times 10^9$ ($m^2 V^{-1} s^{-1}$)				Waveform
			T[b]	KB	PB	TB	
5	20.7	6.11	20.7	25.3	24.7	24.3	Rectangular
10	40.5	6.10	13.6	18.9	18.5	17.6	Rectangular
20	80.8	6.15	11.5	19.8	19.4	18.3	Rectangular

LE = HCl/His 1:2; TE = 4-nitrophenol; $c = 10$ mmol l^{-1}.
[a] Calculated $pH_{LE} = 6.10$.
[b] Calculated $\bar{u}_T = 11.45 \times 10^{-9}$ $m^2 V^{-1} s^{-1}$.

3.2. Optimisation of analysis in the HCl/His (LE)–4-nitrophenol (TE) system

The concentration of the leading ion substantially influences, besides the intensity of the electric field, the quality of separation and the duration of analysis. As expected, the time of analysis is shortened, the column hold-up is diminished and sensitivity is increased with decreasing Cl^- concentration. The results are shown in Table 1. A good agreement between the experimental values of the effective mobilities $\bar{u}_T$ of the terminator and the theoretical values is observed when employing more concentrated solutions of LE. Under these conditions the quality of separation is demonstrated by rectangular waves on the isotachophoregram.

The effect of pH of the solution of the LE was tested in a pH range of 5.58–6.58. The leading electrolyte was a buffer system of the salt and the free base in the ratio 1:0.3, 1:1 and 1:3. The effective mobility and the quality of separated zones of the individual drugs increased with increasing pH of the LE, depending on the increase in the degree of dissociation of the drugs (see Table 2).

Parameters of the optimal electrolyte system for the drugs under study including their principal ITP characteristics determined with the use of potassium iodate as the internal standard are presented in Table 3. The $\bar{u}$ values range from 18.1×10^{-9} to 19.4×10^{-9} $m^2 V^{-1} s^{-1}$ and the highest electrophoretic mobility is shown by kebuzone; this is in accordance with its lowest pK_a value. The small difference between the drug mobilities (about 7% between the slowest and the fastest one) does not allow their reliable separation from each other, but this is not a serious drawback since in real dosage forms the given drugs occur separately. The effective mobility of the terminating 4-nitrophenolate is 16.2×10^{-9} $m^2 V^{-1} s^{-1}$ and therefore the zones of the drugs migrate in the isotachophoretically correct way in the given system. The isotachophoregrams of drugs are shown in Fig. 1; it can be seen that a single ITP analysis takes about 15 min.

3.3. Calibration graphs

Calibration dependences of drug standards $t = f(c)$ were examined at concentration ranges of 20–600 μmol l^{-1} of an analyte and evaluated by linear regression. By transforming the regression equations the following relations for the calculation of the concentration of the analyte from the measured times of the passage of the zone through the detector t(s) were obtained:

PB: $c(\mu mol\ l^{-1}) = 4.077t - 6.920$; $r = 0.9984$; RSD = 1.27% ($n = 6$) at 200 μmol l^{-1}

KB: $c(\mu mol\ l^{-1}) = 3.389t + 0.111$; $r = 0.9997$; RSD = 1.59% ($n = 7$) at 200 μmol l^{-1}

TB: $c(\mu mol\ l^{-1}) = 3.352t + 10.161$; $r = 0.9995$; RSD = 1.88% ($n = 5$) at 200 μmol l^{-1}

The low values of the intercepts and the high values of the correlation coefficients are positive signs of the analytical stability of the zones and rectilinearity of the calibration curves respectively. Good precision of the method is characterised by favourable relative standard deviations (RSDs) shown above.

3.4. Determination of drugs in pharmaceutical formulations

The preparation of the sample for analysis is straightforward for injections where the drug is present in the form of a water-soluble sodium salt. In the case of other formulations

Table 2
Effect of pH of the leading electrolyte solution; calculated values in parentheses

c_{HCl}:c_{His}	pH_{LE}	$\bar{u} \times 10^9$ ($m^2 V^{-1} s^{-1}$)				Zone separation quality order
		T	KB	PB	TB	
1:1.3	5.43 (5.58)	12.9 (9.0)	19.3	17.9	17.5	3rd
1:2	6.10 (6.10)	13.7 (11.5)	18.9	18.5	17.8	2nd
1:4	6.63 (6.58)	16.2 (15.6)	19.4	18.9	18.2	1st

$c_{HCl} = c_{TE} = 10$ mmol l^{-1}; TE = 4-nitrophenol.

it was necessary to find a time sufficient for the quantitative release of the drug (kinetics of elution) by means of 0.1 M sodium hydroxide. In all cases, a 15 min interval was sufficient for complete liberation of the drug from the dosage form. The results of the ITP analyses of pharmaceuticals together with those of reference pharmacopoeial methods and appropriate inter-assay validation [19] are summarised in Table 4.

Phenylbutazone

Two-component PB preparations shown in Table 4 were analysed; the other active compound (isopyrine base) did not interfere with the anionic ITP. In the case of Rheumanol (coated tablets) an increased amount of NaOH for the neutralisation of isopyrine hydrochloride had to be considered to ensure quantitative extraction of PB in the form of sodium salt. The results agreed well with those of potentiometric bromatometric titration after selective extraction of the drug.

Kebuzone

In the composite preparations Ketazon comp., Ketazon mix. and Ketazon inj., other active ingredients (heparin S, benzyl nicotinate, trimecaine) did not interfere with the ITP analysis. The content of KB found by ITP was in good agreement with the nominal content, the reproducibility of determination being better for injections and tablets as compared to ointments. A good agreement with the results of bromatometry was observed.

Table 3
Optimised operational system used for ITP of KB, PB and TB

c (mol l^{-1})		pH_{LE}	$\bar{u} \times 10^9$ ($m^2 V^{-1} s^{-1}$)		
Cl^-	His		KB	PB	TB
0.01	0.04	6.63	19.4 ± 0.2	18.9 ± 0.2	18.1 ± 0.2

TE = 4-nitrophenol; $c = 10$ mmol l^{-1}.

Tribuzone

The ITP determination of the content of the original Czechoslovak drug tribuzone in Benetazon tablets gave practically the same results as the reference method (alkalimetric titration in acetone with visual end-point detection using bromothymol blue) according to PhBs 4 [18].

4. Conclusions

The results presented in this work corroborate the fact that the capillary ITP is a suitable tool for determining KB, TB or PB in pharmaceutical preparations. Most of the previous papers deal with determining such anti-

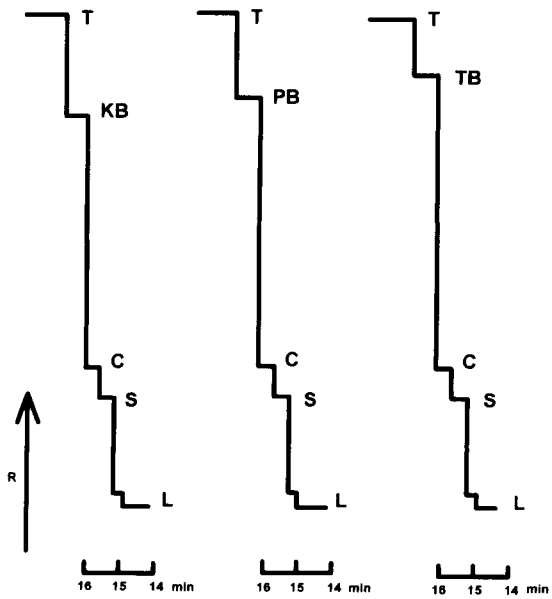

Fig. 1. Isotachophoregram of kebuzone (KB), phenylbutazone (PB) and tribuzone (TB) Operational system: HCl/His, pH 6.6 (L) and 4-nitrophenol (T); S = standard KIO_3; C = hydrogen carbonate.

Table 4
Determination of KB, PB and TB in pharmaceuticals

Preparation	Labelled active ingredient, nominal content	Content (% ± RSD)		Student t-test[a]
		ITP method ($n_A = 6$)	Standard method ($n_B = 3$)	
Rheumanol (tablets)	Phenylbutazone, 100 mg	101.3 ± 2.0	98.4 ± 1.7	1.898
Ketazon (dragées)	Kebuzone, 250 mg	98.3 ± 1.4	98.1 ± 1.4	0.178
Ketazon (injection sol.)	Kebuzone, 200 mg per ml	97.8 ± 0.9	100.5 ± 0.95	3.672
Ketazon H (ointment)	Kebuzone, 50 mg per g	97.2 ± 2.0	101.4 ± 2.1	2.575
Ketazon 10% (ointment)	Kebuzone, 100 mg per g	102.5 ± 1.7	103.1 ± 1.4	0.466
Ketazon mix (ointment)	Kebuzone, 100 mg per g	103.3 ± 1.3	102.0 ± 1.5	1.184
Ketazon comp. (ointment)	Kebuzone, 100 mg per g	98.3 ± 2.5	103.3 ± 1.9	2.691
Benetazon (tablets)	Tribuzone, 250 mg	100.2 ± 2.0	100.5 ± 2.4	0.175

[a] 95% confidence level; $t_c = 2.365$ ($\nu = n_A + n_B - 2 = 7$); Ref. [19].

inflammatory drugs as bulk substances [2,5–10,14,15] or as minor components in biological matrices [11–13]. Although the proposed ITP method is less sensitive than spectrophotometry [3,4], this issue is not of key importance when analysing commercially available pharmaceuticals containing milligram amounts of the drugs. The ITP method is characteristic of good selectivity; other active ingredients and excipients present in pharmaceutical preparations do not interfere with ITP determination because their soluble anions (if produced at all) remain in the zone of impurities separated from the zones of analytes. Moreover, no analyte derivatisation is needed and the sample handling (a single-step ionisation and extraction of the analyte from a pharmaceutical formulation) takes place in aqueous medium. The ITP migration medium employed is purely aqueous without any of the organic solvents that are mandatory in most HPLC analyses.

The proposed method is acceptably time efficient: a single analysis takes less than 20 min for injections and does not exceed 30 min for solid dosage forms and ointments (including the sample preparation steps). The precision of the method, expressed as RSD, was 0.9–2.5% (six replicates) when analysing eight commercial preparations. For five out of eight preparations analysed, no significant differences were found between the results obtained by ITP and pharmacopoeial titrimetric or spectrophotometric methods for the same batch, at the 95% confidence level (Student t-test, see Table 4).

References

[1] Czechoslovak Pharmacopoeia (PhBs), 4th edn., Vol. II, Avicenum, Prague, 1987, pp. 474, 739 and 911.
[2] M. Peterková, B. Kakáč and O. Matoušková, Českoslov. Farm., 26 (1977) 255–258.
[3] G. Morait, V. Tarculet, C. Popa and L. Petroniu, Farmacia (Bucharest), 37 (1989) 1–8.
[4] K.M. Emara and M.E. El-Kommos, J. Pharm. Sci., 4 (1990) 67–72.
[5] J. Kráčmar, J. Kráčmarová, M. Remsová and A. Kovářová, Pharmazie, 43 (1988) 681–686.
[6] R. Fricoteaux, J.J. Aaron and M.G. Quaglia, J. Pharm. Biomed. Anal., 7 (1989) 1585–1590.
[7] G.T. Santasania, J. Liq. Chromatogr., 13 (1990) 2605–2631.
[8] H.G. Eigendorf, Pharmazie, 43 (1988) 287–288.
[9] H.G. Eigendorf, G. Moeschwitzer and R. Budde, Pharmazie, 44 (1989) 645–646.
[10] H.G. Eigendorf, R. Budde, G. Moeschwitzer and H. Koenig, Pharmazie, 45 (1990) 219–220.
[11] Ch. Herrenknecht, D. Ivanovic, E. Guernet-Nivaud and M. Guernet, J. Pharm. Biomed. Anal., 8 (1990) 1071–1074.
[12] R.N. Gupta, J. Chromatogr., 530 (1990) 160–163.
[13] R. Caldwell and H. Challenger, Ann. Clin. Biochem., 26 (1989) 430–443.
[14] J. Čižmárik and A. Lyčka, Pharmazie, 43 (1988) 794–795.
[15] C. Herrenknecht, E. Guernet-Nivaud, M. Guernet and C. Guentin, Ann. Pharm. Fr., 43 (1985) 557–563.

[16] V. Jokl, J. Pospíchalová and M. Polášek, Chem. List., 80 (1986) 1305–1309.
[17] F.M. Everaets, J.L. Beckers and Th.P.E.M. Verheggen, Isotachophoresis, Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[18] Czechoslovak Pharmacopoeia (PhBs), 4th edn., Vol III, Avicenum, Prague, 1987, pp. 273 and 496.
[19] K. Eckschlager, I. Horsák and Z. Kodejš, Vyhodnocování analytických výsledků a metod (Evaluation of analytical results and methods), SNTL, Prague, 1980, p. 44.