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Note

Assay of semi-synthetic codeine base with simultaneous determination of α-codeimethine and O^6-codeine methyl ether as by-product impurities by high-performance liquid chromatography[a]

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Codeine is commercially the most important of the gum opium alkaloids. However, being a minor constituent (*ca.* 0.7 3.8%, w/w) [1] of gum opium, most commercial codeine is derived semi-synthetically by O^3-methylation of morphine using trimethylphenylammonium hydroxide [2]. Recently, we successfully developed a process for the methylation of morphine, using trimethylphenylammonium chloride and potassium carbonate in toluene, to obtain codeine in *ca.* 99% yield [3].

Two by-products of this methylation reaction, namely O^6-codeine methyl ether (CME) and α-codeimethine (CDM), were isolated [4] earlier from the mother liquor remaining after the crystallization of codeine hydrochloride. Traditional liquid chromatography using a cation-exchange resin [4] permitted the separation of CDM while CME was isolated on an alumina column [5].

Thin-layer chromatography (TLC) has been used for assaying codeine phosphate [6,7] in the presence of decomposition products and also for the determination of CDM [8] and CME [9]. These and other by-products of a methylation reaction were also determined by gas chromatography (GC) on a cyanoethyl siloxane (5% XE-60) [8] stationary phase.

Proksa and Cerny [8] used high-performance liquid chromatography (HPLC) with a bonded octadecylsilane stationary phase for the separation of a number of by-products of codeine synthesis using methanol–0.5 *M* aqueous ammonia (13:17) as the mobile phase. However, owing to the high pH (> 10) of the aqueous component of the mobile phase, the useful life span of the stationary phase is short on account of the considerable dissolution of the silica backbone. Sisco *et al.* [10] assayed natural codeine drug substance derived from poppy straw and opium concentrate by reversed-phase gradient HPLC on a bonded octadecylsilane stationary phase. However, this method involves a ternary mobile phase and a two-segment gradient elution requiring more

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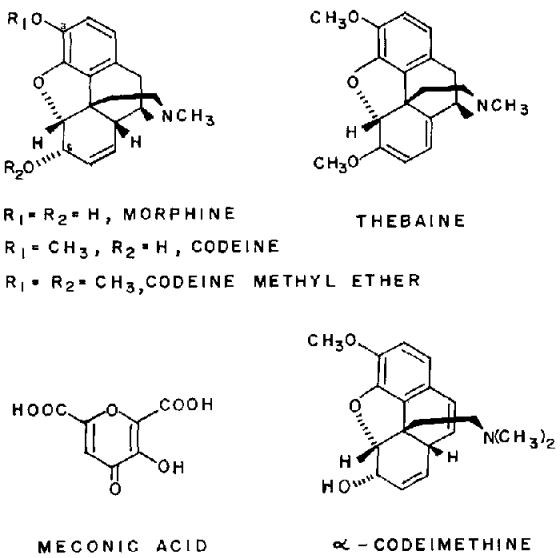


Fig. 1. Structures of codeine and the likely carry-over and synthesis by-product impurities.

than 23 min between two successive injections. Papadoyannis and Caddy [11] reported the separation and determination of codeine alone in pharmaceuticals and body fluids on a reversed-phase octylsilane stationary phase.

In this paper we report the simultaneous assay of semi-synthetic codeine and the determination of potential carry-over (morphine and meconic acid) and synthetic by-product impurities (CME and CDM) (Fig. 1) by isocratic HPLC on a bonded phenyl stationary phase using a simple and inexpensive mobile phase.

EXPERIMENTAL

Liquid chromatograph

The method was developed on a Millipore–Waters (Milford, MA, U.S.A.) high-performance liquid chromatographic system consisting of two Model 6000 series dual-head reciprocating solvent-delivery pumps controlled by a Model 660 solvent programmer, a Model U6K universal injector and a Model 440 filter photometric absorbance detector operating at 254 nm. The analogue output of the detector was recorded and processed with a Waters Model 730 data module (printer, plotter and integrator). A Waters μBondapak phenyl (10 μm) stainless-steel column (30 cm × 3.9 mm I.D.) was used.

Standard samples

Authentic samples of morphine, codeine, O^6-codeine methyl ether and thebaine, as free bases, were obtained from the Government Opium and Alkaloid Works Undertaking (Neemuch, India). α-Codeimethine base was prepared from codeine according to a modified literature method [12]. The crude base was recrystallized from toluene. Meconic acid, trihydrate was isolated from gum opium according to a literature method [13] and was purified by recrystallization from methanol.

Reagents and chemicals

Analytical-reagent grade orthophosphoric acid and triethylamine were used. Methanol was purified to HPLC quality in our laboratory. A Milli-Q system (Millipore, Bedford, MA, U.S.A.) was used to purify water.

A stock buffer solution of triethylammonium phosphate (*ca.* 0.7 *M*) was prepared by mixing 10.0 ml of triethylamine with about 80 ml water and the pH was adjusted to 2.2 with 85% (w/w) orthophosphoric acid (*ca.* 8 ml). The solution was cooled to room temperature and diluted to the volume in a 100-ml volumetric flask. A 1-ml volume of this stock solution was diluted to 100 ml with water to obtain the working buffer solution (7 m*M*; pH 3.1).

Mobile phase

Methanol–7 m*M* triethylammonium phosphate buffer (pH 3.1) (20:80) was used as the mobile phase at a flow-rate of 1.0 ml/min.

Column dead time (t_0)

This was determined by injecting 50 μl of methanol–7 m*M* triethylammonium phosphate buffer (pH 3.1) (30:70) into the mobile phase stream. The peak–trough combination caused by the change in refractive index was used as a marker and the point of baseline crossover was taken as t_0.

External standard calibration graph

Stock standard solution. About 200 mg of codeine, 25 mg of CDM and 8 mg of CME were weighed as free bases, dissolved with the help of a few drops of glacial acetic acid and diluted to volume with the mobile phase in a 50-ml volumetric flask.

Working standard solution. Aliquots of the above stock solution ranging from 1 to 9 ml were diluted to volume with the mobile phase in different 10-ml volumetric flasks to give a series of working standard solutions. A 25-μl volume of each of these calibration solutions was injected into the liquid chromatograph to check the linearity of peak-area response of the UV detector (254 nm) with respect to the amount of each solute injected (μg). Thus, the calibration solution range corresponds to 10–99 μg of codeine, 1.25–11.25 μg of CDM and 0.4–3.6 μg of CME, each expressed as the free base injected per injection. The linearity for each base was verified by a linear regression analysis by the least-squares method.

Sample preparation for assay

About 100 mg of codeine base sample was accurately weighed, dissolved and diluted to volume with the mobile phase in a 50-ml volumetric flask. The sample solution was filtered through a 0.45-μm Millipore PTFE membrane disc filter and a 25-μl aliquot was repeatedly injected into the liquid chromatograph to ensure repeatability.

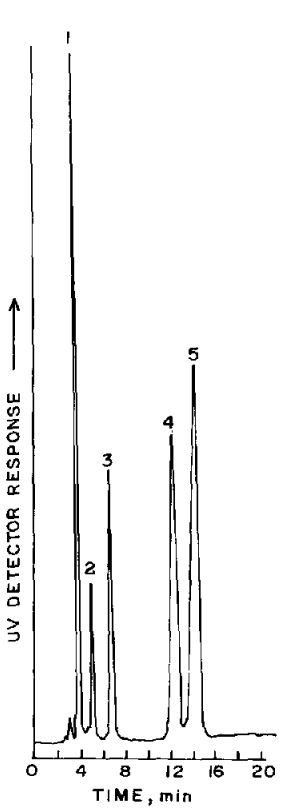
RESULTS AND DISCUSSION

We required a simple, rapid, reliable and inexpensive HPLC method for our process development and optimization work on the methylation of partially purified morphine (*ca.* 80%, w/w) to produce semi-synthetic codeine using trimethylphenyl-

ammonium chloride [3] as the methylating reagent. The codeine thus produced was expected to contain a small amount of unreacted morphine and meconic acid as the carry-over impurities together with by-product impurities such as CDM [4,8] and CME [4,5,8,9]. Our selection of a bonded phenyl stationary phase for this separation was based on past experience with the simultaneous separation of gum opium alkaloids [1,14].

We achieved a baseline separation of codeine and the potential impurities using inexpensive methanol–7 m*M* triethylammonium phosphate buffer(pH 3.0) (20:80) as the mobile phase in about 16 min (Figs. 2 and 3). The HPLC data in Table I indicate good selectivity. We studied the solvent selectivity further by using an isoeluotropic mobile phase prepared by replacing methanol with tetrahydrofuran (4%, v/v). However, tetrahydrofuran did not offer any particular advantage over methanol for the overall separation. Further, there was no incentive to replace cheap methanol with more expensive acetonitrile.

The effect of pH on the separation was studied, keeping the other chromatographic parameters unchanged. Morphine, codeine and CME have similar basicities



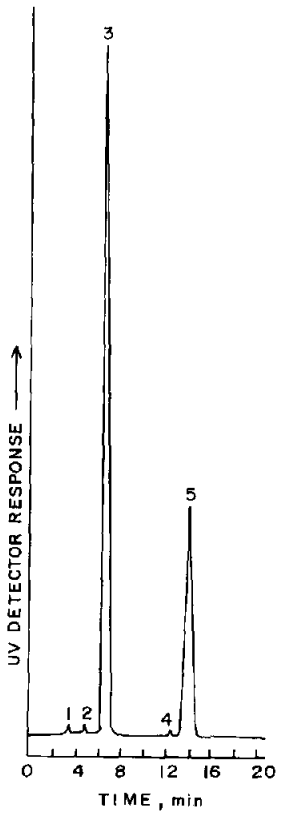


Fig. 2. Isocratic HPLC separation of an artificial mixture of codeine and the likely carry-over and synthesis by-product impurities. For conditions, see Experimental. Peaks: 1 = morphine; 2 = meconic acid; 3 = codeine; 4 = O^6-codeine methyl ether (CME); 5 = α-codeimethine (CDM).

Fig. 3. Chromatogram of a real sample of semi-synthetic codeine (spiked with CME). Details as in Fig. 2.

and CDM is slightly more basic, whereas meconic acid is a dicarboxylic acid (Table I). At pH > 3.0, the capacity factors of all the compounds increased substantially, except for morphine and meconic acid, without a concomitant improvement in overall separation. This is in accordance with the respective pK_a values.

A regression analysis by the least-squares method for the plots of peak area (area units), y, against the corresponding mass of solute injected (μg per 25 μl), x, yielded the following calibration equations, with the correlation coefficients, r, indicating excellent linearity:

$$\text{Codeine: } y = 1.395 \cdot 10^6 x - 0.520 \cdot 10^6;\ r = 0.99928$$
$$\text{CME : } y = 2.009 \cdot 10^6 x + 0.619 \cdot 10^6;\ r = 0.99957$$
$$\text{CDM : } y = 6.976 \cdot 10^6 x + 0.434 \cdot 10^6;\ r = 0.99934$$

A linear relationship was observed at least up to 100 μg of codeine and 10 μg each of CME and CDM.

The calibration equations for codeine, CME and CDM show significant intercepts on the ordinate, which were 0.69%, 1.1% and 5.3%, respectively, of the corresponding peak-area responses at the mid-point of the each calibration graph. Any gross errors in quantification, especially for CME and CDM, were avoided by correcting the results for the respective "blank" values using the "offset calibration graph" feature of the Waters Model 730 data module. The reliability of the quantification may be improved further by selecting the wavelength of maximum absorption (Table I) for each analyte using a progammable UV detector.

In this analysis the intercepts of the calibration graphs on the ordinate governed the limits of determination of impurities. The actual limits of determination of CME and CDM for the simultaneous assay and analysis of semi-synthetic codeine were 0.35% and 0.075% (w/w), respectively. For these evaluations the codeine sample size was 100 μg per 25-μl injection volume, which corresponds to the highest point on the linear part of the calibration graph for codeine. The limits of determination of the two by-product impurities can easily be improved by injecting either the maximum permissible injection volume or even a much larger volume, if a weaker solvent is used to dissolve the sample.

TABLE I

HPLC OF SEMI-SYNTHETIC CODEINE AND THE LIKELY ASSOCIATED IMPURITIES ON A BONDED PHENYL STATIONARY PHASE AT pH 3.1

Void volume time, $t_0 = 3.05$ min at 1.0 ml/min.

Compound	pK_a	λ_{max} (nm)	Retention time (min)	Capacity factor, k'	Selectivity factor, α
Morphine	7.87	285	3.83	0.26	2.65
Meconic acid	–	237	5.16	0.69	
		305			1.68
Codeine	7.95	285	6.59	1.16	2.66
CME	7.32	285	12.47	3.09	1.17
CDM	8.25	275	14.10	3.62	

TABLE II

REPEATABILITY OF RETENTION TIME AND PEAK AREA IN HPLC OF A REAL SAMPLE OF CODEINE

Number of HPLC experiments, $n = 8$. The amount of CME by-product impurity was below the limit of determination (0.35%, w/w).

Component	Mean (%, w/w)	Retention time		Peak area	
		Mean ± S.D. (min)	R.S.D. (%)	Mean ± S.D. (area units) × 10^6	R.S.D. (%)
Codeine	89.45	6.54 ± 0.035	0.54	80.3 ± 1.83	2.28
α-Codeimethine	5.06	13.91 ± 0.088	0.63	32.8 ± 0.821	2.51

We used a real sample of codeine to evaluate the repeatability of the retention times and peak areas of codeine, CME and CDM (Table II). However, the amount of CME in this sample was below the limit of determination (0.35%, w/w). We aimed at a $<3\%$ relative standard deviation (R.S.D.) for codeine and CDM, whereas a $<5\%$, R.S.D. was allowed for CME because its amount in real samples was expected to be low ($<0.5\%$, w/w) owing to the reaction conditions used.

The method was found to be satisfactory for the routine assay of codeine. Moreover, HPLC proved to be a valuable aid in monitoring the progress of methylation reactions. Thus, we found that on methylation of morphine with dimethyl sulphate, the codeine produced was grossly contaminated with CDM and other impurities.

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