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Short communication

Use of *N*,*N*-diethyl-*p*-phenylenediamine sulphate for the spectrophotometric determination of some phenolic and amine drugs

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Spectrophotometric methods are proposed for the determination of drugs containing a phenol group [salbutamol sulphate (SLB), ritodrine hydrochloride (RTD), isoxsuprine hydrochloride (IXP)] and drugs containing an aromatic amine group [dapsone hydrochloride (DAP), sulfamethoxazole (SFM), and sulfadiazine (SFD)] in pharmaceutical dosage forms. The methods are based on coupling of *N*,*N*-diethyl-*p*-phenylenediamine sulphate with the drugs in the presence of KIO_4 to give a green colored product (λ_{max} at 670 nm) and a red colored product (λ_{max} at 550 nm), respectively. Linear relationships with good correlation coefficients (0.9986–0.9996) were found between absorbance and the corresponding concentration of drugs in the range 1–7, 2–22, 1–17, 1.5–12, 2–25, and 2–21 μg mL^{-1} for SLB, RTD, IXP, DAP, SFM and SFD, respectively. Variable parameters such as temperature, reaction time and concentration of the reactants have been analyzed and optimized. The RSD of intra-day and inter-day studies was in the range of 0.2–1.0 and 0.4–1.0 %, respectively. No interference was observed from common pharmaceutical adjuvants. The reliability and performance of the proposed methods was validated statistically; the percentage recovery ranged from 99.5 ± 0.1 to 99.9 ± 0.3 %. Limits of detection were 0.14, 0.21, 0.51, 0.44, 0.33 and 0.37 μg mL^{-1} for SLB, RTD, IXP, DAP, SFM, and SFD, respectively.

Keywords: *N*,*N*-diethyl-*p*-phenylenediamine sulphate, UV/Vis spectrophotometry, oxidation, coupling reaction

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Salbutamol sulfate, 4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol (SLB) and ritodrine hydrochloride, 4-(1*R*,2*S*)-1-hydroxy-2-{[2-(4-hydroxyphenyl)ethyl]amino}propyl) phenol (RTD) are used as antiasthmatic and uterine relaxant drugs, respectively. Isoxsuprine hydrochloride, 4-{1-hydroxy-2-[(1-phenoxypropan-2-yl)amino]

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propyl}phenol (IXP), a derivative of adrenaline, is an alpha receptor antagonist and beta receptor agonist, which is used as a vasodilator and an uterine relaxant.

Dapsone hydrochloride, 4-[(4-aminobenzene)sulfonyl] aniline (DAP), inhibits folic acid synthesis in bacteria and is used as an antileprotic agent. It is also used as an antiacne agent. Sulfamethoxazole, 4-amino-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide (SFM), an antibacterial agent, is one of the components of co-trimoxazole, a pharmaceutical preparation that inhibits dihydrofolate synthesis in bacteria. Sulfadiazine, 4-amino--*N*-pyrimidin-2-yl-benzenesulfonamide (SFD), is also an antibacterial agent that inhibits synthesis of folic acid in bacteria.

Among the various methods available for the estimation of these drugs, such as HPLC, electrophoresis and gas chromatography, spectrophotometry is still the preferred technique due to its simplicity. Several spectrophotometric methods are available for the estimation of salbutamol (1, 2), ritodrine (2–4), isoxsuprine (2, 5, 6), dapsone (7, 8), sulfamethoxazole, and sulfadiazine (9, 10). The aforementioned methods have limitations such as the use of organic solvents (1) extraction by evaporation (2), being time consuming (5) and use of surfactants (8).

Phenylenediamine and its derivatives have been widely used in the estimation of enzymes and drugs (11, 12). This paper, for the first time, describes a simple and sensitive method using *N*,*N*-diethyl-*p*-phenylenediamine sulphate (PADMA) and KIO_4, to assay these drugs in bulk samples and in a wide variety of pharmaceutical preparations. The analytical procedure involves oxidation of PADMA by KIO_4 followed by coupling with drugs containing phenolic and aromatic amino groups to produce green and red colored products having maximum absorbance at 670 nm and 550 nm, respectively.

EXPERIMENTAL

Instrument

A JASCO Model UVIDEC-610, UV-VIS spectrophotometer (JASCO, Japan) with 1-cm matched glass cell was used for absorbance measurements.

Chemicals, reagents and drugs

Analytical reagent grade chemicals and bidistilled water were used throughout the experiment. Salbutamol sulfate (Fluka, Switzerland), ritodrine · HCl (Duphar – Interfran Ltd, India), isoxsuprine HCl (Sigma, USA), dapsone hydrochloride (Sigma) sulfamethoxazole (Sigma, Belgium), sulfadiazine (Sigma, China) were procured and used as received to prepare standard solutions (0.1 mg mL^{-1}) in water. Sulfamethoxazole and sulfadiazine were initially dissolved in 1 mL of 1 mol L^{-1} HCl before making up to the volume. *N*,*N*-diethyl-*p*-phenylenediamine sulphate (Fluka, Germany) solution (0.5 mg mL^{-1}) and KIO_4 (Fluka, Germany) solution (4 mg mL^{-1}) were freshly prepared in water.

Pharmaceutical formulations

Asthalin tablet (Cipla, India) is labeled to contain 4 mg SLB per tablet. Yutopar tablet (Alimbic, India) and Duvadilon tablet (Duphar-Interfran, India) are labeled to contain 10 mg of RTD and IXP per tablet, respectively. Dapsone tablet (GlaxoSmithKline, India) and Septran tablet (GlaxoSmithKline) are labeled to contain 100 mg of DAP and SFM per tablet, respectively. Aubril tablet (Novartis Pharm, India) is labeled to contain 410 mg SFD per capsule.

General procedure

For SLB, RTD, and IXP. – Accurately measured volume of stock solution was transferred to a 10-mL volumetric flask and diluted to obtain a working concentration range of 1–7, 2–22, and 1–17 μg mL^{-1} of solution of SLB, RTD, and IXP, respectively. To the flask containing SLB, 3.8×10^{-4} mol L^{-1} of PADMA, 3.0×10^{-3} mol L^{-1} of NaOH and 8.7×10^{-4} mol L^{-1} KIO_4 were added. Similarly, 4.7×10^{-4} mol L^{-1} of PADMA, 5×10^{-3} mol L^{-1} of NaOH and 7.2×10^{-4} mol L^{-1} of KIO_4 were added to the flasks containing RTD and IXP. The flasks were swirled immediately; when the red colour formed changed to green colour, they were made up to the volume with water. Absorbance was measured against a reagent blank at 670 nm.

For DAP, SFM, and SFD. – Suitable volume of stock solution of DAP, SFM, and SFD was transferred to a 10-mL volumetric flask and diluted to 1.5–12, 2–25, and 2–21 μg mL^{-1}, respectively. To each flask, 4.75×10^{-4} mol L^{-1} of PADMA, 0.01 mol L^{-1} of HCl and 1.7×10^{-4} mL of KIO_4 were added. The flasks were heated on a water bath at 60 ± 5 °C for 10 min. After the flask had been cooled to room temperature, the solution was made up to the volume with water. The absorbance of the red solution was measured against a reagent blank at 550 nm.

Analysis of drugs in pharmaceutical formulations

The proposed method was applied to the quantification of drugs in dosage forms obtained from the local market. Twenty tablets of each drug were weighed and finely powdered using a mortar and pestle. Similarly, ten capsules of SFD were carefully evacuated, and the contents were mixed. A quantity equivalent to 10 mg of each drug was transferred to a 100-mL volumetric flask. The mixture was shaken mechanically in a small quantity of water for 5 min, sonicated in an ultrasonic bath, diluted to the volume with water, mixed and filtered. An appropriate aliquot of the filtrate covering the working concentration range mentioned in the general procedure was transferred to 10-mL volumetric flasks. For SFM and SFD, the powders were treated with 1 mL of 1.0 mol L^{-1} HCl before being subjected to the above mentioned extraction steps. The amount of drug in the formulation was analyzed according to the procedure mentioned above.

RESULTS AND DISCUSSION

Fig. 1 shows the absorption spectra of RTD and SFM. PADMA is oxidized by KIO_4 losing two electrons to yield reactive diethylbenzoquinone-diimine, which couples with the drugs by electrophilic attack at their nucleophilic site, preferably at *p*-position, or to the *o*-position of phenol or aromatic amine to give a leuco-dye, which is oxidized to an indo-dye (14). The coupling reaction takes place under slightly alkaline conditions for phenolic drugs because phenol is present as a more reactive phenoxide ion. Similarly, the reaction occurs in a slightly acidic environment for amine drugs because the concentration of quinine-diimine cation is maximal and at the same time an excessive amount of amine has not been converted to a nonreactive aminium salt (Scheme 1). The indo--dye product was stable for one hour.

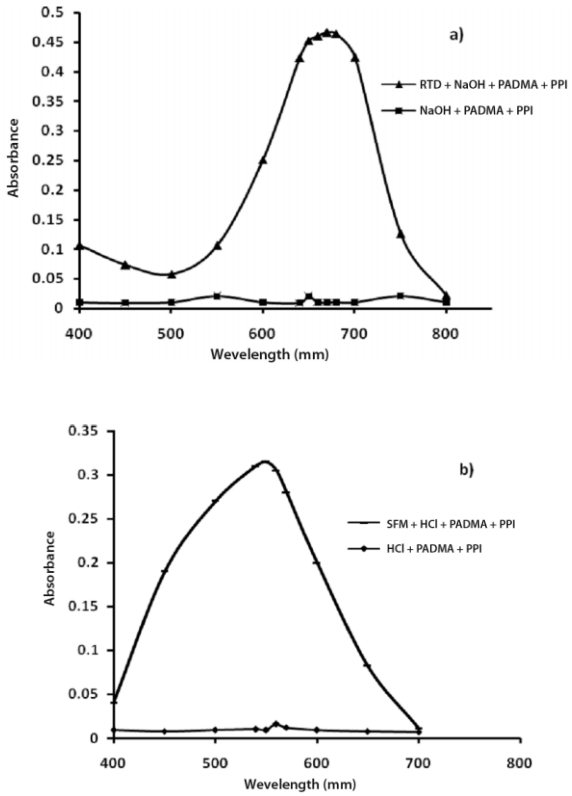
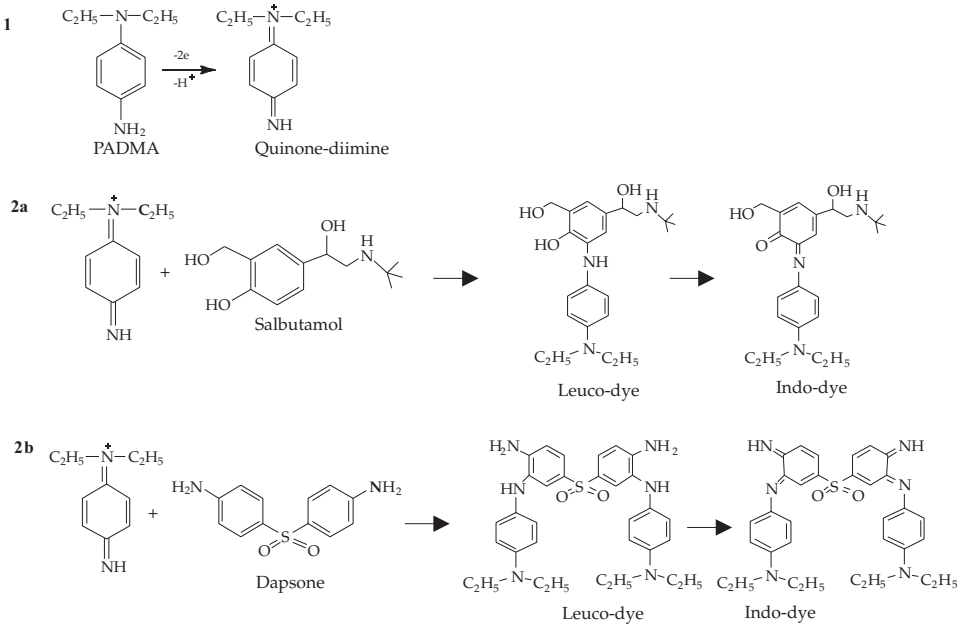


Fig. 1. Absorption spectra of: a) ritodrine (15 μg mL^{-1}) and b) sulfamethoxazole (10 μg mL^{-1}).



Scheme I. Proposed reaction mechanism for the formation of an indo-dye.

Validation

The Beer's law range, molar absorptivity, regression equation, and correlation coefficient were determined for each method. Confidence limits for the slope of the regression line and the intercept were computed using the relation, $b \pm t \cdot \mathrm{SD}$ and $a \pm t \cdot \mathrm{SD}$ at 95 % confidence level (12).

The limit od detection (*LQD*) and the limit of quantification (*LOQ*) values were determined according to the ICH guideline (13) using the formula

$$LOD \text{ or } LOQ = K \times \mathrm{SD}/b$$

were $K = 3$ for *LOD* and 10 for *LOQ*, SD and b stand for standard deviation of the intercept and slope, respectively.

The effects of common excipients used in the pharmaceutical preparations such as dextrose, sucrose, lactose, starch, magnesium stearate, talc, carboxymethyl cellulose and sodium chloride were studied by analyzing synthetic sample solutions containing drugs in the presence of 100-fold concentration excess of each excipient. The tolerance limit was defined as the concentration which gave an error of ± 3.0 % in the drug assay.

In order to establish the molar ratio between the drug and PADMA, the continuous variation method was applied, which indicated that the coupled products formed in the ratios of 1:1, 1:2, 1:1, 1:2, 1:1 and 1:1 for SLB, RTD, IXP, DAP, SFM, and SFD, respectively.

Optimization of reaction variables

Investigations were carried out to establish the most favorable conditions to achieve maximum color. The effect of PADMA concentration was studied over the range of (1.9–5.7) × 10^{-4} mol L^{-1}. The absorbance was maximal at 3.8 × 10^{-4} mol L^{-1} for SLB and 4.7 × 10^{-4} mol L^{-1} for the other drugs. Similarly, the effect of KIO_4 concentration was studied over the range of (1.4–10.4) × 10^{-4} mol L^{-1}. It was found that 8.7 × 10^{-4} mol L^{-1} for SLB, 7.2 × 10^{-4} mol L^{-1} for RTD and IXP and 1.7 × 10^{-4} for DAP, SFX and SFD was needed to obtain maximal absorbance. Alkaline condition was needed to develop the color in SLB, RTD and IXP. For DAP, SFM, and SFD, acidic environment was needed, which also decolorized the red colour developed in blank solution. The concentration of 3 × 10^{-3} mol L^{-1} NaOH for SLB, 5 × 10^{-3} mol L for RTD and IXP and 0.01 mol L^{-1} HCl for DAP, SMF and SFD, as mentioned in the general procedure, was taken for maximal color development. The oxidation of PADMA by various oxidants such as IO_3^-, $[Fe(CN)_6]^{4-}$, $Cr_2O_7^{2-}$, Ce^{4+} was examined and PADMA-IO_4^- was preferred because of the sensitivity achieved. The reaction was carried out at room temperature for phenolic drugs but the color development was slow in the case of drugs containing aromatic amine, so the reaction was carried out on a water bath at 60 ± 5 °C for 10 min. Higher temperature did not affect the sensitivity and duration of the reaction.

Table I. Optical characteristics and statistical data of the regression analysis

Parameter	SLB	RTD	IXP	DAP	SMF	SFD
Colour	Green	Green	Green	Red	Red	Red
λ_{max} (nm)	670	670	670	550	550	550
Beer's law range (μg mL^{-1})	1–7	2–22	1–17	1.5–12	2–25	2–21
Molar absorptivity × 10^{-4} (L mol^{-1} cm^{-1})	2.42	0.920	1.45	1.66	0.831	0.893
Limit of detection (μg mL^{-1})	0.14	0.21	0.51	0.44	0.33	0.37
Limit of quantification (μg mL^{-1})	0.48	0.72	1.71	1.45	1.10	1.24
Regression equation[a]						
Slope b ± CI[b]	0.102 ± 0.003	0.030 ± 0.002	0.036 ± 0.002	0.057 ± 0.002	0.028 ± 0.002	0.033 ± 0.002
Intercept a ± CI[c]	–0.004 ± 0.012	0.015 ± 0.026	0.047 ± 0.002	0.008 ± 0.011	0.036 ± 0.082	0.022 ± 0.024
R	0.9996	0.9980	0.9986	0.9995	0.9992	0.9989

[a] $A = a + bc$, where c is the concentration of the measured solution in μg mL^{-1}.
[b] Confidence interval for slope at 95 % confidence limit for five degrees of freedom.
[c] Confidence interval for intercept at 95 % confidence limit five degrees of freedom.

Analytical performances

Linearity and limiting values. – A linear relationship was found between the absorbance at 670 nm for SLB, RTD and IXP in the range 1–7, 2–22 and 1–17 μg mL^{-1}, respectively, and at 550 nm for DAP, SFX and SFD in the range, 1.5–12, 2–25 and 2–21 μg mL^{-1}, respectively. Regression analysis of the Beer's law plots revealed a good correlation (R = 0.9980–0.9996). High moral absorptivities of the resulting colored solutions indicated high sensitivity of the metod. The *LOD* and *LOQ* values ranged from 0.14–0.51 and 0.48–1.45 μg mL^{-1}, respectively (n = 7). The optical characteristics are shown in Table I.

Interference study. – The results of the interference study are shown in Table II. For sodium chloride, starch, talc, carboxymethyl cellulose, magnesium stearate, sucrose, the tolerance limit was studied by taking the 100-fold concentration of drug. Recovery was 99.1–100.0 %. Dextrose and lactose were tolerated up to 10-fold and 6-fold of that of the drug concentration taken, respectively; recovery was 99.6–100.9 %. Trimethoprim, which is present along with SFM in co-trimoxazole, a sulfonamide formulation, did not interfere with the assay at a 100-fold concentration of the drug, the recovery being 100.3 %.

Table II. Recovery of drugs in the presence of 100-fold excess of various additives[a]

Excipients	Recovery (%)[a]					
	SLB[b]	RTD[c]	IXP[d]	DAP[e]	SFM[f]	SFD[g]
Dextrose	99.3 ± 0.4	99.2 ± 0.3	99.5 ± 0.3	99.8 ± 0.4	99.9 ± 0.2	99.9 ± 0.5
Lactose	100.0 ± 0.8	100.9 ± 0.5	99.8 ± 0.3	99.9 ± 0.5	100.0 ± 0.7	99.9 ± 0.6
Starch	99.8 ± 0.2	99.1 ± 0.7	99.9 ± 0.5	99.5 ± 0.2	99.1 ± 0.3	99.8 ± 0.3
Sucrose	99.9 ± 0.2	99.9 ± 0.8	99.7 ± 0.3	99.9 ± 0.3	99.9 ± 0.3	99.8 ± 0.3
Carboxymethyl cellulose	99.8 ± 0.4	100.0 ± 0.6	99.8 ± 0.1	99.3 ± 0.1	99.8 ± 0.4	99.4 ± 0.7
Talc	99.8 ± 0.3	100.0 ± 0.4	99.6 ± 0.6	99.7 ± 0.8	99.2 ± 0.5	99.4 ± 0.6
Magnesium sterate	99.6 ± 0.6	99.4 ± 0.7	99.5 ± 0.2	99.2 ± 0.1	99.4 ± 0.1	99.6 ± 0.3
Sodium chloride	100.0 ± 0.1	100.0 ± 0.5	100.0 ± 0.1	100.0 ± 0.2	100.0 ± 0.2	100.0 ± 0.4
Trimethoprim	–	–	–	–	100.3 ± 0.2	–

[a] Mean ± SD, n = 3.
[b] Concentration of SBM: 4 μg mL^{-1}.
[c] Concentration of RTD: 13 μg mL^{-1}.
[d] Concentration of IXP: 10 μg mL^{-1}.
[e] Concentration of DAP: 6 μg mL^{-1}.
[f] Concentration of SFM: 14 μg mL^{-1}.
[g] Concentration of SFD: 12 μg mL^{-1}.
[h] The tolerance limit for dextrose and lactose is 10- and 16-fold excess over the drugs.

Table III. Intra-day and inter-day precision data

	Concentration taken ($\mu g\ mL^{-1}$)	Recovery	
		Intra-day[a]	Inter-day[b]
SBM	2.0	2.0 ± 0.9	2.0 ± 0.9
	4.0	4.0 ± 1.0	4.0 ± 1.0
	6.0	6.0 ± 0.5	6.0 ± 0.8
RTD	6.0	6.0 ± 0.6	6.0 ± 0.7
	12.0	12.0 ± 0.3	12.0 ± 0.7
	18.0	18.0 ± 0.4	18.0 ± 0.7
IXP	4.0	4.0 ± 0.6	4.0 ± 0.8
	8.0	8.0 ± 0.4	8.0 ± 0.7
	12.0	12.0 ± 0.6	12.0 ± 0.5
DAP	3.0	3.0 ± 0.5	3.0 ± 0.7
	6.0	6.0 ± 0.4	6.0 ± 0.6
	9.0	9.0 ± 0.4	9.0 ± 0.5
SFM	5.0	5.0 ± 0.5	5.0 ± 0.8
	10.0	10.0 ± 0.6	10.0 ± 0.5
	15.0	15.0 ± 0.4	15.0 ± 0.8
SFD	5.0	5.0 ± 0.9	5.0 ± 0.8
	10.0	10.0 ± 0.8	10.0 ± 0.8
	15.0	15.0 ± 0.2	14.0 ± 0.4

[a] Mean ± RSD (%), n= 5.
[b] Mean ± RSD (%), n = 5, performed over a period of 5 days.

Table IV. Analysis of drugs in pharmaceutical formulations

Formulation	Mass per dosage form (mg)	Mass found (mg)		t-value[c]	F-value[c]	Recovery (%)[d]	Error (%)
		Proposed method[a]	Reported method[a,b]				
Asthalin	4.0	3.9 ± 2.2	3.9 ± 2.5 (1)	1.120	1.21	99.5 ± 0.1	1.5
Yutopar	10.0	9.9 ± 1.4	9.9 ± 1.1 (3)	0.137	1.62	99.9 ± 0.3	0.1
Duvadilon	10.0	9.9 ± 1.0	9.8 ± 1.1 (2)	0.150	1.48	99.9 ± 0.1	0.9
Dapsone	100.0	100.0 ± 0.1	99.9 ± 0.1 (7)	1.330	1.35	99.9 ± 0.2	0.1
Septran	100.0	100.5 ± 0.4	100.0 ± 0.5 (9	1.860	1.10	99.8 ± 0.2	0.5
Aubril	410.0	410.1 ± 0.1	$410.2 \pm 0.0_5$ (9)	0.754	1.22	99.8 ± 0.4	0.0_2

[a] Mean ± SD, n = 6.
[b] Numbers inside the brackets indicate the reference number of reported methods.
[c] t- and F- values after comparison to the reference methods; theoretical values at 95 % confidence limit: t = 2.44, F= 5.05.
[d] Mean ± RSD, n = 6. After adding four different amounts of pure drugs to a fixed concentration of pre-analyzed pharmaceutical formulations.

Table V. Comparison of the proposed with the reported method

Drug studied	Reagent used	λ_{max}	Beer's law range (μg mL^{-1})	Remark	Reference
SLB	2,6-dichloroquinone chlorimide and 7,7,8,8-tetracyanoquino dimethane	602 and 842	1.0–30 and 8.0–20	Use of organic solvent	1
	Nitric acid, sulphuric acid and acetone	386	4.8–16	Tedious steps involving extraction by evaporation	2
RTD	Dapsone	460	0.5–18	Multiple steps; diazotisation followed by coupling reaction	3
IXP	4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole	395	2.0–20	Kinetic determination, time consuming	5
DAP	Iminodibenzyl	570	0.1–2.5	Multiple steps; diazotisation followed by coupling reaction in alcohol medium	7
	Sodium 1, 2-naphthoquinone-4-sulfonic	525	0.4–10	Use of surfactant	8
	Dopamine, molybdic acid	500, 490	0.1–7.0, 0.1–7.0	Multiple steps; diazotisation followed by coupling reaction, in sulphuric acid medium	9
SFM, SFD	3-aminophenol	460		Multiple steps; diazotisation followed by coupling reaction	10
SLB RTD IXP DAP SMF SFD	*N*,*N*-diethyl-*p*-phenylenediamine	670/550	1–7, 2–22,1–17, 1.5–12, 2–25 and 2–21 for SLB, RTD, IXP, DAP, SMF and SFD, respectively	No pH control, use of organic solvents or tedious sample preparation steps	This paper

Precision and accuracy. – The short term precision (intra-day precision) of the drugs evaluated by measuring 5 independent samples at 3 different concentration levels of SLB, RTD, IXP, DAP, SFM and SFD. Similarly, the assay for inter-day precision at the same concentration level was repeated for 5 consecutive days (Table III). The relative standard deviation was between 0.2–1.0 and 0.4–1.0 %, respectively, indicating good precision of the proposed methods.

Available pharmaceutical dosage forms of the investigated drugs were analyzed by the proposed methods. The precision of the method was checked by taking six replicate measurements. The results obtained by the proposed and the reference methods (1–3, 7, 9) for the dosage forms were compared statistically by means of *F*- and *t*-tests and were

not found to differ significantly at the 95 % confidence level. The relative standard deviation ranged from 0.1–2.2 %. The reliability and accuracy of the proposed methods were further ascertained through recovery studies using the standard addition method by adding different amounts of standard drugs to the preanalyzed dosage forms. Recovery value was 99.5–99.9 % and the standard deviation was 0.1–0.3 % (Table IV). The error ranged between 0.0_2–1.5 %.

CONCLUSIONS

The proposed methods offer simple and accurate procedures, based on coupling of oxidized PADMA, for the determination of drugs containing phenolic and aromatic amine groups. The optical parameters and statistical comparison justify the use of these methods in routine drug estimation in pure and dosage forms. Also, the comparison with the existing methods given in Table V shows that the proposed procedures do not involve any critical reaction conditions such as pH control, use of organic solvents or tedious sample preparation steps and can be recommended for the routine analysis of drugs.

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REFERENCES

1. G. G. Mohamed, M. S. Khalil, M. A. Zayed and A. H. M. El-Shall, 2,6-Dichloroquinone chlorimide and 7,7,8,8-tetracyanoquinodimethane reagents for the spectrophotometric determination of salbutamol in pure and dosage forms, *J. Pharm. Biomed. Anal.* **28** (2002) 1127–1133; DOI: 10.1016/S0731-7085(01)00718-X.
2. R. S. Bakry, A. F. El Walily and S. F. Belal, Spectrophotometric determination of etilefrine, ritodrine, isoxsuprine and salbutamol by nitration and subsequent Meisenheimer complex formation, *Anal. Lett.* **28** (1995) 2503–2519; DOI: 10.1080/00032719508004031.
3. P. Nagaraja, K. Sunitha, R. Vasantha and H. Yathirajan, Novel method for the spectrophotometric determination of isoniazid and ritodrine hydrochloride, *Turk. J. Chem.* **26** (2002) 743–750.
4. H. D. Revansiddappa, B. Manjula and P. G. Ramappa, Spectrophotometric method for the determination of ritodrine hydrochloride and amoxicillin, *Anal. Sci.* **15** (1999) 661–664.
5. N. El-Enany, F. Belal and M. Rizk, Kinetic spectrophotometric determination of isoxsuprine in dosage forms through derivatisation with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), *Sci. Pharm.* **74** (2006) 99–119.
6. N. W. Beyene, J. F. V. Staden, R. I. Stefan and H. Y. Aboul-Enein, Determination of isoxsuprine hydrochloride by sequential injection visible spectrophotometry, *Farmaco* **60** (2005) 613–619; DOI: 10.1016/j.farmac.2005.05.001.
7. P. Nagaraja, H. S. Yathirajan, K. R. Sunitha and R. A. Vasantha, Novel methods for the rapid spectrophotometric determination of dapsone, *Anal. Lett.* **35** (2002) 1531–1540; DOI: 10.1081/AL-120006728.
8. H. Y. Wang, L. X. Xu, Y. Xiao and J. Han, Spectrophotometric determination of dapsone in pharmaceutical products using sodium 1,2-naphthoquinone-4-sulfonic as the chromogenic reagent, *Spectrochim. Acta*, Part A **60** (2004) 2933–2939; DOI: 10.1016/j.saa.2004.02.013.

9. P. Nagaraja, H. S. Yathirajan, K. R. Sunitha and R. A. Vasantha, A new, sensitive, and rapid spectrophotometric method for the determination of sulfa drugs, *J AOAC* **85** (2002) 869–874.
10. P. Nagaraja, H. S. Yathirajan, C. R. Raju, R. A. Vasantha, M. S. Hemanthakumar and P. Nagendra, 3-Aminophenol as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives, *Farmaco* **58** (2003) 1295–1300; DOI: 10.1016/S0014-827X(03)00093-4.
11. K. Jiao, W. Sun, S. Zhang and G. Sun, Application of *p*-phenylenediamine as an electrochemical substrate in peroxidase-mediated voltammetric enzyme immunoassay, *Anal. Chim. Acta*. **413**, (2000) 71–78; DOI: 10.1016/S0003-2670(00)00820-5.
12. *Vogel's Textbook of Quantitative Chemical Analysis* 6th ed., J. Mendham, R. C. Denney, J. D. Barnes and M. J. K. Thomas Pearson Education Ltd, Singapore 2000, p. 165.
13. I*CH Harmonized Tripartite Guideline prepared within the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) Q2B, Validation of Analytical Procedures: Methodology*, ICH, London 1996.
14. C. S. P. Sastry, M. V. Suryanarayan and A. S. R. P. Tipirneni, Application of *p*-N,N-dimethylphenylenediamine dihydrochloride for the determination of some diuretics, *Talanta* **36** (1989) 491–494; DOI: 10.1016/0039-9140(89)80234-6.

S A Ž E T A K

Upotreba *N,N*-dietil-*p*-fenilenediamin sulfata za spektrofotometrijsko određivanje lijekova iz skupine fenola i amina

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U radu je predložena spektrofotometrijska metoda za određivanje lijekova s fenolnom skupinom [salbutamol sulfat (SLB), ritodrin hidroklorid (RTD), izoksuprin hidroklorid (IXP)] i lijekova s aromatskom amino skupinom [dapson hidroklorid (DAP), sulfametoksazol (SFM) i sulfadiazin (SFD)] u farmaceutskim dozirnim pripravcima. Metode se temelje na reakciji ljekovitih tvari s *N,N*-dietil-*p*-fenilendiamin sulfatom u prisutnosti KIO_4, pri čemu nastaje zeleni (λ_{max} pri 670 nm), odnosno crveni produkt (λ_{max} pri 550 nm). Apsorbancije linerano ovise o koncentrancijama lijekova uz visok koeficijent korelacije (0,9986–0,9996) u koncentracijskom području 1–7, 2–22, 1–17, 1,5–12, 2–25 i 2–21 μg mL^{-1} za SLB, RTD, IXP, DAP, SFM i SFD. Analizirani su i optimirani promjenjivi parametri kao što su temperatura, reakcijsko vrijeme i koncentracija reaktanata. Repetibilnost i intermedijarna preciznost iznosile su 0,2-1,0, odnosno 0,4-1,0 %. Nije primjećena nikakva interferencija s uobičajenim farmaceutskim pomoćnim sredstvima. Pouzdanost i izvedbene značajke predložene metode validirane su statistički. Povrat analitičke metode bio je od 99,5 ± 0,1 do 99,9 ± 0,3 %. Granice detekcije bile su 0,14, 0,21, 0,51, 0,44, 0,33 i 0,37 μg mL^{-1} za SLB, RTD, IXP, DAP, SFM, odnosno SFD.

Ključne riječi: *N,N*-dietil-*p*-fenilenediamin sulfat; UV/Vis spektrofotometrija; oksidacija; reakcija konjugacije

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