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Analysis of some dosage forms containing pyridine derivatives using a cyclodextrin bonded stationary phase in HPLC

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Abstract: The high performance liquid chromatography of some pyridine derivatives using a silica column to which β-cyclodextrin has been bonded, has been investigated. In spite of the low affinity constants of the drugs for cyclodextrin (of the order of 10^2 M^{-1}) good separations can be achieved using a mobile phase of methanol and pH 7.0 phosphate. Good analysis of some dosage forms are achieved, including one on a tablet containing pheniramine maleate, pyrilamine maleate and phenylpropanolamine hydrochloride. Extractions and chromatography are quick and simple.

Keywords: *High performance liquid chromatography; pyridine derivatives; β-cyclodextrin.*

Introduction

In the preceding paper [1], it has been shown that pyridine derivatives bind to cyclodextrins especially β-cyclodextrin. In this report, the use of a chromatographic column of β-cyclodextrin bonded to silica gel is investigated for the separation and analysis of drugs which are pyridine derivatives. The application to the analysis of several dosage forms is investigated. This type of column has been used to separate a wide variety of structural, positional and optical isomers [2], these isomers having different affinity for the cyclodextrins. The molecules that interact strongly with the cyclodextrin (large binding constant) can be expected to be retained longer on the column than molecules with smaller binding constant. The binding constant for a drug will also depend upon the solvent composition and pH. Of the water miscible solvents frequently used in mobile phases, acetonitrile and ethanol bind more strongly to cyclodextrin than methanol. As drugs usually bind to cyclodextrin when unionised, i.e. have low water solubility, and as a result are more soluble in the solvents than in water, the addition of the solvents to an aqueous buffer should greatly lower retention times and at high concentrations the drug can be eluted with the solvent front. In the earlier work [1] a pH 7.0 phosphate buffer was found to be a good medium for complex formation between the pyridine derivatives and cyclodextrin and so in the present work this was used as a mobile phase with the addition of methanol.

Experimental

Materials

The pyridine derivatives were as described in the preceding paper [1]. The hydrochlorides of phenylephrine, pseudoephedrine and phenylpropanolamine were obtained from Sigma, St. Louis, MO. An ophthalmic solution containing 0.5% tropicamide (Mydrialcyl Alcon, Fort Worth, TX), a nasal spray containing 0.2% pheniramine maleate and 0.5% phenylephrine hydrochloride (Dristan Whitehall Labs, New York, NY), capsules containing 12.5 mg pheniramine maleate and 50 mg nicotinic acid, (Verstat, Saron Pharmacal, St. Petersburg, FL), tablets containing 2.5 mg tripolidine hydrochloride and 60 mg pseudoephedrine hydrochloride (Actifed, Burroughs Wellcome, Research Triangle Park, NC) and tablets containing 50 mg phenylpropanolamine hydrochloride, 25 mg pheniramine maleate and 25 mg pyrilamine maleate (Triaminic, Dorsey Lincoln, NE) were purchased from a local pharmacy.

Apparatus

A spectroflow 400 Solvent Delivery System, Kratos Analytical Inst., Ramsey, NJ and a stainless steel column (4.6 × 100 mm) packed with a silica gel to which β-cyclodextrin had been chemically bonded (Cyclobond 1, Advanced Separation Technologies Inc., Whippany, NJ). Detection was effected at 254 nm using a LDC Spectromonitor D, Milton Roy, Riviera Beach, FL and a 3392-A integrator (Hewlett–Packard, Avondale, PA). The mobile phase was a 0.05 M sodium acid phosphate solution adjusted to pH 7.0 with 0.1 M sodium hydroxide, mixed with methanol. Samples (50 μl) in methanol were injected onto the column using a 7125 manual injector from Rheodyne, Cotato, CA.

Preparation of samples from dosage forms

A. *Opthalmic solution and nasal spray.* One millilitre of the product was diluted stepwise with the mobile phase of 30:70 methanol:0.05 M phosphate, pH 7.0, to give final concentrations of 20 μg ml^{-1} of tropicamide and 20 μg ml^{-1} of pheniramine maleate and 50 μg ml^{-1} of phenylephrine hydrochloride, respectively.

B. *Capsules.* The contents of the capsule were transferred to a 100 ml-volumetric flask. The capsule shell was washed with the mobile phase of 50:50 methanol:0.05 M phosphate, pH 7.0, and the washings transferred to the flask. After adjustment to volume with the mobile phase, the mixture was sonicated for 15 min, cooled then sonicated again for 15 min. An aliquot of the supernatant was centrifuged (400 **g**) for 15 min. One millilitre of the clear supernatant was diluted to 10 ml with mobile phase. The final concentration was 12.5 μg ml^{-1} pheniramine maleate and 50 μg ml^{-1} nicotinic acid.

C. *Tablets.* (a) A tablet was placed in a 100 ml-volumetric flask and 90 ml mobile phase, 40:60 methanol:0.05 M phosphate, pH 7.0, added. After sonication for 15 min, the flask was cooled and the volume adjusted. An aliquot was centrifuged (400 **g**), and 1 ml of the supernatant diluted to 10 ml with mobile phase. The final concentration was 2.5 μg ml^{-1} triprolidine hydrochloride and 60 μg ml^{-1} pseudoephedrine hydrochloride.

(b) As (a) but the mobile phase was 30:70 methanol 0.05 M phosphate buffer, pH 7.0. The final concentration is 25 μg ml^{-1} pyrilamine maleate, 26 μg ml^{-1} pheniramine maleate and 50 μg ml^{-1} phenylpropanolamine hydrochloride.

Results and Discussion

Table 1 clearly shows that increasing the methanol concentration of the mobile phase reduces the retention time of all the drugs, and that with a suitable choice of mobile phase composition, separation can be achieved for any pair of drugs investigated. The structurally related pyridine derivatives, methapyrilene, thenyldiamine, pheniramine and pyrilamine show elution times which parallel their binding constants to cyclodextrin [1], whereas the less related molecules sulphapyridine, triprolidine and niflumic acid do not. Figure 1 shows the separation, using methanol, buffer 30:70, of pheniramine and pyrilamine maleates (a) and of thenyldiamine and triprolidine hydrochlorides (b). Many pharmaceutical products containing pyridine derivatives, such as antihistamines, also contain either phenylephrine, pseudoephedrine or phenylpropanolamine hydrochlorides. The retention times of these molecules with the 30:70 mobile phase are 1.26, 1.66

Table 1
Effect of mobile phase on the capacity factor

Compound	Methanol:phosphate buffer (v/v) 50:5	40:60	35:65	30:70	25:75	20:80
Pyrilamine maleate	5.06	9.76	11.87	16.21	25.10	40.60
Niflumic acid	0.53	1.42	2.00	2.79	4.85	8.56
Sulphapyridine	0.48	0.75	0.97	1.31	2.04	3.32
Tropicamide	0.33	0.65	0.91	1.35	2.21	4.31
Methapyrilene HCl	4.30	6.96	8.40	10.94	16.60	23.80
Pheniramine maleate	6.08	8.92	10.14	12.35	17.18	23.59
Triprolidine HCl	5.33	8.92	10.95	15.30	24.44	40.54
Thenyldiamine HCl	4.21	6.77	8.14	10.43	15.31	21.59
Piroxicam	0.39	0.72	0.96	1.29	2.26	4.42

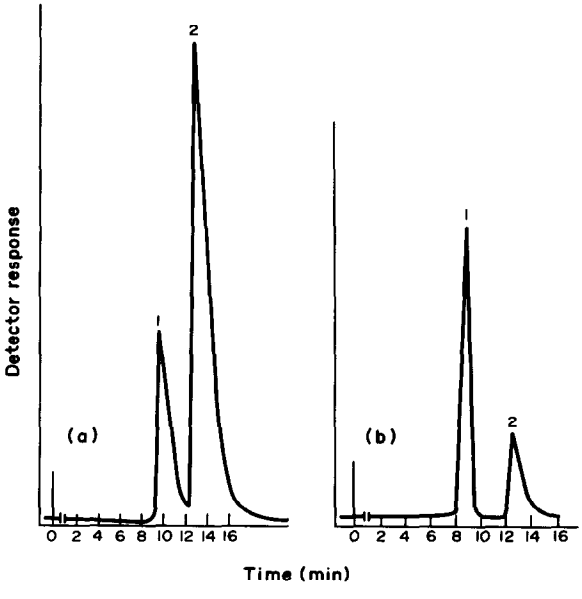

Figure 1
a. Chromatogram of a mixture of pheniramine maleate (1) and pyrilamine maleate (2). b. Chromatogram of thenyldiamine hydrochloride (1) and tripolidine hydrochloride (2). For the conditions see Experimental.

and 1.77 min, respectively. Table 2 shows the statistics for the linear dependence of peak area against concentration for the drugs found in the four dosage forms. Excellent linear relationships are found for the drugs over the concentration range found in the dosage forms. Table 3 shows the analysis of laboratory prepared mixtures of pheniramine maleate and phenylephrine hydrochloride using the 30:70 mobile phase. Clearly both drugs are adequately assayed by this procedure.

The summary of the analysis of the five dosage forms is shown in Table 4. The

Table 2
Regression analysis of standard curves

Compounds	Concentration range used μg ml^{-1}	Regression coefficient r	Slope	Intercept
Triprolidine HCl	1–5	0.9996	0.5859	−0.0130
Pyrilamine maleate	5–35	0.9995	0.8600	−0.0140
Pheniramine maleate	10–80	0.9993	0.1617	−0.0620
Tropicamide	15–50	0.9995	0.1534	−0.0050
Pseudoephedrine HCl	10–50	0.9997	0.0137	0.0430
Phenylephrine HCl	10–60	0.9991	0.0375	0.0888
Phenylpropanolamine HCl	20–100	0.9998	0.0153	0.0505

Table 3
Analysis of pheniramine maleate and phenylephrine hydrochloride in known mixtures

Mixture	Added μg ml^{-1}	Found (*) μg ml^{-1}	Recovery % (±SD)
1. Pheniramine maleate	8.0	8.13	101.63 (±1.50)
Phenylephrine HCl	20.0	20.42	102.10 (±2.80)
2. Pheniramine maleate	16.0	15.82	98.88 (±1.68)
Phenylephrine HCl	40.0	39.10	97.75 (±2.90)
3. Pheniramine maleate	20.0	19.79	98.95 (±1.67)
Phenylephrine HCl	50.0	49.30	98.60 (±2.85)

* Average of five determinations.

Table 4
Analysis of some dosage forms containing pyridine derivatives

Preparations	Label	Assay	Recovery (%)	SD (±)	CV* (%)
Eye drops					
Tropicamide	5 mg ml^{-1}	4.97	99.40	0.35	1.84
Nasal spray:					
Pheniramine maleate	2 mg ml^{-1}	1.92	96.0	0.45	2.33
Phenylephrine HCl	5 mg ml^{-1}	4.93	98.6	2.49	5.05
Capsules:					
Pheniramine maleate	12.5 mg per capsule	12.06	96.48	0.39	3.23
Tablets: A					
Triprolidine HCl	2.5 mg per tablet	2.44	97.60	0.05	2.05
Pseudoephedrine HCl	60 mg per tablet	59.35	98.92	2.07	3.49
Tablets: B					
Pheniramine maleate	25 mg per tablet	25.00	100.00	0.24	0.96
Pyrilamine maleate	25 mg per tablet	25.00	104.56	0.49	1.87
Phenylpropanolamine HCl	50 mg per tablet	49.30	98.60	0.78	1.59

* Standard deviation from the mean of 5 determinations divided by the mean expressed as a percent.

tropicamide eye drops as a single component were readily assayed. The capsules contained nicotinic acid and pheniramine maleate. However, only pheniramine was determined because nicotinic acid, as shown in the preceding paper, does not complex with cyclodextrin and so was eluted in the solvent front. The tablets containing triprolidine and pseudoephedrine hydrochlorides were readily determined as the drugs had retention times of 7.9 and 1.96 min, respectively. Again no interference was found. The analysis of the tablets containing maleate salts of pheniramine and pyrilamine as well as phenylpropanolamine hydrochloride has received considerable attention because of the widespread use of the mixture as antihistaminic decongestants. An early attempt to use HPLC for the mixture using ODS columns and methanol phosphate buffers as the mobile phase failed to resolve the peaks of the maleates [3]. More recently the HPLC determination of triamine tablet formulations using ODS columns and a mobile phase precisely adjusted to pH 7.44 [4] was reported. The method involved heating of the samples over a steam bath, detection at 216.5 nm and retention times of 20 min. In the current investigation the formulations were efficiently analysed on the Cyclobond 1 column with the 30:70 mobile phase at pH 7.0. The three peaks are well separated, as shown in Fig. 2, allowing excellent quantification. Pheniramine (K = 310 M^{-1}) elutes before pyrilamine (K = 731 M^{-1}) as expected, which is the reverse order of that found with the ODS column [4]. This procedure for successful quantitative analysis of the triamine product is extremely simple. The Cyclobond column is readily washed with buffer of the appropriate pH and water. In the analysis of all of the five dosage forms no interference from any adjuvant was found. Apparently none of them bound significantly to the bonded cyclodextrin and as a result ran with the solvent front. These investigations on the analysis of pyridine derivatives clearly show excellent applications of cyclodextrins, either in the mobile phase or bonded to a stationary phase in pharmaceutical analysis.

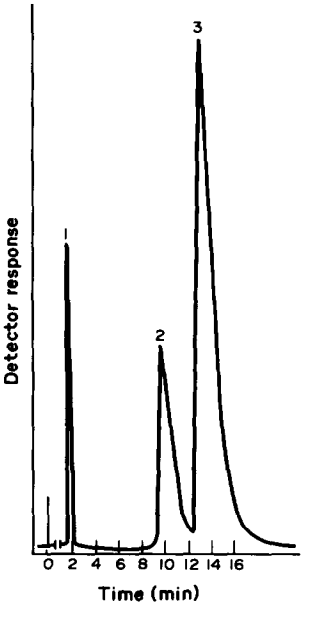

Figure 2
Chromatogram of tablet containing phenylpropanolamine hydrochloride (1), pheniramine maleate (2) and pyrilamine maleate (3). For the conditions see Experimental.

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References

[1] S. L. Gezawi, N. Omar, N. El Rabbat, H. Ueda and J. H. Perrin, *J. Pharm. Biomed. Anal.* **6**, 393–398 (1988).
[2] W. L. Hinze, *Sep. Purif. Methods* **10**, 159–237 (1981).
[3] V. D. Gupta and A. G. Chanekar, *J. Pharm. Sci.* **66**, 895–897 (1977).
[4] D. R. Heidemann, *J. Pharm. Sci.* **70**, 820–822 (1981).

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