Journal of Pharmaceutical & Biomedical Analysis
Vol. 2, No. 1, pp. 45–52, 1984
Printed in Great Britain

0731–7085/84 $3.00 + 0.00

Difference spectrophotometric assay of 1,2-diphenolic drugs in pharmaceutical formulations — I. Boric acid reagent

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Abstract: A new procedure is described for the selective determination of drugs containing a 1,2-diphenolic moiety. The assay is based upon the measurement of difference absorbance between two equimolar solutions of the drug in pH 7 phosphate buffer, one of which also contains 0.1 M boric acid. The difference absorbance, which is maximum at about 292 nm, is due to the different spectral characteristics of the boric acid ester of the drug and of the unesterified drug and is proportional to the concentration of the drug. The accuracy, precision, sensitivity and specificity of the procedure are discussed. Applications of the assay are described for adrenaline, isoetharine, isoprenaline, levodopa and methyldopa in pharmaceutical formulations.

Keywords: *1,2-Diphenols; adrenaline; isoetharine; isoprenaline; levodopa; methyldopa; difference spectrophotometric assay; pharmaceutical formulations.*

Introduction

Interference in the ultraviolet spectrophotometric determination of drugs in formulations by other absorbing components such as co-formulated drugs, decomposition products and excipients, occurs commonly. Modifications to the normal procedure of measuring the absorbance at a single wavelength permit the assay of one or more substances in a sample with a reduction or elimination of the interference from other components. Examples of these modifications include the baseline [1] and Morton-Stubbs [2] correction techniques, simultaneous equations [3], absorbance ratios [4], orthogonal polynomials [5, 6], compensation spectrophotometry [7, 8], derivative spectrophotometry [9–11] and multi-channel spectrophotometry [12].

The technique of difference spectrophotometry has also proved to be extremely useful for eliminating both non-specific matrix interference and specific interference from co-formulated drugs and decomposition products in a wide variety of formulations [13]. The criterion for applying difference spectrophotometry to the assay of a substance in the presence of interfering components is that an absorbance difference can be induced between two equimolar solutions of the substance by the addition of reagents to one or both of the solutions, provided that the absorbance of the interfering components remains unaltered by the reagents. The measured difference absorbance (ΔA) is

proportional to the concentration of the substance if Beer's law is obeyed by the individual solutions, and is unaffected by the other absorbing components, the $\triangle A$ of which is zero. Many difference spectrophotometric procedures are based upon the induction of the $\triangle A$ by altering the pH of the solution of the analyte [14–16], although other procedures have been developed that involve oxidation [17], reduction [18, 19] and competitive condensation [20].

One group of substances that has not been determined by difference spectrophotometry is the ortho-diphenols including the important drugs adrenaline, isoprenaline, methyldopa and levodopa. Whereas monophenolic substances are readily assayed by difference spectrophotometry [21, 22] in which the $\triangle A$ is measured between two equimolar solutions of the phenol buffered at different pH values, one above and one below the pK_a of the phenol, the rapid oxidation of *o*-diphenols at high pH prohibits the application of pH-induced difference spectrophotometry to this class of compounds. Esterification of *o*-diphenols with boric acid, which results in a bathochromic shift of their absorption bands and an increase in absorbance [23–25], has been used in the present study to provide a rapid difference spectrophotometric assay that is selective for catechol derivatives in the presence of other absorbing substances including monophenols.

Experimental

Reference substances

Adrenaline hydrogen tartrate (BDH Chemicals, Poole, UK)
Isoetharine hydrochloride (Riker Laboratories, Loughborough, UK)
Isoprenaline sulphate (Halewood Chemicals, Staines, UK)
Levodopa (Roche Products, Welwyn Garden City, UK)
Methyldopa (Merck Sharp & Dohme, Hoddesdon, UK)

Reagents

Phosphate buffer pH 7. Dissolve 17.0 g potassium dihydrogen orthophosphate A.R. in 800 ml water, adjust to pH 7.0 with 1 M sodium hydroxide using a pH meter and dilute to 1 l.

Phosphate–boric acid buffer pH 7. Dissolve 17.0 g potassium dihydrogen orthophosphate A.R. and 15.45 g boric acid A.R. in 800 ml water, adjust to pH 7.0 with 1 M sodium hydroxide and dilute to 1 l.

Spectrophotometer

Absorbance values were measured in 1 cm silica quartz cells matched for equal transmission and pathlength, using a Perkin–Elmer 552 UV-visible double-beam recording spectrophotometer. The spectral slitwidth was 2 nm and the response (time constant) 2 sec.

Standard solutions

Dissolve the appropriate reference substance (about 50 mg accurately weighed) in a 250 ml volumetric flask containing 200 ml water and 2 ml 0.1 M hydrochloric acid and dilute to 250 ml with water. Transfer 5 ml of the solution to each of two 25 ml volumetric flasks, one containing phosphate buffer (10 ml) and the other containing phosphate–

boric acid buffer (10 ml) and dilute each solution to 25 ml with water. Measure the absorbance of the solution containing boric acid at the wavelength of maximum difference absorbance near 292 nm relative to that of the solution of the uncomplexed drug in the reference cell. Correct the measured difference absorbance ($\triangle A$) for any small difference absorbance of the buffers only, diluted with water (2:3 v/v), as described for the standard solutions.

Sample solutions

Aqueous formulations. Dilute the sample with water to give a concentration of the drug of 0.2 mg ml^{-1} based upon the declared concentration. Continue the assay as described above for the standard solutions from the words "transfer 5 ml of the solution . . .".

Tablets. Weigh and powder 20 tablets. Shake an accurately weighed quantity of the powder containing about 20 mg of the drug, with 80 ml of water and 1 ml of 0.1 M hydrochloric acid for 30 min and dilute to 100 ml with water. Clarify the solution by passing it through No. 1 filter paper, discarding the first 10 ml of filtrate. Continue the assay as described above for the standard solutions from the words "transfer 5 ml of the solution . . .".

Treatment of the results

Calculate the concentration of the drug in the sample solutions and hence in the sample from the proportional relationship that exists between the measured difference absorbance and the concentration.

Results and Discussion

The complexation of certain carbohydrates and other polyhydroxylic compounds with boric acid, which has been known for about a century, has found many applications in pharmaceutical and biomedical analysis [26–32], chemical synthesis [33, 34], structure elucidation [35] and pharmaceutical stabilization [23].

Esterification of catechols with boric acid occurs extremely rapidly to produce a complex that has a longer wavelength of maximum absorbance and higher absorptivity than the parent compound. Figure 1a shows the change in the UV absorption spectrum of a solution of levodopa at pH 7 induced by boric acid. The difference absorption spectrum of levodopa in the presence of boric acid relative to an equimolar solution of unesterified levodopa (Fig. 1b) shows maximum difference absorbance at 292 and 240 nm. Two isosbestic points at 261 and 270 nm occur close to the trough between the difference absorption bands owing to the equal absorbance of the two species at these wavelengths. Almost identical values of λ_{max} and isosbestic points were observed in the difference spectra of other catechol drugs.

In order to determine the optimum pH and concentration of boric acid for the assay, the absorbance at 292 nm of equimolar solutions of levodopa (2×10^{-4}M), varying in boric acid concentration (0–0.2 M) and at different pH values in the range 6–8, was measured. The maximum complex formation was obtained with a high concentration of boric acid and at a high pH value (Fig. 2). However, in solutions of levodopa without boric acid at pH values above 7, discolouration and an increase in absorbance were observed; these changes were attributed to slow oxidation of the drug. A pH of 7 and a

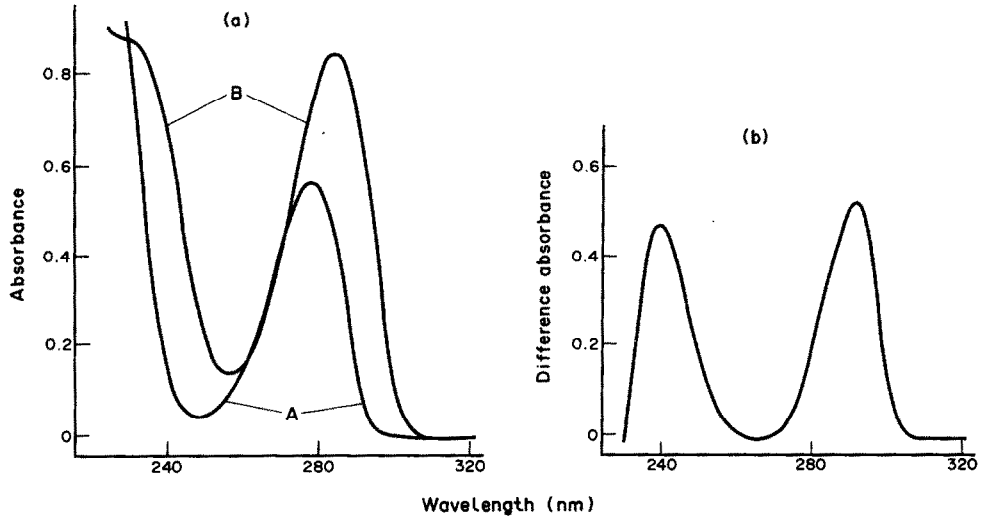

Figure 1
(a) The UV absorption spectra of levodopa (40 μg ml^{-1}): A, in phosphate buffer pH 7; and B, in phosphate–boric acid buffer pH 7. (b) The difference absorption spectrum of solution B relative to solution A.

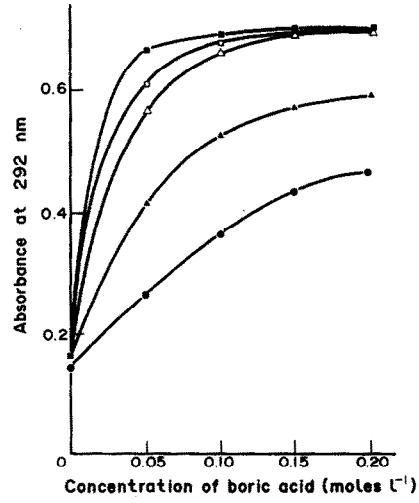

Figure 2
The effect of the variation of pH and concentration of boric acid on the absorbance at 292 nm for a solution of levodopa (40 μg ml^{-1}). ■ pH 8.0, □ pH 7.5, △ pH 7.0, ▲ pH 6.5, ● pH 6.0. Some data points have been omitted for clarity.

boric acid concentration of 0.1 mol l^{-1} were selected as conditions that provide almost complete formation of the esters and satisfactory stability of the unesterified compounds.

Adjustment of both phosphate and phosphate–boric acid buffers to exactly the same pH, 7.0, avoids the interference that may arise from pH-induced difference absorbance of co-formulated drugs that have pK_a values within 2–3 pH units of pH 7, if there was even a small difference in pH between the two buffers [13]. Thus, the absorbance of monophenols, which in general have pK_a values in the range 8–10, and of other ionizable substances that have a pK_a in the range 4–10, is identical in the two buffers.

Precision and compliance with Beer's law

Beer's law graphs for the five catechol derivatives assayed by the procedure (Table 2) showed that a proportional relationship exists between the measured $\triangle A$ and concentration of the drug in the range 0–0.08 mg ml^{-1}. For example, the linear regression equation for levodopa was $y = 0.0129x + 0.001$ where y is the $\triangle A_{292}$ and x $\mu g\ ml^{-1}$ is the concentration of the drug in the standard solutions ($n = 6$; correlation coefficient = 0.9998).

To assess the precision of the procedure, a sample of Adrenaline Solution B.P. was assayed ten times. The mean concentration was found to be 100.9% of the stated amount and the relative standard deviation was 0.63%, indicating satisfactory precision.

Specificity and sensitivity

The specificity of the difference spectrophotometric procedure for *o*-diphenols was investigated by examining a number of monophenols, diphenols and triphenols under the conditions of the assay. The results in Table 1 show that boric acid induces an absorbance difference in solutions at pH 7 of catechol (compound **1**) and its derivatives (**2–10**), the maximum difference occurring at 291–292 nm except that of catechol and 3-methylcatechol which occur at 287 and 285 nm respectively. 1,2,3-Triphenols (**11–13**) also display a bathochromic shift in boric acid solution although the wavelength of maximum difference absorbance and molar difference absorptivity ($\triangle\epsilon$) are less consistent than those of the catechol derivatives. Boric acid has no effect on the UV spectra of other di- and triphenols (**14–16**) lacking the *o*-diphenolic moiety or of monophenolic derivatives (**17–29**).

Salicylic acid (**30**) and certain of its derivatives (**31, 32**) are known to form boric acid esters [36] and are the only substances in this study, other than *o*-diphenols, that were found to give a measurable difference absorbance, particularly above 300 nm. Interference by salicylic acid derivatives or any other non-catecholic substance in the assay of *o*-diphenols is readily detected by comparing the difference absorption spectrum of the sample with that of the appropriate reference substance. Difference absorbance in the sample spectrum above 310 nm, where the *o*-diphenols exhibit none, or distortion of the sample spectrum would indicate that interference occurs also at 292 nm, the wavelength of measurement. Zero difference absorbance above 310 nm by the sample and co-incident isosbestic points in the spectra of the sample and appropriate reference substance are reasonable evidence of specificity of the assay for the *o*-diphenolic substance.

Chlorocresol, atropine, papaverine and hydrochlorothiazide present in certain formulations of *o*-diphenolic drugs (Table 2) exhibit zero difference absorbance at 292 nm and so do not interfere in the assay of these formulations.

The ratios of difference molar absorptivity at 292 nm to the molar absorptivity at the λ_{max} near 280 nm of the unesterified drug at pH 7 for eight drug substances (**3–10**) containing a 1,2-diphenolic group are also reported in Table 1 to enable a comparison to be made of the relative sensitivity of the $\triangle A$ procedure with that of a conventional UV spectrophotometric assay of the drug. The ratios are in the range 0.70–0.94 and demonstrate that little loss of sensitivity is experienced in the use of the proposed procedure rather than a conventional UV assay for these drugs.

Assay results

A number of formulations of catechol derivatives, either prepared extemporaneously

Table 1
Substances displaying a difference absorbance at 292 nm

Substance	Phenol classification	$\Delta\epsilon_{292}$	$(\frac{\Delta\epsilon_{292}}{\epsilon_{280}})^*$
1. Catechol	1,2-Diphenol	1118	
2. 3-Methylcatechol	1,2-Diphenol	535	
3. Adrenaline	1,2-Diphenol	2506	0.94
4. Noradrenaline	1,2-Diphenol	2360	0.91
5. Isoetharine	1,2-Diphenol	2674	0.89
6. Isoprenaline	1,2-Diphenol	2619	0.88
7. Levodopa	1,2-Diphenol	2544	0.91
8. Methyldopa	1,2-Diphenol	2622	0.85
9. Carbidopa	1,2-Diphenol	1863	0.70
10. Dopamine	1,2-Diphenol	2331	0.82
11. Pyrogallol	1,2,3-Triphenol	−61	
12. *n*-Propylgallate	1,2,3-Triphenol	4622	
13. Benserazide	1,2,3-Triphenol	138	
14. Resorcinol	1,3-Diphenol	0	
15. Quinol	1,4-Diphenol	0	
16. Phloroglucinol	1,3,5-Triphenol	0	
17. *o*-Cresol	Monophenol	0	
18. *m*-Cresol	Monophenol	0	
19. *p*-Cresol	Monophenol	0	
20. *o*-Aminophenol	Monophenol	0	
21. *m*-Aminophenol	Monophenol	0	
22. *p*-Aminophenol	Monophenol	0	
23. *m*-Nitrocresol	Monophenol	0	
24. 2-Methoxyphenol	Monophenol	0	
25. Chlorocresol	Monophenol	0	
26. *p*-Hydroxybenzoic acid	Monophenol	0	
27. Orciprenaline	Monophenol	0	
28. 2,7-Dihydroxynaphthalene	Monophenol	0	
29. Paracetamol	Monophenol	0	
30. Salicylic acid	Monophenol	−369	
31. Salicylaldehyde	Monophenol	0	
32. Salicylamide	Monophenol	47	
33. Papaverine	—	0	
34. Hydrochlorothiazide	—	0	
35. Atropine	—	0	

* Ratio of the difference molar absorptivity at 292 nm ($\Delta\epsilon_{292}$) to the molar absorptivity of the unesterified drug at its λ_{max} near 280 nm.

in the laboratory according to compendial recipes or commercial products purchased locally, were assayed by the procedure. For comparison the compendial formulations were also assayed by the official methods [37–40]. The pharmacopoeial procedure for Adrenaline Injection and Adrenaline Solution comprises a gravimetric assay of total adrenaline as its O^3, O^4-*N*-triacetyl derivative and a polarimetric measurement at the D-line for the (-) isomer. Since the ΔA procedure does not distinguish between the optical isomers of adrenaline, comparison of the results by the ΔA procedure was made with those obtained by the gravimetric stage only of the official procedure. A combination of the ΔA procedure for total adrenaline and a simple spectropolarimetric assay [41] provides an assay, specific for (-) adrenaline, that is more rapid than the time-consuming pharmacopoeial procedure.

The results in Table 2 show that excellent recoveries of the active ingredient were obtained in both the extemporaneously prepared and commercial samples, including

Table 2
Comparative assay results by proposed and official methods

Drug	Formulation	Declared amount	Source*	Other UV-† absorbing components	Found (as % of declared amount)	
					ΔA method	‡Official method
Adrenaline hydrogen tartrate	Injection B.P.	1.8 mg ml^{-1}	E	—	100.0	98.1
	Injection B.P.	1.8 mg ml^{-1}	C	—	100.9	98.6
	Solution B.P.	1.8 mg ml^{-1}	E	Chlorocresol, 1 mg ml^{-1}	99.8	100.0
	Solution B.P.	1.8 mg ml^{-1}	C	Chlorocresol, 1 mg ml^{-1}	100.9	97.1
	Eye-drops with zinc sulphate B.P.	0.9 mg ml^{-1}	E	—	99.5	99.0
	Eye-drops with zinc sulphate B.P.	0.9 mg ml^{-1}	C	—	99.5	99.0
	Spray, compound with atropine B.P.C. 1973	8.0 mg ml^{-1}	E	Papaverine HCl, 8 mg ml^{-1}	100.4	99.0
	Spray, compound with atropine B.P.C. 1973	8.0 mg ml^{-1}	C	Papaverine HCl, 8 mg ml^{-1}	101.6	98.7
Isoetharine hydrochloride	Tablet	10 mg	C	—	97.8	—
Isoprenaline sulphate	Tablet B.P.	10 mg	C	—	102.1	100.5
	Spray B.P.C. 1968	10 mg ml^{-1}	E	—	99.2	99.5
	Spray B.P.C. 1968	10 mg ml^{-1}	C	—	98.9	99.6
Levodopa	Tablet B.P.	500 mg	C	—	98.3	99.0
Methyldopa	Tablet B.P.	125 mg	C	—	102.2	102.7
	Tablet B.P.	250 mg	C	—	102.2	101.8
	Tablet	250 mg	C	Hydrochlorothiazide, 15 mg	99.4	—

* E = Extemporaneously prepared sample; C = Commercial sample.
† Compounds absorbing at λ_{max} of the unesterified drug, near 280 nm.
‡ Official methods used: BP 1980, BPC 1973 or BPC 1968 as indicated.

those containing other UV-absorbing components that would interfere in a conventional UV spectrophotometric procedure. In addition, good agreement was observed between the results of the $\triangle A$ procedure and those given by the official methods, confirming the selectivity and accuracy of the proposed procedure.

Acknowledgements: The technical assistance of Mrs Pauline Constable is gratefully acknowledged.

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[*Received for review 9 May 1983*]