J. Pharm. Pharmac., 1974, **26**, 197–200 *Received October* 16, 1973

An assay for phenylephrine applicable to stability studies

E. SHOTTON AND D. J. PRIAULX

Department of Pharmaceutics, The School of Pharmacy (University of London), Brunswick Square, London WC1N 1AX, U.K.

An assay specific for phenylephrine, in the presence of decomposition products, is described. The procedure depends on the separation of the active constituent by cationic exchange resin and the subsequent determination of the ultraviolet absorbance at two selected wavelengths. The method was evaluated using a grossly decomposed buffered solution of phenylephrine hydrochloride, and it was found that phenylephrine could be assayed selectively under these severe conditions. Several other reported methods were also evaluated under these conditions.

The assay of phenylephrine has been frequently referred to in the literature (Auerbach, 1950; Koshy & Mitchner, 1963; Levine & Doyle, 1967). One method has been specifically recommended for use in stability studies (Schriftman, 1959); this involved paper chromatographic separation of the intact phenylephrine followed by determination by photoelectric densiometry. Kelly & Auerbach (1961) used a cationic exchange resin to effect separation of phenylephrine from interfering substances, followed by a colorimetric assay employing diazotized *p*-nitroaniline or Millon's reagent. A colorimetric assay has been used to study the effect of acetylsalicylic acid on phenylephrine degradation in solid formulations (Troupe & Mitchner, 1964). The B.P. 1968 assay for phenylephrine hydrochloride injection is dependent upon the absorbance of an accurately diluted sample measured at the absorption maximum at 273 nm. Any assay method used for stability studies must differentiate between phenylephrine and its decomposition products; two of these products have recently been identified as isoquinoline derivatives (Millard, Priaulx & Shotton, 1973). An assay has been developed based on spectrophotometric measurements following an initial separation with a cationic exchange resin. It was compared with several reported methods, using a decomposed solution.

METHOD AND RESULTS

Chromatography column

This was prepared from a 100 ml pipette. Dimensions: overall length 18·5 cm, reservoir (50 ml capacity) 7·5 cm × 3 cm, column 10 cm × 5 mm i.d., with glass wool plug at base, connected via plastic sleeving to a capillary outlet; this controlled the flow rate and was required to be within the range 0·6–0·9 ml min^{-1}.

Ion exchange resin

500 g of the ion exchange resin, Zeokarb "225", 50–100 mesh, 1% DVB (The Permutit Co. Ltd.), was washed with 2 litres of 3M hydrochloric acid for 15 min. The acid was filtered and the resin washed twice more with fresh acid after which the

resin was washed repeatedly with distilled water until acid free. This washed resin was used within two weeks.

Separation procedure

About 2 g of the washed resin was packed into the column and 20 ml of distilled water was used to wash the packed resin. A phenylephrine solution containing 5 mg of phenylephrine hydrochloride in 2 ml was diluted to 20 ml with distilled water and passed through the column at a flow rate of approximately 0·75 ml min^{-1}. The column was washed with 100 ml of distilled water, added in 10 ml aliquots, draining each wash just to the top of the resin. The phenylephrine was eluted with 0·2N hydrochloric acid AR, and the first 50 ml collected. The efficiency of this separation procedure was checked using the t.l.c. system described previously (Millard & others, 1973). The main decomposition product (a mixture of 1,2,3,4-tetrahydro-4,6-dihydroxy 2-methylisoquinoline and the -4,8-dihydroxy analogue) was completely retained on the resin, as were the other trace constituents of a decomposed solution except that a small amount of 2-methylisoquinoline-6(2H)-one and the -8(2H)-one was eluted with the phenylephrine. The conditions for the separation are such that a minimal amount of contaminant is eluted along with total recovery of phenylephrine.

Spectrophotometric assay procedure

A Hilger Watts, model H700, manual ultraviolet spectrophotometer was used for the quantitative determination of the eluted phenylephrine, using matched 1 cm silica cells and 0·2N hydrochloric acid in the blank cell.

(*a*) *Freshly prepared solutions.* The absorbance of the collected eluent was measured at 273 nm and compared to a series of standard solutions of phenylephrine hydrochloride in 0·2N HCl. This confirmed that Beer's Law was obeyed, and the percentage recovery from the column was determined at 273 nm.

(*b*) *Degraded solutions.* The absorbance of the collected eluent was measured at 273 nm and 300 nm. The absorbance of the interfering decomposition product (2-methyl isoquinoline 6(2H)-one and its 8(2H) analogue) had the same value at both 273 nm and 300 nm while the absorbance of phenylephrine was negligible at 300 nm (Fig. 1). The corrected absorbance reading was obtained by direct subtraction.

i.e. Absorbance (corrected) = Absorbance (273 nm) − Absorbance (300 nm).

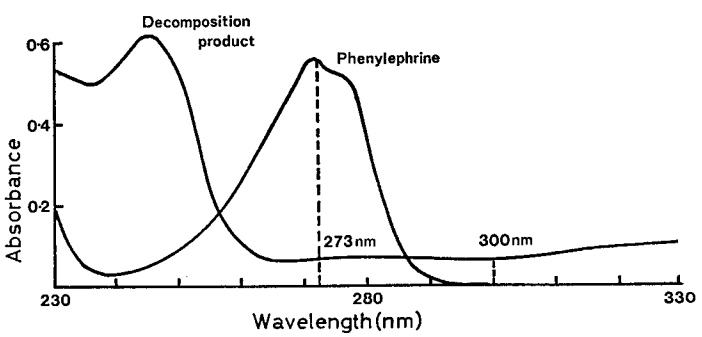

FIG. 1. Ultraviolet absorption spectra of phenylephrine and a minor decomposition produc (solvent: 0·2N HCl in distilled water).

The spectrum of this contaminant remained unchanged throughout our studies; where a correction was necessary it was usually small and only accounted for about 2% of the total absorbance at 273 nm. The corrected absorbance was then used to determine the concentration of phenylephrine from a suitable calibration curve, as in (*a*) above.

A sample from a freshly prepared solution was passed through the resin column and assayed. After seven repetitions the mean recovery was found to be 100·1% with a coefficient of variation of 0·4%.

Comparison of the described assay with other methods

A degraded solution of phenylephrine was prepared. 500 mg of phenylephrine hydrochloride was heated at pH 6·8 and 95° until approximately 50% of the phenylephrine content had decomposed. The oxidation products were separated from the phenylephrine by the column chromatographic separation described by Millard & others (1973) and the various fractions were shown by t.l.c. to be free from phenylephrine. A "synthetic" degraded solution was prepared by dissolving the whole of the isolated oxidation products and 250 mg of fresh phenylephrine hydrochloride in phosphate buffer at pH 6·8 to a total volume of 100 ml. The ultraviolet spectrum of this solution over the range 230–330 nm was similar to that of a phenylephrine solution which was half decomposed, and the prepared solution was assayed by several reported methods (Table 1).

Table 1. *Analysis of a "synthetic" degraded phenylephrine solution containing 2·5 mg of phenylephrine hydrochloride per ml using various assay procedures.*

Source of assay	Assay type	Det. concn of phenylephrine HCl mg ml^{-1}	Number of determinations	Coefficient of variation
Koshy & Mitchner (1963)	colorimetric	3·98	8	0·37
Kelly & Auerbach (1961)	Ion exchange, colorimetric	4·03	5	0·96
Levine & Doyle (1967), as modified by Kaistha (1970)	Ion pair-formation, partition, and ultraviolet	5·35	3	—
Troupe & Mitchner (1964)	Colorimetric, solvent extraction	2·19	9	11·2
B.P. 1968	Ultraviolet	12·2	7	0·35
This work	Ion exchange ultraviolet	2·48	7	1·00

The 4-amino antipyrine colorimetric method (Koshy & Mitchner, 1963). 2% sodium tetraborate was used to maintain a constant pH for maximum colour stability. Variations in colour stability were overcome by the measurement of colour intensity 15 min after colour development.

An ion exchange chromatographic method (Kelly & Auerbach, 1961). Amberlite 1R–120 (50–100 mesh) was used to achieve separation of the drug. Using a freshly prepared solution, recovery of the phenylephrine from the column was 98·9 $\pm$ 0·9%. Colour development was obtained for assay purposes using Millon's reagent due to the possibility of catecholamines being present.

Ion pair formation method (Levine & Doyle, 1967, as modified for isoprenaline by Kaistha, 1970). This method involved the extraction of phenylephrine quantitatively by formation of an ion-pair with di-(2-ethylhexyl) phosphoric acid. Complete extraction into the ether phase occurred in the presence of a potassium phosphate buffer (pH 6·0). The phenylephrine was re-extracted into the aqueous phase using 0·1N HCl and measured spectrophotometrically at 273 nm.

DISCUSSION

Zeokarb "225" ion exchange resin completely separated phenylephrine from the main decomposition product, 1,2,3,4-tetrahydro-4,6 (and 4,8) -dihydroxy-2-methyl isoquinoline, but occasionally a small percentage of another decomposition product could not be separated from the phenylephrine so the subsequent assay was designed to allow for any necessary correction. The assay of a degraded solution of known phenylephrine strength showed that the procedure was selective even in the presence of large quantities of decomposition products. Other assays were less accurate under these extreme conditions. Troupe and Mitchner's method was selective; this was to be expected as the secondary amino-group is lost during the oxidation process. The method is based on the formation of a coloured dithiocarbamate using a carbon disulphide reagent. It is difficult to carry out as the organic layer, composed of benzene, isopropyl alcohol, and carbon disulphide, is filtered through Whatman No. 42 filter paper, before the spectrophotometric determination of the coloured dithiocarbamate present in this phase. Colour stability was variable and evaporation of the volatile solvent can occur during this procedure. This is reflected in the large standard deviation limiting the value of the method. The separation procedures in the Kelly and Auerbach, and the ion pair formation methods were less efficient at removing the decomposition products; in each of the above methods one decomposition product was not removed at all, and the subsequent quantitative determinations were not selective, resulting in large errors when grossly decomposed solutions were assayed. The B.P. method, based solely on spectrophotometric measurement of the active drug at 273 nm, without any separation stage, was especially susceptible to the absorbance characteristics of the decomposition products, and could not be used to detect decomposition in even slightly decomposed solutions. In the Koshy and Mitchner method, although the colorimetric procedure is fairly selective for phenols with a free *para* position, the method cannot be used where very large amounts of decomposition products are present.

The method described in this paper is to be preferred for use in a quantitative stability study at elevated temperatures where decomposition is appreciable.

Acknowledgement

We are grateful to the Guernsey Education Council for a research grant to D.J.P.

REFERENCES

AUERBACH, M. E. (1950). *J. Am. pharm. Ass. (Sci. Edn)*, **39**, 50–52.
British Pharmacopoeia (1968). London: The Pharmaceutical Press.
KAISTHA, K. K. (1970). *J. pharm. Sci.*, **59**, 241–248.
KELLY, C. A. & AUERBACH, M. E. (1961). *Ibid.*, **50**, 490–493.
KOSHY, K. T. & MITCHNER, H. (1963). *Ibid.*, **52**, 802–803.
LEVINE, J. & DOYLE, T. D. (1967). *Ibid.*, **56**, 619–622.
MILLARD, B. J., PRIAULX, D. J. & SHOTTON, E. (1973). *J. Pharm. Pharmac.*, **25**, *Suppl.*, 24P–31P.
SCHRIFTMAN, H. (1959). *J. Am. pharm. Ass. (Sci. Edn)*, **48**, 111–113.
TROUPE, A. E. & MITCHNER, H. (1964). *J. pharm. Sci.*, **53**, 375–379.