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Determination of fenoprofen in serum by capillary isotachophoresis

J. Sádecká, A. Hercegová, J. Polonský*

Department of Analytical Chemistry, Faculty of Chemical Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

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Abstract

A isotachophoretic method with conductivity detection was developed and validated to directly determine fenoprofen in human serum. The leading electrolyte contained hydrochloric acid (10 mmol/l), 6-aminocaproic acid (pH 4.8) and polyvinylpyrrolidone (0.1%). The terminating electrolyte was 4-morpholineethanesulfonic acid (5 mmol/l). The calibration curve was linear over the concentration range 0.02–0.40 mmol/l. Within-day standard deviation ranged from 0.001 to 0.004 and between-day standard deviation ranged from 0.001 to 0.004. The limit of determination was 0.02 mmol/l. The assay was employed to determine serum concentration of fenoprofen in patients.

Keywords: Fenoprofen

1. Introduction

Fenoprofen is a non-steroidal anti-inflammatory drug which has a wide therapeutic range of 16–65 mg/l (0.062–0.268 mmol/l) in serum and a toxic concentration is not available [1]. A number of HPLC methods for determination of fenoprofen in plasma [2–7], serum [3] and urine [5,6] appeared in the literature. Each of these methods requires a sample preparation based on simple acetonitrile deproteinization [2,3], liquid–liquid extraction [4–6] or on-line dialysis [7]. HPLC was also used to study binding of fenoprofen to human serum albumin [8,9].

Capillary isotachophoresis (ITP) has proven to be an useful tool for the control of the composition and purity of various pharmaceutical preparations [10–18]. ITP was also used for the determination of drugs in human body fluids [19–21], as well as in studying their metabolism and excretion [22,23]. The main problem in the application of ITP in medical and biological research is the variability and complexity of the sample matrix. Hence the ITP analysis is combined with sample pretreatment, and/or other separation technique. A complicated extraction procedure was necessary for the separation of urinary tiols as tributyltinmercaptides [24]. By applying the spacer technique, lipoproteins [25,26], proteins [27] and peptides [28] in human serum were analyzed by ITP. In recent years, the combination of ITP with CZE is most promising, where ITP serves as the preseparation and preconcentration step. The combination of ITP–CZE is a technique that is especially suitable for the analysis of microcomponents in complicated matrices of varying composition [29–31].

This paper describes a simple and sensitive method based on isotachophoresis for the quantitation of fenoprofen in serum. The method was validated according to validation procedures, parameters and acceptance criteria based on recommendations of the

*Corresponding author.

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Washington Conference of Analytical Methods Validation [32].

2. Experimental

2.1. Materials and reagents

All chemicals were of analytical-reagent grade. Deionized, redistilled water was used in the preparation of the electrolyte systems and solution of model mixtures.

Fenoprofen calcium salt, hydrate (Sigma) was obtained as a gift from Dr. H. Shintani (National Institute of Health Sciences, Tokyo, Japan). Control serum was obtained from Imuna (Slovak Republic). A stock solution of fenoprofen (20 mmol/l) was prepared by dissolution of fenoprofen in 100 mmol/l sodium hydroxide solution and dilution to volume with water, and then storage at 4°C. Standard solutions of fenoprofen were obtained from the stock solution by dilution with purified water. Serum standards were prepared daily by adding a known amount of the standard to drug-free control serum, these standards were used to create ITP-calibration curves as a control and to determine analytical recoveries.

2.2. Instrumentation

Isotachophoretic separations were performed using a Villa Labeco ZKI 02 column-coupling isotachophoretic analyzer equipped with a conductivity detector. The analytical capillary (90 mm×0.3 mm I.D.) was connected with a preseparation capillary (90 mm×0.8 mm I.D.). Capillaries made of fluorinated ethylene–propylene copolymer (FEP) were used.

2.3. Isotachophoretic conditions

The electrolyte systems are given in Table 1. The driving current for the preseparation column was 100 μA and for the analytical column was 30 μA. The samples was injected with a 10-μl microsyringe (Hamilton, Switzerland).

3. Results and discussion

3.1. Method development

The main problem in the direct ITP analysis of serum for drugs is dealing with the high and variable concentrations of proteins (6–8 g/l) and ions (150–200 mmol/l) present.

Proteins may interfere in three ways: by adsorbing to the capillary wall and detector affecting reproducibility, by binding the drug, and by interfering with detection of the analyte zone.

If undiluted serum is injected, the large quantity of adsorbed serum proteins can cause a change in conductivity and washing between runs is needed. Sodium dodecylsulfate (100 mmol/l) dissolved in a leading electrolyte can provide excellent removal of adsorbed proteins.

Like many other drugs, fenoprofen tends to bind to the serum proteins (human serum albumin) and needs to be released from them during the sample preparation. In HPLC and CZE this can be done by

Table 1
Electrolyte systems for ITP

Parameter	Conditions
Leading electrolyte	
LE1	10 mmol/l hydrochloric acid including 0.1% PVPD[a] adjusted with 6-aminocaproic acid to pH 4.5–5.0
LE2	10 mmol/l hydrochloric acid including 0.1% PVPD[a] adjusted with creatinine to pH 5.0–5.5
Terminating electrolyte	
TE1	5 mmol/l 4-morpholineethanesulfonic acid (MES)
TE2	5 mmol/l MES adjusted with TRIS[b] to pH 7.0

[a] PVPD=Polyvinylpyrrolidone.
[b] TRIS=Tris(hydroxymethyl)aminomethane.

liquid–liquid extraction, or a more attractive approach is on-line dialysis. The simple way to overcome this problem is to add organic solvent (acetonitrile) to the sample. In ITP separations the problems with protein-binding of the drugs do appear to be of a lesser extent than in e.g. HPLC analysis. Since the electric field strength is high during ITP separation, the drug may be desorbed by electrodesorption from the matrix. It is important that the drug is reversibly adsorbed and the rate of desorption is high in comparison with the time needed for analysis. In addition a proper choice of the composition of the leading electrolyte can also lead to the release of the drug from the drug–protein complex. In the development stage, different leading electrolytes and driving currents were tested in the search for a suitable desorption method. Based on our results it appeared that the optimum experimental conditions for the desorption can be obtained if the pH of the leading electrolyte is in the range 4.5–5.0 and the driving current in the 9-cm long preseparation capillary is 100 μA. Recovery results for the validated method are given below.

To find conditions giving a satisfactory separation in reasonable time, we studied the effect of the pH of LE and the injection volume on the separation. The effect of pH was studied from 4.5 to 5.0 with 6-aminocaproic acid and from 5.0 to 5.5 with creatinine. The pH was increased in steps of 0.5 unit or less. In the pH range studied, the carboxylic group of fenoprofen is dissociated and thus the analyte migrates as the anion. In the pH range 4.5–5.0 fenoprofen was well separated from serum and the leading electrolyte at pH 4.8 produced the best separation from the endogenous compounds. At pH 5.5 and higher, fenoprofen migrated in the zone of proteins.

One difficulty in the direct analysis of serum is the variable, but generally high, concentrations of electrolytes in serum because, in ITP, the ionic strength of the sample to be analyzed is limited. For instance, high concentrations of chloride (another anion) result in a prolonged time of analysis and poor separation. The excessive amounts of ions may be removed either by sample preparation or by using column-coupling ITP. In the commercial instrument used for our ITP experiments, the analytical capillary of 0.3 mm I.D. is combined with the preseparation capillary of 0.8 mm I.D. which increases the load capacity of the system without a considerable increase in the time of analysis. The matrix components were almost totally (97%) removed from the separation system prior to entering the analytical capillary, where further separation and detection take place. The detector in the preseparation capillary ensures that the time of current switching can be adjusted precisely with respect to the actual composition of the separated sample, so that always the same size of the sample segment can be transferred into the analytical capillary, irrespective of how long the zones of matrix preceding the analyte are. Furthermore, a proper switching of the current leads to the ITP analysis (in the analytical column) of a very simplified part of the sample, which further eliminates interferences from the matrix. Hence in column-coupling ITP the problems with variable concentrations of ions in the samples do appear to be of a lesser extent than in single-column ITP. The maximum volume of serum that can be completely separated before the zone of fenoprofen reaches the preseparation capillary detector was confirmed experimentally to be 5 μl for a 9-cm long capillary and a driving current of 100 μA.

3.2. Method validation

3.2.1. Selectivity

Several free human serum samples from different healthy subjects and synthetic serum samples were tested for the absence of interfering compounds. The relative step heights of endogenous compounds in serum were compared with that of fenoprofen. As can be seen in Fig.1 at pH 4.8, none of the endogenous compounds in serum interfered with analysis.

3.2.2. Linearity

Linearity was evaluated using two sets of standard solutions: one prepared from reference standard at levels 0.02, 0.05, 0.10, 0.20, 0.30 and 0.40 mmol/l and one prepared by spiking 0.5 ml of blank serum with appropriate volumes of standard in order to obtain concentrations of 0.02, 0.05, 0.10, 0.20, 0.30 and 0.40 mmol/l. The volume added was always less than 2% of the total volume of the samples, so that the integrity of the serum was maintained. Each

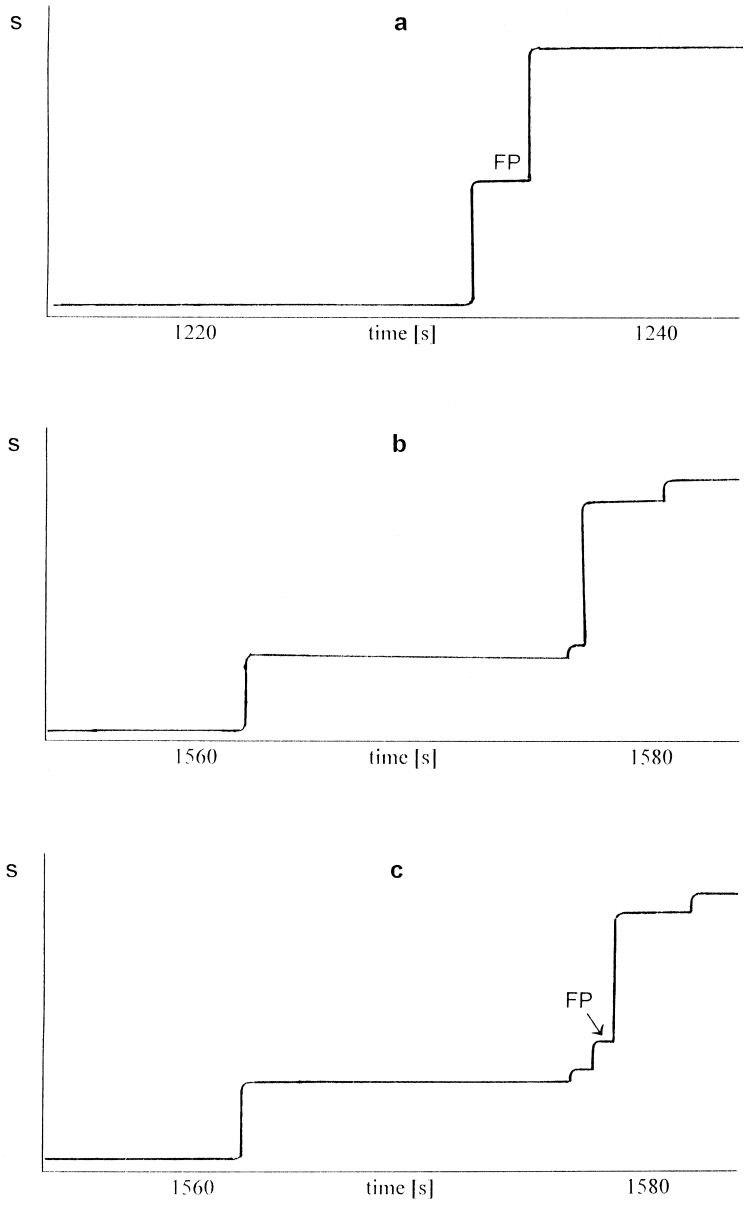

Fig. 1. Isotachopherograms of fenoprofen (FP) in (a) aqueous standard (0.05 mmol/l), (b) serum from a patient before therapy and (c) serum from the same patient 1 h after a dose of 600 mg of fenoprofen. Leading electrolyte: 10 mmol/l hydrochloric acid including 0.1% PVPD, adjusted with 6-aminocaproic acid to pH 4.8. Terminating electrolyte: 5 mmol/l 4-morpholineethanesulfonic acid.

solution was injected three times and the procedure was repeated on different days ($n=5$). Standard calibration curves (Table 2) were obtained from unweighted least-squares linear regression analysis of the data. The standard curves showed good linearity in the range from 0.02 to 0.40 mmol/l ($r^2=0.996$) For each calibration curve, the intercept was not statistically different from zero. The differ-

Table 2
Method linearity

Parameter	Fenoprofen-standard			Fenoprofen in serum		
	Mean	Within-day SD	Between-day SD	Mean	Within-day SD	Between-day SD
Linear slope (zone length/mmol/l)	50.23	0.05	0.01	47.90	0.13	0.35
y-Intercept (zone length)	0.10	0.03	0.08	0.04	0.04	0.05
r^2	0.998			0.996		
Linear range (mmol/l)	0.02–0.40			0.02–0.40		
LOD (mmol/l)	0.006			0.007		
LOQ (mmol/l)	0.018			0.020		

ent slopes from both sets indicate that interference from serum may be due to partial protein-binding of fenoprofen.

3.2.3. Recovery

The recovery of fenoprofen was determined by comparing zone length from drug-free serum spiked with known amounts of drug (0.02, 0.20 and 0.40 mmol/l) versus zone length of the same concentrations prepared in purified water injected directly on the column. Five replicate analyses were carried out at each concentration during three days. Data on the analytical recovery are given in Table 3. The results of the recovery experiments were satisfactory, the mean recovery of fenoprofen was found to be consistent over the evaluated concentration range, and was 93.1%.

Table 3
Recovery of fenoprofen

Nominal concentration (mmol/l)	Mean recovery (%)	Within-day SD	Between-day SD
0.02	89.5	3.9	4.5
0.20	95.2	1.7	1.2
0.40	94.6	0.7	0.8
Mean	93.1	2.1	2.2

3.2.4. Accuracy and precision

The accuracy and precision of the method were evaluated by analyzing five replicates of spiked samples at each of three concentrations (0.02, 0.20 and 0.40 mmol/l) against a calibration curve. Accuracy was given by the % bias [(mean of measured−mean of added)/mean of added]×100. Precision was evaluated as the within-day and the between-day standard deviations (SD) (Table 4). The within-day and between-day SDs were below 0.005 at all concentration levels. The bias varied between −5% and 1%. The presented results indicated that ±15% criteria are achieved [32].

3.2.5. Limit of detection and limit of quantitation

With an injection volume of 5.0 μl, the detection limit (defined as the sample concentration resulting in a zone length of 0.3 s) was approximately 0.007 mmol/l. The limit of quantitation was defined as the sample concentration resulting in a zone length of 1.0 s. The lowest concentration of the calibration graph was 0.02 mmol/l which was therefore the practical lower limit of quantitation.

3.2.6. Stability

A stock solution of fenoprofen (20 mmol/l) stored at 4°C was stable for at least three months. For stability studies, control serum was spiked with 0.05, 0.20 and 0.40 mmol/l of fenoprofen. The stability of

Table 4
Summary of accuracy and precision of the analytical method for fenoprofen in serum

Nominal concentration (mmol/l)	Measured concentration (mmol/l)	Bias (%)	Within-run SD	Between-run SD
0.02	0.019	−5.0	0.001	0.001
0.20	0.201	0.5	0.004	0.002
0.40	0.404	1.0	0.002	0.004

the analyte in serum was conducted at the ambient temperature (22°C) and at 4°C. Fenoprofen in serum samples at the ambient temperature was stable during 8 h, whatever the concentration; at 4°C it was stable for at least 5 days.

3.2.7. Ruggedness

For this study, LE (pH 4.8) preparation to LE (pH 4.8) preparation reproducibility of RSHs values were tested. Three different LEs pH 4.8 were tested and five replicate analyses were carried out with a standard solution of fenoprofen and fenoprofen in serum samples. RSH was calculated using the equation: RSH=h_x/h_t, where h_x is the height of the step of the fenoprofen zone and h_t is the height of the step of the terminating zone. As seen in Table 5, the method proved to be rugged with respect to small changes in electrolyte composition. A capillary from a different batch was also used to generate validation data. The results were consistent between the three capillaries obtained from the same supplier.

3.2.8. Real samples

The method developed was found to be suitable for the analysis of all samples collected during investigations in humans. Since the method was developed, 27 samples have been analyzed from patients (some results are given in Table 6). No interfering metabolite zones were observed in the serum of patients who had been prescribed fenoprofen. In humans the parent drug undergoes hydroxylation forming the metabolite 4′-hydroxyfenoprofen. 4′-Hydroxyfenoprofen (standard, Eli Lilly) has a low effective mobility in comparison with fenoprofen. In real samples this metabolite was not found, probably due to the fact that the concentration of 4′-hydroxyfenoprofen is low when compared to the parent drug and lies below the detection limit of the ITP method. Both the parent drug and its 4′-hydroxymetabolite are also conjugated to their acylglucuronides. The effective mobilities of acylglucuronides are too low, therefore, interference due to the presence of acylglucuronides is not expected. Interference from other drugs was examined by analyzing serum samples from patients taking various drugs. None of these patients′ serum showed zones which would interfere with fenoprofen. Pure standards of some commonly administered

Table 5
Reproducibility of method ruggedness

Sample	Mean $_{RSH}$[a]	Within-run RSD (%) $_{RSH}$[a]	Between-run RSD (%) $_{RSH}$[a]
Fenoprofen-standard	0.47	1.03	1.09
Fenoprofen in serum	0.47	0.95	1.50

[a] RSH=h_x/h_t, where h_x is the height of the step of the fenoprofen zone and h_t is the height of the step of the terminating zone.

Table 6
Isotachophoretic determination of fenoprofen in serum samples obtained at various hours after a dose of 600 mg of fenoprofen

Time (h)	Mean concentration (mmol/l)	Within-run SD (n=7)	Between-run SD (n=5)
Patient 1			
1	0.142	0.007	0.008
3	0.126	0.004	0.006
5	0.105	0.004	0.004
8	0.071	0.003	0.003
10	0.042	0.003	0.003
12	0.025	0.001	0.002
Patient 2			
1	0.215	0.005	0.006
3	0.183	0.005	0.006
5	0.149	0.004	0.004
8	0.096	0.004	0.004
10	0.064	0.002	0.003
12	0.052	0.002	0.003

anti-inflammatory drugs (flurbiprofen, diclofenac, ibuprofen, ketoprofen and naproxen) were also assayed under the described isotachophoretic conditions. Drugs which were found not to interfere with the assay are: amiloride, amitriptyline, bisoprolol, clomipramine, chlorthalidone, diclofenac, flurbiprofen, furosemide, hydrochlorothiazide, ibuprofen, imipramine, ketoprofen, labetalol, methyldopa, metoprolol, mianserin, naproxen and verapamil.

4. Conclusion

The feasibility of validating a ITP method for the determination of fenoprofen in serum is shown. This method, validated for concentrations ranging from 0.02 to 0.40 mmol/l has a good selectivity, reproducibility and accuracy. The limit of detection was lower than the LOD obtained with the HPLC method that includes acetonitrile deproteinization. [3]. The possibility of determining fenoprofen by direct injection of serum and low running costs make ITP a good alternative to existing methods.

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References

[1] TIAFT, The Bulletin of The International Association of Forensic Toxicologists, Vol. 26, No. 1 Suppl., January 1996, p. 13.
[2] S.G. Owen, M.S. Roberts, W.T. Friesen, J. Chromatogr. 416 (1987) 293–302.
[3] P.J. Streete, J. Chromatogr. 495 (1989) 179–193.
[4] F. Lapicque, P. Netter, B. Bannwarth, P. Trechot, P. Gillet, H. Lambert, R.J. Royer, J. Chromatogr. 496 (1989) 301–320.
[5] Ch. Volland, H. Sun, L.Z. Benet, J. Chromatogr. 534 (1990) 127–138.
[6] F.T. Delbeke, M. Debackere, Biomed. Chromatogr. 8 (1994) 29–31.
[7] R. Herraez-Hernandez, N.C. van de Merbel, U.A.Th. Brinkman, J. Chromatogr. B 666 (1995) 127–137.
[8] A. Shibukawa, M. Nagao, A. Terakita, J.Y. He, T. Nakagawa, J. Liq. Chromatogr. 16 (1993) 903–914.
[9] H.L. Wang, H.F. Zou, Y.K. Zhang, Biomed. Chromatogr. 12 (1998) 4–7.
[10] D. Tsikas, J. Fauler, S. Bracht, G. Brunner, J.C. Frolich, Electrophoresis 14 (1993) 664–666.
[11] T. Buzinkaiova, J. Sadecka, J. Polonsky, E. Vlasicova, V. Korinkova, J. Chromatogr. 638 (1993) 231–234.
[12] Z. Mala, L. Krivankova, P. Bocek, Electrophoresis 17 (1996) 125–129.
[13] R.T. Wang, C.T. Wang, K.C. Huang, T.C. Lin, T.C. Pan, Jpn. J. Toxicol. Environ. Health 42 (1996) 110–113.
[14] M. Pospisilova, M. Polasek, J. Prochazka, J. Chromatogr. A 772 (1997) 277–282.
[15] I. Valaskova, J. Balazova, E. Havranek, Ceska Slov. Farm. 45 (1996) 129–131.
[16] M. Pospisilova, J. Dolejsova, Ceska Slov. Farm. 46 (1997) 265–268.
[17] I. Buben, E. Niklickova, N. Novotna, Ceska Slov. Farm. 46 (1997) 261–264.
[18] M.T. Ackermans, J.L. Beckers, F.M. Everaerts, I.G.J.A. Seelen, J. Chromatogr. 590 (1992) 341–353.
[19] I. Valaskova, J. Balazova, E. Havranek, J. Chromatogr. B 674 (1995) 310–313.
[20] T. Buzinkaiova, V. Korinkova, Farm. Obz. 64 (1995) 255–259.
[21] J. Sadecka, J. Polonsky, J. Chromatogr. A 735 (1996) 403–408.
[22] J. Sadecka, J. Polonsky, H. Shintani, Pol. J. Environ. Science 6 (1997) 47–51.
[23] D. Tsikas, R.H. Boger, S.M. Bode-Boger, G. Brunner, J.C. Frolich, J. Chromatogr. A 699 (1995) 363–369.
[24] M. Wronski, J. Chromatogr. B 676 (1996) 29–34.
[25] F. Tallet, M.P. Vasson, R. Couderc, G. Lefevre, D. Raichvarg, Clin. Chim. Acta 244 (1996) 1–15.
[26] G. Schmitz, C. Moolers, V. Richter, Electrophoresis 18 (1997) 1807–1813.
[27] J. Caslavska, P. Gebauer, W. Thormann, Electrophoresis 15 (1994) 1167–1175.
[28] V. Kasicka, Z. Prusik, Prog. HPLC–HPCE 5 (1997) 173–197.
[29] A. Prochazkova, L. Krivankova, P. Bocek, Electrophoresis 19 (1998) 300–304.
[30] L. Krivankova, A. Vrana, P. Gebauer, P. Bocek, J. Chromatogr. A 772 (1997) 283–295.
[31] S. Auriola, I. Jaaskelainen, M. Regina, A. Urtii, Anal. Chem. 68 (1996) 3907–3911.
[32] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309–312.