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Note

Determination of pseudomorphine in morphine injection by high-performance liquid chromatography

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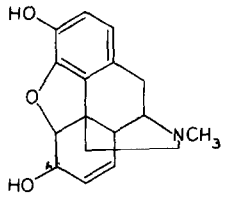
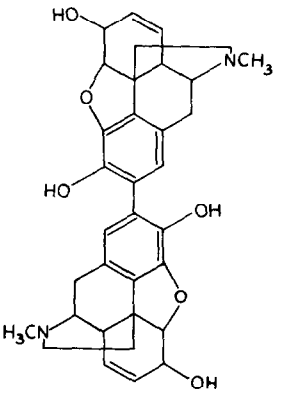
Morphine (I) is known to be oxidised in aqueous solution; it will decompose on heating in strong base[1] or at milder pH conditions on storage[2] to give pseudomorphine (II) as the major degradation product. Antioxidants would normally be added to morphine injections prior to autoclaving to prevent oxidation but their use is precluded in intrathecal injections and so when manufacturing morphine intrathecol injections it is necessary to monitor pseudomorphine production.



I



II

High-performance liquid chromatography (HPLC) is the most suitable technique for the determination of morphine and its degradation compounds and two methods using ion-exchange[2] and ion-pair chromatography[3] have been reported. However, both these methods have their problems. The ion-exchange method needs a gradient elution to affect separation and the pH of the buffer has to be very closely controlled. The ion-pair chromatography is highly pH dependent and so very small changes in pH can lead to loss of resolution and imprecise quantitative measurement. In addition, the low pH necessary for ion-pairing has a deleterious effect on the bonded phase.

This report will describe a relatively simple method for the analysis of morphine and pseudomorphine. The method utilises a cyano bonded column operating in the reversed-phase mode with acetonitrile–phosphate buffer as the mobile phase.

The method is shown to be rapid and sensitive and resolution is not effected by relatively large changes in pH.

MATERIALS AND METHODS

Potassium dihydrogen phosphate and HPLC-grade acetonitrile were purchased from BDH. Morphine was purchased from Macfarlane Smith. Pseudomorphine was synthesised by the method of Bentley and Dyke[4] and the structure confirmed by melting point and mass spectrometry.

Chromatography was carried out on a Pye Unicam LC3 chromatograph. A Radial-Pak μBondapak-CN column (10 μm; 10 cm × 5 mm I.D.), Waters Assoc. was used and the mobile phase was potassium dihydrogen phosphate 0.05 *M*, pH 4.5–acetonitrile (80:20) at a flow-rate of 1 ml/min. Detection was by UV at 240 nm and the injection volume of 50 μl was delivered by a valve loop injector. The pH of the buffer was adjusted when necessary with 5 *M* sodium hydroxide or 5 *M* phosphoric acid.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of morphine and pseudomorphine on the μBondapak-CN column. Unlike the analysis on a C_{18} reversed-phase column the two peaks are clearly resolved without the use of ion-pair reagents. Between pH 3.5–5.5 resolution is not affected by changes in pH but above pH 5.5 resolution deteriorates until at pH 7.0, $R_s = 0.4$ (Table I).

When using the ion-pair method it was found that changes of 0.2 in pH would

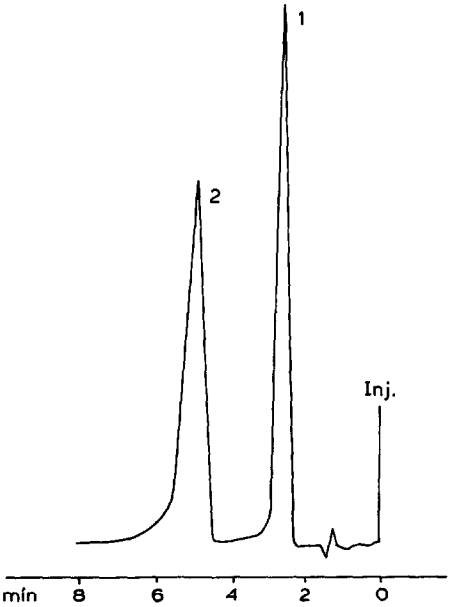


Fig. 1. HPLC of morphine (1) and pseudomorphine (2) on μBondapak-CN.

TABLE I

RETENTION VOLUMES (V_R) CAPACITY FACTORS (k') AND RESOLUTION (R_s) OF MORPHINE AND PSEUDOMORPHINE AT DIFFERENT pH VALUES

pH	*Morphine*		*Pseudomorphine*		R_s
	V_R *(ml)*	k'	V_R *(ml)*	k'	
3.57	2.2	0.7	4.2	2.1	2.1
4.48	2.4	0.8	4.8	2.6	2.3
5.38	2.8	1.09	5.3	3.0	2.1
6.18	3.6	1.7	4.7	2.5	0.84
7.03	3.8	1.9	4.3	2.3	0.38

significantly alter resolution and sensitivity giving poor precision and reproducibility in quantitative analysis. The authors themselves reported that a change of pH between 1.9–2.4 can markedly affect the chromatographic performance[3]. With the CN-column such a small change in pH did not affect the chromatograph and resolution was maintained over a wider pH range (3.5–5.5).

The method has been used to measure the pseudomorphine content of morphine intrathecal injections. By monitoring at 240 nm a pseudomorphine concentration of 100 ng/ml in 1% morphine solutions could be detected. A calibration curve in the range 5–100 μg/ml was linear ($r = 0.99$). Samples of the injections which were not purged with nitrogen showed 0.2% degradation after autoclaving whereas purging the solution with nitrogen to remove oxygen prior to autoclaving reduced the level of degradation to 0.01%. This was considered to be an acceptable level.

REFERENCES

1 S. Yeh and J. L. Lach, *J. Pharm. Sci.*, 50 (1961) 35.
2 P. O. Roksuagg, J. B. Fredrickson and T. Waaler, *Pharm. Acta Helv.*, 55 (1980) 198.
3 I. Beaumont and T. Deeks, *J. Chromatogr.*, 238 (1982) 520.
4 K. W. Bentley and S. F. Dyke, *J. Chem. Soc.*, (1959) 2574.