Stability Indicating RP-HPLC Method for The Simultaneous Estimation of L-Methyl Folate and Escitalopram in Bulk and Pharmaceutical Dosage Form

G Priyanka[1], Raavi Sujitha[2]
[1,2]*Department of Pharmaceutical Analysis, Sun Institute of Pharmaceutical Education and Research, Kakupalli, Nellore, Andhra Pradesh - 524346*

***Abstract*—A simple, precise, accurate, robust and stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for simultaneous estimation of L-methyl folate and escitalopram in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved on a BDS C8 column (150 × 4.6 mm, 5 µm) using 0.01 N potassium dihydrogen phosphate buffer and acetonitrile as mobile phase in the ratio 55:45 v/v. The flow rate was maintained at 1.0 mL/min and detection was performed at 212 nm. Retention times were found to be 2.202 min for L-methyl folate and 2.962 min for escitalopram. The method was validated according to ICH guidelines. Linearity was observed over the concentration range of 18.75–112.5 µg/mL for L-methyl folate and 25–150 µg/mL for escitalopram with correlation coefficients of 0.9999. Recovery studies showed mean recoveries of 99.56% and 99.58% for L-methyl folate and escitalopram respectively. The developed method was found to be specific, precise, accurate, robust and suitable for routine quality control analysis.**

***Index Terms*—RP-HPLC, L-Methyl Folate, Escitalopram, Method Validation, Stability Indicating Method.**

I. INTRODUCTION

Depression is one of the most prevalent psychiatric disorders worldwide and represents a significant public health concern due to its impact on quality of life, social functioning, and overall health outcomes[1]. Pharmacological management of depression commonly involves selective serotonin reuptake inhibitors (SSRIs), among which escitalopram is considered one of the most effective and well-tolerated agents. Escitalopram is the S-enantiomer of citalopram and exerts its therapeutic action by selectively inhibiting serotonin reuptake in the central nervous system, thereby increasing serotonergic neurotransmission. Although escitalopram has demonstrated significant clinical efficacy, treatment response can vary among patients because of multiple physiological and biochemical factors[2].

Recent clinical studies have emphasized the importance of folate metabolism in the pathophysiology and treatment of depression[3]. L-methyl folate, the biologically active form of folic acid, serves as a critical cofactor in one-carbon transfer reactions and neurotransmitter biosynthesis. Unlike folic acid, L-methyl folate readily crosses the blood-brain barrier and directly participates in the synthesis of serotonin, dopamine, and norepinephrine. Deficiency of folate has been associated with poor antidepressant response and increased severity of depressive symptoms[4]. Consequently, combination therapy involving escitalopram and L-methyl folate has gained considerable attention in the management of depressive disorders[5].

The increasing therapeutic importance of this combination necessitates the development of reliable analytical methods capable of simultaneously quantifying both drugs in pharmaceutical dosage forms[6]. Analytical methods employed in pharmaceutical quality control must possess adequate sensitivity, specificity, precision, accuracy, and robustness. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) has emerged as one of the most versatile analytical techniques for pharmaceutical analysis because of its superior separation efficiency, reproducibility, and

applicability to a broad range of pharmaceutical compounds[7].

Although several analytical methods have been reported for individual estimation of escitalopram and folate derivatives, only limited information is available regarding simultaneous estimation of L-methyl folate and escitalopram using a stability-indicating RP-HPLC method[8]. Therefore, the present study was undertaken to develop and validate a simple, rapid, economical, and stability-indicating RP-HPLC method for simultaneous estimation of L-methyl folate and escitalopram in bulk drug substances and pharmaceutical dosage forms in accordance with International Conference on Harmonisation (ICH) guidelines[9-15].

II. DRUG PROFILE

L-methyl folate, chemically known as levomefolic acid, is the biologically active metabolite of folic acid. It possesses a molecular formula of C20H25N7O6 and a molecular weight of 459.46 g/mol. L-methyl folate plays a fundamental role in numerous metabolic pathways involving DNA synthesis, amino acid metabolism, and neurotransmitter production. It acts as a methyl donor in the conversion of homocysteine to methionine, thereby supporting methylation reactions essential for normal physiological function. Clinically, L-methyl folate is indicated in folate deficiency, megaloblastic anemia, pregnancy-associated nutritional supplementation, and as an adjunctive therapy in depressive disorders. Because of its ability to cross the blood-brain barrier, it has become increasingly important in psychiatric pharmacotherapy.

Escitalopram is chemically designated as S-(+)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile. It possesses a molecular formula of C20H21FN2O and a molecular weight of 324.39 g/mol. Escitalopram belongs to the class of selective serotonin reuptake inhibitors and is widely prescribed for major depressive disorder, generalized anxiety disorder, panic disorder, and obsessive-compulsive disorder. The drug exhibits high selectivity for serotonin transporters, resulting in increased serotonin concentrations within synaptic clefts. Escitalopram demonstrates excellent oral bioavailability, extensive hepatic metabolism, and an elimination half-life ranging between 27 and 32 hours. Owing to its favorable safety profile and therapeutic effectiveness, escitalopram remains one of the most frequently prescribed antidepressants worldwide.

III. MATERIALS AND METHODS

Materials

Reference standards of L-methyl folate and escitalopram were obtained as gift samples. Acetonitrile, methanol, potassium dihydrogen phosphate and orthophosphoric acid of HPLC grade were used throughout the study.

Instrumentation

Chromatographic analysis was performed using a Waters HPLC 2695 system equipped with PDA detector and Empower software.

Optimized Chromatographic Conditions

Table 1: Optimized Chromatographic Conditions

Parameter	Condition
Column	BDS C8 (150 × 4.6 mm, 5 µm)
Mobile Phase	0.01 N KH_2PO_4: Acetonitrile (55:45 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	212 nm
Injection Volume	10 µL
Column Temperature	30°C
Run Time	6 min
Diluent	Water: Acetonitrile (50:50)

Preparation of Standard Solution

Accurately weighed quantities of 37.5 mg of L-methyl folate and 50 mg of escitalopram were transferred into separate 50 mL volumetric flasks and diluted with diluent to obtain stock solutions. Appropriate dilutions were made to obtain working standards containing 75 µg/mL of L-methyl folate and 100 µg/mL of escitalopram.

IV. METHOD DEVELOPMENT

Several chromatographic trials were performed using different mobile phase compositions and stationary phases. Initial trials resulted in poor peak shape; low plate counts and tailing. Optimization led to the selection of KH_2PO_4 buffer and acetonitrile (55:45 v/v) on a BDS C8 column. Under optimized conditions, both analytes showed excellent peak symmetry and satisfactory resolution.

V. METHOD VALIDATION

The developed chromatographic method was validated according to ICH Q2(R1) guidelines with respect to specificity, system suitability, linearity, precision, accuracy, robustness, limit of detection, and limit of quantification.

System suitability testing demonstrated satisfactory chromatographic performance. Theoretical plate counts exceeded the minimum requirement of 2000, tailing factors were below 2.0, and resolution values between the analyte peaks exceeded 2.0, confirming adequacy of the chromatographic system.

Specificity studies were performed using blank and placebo solutions. No interfering peaks were observed at the retention times corresponding to L-methyl folate and escitalopram, demonstrating the ability of the method to selectively quantify both analytes in the presence of formulation excipients.

Linearity was evaluated across concentration ranges of 18.75–112.5 µg/mL for L-methyl folate and 25–150 µg/mL for escitalopram. Calibration plots exhibited excellent linear relationships between peak area and concentration with correlation coefficients greater than 0.999. The regression equations obtained further confirmed the proportional response of the detector over the studied concentration ranges.

Precision studies included system precision, repeatability, and intermediate precision. In all cases, percentage relative standard deviation values were below 2%, indicating excellent reproducibility of the developed method. Accuracy was assessed through recovery studies performed at 50%, 100%, and 150% concentration levels. Mean recoveries were found to be 99.56% for L-methyl folate and 99.58% for escitalopram, demonstrating the accuracy of the method.

Robustness was evaluated by introducing deliberate variations in flow rate, mobile phase composition, and column temperature. The method remained unaffected by these changes, and all system suitability parameters remained within acceptable limits. These results confirmed the robustness and reliability of the analytical procedure.

Table 2: System Suitability Results

Parameter	L-Methyl Folate	Escitalopram
Retention Time (min)	2.202	2.962
Plate Count	2086	2632
Tailing Factor	1.32	1.28
Resolution	-	3.5

Table 3: Linearity Data of L-Methyl Folate

L-Methyl Folate (µg/mL)	Peak Area
18.75	821537
37.5	1653023
56.25	2422925
75	3232723
93.75	4028001
112.5	4849908

Table 4: Linearity Data of Escitalopram

Escitalopram (µg/mL)	Peak Area
25	1844051
50	3796335
75	5673893
100	7565111
125	9323798
150	11277386

Table 5: Precision Results

Parameter	L-Methyl Folate	Escitalopram
System Precision (%RSD)	0.8	0.3
Repeatability (%RSD)	0.8	0.5
Intermediate Precision (%RSD)	0.5	0.9

Table 6: Recovery Results

Drug	Mean Recovery (%)
L-Methyl Folate	99.56
Escitalopram	99.58

Table 7: LOD and LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
L-Methyl Folate	0.42	1.26
Escitalopram	0.43	1.27

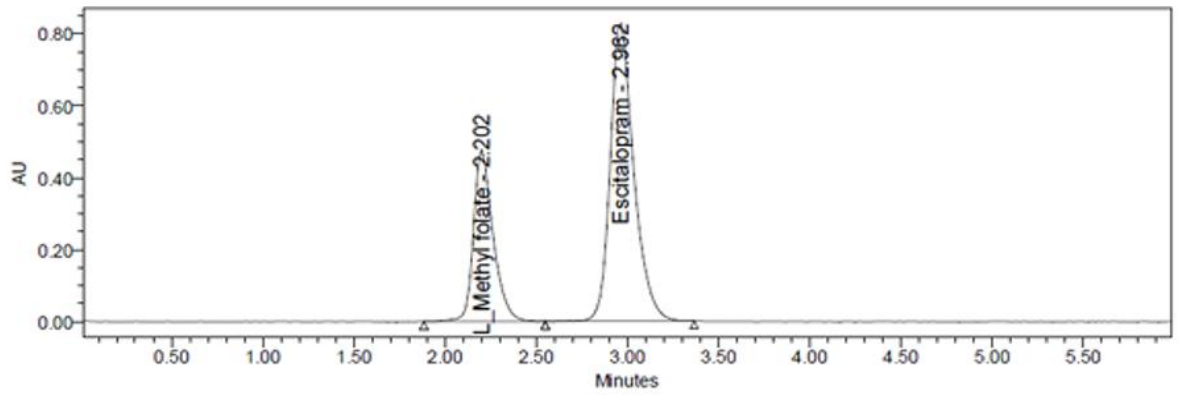

Figure 1 : Chromatograms of Metthyl Folate and Escitalopram

VI. STABILITY STUDIES

Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, photolytic and hydrolytic conditions. The developed method successfully separated degradation products from the principal drug peaks, confirming the stability-indicating nature of the analytical method.

VII. RESULTS AND DISCUSSION

The primary objective of the present investigation was to develop a simple and reliable RP-HPLC method for simultaneous estimation of L-methyl folate and escitalopram. The optimized chromatographic conditions successfully achieved complete separation of the two analytes within a short analysis time. The retention times obtained for both compounds were highly reproducible and allowed rapid routine analysis.

Validation studies confirmed the suitability of the developed method for pharmaceutical applications. Excellent linearity was demonstrated by correlation coefficients of 0.9999 for both analytes. Precision studies yielded percentage relative standard deviation values well below the acceptance limit of 2%, indicating outstanding reproducibility. Recovery studies confirmed the accuracy of the method, with recoveries approaching 100%.

Sensitivity studies revealed low limits of detection and quantification, indicating the capability of the method to detect and quantify trace amounts of the analytes. Robustness studies established that minor variations in chromatographic parameters did not significantly influence analytical performance. These observations collectively demonstrate the reliability of the developed RP-HPLC method.

Furthermore, forced degradation studies indicated that degradation products did not interfere with the principal drug peaks, confirming the stability-indicating nature of the analytical procedure. Such capability is particularly important for stability testing and regulatory compliance in pharmaceutical quality control laboratories.

VIII. CONCLUSION

A novel, simple, economical and stability-indicating RP-HPLC method was successfully developed and validated for simultaneous estimation of L-methyl folate and escitalopram in bulk and pharmaceutical dosage forms. The method exhibited excellent specificity, linearity, precision, accuracy and robustness according to ICH guidelines. Owing to its short run time and reliability, the method is suitable for routine quality control and stability studies in pharmaceutical industries.

REFERENCES

[1] S. L. Snyder, J. J. Kirkland, and J. W. Dolan, Introduction to Modern Liquid Chromatography, 3rd ed. New York, NY, USA: Wiley, 2010.

[2] D. A. Skoog, F. J. Holler, and S. R. Crouch, Principles of Instrumental Analysis, 6th ed. Belmont, CA, USA: Thomson Brooks/Cole, 2007.

[3] G. R. Chatwal and S. K. Anand, Instrumental Methods of Chemical Analysis, 5th ed. Mumbai, India: Himalaya Publishing House, 2012.

[4] International Conference on Harmonisation (ICH), Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva, Switzerland: ICH, 2005.
[5] United States Pharmacopeial Convention, USP 43–NF 38. Rockville, MD, USA: United States Pharmacopeial Convention, 2020.
[6] A. H. Beckett and J. B. Stenlake, Practical Pharmaceutical Chemistry, 4th ed. New Delhi, India: CBS Publishers, 2005.
[7] H. H. Willard, L. L. Merritt, J. A. Dean, and F. A. Settle, Instrumental Methods of Analysis, 7th ed. New Delhi, India: CBS Publishers, 2004.
[8] M. E. Swartz and I. S. Krull, Analytical Method Development and Validation. New York, NY, USA: Marcel Dekker, 2012.
[9] Y. Kazakevich and R. Lobrutto, HPLC for Pharmaceutical Scientists. Hoboken, NJ, USA: Wiley-Interscience, 2007.
[10] J. Ermer and J. H. M. Miller, Method Validation in Pharmaceutical Analysis. Weinheim, Germany: Wiley-VCH, 2005.
[11] M. Blessy, R. D. Patel, P. N. Prajapati, and Y. K. Agrawal, "Development of forced degradation and stability indicating studies of drugs—A review," Journal of Pharmaceutical Analysis, vol. 4, no. 3, pp. 159–165, 2014.
[12] M. Bakshi and S. Singh, "Development of validated stability-indicating assay methods," Journal of Pharmaceutical and Biomedical Analysis, vol. 28, no. 6, pp. 1011–1040, 2002.
[13] M. W. Dong, Modern HPLC for Practicing Scientists. Hoboken, NJ, USA: Wiley, 2006.
[14] R. N. Rao and V. Nagaraju, "An overview of analytical method development and validation by HPLC," Journal of Pharmaceutical and Biomedical Analysis, vol. 33, no. 4, pp. 335–343, 2003.
[15] L. R. Snyder, J. L. Glajch, and J. J. Kirkland, Practical HPLC Method Development, 2nd ed. New York, NY, USA: Wiley, 1997.