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Spectrophotometric determination of clobetasol propionate, halobetasol propionate, quinagolide hydrochloride, through charge transfer complexation

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Abstract

Two spectrophotometric procedures are described for the determination of clobetasol propionate(I), halobetasol propionate(II) (corticosteroids) and quinagolide hydrochloride(III) (prolactin inhibitor). For corticosteroid drugs, the procedures are based on the formation of phenyl hydrazones of the corticosteroids which are subsequently subjected to charge transfer complexation reaction with either 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) as π-acceptor or with iodine as σ-acceptor. Prolactin inhibitor was reacted directly with the previous reagents. The molar ratios of the reactants were established and the experimental conditions were studied giving maximum absorption at 588 and 290 nm with DDQ and iodine methods, respectively for the three drugs. The concentration ranges were 20–150, 50–300, and 20–80 μg ml^{-1} in DDQ method for (I), (II), and (III), respectively and 13–20, 15–40, and 8–32 μg ml^{-1} in iodine method for (I), (II) and (III), respectively. © 2002 Published by Elsevier Science B.V.

Keywords: Spectrophotometry; Charge-transfer complex; π- and σ-acceptor; Clobetasol propionate; Halobetasol propionate; Quinagolide hydrochloride; Drug formulations

1. Introduction

Clobetasol propionate(I), and halobetasol propionate(II) are widely used as anti-inflammatory drugs. Clobetasol propionate(I) is (11B,16B)-21-chloro-9-fluoro-11,17-dihydroxy-16-methylpregna-1,4-diene-3,20-dione [1]. Halobetasol propionate(II) is (6α,11B,16B)-21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)pregna-1,4-diene-3,20-dione [1]. Several methods have been reported for their determination in pharmaceutical formulations and biological fluids, including spectrophotometry [2–7], HPTLC [8], HPLC [9–12].

Quinagolide hydrochloride(III) is used as prolactin inhibitor and it is (3α,4aα,10aB)-(±)-*N*,*N*-Diethyl-*N*-(1,2,3,4,4a,5,10,10a-octahydro 6-hydroxy-1-propylbenzo[g]-quinolin-3-yl) sulfamide [1]. Only HPLC method [13] has been reported for its

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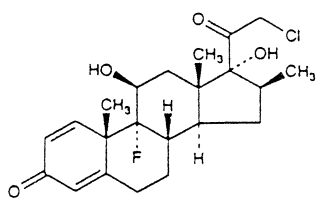
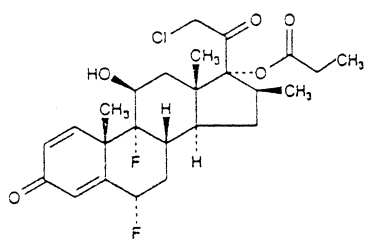
determination in bulk powder and in pharmaceutical formulation. The structure of the drugs are shown in Scheme 1.

Charge transfer complexation reaction have been extensively utilized for the determination of electron donating basic nitrogenous compounds using either π- or σ-acceptor such as (DDQ) or (iodine), respectively [14,15].

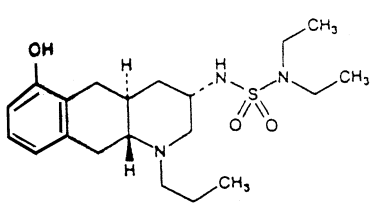
The reaction of corticosteroids with phenyl hydrazine results in the introduction of a basic nitrogen moiety to the corticosteroid molecule rendering it capable of participating in charge transfer complexation reactions with the π- or σ-acceptor [16].



Clobetasol propionate I



Halobetasol propionate II



Quinagolide hydrochloride III

Scheme 1.

A favorable characteristic of the proposed procedures are the speed, selectively, and ease of performing the assay. Searching the published methods for the determination of quinagolide hydrochloride, and halobetasol propionate shows that the colorimetric techniques have not been previously applied, consequently the present work describes new colorimetric methods which are cheaper than the published HPLC [12,13]. In the determinations of corticosteroids drugs, the newly proposed methods are simple, fast, of low cost, saving time and require minimum chemicals than the published colorimetric methods [2–7].

Also, the proposed methods, especially the iodine methods have nearly the same sensitivity with respect to the published methods. Hence, the proposed methods are more suitable for routine control analysis in a less equipped quality control laboratory.

This paper describes the application of to reaction with π- and σ-acceptors (DDQ and iodine, respectively) in the spectrophotometric determination of the cited drugs in pure, and dosage forms.

2. Experimental

2.1. Apparatus

1-SHIMADZU 1601 PC UV–Vis spectrophotometer.

2.2. Materials

2.2.1. Pure samples

Clobetasol propionate, working standard, kindly supplied by Glaxo Wellcome, Cairo, Egypt. Its purity was found to be a 99.76 ± 0.86%, according to the official method [10].

Halobetasol propionate, working standard, kindly supplied by Novartis Pharma S.A.E., Cairo, Egypt. Its purity was found to be 100.40 ± 1.34% according to the compendial method [12].

Quinagolide hydrochloride, working standard, kindly supplied by Novartis Pharma S.A.E., Cairo, Egypt. Its purity was found to be 99.88 ± 1.70% according to the compendial method [13].

2.2.2. Market samples

1. Dermovate cream (Glaxo Wellcome pharmaceuticals), batch number 000210A. Each 100 g cream was labeled to contain clobetasol propionate 0.05 g, cetostearyl alcohol 8.40 g, glyceryl monostearate 11.00 g, arlacel 1.50 g, beeswax substitute 1.15 g, propylene glycol 47.50 g, chlorocresol 0.075 g, sodium citrate 0.05 g, citric acid monohydrate 0.05 g, purified water to 100.00 g.
2. Micracorten cream (Novartis pharma), batch number 024. Each 100 g cream was labeled to contain halobetasol propiorate 0.05 g, cetyl alcohol 6.00 g, glycerin pure 2.00 g, isopropyl isosterate 3.00 g, isopropyl palmitate 2.00 g, polyoxyethylene-21-stearyl ether 3.00 g, water 83.95 g.
3. Norprolac Tablet (Novarits pharma), batch number 033/017. Each tablet was labeled to contain quinagolide hydrochloride 0.075 mg, silica colloidal anhydrous 0.30 mg, magnesium stearate 0.70 mg, methyl hydroxylpropyl cellulose 12.90 mg, cellulose microcrystalline 25.80 mg, lactose 87.00 mg.

2.2.3. Reagents and chemicals

All chemicals used were of analytical grade, solvents were of spectroscopic grade.

1. Phenylhydrazine hydrochloride, 2.0% w/v in ethanol.
2. DDQ (Aldrich Co.), 0.5% w/v solution in acetonitrile, freshly prepared.
3. Iodine (Chemiefarma, Holland), 2×10^{-3} M solution in chloroform, freshly prepared.
4. Absolute ethanol (Analar, BHS, England).
5. Sodium hydroxide, 0.5 M aqueous solution.

2.2.4. Standard stock solutions

Standard stock solutions must be freshly prepared for all cited drugs.

1. For (I) and (II): 1 mg ml^{-1} in ethanol.
2. For (III): 0.4 mg ml^{-1} base in acetonitrile and in chloroform for DDQ and iodine methods, respectively.

2.2.4.1. Preparation of quinagolide base. An accurately weighed amount of quinagolide hydrochloride equivalent to 40 mg was transferred quantitatively into 125 ml separating funnel containing 10 ml of 0.5 M sodium hydroxide. The solution was extracted with 4×20 ml chloroform and washed with 20 ml water. The chloroform extract was filtered through anhydrous sodium sulphate into a 100 ml volumetric flask and the volume was completed to the mark with chloroform. Twenty-five milliliters of the chloroform extract was evaporated under nitrogen and dissolved in 25 ml acetonitrile (0.4 mg ml^{-1}).

2.3. Hydrazone formation of corticosteroid drugs

To 25 ml ethanolic solution (1 mg ml^{-1}), of each of the corticosteroid drugs, 15 ml of phenylhydrazine solution, and the mixture was refluxed for 45 min. Ethanol was evaporated at low temperature and the residue was dissolved in 2×10 chloroform. The chloroform solution was filtered, transferred into a 25 ml volumetric flask and completed to volume with chloroform, (for iodine method), (1 mg ml^{-1}). Ten milliliters of chloroform solution was evaporated under nitrogen and the residue was dissolved in 10 ml acetonitrile (for DDQ method) (1 mg ml^{-1}).

2.3.1. Construction of calibration curves

Calibration curves were constructed according to the optimum conditions in Table 1.

2.3.1.1. DDQ method. Different aliquots of each of the hydrazone standard solution in acetonitrile (0.2–1.5 mg), (0.5–3.0 mg) for I and II, respectively and (0.2–0.8 mg) of III were transferred into separate 10 ml volumetric flasks. Four milliliters DDQ reagent was added for I, II and 1 ml for III and the volume was completed to the mark with the same solvent. The absorbance was measured at 588 nm for the cited drugs against a reagent blank.

2.3.1.2. Iodine method. Different aliquots of each of hydrazone standards solution and quinagolide standard stock solution in chloroform (0.13–0.20 mg), (0.15–0.40 mg), and (0.08–0.32 mg) for I, II

Table 1
Optimum conditions used for the proposed methods

Parameters	Clobetasol propionate		Halobetasol propionate		Quinagolide hydrochloride	
	DDQ	Iodine	DDQ	Iodine	DDQ	Iodine
Amount of standard taken (μg/10 ml)	200–1500	130–200	500–3000	150–400	200–800	80–320
Amount of reagent	4 ml	1 ml	4 ml	1 ml	1 ml	1 ml
Time	Immediately	Immediately	Immediately	After 45 min	Immediately	After 1 h
λ_{max}	588	290	588	290	588	290
Stability of colored product	20 min	20 min	20 min	20 min	20 min	Constant for more than 2 h

and III, respectively, were transferred into separate 10 ml volumetric flasks, 1 ml iodine solution was added to each flask and the volume was completed to the mark with chloroform.

The absorbance was measured immediately, after 45 min, and after 1 h at 290 nm for I, II and III, respectively, against a reagent blank.

2.3.2. Dosage forms

2.3.2.1. Creams for (I) and (II). Two grams of cream in 200 ml water was melted in water bath, cooled, and extracted with 3×15 ml chloroform. Each chloroform extract was cooled in a refrigerator, filtered, and washed each time. The chloroform was collected and evaporated. The residue was dissolved in 10 ml ethanol and was refluxed as under 2.3 for hydrazone formation.

2.3.2.2. Tablet for (III). Twenty tablets of norprolac were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 1 mg quinagolide base was dissolved in about 50 ml warm distilled water. The solution was rendered alkaline with 10 ml 0.5 N sodium hydroxide, and then extracted with chloroform as under Section 2.2.4. After preparation of test solution, proceed as described under Sections 2.3.1.1 and 2.3.1.2

3. Results and discussion

It is assumed that under the described experimental conditions, phenylhydrazine condenses with C_{20} keto group in the corticoid molecule to give yellow product according to the following reaction [16].

$$>C{=}O + H_2N{-}\underset{H}{N}{-}\Phi \rightarrow C{=}N{-}\underset{H}{N}{-}\Phi + H_2O$$

Fifteen milliliters of phenylhydrazine reagent was sufficient to ensure a quantitative reaction. The optimum time of refluxing the ethanolic corticoid solution with phenylhydrazine reagent was 45 min, the ethanol then being evaporated from the reaction mixture prior to extraction of the hydrazone with chloroform in which excess free phenyl hydrazine hydrochloride is soluble [16]. However, the very low concentration of the latter that may escape into the chloroform, is assumed to be unreactive in charge transfer complexation reactions. It was confirmed experimentally that no absorbance at wavelengths with DDQ or iodine reagent was observed [16].

4. DDQ method

π-acceptors are known to yield charge transfer complexes with a variety of electron donors [17]. In non-polar solvent, the molecular charge transfer complexes are formed, whereas in polar solvent, the radical anions are the predominant species [16]. When the phenylhydrazone of the corticocosteroid drugs in acetonitrile was mixed with DDQ solution, a radical anion is formed as in the following equation:

$$\underset{\text{Donor}}{\ddot{D}} + \underset{\text{Acceptor}}{A} \rightarrow (D-A) \overset{\text{polar}}{\underset{\text{solvent}}{\rightarrow}} \dot{D}^{+} + \underset{\text{Radical anion}}{\dot{A}^{-}}$$

The optimum reaction time was determined by following color development at ambient temperature (30 °C), complete color development was attained immediately and was stable for 20 min for all the studied drugs as shown in Table 1.

The cited drugs exhibit approximately the same absorption maxima at 588 nm but with different intensities as shown in Fig. 1.

The stochiometry of the reactions was studies by Job's method, it was found that the ratios were 1:1 (donor/acceptor) for I, II, and III [18]. This result indicates that the cited drugs possess only one strongly basic nitrogen, whose lone electron pair are readily available for complexation with the acceptor.

The spectrophotometric properties of the color species formed with DDQ as well as the different parameters affecting the color development were extensively studied to determine the optical conditions for the assay procedure. The reaction was studied as a function of the volume of the reagent, and the nature of the solvent as shown in Table 1. Stability of colors and the molar ratio were also studied. Thus the relationship between the concentration of the studied drugs and the absorbency of the color formed was determined.

Beer's law was obeyed in the concentration ranges of 20–150, 50–300, and 20–80 μg ml^{-1} with mean percentage recoveries of 100.43, 100.05, and 100.28% and RSD of 1.15, 1.35, and 1.59% for I, II, and III, respectively, as shown in Table 4.

Also, Table 2 illustrates sensitivity ranges, molar absorbtivity, regression equation, correlation coefficient, and mean accuracy percentage for the proposed methods.

4.1. Iodine method

The phenylhydrazone of corticosteroids I, II, and *n* donor quinagolide III react with σ electron acceptor iodine forming charge transfer complex followed by tri-iode ion pair formation [15,16].

Charge transfer complexes formed have a high absorption band at 290 nm and a lower band has a maximum at 365 nm followed by the formation of tri-iode ion pair which is accompanied by variation in maximum absorption to wavelength ranging from 270 to 310 nm [15].

It is suggested that the cited drugs react with iodine to form a tri-iode ion pair with a higher band absorption at 290 nm and a lower band at 365 nm, for all drugs as shown in Fig. 2. The reaction is represented in Scheme 2.

This was postulated on the basis of the molar ratio of cited drugs to iodine (1:1) and consideration of previous reports [19] on similar reaction. Regarding the third step in equation, iodine alone does not absorb at wavelength of maximum absorption, hence the stoichiometry will show only the iodide ion released as a result of 1 mol of iodine being consumed in the second step [16].

The optimum conditions for the reaction between iodine and the cited drugs were carefully studies, and the results are presented in Table 1. Beer's law is obeyed in concentration ranges 13–20, 15–40, and 8–32 μg ml^{-1}, with mean percentage accuracy of 100.15, 99.75, 100.47% and RSD of 1.76, 1.71 and 1.47% for I, II and III, respectively.

Spectral data for reaction products of studies drugs are given in Table 2.

In order to determine the accuracy, and precision of both procedures solutions containing three different concentrations of each of the corticosteroids clobetasol propionate, halobetasol propionate, and the prolactin inhibitor quinagolide hydrochloride were prepared, and analyzed in five replicates. The results were 100.24 ± 0.32, 99.98 ± 0.41, 99.95 ± 0.043% in DDQ for I, II, III, respectively and 100.50 ± 0.05, 100.72 ± 0.02, 100.19 ± 0.09% in iodine methods for I, II, III, respectively.

The proposed methods were applied for the determination of pharmaceutical formulations and the results are shown in Table 3. The validity of the proposed procedures was assessed by applying the standard addition technique. The results obtained were reproducible with low standard deviation as shown in Table 3 and the mean recovery was compared to that obtained by the reported methods, in order to demonstrate the validity and applicability of the proposed methods.

Table 2
Spectral data for the cited drugs used in the proposed methods

Parameters	Clobetasol propionate			Halobetasol propionate			Quinagolide hydrochloride		
	DDQ	Iodine	Official method [10]	DDQ	Iodine	Compendial method [12]	DDQ	Iodine	Compendial method [13]
Concentration range (μg ml^{-1})	20–150	13–20	10–60	50–300	15–40	10–60	20–80	8–32	4−12
Intercept (*a*)	0.062	−1.475		0.008	−0.444		−0.158	−0.0166	
RSD of intercept (*a*)	2.5×10^{-3}	1.74×10^{-2}		0.5×10^{-6}	2.29×10^{-2}		4.9×10^{-3}	2.34×10^{-3}	
Slope (*b*)	0.0063	0.128		0.0033	0.049		0.0143	0.0168	
RSD of slope (*b*)	1.5×10^{-8}	1.87×10^{-3}		1.5×10^{-8}	1.2×10^{-5}		2.7×10^{-4}	6.3×10^{-5}	
Correlation coefficient (*r*)	0.998	0.989		0.983	0.998		1.042	1.042	
Molar absorbitivity	2.95×10^{3}	17.29×10^{3}		1.63×10^{3}	8.81×10^{3}		4.45×10^{3}	6.63×10^{3}	
LOD (μg ml^{-1}) [20]	19.50	8.00		38.27	10.00		18.00	6.00	
LOQ (μg ml^{-1}) [21]	18–100	10–18		40–250	6–20		16–63	5–28	

There was no evidence of interference from the excipients. The results of the proposed methods were statistically compared with those obtained by the reported methods. Table 3 shows that the calculated t- and F-values are less than the theoretical ones, confirming accuracy, and precision at 95% confidence level.

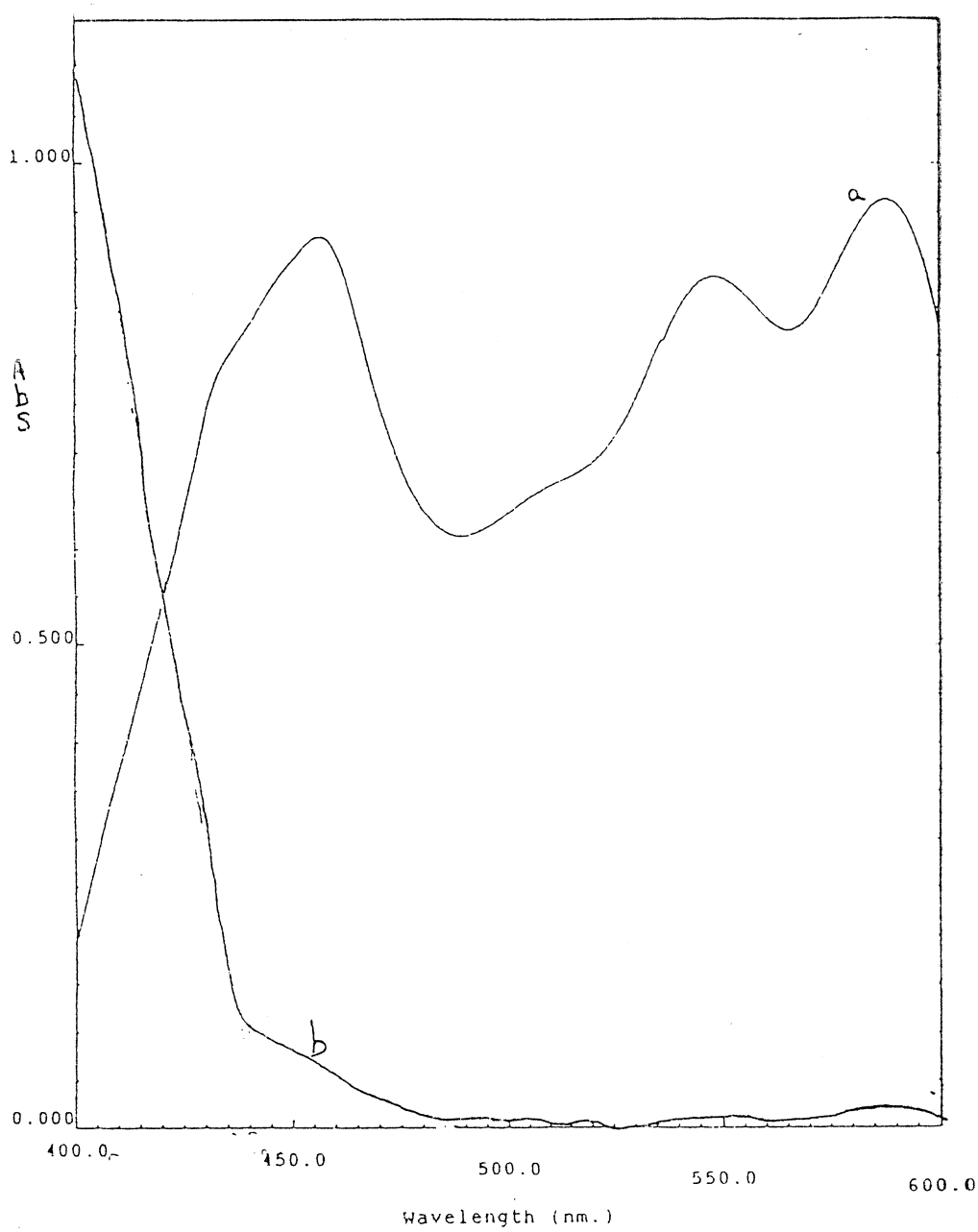


Fig. 1. Absorption spectra of: (a) hydrazone complex (150 μg ml^{-1}) in acetonitrile; (b) DDQ reagent (0.5% w/v) in acetonitrile.

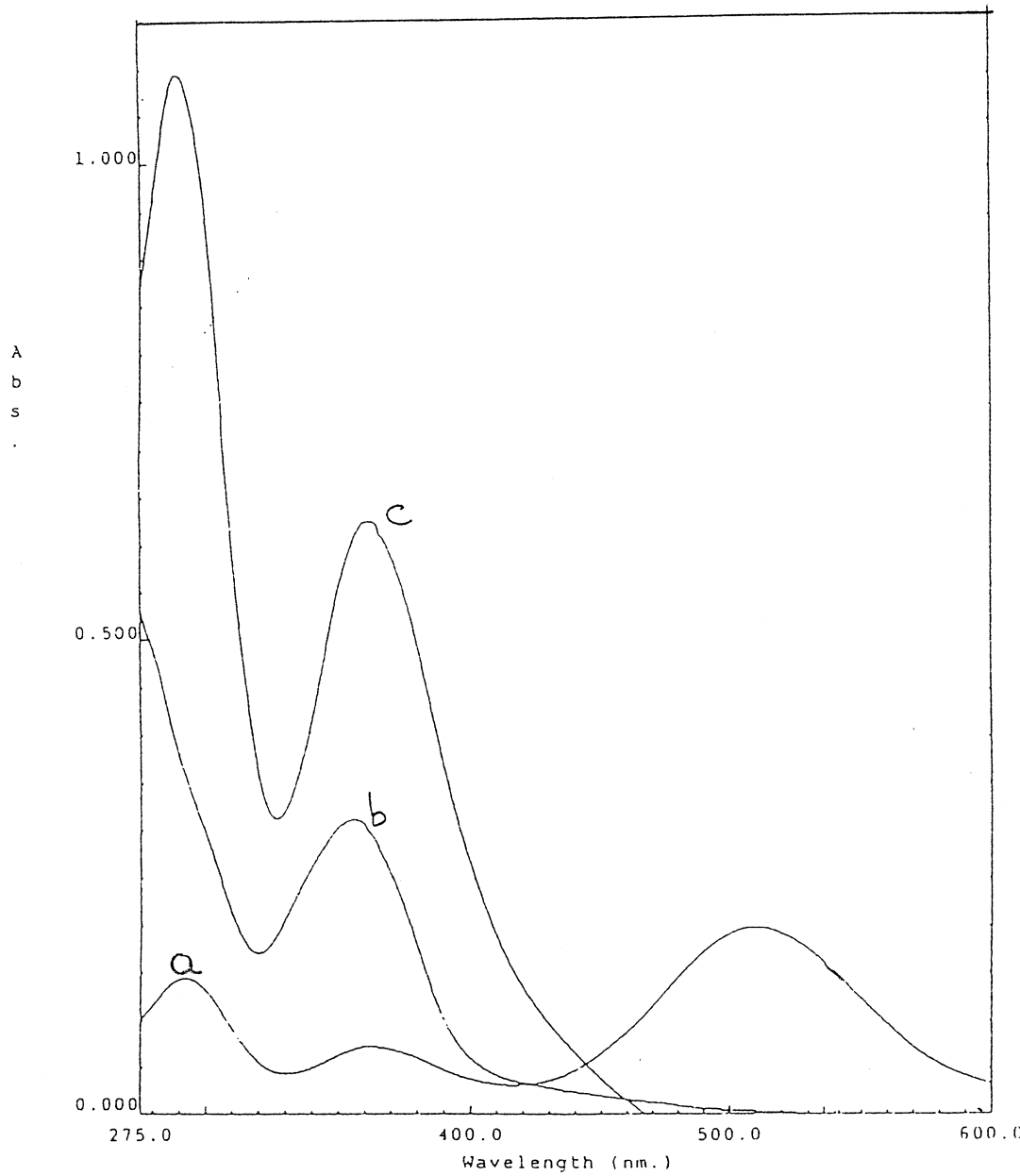


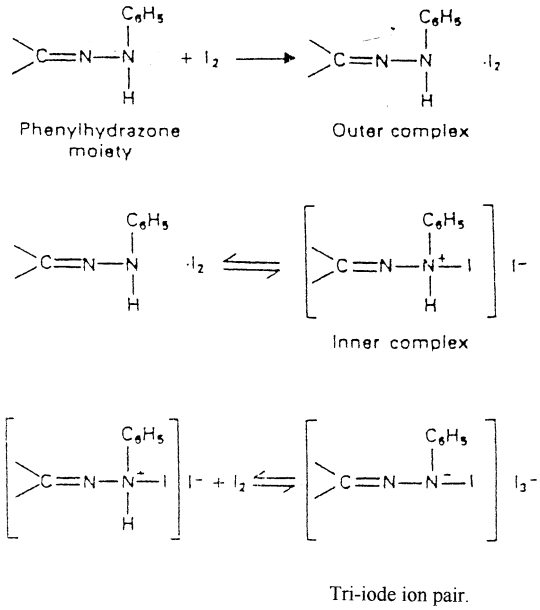
Fig. 2. Absorption spectra of: (a) iodine reagent (2×10^{-3} M) in chloroform; (b) hydrazone (20 μg ml^{-1}) in chloroform; (c) hydrazone complex (20 μg ml^{-1}) in chloroform.

5. Method validation

5.1. Linearity range

In quantitative analysis of the corticosteroids clobetasol propionate(I) halobetasol propionate(II), and the prolactin inhibitor quinagolide hydrochloride(III), a calibration curves were plotted, representing the relationship between the absorbance, and the corresponding concentration.

Beer's law was obeyed in concentration ranges 20–150, 50–300, 20–80 μg ml^{-1} in DDQ method



Scheme 2.

for (I), (II), and (III), respectively and 13–20, 15–40, and 8–32 μg ml^{-1} in iodine method for (I), (II), and (III), respectively where linearities were achieved.

5.2. Sensitivity

Sensitivity of the methods can be determined, through the limit of quantitation (LOQ), and limit of detection (LOD), in which determined by the analysis of samples with known concentration of analyte, and by establishing the minimum level at which the analyte can be reliably detected. LOD and LOQ are shown in Table 2.

5.3. Specificity/selectivity

Specificity is the ability of the method to measure the analyte response in the presence of the additives, and excipients.

It was found that assay results were not changed in pure form and drug formulations. In the proposed methods, there was no need of pre-separation and only filteration was applied to make the solution clear.

5.4. Accuracy

Standard addition, and recovery experiments were applied to determine the accuracy of the

Table 3
Analysis of dosage forms of the cited drugs, using the proposed and reported methods

Preparation	DDQ method		Iodine method		Official and compendial methods
	Found %	Recovery %	Found %	Recovery %	
Dermovate cream: clobetasol propionate 0.05 mg/100 g cr. B.N. 000210 A	99.54 ± 0.44% $F = 1.82(6.39)$ $t = 1.71(2.306)$	99.68 ± 0.07%	100.58 ± 0.04% $F = 2.2(6.39)$ $t = 0.129(2.306)$	99.28 ± 0.05%	99.32 ± 0.34%
Miracorten cream: halobetasol propionate 0.05 mg/100 g B.N.024	98.96 ± 0.13% $F = 1.95(6.39)$ $t = 0.29(2.306)$	99.08 ± 0.20%	100.97 ± 0.02% $F = 1.47(6.39)$ $t = 0.055(2.306)$	100.16 ± 0.23%	100.15 ± 0.28%
Norprolac tablet: quinagolide hydrochloride 0.075 mg/tab B.N.033/017	100.28 ± 0.04% $F = 2.06(6.39)$ $t = 1.64(2.306)$	100.01 ± 0.08%	99.97 ± 0.02% $F = 1.77(6.39)$ $t = 0.43(2.306)$	99.94 ± 0.07%	99.92 ± 0.06%

Table 4
Statistical comparison between the result of analysis of the cited drugs in pure form applying the proposed and reported methods

Parameters	Clobetasol propionate			Halobetasol propionate			Quinagolide hydrochloride		
	DDQ	Iodine	Official method	DDQ	Iodine	Compendial method	DDQ	Iodine	Compendial method
Mean[a] ± RSD%	100.43 ± 1.15%	100.15 ± 1.76%	99.76 ± 0.86%	100.05 ± 1.35%	99.75 ± 1.71%	100.40 ± 1.34%	100.28 ± 1.59%	100.47 ± 1.47%	99.88 ± 1.72%
Variance	1.32	3.10		1.82	2.92		2.53	2.16	
N	5	5		5	5		5	5	
F	1.78(6.39)	4.19(6.39)		1.011(6.39)	1.62(6.39)		1.17(6.39)	1.37(6.39)	
t	1.05(2.306)	0.443(2.306)		0.36(2.306)	0.60(2.306)		0.34(2.306)	0.52(2.306)	

Figures in parentheses are the theoretical *t* and *F* values at the confidence limit.
[a] The average of five determinations.

proposed methods, and in order to detect any interactions of the excipients.

Results shown in Table 3, compared with that in Table 4, show no interference from the excipients.

5.5. Precision

In order to determine the precision of both procedures, solutions containing three different concentrations of each of the corticosteroids, and the prolactin inhibitor were prepared, and analysed in five replicates, results are shown in Section 6.

The results were reproducible also, with low standard deviation as shown in Table 3, and this indicates that the developed methods have a good precision.

5.6. Robustness/ruggedness

Interlaboratory testing of the methods could not be performed, due to high expenses, and non-availability of accredited co-operative laboratories.

5.7. Stability

The solution of the corticosteroids were stable, if kept in the refrigerator for about 1 week, but the hydrazone solutions of the corticosteroid drugs were unstable, so they must be freshly prepared.

The prolactin inhibitor quinagolide hydrochloride, was stable for more than 1 week in the refrigerator.

6. Conclusion

The suggested methods have the advantages of being simple, accurate, time saving, inexpensive, sensitive, and requires minimum equipments and chemicals. DDQ and iodine method utilize a single step reaction and single solvent. The iodine method was more sensitive than the DDQ method, and had nearly the same sensitivity, when compared with the reported one.

These methods cannot be used as stability indicating methods, and they are also selective, but not specific.

The results are reproducible, and when compared with the reported methods, using the student's t-test, and variance ratio F-test no significance differences are observed in respect to accuracy and precision.

These methods can be used as general methods for spectrophotometric determination of the cited drugs in bulk powder and in dosage forms, have many advantages over other separation techniques such as HPLC, are reduced cost, and speed with high accuracy. The proposed methods are suitable for routine quality control.

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