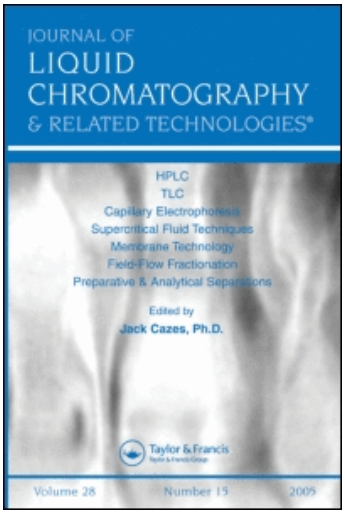

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A Stability Indicating HPLC Assay for Diamorphine in Aqueous Solution

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A STABILITY INDICATING HPLC ASSAY FOR DIAMORPHINE IN AQUEOUS SOLUTION

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ABSTRACT

A method is described for the rapid and simultaneous determination of diamorphine, and its hydrolysis products, 6-acetylmorphine and morphine using high-performance liquid chromatography. The column used was a Hypersil BDS C18 (100 x 4.6 mm) and the mobile phase comprised 80% aqueous potassium hydrogen phosphate (0.05M), 20% acetonitrile, adjusted to pH 3.0 with orthophosphoric acid. The flow rate was 1.0 $ml.min^{-1}$ and the limit of detection of each compound was 0.01 $\mu g.ml^{-1}$. The assay was linear over the range of 0.01 to 100 $\mu g.ml^{-1}$. The inter and intra-assay reproducibilities were better than 4% over this range of concentrations. The assay method was used to determine the effect of temperature on the degradation of diamorphine at pH 7.4 in aqueous solution.

INTRODUCTION

Diamorphine (3,6-diacetylmorphine, heroin) is a potent synthetic opiate analgesic which is commonly used for the relief of acute and chronic pain in man as well as being a drug of abuse [1]. Aqueous solutions of diamorphine hydrochloride are much less stable than solutions of other opiates such as morphine and codeine. Previous studies have shown that the drug is rapidly deacetylated in aqueous solution at alkaline pH to form 6-monoacetylmorphine [2-6] and is further hydrolysed slowly to form morphine [7]. Hydrolysis of the 3-acetyl group takes place both in the dark and in the cold or on exposure to daylight [8]. Because of its instability in aqueous solution, the use of diamorphine in *in vitro* experiments requires the careful monitoring of its breakdown products.

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A major problem in determining the stability of diamorphine in solution has been the lack of a specific assay to separate the 6-acetylmorphine and morphine. Early studies using quantitative TLC and GLC [9, 10] focussed on the breakdown of diamorphine but had difficulty in accurately measuring concentrations of the two hydrolysis products. HPLC methods have been more successful, but have often required derivatisation or dilution [4] or have required the use of a complex HPLC mobile phase containing ion-pairing agents or other additives [5,11-13].

In this study several types of HPLC column and mobile phase have been evaluated in order to develop a rapid and simple method for the separation of diamorphine and its hydrolysis products.

MATERIALS AND METHODS

Chemicals and reagents

Diamorphine hydrochloride and 6-acetylmorphine hydrochloride were purchased from Macfarlane Smith (Edinburgh, UK) and morphine hydrochloride was obtained from Boots Co. Ltd (Nottingham, UK). Acetonitrile, triethylamine and potassium dihydrogen orthophosphate were all HPLC grade (Fisons plc, Loughborough, UK). All other chemicals were of analytical grade or better.

Stock solutions

Diamorphine, morphine and 6-acetylmorphine stock solutions were prepared in acetonitrile : water (20:80 v/v) and stored at -30°C. Calibration standards were prepared by diluting the stock solutions of the drugs with HPLC mobile phase. A 10-point calibration line was used with concentrations in the range 0.01 to 100.0 $\mu g.ml^{-1}$ for all three compounds.

Chromatographic conditions

The HPLC apparatus consisted of two Gilson 305 pumps (Gilson Medical Electronics, Villiers-le-Bel, France), a Gilson 805 Manometric module, a Rheodyne model 7125 injector with 200 µl loop (Rheodyne, Cotati, CA, U.S.A.), a Gilson 115 UV detector, and a Gilson 712 data handling system. Several types of HPLC column packing materials were evaluated for an optimum separation of diamorphine and its hydrolysis products: Hypersil ODS, Hypersil BDS C18 and Hypersil CPS (Shandon HPLC, Runcorn, UK), Spherisorb ODS and Spherisorb CN (Phase Separations, Queensferry, UK). All the HPLC columns were 150 mm

x 4.6 mm i.d., with 5 µm particle size. For the BDS C18 column, the mobile phase consisted of 80 % (v/v) aqueous 0.05M potassium hydrogen phosphate (adjusted to pH 3.0 with orthophosphoric acid), and 20 % (v/v) acetonitrile. For the remaining three columns the mobile phase was 60 % (v/v) aqueous 0.05M potassium hydrogen phosphate (adjusted to pH 3.0 with orthophosphoric acid), and 39.9 % (v/v) acetonitrile containing 0.1% triethylamine. The mobile phase flow rate was 1.0 ml.min^{-1} and the injection volume was 50 µl.

Assay validation

Standard calibration lines for each compound were generated from the HPLC analysis of a range of calibration standards (see stock solutions section). Peak-areas of all three compounds (diamorphine, 6-acetylmorphine and morphine) were recorded. Linear regression analysis was performed to determine the slope, intercept and the correlation coefficient of the calibration lines.

The intra-assay precision was evaluated by analysing six replicate samples at each of three different concentration levels for each compound, as shown in Table 1. The inter-assay precision was evaluated by analysing replicate samples, identical to those used for the intra-assay validation, on three different days. The precision of the assay is expressed as a coefficient of variation (CV%) at each concentration point.

Stability of diamorphine in aqueous solution

The stability of diamorphine (100 µg.ml^{-1}) in aqueous solution (pH 7.4) was assessed at temperatures of 4°C, 25°C and 37°C. Aliquots (1.0 ml) of freshly made up diamorphine solution in aqueous sodium phosphate buffer (0.1 M, pH 7.4) were sealed in amber glass vials, which were stored at 4°C, 25°C or 37°C. Vials were removed at appropriate time intervals for analysis over a period of 14 days. The HPLC system was calibrated at the start, during, and at the end of the stability study. The decrease in diamorphine concentration and the increase in 6-acetylmorphine and morphine concentrations were determined by interpolation from the appropriate averaged calibration line.

RESULTS AND DISCUSSION

Chromatographic separation and validation of assay

The order of elution of the compounds, for all the HPLC columns evaluated was: morphine, 6-acetylmorphine and diamorphine. The Hypersil ODS and Spherisorb ODS columns produced

TABLE 1

Intra- and inter-day reproducibilities for the analysis of diamorphine, morphine and 6-acetylmorphine

Compound	spiked conc ($\mu g.ml^{-1}$)	intra-day variation (n=6) CV%	inter-day variation (n=18) CV%
diamorphine	0.1	2.8	4.2
	1.0	2.3	3.7
	10.0	0.8	2.3
	100.0	1.2	2.8
6-acetylmorphine	0.1	2.9	3.8
	1.0	2.0	2.1
	10.0	0.9	2.5
	100.0	1.1	1.9
morphine	0.1	2.6	2.7
	1.0	1.4	2.0
	10.0	1.6	2.8
	100.0	0.8	1.7

a very long retention time for diamorphine (>60 min), which was required in order to separate morphine from the solvent front. The Spherisorb CN column gave broad and tailing peak shapes for the compounds, particularly for diamorphine. Optimum separations were obtained with the Hypersil CPS or the Hypersil BDS HPLC columns, both of which gave baseline separation of the three compounds with symmetrical peaks shapes, within a run time of 5 min. The Hypersil BDS column was chosen in preference to the Hypersil CPS since the addition of triethylamine was not necessary in order to obtain non-tailing peaks.

The retention times on the Hypersil BDS column for diamorphine, 6-monoacetylmorphine and morphine were 4.2 min, 3.4 min and 2.8 minutes respectively. All the validation analyses were performed using the Hypersil BDS column. The intra-day and inter-day precision (expressed as a coefficient of variation) for the assay was better than 4% (n=6) for each compound at concentrations of 0.1, 0.5, 1.0, 5.0, 10, 25, 50, 75, 100 $\mu g\ ml^{-1}$. Details of the validation at 0.1, 1.0, 10.0, and 100.0 $\mu g\ ml^{-1}$ are shown in Table 1 . The limit of detection was defined as three times the signal-to noise-ratio, and was found to be 0.01 $\mu g\ mL^{-1}$ for all three compounds. Calibration lines showed good linearity over the range of concentrations between 0.1 $\mu g\ ml^{-1}$ and 100 $\mu g\ ml^{-1}$ with the R^2 value being greater than 0.99 for each compound. A typical chromatogram showing the separation of diamorphine, 6-monoacetylmorphine and morphine is shown in Figure 1.

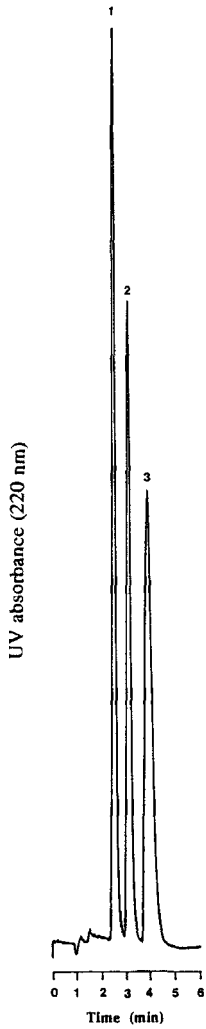

Figure 1: HPLC chromatogram of a standard solution containing 10.0μg.ml^{-1} of morphine (1), 6-acetylmorphine (2) and diamorphine (3).

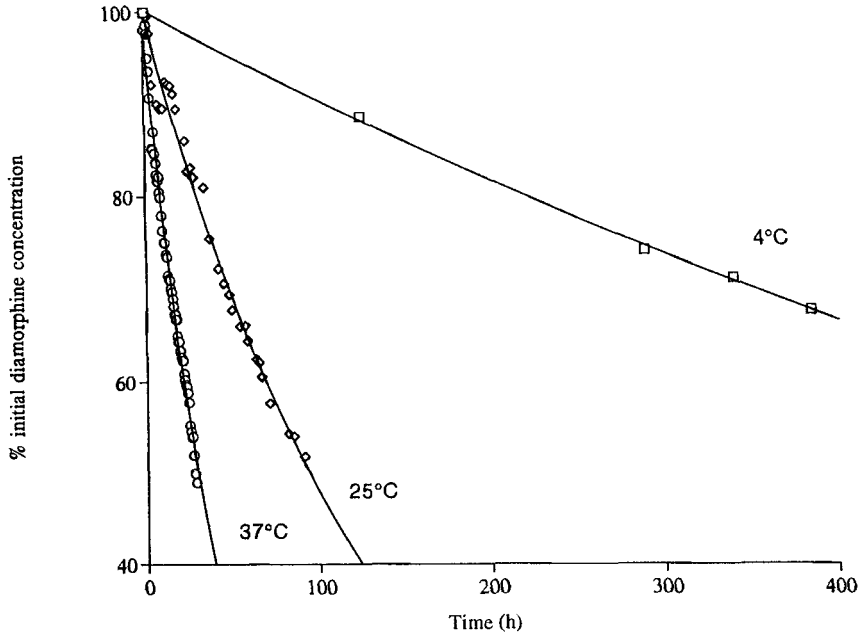

Figure 2: The effect of temperature on the stability of diamorphine in pH 7.4 buffer.

Assay application: stability of diamorphine in aqueous solution

The rate of deacetylation of diamorphine in aqueous solution was studied at 4°C, 25°C, 37°C. First order kinetics were apparent in all cases and the rate of degradation was substantially increased at higher temperature (Figure 2), with 6-monoacetylmorphine being the major degradation product and morphine a minor product. In all cases, the rate of appearance of 6-monoacetylmorphine mirrored the disappearance of diamorphine. The mean half-life of diamorphine in aqueous solution at pH 7.4 was 774 h at 4°C, 96.8 h at 25° C, 32.9 h at 37°C. A previous study of diamorphine stability at pH 4.9 in aqueous solution [2] indicated that the drug was considerably more stable than at neutral pH. Other studies of diamorphine stability at acid pH values have also concluded that a small but significant degradation of diamorphine will occur over the prolonged periods when the drug is made up in aqueous solution before use [13-16]. The data from this study suggests that there is a significant increase in the rate of hydrolysis of diamorphine at neutral pH compared with the acid pH values used in previous stability studies.

CONCLUSION

The method described provides a sensitive and specific assay for assessing the stability of diamorphine in aqueous solution. It has been successfully applied to study the stability of diamorphine over a range of temperatures.

REFERENCES

[1] D.B. Scott, in: Diamorphine: its chemistry, pharmacology and clinical use, D.B. Scott, ed. Woodhead-Faulkner, London, 1988, pp 55-68.

[2] E.A. Davey, and J.B. Murray, Pharmaceutical Journal 203: 737 (1969).

[3] G.R. Nakamura, J.I. Thornton, T.T. Nogchi, J. Chromatogr. 110: 81-89 (1975).

[4] H. Cooper, A.C. Mehta, R.T. Calvert, Pharmaceutical Journal 226: 682-683 (1981).

[5] I.M. Beaumont, Pharmaceutical Journal. 229: 39-41 (1982).

[6] D.A. Barrett, A.L.P. Dyssegaard, P.N. Shaw, J. Pharm. Pharmacol., 44: 606-608, (1992).

[7] D.A. Barrett, P.N. Shaw, S.S. Davis, J. Chromatogr. Biomed. Applic. 556: 135-145 (1991).

[8] L.A. Stevens and C.M. Wootton, in Diamorphine: its chemistry, pharmacology and clinical use, D.B. Scott (ed.), Woodhead-Faulkner, London, (1988), pp 15-43.

[9] E.W. Gold, J. Hosp. Pharmacy, 31, 12 (1973).

[10] H. Lim and S. Chow, J. Forensic Science, 23, 319 (1978).

[11] S.P. Joel, R.J. Osbourne, M.L. Slevin, J. Chromatogr. 430: 394-399 (1988)

[12] J.P. Davignon, G.K. Poochikian, J.C. Cradock, Int. J. Pharm. 13: 219-226 (1983).

[13] V.A. Jones, P.J. Haskin, O.A. Omar, A. Johnston, G.W. Hanks, P. Turner, Br. J. clin. Pharmacol. 23: 651 (1987).

[14] M.L. Kleinberg, M.E. DuaFala, C. Nacov, K.P. Flora, J. Hines, K. Davis, A. McDaniel, D. Scott, Am. J. Hosp. Pharm. 47: 377-81 (1990).

[15] M. Northcott, M.A. Allsopp, H. Powell, G.J. Sewell, J. Clin. Pharm. Ther. 16: 123-129 (1991).

[16] O.A.Omar, P.J. Hoskin, A. Johnston, G.W. Hanks, P. Turner, J.Pharm. Pharmacol. 41: 275-277 (1989).

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