J. Pharm. Pharmac., 1969, **21**, *Suppl.*, 49 S–51 S *Received April* 30, 1969

SHORT COMMUNICATION

The gas chromatographic determination of adrenaline in pharmaceutical products

P. F. G. BOON AND A. W. MACE

Analytical Department, CIBA Laboratories Ltd., Horsham, Sussex, England

The determination of adrenaline in formulated products is complicated by the possibilities of decomposition, racemization and reaction with the bisulphite ion commonly incorporated in such products. Of the many available methods that of Welsh (1955) attempts to deal with all these points by a combination of acetylation and solvent extraction followed by measurement of weight and optical rotation; it has been adopted by the B.P. and the U.S.P. The procedure is rather insensitive and its specificity has been questioned by Higuchi, Sokoloski & Schroeter (1959) who suggest the addition of a liquid - liquid chromatographic purification stage.

The gas chromatography of catecholamines has been described by several workers, though not its direct application to formulated products. The potential specificity and sensitivity of the method lead to its selection for further study. Formulated products are usually aqueous and often dilute (e.g. injection of lignocaine and adrenaline 0·00125%) hence a concentrating stage must be introduced before chromatography.

Solvent extraction of adrenaline

Temple & Gillispie (1966) have described the solvent extraction of adrenaline with the aid of the ion-pairing compound di-(2-ethylhexyl)phosphoric acid (DEHP). We have shown that the extraction of adrenaline (2·5 mg) from aqueous buffer (pH 7·4) is essentially complete with four equal volumes of 1% DEHP in chloroform.

Gas chromatography of adrenaline

Derivative formation is essential and the silanization procedure of Capella & Horning (1966) with NO-bis(trimethylsilyl)acetamide was selected for this work; the reaction is said to give the tri-*O*-trimethylsilyl derivative. Rates of silanization in the absence and presence of DEHP are shown in Fig. 1. The reaction was shown to be essentially stoichiometric and complete after 120 min by the determination of the derivative content of reaction mixtures in terms of a purified specimen of the tri-*O*-trimethylsilyl derivative obtained from a preparative scale reaction. The gas chromatographic separation of a mixture of noradrenaline, adrenaline and isoprenaline as their trimethylsilyl derivatives is shown in Fig. 2.

Proposed method

Materials, phosphate buffer (pH 7·4): add 0·2M potassium dihydrogen phosphate (500 ml) to 0·2N sodium hydroxide (391 ml), dilute to 1 litre with water and mix. *Extracting solvent:* 1% v/v di-(2-ethylhexyl)phosphoric acid in chloroform. *Silanizing reagent:* mix equal volumes of dry pyridine and NO-bis-(trimethylsilyl)acetamide, add an appropriate amount of methyl myristate as an internal standard.

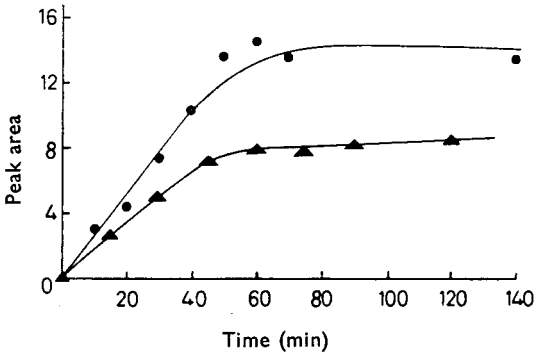

FIG. 1. Rate of silanization of adrenaline in the presence; —●—, and absence; —▲—, of DEHP.

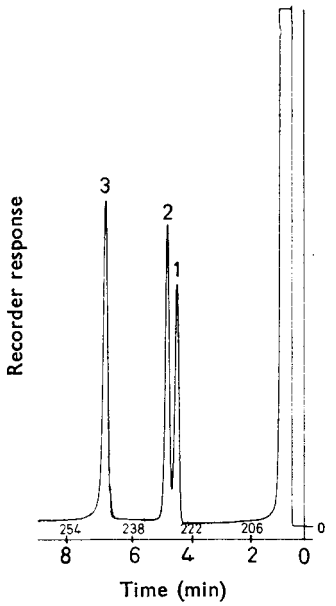

FIG. 2. Gas chromatographic separation of (1) Noradrenaline, (2) Adrenaline, (3) Isoprenaline as their silanized derivatives.

Procedure (suitable for concentrations down to 0·01%). To a volume of sample containing 10 mg of adrenaline add sufficient phosphate buffer to produce pH 7·4 and a minimum volume of 10 ml. Extract with 4 × 10 ml of extracting solvent, combine the extracts and evaporate them to near dryness on a water bath, removing the last 1–2 ml of chloroform in a current of air. Add 2·0 ml of silanizing reagent to the residue, stopper and set aside for 2 h with occasional shaking. Concomitantly prepare a standard by treating a suitable quantity of adrenaline similarly. Gas chromatograph suitable volumes of standard and sample under the following conditions:

Column: 5% O.V. 17 (a partially phenylated polysiloxane) 5 ft supported on acid-washed, silanized Gas Chrom P packed in a glass column, internal diameter 4 mm; column temperature, initially 190°, programming at 8°/min to 250°; injection port temperature, 210°; gas flow, 45 ml nitrogen/min; load, 2 μl.

(The instrument used for this investigation was a Pye 104 Chromatograph with flame ionization detector.)

Obtain the adrenaline content of the sample by comparison of the area of the peaks due to methyl myristate and silanized adrenaline in both the sample and standard chromatograms.

The general method is modified to deal with preparations containing less than 0·01% adrenaline. The sample aliquot may be reduced to as low as 0·25 mg adrenaline and extracted with 0·1% DEHP in chloroform. The chromatography conditions are adjusted to (i) column temperature 180°, (ii) temperature programming 4°/min.

Table 1. *Application of the proposed method to formulated products*

Formulation	Adrenaline % w/v Declared	Found
Adrenaline injection B.P.	0·1	0·098
Adrenaline solution B.P.	0·1	0·105
Compound spray of adrenaline and atropine B.P.C. ..	0·444	0·438
Zinc sulphate and adrenaline eye drops B.P.C.	0·05	0·0514
Injection of lignocaine and adrenaline	0·0005	0·00047
Injection of procaine and adrenaline	0·002	0·00198

Table 2. *Comparison of proposed method with U.S.P. XVII and biological methods on degraded preparations*

	Adrenaline % w/v			
	Initial	GLC	U.S.P.	Biological
Sample 1 ..	1·00	0·95	0·89	0·90
2 ..	1·00	0·51	0·43	0·50
3 ..	2·00	1·42	—	1·25
4 ..	1·00	0·99	0·93	—

Results

The scope of the method for control purposes has been demonstrated by its application to a number of freshly prepared formulations with adrenaline contents in the range 0·5 to 0·0005%, the results are given in Table 1. Whilst the method will not detect racemization in preparations that have undergone decomposition as a result of prolonged or unsatisfactory storage, it is of value in the examination of degraded preparations which are not susceptible to racemization. The results obtained by applying the method to degraded preparations, formulated to be optically stable, are compared in Table 2 with those obtained by the U.S.P. method and a biological method (pithed rat). Thus the method is valid in preparations where up to 50% degradation has occurred.

The reproducibility of the method is estimated to be $\pm 5\%$ for preparations containing 0·05% adrenaline and above and $\pm$ 10% for lower concentrations.

Acknowledgment

We wish to thank N. J. P. Winsey for the biological determinations.

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