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Application of Capillary Isotachophoresis in Phenothiazines Determination

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Abstract

A capillary isotachophoretic method (cITP) using three electrolyte systems for determination of promethazine, thioridazine and chlorpromazine hydrochlorides in pharmaceutical preparations was demonstrated. Proposed systems were characterised by linearity range 5-100 mg/L with R^2 in all cases were higher than 0.999. The elaborated method was tested on pharmaceutical preparations. The recovery values were from 96.5% to 101.3%. The proposed isotachophoretic method is simple with acceptable precision and accuracy and can be suitable for routine analysis of studied biological active compounds.

Keywords: Capillary isotachophoresis; Chlorpromazine hydrochloride; Promethazine hydrochloride; Thioridazine hydrochloride

Introduction

The phenothiazine derivatives such as: promethazine, chlorpromazine and thioridazine hydrochlorides are used as psychotropic, neuroleptic, local anesthetics, anti-allergic and antiyomiting drugs [1]. The therapeutic interest in these compounds group justifies research to establish different analytical methods for the determination of these drugs in pharmaceutical preparations and biological samples.

Many methods have been reported for the analysis of these compounds, including spectrophotometric, fluorimetric, electrochemical and chromatographic. Spectrophotometric methods were based on the reaction between phenothiazine derivative and oxidizing agent such as: cerium (IV), potassium dichromate, chloramines T and ammonium molybdate [2-4]. Moreover some authors described spectrophotometric determination of phentothiazine as ion-pairs or complexes [5-7]. Other spectroscopic method used for phentothiazine derivatives determination is fluorimetry and luminescence [8-10]. Electrochemical methods [11-13] have been applied to the determination of phenothiazine drugs in pharmaceuticals and biological fluids. The most often in phenothiazine derivatives analysis liquid chromatography is used. This technique was applied for analysis of biological active substances in differ dosage forms [14-16] and biological fluids [17-21].

Many of papers described application of electromigration methods for determination of phenothiazines. Among these there is capillary electrophoresis (CE) [22-24]. Last time besides CE the capillary isotachophoresis was most often used in pharmaceutical analysis. Isotachophoresis is the separation technique for qualitative and quantitative analysis of ionic compounds, based on differences in their effective mobility in solution. This method was tested as a complementary method to HPLC for the determination of many pharmaceuticals [25-27]. In our earlier papers capillary isotachophoresis was successfully applied for determination of neomycin trisulphate and metoprolol tartrate [28-30].

Because of we found only several papers [31-33] about application of capillary isotachophoresis for phenothiazine derivatives analysis we decided use this technique in our studies. In our paper we described the isotachophoretic method for determination of three phenothiazine derivatives: thioridazine hydrochloride (TDZ), promethazine hydrochloride (PMT) and chlorpromazine hydrochloride (CPM). The linear calibration range was studied for eventual application of elaborated method to pharmaceutical preparations analysis. The obtained results were discussed with regards of accuracy and precision.

Material and Methods

Chemical and reagents

All used reagents were analytical grade. ε-aminocaproic acid (EACA), morpholinoethanosulphonic acid (MES), β-alanine and hydroxyethylcellulose (HEC) were purchased from Sigma and others reagents from Alchem, Toruń (Poland). Thioridazine hydrochloride, promethazine hydrochloride, chlorpromazine hydrochloride and erythromycin were purchased from Sigma, (Germany), whereas the pharmaceutical preparations: Diphergan, Thioridazin and Fenactil were bought in local pharmacy. Demineralized water (conductivity below 0.05 μS) was obtained by HLP Smart 2000 purification system (Hydrolab, Poland) and was used for sample solution preparation.

Isotachophoretic instrumentation and analysis conditions

Isotachophoretic separations were performed using a Villa Labeco EA 100/101 isotachophoretic analyzer equipped with a conductivity detector. The PTFE pre-separation capillary (90 mm×0.8 mm I.D.) was connected with PTFE analytical capillary (160 mm×0.3 m I.D.). Samples were injected via a sample valve of 30 μL fixed volume by internal sample loop. The isotachopherograms were evaluated with the PC software package supplied with analyser (KasComp, Slovakia).

Phenothiazines were analyzed applying three electrolytes systems: leading (LE)-10 mM sodium acetate+0.08% hydroxethylocelulose (HEC)+acetic acid to pH=5.5 and terminating (TE)–10 mM β-alanine (I); LE-10 mM morpholinoethanosulphonic acid (MES)+5 mM ammonium (pH=6.5) and TE-10 mM ε-aminicaproic acid (EACA)+0.08% HEC (II) and LE-10 mM sodium acetate+0.08%

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HEC+acetic acid to pH=5.5 and TE-5 mM erythromycin (III). Previously, the system I was applied for neomycin analysis [29].

In this paper, new electrolyte systems (II and III) based on cationic mode separation was discussed. Leading electrolyte in system (III) was the same as in system (I), whereas as terminating ion we tested erythromycin, which characterized low effective mobility. Moreover, we proposed 10 mM MES as leading ion with addition 5 mM ammonium to pH=6.5 in system (II). This pH value permitted optimizing phenothiazines stabilization and dissociation. It should be noted; these electrolytes composition was not used in phenothiazines analysis. The choice of electrolytes composition was discussed in details by Kurzawa [30].

The driving current of the pre-separation column was changed from 250 μA to 150 μA during the analysis in all causes. The determination conditions (HEC concentration, current intensity and pH) were optimized by an analysis of phenothiazines standard solution.

Phenothiazines were identified using the relative step height (RSH) parameter which was calculated from the relation: RSHX=(HX-HL)/(HT-HL), where HX is the zone height of analyzed substance, HL and HT is step height of leading and terminating ion, respectively [34].

Preparation of standards

Stock solutions of analyzed phenothiazines were prepared by dissolving appropriate amounts of the analyzed compounds in 100 mL demineralized water resulting concentration 100 mg/L. Working standard solutions were prepared by dilution of stock solution obtaining concentration of each drug from 5.0 to 100 mg/L.

Preparation of pharmaceutical preparations

Diphergan, Thioridazin and Fenactil solutions were prepared as follow: one, two and 3 tablets were crushed, dissolved in beaker with 150 ml water and placed in ultrasonic bath. After sonification, the solutions were filtered and transferred to 1L volumetric flask and made up to volume by water. The final concentrations of biological active compounds were 10.0; 20.0 and 30.0 mg/L.

Method validation

The elaborated method was validated in terms of linearity, within-day and between days precision, accuracy and limits of detection (LOD) and quantification (LOQ). Calibration curves were constructed as dependence of zone length on the concentration. The accuracy was tested by recovery value at three level of concentration. The within-day precisions were studied at three concentration levels by three time repetitions whereas the between-days precisions were evaluated by five consecutive days.

Results and Discussion

RSH stability

As was earlier described, the studied phenothiazines were characterized by RSH parameter which was calculated from the relation: RSH=(HX-HL)/(HT-HL), where HX is the zone height of analyzed phenotiazines cation, HL and HT are step heights of the leading and terminating ion, respectively. The typical isotachopherograms for the studied phenothiazines are presented in figure 1.

The mean RSH values of each studied pharmaceutical are presented in table 1.

The stability of RSH is one important parameter in isotachophoretic analysis. Relative standard deviations of RSH were also reasonable and amount below 1.6%. The applied three electrolytes systems seem to be well adjusted to provide good performance of the method.

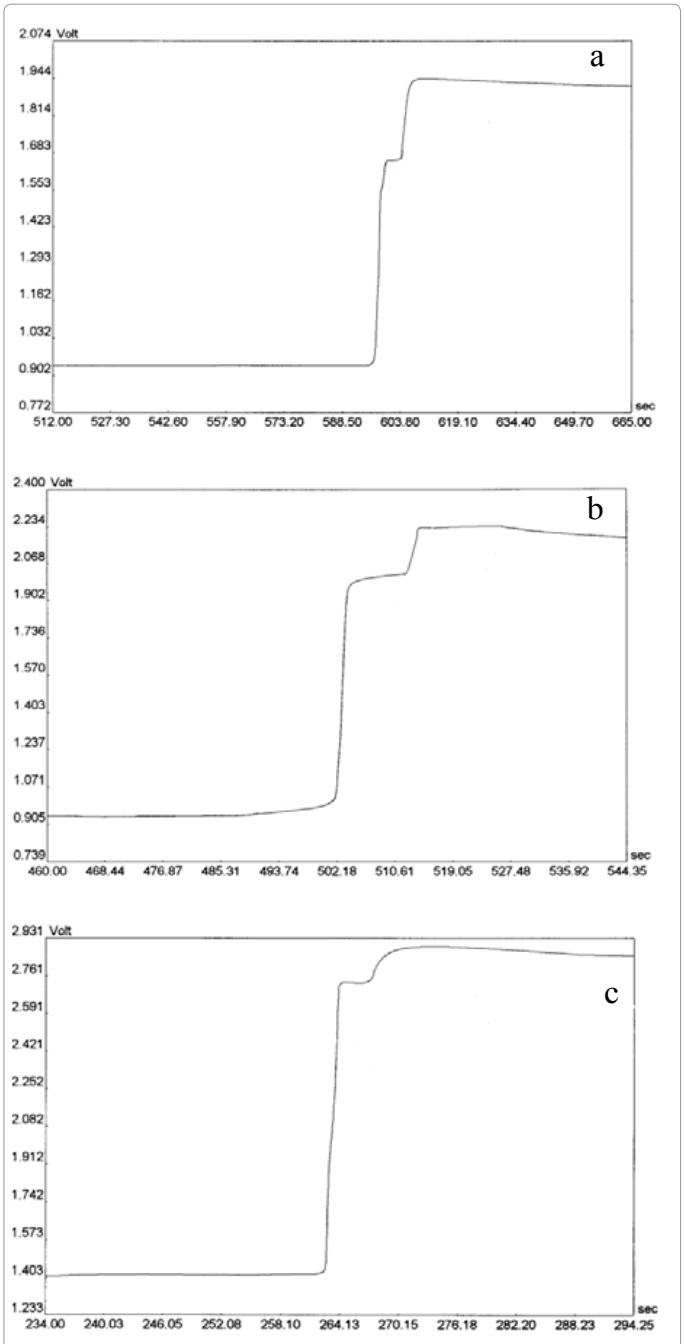

Figure 1: Isotachopherograms of: (a) promethazine (c=90 mg/L), (b) thioridazine (c=90 mg/L) and (c) chlorpromazine (c=70 mg/L) at I electrolyte system.

The analysis times were depending on the used electrolyte system and ranged from 5.33 to 13.55 min.; from 8.78 to 12.20 min. and from 4.60 to 11.90 min. for promethazine, thioridazine and chlorpromazine, respectively.

Calibration curves

The calibration curves were constructed as the dependence

Electrolite system	PMT	TDZ	CPM
	RSH ± μ.	RSH ± μ	RSH ± μ
I	0.666 ± 0.006	0.608 ± 0.012	0.506 ± 0.007
II	0.551 ± 0.010	0.594 ± 0.010	0.487 ± 0.008
III	0.632 ± 0.009	0.612 ± 0.006	0.495 ± 0.005

Where: μ - Confidence limit, *p*=95%;

Table 1: The mean RSH value of promethazine, thioridazine and chlorpromazine (n=5).

	Equation of calibration curve	R^2	LOD, mg/L	LOQ, mg/L
	System I			
PMT	y=(0.1870 ± 0.0005)x+(0.300 ± 0.002)	0.9998	2.93	9.76
TD	y=(0.0480 ± 0.0002)x-(0.1760 ± 0.0004)	0.9990	2.34	7.82
CPM	y=(0.0980 ± 0.0004)x-(0.584 ± 0.001)	0.9990	1.93	6.45
	System II			
PMT	y=(0.1080 ± 0.0005)x-(0.298 ± 0.001)	0.9998	2.70	9.01
TD	y=(0.1600 ± 0.0007)x-(1.267 ± 0.001)	0.9998	2.39	7.97
CPM	y=(0.1290 ± 0.0006)x+(0.925 ± 0.001)	0.9998	2.67	8.90
	System III			
PMT	y=(0.1800 ± 0.00030)x-(1.048 ± 0.006)	0.9975	3.00	9.99
TD	y=(0.1650 ± 0.0008)x-(1.277 ± 0.002)	0.9998	2.69	8.98
CPM	y=(0.1570 ± 0.0008)x-(1.073 ± 0.002)	0.9998	2.60	8.66

Table 2: The statistical parameters of calibration curves.

of zone length on the analyzed substances concentration. At each concentration levels five repetitions of measurements were made. The calibration curves are expressed as $y=(b\pm S_b).x+(a\pm S_a)$, where S_b, S_a – standard deviations of slope and intercept. The statistical parameters of calibration curves are collected in table 2.

Linear regression of elaborated procedure for each used electrolyte systems revealed good correlation. The calculated determination coefficients were from 0.9975 to 0.9998. The determination coefficient values for analysed PHs were close to one what indicated satisfactory linearity between zone length and the concentration of studied pharmaceuticals.

The detection limit (LOD) of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically taken as three times the noise level. The quantitation limit (LOQ) is the lowest level of analyte that can be accurately measured, and it is often evaluated as ten times the noise level. In our studies we have evaluated both quantities according to the procedure of Miller and Miller [35]. Limit of detections were calculated from $(y+3\cdot s_{y/x})/b$, where the calculated intercept of the calibration curve can be used as estimate of y, $s_{y/x}$ being the standard deviation in the *y*-direction of the calibration curve and *b* being the slope of the calibration curve. The $10.s_{y/x}/b$ expression was used for estimation of the quantification limit. The LOQ is very important parameter, with respect to the potential applications of elaborated method. The obtained value suggested that our three electrolyte systems can be suitable for the analysis of pharmaceutical preparation containing analyzed phenothiazines (in all cases LOQ<10 mg/L). Taking into account the fact that the lowest dose tested active substances in one tablet is 10 mg, they can be determined with sufficient accuracy and precision using the developed methods.

Analysis of pharmaceutical preparations

The elaborated methods of the phenothiazine derivatives determination were tested on the pharmaceutical preparations: Diphergan, Thioridazin and Fenactil. Each sample was analyzed 5 times at three concentration level (10.0; 20.0 and 30.0 mg/L). The analyses were performed at the same conditions as calibration curve and results listed in table 3.

The accuracy of the method was evaluated from recovery assays. In all cases the recovery values are close to 100 % and fall within the scope required by British Pharmacopoeia [1].

Moreover, the relative standard deviation values are lower than 3% what confirms good precision of the elaborated methods. The obtained results are similar as those obtained by Kubacak et al. [31]. Authors tested several electrolyte systems for promethazine determination and the best results were obtained for the following one: LE: 10 mmol/L of potassium acetate with acetic acid to pH 4.8 and TE: 5 mmol/L of β-alanine with additive 0.2% methylhydroxyethylcellulose (m-HEC). The calibration graph was r in the range of 40-200 mg/L with R^2=0.9992. The RSD value was 1.12% and total analysis time was 6 min. The recoveries of drug in pharmaceutical preparations were from 97.22% to 99.72% depending on their therapeutic form.

The obtained results in respect to accuracy and precision can be compared with those obtained in our earlier papers [28-30] where we described determination of metoprolol tartrate and neomycin trisulfate with use of capillary isotachophoresis.

Conclusion

The developed isotachophoretic method for the determination of promethazine, thioridazine and chlorpromazine is precise, accurate, reproducible and relatively fast. Due to no consumption of organic

pharmaceuticals	C_{det} ± μ mg/L	Recovery, %	$RSD_{intra\text{-}day}$ %	$RSD_{inter\text{-}days}$ %
	System I			
Diphergan	10.04 ± 0.05	100.4	0.52	1.42
	20.07 ± 0.32	100.3	1.28	1.32
	29.98 ± 0.13	99.93	0.72	0.95
Thioridazin	10.02 ± 0.13	100.2	1.15	1.11
	19.95 ± 0.10	99.75	0.67	0.83
	29.93 ± 0.12	99.77	0.34	0.56
Fenactil	9.91 ± 0.05	99.10	0.74	0.98
	19.89 ± 0.08	99.45	0.69	0.57
	29.94 ± 0.14	99.80	0.53	0.43
	System II			
Diphergan	10.12 ± 0.08	101.2	0.58	0.68
	20.07 ± 0.09	100.3	0.51	0.49
	30.02 ± 0.16	100.1	0.49	0.75
Thioridazin	10.13 ± 0.07	101.3	0.67	0.92
	20.17 ± 0.07	100.8	0.38	0.67
	30.20 ± 0.10	100.7	0.34	0.36
Fenactil	10.10 ± 0.05	101.0	0.52	0.46
	20.16 ± 0.08	100.8	0.73	0.95
	30.22 ± 0.07	100.7		
	System III			
Diphergan	9.87 ± 0.07	98.70	0.68	0.79
	19.68 ± 0.13	98.40	0.70	0.82
	29.58 ± 0.13	98.60	0.46	0.44
Thioridazin	9.85 ± 0.10	98.50	0.98	0.92
	19.66 ± 0.15	98.30	0.87	0.32
	29.60 ± 0.10	98.67	0.72	0.61
Fenactil	9.65 ± 0.20	96.50	1.56	1.24
	19.56 ± 0.22	97.80	0.94	1.04
	29.65 ± 0.32	98.83	0.94	0.99

Where: μ - Confidence limit, *p*=95%; RSD - relative standard deviation.

Table 3: Determination of active substances in pharmaceuticals.

solvents and low consumption of electrolytes it is environmental friendly method. The obtained results clearly indicated that the method may be well suited for the routine pharmaceutical preparation analysis and can as an alternative to the commonly used titrimetric and chromatographic methods.

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