RJC

RASĀYAN *J. Chem.*
Vol. 12 | No. 4 |2338 - 2347| October - December | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND L-METHYLFOLATE IN BULK AND TABLET DOSAGE FORM

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**ABSTRACT**

A new RP-HPLC method was developed for the simultaneous estimation of Escitalopram and L-methyl folate in bulk and pharmaceutical dosage forms. The chromatographic separation was carried out on DSC-18column(4.6x250mm,5µparticlesize) with an isocratic mobile phase composed of an orthophosphoric acid buffer, Acetonitrile, (45:55v/v) at a flow rate of 1mL/min. The temperature of the column was maintained at30°C and PDA detector at 215 nm was used during separation. The parameters like system suitability, specificity, linearity, precision, accuracy, LOD, LOQ and robustness were validated as stated in the ICH guidelines. The retention times for Escitalopram and L-methyl folate were2.158minand 3.899minrespectively. The % recoveries of Escitalopram and L-methyl folate were100.42 % and 100.14% respectively. The% RSD for assay of tablets was found to be less than 2%. Forced degradation studies were also noted. The proposed method was accurate, sensitive and precise. Hence the method can be used for quality control of tablets containing both drugs escitalopram and L-methyl folate in quality control laboratories and pharmaceutical industries.

**Keywords:** Escitalopram, L-methyl folate, RP-HPLC and Orthophosphoric Acid buffer.



## INTRODUCTION

Escitalopram is chemically named as (1S)-1- [3-(dimethyl amino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile[1] shown in Fig.-1.It comes under antidepressant agent known as selective serotonin reuptake inhibitors (SSRIs) and it can also be used as S-enantiomer of citalopram. SSRIs have same pharmacological activity but with different structural differences between the compounds in their class. Like other antidepressant agents[2], a clinical effect is observed after a few weeks of therapy. SSRIs[3,4] are potent inhibitors of neuronal serotonin reuptake and they effect on norepinephrine or dopamine reuptake very little and do not antagonize α- or β-adrenergic, dopamine D2 or histamine H1 receptors. SSRIs block serotonin reuptake and increase serotonin stimulation of somatodendritic[5] 5-HT1A and terminal auto receptors take place when it is used in acute conditions. Desensitization of somato dendritic 5-HT1A and terminal auto receptors occurs when it is in chronic use. Adaptive variations in neuronal function and leads to improved serotonergic neurotransmission occurs due to increased mood or decreased anxiety of overall clinical effect. Generally, within first two weeks of therapy side effects include dry mouth, nausea, dizziness, drowsiness, sexual dysfunction and headache may happen. They are usually less severe compared to tricyclic antidepressants. To treat major depressive disorder (MDD) and generalized anxiety disorder (GAD) Escitalopram can be used.

L-methylfolate is chemically named as (2S)-2- [[4-[(2-Amino-5-methyl-4-oxo-1,6,7,8-tetrahydropteridin-


http://dx.doi.org/10.31788/RJC.2019.1245438

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6-yl) methylamino [benzoyl] amino] pentane dioic acid shown in Fig.-2. L-methyl folate is used in the methylation of homocysteine to form methionine and tetrahydrofolate (THF). THF is the immediate acceptor of one-carbon units for the synthesis of thymidine-DNA, purines (RNA and DNA) and methionine. The un-methylated form, folic acid (vitamin B9), is a synthetic form of folate and must undergo enzymatic reduction by methylenetetrahydrofolate reductase (MTHFR)[6,7] to become biologically active.[8]

Several assay methods including UV [9-14], HPLC[15-24] and HPTLC[25-27] are stated in the literature for the estimation of Escitalopram and L-methyl folate separately and in combined dosage forms. Hence, a new method was developed for the simultaneous estimation and validation of Escitalopram and L-methyl folate in a tablet dosage form as per ICH guidelines.[28]

## EXPERIMENTAL

### Chemicals

The standard samples of Escitalopram and L-methyl folate were obtained from Spectrum pharma research solutions, Hyderabad. The chemicals used for separation were HPLC grade acetonitrile, HPLC grade methanol was provided by Merck chemical division, Mumbai. Throughout the separation HPLC grade water obtained from Milli-Q water was used. REMFOLET, a commercial tablet contains dosage of Escitalopram-7.5mg & L-methyl folate- 10mgwerebroughtfromthe local pharmacy.

### Chromatographic Conditions

The separation was carried out by RP-HPLC waters 2695 with 2996 Photodiode Array Detector. The chromatographic peak integration along with data acquisition and data processing was obtained by using Empower 2 software. The separation of analytes was carried out using Hypersil ODSC18, (250 x 4.6 mm, 5µ) column and mobile phase comprising of Perchloric acid Buffer: Acetonitrile (45:55 % v/v) at a flow rate of 1.0 ml/min. The mobile phase used was sonicated for 5 min in ultrasonic bath before using for separation and filtered through a 0.45µm nylon filter. The sample solutions were studied at 215nm at an injection volume of 10 µL.

### Perchloric Acid Preparation

About 1ml of perchloric acid in 1L of milli-Q water.

### Preparation of Diluent

A mixture of water: acetonitrile 50:50 v/v.

### Preparation of Stock Solutions

**Escitalopram (750µg/ml):**

Escitalopram of about 18.75 mg was dissolved in 25 ml of diluent.

**L-methyl folate (1000µg/ml)**

L-methyl folate of about 25mg was dissolved in 25 ml of diluent.

### Standard Preparations

**Escitalopram Standard Preparation** (75µg/ml):

From the stock solution of Escitalopram 1 ml was dissolved in 10 ml of diluent.

**L-methyl folate standard Preparation** (100µg/ml):

From the stock solution of L-methyl folate, 1ml was dissolved in 10ml diluent.

### Method Validation

Validation parameters specificity, accuracy, linearity, precision, robustness, the stability of sample at & 24 hours, LOD & LOQ were assessed as per ICH guidelines.

**Specificity**
The measure of analyte response with degradation products shows the ability of an analytical method specificity.

**Accuracy**
The percentage of recovery of Escitalopram and L-methyl folate was calculated to get accuracy of proposed method. Each analyte was examined at three concentration levels 50%, 100% & 150% and six determinations were carried out at each level to get accurate results.

**Precision**
The intra-day and inter-day precision were carried out to estimate the response of an analyte and to express the standard deviation of an analytical method. To estimate the response of intra-day precision of Escitalopram and L-methyl folate repeatability studies were carried out six times and inter-day precision of Escitalopram and L-methyl folate was carried out at three different concentrations for three different days and three times on each day.

**Linearity**
The linearity test was performed to validate the analytical system linear response and is directly proportional to the range of concentration of analyte. The linear regression data of Escitalopram and L-methyl folate over a wide range of concentration of displays corresponding peak area, a calibration curve indicates good linear relationship with correlation coefficient.

**Robustness**
To validate the robustness of an analytical method certain changes like concentration ratio of mobile phase solvents, the flow rate of an analyte and temperature of the column were done to the proposed method and it does not show any effect on the peak tailing, peak area, and theoretical plates.

**Limit of Detection & Limit of Quantitation**
The smallest level of analyte that gives a measurable response as LOD and the lowest amount of analyte that was reproducibly quantified gives LOQ.
LOD and LOQ were calculated by using equations, LOD=3.3 × sd/s and LOQ=10 × sd/s, where sd = standard deviation, S= slope of the calibration curve.

**Assay of Escitalopram and L-methyl folate in Tablet**
The assay of REMFOLET, a commercial tablet contains a dosage of Escitalopram-7.5mg & L-methyl folate-10mgwas found out by injecting sample solutions and scanning at 215 nm. The chromatogram of commercial tablets of Escitalopram and L-methyl folate was observed with retention times of 2.158 and 3.899 min.

**Forced Degradation Studies**
According to ICH guidelines forced degradation studies of hydrolysis (acidic and alkaline), photolysis, oxidation, and thermal degradations were performed for Escitalopram and L-methyl folate.

**Oxidation**
The degradation of oxidation was carried out by adding 1 ml of 20% hydrogen peroxide to1 ml of stock solutions of Escitalopram and L-methyl folate separately and kept for 30 min at $60^0$ C.
The solutions obtained were dilutedto75µg/ml of Escitalopram& 100µg/ml of L-methyl folate. 10µlof each solution was injected into the system to get chromatograms and to assess the stability of the sample.

**Acid Degradation Studies**
Acid degradation of escitalopram and L-methyl folate was studied by adding1 ml of 2N Hydrochloric acid to 1 ml of stock solutions separately and refluxed for 30mins at $60^0$C. The solutions obtained were dilutedto75µg/ml of Escitalopram& 100µg/ml of L-methyl folate. 10µlof each solution was

injected into the system to get chromatograms and to assess the stability of sample.

**Alkali Degradation Studies**

Alkali degradation of escitalopram and L-methyl folate was studied by adding1 ml of 2N of NaOH to 1 ml of stock solutions separately and refluxed for 30mins at $60^0$C. The solutions obtained were dilutedto75µg/ml of Escitalopram& 100µg/ml of L-methyl folate. 10µl of each solution was injected into the system to get chromatograms and to assess the stability of the sample.

**Thermal Degradation Studies**

The dry heat degradation was carried out by placing Escitalopram and L-methyl folate solutions in oven at$105^0$ C for6hourstostudydryheat degradation. The solutions obtained were dilutedto75µg/ml of Escitalopram& 100µg/ml of L-methyl folate. 10µlof each solution was injected into the system to get chromatograms and to assess the stability of sample.

**Photo Stability Studies**

The photochemical stability of Escitalopram and L-methyl folate was studied by exposing them to UV Light by keeping it in a UV Chamber for 7days or 200-watt hours/$m^2$ in photostability chamber. The solutions obtained were dilutedto75µg/ml of Escitalopram& 100µg/ml of L-methyl folate. 10µlof each solution was injected into the system to get chromatograms and to assess the stability of the sample.

**Neutral Degradation**

In neutral conditions, the drugs Escitalopram and L-methyl folate were exposed to water for 6 hrs at a temperature of 60ºC. The solutions obtained were dilutedto75µg/ml of Escitalopram& 100µg/ml of L-methyl folate. 10µlof each solution was injected into the system to get chromatograms and to assess the stability of sample.

## RESULTS AND DISCUSSION

**Optimized Chromatographic Conditions**

The best method was developed and validated for the estimation of Escitalopram and L- methyl folate to use in pharmaceutical formulations. To analyze the drugs several chromatographic conditions were used. Finally, the optimized chromatographic conditions employed were Perchloric acid Buffer: Acetonitrile in the ratio of 50:50 % v/ v mobile phase at a flow rate of 1.0 ml/min analyzed at 215nm at an injection volume of 10 µl by injecting into ODS C18, (250 x 4.6 mm, 5µ) column. Escitalopram and L-methyl folate standard and sample chromatograms were noted as sharp peaks shown in Fig.-3 and Fig.-4. The optimized conditions were given in Table-1.

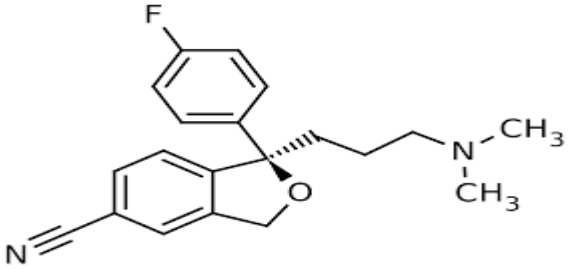


Fig.-1: Chemical Structure of Escitalopram

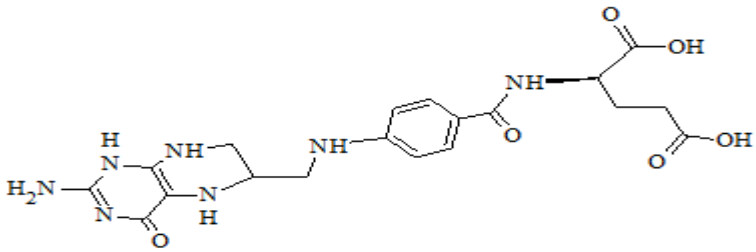


Fig.-2: Chemical Structure of L-methyl folate

Table-1: Optimized Chromatographic Conditions

| Parameters | Chromatographic conditions |
|---|---|
| Mobile Phase | Perchloric acid buffer: Acetonitrile (45:55 v/v) |
| Column | ODS C18, 250 x 4.6 mm, 5 µ. |
| Wave length | 215 nm |
| Flow rate | 1.0 ml/min |
| Injection volume | 20 µl |
| Run time | 7 min |
| Diluent | Water: Acetonitrile (50:50 v/v) |

The percentage of recoveries of Escitalopram and L-methyl folate ranges from 100.14%and 100.42% at three concentration levels 50%, 100% and 150% and shows % RSD < 2. The results were shown in Table-2.

Table-2: % Recovery Data for Escitalopram and L-methylfolate

| Drug | Spiked Level % | % Recovery | % RSD |
|---|---|---|---|
| Escitalopram | 50 | 99.77 | 1.51 |
| | 100 | 99.36 | 0.81 |
| | 150 | 100.8 | 1.05 |
| L-Methylfolate | 50 | 98.79 | 1.03 |
| | 100 | 100.4 | 1.53 |
| | 150 | 99.97 | 1.69 |

The repeatability and intermediate precisions of Escitalopram and L-methyl folate were recorded and shows % RSD < 2. The results were shown in Table-3.

Table-3: Precision Method of proposed RP-HPLC method

| Drug | Mean Area | % RSD |
|---|---|---|
| Escitalopram | 814793.2 | 0.7 |
| L-methyl folate | 1156745 | 0.75 |

The linear response of Escitalopram and L-methyl folate was obtained by choosing range between 18.75-112.5µg/mL of Escitalopram and 25-150µg/mL of L-methyl folate. The regression equation and correlation coefficient for Escitalopram and L-methyl folate were found to be y = 10861x + 20789; $R^2$=0.9990 and y = 11294x + 32742; $R^2$=0.9990 respectively and results were given in Table-4. Calibration curves were noted in Fig.-5 and Fig.- 6.

Table-4: Results of Linearity

| S. No. | Escitalopram | | L-Methylfolate | |
|---|---|---|---|---|
| | Conc. (µg/ml) | Peak Area | Conc. (µg/ml) | Peak Area |
| 1 | 18.75 | 231681 | 25 | 341517 |
| 2 | 37.5 | 440511 | 50 | 610782 |
| 3 | 56.25 | 646843 | 75 | 886452 |
| 4 | 75 | 828672 | 100 | 1164400 |
| 5 | 93.75 | 1045932 | 125 | 1430712 |
| 6 | 112.5 | 1228496 | 150 | 1724533 |

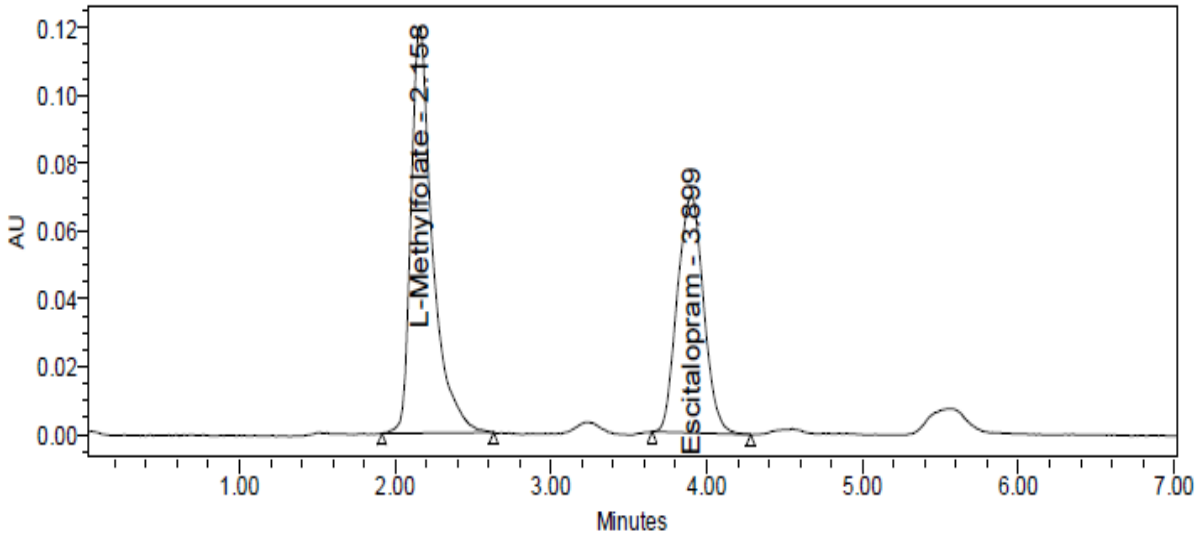


Fig.-3: Standard Chromatogram of Escitalopram and L-methyl folate

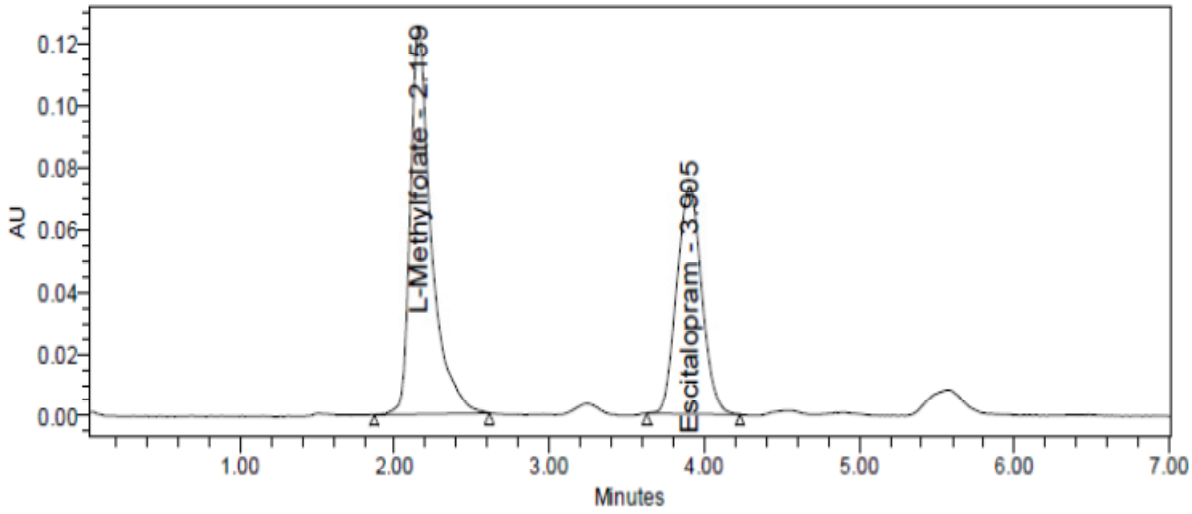


Fig.-4: A Typical Chromatogram of Escitalopram and L-methyl folate in Tablet Dosage Form

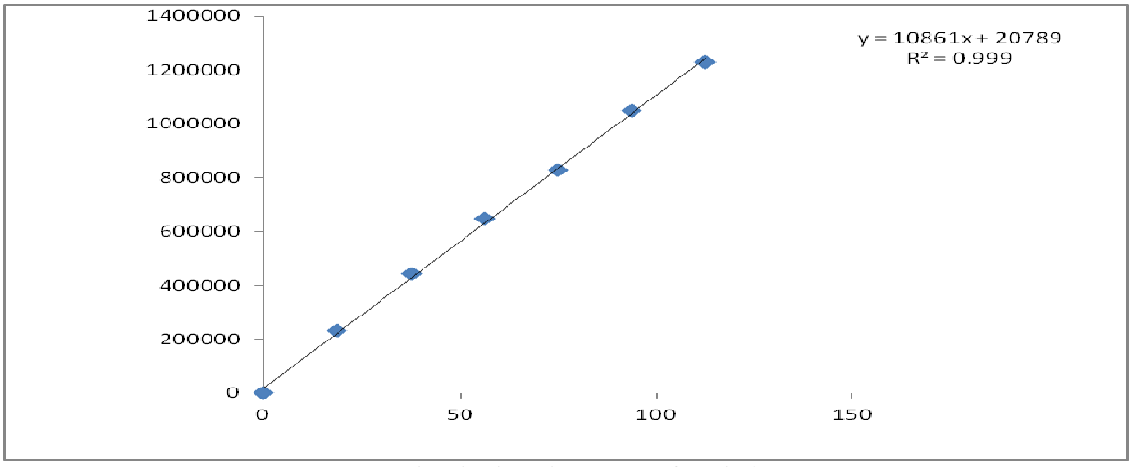


Fig.-5: Linearity Curve of Escitalopram

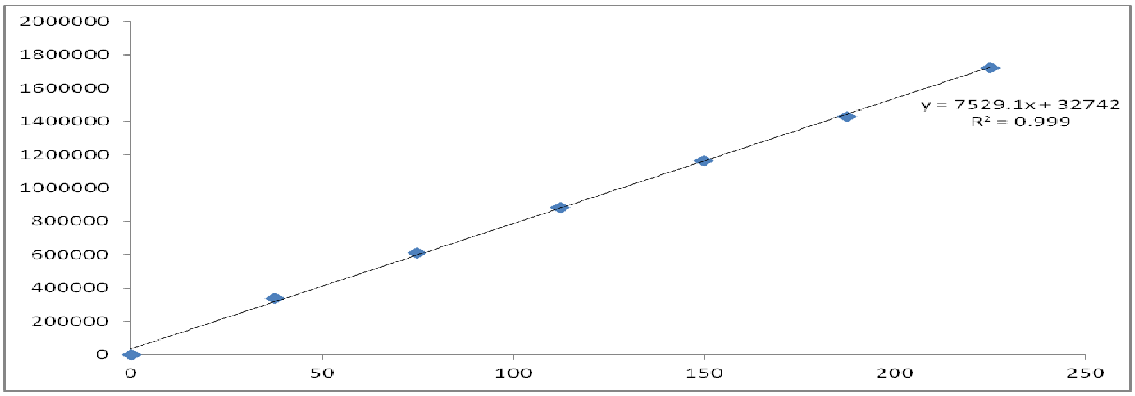


Fig.-6: Linearity Curve of L-methyl folate

Robustness of Escitalopram and L-methyl folate was obtained by changing the mobile phase composition by 5% variation, flow rate by 0.1 units and column temperature by 5°C. It has no significant effect on chromatographic behavior of the samples and results were given in Table-5.

Table-5: Robustness Data

| Parameters | Changed condition | Mean Peak Area | | USP Plate count | |
|---|---|---|---|---|---|
| | | Escitalopram | L-methylfolate | Escitalopram | L-methylfolate |
| Flow rate (ml/min) | 0.9 | 666540 | 734095 | 2706 | 1869 |
| | 1 | 824280 | 1161199 | 9316 | 9419 |
| | 1.1 | 947853 | 1079060 | 3275 | 2187 |
| Temperature (±5 0C) | 25 | 761330 | 1056087 | 3728 | 1956 |
| | 30 | 824280 | 1161199 | 9316 | 9419 |
| | 35 | 1273521 | 1491055 | 3935 | 1896 |
| Mobile Phase (±5%) | 70:30 (%v/v) | 880927 | 957504 | 2932 | 1962 |
| | 75:25(%v/v) | 824280 | 1161199 | 9316 | 9419 |
| | 80:20 (%v/v) | 793424 | 925800 | 2921 | 2010 |

LOD and LOQ of Escitalopram and L-methyl folate were evaluated based on relative standard deviation of the response and slope of the calibration curve. The detection limits were found to be 0.04µg/mL and 0.01µg/mL for Escitalopram and L-methyl folate respectively. The quantitation limits were found to be 0.13µg/mL and 0.04µg/mL for Escitalopram and L-methyl folate respectively. The results were given in Table-6.

Table-6: Results of LOD and LOQ

| Drug | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| Escitalopram | 0.04 | 0.13 |
| L-methylfolate | 0.01 | 0.04 |

Forced degradation studies of Escitalopram and L-methyl folate were carried out under conditions of hydrolysis (acid and base), dry heat, oxidation, UV light, photolysis and neutral medium.
It was clearly showed that Escitalopram and L-methyl folate were degraded at acid condition about 4.67% and 4.66% & alkali 3.30% and 3.72% respectively. Both Escitalopram and L-methyl folate are sensitive to acid and alkali and there was no degradation occurs under UV light and neutral conditions. The respective chromatograms were shown from Fig.-7 to Fig.-12 and the results of forced degradation studies were given in Table-7.

Table-7: Results of Forced Degradation Studies

| S. No. | Degradation condition | Escitalopram | | L-methylfolate | |
|---|---|---|---|---|---|
| | | % Assay | % Degradation | % Assay | % Degradation |
| 1 | Acid Degradation | 95.33 | 4.67 | 95.33 | 4.67 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | Base Degradation | 96.7 | 3.3 | 96.27 | 3.73 |
| 3 | Peroxide Degradation | 97.76 | 2.24 | 97.43 | 2.57 |
| 4 | Thermal Degradation | 98.69 | 1.31 | 98.15 | 1.85 |
| 5 | UV Degradation | 99.59 | 0.41 | 99.54 | 0.46 |
| 6 | Neutral Degradation | 99.89 | 0.11 | 99.49 | 0.51 |

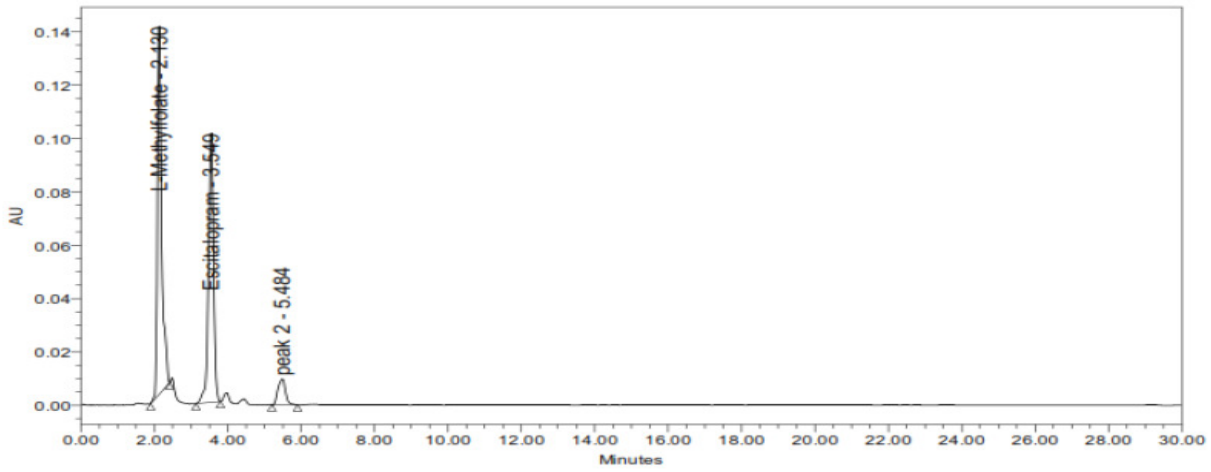


Fig.-7. Peroxide Degradation of Escitalopram and L-Methylfolate

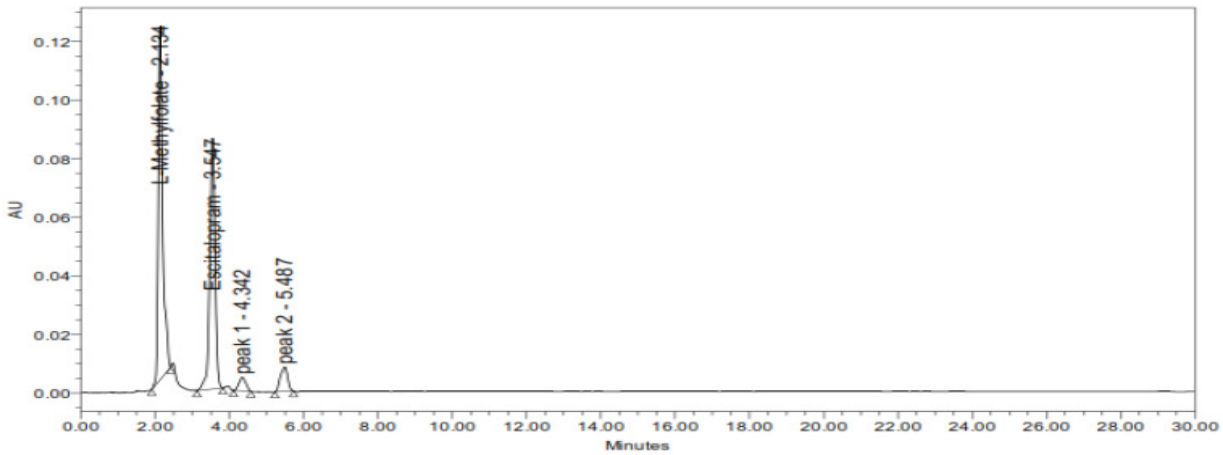


Fig.-8. Acid Degradation of Escitalopram and L-Methylfolate

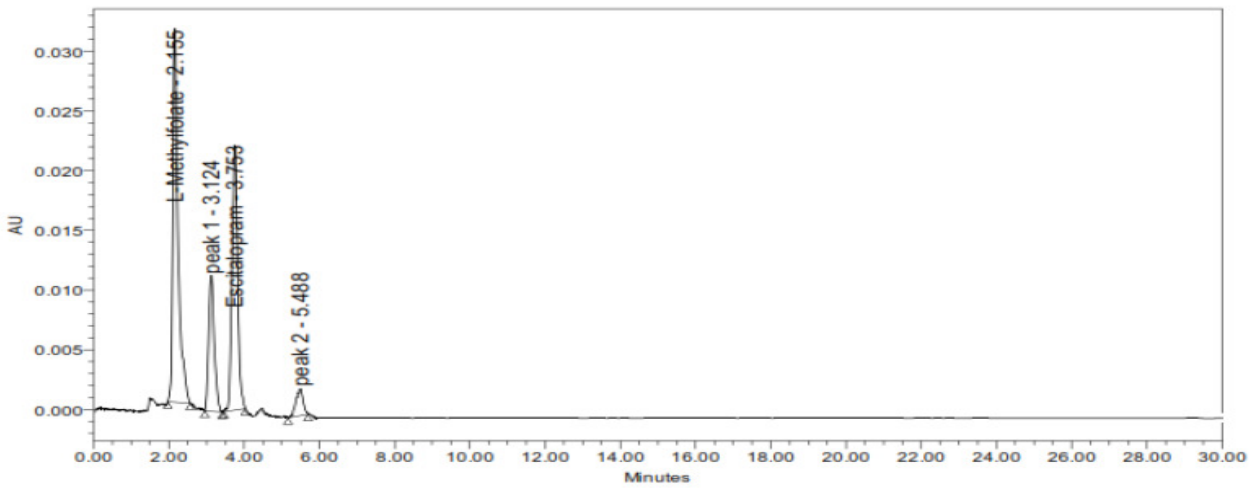


Fig.-9. Alkali Degradation of Escitalopram and L-Methylfolate

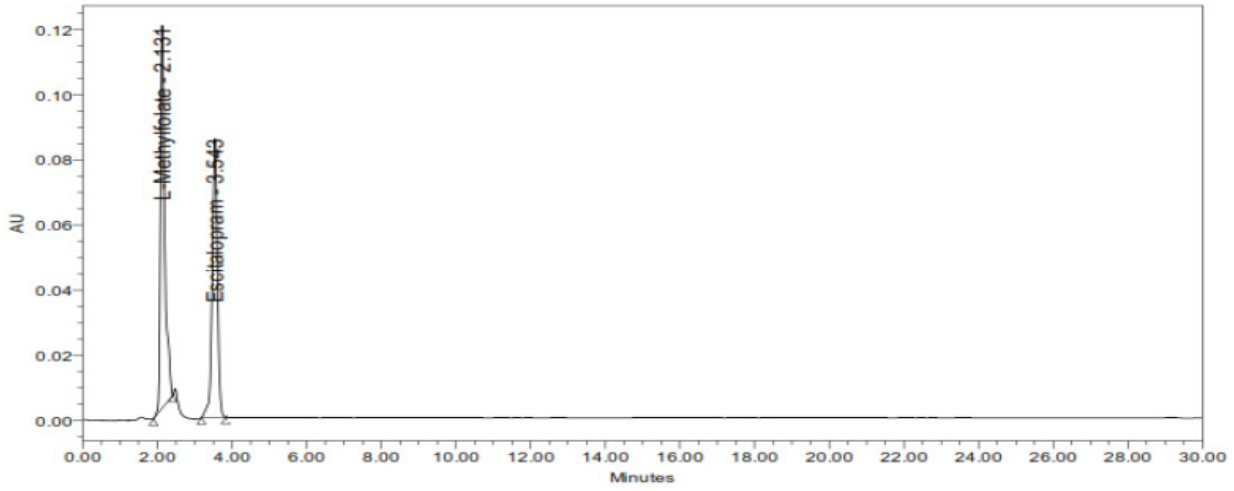


Fig.-10: Thermal Degradation of Escitalopram and L-Methylfolate

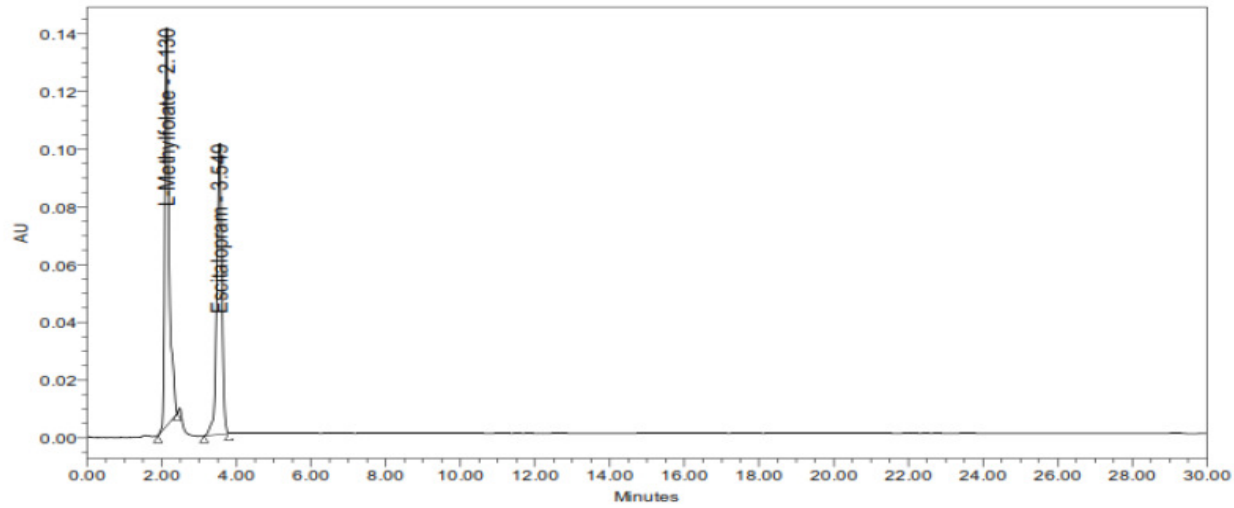


Fig.-11. UV Degradation of Escitalopram and L-Methylfolate

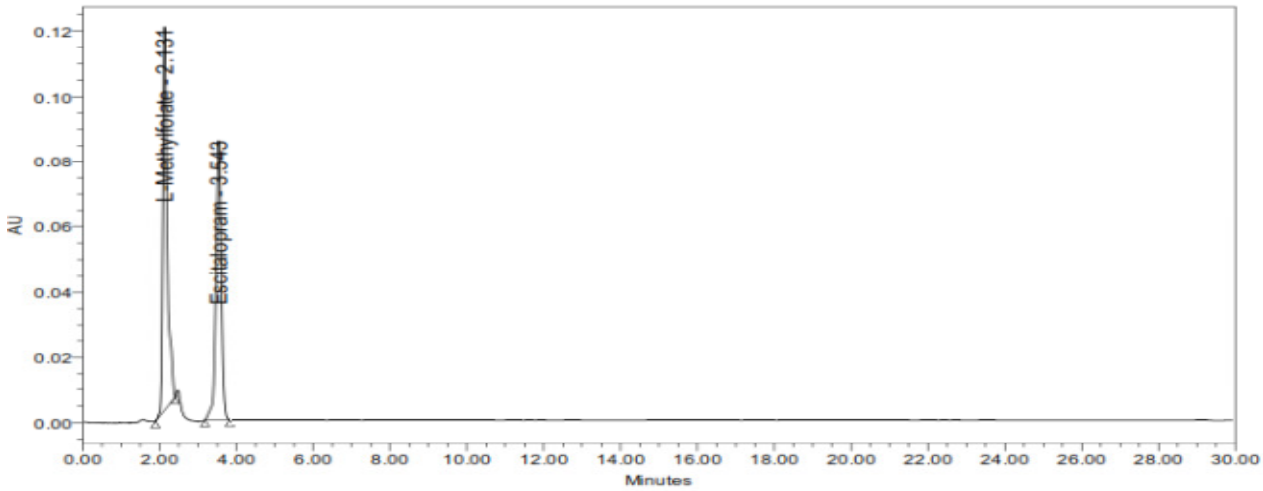


Fig.-12. Neutral Degradation of Escitalopram and L-Methylfolate

## CONCLUSION

The new stability-indicating RP-HPLC method has been developed for estimation of Escitalopram and L-methyl folate in bulk and pharmaceutical dosage form. The developed method was validated, and it was

found to be simple, sensitive, precise, robustness and it can be used for the routine analysis of Escitalopram and L-methyl folate in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Escitalopram and L-methyl folate under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. Finally, it was concluded that the method is simple, sensitive and can separate the drug from degradation products and excipients found in the dosage form.

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[RJC-5438/2019]