ELSEVIER

Journal of Chromatography B, 757 (2001) 215–220

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of fendiline and gallopamil by capillary isotachophoresis

Tatiana Buzinkaiová*, Jozef Polonský, Ivan Skačáni

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

Received 20 October 2000; received in revised form 19 January 2001; accepted 22 February 2001

Abstract

Capillary isotachophoresis was applied for the determination of fendiline and gallopamil – calcium antagonists – in serum. The cationic electrolyte system containing Na^+ with acetic acid as a counter constituent was used as a leading electrolyte with the pH 4.7 and the terminating electrolyte was β-alanine. Most of the proteins were precipitated with methanol, ethanol and dimethylketone. The lowest limits of quantitation were obtained for the pretreatment of serum with methanol. The recoveries of both compounds varied from 91.3 to 97.5%. The relative standard deviations varied from 0.6 to 7.7%. © 2001 Published by Elsevier Science B.V.

Keywords: Fendiline; Gallopamil

1. Introduction

Coronary heart diseases have reached an alarming level in the world. Fendiline is one of the anti-anginal agents for the treatment of these diseases. This drug belongs to the group of calcium antagonists. Fendiline is a synthetic substance of the diphenylpropylamine derivative group: *N*-(1-phenylethyl)-3,3-diphenylpropylamine. Two hundred and twenty-seven compounds of the amine type, including fendiline, were analysed by thin-layer chromatography (TLC) and results were compared with results obtained by gas chromatography (GC) [1]. The complex quantitative assay for fendiline has used capillary GC with nitrogen–phosphorus selective detection [2]. The first review of the pharmacological and clinical properties of fendiline was published in 1987 [3] and various authors completed it from other points of view [4–6].

Gallopamil, α-[3-{[2-(3,4-dimethoxyphenyl)ethyl]-methylamino}propyl] - 3,4,5 - trimethoxy - α - (1-methylethyl)-benzeneacetonitrile, belongs to the calcium antagonists, too. Chromatographic techniques are dominant for the determination of gallopamil. One of the possibilities for gallopamil determination is high-performance liquid chromatography (HPLC) with fluorescence detection. The method is based on the direct HPLC analysis of diethyl ether plasma extract of a relatively small sample volume using a counter-ion technique [7]. Chiral HPLC for the determination of gallopamil with derivatization and without prior derivatization was used [8]. Simultaneous baseline separation of the enantiomers of verapamil, norverapamil and gallopamil using micellar electrokinetic capillary

*Corresponding author. Fax: +421-7-5292-6043.
E-mail address: tanab@chtf.stuba.sk (T. Buzinkaiová).

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00146-3

chromatography was published [9,10]. Both drugs are produced in the hydrochloride forms. Despite this, the use of capillary isotachophoresis (ITP) for their analyses has not been published. This paper describes the determination of fendiline and gallopamil in tablets and serum by capillary ITP. The method was validated according to the validation procedure.

2. Experimental

2.1. Instrumentation

The isotachophoretic separations and determinations were carried out using a Labeco ZKI 02 (Slovak Republic) equipped with a column-coupling configuration of the separation unit. The preseparation and analytical columns of, respectively, 90 mm ×0.80 mm I.D. and 160 mm×0.30 mm I.D. capillary tubes made of fluorinated ethylene-propylene copolymer were connected with conductometric detectors. The results were processed with the ITP-PC version 2 program by KasCom (Slovak Republic). For the measurement of pH a Model OP-211 pH meter with a combined glass electrode was used.

2.2. Reagents

All used chemicals were of analytical grade and commercially available, additionally purified by the usual methods, respectively. Fendiline as hydrochloride was obtained from Thiemann Arzneimittel (Germany) and gallopamil (hydrochloride) from Knoll (Germany) with the full chemical specifications. Deionized and 2× redistilled water was used for the preparation of the electrolyte systems and solutions. The standard dried serum (Imuna, Slovak Republic) was used for the optimization of experimental conditions for fendiline and gallopamil ITP analysis.

2.3. Isotachophoretic conditions

The cationic system contained a leading electrolyte (LE) with Na^+ and acetic acid as a counter constituent and 0.01 mol/l β-alanine as the terminating electrolyte (TE). The simplest way for lowering the limit of quantitation (LOQ) in ITP is achieved by changes of the driving current and the concentration of LE in the columns [11]. The driving current for the preseparation column was 200 μA and for the analytical column 40 μA. For the chosen analyses the driving current in the analytical column was decreased to 10 μA in the 25th minute of analysis. The concentration of LE in the analytical column was changed from 0.01 mol/l to 0.005 mol/l. The LOQ is in this work defined as the amount of the fendiline or gallopamil which produced a zone length three times longer than the minimum zone length recommended by the ZKI 02 producer. It corresponds to the 0.3 mm length with the injected volumes of serum sample of 5 and 30 μl.

2.4. Preparation of stock solutions

Standard stock solutions of fendiline and gallopamil (0.1 mg/ml) in water were used for the preparation of the working solutions of lower concentrations with their dilution.

2.5. Solutions of drug Sensit

A tablet of Sensit containing 50, 75, 100 mg of fendiline/tablet was crushed in an agate bowl and dissolved in water–methanol (1:1), because of its limited dissolubility in water, to obtain the concentration of fendiline 0.1 mg/ml.

2.6. Serum

Spiked serum standards for the recovery with precipitation were obtained by serial dilution of stock solutions of fendiline and gallopamil. The standards to obtain the concentrations of 10, 50, 100, 150, 200 ng/ml of these substances in serum were added to it for the calibration curves: 0.5 ml of standard serum was spiked with various amounts of fendiline and gallopamil and then the certain volume of methanol, ethanol or dimethylketone respectively, was added for the protein precipitation. After mixing and centrifugation at 10 000 *g* for 5 min the solutions could be injected into the ITP analyser.

Table 1
Experimental conditions, LOQ and R_{SH} for analysis of fendiline and gallopamil in standard solutions by capillary ITP

	Column	System			
		I	II	III	IV
Concentration of LE (mol/l)	Preseparation	0.01	0.01	0.01	0.01
	Analytical	0.01	0.01	0.005	0.005
Driving current (μA)	Preseparation	200	200	200	200
	Analytical	40	40, 10	40	40, 10
R_{SH} fendiline		0.43	0.46	0.49	0.49
RSD (%)		0.71	0.68	0.70	0.73
LOQ fendiline (ng/ml)		500	156	130	100
R_{SH} gallopamil		0.85	0.87	0.88	0.93
RSD (%)		0.68	0.67	0.67	0.71
LOQ gallopamil (ng/ml)		758	203	198	184

2.7. Intra- and inter-day validation

Intra- and inter-day validation studies for accuracy and precision of fendiline and gallopamil determinations were performed on three standard solutions in serum analysed five times. The analyses were repeated on 3 separate days.

3. Results and discussion

Fendiline and gallopamil were analysed by capillary ITP as cations.

3.1. Standard solutions of fendiline and gallopamil

The electromigration of fendiline and gallopamil depends on the pH of the LE. The influence of the pH of LE in the interval 4.1–5.3 on the level of their protonization, the basic qualitative parameter (R_{SH}) – relative step high – was studied. The optimum pH was 4.7 when these compounds were well separated from endogenous compounds with the lowest LOQ. The LOQ and R_{SH} obtained with LE, pH 4.7 in systems I–IV are shown in Table 1 supporting the influence of experimental conditions for the ITP analysis of fendiline and gallopamil.

For the determinations of fendiline and gallopamil as basic substances in drugs calibration curves were used and were statistically evaluated by linear regression. The linearity is defined in Table 2.

3.2. The determination of fendiline in drugs

Fendiline was determined in the drug Sensit (Thiemann Arzneimittel). Six calibration points repeated five times were measured with standard solutions. Ten tablets for each type of Sensit were analysed and every analysis was repeated three times. The relative standard deviation (RSD) for a drug solution in the concentration 0.1 mg/ml was less than 0.4%. Table 3 shows the content of fendiline in Sensit.

Table 2
Linearity of the method in the range 500–1200 ng/ml for fendiline and gallopamil in systems I–IV

System	a_f	b_f	r_f^2	a_g	b_g	r_g^2
I	0.4031	19.6943	0.9974	2.2286	1.7386	0.9992
II	0.5573	2.6870	0.9978	1.2550	1.5110	0.9990
III	1.4098	1.0890	0.9982	1.3096	1.6090	0.9982
IV	1.6711	1.1240	0.9978	−0.8310	1.8021	0.9986

Equation for the straight line: $y=a+bx$ where y is the zone length (mm), a is the intercept on the ordinate, b is the slope, r^2 is the coefficient of the determination, subscript f denotes fendiline and subscript g denotes gallopamil.

Table 3
Amount of fendiline found in drugs

Drug	Declared (mg/tablet)	Found (mg/tablet)
Sensitivity 50	50.0	49.48±0.15
Sensitivity 75	75.0	74.40±0.28
Sensitivity 100	100.0	100.04±0.27

3.3. Compounds in serum

The lowest LOQ of fendiline and gallopamil obtained by ITP analyses of their standard solutions (fendiline 100 ng/ml and gallopamil 184 ng/ml – see Table 1) corresponds with their upper steady-state therapeutic level in serum. The therapeutic level for fendiline is 10–100 ng/ml and for gallopamil 100–200 ng/ml. One of the possibilities of the lowering of absolute LOQ in ITP is the injection of larger serum volumes. But the injection of relative larger volumes of serum could be a problem in the ITP system because of the possibility of protein precipitation in ITP columns. The sample pretreatment to remove proteins was necessary for the lowering LOQ. Three precipitation agents were tested: methanol, ethanol and dimethylketone in various volumes added to serum (1:1, 1.5:1, 2:1). With the addition of these solvents the influence of their volumes on the elimination of the majority of proteins and detectable compounds was significant (Table 4). The basic qualitative parameter R_{SH} of fendiline in these analyses was changing in comparison with standard solutions in water probably with the influence of solvents on the fendiline effective mobility (solvolysis). The same R_{SH} of fendiline was obtained in its standard solutions in the mixture of organic solvent–water. This change was not evident for gallopamil. Table 5 shows that the response is linear for both compounds.

The precipitation of serum and follow-up ITP analysis in electrolyte system IV is lowering LOQ for fendiline and gallopamil. The lowest LOQ was obtained for the precipitation with methanol (1:2): fendiline 9 ng/ml, gallopamil 15 ng/ml with the injection 30 μl of sample. Fig. 1a and b shows ITP analyses of serum precipitated with methanol spiked with analysed compounds in electrolyte system IV.

The results of intra- and inter-day precision and accuracy for fendiline and gallopamil in serum are given in Table 6. All RSDs were less than 8% with the lowest accuracy 93.7% for gallopamil (concentration 300 ng/ml) and 91.3% for fendiline (concentration 300 ng/ml).

Table 4
Changes of R_{SH} and LOQ for fendiline and gallopamil in serum with the precipitation in system I

Precipitant serum:precipitant	Fendiline		Gallopamil	
	R_{SH}	LOQ (ng/ml)	R_{SH}	LOQ (ng/ml)
Ethanol				
1:1	0.88	80	0.90	56
RSD (%)	0.76		0.69	
1:1.5	0.89	111	0.90	71
RSD (%)	0.74		0.67	
1:2	0.89	130	0.90	89
RSD (%)	0.70		0.69	
Dimethylketone				
1:1	0.87	78	0.0.83	53
RSD (%)	0.68		0.73	
1:1.5	0.88	97	0.83	65
RSD (%)	0.64		0.74	
1:2	0.88	110	0.83	83
RSD (%)	0.61		0.69	
Methanol				
1:1	0.89	66	0.83	48
RSD (%)	0.68		0.65	
1:1.5	0.90	76	0.84	56
RSD (%)	0.69		0.64	
1:2	0.91	83	0.84	72
RSD (%)	0.72		0.67	

4. Conclusion

It is shown that the ITP method is suitable for the quick determination of fendiline and gallopamil in drugs without pretreatment. The simple precipitation of proteins with methanol, ethanol and dimethylketone was used because of LOQ of fendiline and gallopamil without pretreatment in serum (LOQs did not correspond with their the lowest therapeutic levels). The lowest obtained LOQ in serum was for the precipitation with methanol 9 ng of fendiline/ml and for gallopamil 15 ng/ml. With this simple

Table 5
The linearity of ITP analyses of fendiline and gallopamil with the precipitation in system I and the ratio of precipitant 1:2

Precipitant	Fendiline			Gallopamil		
	a	*b*	r^2	*a*	*b*	r^2
Ethanol	−0.8898	0.9701	0.9910	−0.0020	0.8958	0.9950
Dimethylketone	4.9989	0.6021	0.9896	4.1702	0.5822	0.9944
Methanol	2.3313	0.8898	0.9940	2.9990	0.7054	0.9934

Equation for the straight line: $y=a+bx$ where y is the zone length (mm), a is the intercept on the ordinate, b is the slope and r^2 is the coefficient of the determination.

pretreatment ITP analyses of fendiline and gallopamil in the cationic electrolyte system with conductometric detection is suitable to determine these calcium antagonists in serum. The difference between R_{SH} of fendiline and gallopamil in serum was not explicit. However, these two compounds are not used together in multiple therapy, nor are they found together in drugs.

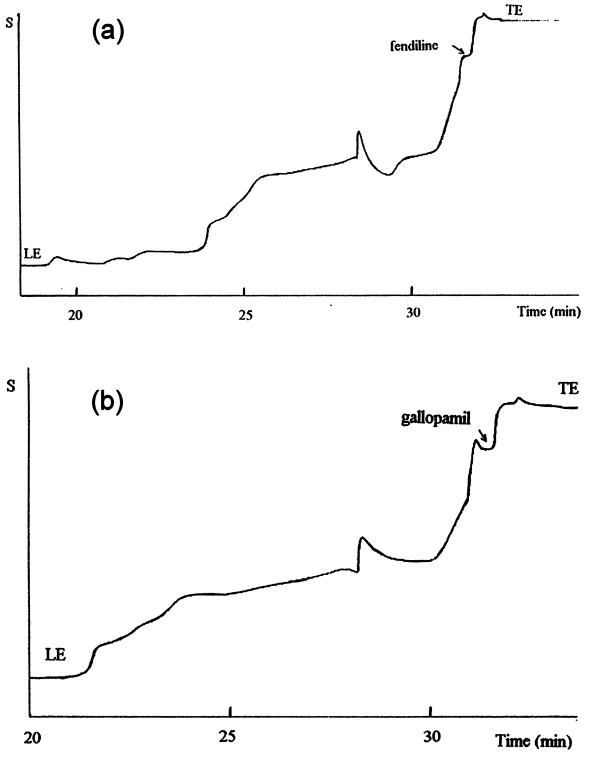

Fig. 1. Isotachopherograms of serum precipitated with methanol in electrolyte system IV. (a) Serum spiked with fendiline in the concentration 100 ng/ml, injection 30 μl, (b) serum spiked with gallopamil in the concentration 200 ng/ml, injection 30 μl.

Acknowledgements

The authors thank Dr. Teuber (Thiemann Arzneimittel GmbH) and Dr. Brode (Knoll AG, BASF

Table 6
Intra- and inter-day precision and recovery of fendiline and gallopamil (injected volume 30 μl of spiked serum precipitated with methanol)

	Concentration (ng/ml)		
	300	350	400
Intra-day fendiline			
n	5	5	5
Mean	274	335	384
RSD (%)	7.1	4.6	4.1
Recovery (%)	91.3	95.7	96.0
Inter-day fendiline			
n	5	5	5
Mean	285	337	390
RSD (%)	7.7	5.0	4.9
Recovery (%)	95.0	96.3	97.5
Intra-day gallopamil			
n	5	5	5
Mean	285	337	387
RSD (%)	6.0	6.2	5.7
Recovery (%)	95.0	96.3	96.8
Inter-day gallopamil			
n	5	5	5
Mean	281	333	382
RSD (%)	6.8	6.6	6.0
Recovery (%)	93.7	95.1	95.5

Gruppe) for the standard of fendiline, drug Sensit and the standard of gallopamil.

References

[1] A. Lohmann, N. Diekmann, J. Chromatogr. 564 (1991) 289.
[2] G. Musumarra, J. Anal. Toxicol. 11 (4) (1987) 160.
[3] R. Bayer, R. Mannhold, Pharmatherapeutica 5 (2) (1987) 103.
[4] P. Meera, W. Schreibmayer, O. Tripathi, J. Physiol. 467 (1993) 260.
[5] V.L. Kozlovskij, I.V. Prakhe, Eksp. Klin. Farmakol. 58 (1) (1995) 18.
[6] C. Schlatterer, R. Schaloske, J. Biochem. 313 (2) (1996) 661.
[7] D.R. Rutledge, J. Chromatogr. 421 (1) (1987) 165.
[8] H. Fieger, G. Blaschke, J. Chromatogr. 575 (2) (1992) 255.
[9] J.G. Clothier, L.M. Daley, T.A. Sterling, J. Chromatogr. 683 (1) (1996) 37.
[10] J.G. Clothier, T.A. Sterling, J. Chromatogr. 723 (1) (1996) 179.
[11] F.M. Everaerts, J.L. Becker, Th.P.E.M. Verheggen, Isotachophoresis: Theory, Instrumentation and Applications, Journal of Chromatography Library 6, Elsevier, New York, 1976.