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Study of the stability of promethazine enantiomers by liquid chromatography using a vancomycin-bonded chiral stationary phase

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Abstract

Three chiral stationary phases based on macrocyclic antibiotics (teicoplanin, vancomycin and ristocetin A) have been tested for chiral separations of promethazine. The vancomycin phase permits the best, baseline enantioseparation of promethazine, with a mobile phase of a 80:20 (v/v) mixture of methanol with a 1% aqueous triethylamine acetate buffer of pH 4.1 and with the analysis time not exceeding 15 min. The limits of detection amount to 27.5 and 31.0 ng/ml for the earlier and later eluting enantiomers, respectively. This separation system, that also permits a sufficient resolution between the promethazine enantiomers and their degradation products, has further been used for the monitoring of the effects of light, temperature and the promethazine concentration in solution on the stability of methanolic promethazine solutions over a period of 19 days. It has been found that the stability of more concentrated solutions is primarily affected by the temperature, whereas the effects of the temperature and light are comparable with more dilute solutions. After 19 days, a solution of 0.5 mg/ml promethazine stored in darkness at a low temperature still contained 84.0% of the original amount of the enantiomers; this value was 89.6% for a solution with the ten times lower promethazine concentration. If the solutions were stored in darkness but at laboratory temperature, the respective values decreased to 38.1 and 62.6% and for the solutions exposed to light at laboratory temperature they decreased even more to 36.7 and 52.6% of the initial promethazine amount.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Promethazine; Vancomycin

1. Introduction

Promethazine belongs to the group of phenothiazine drugs that are extensively used as psychopharmaceuticals in human medicine. Phenothiazines are primarily known as antidepressive agents but they also have antiallergic effects [1,2]. Over 100 different compounds have been derived from the basic phenothiazine skeleton and several of them, such as promethazine, thioridazine, levomepromazine, atenolol, oxprenolol, etc. are chiral. As with other chiral drugs, the individual enantiomers can exhibit different pharmaceutical effects.

Many methods can be found in the literature for the separation and quantification of promethazine in various biological and pharmaceutical samples. Attention has also been paid to enantioselective separations of this drug.

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Flow-injection analysis combined with photochemically induced fluorescence (PIF) was applied to the determination of aminophenothiazines in urine samples and pharmaceutical preparations. The limit of detection obtained with a sensitive PIF detector ranged between 60 and 80 ng/ml [3]. Solid-phase extraction (SPE) followed by a spectrophotometric measurement was used in analysis of promethazine and other drugs in pharmaceutical creams, the SPE procedure serving to remove the interfering cream components from the samples and allow more accurate spectrophotometric analysis [4].

A high-performance liquid chromatography (HPLC) method of quantification of promethazine in human serum, suitable for bioavailability studies, was developed using an electrochemical detector. The advantages of the electrochemical detection are an improved precision and sensitivity of the method and ease of sample preparation [5]. The absolute bioavailability (AB) of three phenothiazine drugs was studied by means of HPLC. The results of analyses of human sera have demonstrated that the AB of promethazine is almost twice that of chlorpromazine and promazine [6]. Another separation procedure by RP HPLC coupled with a coulometric electrochemical detector has also been shown to suit well for the determination of promethazine, levomepromazine and chlorprothixene in human serum. The authors reported that the studied drugs were stable both in acetonitrile and in human serum for a minimum of 6 months when stored at −20 °C [7]. Posibilities of storaging promethazine and some other drugs was studied in plastic syringes (compared to glass containers). The stability of promethazine for 24 h was confirmed [8]. Short-term stability of promethazine under exposure to light at selected wavelength regions was studied in aqueous solution. In addition, application of plastic films as light protecting agents was tested [9]. A detailed study of the mechanism of promethazine degradation under both aerobic and anaerobic conditions was performed by Underberg [10,11].

Both HPLC and capillary electrophoresis (CE) have been used for chiral separations of phenothiazines, especially promethazine. The first attempts at separating the enantiomers of promethazine by HPLC employed a derivatization procedure prior to separation in an achiral environment [12]. Further on, chiral separation systems with various chiral selectors (CSs) have mostly been used. Whereas β-cyclodextrin and its derivatives, with an exception of sulfobutyl ether-β-cyclodextrin in CE [15], have not been able to adequately resolve the enantiomers of promethazine [13,14], amino acid derivative [16] or protein-based CSs, such as human serum albumin, bovine serum albumin [14,17,18] or human serum transferrin [19], have yielded somewhat better results. Other possibilities have been offered by Pirkle-type chiral stationary phases (CSPs) combined with mobile phases of a low polarity (hexane, an alcohol with an ion-pairing agent) [14,20], or CSPs with cellulose esters as CSs [14,17,21]. A combination of two chiral selectors—α_1-acid glycoprotein column and sparteine as a cationic chiral mobile phase modifier—gave good enantioresolution of promethazine, especially if 2-propanol was added to the mobile phase [22]. Macrocyclic antibiotics (MAs), namely vancomycin as a CS, have been studied for CE enantioseparations of promethazine [17].

Macrocyclic antibiotics are a group of structurally diverse naturally occurring compounds that have widely been used as efficient chiral selectors in both liquid chromatography and capillary electrophoresis [23–25]. Glycopeptides combine the advantages of protein-based, cavity-forming and π-donor π-acceptor-interacting CSs. Vancomycin, teicoplanin, ristocetin A and avoparcin bonded to a silica surface are even available as commercial chiral stationary phases [26].

The aim of this work is: (i) to evaluate the suitability of macrocyclic antibiotic-based CSPs (used in a reversed-phase mode) for enantioselective separations of promethazine; and (ii) to study the stability/decomposition of promethazine enantiomers in solution at two different concentration levels.

2. Experimental

2.1. Chemicals

Promethazine was obtained from the State Institute for Control of Drugs (Praha, Czech Republic), purity >99%.

The mobile phases were prepared from the following compounds and solvents: triethylamine, purity

>99% (Sigma), glacial acetic acid, analytical grade (Lachema, Brno, Czech Republic), methanol, purity for chromatography and acetonitrile, gradient-grade purity (Merck, Darmstadt, Germany). Distilled and deionized water was used in the experiments.

2.2. Instrumentation and chromatographic conditions

The UV/VIS spectra were recorded on a Philips PU 8800 instrument (Pye Unicam, Cambridge, UK)

The HPLC equipment (Dionex Corporation, Sunnyvale, CA, USA) consisted of a P 580 Pump, an UVD 170S detector and Rheodyne injection valve Model 7125 (Cotati, CA, USA) with a 10-μl sample loop. Signal acquisition and data handling were performed with the PC Chromeleon PeakNet 6 software.

Commercially available steel columns (250×4.6 mm I.D.), particle size 5 μm, Chirobiotic V (with vancomycin bonded to silica gel), Chirobiotic T (with teicoplanin bonded to silica gel) and Chirobiotic R (with ristocetin A bonded to silica gel), were utilized. These columns were manufactured by ASTEC (Whippany, NY, USA).

The detection wavelength was 254 nm. The measurements were carried out at a temperature of 22 °C.

The mobile phases consisted of a 1.0% (v/v) triethylamine acetate (TEAA) buffer, pH 4.1 or 6.0, with different portions of methanol (MeOH) as the organic modifier. The buffer pH was adjusted with acetic acid to required values before the addition of the organic modifier. The buffers were filtered through a 0.45 μm filter. The mobile phase flow-rate was 0.7 ml/min. The column void volumes were determined with a 5×10^{-4} mol/l aqueous solution of KI.

2.3. Calibration

The response factors of the promethazine decomposition products were unknown and thus the loss in the promethazine content could not be found by simple application of the internal normalization method, as this requires that the response factors of all the substances to be separated be identical. Therefore, the calibration dependence was obtained for a fresh methanolic solution of promethazine, within a range from 1 to 0.001 mg/ml. The areas of the peaks of the two enantiomers were identical at any concentration measured; therefore, the sum of the peak areas was treated by linear regression as the function of the promethazine concentration, whereas the dependences of the peak height on the concentration were obtained separately for the two enantiomers. The points of the calibration plot are the arithmetic means of four measurements. The calibration plot is only linear within a concentration range of 0.08 to 0.001 mg/ml; the results of the regression analysis for this range are listed in Table 1. The limit of detection (equal to the triple of the standard deviation of the noise) and the limit of quantification (ten times the standard deviation of the noise) were obtained from the regression parameters of the peak height dependence on the enantiomer concentration (Table 1).

3. Results and discussion

3.1. Enantioselective separation on macrocyclic antibiotic-based chiral stationary phases

Three chiral stationary phases with macrocyclic antibiotics—vancomycin, teicoplanin and ristocetin

Table 1
Parameters of the calibration dependences and values of the limits of detection (LD) and the limits of quantification (LQ)

Plotted value	Intercept (mAU min)	Slope (mAU min ml/mg)	Correlaction coefficient	LD (ng/ml)	LQ (ng/ml)
Peak area – both enantiomers	0.046	1839.3	0.9999		
Peak height – the first enantiomer	0.08	2544.0	0.9996	27.5	165.0
Peak height – the second enantiomer	0.08	2216.0	0.9997	31.0	190.0

A—as the chiral selectors have been used in enantioseparations of promethazine. The mobile phases contained a 1% triethylamine acetate buffer of a pH of either 4.1 or 6.0; these pH values were selected as the limits ensuring the stationary phase stability. In view of the promethazine structure and its pK_a value of 9.07, it could not be expected that the mobile phase pH might affect the promethazine retention and enantioseparation through a change in the promethazine protonation. However, the pH might influence the dissociation and protonation of the functional groups of the macrocyclic antibiotics and thus also their interactions with the analyte. Methanol was used as the organic modifier of the mobile phase, as acetonitrile was found less suitable [27]. The results obtained with the studied stationary phases and optimized mobile phases are summarized in Table 2. The teicoplanin stationary phase is not suitable for reversed-phase chiral separation of promethazine at any pH value and mobile phase composition. The enantiomers are partially separated on the ristocetin A column with a mobile phase of pure methanol. The vancomycin based stationary phase yielded the best results in the enantioseparation of promethazine, with a good resolution and a time of analysis not exceeding 15 min. (Fig. 1). However, the use of vancomycin as the chiral selector in capillary electrophoresis was unsuccesful for chiral separation of promethazine [17].

3.2. A study of the stability of promethazine in a methanolic solution

Earlier measurements indicated that promethazine is unstable in methanolic solutions. The decomposition process could also be observed visually because a fresh, colourless promethazine solution began to turn purple after a few days and the colouration gradually deepened. Therefore, the UV/VIS spectra

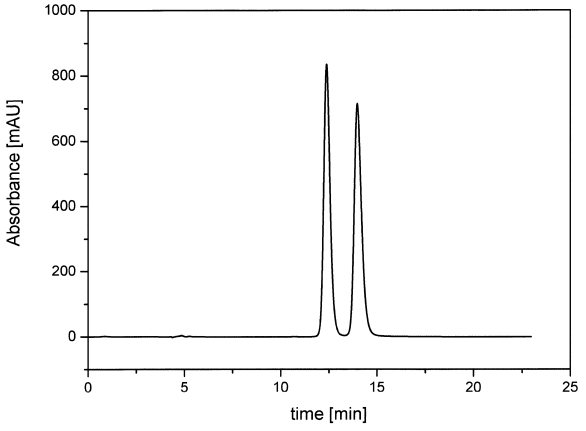

Fig. 1. Enantioseparation of a freshly prepared methanolic solution of promethazine, concentration, 0.5 mg/ml.

were obtained (Fig. 2) for a fresh methanolic solution with a promethazine concentration of 0.5 mg/ml and for the same solution after 19 days of storage in daylight and at laboratory temperature. It can be seen that the spectrum changes due to the effects of light and temperature: whereas the fresh solution exhibits only two maxima at 300 and 245 nm (Fig. 2a), the aged solution yields another local maximum at 530 nm and its spectrum has a different course within the region 350–270 nm (Fig. 2b).

The further study of the promethazine stability employed the conditions found in the previous experiments, permitting a baseline enantioseparation of the promethazine racemate in a sufficiently short elution time and yielding an adequate resolution between the promethazine enantiomers and their decomposition products. Therefore, the vancomycin chiral stationary phase was used, with the mobile phase of a 80:20 (v/v) mixture of methanol with a 1% aqueous triethylamine acetate buffer of pH 4.1 (see Table 2). The chromatogram in Fig. 1, which corresponds to the enantioseparation of a fresh

Table 2
Enantioselective separation of promethazine on three MA-based CSPs in optimized mobile phases

CSP	Mobile phase composition	Resolution	Retention factors
Vancomycin	MeOH:1% TEAA, pH 4.1=80:20	1.11	1.53/1.82
	MeOH:1% TEAA, pH 6.0=80:20	1.49	2.55/3.01
Teicoplanin	Various compositions[a]	0.00	⟨2.81, 9.19⟩
Ristocetin A	MeOH	0.68	2.25/2.44

[a] Methanol or acetonitrile 100–10%; buffer 1% TEAA, pH=4.0 or 6.0.

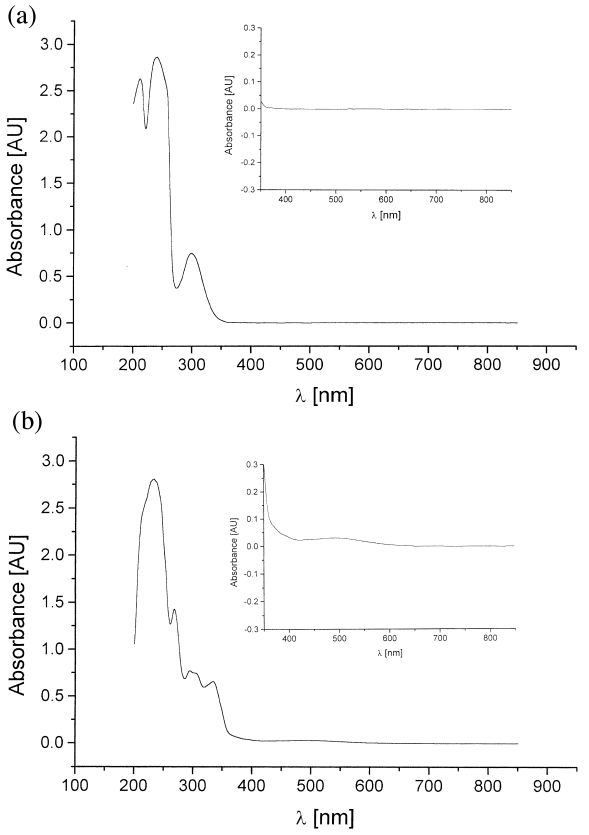

Fig. 2. (a) UV–VIS spectrum of a freshly prepared methanolic solution of promethazine, concentration, 0.5 mg/ml. (b) UV–VIS spectrum of methanolic solution of promethazine (from Fig. 2a) after 19 days of exposure to daylight at laboratory temperature.

methanolic solution of 0.5 mg/ml promethazine, demonstrates that no decomposition product is present.

The previous experiments seemed to indicate that the extent of promethazine decomposition in a methanolic solution depended on the promethazine concentration. Therefore, the stability study employed two different promethazine concentrations, 0.5 and 0.05 mg/ml, under three different sets of conditions. One pair of the solutions was stored in darkness and at a low temperature, another pair in darkness and at laboratory temperature (22±2 °C), and the third pair was exposed to daylight at laboratory temperature. The loss in the amounts of the promethazine enantiomers and the increase in the contents of the decomposition products were monitored by measuring the areas of the appropriate chromatographic peaks. The promethazine stability was monitored on a short time scale (during the first 5 days, the chromatograms were measured daily), and over a longer time range (after 12 and 19 days). It was found in all the cases that the ageing process led to the same decrease in the peak areas of the two enantiomers and thus the sum of the two areas was plotted graphically for a greater lucidity. The more concentrated solution (0.5 mg/ml) was diluted ten times immediately prior to the measurement in order that the concentration be located in the linear part of the calibration plot, and the values corresponding to the dilute solution were represented graphically. Fig. 3 depicts the time dependence of the stability of the 0.05 mg/ml solution. It can be seen that the promethazine decomposition is equally affected by the temperature and light in the beginning, whereas the effect of the temperature is somewhat more pronounced at later stages. The decomposition can also be observed in the solutions stored in darkness at a low temperature, but its extent is substantially smaller.

The dependences obtained for the 0.5 mg/ml promethazine solution are given in Fig. 4, from

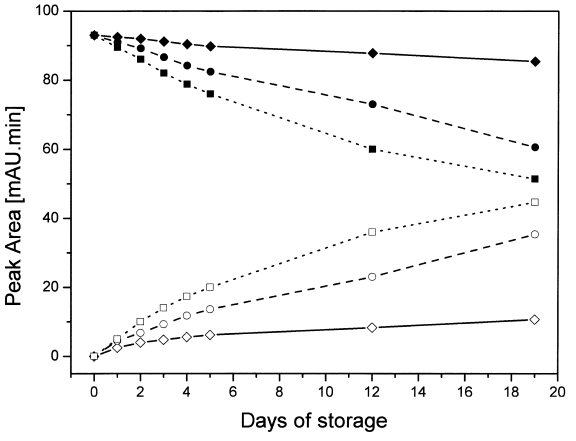

Fig. 3. Stability (decomposition) of a 0.05 mg/ml solution of promethazine: – ♦ –, peak area of the two enantiomers (dark, cold); – ◇ –, peak area of the products of decomposition (dark, cold); - - ● - -, peak area of the two enantiomers (dark, laboratory temperature); - - ○ - -, peak area of the products of decomposition (dark, laboratory temperature); . ■ . . . , peak area of the two enantiomers (light, laboratory temperature); . . . □ ., peak area of the products of decomposition (light, laboratory temperature).

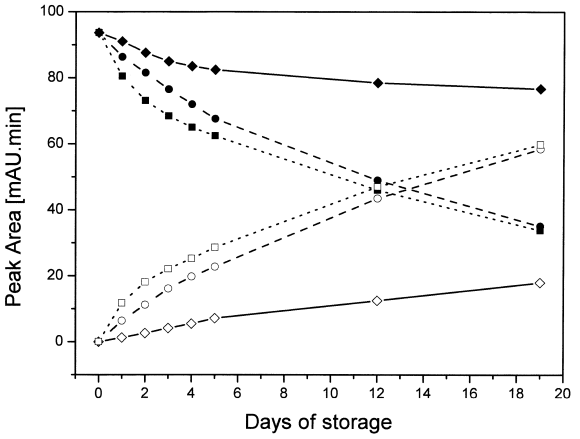

Fig. 4. Stability of a 0.5 mg/ml solution of promethazine; for symbols see Fig. 3.

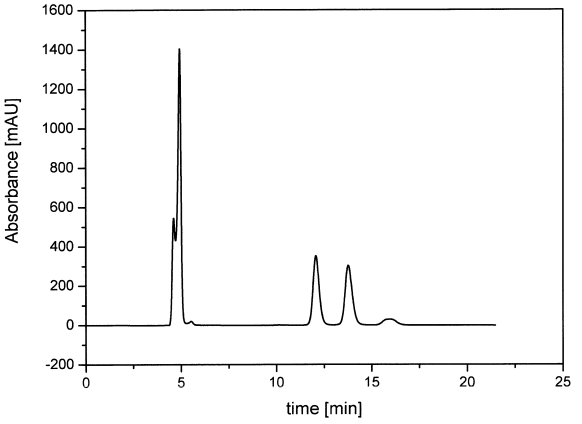

Fig. 5. Enantioseparation of a methanolic solution of promethazine (0.5 mg/ml) stored (daylight, laboratory temperature) for 19 days.

which it follows that the effect of the temperature on the decomposition is much larger than that of light. Table 3 lists the promethazine concentrations in its methanolic solutions after 19 days. It can be seen from Table 3 that the more concentrated solution decomposes much more than the dilute solution, especially during storage at laboratory temperature. The stability of promethazine in solutions differing ten times in the concetration is similar if the solutions are stored in darkness and at a low temperature.

Fig. 5 shows a chromatogram of the enantioseparation of a 19 days old methanolic solution of 0.5 mg/ml promethazine that was exposed to light at laboratory temperature. The peaks of the decomposition products are quite pronounced (cf. Fig. 1). These peaks probably correspond to the oxidation products which were isolated and identified previously [10,11].

4. Conclusions

The vancomycin stationary phase has yielded very good results in chiral separations of promethazine. With the mobile phase of a 80:20 (v/v) mixture of methanol with 1% aqueous TEAA of a pH of 4.1, baseline enantioseparations have been attained, with simultaneous adequate resolution of the promethazine enantiomers from their decomposition products. It has been found that the extent of the promethazine degradation is not only affected by external factors such as light and temperature, but

Table 3
Promethazine concentrations found in its methanolic solutions after 19 days of storage

Storing conditions	Darkness, cold	Darkness, laboratory temperature	Light, laboratory temperature
Original concentration		0.050 mg/ml	
Concentration found after 19 days[a] (mg/ml)	0.045	0.031	0.026
Found (%)	89.6	62.6	52.6
Original concentration		0.500 mg/ml	
Concentration found after 19 days[b] (mg/ml)	0.420	0.191	0.184
Found (%)	84.0	38.1	36.7

[a] The resultant concentration was obtained by comparing the sum of the peak areas for the two enantiomers with the regression parameters of the linear calibration plot.

[b] The resultant concentration was calculated as ten times the value obtained from the comparison of the sum of the enantiomer peak areas with the calibration plot (the solution was diluted ten times prior to measurement—see the text above).

also by the promethazine concentration in the solution.

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