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Short communication

HPLC resolution of thioridazine enantiomers from pharmaceutical dosage form using cyclodextrin-based chiral stationary phase

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Abstract

Resolution of racemic thioridazine obtained from Thioril® tablets (Cipla Ltd., Goa, India) into its enantiomers has been achieved by HPLC using a β-cyclodextrin (CD)-bonded stationary phase. Thioridazine was isolated from commercial formulations and was purified using preparative TLC. The purity was ascertained by RP-HPLC. For the resolution of *rac*-thioridazine using cyclodextrin based CSP and mobile phase of 0.05 M phosphate buffer (pH 6.5)–acetonitrile (50:50) was found to be successful. The optimum conditions of resolution were established by systematically studying the effect of organic modifier, concentration of buffer, pH and flow rate of mobile phase. The detection limit was found to be 10 μg (5 μg of each enantiomer). The enantiomeric purity of each of the resolved isomers was verified by optical rotation.

Keywords: Thioridiazine; Cyclodextrin-based CSPs; RP-HPLC; Enantiomeric separation

1. Introduction

Several drugs are administered as racemates. Sometimes the pharmacological activities of the two enantiomers are different. And, one of them may be toxic. During the last decade, resolution of racemic compounds attracted a great attention in analytical chemistry, especially in pharmaceutical analysis. Thioridazine is marketed in the racemic form as the hydrochloride of 10-[2-(-methyl-2-piperidyl) ethyl]-2-methylthiophenothiazine (Fig. 1). It belongs to the antipsychotic phenothiazine group and is mostly prescribed for the symptomatic management of psychotic disorder [1]. Thioridazine possesses a stereogenic centre at position 2 in the piperidyl ring. (*R*)-Thioridazine has a 2.7 times higher affinity than (*S*)-thioridazine for D2 receptors in isolated rat brain preparation while (*S*)-thioridazine has a 10 times higher affinity for the D1 receptors [2]. The antipsychotic effect is believed to be associated with (*R*)-thioridazine [3].

Thioridazine has been resolved into its enantiomers either directly or indirectly by different chromatographic techniques. Use of β-acetylated cyclodextrin column [4], hydroxypropyl ether-β-cyclodextrin [5], chiralpak AD [6], phenylcarbamate-β-cyclodextrin-bonded phase [7] and (*R*)-*N*-(α)-phenylethyl-*N*-propyl urea based CSP [8] has been reported for direct HPLC resolution. Use of cyclodextrin [9], hydroxypropyl-β-cyclodextrin [10], ovoglycoprotein [11], sulphated-β-cyclodextrin [12] and heptakis (2,6-di-*O*-methyl)-β-cyclodextrin [13] has been cited in literature for resolution of racemic thioridazine by capillary electrophoresis.

The present paper describes resolution of racemic thioridazine on β-cyclodextrin (CD)-bonded phase by HPLC. Results of experimental study of the effect of volume ratio of constituents of the mobile phase, pH and flow rate on resolution have also been discussed. To the best of authors' knowledge there is no earlier report on the HPLC resolution of thioridazine using cyclodextrin based chiral stationary phase.

2. Experimental

2.1. Equipment

The HPLC system consisting of an L-6200 intelligent pump, L-4250 UV–vis variable wavelength absorption detector and D-2500 chromato-integrator was from Merck-Hitachi (Darmstadt, Germany). Cyclodextrin-bonded chiral phase column (250 mm × 4.0 mm i.d., 5 μm particle diameter) known as LiChroCART® 250-4 ChiraDex®, and reversed phase RP-18

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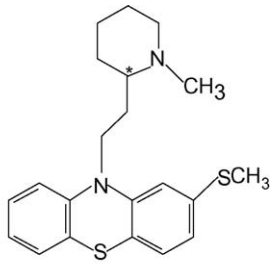

Fig. 1. Structure of (*rac*)-thioridazine.

column (250 mm × 4.0 mm i.d., 5 μm particle diameter) known as LiChroCART® 250-4 were used for HPLC studies. The other equipment used were, pH meter (Cyberscan 510), UV–vis spectrophotometer (Hitachi U 2001), Polarimeter (model Krüss P3002, Germany) and Elementar Analysensysteme GmbH VarioEL for elemental analysis.

2.2. Chemicals and reagents

Thioridazine obtained from Aldrich fine chemical (USA) was used as the standard reference. Silica gel G, with 13% calcium sulphate as binder, having chloride, iron and lead impurities up to 0.02% and showing pH 7.0 in a 10% aqueous suspension, and HPLC grade acetonitrile and methanol were from E. Merck (Mumbai, India), while disodium hydrogen phosphate, monosodium hydrogen phosphate and phosphoric acid were from E. Merck (Darmstadt, Germany).

2.3. Isolation and purification

Each film-coated label contains thioridazine hydrochloride 25 mg, colour sunset yellow ponceau 4R, indigo carmine. Thioril® tablets (Cipla Ltd., Goa, India), labelled to contain 25 mg (±)-thioridazine hydrochloride per tablet were extracted with ethanol and filtered through Whatman No. 42 filter paper. The residue was washed several times with small volumes of absolute ethanol for complete recovery. The combined extract was concentrated in vaccuo. The sample so obtained was recrystallized with ethanol–acetone.

2.4. Preparation of solutions

Stock solutions (2×10^{-3} M) of the standard and the sample (extracted and purified from tablets) of thioridazine were prepared in methanol. All solutions were stored in refrigerator.

2.5. TLC

A slurry of silica gel G (20 g) was prepared in distilled water (40 mL). It was than applied to glass plates with Stahl type applicator to give layers of 20 cm × 10 cm × 0.5 mm. The plates were activated overnight at 60 °C. The chromatograms were developed up to 15 cm at 20 ± 2 °C in paper-lined rectangular glass chambers, pre-equilibrated with the mobile phase for 15 min. The plates were allowed to dry at room temperature and then exposed to iodine vapours to locate the spots. For preparative TLC, 5 μL of each of the stock solutions (2×10^{-3} M) was applied. After developing the chromatogram in CH_3CN–CH_3OH (8:2), spots were marked and cut. Silica gel was extracted several times with small volumes of 70% ethanol. The combined extract was filtered through Whatman No. 42 filter paper. Preparative TLC plates were run to collect sufficient amount. The sample was further recrystallized with ethanol–acetone.

2.6. HPLC

RP-18 column was used for establishing the purity of thioridazine while cyclodextrin-bonded phase column (known as ChiraDex®) was used for enantiomeric separation of thioridazine. Samples were introduced into either of the columns using a model injection valve with a 20-μL loop. Column temperature ambient (≈20 °C), chart speed 5 cm/min, injection mode (25 μL Hamilton syringe), U6-K universal injector (Syringe-loop injector), recorder range 10 mV. Filtration assembly used was from Millipore (Milford, MA, USA) with solvent compatible filters of pore size 0.45 and 0.50 μm. Phosphate buffer, to which organic modifier like methanol and acetonitrile were added, was used as mobile phase at a flow rate of 1 mL/min. The solution was filtered through a 0.45 μm cellulose nitrate membrane (Whatmann International Ltd, Maidstone, England), and degassed prior to use. The pH was adjusted to the desired level by addition of NaOH or phosphoric acid.

2.7. UV spectrophotometry

The stock solutions were diluted to 10^{-4} M and the solutions were scanned to determine λ_{max}. Solutions in the range of 1×10^{-5} to 5×10^{-5} M were prepared by dilution. Their absorbance was recorded and standard plots were made. Absorbance of the compound recovered from TLC experiment was determined.

3. Result and discussion

3.1. Purification and characterization of thioridazine

The weight of thioridazine obtained by extraction and crystallization was little more than the value on labels. Thus, it was concluded that the commercial tablets of thioradazine contained some ethanol soluble impurities that were co-extracted.

3.1.1. TLC

To purify further, preparative TLC was carried out on silica gel plates using several different combinations of solvent system. Extensive TLC experiments were carried out by varying one solvent at a time systematically in order to work out a successful one. The solvent system CH_3CN–CH_3OH (8:2) was found to be successful. Concentration of the sample so recovered was determined using standard plots. The recovery was 93%.

Purity of (±)-thioridazine was confirmed by m.p., optical rotation, λ_{max} determination, CHN analysis and RP-HPLC. The m.p. was found to be 155 °C [14] and the λ_{max} (ethanol) was found to be 280 nm [14]. Further characterization included determination of hR_f (72 in CH_3CN–CH_3OH (8:2)) on silica gel plates, R_t of 4.48 min using RP-HPLC LiChroCART (250 mm × 4.6 mm) column, and zero optical rotation (C = 1, ethanol). CHNS Calcd. for $C_{21}H_{26}N_2S_2$: C, 61.0; H, 6.40; O, 10.10; S, 15.70; N, 6.80; Found: C, 61.0; H, 6.31; O, 9.90; S, 15.55; N, 6.77. Polarimetry of the sample (C = 1, ethanol) showed it to be optically inactive and thus racemic.

3.1.2. RP-HPLC

LiChroCART RP-18 column was equilibrated with 0.05 M phosphate buffer and acetonitrile (50:50) at a flow rate of 1.0 mL/min at room temperature. Detection was carried out by UV at a fixed wavelength of 280 nm and the mobile phase consisted of 0.05 M phosphate buffer (pH 6.5)–acetonitrile (50:50, v/v), when a single peak was observed which confirmed the purity of the thioridazine drug extracted from tablets. The purified sample was subjected to enantiomeric resolution on ChiraDex® column.

3.2. Separation of enantiomers

A typical chromatogram for the resolution of *rac*-thioridazine using cyclodextrin based CSP and mobile phase of 0.05 M phosphate buffer (pH 6.5)–acetonitrile (50:50) is shown in Fig. 2. The two enantiomers clearly appear at R_t 4.66 and 5.10 min. Mobile phase consisting of acetonitrile–water (50:50), and 0.05 M phosphate buffer (pH 6.5)–methanol (50:50, v/v) were not successful in resolving the enantiomers on cyclodextrin based chiral stationary phase as there was observed a single peak with R_t of 4.48 and 4.82 min, respectively. It is known that organic modifier of buffer and pH play very important role in enantiomeric separation. The experimental strategy for the resolution of enantiomers of thioridazine is briefly discussed below.

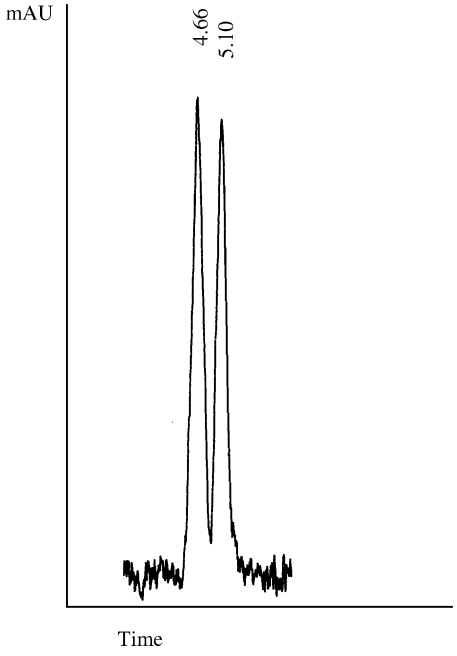

Fig. 2. Chromatogram showing resolution of *rac*-sample of thioridazine. Column: ChiraDex, cyclodextrin-bonded chiral phase (5 μm), LiChroCART 250 mm × 4.0 mm i.d.; temperature 20 °C; injection volume 20.0 μL; sample concentration 0.5 mg/mL; detection absorption at 280 nm; mobile phase, acetonitrile–0.05 M phosphate buffer pH 6.5 (50:50, v/v).

3.2.1. Effect of organic modifier

Two organic modifiers, acetonitrile and methanol in the range of 10–80% in phosphate buffer (0.05 M, pH 6.5) were tried. Enantioseparation was observed with acetonitrile and not with methanol because acetonitrile had greater solvent strength in the reverse phase mode and had a higher affinity for the cyclodextrin cavity. On increasing the acetonitrile percentage up to 50%, resolution increased, further increase in acetonitrile percentage resulted in poor resolution. For thioridazine, the plot of Rs versus % organic modifier showed that the best Rs value was obtained with acetonitrile at 50%. Thus, less retention and high enantioselectivity were observed on using acetonitrile as compared to methanol at equivalent volume-based mobile phase composition.

3.2.2. Effect of concentration of buffer

Effect of phosphate buffer in the range of 0.01–0.08 M was investigated. The results are summarized in Table 1. For (±)-thioridazine, the concentration of 0.05 M exhibited the maximum resolution value. Poor resolution was observed when the molarity of phosphate buffer was less/more than 0.05 M. The selectivity, however, remained unaffected. The plot of Rs versus concentration of buffer showed that the best resolution occurred at 0.05 M phosphate buffer. With acetonitrile as the other component of mobile phase the effect of buffer concentration in the range of 0.01–0.08 M on separation of the two enantiomers clearly indicates that on increasing the buffer molarity up to 0.05 M the resolution increased as shown by increasing base line separation; it decreased on reaching 0.08M, when no base line separation and only a V-shaped peak was observed.

3.2.3. Effect of buffer pH

An important factor in chiral chromatography is the pH of the buffer that affects the binding affinity of both chiral selector

Table 1
Influence of buffer concentration on retention, enantioselectivity and resolution

Buffer molarity (M)	Rs	k'		α
		Peak 1	Peak 2	
0.01	0.71	4.53	4.91	1.08
0.02	0.83	4.23	4.53	1.07
0.03	1.09	4.39	4.74	1.07
0.04	1.16	5.30	5.72	1.07
0.05	1.42	4.66	5.10	1.09
0.06	1.07	4.12	4.62	1.12
0.07	1.03	4.53	4.84	1.06
0.08	0.80	4.64	5.04	1.08

Rs, resolution; k', capacity factor; α, selectivity.

and the analyte in terms of the formation of inclusion complex. The buffer pH is very effective for determining selectivity and chiral recognition as the interaction between the analyte and chiral selector is largely controlled by pH causing ionization. The most stable pH range specified for the LiChroCART® 250-4 ChiraDex® phase is 3.0–7.5. The dependence of resolution of (±)-thioridazine on pH of the mobile phase was studied. It was found that resolution increased when pH was increased from 3.5 to 6.5 and it was the best at pH 6.5 as shown by increasing base line separation; resolution decreased above pH 6.5. The pH of buffer below 4.5 was adjusted by adding phosphoric acid because phosphate buffer range is approximately between pH 4.5 and 9.5. In general, the binding strength of the charged species to the CD cavity is weaker than that of the corresponding neutral species, presumably owing to diminished hydrophobic interaction between the charged guest molecule and the non-polar cyclodextrin cavity.

3.2.4. Effect of flow rate

According to the column plate theory, the plate number is dependent on the flow rate of the mobile phase, and thus resolution is affected by flow rate,

$$H_{\min} = A + 2(BC)^{1/2}$$

$$N_{\max} = \frac{1}{H_{\min}}$$

$$u_{\text{opt}} = \left(\frac{B}{C}\right)^{1/2}$$

where A, B and C are constants, $H_{\min}$ is the plate height, $N_{\max}$ the plate number and u_{opt} the optimum flow rate. The influence of flow rate was examined under the optimum mobile phase concentration of buffer and pH. The flow rate was increased from 0.50 to 1.30 mL/min as shown in Table 2. It was found that the flow rate had little effect on α but it affected Rs greatly which was maximum at 1 mL/min. It was also found that on decreasing the flow rate, capacity factor increased. Resolution increased with increasing flow rate and it was the best at a flow rate of 1 mL/min; it then decreased above flow rate 1 mL/min. A flow rate of 1.0 mL/min was found to be the optimum flow rate.

Table 2
Influence of flow rate on retention, enantioselectivity and resolution

Flow rate (mL/min)	Rs	k'		α
		Peak 1	Peak 2	
1.3	0.98	3.14	3.40	0.98
1.2	1.03	3.98	4.30	1.03
1.1	1.11	4.29	4.58	1.11
1.0	1.42	4.66	5.10	1.09
0.9	1.10	5.05	5.60	1.10
0.8	1.05	5.97	6.40	1.05
0.7	0.96	6.67	7.14	0.96
0.6	0.90	7.62	8.19	0.90
0.5	0.87	7.80	8.76	0.87

The analyte fits into the CD cavity possessing hydrophobic character as is generally accepted for CD based chiral recognition. Analyte with higher hydrophobicity exhibits higher affinity for the cavity, forming more stable complex then the compound possessing lower hydrophobicity. Hydrogen bonds are formed between the hydroxyl group on the rim of the CD and the nitrogen on in chiral carbon and nitrogen, respectively. The results, as discussed above, indicate that the resolution occurs due to formation of inclusion complex, and hydrogen bonding and electrostatic interaction play a significant role.

3.3. Detection limit

The detection limit (LOD) was estimated from the signal to noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The limit of detection was found to be 10 μg (5 μg of each enantiomer).

4. Conclusion

The method involves direct separation of the enantiomers of thioridazine on a chiral stationary phase with cyclodextrin as chiral selector at specific pH and mobile phase. The chromatographic conditions described herein provide a novel, rapid and reliable approach for separation and analysis of thioridazine enantiomers from commercial samples. Pure sample in quantitative yield from pharmaceutical dosage form can also be obtained. The selectivity and migration behaviour of thioridazine are greatly influenced by interaction of thioridazine with β-cyclodextrin in which inclusion complex formation and hydrogen bonding play a significant role. The newly developed reversed-phase HPLC method provides a separation superior to other HPLC methods. It is a simple method with good linearity, precision and detection sensitivity for separation of thioridazine stereoisomers. Furthermore, this method is applicable and useful for evaluation of thioridazine stereoisomers in pharmaceutical formulations.

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