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RP-HPLC stability indicating method development for the estimation of drug marketed formulation

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***Abstract*---**In this study our idea is to developed RP-HPLC method for analysis and method of development for pure drug and their pharmaceutical formulation and their stability indicating its RP-HPLC method. It is used to validate and analysis for olanzapine drugs. By RP-HPLC method the drug olanzapine passes through various quality test and ensure the identity, strength, quality, purity and potency of the drug substance and drug products.

***Keywords*---**RP-HPLC, Olanzapine, ICH, ANDA, NDA.

Introduction

The main aim of this method is to separate the main active compounds with any other impurities which is synthetic intermediates and degradants. (Sethi P.D et al 1996). The analysis can be divided in two types: -

a. Qualitative analysis (Dolan J.W et al, 2002): It is used for identification of chemical species which is present in the formulation.
b. Quantitative analysis: It estimates quantity which is present in a mixture.

These methods are easy to apply, low-cost and does not use polluting reagents and which require relatively in-expensive instruments. The methods were found is simple, rapid accurate and economic. (Dan W, et al 20020) (Sahu Ret al 2006)

Materials and Methods (Sahu Ret al 2006)

Material used

The Drug Olanzapine is used .it is a marketed preparation. other chemicals are Methanol, Acetonitrile, Methanol and Water. All reagents are AR grade and HPLC grade.

Instruments:

HPLC, Double beam UV Visible Spectrometer, FT-IR.

Methods: (Vilas Chaudhary et al, 2013)

- Marketed Formulation of Olanzapine:
  Marketed formulation Olenz-5 is used which is manufactured by sun pharma.
- Organoleptic evaluations
  It refers to taste, appearance, odor etc.
- Identification of Olanzapine
- ***FTIR spectrum***
  IR absorption spectrum of Olanzapine was obtained by KBr pellet method.
- ***Solubility***
  Solubility of Olanzapine was performed in various solvents
- ***Determination of λ_{max} of Olanzapine***
  λ_{max} was determined by using U.V. Spectrophotometer
- Determination of Melting Point of Olanzapine
  - It was determined by using Melting Point apparatus and the M.P. of drug was foundto be195-198 °C
  - Analytical Method Development by HPLC

Mobile Phase Selection (Mahmoud A.,et al 2013)

The mobile phase of drug olanzapine was taken in different ratio. it is more suitable for analysis. 1.75 gm dihydrogen potassium phosphate into 1L of purified

water and then add Tri ethanolamine upto 1ml then adjust the pH between 5- 6. Then filtered it.

Selection of wavelength

Accurately weigh the 100 mg of drug and transferred to a 100 ml volumetric flask, then volume was adjusted to the mark with mobile phase. From above solutions 0.1 ml was transferred into 10 ml volumetric flask , volume made up with mark .then observed with maximum absorbance was found at 260.00 nm.

Selection of Separation Variable

The separation of various variables are selected which is constant in whole experiment while performing.

System Suitability Parameters

After complete saturation of three column and replicate and injected separately .it is reported on chromatogram.

Preparation of Standard Stock Solution

Accurately weigh 10 mg of drug and transfer in to 10ml volumetric flask, and then make up the volume with the H_2O: ACN to formed stock preparation.

Preparation of Working Standard Solution

From stock solutions take 1ml drug and dilute up to the 10 ml of solution 0.5-2.5 ml solutions and make up the volume with the H_2O: ACN , which formed standards solution of 5-25 µg/ ml concentration.

Preparation of Calibration Curves of olanzapine

For preparation of standard solution , solution is injected thrice peak area was calculated and plotted against the concentration in range and then regression value was found by using curve analysis .

Analysis of Tablet Formulation (A. Rukmangada et al 2013)

- Assay of formulations
  For analysis take 10 mg of drug and transfer in to volumetric flask and dissolve it into the mobile phase. Then shake for 15 minutes and filter it and then volume was made up. Take 1.0 ml of solutions and dilute with 10.0 ml.it is 100 mg/ml .In above solution 1 ml was withdrawn , dilute with 10 ml with mobile phase to form solution containing 10 µg/ml of drug
- Linearity (Rubesh kumaret 2011)
  Linearity of analytical procedure is its ability to obtain test. The calibration curve was plotted for different concentration and it was recorded and calculated.

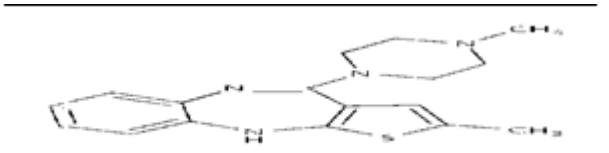
- Accuracy
  Four performing the Recovery studies is to validate the accuracy of developed Stability indicating RP-HPLC method.
- Precision: -
  It is used to evaluate the inter and intraday precision
- Repeatability
  Standard solutions of drug were analyzed in different day and by different analyst
- Thermal degradation
  Weigh accurately 50mg of drug in petri dish and kept in oven for 4 weeks at 50°C. Samples were withdrawn and diluted for 10 µg/ml and tested in HPLC .Calculate the percentage of degradation using calibration curve of drug.
- Photolytic degradation
  The olanzapine was exposed to sunlight during the daytime (70,000–80,000 lux) for 2 days. Then Samples were withdrawn and diluted at 10 µg/ml and tested in HPLC to calculate the percentage degradation using calibration curve of drug.

Results

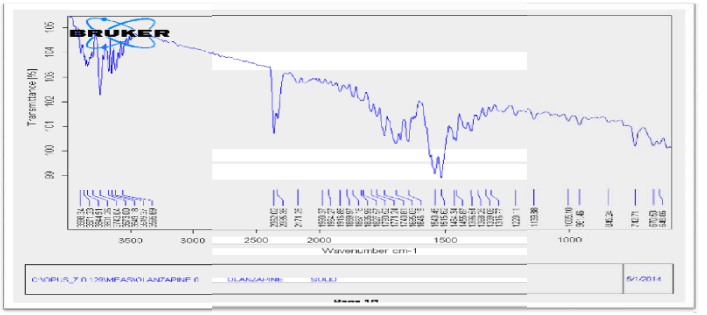
Organoleptic property

The drug olanzapine is yellow in colour and solid crystalline in nature

Structure of Olanzapine



Result of FTIR of Olanzapine



Interpretations of FTIR

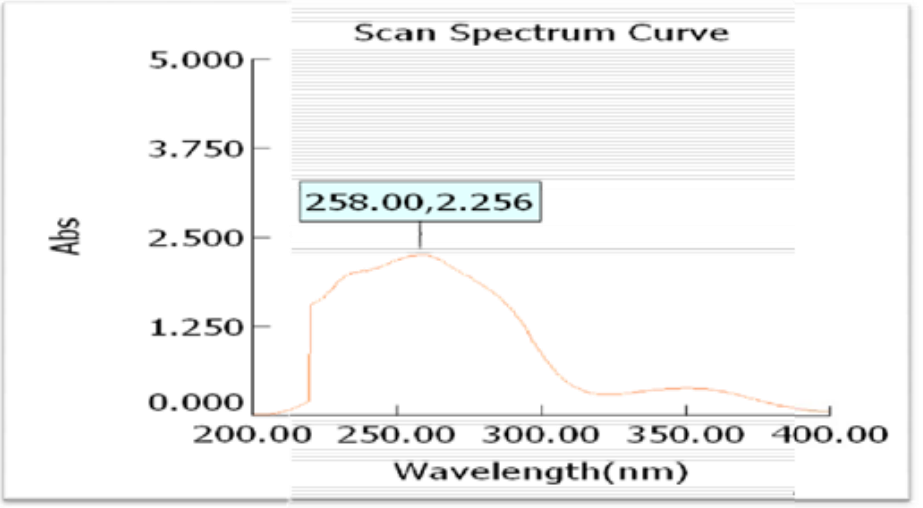
Table 1
Interpretations spectra of FTIR of drug

S.N.	Groups	Experiment peak cm $^{-1}$	Theoretical peak cm $^{-1}$
1	OH - bonds	3564.69	3570-3450
2	-N=C=N-bonds	2173.15	2175-2130
3	-C-C- multiple bond	1954.17	1960
4	Anhydride stretching vibrations	1879.27	1890-1750
5	Anhydride stretching vibrations	1761.24	1791-1747
6	-C=N- stretching vibrations	1642.20	1661-1634
7	N-H bending vibrations	1514.62, 1542.45	1550-1510
8	Sulfur	1129.00	1200-1050

Results of Solubility Study

The solubility of drug is that it is insoluble in water , 0.1 N sodium hydroxide ,Phosphate buffer pH 7.4 and soluble in0.1 N hydrochloric acid Methanol and Acetonitrile

Determination of λ_{max} of Drug at the range of 258.0 nm



Selection of λ_{max} of drug Olanzapine

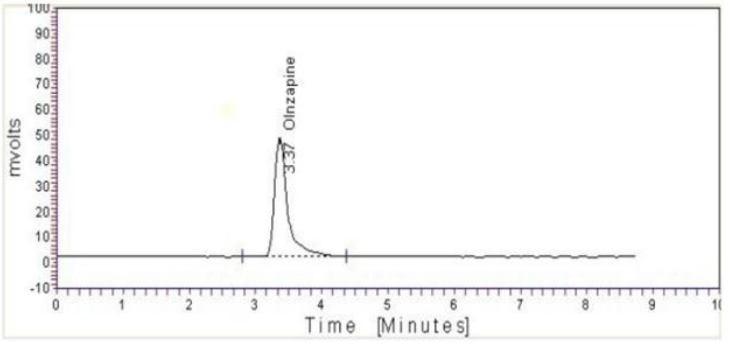
Results of RP-HPLC Methods
Mobile Phase Selection

Mobile phase	Ratio	Flow rate	Results
H_20:C_2H_5OH	50:50	1ml/min	No peak Obtain
C_2H_5OH: H_20	80:20	1ml/min	Peak Broadening
Acetonitrile:Water	50:50	1ml/min	No Peak obtain
20 mM KH_2PO_4 : Acetonitrile	70:30	1.0ml/min	Tailing
20 mM KH_2PO_4 (pH adjust with OPA 3.0): Acetonitrile	70:30	1.0ml/min	Tailing
20 mM KH_2PO_4 add 1 ml TEA (pH adjust with OPA 4.0): Acetonitrile	70:30	1.0ml/min	Tailing
Buffer:-1.75 gm KH_2PO_4 in 1000 ml water. Add 1 ml of TEA adjust the pH – 6 with OPA. Mobile phase – Buffer : Acetonitrile (60:40)	60 : 40	1.0ml/min	Most Suitable

System Suitability Parameters

System suitability Parameter	Retention time	Area under the curve	Theoretical plates	t.factor
Rep-1	2.37 5	1251.23	3078	1.18
Rep-2	2.372	1252.45	3056	1.20
Rep-3	2.374	1253.65	3098	1.15
Mean	2.374667	1250.78	3077.333	1.176667
S.D.	0.000577	3.378835	21.00794	0.025166

Linearity and Calibration Graph

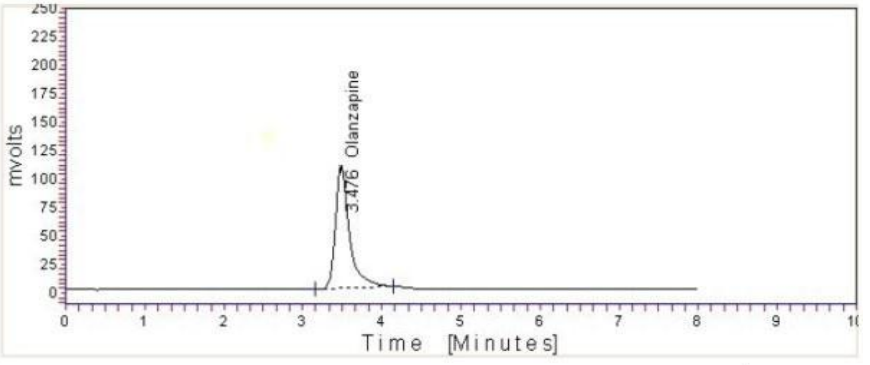


Result-A Table

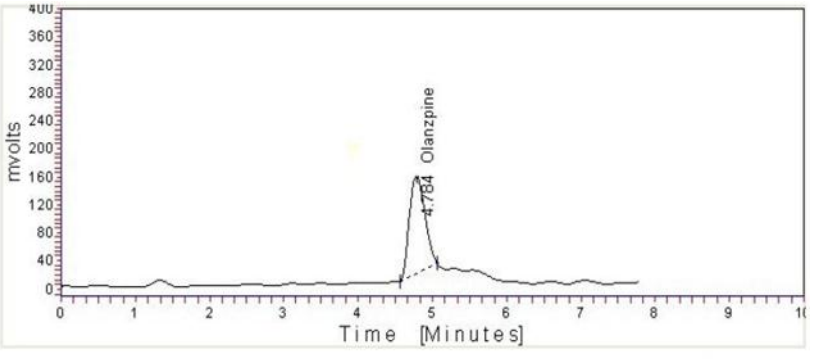
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	3.37	609.517	46.432	100	100	0.033
Total		609.517	46.432	100	100	

Chromatogram of 5 ppm

Chromatogram of 10 ppm

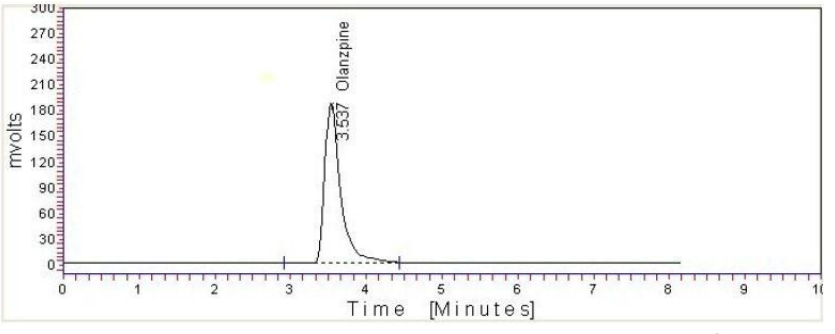


Result-A Table						
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	3.476	1251.23	108.474	100	100	0.05
Total		1251.23	108.474	100	100	



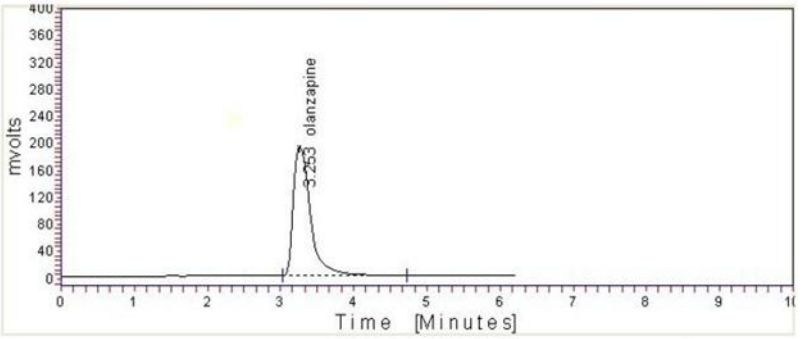
Result-A Table						
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	4.784	1863.78	138.662	100	100	0.05
Total		1863.78	138.662	100	100	

Chromatogram of 15 ppm



Result-A Table						
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	3.538	2483.691	187.024	100	100	0.05
Total		2483.691	187.024	100	100	

Chromatogram of 20 ppm



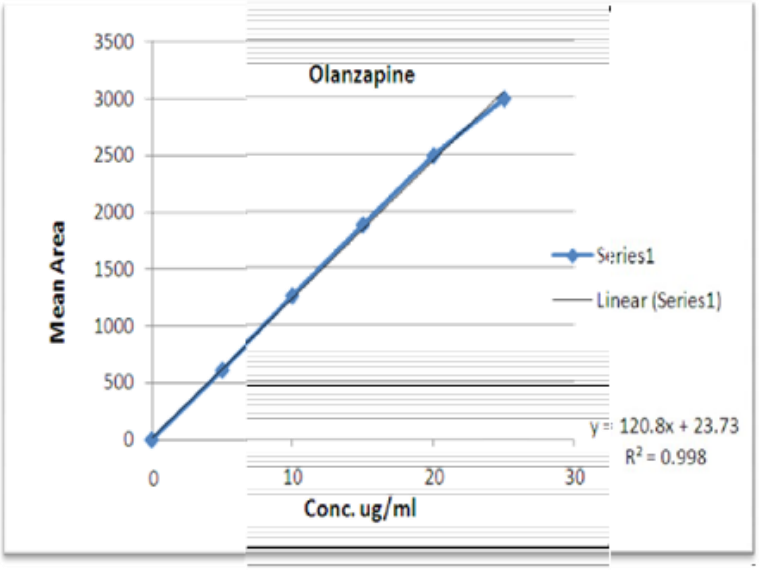
Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	3.254	2979.817	191.205	100	100	0.05
Total		2979.817	191.205	100	100	

Chromatogram of 25 ppm

Result of Linearity

Conc. µg/ml	0	5	10	15	20	25
Rep.	0	0	0	0	0	0
1	0	608.517	1252.23	1862.78	2481.691	2977.817
2	0	610.258	1251.458	1864.589	2483.985	2981.715
3	0	613.547	1257.658	1897.564	2476.985	2984.855
Mean	00	610.774	1252.782	1871.978	2482.22	2982.129
S.D.	00	3.218452	3.378835	19.58124	4.020715	3.257899
R.S.D%	000	0.537527	0.269707	1.043789	0.161915	0.109247



Calibration Curve of Olanzapine

Regression Equation

Y

=
m
x

+

c

Y
=

A
U
C
AUC + 120.8x +23.73

m= slope = 120.8

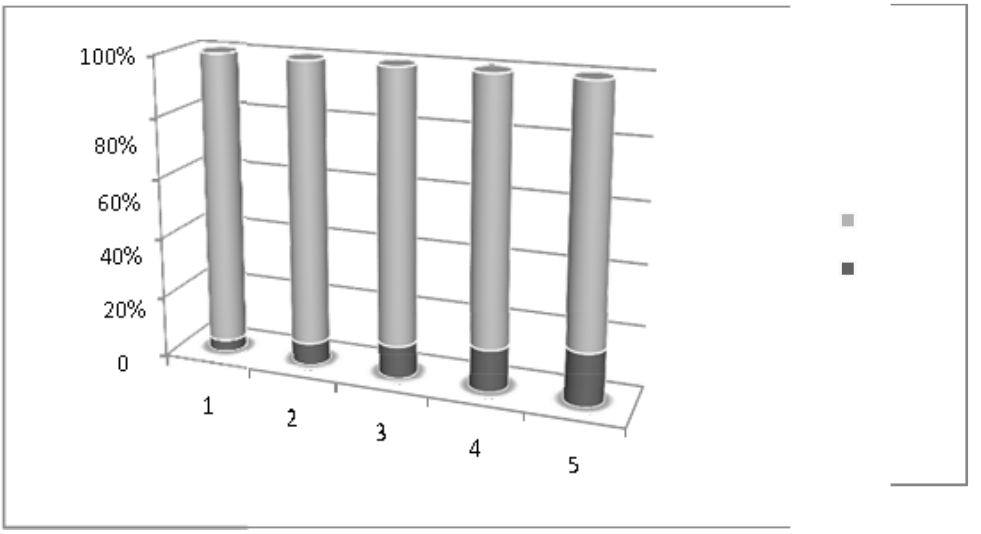
c
=
Intercept = 23.73X= Conc. in □g/ml r^2= 0.998

Assay of tablet Formulations

Standard Concentration µg/ml	Drug Olanzapine 10 (µg/ml)
Re-1	10.11
Re-2	10.06
Re-3	10.04
%	
Re-1	103
Re-2	98.504
Re-3	100.9
Mean	10.076
SD	0.746
% RSD	0.743

Validation of Developed Method
Linearity

Replicates	Concentration (□g/ml)	Mean AUC	Response Ratio
Rep-1	5	612.772	123.3547
Rep-2	10	1252.21	124.122
Rep-3	15	1874.96	124.0642
Rep-4	20	2481.32	123.164
Rep-5	25	2984.132	118.2832
Mean			122.196
S.D.			1.454
R.S.D.			2.993



Response Ratio Curve of Olanzapine

Result of Accuracy: -

Level of %Recovery	80	100	120
	Olanzapine		
Amt .present	10mg 10mg 10mg	10mg 10mg 10mg	10mg 10mg 10mg
Amt of Std. added	8 mg 8 mg 8 mg	10 mg 10 mg 10 mg	12.0 mg 12.0 mg 12.0 mg
Amt recovered	7.98 mg 8.01 mg 8.00 mg	10.05 mg 9.00 mg 8.98 mg	12.94 mg 11.02 mg 12.97 mg
Recovery	99.75 100.12	100.50 100.00	99.57 100.03

percentage	100.00	99.80	99.55

Recovery Studies validation

Level	Drug olanzapine	Recovery percentage	Standard Deviation	% RSD
80	Olanzapine	98.957	0.192	0.192
100		100.100	0.361	0.360
120		99.750	0.289	0.289

Precision:

Repeatability

Drugs	Label claims	Amount	% Label claim	Standard deviation	% RSD
Olanzapine	10 mg	9.86	98.40	0.253	0.124

Intermediate Precision- (Inter-day and Intra-day Precision)

For Intra-day		For Inter-day	
Time (hrs)	% Label Claim		
	Olanzapine		
1	98.20	I day	98.50
2	98.10	II day	98.00
3	99.00	III day	97.80
4	99.85		
5	99.70		
6	99.30		
Mean	99.93	Mean	96.6
SD	0.19436	SD	0.35055
% RSD	0.20381 4	% RSD	0.36436

Results of Analyst to Analyst

Analyst	Label claim	Amount	%Label claim	Standard deviation	% RSD
1	10	9.95	99.84	0.254	0.229

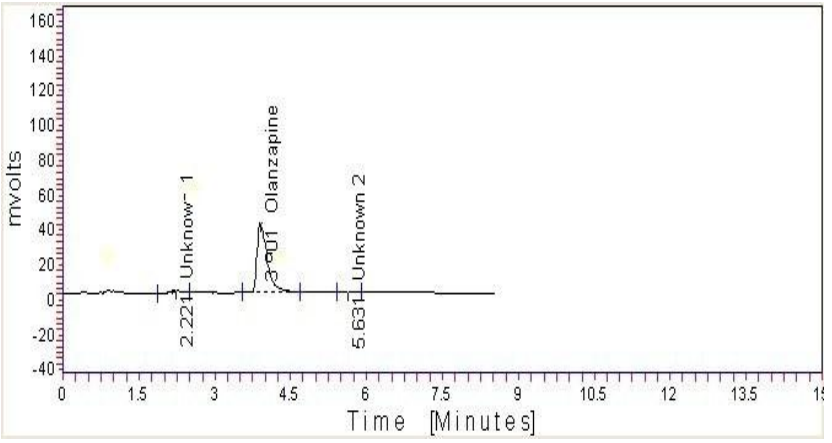
Robustness

Compounds	% RSD	Condition	
Temp.		- 5	+ 5
Olanzapine	0.52	0.68	0.51
Flow rate		10	

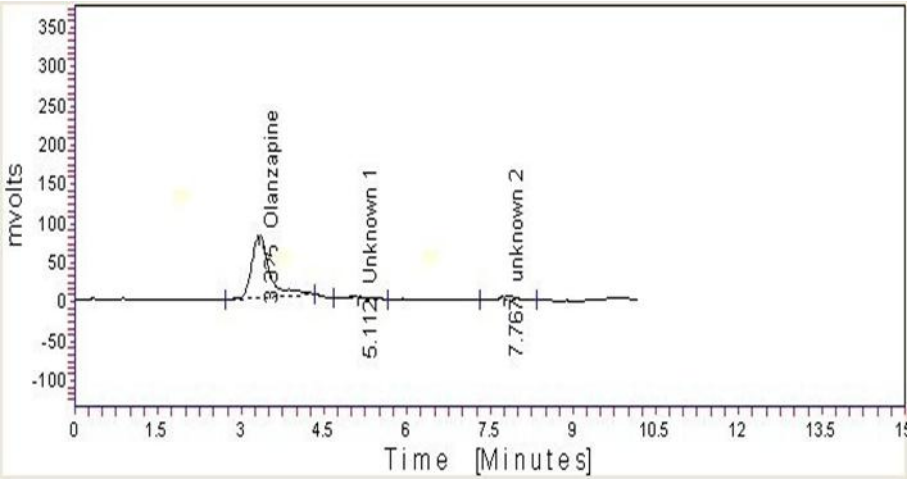
Olanzapine	0.42	0.46	0.87
Mobile phase ratio percentage		- 2	+ 2
Olanzapine	0.33	0.75	0.16

Forced Degradation Studies

conditions	%D.R	% Drug retain
drugs	97.91	0
Acid hydrolysis	82.36	15.50
Alkaline hydrolysis	87.25	12.23
Oxidative degradation	90.53	7.47
Thermal degradation	94.39	2.21
Photolytic degradation	95.46	4.54



Chromatogram of 10 µg/ml of Olanzapine after Acidic hydrolysis



Chromatogram of 10 µg/ml of Olanzapine after Alkaline hydrolysis

Selection of Separation Variable

Variable	Condition
Columns	
Dimensions.	240mm x 4.50mm
Particle Size of drug	5 µm
Bonded Phase	Octadecyl-silane
Phase preparation	
Buffer solution	50
Acetonitrile	30
F.R	1ml/min
Temp	R.T
Drug Sample	20 □1
Detected wavelength	257.0 nm
RT Olanzapine	3.576 + 0.5 min

Conclusions

The RP-HPLC methods was developed for estimation of LEVO validated as per ICH norms. The results of this study were found was more reproducible and rapid simple method to fulfil the objectives of this research

References

1. Sethi P.D.; HPLC: Quantitative Analysis of Pharmaceutical Formulation; CBS Publishers and Distributors, New Delhi; 1996; 113-202.
2. Davidson A.G., Beckett A.H. and Stenlake J.B.; Practical Pharmaceutical Chemistry; 4th edition; CBS Publishers and Distributors, New Delhi; 1989; 276-99.
3. Jeffery G.H., Bassett J., Mendham J. and Denrey R.C.; Vogel's Textbook of Quantitative Chemical Analysis; 5th edition; Longman Group UK Ltd, England; 1989; 6-14.
4. http,//www. youngincom/application/AN-0608-0115ENpdf.
5. Swarbrick, James B. and James. C.; Encyclopedia of pharmaceutical technology; Volume I; Marcel Dekker Inc., New York; 1998; 217 - 224.
6. Sahu R. Nagar P. and Jain D.; Indian J. Pharm. Science; 2006; 68(4); 503-506.
7. Jain N., Jain R., Swami H. and Pandey S.; Indian Journal of Pharmacy and Pharmaceutical sciences ;volume1;Issue1;July-Sept 2009;189-191.
8. Khan, M.R. and Jain, D.; Indian Journal of Pharmaceutical sciences; 2006;64(6);546-548.
9. Meyer Veronica R.; Practical High Performance Liquid Chromatography; 2nd edition, John wiley and sons, London;1993;56-58.
10. Braumann T., Weber G. and Grimme L.H.; J.Chromatogr; 1983;48-65.
11. Snyder L.R.; Dolan J.W. and Dolan J.W.; J.Chromatogr; 1989; 48-65.
12. Aitzemuller K.; Practice of High Performance Liquid Chromatography, springer –Verlag; 1986; 301.
13. SGE International Pty. Ltd, TA-0010-H, 2001.
14. FDA; Draft Guidance for Industry, Stability Testing of Drug Substances and Drug Products; (FDA, Rockville, MD, June 1998); glossary.

15. ICH; Guidance for Industry, Q1A(R2); Stability Testing of New Drug Substances and Products; November; 2003; 1-17.
16. European Council (1993) Directive 93/42/EEC of 14 June 1993.
17. European Council (1990) Directive 90/385/EEC of 20 June 1990 on active implantable medical devices. Chapter 8 References Lakshmi Narain College of Pharmacy, Bhopal (M.P.) Page 58
18. Dan W., Reynolds Kevin L., Facchine June F. and Mullaney; Conducting Forced Degradation Studies; Pharmaceutical Technology;2002.
19. FDA; ICH: Guideline on the Validation of Analytical Procedures: Methodology, Availability, Notice; Federal Register 62 (96); 19 May 1997; 27463–27467.
20. Conners K.A., G.L. Amidon, and Stella, V.L.; Chemical Stability of Pharmaceuticals; Wiley and Sons; New York; 2d Ed.; 1986; 375-384.
21. FDA; ICH: Guideline for the Photostability Testing of New Drug Substances and New Drug Products; ICH Q1B; Federal Register 62 (95); 16 May 1997; 27115–27122 .
22. FDA; Center for Drug Evaluation and Research; Submitting Documentation for the Stability of Human Drugs and Biologics; Rockville, MD; February 1987; 38.
23. FDA; ICH: Guideline on Impurities in New Drug Products; ICH Q3B; Federal Register (Notices); 62 (96); 19 May 1997; 27453–274561.
24. FDA; Draft Guidance for Industry, Stability Testing of Drug Substances and Drug Products FDA, Rockville, MD, June 1998; glossary.
25. FDA; ICH: Guidance on Q6A Specifications; ICHQ6A; Federal Register (Notices); 65 (251); 29 December 2000; 83041–83063.
26. Reynolds, DW; Forced degradation of pharmaceuticals; Am Pharm Rev 2004; 56–61.
27. Dolan J.W.; Stability-indicating assays; LCGC N Am; 2002; 20; 346–349.
28. Baertschi S.W.; Pharmaceutical stress testing: predicting drug degradation; Taylor & Francis, Boca Raton; 2005.
29. Yoshioka S. and Stella V.; Stability of drugs and dosage forms; Plenum Pub Corp; 2000.
30. Kanakapura Basavaiah, Anil kumar Urdigere Rangachar, and Kalsang Tharpa; Quantitative Determination of Olanzapine in Pharmaceutical Preparations by HPLC; J. Mex. Chem. Soc.; 2008; 52(2); 120-124.
31. A. Pathak and S.J. Rajput; Development of a Stability-Indicating HPLC Method for Simultaneous Determination of Olanzapine and Fluoxetine in Combined Dosage Forms; Journal of Chromatographic Science; Vol. 47; August 2009; 605-611.
32. Prameela Rani A. and Bala Sekaran C.; Development of HPLC method for the determination of olanzapine in bulk and dosage forms. International Journal of PharmTech Research Coden(USA); IJPRIF ISSN : 0974-4304; July-Sept 2009; Vol.1, No.3; 654-657.
33. Zhang Meng-qi, JIA Jing-ying, LU Chuan, et al; Development and validation of a liquid chromatography-isotope dilution tandem mass spectrometry for determination of olanzapine in human plasma and its application to bioavailability study; Acta Pharmaceutica Sinica 2010; 45 (6); 767-771.
34. Basavaiah K., Zenita O., Rajendraprasad N., Kalsang Tharpa and Vinay K.B.; Simple and sensitive spectrophotometric determination of olanzapine in pharmaceuticals using hexacyanoferrate (III) an improved protocol; Journal of Advanced Pharmaceutical Research; 2010, 1(2); 146-156.

35. Rubesh kumar S., P. Gayathri, Duganath N., Kiran C.H., Sridhar C. and Jayaveera K.N.; Simultaneous Estimation of Fluoxetine HCl and Olanzapine in Bulk Drug and Pharmaceutical Formulation by Using UV-VisibleSpectroscopy Method; International Journal of Pharmaceutical Sciences and Drug Research; 2011; 3(1); 52-55.
36. A. Rukmangada Rao, Delhi Raj N. and Jagadeesh; RP-HPLC method for Quantitative Estimation of Olanzapine in tablet Dosage forms; International Journal of Pharma and Bio Sciences; 2012 July; 3(3); 267 – 272.
37. Mahmoud A. Tantawy, Nagiba Y. Hassan, Nariman A. Elragehy and Mohamed Abdelkawy; Simultaneous determination of Olanzapine and Fluoxetine hydrochloride in capsules by spectrophotometry, TLC-spectrodensitometry and HPLC; Journal of Advanced Research 2013(4), 173–180.
38. Vilas Chaudhary and Milind Ubale; Simultaneous High-Performance Liquid Chromatographic Determination of Olanzapine hydrochloride in Pharmaceutical Dosage Form; International Journal of Advances In Pharmacy, Biology and Chemistry; Vol. 2(1); Jan- Mar, 2013; 57-62.