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A New stability indicating RP-HPLC method for simultaneous estimation of Escitalopram and L-methylfolate in bulk and tablet dosage form

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Abstract

The Present Study, A New Stability- Indicating RP-HPLC Method Has Been Developed For Simultaneous Estimation Of Escitalopram And L-Methylfolate In Bulk And Tablet Dosage Form. The Developed Method Was Considered A Sensitive, Precise, And Accurate RP-HPLC Method For The Analysis Of Escitalopram And L-Methylfolate. To Optimize The Mobile Phase, Various Combinations Of Buffer And Organic Solvents Were Used On Kromosil-250x4.6mm, 5µ Column. Then The Mobile Phase Containing A Mixture Of 0.1% OPA (Ph 4.0) And Acetonitrile 60:40 % V/V Was Selected At A Flow Rate Of 1.0 Ml/Min For Developing The Method And The Peaks With Good Shape And Resolution Were Found Resulting In Short Retention Time, Baseline Stability And Minimum Noise. The Retention Times Of Escitalopram And L-Methylfolate Were Found To Be 3.045min And 3.661min Respectively. No Interfering Peaks Were Found In The Chromatogram Indicating That Excipients Used In Formulations Didn't Interfere With The Estimation Of The Drugs By The Proposed HPLC Method.

Keywords: Stability RP-HPLC, Epalrestat And Pregabalin, Validation.

Introduction

Escitalopram [(1-9),] The S-Enantiomer Of Citalopram, Belongs To A Class Of Antidepressant Agents Known As Selective Serotonin-Reuptake Inhibitors (Ssris). Despite Distinct Structural Differences Between Compounds In This Class, Ssris Possess Similar Pharmacological Activity. As With Other Antidepressant Agents, Several Weeks Of Therapy May Be Required Before A Clinical Effect Is Seen. Ssris Are Potent Inhibitors Of Neuronal Serotonin Reuptake. They Have Little To No Effect On Norepinephrine Or Dopamine Reuptake And Do Not Antagonize A- Or B-Adrenergic, Dopamine D2 Or Histamine H1 Receptors. Chemically, Escitalopram Is (1S)-1-[3-(Dimethylamino) Propyl]-1-(4-Fluorophenyl)-1, 3-Dihydro-2-Benzofuran-5-Carbonitrile. The Antidepressant, Antiobsessive-Compulsive, And Antibulimic Actions Of Escitalopram Are Presumed To Be Linked To Its Inhibition Of CNS Neuronal Uptake Of Serotonin.

Escitalopram Blocks The Reuptake Of Serotonin At The Serotonin Reuptake Pump Of The Neuronal Membrane, Enhancing The Actions Of Serotonin On 5HT1A Autoreceptors. Ssris Bind With Significantly Less Affinity To Histamine, Acetylcholine, And Norepinephrine Receptors Than Tricyclic Antidepressant Drugs.

Epalrestat Is The Only ARI Commercially Available. It Is Easily Absorbed Into The Neural Tissue And Inhibits The Enzyme With Minimum Side Effects. The Chemical Structure Of Was Given In Fig 1.

5-Methyltetrahydrofolic Acid [10-14] 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. Chemically It Is (2S)-2-[[4-[[(6S)-2-Amino-5-Methyl-4-Oxo-1, 6, 7, 8-Tetrahydropteridin-6-Yl] Methylamino] Benzoyl] Amino] Pentanedioic Acid

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. The Chemical Structure Of L-Methylfolate Was Given In Fig 2.

The Review Of Literature Revealed That Several Analytical Methods Have Been Reported For Escitalopram And L-Methylfolate [18-21] In Spectrophotometry, HPLC, HPTLC And LC/MS Individually And In Combination. To Date, There Have Been No Published Reports About The Stability Indicating Studies And Simultaneous Estimation Of Escitalopram And L-Methylfolate By HPLC In Bulk Drug And In Tablet Dosage Forms. This Present Study Reports For The First Time Stability Indicating Simultaneous Estimation Of Escitalopram And L-Methylfolate By RP-HPLC In Bulk Drug And In Tablet Dosage Form.

Materials and Methods

Chemicals and Reagents

Escitalopram And L-Methylfolate Were Obtained As Gift Sample From Spectrum Pharma Research Laboratory In Hyderabad. Tablets (Escitafol, Intas Pharmaceuticals Ltd.) Containing Escitalopram (7.5 Mg) And L-Methyl Folate(10 Mg)Marketed Formulation Was Purchased From Local Market. Acetonitrile, Water HPLC Grade (Merck. Mumbai, India) Potassium Dihydrogen Ortho Phosphate, Triethylamine (RANKEM, Mumbai, India.) Ortho Phosphoric Acid HPLC (Merck., Mumbai, India) All Solvents Used In This Work Are HPLC Grade.

Instrument And Chromatographic Conditions

RP-HPLC Waters 2695 Separation Module Equipped With 2996Photodiode Array Detector Was Employed In This Method. The Empower 2 Software Was Used For LC Peak Integration Along With Data Acquisition And Data Processing. The Column Used For Separation Of Analytes Kromosil-(250x4.6mm, 5µ). Mobile Phase Consisting Of 0.1% OPA (Ph 4.0) And Acetonitrile 60:40 %V/V At A Flow Rate Of 1.0 Ml/Min. It Was Filtered Through 0.45µm Nylon Filter And Sonicated For 5 Min In Ultrasonic Bath The Total Runtime Was Set As 8min. Samples Were Analysed At 210 Nm At An Injection Volume Of 10 Ml.

Preparation of Mobile Phase

Preparation Of 0.1% OPA (Ph 4.0)

Accurately 1ml Of OPA 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 Adjust Ph 4.0 With Triethylamine. Sonicate To Degas.

Preparation of Solutions

Preparation of Escitalopram Stock Solution

Accurately Weighed And Transferred 7.5mg Of Escitalopram In To 10ml Of Clean Dry Volumetric Flask, Add 7ml Of Diluent, Then Sonicated For 10min And Make Up The Volume With Diluent.

Preparation Of L-Methylfolate Stock Solution

Accurately Weighed 10mg Of L-Methylfolate And Transferred Into 10ml Of Clean Dry Volumetric Flask, Add 7ml Of Diluent, Then Sonicated For 10 Min And Make Up The Final Volume With Diluent.

Preparation Of Escitalopram Standard Solution

From The Above Escitalopram Stock Solution 1ml Was Pipette Out Into 10ml Of Clean Dry Volumetric Flask And Make Up The Final Volume With Diluent.

Preparation Of L-Methylfolate Standard Solution

From The Above L-Methylfolate Stock Solution 1ml Was Pipette Out Into A 10ml Clean Dry Volumetric Flask And Make Up The Final Volume With Diluent.

Method Validation

The Validation Of The Method Was Carried Out As Per ICH Guidelines**.** The Parameters Assessed Were Precision, Accuracy And Stability.

Accuracy

The Accuracy Was Determined By Calculating % Recoveries Of Escitalopram And L-Methylfolate. It Was Carried Out By Adding Known Amounts Of Each Analyte Corresponding To Three Concentration Levels (50, 100, And 150%) Of The Labelled Claim To The Excipients. At Each Level, Six Determinations Were Performed And The Accuracy Results Were Expressed As Percent Analyte Recovered By The Proposed Method.

Precision

Precision Of An Analytical Method Is Usually Expressed As The Standard Deviation. The Repeatability Studies Were Carried Out By Estimating Response Of Escitalopram 75µg/Ml And L-Methylfolate 100µg/Ml Six Times. The Intra-Day And Inter-Day Precision Studies (Intermediate Precision) Were Carried Out By Estimating The Corresponding Responses Three Times On The Same Day And On Three Different Days For Three Same Concentrations And The Results Are Reported In Terms Of Relative Standard Deviation.

System Suitability

System Suitability Was Performed By Freshly Preparing Standard Solutions Containing Escitalopram 75µg/Ml And L-Methylfolate 100µg/Ml. From The Prepared Solutions 10µl Solution Of Each Was Injected 6 Times Into The HPLC System And The Suitability Of The System Was Evaluated.

Assay of Escitalopram and L-Methylfolate in Injection

Assay Of Marketed Product Was Carried Out By Using The Developed Method. Sample Solutions Were Prepared And Injected Into RP-HPLC System. The Sample Solution Was Scanned At 235 Nm. The % Drug Estimated Was Found To Be 99.85 And 99.72% Escitalopram And L-Methylfolate Respectively. The Chromatogram Showed Two Single Peaks Of Escitalopram And L-Methylfolate Was Observed With Retention Times Of 3.042 And 3.657 Min (Figure 3).

Results & Discussions

Optimized Chromatographic Conditions

To Establish And Validate An Efficient Method For Analysis Of These Drugs In Pharmaceutical Formulations, Preliminary Tests Were Performed. Different Chromatographic Conditions Were Employed For The Analysis Of The Escitalopram And L-Methylfolate In Both Bulk And Tablet Dosage Form. Finally The Analysis Was Performed By Using 0.1% OPA (Ph 4.0) And Acetonitrile 60:40 %V/V At A Flow Rate 1.0 Ml/Min. Samples Were Analysed At 230nm At An Injection Volume Of 10 Ml And Separation Was Carried By Using Kromosil-250x4.6mm, 5µ Column. The Proposed Method Was Optimized To Give A Sharp Peak With Minimum Tailing For Escitalopram And L-Methylfolate (Fig 4). The Optimized Conditions Were Given In Table 1.

Precision Was Evaluated By A Known Concentration Of Escitalopram And L-Methylfolate Was Injected Six Times

And Corresponding Peaks Were Recorded And % RSD Was Calculated And Found Within The Limits. The Low % RSD Value Was Indicated That The Method Was Precise And Reproducible And The Results Were Shown In The Table (Table 2). Accuracy Of The Method Was Proved By Performing Recovery Studies On The Commercial Formulation At 50, 100 And 150% Level. % Recoveries Of Escitalopram And L-Methylfolate Ranges From 98.33 To 101.79% And 99.7 To 101.2% For Escitalopram And L-Methylfolate Respectively In Simultaneous Equation Method And The Results Were Shown In The (Table 3).

Conclusion

A New Stability- Indicating RP-HPLC Method Has Been Developed For Estimation Of Escitalopram And L-Methylfolate In Bulk And Tablet Dosage Form. The Developed Method Was Validated And It Was Found To Be Simple, Sensitive And Precise And It Can Be Used For The Routine Analysis Of Escitalopram And L-Methylfolate In Both Bulk And Pharmaceutical Dosage Forms. Finally It Was Concluded That The Method Is Simple, Sensitive And Has The Ability To Separate The Drug From Excipients Found In The Dosage Form.

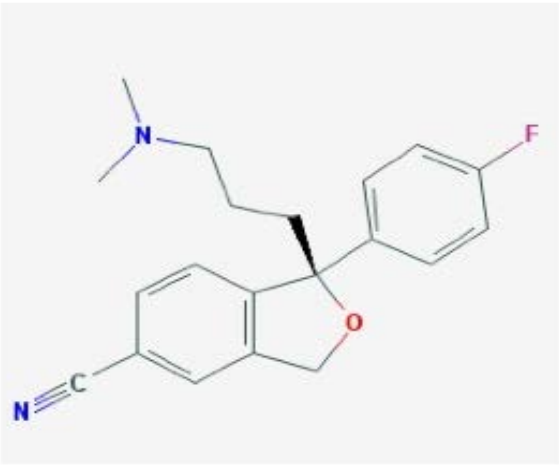

Fig 1: Chemical Structure of Escitalopram

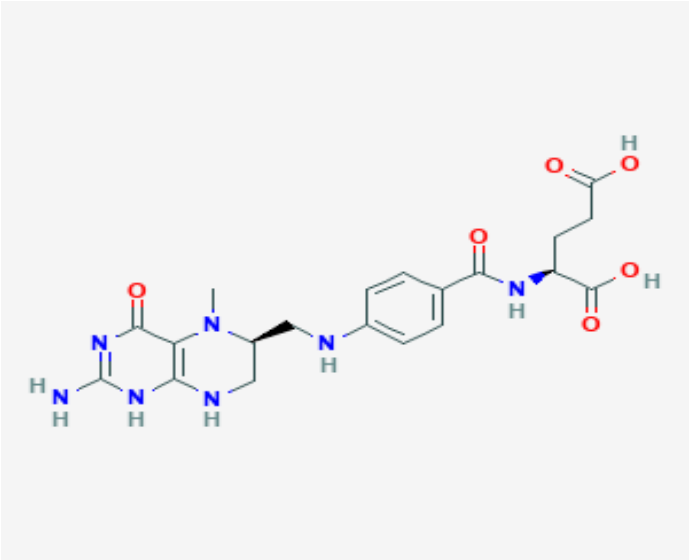

Fig 2: Chemical Structure of L- Methylfolate

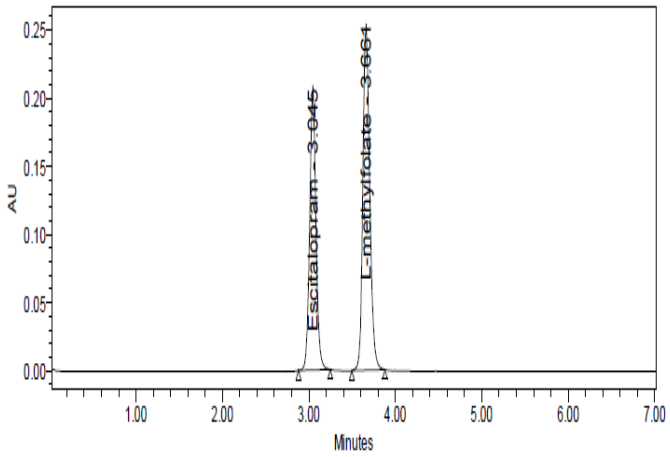

Fig 4: Standard Chromatogram Of Escitalopram And L-Methylfolate (API)

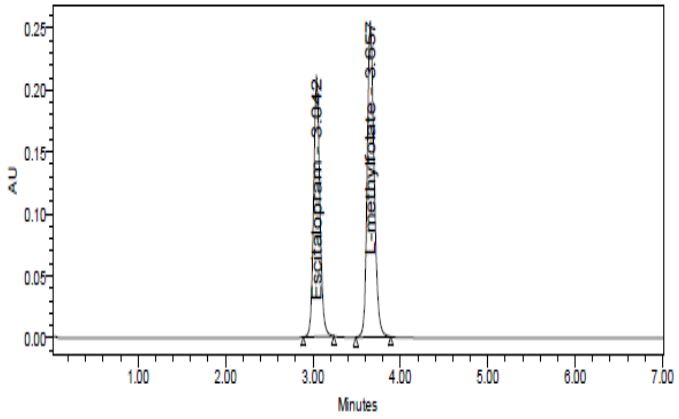

Fig 3: A Typical Chromatogram Of Escitalopram And L-Methylfolate In Tablet Dosage Form

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	Water 2695 Separation Module With PDA Detector
Mobile Phase	0.1% OPA Ph 4.0:Acetonitrile 65:35%V/V
Column	Kromosil-250x4.6mm, 5µ Column
Column Temperature	30 ^{0}C
Wavelength	230nm
Diluent	Water:ACN (50:50)
Injector Volume	10µl
Flowrate	1ml/Min
Runtime	7min
Retention Time	Escitalopram -3.045min And L-Methylfolate -3.661min
Theoretical Plates	Escitalopram -8669 And L-Methylfolate -10198

Table 2: Precision Method Of Proposed RP-HPLC Method

Injection	Escitalopram Concentration	Area	L-Methylfolate Concentration	Area
1	75µg/Ml	1036318	100µg/Ml	1401288
2		1029223		1403067
3		1031609		1408727
4		1022751		1399220
5		1035510		1402039
6		1038562		1393429
Mean		1032329		1401295
STDV		5775.4		5002.4
%RSD		0.6		0.4

Table 3:% Recovery Results of Escitalopram And L-Methylfolate

Conc.	Escitalopram			L-Methylfolate		
	Amount Added (µg/Ml)	Amount Recovered (µg/Ml)	% Recovery	Amount Added (µg/Ml)	Amount Recovered (µg/Ml)	% Recovery
50%	37.5	37.59	100.23	50	49.63	99.3
	37.5	38.17	101.79	50	50.12	100.2
	37.5	37.04	98.77	50	50.12	100.2
100%	75	74.73	99.64	100	99.12	99.1
	75	74.57	99.43	100	100.1	100.1
	75	75.07	100.09	100	101.00	101.0
150%	112.5	110.62	98.33	150	148.04	98.7
	112.5	111.02	98.68	150	148.95	99.3
	112.5	110.74	98.43	150	151.8	101.2

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