J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 51S–53S Received May 1, 1968

SHORT COMMUNICATION

The determination of diamorphine by thin-layer chromatography and spectrophotometry

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THE assay of diamorphine in Diamorphine Injection B.P. is based on its hydrolysis to morphine followed by a colorimetric determination (Allport & Jones, 1942; Stephens, 1951; Hooper, Shaw & Tims, 1967). Stored solutions of diamorphine may contain O^3- and O^6-acetylmorphines as well as diamorphine and morphine (Davey, Murray & Rogers, 1967); all are converted into morphine in the first stage of the British Pharmacopoeial assay of the injection and thus contribute to the final morphine content.

Separation of opium alkaloids and their acetylated derivatives by thin-layer chromatography has already been reported (Mary & Brochmann-Hanssen, 1963; Vignoli, Guillot & others, 1965, 1966a, b) and the application of this technique to diamorphine, the acetylmorphines and morphine is discussed below.

EXPERIMENTAL

Aluminiumoxid DS-5 (Camag), pH 7·5–7·8, was heated at 100° (30 min) with distilled water. It was filtered through glass, then washed repeatedly with distilled water. This procedure removed absorbing impurities and reduced the "blank" absorbances at both wavelengths (see below) to 0·05 or less. Plates (20 × 20 cm) were spread with this material in the usual way to a thickness of 0·25 mm and were air-dried then activated at 120° for 1 hr.

The assay is carried out as follows.

Apply the sample in aqueous solution (10 μl containing 250–750 μg of diamorphine hydrochloride) by means of an Agla micrometer syringe, leaving about 2·5 cm on each side of the plate to eliminate edge effects; this allows room for the development of two sample solutions, a standard solution and a blank. Tanks lined with Whatman No. 1 paper impregnated with the running solvent are used and chromatograms are developed (ascending technique) with benzene–methanol–aqueous ammonia (0·880) (90 : 10 : 0·2 by vol.).

Solvent is allowed to ascend 15 cm from the starting line (45 min) then plates are air-dried (5 min). Alkaloids are located by viewing in ultraviolet light (254 mμ). Diamorphine appears as a faint yellow fluorescent spot at Rf 0·63; O^3-acetylmorphine, O^6-acetylmorphine and morphine, if present, appear at Rf 0·53, 0·35 and 0·06, respectively.

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Scrape the alkaloid spot into water (5 ml), mix (2 min) and centrifuge. Filter the solution through a MF Millipore membrane filter (no diamorphine was adsorbed on the filter) of mean pore size 0·22 μ in a Swinny filter holder. Elute similarly a blank area of alumina from the same plate, equal in size to that of the sample spot, and at a location on the plate with identical Rf. Measure the absorbance of the sample filtrate against the blank at 278·5 and 252 mμ, which are wavelengths of maximum and minimum absorption of diamorphine.

Calculate the concentration of diamorphine in the sample solution by comparison with the results obtained from a standard solution run on the same plate.

$$C_{sample} = C_{standard} \frac{(A_{278\cdot5} - A_{252})_{sample}}{(A_{278\cdot5} - A_{252})_{standard}}$$

where C represents the concentration of diamorphine hydrochloride, and $A_{278\cdot5}$ and A_{252} are the absorbances at 278·5 and 252 mμ, respectively.

RESULTS

The procedure has been applied to solutions containing 10 mg of diamorphine hydrochloride per ml. Sample A, of pH 4·02, was sterilized by filtration through a MF Millipore filter. Sample B was sterilized by heating with a bactericide (0·002% phenylmercuric nitrate); its pH was 4·45 before and 2·65 after sterilization. The chromatograms from sample A showed only one spot, at Rf 0·6, whereas sample B showed two spots, at Rf 0·6 and 0·3, corresponding to diamorphine and O^6-acetylmorphine. Neither phenylmercuric nitrate nor sodium metabisulphite caused any interference. The results of six replicate assays of each sample are given in Table 1. The absorbance difference, $A_{278\cdot5} - A_{252}$, was directly proportional to the concentration of diamorphine in the range of concentrations studied.

TABLE 1. RESULTS FOR DIAMORPHINE INJECTION
(mg of diamorphine hydrochloride per ml)

	Sample A	Sample B
Plate	Sterilized by filtration	Sterilized by heat
I	9·97	9·37
II	10·09	9·46
III	9·75	9·25
IV	9·90	9·55
V	10·05	9·42
VI	10·05	9·60

The concentrations before sterilization were 10·00 mg/ml.
The coefficient of variation, based on 12 determinations of degraded and undegraded solutions, was 1·3%.

The percentage recovery of diamorphine from the chromatograms varied with each plate and with different batches of alumina, but it was always in the range 93–98%.

Acknowledgement. We thank Macfarlan Smith Limited for samples of diamorphine hydrochloride.

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