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Note

Gas chromatographic assay of codeine in human plasma utilizing nitrogen-selective detection

NED L. RENZI, Jr., SUSAN M. STELLAR and KUNG T. NG*

McNeil Pharmaceutical, Department of Drug Metabolism, Spring House, PA 19477 (U.S.A.)

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Codeine is a widely prescribed narcotic analgesic and antitussive drug. The determination of codeine in human plasma samples following ingestion of therapeutic doses of codeine requires assays which are both highly sensitive and selective. Various analytical methods [1—10] have been developed for this purpose. They include radioimmunoassay (RIA) (1), gas chromatography—mass spectrometry (GC—MS) (2) and gas chromatography (GC) [3—10]. Among these methods, GC is the technique commonly available in most laboratories. Previously reported GC methods employed either flame ionization detection (FID) [3—7] or electron-capture detection (ECD) [8—10]. These methods require derivatization for improvement of GC properties and/or sensitivity. They are usually time-consuming and difficult to establish.

This paper describes a GC method employing nitrogen-selective detection. No derivatization step is necessary with the present method. The procedure is rapid and sensitive and has been used successfully in analyzing plasma samples from clinical studies.

EXPERIMENTAL

Material

Nanograde-quality methanol and chloroform were purchased from Mallinckrodt (St. Louis, MO. U.S.A.) and glass-distilled grade *n*-butanol from Burdick and Jackson (Muskegon, MI, U.S.A.). All solvents were used without further purification. Glass bottles (15 ml) from Wheaton Company (Millville, NJ, U.S.A.) with polyethylene screw-caps from Poly-Seal Corp. (Baltimore, MD, U.S.A.) were used for the plasma extraction and 12-ml glass-stoppered centrifuge tubes (Arthur H. Thomas, Philadelphia, PA, U.S.A.) were used for the final solvent evaporation. Volumetric glass pipettes were utilized for all solvent transfers. All glassware was soaked in chromic acid for at least 1 h,

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rinsed thoroughly with distilled water and heat treated for 3 h at 270°C. Polyethylene screw-caps were soaked in *n*-heptane for at least 1 h and dried at 60°C prior to use. Codeine phosphate and oxycodone hydrochloride (McNeil Pharmaceutical, Spring House, PA, U.S.A.) were used as received from the stock room. Codeine phosphate was of U.S. Pharmacopoeial quality and oxycodone hydrochloride met all U.S. New Drug Application requirements.

Stock solutions

Stock solutions of codeine phosphate were prepared in methanol in concentrations of either 10 or 1 μg/ml. A stock solution of oxycodone hydrochloride, the internal standard, was first prepared in methanol (10 μg/ml), which was further diluted to 3 ng/ml with 10% butanol in chloroform. All concentrations were expressed in terms of the free base. Clinical control plasma obtained from Interstate Blood Bank (Philadelphia, PA, U.S.A.) was used in the preparation of the codeine plasma standards. Plasma standards (volume 10.0 ml) ranging from 5 to 200 ng/ml were prepared by the addition of various amounts of codeine stock solutions (volume 0.05—0.20 ml).

Apparatus

A Hewlett-Packard Model 5710A (Avondale, PA, U.S.A.) gas chromatograph equipped with dual nitrogen—phosphorus-selective detectors was used. The column was a 0.91 m (3 ft.) × 2 mm I.D. silanized glass column packed with 1% OV-17 on Chromosorb W HP (100—120 mesh) from Supelco (Bellefonte, PA, U.S.A.). The column was conditioned at 270°C overnight with a helium flow of 30 ml/min.

Chromatographic conditions were: column oven temperature, 235°C; injection port temperature, 260°C; detector temperature, 300°C. The helium carrier gas flow-rate was 30 ml/min, hydrogen flow-rate was 3 ml/min and air flow-rate was 100 ml/min. The voltage of the detector was set at 18 V. Under these conditions, the retention times for codeine and oxycodone were 2.3 and 3.8 min, respectively.

Assay procedure

A 2-ml volume of plasma containing codeine as standard or unknown, 10.0 ml of internal-standard solution (30 ng of oxycodone in 10% butanol in chloroform) and 1.0 ml of 1 *N* sodium hydroxide were added to a 15-ml bottle, and the mixture was shaken on an Eberbach table-top shaker, Eberbach (Ann Arbor, MI, U.S.A.) at 120 oscillations/min for 20 min. After centrifugation at 681 *g*, the supernatant aqueous phase was aspirated and discarded. An 8-ml volume (± 0.5 ml) of the organic layer was transferred to a 15-ml bottle containing 5.5 ml of 0.1 *N* sulphuric acid. The mixture was then shaken for 20 min and centrifuged for 10 min. A 5-ml volume (± 0.5 ml) of the aqueous layer was transferred to a 15-ml bottle containing 8.0 ml of 10% butanol in chloroform and 1.0 ml of 1 *N* sodium hydroxide. The mixture was shaken for 15 min and centrifuged for 10 min. The aqueous phase was removed and discarded. A 7-ml volume (± 0.5 ml) of the remaining organic phase was pipetted into a 12-ml centrifuge tube, and the solvent was evaporated to dryness under a stream of nitrogen at 40°C. A 1-ml volume of chloroform was added to each

tube to wash down the sides of the tube by vortex action. The chloroform was then evaporated to dryness. The residue was reconstituted in 20 μl of methanol, and 5 μl of the solution were injected into the gas chromatograph.

Clinical study samples

The mean plasma concentration data were taken from the results of a bioavailability study comparing the bioavailability of three drug products all containing codeine and acetaminophen. (The products were found to be bioequivalent.) In this study 24 healthy male adult volunteers were fasted for at least 12 h before and 1 h after administration. Two tablets (each containing 325 mg of acetaminophen and 30 mg of codeine phosphate) and 200 ml of distilled water were administered to each subject.

Blood samples of 15 ml were collected in heparinized vacuum tubes by venipuncture at 0 (pre-dose), 10, 20, 40, 60 and 90 min and 2, 3, 4, 6 and 8 h after administration. The plasma was separated by centrifugation immediately after collection and was stored frozen at -10°C in polypropylene snap-cap tubes until analysis.

RESULTS AND DISCUSSION

Codeine is fairly water-soluble and thus a polar extraction solvent such as 10% butanol in chloroform was required. Other solvents, such as ether, ethyl acetate and chloroform, were found to give unsatisfactory extraction efficiencies (<50%) in a three-step extraction procedure. The present procedure has an extraction efficiency of 84 ± 4% (8 determinations) at 50 ng/ml and 81 ± 3% (10 determinations) at 150 ng/ml of codeine in plasma.

Typical chromatograms from plasma samples with and without codeine are

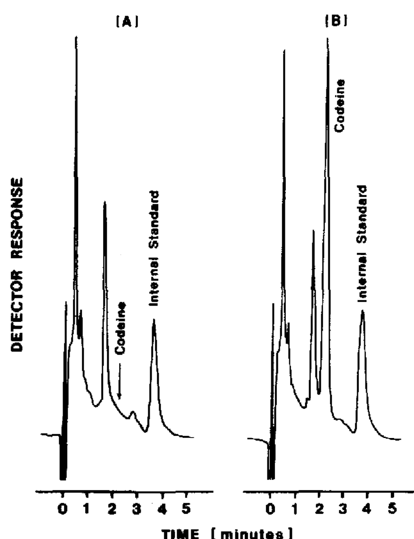

Fig. 1. Typical gas chromatograms from (A) 2 ml of blank plasma containing 30 ng of internal standard (blank without internal standard showed no peak at around 3.8 min and (B) 2 ml of plasma containing 300 ng of codeine and 30 ng of internal standard.

shown in Fig. 1. From these chromatograms, it is clear that there are no peaks in blank plasma samples which interfere with codeine.

The measurable metabolites of codeine in human plasma are morphine, norcodeine and their conjugates [4, 11]. Morphine and the conjugates of morphine and norcodeine are not extracted using this procedure; therefore, they cannot interfere with the assay. Norcodeine is extracted by the procedure, but the concentration of norcodeine in plasma is very low (<10 ng/ml at 1 h) [4] and its retention time is longer than that of codeine. Under the present GC conditions, norcodeine, codeine and oxycodone have retention times of 2.9, 2.3 and 3.8 min, respectively. Drugs that are co-administered with codeine, such as acetaminophen and aspirin, do not interfere in the assay since neither compound is extracted using the present procedure.

The standard curve is linear between 20 and 200 ng/ml. The peak height ratios of replicate samples (expressed as codeine/oxycodone) obtained from the addition of known amounts of codeine to control blank plasma, followed by processing according to the described procedure, are summarized in Table I. A least-squares regression analysis of the data gives a straight-line standard curve with a correlation coefficient of 0.98, a slope of 0.022 and an intercept of -0.06 ± 0.06, which, within experimental error, shows that the line passed through the origin. The assay is reproducible (inter-run) as can be seen in Table I. The coefficients of variation (C.V.) range from 7 to 12% for all selected concentrations.

The concentrations of codeine in plasma following ingestion of a therapeutic dose (60 mg) of codeine are in the range of 5 to 200 ng/ml [11]. At these concentrations, previously reported GC methods using FID or ECD require derivatization for improvement of chromatographic properties and/or sensitivity [3—10]. The nitrogen—phosphorus-selective detector used in the nitrogen mode, however, shows good sensitivity in detecting codeine. The detection limit for codeine was 3 ng injected, and the detector response was linear between 3 and 150 ng. The lowest concentration of codeine in a 2-ml plasma sample that could be detected was 5 ng/ml. However, the lowest level of codeine in plasma (2 ml sample) that could be quantitated both accurately and

TABLE I

MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF PEAK HEIGHT RATIOS AT VARIOUS CODEINE CONCENTRATIONS GENERATED OVER A PERIOD OF TWO WEEKS

Codeine plasma concn. (ng/ml)	No. of determinations	Peak height ratios		
		Mean	S.D.	C.V.
20	10	0.33	0.02	0.07
50	18	1.00	0.11	0.12
75	17	1.66	0.18	0.10
125	15	2.69	0.28	0.10
150	19	3.28	0.37	0.11
200	18	4.43	0.39	0.08

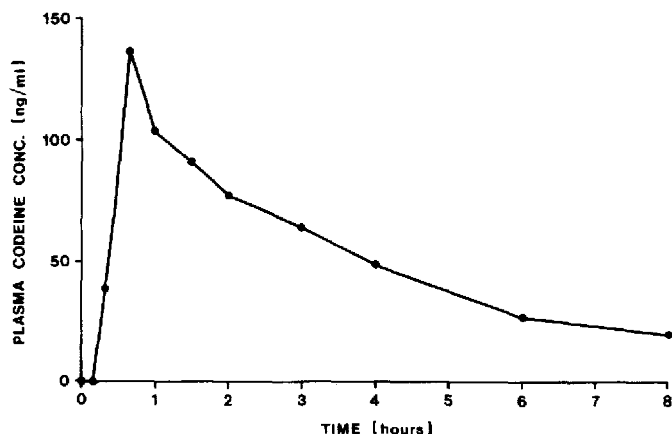

Fig. 2. Plasma-concentration profile of codeine in a human subject following oral administration of 60 mg of codeine phosphate and 650 mg of acetaminophen as two tablets.

TABLE II

MEAN PLASMA CODEINE CONCENTRATIONS FROM A CLINICAL STUDY DETERMINED BY THE PRESENT GC METHOD

Time after drug administration	Mean codeine concn. (ng/ml) from the study (24 subjects)	
	Mean	± S.E.M.
10 min	2.0	1.6
20 min	46.0	8.4
40 min	147.0	12.6
1 h	136.0	10.4
1.5 h	111.0	7.6
2 h	85.0	4.1
3 h	65.0	3.3
4 h	55.0	3.7
6 h	29.0	2.4
8 h	7.0	2.9

precisely (C.V. <15%) was 20 ng/ml. We find that this is adequate sensitivity for clinical samples.

To date, this procedure has been employed successfully in analyzing over 1000 clinical samples for codeine. No interference peaks due to metabolites have ever been observed with the clinical samples. A typical plasma-concentration profile of codeine in man is shown in Fig. 2.

Mean plasma codeine concentrations (± S.E.M.) from a portion of the clinical study as obtained by the present method are shown in Table II. The mean plasma concentration vs. time profile is almost identical to that reported by Findlay et al. [11] for a similar clinical study using the RIA method [1].

The present procedure is rapid since no derivatization step is required. Up to 60 samples can be analyzed routinely in a 8-h working day. Also, the procedure is sensitive enough to have utility in handling clinical plasma samples and a variety of other types of samples, such as urine and animal plasma samples.

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