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Short Communication

Determination of impurities in dextropropoxyphene hydrochloride by high-performance liquid chromatography on dynamically modified silica

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ABSTRACT

A high-performance liquid chromatographic method was developed for the separation and determination of small amounts of impurities in dextropropoxyphene hydrochloride. The separation was achieved on a column of bare silica (LiChrosorb Si 100), using as the eluent methanol–tetrahydrofuran–water–0.2 *M* potassium phosphate buffer (pH 7.5) (350:84:516:50) containing 2.5 m*M* cetyltrimethylammonium bromide. The selectivity of the system towards a mixture of dextropropoxyphene hydrochloride and two possible impurities was investigated using different brands of silica. Only minor variations were found relative to those of a chromatographic system based on chemically bonded ODS-silica as the column material. The method is well suited for pharmacopoeial purposes, especially as the utilization of reference substances is not necessary.

INTRODUCTION

Dextropropoxyphene hydrochloride is a narcotic analgesic and monographs fixing the quality of the substance can be found in several modern pharmacopoeias. In the monographs in both the U.S. Pharmacopeia (USP XXII) [1] and the British Pharmacopoeia (B.P. 88) [2] a test for related substances is prescribed by means of gas chromatography (GC) using authentic samples of two known impurities as the reference substances. The two substances are an acetoxy compound, which is a possible impurity originating from the route of synthesis, and a carbinol compound, which may originate both from the route of synthesis and from degradation. The structures of dextropropoxyphene and the two impurities are given in Table I. As the drug substance itself and probably also the related substances are considered to be narcotics, the exchange of reference substances between countries may be difficult or even impossible owing to national and international restrictions. Hence it would be useful to have at the disposal of the authorities a method that allows the investigation of the content of related substances without the use of reference substances.

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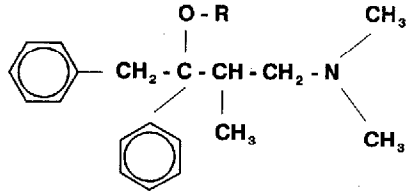
TABLE I

STRUCTURES OF DEXTROPROPOXYPHENE AND TWO POSSIBLE IMPURITIES



No.	Substance	R
I	Dextropropoxyphene	$-COCH_2CH_3$
II	Acetoxy compound	$-COCH_3$
III	Carbinol compound	-H

A separation method based on high-performance liquid chromatography (HPLC) with chemically bonded ODS-silica as the column material is an obvious possibility for a basic drug substance such as dextropropoxyphene. Previously published methods using bonded-phase materials as the column packing has not been specifically intended for purity testing of the raw material [3–5]. Further, the selectivity of such chromatographic systems has previously been shown to depend strongly on the brand of column material [*e.g.*, 6, 7] and, therefore, a suitability test will most often be needed involving the use of one or more reference substances.

This present investigation was performed with a view to developing an HPLC method suitable for separating and determining possible impurities in dextropropoxyphene hydrochloride for pharmacopoeial purposes and without the need for reference substances. For this purpose, the dynamically modified silica approach was chosen. This technique has been shown to be independent of the brand of column material [7].

EXPERIMENTAL

Chemicals

Samples of dextropropoxyphene hydrochloride were of pharmacopoeial quality. Dextropropoxyphene impurities were B.P. Chemical Reference Substances. All reagents were of analytical-reagent grade from E. Merck (Darmstadt, F.R.G.).

Chromatography

A liquid chromatograph consisting of a Model 410 LC pump (Kontron, Zürich, Switzerland), a Model 2012 spectrophotometer detector (Cecil, Cambridge, U.K.) operated at 220 nm and a Model 7125 injection valve (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 20-μl loop was used. Chromatograms were recorded on a Model BD 8 recorder (Kipp and Zonen, Delft, The Netherlands) and retention data were measured and processed by means of a Model 3353A laboratory data system (Hewlett-Packard, Cuperline, CA, U.S.A.).

All experiments were performed on columns of 120 × 4.6 mm I.D. (Knauer, Oberursl, F.R.G.) packed by the dilute slurry technique with silica or chemically

bonded octadecylsilyl (ODS) silica of 5 μm particle size. For chromatography on dynamically modified silica the eluents were mixtures of methanol, acetonitrile or tetrahydrofuran with water and a fixed amount (5%) of 0.2 *M* potassium phosphate buffer (pH 7.5), and with the addition of 2.5 m*M* cetyltrimethyl ammonium (CTMA) bromide. The columns were equilibrated by eluting overnight. During chromatography, the analytical column was guarded by a silica precolumn situated between the pump and the injection valve. Following each series of experiments the column was brought to its initial state by rinsing with methanol–0.05 *M* nitric acid (1:1) and finally with methanol. For bonded-phase chromatography the eluent was acetonitrile–0.2 *M* sodium perchlorate (adjusted to pH 2.0 with hydrochloric acid) (40:60).

Test and standard solutions

For impurity testing, 0.5% solutions of the individual samples of dextropropoxyphene hydrochloride in the eluent were used. A 0.0025% solution of dextropropoxyphene hydrochloride in the eluent was used as an external standard. Volumes of 20 μl of each solution were injected.

For system suitability testing, 50 mg of dextropropoxyphene hydrochloride were boiled for 30 min in 5.0 ml of 1 *M* alcoholic potassium hydroxide, after cooling 2.5 ml of 2 *M* hydrochloric acid were added and the mixture was diluted to 50 ml with eluent.

RESULTS AND DISCUSSION

The HPLC method was elaborated using a sample of dextropropoxyphene hydrochloride (I) containing two potential impurities (II and III). Several parameters will influence the retention and selectivity in a chromatographic system based on the dynamically modified silica approach [7–9]. The starting point in choosing the actual eluent was a previously used mixture, methanol–water–0.2 *M* potassium phosphate buffer (pH 7.5) (50:45:5) containing 2.5 m*M* CTMA [8]. The separation of III and dextropropoxyphene hydrochloride using this eluent was insufficient. On replacing 50% of methanol by 30% of acetonitrile, resulting in an isoeluotropic eluent, the shape of the dextropropoxyphene peak became unacceptably poor. Also, the use of tetrahydrofuran as the organic modifier proved unsuccessful. It was not possible to achieve a sufficient separation unless a percentage of tetrahydrofuran was used that resulted in an unacceptably long retention time. Furthermore, problems were encountered in dissolving the drug substance in such eluents.

Optimization of eluent compositions by using ternary mixtures of solvents has been described previously in chromatography on chemically bonded phases by Schoenmakers *et al.* [10] and also in reversed-phase chromatography on dynamically modified silica by Hansen and Helboe [9]. When performing optimization experiments using methanol at concentrations between 0 and 50% and tetrahydrofuran at concentrations between 32 and 0%, the optimum mixture turned out to be methanol–tetrahydrofuran–water–0.2 *M* potassium phosphate buffer (pH 7.5) (350:84:516:50).

In order to compare possible variations in selectivity using different brands of column materials to those of a chromatographic system based on the use of chemically bonded ODS-silica, a separation method utilizing such column materials was required. The method used is a proposal from the BP Laboratory discussed during the elaboration of a draft monograph on dextropropoxyphene hydrochloride for the

TABLE II

SEPARATION FACTORS (α) BETWEEN DEXTROPROPOXYPHENE AND TWO POSSIBLE IMPURITIES MEASURED ON SIX DIFFERENT SILICA COLUMNS AND FIVE DIFFERENT ODS-SILICA COLUMNS

Column material	Separation factor	
	II	III
Bare silica		
LiChrosorb Si 100	1.69	1.23
Spherisorb S 5 W	1.63	1.27
Partisil 5	1.64	1.32
Zorbax SIL	1.66	1.29
Nucleosil 50-5	1.63	1.28
Nucleosil 100-5	1.62	1.20
ODS-silica		
LiChrosorb RP-18	1.22	1.34
Hypersil ODS	1.30	1.42
Spherisorb ODS-1	1.35	1.44
Spherisorb ODS-2	1.49	1.49
Partisil ODS-3	1.49	1.70

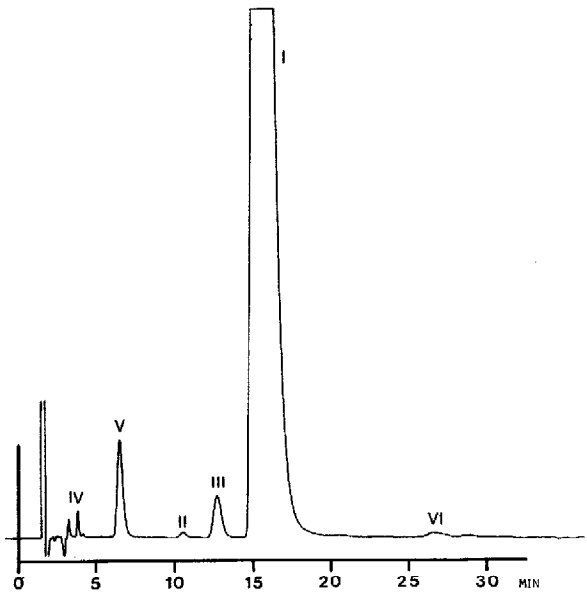


Fig. 1. Chromatogram of a sample of dextropropoxyphene hydrochloride solution that had been stored for several days. Column, Partisil 5 (120 × 4.6 mm I.D.); eluent, methanol–tetrahydrofuran–water–0.2 *M* potassium phosphate buffer (350:84:516:50) containing 2.5 m*M* CTMA; detection wavelength, 220 nm (0.1 a.u.f.s.); flow-rate, 1 ml/min. Peaks I–III as in Table I; IV–VI = unknown impurities.

European Pharmacopoeia [11]. The eluent for this chromatographic system was acetonitrile–0.2 *M* sodium perchlorate (pH 2.0) (40:60).

The two separation methods were tested using six different brands of bare silica and five different brands of ODS-silica. The selectivities of the individual chromatographic systems towards a mixture of dextropropoxyphene hydrochloride and impurities II and III as expressed by the separation factor of each compound from the dextropropoxyphene peak are given in Table II. It can be seen that the variations in selectivity of the system based on bonded-phase materials are considerably larger than those of the dynamically modified silica system, and in one instance the two impurity peaks coincide. This is in accordance with experience from previous investigations on the determination of impurities in propranolol [12] and on separations of corticosteroids [13]. The possible lack of separation of the two impurities in the bonded-phase system might constitute a serious drawback with regard to the requirement for the content of related substances to be expressed as is often done in pharmacopoeial monographs, *e.g.*, "not more than 0.5% of each individual impurity". A batch containing the allowed amount of each of II and III would appear in the test as containing 1% of a single impurity, hence the batch would be rejected, Another drawback to the bonded-phase system is that the order of elution of the two possible impurities has reversed in comparison with that in the dynamically modified silica system (see below).

In Fig. 1 shows a chromatogram of a sample of dextropropoxyphene hydrochloride that had been stored in solution at room temperature for several days. Apart from the known impurities (II and III), several unknowns are seen. Unknown V was found in detectable amounts only in stored solutions and, further the peak corresponding to carbinol impurity III increases on standing. Therefore, sample solutions should be prepared fresh daily. It appears that the peak corresponding to impurity III is closer to the main peak than that corresponding to II. As impurity III is a degradation product, it may be prepared directly from the sample to be investigated (see Experimental) and used for a suitability test, as the order of elution in a chromatographic system based on dynamically modified silica is independent of the brand of column material used. This possibility is not available when using the chromatographic system based on bonded-phase material, as impurity II, the one originating solely from the route of synthesis, is that which elutes most closely to the main peak.

Using the chromatographic system based on dynamically modified silica as described above, a series of samples of dextropropoxyphene hydrochloride were analysed. For quantification, external standardization relative to a dilute solution of the sample to be investigated was used. Linearity of the detector response for the external standard was ensured up to a content corresponding to 2% of the amount in the main peak in the chromatogram of the test solution, injecting amounts ranging between 0.5 and 10 μg ($n = 5$), $y = 1.05x + 0.05$ ($r = 1.0000$). The limit of quantification was *ca.* 0.2 μg (corresponding to 0.05% of the amount in the main peak). The response factor of impurities II and III was shown to be comparable to that of dextropropoxyphene hydrochloride in the range 215–220 nm. Hence the amount of impurity can be taken as that determined relative to the external standard, which is a dilution of dextropropoxyphene hydrochloride. The absorption maximum for compounds I–III is *ca.* 215 nm. However, the detection wavelength used was 220 nm in

TABLE III

RESULTS OF THE ANALYSIS OF EIGHT SAMPLES OF DEXTROPROPOXYPHENE HYDROCHLORIDE

Sample	Amount of impurity (%)[a]	
	II	III
A	0.15	0.05
B	0.18	0.05
C	–	–
D	0.12	0.05
E	0.17	0.05
F	–	–
G	–	0.05
H	–	–

[a] Each determination was performed in triplicate.

order to improve the signal-to-noise ratio, as 215 nm is close to the UV cut-off limit of the eluent.

The precision of the method was investigated by analyzing separately ten portions of a 0.0025% solution of dextropropoxyphene hydrochloride (corresponding to a 0.5% impurity level). The relative standard deviation was 0.9%. The results of the analyses are given in Table III.

CONCLUSION

An HPLC method based on dynamically modified silica has been developed and shown to be suitable for the separation and determination of possible impurities in dextropropoxyphene hydrochloride. The efficiency of the column can be ensured by means of a system suitability test without using any reference substances. The method implies a requirement on the minimum resolution between the peak corresponding to the substance to be examined and a degradation product which can be prepared *in situ*. Hence the method has been shown to be superior to an alternative HPLC method based on chemically bonded ODS-silica, as the latter exhibited large brand-to-brand variations in selectivity towards a test mixture containing dextropropoxyphene hydrochloride and two possible impurities. The method is, therefore, deemed well suited for pharmacopoeial purposes.

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