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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTIFICATION OF ANTIHISTAMINIC & ASTHMATIC DRUG IN BULK AND TABLET DOSAGE FORM

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ABSTRACT:
This research describes the stability indicating RP-HPLC method in pharmaceutical tablet dosage forms for simultaneous estimation of Levocetirizine dihydrochloride (LEV) and Montelukast Sodium (MON). The proposed RP-HPLC method was developed using separation module Waters 2695 with PDA detector and chromatographic separation was performed at a flow rate of 1 mL / min using column Hypersil BDS C18 (250/4.6 mm, 5 µm) with a run time of 10 min. The mobile phase consisted of a 40:60% v / v Phosphate buffer and acetonitrile, pH with orthophosphoric acid was adjusted to 7.0 and the eluents were scanned at 230 nm using a PDA detector. Retention times for LEV and MON were 3.06 min, and 6.76 min, respectively. A linearity response was observed with a concentration range of 12.56–37.68 µg / mL for LEV and 23.78–71.20 µg / mL for MON. Limit of detection and limit of quantification for LEV are 0.079 µg/mL and 0.239 µg/mL and for MON are 0.156 µg/mL and 0.473 µg/mL, respectively. The stability indicating method was developed by subjecting the drugs to stress conditions such as acid, base hydrolysis, oxidation, neutral, photo- and thermal degradation, and the degraded products produced from the samples were successfully solved.
Keywords: Levocetirizine dihydrochloride, Montelukast Sodium, RP-HPLC, stability indicating

INTRODUCTION

In Introduction to Analytical Chemistry Analytical chemistry can be termed as the science and art of determining the components of materials in related elements or compound contained. Analytical chemistry pursued to improved means of measuring the chemical composition of natural as well as artificial materials.

The techniques of this science are used to determine the exact amounts of the identified substances which may be present in a material. Analytical chemistry is important in agricultural, clinical, environmental, juristic, productive metallurgical and pharmaceutical chemistry.

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Analytical techniques show important aspects mainly in assuring and maintaining the quality of substance and are critical components of Q.A. / Q.C / An analytical chemist has the duty to calculate the reliability, benefit, accuracy, specificity and interception.

In general terms pharmaceutical analysis comprises of those procedures which are necessary to determine the identity, strength, quality and purity of products.

The study of analytical chemistry shall consist of:

a) Qualitative Analysis: Qualitative analysis is concerned with the identification of the elements or functional groups present in the compounds.

b) Quantitative Analysis: Quantitative analysis deals with the calculation of the quantity of one or more constituents present in the sample. [1]

Selection of Analytical Method:-

Method should be,

1. As simple as possible

2. Most specific

3. Most effective, convenient and economical

4. As accurate and precise as required

5. Should be fully optimized before conduction for validation of its characteristics such as accuracy, precision, sensitivity etc. [2]

Importance of Analytical Chemistry

- Development of theory of analytical method in every possible way.

- Improvement and monetization of the exiting analytical method scientifically.

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• Scientific elaboration of new analytical methods, which meet the requirementof advancing science and modern production.

• Analysis of natural substances, environment and also industrial material. [2, 3]

Classification of Analytical Methods:

Analytical methods categories into two;

Classical methods and Instrumental methods:-

Volumetric Methods: In volumetric, also known as titrimetric, procedures, the mass or volume of a standard reagent required to react completely with the analyte is measured.

Gravimetric Methods: In gravimetric measurements, the mass of the analyte or compounds produced from it is determined. The extent of their general application is, however, decreasing with the required time and with the development of instrumental methods to supplant them.

Instrumental Methods: These methods measure some physical properties as conductivity, electrode potential, mass-to-charge, ratio light absorption or emission and fluorescence of substance. The various techniques available for the analysis of analytes, which can be broadly classified as,

a) Spectroscopic Techniques

1. Ultraviolet and visible spectrophotometry

2. Infra-red spectrophotometry

3. Raman spectroscopy

4. Fluorescence and phosphorescence spectrophotometry

5. Atomic spectrophotometry (emission & absorption)

6. Fluorescence and phosphorescence spectrophotometry

7. X-ray fluorescence spectroscopy

8. Radiochemical techniques including activation analysis

9. NMR spectroscopy ESR spectroscopy

b) Electrochemical Techniques

1. Potentiometry

2. Voltammetry

3. Stripping techniques

4. Amperometry techniques

5. Coulometry

6. Electrogravimetry

7. Conductance technique

c) Chromatographic Techniques

1. Gas chromatography (GC)

2. High performance liquid chromatography (HPLC)

3. High-performance thin layer chromatography (HPTLC)

4. Supercritical fluid chromatography (SFC)

5. Ultra-pressure liquid chromatography (UPLC) d) Miscellaneous Techniques

6. Dry heat analysis

7. Mass spectrometric

8. Kinetic techniques [4] d) Hyphenated Techniques

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9. GC-MS

10. GC-IR

11. CE-MS

12. LC-MS

13. LC-NMR

14. MS-MS

15. ICP-MS

16. L C- MS- NMR Amongst all the techniques stated above UV-Visible spectrophotometry and High Performance Liquid Chromatography (HPLC) are the most widely used techniques of pharmaceutical substances for quantitative analysis, and are briefly discussed further. [5]

Introduction to Chromatography

1 Chromatography Chromatography is essentially a set of techniques for identification, separation and determination of different compounds. This technique is based on difference in migration rated of the components via a stationary phase by a gaseous or liquid mobile phase.

2 The Chromatographic Separation Theory In chromatographic separations, the sample is subjected to a mobile flow into or through and then forced through a stable stationary phase, which is fixed in place on a column or on a solid surface. The two phases are selective, such that the sample components are distributed to different degrees between mobile phase and stationary phase. These compounds travel slower to a stationary layer and at a shorter distance with a higher affinity. In contrast, components with a lower affinity travel faster and longer, the sample components are separated into discreet bands or zones that can be qualitatively or quantitatively analyzed.

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3 Classification of Chromatographic Methods Chromatographic methods are classified based on mechanism or nature of mobile phase or stationary phase.[8] that are used in chromatography are defined as follows:

♣ The baseline is any portion of the chromatogram in which only the mobile phase of the column appears.

♣ The peak maximum is the highest point of the peak.

♣ The injection point is the time point at which the sample is placed on the column.

♣ Dead point is the position of the peak-maximum of the unretained solute.

♣ The dead time (to) be the time elapsed between the injection point and the dead point.

♣ The dead volume (Vo) is the volume of mobile phase passed through the column between the injection point and the dead point. Thus, Vo=Qto When, Q is the flow rate in ml / min and to is dead time.

♣ The retention time (tr) is the time elapsed between the injection point and the peak maximum. Each solute has its own characteristic retention time

High Performance Liquid Chromatography (HPLC)

Introduction to HPLC High performance liquid chromatography (HPLC) is one of the best method of chromatography; the most widely used analytical technique as shown in figure no-1.2. HPLC uses a liquid mobile phase to separate the components of the mixture. In this method, solute first dispersed in a solvent, then forced to pass through a chromatographic column under high pressure. In the column, this mixture is resolved into its various components. The amount of resolution is important and it is depends on the extent of interaction between the both phases.

HPLC is a flexible analytical tool for pharmaceutical, ionic, polymer and many organic and biomolecules research.

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Advantages of HPLC

- Separation of mixed components.
- Quantitative analysis/Qualitative analysis.
- Preparation of interest components.
- Precise and rapid quantitative analysis.
- Automated operation. [10]

Types of HPLC

There are various ways to classify liquid column chromatography, three modes can bespecified.

a) **Adsorption Chromatography:**

Packing and separation is based on repeated adsorption-desorption steps in whichsilica gel is used as an adsorbent in the stationary phase.

b) **Ion-Exchange Chromatography:**

The stationary phase has an ionically charged surface of to the sample ions which has opposite charged surface. This technique is almost exclusively used for ionic orionizable specimens. The stronger charge on the sample, then it will be attracted tothe ionic surface stronger and hence it takes long time to elute. Both pH and ionic strength are used to control elution time, where the mobile phase is an aqueous buffer.

c) **Size Exclusion Chromatography:**

The material is filled in the column having precisely controlled pore sizes. The sample is simply filtered according to its solvated molecular size. Larger molecule are immediately washed through the column while smaller molecules penetrate inside the pores of the packing particles and then elute. It is also said to be a gel filtration or gel permeation chromatography but nowadays, the stationary phase is not restricted to a "gel"

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Modes of HPLC

LC mode	Packing materials	Mobile phase	Interaction
Normal phase chromatography	Silica gel	n-Hexane	Adsorption
Reversed phase chromatography	Silica-C 18 (ODS)	Methanol/water	Hydrophobic
Size exclusion chromatography	Porous polymer	THF	Gel permeation
Ion exchange chromatography	Ion exchange gel	Buffer solution	Ion exchange

Stationary Phase (Adsorbents)

The stationary phase can be a solid or liquid which adsorbed on a solid. In liquid chromatography, immobilization occurs by reaction of liquid with a solid. The stationary phase cab be consists of particles (solid or porous), a fibrous material (e.g. paper) or the walls of a tube (e.g. capillary). HPLC separations depend on the surface interactions and the types of the adsorption sites. Advanced HPLC adsorbents are small rigid porous particles materials with high surface area.

Important features of adsorbent parameters are:

- Particle size : 3 to 10 µm
- Pore size : 70 to 300 Å.S
- Surface area : 50 to 250 m^2/g.
- Particle size distribution : As narrow as possible, usually within 10% ofthe Mean.
- Bonding phase density : 1 to 5 per 1 nm^2

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2.5.7.1. Mobile Phase

The mobile phase moves the solutes through the stationary phase which is the part of the chromatographic system. The mobile phase are either liquids or gases. To adjust the retention and chromatographic separation in liquid chromatography used theliquid mobile phases and the gas mobile phase temperature is used to adjust the retention in gas chromatography. [12]

MATERIALS AND METHODS

Drug samples: Drug sample WS/LVCPC18348 (99.38 % purity) and WS/MIT8192 (98.66 % purity) were kindly supplied by Emcure Pharmaceuticals Ltd., Pune.

Marketed Tablet: Tablets Nukast-10 containing 5 mg of Drug A and 10 mg of Drug B were supplied by Emcure Pharmaceuticals Ltd., Pune.

Reagent & chemical used: Methanol HPLC grade (Merck specialties Pvt. Ltd. Mumbai)

Acetonitrile HPLC grade (Merck specialties Pvt. Ltd. Mumbai), Double distilled water (Milli Q Water)

Disodium hydrogen orthophosphate dihydrate (SD fine chem limit Mumbai),Sodium Hydroxide (Merck specialties Pvt. Ltd. Mumbai)

Instruments used: UV Visible double beam spectrophotometer model-serial No. 1800, A114545, 00377 (Shimadzu corporation Japan), RP-HPLC system model-serial No. SCM 1000, P4000, UV2000, SN4000 (Thermo Technologies, New Delhi), Fast clean ultrasonic cleaner (Equitron Pvt. Ltd)

Single Pan electronic balance (Startorious Pvt. Ltd), pH meter (LABINDIA instruments)

Distillation water system model-serial No. 3361041 (Borosil, Pune), Hot Air Oven model-serial No. BT129 (Universal Instruments)

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EXPERIMENT

Experimental Procedure

UV Method

Drug Characterization

A. *Description*

The sample of drug was visually observed for the color, odor and physically appearance.

B. *Melting Point*

Melting points of Drug A and Drug B were determined by taking a small amount of pure drug in a capillary tube closed at one end placing it in melting point apparatus. The temperature at which the drug is melting has been noted. Average reading of the triplicate was taken and compared to literature.

C. *Solubility studies*

Drug A and Drug B pure samples were taken in amount of pinch and added to the test tubes containing different solvents, Buffers and solubility was evaluated.

Selection of common solvent

After assessing the solubility of drugs in different solvents Buffer pH 7.0 has been selected as common solvent for further method development studies.

Preparation of Buffer solution pH 7.0 / Diluent

Buffer solution was prepared by dissolving 2.8 gm of Disodium hydrogen orthophosphate dihydrate in 1000 ml of water, and later adjust pH to 7.0 with OPA.

Standard stock solution of Drug A and Drug B

To prepare standard stock solution accurately and separately weighed quantity of about 50mg of Drug A and Drug B were taken in 100 ml volumetric flask dissolved in sufficient quantity of methanol and diluted to 100 ml with the same solvent. From the above stock solution, 1 ml was withdrawn to 100 ml of volumetric flask and diluted with the same solvent.

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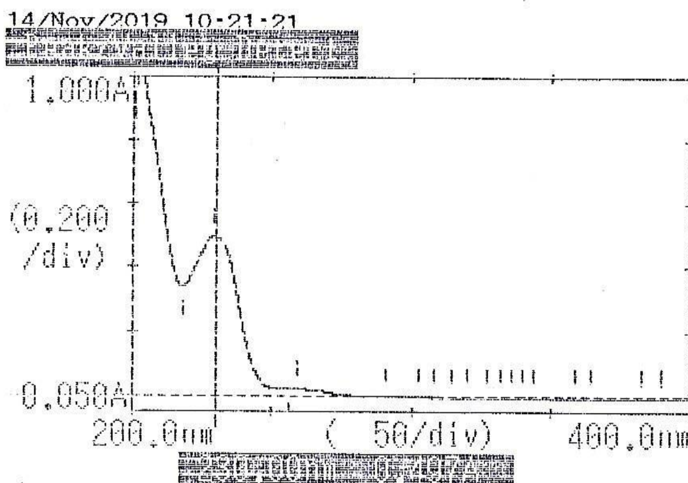
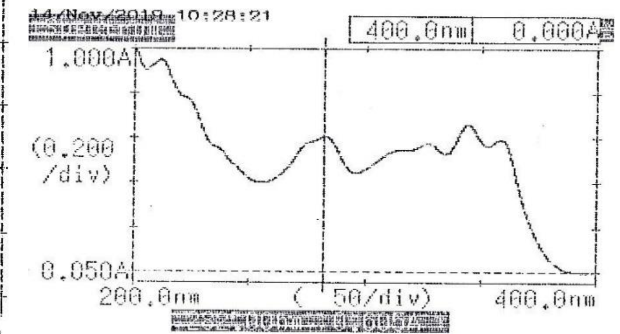
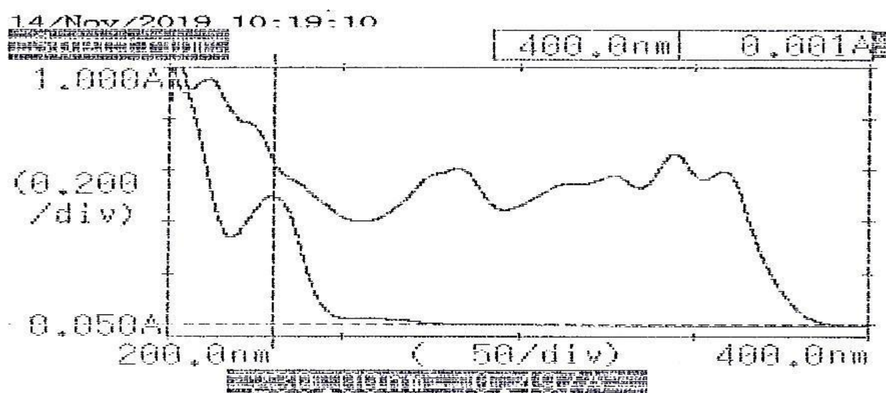
Determination of λ max of Drug A and Drug B

he standard solution of Drug A was scanned in the range of 200-400 nm andλ max was found to be 230 nm against methanol. Similarly, the standard solution of Drug B was scanned in the range of 200-400 nm and the λ max was found to be 282 nm





λ max of Drug A **λ max of Drug B**



λ max of Drug A & Drug B

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Simultaneous estimation of Drug A and Drug B in bulk drug and tablet dosage form by using RP-HPLC method.

Solubility of drugs in different solvents

Solvent	Solubility	
	Drug A	**Drug B**
Water	freely soluble	freely soluble
Methanol	freely soluble	freely soluble
Acetonitrile	Soluble	Insoluble
0.1 M KH_2PO_4	Soluble	Insoluble
0.1 M NaH_2PO_4	Soluble	Insoluble
0.1 M Ammonium acetate	Soluble	Insoluble
0.1 M K_2HPO_4	Soluble	Insoluble
0.1 M $Na_2HPO_4.2H_2O$	Soluble	Soluble
0.1 M Ammonium dihydrogen phosphate	Soluble	Insoluble
0.1 M $Na_3PO_4.2H_2O$	Soluble	Insoluble

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Optimization of mobile phase and chromatographic conditions

Sr. No	Mobile phase	pH	Flow Rate (ml/min)
1	Buffer: Acetonitrile: Methanol (40:30:30)	7.0	1
2	Buffer: Methanol : Acetonitrile (25:25:50)	5.5	1
3	Buffer: Methanol (25:75)	5.5	1
4	Buffer: Acetonitrile (90:10)	7.0	1
5	Buffer: Acetonitrile (50:50)	6.5	1
6	Buffer: Acetonitrile (50:50)	7.0	1
7	Buffer: Acetonitrile (50:50)	7.5	1
8	Buffer: Acetonitrile (50:50)	6.5	1.2
9	Buffer: Acetonitrile (40:60)	7.0	1

1. *Selection of chromatographic parameters*

Table No. 9.3. Chromatographic parameters

Parameters	Chromatographic conditions
HPLC system	Waters 2695
Detector	2996 PDA
Column	Hypersil BDS C18 250 x 4.6 mm
Mobile Phase	Buffer: Acetonitrile (pH 7.0 with OPA) (40:60 v/v)
Detection Wavelength	230 nm
Flow rate	1 ml/min
Temperature	30°C
Sample volume	10 µl
Run Time	10 min
Isocratic elution	

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2. *Checking the resolution of two drugs and internal standard*

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Mixed standard solution of Drug A and Drug B was injected to get chromatogram. The retention time for the two drugs were found to be:

Drug A : 3.06 ± 0.5 min Drug B : 6.76 ± 0.5 min

RESULTS AND DISCUSSION

A simple precise, accurate and economic stability indicating RP-HPLC method was developed and validated for estimation of Drug A and Drug B from bulk and marketed formulation. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, ruggedness and robustness.

10.1. HPLC Method

10.1.1. Solubility of drugs in different solvents.

The selection of solvents was made assessing solubility of both drugs indifferent solvents like methanol, ethanol, acetonitrile and different listed buffer. Solubility of drugs in different solvents are given in **Table 10.1**

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Table 10.1: Solubility of drugs in different solvents

Solvent	Solubility	
	Drug A	**Drug B**
Water	freely soluble	freely soluble
Methanol	freely soluble	freely soluble
Acetonitrile	Soluble	Insoluble
0.1 M KH_2PO_4	Soluble	Insoluble
0.1 M NaH_2PO_4	Soluble	Insoluble
0.1 M Ammonium acetate	Soluble	Insoluble
0.1 M K_2HPO_4	Soluble	Insoluble
0.1 M $Na_2HPO_4.2H_2O$	Soluble	Soluble
0.1 M Ammonium dihydrogen phosphate	Soluble	Insoluble
0.1 M $Na_3PO_4.2H_2O$	Soluble	Insoluble

Table 10.2 : Chromatographic Trial 01

Mobile Phase	Buffer: Acetonitrile: Methanol
Mobile phase concentration	40:30:30 % v/v/v
Injection volume	10 µl
Diluent	Methanol
Flow rate	1 ml/min
Colum temperature	30°C

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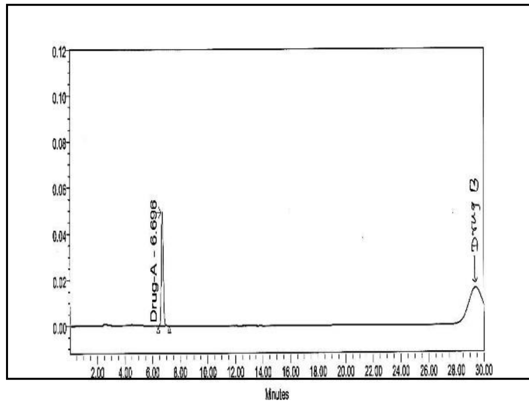
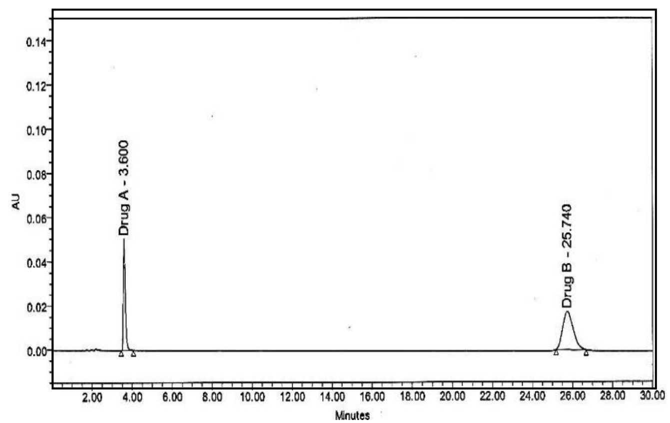
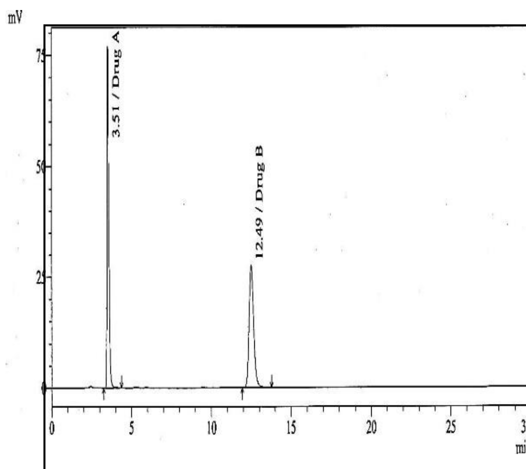
Chromatographic Trial

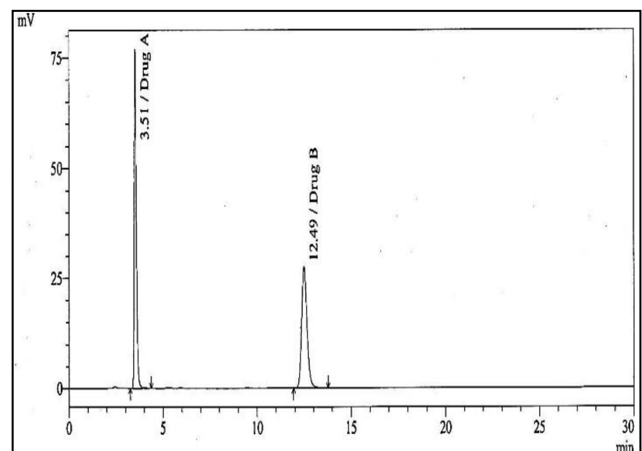




Chromatographic Trial 01

Chromatographic Trial





Chromatographic Trial 03

Chromatographic Trial 04

Analysis of marketed formulation

Formulation tablets	Drug Label claim (mg)	% found ± RSD	% Recovery ± RSD
Drug A	5	98.55 ± 0.30	99.45 ± 0.10
Drug B	10	101.52 ± 0.41	98.22 ± 0.15

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System suitability of Drug A

Sr. No.	Injection No.	RT	Tailing Factor	Theoretical Plate	Peak Area
1	1	3.03	1.24	38179	504381
2	2	3.03	1.24	37816	504966
3	3	3.03	1.25	37808	505689
4	4	3.03	1.25	37746	503164
5	5	3.03	1.25	37816	508937
Mean		3.03	1.25	37873	505427
SD					2168.90
%RSD					0.43

System suitability of Drug B

Sr. No.	Injection No.	RT	Tailing Factor	Theoretical Plate	Peak Area
1	1	6.79	1.17	72006	1439678
2	2	6.79	1.16	71677	1440397
3	3	6.79	1.16	71649	1440443
4	4	6.79	1.15	71568	1435168
5	5	6.78	1.21	71274	1456754
Mean		6.79	1.17	71635	1442488
SD					8269.70
%RSD					0.57

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: Linearity studies of Drug

Linearity Solution	Concentration (μg/ml)	Area of Drug A	Mean area of Drug A	Std. deviation	%RSD
Linearity L-1 (50%)	12.56	251989	251813	200.226	0.08
		251854			
		251595			
Linearity L-2 (75%)	18.84	368395	368600	377.811	0.10
		368369			
		369036			
Linearity L-3 (100%)	25.12	494865	495138	469.104	0.09
		494870			
		495680			
Linearity L-4 (125%)	31.40	617052	616357	1002.106	0.16
		616810			
		615208			

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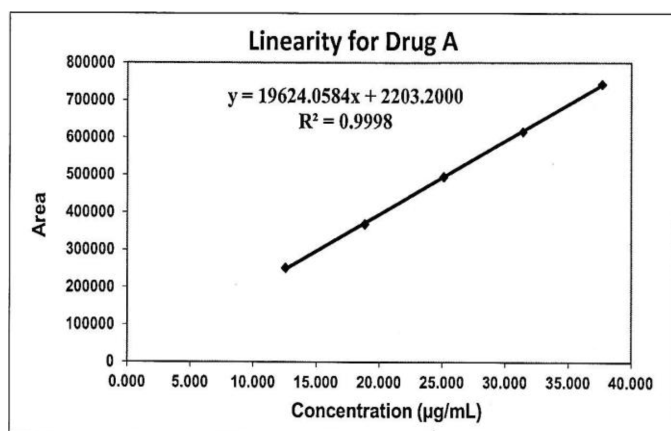
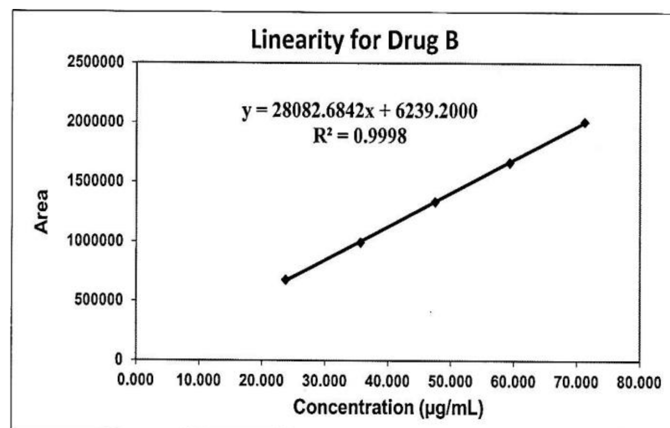
Table 10.11: Regression data of Drug A

Slope	Intercept	Regression Equation	Coefficient of Correlation
19624.0584	2203.2000	y=19624.0584x + 2203.2000	0.9998





Linearity of Drug A

Linearity of Drug B

Regression studies of Drug B

Slope	Intercept	Regression Equation	Coefficient of correlation
28082.6842	6239.2000	y=28082.6842x + 6239.2000	0.9998

Parameter Count	Change level	Mean	% Assay	% RSD
Flow rate ± 0.1 (ml/min)	0.9	560587	101.56	0.33
	1.1	458881	101.66	0.32
pH (7.0 ± 0.2)	6.8	510992	102.41	0.46

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	7.2	508326	101.86	0.33
Wavelength (230 ± 2nm)	228	494758	101.74	0.32
	232	498481	101.84	0.33

Table 10.19- Robustness study of Drug B

Parameter Count	Change level	Mean	% Assay	% RSD
Flow rate ± 0.1 (ml/min)	0.9	1592893	100.87	0.41
	1.1	1304626	101.01	0.40
pH (7.0 ± 0.2)	6.8	1456552	101.75	0.48
	7.2	1442763	101.27	0.40
Wavelength (230 ± 2nm)	228	1536167	101.06	0.39
	232	1363403	101.17	0.39

Solution stability for Drug A

Time interval(hrs)	Area of standard	Area of sample	Cumulative Mean for standard	Cumulative Mean for sample
Initial	488575	469903	488575	469604
		469304		
12 Hrs	493067	474976	489985	471803
	493953	473028		
18 Hrs	495963	476010	491242	472620
	495324	472501		
24 Hrs	495279	475158	491956	473451
	495054	476730		
36 Hrs	498936	478473	492950	474387

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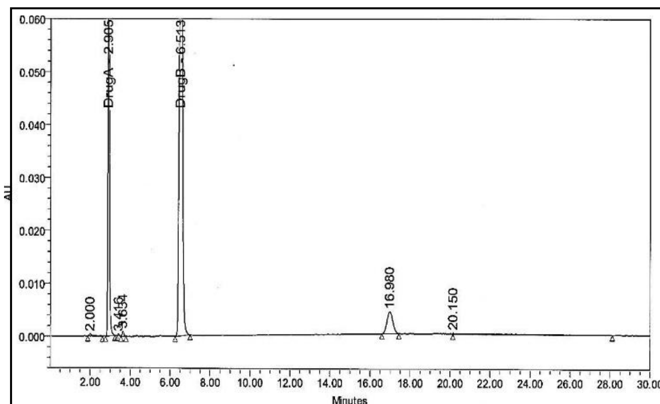
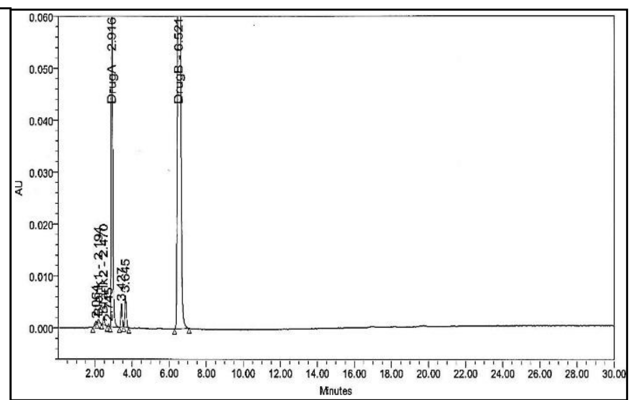
	497902	477782		
48 Hrs	501601	480925	494194	475641
	502953	482900		
SD			**4896.313**	**4100.832**
%RSD			**0.99**	**0.86**

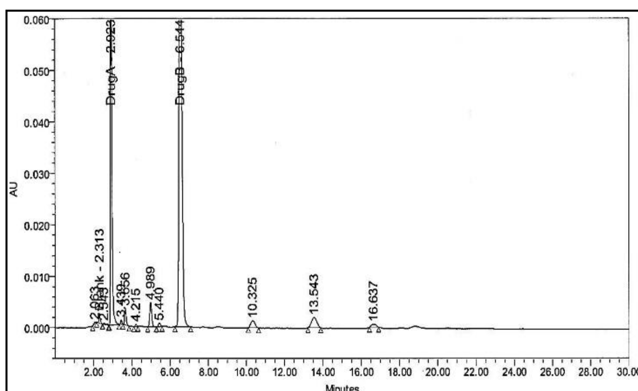
10.3. Forced Degradation Studies



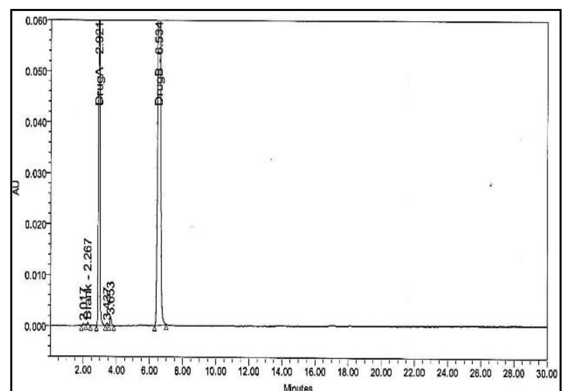
Chromatogram of Acid degradation



Chromatogram of Alkali degradation



Chromatogram of photo chemically



Chromatogram of Dry Heat

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All the results obtained from forced degradation studies

Stress condition		Acid stress	Base stress	Oxidation stress	UV stress	Dry Heat stress	Neutral stress
% Degraded	Drug A	10.62	5.11	3.59	5.91	0.75	8.75
	Drug B	12.27	-5.90	-1.02	27.84	2.63	-2.77
Amount Recovered %	Drug A	88.11	93.61	95.13	92.81	97.97	89.98
	Drug B	89.67	107.84	102.96	74.10	99.30	104.70
Degraded products t_R (min)		3.41, 3.63, 16.98, 20.15	2.74, 3.42, 3.64	1.99, 3.41, 3.63	3.43, 3.65, 4.21, 16.63	2.01, 3.43, 3.63	3.42, 3.63, 4.34
Peak angle	Drug A	0.22	0.29	0.22	0.35	0.21	0.23
	Drug B	0.10	0.09	0.09	0.12	0.09	0.09
Peak threshold	Drug A	0.42	0.41	0.42	0.42	0.41	0.42
	Drug B	0.27	0.27	0.27	0.29	0.26	0.26

t_R = Retention time, peak purity = Peak angle > peak threshold

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