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Short Communication

p-Benzoquinone as a reagent for determining some catecholamines

M. A. KORANY[1]*, A. M. WAHBI[2] and M. H. ABDEL-HAY[1]

[1] *Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt*
[2] *College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*

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Introduction

p-Benzoquinone has been widely used in organic synthesis, but has found few applications in drug analysis. It is characterized by its yellow colour and can be reduced in mild conditions to the colourless hydroquinone. Hofmann [1] described the first 1,4-addition reaction to a quinone: *p*-quinone was heated with aniline in alcohol to give 2,5 dianilino-1,4-benzoquinone and hydroquinone. The reactions of *p*-benzoquinone with nitroanilines [2] and with chloromethylanilines [3] have been studied. *p*-Benzoquinone reacts with *o*, *m* and *p* nitroanilines in dilute HCl to give reddish brown adducts which, if kept in contact with the mother liquor for some weeks, change into the nitroanilino-*p*-benzoquinones [2].

The use of *p*-benzoquinone for the determination of some drugs has been little studied. Coloured quinhydrone complexes formed during its freezing with indole, tryptophan and indoleacetic acid have been described [4]. Johnson and Nunn [5], using an earlier method [6], developed a technique for determining calcium or sodium cyclamate in canned fruits and fruit products. The method was based on the acid hydrolysis of cyclamate to cyclohexylamine, extraction of the amine into chloroform, and reaction with *p*-benzoquinone. The product, 2-(cyclohexylamino)-1,4-benzoquinone, was determined spectrophotometrically at 493 nm. A modified method for the determination of sodium cyclamate used quinhydrone after hydrolysis in acid dimethylsulphoxide [7, 8]. *p*-Benzoquinone was used for the colorimetric identification and determination of phenothiazine derivatives [9, 10]. The intensity of the colour developed depended on the acid used and its concentration. The most stable and sensitive colour was obtained with pure or 75% aqueous phosphoric acid. Recently, Benson and Spillane [11] investigated the reaction of *p*-benzoquinone with a wide range of amines. The method was based on the dissolution of the amine in an organic solvent (e.g.

* To whom correspondence should be addressed.

chloroform), the absorbance (λ_{max} 478–510 nm) of the product of its reaction with 1% ethanolic *p*-benzoquinone at 60°C being determined.

This paper describes the use of *p*-benzoquinone for the colorimetric determination of adrenaline, noradrenaline, isoprenaline sulphate and methyldopa. The coupling reaction between these catecholamines and *p*-benzoquinone was carried out in buffered aqueous/alcoholic solutions at room temperature.

Experimental

Apparatus

A Beckman double-beam spectrophotometer (Model 24) was used with glass cuvettes of 10 mm pathlength.

Reagents

p-Benzoquinone solution, 1% w/v in ethanol, was freshly prepared. McIvaine's citric acid–phosphate buffer solutions [12] were used. Adrenaline, or noradrenaline, and/or methyldopa (20 mg) were dissolved in 5 ml 0.1 M HCl. The solution was made up to 100 ml with pH 6 buffer. For isoprenaline sulphate, pH 5.4 buffer was used.

Procedure for drug solutions

To a 5-ml portion of drug solution in a 25 ml volumetric flask 2 ml pH 6 buffer (for isoprenaline sulphate pH 5.4 buffer) and 2 ml of *p*-benzoquinone reagent were added. The blank solution used 5 ml of the appropriate buffer solution in place of the sample. After 30 min at room temperature, the flask was made up to volume with ethanol and the absorbance of the solution measured against the blank at 502 nm for isoprenaline sulphate, at 485 nm for methyldopa and noradrenaline, and at 495 nm for adrenaline. The concentration of the drug was calculated from the corresponding calibration graph prepared using a reference substance, or from the value of $A^{1\%}_{1\,cm}$.

Procedure for isoprenaline sulphate and methyldopa tablets

Twenty tablets were weighed and powdered. An accurately weighed amount of the powder, equivalent to *ca.* 200 mg isoprenaline sulphate or methyldopa, was extracted with 0.1 M HCl by washing through a filter paper into a 100 ml volumetric flask. The extract was diluted to the mark with 0.1 M HCl, and 5 ml of this solution transferred to a 100 ml calibrated flask and diluted to the mark with pH 5.4 buffer (for isoprenaline sulphate) or pH 6 buffer (for methyldopa). The procedure described above for drug solutions was then used, starting with 10 ml of the diluted extract.

Procedure for adrenaline or noradrenaline in ampoules

An accurately measured volume of adrenaline or noradrenaline solution from the ampoules, equivalent to 20 mg adrenaline or noradrenaline, was placed in a 100 ml calibrated flask, and added up to the mark buffer pH 6. Starting with 5 ml of this solution the procedure for drug solutions described above was applied.

Results and Discussion

Isoprenaline sulphate, methyldopa, adrenaline and noradrenaline are phenolic drugs having a side chain with either a free amino group or a partially substituted alkylamine

group. When allowed to react with ethanolic *p*-benzoquinone solution, they were found to give a reddish-brown colour. This reaction provided a quantitative procedure for the colorimetric determination of the drugs. The effect of the buffer pH on the drug–benzoquinone reaction was examined for each compound. Figure 1 and Table 1 show the optimum pH value for each drug. Figure 2 shows the absorption spectra in the range 430–550 nm of the coloured products. The maximum wavelengths are given in Table 1.

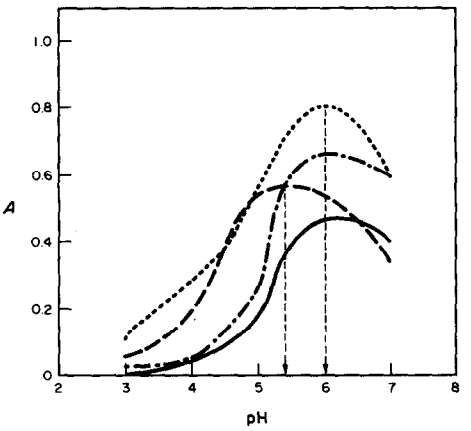

Figure 1
Effect of pH on the absorbance at the optimum wavelength produced in the reaction of *p*-benzoquinone with methyldopa (———); isoprenaline sulphate (– – – –); adrenaline (. . . .); and noradrenaline (–.–.–.).

Table 1
Catecholamine colour reaction with *p*-benzoquinone

Compound	λ_{max} (nm)	Linear range (mg/25 ml)	Correlation coefficient (*r*)	Optimum pH	$A_{1\,cm}^{1\%}$	log ε	R.S.D. (%)
Isoprenaline sulphate	502	0.2–1.2	0.9999	5.4	548	4.1839	0.51
Methyldopa	485	0.2–1.2	0.9998	6.0	458	4.0385	0.52
Adrenaline	495	0.2–1.2	0.9996	6.0	748	4.1369	0.37
Noradrenaline	485	0.2–1.2	0.9998	6.0	601	4.0076	0.43

* Mean of six separate determinations.

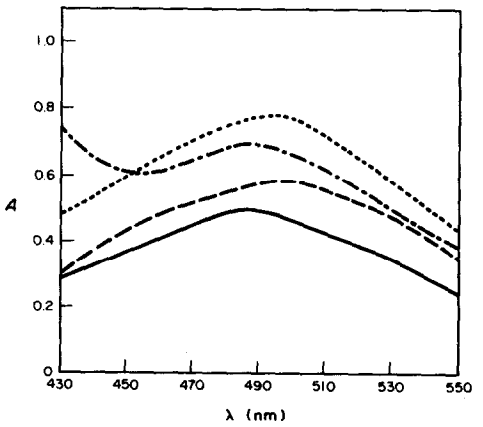

Figure 2
Absorption spectra for the reaction products of *p*-benzoquinone with methyldopa (———); isoprenaline sulphate (– – – –); adrenaline (. . . .); and noradrenaline (–.–.–.).

The reddish brown colour produced in the conditions described was stable for 2 h. The absorbance at λ_{max} was found to obey Beer's law over the concentration range 0.2–1.2 mg/25 ml for each drug. The correlation coefficients were found to be >0.999 (Table 1). Replicate determinations of $A_{1\,cm}^{1\%}$ values for each drug gave relative standard deviations of less than 1% (Table 1). The values of log ϵ were in the narrow range 4.007–4.183 indicating that the sensitivity of the method is similar for the four catecholamines studied (Table 1).

The method was applied to the determination of catecholamines in their pure state, and to commercially available tablets of isoprenaline sulphate and methyldopa, and injections of adrenaline and noradrenaline. The results obtained are given in Table 2. The possibility of interfering constituents in tablets cannot be overlooked. The proposed method was thus compared with an ultraviolet absorption method in the determination of tablets of isoprenaline sulphate and methyldopa. The absorbance was measured in 0.05 M H_2SO_4 at 278.5 and 279 nm for isoprenaline sulphate and methyldopa respectively. (Control experiments showed that tablet fillers and excipients had no effects on the UV determination). Using the *F*- and *t*-tests (Table 2), no significant differences in precision and accuracy between the proposed method and the UV method were found. Because of the low recoveries obtained in the analysis of adrenaline and noradrenaline ampoules, the standard addition principle was used to evaluate the accuracy of the proposed method for such samples, and to test for interferences from the ampoule constituents. The mean percentage recoveries (± standard deviation) for added adrenaline and noradrenaline in the concentration range 0.2–0.6 mg ml^{-1} were 100.1 ± 0.6 and 100.1 ± 0.8, respectively. This simple method thus compares favourably with

Table 2
Determination of catecholamines in pure form and in pharmaceutical preparations

Compound	Recovery (%)*	
	Proposed method	UV method
Isoprenaline sulphate		
Powder	99.69 ± 0.77	—
Tablets (Prenasma)†	100.23 ± 0.94 (F = 2.48; t = 1.06)	100.76 ± 0.60
Methyldopa		
Powder	100.07 ± 0.92	—
Tablets (Aldomet)‡	99.99 ± 0.65 (F = 1.79; t = 1.43)	100.51 ± 0.49
Adrenaline		
Powder	99.77 ± 0.56	—
Ampoules (Adrenaline)§	87.44 ± 0.54	—
Noradrenaline		
Powder	99.85 ± 0.69	—
Ampoules (Levophed)‖	87.32 ± 0.60	—

* Each result is the mean of five experiments ± standard deviations.
† Labelled to contain 20 mg of isoprenaline sulphate per tablet (Misr, Egypt).
‡ Labelled to contain 250 mg of methyldopa per tablet (Kahira, Egypt).
§ Labelled to contain 1 mg ml^{-1} of adrenaline (Misr, Egypt).
‖ Labelled to contain 1 mg ml^{-1} of noradrenaline (Winthrop, USA).

colorimetric methods based on the oxidation of catecholamines [13]. It may be applicable to other drug substances containing amino groups.

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