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A new colorimetric method for the determination of bendrofluazide in pharmaceutical preparations

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Summary

A rapid, convenient and specific colorimetric method is described for the determination of bendrofluazide in pharmaceutical preparations. The method is based on heating an aqueous solution of the thiazide with 1,2 naphthaquinone-4-sulphonic acid sodium salt in the presence of tetramethyl ammonium hydroxide solution. The absorbance of the resulting yellow-orange solution is then measured at 450 nm. The method is specific for bendrofluazide since other diuretics of similar chemical structure do not give the colorimetric assay.

Introduction

A rapid, convenient and specific colorimetric method is described for the determination of bendrofluazide in pharmaceutical preparations. An aqueous solution of the compound is heated for 30 min and subsequently reacted with 1,2 naphthaquinone-4-sulphonic acid sodium salt in presence of tetramethyl ammonium hydroxide solution. The absorbance of the resulting yellow-orange solution is then measured at 450 nm against a blank which is prepared in the same way as the sample solution but omitting the heating. The method is specific for bendrofluazide since other diuretics of similar chemical structure do not give the colorimetric assay. Bendrofluazide (3-benzyl-3,4-dihydro-6-trifluoromethyl benzo-1,2-4-thiodiazine-7-sulphonamide-1,1-dioxide) is one of the most potent diuretic and antihypertensive agents. The diuretic effect of bendrofluazide is due largely to its ability to inhibit the renal tubular reabsorption of sodium and chloride ions and, to a lesser extent potassium and bicarbonate ions.

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The BP (1980) method for the determination of bendrofluazide powder is by a non-aqueous titration with tetrabutyl ammonium hydroxide determining the end-point potentiometrically. For the tablets the BP (1980) used a spectrophotometric method based on measuring the absorbance at 275 nm. However, direct spectrophotometry does not allow the unequivocal determination of the drug. The BP method for the determination of chlorothiazide, hydrochlorothiazide and hydroflumethiazide in their tablet forms is based on a colorimetric determination by first hydrolyzing the thiazide with sodium hydroxide for 1 h, diazotizing and then coupling with N-(1-naphthyl)ethylene diamine. Chromotropic acid has been reported as a coupling agent (Bermejo, 1961). The same method but extending the hydrolysis period to 3 h was applied by Ghelarodoni and Fedi (1962).

These methods have the disadvantage that they are non-specific and time-consuming.

Other methods for the determination of bendrofluazide have been described by Chiang (1961) who used a non-aqueous titration with sodium methoxide using 4-(*p*-nitrophenylazo)resorcinol as indicator, and by Marques Leal and Ramos Lopes (1963) who used the same method with azo-violet as the indicator.

These methods proved not to be satisfactory for bendrofluazide in pharmaceutical preparations and in microquantities.

Hence, the development of a simple time-saving and specific method for the determination of bendrofluazide in pharmaceutical preparations in highly desirable.

Experimental

Materials and reagents

Bendrofluazide BP—obtained from Boots.

Bendrofluazide tablets B.P.—obtained from Boots

1,2-Naphthaquinone-4-sulphonic acid sodium salt reagent (E. Merck)—dissolve 100 mg of the salt in 100 ml water. A freshly prepared solution should be used.

Tetramethyl ammonium hydroxide reagent—dilute 1 ml of tetramethyl ammonium hydroxide (25%) to 10 ml with water.

All the chemicals used were analytical reagent grade.

Absorbance measurements. The absorbance measurements were made with a Beckman Model DU-2 spectrophotometer using 1-cm cuvettes.

Procedure

Calibration graph

Measure 5 ml of bendrofluazide ethanolic solution (1 mg/ml) into a conical flask. Dilute to 40 ml with water. Heat on a boiling water bath for 30 min under reflux. Transfer to a 50 ml calibrated flask and fill to volume with water. Pipette several accurately measured aliquots in the range of 1–6 ml into 25 ml calibrated flasks. Dilute each to 15 ml with water and then add in the following order 1.0 ml of 1,2-naphthaquinone sulphonate sodium reagent and 0.6 ml of the tetramethyl

ammonium hydroxide. Mix well and fill to volume with water. Leave to stand for 10 min and measure the absorbance at 450 nm in 1-cm cell against a blank. The blank is prepared by diluting a freshly prepared ethanolic solution of bendrofluazide (1 mg/ml) with water containing bendrofluazide as in the hydrolyzed solutions, 1.0 ml of 1,2-naphthaquinone sulphonate sodium reagent is added, followed by 0.6 ml of the tetramethyl ammonium hydroxide solution.

Sample preparation and assay

Weigh and powder 20 tablets. Dissolve an accurately weighed amount of the powder equivalent to 25 mg bendrofluazide in sufficient ethanol (95%) to give 25 ml of solution. Filter and reject the first 10 ml of the filtrate. Proceed as described for the calibration graph using 5 ml of the filtrate.

Calculate the amount of bendrofluazide from the calibration graph.

Results and Discussion

A characteristic yellow colour with an absorption maximum at 450 nm develops when an aqueous solution of bendrofluazide is heated for 30 min and allowed to react with 1,2-naphthaquinone-4-sulphonic acid sodium salt in presence of tetramethyl ammonium hydroxide. A standard graph was plotted for various concentrations of bendrofluazide—the colour was found to obey Beer's law over the concentration of 4–24 $\mu g \cdot ml^{-1}$.

The formation of the yellow-orange colour can be explained on the basis that on heating the bendrofluazide solution, partial decomposition occurs to yield 4-amino-6-trifluoro-methyl-benzene-1,3-disulphonamide and phenylacetaldehyde. The latter reacts with 1,2-naphthaquinone-4-sulphonic acid sodium salt in presence of tetramethyl ammonium hydroxide forming a yellow-orange compound; 1,2-naphthaquinone-4-sulphonic acid sodium salt has not been reported to react with phenylacetaldehyde, but it has been reported for the determination of primary aromatic amines (Salama and Omer, 1980).

Factors that affect colour formation

Effect of heating time. Samples heated for 25–50 min on a boiling water bath

TABLE 1

ABSORBANCE OF THE CHROMOPHORE DERIVED FROM 8 μg BENDROFLUAZIDE PER ML HEATED ON A BOILING WATER BATH FOR VARIOUS PERIODS OF TIME

Time (min)	Absorbance at 450 nm
10	0.180
15	0.194
20	0.198
25	0.200
30	0.200
40	0.199
50	0.201

TABLE 2

EFFECT OF REACTION TIME ON ABSORBANCE OF CHROMOPHORE DERIVED FROM BENDROFLUAZIDE [a]

Time (min)	Absorbance at 450 nm
5	0.198
8	0.200
10	0.200
20	0.200
30	0.197
60	0.190

[a] Concentration of 8 μg bendrofluazide/ml.

and then processed as described had absorbance that did not differ from each other by more than 0.5% (Table 1).

Effect of reaction time on colour intensity. The colour intensity reached a maximum within 5 min after the addition of the tetramethyl ammonium hydroxide reagent, and remained constant for approximately 20 min and then decreased slowly (Table 2).

Amount of tetramethylammonium hydroxide. Fig. 1 shows that the addition of 0.4–0.8 ml of 2.5% tetramethyl ammonium hydroxide to a solution containing 16 μg/ml gives the optimum base concentration for production of maximum intensity and stability of colour. Increasing the concentration of tetramethyl ammonium hydroxide to 1.6 ml leads to a loss of absorbance of about 5%.

Reproducibility. The precision study was performed by running replication studies on 8 samples of bendrofluazide, each containing 16 μg/ml. Each sample was analyzed by the proposed method. The coefficient of variation was 0.3% (Table 3).

Comparative analyses. Comparison between the suggested method and the BP method was carried out on bendrofluazide tablets. The results are tabulated in Table 4.

Specificity. Chlorothiazide, hydrochlorothiazide, hydroflumethiazide and cyclopenthiazide which belong to the same series of benzo-1,2,4-thiadiazines-7-sulphonamide did not give the colorimetric assay under the experimental conditions stated and therefore the method is very specific for bendrofluazide.

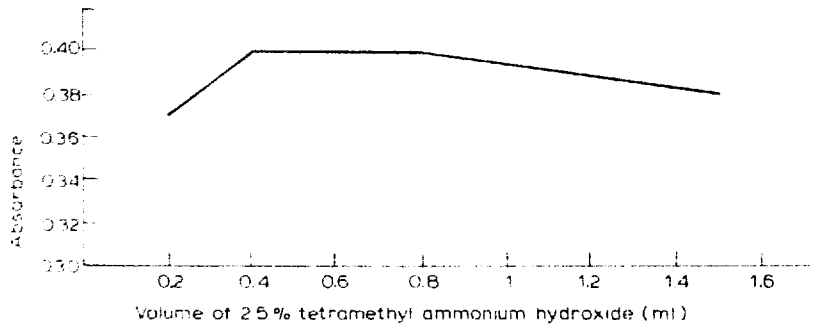

Fig. 1. Effect of the amount of tetramethyl ammonium hydroxide on the colour intensity.

TABLE 3

REPRODUCIBILITY OF ABSORBANCE OF CHROMOPHORE DERIVED FROM REPLICATE SAMPLES OF BENDROFLUAZIDE CONTAINING 16 μg/ml

Solution	Absorbance at 450 nm
1	0.400
2	0.401
3	0.399
4	0.400
5	0.398
6	0.402
7	0.400
8	0.401
Average	0.400
Standard deviation	±0.0012
Coefficient of variation	0.3%

TABLE 4

COMPARATIVE ANALYSIS OF BENDROFLUAZIDE TABLETS

Compound	Amount of bendrofluazide in mg [a] found		
	Labelled	BP method	Proposed method
Bendrofluazide tablets	2.5	2.45	2.5
Bendrofluazide tablets	5	5.0	5.0

[a] Average of 3 determinations.

Free amine. The free amine (5-trifluoromethyl-2,4-disulphamoylaniline) which may be present in bendrofluazide as an impurity does not react with 1,2-naphthaquinone sulphonate sodium reagent under the experimental conditions. Therefore the method has the advantage that only bendrofluazide is determined.

Phenylacetaldehyde. Phenylacetaldehyde would interfere with the method of assay, but the interference has been eliminated by difference spectrophotometry.

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