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STABILITY-INDICATING HPLC-DAD AND TLC-DENSITOMETRIC METHODS FOR DETERMINATION OF CROTAMITON IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

Mohammed Wafaa Nassar[1], Khalid A.M. Attia[2], Ragab A. Said[3], Ahmed El-Olemy[3] and Mohamed A. Hasan[4]*

*Assistant Lecturer of Analytical Chemistry, Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al- Azhar University, 11751 Nasr City, Cairo, Egypt.

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***Corresponding Author**
Mohamed A. Hasan
Assistant Lecturer of Analytical Chemistry, Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.

ABSTRACT

In the present work, a rapid, specific and reproducible reversed phase high performance liquid chromatography with diode array detection (HPLC-DAD) method has been developed and validated for the determination of crotamiton in the presence of its degradation products. The drug was found to be labile to acidic hydrolysis, alkaline hydrolysis, oxidation, photolysis, thermal and neutral hydrolytic conditions. The acidic hydrolysis gives the highest degradation percentage, so complete degradation of the drug was done using reflux with 1N HCl for 3 hours and the degradation pathway was confirmed using TLC, IR, ^{1}H NMR and mass spectrometry. Then, thin-layer chromatography (TLC) - densitometry method has been developed and validated for determination of crotamiton in the presence of its acid-induced degradation product. The developed methods were validated according to the International Conference on Harmonization (ICH) guidelines demonstrating good accuracy and precision. The results were statistically compared with those obtained by the reported method, and no significant difference was found.

KEYWORDS: Crotamiton; HPLC-DAD; TLC-densitometry and degradation product.

INTRODUCTION

Crotamiton is chemically designated as *N*-ethyl-*N*-(2-methylphenyl) but-2-enamide, **figure (1)**. Crotamiton is an antiparasitic that is toxic to the scabies mite. Crotamiton also relieves itching by producing what is called a counter-irritation. As crotamiton evaporates from the skin, it produces a cooling effect.[1] The United States Pharmacopeia (USP) describes a spectrophotometric method for determination of crotamiton in raw material and HPLC method for determination of crotamiton in cream.[2] Literature survey reveals that HPLC methods were reported for determination of crotamiton in ointments and biological fluids [3-5], also for separation of crotamiton *cis* and *trans*-isomers.[6,7] Polarographic and photocatalytic decomposition methods were developed for determination of crotamiton.[8,9] Reviewing the literature on the determination of crotamiton revealed the lack of any stability indicating HPLC and TLC-densitometric methods for the determination of crotamiton in presence of its possible degradation products. The aim of this work is to develop and validate simple, sensitive and selective chromatographic methods for the determination of crotamiton in presence of its degradation products. Moreover, DAD technology facilitate the detection of all possible degradation products through multiple monitoring process.

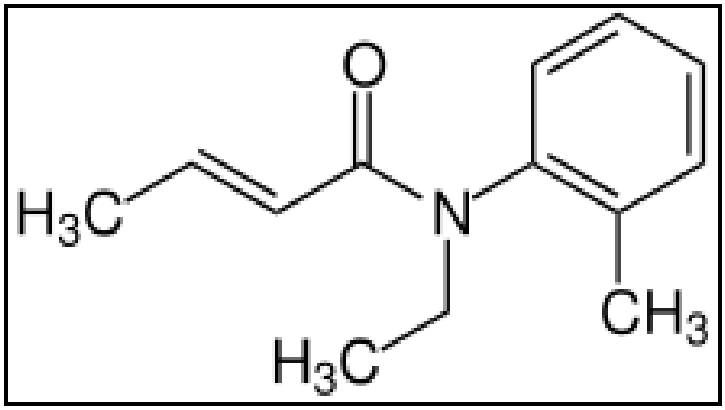


Figure (1): Structural formula of crotamiton.

2. Experimental

2.1. Instruments

- HPLC, constaMetric® 4100 LDC Analytical pump (Milton Roy, USA), equipped with spectra system UV3000 diode-array UV-visible detector and spectra system AS3000 auto sampler. The chromatographic analysis was carried out using (ChromQuest 4.2.34, version 3.1.6) data analysis program.

- Camag Linomat autosampler (Muttenzl, Switzerland), a Camag microsyringe (100 µL) and a Camag 35/N/30319 TLC scanner with win CATS software; an ultraviolet (UV) lamp with a short wavelength at 250 nm (Desaga, Wiesloch, Germany).
- Aluminum TLC plates precoated with silica gel 60 GF_{254} (20×20 cm), (Merck, Darmstadt, Germany).
- Chromatographic tank (25 × 25 × 9 cm).
- Hot plate (Torrey pines Scientific, USA).
- Jenway, 3510 pH meter (Jenway, USA).
- Rotatory evaporator (Scilogex-RE 100-pro, USA).
- NMR, Gemini-400 BB (Agilent, USA).
- FT-IR, Nicolet IR 200 (Thermo electron corporation, USA).
- GCMS-QP-1000 EX mass spectrometer at 70 ev (Shimadzu, Tokyo, Japan).

2.2. Materials and reagent

- Pure crotamiton (99.70 %) was kindly provided by Novartis Pharma S.A.E., Cairo, Egypt.
- Eurax® cream: (batch number Y1054) manufactured by Novartis Pharma S.A.E., Cairo, Egypt under licence from: Novartis Consumer Health SA - Switzerland; labeled to contain 100 mg of crotamiton per 1 gm of cream.
- Acetonitrile, dichloroethane, methanol, petrolium ether and toluene, all of HPLC grade, (Sigma-Aldrich, Germany).
- Hydrochloric acid, analytical grade (El-Nasr Co., Egypt), prepared as 0.1N and 1N aqueous solutions.
- Sodium hydroxide, analytical grade (El-Nasr Co., Egypt), prepared as 0.1N and 1N aqueous solutions.
- Hydrogen peroxide (30%), analytical grade (El-Nasr Co., Egypt), prepared as 6% aqueous solution.

2.3. Standard solutions

Stock solution of crotamiton (1 mg/ml) was prepared by diluting 0.1 ml (equal to 100 mg) of pure crotamiton in 50 ml of methanol and the volume was completed to 100 ml with methanol. Different sets of working solution at various concentrations were prepared by appropriate dilution of the stock solution.

2.4. Preparation of forced degradation solutions

Working standard solution (15 µg/ml) was used during forced degradation studies. For each degradation sample, 2ml of crotamiton working standard solution (15 µg/ml) were transfered to 10 ml volumetric flask and completed to volume with methanol to obtain a concentration of 3 µg/ml for each sample. Acidic and basic hydrolysis were carried out using 2 ml of 0.1 N HCl, and 0.1 N NaOH for 2 hours at room temperature. Each sample was neutralized with alkali or acid before dilution. Oxidative degradation was carried out using 1 ml of 6 % H_2O_2 for 2 hours at room temperature. Neutral hydrolysis was done using 2ml deionized water for 5 hours at room temperature and for 3 hours at 80°C. Also thermal study without water was carried out for 3 hours at 80°C. Photolytic study was done by exposing solutions of the drug to sunlight for 24 hours. The prepared solutions were kept away from light to prevent possible photodegradation. Then, the procedure described under chromatographic conditions was followed. From the peak area of crotamiton in each chromatographed sample, the % degradation was then calculated. After the previous treatments, all forced degradation solutions were filtered with 0.45 µm membrane filters.

2.5. Preparation of acidic-induced degradation product

0.1 ml (equal to 100 mg) of pure crotamiton solution was treated with 50ml of 1N HCl in a 100-ml round bottomed flask and the solution was heated under reflux for 3 hours and 2-butenoic acid started to precipitate. After cooling, the solution was filtered, the precipitate was dried and the filtrate containing *N*-ethyl-2-methylaniline was neutralized to pH = 7 using 1N NaOH and evaporated under vacuum. The obtained residue was extracted three times with 25 ml of methanol, filtered into 100-ml volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradation product derived from 1 mg/ml of crotamiton.

2.6. Procedures

2.6.1. Construction of calibration curves

- **RP-HPLC method**

Aliquots of crotamiton equivalent to (10-100 µg), were accurately transferred from its respective working standard solution (100 µg/ml) into a set of 10-ml volumetric flasks and the volume was then completed to the mark with the mobile phase. A 20-µL of each solution was injected into Discovery® HS C_{18} column (250 mm x 4.6 mm, 5µm particle size) using mobile phase consists of acetonitrile : water : methanol (50:30:20, by volume) at flow rate 1.0

ml/min and UV detection at 254 nm. Calibration curve was constructed by plotting the peak area against the corresponding concentrations of crotamiton in µg/ml.

- **TLC-densitometry method**

Aliquots of crotamiton stock standard solution (1mg/ml) equivalent to (1-6 mg) were transffered to a set of 10-ml volumetric flasks and the volume was then completed to the mark with methanol. A 10 µL of each solution were applied to TLC plate using Camag Linomat auto sampler with micro syringe (100 µL). The plate was then developed by the ascending technique using toluene : petroleum ether : acetonitrile : dichloromethane (14:5:3:1, by volume) as a mobile phase. The plate was then removed and air-dried. The chromatogram was scanned at 250 nm. Calibration curve representing the relationship between integrated peak area and the corresponding concentrations in µg/spot of crotamiton per spot was plotted.

2.6.2. Application to pharmaceutical formulation

Transfer a portion of Eurax® Cream (10% crotamiton) equivalent to 50mg crotamiton to 50 ml volumetric flask. Add 25 ml of methanol, shake and sonicate to disperse the cream. Dilute with methanol to volume. Pass through moderately retentive filter paper to obtain a solution labeled to contain (1mg/ml) of crotamiton. Transfer 10 ml into 100-ml volumetric flask then complete to volume with methanol to obtain a solution labeled to contain (100 µg /ml) of crotamiton. The solution was analyzed using the procedures described previously.

3. RESULTS AND DISCUSSION

Forced degradation studies were carried out on crotamiton to evaluate its degradation behaviour by monitoring the chromatograms of crotamiton degradation products using the diode array detector to detect any degradation present in the samples over the entire scanning area and to check the purity of the eluted peaks. **Figure (2)** shows a typical chromatogram of crotamiton with retention time of 6.972±0.062 min. For more confirmation of the separation process the UV absorption curve of crotamiton have been recorded and it shows typical matching with the curve extracted from the DAD as shown in **figures (2,3)**.

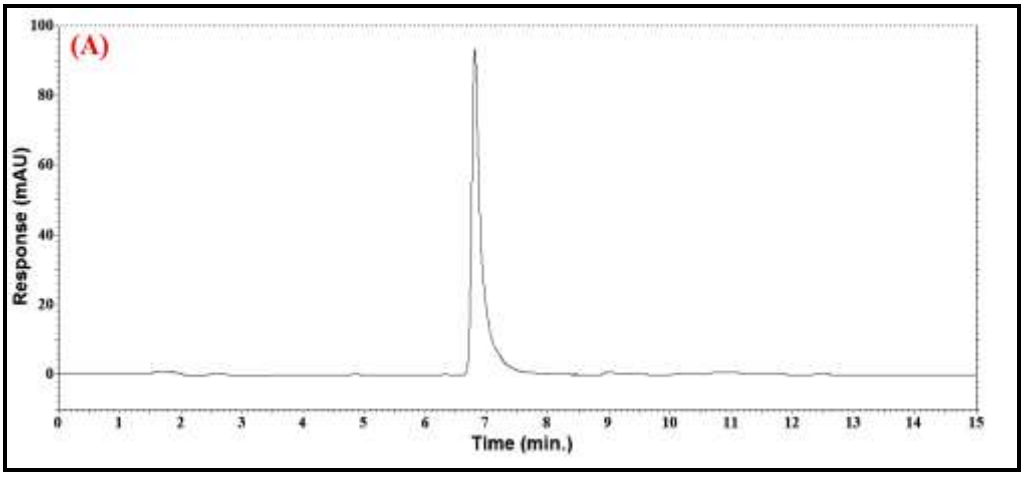


Figure (2): HPLC chromatograms of 3 µg/ml crotamiton (A) 2D chromatogram at 254 nm and (B) 3D chromatogram in scanning mode using DAD.

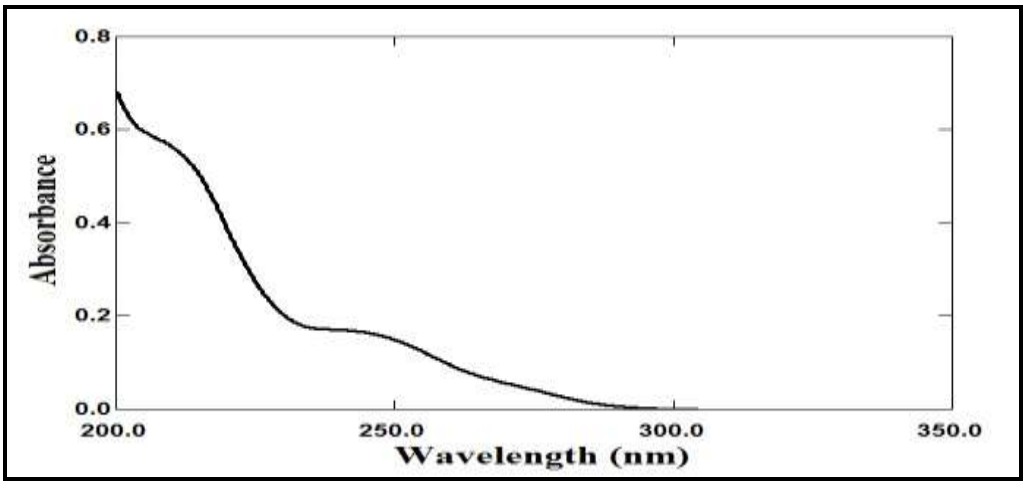
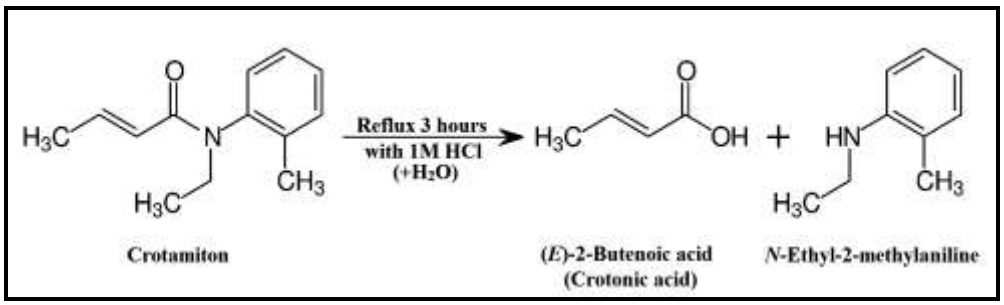


Figure (3): UV absorption spectrum of 3 µg/ml of crotamiton.

HPLC method revealed that crotamiton is sensitive to acidic, alkaline and oxidative degradation at room temperature, but the acidic hydrolysis gives the highest degradation percentage, **figures(4-6)**. No degradation products were found by neutral hydrolysis after using of 2ml deionized water at room temperature for 5 hours, but when the neutral hydrolysis was done using 2 ml deionized water for 3 hours at 80°C, the degradation product peak appeared, **figure(7)**. Thermal degradation was carried out for 3 hours at 80°C to give nearly the same results as hydrolytic degradation, **figure(8)**. Crotamiton was also found to be sensetive to photolytic degradation by exposing solutions of the drug to sunlight for 24 hours, **figure(9)**. The results of crotamiton stability studies are given in **table(1)**. It should be noted that acidic hydrolysis using 0.1 NHCl produce the highest degradation product and maximum reduction in peak area of intact crotamiton. Therefore, complete degradation of crotamiton was obtained after reflux the drug with 1N HCl for 3 hours to give 2-butenoic acid which precipitate in acidic medium and *N*-ethyl-2-methylaniline which is extracted in methanol as shown in the following scheme.



Proposed degradation pathway of intacat crotamiton.

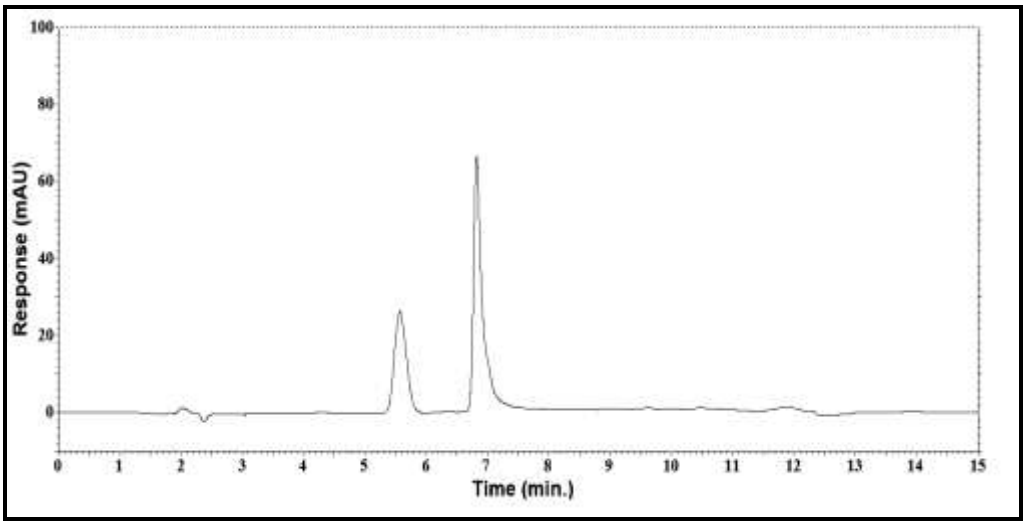


Figure (4): HPLC chromatogram of crotamiton (3 µg/ml) and its acid-induced degradation product using 0.1 N HCl for 2 hours at room temperature.

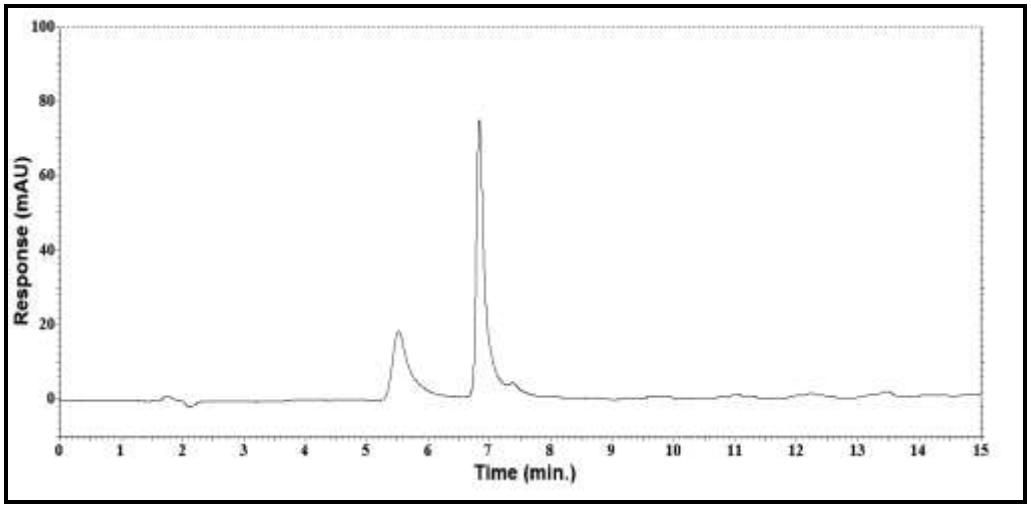


Figure (5): HPLC chromatogram of crotamiton (3 µg/ml) and its alkaline-induced degradation product using 0.1 N NaOH for 2 hours at room temperature.

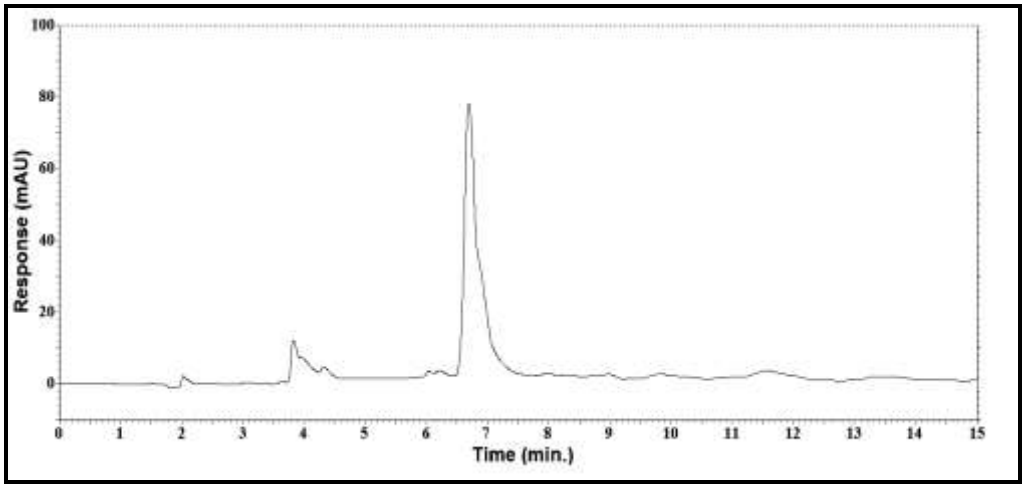


Figure (6): HPLC chromatogram of crotamiton (3 µg/ml) and its oxidative-induced degradation product using 6% H_2O_2 for 2 hours at room temperature.

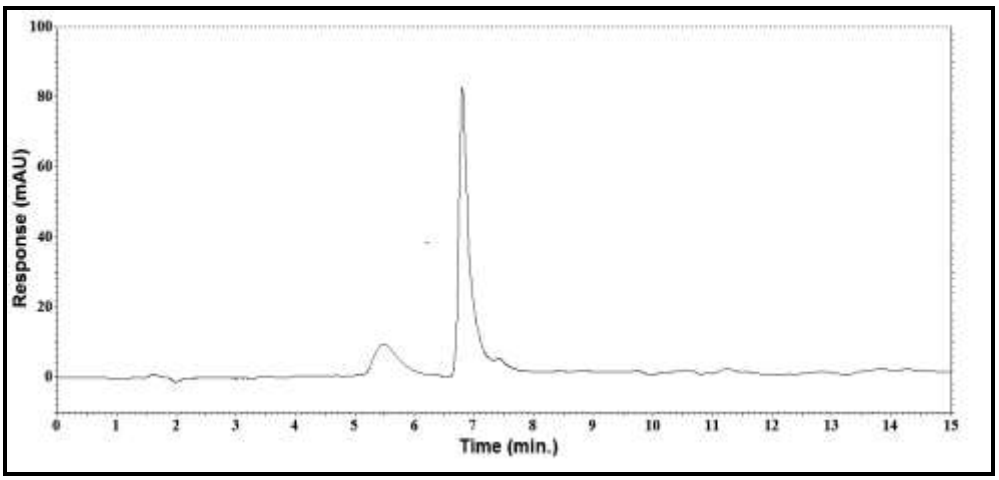


Figure (7): HPLC chromatogram of crotamiton (3 µg/ml) and its neutral hydrolytic degradation product using deionized water at 80 °C for 3 hours.

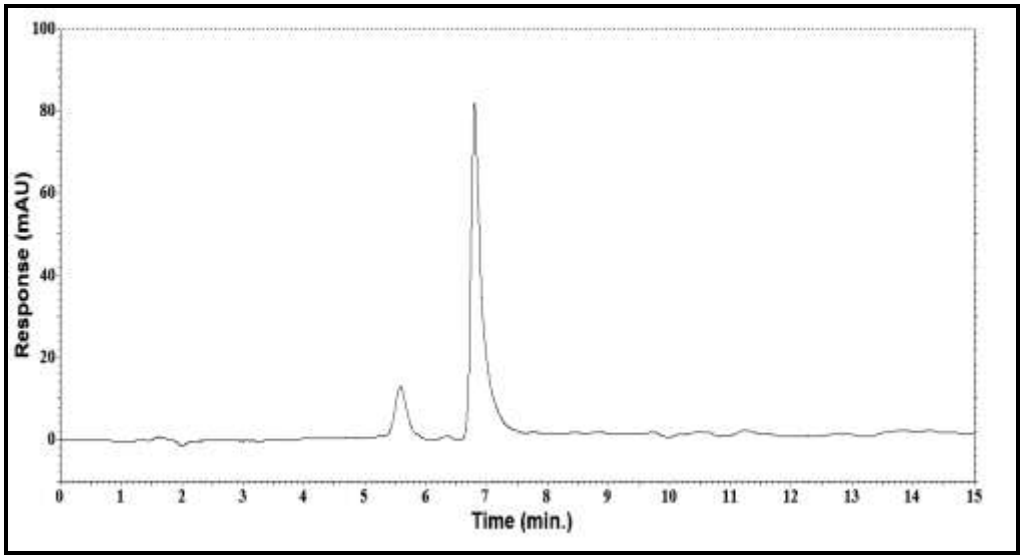


Figure (8): HPLC chromatogram of crotamiton (3 µg/ml) and its thermal degradation product at 80 °C for 3 hours.

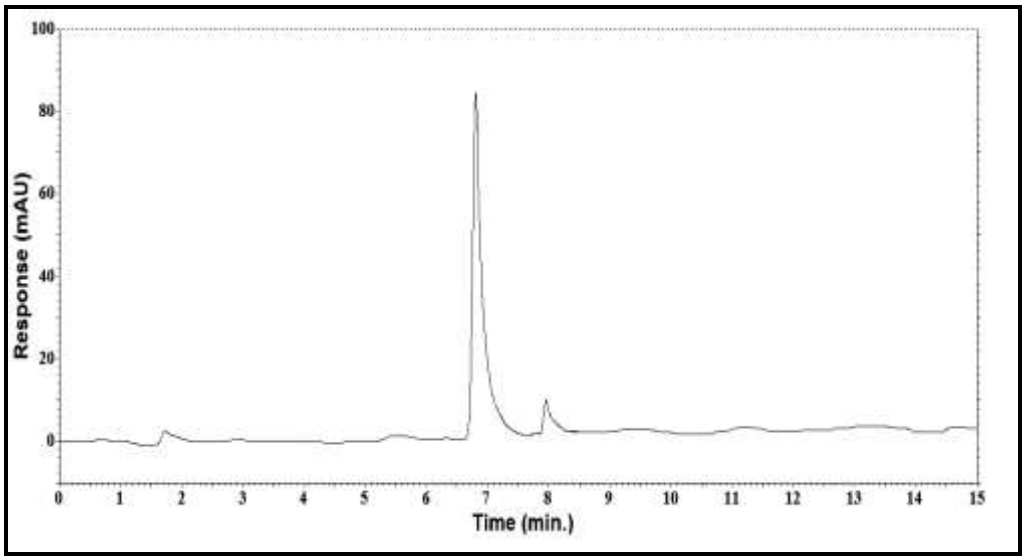


Figure (9): HPLC chromatogram of crotamiton (3 µg/ml) and its photolytic degradation product after exposing to sunlight for 24 hours.

Table (1): Summary of forced degradation studies.

Stress conditions	Number of degradates (t_R)	% Degradatin
0.1 N HCl at room temperature for 2 hours	1 (5.61)	28.91
0.1 N NaOH at room temperature for 2 hours	1 (5.49)	20.34
6% H_2O_2 at room temperature for 2 hours	1 (3.82)	14.67
Neutral hydrolytic degradation at room temperature for 5 hours	0	0
Neutral hydrolytic degradation at 80 °C for 3 hours	1 (5.47)	12.58
Thermal at 80 °C for 3 hours	1 (5.58)	12.49
Sunlight for 24 hours	1 (7.96)	11.53

3.1. Chromatographic conditions

HPLC method was applied to separate crotamiton and its degradation products, therefore several trials have been undertaken to reach the optimum stationary/mobile phases matching. Good chromatographic separation could be achieved by using Discovery® HS C_{18} column (250 mm x 4.6 mm, 5µm particle size) using mobile phase consists of acetonitrile : water : methanol (50:30:20, by volume) at flow rate 1.0 ml/min. The eluents were monitored by the diode array detector (DAD) from 200 to 350 nm and peak area values were measured at 254 nm. All determinations were performed at ambient temperature.

While, TLC densitometric method was applied to separate crotamiton and its acidic-induced degradation products and results were satisfactory when using toluene : petrolium ether : acetonitrile : dichloroethane (14:5:3:1, by volume) as a developing system and UV detection at 250 nm. R_f values were found to be 0.34 and 0.49 for crotamiton and its degradation product, respectively as shown in **figures(10-13)**. This separation allows the determination of crotamiton at 250 nm without any interference from its degradation product.

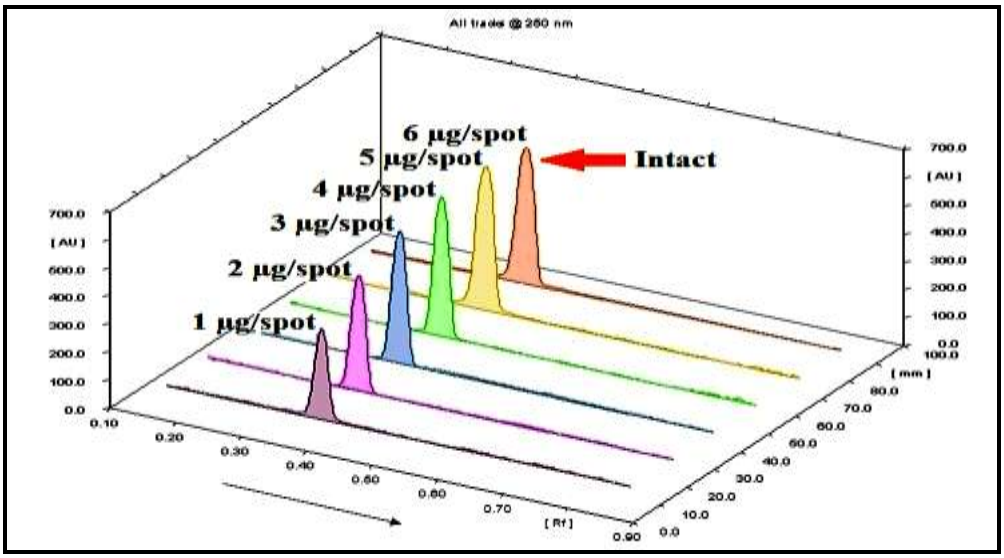


Figure (10): 3D densitometric chromatogram of crotamiton (1 – 6 µg/spot) at 250 nm.

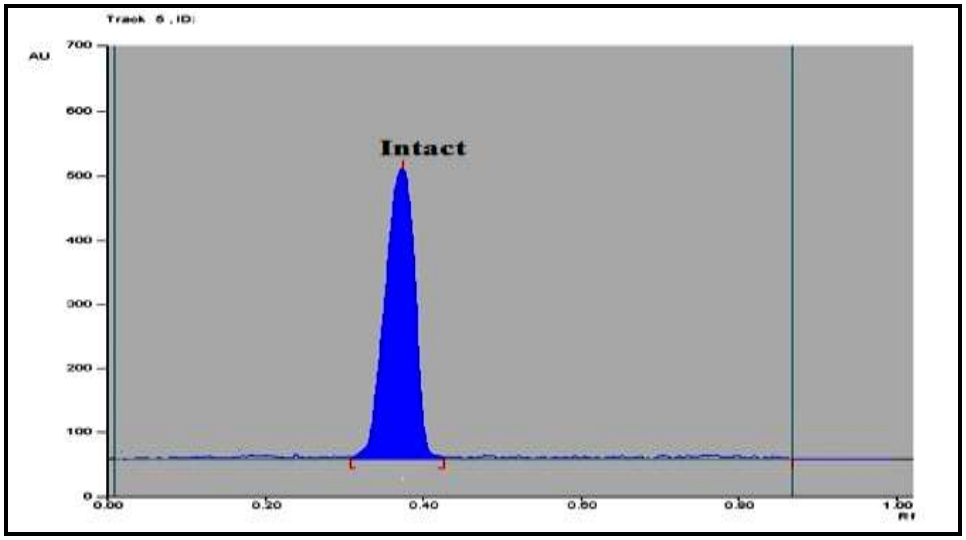


Figure (11): 2D densitometric chromatogram of crotamiton (5 µg/spot) at 250 nm.

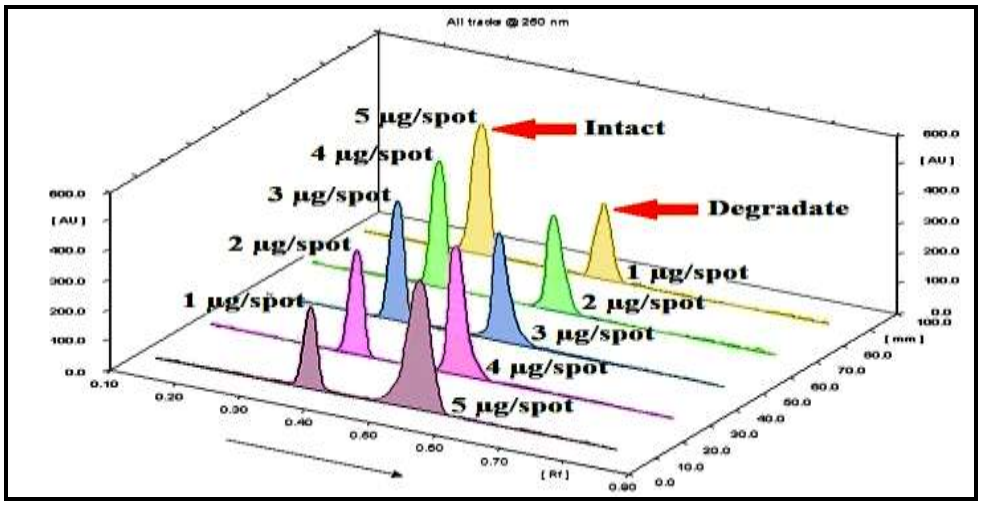


Figure (12): 3D densitometric chromatogram of crotamiton (1 – 5 µg/spot) and its acid-induced degradation product (5 – 1 µg/spot) at 250 nm.

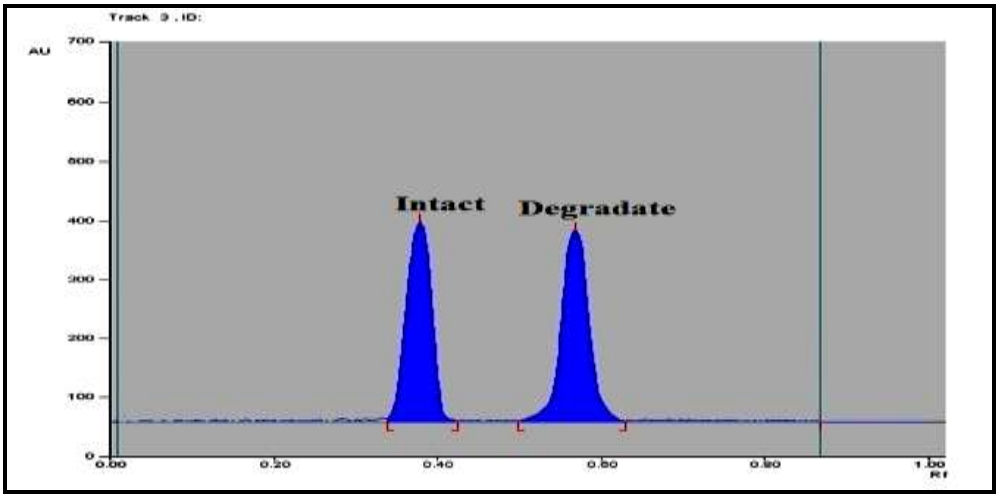


Figure (13): 2D densitometric chromatogram of crotamiton (3 µg/spot) and its acid-induced degradation product (3 µg/spot) at 250 nm.

3.2. Confirmation of complete acidic degradation

3.2.1. Confirmation of degradation product using TLC technique

Time required for complete degradation was exactly determined by spotting on TLC plates every 30 minutes using mobile phase system consists of toluene : petrolium ether : acetonitrile : dichloroethan (14:5:3:1, by volume), complete degradation of crotamiton was confirmed by absence of spot in the region of the degradation product corresponds to the spot of the intact drug.

3.2.2. Confirmation of degradation product using IR spectroscopy

IR spectrum of the intact crotamiton in **figure (14)**, showed peak of carbonyl group of amide bond (C=O) at 1668.12 cm^{-1}, while IR spectrum of degradation product in **figure(15)**, showed disappearance of (C=O) stretch of amide which indicate the cleavage of amide linkage.

3.2.3. Confirmation of degradation product using ^{1}H NMR spectroscopy

The ^{1}H NMR of the intact crotamiton in dimethyl sulfoxide (DMSO) in **figure (16)**, showed triplet signal of three protons of aliphatic ($-CH_3$) in ethyl group attached to nitrogen atom at 1.258-1.293 ppm, doublet signal of three protons of aliphatic ($-CH_3$) attached to ethylene group at 1.975 - 1.998 ppm, singlet signal of three protons of aromatic ($-CH_3$) attached to benzen ring at 2.239 ppm, multiplet signal of two protons of ($-CH_2-$) in ethyl group attached to nitrogen atom at 4.227 - 4.280 ppm, multiplet signals of two protons of (-CH=CH-) in ethylene group at 6.251 - 6.607 ppm and multiplet signals of four aromatic protons at 7.045-7.567 ppm.

The ^{1}H NMR of the degradate in dimethyl sulfoxide (DMSO) in **figure (17)**, showed appearance of (-NH-) secondary amino group singlet signal at 4.352 ppm indicating the cleavage of amide linkage with formation of amino group.

3.2.4. Confirmation of degradation product using mass spectrometry

Mass spectrometry was performed for the intact drug and its degradation product and molecular ion peak was obtained at m/z = 204.13 and m/z = 136.11, respectively indicating that the molecular weight of the degradation product is 136.11 as shown in **figures (18,19)**.

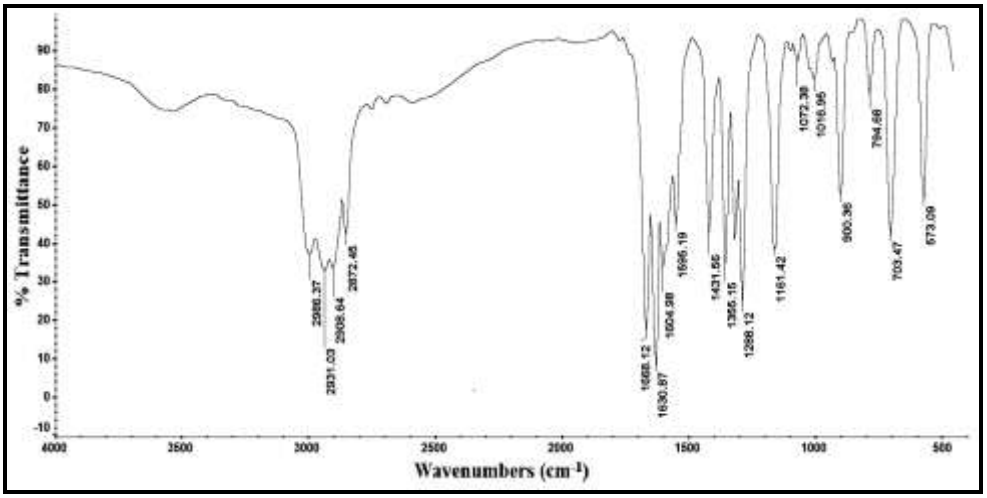


Figure (14): IR spectrum of intact crotamiton.

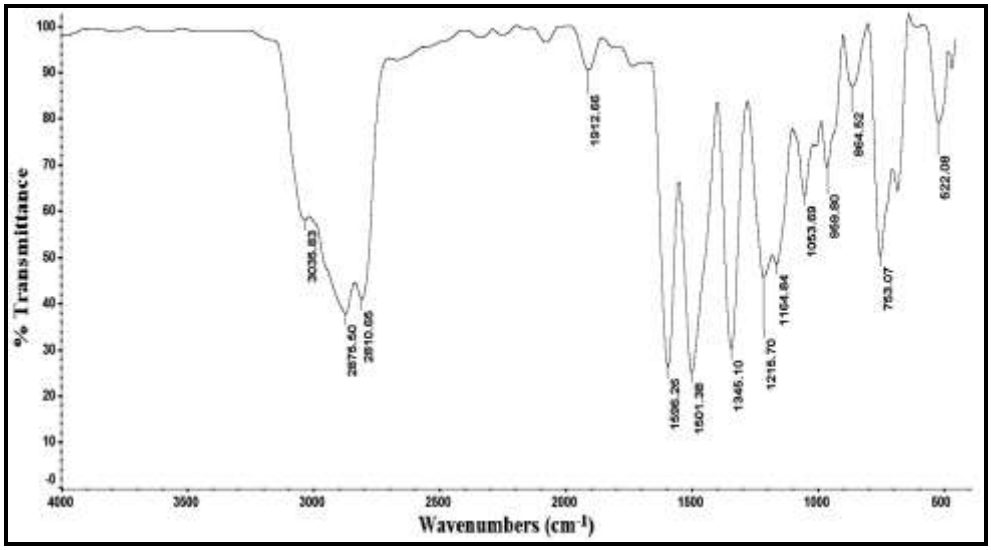


Figure (15): IR spectrum of crotamiton degradation product.

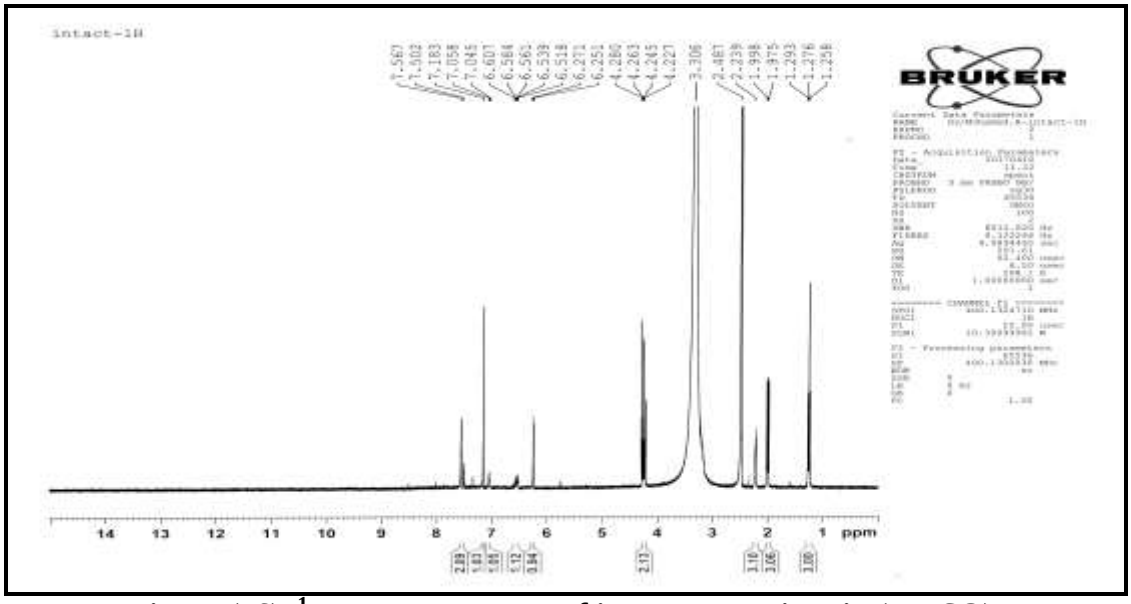


Figure (16): ^{1}H NMR spectrum of intact crotamiton in (DMSO).

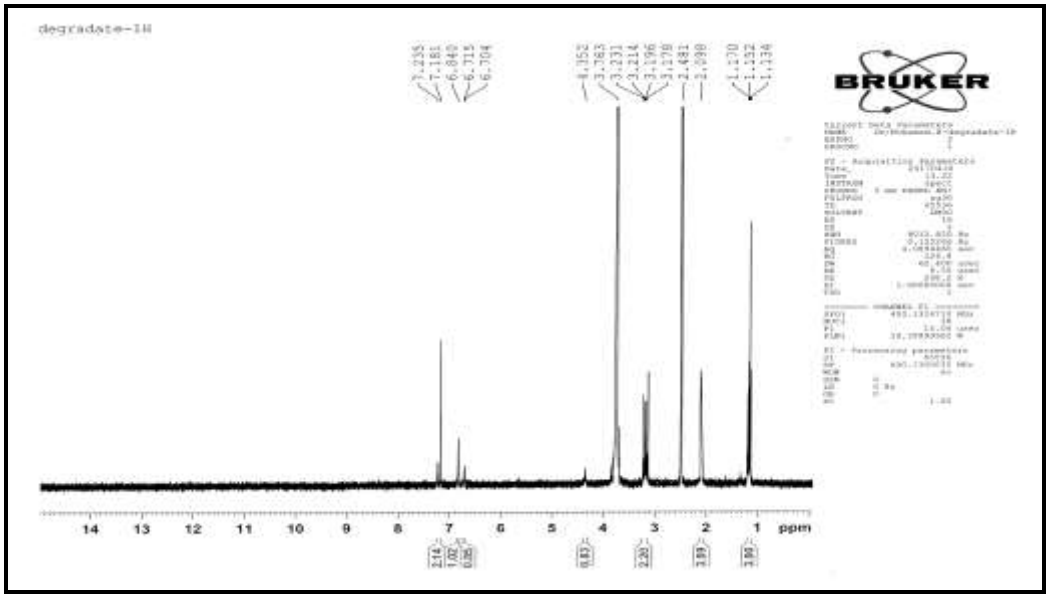


Figure (17): ^{1}H NMR spectrum of crotamiton degradation product in (DMSO).

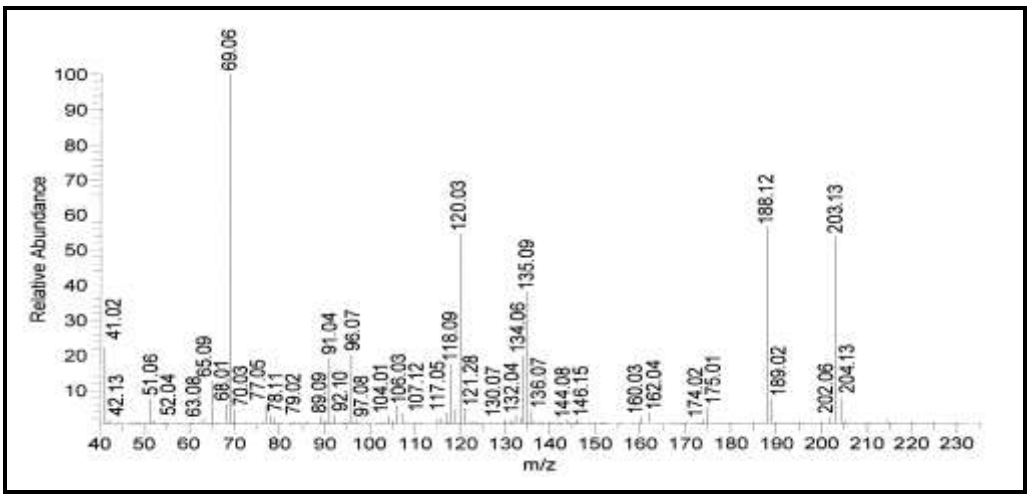


Figure (18): Mass spectrum of intact crotamiton.

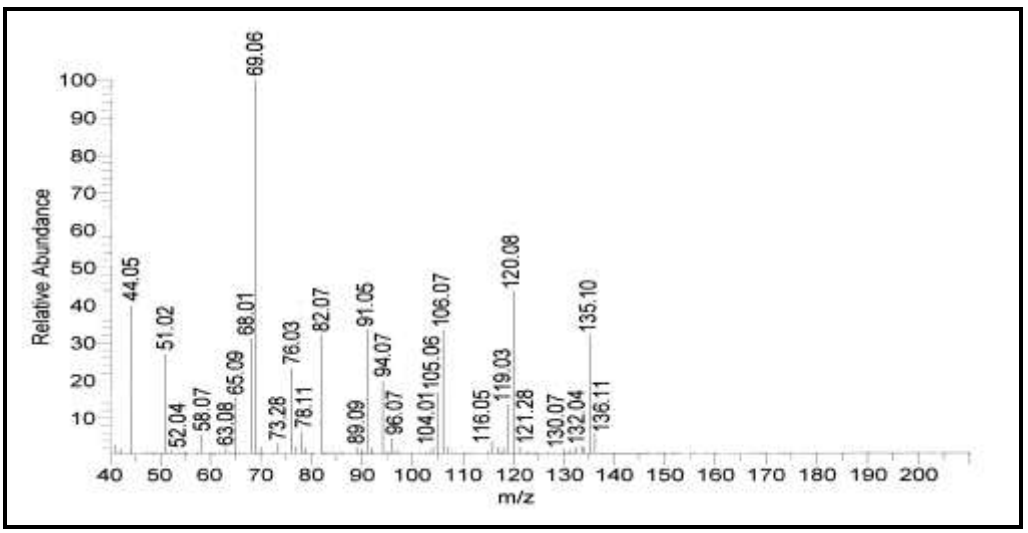


Figure (19): Mass spectrum of crotamiton degradation product.

3.3. Methods validation

Validations of the proposed methods were assessed as per the ICH guidelines.[10]

- **Linearity and range**

Calibration graphs were constructed by plotting the area under peak versus drug concentrations. The regression plots were found to be linear over the range of (1-10 µg/ml) for RP-HPLC method and over the range of (1-6 µg/spot) for TLC-densitometric method. The linear regression equations for the graphs were:

***y* = 578796.98 *x* + 43056.10** …… **(*r* = 0.9997), for RP-HPLC method.**

***y* = 3454.21 *x* + 468.72** …… **(*r* = 0.9995), for TLC-densitometric method.**

Where ***y*** is the area under peak values, ***x*** is the drug concentration and ***r*** is the correlation coefficient. Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **table(2)**.

- **Limits of detection and quantitation**

The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

$LOD = 3.3\ \sigma / S$ $LOQ = 10\ \sigma / S$

Where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve. LOD and LOQ values were mentioned in **table(2)**.

- **Accuracy and precision**

Accuracy and precision of the methods were determined by applying the proposed procedure for determination of three different concentrations, each in triplicate, of crotamiton in pure form within linearity range in the same day (intraday) and in three successive days (interday). Accuracy as percent recovery (%R) and precision as percent relative standard deviation (%RSD) were calculated and results are listed in **table(2)**. To ascertain the accuracy of the suggested methods, recovery studies were carried out by standard addition technique at four different levels in **table(3)**.

- **Specificity**

The specificity of the stability-indicating HPLC DAD method was assessed by resolving crotamiton from its possible degradation products. The results revealed that the proposed

method was able to completely discriminate crotamiton from all of its degradation products, confirming the specificity of the method. Moreover, the peak purity was checked using DAD and the purity of crotamiton was found to be more than 0.992 indicating that no additional peaks were co-eluted with the main compound.

The specificity of the TLC- densitometric method was assured by applying it to laboratory prepared mixtures of the intact crotamiton together with its acidic-induced degradation product. The proposed procedure was adopted for the selective determination of intact crotamiton in presence its degradation product.

- **Robustness**

The robustness of the methods was evaluated by slight changes in the chromatographic parameters such as flow rate (±0.1 mL/min.), the working wavelengths (± 2 nm) and mobile phase contents ratio (±2%). In each case only one parameter was changed while other parameters were kept constant. These minor changes did not have any significant effect on the the peak area or separation of crotamiton from its degradation products and % RSD of the responses were < 2 % confirming robustness of the procedures, as shown in **table(2)**.

- **System suitability**

System suitability was checked by calculating different parameters including resolution, tailing factor, capacity factor and number of theoretical plates. The obtained values were found to be in the acceptable ranges when compared to USP reference values as shown in **table(4)**.

Table (2): Regression and validation data for the determination of crotamiton by the proposed methods.

Parameters	**HPLC method**	**TLC densitometric method**
Wavelength (nm)	254	250
Linearity range	1 ─ 10 (µg/ml)	1 ─ 6 (µg/spot)
LOD	0.182 (µg/ml)	0.202 (µg/spot)
LOQ	0.552 (µg/ml)	0.612 (µg/spot)
- Regression Equation - Slope (b) - Intercept (a)	$y^{a} = b\,x^{b} + a$ 578796.98 43056.10	$y^{a} = b\,x^{b} + a$ 3454.21 468.73
Correlation coefficient (r)	0.9997	0.9995
Accuracy (% R)	100.43	99.68
Precision (% RSD) Repeatability[c] Intermediate precision[d]	 1.216 1.455	 1.225 1.039

Robustness (% RSD)		
- Mobile phase contents ratio (±2%)	0.812	1.386
- Detection wavelengths (±2 nm)	1.349	1.526
- Flow rate (±0.1 ml/min.)	1.778	—

a Peak area of crotamiton.

b Concentration in µg/ml for HPLC method and in µg/spot for TLC densitometric method.

c The intraday (n = 3), average of three concentrations of crotamiton (2, 6 and 8 µg/ml) for HPLC method and (3, 4 and 5 µg/spot) for TLC densitometric method repeated three times within the day.

d The interday (n = 3), average of three concentrations of crotamiton (2, 6 and 8 µg/ml) for HPLC method and (3, 4 and 5 µg/spot) for TLC densitometric method repeated three times in three days.

Table (3): Recovery study of crotamiton by adopting standard addition technique using the proposed methods.

Pharmaceutical taken (µg/ml)	HPLC method			TLC densitometric method		
	Pure added (µg/ml)	Pure found (µg/ml)	% Recovery	Pure added (µg/spot)	Pure found (µg/spot)	% Recovery
2	1	0.98	98.26	1	1.02	101.72
	3	2.95	98.45	2	2.01	100.39
	5	5.07	101.39	3	2.96	98.51
	7	7.06	100.87	4	4.03	100.63
Mean			99.74			100.31
% RSD			1.624			1.329

Table (4): System suitability parameters for determination of crotamiton by the proposed methods.

parameters	Stability HPLC method	TLC densitometric method	Reference value
Resolution (R)	Acidic hydrolysis (2.83) Basic hydrolysis (2.47) Oxidative hydrolysis (3.69) Neutral hydrolysis (2.15) Thermal degradation (2.28) Photodegradation (2.11)	2.08	**R > 2**
Tailing factor (T)	1.73	0.96	**T < 2**
Capacity factor (K)	1.56	1.94	**1–10 acceptable**
Theoretical Plates (N)	2248	2117	**N > 2000**

3.4. Application for pharmaceutical preparation

The proposed methods were applied to the determination of crotamiton in Eurax® cream. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared tothose obtained by the reported method[2] indicating good accuracy and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **table(5)**. No significant differences were found by applying student's *t*-test and F-test at 95 % confidence level.

Table (5): Determination of crotamiton in Eurax® cream by the proposed methods and the reported method.

Parameters	Stability HPLC method	TLC densitometric method	Reported method* [2]
Number of measurements	5	5	5
Mean % recovery of crotamiton	100.28	99.79	99.43
% RSD	0.910	1.085	1.380
Student's *t*-test**	1.153 (2.306)	0.457 (2.306)	——
F-value**	2.260 (6.388)	1.606 (6.388)	——

* Reference method is HPLC using C18 column, mobile phase was acetonitrile and water (3:2, v/v) , flow rate (1 ml/min) and UV-detection at 254 nm.

** The values in parenthesis are tabulated values of "*t* " and "*F* " at (P = 0.05).

CONCLUSION

In this work, two chromatographic methods were developed and applied for the determination of crotamiton. The first method was stability indicating HPLC method for the determination of crotamiton in the presence of its possible degradation products and to evaluate its degradation behaviour in different stress conditions. The second method was TLC densitometric method for the determination of crotamiton in the presence of its acid-induced degradation product. The proposed methods are simple, accurate and precise and can be used for the analysis of crotamiton in pure form and in pharmaceutical dosage form.

REFERENCES

1. Sweetman S. Martindale: the complete drug reference. 36th ed. London: The Pharmaceutical Press; 2009.
2. The United States Pharmacopeia, National Formulary 35, United States Pharmacopeia Convention Inc., 30th edn, 2012.

3. Izumoto SI, Machida Y, Nishi H, Nakamura K, Nakai H, Sato T. Chromatography of crotamiton and its application to the determination of active ingredients in ointments. J Pharmaceut Biomed. 1997; 15(9-10): 1457-1466.
4. Jie S, An-dong C, Li-chun Z. Study and improvement of the control method for compound diphenhydramine and crotamiton rincture. Chinese J Appl Mon. 2006;05.
5. Sioufi A, Sandrenan N, Dubois JP. Determination of crotamiton in plasma and urine by high-performance liquid chromatography. J Chromatogr B. 1989; 494: 361-367.
6. Zhang CY, Huang HW, Wu JM, Yao J, Hu CQ. Content determination method of crotamiton. cpj. 2015; 50: 1721-1725.
7. Qun X, Xiongfeng H, Rohrer J. Separation of cis and trans isomers of crotamiton using an Acclaim C30 column. Thermo Fisher Scientific. 2016.
8. Babu TR, Sivasankar K, Sujana P. Polarographic behavior of crotamiton and determination of its residues in water samples. Anal Chem Ind J. 2012; 11(4): 146-148.
9. Fukahori S, Fujiwara T, Ito R, Funamizu N. Photocatalytic decomposition of crotamiton over aqueous TiO_2 suspensions:Determination of intermediates and the reaction pathway. Chemosphere. 2012; 89: 213-220.
10. International Conference on Harmonization, ICH Harmonised Tripartite Guideline. Validation of analytical procedure: text and methodology, Q2 (R1). Geneva: International Conference on Harm-onization; 2005.