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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF ESCITALOPRAM AND CLONAZEPAM IN COMBINED DOSAGE FORM BY RP-HPLC

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ABSTRACT

The present work involved the development of accurate, precise, simple and suitable RP-HPLC method for estimation of the drugs in multicomponent tablet formulations. A thorough literature survey revealed few spectrophotometric methods were reported like, UV-Visible and colorimetric method for simultaneous estimation of these drugs in pharmaceutical formulations.Simple, sensitive and reliable spectroscopic methods for estimation of Escitalopram and Clonazepam in combined dosage form have been attempted. In RP-HPLC method, the analyte were resolved using KH2PO4 Buffer (20mM): and a mixture of 50:50 of Methanol and Acetonitrile (55:45), pH 6.0 at a flow rate of 1.2ml/min, on HPLC autosampler system containing UV- visible detector, and Photo diode array detector with LC-Solution Software and Nucleosil C8 column (4.6 x 150 mm). The detection was carried out at 240 nm. The method gave the good resolution and suitable retention time and an optimum suitability parameter. From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of Escitalopram and Clonazepam in their combined dosage tablet formulations. The method shows good reproducibility Compared to UV-spectrophotometric methods. The RP-HPLC method is accurate, precise, specific, reproducible and sensitive. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. The methods were found to be, rapid and economic and given optimum chromatographic parameter.

Key words: Method Development, Method Validation, Precision, HPLC, Escitalopram.

INTRODUCTION

A variety of methods are available for analyzing pharmaceutical compounds; however, high-pressure liquid chromatography is currently the method of choice for the analysis of these compounds. High-pressure liquid chromatography is at times called high-performance liquid chromatography because it offers high performance over ambient pressure or low-pressure liquid chromatography. The use of the term high performance can be debated; however, its use in the context described above is acceptable.

HPLC is used in the pharmaceutical industry for a wide variety of samples. It is the method of choice for checking the purity of new drug candidates, monitoring changes or the scale up of synthetic procedures, in-process testing for developing new formulations, and quality control/assurance of the final Drug Product.

HPLC is short for the High Performance Liquid Chromatography. HPLC is an analysis method that yields high performance and high speed compared with traditional column chromatography because of the forcibly pumped mobile phase. Recently, ultrafast analysis using a high-pressure-resistant apparatus has been attracting attention. UHPLC (Ultra High Performance LC) is becoming established as an abbreviation for this ultrafast LC method.

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This technique is based on the same method of separation as classical column chromatography i.e. adsorption, partition, ion exchange and gel permeation but it differ from column chromatography, in that mobile phase is pumped through the packed column under high pressure.

It is the most popular technique today among the different chromatographic procedures. Due to significant evolution of Liquid Chromatography (LC) instruments providing the superior qualitative and quantitative results. The development of HPLC has enabled LC to achieve great success in providing following features. [3]

- Speed of separation.
- High resolving power.
- Monitoring the column effluent.
- Repetitive and reproducible analysis.
- Automation of analytical procedure.

In all types of chromatography the two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phases to varying degrees. Those components strongly retained by the stationary phase move slowly with the flow of mobile phase and vice versa. As a consequence of these differences in migration rates, sample components separate into discrete bands, or zones, that can be analyzed qualitatively and quantitatively.[4]

DRUG PROFILE

A) **Escitalopram.**[18-19]

Structure

Chemical name: (1S)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-3H-2 benzofuran-5-carbonitrile

Description : White to slightly yellow fine powder

Molecular formula : $C_{20}H_{21}FN_2O$

Molecular weight : 324.3

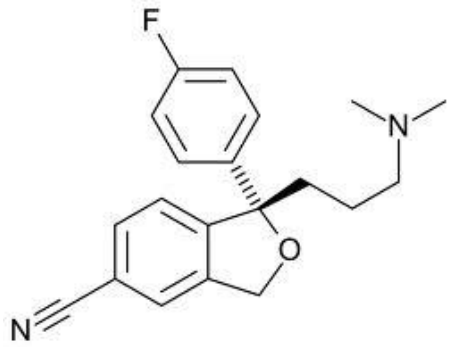
Melting point : 146-149°C

BCS Class : Class I (High Solubility and High Permeability)

Solubility : Freely soluble in Acetonitrile, methanol and dimethylsulfoxide.

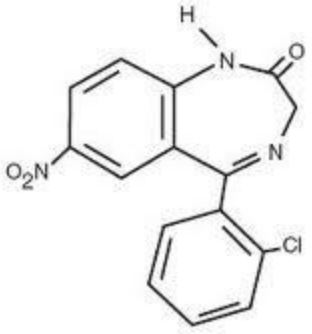
Category : Antidepressant.

Mechanism of action: Selective serotonine reuptake inhibitor.



B) **Clonazepam.**[20-21]

Structure :



Chemical name : 5(2-chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one.

Description : A light yellow, crystalline powder with faint odour.

Molecular formula : $C_{15}H_{10}ClN_3O_3$

Molecular weight : 315.7

Melting point : 236 – 238°C

BCS Class : Class II (Low Solubility and High Permeability)

Solubility : Soluble in Acetonitrile, Slightly soluble in alcohol and methyl alcohol, sparingly soluble in acetone and chloroform, Insoluble in water.

Category : Sedative, Hypnotic and Anticonvulsant.

Mechanism of action : Modulating GABA function in brain via benzodiazepine receptor

EXPERIMENTAL WORK

Material and Instruments.

Materials:

The drugs used for the present investigation were available at Mepro Pharmaceuticals Pvt. Ltd, Surendranagar, Gujarat, and obtained as follows,

A. Details Of Standards and Finished Formulation:

1. Escitalopram Oxalate

Quantity - 10.0 g

Purity (Assay) - 99.10 % w/w

2. Clonazepam

Quantity - 10.0 g

Purity (Assay) - 99.70 % w/w

3. Finished Formulation

Brand Name - Product-X

Mfd by - Mepro Pharmaceuticals Pvt Ltd

Content - 1. Escitalopram (10mg)

2. Clonazepam (0.5mg)

The Finished Formulation was obtained from Mepro Pharmaceuticals and is referred here after in this thesis by the name as Product-X.

B. Reagents and chemicals

All reagents and chemicals used were of AR grade and HPLC grade.

- Methanol (HPLC grade).
- Acetonitrile (HPLC grade).
- Potassium Dihydrogen Phosphate (AR grade).
- Water (HPLC grade)
- Triethylamine (AR grade).
- Ortho Phosphoric Acid (AR grade).
- Hexane Sulphonic Acid Sodium Salt (AR grade).

Analytical Method Development for the Estimation of Escitalopram and Clonazepam in Combined Dosage Form by HPLC Method

Method Development Strategy.

Selection of Common Solvent (Diluent):

Acetonitrile of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in mixture of Acetonitrile Methanol and Water in the ratio 40:40:20. The selection was made after assessing the solubility of both the drugs in different solvents.

Determination of λmax of ESCI and CLO:

- **Escitalopram Oxalate standard stock solution:** An accurately weighed quantity of Escitalopram Oxalate 64 mg equivalent to 50 mg was transferred to the 100 ml volumetric flask and dissolved in Acetonitrile. The volume was made up to the mark with Acetonitrile (500 µg/ml).
- **Clonazepam standard stock solution:** An accurately weighed quantity of Clonazepam 50 mg was transferred to the 100 ml volumetric flask and dissolved in Acetonitrile. The volume was made up to the mark with Acetonitrile (500 µg/ml).

  The aliquot portions of stock standard solutions of Escitalopram and Clonazepam were diluted appropriately with Diluents (Acetonitrile: Methanol: Water 40:40:20) to obtain concentration 100 µg/ml of Escitalopram and 5 µg/ml of Clonazepam. The UV scan of the solution in the range of 400 – 200 nm is shown in Fig. 15.

1. Selection of chromatographic condition for Simultaneous estimation of drugs.

a) Preparation of standard solutions:

- **ESCI stock solution:** Weighed accurately 32mg of Escotalopram oxalate working standard equivalent to Escitalopram 25mg into a 25ml volumetric flask, add 10ml acetonitrile and sonicate to dissolve. Make it up to the mark with acetonitrile.
- **Clonazepam stock solution:** Weighed accurately 50mg of Clonazepam working standard into a 50ml volumetric flask, add 10ml acetonitrile and sonicate to dissolve. Make it up to the mark with acetonitrile.
- **Escitalopram standard solution:** Pipette 2ml of Escitalopram stock solution into a 20ml volumetric flask, add 10 ml diluent (Acetonitrile: Methanol: Water 40:40:20) and mix. Finally make up to the mark with diluent.
- **Clonazepam standard solution:** Pipette 2ml of Clonazepam stock solution into a 20ml volumetric flask, add 10 ml diluent (Acetonitrile: Methanol: Water 40:40:20) and mix. Finally make up to the mark with diluent.
- **Mix standard solution:** Pipette 2ml of Escitalopram stock solution (10 µg/ml) and 2 ml of Clonazepam stock solution (10 µg/ml) into a 20ml volumetric flask, add 10 ml diluents (Acetonitrile: Methanol: Water 40:40:20) and mix. Finally make up to the mark with diluent.

b) Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing mixture of ESCI and CLO was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Nylon membrane filter 0.45µ.

Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The mobile phases tried are as follows:

Trial 1:

- **Chromatographic Parameters:**

Column	:	Inertsil C_{18}, 150 × 4.6mm, 5µm
Flow Rate	:	1 ml/min
Wavelength	:	240 nm
Injection volume	:	20µl
Column oven Temperature:		Ambient (25˚c)
Run Time	:	20 minutes
Mobile Phase	:	Mixture of Buffer, Methanol and Tetrahydrofuran in the ratio of 45:40:15

- **Mobile phase-Buffer:** Dissolved 6.8gms of anhydrous dibasic ammonium phosphate in 950 ml of HPLC water, adjusted pH 7.0 with diluted NaOH, and finally made up to 1 ltr with water.

- **Preparation of diluent:**
  Use mobile phase as diluent Resolution between two peaks is very less. Hence this method is not suitable.

Trial 2: As per USP Method of Escitalopram

- **Chromatographic Parameters:**

Column : Inertsil C8, 250 × 4.6mm, 5µm
Flow Rate : 1.5 ml/min
Wavelength : 240 nm
Injection volume : 20µl
Column oven Temperature: Ambient (25°C)
Run Time : 20 minutes
Mobile Phase : Mixture of Buffer, Methanol and Acetonitrile in the ratio of 55:25:20.To the mixture slowly added 0.94 gm of Hexane sulphonic acid sodium salt.

- **Mobile phase-Buffer:**
  Dissolved 6.8gms of monobasic potassium dihydrogen phosphate in 995 ml of HPLC water, added 5 ml of triethylamine and adjusted pH 6.0 with dilute ortho-phosphoric acid.
- **Preparation of diluent:**
  Used mobile phase as diluents
- **Escitalopram as per USP Method of Escitalopram:**
  By using above method Retention time for clonazepam is too far. Hence this method is not suitable.

Trial 3:

- **Chromatographic Parameters:**

Column : Inertsil ODS (C_{18}), 250 × 4.6mm, 5µm
Flow Rate : 1.5 ml/min
Wavelength : 240nm
Injection volume : 20µl
Column oven Temperature: Ambient
Sample cooler Temperature : Ambient
Run Time : 12 minutes
Mobile Phase : Buffer: Methanol: Acetonitrile (55:25:20)

- **Mobile phase-Buffer:**
  Dissolve 6.8gms of monobasic potassium dihydrogen phosphate in 995 ml of HPLC water, add 5ml of triethylamine and adjust pH 6.0 with orthophosphoric acid.
- **Preparation of diluent:**
  Acetonitrile of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the mixture of Acetonitrile: Methanol: Water 40:40:20.
- **Mix Standard of Escitalopram and Clonazepam:**

Trial 4:

- **Chromatographic Parameters:**

Column : Inertsil C_8, 4.6 × 250mm, 5µm
Flow Rate : 1.5 ml/min
Wavelength : 240 nm
Injection volume : 20µl
Column oven Temperature: Ambient
Sample cooler Temperature : Ambient
Run Time : 20 minutes
Mobile Phase : Buffer Methanol: Acetonitrile (55:25:20)

- **Mobile phase-Buffer:**
  Dissolve 6.8gms of monobasic potassium dihydrogen phosphate in 995 ml of HPLC water, add 5ml of triethylamine and adjust pH 6.0 with ortho phosphoric acid.

Table 1.

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Gas Chromatography (GC)	Gas-Liquid	Liquid bonded to a solid surface	Partition between gas & liquid
	Gas-Solid	Solid	Adsorption
Liquid Chromatography (LC)	Liquid-Liquid	Liquid bonded to a solid surface	Partition between immiscible liquids
	Liquid-Solid	Solid	Adsorption
	Ion exchange	Ion exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid & mobile liquid
Supercritical fluid Chromatography (SFC)		Organic species bonded to a solid surface	Partition between supercritical fluid & bonded surface

Table 2. Analysis in Tablet formulation.

Sr.No	Peak Area		Retention Time		Asymmetry (Tailing)		No. of Theorotical plates		Resolution
	CLO	ESCI	CLO	ESCI	CLO	ESCI	CLO	ESCI	
1	580011	18295870	3.53	5.97	1.062	1.645	5951	4666	7.37
2	589000	18287120	3.55	6.01	1.008	1.604	5983	4612	7.38

3	570150	17998099	3.51	6.09	1.06	1.681	5901	4620	7.51
Mean	584506	18291495	3.5	6.0	1.0	1.6	5967.0	4639.0	7.40
±S.D.	6356.2	6187.2	0.0141	0.0283	0.0382	0.0290	22.6274	38.1838	0.0071
%RSD	1.09	0.03	0.40	0.47	3.69	1.78	0.38	0.82	0.10

Table-12: Summary of system suitability of Test results.

Sr. No.	Escitalopram		Clonazepam	
	Assay (mg)	Assay % of LC	Assay (mg)	Assay % of LC
1	10.180	101.8	0.507	101.4
2	10.153	101.5	0.505	101.0
3	9.965	99.7	0.504	100.8
Average	10.099	101.0	0.505	101.1
SD	0.117	1.1358	0.0015	0.3055
% RSD	1.16	1.12	0.30	0.30

Figure-1 max of ESCI and CLO.

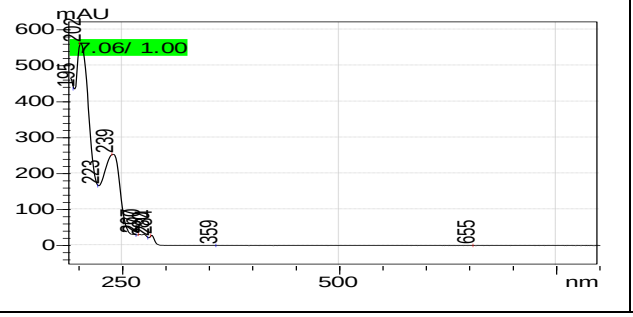
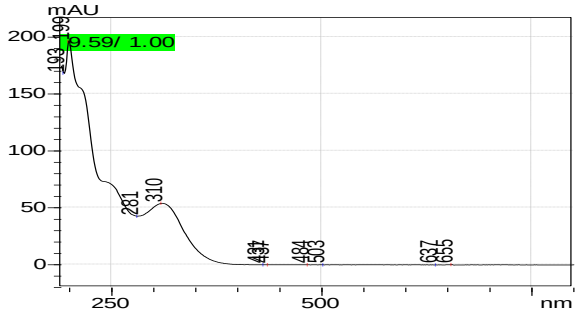
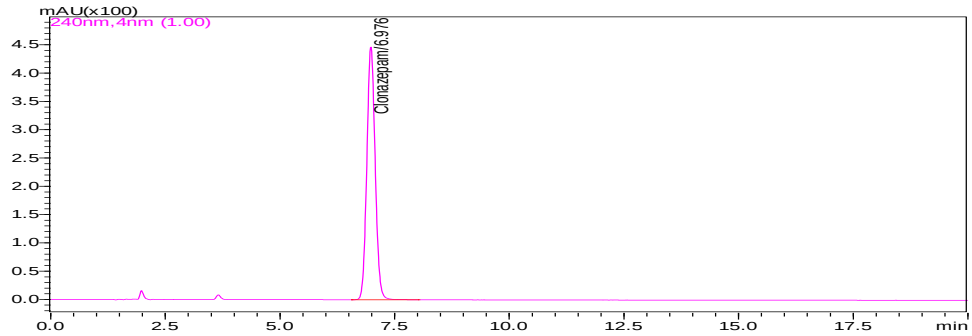
Escitalopram	Clonazepam
	

Figure-2 Chromatograms of Trial 1.

Clonazepam Standard:



Escitalopram Standard:

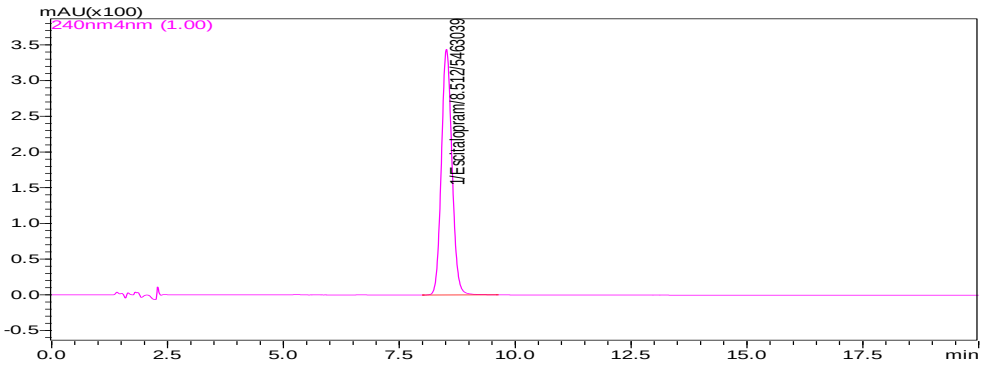
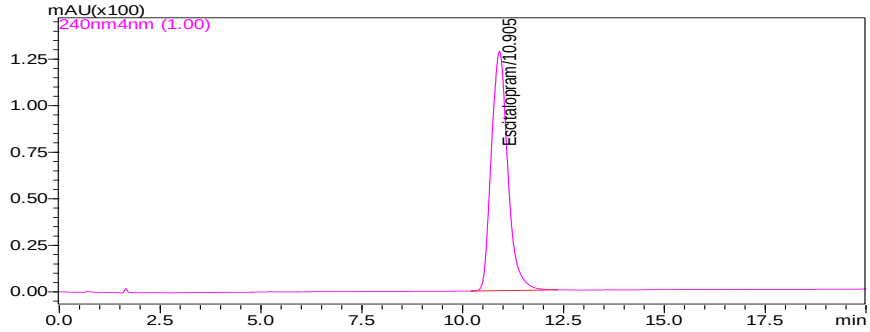


Figure-03 Chromatograms of Trial 2.
Escitalopram as per USP Method of Escitalopram:



Clonazepam as per USP Method of Escitalopram:

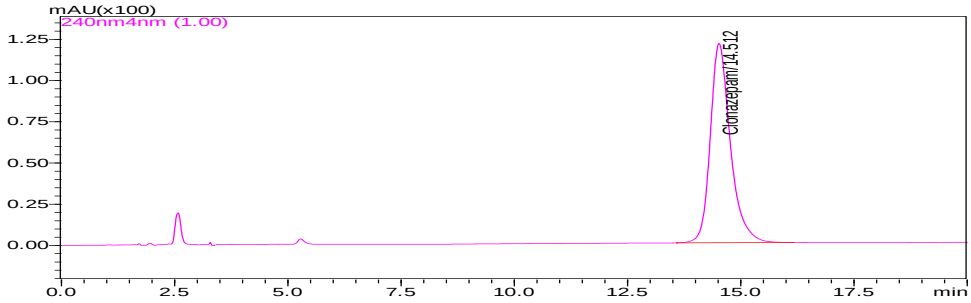


Figure-04: Chromatogram of Trial 3.
Mix Standard of Escitalopram and Clonazepam:

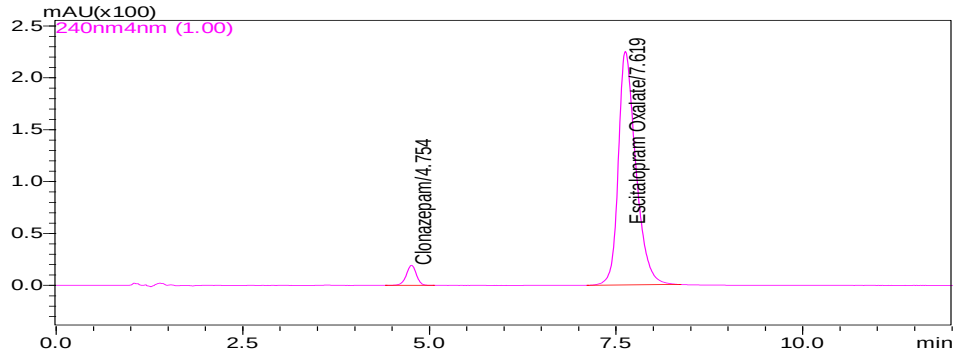
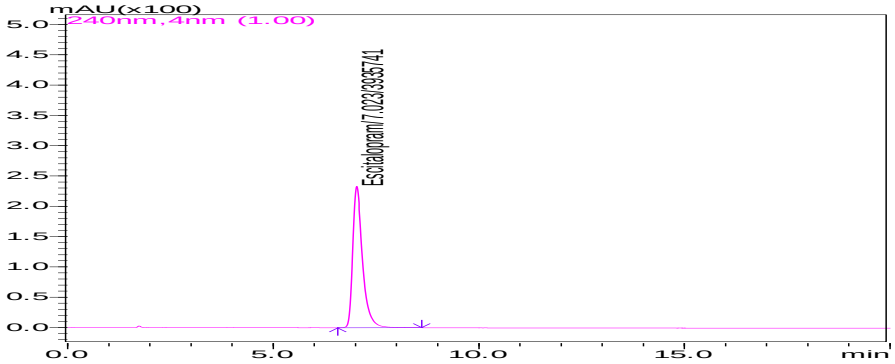
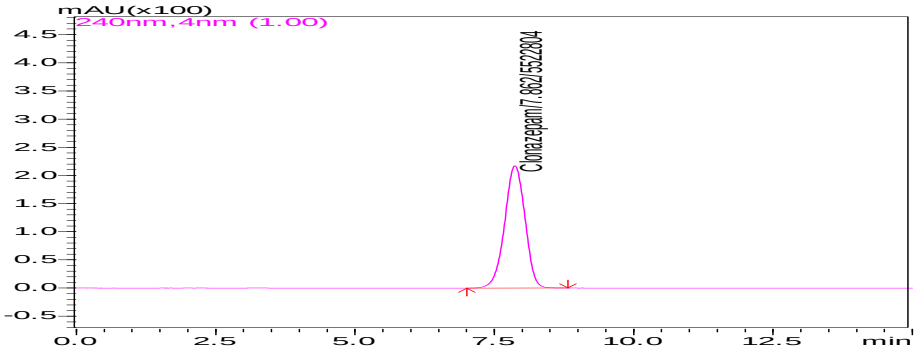


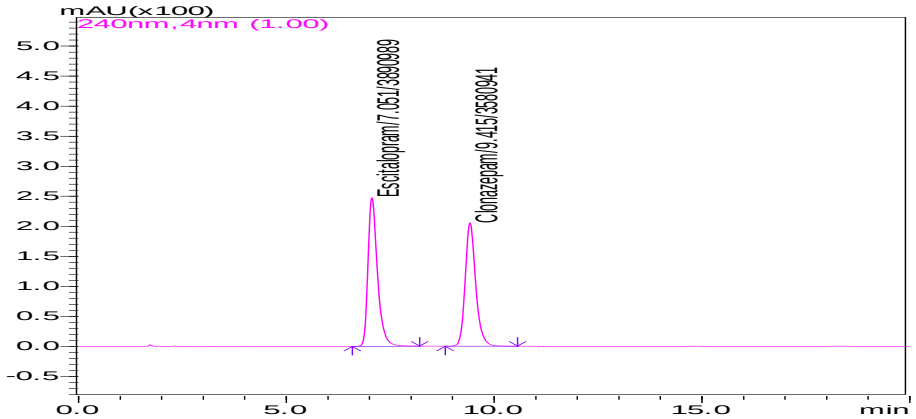
Figure-05: Chromatogram of Trial 4.
Escitalopram Standard :



Clonazepam Standard :



Escitalopram and Clonazepam mix Standard:



RESULTS AND DISCUSSION

High Performance Liquid Chromatography which is a highly sophisticated technique which can be used for single, or multiple component estimation, these method produce highly reproducible and accurate results.

Thus one such method was developed containing two components in a single formulation. In the current drug market scenario multiple drug therapy is replacing the single drug therapy; therefore the development of method for estimation of multiple components in a single method with relatively shorter analysis time is the requirement of the hour.

An antidepressant solid dosage form containing Escitalopram and Clonazepam is selected. A fixed dose combination containing Escitalopram 10mg and Clonazepam 0.5mg is recently available in market as tablet dosage form and is indicated in depression disorder.

The literature reported that following methods of analysis were available for the estimation of individual drugs.

- **Escitalopram Oxlate:**

1. zero order spectrophotometric method for estimation of escitalopram oxalate in tablet formulations
2. High performance liquid chromatographic method
3. Thin layer chromatographic method
4. Colorimetric Method for the Estimation of Escitalopram Oxalate in Tablet Dosage Form

- **Clonazepam:**

1. High performance liquid chromatographic method.
2. UV-Spectroscopic method.
3. Thin layer chromatographic method.
4. Reverse phase high performance liquid chromatographic method.

No method is so far reported for estimation of both drugs in combination.

Hence in the present work an attempt has been made to develop methods using RP-HPLC for their estimation.

Pure standards of Escitalopram Oxalate were obtained from the M/S Mepro pharmaceutical Pvt. Ltd.

Percent purity of above mentioned drugs were reported by Supplier Company as follows-

- Escitalopram Oxalate - 99.10 %w/w.
- Clonazepam- 99.70 %w/w.

These were not analyzed in our study and the % purity stated by the suppliers was taken as standard for comparison studies.

1. RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature.

This technique is commonly used for the quantitative estimation of the drugs from their formulation

this method offers advantages of estimating the constituents for the multicomponant system.

This technique was employed in the present investigation for estimation of Escitalopram and Clonazepam in tablet dosage form. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

HPLC Column Selected:

HPLC (Shimadzu) system with Nucleosil C_8 (4.6 x 150 mm, 5 µm) column and UV detector was used for the study. The standard and sample solution of ESCI and CLO were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

Mobile Phase selected:

A) Buffer and B) Methanol:Acetonitrile (50:50)
Solution-A and Solution-B in the ratio of (55:45)

- **Mobile phase-Buffer:**

Dissolve 5.46gms of monobasic potassium dihydrogen phosphate and 2.0 gm hexane sulphonate Sodium salt in 995 ml of HPLC water, added 5ml of triethylamine and adjusted pH 6.0 with dilute ortho-phosphoric acid. The wavelength 240 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λmax obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table 12.
The following chromatographic conditions were established by trial and error.

- **Chromatographic Parameters:**

Column : Nucleosil C_8, 4.6 × 150mm, 5µm
Flow Rate : 1.2 ml/min
Wavelength : 240 nm
Injection volume : 20µl
Column oven Temperature: Ambient
Sample cooler Temperature : Ambient
Run Time : 10 minutes
Mobile Phase: A) Buffer and
B) Methanol:Acetonitrile (50:50)
Solution-A and Solution-B in the ratio of (55:45).

- **Mobile phase-Buffer:**

Dissolve 5.46gms of monobasic potassium dihydrogen phosphate and 2.0 gm hexane sulphonate Sodium salt in 995 ml of HPLC water, added 5ml of triethylamine and adjusted pH 6.0 with dilute ortho-phosphoric acid

- **Preparation of diluent:**

Acetonitrile of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the mixture of Actonitrile, Methanol and Water in the ratio 40:40:20.

- **System suitability acceptance criteria:**
  1) Relative standard deviation of the area of Escitalopram and Clonazepam peaks in standard chromatograms should not be more than 2.0 %.
  2) Theoretical plate of Escitalopram and Clonazepam peak in Std chromatograms should not be less than 2000.
  3) Tailing Factor (Asymmetry) of Escitalopram and Clonazepam peaks in Standard Chromatograms should be less than 2.0.
  4)
- **Thus the results obtained for such method are given as follow:**

After establishing the chromatographic conditions, Mix standard and sample were prepared and analysed by following procedure described under experimental work. It gave accurate, reliable results and can be extended for estimation of drugs in marketed tablet formulation.

Thus the above analysis, passes the limit of % L.C. as between 98 – 102%

1. Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of Escitalopram and Clonazepam. Thus no interference was found at the Retention of ESCI and CLO which is and respectively.

CONCLUSION.

The antidepressant combination of Escitalopram and Clonazepam is available in market as a tablet formulation.

Multicomponent formulations are gaining precedence over single component formulations owing to the following reasons:

- Synergism of effects.
- Reduction of cost of treatment.
- Increased patient compliance.

Due to this rise in the multicomponent formulations, the challenges faced by the analytical chemist are on the rise. Estimation of drugs from a multicomponent formulation requires a method capable of discriminating the two or more components. Approaches to multicomponent analysis can be broadly categorized into those which rely on physical separation of components prior to analysis (e.g. chromatographic methods) and those

that do not actually separate the components (e.g. simultaneous equations method in spectroscopy).

References

1. Skoog DA, Holler FJ, Crouch SR.*et al.* Principle of Instrumental Analysis. India: *Thomson Publications*; 6, 1-3, 2007, 145-147, 180.
2. Scott RPW, Technique and Practice of chromatography. New York: *Marcel Dekker;* Vol. 70. p. 1-12.
3. Mendham J, Denney RC, Barnes JD, Thomas M.*et al.,* Vogel's Textbook of Quantitative Analysis. Singapore: *Pearson Education*; 2003, 8-9.
4. Gennaro AR, Karen BM, Medwick T.*et al,* Remington: The Science and Practice of Pharmacy. 21st ed. Pennsylvania: *Lippincott Williams & Wilkins*; 2005, 599.
5. Sepration modes in HPLC. (cited 2012 January 1); Obtained from URL-http://www.waters.com/waters/nav.htm?cid=10049076.
6. Christen GD. Analytical Chemistry. 5th ed. Massachusetts: John Wiley & Sons: 2001. p.505
7. Gennaro AR, Karen BM, Medwick T.*et al.,* Remington: The Science and Practice of Pharmacy. 21st ed. Pennsylvania: Lippincott Williams & Wilkins; 2005, 600-633.
8. Willard HH, Merritt LL, Dean JA' Settle FA.*et al.,* Instrumental Methods of Analysis. 7th Edn. New Delhi: *CBS Publisher and Distributors:* 2002, .617.
9. Sethi PD. High Performance Liquid Chromatography-Quantitative Analysis of Pharmaceutical Formulations. New Delhi: *CBS Publishers and Distributors*; 1, 3-11, 2001, 116-120.
10. Weston A, Brown PR. High Performance Liquid Chromatography- Separations in High Performance Liquid Chromatography- Instrumentation for HPLC. In: HPLC and CE – Principles and practice. USA: Academic Press: 1997. p.1-4, 8-11, 24-26, 29-32, 71.
11. Kasture AV, Mahadik KR, Wadodkar SG, More HN. Pharmaceutical Analysis- Instrumental Methods. 14th ed. Pune, India: *Nirali prakashan;* 2006, 6-7, 28-30, 49.
12. Sharma BK. Instrumental Methods Of Chemical Analysis. Meerut, India: Goel Publication Co.; 1983. p.25, 3, 6.
13. Michael WD. Modern HPLC for practicing Scientists. Massachusetts: A John Wiley & Sons Inc. Publication; 2006. p.194-205.
14. Snyder LR, Kirkland JJ. Introduction to Modern Liquid Chromatography. New York: *John Wiley & Sons*; 1979, 16-37.
15. United State Pharmacopeia, USP 29/NF 24, US Pharmacopeial Convention, *Inc, Rockville MD*, 2006, 555-556, 1777, 1861, 3050-3052.
16. The tripartite harmonized ICH Guideline Q2 (R1), Validation of analytical process: Text and methodology. Current step 4 version, (cited 2011 jan 10); Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.
17. Michael WD. Modern HPLC for practicing Scientists. Massachusetts: A John Wiley & Sons Inc. *Publication;* 2006, 194-205.
18. Biopharmaceutics Classification System (BCS) Results-Escitalopram. (cited 2011 Dec 1); Available from:
19. Escitalopram search. (cited 2011 Dec 1); Available from:
20. Evaluation of Effectiveness and safety of Antiepileptic medication in patient with epilepsy. (cited 2011 Dec 1); Available from: http://www.effectivehealthcare.ahrq.gov/index.cfm/search-for-guides- reviews-and-reports/?productid=463&pageaction=displayproduct
21. Clonazepam search. (cited 2011 Dec 1); Available from:http://www.drugbank.ca/drugs/DB01068
22. Agarwal S, Kadajji VW, Perumal SS, Tapas K,Chattaraj, Tapan KP. *et al*, Bioequivalence of two commercial preparations of escitalopram oxalate/clonazepam using a liquid chromatography-electrospray mass spectrometry method. Thieme e-journal. 58(11), 2008, 551-556.
23. Kakde R, Satone D, Bawane N. *et al.,* High Performance Thin Layer Chroatography method for simultaneous analysis of escitalopram oxalate and clonazepam in pharmaceutical preparations. *Indian J Pharm Sci.* 71(6), 2009, 702–705.
24. Gandhi SV, Dhavale ND, Jadhav VY, Sabnis SS.*et al.,* Spectrophotometric and reversed-phase high-performance liquid chromatographic methods for simultaneous determination of escitalopram oxalate and clonazepam in combined tablet dosage form. *Journal of AOAC International.* 91(1), 33-38.
25. Dhavale N, Gandhi S, Sweta S, Bothra K. Simultaneous High Performance Thin Layer Chroatography Determination of Escitalopram and Clonazepam in Combined tablets. *Journal of Chromatographia.* Vol 67, Numbers 5-6, 487-490.
26. Tapobana S, Dey S, Samal HB, Kumar DB, Mohanty DL, Bhar K. RP-HPLC method for the estimation of Escitalopram in bulk. International Journal of Chemistry Research, Vol 2, Issue 2, 2011, p.11-15.
27. Suneethaa A. Liquid chromatographic method for estimation of Ecitalopram oxalate in tablet. Vol 2/Issue 1/Jan-Feb 2011, p. 140.

28. Isabelle FB, Fabiane P, Christian J. Development of a rapid RP-HPLC method for the determination of clonazepam in human plasma. Journal of Pharmaceutical and Biomedical Analysis Volume 36, Issue 4, 19 November 2004, p. 865–869.
29. Vetrichelvan T, Arul K, Sumithra M, Umadevi B. Colorimetric method for the estimation of escitalopram oxalate. Indian Journal of Pharmaceutical Sciences 2010 Mar-Apr; 72(2): p. 269–271.
30. Taylor EH, Sloniewsky D, Gadsden RH. Automated extraction and high-performance liquid chromatographic determination of serum clonazepam. Therapeutic drug monitoring 1984; 6 (4):p. 474-477.
31. Spell JC, Steward JT. Analysis of clonazepam in a tablet dosage form using smallbore HPLC. Journal of Pharmaceutical and Biomedical Analysis Volume 18, Issue 3, November 1998, p. 453–460
32. R B Kakde, Satone DD. Spectrophotometric Method for Simultaneous Estimation of Escitalopram Oxalate and Clonazepam. Indian Journal of Pharmaceutical Sciences. 2009 Nov-Dec; 71(6): p. 702–705.