Determination of the anhydrous morphine content of kaolin and morphine mixture B.P.C.

KEITH HELLIWELL*, PENNY GAME, *Research and Development Laboratories. William Ransom & Son Ltd., Hitchin, Hertfordshire, U.K.*

Despite the widely accepted usage of kaolin and morphine mixture B.P.C., a satisfactory method of determining its anhydrous morphine content has never been proposed. This is attributed to a portion of the morphine being adsorbed onto the kaolin from which it cannot be quantitatively eluted (Dr D. C. Garratt personal communication).

The anhydrous morphine content of chloroform and morphine tincture B.P. is determined by a modified method (Helliwell & Sanders 1978) based upon the Radulescu colour reaction (Radulescu 1905) as modified by Adamson & Handisyde (1946). We have adopted this method for the determination of the anhydrous morphine content of the B.P.C. mixture. The limits for the anhydrous morphine content of chloroform and morphine tincture B.P. are 0·157–0·191% w/v, and from the incorporation rate of this tincture into kaolin and morphine mixture B.P.C., we would propose a limit for the content of anhydrous morphine of 0·0061–0·0078% w/v.

A simple, quantitatively reproducible method for determining the total anhydrous morphine content of the B.P.C. mixture is now described. We have also examined the ratio of 'free' to total morphine in this preparation. For this purpose two commercially available pre-packaged kaolin and morphine mixtures (200 ml bottles intended for direct sale) and three laboratory prepared samples; two from commercially available powdered premixes and a third from the individual components of the mixture were tested. The laboratory prepared samples were allowed to stand for not less than 12 h before assay to allow component equilibrium to occur.

For total anhydrous morphine content, a 20·0 ml portion from the homogeneous mixture was centrifuged and the supernatant transferred to a separator. 20 ml ethanol (96% v/v) was added to the residue and the whole mixed to a homogeneous paste. This was re-centrifuged and the supernatant liquid again transferred to the same separator as before. The process was repeated with a further 2 × 20 ml ethanol. To the contents of the separator were added 30 ml water and 5 ml dilute ammonia solution (10% w/w). This mixture was extracted with 3 × 25 ml chloroform, each chloroform fraction, after separation, being washed with the same 15 ml of a 2:1 mixture of water and ethanol (96% v/v). The combined chloroform fractions were evaporated just to dryness on a waterbath and 10 ml MHCl added with gentle warming to dissolve the residue as completely as possible. This acid extract was transferred to 100 ml volumetric flask with the aid of water and made to volume. To 20·0 ml of a filtered portion of this solution was added 8 ml of a freshly prepared 1·0% w/v solution of sodium nitrite in water. This mixture was allowed to stand in the dark for 15 min, after which 12 ml of dilute ammonia solution were added and the volume made to 50 ml with water. The extinction of a 4 cm layer of this solution was measured at the maximum at about 442 nm, using as a blank a solution prepared in the same manner and at the same time, but replacing the sodium nitrite solution with water. The content of anhydrous morphine was determined by reference to a calibration curve prepared from suitable portions of an accurately prepared 0·008% w/v solution of morphine in 0·1 M HCl, each being diluted to 20·0 ml with 0·1 M HCl and using the method described above commencing at the addition of sodium nitrite solution.

The total anhydrous morphine content of the mixtures is given in Table 1(a); replicate determinations were performed on all samples. Mixtures (i), (ii) and (iii) were prepared from chloroform and morphine tinctures with known anhydrous morphine contents and gave 100 ± 15% expected recovery of anhydrous morphine.

Table 1. Results from replicate assays for the anhydrous morphine content of kaolin and morphine mixture B.P.C.

Sample	(a) Total anhydrous morphine content of mixture by proposed assay method. (Suggested limits: 0·0061–0·0078% w/v) Individual results	Mean	(b) 'Free' anhydrous morphine content of dekaolinated soln. (% w/v) Individual results	Mean	(c) Ratio: 'free' total morphine in mixture
(i)	0·0071		0·0055		
	0·0071	0·0070	0·0055	0·0055	0·79
	0·0070		0·0055		
	0·0069		0·0054		
(ii)	0·0073		0·0052		
	0·0072	0·0073	0·0052	0·0052	0·71
(iii)	0·0073		0·0055		
	0·0072	0·0072	0·0055	0·0055	0·76
	0·0071				
(iv)	0·0064		0·0057		
	0·0064	0·0064	0·0057	0·0057	0·89
(v)	0·0074		0·0058		
	0·0072	0·0073	0·0058	0·0058	0·79

Key:
(i) Mixture made in laboratory from a commercially available powdered premix.
(ii) Mixture made in laboratory from a commercially available powdered premix.
(iii) Mixture made in the laboratory from the individual components of the mixture.
(iv) Commercially available pre-packaged mixture.
(v) Commercially available pre-packaged mixture.

* Correspondence.

The constituents of mixtures, such as kaolin and morphine mixture B.P.C., with high solids content are usually determined on a weight in weight basis. We were able to pipette the homogeneous mixture providing that the pipette was allowed to drain thoroughly. This is reflected in the recoveries of morphine from the mixtures, and we therefore recommend that the determination for total anhydrous morphine be on a weight in volume basis.

The 'free' morphine content (that in the dekaolinated solution) of the mixtures was determined by taking a portion of the homogeneous mixture and centrifuging it in such a way that 20·0 ml of the supernatant liquid could be pipetted into a separator. To this were added 5 ml ethanol (96% v/v), 5 ml water and 2 ml dilute ammonia solution (10% w/w). This mixture was extracted with 3 × 30 ml of a 2:1 mixture of chloroform and ethanol. Each chloroform fraction, after separation, was washed with the same 20 ml of a 1:1 mixture of ethanol and water. The method of assay was continued as previously described for the determination of the total anhydrous morphine content commencing at the words '. . . The combined chloroform fractions were evaporated . . .'.

The 'free' morphine content of the mixtures under test is given in Table 1(b). We found that only about 75% of the morphine content appears to be 'free' in solution.

March 20, 1980

REFERENCES

Adamson, D. C. M., Handisyde, F. P. (1946) Quart. J Pharm. 19: 350

Helliwell, K., Sanders, L. (1978) Pharm. J. 220: 224

Radulescu, D. (1905) Bul. Soc. Stinte Bucuresti 14: 602

On the mechanism of metabolic *N*-dealkylation. Isolation of a relatively stable carbinolamine

GERARD HUIZING*, JORDI SEGURA**, ARNOLD H. BECKETT***, *Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW 3 6LX, U.K. and **Division of Biochemistry and Biopharmaceutics, Institute of Research, Laboratorios Almirall, SA, Barcelona, Spain*

Current evidence does not indicate whether tertiary amines are dealkylated by initial α*C*-oxidation, or by initial *N*-oxidation and subsequent *N*-oxide dealkylation (Bickel 1969; Hucker 1973). In the case of clebopride (I, Table 1; Cleboril), a new benzamide drug (Prieto et al 1977; Roberts et al 1978), previous identification of in vitro metabolic products had indicated amide hydrolysis (product III), *N*-oxidation (product VI), *N*-dealkylation (product II), and *N*-dealkylation, followed by *C*-oxidation (product V) or *N*-oxidation (products VII and VIII) (Huizing & Beckett 1980; Huizing et al 1979b, 1980).

We now report that when I was incubated with 9000 *g* supernatant of liver homogenates of male NZW rabbits (Cowan et al 1976), and an extract of the incubation mixture subjected to t.l.c. (Huizing et al 1979a), metabolite IV was observed, in addition to the products mentioned above (R_F values are given in Table 1). A positive diazo-coupling reaction for IV indicated an intact aromatic amino group. Unlike the basic metabolic product III, but like the *N*-oxide VI, compound IV was extractable from incubation mixtures into chloroform, at all pH values, suggesting a neutral character for IV. Although IV is unstable at room temperature (one of the breakdown products of IV is I), compound IV may be purified by preparative t.l.c.

Treatment overnight with 2M sulphuric acid at 95 °C, decomposes IV to various unidentified products. Treatment with Zn/HCl of mixtures containing IV and VI, resulting from incubation of I with 9000 *g* supernatant of rabbit liver, gave a significant reduction of IV and VI, whereas treatment with $KMnO_4$ did not produce important changes (for reduction and oxidation procedures, see Huizing et al 1979b).

Field desorption (FD)*-mass spectrometry of IV gave an abundant molecular ion at *m/z* 389 (wire current 17 mA) (i.e. 16 atomic mass units higher than that of the parent compound I; see Huizing et al 1980), further ions were observed at *m/z* 390 (M + 1) and at *m/z* 412 (M + 23, i.e. M + Na) at a wire current of 13 mA; such ions are commonly observed in FD-mass spectrometry (Kirk et al 1976; Wilson 1977). The observed decrease in relative abundance for the ions at M + 1 and M + 23 and the initial appearance and further increase in abundance for the M^+ ion, when the wire current was increased from 13 to 17 mA, is in agreement with the rules proposed by Schulten & Beckey (1974). The mass spectrum of IV, determined under

* Present address: Laboratory of Clinical Pharmacy and Toxicology, Central Hospital Pharmacy for The Hague, P.O. Box 43100, 2504 AC The Hague, The Netherlands.

*** Correspondence.

* For the FD-mass spectrometry studies a double beam AEI MS 30 and a single beam AEI MS 50 instrument were used, in the direct inlet mode; the samples were coated on a tungsten emitter wire by the dipping technique of Schulten & Beckey (1974) from solutions in methanol; the reference beam was used for mass marking, using the electron impact mass spectrum of perfluorokerosene No. 4, high boiling grade.