

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF L-METHYL FOLATE & ESCITALOPRAM BY RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the L-methyl folate and Escitalopram in Tablet dosage form. Chromatogram was run through Waters C18 150 x 4.6 mm, 5 μ . Mobile phase containing Buffer 0.01N KH₂PO₄ (3.45pH): Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.01N KH₂PO₄. Temperature was maintained at 30°C. Optimized wavelength selected was 212 nm. Retention time of L-methyl folate and Escitalopram were found to be 2.263min and 3.079min. %RSD of the L-methyl folate and Escitalopram were and found to be 0.2 and 0.5 respectively. %Recovery was obtained as 99.79% and 100.54% for L-methyl folate and Escitalopram respectively. LOD, LOQ values obtained from regression equations of L-methyl folate and Escitalopram were 0.24, 0.29 and 0.72, 0.87 respectively. Regression equation of L-methyl folate is $y = 22979x + 8347$. and $y = 31569x + 3873$. of Escitalopram. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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

INTRODUCTION

Chemically L-MethylFolate (L-MF) was an 5-methyltetrahydrofolic acid is a methylated derivate of tetrahydrofolate. It is generated by methylenetetrahydrofolate reductase from 5,10-methylenetetrahydrofolate and used to recycle homocysteine back to methionine by 5-methyltetrahydrofolate-homocysteine methyltransferases Molecular weight and Molecular formula of L-MF were 459.463 g/mol, C₂₀H₂₅N₇O₆ respectively. 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. Structure of the L-MF was shown in figure 1 (A).^[1]

Chemically Escitalopram (EST) was an (1S)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile. Molecular weight and Molecular formula of EST were 324.3919g/mol C₂₀H₂₁FN₂O respectively. The upper, antiobsessive-urgent, and antibulimic activities of escitalopram are attempted to be connected to its hindrance of CNS neuronal take-up of serotonin. Escitalopram obstructs the

reuptake of serotonin at the serotonin reuptake pump of the neuronal film, improving the activities of serotonin on 5HT_{1A} autoreceptors. SSRIs tie with altogether less liking to histamine, acetylcholine, and norepinephrine receptors than tricyclic energizer drugs. Structure of the EST was shown in figure 1 (B).^[2]

Literature survey reveals there are several methods to estimated thee drugs in single or in combination of two drugs.^[5-9] but there is only very few HPLC methods are available for simultaneous estimation of L-MF and EST, so the scope of developing and alidating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

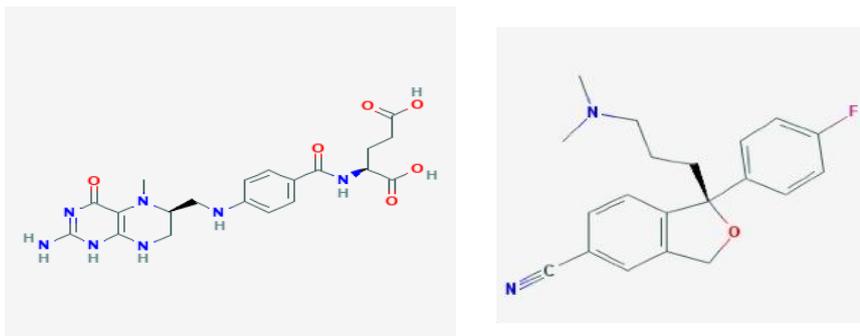


Figure 1: Structure of (A) L-Methyl Folate (B) Escitalopram.

MATERIALS AND METHODS

Reagents and Chemicals: The pharmaceutical drug samples of L-MethylFolate and Escitalopram were obtained from Spectra Pharma Pvt. Ltd., Hyderabad. All the chemicals and solvents were used as HPLC grade. The pharmaceutical dosage form was purchased from local pharmacy.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Discovery C18 150x4.6mm, 5m. As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Discovery C18 150x4.6mm, 5m column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of buffer solution (pH3.0) and Acetonitrile was taken in the ratio of (50:50% v/v) mobile phase for the investigation with a flow rate of a 1.0ml/min. The temperature was maintained at 30°C. The injection volume was 10µl and the UV detection was achieved at 212nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Mixture of 600 ml of 0.01N KH₂PO₄ buffer (pH-3.5) and 400 ml of Acetonitrile in the ration of 50:50 v/v were mixed and degased in ultrasonic water bath for 15 minutes and filtered through 0.45 µ filter paper. Mobile phase was used as a diluent

Preparation of mixture Standard stock solution: Accurately weighed 7.5 mg of L-methyl folate, 10 mg of Escitalopram and transferred to individual 25 ml volumetric flasks separately. 3/4 th of 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. (300µg/ml of L-methyl folate and 400µg/ml of Escitalopram).

Preparation of Sample (Tablet) 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 50 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 (150µg/ml of L-methyl folate and 200µg/ml of Escitalopram).

Optimized chromatographic conditions

Column Used: Discovery C₁₈ 150 x 4.6 mm, 5µ.

Mobile phase: buffer: Acetonitrile (50:50 v/v)

Flow rate: 1 .0ml/min

Wavelength: 212.0 nm

Temperature: 30°C

Injection Volume: 10.0µl

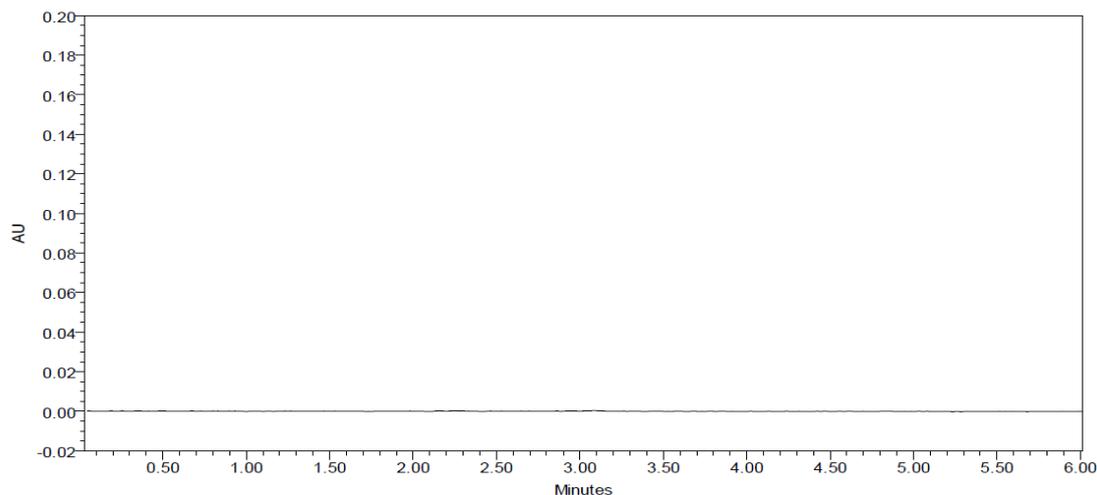


Figure 2: Blank chromatogram.

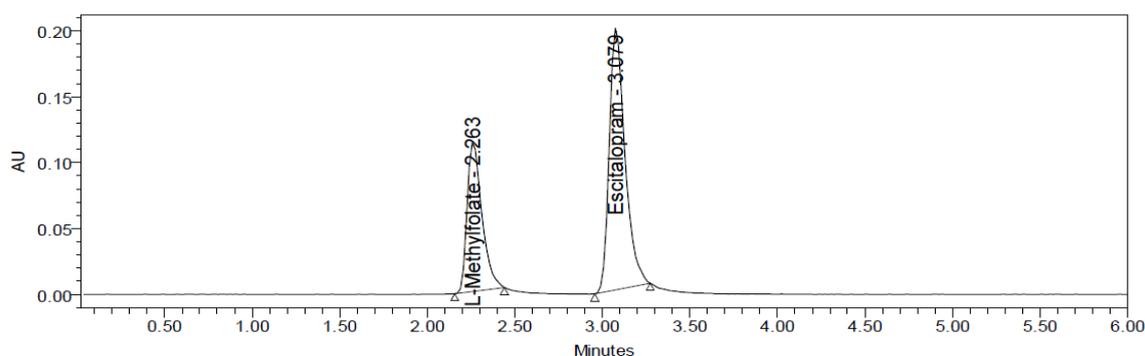


Figure 3: Chromatogram of standard mixture of L-MF & EST

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	L-Methyl Folate	2.263	669291	1.43	5	3758.0
2	Escitalopram	3.079	1279118	1.35	4.9	6040.0

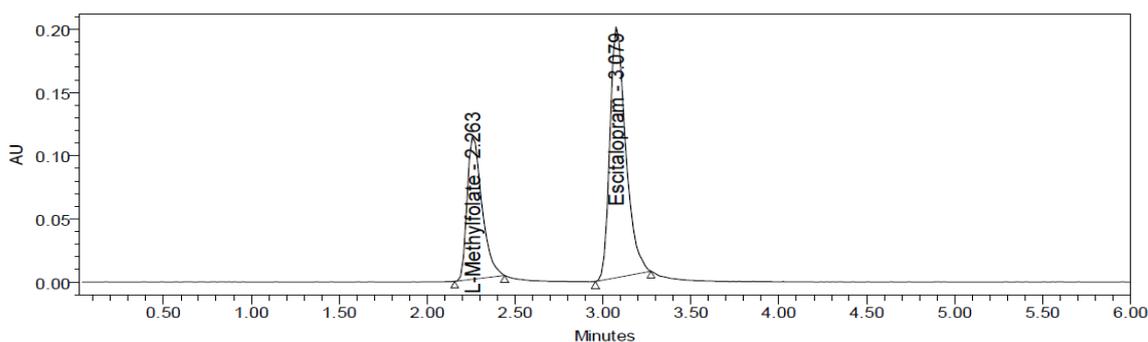


Figure 4: Chromatogram of sample mixture of L-MF & EST.

Validation

The above optimized chromatographic method has been validated for the assay of L-MF & EST using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of L-Methylfolate and Escitalopram (L-MF & EST) drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the

final concentration of the each drug ($\mu\text{g/ml}$) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of L-Methylfolate (7.5mg) + Escitalopram (10mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of drug L-Methylfolate (7.5mg) + Escitalopram (10mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the

model mixtures containing 80,100 and 120% of L-Methylfolate & Escitalopram. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae $LOD = 3.3 \times \text{standard deviation} / \text{slope}$; $LOQ = 10 \times \text{standard deviation} / \text{slope}$. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for L-MF was found to be $y = 22979x + 8347$ (slope, intercept and correlation coefficient were found to be 22979, 8347 and 0.999 respectively) and linear over beer's range of 5-30 $\mu\text{g/ml}$. The regression equation for EST was found to be $y = 31569x + 3873$ (slope, intercept and correlation coefficient were found to be 31569, 3873 and 0.999 respectively) and linear over beer's range of 12.5-75 $\mu\text{g/ml}$. Linearity graph of L-MF & EST were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for L-Methylfolate and Escitalopram were and found to be 0.2 and 0.5 respectively. %RSD of method

precision for L-Methylfolate and Escitalopram were and found to be 0.3 and 0.3 respectively. % recovery was obtained as 99.78% and 100.54% for L-Methylfolate and Escitalopram respectively. The calculated RSD values were less than 1. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug L-MF & EST and along with 5 $\mu\text{g/mL}$ of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.78% and 100.54% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for L-MF and EST was found to be 0.24 μg and 0.29 μg respectively. LOQ for L-MF and EST was found to be 0.72 μg and 0.87 μg respectively. Summary of all the validation parameter shown in table 4.

Degradation

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.

Table 1: Linearity table for L-MF and EST.

L-methyl folate		Escitalopram	
Conc ($\mu\text{g/mL}$)	Peak area	Conc ($\mu\text{g/mL}$)	Peak area
0	0	0	0
7.5	186908	10	318966
15	353809	20	633426
22.5	536247	30	953781
30	691791	40	1280934
37.5	865185	50	1578645
45	1043683	60	1890790

Table 2: System precision table of L-MF & EST.

S. No	Area of L-methyl folate	Area of Escitalopram
1.	670443	1278518
2.	667529	1275071
3.	669678	1273444
4.	668112	1286570
5.	670475	1281259
6.	669511	1270440
Mean	669291	1277550

Table 3: degradation data of L-MF & EST.

Type of degradation	L-methyl folate			Escitalopram		
	AREA	% Recovered	% Degraded	AREA	% Recovered	% Degraded
Acid	621255	92.45	7.55	1176708	91.92	8.08
Base	643728	95.80	4.20	1180516	92.22	7.78
Peroxide	646620	96.23	3.77	1203381	94.01	5.99
Thermal	653460	97.24	2.76	1248035	97.49	2.51
Uv	658733	98.03	1.97	1257566	98.24	1.76
Water	665819	98.03	1.97	1271780	99.35	0.65

Table 4: summary of validation data of L-MF & EST.

Parameters		L-methyl folate	Escitalopram	LIMIT
Linearity Range ($\mu\text{g/ml}$)		7.5-30 $\mu\text{g/ml}$	10-40 $\mu\text{g/ml}$	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		22979	31569	
Intercept(c)		8347	3873	
Regression equation (Y=mx+c)		$y = 22979x + 8347.$	$y = 31569x + 3873.$	
Assay (% mean assay)		99.41%	99.58%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.2	0.5	NMT 2.0%
Method precision %RSD		0.3	0.3	NMT 2.0%
Accuracy %recovery		99.78%	10054%	98-102%
LOD		0.24	0.29	NMT 3
LOQ		0.72	0.87	NMT 10
Robustness	FM	0.4	0.7	% RSD NMT 2.0
	FP	0.4	0.9	
	MM	1.7	1.0	
	MP	0.5	0.7	
	TM	0.5	0.4	
	TP	0.3	0.6	

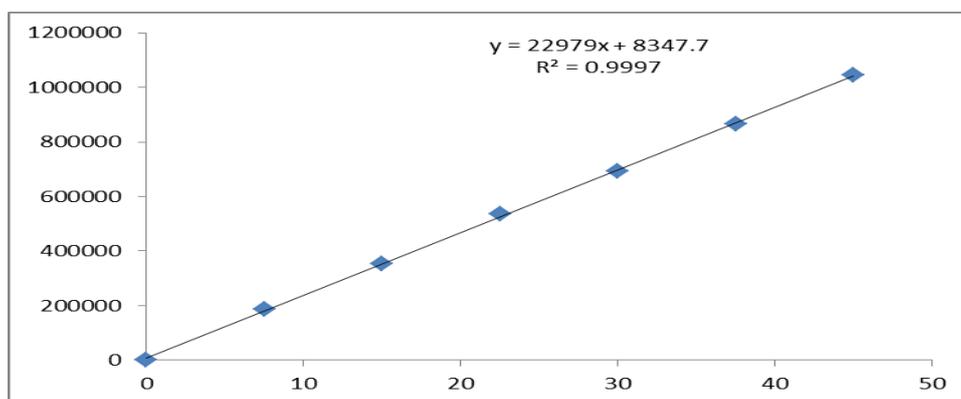


Fig. 5: Linearity curve of L-Methyl Folate.

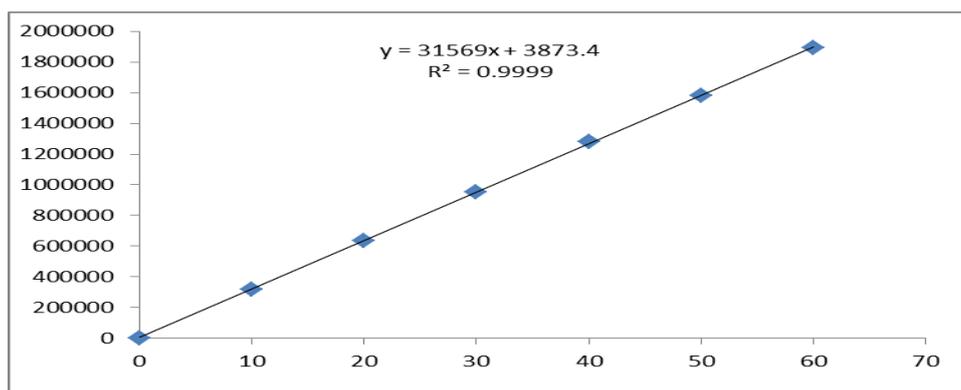


Fig. 6: Linearity curve of Escitalopram.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the L-Methylfolate and Escitalopram in Tablet dosage form was developed and the proposed method as suitable for routine analysis of L-MF & EST.

A) L-MethylFolate (B) Escitalopram

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