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Improved high-performance liquid chromatographic resolution of the geometric isomers of 6-hydroxy-4-(1-hydroxy-1-methylethyl)-1-cyclohexene-1-ethanol and by-products with β-cyclodextrin

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ABSTRACT

The aim of this study was to optimize the high-performance liquid chromatographic separation of (6*S*,4*R*)-(−)-6-hydroxy-4-(1-hydroxy-1-methylethyl)-1-cyclohexene-1-ethanol and its potential impurities to determine them in the active ingredient and in pharmaceutical formulations for purity and stability analysis. A comparison of conventional normal- and reversed-phase high-performance liquid chromatographic analysis and a method employing β-cyclodextrin was made. The reversed-phase analysis without β-cyclodextrin was undoubtedly unsuitable for an acceptable high-performance liquid chromatographic separation. On the other hand the other two methods were more selective and showed good precision, accuracy and linearity in the range investigated. However the use of β-cyclodextrin as eluent modifier with a reversed-phase was preferable for its improved selectivity and also for allowing the use of a non-toxic and less expensive eluent. Also, the limits of detection and quantitation obtained for the impurities made the β-cyclodextrin method very suitable for purity and stability analysis.

INTRODUCTION

(6*S*,4*R*)-(−)6-Hydroxy-4-(1-hydroxy-1-methylethyl)-1-cyclohexene-1-ethanol (CO/1408) is a polyol compound used in the treatment of some pulmonary diseases [1].

Diastereomeric and enantiomeric chromatographic separation of *cis* and *trans* and (+) and (−) *trans* forms of this compound has been achieved with β-cyclodextrin (β-CD), either adsorbed on to the stationary phase or dissolved in the mobile phase, as previously described [2]. Beside the *cis* isomer (II), two additional impurities might be present in the active ingredient: the starting manufacturing material Nopol (I), (1*R*)-(−)-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol, and the bicyclic degradation product (III), (6*R*, 7a*S*)-6-(1-hydroxy-1-methylethyl)-2,3,5,6,7,7a-hexahydrobenzofuran. Their structures, identified by nuclear magnetic resonance and mass spectrometric analysis, are shown in Fig. 1.

The present work describes the simultaneous determination of the active drug and its potential impurities by a rapid and inexpensive routine analysis employing β-CD as a selective eluent modifier.

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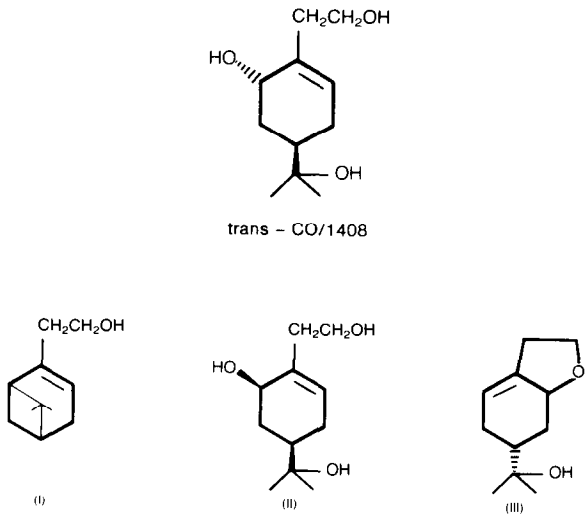

Fig. 1. Structures of *trans*-CO/1408 and its potential impurities: (I) Nopol, (II) *cis*-CO/1408 and (III) bicyclic degradation product.

A study of this inclusion complex separation in comparison with conventional normal- and reversed-phase high-performance liquid chromatographic (RP-HPLC) analysis is reported and discussed.

EXPERIMENTAL

A Varian 2010 liquid chromatograph with a 2050 variable-wavelength UV detector and a Perkin Elmer Series 4 liquid chromatograph with an LC-85 variable-wavelength UV detector were used. Spectrophotometric detections were recorded at 205 nm (range 0.32 a.u.f.s.). Experiments were carried out with a prepacked LiChrosorb reversed-phase RP-8 (10 μm) and a normal-phase Si_{60} (5 μm), 250 × 4.0 mm I.D. column (Merck, Darmstadt, Germany) at room temperature.

The eluents were prepared with Merck HPLC-grade solvents and were filtered and degassed prior to use. β-CD was of analytical reagent grade and supplied by Fluka (Buchs, Switzerland). CO/1408 and its impurities were synthesized in our laboratories, except Nopol, which was supplied by Fluka.

RESULTS AND DISCUSSION

A conventional normal-phase chromatographic system using an Si_{60} column and a non-aqueous eluent (*n*-hexane–diethyl ether–methanol 75:10:15) allowed a satisfactory baseline separation of the active ingredient CO/1408 and its potential impurities (see Fig. 2). However, the toxicity and expense of the mobile phase with a non-optimal separation of *cis* impurity induced us to develop an alternative RP chromatographic method.

Conventional RP analysis, using a LiChrosorb RP-8 (10 μm) column and an aqueous eluent was not suitable for an acceptable HPLC separation of the active

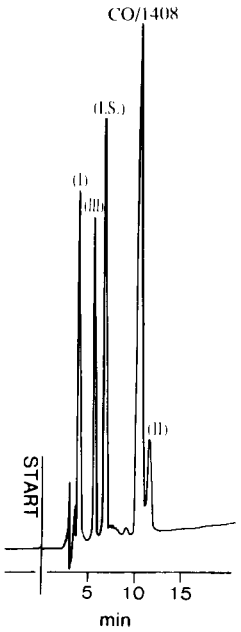

Fig. 2. Separation of CO/1408 and its potential impurities I, II and III using sobrerol (5-hydroxy-α,α,4-trimethyl-3-cyclohexene-1-methanol) as internal standard (I.S.) on a LiChrosorb Si_{60} (5 μm) 250 × 4.0 mm I.D. column. Mobile phase: *n*-hexane–diethyl ether–methanol (75:10:15). Flow-rate: 0.8 ml/min.

ingredient and its impurities, as shown in Fig. 3a. As a previous work [2] reported that β-CD is able to form a relatively stable complex with CO/1408, we decided to verify the utility of β-CD inclusion properties in this case also. The addition of β-CD to the mobile phase effectively decreased the retention times both of CO/1408 and of impurities, improving their separation in comparison with the above-mentioned conventional RP-HPLC analysis (see Fig. 3a and b).

As foreseen, an inversion of the elution order can be observed when comparing the normal-phase chromatogram with the reversed-phase chromatogram, with or without β-CD. Hence the more probable reaction impurity, that is the *cis* form of CO/1408, elutes first and is better quantified in RP than in normal-phase chromatography, where it is on the tail of CO/1408. This analytical method can therefore be used for quantitative CO/1408 determination without any interference with the three potential impurities and internal standard. In normal-phase analysis *trans*-sobrerol (5-hydroxy-α,α,4-trimethyl-3-cyclohexene-1-methanol) has been used as internal standard, while in RP analysis the inferior homologue of CO/1408 [6-hydroxy-4(1-hydroxy-1-methylethyl)-1-cyclohexene-1-methanol] has been employed. The two quantitative methods are both rapid and specific (see Figs. 2 and 3b).

Accuracy and precision were evaluated by ten injections of the same standard solution (3 μg of CO/1408 dissolved in the mobile phase) on two different days. Linearity was determined by six replications of five points between 2.5 and 3.7 μg and was significant for both methods. These results are summarized in Table I.

To evaluate the limit of detection and quantitation of the impurities, 80 μg of

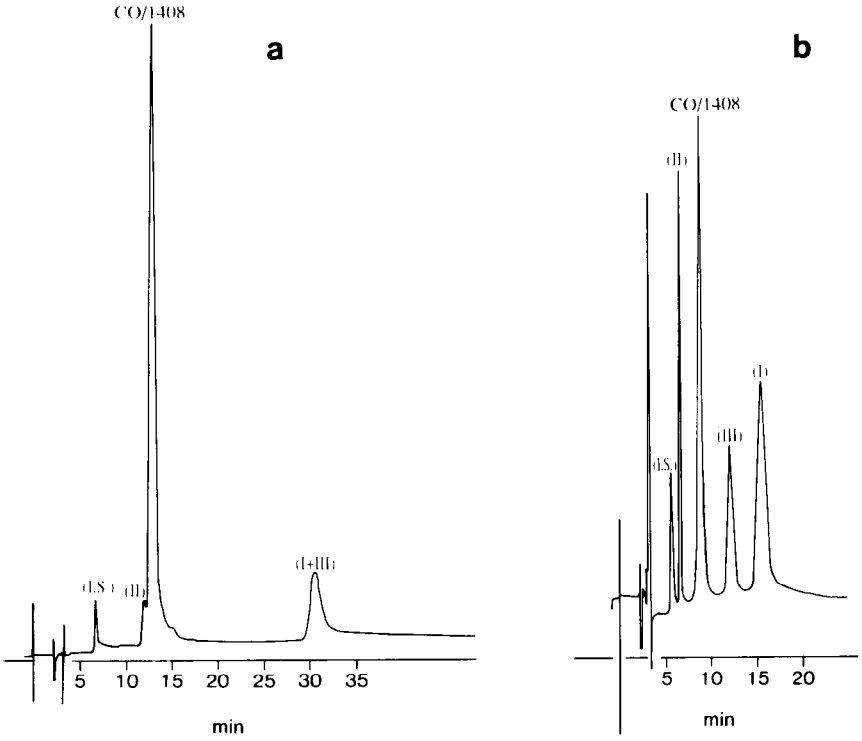

Fig. 3. (a) Separation of CO/1408 and its potential impurities I, II and III using CO/1470 [6-hydroxy-4-(1-hydroxy-1-methylethyl)-1-cyclohexene-1-methanol] as internal standard (I.S.) on a LiChrosorb RP-8 (10 μm) 250 × 4.0 mm. I.D. column. Mobile phase: potassium phosphate buffer solution (0.025 *M*, pH 7.4)–acetonitrile–methanol (90:5:5). Flow-rate: 1 ml/min. (b) As (a) but with the addition of β-CD to the mobile phase (1%, w/v).

CO/1408 were spiked with 0.1 μg of Nopol and bicyclic compound and 0.032 μg of *cis*-CO/1408. This mixture was dissolved in the eluent and injected with the β-CD HPLC method. The limits of detection and quantitation, evaluated for the three impurities, were therefore: Nopol and bicyclic compound < 0.15% (w/w) and *cis*-CO/1408 < 0.04% (w/w).

We can therefore conclude that the two methods are qualitatively and quantita-

TABLE I

Column phase	Precision[a] (%)	Accuracy[b] (%)	Intercept	r[c]	F_{ratio} (ANOVA)
Normal	± 1.93	+0.05	−0.020 ± 0.181	0.997	2399.5 ($\alpha \ll 0.01$)
Reversed[d]	± 1.65	+0.14	+0.059 ± 0.088	0.999	11792.5 ($\alpha \ll 0.01$)

[a] Precision (%) = $\pm\ 100 \cdot t_{(0.05,v)}\, s / \bar{x}$ (v = degree of freedom).
[b] Accuracy (%) = $100\ (\bar{x} - \mu)/\mu$ (μ = true value).
[c] r = Coefficient of correlation.
[d] β-CD has been used as a selective eluent modifier.

tively comparable, but the analysis employing β-CD is preferable for a better HPLC separation of *cis*-CO/1408 and for the use of a non-toxic and less expensive eluent. Many authors have successfully used CDs for routine separations that are difficult to achieve by conventional methods [3–6]; this work is another significant example of the chromatographic application of β-CD in routine analysis.

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