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Note

Determination of morphine, diamorphine and their degradation products in pharmaceutical preparations by reversed-phase high-performance liquid chromatography

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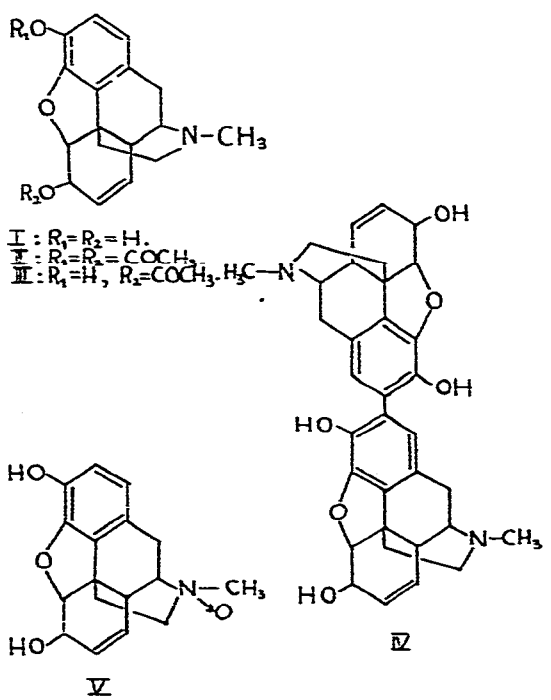
Morphine (I) and its diacetyl derivative diamorphine (II) are frequently used in aqueous oral and parenteral medicines. Diamorphine is known to undergo hydrolysis in aqueous solution to the monoacetyl derivative (III) and morphine itself[1]. Morphine is more stable in aqueous solution than diamorphine, but does decompose on



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heating in strong base[2] and at milder pH conditions on storage[2,3]. The degradation products of morphine are pseudomorphine (IV) and morphine-N-oxide (V)[2], pseudomorphine being by far the major degradation product.

Methods have been described for the selective determinations of morphine and its degradation products[3] and for the determination of diamorphine and morphine mixtures[4] by high-performance liquid chromatography (HPLC). However, the method described in this report can be used to determine both diamorphine and morphine and their degradation products with only minor variations to the liquid phase. The method, which involves an ion-pair technique, is rapid, easy to perform and highly pH dependant. This method has been used to study the stability of morphine and diamorphine oral mixtures and morphine intrathecal injections.

MATERIALS AND METHODS

Apparatus and materials

The separations of diamorphine and morphine mixtures were carried out on a Pye Unicam LC3 chromatograph coupled to a Hypersil ODS (particle size 5 μm) column, 10 cm × 5 mm I.D., via a Rheodyne 7125 injection valve with a 20-μl loop. Flow-rate was set at 2.0 ml min^{-1} and detection wavelength was 284 nm with a sensitivity of 0.32 a.u.f.s. Peaks were recorded on a Bryans 28000 chart recorder.

Diamorphine hydrochloride and morphine hydrochloride were purchased from Macfarlan Smith Ltd., and 6-monoacetylmorphine was kindly supplied by the Laboratory of the Government Chemist. All standards were used as supplied. The mobile phase, which consisted of 0.01 *M* aq. sodium pentanesulphonate–acetonitrile–orthophosphoric acid (69.5:30:0.5), was prepared freshly each day and degassed before use.

The separations of morphine sulphate and pseudomorphine were performed using an Ultrasphere ODS (particle size 5 μm) column, 25 cm × 4.6 mm I.D., coupled via a Rheodyne 7125 injection valve with 20-μl loop to a single-piston Altex 110A metering pump. The variable wavelength Pye Unicam LC3 UV detector was set at 281 nm and peaks were recorded on a potentiometric Tekman TE200 pen recorder.

Morphine sulphate B.P. (May and Baker) was used as supplied. Pseudomorphine was prepared by the method of Bentley and Dyke[5]; melting point and infrared spectrum agreed with published data[5]. Acetonitrile (Fisons, HPLC grade), sodium pentanesulphonate (Fisons, HPLC grade) and orthophosphoric acid (BDH, AnalaR grade) were used as supplied.

The mobile phase which afforded the best separation of morphine sulphate and pseudomorphine consisted of 0.01 *M* aq. sodium pentanesulphonate–acetonitrile–orthophosphoric acid (69.95:30:0.05) at a flow-rate of 1.0 ml min^{-1} (2000 p.s.i.). The recorder was set at 0.16 a.u.f.s. to record morphine peaks at 80–90% of the full scale deflection.

Procedure

In the study of diamorphine solutions, the following procedure was carried out. Serial dilutions of standard solutions of diamorphine, monoacetylmorphine and morphine were prepared freshly each day in the range of 0.1–2.0 mg ml^{-1}, and calibration curves were produced.

For the calibration of pseudomorphine concentration *versus* peak height and peak height ratio (pseudomorphine/morphine), the following solutions were prepared: A, containing 200 mg of morphine sulphate in 100 ml of water; B, containing 20 mg of pseudomorphine in 100 ml of the mobile phase. From solutions A and B, calibrator solutions C1–C6 were prepared, containing 0, 4, 8, 12, 16 and 20 μg ml^{-1} pseudomorphine respectively and each containing 200 μg ml^{-1} morphine sulphate.

In both studies, each standard solution was injected onto the column twice to give data points which were mean values of two measurements. When assaying samples under test, a full set of calibration points was plotted for each set of samples. The "best fit" straight line being plotted by regression analysis.

The relationship between acid concentration in the mobile phase and retention times of morphine and pseudomorphine was investigated, as was the effect of varying the concentration of the ion-pair reagent.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram produced by a mixture of the three standard materials morphine, diamorphine and 6-monoacetylmorphine under the conditions described for the separation of diamorphine and morphine mixtures. Total analytical time is short, retention times being morphine 37 sec, 6-monoacetylmorphine 58 sec and diamorphine 111 sec. The resolution is good, with an R_s value of 2.79 between 6-monoacetylmorphine and diamorphine, and a capacity factor, k', of 3.11. No internal standard is necessary as the reproducibility of the injection valve system is excellent; a co-efficient of variation of only 0.78% was found between 20 repeat injections of the same standard. Linearity of the calibration curves is good in the range studied and the limit of detection is 2.0 μg ml^{-1}.

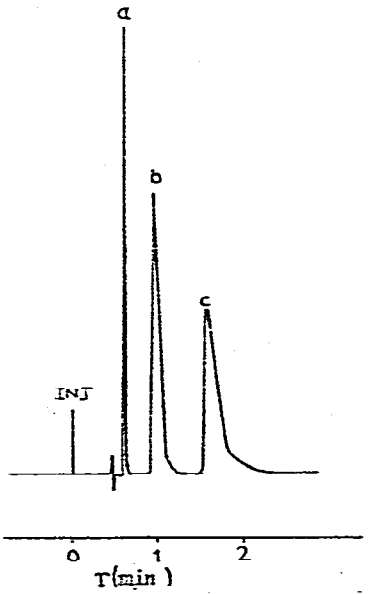

Fig. 1. Typical chromatogram of a mixture of morphine (a), 6-monoacetylmorphine (b) and diamorphine (c) standard solutions.

The plots of peak height ratio (pseudomorphine/morphine) and pseudomorphine peak height *versus* pseudomorphine concentration both gave direct linear relationships, under the adapted conditions described for this assay, with standard deviations of 0.00174 for peak height ratios ranging from 0 to 0.124 and 0.274 mm for pseudomorphine peak height values ranging from 0 to 23.5 mm. The reproducibility of the method, was assessed by running the standard solution C6 (20 μg ml^{-1} of pseudomorphine) five times. The standard deviation of five results was 0.31 μg ml^{-1} and the co-efficient of variation was 1.55%.

The limit of detection of pseudomorphine is 0.4 μg ml^{-1}. At the higher concentrations of pseudomorphine measured a further peak is detectable which is thought to be morphine-N-oxide.

The plot of pseudomorphine peak height (at 0.02 a.u.f.s.) *versus* concentration, between 0 and 1.0 μg ml^{-1} also gives a straight line.

Fig. 2 shows a chromatogram produced by a mixture of morphine and pseudomorphine standard solutions. Morphine-N-oxide is thought to be present as an impurity of pseudomorphine.

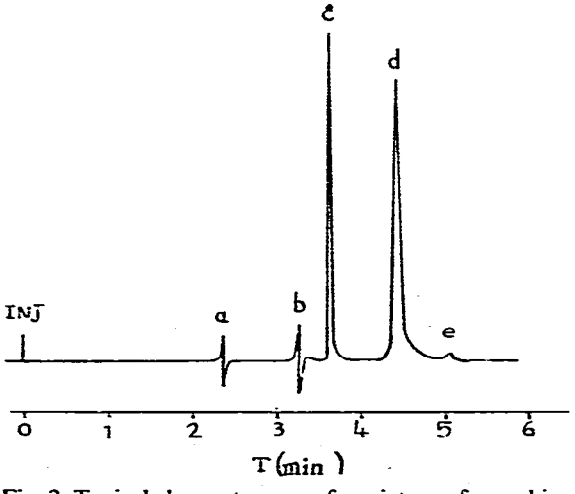

Fig. 2. Typical chromatogram of a mixture of morphine (c) and pseudomorphine (d) standard solutions. Other peaks: a = solvent front; b = unidentified; e = probably morphine-N-oxide.

Table I illustrates the effects of varying the mobile phase. The effect of acid concentration is highly significant giving the optimum R_s value at a pH of about 1.9. However, under these conditions the pseudomorphine is virtually non-retained and the solvent front interferes with the measurement of peak heights. The best chromatographic conditions exist at a pH of about 2.6. At this pH the pseudomorphine is well retained, a good separation of morphine and pseudomorphine occurs and peaks are sharp as shown by the R_s value. A value for N (the number of theoretical plates) of 5100 plates per metre was obtained under these conditions. Above this pH value the peaks tend to broaden as the retention volumes increase, leading to inaccuracies in quantitative measurements. At pH values between 2.10 and 2.20 peaks become inseparable and low R_s values result.

When the ion-pair reagent is removed from the mobile phase, both the mor-

TABLE I

THE EFFECTS OF VARYING THE MOBILE PHASE AND RESOLUTION OF MORPHINE AND PSEUDOMORPHINE

Solvent: acetonitrile–water–orthophosphoric acid.

$$R_s = \frac{t_R\,(B) - t_R\,(A)}{\frac{1}{2}\,[W\,(B) - W\,(A)]}$$

$$k' = \frac{t_R - t_m}{t_m}$$

where t_R (B) = retention time of pseudomorphine in seconds, t_R (A) = retention time of morphine in seconds, W (B) = peak width of pseudomorphine in seconds, W (A) = peak width of morphine in seconds and t_m = retention time of non-retained solute, taken as solvent front.

Solvent	*Concentration of sodium pentane-sulphonate (M)*	*pH*	*Retention volume (ml)*		R_s	*Capacity factor (k') for pseudomorphine*
			Morphine	*Pseudomorphine*		
30:70:0.00	0.01	5.95	4.02	6.18	5.31	1.75
30:70:0.05	0.01	2.58	3.78	4.47	5.13	0.96
30:70:0.10	0.01	2.40	3.53	3.95	4.17	0.77
30:70:0.25	0.01	2.14	3.27	3.28	0.50	0.52
30:70:0.50	0.01	2.10	3.18	3.10	−1.67	0.44
30:70:2.50	0.01	1.92	3.17	2.45	−28.67	0.15
30:70:0.05	Nil	2.20	4.53	8.75	4.96	3.01
30:70:0.05	0.005	2.30	4.02	5.30	3.67	1.39
30:70:0.50	Nil	1.90	3.15	3.05	−1.00	0.408

phine and the pseudomorphine are retained on the column for much longer periods of time and peaks become broader and start to tail. On adding more acid to the mobile phase without ion-pair reagent, peaks again become sharp and less well retained, but their separation is poor. The ion-pair reagent, consequently, is very important in this particular separation.

This method has been used in assessing the stability of an intrathecal morphine injection 2 mg in 10 ml. It has already been shown that this preparation is stable after three autoclave cycles and on storage at ambient and elevated temperatures (37°C) for greater than 3 months. The stability of the product is improved by sealing the ampoules under nitrogen.

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