TECHNICAL NOTE

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Determination of Hydrocodone in Tussionex® Extended-Release Suspension by High-Performance Liquid Chromatography (HPLC)

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ABSTRACT: The high-performance liquid chromatographic (HPLC) method presented is a simple and rapid analytical procedure for the determination of hydrocodone in Tussionex Pennkinetic® extended-release suspension in the presence of chlorpheniramine and several excipients. Hydrocodone was extracted and separated from the suspension with methylene chloride under alkaline pH conditions. Sample recoveries were from 99.6 to 104.3%. Satisfactory resolution was obtained using a C_8 column (Alltech 10μ*M*, 25-cm by 4.6-mm inside diameter) and a mobile phase consisting of acetonitrile buffered at pH 4.5 (0.01*M* KH_2PO_4 and 0.05*M* KNO_3) maintaining a flow rate of 1.4 mL/min. Corresponding run time for the complete separation of hydrocodone was around 14 min. The method was used to determine the concentration of HCD in five Tussionex® extended-release suspension samples that have been tampered with and in one control sample.

KEYWORDS: forensic science, toxicology, high-performance liquid chromatography, hydrocodone, Tussionex Pennkinetic, product tampering

Tussionex Pennkinetic® is an extended-release suspension that contains hydrocodone polistirex equivalent to 10 mg of anhydrous hydrocodone bitartrate (HCD) and chlorpheniramine polistirex equivalent to 8 mg of chlorpheniramine maleate in each 5 mL of the suspension. Some of the other ingredients are: ascorbic acid, ethylcellulose, high fructose corn syrup, PEG 3350, propylene glycol, polysorbate 80, sucrose, kathan gum, methylparaben, propylparaben, and dyes. The suspension is used for the relief of cough and respiratory symptoms associated with allergy or colds. The drugs in this medication are in a resin-complex base that produces a slow release and a prolonged effect of the active ingredients (1). Medicines containing narcotic substances are frequently abused and are subject to analysis as was the above product, although few references are known about the toxic effects of Tussionex (2).

Six samples of Tussionex were brought by the State Bureau of Investigation (SBI) to determine their content of HCD, it was suspected that the samples were tampered with and the amount of HCD was substantially reduced. Quantitation of HCD in Tussionex includes its removal from polistirex binding followed by separation from other components, extraction, and measurement of HCD with an analytical procedure.

Several gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods have been published for the quantitation of hydrocodone in biological fluids (3,4) or in formulations (5,6). However, none of the above methods was suitable because they either consisted of a two-step extraction procedure (3,6) that was time consuming or they used solvents that we found to have less than optimal extraction power (4,6). One method (5) used water for the extraction of HCD from tablets followed by the direct injection into the HPLC instrument. This method could not be used in the case of Tussionex because of drug binding on the polistirex and the presence of several interfering substances.

We have, therefore, designed a simple and quick HPLC method for the separation and quantitation of HCD for application to the analysis of the drug in the sustained-release suspension of Tussionex Pennkinetic®. The method adapts some of the steps from the above methods and it was found to be suitable in practice.

Experimental Procedure

Materials and Reagents

Six samples of Tussionex (Fisons Corporation, PO Box 1766, Rochester, NY 14603) were brought for analysis, five of which were the object of a complaint of tampering with the content of HCD. Acetonitrile, methylene chloride (Fisher Scientific, Fair Lawn, NY), and methanol (Burdick and Jackson Laboratories, Muskegon, MI) were of HPLC-grade purity. All aqueous solutions were made with purified water (Nanopure Ultrapure Water System, Barnstead/Thermolyne Corp., Dubuque, IA). Anhydrous hydrocodone bitartrate (H-4516 Lot 47F0128) was purchased from Sigma Chemical Company, St. Louis, MO 63178.

Instrumentation

For the extraction of Tussionex samples, a Multipurpose Rotator Model 150V with variable speed (Scientific Industries Inc., Spring-

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field, MA 01103) was used. The evaporation of the extraction solvents was carried out with a N-EVAP analytical Evaporator Model 112 (Organomation Associates Inc., PO Box 5, Shrewsbury, MA 01545). The centrifuge used for the separation of the layers after extraction was IEC Model CENTRA 4 capable of 12000 rpm (International Equipment Company, 300 Second Ave., Needham Heights, MA 02194). The basic chromatographic system consisted of an ISCO V_4 variable wavelength detector at 280 nm, a Beckman 110B solvent delivery system, and a manual injector (Rheodyne 7125, Cotati, CA) fitted with a 100 μL sample loop.

Chromatographic Conditions

Chromatographic separation of hydrocodone was accomplished using an Alltech C-8 column (10μ*M*, 25-cm by 4.6-mm inside diameter [ID]) (Alltech Associates, Inc. 2051 Waukegan Rd., Deerfield, IL 60015). The mobile phase consisted of acetonitrile 18% and an aqueous solution of 0.01*M* KH_2PO_4 and 0.05*M* KNO_3 (pH 4.5) 82%. The flow rate was maintained at 1.4 mL/min.

Preparation of Stock Solutions and Standard Solutions

A stock solution of HCD was prepared by introducing 250 mg of HCD into a 50-mL volumetric flask followed by addition of purified water to volume (5 mg/mL). Four standard solutions encompassing a range from 0.5 to 3.0 mg/mL of HCD were prepared by serial dilution of the stock solution with purified water. When not in use, all the solutions were stored in airtight containers at 4°C. HCD is degradable in aqueous solutions, and therefore, the stock and standard solutions were freshly prepared at weekly intervals because it was established that negligible deterioration occurred under the conditions of storage.

Sample Preparation

The six Tussionex samples were stored in capped borosilicate glass test tubes at 4°C before analysis that was within two days of receipt.

Extraction Procedure

The extraction of hydrocodone was carried out as follows. To each screw-cap 20-mL test tube was added 0.5 or 1.0 mL of Tussionex samples, 1.0 mL of water, 0.5 mL of 1 *N* sodium hydroxide solution and 15 mL of methylene chloride. The test tubes were capped and shaken at a moderate rate (100 cpm) on the automatic rotator for 20 min. After centrifugation at 2500 rpm for 5 min, the organic layer was separated by aspiration and it was transferred into a clean and dry conical glass test tube. The organic solution was evaporated to dryness at 35 to 40°C under a gentle stream of nitrogen. The residue was dissolved in 5 mL of methanol and a 20- to 80-μL aliquot was injected into the HPLC using a Hamilton 25- or 100-μL syringe. Resulting chromatograms were subsequently analyzed by comparing sample peak areas under the curve (AUCs) to those of the external standards.

Recovery

The recovery of hydrocodone was determined by adding known amounts of the drug to water (concentration 0.5 to 3 mg/mL) and comparing the resultant chromatograms to those obtained from authentic (unextracted) standard solutions.

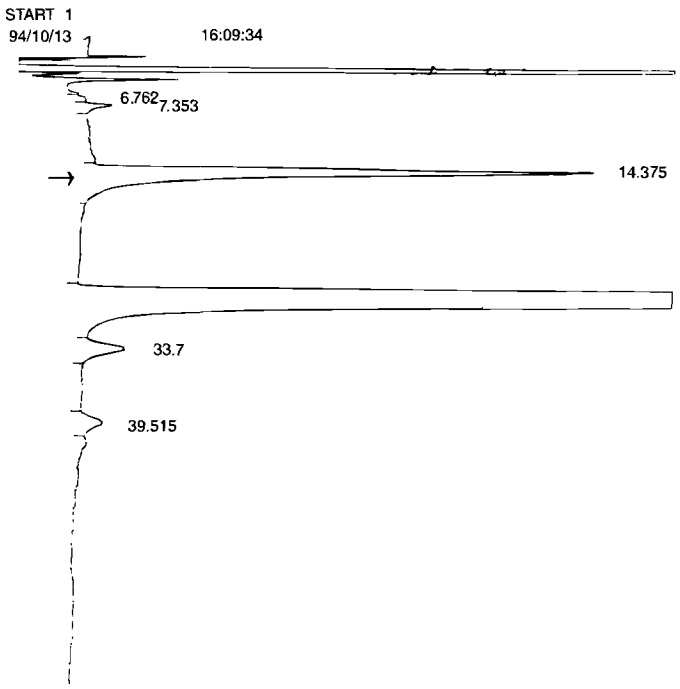

FIG. 1—*Chromatographic separation of hydrocodone following extraction from an extended-release suspension (Tussionex® Pennkinetic).*

Results and Discussion

The peaks of hydrocodone were symmetrical and well resolved from additional peaks from extracts of Tussionex samples.

Figure 1 illustrates the on-column chromatographic separation of the peaks of hydrocodone from the other peaks from Tussionex extracts. Several reversed-phase columns and mobile-phase compositions were evaluated to optimize the separation of hydrocodone while minimizing retention time. The popular C_{18} and C_8 columns yielded the best results and a satisfactory (baseline or near-baseline)

TABLE 1—*Recovery of hydrocodone from spiked water samples.*

Amounts Added to Water, mg/mL	Number of Samples	Recovery, mg/mL	Percent Recovery, ±SD
0.5	4	0.515	103.0 ± 2.08
1.0	4	1.043	104.3 ± 1.78
2.0	4	2.014	100.7 ± 0.93
3.0	4	2.987	99.6 ± 0.95

TABLE 2—*HCD in Tussionex extended release suspension (n = 4).*

	Anhydrous	
Suspension Sample	Hydrocodone, mg/mL	Bitartrate Found, % ± SD
1	0.81 ± 0.00	40.5 ± 0.00
2	1.08 ± 0.52	54.0 ± 25.9
3	1.01 ± 0.32	50.5 ± 16.0
4	1.02 ± 0.19	51.0 ± 9.3
5	1.86 ± 0.41	93.0 ± 20.4
6	0.88 ± 0.09	44.0 ± 7.7

resolution of hydrocodone in all samples was achieved with well defined, symmetric peaks at retention times of ≈14 min.

Calibration Curve

Duplicate injections of known HCD standards (0.5 to 3 mg/mL) were made each day of analysis. The areas under the curve (AUCs) were averaged for each known standard, and plotted against hydrocodone concentration (hydrocodone is the measured analyte, not HCD). R^2 values ranged from 0.96 to 0.99. A greater dilution or a larger aliquot of the final solution was used to bring the concentration of the samples within the desired range. Calculations were performed to convert the concentrations of hydrocodone to HCD concentrations.

Recovery

Recoveries of hydrocodone from spiked water samples were calculated after extraction using the calibration curve previously established. Results of the extraction procedure are summarized in Table 1 showing recovery from 99.6 to 104.3%. The efficiency of the extraction procedure was found to be highly dependent upon the nature of the extraction solvent; e.g., recoveries as low as 41.3% resulted from the use of diethylether. The best results were obtained with methylene chloride as the extracting solvent. The addition of isopropanol to ethyl ether improved the recovery of hydrocodone but it was necessary to limit the proportion of the former solvent to 10% by volume. The presence of this polar organic modifier at higher concentrations frequently resulted in the elution of water (and water-soluble contaminants) from the samples with the compound of interest i.e., hydrocodone. A number of solvents, both halogenated and nonhalogenated, were initially screened for the ability to extract hydrocodone from Tussionex; methylene chloride gave optimum results.

Determination of HCD in the Tussionex Samples

The amounts of HCD recovered from the six samples of Tussionex under investigation were determined. These results are presented in Table 2. All samples under investigation except Sample 5 had much less concentration of HCD than the label claim (2.0 mg/mL); the amounts varied between 0.81 and 1.08 mg/mL. According to the SBI, Sample 5 was a genuine nontampered suspension that was included with the other samples as a blind control.

The proposed analytical method for HCD in Tussionex extended-release suspension is specific, accurate, and rapid with complete separation from chlorpheniramine and other ingredients, and is suitable for routine analysis of Tussionex formulations.

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