International Journal of Pharmaceutics, 93 (1993) 13–19

IJP 03011

Application of *p*-*N*,*N*-dimethylphenylenediamine dihydrochloride for the determination of some antiarrhythmic agents and anticoagulants

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(Received 3 October 1990)
(Modified version received 15 July 1992)
(Accepted 4 August 1992)

Key words: Spectrophotometry; Procainamide hydrochloride; Acebutolol hydrochloride; Nicoumalone; *p*-*N*,*N*-Dimethylphenylenediamine dihydrochloride; Routine analysis

Summary

A simple and sensitive spectrophotometric method is described for the determination of procainamide hydrochloride (PAH), acebutolol hydrochloride (ACBH) and nicoumalone (NIC) in bulk samples and pharmaceutical formulations. The method is based on the oxidative coupling reaction through the involvement of an aromatic primary amino group in the drug (existing free in PAH or released through hydrolysis in ACBH or reduction in NIC) with *p*-*N*,*N*-dimethylphenylenediamine dihydrochloride (DMPD) in the presence of an oxidant ($S_2O_8^{2-}$ or Fe(III)) resulting in the formation of an intensely colored product having maximum absorbance at 550 nm (PAH) or 560 nm (ACBH and NIC). Beer's law is obeyed in the concentration ranges 2–20, 3–36 and 4–40 μg/ml of the three drugs studied. The proposed method is applied to the determination of these drugs in bulk samples and formulations and the results compare favourably with those from the reference methods.

Introduction

Procainamide hydrochloride (PAH, benzamide, 4-amino-*N*-{2-(diethylaminoethyl)} hydrochloride) and acebutolol hydrochloride (ACBH, butanamide, *N*-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino[propoxyl]phenyl] hydrochloride) are two widely used antiarrhythmic agents, while nicoumalone (NIC, 4-hydroxy-3-(1-*p*-nitrophenyl-3-oxobutyl)coumarin) is a widely used anticoagulant which inhibits the hepatic biosynthesis of clotting factors by impairing the vitamin K dependent reactions involved in blood coagulation.

This inhibition results in biologically inactive forms of these clotting factors.

Due to their medicinal importance several methods have been reported for their determination, either per se or in dosage forms. Among the three drugs, only PAH (Pharmacopoeia India, 1985; British Pharmacopoeia, 1988; USP XII, 1990) and NIC (Pharmacopoeia India, 1985;

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British Pharmacopoeia, 1988) have official methods. NIC has been detected by reaction with Zn-$CaCl_2$/*N*-benzoylation/ hydroxylamine/ $FeCl_3$ (Auterhoff and Beinroth, 1980). ACBH has been determined colorimetrically using sodium-1,2-napthaquinone-4-sulfonate (Hakyemez, 1988), 3-methyl-2-benzothiazolinone hydrazone hydrochloride-cerium(IV) (Ramana Rao et al., 1990b), chloroanilic acid (Elsayed et al., 1988) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (Elsayed et al., 1988). Chromogenic reagents such as folin-Ciocalteu reagent (Ramana Rao et al., 1990a), 3-α,γ-dicarboxypropyl rhodamine (Kvach and Aleksandrova, 1987), *o*-aminophenols-potassium iodate (Sastry et al., 1985) and *N*-alkylaminophenol-iodine (Sastry et al., 1984) have been suggested in the estimation of PAH. Most of these methods suffer from low selectivity and sensitivity and the stability of the colored species formed is less than 20 min in many instances. A sensitive method would greatly aid in determining the above drugs in their pharmaceutical formulations.

Kramer and Tolentino (1971) described a visible spectrophotometric method for the assay of phenols and aryl amines via oxidative coupling reaction using *p*-*N*,*N*-dimethylphenylenediamine (DMPD) and dichromate-ferricyanide. The authors have noticed that the same combination is not suitable for the assay of the mentioned drugs in low concentrations. However, the use of persulfate (PAH, ACBH) or Fe(III) (NIC) in place of dichromate-ferricyanide permits their determination up to 2, 3 and 4 μg/ml, respectively. The stability of the colored species formed is remarkably longer (PAH, 8 h; ACBH, 6 h; NIC, 30 min) in two of the proposed methods.

Materials and Methods

Preparation of standard solutions

Procainamide hydrochloride (200 μg/ml; Natco Fine Pharm. Pvt. Ltd, India) was prepared by dissolving 20 mg of the drug in 100 ml of distilled water. Acebutolol hydrochloride (250 μg/ml; May & Baker (India) Ltd) was prepared by dissolving 50 mg of ACBH in 15 ml of distilled water, and then adding 5 ml of concentrated hydrochloric acid. The solution was refluxed for 45 min in a water bath, cooled and the excess of hydrochloric acid was removed under vacuum and diluted to 200 ml with distilled water. For the preparation of nicoumalone solution (400 μg/ml; Sarabhai Chemicals Ltd), 40 mg of NIC was treated with 10 ml of 1 M methanolic hydrochloric acid and 0.4 g of zinc dust was added in portions. After standing for 45 min at room temperature, the solution was filtered through cotton wool, the residue was washed with methanol (3 × 5 ml) and the filtrate was made up to 100 ml with methanol (Qualigens, Excellar) after adjusting the pH to a value between 6.0 and 7.0 with sodium hydroxide.

Aqueous solutions of DMPD dihydrochloride (Johnson Chemicals Ltd, 4.78×10^{-3} M), potassium persulfate (E. Merck, 1.0×10^{-2} M) and ferric chloride hexahydrate (Wilson, 7.399×10^{-2} M) were freshly prepared. Analytical grade compounds and double-distilled water were used throughout the investigations.

Instrumentation

A Systronics model 106 digital spectrophotometer equipped with 1-cm matched glass cells was used for all absorbance measurements. All pH measurements were performed on a Elico model LI-120 digital pH meter.

Procedure for bulk samples

Volumes of standard solutions of PAH (50–500 μg) or hydrolysed ACBH (75–900 μg) or reduced NIC (100–1000 μg) were placed into a series of 25 ml graduated tubes. Solutions of oxidant (1 ml of $S_2O_8^{2-}$ for PAH and ACBH, 1 ml of Fe(III) for NIC) and DMPD dihydrochloride (1.5 ml for PAH, 2.0 ml for ACBH and NIC) were added to each tube, allowed to stand for 30 min (PAH and NIC) or 40 min (ACBH) and diluted to volume with distilled water. After color development and final dilution the absorbances were measured at 550 nm (PAH) or 560 nm (ACBH and NIC) against a reagent blank during the stability period (1 min–8 h for PAH, 1 min–6 h for ACBH or 1–30 min for NIC). The drug concentration was read from its appropriate calibration curve.

Procedure for pharmaceutical formulations

Tablet powder equivalent to 20 mg of PAH or 50 mg of ACBH was solubilized with 3×5 ml portions of distilled water and filtered. The filtrate was treated as in the preparation of standard drug solution and analyzed as under the procedure for bulk samples. For NIC, tablet powder equivalent to 40 mg of NIC was solubilized with 3×10 ml portions of acetone, the combined acetone filtrate was evaporated on a steam bath and the residue was treated as in the preparation of standard drug solution and analyzed as under procedure for bulk samples.

Results and Discussion

Reaction parameters

The spectral characteristics of the three drugs (PAH, ACBH and NIC) with DMPD-oxidant are shown in Fig. 1. In each system the characteristic λ_{max} appeared only when all the three components (drug, oxidant and DMPD) were present. Mixtures of DMPD and oxidant (reagent blank) or drug and oxidant showed low or no absorption in this region. However, all the measurements were carried out against a reagent blank. Of the various oxidants ($S_2O_8^{2-}$, Fe(III), CAT, OCl^-, Cr(VI), Ce(IV), $Fe(CN)_6^{3-}$ or Cr(Vi)-$Fe(CN)_6^{3-}$) tested in combination with DMPD, $S_2O_8^{2-}$ (for PAH and ACBH) or Fe(III) (for NIC) were found to be best suited for the determination of these drugs.

The effect of concentration of reagents and other variables on sensitivity, stability and obedience of Beer's law have been studied. The conditions were optimized by variation of one parameter at a time. 0.7–1.2 ml of 1.0×10^{-2} M potassium persulphate, 0.8–1.2 ml of 7.399×10^{-2} M ferric chloride solutions and 1.3–1.8 ml (PAH) or 1.8–2.3 ml (for ACBH and NIC) of 4.78×10^{-3} M DMPD solutions were determined to be the optimal quantities for maximum color formation. From the experiments in which the reagents were added in all possible sequences, but with the drug first, it was concluded that the order of addition of reactants has no influence when mixing of the reactants is carried out one after another without any delay. The maximum color intensity and stability were obtained after 30 min for PAH and 40 min for ACBH after mixing the reactants and remained stable for further periods of 8 and 6 h, respectively. However, in the case of NIC, the maximum intensity was attained after 30 min, remained stable for the next 30 min and afterwards slowly increased by 0.001 absorbance unit for every 10 min up to 180 min. Therefore, the spectral measurements were carried out between 30 and 60 min. Heating the reaction mixture at 60°C even for 10 min causes a marked decrease in the absorbance. Hence, storage for 30 or 40 min at room temperature is recommended.

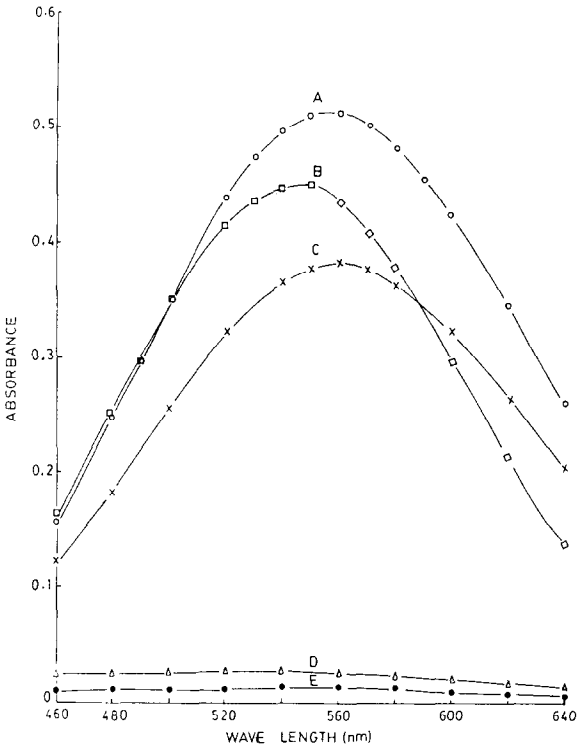


Fig. 1. Absorption spectra of DMPD - $S_2O_8^{2-}$ systems for procainamide hydrochloride and acebutolol hydrochloride and DMPD-Fe(III) system for nicoumalone. [DMPD] = 2.869×10^{-4} M for PAH and 3.825×10^{-4} M for ACBH and NIC; [$S_2O_8^{2-}$] = 4.0×10^{-4} M; [Fe(III)] = 2.959×10^{-4} M; [PAH] = 5.886×10^{-5} M; [ACBH] = 8.045×10^{-5} M; [NIC] = 1.132×10^{-4} M. (○——○) (A) ACBH - $S_2O_8^{2-}$-DMPD/$S_2O_8^{2-}$-DMPD; (□——□) (B) PAH -$S_2O_8^{2-}$-DMPD/$S_2O_8^{2-}$-DMPD; (×——×) (C) NIC-Fe(III)-DMPD/Fe(III)-DMPD; (△——△) (D) Fe(III)-DMPD/distilled water; (○——○) (E) $S_2O_8^{2-}$-DMPD/distilled water.

TABLE 1

Optical characteristics, precision and accuracy

Parameters	PAH	ACBH	NIC
Beer's law limits (μg/ml, C)	2–20	3–36	4–40
Molar absorptivity (l mol^{-1} cm^{-1})	7.61×10^3	6.34×10^3	3.36×10^3
Sandell's sensitivity (μg cm^{-2} 0.001 absorbance unit^{-1})	0.0357	0.0588	0.1053
Regression equation ($a + bC$)			
Slope (b)	2.78×10^{-2}	1.69×10^{-2}	0.95×10^{-2}
Intercept (a)	1.22×10^{-3}	2.01×10^{-3}	-3.39×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999
Relative standard deviation [a](%)	0.21	0.34	0.52
Range of error [a] (%, 95% confidence limit)	0.22	0.36	0.55
% error in bulk samples [b]	+0.46	+0.23	−0.67
Stability of the colored species	8 hr	6 hr	30 min

[a] Calculated from six determinations.

[b] Sample size: 200 μg for PAH and 500 μg for ACBH and NIC. Average error for three determinations.

The colored species resulting from ACBH or PAH were not extractable into water immiscible organic solvents (chloroform or butan-1-ol). However, in the case of NIC, the colored species was extractable into butan-1-ol but there was no significant improvement in the optical characteristics. Even though final dilution with isopropanol instead of water during color development in

TABLE 2

Analysis of pharmaceutical dosage forms

Drug	Nominal amount (mg)	Proposed method, found (%) [a]	Added (mg)	Recovery (%) [a]	Reference method, found (%) [b]
Tablets					
PAH (250 mg)	250	99.9 ± 0.2 $t = 0.235$, $F = 1.326$	50	99.3 ± 0.6	99.6 ± 0.2
PAH (250 mg)	250	99.8 ± 0.3 $t = 0.278$, $F = 2.589$	50	99.4 ± 0.3	99.2 ± 0.2
ACBH (400 mg)	400	99.9 ± 0.5 $t = 0.537$, $F = 1.954$	50	99.8 ± 0.4	100.1 ± 0.7
ACBH (200 mg)	200	99.6 ± 0.6 $t = 0.644$, $F = 2.046$	50	100.1 ± 0.5	99.3 ± 0.4
ACBH (200 mg), HCT (12.5 mg)	200	99.8 ± 0.4 $t = 0.420$, $F = 1.732$	50	99.4 ± 0.38	100.2 ± 0.5
NIC (4 mg)	4	99.6 ± 0.6 $t = 0.620$, $F = 1.556$	5	99.2 ± 0.3	99.2 ± 0.7
NIC (1 mg)	1	99.2 ± 0.4 $t = 0.467$, $F = 2.079$	5	99.6 ± 0.6	98.8 ± 0.6

[a] Average $\pm$ S.D. of six determinations; the t and F values refer to comparison of the proposed method with the reference method ([b]). Theoretical values at 95% confidence limit: $t = 2.57$, $F = 5.05$.

[b] Reported method for ACBH (Hakyemez, 1988); B.P. method for PAH and NIC (British Pharmacopoeia, 1988) HCT, hydrochlorothiazide; SYFCF, Sunset Yellow FCF.

NIC causes an increase in the absorbance value, linearity is not attained. Therefore, complete aqueous medium is preferred.

Analytical data

The optical characteristics such as Beer's law limits (Fig. 2), molar absorptivity and Sandell's sensitivity are given in Table 1. The slope (b), intercept (a) and correlation coefficient (r) data obtained by linear-least squares treatment of the results at different concentrations of each drug are incorporated in Table 1. The precision and accuracy were determined by the analysis of six separate samples containing 3/4ths the amount of the upper Beer's law limit of each drug and the results are summarized in Table 1. The determination of each drug gave a coefficient of variation of less than 1%.

Application to the analysis of formulations

In the analysis of pharmaceutical formulations, the results obtained from the proposed and reference methods were compared (Table 2) statistically by means of Student's t-test and by the variance ratio F-test and no significant difference was observed. To evaluate the validity and reproducibility, a known amount of the pure drug was

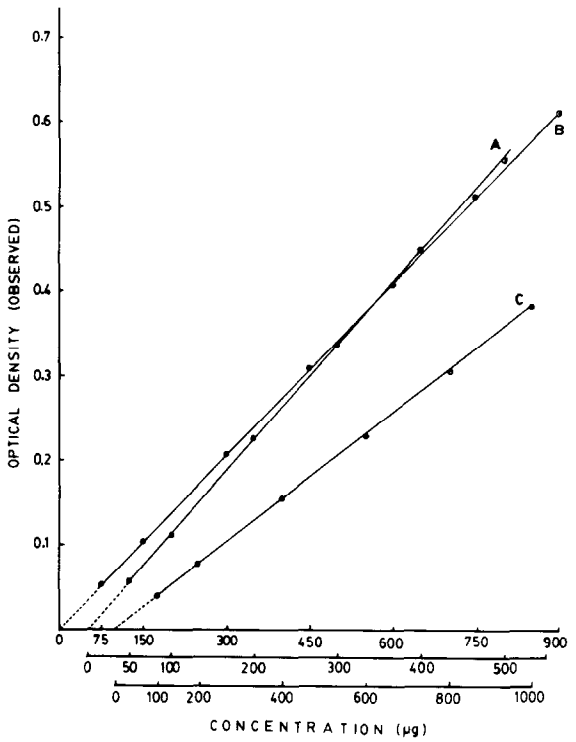


Fig. 2. Beer's law plots of procainamide hydrochloride (A) and acebutolol hydrochloride (B) with DMPD-$S_2O_8^{2-}$ and nicoumalone with DMPD-Fe(III) systems. [DMPD] = 2.868×10^{-4} M for A and 3.824×10^{-3} M for B and C; [$S_2O_8^{2-}$] = 4.0×10^{-4} M for A and B; [Fe(III)] = 2.959×10^{-4} M for C.

TABLE 3

Intra-day and inter-day precision data of PAH, ACBH and NIC in pharmaceutical formulations

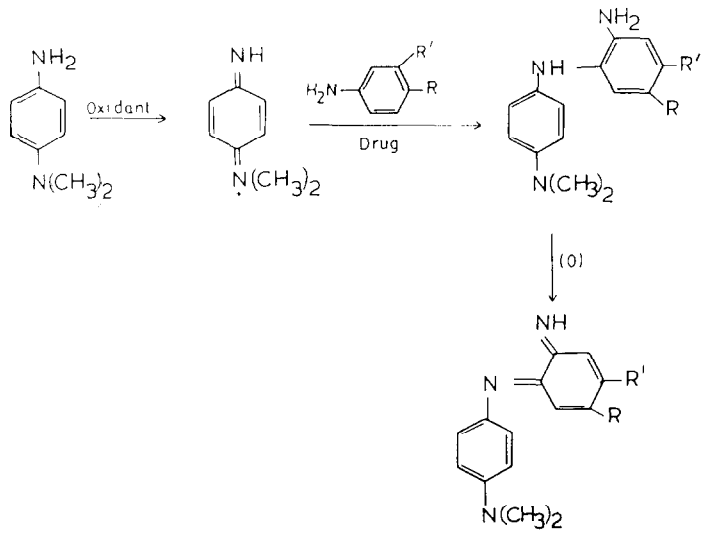
Drug concentration (μg)	Amount obtained through absorbance measurements (n = 6)					
	Intra-day			Inter day		
	Mean	S.D.	C.V. (%)	Mean	S.D.	C.V. (%)
PAH						
300.0	300.8	3.17	1.06	301.3	2.34	0.78
400.0	401.0	1.80	0.45	402.9	2.41	0.60
ACBH						
450.0	447.8	3.54	0.79	446.8	4.06	0.90
750.0	752.2	2.18	0.29	749.3	4.79	0.64
NIC						
600.0	595.6	7.24	1.21	594.3	8.44	1.42
800.0	799.1	4.86	0.61	790.3	8.70	1.09

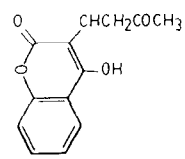
C.V., coefficient of variation.

added to previously analysed samples and the mixtures were analysed by the proposed method. The percent recovery values obtained are listed in Table 2. Recovery experiments indicated the absence of interference from the active ingredients such as hydrochlorothiazide and Sunset Yellow FCF and the commonly encountered pharmaceutical additives and excipients such as lactose, glucose, starch, propylene glycol, boric acid, sodium lauryl sulfate, magnesium carbonate, magnesium stearate, gum acacia and talc.

The imprecision of the assay was checked by running six separate analyses of the same sample on the same day and on separate days. As shown in Table 3, the formulations had coefficient of variations (C.V.) below 2% at the concentrations tested.

Comparison of the proposed and reported visible spectrophotometric methods data concerning simplicity, sensitivity and selectivity reveals the following information. In the case of NIC, the sole method available is cumbersome and involves lengthy procedure for color development ($Zn\text{-}CaCl_2$ (reduction)/C_6H_5COCl (benzoylation)/NH_2OH (oxamic ester formation)/Fe^{3+} (complexation)) and possesses poor sensitivity, while the proposed method is simple and highly sensitive. The chromogenic reagents used in the four reported methods for the determination of ACBH, namely, MBTH-Ce(IV)/λ_{max}, 465 nm; oxidative coupling), sodium 1,2-naphthaquinone-4-sulfonate (λ_{max}, 477 nm; nucleophilic displacement), DDQ or chloranilic acid (λ_{max}, 460 nm; complexation) are less sensitive and possess low



DRUG	R	R′
PAH	$CONHCH_2CH_2N(C_2H_5)_2$	H
ACBH	$OCH_2CHOHCH_2NHCH(CH_3)_2$	$COCH_3$
NIC		H

Scheme 1.

λ_{max} values when compared to the proposed method. Even though there are several reported methods for the estimation of PAH employing different chromogenic reagents (reduction of folin-Ciocalteu reagent – general; Schiff base formation with aldehydes such as PDAB, PDAC and vanillin – equilibrium reaction requires maintenance of stringent conditions; diazo coupling reaction with different couplers such as *N*-1-naphthylethylenediamine, 3-α,γ-dicarboxypropyl rhodamine and 3-α,β-dicarboxyethyl rhodamine – general for primary aromatic amines; oxidative coupling reaction with *o*-aminophenols-IO_3^-; complex formation with *N*-alkylaminophenol -I_2), most of them are less sensitive than the proposed method.

The proposed method is advantageous especially in the case of NIC and ACBH in comparison to the reported methods which possess lower sensitivity and selectivity. Hence, the proposed method can be preferred over the reported methods for the routine determination of NIC, ACBH and PAH in their pharmaceutical formulations.

Chemistry of the colored species

The course of the reaction in the formation of colored species was postulated by analogy (Kramer and Tolentino, 1971). DMPD undergoes oxidation in the presence of $S_2O_8^{2-}$ or Fe(III) with a two-electron transfer to the less stable and highly reactive *p-N,N*-dimethylbenzoquinone diimine (PDBQDI). This species reacts with primary amino compounds under the experimental conditions by an electrophilic attack on the most nucleophilic site of the substrate (i.e., *ortho* position to the aromatic primary amino group, since the *para* position is blocked). The resulting leucodye is oxidized to the indodye. The variation in λ_{max} and ϵ_{max} of the colored species formed may be attributed to the inductive effects of the substituents (electron-withdrawing or electron-releasing) attached to the aromatic nucleus. These are given in Scheme 1.

Acknowledgement

One of the authors (T.T.R.) is grateful to the C.S.I.R., New Delhi, for the award of a Senior Research Fellowship.

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