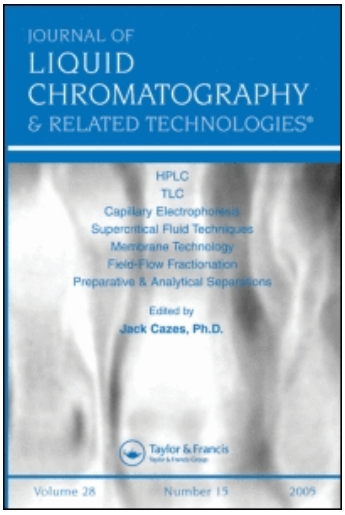

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OPTIMIZED AND VALIDATED HPLC METHODS FOR COMPENDIAL QUALITY ASSESSMENT. II. OPIUM ALKALOIDS

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ABSTRACT

The main opium alkaloids, such as morphine, codeine and papaverine have their own monographs in the modern pharmacopoeias. This paper includes reversed phase high performance liquid chromatographic procedures for the purity control of the mentioned opiates. The optimized and validated methods make possible the separation of morphine, codeine, thebaine, noscapine, papaverine and the detection of the four opiate impurities in morphine, codeine and papaverine. The limit of detection ranges from 0.01 up to 0.1%. Also, morphine content in codeine chloride can be quantitated with good accuracy.

INTRODUCTION

The most important opium-alkaloids, morphine, codeine, papaverine, are permanent members of the standard medical stock. Morphine itself, as an analgesic, is essential to endure severe pains. Codeine is one of the most often

used antitussives even nowadays, and the same is valid for papaverine in the field of smooth musculature relaxation. Though, its therapeutical significance is less, noscapine also has its own monograph in the modern pharmacopoeias. Almost all of these monographs prescribe purity test(s) for the detection of "related" or "foreign" alkaloids (substances). Table 1 summarizes the prescribed tests of four pharmacopoeias (European Pharmacopoeia Ed.II., United States Pharmacopeia Ed.XXIII, Deutsches Arzneibuch Ed.10., Pharmacopoea Hungarica ed.VII). It may be seen that, only non-specific chemical reactions, dissolution tests (sulfuric acid) or TLC procedures under general addressing, as detection of "foreign" ("related") alkaloids, are prescribed. In conclusion, specific testing for an individual (i.e. named) opiate-impurity (e.g. codeine in morphine and vice versa) only exceptionally occurs. The general suitability of HPLC for compendial quality control was raised and illustrated in a previous paper[1] by the HPLC purity test of methylxanthine alkaloids . The present publication provides RP-HPLC methods for the quality testing of the three main natural opiates.

The HPLC separation of the major alkaloids in opium and in plant material has been the subject of several works.[2-5] Recently, capillary electrophoresis was used for the same purpose.[6] For the HPLC determination of the main opium alkaloid, morphine, in biological samples, numerous papers could be found. A few years ago Tagliaro et al.[7] gave an excellent overview of such separations and detection techniques. Similarly, HPLC separation of codeine[8,9] and also its metabolites[10] was the subject of publications. As morphine and codeine are common biological degradation products of each other, their separation and the HPLC separation of some of their other metabolites frequently emerge in the analysis of biological samples.[11-18] However, only a few works were found dealing with HPLC separation and determination of morphine,[19] codeine,[20,21] or papaverine in pharmaceutical preparations. Purity tests, by HPLC, suitable for compendial use, could not be found in the literature. In the present paper, optimized and validated RP-HPLC systems are suggested for the purity control of morphine, codeine, and papaverine. In each of these three substances the other two opiates, as well as noscapine and thebaine, can be selectively and sensitively detected.

EXPERIMENTAL

Chromatography

The HPLC apparatus is comprised in an ISCO pump Model 2350 (USA) combined with a Valco injector unit (10 µL loop). An ISCO variable

Table 1

Pharmacopoeial Methods for Testing Related Substances in Opium Alkaloids

Tested Alkaloid	Impurity Investigated (Method)			
	Eur. Ph. II	**USP XX III**	**DAB 10**	**Ph. Hg. VII**
Codeine	Foreign alk. (TLC) Morphine ($NaNO_2$)	Chromatographic purity (TLC) Morphine ($K_3Fe(CN)_6$ + $FeCl_3$)	Foreign alks. O,O-dimethyl-morphine (TLC) Morphine ($NaNO_2$)	Foreign alks., degradation prods. (TLC) Morphine ($NaNO_2$)
Morphine	Related subs., Codeine (TLC)	Foreign alks. (isolation of, detn. by acidi-alkalimetry)	Related subs., Codeine (TLC)	Related alks. (as in USP) Organic impurities (TLC)
Noscapine	Related subs. (TLC)	Ordinary impts. (TLC) Morphine ($K_3Fe(Cn)_6$ + $FeCl_3$)	Related subs. (TLC)	Related alks., degradation prods. (TLC) Morphine ($KH(IO_3)_2$
Papaverine	Foreign alks., Codeine (TLC)	Organic impts. (cryptopine, thebaine, etc.) (H_2SO_4 dissoln.)	Foreign alks. Codeine (TLC)	Related subs., degradation prods. (TLC)

absorbance detector (230-800 nm) was used. The equipment units, subsequent to the pump, was thermostatted (Column Heater-Chiller, Model 7955 Jones Chromatography Ltd.,Wales) at 23° - 30° ± 1°C. The chromatograms were recorded, the data handling was effected by a Hewlett-Packard integrator Model 3396 Ser.II. The C_{18} sorbent, Hypersil 5-ODS (Shandon) was packed in a

stainless steel column 250x4.0 mm I.D., BST, Budapest, Hungary. As mobile phases for optimization, sonically degassed and filtered mixtures of methanol and phosphate buffer solutions pH 3 and pH 8, methanol-water mixtures, occasionally containing pentanesulfonic acid or tetrabutylammonium bromide in different concentrations, were used.

The column void time was signalled by the injection of methanol. Each data of retention was calculated as an average of at least three parallel runs. The eluent flow rate for optimization was adjusted to 0.6-0.8-1.0-1.5 mL/min. The effluent was monitored at the wavelength of optimal detectability of the tested compound. After each experiment, the column was brought to the initial state by washing with 50 mL of methanol-water mixture, in which the amount of water corresponded to that of the buffer solution in the eluent.

A final purging of the column was performed with 50mL of methanol. In the case of the column being loaded by an eluent with pH 8, a prewash with 50 mL 10:90 mixture of methanol- phosphoric acid (pH 2.5) was made.

Materials

Tetrabutylammonium hydrogensulfate 97%, was from Aldrich. Pentanesulfonic acid natrium 98%, was also from Aldrich.

Buffer solutions at pH 3 and 8 were prepared by mixing the proper volumes of 0.067 M aqueous solution of potassium dihydrogen phosphate and disodium hydrogenphosphate (KH_2PO_4,$Na_2HPO_4.2H_2O$) anal. grade, Reanal, Budapest. The pH of the solutions was tested by potentiometry with an accuracy ± 0.02 unit.

Methanol RS for HPLC, Carlo Erba. Water, deionized, double distilled. Sodium chloride 99.99%, Aldrich.

Model Substances

Morphine chloride, Codeine chloride, Papaverine chloride, Noscapine chloride, met the requirements of the Hungarian Pharmacopoea ed. VII. Thebaine chloride was generously donated by Alkaloida (Tiszavasvári, Hungary) and was used without further purification.

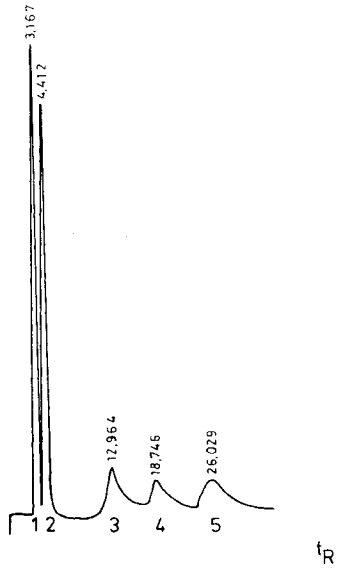

Figure 1. The detection of "related alkaloid" impurities in morphine. (Procedure see in the text under "Prescription."). (1) Morphine (extracted amount: 0.5 g). Impurities, added % (injected μg / 10 μL):(2) codeine 0.1 (0.5), (3) thebaine 0.1 (0.5) (4) noscapine 0.1 (0.5) (5) papaverine 0.02 (0.1).

RESULTS AND DISCUSSION

Morphine Chloride

The optimization of the procedure was performed by successive changing of the methanol content, pentanesulfonic acid concentration, and pH of the phosphate buffer in the eluent, as well as the flow rate and temperature.

None of the proven systems separated the peaks of morphine and codeine sharply enough for purity test purposes. The unique, amphoteric character of morphine, allowed its preseparation from the four related alkaloids by solubility difference, i.e.: from an appropriately alkalinized medium the non-phenolic related compounds can be extracted with chloroform, while morphine mostly remains, as phenolate, in the aqueous solution. Figure 1 shows the HPLC-separation of the four related alkaloids and morphine, when the latter was spiked with 0.1 % w/w of codeine, thebaine, noscapine, and 0.02 % w/w of

Table 2

Retention Time and Limit of Detection of Opium Alkaloids

Compound	Retention Time Min. Methanol Content, %*			Limit of Detn., μg**
	30	40	50	
Morphine	3.1	2.6	2.5	0.1
Codeine	4.4	3.3	2.7	0.1
Thebaine	21.7	8.1	4.3	0.25
Noscapine	41.3	10.9	4.9	0.4
Papaverine	54.3	13.9	5.3	0.05

* Eluent composition: Phosphate buffer, pH=3 - Methanol + 0.005M pentane-sulphonate Na

** At signal-to-noise ratio min. 3:1.

papaverine. It can be seen, that the peaks of codeine and morphine, as a consequence of the reduced amount of the latter, separate sharply, followed by the peaks of the other three substances moving with rather high retention. It was established, that chloroform extracts only about 0.5 % w/w (2.5 mg) of the examined morphine amount (500 mg).

Prescription

Test solution

0.5 g of the tested morphine chloride is dissolved in 10 mL of water and then, by the dropwise addition of 2M sodium hydroxide, the pH of the solution is adjusted to 11. This alkaline aqueous solution is extracted three times with ten mL portions of chloroform.

The combined chloroformic phase is filtered through a layer of anhydrous sodium sulfate and evaporated to dryness. The dry residue is dissolved in 10 mL of the eluent. 10 μL of this test solution is injected and chromatographed.

Mobile phase

The mobile phase was Phosphate buffer pH 3 - methanol (65:35) + 0.005M pentanesulfonic acid natrium.

Eluent flow rate

Eluent flow rate was 1 mL/min., the rather high retention time of thebaine, noscapine and papaverine (retention times see Table 2) is reduced by increasing the flow rate, after the complete elution of codeine, to 1.5 mL/min. (Also, gradient elution may be applied with the increasing of the methanol content of the eluent up to 40 % v/v after the first five minutes of development).

Evaluation

The appearance of a definite peak of codeine, thebaine, noscapine, and papaverine indicates the presence of as much, or more than, 0.1 μg (0.02%) 0.25 μg (0.05%) 0.37 μg (0.074%) 0.02 μg (0.004%) impurity respectively (see Table 2). It is to be noted, that codeine impurity, as much as 0.1 %, also can be quantitatively measured, using standard solutions.

Codeine Chloride

In case of the codeine a pretreatment, similar to that of morphine, to reduce the tested substance/impurity ratio, was not possible. Therefore, an unusually great amount of codeine, allowed by the loading capacity of the column and the suitable resolution from the adjacent peak (morphine) had to be chromatographed. The optimization of the chromatographic system was performed in the same manner as that of morphine. A chromatogram is shown by Figure 2.

It can be seen, though the complete elution of the large, tailing peak of 100 μg codeine takes about 10 minutes, the system is suitable for the detection of the four opiate impurities.

It is to be noted, that morphine can be detected and also quantitated with a good precision in the range from 0.2 up to 1.0 μg (Table 3). A higher amount of morphine may cause an overlapping with the peak of codeine.

Prescription

Test solution

0.1 g of codeine chloride is dissolved in 10 mL of the eluent. 10 μL of the solution is injected and chromatographed in the chromatographic system as that used for morphine.

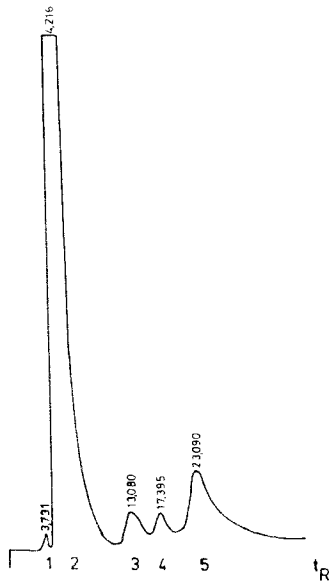

Figure 2. The detection of "related alkaloid" impurities in codeine (Procedure see in the text under "Prescription"). (2) Codeine (examined amount: 100 μg/10 μL). Impurities, added % = injected μg/10 μL: (1) morphine 0.1 (3) thebaine 0.25 (4) noscapine 0.25 (5) papaverine 0.1.

Table 3

Precision* (Repeatability) of the Morphine Content Determination in Codeine

Morphine in Codeine % (μg/100 μg)	S.D. %	Linearity (peak area/ concn.)
0.2	3.58	
0.4	3.60	
0.6	2.63	r = 0.9991
0.8	1.85	n = 5
1.0	2.28	

* It was determined by assaying 5 aliquots at each concentration.

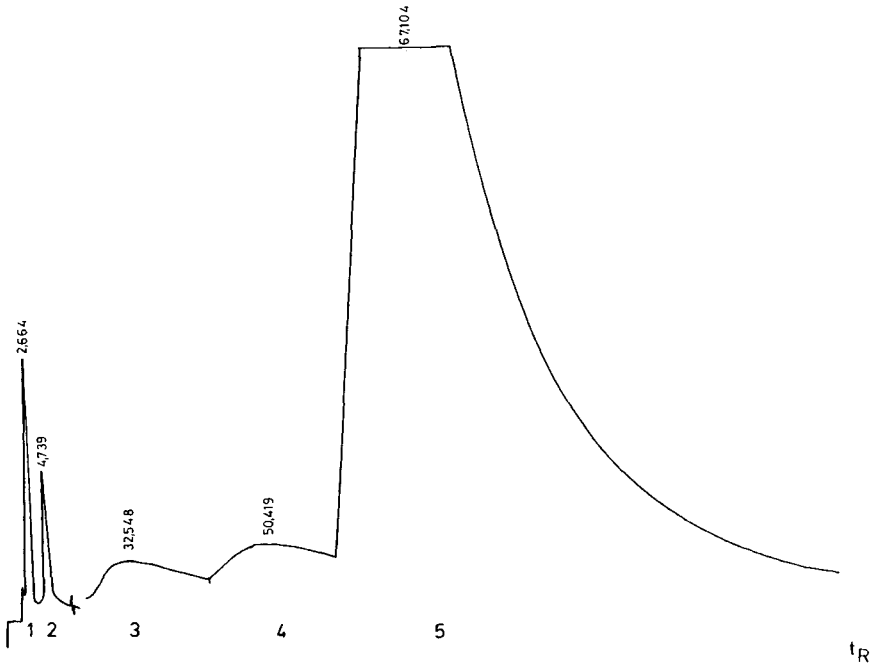

Figure 3. The detection of "related alkaloid" impurities in papaverine. (Procedure see in the text under "Prescription".) (5) Papaverine (extracted amount 0.5 g). Impurities: (1) morphine (2) codeine (3) thebaine (4) noscapine injected:1-1 μg.

Eluent flow rate

The eluent flow rate is 0.8 mL/min., and it changed to 1.5 mL/min. after the elution of codeine's peak.

Evaluation

The appearance of a definite peak of morphine, thebaine, noscapine and papaverine indicates the presence of the impurities as much or more than 0.1 μg (0.1%) 0.25μg (0.25%) 0.37 μg (0.37%) 0.004 μg (0.004%) respectively.

Papaverine Chloride

As the purity control of papaverine had to be considered at a reasonable wavelength of detection, 254 nm, papaverine exhibits an extreme sensitivity, i.e., with a large, tailing peak by which the small adjacent peak of noscapine impurity is overlapped. However, the different solubilities of papaverine and its potential impurities in ethanol offered a way to reduce the final concentration of papaverine. It was established that only 4.2% of the tested papaverine dissolves in the ethanol, while the impurities, being present in an amount 0.1-1%, are completely extracted into the ethanol. The chromatogram of a purity test, was made in the optimized chromatographic system, is shown by Fig. 3. The optimization was performed by the same manner as in case of morphine.

Prescription

Test solution

0.5 g of papaverine chloride is shaken in 3 mL of 96 % ethanol for 1 minute and filtered into a 50.0 mL volu-metric flask, diluted to volume with the eluent and mixed. 10 μL of the solution is injected and chromatographed.

Mobile phase

The mobile phase consists of phosphate buffer pH=3 - methanol 70:30 + 0.005 M pentanesulfonic acid natrium.

Eluent flow rate

The eluent flow rate is 1.5 mL/min. To enhance the elution of thebaine, noscapine, and papaverine, gradient elution up to 40% v/v of methanol content may be applied, the same as that of the testing of morphine.

Evaluation

The appearance of a definite peak indicates the presence of as much or more than 0.1 μg (0.1%) morphine and/or codeine, 0.25 μg (0.25%) of thebaine, 0.037 μg (0.037%) of noscapine.

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