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Short communication

Determination of phenothiazine derivatives by high performance thin-layer chromatography combined with densitometry

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Abstract

A high performance thin-layer chromatography (HPTLC) method combined with densitometry for determination of phenothiazine derivatives is described.

Quantitation was performed in reflectance mode by using a computer-controlled densitometer Desaga CD 60. Established calibration curve ($r > 0.999$), precision (RDS values: 0.95–2.53%), detection limits as well as recovery values (101.1–102.8%) were found to be satisfactory.

The presented method is rapid, precise and sensitive, and may be alternative to traditionally used HPLC.

The method has been successfully applied in the analysis of pharmaceutical formulations.

Keywords: HPTLC-densitometry; Phenothiazine derivatives; Promethazine hydrochloride

1. Introduction

European and United States Pharmacopoeias suggest the elaboration of analytical methods reducing the amount of reagents and material used in pharmaceutical assays. It is closely related to the harmful influence of chemicals on human health and protection of environment. Currently, many monographs include tests for pharmaceutics based on HPLC method where the mobile phases contain significant amounts of organic solvents. High performance thin-layer chromatography (HPTLC) combined with densitometry is a highly efficient method in which numerous samples can be run simultaneously using small quantities of solvents. This reduces the time and cost of analysis and the possibilities of pollution of the environment. The progress in instrumentation (e.g., automated application) cause that HPTLC combined with densitometry should be taken into consideration as an alternative to HPLC.

Promazine hydrochloride, promethazine hydrochloride and thioridazine hydrochloride belong to the phenothiazine derivatives including a phenothiazine ring with different substituents attached at the 2- and 10-positions. They are used as psychotropic, neuroleptic, local anaesthetic, anti-allergic and antivomiting drugs [1]. Their wide application in medicine requires the methods for their determination in different dosage forms.

Many methods have been reported for the analysis of these compounds, including spectrophotometry with UV, fluorimetric and chemiluminescence detection [2–7], voltammetry [8], capillary electrophoresis [9–11] and gas chromatography [12]. HPLC methods are now widely used for phenothiazines determination [13–17].

The purpose of our paper was investigation of possibilities of quantitative analysis of phenothiazine derivatives with the use of HPTLC combined with densitometry. The proposed method was validated according to international requirements [18,19] and compared with traditionally used HPLC.

2. Experimental

All phenothiazine derivatives standards, pro-analytical grade, were obtained from Sigma–Aldrich (USA). All organic solvents used in the study, of analytical grade, were purchased from Merck (Darmstadt, Germany). In HPLC experiments ultra-pure distilled water and methanol of HPLC grade were used.

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Diphergan ampoules, containing in 2 mL 0.05 g of promethazine hydrochloride, 0.01 g of phenol and water were obtained from Jelfa (Jelenia Góra, Poland). Diphergan syrup, containing in 5 mL, 5 mg of promethazine hydrochloride and supportive substances (citric acid, natrium benzoate, gentisic natrium salt, orange oil and sugar), were obtained from Altana Pharma (Warsaw, Poland).

Stock solutions of each phenotiazine derivative were prepared in methanol. Working solutions were prepared by dilution of the stock solutions in the range of 0.0265–0.265 mg/mL for promethazine hydrochloride, 0.036–0.22 mg/mL for thioridazine hydrochloride and 0.04–0.12 mg/mL for promazine hydrochloride.

Sample of Diphergan ampoules was prepared by dilution of 0.1 mL in 100 mL of methanol. Sample of Diphergan syrup was prepared by dilution of 1 mL in 10 mL of methanol.

In chromatographic experiments, several stationary phases were tested: silica (TLC, HPTLC and LiChrospher) and silica modified with diol, aminopropyl and octadecyl groups (Merck, Darmstadt, Germany). All plates were previously washed with methanol and acetone and dried in a stream of warm air. Samples were applied with use of Desaga AS 30 applicator (Heidelberg, Germany). Five microlitres samples were applied as streaks 6 mm long under nitrogen at 2533 hPa for 5 s. To quantitative determination a chromatographic system composed of: LiChrospher Si 60 as the stationary phase, and mixture of acetone, methanol and ammonia (25%) (90:10:2, v/v/v) as the mobile phase was used. The plates were developed "face down" in horizontal Teflon DS chambers (Chromdes, Lublin, Poland). The distance was 50 mm.

The densitograms were obtained using Desaga CD-60 densitometer (Heidelberg, Germany) controlled by a Pentium computer with Windows Software ProQuant. In quantitative analysis meander scanning of 1.0 mm wide paths at 247 nm was used.

HPLC experiments were performed on a Waters chromatograph (USA) with a Dual Absorbance Detector (detection wavelength set at 247 nm). Waters C18 steel column 150 mm × 4.6 mm filled with adsorbent with particle diameter 5 μm was used. Twenty microlitres of each solution was injected. For the elution of the investigated compounds the mobile phase composed of methanol and water with addition of ammonia (25%) (90:10:0.1, v/v/v) was used (Fig. 1).

Elution was performed at 0.5 mL/min. flow-rate and at the temperature 25 °C.

3. Results and discussion

The procedure for the determination of phenothiazine derivatives using HPTLC-densitometry in comparison with HPLC method is reported.

All chromatographic systems were chosen experimentally. To obtain satisfactory results an optimization of the HPTLC method was carried out using several different stationary and mobile phases. Chromatographic experiments were performed on silica (TLC, HPTLC and LiChrospher) and silica modified with diol, aminopropyl or octadecyl groups. The best sensitivity (the lowest detection limits) and repeatability were obtained on

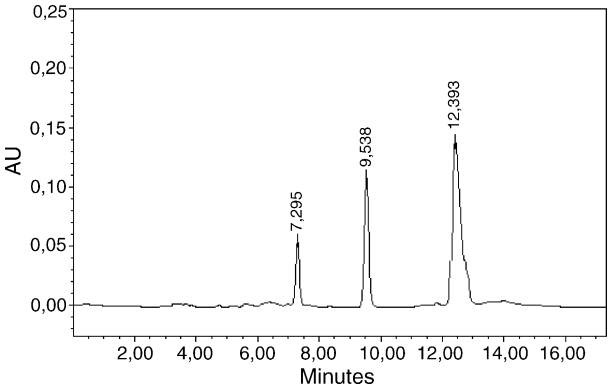

Fig. 1. The HPLC chromatogram of promethazine (t_R, 7295), promazine (t_R, 9538) and thoiridazine (t_R, 12,393).

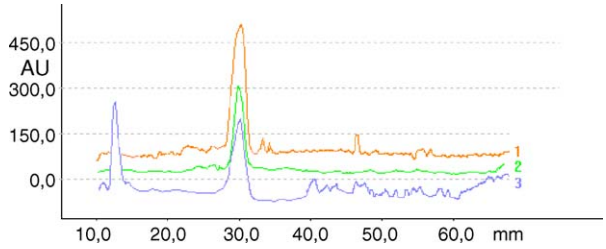

Fig. 2. HPTLC densitograms of promethazine hydrochloride standard (2), Diphergan-ampoules (1) and Diphergan-syrup (3) obtained at λ = 247 nm.

Si 60 LiChrospher plates. The spherical shape of particles and their small size (6–8 μm) ensure also short development times and excellent separation performance. To improve the shape of chromatographic bands the addition of small amount of ammonia to mobile phase was necessary. The other components of investigated preparations have no influence on the result of analysis. They were well separated from the main compound (Fig. 2). The purity of the determined compound was verified by comparison of spectra (Fig. 3).

The validity of the chromatographic assay was established through a study of linearity, precision and accuracy.

The linearity was established with a series of working solutions in the concentration range of 0.0265–0.265 mg/mL for promethazine, 0.036–0.22 mg/mL for thioridazine and 0.04–0.12 mg/mL for promazine. Five levels of calibration standards were prepared at various concentration levels. Solution

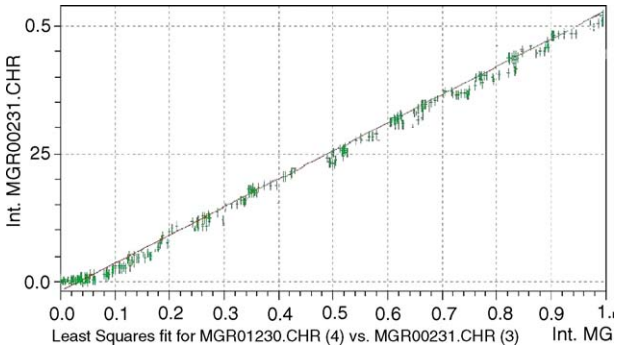

Fig. 3. Peak–purity correlation of ptomethazine hydrochloride standard and promethazine hydrochloride in the samples of pharmaceutical preparations.

Table 1
Summary of linear regression data for calibration standards

Compound	Method	Concentration range (mg/mL)	Slope	Intercept	Correlation coefficient (r)	LOD (mg/mL)
Promethazine	HPLC	0.0265–0.265	172494466	1984512	0.99991	0.0035
	HPTLC		4784.3	57.4	0.99975	0.0058
Thioridazine	HPLC	0.036–0.22	195922577	−413058	0.99995	0.0022
	HPTLC		6323.9	81.234	0.99977	0.005
Promazine	HPLC	0.04–0.12	264314151	−814880	0.99991	0.0019
	HPTLC		6486.0	14.0	0.99987	0.0024

Table 2
Precision of HPTLC and HPLC method

Compound	Concentration (mg/mL)	Method			
		HPLC		HPTLC	
		Peak area ($n=6$)	RDS (%) ($n=6$)	Peak area ($n=6$)	RDS (%) ($n=6$)
Promazine	0.04	9875624	0.95	277.1	1.67
	0.06	14989600	0.84	399.3	2.48
	0.08	20183686	0.81	533.0	1.99
	0.1	25601243	1.54	660.0	2.11
	0.12	31001254	1.04	795.3	0.95
Promethazine	0.0265	6511232	0.98	191.0	2.53
	0.053	10921794	0.58	296.9	1.88
	0.106	20655091	1.13	565.3	0.95
	0.159	29341597	1.27	830.1	2.18
	0.265	47628224	0.81	1320.0	1.42
Thioridazine	0.036	6677117	1.44	304,3	2.40
	0.055	10362684	1.30	429.0	1.52
	0.11	21012365	1.09	770.8	2.06
	0.147	28612354	0.80	1026.5	1.17
	0.22	42611753	1.04	1465	1.43

of each concentration was injected six times and the mean peak areas were taken for the construction of calibration curve. Correlation coefficients (r) were found to be >0.999 for both methods. It means that the curves are linear in these ranges of concentrations and the correlations are suitable for quantitative determinations. Linear regression data are given in Table 1.

The precision of the method was determined by running replicate samples of five concentrations of each compound; the relative standard deviations were 0.58–1.54% for HPLC and 0.95–2.53% for HPTLC method (Table 2).

The accuracy of the methods was checked by determination of promethazine, promazine and thioridazine in the laboratory-prepared dosage containing defined quantities of the substances. The recoveries obtained were 99.8–101.7% for HPLC and 101.1–102.8% for HPTLC (Table 3).

The limit of detection (LOD) of each compound was also calculated and was found to be satisfactory for both methods (Table 1). It amounts to 1.9 μg/mL for promazine, 2.2 μg/mL for thioridazine, 3.5 μg/mL for promethazine (HPLC method) and 2.4, 5.0, 5.8 μg/mL, respectively (HPTLC method).

Table 3
Accuracy of HPTLC and HPLC method

Compound	Applied (μg/mL)	Method					
		HPLC			HPTLC		
		Found (μg/mL)	Recovery (%)	RDS (%)	Found (μg/mL)	Recovery (%)	RDS (%)
Promazine	56.0	55.90	99.8	0.22	57.12	102.0	1.02
	94.0	94.2	100.2	0.21	95.04	101.1	1.75
Promethazine	80.0	81.35	101.7	0.95	82.10	102.6	0.94
	210.0	212.49	101.2	0.41	215.90	102.8	2.11
Thioridazine	73.0	72.34	99.1	1.01	74.10	101.5	1.52
	154.0	155.69	101.1	0.82	157.23	102.1	1.11

Table 4
HPTLC determination of promethazine hydrochloride in pharmaceutical preparations

Sample	Nominal concentration (mg/mL)	Found concentration (mg/mL)	*n*	RDS (%)	Recovery (%)
Diphergan (ampoules)	25	26.0874	10	2.8	104.3
Diphergan (syrup)	1	1.0491	10	2.3	103.9

The applicability of the method was verified by determination of promethazine hydrochloride in pharmaceutical preparations. The selectivity of the separation and the specificity of the detection were shown by the densitograms (Fig. 2) and comparison of spectra (Fig. 3). The obtained results are given in Table 4. RDS values obtained within 2.3–2.8% and recovery values 103.9–104.3% were found to be satisfactory and confirm the suitability of HPTLC-densitometry method.

The acceptable results show the possible application of the HPTLC-densitometry method to the control of the drugs as an alternative to traditionally used HPLC.

4. Conclusion

HPTLC combined with densitometry was found suitable for the determination of phenothiazine derivatives and can be used in the routine control. Its advantages are the low cost of reagents, simplicity, speed, satisfactory accuracy, precision and high efficiency.

This method should be taken into consideration as an alternative to HPLC in phenothiazine derivatives investigations.

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