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Validated stability indicating methods for determination of dothiepin hydrochloride

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ABSTRACT

Two simple, accurate, sensitive and reproducible methods have been developed and subsequent validated for the determination of Dothiepin Hydrochloride in presence of its oxidative and photo-degradates, as stability-indicating studies. In the spectrophotometric method, zero-crossing technique was adopted for determination of the investigated drug in presence of its oxidative and photo-degradates, by the use of derivative and derivative ratio techniques, respectively. While, the second method was isocratic reversed-phase (RP) stability-indicating high-performance liquid chromatographic method, which was adopted for determination of Dothiepin Hydrochloride in presence of its oxidative and photo-degradates. The chromatographic separation was achieved isocratically by using a mobile phase of acetonitrile: phosphate buffer (50:50, v/v), adjusted to pH 7.0 using orthophosphoric acid and 0.5 % triethylamine. The analysis was carried out using Agilent eclipse XDB C18 column [150 mm x 4.6 mm, 5 µm] at flow rate of 1.2 ml.min^{-1} and the UV detection at 230 nm. All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines reference and successfully applied for determination of Dothiepin Hydrochloride in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference method of analysis for Dothiepin Hydrochloride and no significant differences were found.

KEYWORDS

Dothiepin hydrochloride;
Derivative spectrophotometry;
Ratio spectra;
Difference spectrophotometry;
High-performance liquid chromatography;
Stability indicating methods.

INTRODUCTION

Dothiepin hydrochloride, (3(6*H*)-dibenzo [b,e] thiepin-11-ylidene) propyl dimethylamine hydrochloride. It is a tricyclic antidepressant with a noticeable action[1], indicated in the treatment of depression and anxiety. The ICH-guidelines[2] requires performing stress-testing of the drug substance that can help in identifying the likely degradation-products, also can be useful in establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used. Moreover, validated stability-indicating method should be applied in the stability study[3]. Stability-indicating methods can be specific one that evaluates the

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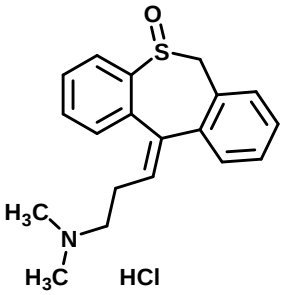

Figure 1 : Chemical structure of dothiepin hydrochloride

drug in the presence of its-degradation products, excipients and additives[4].

Several methods have been reported for its determination, including extractive colorimetric ion pair complex formation[5], kinetic spectrophotometry using alkaline potassium permanganate or 4-chloro-7-nitrobenzofurazon[6], charge transfer and ion-associate complex formation[7,8]. Other techniques such as HPLC[9,10], GC[11] and voltammetry[12] were reported for Dothiepin analysis.

All the reported literatures were not involving the use of either derivative or derivative-ratio methods have been reported for determination of Dothiepin hydrochloride in pharmaceutical Formulations. But, the present work was successfully developed novel simple, rapid, accurate and sensitive derivative and derivative-ratio spectrophotometric methods for the determination of Dothiepin hydrochloride in pharmaceutical Formulations. Those novel methods could be adopted for the determination of Dothiepin hydrochloride in presence of its oxidative and photo-degradates, respectively, this beside a simple isocratic high performance liquid chromatographic method was also developed.

The developed work can be used for the routine quality control analysis of the investigated drug in raw material and pharmaceutical formulations and for stability-indicating studies.

MATERIALS AND METHODS

(A) Chemicals and reagents

Dothiepin Hydrochloride was kindly supplied by El-Kahira for pharmaceuticals and chemical industry company and certified to contain 98.1%. Prothiaden® tablets and capsules: Batch number 57043/3J and 33476/3J, manufactured by Kahira for pharmaceuticals and chemical industry company. Each tablet contains 75 mg Dothiepin Hydrochloride and each capsule contains 25 mg Dothiepin Hydrochloride.

Acetonitrile, methanol and bi-distilled water (Riedel-dehaen, Sigma-Aldrich, Germany), Hydrochloric acid (Adwic), aqueous (0.01M), chloroform (Adwic), ammonia (Adwic), hydrogen peroxide 30% (E. Merck, Germany) and TLC aluminium plates pre-coated with silica gel 60 F_{254} (E.Merck). Phosphate buffer prepared by dissolving 1.74 g of dipotissium hydrogen orthophosphate in 1.0 liter water and then adjusted to pH 7.0 by using phosphoric acid and triethylamine.

All chemical and reagents used through this work are of spectroscopic and chromatographic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word water.

(B) Instruments

A double-beam Shimadzu (Japan) UV-VIS Spectrophotometer (UV-1601 PC), model TCC-240 A; connected to an IBM compatible computer and HP 695 C DeskJet printer is used. The bundled software is UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth is 2 nm and the wavelength scanning speed was 2800.0 $nmmin^{-1}$. The absorption spectra of the reference and the test solutions are recorded in1.0-ml quartz cells at 25.0°C, using '$\Delta\lambda$ = 4 nm and scaling factor of 10 for computing first derivative (D^1)' and '$\Delta\lambda$ = 8 nm and scaling factor of 100 for second (D^2) third (D^3) derivatives'.

The HPLC (Agilent Hewlett Packard series) instrument was equipped with a model series 1100 pump, manual injector Agilent 1100 series, 20 µl loop and a UV-visible wavelength detector Agilent 1100 series. The chromatographic separation was performed using (150×4.6 mm I.D.) Agilent eclipse XDB C18 column (5µm particle size) at ambient temperature. Ultrasonic vibrator, (J.P Selecta´S-a; CD 300513 Espain). Disposible membrane filters, 0.45µm, (Agilent 3150-0576).

(C) UV illumination

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

TABLE 1 : Validation of the proposed methods for determination of dothiepin hydrochloride in presence of its oxidative-degradate

Parameters	D^1	D^2	D^3	DR^1	HPLC
Linearity (μgml^{-1})	2.0 - 40	2.0 - 40	2.0 - 40	2.0 - 40	1.0 – 14.0
Slope	3×10^{-5}	24×10^{-4}	32×10^{-4}	11.2×10^{-2}	88.70
Standard error of slope	1.81×10^{-5}	1.7×10^{-5}	2.23×10^{-5}	824×10^{-6}	74.4×10^{-2}
R.S.E of slope	5.95×10^{-3}	7.21×10^{-3}	7.12×10^{-3}	7.47×10^{-3}	8.85×10^{-3}
Confidence limit of slope	296×10^{-5}-304×10^{-5}	2357×10^{-6}-2429×10^{-6}	3129×10^{-6}-3223×10^{-6}	0.1103-0.1137	84.07-87.31
Intercept	2×10^{-4}	15×10^{-4}	11×10^{-4}	0.033	3.174
Standard error of intercept	434×10^{-6}	408×10^{-6}	535×10^{-6}	0.007	6.335
Confidence limit of intercept	-69×10^{-5}-114×10^{-6}	685×10^{-6}-2399×10^{-6}	2.38×10^{-5}-2271×10^{-6}	-0.00821-0.0747	-10.628 – 16.975
Correlation coefficient (r)	0.9996	0.9995	0.9995	0.9995	0.9995
Standard error of estimation	935×10^{-6}	878×10^{-6}	1151×10^{-6}	0.0425	11.221
LOD	0.477	0.561	0.552	0.582	0.244
LOQ	1.447	1.700	1.672	1.759	0.739
S.D*[a]	0.565	0.190	0.176	0.934	0.707
S.D*[b]	0.191	0.203	0.125	0.879	0.850

***[a]The intraday (n=5) *[b] the interday (n=9) are the standard deviations of different samples concentrations of dothiepin hydrochloride**

TABLE 2 : Validation of the proposed methods for determination of dothiepin hydrochloride in presence of its photo-degradate

Parameters	D^2	D^3	DR^1	HPLC
Linearity (μgml^{-1})	2.0 - 40	2.0 - 40	2.0 - 40	1.0 – 14.0
Slope	12×10^{-4}	13×10^{-4}	0.0167	88.70
Standard error of slope	9.76×10^{-6}	9.09×10^{-6}	101×10^{-6}	74.4×10^{-2}
R.S.E of slope	7.84×10^{-3}	6.88×10^{-3}	5.96×10^{-3}	8.85×10^{-3}
Confidence limit of slope	1204×10^{-6}- 1245×10^{-6}	132×10^{-5}- 135×10^{-6}	0.0165 - 0.0169	84.07- 87.31
Intercept	1×10^{-4}	2×10^{-4}	55×10^{-4}	3.174
Standard error of intercept	234×10^{-6}	218×10^{-6}	24×10^{-4}	6.335
Confidence limit of intercept	-35×10^{-5}-628×10^{-6}	-26×10^{-5}-66×10^{-5}	44×10^{-5}-106×10^{-4}	-10.628 – 16.975
Correlation coefficient (r)	0.9994	0.9996	0.9996	0.9995
Standard error of estimation	503×10^{-6}	469×10^{-6}	0.00522	11.221
LOD	0.644	0.553	0.474	0.244
LOQ	1.950	1.677	1.437	0.739
S.D*[a]	0.676	0.548	0.209	0.707
S.D*[b]	0.915	0.671	0.147	0.850

***[a]The intraday (n=5) and *[b] the interday (n=9) are the standard deviations of different samples concentrations of dothiepin hydrochloride**

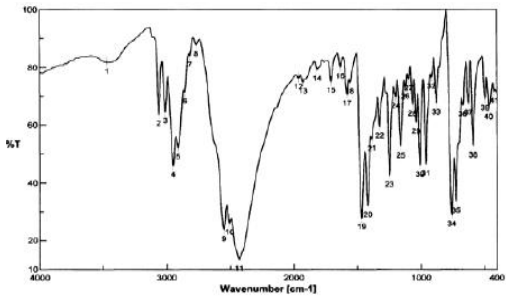

Figure 2a : IR spectrum of intact dothiepin hydrochloride

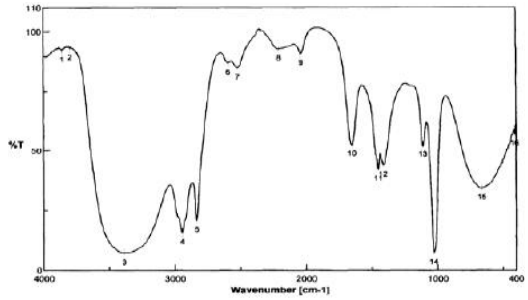

Figure 2b : IR spectrum of intact dothiepin hydrochloride photo-degradate

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(D) Standard solutions

(1) Standard solution of the studied drug

Stock standard solution of Dothiepin Hydrochloride having concentration of (0.1 $mg.ml^{-1}$) was prepared in 0.01 M HCl. This solution was used as working standard solution.

(2) Standard solution of dothiepin hydrochloride oxidative and photo-degradates

Stock standard solutions of Dothiepin Hydrochloride oxidative and photo-degradates, having concentration of (0.1 $mg.ml^{-1}$) were prepared by "adding 10 ml of 30%v/v hydrogen peroxide to 10 mg of Dothiepin hydrochloride for 8 hours" and "subjecting 100 μgml^{-1} of the investigated drug to UV illumination in a UV cabinet ($\lambda = 254$ nm) for 7 hours" and then the volumes were diluted with 0.01 M HCl, respectively, as discussed under (2.3.1.). Those prepared degradated solutions were used as working standard solutions.

Complete degradation was checked by using HPTLC system; silica gel 60 F_{254} plates and chloroform: methanol: ammonia (50:30: 0.5, v/v/v) as a developing system.

(E) Procedures

(a) Spectrophotometric methods

(1) Derivative spectrophotometric method (D^n)

From stock standard solution of Dothiepin Hydrochloride, aliquots were transferred into a series of 50 ml volumetric flasks, and diluted to volume with 0.01 M HCl to obtain a concentration range of 2.0-40.0 $\mu g.ml^{-1}$. The values of 'first (D^1), second (D^2) and third (D^3)' derivative spectrophotometry amplitudes at 314.60 nm, 244.80 nm and 279.20 nm (Zero-crossing of oxidative-degradate)' and 'second (D^2) and third (D^3)' derivative spectrophotometry amplitudes at 284.00 nm and 259.40 nm (Zero-crossing of photo-degradate)' were then computed, plotted versus corresponding concentrations; and the regression equations were then computed, respectively.

(2) Derivative ratio spectrophotometric method (DR^n)

Calibration curve was performed by transferring aliquots from stock standard solution of the analyzed drug into a series of 50 ml volumetric flasks, and diluting to volume with 0.01 M HCl to obtain a concentration range of 2.0-40.0 $\mu g.ml^{-1}$. The spectra of oxidative and photo-degradate solutions, each having concentration 8.0 $\mu g.ml^{-1}$ was scanned and stored in the instrument PC as a devisor. The spectra of Dothiepin Hydrochloride were divided by each devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 304.60 and 241.00 nm, plotted versus concentrations, and the regression equations were computed, respectively.

(b) Chromatographic method

Stationary phase, C18 Agilent eclipse XDB column (5µm, 150×4.6 mm), acetonitrile: phosphate buffer 'pH 7' in a ratio (50:50, v/v) with a flow rate of 1.2 $ml.min^{-1}$ as 'degassed and filtered' mobile phase, and UV detection at 230 nm, were the adopted chromatographic conditions. Construction the calibration curve was performed by transferring aliquots of Dothiepin Hydrochloride stock standard solution into a series of 50 ml volumetric flasks and diluting with the mobile phase to the volume having a concentration range of 1.0-14.0 $\mu g.ml^{-1}$. Under the previously mentioned chromatographic conditions, 20µl-volume from each solution was injected in triplicate, the average peak area obtained for each concentration was plotted versus concentration and the regression equation were then computed.

(F) Assay of the pharmaceutical preparations

(1) Prothiaden® tablets

Twenty tablets were accurately weighed and finely powdered. A portion of the powder equivalent to 10 mg of Dothiepin Hydrochloride was accurately weighed, transferred to beaker with 70.0 ml of 0.01 M HCl, shaken, sonicated, filtered, then completed to the volume with 0.01 M HCl to obtain a concentration of 0.1 $mg.ml^{-1}$ and the procedures mentioned under (2-4) were adopted.

(2) Prothiaden® capsules

Four capsules were emptied, then a portion of the powder equivalent to 10 mg of Dothiepin Hydrochloride was accurately weighed, transferred to beaker with 70.0 ml of 0.01 M HCl, shaken, sonicated, filtered, then completed to the volume with 0.01 M HCl to obtain a concentration of 0.1 $mg.ml^{-1}$ and the procedures

TABLE 3a : Determination of dothiepin hydrochloride in commercial tablets& capsules using the proposed methods and Reference method in presence of oxidative-degradate

Items	D^1	D^2	D^3	DR^1	HPLC	Ref. method
Commercial* tablets	99.77±0.273 t =-1.171(2.160)** F = 1.038(5.998)**	99.99±0.396 t = 0.288(2.160)** F = 2.186(5.998)**	100.17±0.238 t = -1.703(2.160)** F = 1.266(3.633)**	100.18±0.183 t =-1.895(2.160)** F = 1.175(5.998)**	99.77±0.536 t = -0.619(2.310)** F = 3.997(6.388)**	99.94±0.268
Recovery***	99.60±0.345	100.32±0.836	99.60±0.247	98.65±0.701	99.90±0.669	----------
	D^1	**D^2**	**D^3**	**DR^1**	**HPLC**	**Ref. method**
Commercial* capsules	100.26±0.345 t =-0.595(2.160)** F = 4.417(5.998)**	99.99±0.396 t = -1.977(2.160)** F = 5.479(5.998)**	100.17±0.238 t = -1.640(2.160)** F = 1.980(5.998)**	100.20±0.126 t =2.157(2.160)** F = 1.814(3.633)**	99.54±0.236 t = -6.413(2.310)** F = 1.940(6.388)**	100.36±0.169
Recovery***	99.90±0.754	98.99±0.693	99.98±0.316	100.25±1.535	100.07±0.459	----------

***Mean and S.D for five determinations, percentage recovery from the label claim amount. **Theoretical values for t and F at (p = 0.05). ***For standard addition (n=5)**

TABLE 3b : Determination of dothiepin hydrochloride in commercial tablets& capsules using the proposed methods and Reference method in presence of photo-degradate

Items	D^2	D^3	DR^1	HPLC	Ref. method
Commercial* tablets	99.81±0.623 t = -0.447(2.160)** F = 5.142(5.998)**	100.25±0.258 t = -2.132(2.160)** F = 1.076(3.633)**	100.08±0.156 t =-1.259(2.160)** F = 2.953(3.633)**	99.77±0.536 t = -0.619(2.310)** F = 3.997(6.388)**	99.94±0.268
Recovery***	99.43±0.524	99.52±0.771	99.44±0.795	99.90±0.669	----------
	D^2	**D^3**	**DR^1**	**HPLC**	**Ref. method**
Commercial* capsules	100.39±0.389 t = 0.141(2.160)** F = 5.312(5.998)**	100.39±0.387 t = 0.131(2.160)** F = 5.219(5.998)**	100.21±0.139 t = 1.984(2.160)** F = 1.478(3.633)**	99.54±0.236 t = -6.413(2.310)** F = 1.940(6.388)**	100.36±0.169
Recovery***	100.20±0.795	100.39 ±0.529	99.73 ±0.604	100.07±0.459	----------

***Mean and S.D for five determinations, percentage recovery from the label claim amount. **Theoretical values for t and F at (p = 0.05). ***For standard addition (n=5)**

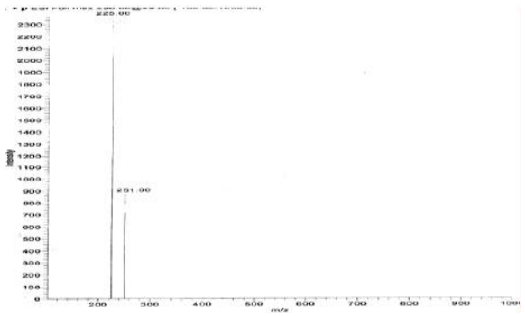

Figure 2c : Mass spectrum of the intact dothiepin hydrochloride

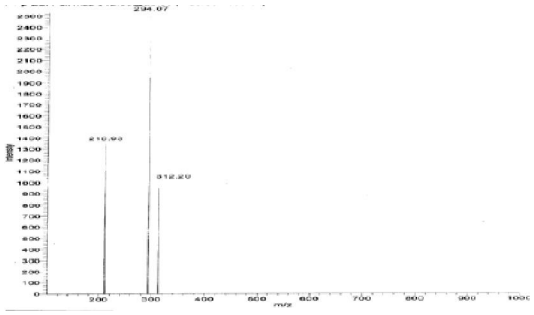

Figure 2d : Mass spectrum of the dothiepin hydrochloride oxidative-degradate

mentioned under (2-4) were adopted.

RESULTS AND DISCUSSION

(A) Method development

(1) Spectrophotometric method

The stability of Dothiepin Hydrochloride was studied until complete degradation was achieved by using 30%v/v hydrogen peroxide for 8 hours and UV illumination at λ = 254 nm for 7 hours, where different concentrations of hydrogen peroxide and different time intervals (for oxidative-degradation) also different time intervals (for photo-degradation) were studied until reaching these optimum conditions which accomplish

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TABLE 4a : Determination of dothiepin hydrochloride in laboratory prepared mixtures with its oxidative-degradate by the proposed methods

Sample no.	Intact in µgml^{-1}	Degradate in µgml^{-1}	D^1	D^2	D^3	DD^1	DR^1
			*%Recovery				
1	18.00	2.00	99.20	100.00	99.02	99.79	100.03
2	18.00	4.00	99.40	99.75	98.62	98.07	100.68
3	18.00	8.00	99.80	98.52	98.43	99.70	100.05
4	18.00	12.00	100.00	98.28	98.62	98.12	100.00
5	18.00	16.00	100.00	99.01	98.81	98.82	99.95
6	18.00	18.00	100.19	99.00	99.02	100.66	100.04
	Mean		99.76	99.09	98.75	99.19	100.13
	±S.D.		0.385	0.672	0.238	1.013	0.274

HPLC

Sample no.	Intact in µgml^{-1}	Degradate in µgml^{-1}	*%Recovery
1	8.00	1.00	100.66
2	8.00	2.00	99.63
3	8.00	4.00	100.17
4	8.00	6.00	99.63
5	8.00	8.00	99.41
	Mean		99.90
	±S.D.		0.509

***The average recovery of 5-separate determinations of the intact drug**

TABLE 4b : Determination of dothiepin hydrochloride in laboratory prepared mixtures with its photo-degradate by the proposed methods

Sample no.	Intact in µgml^{-1}	Degradate in µgml^{-1}	D^2	D^3	DR^1
			*%Recovery		
1	18.00	2.00	100.00	99.5	100.69
2	18.00	4.00	100.49	100.00	99.30
3	18.00	8.00	101.98	100.00	99.65
4	18.00	12.00	101.48	100.49	99.75
5	18.00	16.00	100.49	100.98	99.85
6	18.00	18.00	100.49	100.25	99.75
	Mean		100.82	100.20	99.83
	±S.D.		0.745	0.503	0.462

HPLC

Sample no.	Intact in µgml^{-1}	Degradate in µgml^{-1}	*%Recovery
1	8.00	1.00	100.5 6
2	8.00	2.00	99.9 3
3	8.00	4.00	99.38
4	8.00	6.00	100.20
5	8.00	8.00	99.52
	Mean		99.92
	±S.D.		0.485

***The average recovery of 5-separate determinations of the intact drug**

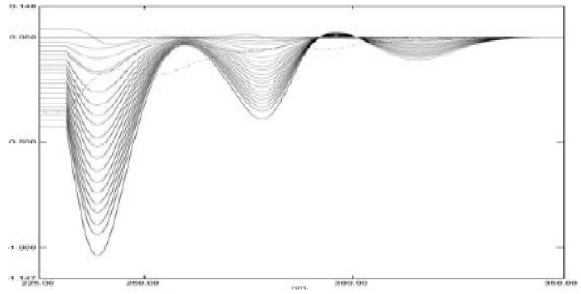

Figure 3a : First derivative (D^1) for different concentrations (2.0-40.0µgml^{-1}) of dothiepin hydrochloride (-) in presence of its oxidative –degradate (….)

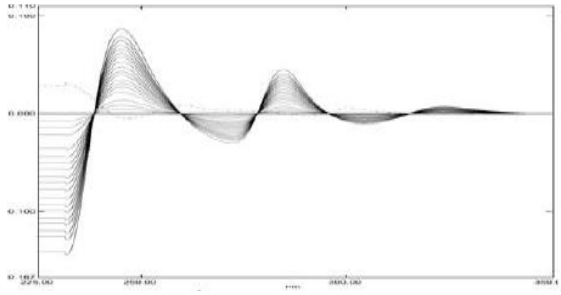

Figure 3b : Second derivative (D^2) for different concentrations (2.0-40.0µg ml^{-1}) of dothiepin hydrochloride (-) in presence of its oxidative-degradate

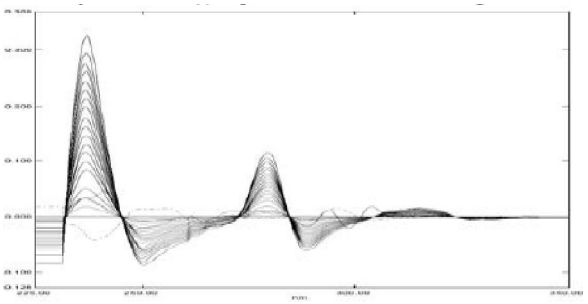

Figure 3c : Third derivative (D^3) for different concentrations (2.0-40.0µg ml^{-1}) of dothiepin hydrochloride (-) in presence of its oxidative-degradate (…)

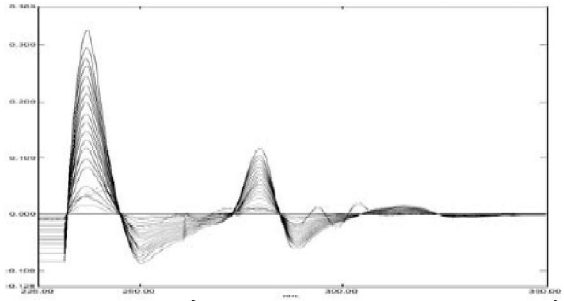

Figure 4a : Second derivative (D^2) for different concentrations (2.0-40.0µg ml^{-1}) of dothiepin hydrochloride (-) in presence of its photo-degradate

TABLE 5a : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations and applications of standard addition technique by the proposed first derivative spectrophotometric methods

Pharmaceutical preparation	Found		Standard addition technique(D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery *
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	25.06	100.26 ± 0.345	10.0	6.00	6.05	100.92
				8.00	8.00	100.00
				10.0	10.03	100.33
				12.0	11.96	99.67
				14.0	14.0	100.00
Mean ± S.D						100.18±0.473
Pharmaceutical preparation	**Found**		**Standard addition technique(D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery ***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	75.13	100.17 ± 0.238	10.0	6.00	5.94	99.07
				8.00	7.96	99.51
				10.0	10.0	100.00
				12.0	11.96	99.67
				14.0	13.96	99.75
Mean ± S.D						99.60±0.345

*** The average recovery of 4-separate determinations for pharmaceutical preparation**

TABLE 5b : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations and applications of standard addition technique by the proposed Second derivative spectrophotometric methods

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	24.75	99.99 ± 0.396	10.0	6.00	5.88	98.09
				8.00	7.92	99.00
				10.0	9.85	98.54
				12.0	11.96	99.66
				14.0	13.95	99.67
Mean ± S.D						98.99±0.693
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	74.25	99.99 ± 0.396	10.0	6.00	6.11	101.90
				8.00	8.08	101.00
				10.0	10.04	100.48
				12.0	11.96	99.66
				14.0	14.04	100.32
Mean ± S.D						100.32±0.836

*** The average recovery of 4-separate determinations for pharmaceutical preparation**

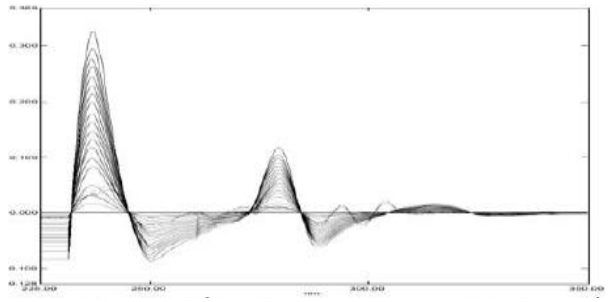

Figure 4b : Third derivative (D^3) for different concentrations (2.0-40.0µg ml^{-1}) of dothiepin hydrochloride (-) in presence of its photo-degradate (…)

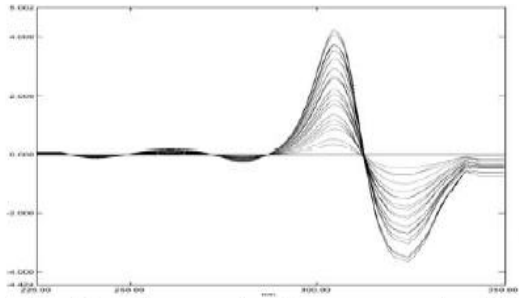

Figure 5a : First – derivative of the ratio- spectra for different concentrations (DR^1) (2.0-40.0µgml^{-1}) of dothiepin hydrochloride, using 8.0 µgml^{-1} of its oxidative-degradate as a divisor

complete degradation. The degradation process under the previously mentioned conditions was followed by using TLC, where there was a single component indicating to the presence of one spot of either oxidative or photo-degradate after complete degradation. Also, complete degradation process was conformed by adopting IR and mass spectrometry, where the suggested degradates were produced as shown in figure 2a-2d. All the degradation products did not interfere with Dothiepin Hydrochloride quantification.

The present work is concerned with determination of Dothiepin Hydrochloride in presence of its oxidative and photo-degradates, where two simple, sensitive and rapid spectrophotometric and chromatographic methods were described. In the spectrophotometric method, 2-different techniques were adopted, including derivative (D^n) and derivative ratio spectrophotometric (DR^n) techniques, where the investigated drug could be determined in presence of its both-degradates.

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TABLE 5c : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations and applications of standard addition technique by the proposed Third derivative spectrophotometric methods

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery *
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	25.04	100.17 ± 0.238	10.0	6.00	6.00	100.00
				8.00	8.03	100.48
				10.0	10.0	100.00
				12.0	11.96	99.67
				14.0	13.96	99.75
Mean ± S.D						99.98±0.316
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery ***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	75.13	100.17 ± 0.238	10.0	6.00	6.00	100.00
				8.00	7.96	99.51
				10.0	9.96	99.66
				12.0	11.92	99.35
				14.0	13.93	99.50
Mean ± S.D						99.60±0.247

*** The average recovery of 4-separate determinations for pharmaceutical preparation**

TABLE 5d : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations and applications of standard addition technique by the proposed Second derivative spectrophotometric methods

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery *
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	25.09	100.39 ± 0.389	10.0	6.00	6.00	100.00
				8.00	8.00	100.00
				10.0	10.09	100.97
				12.0	12.12	100.96
				14.0	13.87	99.07
Mean ± S.D						100.20±0.795
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery ***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	74.86	99.81± 0.623	10.0	6.00	6.00	100.00
				8.00	8.00	100.00
				10.0	9.90	99.02
				12.0	11.88	99.04
				14.0	13.87	99.07
Mean ± S.D						99.43±0.524

***The average recovery of 4-separate determinations for pharmaceutical preparation**

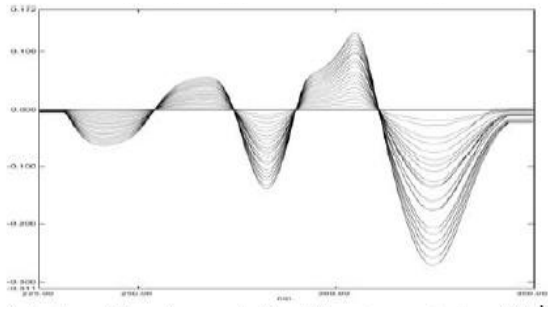

Figure 5b : First – derivative of the ratio- spectra for different concentrations (DR^1) (2.0-40.0μgml^{-1}) of dothiepin hydrochloride, using 8.0 μgml^{-1} of its photo-degradate as a divisor

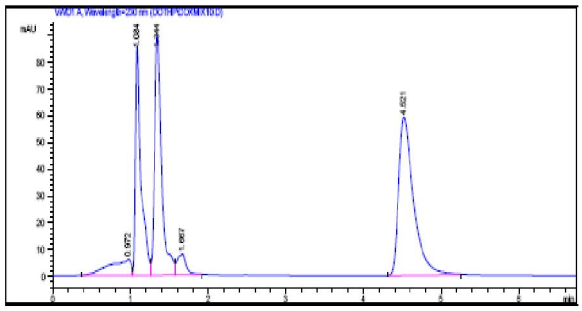

Figure 6b : Scanning profile of HPLC chromatogram of dothiepin hydrochloride & its oxidative-degradate

(2) Derivative spectrophotometric method (D^n)

In the derivative spectrophotometric technique, Dothiepin Hydrochloride could be determined a concentration range of (2.0-40.0 μgml^{-1}) in presence of its oxidative-degradate by computing first (D^1), second (D^2) and third (D^3) derivative spectrophotometry, and in presence of photo-degradate by utilizing second (D^2) and third (D^3) derivative spectrophotometry, where the amplitudes were measured at 314.60 nm, 244.80 nm and 279.20 nm (Zero-crossing of oxidative-degradate) and at 284.00 nm and at 259.40 nm (Zero-crossing of photo-degradate), as shown in figure 3a-3c and 4a-4b, respectively.

(3) Derivative of ratio spectrophotometric method (DR^n)

The advantage of the derivative ratio spectral method may be the chance of doing measurement in correspondence of peaks, so there is a potential for

TABLE 5e : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations and applications of standard addition technique by the proposed Third derivative spectrophotometric methods

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery *
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	25.09	100.39 ± 0.389	10.0	6.00	6.00	100.00
				8.00	8.08	100.99
				10.0	10.0	100.00
				12.0	12.11	100.94
				14.0	14.0	100.00
Mean ± S.D						100.39±0.529
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery ***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	75.19	100.25 ± 0.258	10.0	6.00	6.00	100.00
				8.00	8.00	100.00
				10.0	9.90	99.04
				12.0	11.88	99.05
				14.0	13.74	98.17
Mean ± S.D						99.52±0.771

*** The average recovery of 4- separate determinations for pharmaceutical preparation**

TABLE 5f : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations & applications of standard addition technique by the proposed derivative ratio spectrophotometric method, using 8.0µgml^{-1} of the oxidative -degradates as a divisor

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure found (μgml^{-1})	%Recovery *
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	24.89	99.54% ± 0.236	10.0	6.00	2.01	100.62
				8.00	3.98	99.74
				10.0	8.02	100.23
				12.0	9.95	99.47
				14.0	14.04	100.29
Mean ± S.D						100.07±0.459
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery ***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	74.83	99.77% ± 0.536	10.0	6.00	1.98	98.99
				8.00	3.98	99.46
				10.0	8.05	100.59
				12.0	10.0	100.05
				14.0	14.06	100.42
Mean ± S.D						99.90±0.669

***The average recovery of 4-separate determinations for pharmaceutical preparation**

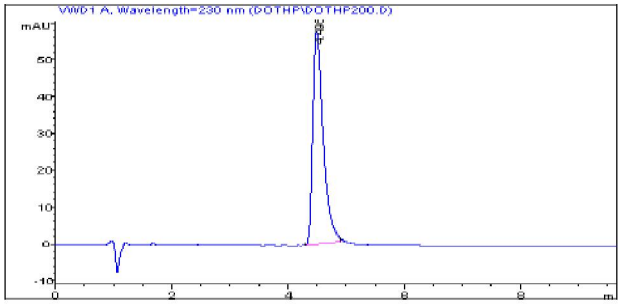

Figure 6a : Scanning profile of HPLC chromatogram of dothiepin hydrochloride

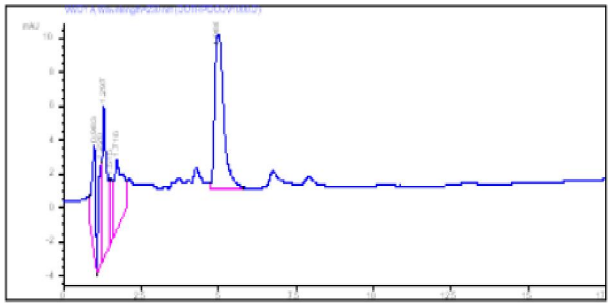

Figure 6c : Scanning profile of HPLC chromatogram of dothiepin hydrochloride & its photo-degradate

greater sensitivity and accuracy. While the main disadvantages of zero-crossing method for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and the circumstance that the working wavelengths generally do not fall in correspondence of peaks of the spectrum. This particularly pronounced disadvantage when the slope of the spectrum is very high with consequent loss of accuracy and precision and the working wavelength is in proximity of the base of the spectrum, which causes poor sensitivity[10].

The main instrumental parameter conditions were optimized for a reliable determination of the investigated drug. Different divisor concentrations of either oxidative or photo-degradate was examined to select an appropriate concentration, which is very important factor in practice, where the best results were obtained by using 8 µg.ml^{-1} concentration of either oxidative or photo-degradate stock standard as a devisor. The first derivative of the ratio spectra (DR^1) at 304.60 nm and

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TABLE 5g : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparations & applications of standard addition technique by the proposed derivative ratio spectrophotometric method, using 8.0μgml^{-1} of the photo-degradates as a divisor

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery *
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	25.05	100.21 ± 0.139	10.0	6.00	6.04	100.69
				8.00	7.92	99.02
				10.0	9.96	99.62
				12.0	11.95	99.58
				14.0	13.96	99.75
Mean ± S.D						99.73±0.604
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery ***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	75.06	100.08 ± 0.156	10.0	6.00	5.88	98.04
				8.00	7.98	99.75
				10.0	10.0	100.00
				12.0	11.95	99.63
				14.0	13.97	99.79
Mean ± S.D						99.44±0.795

***The average recovery of 4-separate determinations for pharmaceutical preparation**

241.00 nm permitted a selective determination of Dothiepin Hydrochloride in a concentration range of (2.0-40.0 μgml^{-1}) in the presence of its oxidative and photo-degradates as shown in figure 5a-5b, where no noise was bserved from the divisor.

(4) Chromatographic method

The separation of Dothiepin Hydrochloride from its degradation-products has been performed on Agilent eclipse XDB (150×4.6 mm I.D., 5 μm particle size). The proportion of the mobile phase components was optimized to reduce each of 'retention time and tailing' and to enable good resolution from its-degradates. At high acetonitrile ratio, retention time of different components decrease but with excessive tailing of its peak. High resolution was obtained by using acetonitrile: phosphate buffer pH 7.0 in a ratio (50: 50, v/v) as a mobile phase, with a flow rate 1.2 ml.min^{-1}, and detection at 230 nm, where the maximum sensitivity was observed. The average retention time was 4.96 ± 0.05 min for 10 replicates as shown in figure 6a-6c.

TABLE 5h : Quantitative determination of dothiepin hydrochloride in the pharmaceutical preparation & application of standard addition technique by HPLC method

Pharmaceutical preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Prothiaden®25 mg dothiepin capsules B.P B.N.57043/3J	25.05	100.21 ± 0.139	10.0	6.00	6.04	100.69
				8.00	7.92	99.02
				10.0	9.96	99.62
				12.0	11.95	99.58
				14.0	13.96	99.75
Mean ± S.D						99.73±0.604
Pharmaceutical preparation	**Found**		**Standard addition technique (D^2)**			
	mg	**%**	**Claimed (μgml^{-1})**	**Pure added (μgml^{-1})**	**Pure Found (μgml^{-1})**	**%Recovery***
Prothiaden®75 mg dothiepin tablets B.P B.N.33476/3J	75.06	100.08 ± 0.156	10.0	6.00	5.88	98.04
				8.00	7.98	99.75
				10.0	10.0	100.00
				12.0	11.95	99.63
				14.0	13.97	99.79
Mean ± S.D						99.44±0.795

***The average recovery of 5-separate determinations for pharmaceutical preparation**

Method validation

ICH guidelines[3] for validation method were followed, where all validation parameters were shown in TABLE 1 and 2. All the obtained results were statistically compared to the reference method[14] and no significant differences were found TABLE 3a and 3b, respectively.

Specificity

Degradation behavior of Dothiepin Hydrochloride was investigated by the proposed methods, where it was determined in solutions containing different amounts (up to 100%) of its oxidative and photo-degradates each separately, by the proposed spectrophotometric and chromatographic methods, as shown in TABLE 4a and 4b where, the recovery % and R.S.D. proved high specificity of the adopted method.

Standard addition technique

To check the validity of the proposed methods, the standard addition method was applied by adding Dothiepin Hydrochloride to the previously analyzed tab-

lets and capsules. The recovery of it was calculated by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of analysis of the commercial 'tablets and capsules' and the standard addition method (recovery study) of the studied drug are shown in TABLE 5a-5h suggested that there is no interference from any excipients, which are normally present in tablets and capsules.

CONCLUSION

The proposed methods are accurate, precise and specific ones, where Dothiepin hydrochloride can be determined in bulk powder, in laboratory prepared mixtures with different ratios of its both-degradates, separately and in pharmaceutical preparations without any interference from common excipients present. ICH guidelines were followed throughout the study for method validation and stress testing, and the suggested methods can be applied for routine quality control analysis and stability studies.

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