International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 4; July 2018; Page No. 35-44

RP – HPLC method development and validation for the determination of escitalopram oxalate and clonazepam in tablet dosage form

PV Subash Chandra Boss[1*], Dr. S Anbazhagan[2], P Rishop Raja[3], S Valarmathy[4], G Winkumar[5], R Suriya[6]
[1] Department of Pharmaceutical Analysis, Surya School of Pharmacy Surya Group of Institutions, Vikravandi, Tamil Nadu, India
[2-6] Surya School of Pharmacy Surya Group of Institutions, Vikravandi, Tamil Nadu, India

Abstract
A simple, specific, precise and accurate revere phase liquid chromatography method has been developed for estimation of Escitalopram oxalate and Clonazepam in solid dosage forms. The chromatographic separation was achieved on a 5 – micron C 18 column (250x 4.6mm) using a mobile phase consisting of a mixture of ORTHO PHOSPHORIC ACID pH 6: Acetonitrile: Methanol (55:20:25 % v/v) was used pH 6.0 The flow rate was maintained at 1.0 ml / min. The detection of the constituents was done using UV detector at 220 nm for Escitalopram oxalate and Clonazepam. The retention time of Escitalopram oxalate and Clonazepam found is eluted 4.06 and 6.34minutes min respectively. The developed method was validated for accuracy, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness as per the ICH guidelines.

Keywords: development and new validation, escitalopram oxalate and clonazepam tablet form and RP

Introduction
Most of the pharmaceutical industries are manufacturing new drug formulations to meet the market demand based on the literature survey Escitalopram oxalate and Clonazepam and their pharmaceutical dosage form. The Escitalopram oxalate and Clonazepam is used for the Depression, Anxiety, and Treatment of panic disorder.
Standard analytical procedure for newer drugs of formulation may not be available in pharmacopoeias; hence it is essential to develop newer analytical methods which are simple, accurate, precise, specific, economic, linear and rapid.
The survey reveals that there are only few methods reported for quantitative analysis of, Escitalopram oxalate and Clonazepam and their pharmaceutical dosage form by High performance liquid chromatography (RP-HPLC).Estimation of Escitalopram oxalate and Clonazepam and their pharmaceutical dosage forms until now made it a worthwhile project. Plan was aimed to presume the present research work by selecting Escitalopram oxalate and Clonazepam as drug.
Therefore in the proposed project, a successful attempt has been made to develop simple, accurate, economic and rapid methods for the estimation of Escitalopram oxalate and Clonazepam in bulk and various capsule formulations and to validate the methods, as a result for simple, economic, precise and accurate methods were developed and validated as follow
Today modern Pharmaceutical analysis has more emphasis to satisfy our query for better understanding of Pharmaceutical compounds, by the use of advanced instrumental methods. It also plays an important tool for quality assurance of Pharmaceutical product throughout the self-life.
Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeia; hence, it is essential to develop newer analytical methods, which are accurate, precise, and specific, linear, simple and rapid.

RP-HPLC Method
An effort has been made to indentify simple, precise, specific and accurate methods for the estimation of aspirin and Escitalopram oxalate and Clonazepam in bulk and in formulation by using RP-HPLC.
The solution of 10µg/ ml and 25 mg of Escitalopram oxalate and Clonazepam in mobile phase a mixture of ortho phosphoric acid pH 6: Acetonitrile: Methanol (55:20:25 % v/v) was used pH 6.0 was prepared and the solution was scanned in the range of 200-400 nm. At 220 nm, the drug showed maximum absorbance with 2 hours stability. Hence in this was selected as a detection wavelength. Quantification of Escitalopram oxalate and Clonazepam was done by external standard calibration method.

Materials and Methods
Materials
Drug Samples
Escitalopram oxalate and Clonazepam was purchased from Tristar Pharma Pudhucherry

Formulation used
Desilam plus (Msn Laboratories Pvt. Limited) containing Metaprolol Tartrate equivalent to 10MG AND 75MGmg was purchased from a Local Pharmacy.

Chemicals and solvents used
Distilled water (RP-HPLC GRADE), Methanol (RP-HPLC grade), Water for RP-HPLC, Acetonitrile (RP-HPLC grade) was purchased from Qualigens India Pvt. Limited and Loba Chemicals India Limited.

Instruments used
1. Shimadzu AUX- 220 Digital balance

2. Shimadzu RP-HPLC system
3. Sonicator – Sonica ultrasonic cleaner
4. Micropipette.

Specifications of instruments

a) Shimadzu AUX- 220 Digital balance (Shimadzu Instruction Manual)

Table 1

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

Model: Shimadzu, UV- 1700; Cuvetts: 1 cm quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism
Monochromator	Aberration- correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10g/l) 0.04% or less (340 nm: $NaNO_2$ 50g/l)
Measurement wavelength range	190~ 1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance: -3.99 ~ 3.99 Abs Transmittance: -399 ~ 399%
Photometric accuracy	± 0.004 Abs (at 1.0 Abs), ± 0.002 Abs (at 0.5 Abs)
Operating Temperature/ Humidity	Temperature range: 15 to 35° C Humidity range: 35 to 80% (15 to below 30° C)35 to 70% (30 to below 35° C)

b) Shimadzu HPLC (Shimadzu Instruction Manual)

Table 2

Detector Specifications	
Light source	Deuterium arc lamp
Wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 40°C (39 to 104°F)
Recording range	0.0001 to 4.000 AUFS
Operating Temperature/ Humidity	4 to 35°C/ 75 %

Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping methods	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 40°C

Methods

Selection of Chromatographic Method

Proper selection of the method depends upon the nature of the sample, molecular weight, and solubility. The drug selected for the present study was polar. Polar compounds can be separated by reverse phase chromatography, as regularly practiced, reverse phase chromatography utilizes a hydrophobic bonded phase packing, usually processing a C_{18} or C_8 functional group and polar mobile phase, usually a practically or fully aqueous mobile phase. From the above considerations for this Reverse phase chromatographic technique, C_{18} column was chosen as stationary phase, different ratios of mobile phases were performed from that a mixture of ORTHO PHOSPHORIC ACID pH 6: Acetonitrile :Methanol (55:20:25 % v/v) was selected as mobile phase.

Selection of Detection wavelength

A solution of Escitalopram oxalate and Clonazepam (10 µg/ml) was scanned in the UV region using ORTHO PHOSPHORIC ACID pH 6: Acetonitrile: Methanol (55:20:25 % v/v). The λ_{max} was found at 220nm. Escitalopram oxalate and Clonazepam have marked absorbance in all the different ionic strength of and ratios of mobile phase. There was no significant change in λ_{max}. Hence, 220nm was selected as detection wavelength for the estimation of Desilam plus by RP-HPLC method.

Initial Separation Conditions

The following chromatographic conditions were fixed initially to improve the separation of Ecosprin-av
Mode of operation: GRADIENT
Stationary phase: C_{18} Column (150 mm × 4.6 mm i.d., 5 µ)
Mobile phase: OPA PH6: Acetonitrile:Methanol
Ratio: 55:20:25 % v/v.
Detection wavelength: 220nm
Flow rate: 1.0 ml/min
Temperature: Ambient
Sample volume: 20 µl
Operating pressure: 210 kgf
Quantification method: External Standard Calibration Method
His mobile phase was allowed to run for 60 minutes to record a steady baseline. Desilam plus drug solution was injected and

chromatogram was recorded. It was observed that the drug was eluted Escitalopram oxalate and Clonazepam at 4.06 and 6.34minutes. Hence the different ratios of mobile phase were tried to get the good peak shape, short retention time and acceptable system suitability parameters.

Effect of Ratio of mobile phase

The mobile phase concentration of buffer was changed in different proportions like 50:25:25 % v/v, 55:25:20 % v/v and 55:20:25 % v/v of ORTHO PHOSPHORIC ACID pH 4: Acetonitrile 55:20:25 % v/v. The chromatograms were recorded for the above ratios. In this ORTHO PHOSPHORIC ACID pH 6: Acetonitrile: Methanol (55:20:25 % v/v), the drug was eluted Escitalopram oxalate and Clonazepam at 4.06 and 6.34minutes. In the ratio of (ORTHO PHOSPHORIC ACID pH 6: Acetonitrile: Methanol (55:20:25 % v/v) the peak shape and system suitability parameters were good. Hence, the ratio was selected for further analysis.

Effect of ratio of mobile phase

In the ratio of ORTHO PHOSPHORIC ACID pH 6: Acetonitrile: Methanol (55:20:25 % v/v) the peak shape and system suitability parameters were good. Hence, the ratio was selected for further analysis.

Buffer; Water previously adjusted to pH6.0 with orthophosphoric acid filter and degas before use.

Preparation of standard solution

A. Preparation of standard solution

Weigh accurately about 100mg of Escitalopram oxalate working standard in a 100 ml volumetric flask. Add about 20 ml of mobilephase, sonicate to dissolve, and make up to the mark with mobile phase.

B. Preparation of standard solution

Weigh accurately about 50 mg of Clonazepam working standard into a 100 ml volumetric flask. Add about 20 ml of mobilephase, sonicate to dissolve, and make up to the mark with mobile phase.

Mixed standard preparation

Take 10ml of standard solution A and 1ml of standard solution B to 100 ml with mobile phase.

Sample Preparation

Weigh and remove capsule and crush the content of 20 tablets, weigh accurately powder sample (10 mg equivalent of Escitalopram oxalate) and 05mg equvallent Clonazepam 118 mg into a 100 ml volumetric flask, add 20 ml of mobilephase. Sonicate for 10 minutes and dilute to the volume with mobile phase sonicate to dissolve too completely. Filter with o.45µ membrane filter and inject.

Preparation of calibration curve

In this method, the aliquots of stock solution of Escitalopram oxalate (6.0- 14.0ml) and Clonazepam (1-5ml) were transferred into a 100 ml of volumetric flask and made up to the mark with mobile phase. A solution contains 10, 20, 30, 40, 50µg / ml of Escitalopram oxalate and 1, 2, 3, 4, 5 µg /ml of Clonazepam in mobile phase were injected and the chromatograms were recorded at 220nm. It was found that the above concentration range was linear. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of escitalopram oxalate and clonazepam in tablet formulation

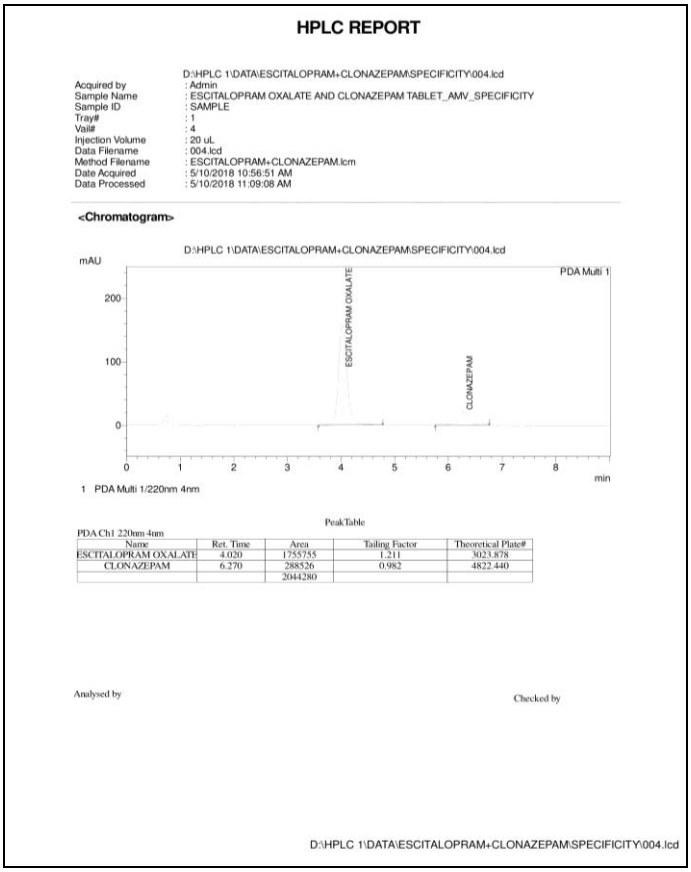
Weigh remove the Tablest and crush the content of 20 tablets, the average weight was found and powdered 10 mg equivalent of escitalopram oxalateand 75mg equvallent Clonazepam 188 mg into a 100 ml volumetric flask, add 20 ml of Mobile phase. Sonicate for 10 minutes and dilute to the volume with mobile phase sonicate to dissolve completely. Filter with o.45 µ membrane filter and inject. Inject the solution and recorded the chromatogram. The concentration of each test solution was determined by using slope and intercept values from calibration graph.

Recovery Studies

To ensure the reliability of the methods, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre – analyzed sample formulation and the content were mixed and made to the volume with mobile phase and re- analyzed by the proposed method, the percentage recovery was calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

Calibration of standard was repeated for three times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of intercept.



HPLC REPORT

D:\HPLC 1\DATA\ESCITALOPRAM+CLONAZEPAM\SPECIFICITY\004.lcd

Acquired by : Admin
Sample Name : ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET_AMV_SPECIFICITY
Sample ID : SAMPLE
Tray# : 1
Vail# : 4
Injection Volume : 20 uL
Data Filename : 004.lcd
Method Filename : ESCITALOPRAM+CLONAZEPAM.lcm
Date Acquired : 5/10/2018 10:56:51 AM
Data Processed : 5/10/2018 11:09:08 AM

<Chromatogram>

D:\HPLC 1\DATA\ESCITALOPRAM+CLONAZEPAM\SPECIFICITY\004.lcd

1 PDA Multi 1/220nm 4nm

PeakTable

PDA Ch1 220nm 4nm

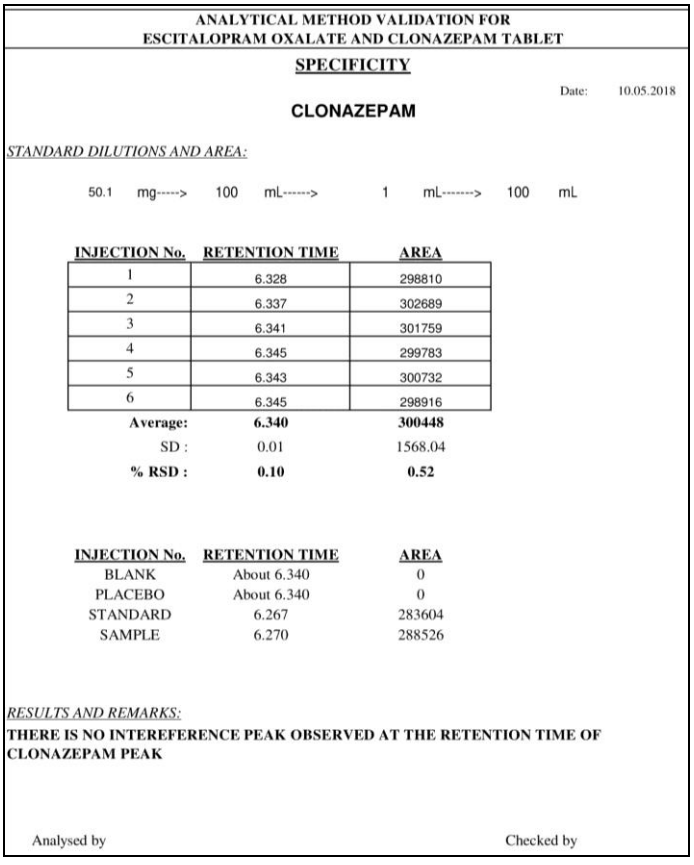
Name	Ret. Time	Area	Tailing Factor	Theoretical Plate#
ESCITALOPRAM OXALATE	4.020	1755755	1.211	3023.878
CLONAZEPAM	6.270	288526	0.982	4822.440
		2044280		

Analysed by

Checked by

D:\HPLC 1\DATA\ESCITALOPRAM+CLONAZEPAM\SPECIFICITY\004.lcd

Fig 1



ANALYTICAL METHOD VALIDATION FOR
ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET

SPECIFICITY

Date: 10.05.2018

CLONAZEPAM

STANDARD DILUTIONS AND AREA:

50.1 mg-----> 100 mL-----> 1 mL-------> 100 mL

INJECTION No.	RETENTION TIME	AREA
1	6.328	298810
2	6.337	302689
3	6.341	301759
4	6.345	299783
5	6.343	300732
6	6.345	298916
Average:	**6.340**	**300448**
SD :	0.01	1568.04
% RSD :	**0.10**	**0.52**

INJECTION No.	RETENTION TIME	AREA
BLANK	About 6.340	0
PLACEBO	About 6.340	0
STANDARD	6.267	283604
SAMPLE	6.270	288526

RESULTS AND REMARKS:

THERE IS NO INTEREFERENCE PEAK OBSERVED AT THE RETENTION TIME OF CLONAZEPAM PEAK

Analysed by Checked by

Fig 2

ANALYTICAL METHOD VALIDATION FOR
ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET

SPECIFICITY

Date: 10.05.2018

ESCITALOPRAM OXALATE

STANDARD DILUTIONS AND AREA:

127.7 mg-----> 100 mL-----> 10 mL------> 100 mL

INJECTION No.	RETENTION TIME	AREA
1	4.045	1836657
2	4.053	1829223
3	4.057	1839799
4	4.061	1839878
5	4.060	1837155
6	4.062	1837777
Average:	**4.056**	**1836748**
SD :	0.01	3923.36
% RSD :	**0.16**	**0.21**

INJECTION No.	RETENTION TIME	AREA
BLANK	About 4.056	0
PLACEBO	About 4.056	0
STANDARD	4.015	1757078
SAMPLE	4.020	1755755

RESULTS AND REMARKS:

THERE IS NO INTEREFERENCE PEAK OBSERVED AT THE RETENTION TIME OF ESCITALOPRAM OXALATE PEAK

Analysed by Checked by

Fig 3

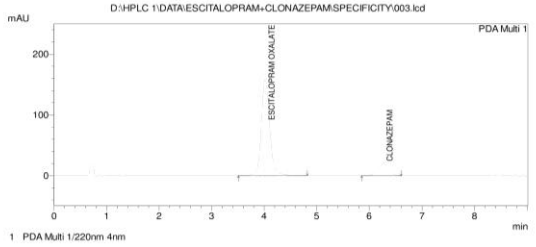
HPLC REPORT

D:\HPLC 1\DATA\ESCITALOPRAM+CLONAZEPAM\SPECIFICITY\003.lcd

Acquired by : Admin
Sample Name : ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET_AMV_SPECIFICITY
Sample ID : STANDARD
Tray# : 1
Vial# : 3
Injection Volume : 20 uL
Data Filename : 003.lcd
Method Filename : ESCITALOPRAM+CLONAZEPAM.lcm
Date Acquired : 5/10/2018 10:47:27 AM
Data Processed : 5/10/2018 11:05:06 AM

<Chromatogram>

D:\HPLC 1\DATA\ESCITALOPRAM+CLONAZEPAM\SPECIFICITY\003.lcd



1 PDA Multi 1/220nm 4nm

PeakTable

PDA Ch1 220nm 4nm

Name	Ret. Time	Area	Tailing Factor	Theoretical Plate#
ESCITALOPRAM OXALATE	4.015	1757078	1.210	3021.142
CLONAZEPAM	6.267	283604	0.993	4815.654
		2040682		

Analysed by Checked by

D:\HPLC 1\DATA\ESCITALOPRAM+CLONAZEPAM\SPECIFICITY\003.lcd

Fig 4

ANALYTICAL METHOD VALIDATION FOR ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET

LINEARITY AND RANGE

Date: **09.05.2018**

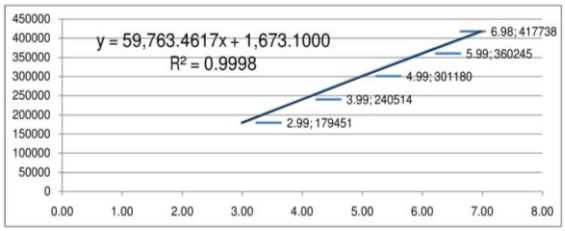
CLONAZEPAM standard purity : 99.38 %

				STANDARD DILUTIONS					FINAL CONCENTRATION
50.2	mg----->	100	mL----->	0.6	mL----->	100	mL		2.99 mcg/mL
50.2	mg----->	100	mL----->	0.8	mL----->	100	mL		3.99 mcg/mL
50.2	mg----->	100	mL----->	1.0	mL----->	100	mL		4.99 mcg/mL
50.2	mg----->	100	mL----->	1.2	mL----->	100	mL		5.99 mcg/mL
50.2	mg----->	100	mL----->	1.4	mL----->	100	mL		6.98 mcg/mL

STANDARD CONCENTRATIONS AND AREA:

Conc. (mcg/mL) (X) :	2.99	3.99	4.99	5.99	6.98
Observed Area (Y) :	179451	240514	301180	360245	417738

LINEARITY GRAPH



Analysed by Checked by

Fig 5

ANALYTICAL METHOD VALIDATION FOR ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET

LINEARITY AND RANGE

Date: **09.05.2018**

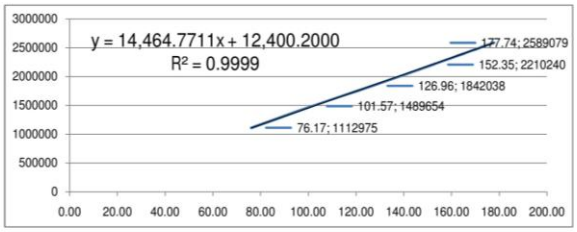
ESCITALOPRAM OXALATE standard purity : 99.34 %

				STANDARD DILUTIONS					FINAL CONCENTRATION
127.8	mg----->	100	mL----->	6.0	mL----->	100	mL		76.17 mcg/mL
127.8	mg----->	100	mL----->	8.0	mL----->	100	mL		101.57 mcg/mL
127.8	mg----->	100	mL----->	10.0	mL----->	100	mL		126.96 mcg/mL
127.8	mg----->	100	mL----->	12.0	mL----->	100	mL		152.35 mcg/mL
127.8	mg----->	100	mL----->	14.0	mL----->	100	mL		177.74 mcg/mL

STANDARD CONCENTRATIONS AND AREA:

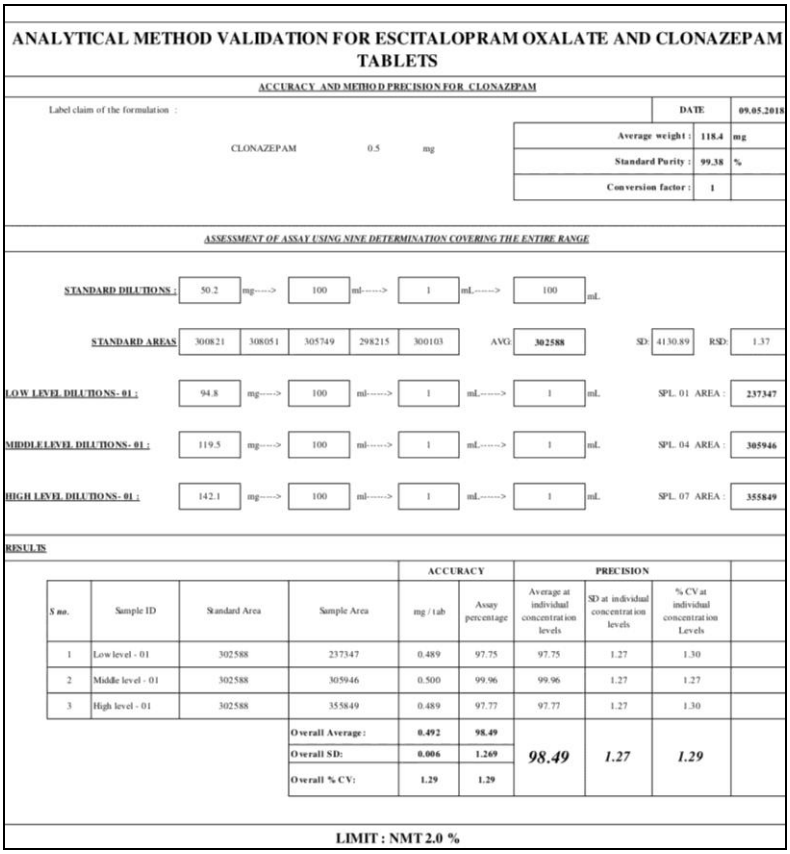
Conc. (mcg/mL) (X) :	76.17	101.57	126.96	152.35	177.74
Observed Area (Y) :	1112975	1489654	1842038	2210240	2589079

LINEARITY GRAPH



Analysed by Checked by

Fig 6



ANALYTICAL METHOD VALIDATION FOR ESCITALOPRAM OXALATE AND CLONAZEPAM TABLETS

ACCURACY AND METHOD PRECISION FOR CLONAZEPAM

Label claim of the formulation :

CLONAZEPAM 0.5 mg

DATE 09.05.2018

Average weight : 118.4 mg

Standard Purity : 99.38 %

Conversion factor : 1

ASSESSMENT OF ASSAY USING NINE DETERMINATION COVERING THE ENTIRE RANGE

STANDARD DILUTIONS : 50.2 mg-----> 100 ml------> 1 mL------> 100 mL

STANDARD AREAS 300821 308051 305749 298215 300103 AVG: 302588 SD: 4130.89 RSD: 1.37

LOW LEVEL DILUTIONS- 01 : 94.8 mg-----> 100 ml------> 1 mL------> 1 mL. SPL. 01 AREA : 237347

MIDDLE LEVEL DILUTIONS- 01 : 119.5 mg-----> 100 ml------> 1 mL------> 1 mL. SPL. 04 AREA : 305946

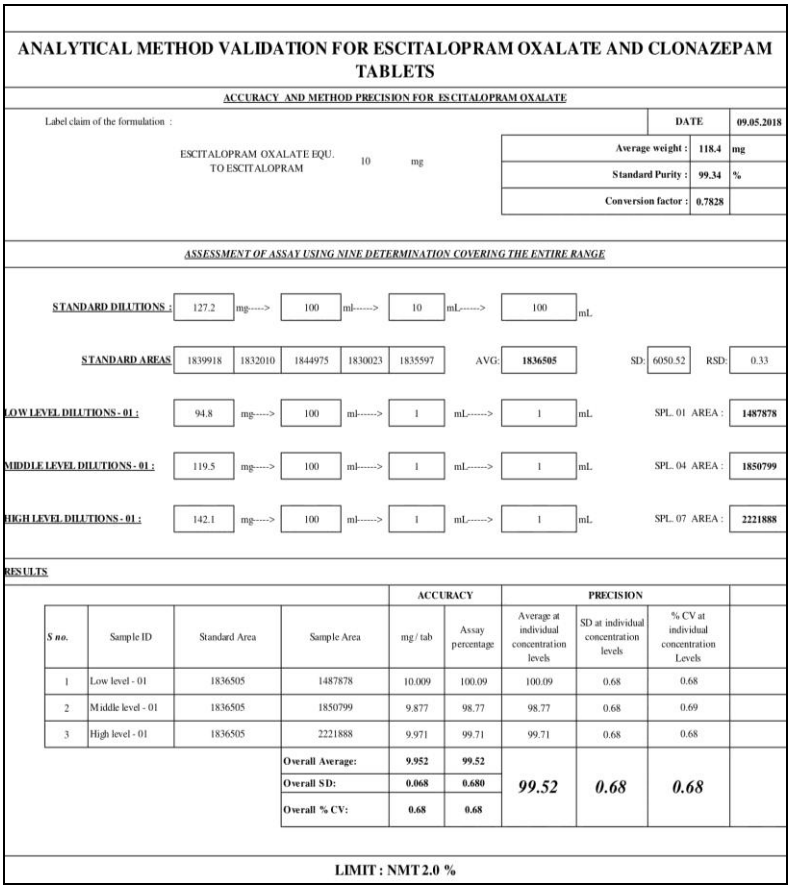
HIGH LEVEL DILUTIONS- 01 : 142.1 mg-----> 100 ml------> 1 mL------> 1 mL. SPL. 07 AREA : 355849

RESULTS

				ACCURACY		PRECISION			
S no.	Sample ID	Standard Area	Sample Area	mg / tab	Assay percentage	Average at individual concentration levels	SD at individual concentration levels	% CV at individual concentration Levels	
1	Low level - 01	302588	237347	0.489	97.75	97.75	1.27	1.30	
2	Middle level - 01	302588	305946	0.500	99.96	99.96	1.27	1.27	
3	High level - 01	302588	355849	0.489	97.77	97.77	1.27	1.30	
			Overall Average:	**0.492**	**98.49**				
			Overall SD:	**0.006**	**1.269**	***98.49***	***1.27***	***1.29***	
			Overall % CV:	**1.29**	**1.29**				

LIMIT : NMT 2.0 %

Fig 7



ANALYTICAL METHOD VALIDATION FOR ESCITALOPRAM OXALATE AND CLONAZEPAM TABLETS

ACCURACY AND METHOD PRECISION FOR ESCITALOPRAM OXALATE

Label claim of the formulation :

ESCITALOPRAM OXALATE EQU. TO ESCITALOPRAM 10 mg

DATE 09.05.2018

Average weight : 118.4 mg

Standard Purity : 99.34 %

Conversion factor : 0.7828

ASSESSMENT OF ASSAY USING NINE DETERMINATION COVERING THE ENTIRE RANGE

STANDARD DILUTIONS : 127.2 mg-----> 100 ml------> 10 mL------> 100 mL

STANDARD AREAS 1839918 1832010 1844975 1830023 1835597 AVG: 1836505 SD: 6050.52 RSD: 0.33

LOW LEVEL DILUTIONS - 01 : 94.8 mg-----> 100 ml------> 1 mL------> 1 mL. SPL. 01 AREA : 1487878

MIDDLE LEVEL DILUTIONS - 01 : 119.5 mg-----> 100 ml------> 1 mL------> 1 mL. SPL. 04 AREA : 1850799

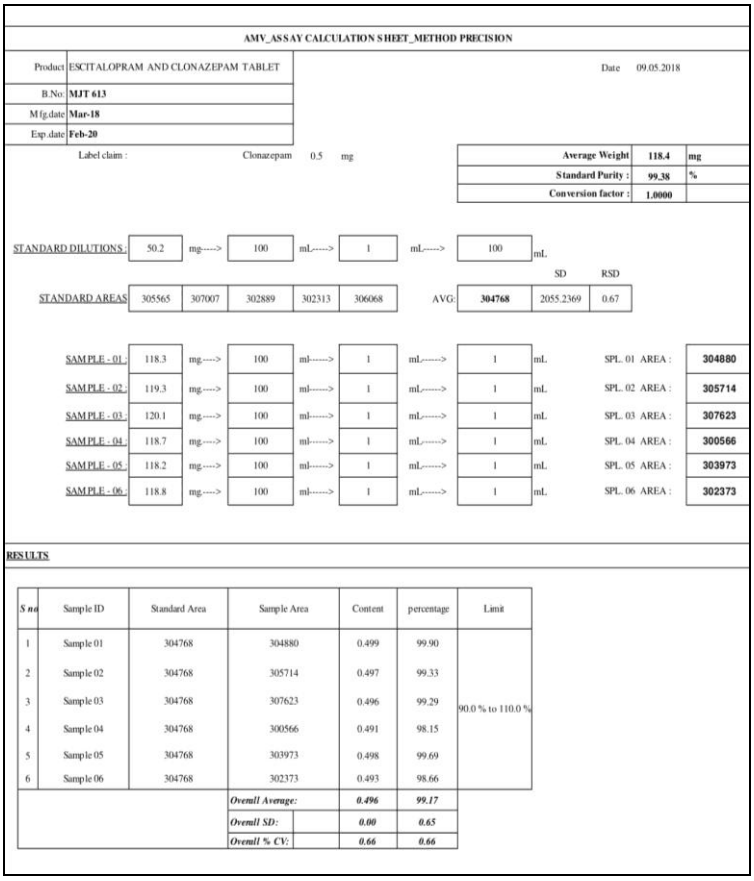
HIGH LEVEL DILUTIONS - 01 : 142.1 mg-----> 100 ml------> 1 mL------> 1 mL. SPL. 07 AREA : 2221888

RESULTS

				ACCURACY		PRECISION			
S no.	Sample ID	Standard Area	Sample Area	mg / tab	Assay percentage	Average at individual concentration levels	SD at individual concentration levels	% CV at individual concentration Levels	
1	Low level - 01	1836505	1487878	10.009	100.09	100.09	0.68	0.68	
2	Middle level - 01	1836505	1850799	9.877	98.77	98.77	0.68	0.69	
3	High level - 01	1836505	2221888	9.971	99.71	99.71	0.68	0.68	
			Overall Average:	**9.952**	**99.52**				
			Overall SD:	**0.068**	**0.680**	***99.52***	***0.68***	***0.68***	
			Overall % CV:	**0.68**	**0.68**				

LIMIT : NMT 2.0 %

Fig 8



AMV_ASSAY CALCULATION SHEET_METHOD PRECISION

Product	ESCITALOPRAM AND CLONAZEPAM TABLET
B.No:	**MJT 613**
Mfg.date	**Mar-18**
Exp.date	**Feb-20**

Date 09.05.2018

Label claim : Clonazepam 0.5 mg

Average Weight	**118.4**	**mg**
Standard Purity :	**99_38**	**%**
Conversion factor :	**1.0000**	

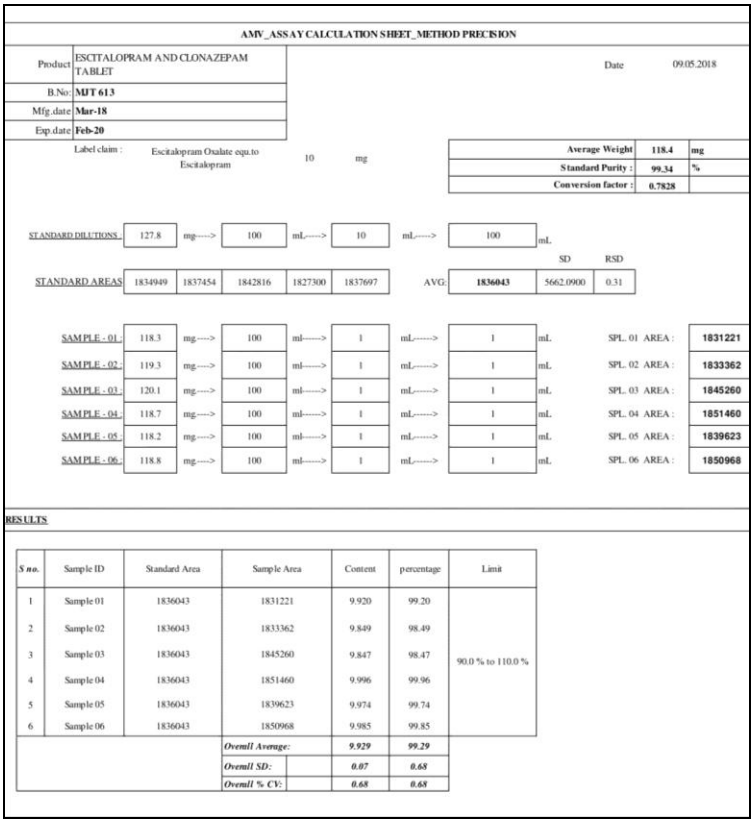
STANDARD DILUTIONS :	50.2	mg---->	100	mL----->	1	mL----->	100	mL	
								SD	RSD
STANDARD AREAS	305565	307007	302889	302313	306068	AVG:	**304768**	2055.2369	0.67

SAMPLE - 01 :	118.3	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 01 AREA :	**304880**
SAMPLE - 02 :	119.3	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 02 AREA :	**305714**
SAMPLE - 03 :	120.1	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 03 AREA :	**307623**
SAMPLE - 04 :	118.7	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 04 AREA :	**300566**
SAMPLE - 05 :	118.2	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 05 AREA :	**303973**
SAMPLE - 06 :	118.8	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 06 AREA :	**302373**

RESULTS

S no	Sample ID	Standard Area	Sample Area	Content	percentage	Limit
1	Sample 01	304768	304880	0.499	99.90	90.0 % to 110.0 %
2	Sample 02	304768	305714	0.497	99.33	
3	Sample 03	304768	307623	0.496	99.29	
4	Sample 04	304768	300566	0.491	98.15	
5	Sample 05	304768	303973	0.498	99.69	
6	Sample 06	304768	302373	0.493	98.66	
			Overall Average:	***0.496***	***99.17***	
			Overall SD:	***0.00***	***0.65***	
			Overall % CV:	***0.66***	***0.66***	

Fig 9



AMV_ASSAY CALCULATION SHEET_METHOD PRECISION

Product	ESCITALOPRAM AND CLONAZEPAM TABLET
B.No:	**MJT 613**
Mfg.date	**Mar-18**
Exp.date	**Feb-20**

Date 09.05.2018

Label claim : Escitalopram Oxalate equ.to Escitalopram 10 mg

Average Weight	**118.4**	**mg**
Standard Purity :	**99_34**	**%**
Conversion factor :	**0.7828**	

STANDARD DILUTIONS :	127.8	mg---->	100	mL----->	10	mL----->	100	mL	
								SD	RSD
STANDARD AREAS	1834949	1837454	1842816	1827300	1837697	AVG:	**1836043**	5662.0900	0.31

SAMPLE - 01 :	118.3	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 01 AREA :	**1831221**
SAMPLE - 02 :	119.3	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 02 AREA :	**1833362**
SAMPLE - 03 :	120.1	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 03 AREA :	**1845260**
SAMPLE - 04 :	118.7	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 04 AREA :	**1851460**
SAMPLE - 05 :	118.2	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 05 AREA :	**1839623**
SAMPLE - 06 :	118.8	mg---->	100	ml----->	1	mL----->	1	mL	SPL. 06 AREA :	**1850968**

RESULTS

S no.	Sample ID	Standard Area	Sample Area	Content	percentage	Limit
1	Sample 01	1836043	1831221	9.920	99.20	90.0 % to 110.0 %
2	Sample 02	1836043	1833362	9.849	98.49	
3	Sample 03	1836043	1845260	9.847	98.47	
4	Sample 04	1836043	1851460	9.996	99.96	
5	Sample 05	1836043	1839623	9.974	99.74	
6	Sample 06	1836043	1850968	9.985	99.85	
			Overall Average:	***9.929***	***99.29***	
			Overall SD:	***0.07***	***0.68***	
			Overall % CV:	***0.68***	***0.68***	

Fig 10

ANALYTICAL METHOD VALIDATION FOR ESCITALOPRAM OXALATE AND CLONAZEPAM TABLET

Date: **09.05.2018**

SYSTEM SUITABILITY

TANDARD DILUTIONS AND AREA:

SCITALOPRAM OXALATE

127.7 mg-----> 100 mL------> 10 mL-------> 100 mL

LONAZEPAM

50.1 mg-----> 100 mL------> 1 mL-------> 100 mL

ESCITALOPRAM OXALATE

INJECTION No.	AREA	RT	TF	TP
1	1836657	4.045	1.221	3144.675
2	1829223	4.053	1.215	3148.857
3	1839799	4.057	1.217	3146.771
4	1839878	4.061	1.216	3143.659
5	1837155	4.060	1.22	3148.162
6	1837777	4.062	1.218	3150.202
Average:	**1836748**	**4.05633**	**1.21783**	**3147.05**
SD :	3923	0.00644	0.0023	2.52
% RSD :	**0.21**	**0.16**	**0.19**	**0.08**

CLONAZEPAM

INJECTION No.	AREA	RT	TF	TP
1	298810	6.328	0.997	4938.502
2	302689	6.337	0.971	4923.112
3	301759	6.341	0.986	4910.913
4	299783	6.345	0.988	4936.202
5	300732	6.343	0.994	4979.82
6	298916	6.345	0.998	4978.936
Average:	**300448**	**6.33983**	**0.989**	**4944.58**
SD :	1568	0.00652	0.0100	28.72
% RSD :	**0.52**	**0.10**	**1.02**	**0.58**

ESULTS AND REMARKS:

he % coeffecient of variation (%RSD) for 6 replicate injections is below 2.0%

HEORETICAL PLATE = NLT 1500 ; TAILING FACTOR = NMT 2.0

Fig 11

System suitability studies

The system suitability studies carried out as specified in ICH guidelines and USP. The parameters like tailing factor, asymmetry factor, number of theoretical plates, capacity factor were calculated.

References

1. Ashutoshkar. Pharmaceutical Drug Analysis. Revised 2nd edn, New Age International Pvt. Ltd., New Delhi. 2005; pp. 461-462.
2. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry, Part B. 4th edn., CBS Publishers and Distributors, New Delhi. 2002; 141:279-298.
3. Alexeyev VN. Quantitative Chemical Semi Microanalysis. 1st Indian edn, CBS Publishers and Distributors, New Delhi. 2004; pp. 16-17.
4. Anonymous. Instruction manual. Shimadzu AX-200 Digital balance, Shimadzu Corporation, Kyoto, Japan. 2001; 42.
5. Anonymous. Instruction manual. Shimadzu solvent delivery module LC-10 AT*VP*, Shimadzu Corporation, Kyoto, Japan. 2001, 11.2.
6. Anonymous. Instruction manual. Shimadzu SPD-10 A_{VP} UV-VIS detector Shimadzu Corporation, Kyoto, Japan. 2001; 11:2.
7. Anonymous. Shimadzu Instruction manual Pharamaspec UV-1700 series operation guide, Shimadzu Corporation, Kyoto, Japan. 2001; pp. 6-2.
8. Anonymous. The Indian Pharmacopoeia Volume I and II. The Controller of Publication, New Delhi, 1996, 709-710.
9. Sethi PD. HPLC Quantitative Analysis of Pharmaceutical Formulations. 1st edn., CBS Publishers and Distributors, New Delhi. 2001; pp. 7-8.
10. Sharma BK. Instrumental Methods of Chemical Analysis. 26th edn., Goel Publishing House, Meerut, India. 2000; S74-S75:S86.
11. Http://www.curehunter.com/public/keywordSummaryC0 05865.do
12. Ashutoshkar. Pharmaceutical Drug Analysis. Revised 2nd edn., New Age International Pvt. Ltd., New Delhi. 2005; pp. 461-462.
13. Chatwal Gurdeep R, Sham K. Instrumental Methods of Chemical Analysis. 5h revised edn. Himalaya Publishing House, Mumbai. 2000; 2:160.
14. Code Q2A. Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines, Geneva,

Switzerland. 1994; pp. 1-3.
15. Code Q2B. Validation of Analytical Procedure: Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland. 1996; pp. 1-8.
16. Douglas Skoog A, Donald M. Analytical Chemistry. 8th edn, Thomson Asia Pvt. Ltd., Singapore. 2006; 695.
17. Francis Rouessac, Annic Rouessac. Chemical Analysis Modern Instrumental Methods and Techniques. English edn, John Willey and Sons, Ltd., New York. 2000; 45:46.
18. Willard H, Hobart Meritt Lynne, John A. Dean. And Frank A. Settle. Instrumental Methods of Analysis. 7th edn., CBS Publishers and Distributors, New Delhi. 1986; 118(141):437-539.
19. William Kemp. Organic Spectroscopy. 3rd edn., Palgrave Publishers, New York. 1996; pp. 258-259.
20. Williams Dudley H. Spectroscopic Methods in Organic Chemistry. 5th edn, Tata McGraw-Hill Publishing Company Ltd., New Delhi. 2007; 3.
21. Douglas A, Skoog Donald M, James Holler F, Timothy Nieman A. Principles of Instrumental Analysis, 5th edn., Thomson Asia Pvt. Ltd., Singapore. 2006; 973:977-979.
22. Sharma YR. Elementary Organic Spectroscopy. 4th Revised enlarged edn., S. Chand and Company Ltd., New Delhi. 2007; 11.