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*Short Communication*

# Liquid chromatographic resolution of the enantiomers of psychotropic drug levomepromazine (methotrimeprazine) with β-cyclodextrin-bonded chiral stationary phase*

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## Introduction

Enantiomeric high-performance liquid chromatographic (HPLC) separation for N-substituted chiral phenothiazine-type drug substances as promethazine [1–9], thioridazine [3, 4, 8], alimemazine [3, 4, 10], ethopropazine [3], propiomazine [4], trimeprazine [11] and cyamemazine [10] have been published and some unsuccessful attempts have also been reported [3].

Levomepromazine (LM), a widely used non-racemic chiral pharmaceutical in this series (Fig. 1) is the active constituent of several pharmaceutical preparations [12] for which no chiral separation method has been published so far. This prompted us to elaborate a simple and multipurpose HPLC method which can be of use for the determination of enantiomeric purity of LM in different media (bulk substance, pharmaceutical dosage forms and biological fluids).

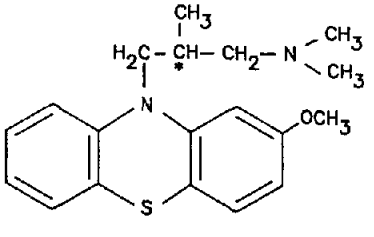

**Figure 1**
Chemical structure of levomepromazine.

We report herein the use of the improved β-cyclodextrin (β-CD) chiral stationary phase (CSP) [13] for separation of LM enantiomers by HPLC.

## Experimental

### *Apparatus*

An isocratic HPLC system consisted of a Beckman (Berkeley, CA, USA) 100 A pump, a 163 model variable wavelength UV detector (containing a 8 μl flow cell) and a Rheodyne 7125 sample injection valve (Rheodyne Inc., Cotati, CA, USA) fitted with 10 μl loop was used. Chromatograms were recorded on a Shimadzu (Tokyo, Japan) C-R3A model reporting integrator. The separations were carried out on a stainless steel Cyclobond I column (250 × 4.6 mm i.d.) packed with 5 μm silicagel with chemically bonded β-CD (Astec, Whippany, NJ, USA).

### *Materials*

Levomepromazine and levomepromazine maleate (both *S*-enantiomer) [14] were products of EGIS Pharmaceuticals (Budapest, Hungary). The racemate and the *R*-(+)-form of LM were synthesized as described in the literature [15] and recrystallized twice from

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acetone. Methanol (HPLC grade), glacial acetic acid and triethylamine (reagent grade) were purchased from Merck (Darmstadt, Germany). Water was double-distilled and stored in a glass container.

*Chromatographic conditions*

The mobile phase used was a mixture of methanol and triethylammonium acetate (TEA) buffer in various proportions. The aqueous component of the mobile phase (prepared from 1.00% v/v of glacial acetic acid and 1.00% v/v triethylamine solutions) was filtered in a G4 sintered glass filter and ultrasonicated for 5 min before use. The pH values of the buffers were measured before dilution with methanol. Isocratic elution was applied at a flow rate of 1.0 ml $min^{-1}$.

LM bulk substance, accurately weighed, was dissolved in the mobile phase (*ca* 0.2 mg $ml^{-1}$) and 10 μl portions of this solution were injected into the HPLC system.

## Results and Discussion

Typical chromatograms obtained for chiral separation of LM enantiomers are shown in Fig. 2.

Linear standard curves were obtained for both enantiomers by performing serial injections from synthetic binary mixtures. The calibration graph (Fig. 3) constructed by plotting peak area/10,000 versus actual concentration (%) of *R*-(+)-enantiomer in the binary mixture was $y = 2.032x + 0.022$ ($r^2 > 0.999$) over the concentration range 0.5–10%.

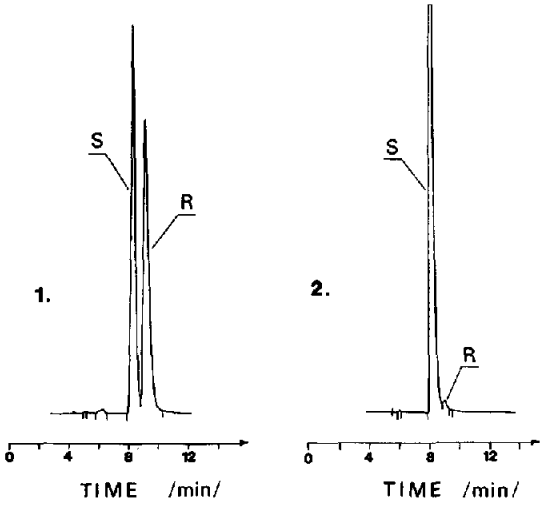


**Figure 2**
HPLC resolution of levomepromazine enantiomers. Column: Cyclobond I, 5 μm (250 × 4.6 mm i.d.); flow rate: 1 $cm^3$ $min^{-1}$; mobile phase: methanol–aqueous TEA buffer (pH 4.5) (46:54, v/v); temperature: 25°C; detection: UV at 254 nm. 1, Racemate; 2, 0.49% *R*-enantiomer in LM (*S*-enantiomer).

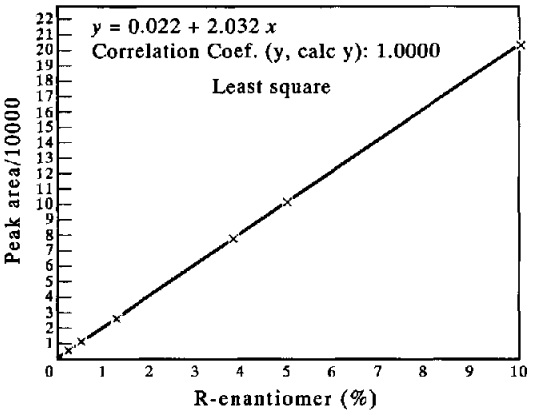


**Figure 3**
Calibration curve for the determination of *R*-enantiomer in LM.

System precision obtained for the *R*-(+)-enantiomer in LM bulk substance at 1.285% actual impurity level was 1.59% RSD ($n = 4$). Accuracy (expressed as the difference of the determined and actual composition at 1.285% impurity level) was 0.012% ($n = 4$, mean) calculated from the enantiomer peak area ratios. The detection limit for the minor component in binary enantiomeric mixtures can be estimated to about 0.1% ($S/N = 2$). The lowest detectable concentration of the *S*-enantiomer as minor component can be even lower because the *R*-form is eluted later. Limit of quantitation for *R*-enantiomer as impurity in LM bulk substance was about 0.5% ($S/N = 10$). Precision and accuracy calculated as above for 0.498% actual impurity level was 3.87% RSD ($n = 4$) and 0.003% ($n = 4$, mean), respectively.

The effects of environmental changes on the separation were studied by varying the temperature, flow rate, the methanol–buffer ratio of the eluent and pH of the aqueous component of the mobile phase.

The comparison of the same separation made between 15 and 40°C (other conditions: as in Fig. 2) shows that the magnitude of the separation factor (α) and resolution ($R_S$) decrease with increasing temperature (from 1.20 to 1.12 and 1.30 to 0.96, respectively) but the marked loss of resolution can be observed above 30°C (from 1.22 to 0.96).

**Table 1**
Influence of the mobile phase composition on the chiral separation of levomepromazine enantiomers. For chromatographic conditions, see Fig. 2

| Mobile phase (volume per cent of methanol) | Retention times ($t_R$ (S) (min) | $t_R$ (R) (min) | Capacity factors $k'_S$ | $k'_R$ | Separation factor | Resolution $R_S$ |
|---|---|---|---|---|---|---|
| 30 | 39.0 | 47.8 | 15.97 | 19.74 | 1.24 | 1.73 |
| 35 | 21.2 | 25.2 | 8.23 | 9.96 | 1.21 | 1.69 |
| 40 | 11.9 | 13.6 | 4.17 | 6.66 | 1.18 | 1.48 |
| 44 | 9.7 | 10.9 | 3.21 | 3.75 | 1.17 | 1.35 |
| 46 | 8.1 | 9.1 | 2.54 | 2.94 | 1.16 | 1.28 |
| 50 | 6.1 | 6.7 | 1.67 | 1.90 | 1.14 | 1.14 |
| 60 | 4.2 | 4.3 | 0.80 | 0.87 | 1.09 | — |

There was no appreciable change in the selectivity factor as a function of flow rate. A significant improvement was obtained, however, in resolution (from 1.08 to 1.42) as the flow rate was reduced (from 1.5 to 0.4 ml $min^{-1}$; other conditions: as in Fig. 2).

With increasing pH of the aqueous component of the mobile phase (from 3.84 to 7.35; other conditions: as in Fig. 2) a small decrease in the selectivity factor could be observed (from 1.21 to 1.15), while the resolution was markedly improved (from 1.00 to 1.38). Unfortunately, retention times ($t_R$) at pH 7.35 became too long (22.7 and 25.6 min for *S*- and *R*-enantiomers, respectively). A significant improvement was obtained, however, in both the separation factor and resolution as the methanol content of the mobile phase was reduced (Table 1). Table 1 shows that an acceptable compromise can be obtained for the separation factor, resolution and retention time using 40–46 volume per cent of methanol (60–54 vol. per cent of aqueous buffer of pH 4.5) in the mobile phase, together with 1.0 ml $min^{-1}$ of flow rate at 25°C.

The described HPLC method has been applied for the determination of enantiomeric purity of levomepromazine maleate (LMM), a widely used form of LM in pharmaceutical preparations [12]. It has been stated that using the same procedure as above, the determination of the *R*-(+) enantiomer in LMM can be carried out with approximately the same precision and accuracy as obtained for LM bulk substance. The well-separated peak of maleic acid ($t_R$ = 4.49 min) does not interfere.

HPLC using CSPs has been proved to be the most powerful technique for separation of enantiomers [16–18]. A great variety of CSPs has been developed and applied to various classes of compounds [19–20]. Among the commercially available CSPs for HPLC columns, the cyclodextrin-bonded phases appear to offer considerable advantages [13, 21–24]. Particularly, the β-CD phase can be used with very polar mobile phase combinations whereas other CSPs show lower stability in these conditions and their use is frequently limited to mobile phases of low polarity.

The first β-CD columns were less efficient than conventional reversed-phase columns. Recently manufactured columns, however, have considerable efficiency, due to the higher loading of CD and the improved column technology. The increased efficiency allows the use of higher methanol or acetonitrile–water ratios [13], as this paper confirms.

As far as the authors are aware, no CD-bonded CSP has yet been applied for enantiomeric separation of N-substituted chiral phenothiazines. The separation of trimeprazine enantiomers is the only case in this series in which β-CD was successfully used as chiral mobile phase additive [10]. The simple and easy-to-use procedure presented here for the separation of LM enantiomers can certainly be applied for pharmaceutical dosage forms containing LM as active ingredient, as well as for stability trials and, possibly, for enantiospecific bioavailability studies.

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