Journal of Chromatography, 171 (1979) 371–376

CHROM. 11,521

SIMPLE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SEPARATION OF 3,6-DIACETYLMORPHINE HYDROCHLORIDE (HEROIN) AND HYDROLYSIS PRODUCTS

G. K. POOCHIKIAN* and J. C. CRADOCK

Pharmaceutical Resources Branch, Developmental Therapeutics Program, Building 37, Room 6D12 National Cancer Institute, NIH, Bethesda, Md. 20014 (U.S.A.)

(Received October 11th, 1978)

SUMMARY

The application of reversed-phase high-performance liquid chromatography to the measurement of 3,6-diacetylmorphine (DAM) hydrochloride and its degradation products is described. This method has been applied to study the kinetics of the DAM hydrolysis at 26 ± 0.1° and 48 ± 0.1°. The hydrolysis of DAM involved a two-step first-order sequential mechanism between pH 3 and 8.6. The first-order rate constants of each step at all pH levels have been determined. The pH rate profile was constructed from kinetic measurements and demonstrated that stability of DAM hydrochloride solutions was optimal at pH 4.3. This information is being applied to the development of parenteral dosage forms of DAM hydrochloride.

INTRODUCTION

The hydrolysis of 3,6-diacetylmorphine (DAM) hydrochloride to morphine (M) hydrochloride has been qualitatively confirmed by various investigators[1–4]. However, these procedures do not provide convenient simultaneous measurement of DAM and its hydrolysis products. A kinetic study of the hydrolysis of DAM hydrochloride in an aqueous system may elucidate the nature of the drug with respect to its structural stability at various pHs. The results of such an investigation ultimately can be used as a guide in the formulation of the drug in a stable delivery system. While gas–liquid chromatographic techniques have been utilized for this purpose, they require laborious extraction procedures from basic media[5–9]. Since DAM is unstable at alkaline pH, this approach is a potential source of error. Alternatively, high-performance liquid chromatography (HPLC) may provide many of the advantages (direct, selective, efficient and precise) that are desirable in quantitative studies.

Liquid chromatography has been used previously to separate morphine derivatives[9–11]. However, these methods generally used gradient elution and an alkaline mobile phase[9–10]. The method described here allows the simultaneous

* To whom correspondence should be addressed.

quantitation of DAM hydrochloride and its hydrolytic products by simple isocratic elution on a single analytical column. The separation was carried out in an aqueous solution at a pH which showed minimal degradation of DAM hydrochloride and eliminated time-consuming extraction procedures.

EXPERIMENTAL

Equipment

A modular high-pressure liquid chromatograph, including a constant-flow pump (Model 3500B, Spectra-Physics, Santa Clara, Calif., U.S.A.) was used to deliver eluent at a rate of 1 ml/min to a stainless-steel column (300 × 3.9 mm I.D.) packed with fully porous 10-μm silica particles bonded with a monomolecular layer of octadecylsilane (μBondapak C_{18}, Waters Assoc., Milford, Mass., U.S.A.). No attempt was made to control the temperature of the column. Injections were made with a rotary valve injector equipped with a 10-μl injection loop (Valco, Houston, Texas. U.S.A.). A variable wavelength ultraviolet detector, set at 235 nm and 0.1 a.u.f.s., was used to detect eluted compounds. The output signal was recorded with a strip chart recorder (OmniScribe, Houston Inst., Austin, Texas, U.S.A.).

Reagents

M hydrochloride, 6-monoacetylmorphine (MAM) hydrochloride, 3-monoacetylmorphine hydrochloride and DAM hydrochloride were provided by the National Institute on Drug Abuse (Rockville, Md., U.S.A.). 1,8-Bis(dimethylamino)naphthalene (Aldrich, Milwaukee, Wisc., U.S.A.) was used, as received, as the internal standard. Acetonitrile, "distilled in glass" quality (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.) and distilled water were filtered through 0.22-μm solvent resistant filters (Millipore, Bedford, Mass., U.S.A.). All other chemicals were of analytical reagent grade and were used without further purification.

Preparation of solutions

DAM hydrochloride was dissolved in 0.1 *M* phosphate buffer to yield a 0.5 m*M* concentration at the following pH values: 3.0, 3.5, 3.9, 4.5, 5.2, 5.9 and 7.0. A 0.01 *M* phosphate buffer was used at pH 8.6. The above solutions were filtered and promptly dispensed (1.2 ml) in 2-ml flint ampules, sealed and stored at 26 $\pm$ 0.1° or 48 $\pm$ 0.1°. Periodically, 400-μl samples were removed and diluted to 2.0 ml with the mobile phase containing 140 μg/ml of the internal standard, 1,8-bis(dimethylamino)naphthalene.

The mobile phase consisted of three volumes of acetonitrile and seven volumes of 0.015 *M* potassium dihydrogen phosphate adjusted to pH 3.5 with 2 *N* phosphoric acid. The column pressure at a flow-rate of 1 ml/min was *ca.* 800 p.s.i. Separations were affected isocratically at room temperature.

Peak height was used to quantitate DAM hydrochloride and its hydrolysis products. Standard curves comparing ratios of peak height of DAM, MAM and M to 1.12 μg of internal standard exhibited linear responses ($r > 0.9995$) in the working concentrations of 10–200 μg/ml. The peak height of DAM hydrochloride and each of its hydrolysis products was converted to concentration by comparison with standard curves. The method has a lower limit when required of 10–15 ng per injec-

tion and is suitable for the quantitation of DAM hydrochloride and its hydrolysis products.

RESULTS AND DISCUSSION

Chromatographic behavior

The hydrochlorides of M, MAM, DAM and the internal standard were eluted in that order. A representative chromatogram of a synthetic mixture of the hydrochlorides of M, MAM, DAM and the reference is shown in Fig. 1 and the retention data for each substance are listed in Table I.

The retention volumes [V_R (ml)], capacity factors (k') and separation factors (α) for DAM, MAM and M in hydrolyzed samples were identical to those determined for the synthetic mixture. 3-Monoacetylmorphine hydrochloride (V_R = 3.83 ml, k' = 0.64) was not detected in any of the hydrolyzed samples. This observation is in agreement with previous studies[1,4,7].

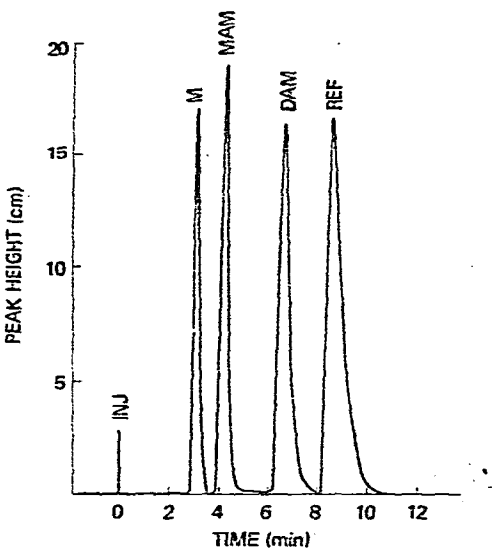


Fig. 1. HPLC chromatogram of morphine (M); 6-monoacetylmorphine (MAM); 3,6-diacetylmorphine (DAM) hydrochlorides, and 1,8-bis(dimethylamino)naphthalene (REF). For column conditions see text.

TABLE I

RETENTION VOLUMES (V_R), CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) OF VARIOUS SOLUTES

Column: 300 × 3.9 mm I.D. stainless-steel, μBondapak C_{18} (10 μm); mobile phase: acetonitrile–0.015 *M* potassium dihydrogen phosphate, pH 3.5 (3:7, v/v); flow-rate: 1 ml/min; detection: UV 235 nm.

Drug	V_R *(ml)*	k'	α
M hydrochloride	3.10	0.33	2.42
MAM hydrochloride	4.20	0.80	2.24
DAM hydrochloride	6.50	1.79	1.45
1,8-Bis(dimethylamino)naphthalene	8.40	2.60	

While searching for the optimum conditions, it was observed that retention of these compounds in the octadecylsilane reversed-phase system was primarily dependent on the proportion of the drug present in one form (ionized *vs.* unionized). Retention was also dependent on the relative lipid solubility of the drug in the stationary phase and the eluent. Elution of the compounds was also affected by changes in the buffer–acetonitrile ratio, pH and ionic strength, as well as the nature of the counter ion of the buffer. In general, an eluent of $5 > pH > 8.7$, less aqueous, and more strongly buffered, resulted in shorter retention times and sharper peaks. The relative order of elution was not affected by these adjustments. The presence of potassium ion *vs.* sodium ion in the aqueous solution exhibited similar phenomena but of smaller magnitude. Successful separation of these compounds was also achieved at high pH (*ca.* 9.0). However, this system was not utilized to avoid on-column deterioration[12]. The optimal eluent was pH 3.5, 0.015 M potassium dihydrogen phosphate buffer in 30% acetonitrile.

Kinetic studies

The main objective of this study was to determine the pH at which DAM hydrochloride was most stable. The information will be applied to the preparation of parenteral formulations of this agent. Also of interest was a determination of rate constants for both reactions since both rate constants involved in the conversion of DAM to morphine had not previously been reported.

Semilog plots of DAM hydrochloride concentration *vs.* time exhibited excellent linearity ($r > 0.99$) at pH 3.0–8.6. The results indicated that the disappearance of DAM hydrochloride was pseudo first-order at all pH levels investigated and was in accord with previous observations[3]. The observed half-lives at $48 \pm 0.1°$, T_{90} values at $26 \pm 0.1°$ and first-order rate constants at both temperatures are presented in Table II. The data obtained at $26 \pm 0.1°$ indicate that DAM is more stable at pH values between 3.5 and 5.2. However, the rate of hydrolysis is too rapid to formulate as a solution. Freeze dried formulations are being prepared.

The log k *vs.* pH profile (Fig. 2) for DAM hydrochloride was constructed from the data obtained at $26 \pm 0.1°$ and $48 \pm 0.1°$. At both temperatures the pH range of maximum stability was 4.0–4.5. At pH 8.6 and pH 3.0 the rates of hydrolysis were approximately 19 and 2.3 times greater than at pH 4.5.

The chromatograms of DAM at various time intervals indicated a two-step

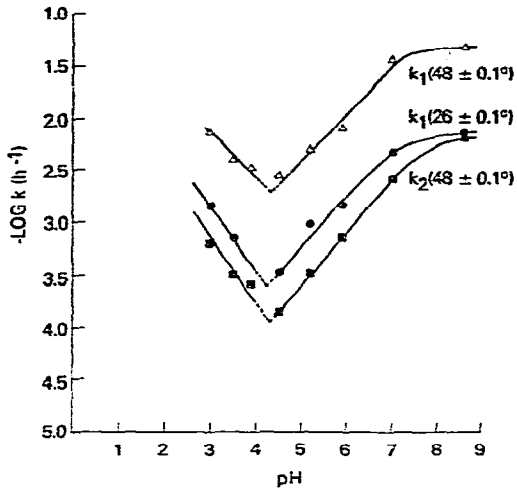
TABLE II

DISAPPEARANCE OF DIACETYLMORPHINE IN AQUEOUS SOLUTION

pH	*48 ± 0.1°*		*26 ± 0.1°*	
	$T_{\frac{1}{2}}$ *(days)* *	k_1 (h^{-1})	T_{90} *(days)* **	k_1 (h^{-1})
3.0	4	0.0072	2.5	0.00140
3.5	8	0.0049	6	0.00070
3.9	8.5	0.0030	—	—
4.5	9.5	0.0028	8	0.00034
5.2	6.25	0.0050	5	0.00097
5.9	3.6	0.0083	2	0.00140
7.0	0.75	0.0372	0.83	0.00480
8.6	0.5	0.0501	0.5	0.00760

* Half-lives of DAM hydrochloride at $48 \pm 0.1°$.

** 10% disappearance of DAM hydrochloride at $26 \pm 0.1°$.



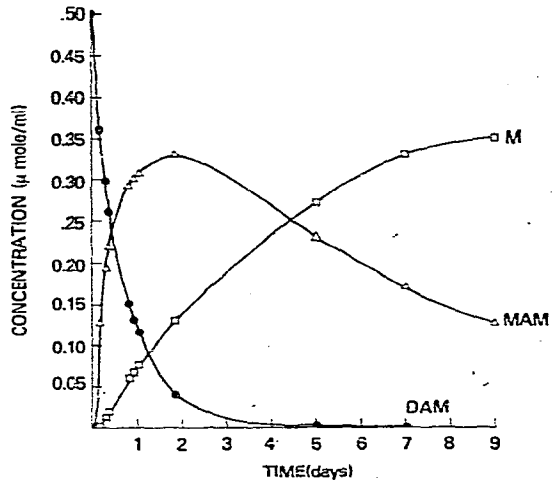


Fig. 2. Log k *vs.* pH profile for the hydrolysis of DAM hydrochloride (0.5 mM) at 26 ± 0.1° and 48 ± 0.1°. The k_1 and k_2 refer to the first and second reaction in consecutive pseudo first-order reactions, respectively.

Fig. 3. Concentration time curves for DAM hydrochloride (●); MAM hydrochloride (△) and M hydrochloride (□) at 48 ± 0.1° and pH 8.6.

sequential mechanism. A typical plot of disappearance of DAM and appearance MAM and M at 48 ± 0.1° and pH 8.6 is presented in Fig. 3. The rapid decrease of DAM is accompanied by an increase in MAM that reached a maximum value after 45 h and then decreased in an approximately linear fashion. After a time-lag of about 2 h the concentration of morphine rose gradually. This pattern suggested that consecutive first-order reactions were involved:

$$DAM \xrightarrow{k_1} MAM \xrightarrow{k_2} M$$

where k_1 and k_2 are pseudo first-order rate constants.

Several methods[13–16] have been described for the determination of the second rate constant of consecutive first-order reactions using dimensionless parameters and variables, such as: $\alpha = A/A_0$, $\beta = B/A_0$, $K = k_2/k_1$ and $\beta_{max.} = K^{K/(1-K)}$ (ref. 16). A_0 is the initial concentration of DAM while A and B are the concentrations of DAM and MAM respectively at time t. $\beta_{max.}$ was the maximum from the graph obtained from the plot of β *vs.* $(1-\alpha)$. The values of $\beta_{max.}$ were used for the determination of the K values graphically[16], which were subsequently used to evaluate the second rate constants, k_2, from $k_2 = K \times k_1$. Table III lists the values of k_2 and the corresponding values of $\beta_{max.}$ and K at 48 ± 0.1°, obtained at pH 3.0–8.6. Examination of Table III revealed that the most stable pH of DAM solution (4.5) had the highest $\beta_{max.}$ value. The k_2 values are also presented graphically in Fig. 2. These results indicate that the rate of conversion at 48 ± 0.1° of DAM to MAM proceeds about one order of magnitude faster than the hydrolysis of MAM to M. Both reactions proceed most slowly at pH 4.0–4.5.

The HPLC method is convenient since aqueous solutions may be directly injected. Extraction procedures[5–9] or alkaline solutions[9–10] are avoided. In addition to the kinetic studies described in this report, the HPLC method may have other pharmaceutical applications. The British Pharmacopoeia (B.P.) [17] does not require

TABLE III

SECOND RATE CONSTANTS — HYDROLYSIS OF DAM HYDROCHLORIDE AT 48 ± 0.1°

pH	$\beta_{max.}$	*K*	k_2 (h^{-1})
3.0	0.79	0.0875	0.00063
3.5	0.80	0.080	0.00032
3.9	0.80	0.080	0.00026
4.5	0.85	0.051	0.00014
5.2	0.82	0.070	0.00035
5.9	0.80	0.080	0.00067
7.0	0.82	0.070	0.00260
8.6	0.74	0.135	0.00676

chromatographic separation of DAM hydrochloride from its hydrolysis products. The 1973 B.P. method utilizes estimation of total phenolic substances as an index of purity. The maximum permissable level is 5% expressed as M base. Qualitatively, DAM hydrochloride is determined spectrophotometrically after hydrolysis to M. In contrast the reversed-phase HPLC method described in this report readily separates and detects less than 1% of MAM and of M in DAM samples. The procedure is also rapid and precise. The coefficient of variation for six individually weighed samples was determined over a six-week period and found to be 1.61% at 0.5 m*M* DAM hydrochloride.

ACKNOWLEDGEMENTS

We thank Dr. K. P. Flora of Pharmaceutical Resources Branch, NCI for helpful discussions and Mrs. Shirley Swindell for assistance in the preparation of this manuscript.

Dr. Robert Willette of National Institute on Drug Abuse generously supplied the necessary samples of DAM hydrochloride, MAM hydrochloride, 3-monoacetylmorphine hydrochloride and M hydrochloride.

REFERENCES

1 G. R. Nakamura and T. Ukita, *J. Pharm. Sci.*, 56 (1967) 294.
2 E. A. Davey, J. B. Murray and A. R. Rogers, *J. Pharm. Pharmacol.*, 20 Suppl. (1968) 515.
3 E. A. Davey and J. B. Murray, *Pharm. J.*, 203 (1969) 737.
4 P. E. Kamp and R. A. van Welsum, *Pharm. Weekbl.*, 108 (1973) 987.
5 J. M. Moore, *J. Chromatogr.*, 147 (1978) 327.
6 G. R. Nakamura, J. I. Thornton and T. T. Noguchi, *J. Chromatogr.*, 110 (1975) 81.
7 E. A. Davey and J. B. Murray, *Pharm. J.*, 207 (1971) 167.
8 D. A. Smith and W. J. Cole, *J. Chromatogr.*, 105 (1975) 377.
9 S. E. Hays, L. T. Grady and A. V. Kreugel, *J. Pharm. Sci.*, 62 (1973) 1509.
10 J. H. Knox and J. Jurand, *J. Chromatogr.*, 87 (1973) 95.
11 P. J. Twitchett, *J. Chromatogr.*, 104 (1975) 205.
12 V. Das Gupta, *J. Pharm. Sci.*, 67, No. 2 (1978) IV.
13 C. G. Swain, *J. Amer. Chem. Soc.*, 66 (1944) 1696.
14 K. R. Heimlich and A . N. Martin, *J. Amer. Chem. Soc.*, 49 (1960) 592.
15 A. A. Frost and R. G. Pearson, *Kinetics and Mechanism*, 2nd ed., Wiley, New York, 1961, p. 166.
16 E. H. Jensen and D. J. Lamb, *J. Pharm. Sci.*, 53 (1964) 402.
17 *The British Pharmacopoeia*, Her Majesty's Stationary Office, London, 1973, p. 153.