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Capillary isotachophoretic determination of flufenamic, mefenamic, niflumic and tolfenamic acid in pharmaceuticals

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Abstract

Anionic capillary isotachophoresis (ITP) with conductimetric detection has been used for determining selected non-steroid anti-inflammatory and analgesic drugs of the phenamate group, namely tolfenamic (**I**), flufenamic (**II**), mefenamic (**III**) and niflumic (**IV**) acid. Initially the pK_a values (proton lost) of **I**–**IV** were determined as 5.11, 4.91, 5.39 and 4.31, respectively, by the UV spectrophotometry in aqueous 50% (w/w) methanol. The optimised ITP electrolyte system consisted of 10 mM HCl + 20 mM imidazole (pH 7.1) as the leading electrolyte and 10 mM 5,5′-diethylbarbituric acid (pH 7.5) as the terminating electrolyte. The driving and detection currents were 100 μA (for 450 s) and 30 μA, respectively (a single analysis took about 20 min). Under such conditions the effective mobilities of **I**–**IV** varied between 23.6 and 24.6 $m^2\ V^{-1}\ s^{-1}$ (evaluated with orotic acid as the mobility standard). The calibration graphs relating the ITP zone length to the concentration of the analytes were rectilinear ($r = 0.9987$–0.9999) in the range 10–100 mg l^{-1} of the drug standard. The R.S.D.s were 0.96–1.55% ($n = 6$) when determining 50 mg l^{-1} of the analytes in pure test solutions. The method has been applied to the assay of the phenamates in six commercial mass-produced pharmaceutical preparations (Mobilisin gel and ointment, Lysalgo capsules, Nifluril cream, Niflugel gel, and Clotam capsules). According to the validation procedure based on the standard addition technique the recoveries were 98.4–104.3% of the drug and the R.S.D. values were 1.25–3.32% ($n = 6$). © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Flufenamic acid; Mefenamic acid; Niflumic acid; Tolfenamic acid; Isotachophoresis; Pharmaceutical analysis

1. Introduction

Tolfenamic acid *N*-(3-chloro-2-methylphenyl)anthranilic acid (**I**), flufenamic acid *N*-(3-trifluoromethylphenyl)anthranilic acid (**II**), mefenamic acid *N*-(2,3-dimethylphenyl)anthranilic acid (**III**) and niflumic acid 2-(3-trifluoromethylphenylamino)nicotinic acid (**IV**) (see Fig. 1) are non-steroid anti-inflammatory and analgesic drugs. These drugs occurring as active components in pharmaceuticals for peroral and/or transcutaneous application are not yet included in

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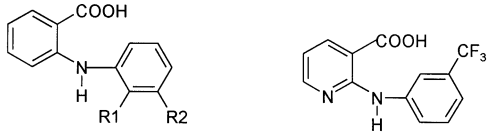
internationally recognised pharmacopoeias, though various mass-produced dosage forms containing **I**, **II**, **III** or **IV** are available on the market.

Over 140 papers dealing with the analysis of such drugs were published during the past two decades. The phenamates were determined in various pharmaceutical preparations as well as in biological materials such as blood plasma, blood serum and urine. The HPLC with UV spectrophotometric detection is the most frequently used technique for their determination, see e.g. [1–5]. The compounds were also assayed directly by UV spectrophotometry [6,7] or, after derivatisation, by spectrophotometry in visual region [8,9]. Recently the **I**–**III** were also determined by spectrofluorimetry [10,11]. Gas chromatography [12] was employed for determining these drugs in blood or plasma. A GC-MS method was used for the identification of **I** and its metabolites in equine plasma and urine [13]. As for electro-migration methods, capillary zone electrophoresis [14,15] and micellar electrokinetic chromatography [16] with UV detection were used earlier just to optimise conditions for the separation and determination of some of these substances in mixtures with other acidic drugs.

To the authors' best knowledge, the **I**, **II**, **III** and **IV** have not yet been determined by capillary isotachophoresis (ITP) though these drugs are weak acids which should undergo ionisation to anionic species in slightly basic medium thus making their determination by ITP possible. In this paper, a simple and selective ITP method for the quantification of **I**, **II**, **III** and **IV** in pharmaceutical preparations is described. Compared to LC methods the migration medium employed in the ITP assays is purely aqueous without any of the organic solvents that are needed in most HPLC analyses, making the ITP procedure environmentally more acceptable; the disadvantage of the ITP is its lower sensitivity which is insufficient for determining low levels of these drugs in body fluids but on the other hand it is quite sufficient for the quantitation of milligram amounts of the **I**–**IV** in pharmaceutical preparations. In comparison to earlier published capillary electrophoresis methods the proposed ITP is somewhat more time consuming and less sensitive but this drawback is compensated by much lower cost of the ITP instrumentation.



I: $R_1 = CH_3$, $R_2 = Cl$; **II**: $R_1 = H$, $R_2 = CF_3$; **III**: $R_1 = R_2 = CH_3$

IV

Fig. 1. Chemical structures of assayed drugs.

2. Experimental

2.1. Apparatus

Isotachophoretic 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 mm × 0.3 mm (i.d.) analytical capillary made of fluorinated ethylene-propylene (FEP) copolymer 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 appropriate ITP software package supplied by the VILLA company.

For the spectrophotometric determination of the pK_a of **I**, **II**, **III** and **IV** the Hewlett-Packard 8453 UV/VIS spectrophotometer equipped with a diode-array detector and a thermostated cell holder housing a 3.5-cm fused silica cell (volume 100 ml) was employed. A Radiometer PHM-64 pH meter with a GK-2041-B combined glass electrode calibrated with standard buffers (hydrogen succinate pH 5.66 and hydrogen phosphate pH 7.88 in aqueous 50% w/w methanol) was used for the pH measurements.

Bandelin SONOREX RK 100 ultrasonic bath (Berlin, FRG) was employed for the sonication of solid dosage forms to facilitate the dissolution or extraction of analysed drugs.

2.2. Reagents

The standards of mefenamic, flufenamic and niflumic acid were obtained from Aldrich (USA), the tolfenamic acid from MEDICA (Finland). Other chemicals (orotic acid, imidazole, poly(vinylalcohol) 49 000 from Fluka AG Buchs, 5,5-diethylbarbituric acid from Aldrich (USA), hydrochloric acid, sodium hydroxide, barium hydroxide, sodium chloride and methanol from Lachema Brno) were of analytical grade. A Millipore Milli-Q RG ultra-pure water was used throughout.

2.3. Dosage forms analysed

MOBILISIN gel (Sankyo, Germany) containing 30 mg g^{-1} of labelled **II** and 20 mg g^{-1} of ammonium salicylate and 2 mg g^{-1} of poly(glycosaminoglycan) sulphate as excipients, MOBILISIN ointment (Luitpold Pharma, Germany) containing 30 mg g^{-1} of labelled **II** and 26.4 mg g^{-1} of hydroxyethyl salicylate and 2 mg g^{-1} of poly(glycosaminoglycan) sulphate as excipients, LYSALGO capsules (Schiapparelli, Italy) containing 250 mg of labelled **III** in a single capsule, CLOTAM capsules (Medika, Finland) containing 100 mg of labelled **I** in a single capsule and also gelatin, feric oxide, magnesium stearate, lactose, maze starch and povidone as excipients, NIFLURIL cream (UPSA, France) containing 30 mg g^{-1} of labelled **IV** and macrogol palmitostearate, macrogol 400, liquid paraffin, stearic acid and parabens as major excipients and NIFLUGEL gel (UPSA, France) containing 25 mg g^{-1} of labelled **IV** and diisopropanolamine and carbomer as excipients.

2.4. Procedure

2.4.1. Ionisation constants pK_a

A solution of **I**, **II**, **III** or **IV** (volume 100 ml in 50% (w/w) methanol, $c = 0.02$ mM, ionic strength $I = 0.01$ adjusted with NaCl) placed in a 3.5-mm fused silica cell thermostated at 25 ± 0.2°C was titrated with μl volumes of 0.01–0.1 M NaOH directly in the spectrophotometric cell and the pH and absorption curves were measured simultaneously [17]. The data of the absorbance (A) versus pH curves measured at 349 and 292 nm for **I**, at 347 and 292 nm for **II**, at 353 and 288 for **III** and at 254 and 289 nm for **IV** (involving typically 25–35 experimental points) were used to compute the apparent pK_a constant of **I**–**IV** by non-linear regression [18]. All the A–pH curves were measured in triplicate and the final pK_a was calculated as the arithmetic mean of six individual results.

2.4.2. The effective mobilities $\bar{u}$ and calibration curves of *I*, *II*, *III* and *IV*

The measurement of the effective mobilities was carried out with 0.2 mM solution of **I**–**IV** and 0.2 mM orotic acid as the standard of mobility. The effective mobility of the drugs was calculated from the waveheights [19] obtained from three repeated ITP measurement taking into account the tabulated values of ionic mobilities of Cl^- and orotic acid 79.1×10^{-9} and 33.1×10^{-9} m^2 V^{-1} s^{-1}, respectively.

The calibration curves were measured with 0.038–0.38 mM–**I**, 0.036–0.36 mM–**II**, 0.041–0.41 mM–**III** and 0.035–0.35 mM–**IV** (five concentrations for each analyte, each measured in triplicate) corresponding to 10–100 mg l^{-1} of **I**, **II**, **III** or **IV**. The time (t, in s) of the passage of the zones of **I**–**IV** 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. The driving and detection currents were 100 (for 450 s) and 30 μA, respectively. The leading electrolyte (LE) was a buffer solution containing 0.01 M HCl and 0.02 M imidazole (pH 7.1). The terminating electrolyte was 0.01 M in 5,5′-diethylbarbituric acid (pH 7.5).

2.4.3. Analysis of dosage forms

All dosage forms examined were processed in such a way that the concentration of **I**–**IV** in the final test solutions or extracts fell within the calibration range. Since these drugs are sparingly soluble in water, equimolar amount of 0.1 M sodium hydroxide was added to each sample and thereafter the solution of the respective sodium salt was diluted with water to achieve the appropriate concentrations.

Table 1
Survey of the isotachophoresis (ITP) electrolyte systems examined

LE 0.01 M	LE counter ion	TE 0.01 M	pH of LE	pH of TE
Cl^-	0.04 M Creatinine	MES	5.31	5.94
Cl^-	0.04 M Creatinine	HEPES	5.31	6.62
Cl^-	0.04 M Creatinine	4-Nitrophenol	5.31	6.38
Cl^-	0.02 M Histidine	4-Nitrophenol	6.01	6.82
Cl^-	0.02 M Histidine	HEPES	6.01	7.01
Cl^-	0.02 M Imidazole	HEPES	7.15	7.73
Cl^-	0.02 M Imidazole	5,5′-Diethylbarbituric acid (VERONAL)	7.15	7.5

2.4.3.1. Capsules. The contents of ten capsules were emptied into an agate mortar, homogenised and appropriate amount of the powdered material corresponding to ≈ 100 mg of **I** or **III** was weighed and dissolved in equimolar amount of 0.1 M sodium hydroxide by applying a 10-min sonication; thereafter the solution was diluted to 200 ml with water, filtered through a dry paper, 10 ml of the filtrate was diluted to 100 ml with water and analysed by ITP.

2.4.3.2. Gel, cream, ointment. An amount of the gel, cream or ointment corresponding to 10 mg of **II** or **IV** was weighed and treated with stoicheiometric amount of sodium hydroxide, the mixture was set aside for 15-min and thereafter it was diluted to 200 ml with water and sonicated for 5 min. The emulsion was cleared by filtration through Schleicher&Schuell SPARTAN 30/B membrane filter (pore size 0.45 μm) before the ITP analysis.

2.4.3.3. Accuracy test. The accuracy of the results obtained by the ITP method was checked by adding known amount of **I**, **II**, **III** or **IV** (standards) corresponding to 100% of the **I**, **II**, **III** or **IV** in the previously analysed dosage form and by determining the recovery of the added analyte by ITP.

3. Results and discussion

3.1. The pK_a values of *I–IV*

The apparent pK_a values (proton lost) were 5.11 ± 0.02 ($n = 6$) for **I**, 4.91 ± 0.03 ($n = 6$) for **II**, 5.39 ± 0.03 ($n = 6$) for **III** and 4.31 ± 0.02 for **IV**; the pK_a value of 1.74 ± 0.03 ($n = 6$) corresponding to the protonation of **IV** was also determined. All the values were determined by UV spectrophotometry at 25°C and $I = 0.01$ (NaCl) in the medium of aqueous 50% (w/w) methanol. These results indicate that the **I**, **II**, **III** and **IV** will be practically completely ionised to anionic species at the $pH \geq 6.5$. Previous literature data on the protolytic characteristics of **I**–**IV** in aqueous media are relatively scarce and the pK_a values could only be found for **II** [20], **III** [21] and **IV** [22–24]; these seem to agree well with the values considering the effect of 50% methanol on the ionisation of the carboxylic group.

3.2. Selection of operational electrolyte system

The ITP operational system has been optimised with respect to the quality of separation, the sensitivity of the ITP determination and time of analysis. Various operational systems with Cl^- as the leading ion and with different counter ions have been tested. The optimisation involved critical selection of the kind and concentration of the counter ion and, consequently, the pH of the leading electrolyte. With these leading electrolytes various terminating electrolytes have been tested. The parameters of the operational electrolyte systems are shown in Table 1.

The first operational system was suitable only for the analysis of niflumic acid which is the strongest acid among the drugs analysed. The next two systems with creatinine as the counter ion were unsuitable because with HEPES as TE

the voltage was too high, causing drop-outs in the analysis run, and with 4-nitrophenol the zones of the analytes were deformed. Problems were encountered also with histidine as counter ion since here the analyte zones were deformed (with HEPES as TE) and/or the analysed drugs migrated in one zone with unidentified impurities occurring in the operational system (with 4-nitrophenol as TE). In the systems with imidazole as the counter ion the migration of the ionised drugs was correct and the boundaries between the zones were sharp. With HEPES as the TE the time of analysis was somewhat shorter compared to Veronal-containing TE but with the imidazole-Veronal system the sensitivity of determination was about twice as high and therefore this system was selected as the most favourable. In this system optimal values of the driving and detection currents were 100 (for 450 s) and 30 μA, respectively. Total time of analysis was about 20 min. Under such conditions the effective mobilities of **I**–**IV** were 24.59×10^{-9}, 23.62×10^{-9}, 23.59×10^{-9} and 24.39×10^{-9} $m^2\ V^{-1}\ s^{-1}$, respectively, with R.S.D. values not exceeding 1.2% ($n = 3$). These values are in fact the ionic mobility values of **I**–**IV** because these drugs are practically completely ionised in the electrolyte system used. Typical isotachophoregram of **I** is shown in Fig. 2.

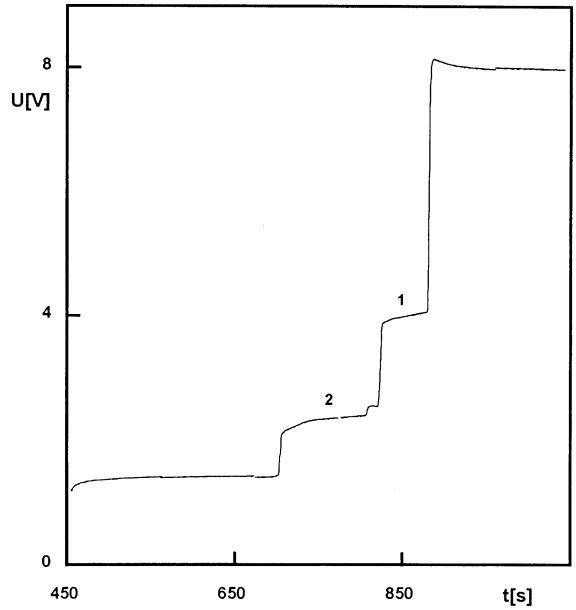


Fig. 2. Isotachophoregram of 0.2 mM tolfenamic acid (1) involving carbonate (2) as impurity. Operational system: 10 mM HCl + 20 mM imidazole (LE) and 10 mM 5,5′-diethylbarbituric acid (TE); time is continuing after switching the driving current from 100 to 30 μA.

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

Analyte	*a*	*b*	*r*	*n*
Tolfenamic acid	1.2604 ± 0.064	4.2679 ± 4.14	0.99872	5
Flufenamic acid	1.3466 ± 0.017	0.6834 ± 1.08	0.99992	5
Mefenamic acid	1.3947 ± 0.023	0.9157 ± 1.50	0.99986	5
Niflumic acid	1.3464 ± 0.014	1.2385 ± 0.78	0.99990	5

[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 five calibration solutions was measured in triplicate).

3.3. Calibration graphs

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 **I**–**IV** in mg l^{-1}) was examined in the concentration range 10–100 mg l^{-1} for each analyte and the ITP data were evaluated by linear regression. The linear regression parameters of the calibration line $t = a \cdot c(\text{analyte}) + b$ are given 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 for replicate analyses of prepared samples of 50 mg l^{-1} of **I**–**IV** ($n = 6$) showed R.S.D. of 0.96–1.55% 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 2.3%.

Table 3
Isotachophoresis (ITP) determination of tolfenamic, flufenamic, mefenamic and niflumic acids in pharmaceutical preparations

Formulation (drug)	Nominal content	ITP method Content ± R.S.D.[a] (%) (mg piece^{-1} or mg g^{-1})
CLOTAM (tolfenamic acid) cps.	100 mg	97.83 ± 1.43
MOBILISIN (flufenamic acid) gel	30 mg g^{-1}	29.5 ± 1.25
MOBILISIN (flufenamic acid) oint.	30 mg g^{-1}	30.9 ± 1.68
LYSALGO (mefenamic acid) cps.	250 mg	248.9 ± 1.35
NIFLURIL (niflumic acid) cream	30 mg g^{-1}	28.5 ± 2.91
NIFLUGEL (niflumic acid) gel	25 mg g^{-1}	26.45 ± 3.32

[a] $n = 6$.

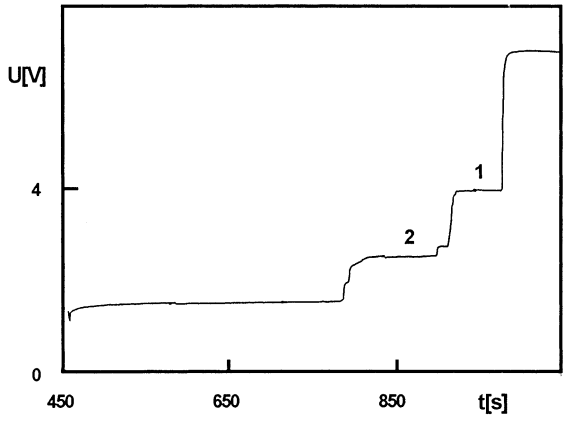


Fig. 3. Isotachophoregram of Niflugel gel; (1) niflumic acid; (2) carbonate as impurity. For operational system see Fig. 2.

3.4. Determination of drugs in pharmaceutical formulations

The ITP was used for determining **I**–**IV** in six pharmaceutical preparations listed in Table 3. In all instances preparations containing appropriate phenamate drug as a single active component have been analysed. It must be noted that there are no official reference methods for determining the **I**–**IV** in pharmaceutical formulations available; therefore only the ITP results are summarised in Table 3 including the repeatability data ($n = 6$). Typical isotachophoregram of a real sample (Niflugel gel) indicating the absence of any interfering zones is presented in Fig. 3. In fact, the only excipient of those listed in Section 2.3 exhibiting electromigration under the conditions of anionic ITP was salicylate which is present in MOBILISIN gel; the ITP zone of salicylate was distinctly separated from that of **II** and located close to the zone of the leading ion and hence salicylate did not interfere with the ITP determination of **II**.

3.4.1. Accuracy and ruggedness/robustness of the ITP method

Since no pharmacopoeial method could be found for the assay of **I**–**IV** to check the accuracy of ITP results the method of the standard addition was utilised. The recoveries of the added **I**–**IV** ranging between 98.4 and 104.3% are shown in Table 4. Hence it can be concluded that the accuracy of the proposed ITP assay is acceptable. As for the ruggedness of the proposed ITP method, no real inter-laboratory assays were carried out. An indication of good ruggedness of the ITP method might be the fact that the R.S.D. values for day-to-day assays of **I**–**IV** in the cited formulations performed in the same laboratory by two analysts within 2 weeks did not exceed 4.5%. Deliberate changes of the pH of the leading electrolyte within the range of pH 6.9–7.3 and those of the driving and detection currents between 50–100 and 10–30 μA, respectively, did not exhibit any significant effects on the accuracy and repeatability of the results of ITP assay of **I**–**IV**. The same was true when the original separation capillary (160 mm × 0.3mm) was replaced by a shorter one (90 mm × 0.3 mm).

Table 4
Isotachophoresis (ITP) recoveries of **I**–**IV** added to pharmaceutical formulations

Formulation	Added (mg l^{-1})	Found ± R.S.D.[a] (%) (mg l^{-1})	Recovery (%)
CLOTAM (tolfenamic acid) cps.	50	49.4 ± 0.65	98.8
MOBILISIN (flufenamic acid) gel	50	49.5 ± 0.55	99.0
MOBILISIN (flufenamic acid) oint.	50	49.2 ± 0.75	98.4
LYSALGO (mefenamic acid) cps.	50	49.3 ± 0.65	98.6
NIFLURIL (niflumic acid) cream	30	31.24 ± 0.87	104.1
NIFLUGEL (niflumic acid) gel	30	31.29 ± 1.68	104.3

[a] $n = 6$.

4. Conclusions

The results presented in this paper corroborate the fact that the anionic capillary ITP is a suitable tool for separating and determining milligram amounts of non-steroid anti-inflammatory and analgesic drugs of the phenamate group, namely tolfenamic (**I**), flufenamic (**II**), mefenamic (**III**) and niflumic acid (**IV**) in various dosage forms. The method is acceptably time efficient; a single analysis takes about 20 min. There might be a minor problem with the presence of carbonate whose zone somewhat lengthens the total time of analysis. The sample preparation is easy. Moreover, the advantage of the ITP is the fact that the migration medium employed is purely aqueous without any of the organic solvents that are needed in most HPLC analyses. The lower sensitivity of the proposed ITP method compared to the HPLC methods published 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 as well as for determining very low concentrations of prospective anionic decomposition products of **I**–**IV** ($\leq 2\%$ relative to the parent compound) that might be formed during drug stability studies. Considering its acceptable reproducibility and accuracy the ITP method devised can be recommended for analytical evaluation of pharmaceutical formulations containing **I**, **II**, **III** or **IV** as the active ingredient.

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