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Original Research Article

ISOLATION AND CHARACTERISATION OF DEGRADATION PRODUCT OF NORTRIPTILINE

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ABSTRACT

A simple, accurate, precise, sensitive& economic stability indicating HPTLC method was developed and validated for nortriptyline. The method was validated as per ICH guidelines, demonstrating to be accurate and precise with in the corresponding linearity range of titled analytes. Inherent stability of the drug was studied by exposing drugs to various stress conditions as per ICH guidelines namely oxidative, photolysis, acidic, basic and thermal. Chromatographic separation was performed on aluminium plate percoated with silica gel using toluene: ethyl acetate: methanol: glacial acetic acid(4:4:1.6:0.4) as mobile phase. The wavelength selected for scanning was 279nm. Regression plots revealed linear relationship in the conc. Range of 200 to 600 ng/band for CAN and HCT respectively. The correlation coefficient curves was found to be 0.998 for analytes. The proposed method can be used for the routine estimation of nortriptyline in bulk and can be employed for stability indicating analysis.

KEYWORDS

Nortryptiline, HPTLC. Validation, ICH guidelines

INTRODUCTION

An impure substance may be defined as any material that affects the purity of the material of interest, viz., an active pharmaceutical ingredient (API) or drug substance. Impurity control in pharmaceutical products is a primary goal of drug development.[1] According to ICH guidelines,1 impurities associated with API's are classified into the following categories:

a. Organic impurities (Process andDrug-related)

b.Inorganic impurities

c. Residual solvents

a. ORGANIC IMPURITIES: Organic impurities may arise during the manufacturing process and/or storage of the drug substance. They may be identified or unidentified, volatile or non-volatile, and these include the starting material, intermediates, degradation products, by-products and reagents, ligands and catalyst used at different stages of synthesis of API and drug products.

b. INORGANIC IMPURITIES: Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

a. Reagents, ligands and catalysts
b. Heavy Metals or other residual metals
c. Inorganic salts
d. Other materials (filter aids, charcoal)

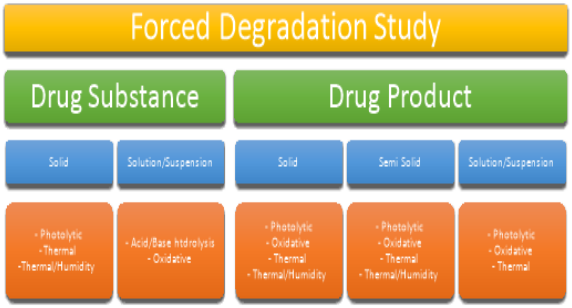
c. RESIDUAL SOLVENTS: Residual solvents are organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products.[2]

Table No. 1: ICH Guidelines on Impurities in an Active Pharmaceutical Ingredient [3-7]

ICH Code	Guideline Title
Q1A	Stability testing of new drug substances and products
Q1B	Stability testing: Photo stability testing of new drug substances and products
Q1C	Stability testing of new dosage froms
Q1D	Bracketing and matrixing designs for stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration application in climatic zones 3 and 4
Q5C	Stability testing of biotechnological/Biological product

FORCED DEGRADATION is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule.

Fig No. 1: Force degradation Studies



OBJECTIVE OF FORCED DEGRADATION STUDIES

a. To develop degradation pathways of drug substances and drug products.

b. To recognize the chemical properties of drug molecules.

c. Identified new stresses, Manufacturing changes, Additional indications

d. To elucidate the structure of degradation products.

e. To resolve stability-related problems

f. To establish the intrinsic stability of a drug substance in the formulation.

g. To reveal the degradation mechanisms of the drug substance and drug product.

h. To distinguish degradation products that is related to drug products from those that are generated from non-drug product in a formulation.

i. To generate stability indicating nature of a developed method.

j. To produce more stable formulations. It also helps in determining the expiry date of a particular formulation.[8]

k. To generate a degradation profile similar to that of what would be observed in a formal stability study under ICH condition.

DEGRADATION CONDITIONS [9]

1. Hydrolytic condition

   Acidic [HCL]

   Basic [NaOH]

2. Oxidative condition
3. Thermal condition
4. Photolytic condition

Materials and methods

- Procurement of drug sample

Table No.2: pure drug procurement

Sr. No.	Drug Sample	Supplier
1	Nortriptyline	INTAS Pharmaceuticals Limited (valia, Gujarat)

- List of Chemicals/Reagents:

Table No.3: Details of Chemicals/Reagents used for research work

Name of Chemical	Reagent Type	Mfg. By
Acetonitrile	HPLC grade	Merck Chemical Ltd.
Methanol		
Water		
Glacial Acetic Acid Toluene Ethyl acetate	-	
Potassium Dihydrogen Phosphate	-	
Sodium Hydroxide	AR grade	Loba Chemicals
Conc. Hydrochloric acid, H_2O_2		

INSTRUMENTS

UV-Vis Spectrophotometer

- Model: Jasco-630 double beam UV –Vis spectrophotometer
- Wavelength range: 200 - 400 nm
- Light sources:
    - ✓ Deuterium lamp
- Detector: Silicon photo-diode (S1337)

HPTLC

High Performance Thin Layer Chromatography (HPTLC)

- Sample applicator: CAMAG Linomat 5
- HPTLC Plate: Silica gel 60F 254 (E. Merck, Germany)
- Development chamber: Twin trough glass chamber, (20×10cm CAMAG)
- Densitometric scanner: CAMAG TLC Scanner IV
- Detector: UV
- Software: Wincats

UV-VISIBLE SPECTROPHOTOMETER [10]

PREPARATION OF STOCK SOLUTION: Accurately weighed quantity of Nortriptyline 10mg was transferred to 100 ml volumetric flask and volume made up to the mark with distilled water to give a stock solution having strength of 100µg/ml.

PREPARATION OF STANDARD SOLUTION: From the stock solution of 100µg/ml pipette out 10 ml in 100 ml volumetric flask and volume made up to the mark with a distilled water to give a standard solution of 10µg/ml. absorbance was taken

Fig No.2: UV. Spectra of Nortriptyline at various concentration 5 □g/ml to 25 □g/ml

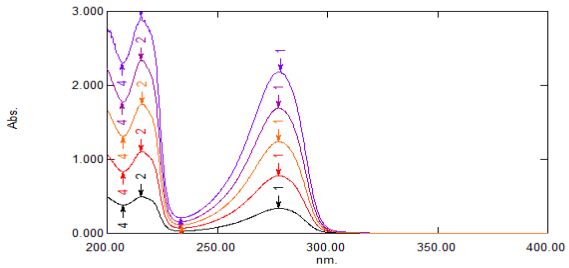
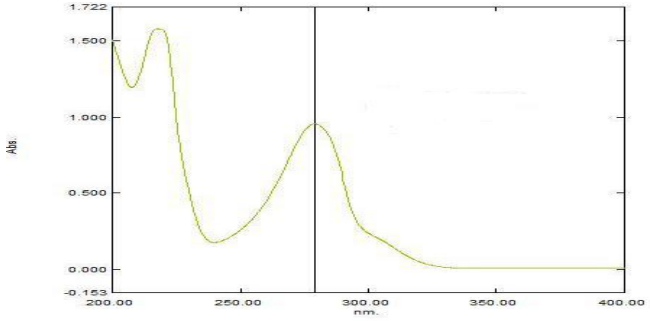


Fig No.3: UV. Spectra of pure drug of nortriptyline



PREPARATION OF CALIBRATION CURVE [11]

Calibration curve for Nortriptyline consist of different concentration of standard solution ranging from 5-25 µg/ml. the solution were prepared by pipetting out 0.5,1,1.5,2 and 2.5 standard solution of Nortriptyline in 10 ml volumetric flask and the volume was adjusted to mark with distilled water. Absorbance of each solution was measured at 279nm against distilled water as a blank and calibration curve was plotted.

Table No.4: Absorbance at 279 nm

Sr. no.	Concentration(ppm)	Absorbance (nm)
1	5	0.167
2	10	0.375
3	15	0.531
4	20	0.735
5	25	0.983

Fig No.4: Standard calibration curve of Nortriptyline

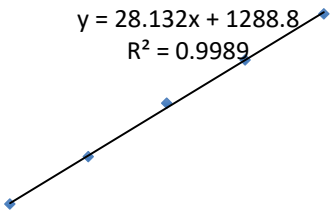


Table No.5: Optical Characteristics of nortriptyline

Sr. No	Parameter	Results
1.	Beer's law limit(µg/ml)	5-25µg/ml
2.	Correlation coefficient	0.994
3.	Regression equation(Y*)	0.0923x - 0.1372
4.	Slope(a)	0. 0923
5.	Intercept(b)	0. 1372

STABILITY OF SOLVENTS The stability of for Nortriptyline was found to be in the 25µg/ml at various time. linearity data for Nortriptyline at 279 nm

Table No.6: Absorance taken at various time interval with fixed concentration

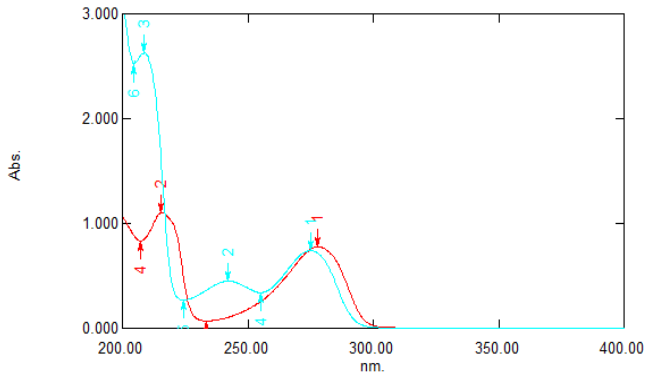
Sr. no.	Concentration (ppm)	Time (min)	Absorbance (at 279 nm)
1.	25	0	0.983
2.	25	30	0.982
3.	25	60	0.984
4.	25	90	0.983
5.	25	120	0.983

ACID HYDROLYSIS

Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 0.1N/1N HCl up to the mark. And it refluxed for 3hr at 80^0c. The degradation sample was cooled at room temperature and neutralized the sample with same strength of 0.1N NaOH. Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water.

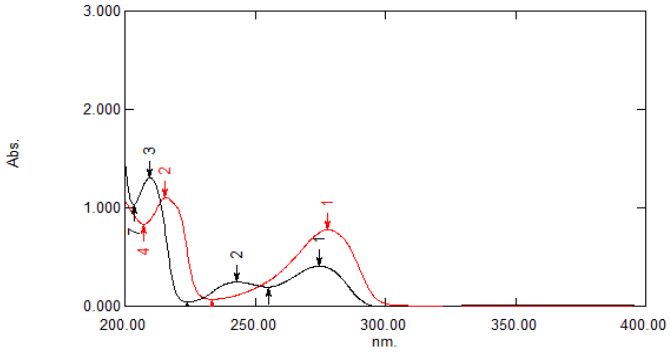
ACIDIC DEGRADATION WITH 0.1 N HCL

Fig No.5: UV. Spectra of acidic degradation in 0.1 N Hcl



DEGRADATION WITH 1 N HCL

Fig No.6: UV. Spectra of acidic degradation in 1 N HCl

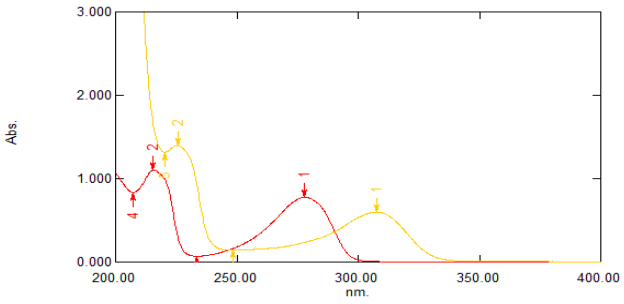


BASIC HYDROLYSIS

Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 0.1/1N NaOH up to the mark. And it refluxed for 3hr at 80^0c. The degradation sample was cooled at room temperature and neutralized the sample with same strength of 0.1N HCL. Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water.

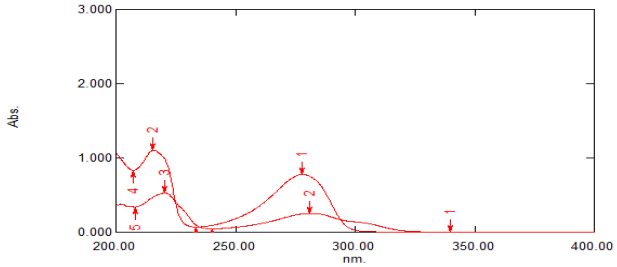
DEGRADATION WITH 0.1 N NAOH

Fig No.7: UV. Spectra of alkali degradation in 0.1N NaOH solution



DEGRADATION WITH 1 N NAOH

Fig No.8: UV. Spectra of alkali degradation 1 N NaOH solution

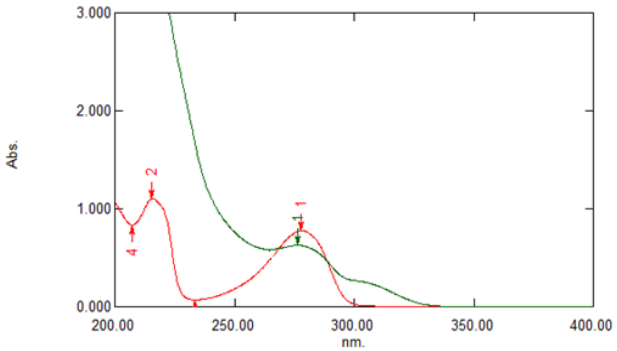


OXIDATIVE CONDITION

Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 3/6 % H_2O_2 up to the mark. And it refluxed for 8hr at 80 ^{O}c. The degradation sample was cooled at room temperature.

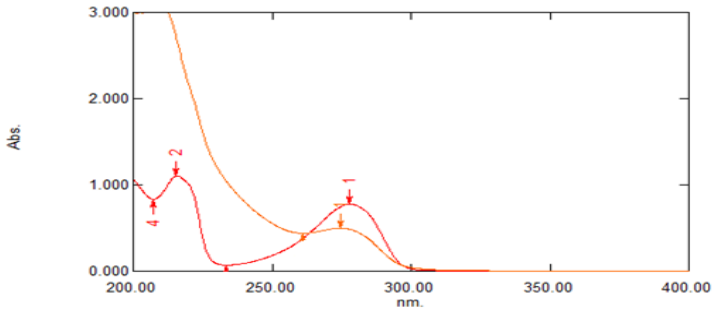
Degradation with 3% H_2O_2

Fig No.9: UV. Spectra of oxidation degradation 3% H_2O_2



Degradation with 6% H_2O_2

Fig No.10: UV. Spectra of oxidation degradation6% H2O2

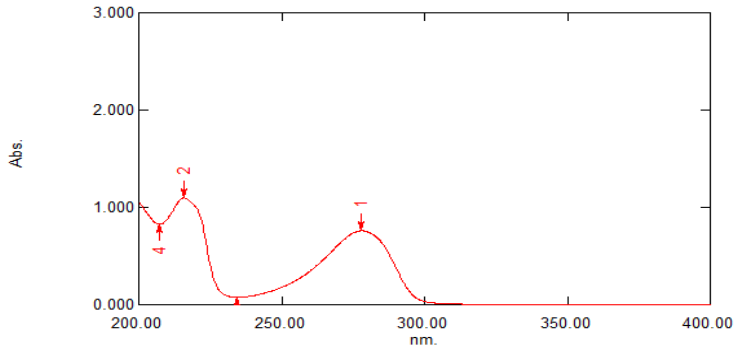


Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water.

THERMAL CONDITION

For dry heat degradation the sample were placed in the oven at 80^{O}c for 24hrs under the dark condition and then cooled at room temperature. Degradation sample were subjected to analysis after its dilution with the distilled water.

Fig No.11: UV. Spectra of Thermal degradation



PHOTOLYTIC CONDITION

For photolytic degradation the sample were placed in the sunlight for 24hrs. Degradation sample were subjected to analysis after its dilution with distilled water.

Fig No.12: UV. Spectra of Photolytic degradation

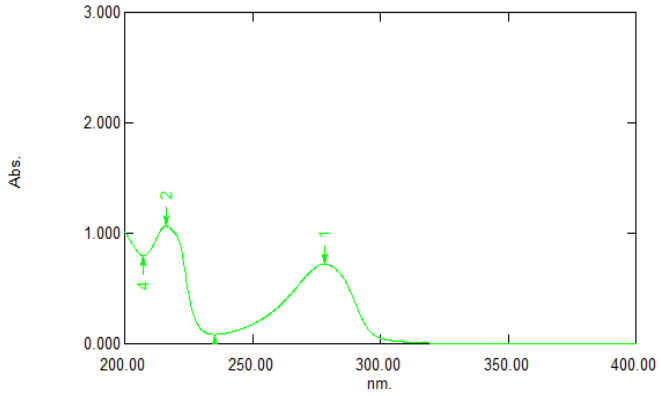


Table No.7: Results of stability of the drug under stress condition

Sr no	Parameter	Absorbance at 279nm	Percentage drug estimated	Percentage Degradation
1.	Normal	0.978	100	00
2.	0.1N HCl	0.740	95.66	34.00
3.	1N HCl	0.473	48.36	51.64
4.	0.1N NaOH	0.600	61.54	38.66

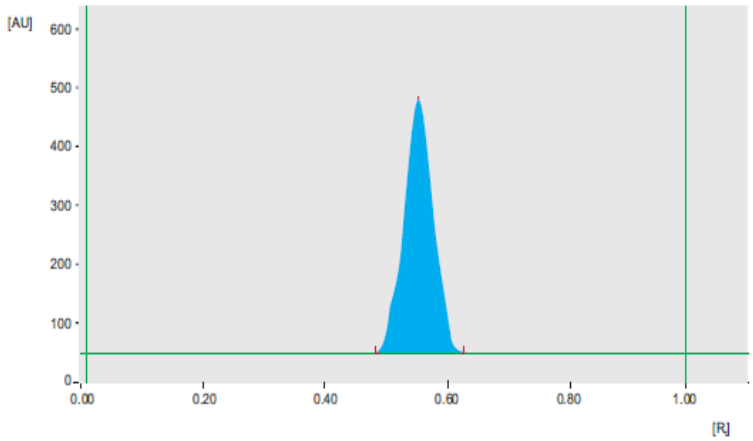
5.	1N NaOH	0.452	46.01	53.99
6.	3% H2O2	0.705	72.08	27.92
7.	6% H2O2	0.498	50.92	49.08
8.	Thermal	0.964	98.56	1.44
9.	Photolytic	0.922	94.27	5.73

HPTLC STUDY [12]

Table. No.8: Linear regression data for the calibration curve

Sr no	Parameters	Value
1.	Detection Wavelength (nm)	279
2.	Beer's Law Limit (ng/band)	200-600
3.	Correlation Coefficient (r2 ± SD)	0.9965 ± 0.00083
4.	Intercept (c) ± SD	0.9965
5.	Confidence limit of intercept	1385.5-1804.1
6.	Slope (m) ± SD	0.00083
7.	Confidence limit of slope	7.901-8.771
8.	SD of residuals from line	58.608

Fig No.13: Densitogram of nortriptyline, Wavelength: 279 nm, Mobile phase: toluene: ethyl acetate: methanol: glacial acetic acid (4:4:1.6:0.4, v/v/v/v) (Rf value is 0.54)



FORCED DEGRADATION STUDIES [13]

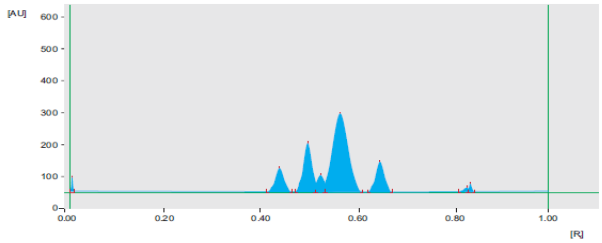
A stock solution containing 10 mg/ml nortriptyline was prepared. This solution was used for stress degradation to indicate specificity of the proposed analytical method and its stability indicating property. In all the degradation studies the average peak area of three replicates was obtained.

ACID AND BASE INDUCED DEGRADATION STUDIES

Degradation studies were carried out by refluxing 5ml of drug solution with 5ml each of 1N HCl and 1N NaOH. The mixtures were refluxed for 3h respectively at 80^0C. The solutions (0.5ml) were taken and neutralized and subsequently diluted to 10 ml with Neutralizing solvent (HCl and NaOH). The resulting solutions were applied to TLC plate in such a way that the final concentration attained was 5000 ng/spot for both acid and base degradation products and the chromatograms were run as described in previous section.

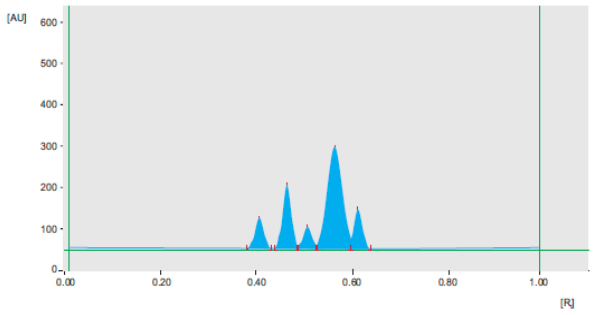
The rate of degradation in acid was slower as compared with alkali. The chromatogram of the acid degraded sample showed peak at Rf value of 0.42, 0.49, 0.53, 0.55, 0.62, 0.82 was observed in acid degradation peak are shown in Fig.

Fig No.14 Densitogram of nortriptaline bulk drug after acidic degradation of 1N HCl



For base degradation study different peak value observed at various Rf values of 0.40, 0.45, 0.49, 0.58, 0.62 showen in fig. The results indicate that nortriptyline undergoes degradation under acidic and basic conditions.

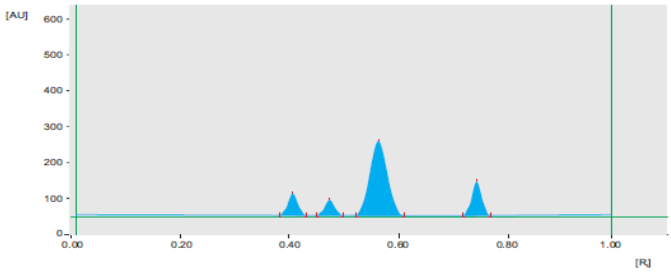
Fig No.15: Densitogram of bulk drug after a base degradation of Nortriptyline



HYDROGEN PEROXIDE INDUCED DEGRADATION PRODUCT [14]

The sample showed an additional peak with 6 % (w/v) at Rf value of 0.39, 0.48, 0.57, 0.73.

Fig No.16: Densitogram of nortriptaline after oxidative degradation (6%H_2O_2)



DRY HEAT DEGRADATION [15]

The standard drug was stored in oven at 50oC for 72h to study dry heat degradation

Fig No.17: Densitogram of Nortriptyline after thermal degradation

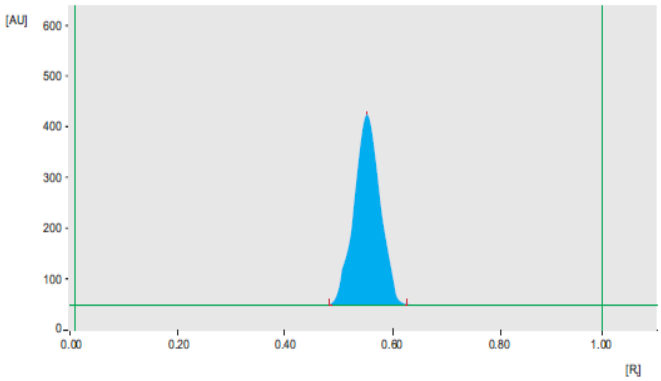


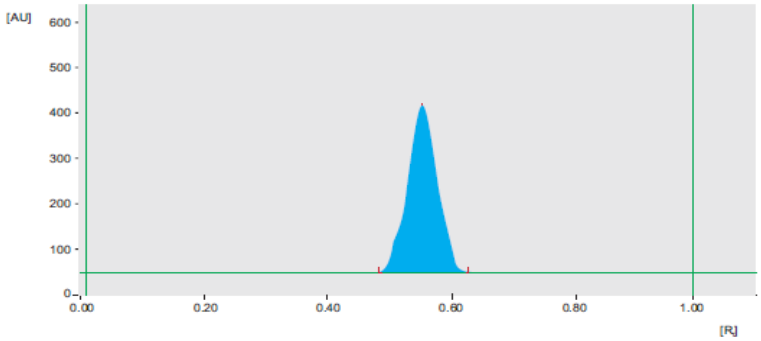
Photo degradation

The photochemical stability of the drug was studied by exposing the stock solution to direct sunlight for 24 hr. After suitable dilution, concentration of 5000ng/spot was applied and the chromatograms were run as described in previous

section. Oxidative degradation (Hydrogen peroxide induced) Studies were performed in 30% (v/v) hydrogen peroxide at room temperature for 48h respectively.

applied to TLC plate to achieve a final concentration of 5000ng/spot and the chromatograms were run and peak obtained RF value at 0.54.

Fig No.18: Densitogram of Nortriptaline after photo degradation



FORCED DEGRADATION

Table No.9: Forced degradation

Sr no	Parameter	% Degradation	Peak area
10.	Normal	100	5786.73
11.	1N HCl	56	3240.16
12.	1N NaOH	34	1967.24
13.	6% H2O2	52	3008.72
14.	Thermal	99.91	5781
15.	Photolytic	98.37	5692

Statistical analysis of the data establishes that the developed HPTLC method is specific, accurate, precise and stability-indicating. The validated method is suitable for analysis of nortriptyline in both bulk without any interference from the excipients. The method can be employed to determine the purity of the drug by detecting any related impurities present. The method is efficient in separating the degradation components from the main analyze nortriptyline and hence can be considered as stability-indicating. This analytical method may be extended for determination of nortriptyline in plasma and other biological fluids and warrant further studies.

RESULTS AND DISCUSSION

A separation process is a method to achieve any phenomenon that converts a mixture of chemical substance into two or more distinct product mixtures, which may be referred to as mixture at least one of which is enriched in one or more of the mixture's constituents. Due to the fact that chemicals usually occur as a combination of various type of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds and its degradation. It is a common practice in isolation of these bioactive compounds and degradation products that a number of different separation techniques such as TLC, column chromatography and flash chromatography, High performance liquid chromatography (HPLC) is a versatile, robust and widely used technique for the isolation of products. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of degradation products. In previous days, Column chromatography was used in many laboratories for preparative purposes as well as for reaction control in organic synthesis. Column chromatography is an extremely time consuming stage in any lab and can quickly become the bottleneck for any process lab. This leads to the development of novel preparative liquid chromatography in which mobile phase flows down by positive air pressure called as Flash chromatography. It is a simple, fast and economical approach to preparative Liquid chromatography. This review try to focus on principle, various components, general procedure and applications of Flash chromatography. Silica gel flash chromatography has become ubiquitous within organic chemistry and since its formal introduction in 1978.All chromatographic methods with the exception of TLC use columns for the separation process. Column chromatography has found its place in many laboratories for preparative purposes as well as for reaction control in organic syntheses. The importance of column chromatography is mainly due to following factors are given below.

1. Simple packing procedure
2. Low operating pressure
3. Low expense for instrumentation

UV spectrophotometer

Accurately weighed quantity of Nortriptyline 10mg was transferred to 100 ml volumetric flask and volume made up to the mark with distilled water to give a stock solution having strength of 100µg/ml. to study the different parameter by UV spectrophotometer, Parameters to be considered for the validation of method arelinearity and range, preparation of calibration curve, stability of solvents, accuracy, precision, robustness, limit of detection and limit of quantization. Optical Characteristics of nortriptyline was found to be Beer's law limit(µg/ml) 5-25µg/ml, Correlation coefficient 0.994, Regression equation 0.0923x - 0.1372, Slope 0. 0923, Intercept 0. 1372, The stability for Nortriptyline was found to be in the 25µg/ml at various time linearity data for Nortriptyline at 279 nm. Accuracy may often be expressed as % recovery by the assay of known and added amount of analyte. It measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by adding previously analysed samples of the Nortriptyline with three different concentration of standard at 80%, 100%, and 120% respectively. Absorbance of solution was measured at 279 nm. The amount of Nortriptyline was calculated at each level and % recoveries were found 99.68%, 99.95%,

99.99% respectively Precision study was carried out at parameter like Repeatability: aliquots of 2, 4, and 8 ml of working standard solution (100µg/ml) were transferred to a series of 10ml volumetric flask. The volume was adjusted up to mark with distilled water. The absorbance of above solution was measured three times and % RSD was calculated. Intraday precision: solutions containing 5, 10, and 15µg/ml of Nortriptyline was prepared and analysed 3 times on the same day and % RSD was calculated. Interday precision: solution containing 5, 10, and 15 µg/ml of Nortriptyline was prepared analysed 3 times on 3 different days and %RSD was calculated the Intra-day precision studies for Nortriptyline was found % RSD 0.598802, 0.408794,0.325573 for the Conc. (µg/ml) 5, 10, 15 respectively. Summary of Validation parameters and Linearity 5-25 µg/ml, Correlation co-efficient 0.994, Slope 0.923, Intercept 0.1372, Limit of detection 0.1123, Limit of quantization 0.3404, Repeatability 0.8822, Interday0.85-1.66 and Intraday 1.01-1.84 respectively

FORCE DEGRADATION STUDY

ACID HYDROLYSIS: Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 0.1N/1N HCl, NaOH, 3/6 % H_2O_2up to the mark and it refluxed for 3hr at 80^0c. The degradation sample was cooled at room temperature and neutralized the sample with same strength of 0.1N NaOH/HCl. Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water degradation was found to be 0.1N HCl34.00%, 1N HCl51.64%, 0.1N NaOH 38.66%, 1N NaOH 53.99%, 3% H2O2 27.92%, 6% $H_2O_2$49.08%, Thermal 1.44, Photolytic 5.73 respectively.

HPTLC STUDY

CHROMATOGRAPHIC HPTLC CONDITIONS

Experimental analysis was performed on silica gel 60F254 HPTLC plates (20cm x 10cm with 250 µm thickness; E Merck, Darmstadt, Germany, Batch-HX011551) using mobile phase consisting of toluene: ethyl acetate: methanol: glacial acetic acid in the ratio of 4:4:1.6:0.4 (v/v/v/v). Prior to chromatographic analysis the plates were washed in methanol, dried in a current of dry air and activated at 110^0C for 5 min. Samples were spotted in the form of bands of width 8mm with a Camagmicrolitre syringe. A constant application rate of 150nl/s was used and the space between two bands were 5mm. Monochromator band width was set at 20nm, each track was scanned six times and baseline correction was used. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (26±2oC) and relative humidity (50±5%). No neckless effect was seen during the development and it took approximately 20 min for the complete development of the TLC plate. The length of each chromatogram run was 80mm. Consequent to development, the plates were dried in current of air by use of an air dryer. Densitometric scanning was performed in the reflectance absorbance mode at 279nm, operated by CamagwinCATS software (V 1.4.5). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400nm. The slit dimension was kept at 6mm x 0.30mm and the scanning speed was 20mm/s. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. Scanned peak areas were recorded for each sample at each concentration level. The average peak areas and variations in peak area obtained were expressed as percent relative standard deviation (% RSD). Linear regression data for the calibration curve was found to

be Detection Wavelength (nm) 279, Beer's Law Limit (ng/band) 200-600, Correlation Coefficient (r2 ± SD) 0.9965± 0.00083, Intercept (c) ± SD 0.9965, Confidence limit of intercept 1385.5-1804.1, Slope (m) ± SD 0.00083, Confidence limit of slope 7.901-8.771, SD of residuals from line 58.608 respectively. Recovery studies of Nortriptyline was found to be for 80,100, 120 level to 101.70 ± 0.25, 101.96 ± 2.88, 101.45 ± 2.58, 101.97 ± 3.29 Degradation studies were carried out by refluxing 5ml of drug solution with 5ml each of 1N HCl and 1N NaOH. The mixtures were refluxed for 3h respectively at 80^0C. The solutions (0.5 ml) were taken and neutralized and subsequently diluted to 10 ml with Neutralizing solvent (HCl and NaOH). The resulting solutions were applied to TLC plate in such a way that the final concentration attained was 5000 ng/spot for both acid and base degradation products and the chromatograms were run as described in previous section. The rate of degradation in acid was slower as compared with alkali. The chromatogram of the acid degraded sample showed peak at Rf value of 0.42, 0.49, 0.53, 0.55, 0.62, 0.82 was observed in acid degradation. For base degradation study different peak value observed at various Rf values of 0.40, 0.45, 0.49, 0.58, 0.62. The results indicate that nortriptyline undergoes degradation under acidic and basic conditions, the sample showed an additional peak with 6 % (w/v) at Rf value of 0.39, 0.48, 0.57, 0.73.

The photochemical stability of the drug was studied by exposing the stock solution to direct sunlight for 24 hr. After suitable dilution, concentration of 5000ng/spot was applied and the chromatograms were run as described in previous section. Oxidative degradation (Hydrogen peroxide induced) Studies were performed in 30% (v/v) hydrogen peroxide at room temperature for 48h respectively applied to TLC plate to achieve a final concentration of 5000ng/spot and the chromatograms were run and peak obtained RF value at 0.54. The degradation of HPTLC was found to in 1N HCl 56 %, 1N NaOH 34 %, 6% H_2O_2 52 %, Thermal 0.09, Photolytic 1.63

SUMMARY

The study describes the simple, sensitive, accurate, rapid and reliable ultra violet spectroscopic method has been developed for determination of Nortriptyline in bulk drug and pharmaceutical formulation. nor is use as second line agent for antidepressant in order to investigate the stability of drug, a stress testing of drug sample by exposing it to variety of force degradation conditions has been recommended. Nortriptyline was subjected to stress degradation under different condition recommended by international conference on harmonization (ICH). Nortriptyline shows maximum absorbance at 279nm and calibration graph linear in the concentration range 5-25 Mcg/ml with correlation co-efficient 0.9997. The stability study indicates appreciable changes were observed by treating the drug with acidic hydrolysis, basic hydrolysis and oxidation. However, there is no appreciable changes were observed for thermal stress and photolytic degradation.

- **UV METHOD**

The study was discussed to develop simple, accurate and precise UV method for estimation of Nortriptyline bulk drug. Standard calibration curve was plotted as absorbance vs. concentration. This straight line obeys linearity in the conc. range of 5 µg/ml to 25 µg/ml in which drug obeyed Beer-Lambert's Law. The correlation was found to be 0.998. The recovery study was carried out by preparing solution of 80%, 100% and 120% and got recovery between 93% to 102%. Precision study was carried out by three different solutions of three different conc. at three different intervals of day time. % RSD of precision studies was found to be 0.4443% for intraday and 0.4430% for interday. Robustness of the

method was determined by carrying out the analysis under different temperature conditions i.e. at ambient temperature and at 18˚C. The % RSD was found to be 0.160%. Ruggedness of the method was determined by carrying out the analysis by different analyst. % RSD was found to be 0.545%. LOD and LOQ were calculated from standard deviation and slope obtained value 0.1123 ppm and 0.3404 ppm respectively. From the above it was concluded that, this method was more accurate, precise, and validated as the linearity followed the Beer-Lambert's Law. Precision robustness ruggedness % RSD was found to be less than 2% and LOD and LOQ were found to be in range.

Degradation study of API drug by UV method were carried out by placing the drug in different stress conditions. The acidic degradation, alkaline degradation, oxidative degradation, dry heat and photo degradation were found to be 34.00%, 38.66%, 27.92%, 1.44% and 5.73% respectively. Changes in max of acidic and alkaline degradant.

- **HPTLC METHOD**

The estimation of different of CEF bulk was carried out by HPTLC using mobile phase having the composition of toluene: ethyl acetate: methanol: glacial acetic acid in the ratio of 4:4:1.6:0.4 (v/v/v/v). on the basis of polarity of drug. Standard calibration curve was plotted as peak area vs. concentration. The straight line obeyed linearity in the conc. range 200-600 ng/band. The correlation was found to be 0.998. The % recovery was found to be 98-100%. The method is found to be precise as %RSD of intraday and interday precision were less than 2%. Robustness was carried out by changing conc. of mobile phase and by changing time from application to development and %RSD was found to be 1.19 % and 1.42 %. LOD and LOQ were found to be 23.20 ng/band and 70.30 ng/band.

Degradation study of API drug by HPTLC method were carried out by placing the drug in different stress conditions. The acidic degradation, alkaline degradation, oxidative degradation, dry heat and photo degradation were found to be 56.00%, 34.00%, 52.00%, 99.91% and 98.37% respectively. The change in peak area was observed.

CONCLUSION

The proposed method is simple, precise, accurate and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis. Results of analysis were validated as per the ICH guidelines. Stability study include effect of acid, base, temperature, oxidation, photolysis and susceptible to hydrolysis across wide range of pH This method is successfully applied for isolation of degraded compound and characterization of it. It also helpful for future Isolation of further degradation Characterization of further degradation, Confirmation of structure of degradant by using LC MS and NMR Study, To study the quality of the marketed drug with this method, To prevent the loss of active ingredient, To prevent alteration in bioavailability, To avoid the formation of toxic degradation products

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