OPTIMIZATION OF STABILITY-INDICATING CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF ROXATIDINE ACETATE IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

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ABSTRACT

Objective: To develop two selective and sensitive stability indicating chromatographic methods, optimized and validated for roxatidine acetate determination in the presence of its degradation products.

Methods: In TLC, the chromatographic separation was achieved using chloroform: methanol: toluene in the ratio (8:2:2 by volume) as a developing system, while in RP-HPLC method, separation was carried out using a mobile phase consisting of 50 mM potassium dihydrogen phosphate: methanol: acetonitrile (5:3:2 by volume) on a C_{18} column and quantification was achieved by UV detection at 276 nm. The elucidation of the degradation pathway is described based on the use of the liquid chromatography /mass spectrometry (LC/MS) technique.

Results: The developed methods were successfully applied for determination of roxatidine in the assay of raw material and tablets as well as in the presence of its degradation products with standard deviation values less than 1.5. Linear regression was obtained in the range of 1.0-10.0 μg/ band and 40–240 μg/mL for TLC-densitometry and HPLC methods, respectively. The methods were validated as per ICH guidelines; accuracy, precision and repeatability are found to be within the acceptable limits.

Conclusion: The suggested methods can be used as stability-indicating methods for determination of bulk powder or pharmaceutical formulations of roxatidine without interference from its degradation products or from excipients. In addition to their stability indicating ability, the proposed methods are sensitive, accurate, precise, and can be used for quality control laboratories.

Keywords: Roxatidine, HPLC, TLC- densitometry, Stability indicating; Forced degradation.

INTRODUCTION

Roxatidine (ROX), 2-(Acetyloxy)-N- [3-[3-(1-piperidinyl methyl) phenoxy] propyl] acetamide is histamine H_2 – receptor antagonist and has as anti-ulcerative activity [1]. Orally administered roxatidine acetate is rapidly metabolized to an active metabolite roxatidine, by esterases enzyme in the small intestine, liver and plasma [2, 3]. Different chromatographic techniques were applied for the detection and determination of ROX in biological fluids. These techniques include high performance liquid chromatography (HPLC) with UV detector [4], liquid chromatography - mass spectrometry (LC-MS) [5, 6], gas chromatography - mass spectrometry (GC-MS) [7] and capillary gas chromatography with nitrogen selective detection [8]. Also ROX was determined in pharmaceuticals spectrophotometry [9] and by capillary zone electrophoresis (CZE) [10].

The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [11]. It suggests that the degradation products that are formed under a variety of conditions should be identified and degradation pathways established.

No stability-indicating methods have been cited in the literature for determination of ROX and its degradation products; therefore the scientific novelty of this work presents a study of the degradation of ROX, identification of the obtained degradation using LC-MS, followed by the development of two stability-indicating chromatographic methods for its determination either in raw material or in pharmaceutical dosage forms without the interference of excipients and their degradation products, which makes them suitable for quality control analysis.

MATERIALS AND METHODS

Instrumentation

- In the TLC system the samples were applied as bands of 6-mm width using the spray-on technique with a Camag 100 μl sample syringe (Muttens, Switzerland) on a precoated silica gel TLC aluminum plates F254; layer thickness 20×20 cm, 0.25 mm, Fluka (Buchs, Switzerland), using a CAMAG Linomat 5, autosampler (Muttens, Switzerland) with a constant application rate 10 μl/s. The slit dimension was kept at 5 x 0.2 mm and 20 mm /s scanning speed was employed. Densitometric scanning was performed on a CAMAG TLC densitometric Scanner 3S/N 130319 in the reflectance absorbance mode for all measurements and operated by WINCATS software (Muttens, Switzerland). The spots were detected under UV short wavelength lamp 254 nm, Spectroline (U.S.A).

- An HPLC system consisted of Agilent 1100series control module by Agilent chemstation for HPLC equipped with a quaternary pump, injector with a 20 μl loop and a UV-variable wavelength detector (Minnesota, U.S.A). Column used was Inertsil ODS-3 C18 RP column (250 x 4.6 mm, 5 μm particle size).

- Structure elucidation of the degradation products was done using Agilent Technologies 6410 triple quadrupole mass spectrometer (U.S.A), fitted with ESI (electrospray ionization) source where ESI-MS analyses were carried out on both positive- and negative- ion modes with scan range from *m/z* 50-500. The MS*n* data were acquired in the Auto MS*n* mode. The ESI source temperature was kept at 350°C and the capillary voltage was maintained at 4.0 Kv, while the fragmentor voltage was 135 and delta EMV was 800. Nitrogen was used as the nebulizing gas at a flow rate of 11 liter/min and the pressure was kept at 40 psi. The Agilent MassHunter software provided with the instrument for instrument control and data acquisition.

Samples

Pure standard

Roxatidine acetate hydrochloride reference standard (batch no: R00200-WS-01) was kindly supplied by Sanofi Aventis Deutshland GmbH (Frankfurt, Germany); and certified to contain 99.4%.

Pharmaceutical formulation

The commercial Gastralgin tablets labeled to contain 150 mg of ROX / tab, (batch no: N 4 33) manufactured by De Angeli, AVENTIS PHARMA S.p.A (Milano, Italy).

Degraded sample

ROX **(**50 mg) was dissolved in the least amount of methanol, refluxed with 50 mL of 1 M HCl, or 1N NaOH solution in a 250-ml round-bottom flask for 1 hour, and tested for complete degradation by TLC using chloroform: methanol: toluene (8:2:2 by volume) as the mobile phase. The degraded solution was then cooled, neutralized with an amount of acid or base equivalent to that of the previously added till pH was 7. The solution was nearly evaporated to dryness, cooled, transferred into a 50-mL volumetric flask with methanol. The volume was then completed to the mark with the same solvent and filtered to obtain solution with final concentration of 1 mg /mL.

Chemicals and solvents

All chemicals and solvents used throughout this work were of analytical grade.

Acetonitrile, HPLC grade (Labscan analytical sciences, New Jersey, U.S.A), Methanol, HPLC grade (Sigam-Aldrich, Labochemikalien GmbH, Germany) and Methanol AR (S.D Fine-Chem Limited, Mumbai, India) were used. Potassium dihydrogenphosphate, chloroform and toluene obtained from (El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt). De-ionized water used; was bi-distilled from "Aquatron" Automatic Water Still A4000, Bibby Sterillin Ltd. (Staffordshire, UK).

Standard solutions

• Stock standard solutions of ROX 1 mg /mL in methanol for TLC densitometry and HPLC methods.

• Stock standard solutions of the acidic and alkaline degradation products derived from complete degradation of standard solutions of ROX (1 mg /mL) in methanol.

• Working solutions of ROX and its acidic and alkaline degradation product, 0.4 mg /mL in methanol for HPLC method.

Laboratory prepared mixtures

Solutions containing different ratios of ROX and its acidic or alkaline degradation products were prepared to contain 5–90% of acidic or alkaline degradation products of ROX.

Construction of calibration curve

For TLC-densitometric method

Aliquots equivalent to 1.0 – 10.0 mg ROX were transferred from its stock solution 1.0 mg /mL into a series of 10 mL volumetric flasks and the volume was completed with methanol. An aliquot equivalent to 10 μL of each solution was applied onto a TLC plate; the samples were applied as bands (bandwidth: 6 mm; spacing: 14.2 mm; 15 mm from bottom edge of the plate) using a Camag Linomat 5 applicator. Linear ascending development was done in a chromatographic tank previously saturated with chloroform: methanol: toluene (8:2:2 by volume) for 1 h at room temperature. The developed plates were air dried and scanned at 276 nm on Camage TLC scanner 3 operated in the absorbance mode; with deuterium lamp as a source of radiation. A calibration curve was constructed by plotting peak areas and the corresponding concentrations of ROX.

For RP-HPLC method

Aliquots equivalent to 0.4 – 2.4 mg ROX were transferred from its working solution 0.4 mg /mL into a series of 10 mL volumetric flasks. The volume was then completed with methanol. An aliquot equivalent to 20 μL of each solution was separately injected. The chromatographic separation was carried out on reversed phase Inertsil ODS-3 column [C_{18} (5 μm, 4.6 x 250 mm)], using 50 mM potassium dihydrogen phosphate pH adjusted to 7 with KOH: methanol: acetonitrile (5:3:2 by volume) as a mobile phase. The

mobile phase was filtered through Millipore filter 0.45 μm, white nylon HNWP 47 mm and was degassed for 15 min in an ultrasonic bath prior to use. UV detection was done at 276 nm. The system was operated at ambient temperature. The flow rate was isocratic at 2 mL /min. The samples were filtered also through a 0.45μm membrane filter, and were injected by the aid of a 20μL Agilent® analytical syringe. The chromatograms were recorded, the peak areas of ROX were determined and the calibration curve relating peak areas to the corresponding concentrations for ROX was constructed.

Analysis of laboratory prepared mixtures

Mixtures containing different concentrations of ROX and its induced acid or alkaline degradation products were analyzed as mentioned under each method. The concentrations were calculated from the corresponding regression equations.

Assay of Pharmaceutical Formulations (Gastralgin tablet)

The contents of ten tablets of Gastralgin ® tablets were powdered and mixed well; an accurately weighed amount of the powder equivalent to 0.1 g of ROX was transferred into 100-mL volumetric flasks, 75 mL of methanol was added as a solvent, stirred well then completed to the volume to obtain 1mg/mL stock solutions, then filtered. The solutions were diluted to the same concentrations of the appropriate working solutions and preceded according to the procedure of each method mentioned above.

Liquid Chromatography- Mass Spectrometry

Aliquots of methanolic standard solution of pure ROX and ROX degradation products were diluted with mobile phase consisted of acetonitrile: 10 mM ammonium formate buffer pH 3.9 at a ratio of (70:30 v/v). An aliquot equivalent to 1.0 μL of each sample was separately injected at flow rate 0.5 mL /min, using Inertsil ODS-3 C_{18} (50 x 4.6 mm, 5 μm particle size) as analytical column). Then samples were subjected to mass spectrometric analysis using positive and negative electrospray ionization modes.

Methods validation

Linearity was determined by using series of ROX standard solutions in the range 1.0-10.0 μg/ band and 40–240 μg/mL for TLC-densitometric and HPLC methods, respectively. The solutions were chromatographed using the specified chromatographic conditions, peak areas were plotted versus concentrations and regression equations were computed.

The intraday and interday precision were evaluated by assaying freshly prepared samples of ROX in triplicate concentrations 2, 6, 8 μg/ band for TLC-densitometric method and 60, 140, 220 μg/ mL for HPLC method.

According to ICH recommendation the approach based on SD of the response and the slope was used for determining the detection and quantitation limits (LOD and LOQ).

The accuracy of the investigated methods was validated by analyzing pure sample of ROX in triplicate. The concentrations of the active drug were calculated from the calculated regression equations.

Selecitivity was ascertained by analyzing different mixtures containing ROX and its acidic or alkaline degradation products in different ratios.

Robustness of HPLC method was investigated by varying chromatographic conditions such as performing separation of ROX and its degradation products on two different HPLC instruments, namely "Agilent" and "Shimadzu", also using different pH values 7 ± 0.2, different flow rates (2 ± 0.2 ml/min) and different organic phase composition by 50 ± 2% as the mobile phase.

System suitability parameters such as resolution, capacity factor and selectivity for the separated peaks were also calculated.

RESULTS AND DISCUSSION

The stability-indicating assay is a method that is employed for the analysis of samples stability in pharmaceutical industry. With the

advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. Examining degradation products under stress conditions is useful in establishing degradation pathways and developing and validating suitable analytical procedures [11]. Therefore, this work which describes stability indicating methods for selective determination of ROX in presence of its degradation products and formulation excipients is considered as a prime requirement, where the drug was subjected to alkaline and acidic forced degradation studies.

Identification of the degradation products

Different concentrations of HCl (0.05 M, 0.5 M and 1 M) and NaOH (0.05 M, 0.5 M and 1 M) were tried. Complete degradation has been achieved by reflux for 1 hour with 1 M HCl for acid degradation, and for 1 hour with with 1 M NaOH for alkaline degradation. Degradation process was followed by TLC method for complete disappearance of the intact drug's spot.

Since impurities and degradation products are usually present in relatively small quantities compared to the drug, an analytical technique capable of separating a mixture containing highly varied concentrations of analytes with sensitive and specific detection is

required. LC-MS is therefore widely used for this purpose [12-15]. The acid and alkaline degradation products were subjected to LC-MS analysis for their identification. The positive ion ESI-MS-scan of the solution of ROX in the mobile phase showed a peak at m/z 349 which refers to the protonated molecular ion of the drug (Figure 1a). Scanning acid or alkaline degradation products solution showed two peaks with the [M+H]+ at m/z 307 and, at m/z 249. It is suggested that the drug undergoes hydrolysis with the cleavage of the ester linkage to produce roxatidine, (Deg I). ROX structure shows an amide group, many amide containing drugs undergo acidic or alkaline hydrolysis with cleavage of the amide linkage [16], thus the probable acidic or alkaline degradation pathway for ROX will be the same, with the production of 3-[3-(1-piperidinyl methyl) phenoxy] propyl amine (Deg II). Deg I and Deg II were eluted at retention time (R_T) of 0.878 min and 0.818 min, respectively and their extracted ion currents of [M+H]+ were shown in Figures.1b, 1c. The UV spectrum obtained of the alkaline or acidic degradation products shows resemblance to that of the drug, this is may be due to that the degradation products retain the same chromophores as the intact drug, which assists the previous assumption. The predicted structural formula of ROX degradation products are shown in the scheme (Figure 2).
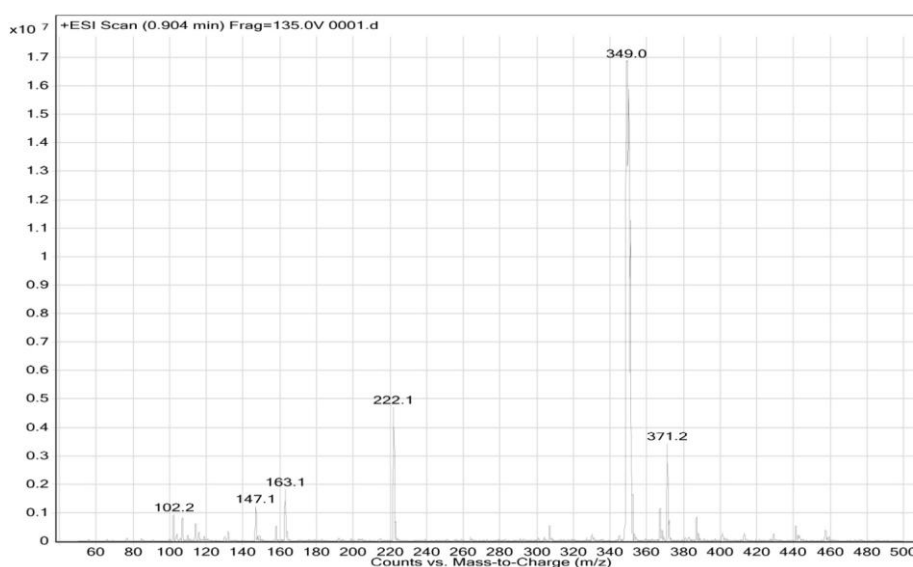

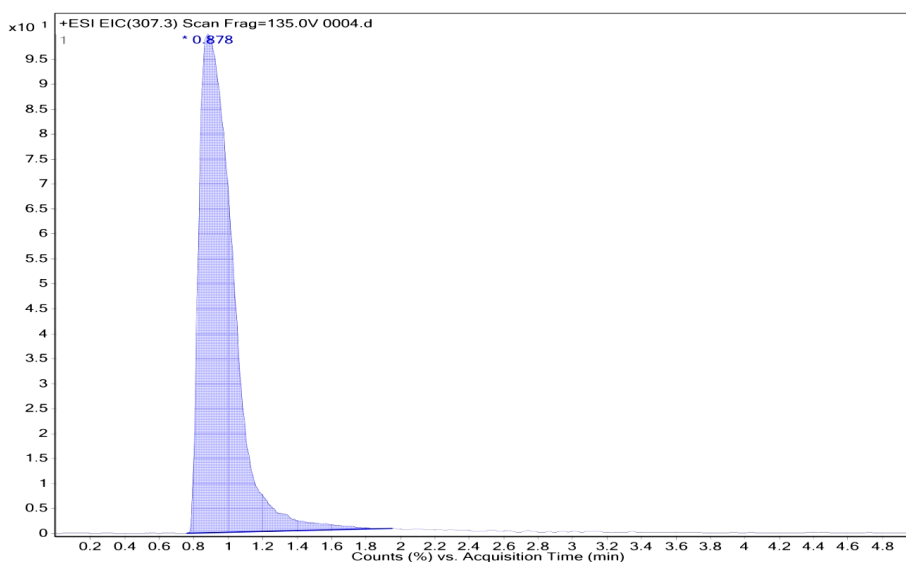**Fig. 1a: Positive ESI- MS of Roxatidine acetate ([M+H]+, m/z 349.0)**

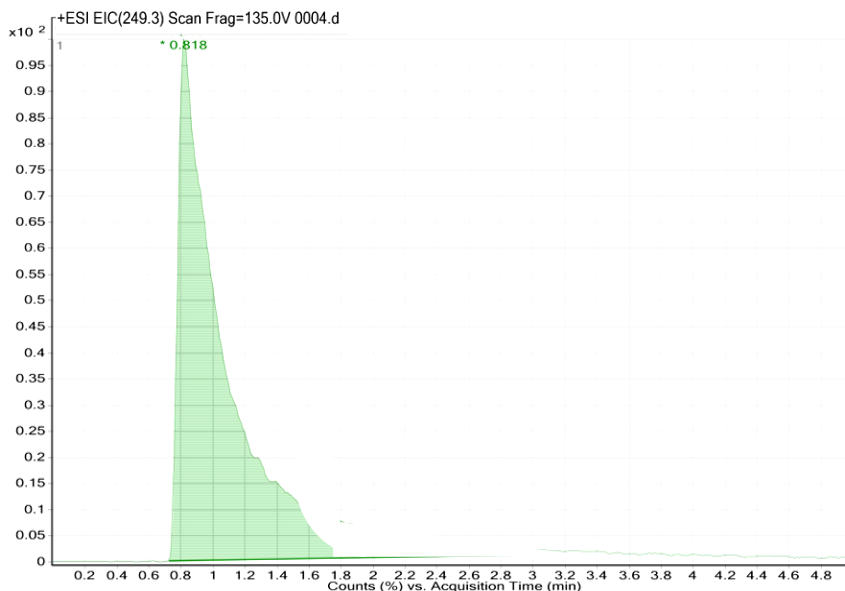**Fig. 1b: HPLC ESI-MS mass chromatogram of Deg I ([M+H]+, m/z 307.3)**

Fig. 1c: HPLC ESI-MS mass chromatogram of Deg II ([M+H]⁺, m/z 249.3)

Roxatidine acetate
M.W.= 348.44

Acidic or alkaline hydrolysis

(Roxatidine)

Deg.I
M.W.= 306.40

Further
Acidic or alkaline hydrolysis
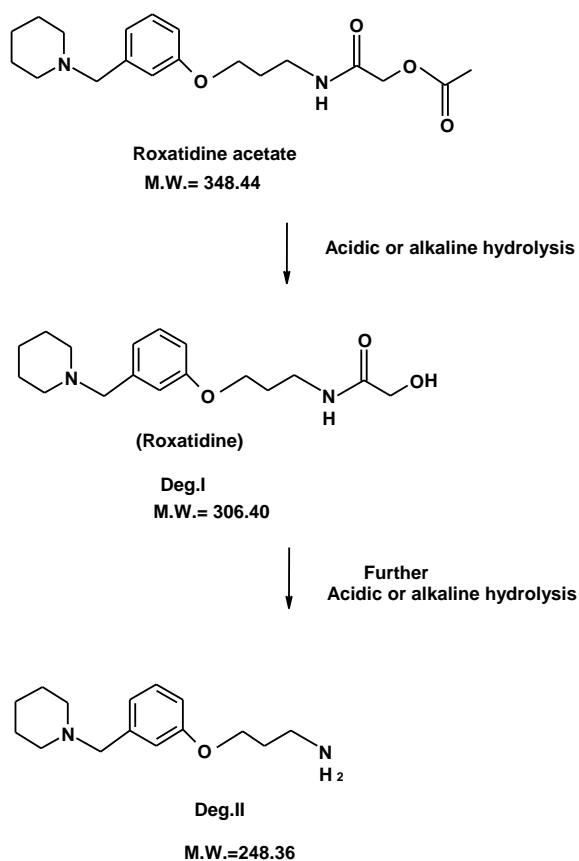
Deg.II

M.W.=248.36

Fig. 2: Scheme of the suggested structures of acid degradation product or alkaline degradation products of roxatidine acetate.

TLC-Densitometric method

A stability-indicating TLC-densitometric method was suggested for the determination of ROX in presence of its acid or alkaline degradation products. The method was based on the difference in the migration rates of the aforementioned compounds using chloroform: methanol: toluene (8:2:2 by volume) as a mobile phase. It was necessary to test the effect of different variables to optimize this method as described below.

1. Mobile phase

Different developing systems of different composition and ratios were tried for separation. The initial separation was developed using methanol, chloroform and glacial acetic acid to (8:2:0.2 by volume) resulted in high R_f values of the drug and the degradation products which were then reduced by changing to non polar developing system; chloroform: methanol (8:2 v/v) and chloroform: methanol: toluene (5:2:5 by volume). Subsequently, changing the ratio of

chloroform, methanol and toluene to (8:2:2 by volume), greatly improved the resolution; and the tailing for all peaks were reduced considerably. The R_f values were 0.04, 0.22 and 0.35 for Deg I, Deg II and ROX, respectively, Figure 3. The drug showed good linearity in range of 1.0-10.0 µg/ band, Figure 4.

2. Band dimensions

Different band dimensions were tested in order to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 6 mm and the inter-space between bands was 14.2 mm.
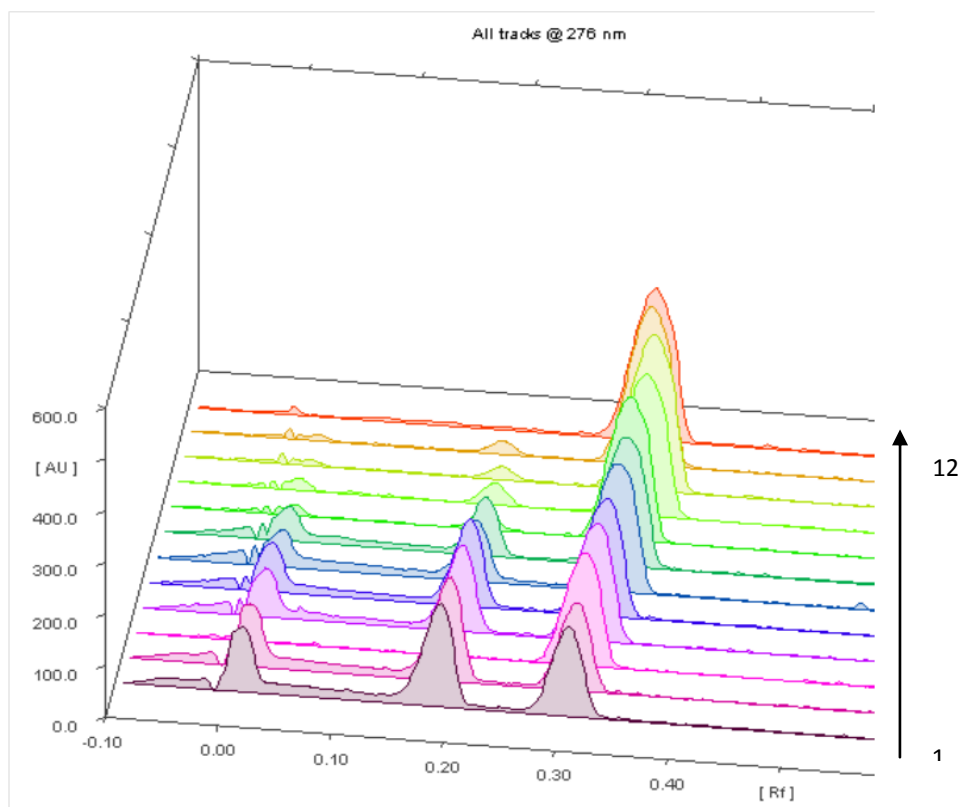

Fig.3: Densitogram of laboratory mixtures of roxatidine and its induced acid or alkaline degradation products in the ratio of 70% - 5% degradation products (from track 1 to track 11), and track 12 is pure roxatidine acetate.
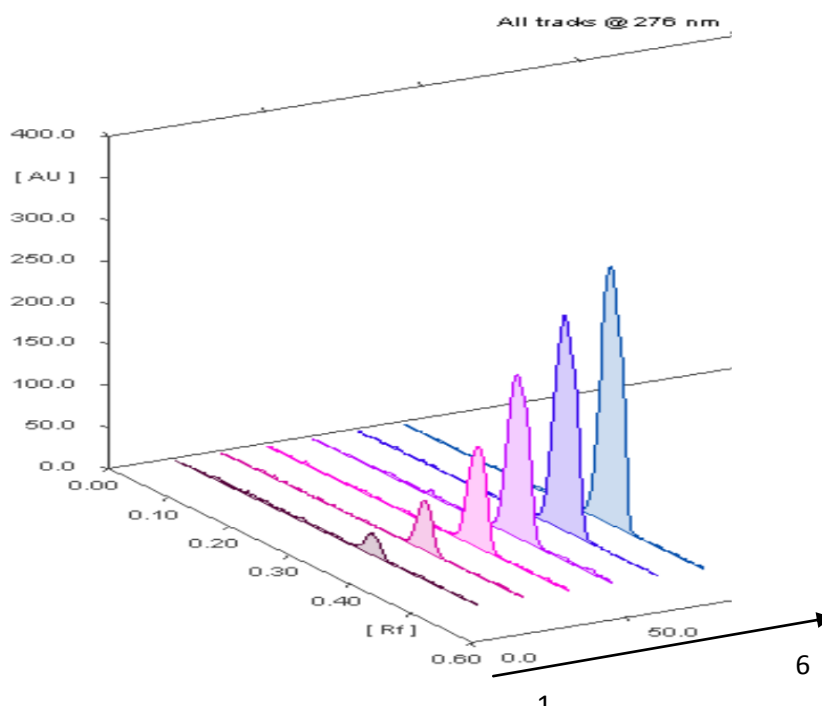

Fig. 4: Densitogram of Roxatidine acetate HCl in the concentration range of 1.0-10.0 µg/ band from track 1 to track 6.

3. Scanning wavelengths

Different scanning wavelengths as 222 nm, 283 nm and 276 nm were tested, where scanning at 276 nm was suitable providing good sensitivity for ROX as the peaks were more sharp and symmetrical with minimum noise.

4. The slit dimensions of the scanning light beam

The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slit dimensions were tried, where 5mm×0.2mm proved to be the slit dimension of choice which provides highest sensitivity.

RP-HPLC Method

Although ROX was determined by different HPLC methods either alone or with its metabolite; none of the methods determined the drug in presence of its hydrolyzed degradation products. The aim of this work was to develop a stability indicating isocratic HPLC assay for the analysis of ROX either in bulk powder or in the pharmaceutical dosage form.

The method was optimized to provide a good separation of the components (acceptable theoretical plates and resolution between peaks) with a sufficient sensitivity and suitable peak symmetry (peak tailing factor < 2) in a short run. For this purpose, the following parameters are established to ensure the efficiency of the chromatographic separation.

1. Mobile phase

Several mobile phases were tried such as methanol: water (65: 35 v/v), acetonitrile: 50 mM potassium dihydrogenphosphate pH 7 (20: 80 v/v) and 50 mM potassium dihydrogenphosphate pH 7: methanol: acetonitrile (5: 3: 2 by volume) this later gave satisfactory separation and peak symmetry for ROX and its degradation products. Adding methanol to the mobile phase did not only improve the separation, the R_t value of the drug was obviously decreased.

2. pH effect

The selected mobile phase was tried along with its pH changed from 2.24 to 7 and adequate resolution obtained, increasing the mobile ionic strength from 20mM to 50 mM of potassium dihydrogenphosphate improved the peak shape.

3. RP-HPLC columns

Various RP-HPLC columns were used and minimum elution time with good resolution was obtained by using Inertsil ODS-3 C_{18} RP column (250 x 4.6 mm, 5 µm particle size).

4. Scanning wavelength

Different wavelengths were studied and maximum sensitivity for the measurement of all compounds obtained at 276 nm.

5. Flow rate

Different flow rates were tested to provide good separation within acceptable time; and the best flow rate was 2 ml /min.
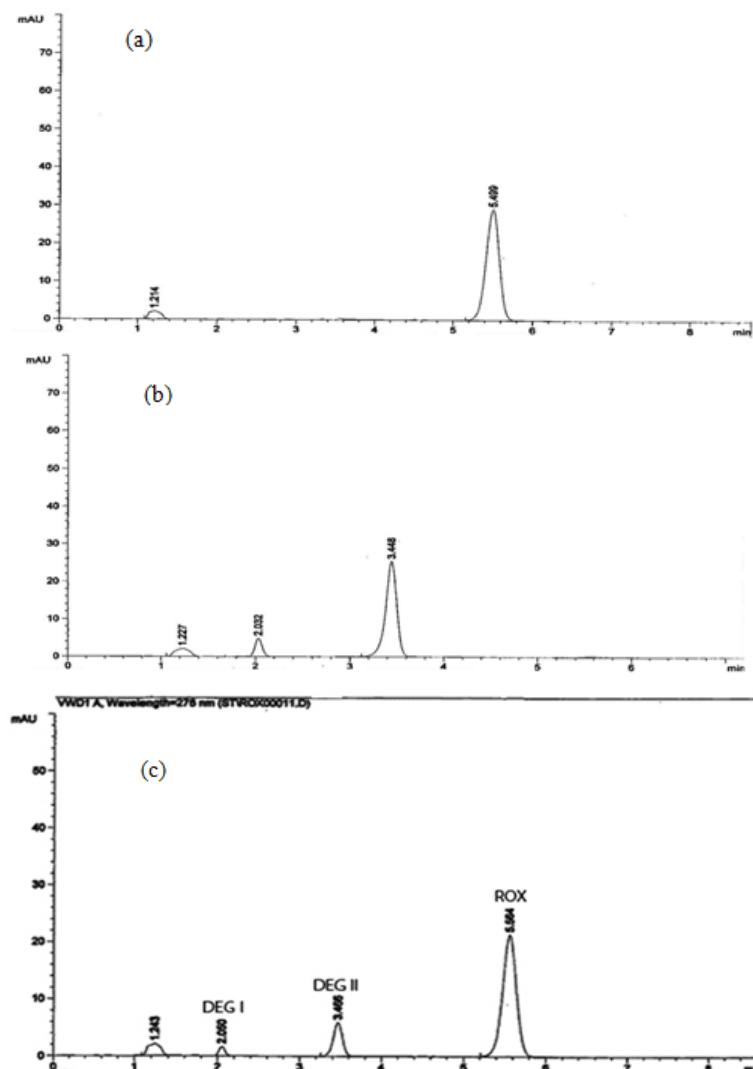

Fig. 5: HPLC chromatograms of (a) Roxatidine acetate, (b) acid or alkaline degradation products and (c) a laboratory mixture of 100 µg/mL of both Roxatidine acetate and its hydrolytic degradation products.

A satisfactory separation obtained using the optimum conditions, where the retention time (R_t) values of ROX was 5.56 while Deg I and Deg II were 2.05 and 3.46, respectively, Figure 5.

Methods validation

The suggested procedures were subjected to the validation scheme according to ICH guidelines [17] and showed good specificity and reproducibility, Table 1.

Linearity

The Calibration curves showed good linearity in the range 1.0-10.0 µg/ band and 40–240 µg/mL for ROX. A linear relationship was obtained between the recorded areas versus the corresponding concentrations of the drug with correlation coefficient (*r*) of 0.9993 and 0.9998 for TLC-densitometric and HPLC methods, respectively, Table 1.

Precision

The intraday and interday variation of the method was carried out and (RSD %) for ROX were found within the the prescribed limit of ICH guidelines (RSD % < 2%). The intraday and interday relative standard deviation (RSD) values obtained, revealed that the proposed methods are precise as shown in Table 1.

LOD and LOQ

The limit of detection (LOD) of ROX were found to be 0.64 (µg/ band) and 4.43 (µg/mL) and the limit of quantitation (LOQ) were found to be 1.93(µg/ band) and 13.42(µg/mL). This testifies the sensitivity of method.

Accuracy

The accuracy of the proposed methods shows good results and is presented in Table1. Moreover, when results obtained by applying the proposed methods for analysis of pure ROX compared to those obtained by applying the reported method [4], they showed no significant difference regarding accuracy and precision. Results were given in Table 2.

Selectivity

The selectivity was proved by the determination of laboratory prepared mixtures containing different ratios of ROX and their degradation products and it was found to be valid up to 70% and up to 90% of degradation products for TLC and HPLC methods, respectively. Satisfactory results were obtained indicating the high selectivity of the proposed methods. Table 3

Table 1: Regression Equation Parameters and Determination of Pure Sample of ROX by the Proposed Methods

Parameters	Method					
	TLC			HPLC		
Wavelength (nm)	276 nm			276 nm		
Linearity range	1.0-10.0 µg/ band			40–240 µg/ mL		
Slope	990.45			3.6671		
Intercept	615.41			9.0667		
*r	0.9993			0.9998		
Mean accuracy	100.57			100.26		
± R.S.D.%	0.48			0.73		
** LOD	0.64 (µg/ band)			4.43 (µg/mL)		
** LOQ	1.93(µg/ band)			13.42(µg/mL)		
Intra-day[a]	2	6	8	60	140	220
Recovery% of Rox	100.05	100.85	100.07	100.25	100.14	100.18
±R.S.D.%	0.90	0.44	0.58	0.67	0.93	0.76
Inter-day[a]	2	6	8	60	140	220
Concentration *n* = 3	100.06	100.56	100.44	100.74	100.87	100.43
Recovery% of Rox ±R.S.D.%	1.04	0.63	1.43	1.20	0.92	1.22

*Correlation coefficient

** LOD =3.3 (S.D / S), LOQ = 10 (S. D / S); where S.D is the residual standard deviation of the slope and S is the slope for TLC-densitometric and HPLC methods.

[a] Intraday precision (average of 3 different concentrations of / 3 replicate each (n=9) within the same day)

[b] Interday precision (average of 3 different concentrations of / 3 replicate each (n=9) repeated on 3 successive days).

Concentration was measured in (µg/ band) for TLC and in (µg/mL) for HPLC methods.

Table 2: Comparison between the Results of Analysis of ROX Using the Proposed Methods and the Reported Method in Pure Form.

	TLC-densitometric method	HPLC method	Reported Method[4]
Mean±S.D.	100.57±0.48	100.26±0.73	100.63±0.63
n	5	5	5
Variance	0.23	0.53	0.40
t-test (2.306) *	0.32	0.19	—
F ratio (6.39) *	1.74	1.32	—

*Figures in parentheses represent the corresponding tabulated values for *F* and t at *P* =0.05

Table 3: Results of the Proposed Methods for the Determination of ROX in Presence of Its Degradation products

Percentage of added degradation products	TLC	HPLC
5%	100.78	-----
10 %	99.94	98.98
30 %	100.63	100.23
50 %	101.27	101.12
70 %	101.17	100.61
90 %	109.36*	100.96
Mean	100.76	100.38
RSD	0.52	0.85

*Rejected value

Robustness and System suitability

The influence of small changes of chromatographic conditions such as change in flow rate (10%), pH values (± 0.2) and organic phase composition studied to determine the robustness are also in favor (RSD % < 2%) of the developed HPLC method for analysis of ROX.

The Rt values of the separated peaks using the mentioned pH range did not change, while changing the flow rate and mobile phase was accompanied by slight decrease or increase of Rt of all peaks. However, the calculated resolution (Rs) values were always above 2,

ensuring complete separation. Other parameters such as resolution, capacity factor and selectivity for the separated peaks were then calculated in Table 4.

Application to commercial tablets

The suggested methods were successfully applied for determination of ROX in its pharmaceutical formulations (Gastralgin tablets). The results were satisfactory and with good agreement with the labeled amounts. Applying the standard addition technique, no interference due to excipients was observed as shown from the results in Table 5.

Table 4: Parameters of system suitability test of the proposed methods for the determination of ROX in presence of its degradation products.

Parameters	Obtained value of TLC-densitometric			Reference value[18]	Obtained value of HPLC		
	ROX	DEG I	DEG I I		ROX	DEG I	DEG I I
Capacity factor ($K´$)	16.5	—	—	$K´ > 2$	3.48	—	—
Selectivity factor (α)	—	8.75	1.59	$\alpha > 1$	—	2.72	1.61
Tailing factor (T)	1.12	1.4	1.0	$T \leq 2$	1.0	1.25	9.98
Resolution Rs(relative to Roxatidine)	—	4.625	2.27	$Rs > 2$	—	13.80	6.1
Theoretical Plates (N)	—	—	—	$N > 2000$	2970	—	—

Table 5: Determination of ROX in Its Pharmaceutical dosage form using the Proposed Methods and application of Standard Addition Technique

	TLC-densitometric				HPLC			
Gastralgin tablet, B.N. N 4 33, claimed to contain 50 mg of ROX /tablet	Taken µg/mL	Recovery% of Rox ±R.S.D.%	Standard added µg/mL	Recovery%[a] of added standard	Taken µg/mL	Recovery% of Rox ±R.S.D.%	Standard added µg/mL	Recovery%[a] of added standard
	3	100.92 ±0.41	2	100.69	100	100.20 ± 0.86	40	100.39
			3	101.20			100	99.58
			6	99.93			140	101.32
Mean ± SD				100.61 ± 0.64	Mean ± SD			100.43 ± 0.87

[a]Average of three different experiments.

CONCLUSION

The present study represents the first report that deals with the development of a stability-indicating chromatographic method for determination of ROX. Both chromatographic methods were validated in compliance with ICH guidelines, revealed that both were specific, sensitive, fast and reliable. The TLC-densitometric method is simple and uses a minimal volume of solvents, compared to the HPLC method. Statistical tests indicate that the proposed TLC-densitometric and HPLC methods appear to be equally suitable for routine determination of ROX in pharmaceutical formulations. It is one of the useful studies where forced decomposition was undertaken under different suggested conditions. As the methods separate the drug from its degradation products, they can be employed as stability indicating techniques.

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