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Method development and the Simultaneous estimation of Escitalopram and L-methylfolate in tablet dosage form by RP-HPLC Method

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ABSTRACT

Analytical Chemistry seeks ever-improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances, which may be present in a material, and to determine the exact amounts of the identified substances. Analytical chemists work to improve the reliability of existing techniques to meet the demands for better chemical measurements, which arise constantly in our society. They adapt proven methodologies to new kinds of materials or to answer new questions about their composition. Escitalopram is one of a class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs). It is used to treat the depression associated with mood disorders. L-methylfolate is the only metabolite of folate that can cross the blood-brain barrier, and it is this form that can directly impact several important CNS reactions, most notably the synthesis of three important neurotransmitters: serotonin, norepinephrine, and dopamine. Simultaneous estimation of Escitalopram and L-methylfolate in tablet dosage form by RP-HPLC Method. The method was validated as for ICH guidelines and it can be used for the routine analysis of Escitalopram and L-methylfolate in tablet dosage form.

Keywords: Escitalopram, methylfolate, estimation, RP-HPLC, method development

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds (1). The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products (2).

Analytical Chemistry seeks ever-improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances, which may be present in a material, and to determine the exact amounts of the identified substances. Analytical chemists work to improve the reliability of existing techniques to meet the demands for better chemical measurements, which arise constantly in our society. They adapt proven methodologies to new kinds of materials or to answer new questions about their composition (3). They carry out research to discover completely new principles of measurement and are at the forefront of the utilization of major discoveries such as lasers and microchip devices for practical purposes. They make important contributions to many other fields as diverse as forensic chemistry, archaeology, and space science (4). Analytical Techniques used are as follows (5):

Spectrometric techniques

- Ultraviolet and visible spectrophotometry
- Fluorescence and phosphorescence spectrophotometry
- Atomic Spectrometry (emission and absorption)
- Infrared Spectrophotometry
- Raman Spectroscopy

- X-Ray Spectroscopy
- Radiochemical Techniques including activation analysis
- Nuclear Magnetic Resonance Spectroscopy
- Electron Spin Resonance Spectroscopy

Electrochemical techniques

- Potentiometry
- Voltametry
- Voltametric Techniques
- Stripping Techniques
- Amperometric Techniques
- Colorimetry
- Electrogravimetry
- Conductance Techniques

Chromatographic techniques

- Gas Chromatography
- High Performance Liquid Chromatography
- High Performance Thin Layer Chromatography

Miscellaneous techniques

- Thermal Analysis (DTA, DTG)
- Mass Spectrometry
- Kinetic Techniques

Hyphenated techniques

- GC-MS (Gas Chromatography – Mass Spectrometry)
- ICP-MS (Inductively Coupled Plasma - Mass Spectrometry)
- GC-IR (Gas Chromatography – Infrared Spectroscopy)
- MS-MS (Mass Spectrometry – Mass Spectrometry)

Methods In Chromatography

According to nature of stationary and mobile phase

- Solid liquid chromatography
- Liquid-liquid chromatography
- Gas Solid chromatography
- Gas liquid chromatography

According to principle of separation

A. Adsorption chromatography

- Gas Solid chromatography
- Thin layer chromatography
- Column chromatography
- High performance liquid chromatography
- Affinity phase chromatography
- Hydrophobic Interaction chromatography (HIC)

B. Partition chromatography

- Gas liquid chromatography
- Paper partition chromatography
- Column partition chromatography

Based on modes of chromatography

- Normal phase chromatography
- Reversed phase chromatography

Other types of chromatography

- Size exclusion chromatography (SEC)
- Gel permeation chromatography
- Gel chromatography
- Gel Filtration
- Gel permeation chromatography
- Ion exchange chromatography
- Chiral chromatograph

Instrumentation of High Performance Liquid Chromatography

The features of modern HPLC are illustrated in the block diagram compromise of components (6)

1. Pumping system
2. Injector
3. Chromatographic column
4. Detector
5. Data collection device (computer, integrator, or recorder)

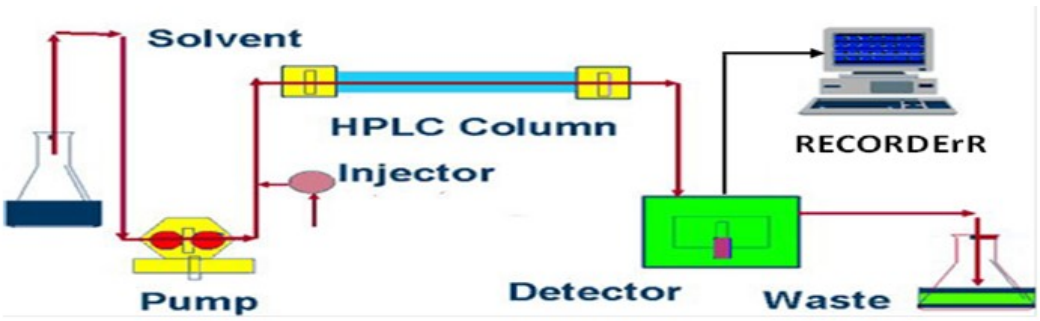


Fig 1: Schematic Diagram of HPL

Pumping system

HPLC pumping systems are required to deliver metered amounts of mobile phase at a constant flow rate. Pumping systems that deliver solvent from one or more reservoirs are available. Pressure fluctuations should be reduced, e.g. by passing the pressurized solvent through a pulse dampning device.Tubing connections should be capable of withstanding the pressures developed by the pumping systems. Many HPLC pumps are fitted with a facility for "bleeding" the system of entrapped air bubbles (7). Modern computer- or micro processor- controlled pumping systems

are capable of delivering a mobile phase of either constant(isocratic elution) or varying (gradient elution) composition, according to a defined programmed. In the case of gradient elution, solvent mixing can be achieved on either the low or high- pressure side of the pump(S) depending on the flow rate composition of mobile phase, operating pressure of up to 42000 kpa(6000 psi) can be generated during routine analysis

Injector

The sample solution is usually introduced into the flowing mobile phase at or near the head of the column using an injection system based on an injection valve design which can operate at high pressure. Such an injection system has a fixed –loop or a variable column device which can be operated manually or by an auto sampler. Partially filling of tuber may lead to poorer injection volume precision (8).

Chromatographic column

Columns are usually made of polished stainless steel, or between 50 - 300 mm long, have an internal diameter of between 2 to 5 mm. They are commonly filled with a stationary phase with a particle size of 5 -1o µm. columns with internal diameters of less than 2 mm are often reoffered to as micro bore columns (9). Desarible, the temperature of the mobile phase the column should be kept to constant during an analysis. Most separations are performed at an ambient temperature, but columns may be heated to give better efficiency normally, columns should not be heated above 6ooC because of the potential for stationary phase degradation or changes occurring to the composition of the mobile phase.

Detector

Ultraviolet/visible (UV/vis) absorption spectrometer are the most commonly used detectors for pharmaceutical analysis. In specific cases fluorescence spectrophotometers, differential refracting meters, electro chemical detectors, light scattering detectors, mass spectrometers, or other special detectors may be used. Here an analyte possesses a chromophoric group that absorbs UV/vis radiation. The UV/vis detector is the most appropriate because of its sensitivity stability. Such a detector is not suitable for detecting analytes with very weak chromophores.

Data Collection Devices

Signals from detector may be collected on chart recorders or electronic integrators that vary in complexity in their ability to process, store reprocess chromatographic data. The data storage capacity of these devices is usually limited. Modern data stations are computer based have a large storage capacity to collect, process, store data for possible subsequent reprocessing. Analytical reports can be customized to the needs of the analyst (10).

Selectivity in RP-HPLC

Three main variables can be used in RPC to change selectivity (α) for neutral samples like mobile phase composition, column type temperature. Overall sample retention acceptable is ($0.5 < K < 20$).

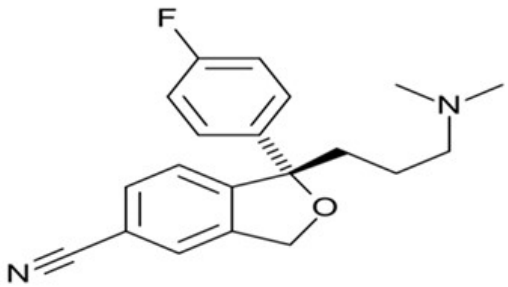
a) Solvent-strength selectivity: The best sample resolution will occur for a % B value, where both pairs have the same resolution peak spacing can be explored while % B is varied for optimum sample retention ($0.5 < K < 20$).

b) Solvent type selectivity: A change in organic solvent type is often used to change peak spacing improve resolution. The selection of RPC solvents for this purpose is guided by solvent properties that are believed to affect selectivity, acidity, basicity dipolarity. Only a slight increase (2 to 5 %) in the selectivity (α value) for a critical b pair may be necessary to achieve acceptable resolution. Changing solvent type in RPC is usually the most effective procedure to alter selectivity achieve the separation of multicomponent neutral samples.

c) Column type selectivity: A change in column type can produce useful changes in selectivity over all sample retention. Retention is greater (run time longer) on the stronger (C8 phenyl column) vs. the weaker cyano column. A change of the column is usually less useful than a change in mobile phase type hence this should be tried only after the (11) use of solvent strength or solvent type selectiveness has failed. Usually a C8 or C18 column should be tried first followed by a cyano, then by a phenyl column. Column padings bonded with cyclodextrin (CD) are useful in separation of enantiomeric isomers.

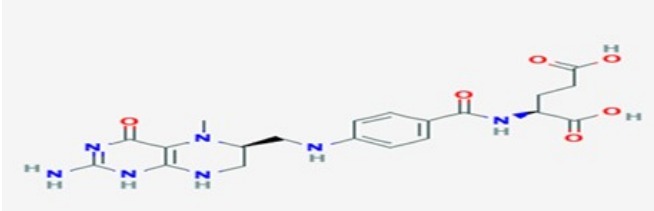
d) Temperature selectivity: Values of K decreases at higher temperature for the RPC separation of neutral compounds. This is less effective for non-ionic compounds as a mean of altering selectivity for improved separation. As the temperature is increased, the relative retention of the polar compounds decreases more rapidly than for the non-polar compounds (12).

Escitalopram



Escitalopram is one of a class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs). It is used to treat the depression associated with mood disorders. It is also used on occassion in the treatment of body dysmorphic disorder and anxiety. The antidepressant, antiobsessive-compulsive, and antibulimic actions of escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin (13). In vitro studies show that escitalopram is a potent and selective inhibitor of neuronal serotonin reuptake and has only very weak effects on norepinephrine and dopamine neuronal reuptake.

L-Methylfolate (5-MTHF)



L-methylfolate is the only metabolite of folate that can cross the blood-brain barrier, and it is this form that can directly impact several important CNS reactions, most notably the synthesis of three important neurotransmitters: serotonin, norepinephrine, and dopamine. Folic acid, a water-soluble B-complex vitamin, is found in foods such as liver, kidneys, yeast, and leafy, green vegetables (14). Folic acid is used to diagnose folate deficiency and to treat topical sprue and megaloblastic and macrocytic anemias, hematologic complications resulting from a deficiency in folic acid.

MATERIALS AND METHODS

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA 2996 Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Escitalopram and L-methylfolate solutions.

Standard Preparation (75µg/ml Escitaloprame and 100µg/ml L-methylfolate)

Accurately Weighed and transferred 7.5mg&10mg of Escitaloprame and L-methylfolate working Standards into 50ml and 50ml clean dry volumetric flasks seperately, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above each stock solution, 1 ml was pipetted out in to a 10ml Volumetric flask and then make up to the final volume with diluent (15).

Sample Preparation

20 tablets were weighed and calculate the average weight of each tablet then the tablet powder weight equivalent to 7.5mg of Escitaloprame and 10mg of L-methylfolate was transferred into a 10ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Degradation studies

Oxidation

To 1 ml of stock solution of Escitaloprame & L-methylfolate, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution of 10µl was injected into the system and the chromatograms were recorded to assess the stability of sample (16).

Acid Degradation Studies

To 1 ml of stock solutions of Escitaloprame & L-methylfolate, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample (17).

Alkali Degradation Studies

To 1 ml of stock solution Escitaloprame & L-methylfolate, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 75µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample (18).

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 75µg/ml&100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample (19).

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 750µg/ml &100µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 75µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

To 1ml of stock solution was tested under neutral conditions by refluxing the drug in water for 1h at a temperature of 60º and the resultant solution of 10 µl were

injected into the system and the chromatograms were recorded to assess the stability of the sample (20).

Method Development

Many trials were done by changing columns and Mobile phases and were reported below.

Trial: 1

Column Used : Discovery 250 x 4.6 mm, 5µ.
Mobile phase : Water: Methanol (50:50A)
Flow rate : 1ml/min
Wavelength : 230nm
Temperature : 25^0 C
Injection Volume : 10µl

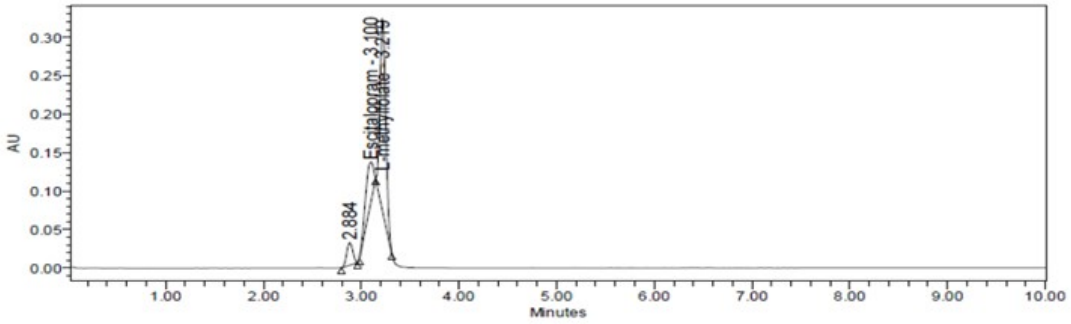


Fig 2: Trial chromatogram 1

Observation: Resolution was low so further trials was continued.

Trial: 2

Column Used : Discovery 250 x 4.6 mm, 5µ.
Mobile phase : Buffer: Acetonitrile (45:55A)
Buffer : 0.1% OPA solution
Flow rate : 1ml/min
Wavelength : 230nm
Temperature : 30^{0}C
Injection Volume : 10µl

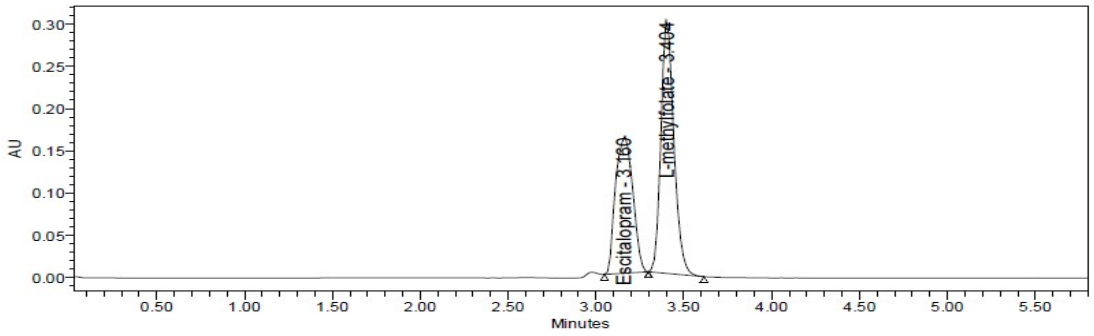


Fig 3: Trial chromatogram 2

Observation: Resolution was low so further trials was continued

Trial: 3

Column Used : Discovery 250 x 4.6 mm, 5µ.
Mobile phase : buffer: ACN (53:47A)
Buffer : 0.1%OPA
Flow rate : 1ml/min
Wavelength : 230nm
Temperature : 30^0 C

Injection Volume : 10µl

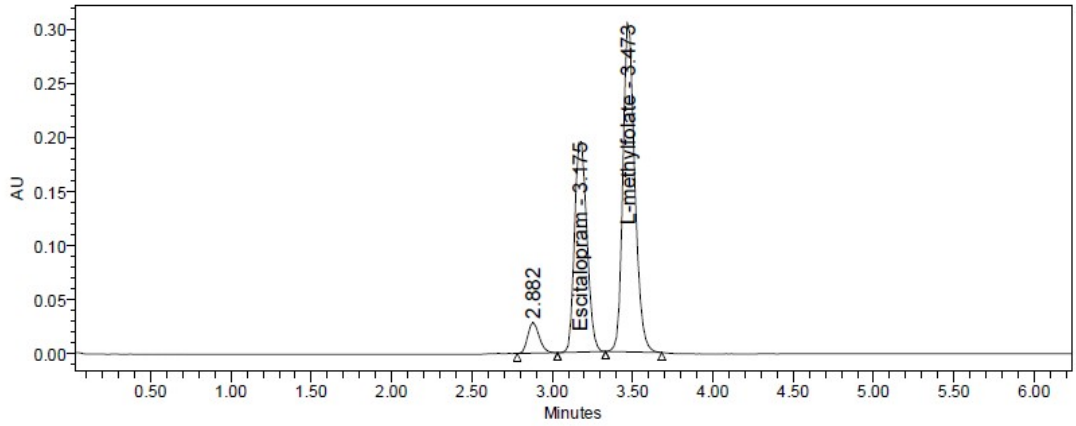


Fig 4: Trial chromatogram 3

Observation: base line noise was observed.

Trial: 4

Column Used : Kromasil 150 x 4.6 mm, 5µ.
Mobile phase : buffer: Acetonitrile (25:75A)
Buffer : 0.1%OPA solution
Flow rate : 1ml/min
Wavelength : 230nm
Temperature : 30^0 C
Injection Volume : 10µl

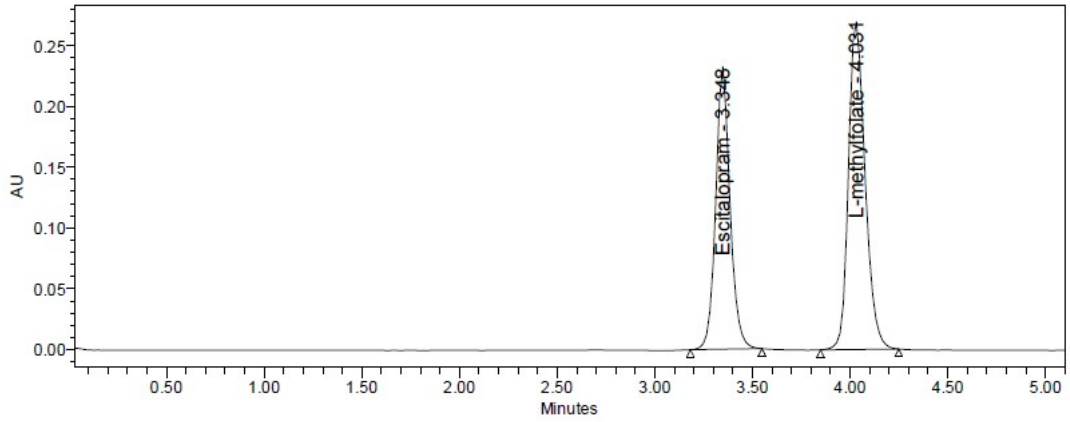


Fig 5: Trial chromatogram 4

Observation: peak shape and retention time was not good.

Trail: 5

Column Used : Discovery 250 x 4.6 mm, 5µ.
Buffer : 0.1% OPA solution
Mobile phase : buffer: Acetonitrile (55:45)
Flow rate : 1.0ml/min
Diluent : water: ACN (50:50)
Wavelength : 230nm
Temperature : 30^0 C
Injection Volume : 10µl

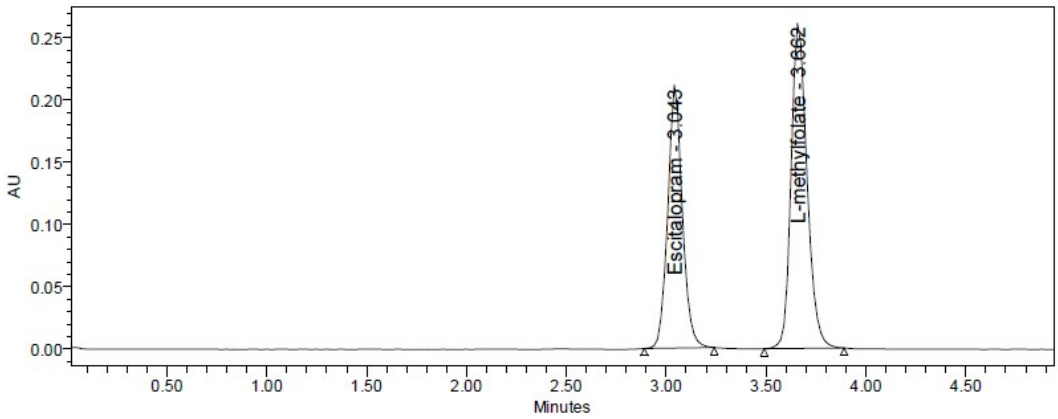


Fig 6: Trial chromatogram 5

Observation: peak shape and retention time is good .
Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : Discovery 250 x 4.6 mm, 5µ.
Buffer : 0.1% OPA solution
Mobile phase : buffer: Acetonitrile (55:45)
Flow rate : 1.0ml/min
Diluent : water:acn: 50:50
Wavelength : 230nm
Temperature : 30^0C
Injection Volume : 10µl

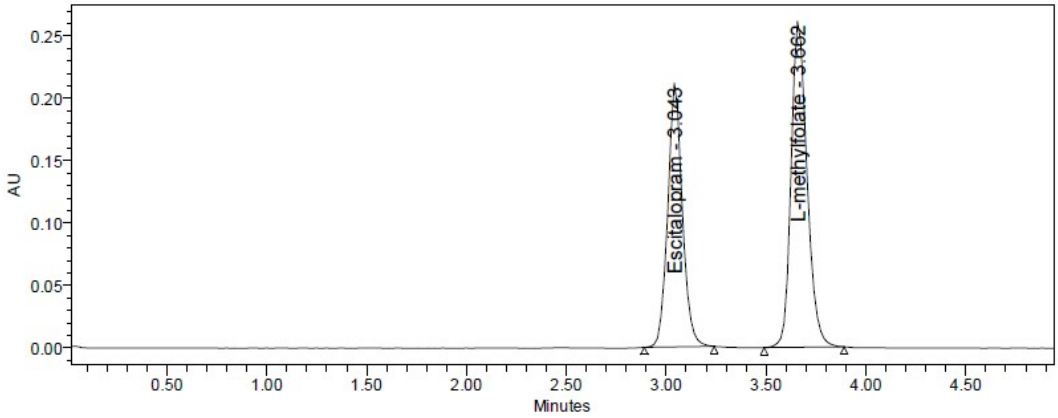


Fig 7: Optimized chromatogram of Escitalopram and L-methylfolate

Observation: peak shape and retention time is good

RESULTS AND DISCUSSIONS

Method Development

For the method development several trials were carried out and reported. These leads to the optimized chromatographic conditions for the estimation of Escitalopram and L-methylfolate in pharmaceutical dosage form. Initially various mobile phase and stationary phase were tested in an attempt to obtain the best resolution for Escitalopram and L-methylfolate. The mobile phase consisting of water pH adjusted to 2 with Orthophosphoric acid, at a flow rate of 1 ml/min was chosen for method development and validation of Escitalopram and L-methylfolate by RP-HPLC method. The detection was selected at 218nm, using reverse phase Discovery C18(250mm x 4.6 mm, 5m)column, the retention time of Escitalopram and L-methylfolate were found to be 3.010min and 3.668min respectively. The total run time was 7 minutes.

A mobile phase consisting of water pH adjusted to 2 with Orthophosphoric acid was selected to achieve maximum separation and sensitivity. The flow rate of 1 ml/min gave an optimal signal to noise ratio with reasonable separation time.

Method Validation

System suitability

All the system suitability parameters are within range and satisfactory as per ICH guidelines

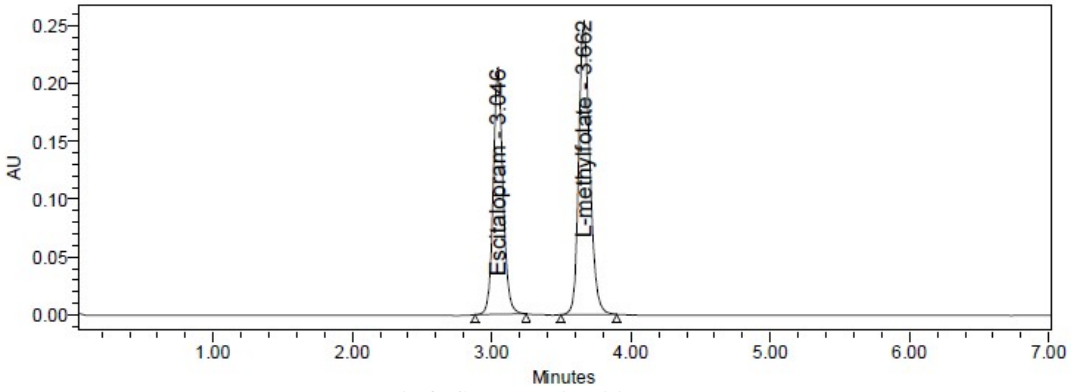


Fig 8: System suitability chromatogram

Table 1: System suitability parameters of chromatogram

Property	Escitalopram	L-methylfolate
Retention time (t_R)	3.046min	3.662min
Theoretical plates (N)	8669 ± 63.48	10198 ± 63.48
Tailing factor (T)	1.07 ± 0.117	1.19± 0.117

Observation

- Retention time for Escitalopram and L-methylfolate 3.046 min and 3.662 min respectively.
- Tailing factor of Escitalopram and L-methylfolate 1.07 ± 0.117 and 1.19± 0.117 respectively.
- Number of theoretical plates for Escitalopram and L-methylfolate 8669 ± 63.48 and 10198 ± 63.48 respectively.

Discussion

All the system suitability parameters are found to be satisfactory. The peak is reasonably symmetrical. High numbers
of theoretical plates indicate efficient performance of the column with reasonable retention times.

Table 2: Calibration data of Escitalopram and L-methylfolate method.

S.no	Concentration Escitalopram (µg/ml)	Response	Concentration L-methylfolate (µg/ml)	Response
1	0	0	0	0
2	18.75	272570	25	381343
3	37.5	528238	50	720007
4	56.25	814528	75	1081548
5	75	1033208	100	1399575
6	93.75	1295106	125	1764674
7	112.5	1533037	150	2061337

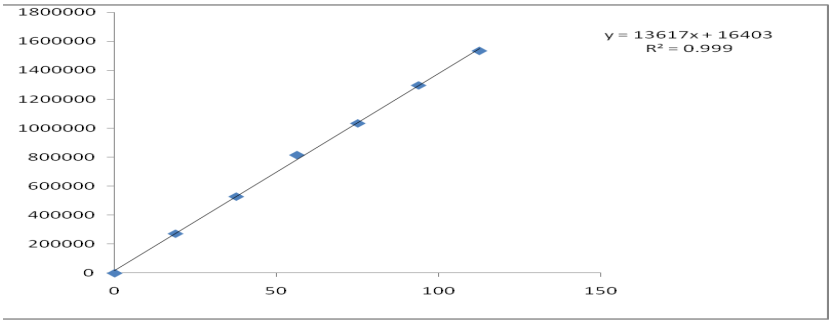


Fig 9: Calibration curve of Escitalopram

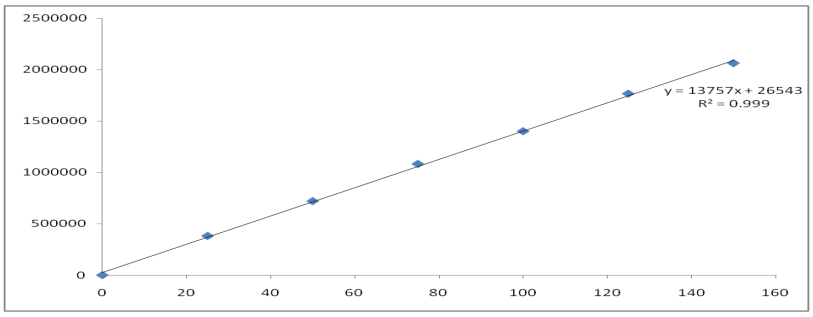


Fig 10: Calibration curve of L-methylfolate

Table 3: Table of Accuracy

Sample	Concentration (%) (µg/ml)	Recovery (%)	% RSD
Escitalopram	37.5	100.26	1.50
	75	99.72	0.33
	112.5	98.48	0.18
L-methylfolate	50	100.04	1.00
	100	99.46	1.58
	150	100.46	0.72

Limit Of Detection

Limit of detection was calculated by std deviation method Escitalopram and L-methylfolate and LOD for Escitalopram and L-methylfolate were found to be 0.29and 0.38 respectively.

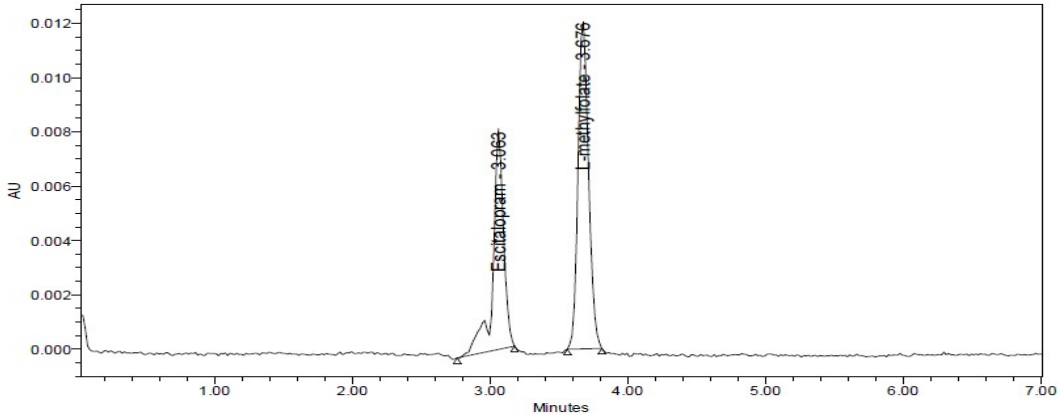


Fig 11: LOD Chromatogram of Escitalopram and L-methylfolate

Table 4: Limit of Detection

Peak Name	RT	Area	s/n	USP Plate count	USP Tailing
Escitalopram	3.063	43578	240.8	10142.1	0.7
L-methylfolat	3.676	63952	364.7	10719.7	1.1

Limit of Quantitation

Limit of Quantification was calculated by std deviation method Escitalopram and L-methylfolate and LOQ for Escitalopram and L-methylfolate were found to be 0.89 and 1.15 respectively.

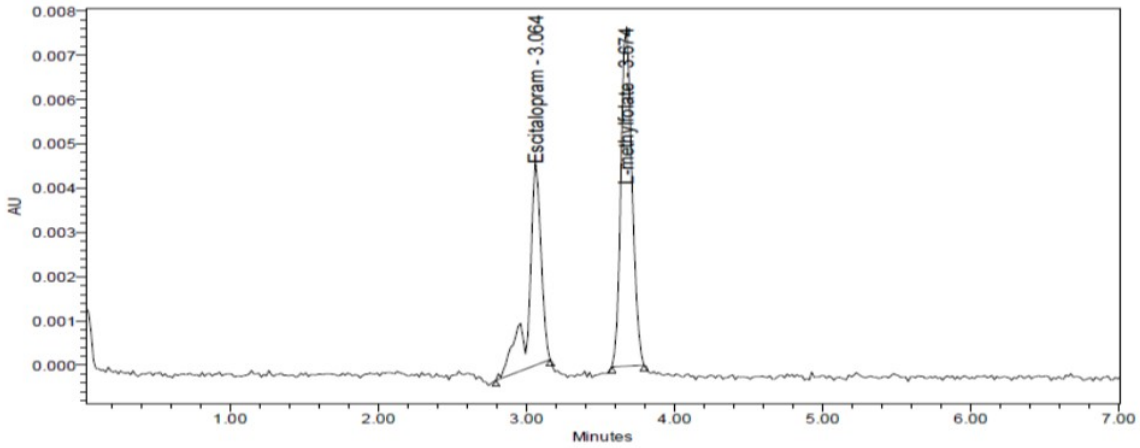


Fig 12: LOQ Chromatogram of of Escitalopram and L-methylfolate

Table 5: limit of quantitation

Peak Name	RT	Area	s/n	USP Plate count	USP Tailing
Escitalopram	3.064	27027	124.7	9278.6	0.7
L-methylfolat	3.676	39883	211.0	10989.4	1.1

Table 6: Robustness data of Escitalopram and L-methylfolate

S.NO	Robustness condition	Escitalopram %RSD	L-methylfolate %RSD
1	Flow minus(0.9ml/min)	0.1	0.1
2	Flow Plus(1.1ml/min)	0.0	0.1
3	Mobile phase minus(50:50)	0.7	0.7
4	Mobile phase Plus(45:55)	0.1	0.1
5	Temperature minus(25^{0c})	0.1	0.1
6	Temperature Plus(30^{0c})	0.1	0.1

DISCUSSION

As per above observations, it was found that the % Relative standard deviation of replicate injections of standard preparations with ±0.1 flow rate and ±5nm wavelength was found to be 2.0%. Also system suitability parameters such as Tailing, Tangent / Column efficiency and %RSD of replicate injections of standard preparation, are meeting the requirements. Hence the proposed method is robust for the estimation of Enalapril and Felodipine even with variation of different flow rate.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.5% and 100.37% for Escitalopram and L-methylfolate respectively

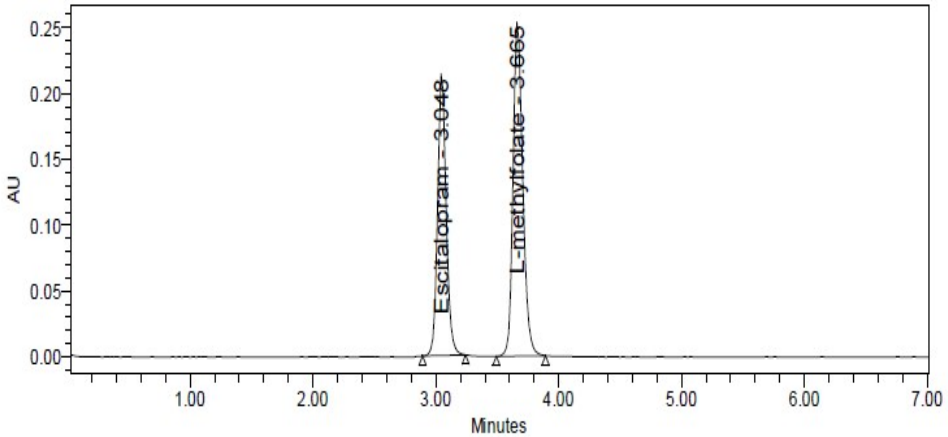


Fig 13: Assay of Tablet

Table 7: Assay of Tablet

S. No.	Escitalopram %Assay	L-methylfolate %Assay
1	99.63	99.60
2	99.59	99.40
3	101.42	101.09
4	100.36	101.36
5	101.80	101.11
6	100.23	99.66
AVG	100.5	100.37
STDEV	0.9171	0.9049
%RSD	0.91	0.90

Degradation studies

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali, oxidative, photolytic, thermal and neutral analysis.

Table 8: Degradation data

Type of degradation	Escitalopram			L-methylfolatee		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	1002308	96.99	3.01	1338711	95.44	4.56
Base	1004372	97.19	2.81	1363642	97.22	2.78
Peroxide	1013347	98.06	1.94	1380153	98.39	1.61
Thermal	1028055	99.49	0.51	1390184	99.11	0.89
Uv	1031605	99.83	0.17	1398277	99.68	0.32
Water	1030472	99.72	0.28	1396733	99.57	0.43

SUMMARY AND CONCLUSION

Table 9: Summary of Validation Parameters

S.No.	Validation Parameters	Requirement	Results		Acceptance Criteria
			Escitalopram	L-methylfolate	
1.	Specificity	No Interference	Pass	Pass	No Interference
2.	Linearity	Correlation Coefficient	0.999	0.999	NLT 0.999
3.	Accuracy	%Recovery	99.48%	99.98%	100 ± 2%
4.	Method Precision	%RSD	0.5	1	NMT 2%
	Ruggedness		0.9	0.9	NMT 1%
5.	Robustness	%RSD	0.97	0.57	NMT 1%
6	LOD		0.29 µg/ml	0.38 µg/ml	
7	LOQ		0.89 µg/ml	1.15µg/ml	
8.	System Suitability	RT	3.046 min	3.662 min	-
		Tailing Factor	1.07	1.19	NMT 2
		Plate Count	8669	10198	NLT 4000
		Assay Value	100.5%	100.37%	100 ± 2%

CONCLUSION

A novel, sensitive, accurate, specific, precise method was developed for the Simultaneous estimation of Escitalopram and L-methylfolate in tablet dosage form by RP-HPLC Method. The method was validated as for ICH guidelines and it can be used for the routine analysis of Escitalopram and L-methylfolate in tablet dosage form.

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