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Stability Indicating Method Development and Validation for the Estimation of Escitalopram and L-Methylfolate in Bulk and Pharmaceutical Dosage Form by RP -HPLC

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ABSTRACT

In the presented work the stability indicating RP-HPLC method was developed for the simultaneous estimation of the L-methyl folate and Escitalopram in bulk and pharmaceutical dosage form .The L methyl folate and Escitalopram were analysed through Std BDS C18 150 x 4.6 mm, 5µ.Using the mobile phase consisting of (0.01N KH_2PO_4 buffer: Acetonitrile)(50:50,v/v) with a flow rate of 0.9ml/min. The wavelength was selected at 230 nm using uv detection. The L Methyl folate and Escitalopram were eluted at 2.232min and 3.279min respectively. The drug was stressed under alkaline, oxidative, thermal, photolytic degradation were analysed. The developed method was validated as per ICH guidelines The Accuracy, Linearity, Precision, and Robustness were within the acceptance limits .Hence this HPLC method was a stability indicating method can be used for routine stability analysis of the Escitalopram and l methyl folate in Pharmaceutical dosage forms.

Keywords: L-methyl folate, Escitalopram, RP-HPLC.

INTRODUCTION

L-methyl folate

The chemically (2S)-2-[[4-[(2-amino-4-oxo-1H-pteridin-6-yl) methylamino] benzoyl] amino] pentanedioic acid is a B complex vitamin containing a pteridine moiety linked by a methylene bridge to Para-amino benzoic acid, which is joined by a peptide linkage to glutamic acid. Levomefolic acid was primary biologically active form of folic acid used at the cellular level for DNA reproduction. A-vitamin (B9) essential to human health and function. One of its most notable functions is its role in creating key neurotransmitters or brain chemicals that regulate human mood, cognitive ability and arousal. **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,

The three primary brain chemicals are dopamine, nor epinephrine and serotonin. Folic acid is soluble in 1 M NaOH (50 mg/ml).The free acid is only slightly soluble in water (0.01 mg/ml) at 0 °C.

Escitalopram

Escitalopram oxalate (trade names Lexapro, Cipralex). The chemically it is a [(s)-1-[3(dimethlyamino) propyl]-1-(4-flurophenyl)-1,3-dihydro isobenzofuran-5-carbonitrile oxalate] is a pure s- enantiomer of the racemic, bicyclic pthalates derivatives citalopram. It is mainly used as an antidepressant agent. ESC is a selective serotonin reuptake inhibitor (SSRI). It is the pure S-enantiomer (single isomer) of the racemic bicyclic phthalane derivative of citalopram. The antidepressant action of ESC, the S-enantiomer of racemic citalopram, is presumed to be linked to the potential of Serotonergic activity in the central nervous system (CNS) neuronal reuptake of serotonin (5HT). Escitalopram is freely soluble in methanol and dimethly sulfoxide (DMSO), sparingly soluble in water and in ethanol, slightly soluble in ethyl acetate, insoluble in Heptane.

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) were purchased from Mark (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of Escitalopram and L Methyl folate as reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instrumentation

In the present study Performed with Waters HPLC 2695 Photo diode array detector and Empower 2 software was used UV-Visible spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of L-methyl folate and Escitalopram. Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises

Determination of maximum absorbance

L-Methyl folate and Escitalopram standard solution was scanned in the range of 200-400 nm against mobile phase as blank. L-Methyl folate and Escitalopram shows maximum absorbance at 230nm.The wave length selected for the determination of L-Methyl folate and Escitalopram is 230nm.

Diluent

Based up on the solubility of the drugs, diluents was selected, Acetonitrile and Water taken the in the ratio of 50:50.

Preparation of Standard stock solutions

Accurately weighed 37.5 mg of L-methyl folate, 50 mg of Escitalopram and transferred to individual 50 ml volumetric flasks separately. 3/4 diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (750µg/ml of L-methyl folate and 1000µg/ml of Escitalopram).

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml L-methyl folate of and 100µg/ml of Escitalopram)

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 25 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters(300µg/ml of L-methyl folate and 400µg/ml of Escitalopram.

Preparation of Sample working solutions (100% solution)

2.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (75µg/ml of L-methyl folate and 100µg/ml of Escitalopram)

Preparation of buffer

0.1% OPA Buffer

1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

0.01N KH_2PO_4

Dissolve 1.36g of potassium dihydrogen Phosphate (**KH_2PO_4**) in 1 liter volume distilled water.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

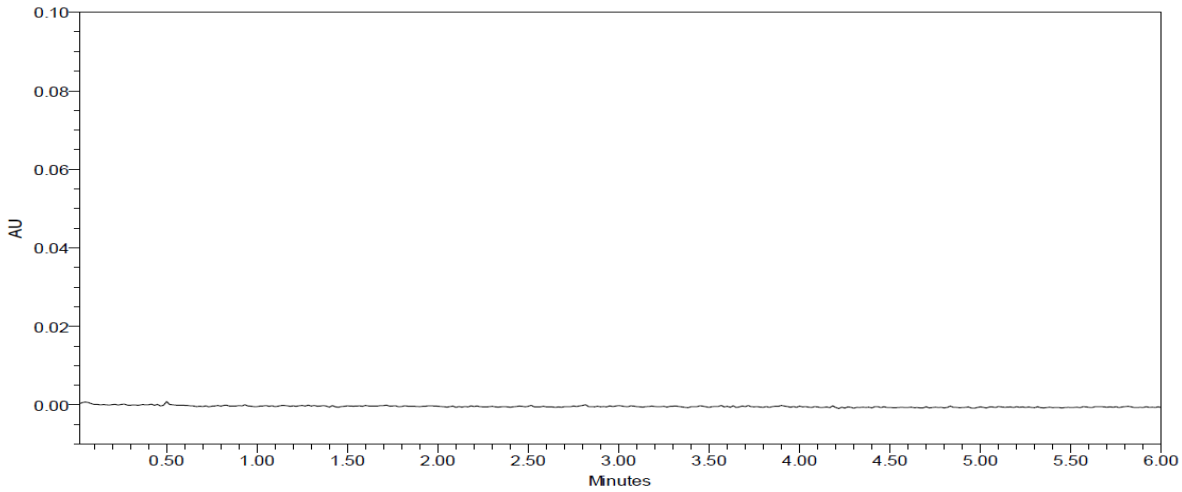
To develop a stability indicating RP-HPLC method for estimation of Escitalopram and L methyl folate in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 50% 0.01N KH2PO4 acid:50% Acetonitrile at a flow rate of 0.9ml/min, samples were analyzed at 230 nm detector wave length and at an injection volume of 10 µL using BDS C18 4.6 x 250mm, 5µm with run time of 6 min. The proposed method Escitalopram and L methyl folate was optimized to give sharp peak with good resolution and minimum tailing effect for the optimized chromatogram was obtained as shown in .

Analytical method validation

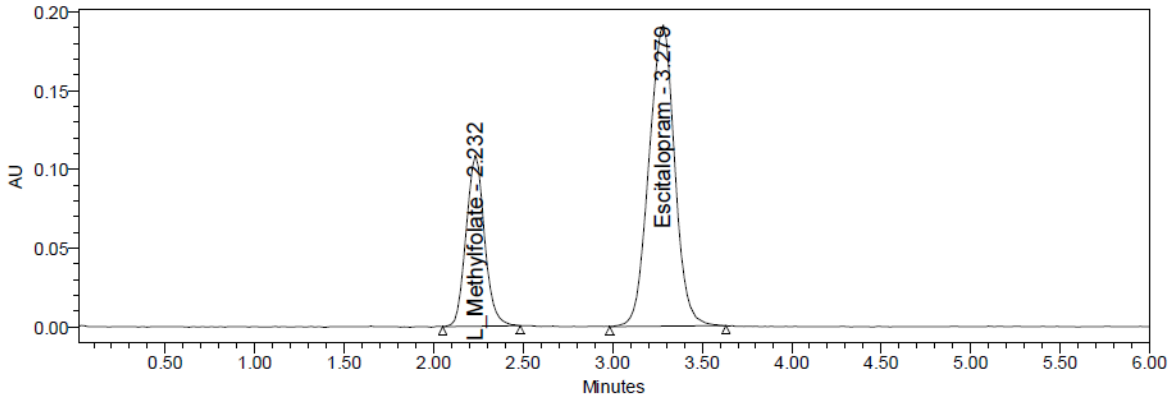
The analytical validation of l methyl folate and Escitalopram by HPLC was carried out with respect to the following parameters.

Table 1: optimized chromatographic conditions

Parameter	Condition
Mobile phase	Acetonitrile:KH2PO4
Flow rate	1ml/min
Column	BDS C18 150*2mm ID,3µm
Detector wave length	230nm
Column temperature	30°C
Injection volume	10µL
Run time	6 min
Diluent	Water and Acetonitrile in the ratio 50:50



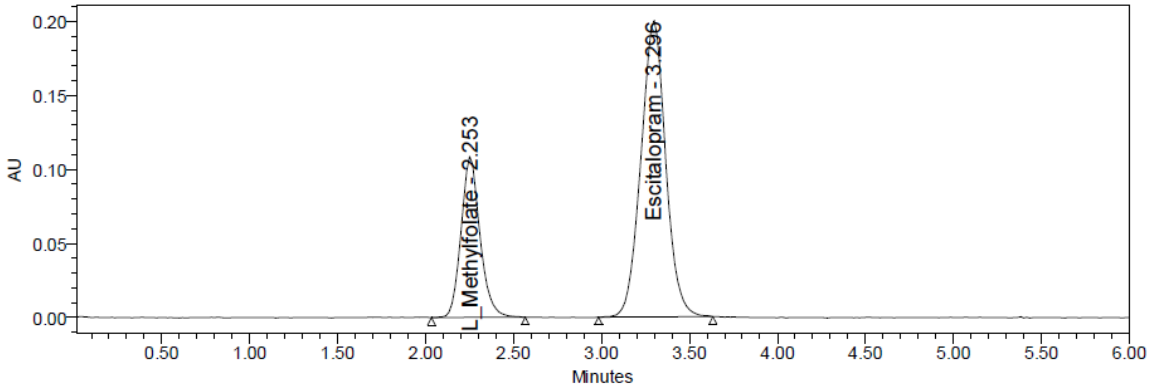
Blank chromatogram of L methyl folate and Escitalopram



Standard chromatogram of L methyl folate and Escitalopram

Table:2 System suitability parameters for L-methyl folate and Escitalopram

S no	L-methyl folate			Escitalopram			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resolution**
1	2.149	2526	1.12	3.347	2048	1.3	3.50
2	2.150	3755	1.28	3.348	2079	1..35	3.52
3	2.152	2010	1.28	3.351	2111	1.35	3.53
4	2.155	2061	1.28	3.356	2016	1.38	3.50
5	2.157	2045	1.32	3.358	2155	1.35	3.51
6	2.233	2033	1.32	3.274	2144	1.32	4.2



Formulation chromatogram

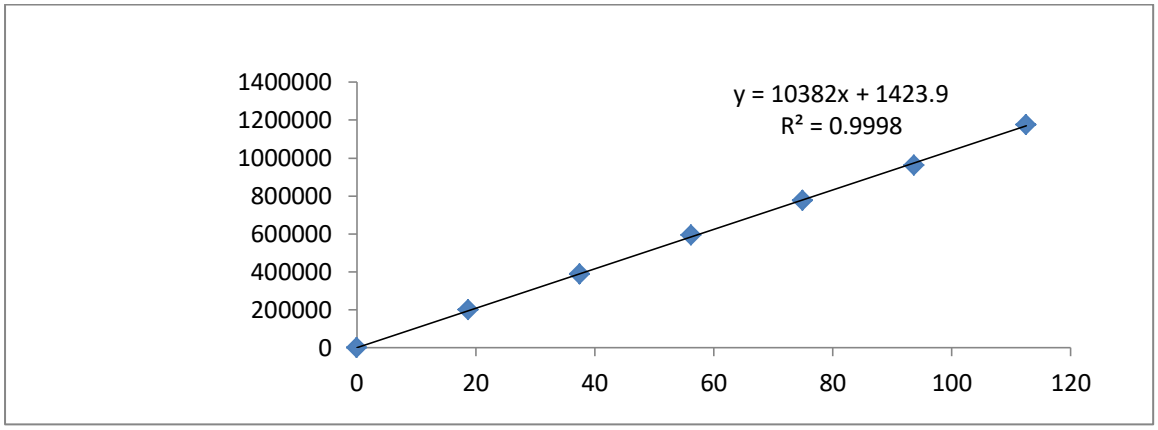
Linearity

Linearity of Escitalopram and l methyl folate were found by injecting six different concentrations of working standard solutions for Lmethyl folate(18.75–112.5 µg/ml)and Escitalopram (25–150 µg/mL).Standard calibration curves were constructed by taking mean peak area on Y-axis and concentrations of drug on X-axis. The linearity equations obtained for L-methyl folate was y = 10382x + 1423 and of Escitalopram was y = 18260x + 7600. Correlation coefficient obtained was 0.999 for the two drugs. The results were shoen in table 3.

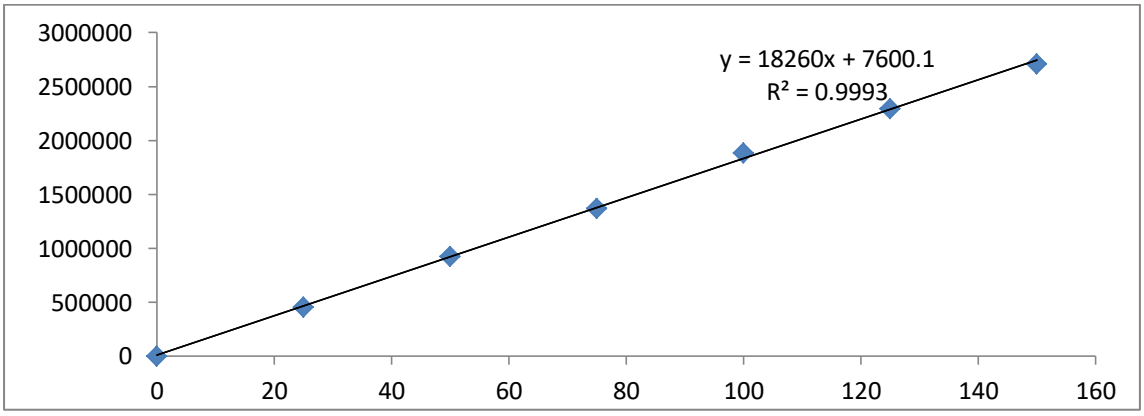
Table 3 : Results for the linearity of L methyl folate and Escitalopram

L-methyl folate		Escitalopram	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
18.75	910966	25	455483

37.5	1845323	50	922662
56.25	2734697	75	1367349
75	3770350	100	1885175
93.75	4594156	125	2297078
112.5	5424428	150	2712214



Calibration curve of L-methyl folate



Calibration curve of Escitalopram

Precision

The system precision was established by six repli-cate injections of the standard solution containing analytes of interest. The value of relative standard deviation of l methyl folate and Escitalopram and was found to be 0.4 and 0.7 within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation of l methyl folate and escitalopram was found to be 0.6 and 0.7 within the limit, indicating the injection repeatability of the method.

Table:4 Precision data for of L-methyl folate and Escitalopram

S. No	L methyl folate	Escitalopram	L-methyl folate	Escitalopram
	System precision		Method precision	

1.	775880	1901818	764441	1901818
2.	777612	1914986	767612	1914986
3.	780619	1899153	770619	1899153
4.	783307	1876148	759307	1880471
5.	775703	1902676	765703	1912392
6.	781867	1885152	771867	1885152
Mean	779165	1896656	766592	1898995
S.D	3217.4	13842.2	4551.3	13985.4
%RSD	0.4	0.7	0.6	0.7

Accuracy

To demonstrate the accuracy of the proposed method a standard addition method was used for analyzing the samples. For this purpose, known amounts of l methyl folate and Escitalopram were supplemented to the working standard sample solution which was previously analyzed and then compared the obtained experimental values to the true values. Each solution was injected in six times and the percentage recovery was calculated. %Recovery was obtained as 99.25% and 99.83% for L-methyl folate and Escitalopram respectively.

Table 5: Accuracy data of L-methyl folate

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	37.5	37.08	98.87	
	37.5	37.51	100.03	
	37.5	37.44	99.85	
100%	75	75.14	100.18	99.25%
	75	74.45	99.27	
	75	74.12	98.82	
150%	112.5	111.75	99.33	
	112.5	111.73	99.31	
	112.5	111.74	99.32	

Table 6 : Accuracy data of Escitalopram

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	50	49.61	99.22	99.83%
	50	49.36	98.73	
	50	49.53	99.07	

100%	100	99.85	99.85
	100	99.45	99.45
	100	100.07	100.07
150%	150	148.42	98.95
	150	148.73	99.16
	150	148.66	99.10

Robustness

The different variations such as variation of pH of the buffer solution, flow rate, wavelength and mobile phase composition. The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in table.

Table7: Robustness data for L-methyl folate and Escitalopram.

S.no	Condition	Change	%RSD of L-methyl folate	% RSD of Escitalopram
1	Flow rate- 1	0.8ml/min	0.3	0.4
2	Flow rate- 2	1.0ml/min	0.3	0.5
2	Mobile phase-1	40:60(%v/v)	0.7	0.7
3	Mobile phase-2	60:40(%v/v)	0.4	0.5
4	Temperature -1	25°C	0.2	0.2
5	Temperature -2	35°C	0.3	0.3

Limit of Detection and Quantification Limit

Determination of the Limit of Detection and Limit of Quantification was performed by standard deviation method. Standard with low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be readily detected

Table8 :Sensitivity table for l methyl folate and escitalopram

Molecule	LOD	LOQ
L-methyl folate	0.15	0.46
Escitalopram	0.15	1.55

Assay of formulation

We were prepared assay sample solution injected into the HPLC **.** Intas Pharmaceuticals Ltd (**Escitafol**), bearing the labels claim L-methyl folate 10mg, Escitalopram 5mg. Assay was performed with the above formulation. Average % Assay for L-methyl folate and Escitalopram obtained was 99.25% and 99.83% respectively =

Table9: Assay Data of L-methyl folate

S.no	% Assay
1	99.01
2	98.83
3	99.26
4	99.03
5	99.64
6	99.71
Avg	99.25
Std Dev	0.36
%RSD	0.4

Table10: Assay data of Escitalopram

S. no	% Assay
1	100.29
2	101.03
3	99.81
4	99.78
5	98.62
6	99.45
Avg	99.83
Std Dev	0.8
%RSD	0.8

Degradation data

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table11: Data of degradation studies

Type of degradation	L-methyl folate			Escitalopram		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	743581	95.05	4.95	1804051	94.93	5.07
Base	753206	96.28	3.72	1815473	95.53	4.47
Peroxide	758790	97.00	3.00	1820734	95.81	4.19
Thermal	762374	97.45	2.55	1835047	96.56	3.44
UV	769501	98.36	1.64	1870879	98.44	1.56
Water	743581	98.36	1.64	1884103	99.14	0.86

CONCLUSION

The stability indicating RP HPLC method was developed and validated for the simultaneous determination of L methyl folate and Escitalopram in bulk and its dosage form , T he proposed method was validated in accordance with ICH guidelines by testing its parameters include linearity, accuracy, precision, robustness, LOD and LOQ. Stress induced studies proves the effectiveness of the proposed stability indicating method.So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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