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HPLC assay of the opiates in opium and cough mixtures using dynamically modified silica and UV absorbance, fluorescence and electrochemical detection

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Summary

A high-performance liquid chromatographic (HPLC) method based on the dynamically modified silica approach has been developed for the separation of the opiates. The chromatographic system consists of bare silica as the column packing material and an eluent consisting of methanol + water + 0.2 M potassium phosphate pH = 7.0 (35 + 60 + 5 v/v) with 2.5 mM of cetyltrimethylammonium (CTMA) bromide added. In this reversed-phase system it is possible to make a separation and quantitation of the opiates ranging in polarity from morphine to papaverine simultaneously within a short time.

Introduction

A great number of methods for the separation of the five main alkaloids in opium involving high-performance liquid chromatography (HPLC) have been published. The methods take advantage of different separation mechanisms and this has previously been summarized (Hansen, 1981a and b).

The difference in polarity between noscapine and morphine makes it difficult to obtain the necessary retention and separation of the first eluted compounds concomitant with a reasonable k′-value (< ca. 10) of the last eluted compound. This problem arises in the normal phase as well as in the reversed-phase and in the ion exchange mode. Recently a method based on a combination of normal phase and anion exchange chromatography was published (Doner and Hsu, 1982). Although claiming to be a significant improvement relative to existing procedures papaverine is eluted close to the solvent front (k′ = 0.16) making the method unsuitable for quantitative determination of this compound in complex mixtures.

A solution to this separation problem might be to use silica chemically modified with cyanopropyl groups (Nobuhara et al., 1980).

Using the dynamically modified silica approach described in detail elsewhere (Hansen, 1981a and b; Hansen et al., 1982, 1983, 1984; Hansen and Helboe, 1984) the problem outlined above can be solved. Combined with suitable detection methods

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more or less selective detection of morphine is achieved.

Experimental

Apparatus

A Waters liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) consisting of a 6000 A pump, a 710 B WISP autoinjector, a 440 ultraviolet (UV) absorbance detector (254 nm), a 730 dato module and a 720 system controller was used. Furthermore a Kontron SFM 23 fluorescence detector (Kontron AG, Zürich, Switzerland) and a Methrom 641VA-656 electrochemical detector (Methrom, Herisau, Switzerland) were used. The electrochemical detection was performed in a flow-cell with a glassy carbon working electrode and a Ag/AgCl reference electrode.

Chemicals

A cough mixture Nirvapon Comp. was obtained from Dumex, Copenhagen and opium was of pharmacopoeial quality. All other reagents were of analytical reagent grade.

Sample preparation

Cough mixture. 5.0 ml of Nirvapon Comp. was diluted to 10.0 ml with water; 20 μl was injected into the liquid chromatograph.

Opium. 100.0 mg of opium was dissolved in 2.0 ml of dimethyl sulfoxide (DMSO) and diluted to 50.0 ml with the eluent. After centrifugation 10 μl was injected into the liquid chromatograph.

Chromatography

A stainless steel (Knauer, Berlin, F.R.G., 120 × 4.6 mm i.d.) packed with LiChrosorb SI 60, 5 μm was used as the analytical column. To prevent the silica in the analytical column from dissolving a saturation column (150 × 4.6 mm i.d.) packed with LiChroprep SI 60, 15–25 μm was installed between the pump and the injection device. As eluent a mixture of methanol + water + 0.2 M potassium phosphate pH = 7.0 (35 + 60 + 5 v/v) with 2.5 mM of CTMA added was used with a flow-rate of 1.0 ml/min. The system was operated at ambient temperature.

Results and Discussion

In the dynamically modified silica approach for reversed-phase chromatography several separation mechanisms are involved (reversed-phase, ion-pairing and to some extent cation exchange (Hansen et al., 1984). The nature of the approach provides additional possibilities if a specific order of elution of solutes is wanted and was therefore applied to the separation of the opiates.

Effect of pH

An increase in the pH-value of the eluent will increase the reverse-phase effect due to an increased adsorption of the hydrophobic quaternary ammonium ion onto the silica surface (Fig. 1). The change in the reversed-phase effect is indicated by the retention of the non-ionic solute benzene. The relatively apolar alkaloids papaverine and noscapine seem to be retained by a pure reversed-phase mechanism, while the retention mechanism of the more polar alkaloids, which exhibits a stronger cationic character, may be explained as a mixture of reversed-phase and cation exchange. At lower

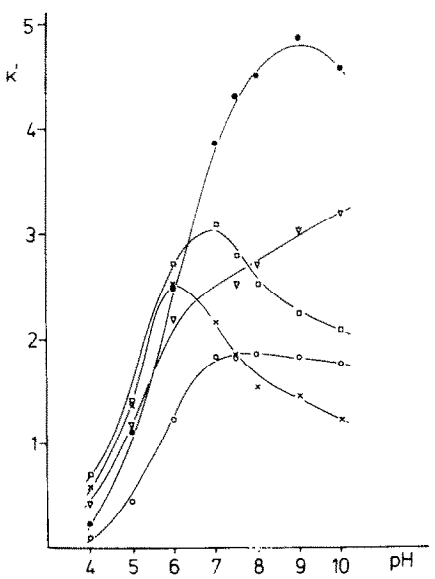

Fig. 1. The k'-value for five opiates versus the pH in the buffer used for the eluent. Column: LiChrosorb SI 60, 5 μm, 120×4.6 mm. Eluent: methanol+0.2 M potassium phosphate buffer +water (50:5:45 v/v) with 2.5 mM of CTMA added. Symbols: ▽, morphine; ×, codeine; □, thebaine; ○, papaverine; and ●, noscapine = narcotine.

pH, where the alkaloids are ionized and the loading of CTMA on the silica surface is low, the retention is primarily due to cation exchange. However, as pH increases, the protonation of the alkaloids decreases and the CTMA-loading on the silica surface increases with the result that the reversed-phase mechanism is dominant. Thus codeine is eluted before thebaine. The reason for the still increasing retention of morphine with increasing pH-value is due to ion-pair formation between the phenolate group in morphine and CTMA.

Effect of modifier concentration

From Fig. 1 a pH-value of 7.0 seemed to be a reasonable choice, and this pH-value was therefore kept constant when investigating the effect of the concentration of the methanol on the retention. Fig. 2 shows that the retention decreases with increasing methanol concentration in the eluent. When the amount of methanol increases, the eluent becomes stronger at eluting, and at the same time the CTMA-loading on the silica surface decreases thus leading to a strong decrease in the reversed-phase retention of papaverine and noscapine. The decrease in retention of the three more polar solutes is less pronounced due to their higher affinity towards the silica — an effect that increases as the CTMA-loading on the silica decreases.

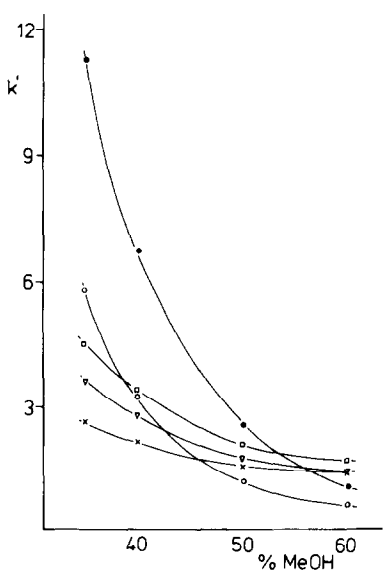

Fig. 2. The k'-value for five opiates versus the concentration of methanol in the eluent. Column as in Fig. 1. Eluent: methanol +0.2 M potassium phosphate pH = 7.0 + water (x : 5 : (95 − x) v/v) with 2.5 mM of CTMA added. Symbols as in Fig. 1.

Choice of final system

As papaverine has a strong UV-absorption, it was preferred to place this compound in the chromatogram after the compounds with lower UV-absorbing properties. The final eluent for the analyses should therefore contain 35% of methanol at pH = 7.0 with 2.5 mM of CTMA added.

Detection

Morphine often appears in low concentrations in pharmaceutical preparations, and as its UV-absorbing properties are weak, the detection limit is high. If morphine is contained in a complex matrix, the problem of selectivity often enhances the detection problems.

Most of these problems can be solved by using

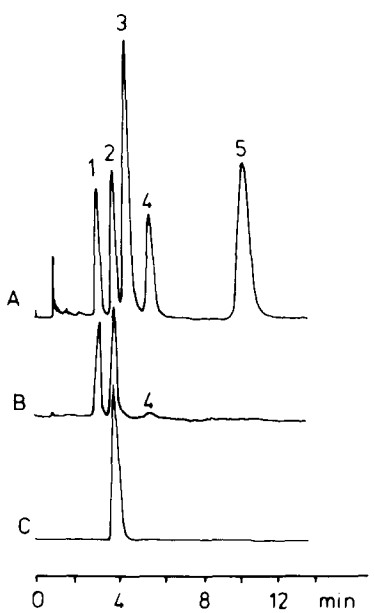

Fig. 3. Separation of five opiates using three different detectors. Column as in Fig. 1. Eluent: methanol + 0.2 M potassium phosphate pH = 7.0 + water (35 : 5 : 60 v/v) with 2.5 mM of CTMA added. A: UV-detection 254 nm. B: fluorescence with excitation 285 nm and emission 325 nm. C: electrochemical detection at 550 mV. Numbers: 1, codeine; 2, morphine; 3, thebaine; 4, papaverine; and 5, noscapine.

more selective and more sensitive detectors. Both fluorescence and electrochemical detection of morphine have been described (Glasel and Venn, 1981, Ishikawa et al., 1982).

In Fig. 3 a comparison of the selectivity of the three detection principles in question is seen. Using electrochemical detection a current–potential curve for morphine similar to those obtained by other workers (Ishikawa et al., 1982) was found. Using an applied potential of 550 mV gives a good compromise between sensitivity and selectivity when detecting morphine. The minimum detectable quantity of morphine, defined as a peak twice the baseline noise, using ultraviolet absorbance at 254 nm, fluorescence at 285 nm as excitation wavelength and 340 nm as emission wavelength and electrochemical oxidation at 550 mV, was 5 ng, 1 ng and 1 ng, respectively.

Application

The present chromatographic system has been applied to the analyses of opiates in a cough-syrup containing 10 mg of morphine, 0.65 mg of codeine, 4.4 mg of noscapine, and 0.9 mg of papaverine per 100 ml of preparation and with a high content of sugar.

All attempts to extract the opiates from this matrix and eventually concentrate them have failed. Using a simple dilution of the syrup and an ordinary reversed-phase system with UV-detection was not feasible due to interfering peaks with the morphine and long retention of papaverine and noscapine.

With the present system it is possible to analyse the 4 opiates in question within a reasonable time of analysis (Fig. 4), and fluorescence or electrochemical detection offers higher selectivity and lower detection limits for the morphine.

Also for the determination of opiates in opium this approach seems to be an advantage as it is possible to dissolve opium in DMSO, dilute it with the eluent and inject this sample into the liquid chromatograph (Fig. 4).

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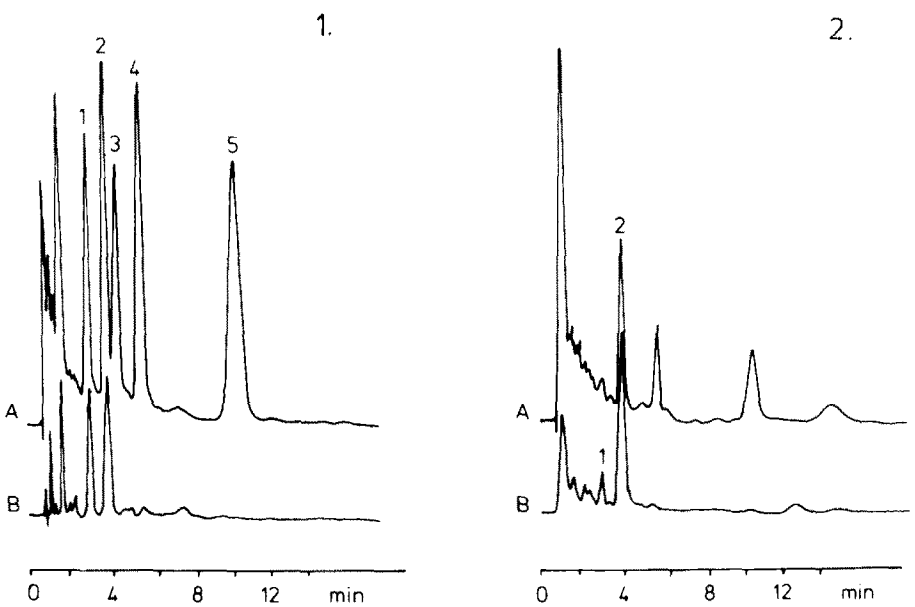

Fig. 4. Determination of opiates in: (1) opium; and (2) a cough mixture. Chromatographic system and numbers as in Fig. 3.

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