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Stability indicating methods for the determination of diloxanide furoate

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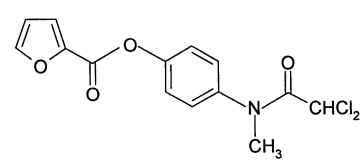
Abstract

Five new selective, precise and accurate methods are described for the determination of diloxanide furoate (DI) in presence of its degradation products. Method A utilizes the first and second derivative spectrophotometry at 270 and 280 nm, respectively. Method B is a RSD_1 spectrophotometric method based on the simultaneous use of the first derivative of ratio spectra and measurement at 270 nm. Method C is a pH-induced difference spectrophotometry using UV measurement at 295 nm. Method D is a densitometric one, after separation on silica gel plate using chloroform: methanol as mobile phase and the spots were scanned at 258 nm. Method E is reversed phase high performance liquid chromatography using methanol: water (80:20% v/v) as mobile phase at a flow rate of 1 ml/min and UV detection at 258 nm. Regression analysis showed good correlation in the concentration ranges 5–30, 5–25, 10–40 μg/ml, 100–500 ng/spot, 2–50 μg/ml with percentage recoveries of 99.92 ± 0.56 and 99.79 ± 0.47, 99.23 ± 0.38, 99.96 ± 0.06, 99.03 ± 0.51, 98.81 ± 0.68 for methods A, B, C, D and E, respectively. These methods are suitable as stability indicating methods for the determination of DI in presence of its degradation products either in bulk powder or in pharmaceutical formulations.

Keywords: Diloxanide furoate; Derivative spectrophotometry; RSD_1 spectrophotometry; pH-induced difference spectrophotometry; Thin-layer chromatographic densitometry; Reversed phase high performance liquid chromatography

1. Introduction

Diloxanide furoate (DI) is '2,2-dichloro-4-hydroxy-*N*-methylacetanilide-2-furoate' and is used as antiamoebic drug [1]. The structural formula is as follows:



Several methods have been reported for the determination of DI including titrimetric [2], electrochemical [3], spectrophotometric [4–19] and chromatographic [20–26] methods. None of these

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reported methods were used for the determination of DI in presence of the investigated degradation products.

The main task of this work is to establish simple and accurate stability indicating methods for the determination of DI in presence of its degradation products, which can be used for the routine and quality control analysis of DI in raw material and pharmaceutical formulations.

2. Experimental

2.1. Instruments

1. SHIMADZU UV-VISIBLE 1601 PC spectrophotometer.
2. UV lamp with short wavelength 254 nm
3. TLC plates coated (20 × 20 cm) with silica gel 60 F_{254} (E.MERCK)
4. SHIMADZU – dual wavelength flying spot CS-9301 densitometer
5. SHIMADZU CLASS-LC 10 liquid chromatographic system equipped with SHIMADZU SPD-10 A diiode array uv-detector, ZORBAX C18 (15 cm × 4.6 mm i.d., 5 μm particle size) column was used as stationary phase.

2.2. Materials

2.2.1. Pure samples

DI was kindly supplied by the National Organization for Drug control and Research. The purity of the sample was found to be 99.98% according to the B.P. method (1998).

2.2.2. Dosage forms

Amoebyl® tablets (Medical Union Pharmaceutical CO.A.R.E.); each tablet was claimed to contain 500 mg of DI; Batch No. 981353

Farcomid® tablets (Pharco Pharmaqceuticals, Egypt); each tablet was claimed to contain 500 mg of DI; Batch No. 151

Furamebe® tablets (SEDICO, Egypt); each tablet was claimed to contain 500 mg of DI; Batch No. 798124

Furamide® tablets (T3A Pharmaceuticals, Egypt); each tablet was claimed to contain 100 mg of DI; Batch No.21548

2.2.3. Reagents

All reagents and chemicals used were of analyticalgrade and were used without further purification

1. Methanol HPLC grade (B.D.H.)
2. Chloroform AR grade (PROLABO)
3. Deionized water
4. 0.1N HCl
5. 0.1N sodium hydroxide
6. 1N sodium hydroxide

2.2.4. Preparation of the degradation products

For 3 h, 0.3 g of pure DI were heated at reflux with 20 ml 1N sodium hydroxide. The solution was allowed to cool and upon cooling, the first degradation product '4-hydroxy-*N*-methyl aniline' separates out. The precipitate was filtered, washed and recrystalized. The filtrate was acidified with 2N sulfuric acid where the second degradation product '2-furoic acid' separates out, which was filtered, washed and recrystalized. The second filtrate contains dichloroacetic acid which is liquid and miscible with water, so it has been difficult to separate it for further quantitation. The obtained powders were used for the preparation of the stock solutions of the degradates.

2.2.5. Standard solutions

2.2.5.1. DI stock solution (1 mg/ml). An accurately weighed amount of DI equivalent to 100 mg was transferred to a 100 ml volumetric flask, 40 ml absolute ethanol was added, shaken for 10 min and completed to volume with absolute ethanol.

2.2.5.2. DI working solutions.

1. *For D_2 & D_3, RSD_1 and pH-induced difference (ΔA) spectrophotometric methods (*100 *μg/ml):* An amount equivalent to 10 ml of the previous stock solution was transferred to a 100 volumetric flask and completed to volume with absolute ethanol.
2. *For densitometric method (Δ* 0.5 *mg/ml):* An amount equivalent to 50 ml of the previous stock solution was transferred to a 100 ml volumetric flask and completed to volume with absolute ethanol.

3. *For HPLC method (*100 *μg/ml):* An amount equivalent to 10 ml of the previous stock solution was transferred to a 100 volumetric flask and completed to volume with methanol:water (80:20% v/v).

The degradates were prepared at the same above-mentioned concentrations and solvents for each of the corresponding methods.

2.2.6. Laboratory prepared mixtures

2.2.6.1. For first (D_1) and second derivative (D_2) spectrophotometric methods. Accurate aliquots equivalent to (50–300 μg) of DI were transferred from its stock solution (100 μg/ml) into a series of 10 ml volumetric flasks, and portions equivalent to 10–80% of the degradates from their stock solutions (100 μg/ml) were added to the same flasks and the volume was completed to the mark with methanol.

2.2.6.2. For RSD_1 spectrophotometric method. Accurate aliquots equivalent to (50–250 μg) of DI were transferred from its stock solution (100 μg/ml) into a series of 10 ml volumetric flasks, and portions equivalent to 10–80% of the degradates from their stock solutions (100 μg/ml) were added to the same flasks and the volume was completed to the mark with methanol.

2.2.6.3. For ΔA spectrophotometric method. Accurate aliquots equivalent to (100–400 μg/ml) of DI were transferred from its stock solution (100 μg/ml) into two sets of 10 ml volumetric flasks, and portions equivalent to 10–60% of the degradates from its stock solution (100 μg/ml) were added to the same flasks and the volume was completed to the mark in one set with 0.1N sodium hydroxide and in the other set with 0.1N HCl.

2.2.6.4. For densitometric method. Accurate aliquots equivalent to (100–500 μg) of DI were transferred from its stock solution (0.5 mg/ml) into a series of 10 ml volumetric flasks, and portions equivalent to 10–80% of the degradates from its stock solution (0.5 mg/ml) were added to the same flasks and the volume was completed to the mark with methanol.

2.2.6.5. For HPLC method. Accurate aliquots equivalent to (20–500 μg) of DI were transferred from its stock solution (100 μg/ml) into a series of 10 ml volumetric flasks, and portions equivalent to 10–90% of the degradates from their stock solutions (100 μg/ml) were added to the same flasks and the volume was completed to the mark with methanol:water (80:20% v/v).

2.3. Procedures

2.3.1. Method A: D_1& D_2 derivative spectrophotometric methods

2.3.1.1. Linearity. Accurate aliquots equivalent to (50–300 μg) of DI were transferred from its working solution into a series of 10 ml volumetric flasks then made up to volume using methanol. The first and second derivative absorption spectra of the UV spectrum of each solution against methanol as a blank were recorded. The peak height using 270 and 280 nm, respectively, as maxima and zero-crossing line as minima were measured. The calibration curves representing the relationship between the measured peak height and the corresponding concentration were constructed.

2.3.1.2. Assay of prepared mixtures. The first and second derivative (D_1 and D_2) spectra of the laboratory-prepared mixtures containing different ratios of DI and its degradates were recorded. The peak heights at 270 and 280 nm, respectively, were measured. The concentration of DI in the prepared mixtures was calculated from the regression equations.

2.3.2. Method B: RSD_1 spectrophotometric method

2.3.2.1. Linearity. Accurate aliquots equivalent to (50–250 μg) of DI were transferred from its working solution into a series of 10 ml volumetric flasks then made up to volume using methanol. The absorption spectra of these solutions were divided by the 'the divisor' (the absorption spectrum of 3 μg/ml of the second degradate), and the ratio spectra thus obtained were smoothed and

the first derivatives of the ratio spectra were recorded. The peak amplitude at 270 nm was measured. The calibration curve representing the relationship between the measured amplitude and the corresponding concentration was constructed.

2.3.2.2. Assay of prepared mixtures. The ratio spectra first derivative curves of the laboratory-prepared mixtures containing different ratios of DI and its degradate were recorded. The peak amplitude at 270 nm was measured, and then the concentration of DI in the prepared mixtures was calculated from the regression equation.

2.3.3. Method C: ΔA spectrophotometric method

2.3.3.1. Linearity. Accurate aliquots equivalent to (100–400 μg) of DI were transferred from its working solution (100 μg/ml) into two sets of 10 ml volumetric flasks. The volume was diluted in one set with 0.1N sodium hydroxide and in the other set with 0.1N HCl. The ΔA spectrum for each concentration was recorded at 296 nm, by placing the alkaline solution in the reference beam and the acidic solution in the sample beam. The calibration curve relating the ΔA at 296 nm to DI concentration was constructed.

2.3.3.2. Assay of prepared mixtures. The ΔA spectra of the laboratory-prepared mixtures containing different ratios of DI and its degradates were recorded. The peak amplitude at 296 nm was measured, then the concentration of DI in the prepared mixtures was calculated from the regression equation.

2.3.4. Method D: densitometric method

2.3.4.1. Linearity. Accurate aliquots equivalent to (100–500 μg) of DI were transferred from its working solution (0.5 mg/ml) to a series of 10 ml volumetric flasks then the volume was completed with methanol. Ten microlitres of each solution was applied to a thin layer chromatographic plate (20×20 cm) using 10 μl micro syringes. Spots were spaced 2 cm apart from each other, 1.5 cm from the bottom edge of the plate, the plate was placed in chromatographic tank previously saturated for 1 h with the developing mobile phase chloroform:methanol (80:20% v/v). The plate was developed by ascending chromatography through a distance of 16 cm, dried at room temperature, the spots were detected under UV lamp, and scanned at 258 nm. (photo mode: reflection; scan mode: zigzag). The calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration was constructed.

2.3.4.2. Assay of prepared mixtures. Ten microliters of different samples of the laboratory prepared mixtures were applied to a thin layer chromatographic plate; proceed as mentioned under linearity starting from 'Spots were spaced…'. The area under the peak was recorded and the concentration of DI from was calculated the regression equation.

2.3.5. Method E: reversed phase high performance liquid chromatographic method

2.3.5.1. Linearity. Accurate aliquots equivalent to (20–500 μg/ml) of DI were transferred from its working solution (100 μg/ml) into a series of 10 ml volumetric flasks. Methanol:water (80:20% v/v) was added to volume to give a final concentration range from 2 to 50 μg/ml. Twenty microliters of the solution from each of the above was injected and the chromatograms recorded maintaining the flow rate at 1 ml/min and monitoring the effluent at 258 nm. Peak area values were then plotted as a function of DI concentration to obtain the calibration curve.

2.3.5.2. Assay of prepared mixtures. The specified HPLC method was followed for the analysis of laboratory prepared mixtures containing different ratios of DI and its degradates. The peak area values for DI were recorded then the concentration of DI in the prepared mixtures was calculated from the regression equation.

2.3.6. Assay of pharmaceutical formulation

The contents of ten tablets of each of the pharmaceutical formulations were thoroughly powdered and mixed, an amount of the powder

equivalent to 100 mg of DI was accurately weighed in 250 ml beaker, 70 ml of absolute ethanol was added, stirred magnetically for about 30 min then filtered through a filter paper into a 100 ml volumetric flask, the beaker and the funnel were washed and the volume was completed with absolute ethanol. The solutions were diluted to the same concentrations of working standard solutions and treated according to linearity for each method.

3. Results and discussion

In this work, DI was determined in thew presence of its degradation products. The degradation products were prepared via alkaline hydrolysis of DI.

The investigated drug and its degradation products were stable under the specified conditions for each of the proposed methods.

3.1. Method A: first and second derivative spectrophotometric methods

Zero order absorption spectra of DI and its degradates in absolute ethanol show severe overlap which interferes with the direct determination of pure DI (Fig. 1).

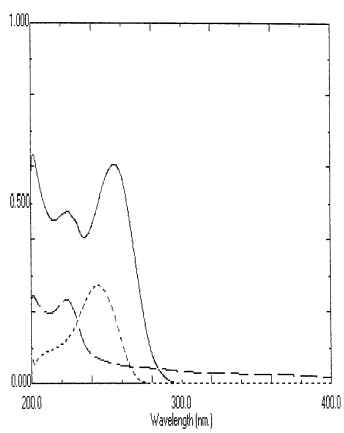

Fig. 1. Zero-order spectra of the methanolic solutions of: intact DI (15 μg/ml) (long line); first degradation product (6 μg/ml) (long-dashed line); and second degradation product (6 μg/ml) (short-dashed line).

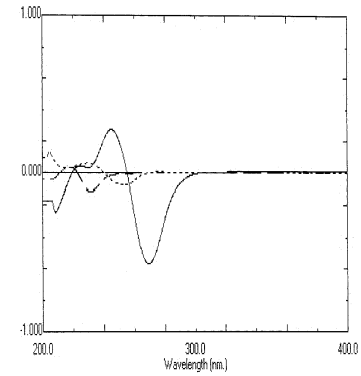

Fig. 2. First derivative spectra of the methanolic solutions of: intact DI (15 μg/ml) (long line); first degradation product (6 μg/ml) (long-dashed line); and second degradation product (6 μg/ml) (short-dashed line).

As shown in Figs. 2 and 3, it is clear that the overlapping observed in the zero-order absorption spectra was eliminated and sharply defined, well separated peak at 270 and 280 nm for the intact molecule which lies at the zero crossing of its degradates was obtained and used for the D_1 and D_2 spectrophotometric determination of intact DI in presence of its degradates.

By applying the D_1 and D_2 spectrophotometric method, a linear correlation was obtained between the peak height and the concentration over the range 5–30 μg/ml for pure DI and the following regression equations were obtained:

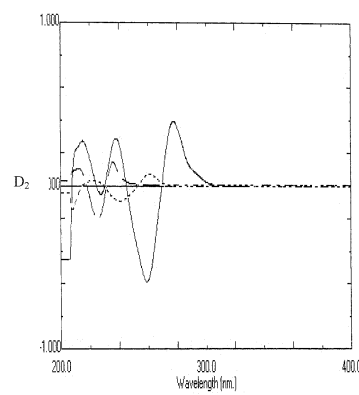

Fig. 3. Second derivative spectra of the methanolic solutions of: intact DI (15 μg/ml) (long line); first degradation product (6 μg/ml) (long-dashed line); and second degradation product (6 μg/ml).

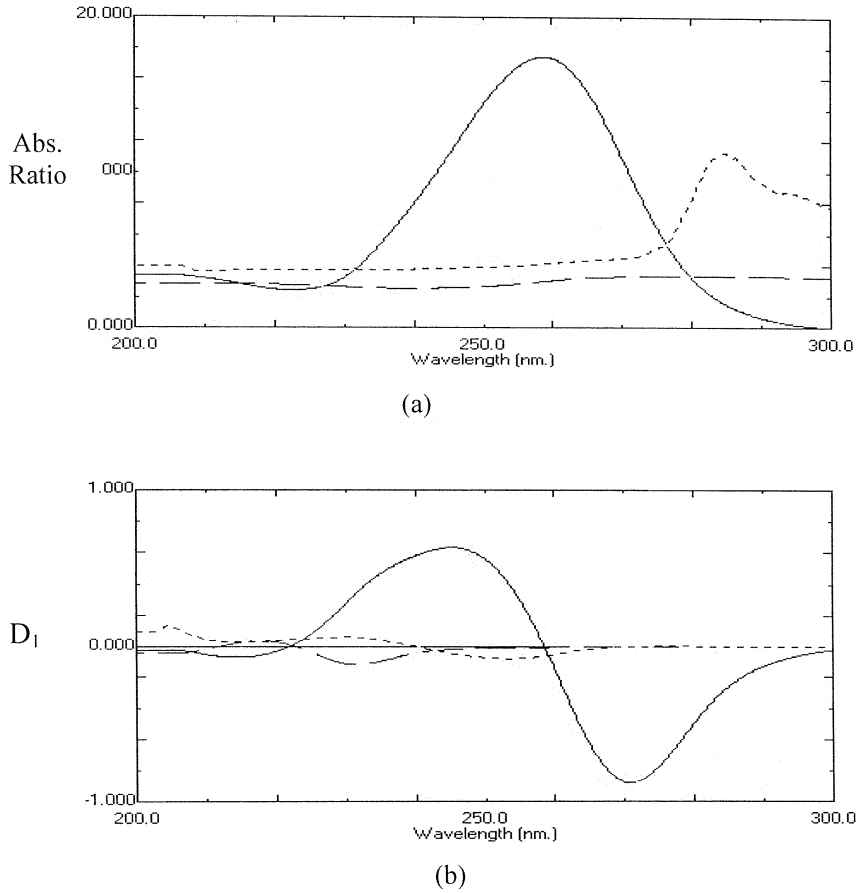

Fig. 4. Ratio-spectra and first derivative curves of the methanolic solutions of: intact DI (10 μg/ml) (long line), first degradation product (5 μg/ml) (long-dashed line), and second degradation product (5 μg/ml) (short-dashed line); using 3 μg/ml of the second degradation product as the divisor.

$H_1 = 0.028C_1 + 0.0117,$

$r_1 = 0.9999 H_2 = 0.018C_2 + 0.0282,$

$r_2 = 0.9999$

where H_1 and H_2 stand for the peak heights in millimeter at 270 and 280 nm, respectively, C_1 and C_2 for the concentrations in μg/ml and r_1 and r_2 for the correlation coefficients.

3.2. Method B: RSD_1 spectrophotometric method

Fig. 1 shows the absorption spectra of DI and its degradates which overlap seriously. Fig. 4 shows the ratio spectra of DI and its degradates (spectra divided by the spectrum of 3 μg/ml of the second degradate) and their first derivatives. As can be seen, the peak at 270 nm for intact DI which lies at the zero-crossing points of its degradates can be adopted for the determination of DI in presence of its degradates and in pharmaceutical formulations.

Calibration curve was obtained by plotting the peak amplitude at 270 nm of the first derivatives of the ratio spectra of DI that shows linear relationship in the range of 5–25 μg/ml and the following regression equation was calculated:

$A = 0.034C + 0.021, \qquad r = 0.9999$

where A stands for the peak amplitude at 270 nm, C for the concentration in μg/ml and r is the correlation coefficient.

3.3. Method C: ΔA spectrophotometric method

The ΔA spectra between 0.1N sodium hydroxide and 0.1N HCl for intact DI and its degradates were recorded and from these spectral characteristics it is clear that the ΔA peak at 295 nm for the intact DI between 0.1N HCl and 0.1N sodium hydroxide could be considered as the λ_{max} most suitable for adopting the ΔA technique for the selective determination of intact DI in presence of its degradates as at this maxima, ΔA for the latter reads zero (Fig. 5).

A calibration curve was constructed relating the ΔA values at 295 nm to drug concentrations showing perfect linearity in the range of 10–40 μg/ml from which the following regression equation was calculated:

$$A = 0.02C + 0.004, \qquad r = 0.9999$$

where A stands for the peak amplitude at 295 nm, C for the drug concentration in μg/ml and r is the correlation coefficient.

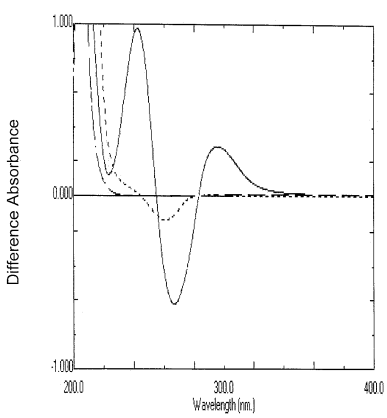

Fig. 5. Difference spectra of: intact DI (20 μg/ml) (long line), first degradation product (5 μg/ml) (long-dashed line), and second degradation product (5 μg/ml) (short-dashed line).

3.4. Method D: densitometric method

This method was applied for the determination of DI. Complete separation of DI was obtained using chloroform:methanol (80:20% v/v) as developing mobile phase. Quantitatively the chromatogram was scanned densitometrically at 258 nm. By applying this technique a linear correlation was obtained between the area under the peak and the concentration of DI in the range of 100–500 ng/spot. The following regression equation was calculated for DI:

$$A = 0.018C + 0.014, \qquad r = 0.9996$$

where A is the area under the peak, C is the corresponding concentration in μg/ml and r is the correlation coefficient.

3.5. Method E: reversed phase high performance liquid chromatographic method

A simple and stability indicating isocratic HPLC method was adopted for the analysis of DI in presence of its degradates and in pharmaceutical formulations. The best peak shape was obtained with methanol:water (80:20% v/v) with retention time of 3.98 min. The final dilution of samples has been done using methanol:water (80:20% v/v) to avoid frontal peak tailing.

A typical chromatogram of pure DI is shown in Fig. 6. The chromatograms as shown in Fig. 6 showed no peak interferences between the drug and its degradates.

The calibration curve for DI was constructed by plotting concentration versus peak area showed good linearity in the range of 2–50 μg/ml. The regression equation was calculated and found to be:

$$A = 0.047C + 0.002, \qquad r = 0.9999$$

where A is the peak area, C is the corresponding concentration and r is the correlation coefficient.

To assess the stability indicating specificity of the proposed methods for the analysis of DI without interference from its degradation products, separate aliquots of the degradation products of DI were mixed with the intact drug in different ratios and analyzed by the proposed

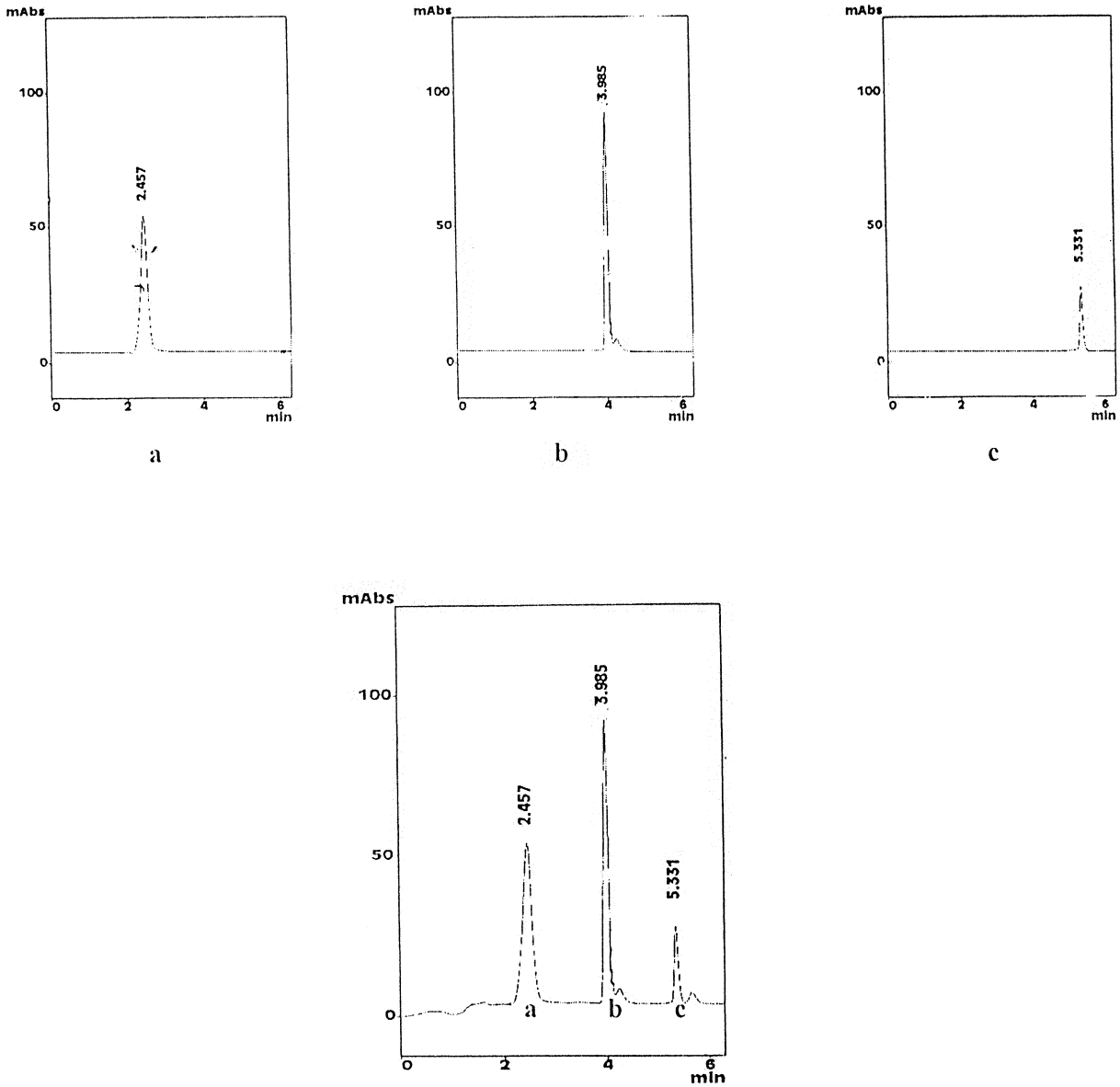

Fig. 6. HPLC chromatograms of (a) first degradation product, 10 μg/ml; (b) pure DI, 10 μg/ml; (c) second degradation product, 10 μg/ml.

methods. The results obtained are shown in Table 1. It is clear that the accuracy of the proposed methods are not affected by the presence of up to 50, 60, 60, 60, 80 and 90% of the degradation product in the D_1, D_2, RSD_1 and ΔA spectrophotometric, TLC-densitometric and RP-HPLC methods, respectively. The proposed methods were applied successfully for the analysis of DI in its dosage form and its validity was further assessed by applying the standard addition technique. Results obtained are presented in Table 2.

Table 3 shows the full validation parameters for the proposed methods.

Table 1
Comparison between the proposed methods and the reported one for the determination of DI in the presence of its degradates

Sample No.[a]	% of degradates	D_1 method (Found %)	D_2 method (Found %)	RSD_1 method (Found %)	ΔA method (Found %)	Densitometric method (Found %)	RP-HPLC method (Found %)	B.P. (1998) method (Found %)
1	10	99.91	99.03	99.12	99.89	100.01	99.89	100.20
2	20	98.74	99.25	99.61	99.10	100.14	99.96	106.96
3	30	99.52	99.28	99.54	99.88	99.97	98.99	113.20
4	40	99.60	98.48	98.97	98.54	99.95	98.25	116.56
5	50	99.90	98.24	98.45	98.87	99.85	99.25	125.12
6	60		99.65	99.22	98.23	99.89	99.42	150.35
7	80					99.79	98.68	176.58
8	90						98.53	188.56
Mean ± S.D.		99.53 ± 0.47	98.98 ± 0.53	99.15 ± 0.42	99.08 ± 0.68	99.98 ± 0.12	98.92 ± 0.50	

[a] Average of four experiments.

Table 2
Comparison between the proposed methods and the reported one for the determination of DI in pure form and in its pharmaceutical formulation

Preparation	D_2 & D_3 method (Found % ± S.D.)	Standard addition (Recovery % ± S.D.)	RSD_1 method (Found % ± S.D.)	Standard addition (Recovery % ± S.D.)	ΔA method (Found % ± S.D.)	Standard addition (Recovery % ± S.D.)	Densitometric method (Found % ± S.D.)	Standard addition (Recovery % ± S.D.)	RP-HPLC method (Found % ± S.D.)	Standard addition (Recovery % ± S.D.)	B.P. method (Found % ± S.D.)
Pure sample	99.92 ± 0.56 & 99.79 ± 0.47		99.23 ± 0.38		99.96 ± 0.06		99.03 ± 0.51		98.81 ± 0.68		99.63 ± 0.54
Amoebyl tablets 25, Batch No. 981353		100.86 ± 0.15 & 100.29 ± 0.36		100.12 ± 0.11		99.77 ± 0.08		99.69 ± 0.17		99.53 ± 0.28	99.96 ± 0.35
Furameb tablets 100, Batch No. 798124		99.98 ± 0.1 & 99.6 ± 0.52		99.68 ± 0.17		99.79 ± 0.3		99.93 ± 0.015		99.01 ± 0.53	99.42 ± 0.63
Farcomid tablets 25, Batch No. 151		99.03 ± 0.17 & 99.55 ± 0.33		99.99 ± 0.09		100.12 ± 0.13		99.84 ± 0.18		100.11 ± 0.11	98.85 ± 0.53
Furamide tablets 100, Batch No. 21548		99.54 ± 0.15 & 99.08 ± 0.09		99.71 ± 0.088		100.006 ± 0.037		99.48 ± 0.07		99.52 ± 0.05	98.42 ± 0.28

The values of all methods in found% ± S.D., and their respective standard additions in recovery% ± S.D.

Table 3
Assay validation of the proposed methods for the determination of DI

Parameter	D_1 spectrophotometric method	D_2 spectrophotometric method	RSD_1 spectrophotometric method	ΔA spectrophotometric method	Densitometric method	HPLC method
Range	5–30 (μg/ml)	5–30 (μg/ml)	5–25 (μg/ml)	10–40 (μg/ml)	100–500 (ng/spot)	2–50 (μg/ml)
LOD	1.60 (μg/ml)	1.63 (μg/ml)	1.59 (μg/ml)	3.25 (μg/ml)	32.85 (ng/spot)	0.65 (μg/ml)
LOQ	4.85 (μg/ml)	4.94 (μg/ml)	4.82 (μg/ml)	9.85 (μg/ml)	99.54 (ng/spot)	1.97 (μg/ml)
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9996	0.9999
RSD%[a]	1.144	1.103	1.270	1.533	1.224	0.756
RSD%[b]	1.321	1.054	0.984	1.015	1.367	1.012

[a] The intraday ($n = 4$) variation for the proposed methods.
[b] The interday ($n = 9$) variation for the proposed methods.

Table 4
Statistical analysis of the results obtained by the proposed methods and B.P. (1998) method for the determination of DI

	B.P. (1998) method	D_1 spectrophotometric method	D_2 spectrophotometric method	RSD_1 spectrophotometric method	ΔA spectrophotometric method	Densitometric method	HPLC method
Concentration range	300 mg	5–30 (μg/ml)	5–30 (μg/ml)	5–25 (μg/ml)	10–40 (μg/ml)	100–500 (ng/spot)	2–50 (μg/ml)
Mean%	99.44	99.92	99.79	99.23	99.96	99.03	98.81
S.D.	0.86	0.56	0.47	0.38	0.06	0.51	0.68
n	8	8	8	8	8	8	8
Variance	0.740	0.412	0.276	0.151	0.007	0.359	0.461
Student's t-test		0.215 (1.761)	0.224 (1.761)	0.698 (1.761)	0.604 (1.761)	0.563 (1.761)	0.747 (1.761)
F test		1.09 (3.575)	1.06 (3.575)	1.01 (3.575)	1.03 (3.575)	1.21 (3.575)	1.08 (3.575)

Values in parenthesis are the theoretical values of t and F (at $P = 0.05$).

The results obtained by applying the proposed methods were statistically compared with those obtained by applying the reference method. Table 4 shows that the values of calculated t and F are less than the tabulated ones indicating that there is no significant difference between the methods. Thus, the proposed methods could be applied as stability indicating methods for the routine and quality control analysis of DI in raw material and pharmaceutical formulations.

4. Conclusion

The suggested methods are simple, accurate, selective and sensitive with no significant difference of the precision. Application of the proposed methods to the analysis of DI in laboratory prepared mixtures and pharmaceutical formulations shows that neither the degradation products nor the excipients interfere with the determination, indicating that the proposed methods could be applied as stability indicating methods for the determination of DI either in bulk powder or in pharmaceutical formulations. Statistical analysis of the results obtained by the five proposed methods and by the non-aqueous titration method of B.P. (1998), revealed no significant difference within a probability of 95%. However, the proposed methods are far more sensitive than the B.P. method. Moreover, the suggested methods are more selective, since the B.P. method does not differentiate between the intact drug and its degradation products.

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