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RESEAECH ARTICLE

RP-HPLC Method Development and Validaton for the Simultaneous Estimation of L-Methyl Folate and EscitalopramIn Bulk and Its Pharmaceutical Dosage Form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the L-methyl folate and Escitalopram in bulk and its pharmaceutical dosage form.Chromatographic separation was carried on Denali C18(150 x 4.6 mm, 5µ) column . Mobile phase consist of Phosphate buffer and Acetonitrile taken in the ratio of 50:50was pumped through column at a flow rate of 1.0 ml/min. Mobile phase PH 6.8 was adjusted with 0.01N KH2PO4.Optimized wavelength selected was 212 nm. Retention time of L-methyl folate and Escitalopram were found to be 2.188min and 3.822min. %RSD of the L-methyl folate and Escitalopram were and found to be 0.5 and 0.6 respectively. %Recovery was obtained as 98.82% and 100.16% for L-methyl folate and Escitalopram respectively. LOD, LOQ values obtained from regression equations of L-methyl folate and Escitalopram were 0.07, 0.22 and 0.84, 2.55 respectively. Regression equation of L-methyl folate is y = 9276.x + 180.2, and y = 9771.x + 4371.. of Escitalopram. The developed method was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: L-methyl folate, Escitalopram, RP-HPLC

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1. Introduction

5-methyltetrahydrofolic acid is a methylated derivate of tetrahydro folate. It is generated by methylene tetrahydro folate reductase from 5,10-methylenetetrahydrofolate and used to recycle homocysteine back to methionine by 5-methyltetrahydrofolate-homocysteine methyltransferases.It cross the blood-brain barrier, and it isthis form that can directly impact several important CNS reactions, most

notably the synthesis of three important neurotransmitters: serotonin, norepinephrine, and dopamine[1-3].

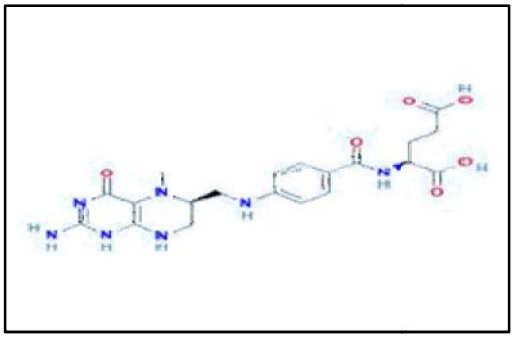

Fig 1: Structure of L-Methyl folate

Escitalopram is one of a class of antidepressants known as specific serotonin reuptake inhibitors (SSRIs).It is utilized to treat the melancholy connected with disposition issue. It is additionally utilized on occassion in the treatment of body dysmorphic turmoil and nervousness. The upper, antiobsessive-impulsive, and antibulimic activities of escitalopram are ventured to be connected to its hindrance of CNS neuronal take-up of serotonin.SSRIs tie with altogether less liking to histamine, acetylcholine, and norepinephrine receptors than tricyclic energizer drugs[4,5].

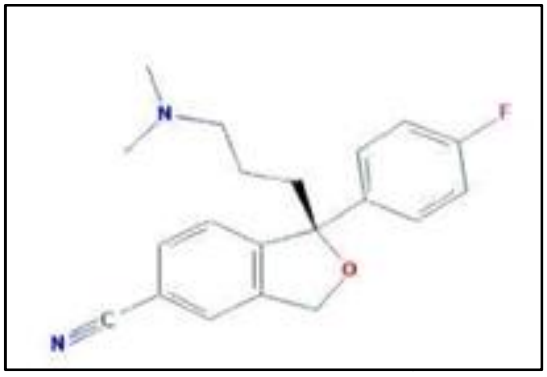

Fig 2: Structure of Escitalopram

Extensive survey of literature few chromatographic methods have been reported for the simultaneous estimation of L-methyl folate and Escitalopram combined dosage form[6-11]. Therefore an attempt has been made to simple, precise, accurate and cost effective RP-HPLC method was developed for the simultaneous estimation of L-methyl folate and Escitalopramin bulk and its dosage form.

2. Materials and Methods

Materials:

Gift sample of L-methyl folate and Escitalopram pure drugs (API), Combination L-methyl folate and Escitalopram tablets (Escitafol) were procured from INTAS Labs, Hyderabad ,India.HPLC grade water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer are obtained from Merck , Mumbai, India.

Instrument:

In this study using liquid chromatographic system was WATERS HPLC 2695 System equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.The analytes were monitored at 212 nm.

Chromatographic conditions:

Mobile phase	:	0.01N KH_2PO_4 buffer: Acetonitrile (50:50%v/v)
Flow rate	:	1.0 ml/min
Column	:	Denali C_{18} (4.6 x 150mm, 5µm)
Detector wave length	:	212nm
Column temperature	:	30°C
Injection volume	:	10 L
Run time	:	6min
Diluent	:	Water and Acetonitrile in the ratio 50:50

Methods

Preparation of Standard solution:

Accurately weighed 37.5 mg of L-methyl folate,50 mg of Escitalopram and transferred to individual 50 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. (750µg/ml of L-methyl folate and 1000µg/ml of Escitalopram). Above the stock solution further respected dilutions were prepared and analysed.

Preparation of Sample solutions:

5 tablets were weighed and the average weight ofeach 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). Further respected dilutions were prepared from the above stock solution.

3. Results and Discussions

Method validation

The developed analytical method was validated as per ICH guidelines for the parameters like specificity, linearity, accuracy, precision, robustness and system suitability.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of L-methyl folate (75ppm) and Escitalopram (100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.Results were Shown in table 1.

Specificity

Checking of the interference in the optimized method. We should not findinterfering peaks in blank and placebo at retention times of these drugs in this method. So this

method was said to be specific.Specificity data was shown in table 2 and fig 3,4.

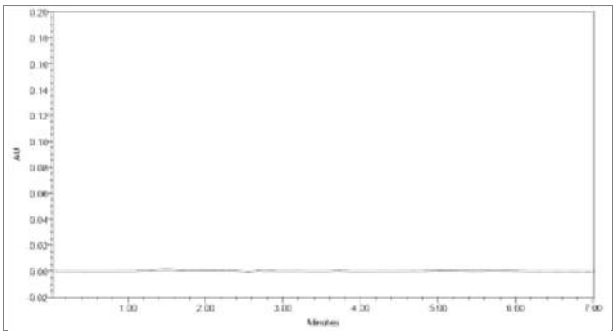

Fig 3: Chromatogram for blank

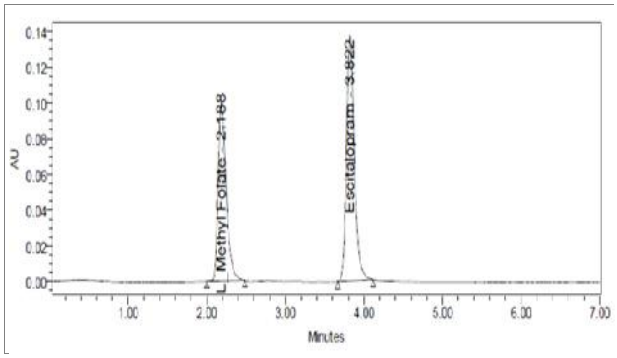

Fig 4: Chromatogram for Standard

Linearity

Preparation 25% Standard solution: 0.25ml each from two standard stock solutions was pipetted outand made up to 10ml. 78.725µg/ml of L-methyl folate and 25 µg/ml of Escitalopram).

Preparation 50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out andmade up to 10ml. (37.5µg/ml of L-methyl folate and 50µg/ml of Escitalopram).

Preparation 75% Standard solution: 0.75ml each from two standard stock solutions was pipetted outand made up to 10ml. (56.25µg/ml of L-methyl folate and 75µg/ml of Escitalopram).

Preparation 100% Standard solution: 1.0ml each from two standard stock solutions was pipetted outand made up to 10ml. (75µg/ml of L-methyl folate and 100µg/ml of Escitalopram).

Preparation 125% Standard solution: 1.25ml each from two standard stock solutions was pipetted outand made up to 10ml. (93.75µg/ml of L-methyl folate and 125µg/ml of Escitalopram).

Preparation 150% Standard solution: 1.5ml each from two standard stock solutions was pipettede outand made up to 10ml (112.5µg/ml of L-methyl folate and 150µg/ml of Escitalopram)

Procedure:

Each standard solution Inject into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. Results were shown in table 3 and fig 5,6.

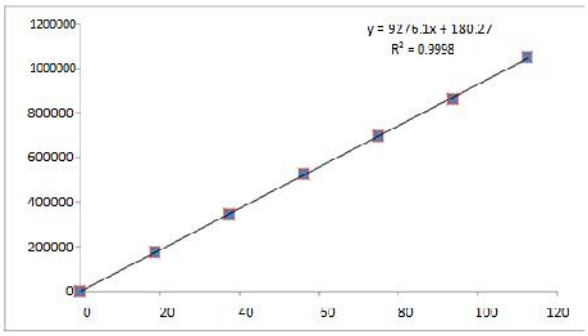

Fig 5: Calibration curve of L-methyl folate

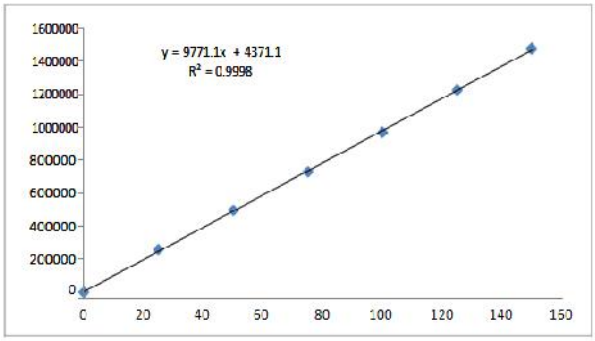

Fig 6: Calibration curve of Escitalopram

Precision

In precision studies intraday and intermediate precision was carried.

Preparation of working solutions (100% solution): 1ml from each stocksolution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml L-methyl folate of and100µg/ml of Escitalopram).

Procedure:

Multiple sampling from a sample stock solution was done and six workingsample solutions of same concentrations were prepared, each injection from each working sample solution was injected into the chromatographic system. Average area, standard deviation and % RSD was calculated. Results were shown in table 4.

Accuracy

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10mlvolumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Procedure:

Three levels of Accuracy samples were prepared by standard addition method.Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 98.82% and 100.16% for L-methyl folate and Escitalopram respectively. Results were shown in table 5.

LOD & LOQ

0.25ml each from two standard stock solutions was pipetted outand transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of L-methyl folate, Escitalopram, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents. Then caluculate the LOD and LOQ. The data was shown in table 6.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, andtemperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Results were shown in table 7.

Table 1: System suitability parameters for L-methyl folate and Escitalopram

S.No	L-methyl folate			Escitalopram			Resolution
Injection	R_t (min)	USP plate count	Tailing	R_t (min)	USP plate count	Tailing	
1	2.188	2321	1.40	3.822	7327	1.26	8.9
2	2.189	2556	1.43	3.826	6953	1.24	8.9
3	2.190	2353	1.39	3.833	6532	1.26	8.8
4	2.191	2371	1.41	3.834	6942	1.25	8.9
5	2.192	2532	1.43	3.837	7296	1.24	8.9
6	2.198	2445	1.46	3.847	8065	1.23	8.8

Table 2: Results for Specificity

S.No	Drug		Observation
1	Blank		Nill
2	Placebo		Nill
3	Standard	L-methyl folate	2.188 min
		Escitalopram	3.822 min

Table 3: Linearity results

L-Methyl folate		Escitalopram	
Concentration(µg/ml)	**Peak area**	**Concentration(µg/ml)**	**Peak area**
0	0	0	0
18.75	176411	25	258167
37.5	344618	50	497209
56.25	525516	75	732105
75	696392	100	969719
93.75	861342	125	1226126
112.5	1049430	150	1477125

Table 4: Results for Precision

	L-Methyl folate		Escitalopram	
S.no	**Intra day**	**Inter day**	**Intra day**	**Inter day**
1	693364	968085	681094	950678
2	694600	969209	688391	953211
3	693209	961879	683319	958298
4	690851	963371	685175	952769
5	698720	965627	688682	952280
6	691528	965952	681427	950481
Avg	693712	965687	684681	952953
STD	2799.9	2760.2	3326.4	2842.8
%RSD	0.4	0.3	0.5	0.3

Table 5: Results for accuracy

Sample	Spike level	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	%Mean Recovery
L-Methyl folate	50%	37.5	37.14	99.06	98.82%
	100%	75	73.93	98.57	
	150%	112.5	111.16	98.81	

Escitalopram	50%	50	50.29	100.58	100.16%
	100%	100	99.71	99.71	
	150%	150	150	99.99	

Table 6: Results for LOD & LOQ

Molecule	LOD	LOQ
L-methyl folate	0.07	0.22
Escitalopram	0.84	2.55

Table 7: Results for Robustness

S.no	Condition	%RSD of L-methyl folate	%RSD of Escitalopram
1	Flow rate (-) 0.9ml/min	1.6	0.8
2	Flow rate (+) 1.1ml/min	1.2	0.5
3	Mobile phase (-) 55B:45A	0.9	0.4
4	Mobile phase (+) 45B:55A	1.4	0.1
5	Temperature (-) 25°C	0.2	0.4
6	Temperature (+) 35°C	0.7	0.6

4. Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the L-methyl folate and Escitalopram in Tablet dosage form. Retention time of L-methyl folate and Escitalopram were found to be 2.188min and 3.822min. %RSD of the L-methyl folate and Escitalopram were and found to be 0.4 and 0.3 respectively. %Recovery was obtained as 98.82% and 100.16% for L-methyl folate and Escitalopram respectively. LOD, LOQ values obtained from regression equations of L-methyl folate and Escitalopram were 0.07, 0.22 and 0.84, 2.55 respectively. Regression equation of L-methyl folate is y = 9276.x + 180.2, and y = 9771.x + 4371 of Escitalopram. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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