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Note

Isolation and identification of hydrocodone in narcotic cough syrups by high-performance liquid chromatography with infrared spectrometric identification

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As part of the work of this laboratory in support of forensic investigation in Canada, it was necessary to develop a preparative high-performance liquid chromatographic (HPLC) procedure to isolate the narcotic hydrocodone (dihydrocodeinone) from pharmaceutical expectorant preparations. The method had to yield the drug component with sufficient purity and quantity for definitive infrared (IR) spectroscopic identification. Such narcotic cough syrups are increasingly abused as heroin substitutes and are submitted for analysis as police exhibits for "double doctoring" and "drugstore break-in" offences.

There are approximately thirty products on the Canadian market containing hydrocodone in a liquid oral dosage form. Hydrocodone bitartrate concentrations vary from 0.33 to 1 mg/ml of syrup. It is formulated as the single active ingredient, or in combination with pheniramine, phenylephrine, diphenylpyraline, guaifenesin, phenylpropanolamine, pyrilamine, doxylamine and etafedrine. The complexity of these products is increased by the inclusion of dyes, preservatives, sugars and other excipients.

Chromatographic methods have been reported for the quantitation of hydrocodone in serum by capillary gas chromatography (GC) with nitrogen–phosphorus detection[1] and GC with electron-capture detection[2] and in tablet formulations by HPLC[3]. A method of analysis employing column chromatography–ultraviolet spectroscopy was developed for hydrocodone in syrup formulations in the presence of antihistamines[4]. However, none of the above methods was suitable for the definitive IR identification of hydrocodone and, furthermore, etafedrine interferes with the A.O.A.C. method[4].

A gradient HPLC method was developed to isolate hydrocodone from liquid oral dosage formulations. An extraction procedure was involved prior to HPLC in order to concentrate hydrocodone and to remove a major portion of guaifenesin where present.

EXPERIMENTAL

Apparatus

HPLC. The chromatographic system consisted of a Spectra-Physics (Santa

Clara, CA, U.S.A.) SP 8100 liquid chromatograph, an SP 8440 UV–VIS detector (254 mm, range 0.16) and an SP 4270 integrator (attenuation 32). The Hamilton PRP-1, 305 mm × 7 mm I.D. column (Chromatographic Specialties, Brockville, Canada) was maintained at 40°C. The mobile phase consisted of various mixtures of solvent A (0.1% triethylamine in water) and solvent B (0.1% triethylamine in acetonitrile) according to the following gradient programme (the flow-rate was 3.5 ml/min):

Run time (min)	*Solvent A (%)*	*Solvent B (%)*
0–7	60	40
7–12	60→20	40→80
12–17	20	80
17–23	20→60	80→40
23–25	60	40

Infrared spectrophotometer. A Beckman Microlab 620 MX computing infrared spectrophotometer (Beckman Instruments, Montreal, Canada) was used.

Reagents and materials

The following reagents and materials were used. Triethylamine (Baker Analysed, J. T. Baker, Phillipsburg, N.J., U.S.A.); acetonitrile (HPLC grade, J. T. Baker); chloroform (glass distilled, Caledon Labs., Georgetown, Canada); ethanol (absolute "Aristar", BDH, Poole, U.K.); cyclohexane (BDH, Toronto, Canada); potassium bromide (Spectrosol, BDH, Poole, U.K.); hydrocodone (dihydrocodeinone bitartrate) (Endo Canada, Vaudreuil, Canada); etafedrine hydrochloride (Lot R6153, Merrell Pharmaceuticals, Concord, Canada); doxylamine succinate (Lot S0799, Merrell Pharmaceuticals).

Solutions

The hydrocodone standard solution was prepared by dissolving dihydrocodeinone bitartrate in distilled water (1 mg/ml).

The synthetic syrup solution was prepared by dissolving 50 mg of etafedrine hydrochloride and 18 mg of doxylamine succinate in 10 ml of distilled water, then adding 5 ml of hydrocodone syrup.

Procedure

An amount of syrup equivalent to approximately 5 mg of hydrocone bitartrate or 5 ml of standard solution was transferred to a separatory funnel along with 5 ml of distilled water and 1 ml of 1.8 *M* sulfuric acid and was extracted twice with 40 ml of chloroform. The chloroform layer was discarded. The aqueous layer was alkalized with 5 ml of 1.0 *M* sodium hydroxide and extracted twice with 25 ml of chloroform. The combined organic extracts were evaporated to dryness, and then dissolved in 0.5 ml of ethanol. The entire extract was injected on to the HPLC column. The effluent was monitored and the peak corresponding to the retention time for hydrocodone was collected and evaporated to dryness. The residue was dissolved in 1 ml of chloroform, diluted with 2 ml of cyclohexane and evaporated to dryness at 50°C under nitrogen. IR spectra were obtained from pellets containing about 0.3% residue in 100 mg of potassium bromide.

TABLE I

HPLC SEPARATION OF ACTIVE INGREDIENTS

Drug	*Retention time (min)*
Phenylephrine	2.0
Guaifenesin	2.8
Phenylpropanolamine	3.2
Hydrocodone	6.9
Pheniramine	12.0
Etafedrine	12.4
Doxylamine	13.6
Pyrilamine	16.0
Diphenylpyraline	16.6

RESULTS AND DISCUSSION

This method addresses the problems associated with active ingredients, dyes, preservatives, sugars and other excipients commonly formulated with hydrocodone syrups. In addition, it is suitable for the identification of hydrocodone in low dosage, pediatric formulations.

Most analytical procedures for hydrocodone involve injection of the diluted syrup on to an analytical HPLC column. This technique was unsuitable for recovery of milligram quantities of hydrocodone due to the low concentration of the drug in pediatric formulations, complex mixtures of excipients, dyes, sugars, preservatives and other active ingredients, and the low loading capacity of analytical columns.

The A.O.A.C. extractive procedure used for hydrocodone in the presence of antihistamines[4] was unsuitable for formulations containing etafedrine since both drugs eluted together from the final column.

Gradient HPLC on a semipreparative column, preceded by an extractive clean-

TABLE II

FORMULATIONS EXAMINED

Formulation	*Active ingredient*	*Concentration (mg/ml)*
A	Hydrocodone bitartrate	1.0
B	Hydrocodone bitartrate	1.0
C	Hydrocodone bitartrate	0.332
	Guaifenesin	20.0
	Phenylephrine hydrochloride	2.0
	Diphenylpyraline hydrochloride	0.2
D	Hydrocodone bitartrate	1.0
	Phenylpropanolamine hydrochloride	5.0
	Pheniramine maleate	2.5
	Pyrilamine maleate	2.5
E	Hydrocodone bitartrate	0.332
	Etafedrine hydrochloride	3.3
	Doxylamine succinate	1.2

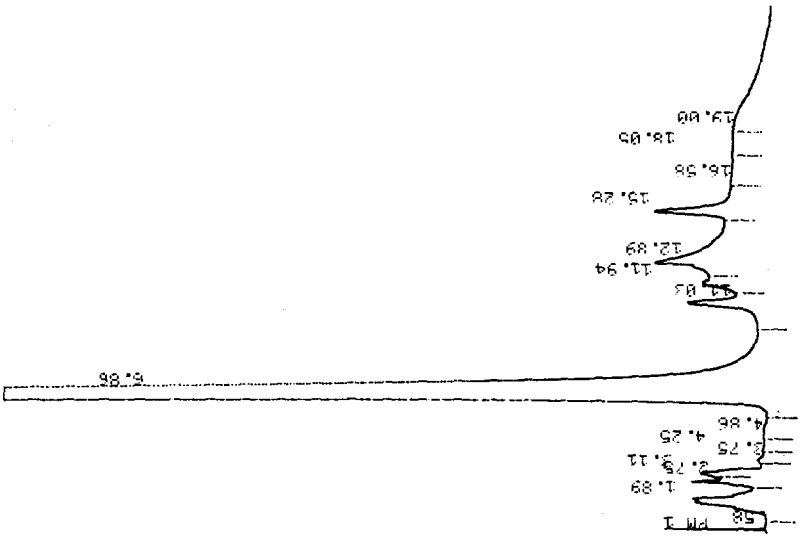

Fig. 1. HPLC chromatogram of hydrocodone standard.

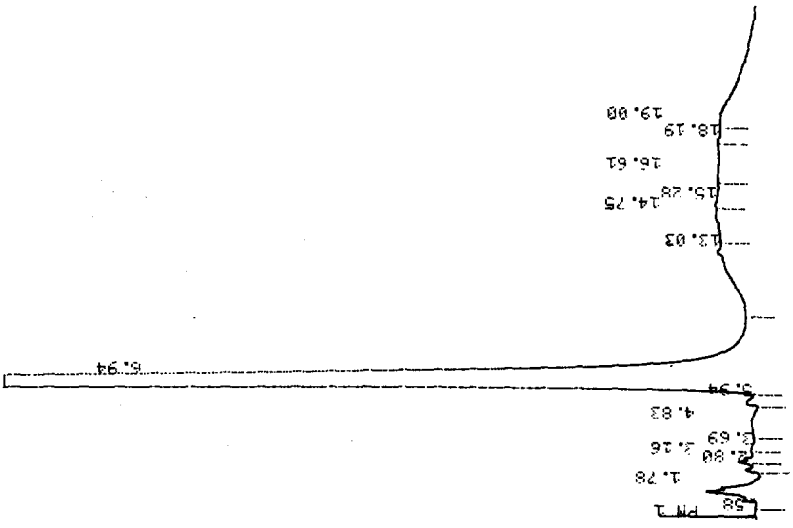

Fig. 2. HPLC chromatogram of a commercial syrup containing hydrocodone.

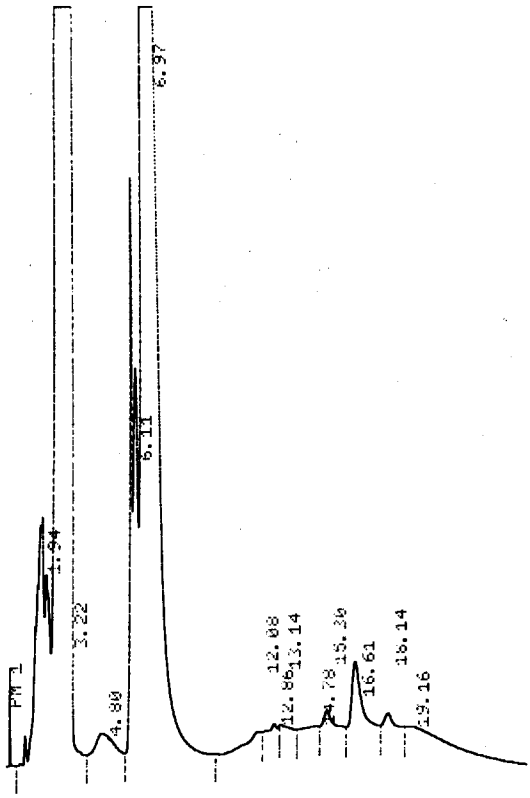

Fig. 3. HPLC chromatogram of a commercial syrup containing hydrocodone plus guaifenesin, phenylephrine and diphenylpyraline.

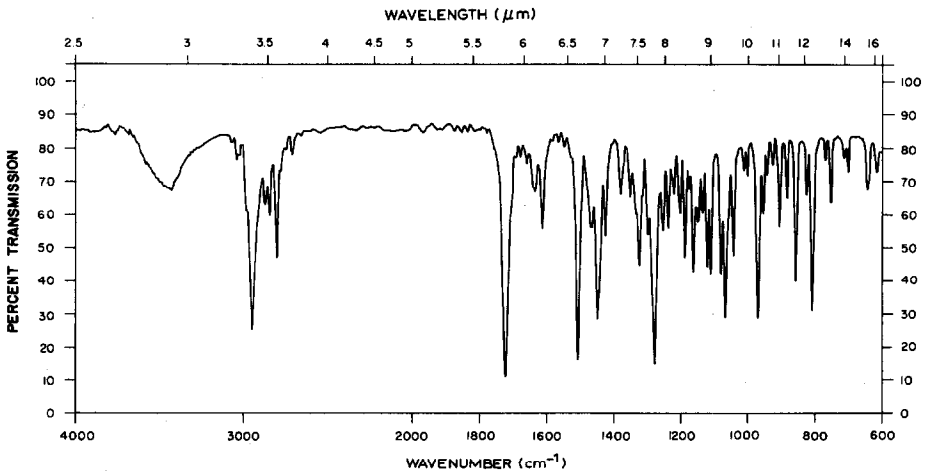

Fig. 4. IR spectrum of hydrocodone residue obtained from a commercial syrup.

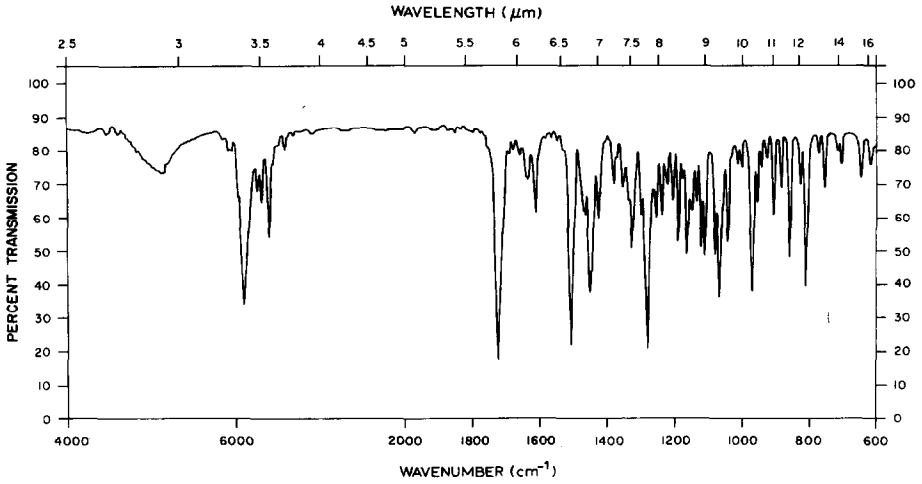

Fig. 5. IR spectrum of reference standard hydrocodone.

up, was chosen to optimize the separation. Guaifenesin can be found in some products in concentrations as high as 40 mg/ml. The first extractions with chloroform removed about 95% of this ingredient. The complete extraction procedure ensured >90% of the hydrocodone was injected onto the HPLC system.

Earlier experiments with a Vydac C_{18} semipreparative column showed that column to be unsuitable because some silica was leached by the mobile phase (pH 9.5). The use of the PRP-1 column overcame this problem. Retention times of the chromatographed compounds are presented in Table I, and some typical co-formulated active ingredients are shown in Table II. The column was thermostated at 40°C to allow a more effective control of operating parameters. Adequate separation is expected at room temperature with minor loss of resolution.

The hydrocodone extract dried from the mobile phase was dissolved in chloroform–cyclohexane and evaporated to yield a highly crystalline material suitable for IR spectra. Chromatograms from formulations are presented together with those of the hydrocodone reference material (Fig. 1–3). Spectra of the isolated hydrocodone (example, Fig. 4) agree with that of the reference material (Fig. 5) treated in an identical manner.

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