A REVIEW ON CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC ESTIMATION OF ESCITALOPRAM OXALATE AND ESZOPICLONE IN BULK AND IN DIFFERENT DOSAGE FORMS

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ABSTRACT
Nowadays antidepressant drugs like selective serotonin reuptake inhibitors (SSRIs) represent the first choice in the treatment of moderate to severe depressive illness, various phobias, and personality disorders and non-benzodiazepine have a demonstrated efficacy in treating sleep disorders. This review includes most of the published analytical methods for estimation of Escitalopram oxalate and eszopiclone based on high-performance liquid chromatography coupled with UV, fluorescence and mass spectrometry detectors, capillary electrophoresis and gas chromatography with mass spectrometry detectors among others. Thus, this paper will help in the selection and development of proper analytical methodologies for estimation of SSRIs and non-benzodiazepine to achieve satisfactory results.

KEYWORDS: Selective Serotonin Reuptake Inhibitors (SSRIs), Non – benzodiazepine, High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC).

INTRODUCTION
Selective serotonin re-uptake inhibitors or serotonin-specific reuptake inhibitors (SSRIs) are a class of compounds typically used as antidepressants in the treatment of major depressive disorder and anxiety disorders.

Escitalopram oxalate is an orally administered selective serotonin reuptake inhibitor (SSRI). Escitalopram utilized as a part of treatment of major depressive disorder and generalized Anxiety Disorder Escitalopram is the pure S-enantiomer (single isomer) of the racemic bicyclic phthalane derivative citalopram. Escitalopram oxalate is designated S-(+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile oxalate.

The mechanism of antidepressant action of Escitalopram, the S-enantiomer of racemic citalopram, is presumed to be linked to potentiation of serotonergic activity in the central nervous system (CNS) resulting from its inhibition of CNS neuronal reuptake of serotonin (5-HT).

Escitalopram oxalate are believed to increase the extracellular level of the neurotransmitter serotonin by limiting its reabsorption into the presynaptic cell, increasing the level of serotonin in the synaptic cleft available to bind to the postsynaptic receptor. They have varying degrees of selectivity for the other monoamine transporters, with pure SSRIs having only weak affinity for the norepinephrine and dopamine transporters.

Use of Escitalopram oxalate is, the main indication for SSRIs is major depressive disorder (also called "major depression", “clinical depression” and often simply “depression”). It is prescribed for anxiety disorders, such as social anxiety disorder, panic disorders, obsessive–compulsive disorder (OCD), eating disorders, chronic pain and occasionally, for posttraumatic stress disorder (PTSD). It is also frequently used to treat depersonalization disorder, although generally with poor results.

Escitalopram oxalate have the power to markedly improve mood, outlook, and behavior in people with depression.

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Escitalopram oxalate have the power to markedly improve mood, outlook, and behavior in people with depression.

The no benzodiazepines are positive allosteric modulators of the GABA-A receptor. Like the benzodiazepines, they exert their effects by binding to and activating the benzodiazepine site of the receptor complex. Many of these compounds are subtype selective providing novel anxiolytics with little to no hypnotic and amnesic effects and novel hypnotics with little or no anxiolytic effects.

Eszopiclone is a no benzodiazepine hypnotic agent that is a pyrrolopyrazine derivative of the cyclopyrrolo
class. The chemical name of eszopiclone is (+)-(5S)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5H-pyrido[3,4-b]pyrazin-5-yl 4-methylpiperazine-1-carboxylate. Its molecular weight is 388.81, and its empirical formula is C_{17}H_{17}ClN_{6}O_{3}. Eszopiclone has a single chiral center with an (S)-configuration.

The precise mechanism of action of eszopiclone as a hypnotic is unknown, but its effect is believed to result from its interaction with GABA-receptor complexes at binding domains located close to or allosterically coupled to benzodiazepine receptors.

Side effect of eszopiclone Memory loss, mental/mood/behavior changes (such as new/worsening depression, abnormal thoughts, thoughts of suicide, hallucinations, confusion, agitation, aggressive behavior, and anxiety).

Allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

This paper gives an overview of various analytical methods for estimation of Escitalopram oxalate and eszopiclone. Different methods have been developed for determination of Escitalopram oxalate and eszopiclone like UV-Spectroscopy, liquid Chromatography, HPTLC and LC-MS.

Reported methods are categorized depending on the following considerations
1. Escitalopram oxalate and eszopiclone analyzed by UV-Spectroscopy methods and Chromatographic method.
2. Analysis of Escitalopram oxalate and eszopiclone from combination formulation with other drug by UV-Spectroscopy methods and Chromatographic method

Analysis of Escitalopram oxalate individual and combination with other drug by spectrophotometric and chromatographic method

Escitalopram oxalate is official in Indian pharmacopoeia.

### TABLE 1.1: OFFICIAL METHODS FOR ESTIMATION OF ESCITALOPRAM OXALATE

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>DRUG</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escitalopram oxalate (IP 2014)</td>
<td>Liquid chromatography</td>
<td>Detection Wavelength: 240 nm Mobile Phase: n-hexane, Ethanol, Trifluoroacetic Acid (50:50 v/v) Stationary Phase: Stainless Steel Column 25 cm × 3.6 mm packed with Octadecylsilane Flow Rate: 0.4 ml/min</td>
</tr>
</tbody>
</table>

### TABLE 1.2 REPORTED METHOD OF ESCITALOPRAM OXALATE

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>DRUG</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escitalopram in Tablet Dosage Forms</td>
<td>Colorimetric Method</td>
<td>Wavelength:417 nm Linearity Range:2-10 µg/ml Correlation Coefficient (R²): 0.9996 LOD: 0.00345 µg/ml LOQ: 0.01045 µg/ml %Recovery: 98-102%</td>
</tr>
<tr>
<td>2</td>
<td>Escitalopram in Tablet Dosage Forms</td>
<td>RP-HPLC Method</td>
<td>Detection Wavelength:226 nm Mobile Phase: Methanol: disodium hydrogen phosphate: acetonitrile (28:44:28v/v) Stationary phase: BDS C8, 5- column (250x4.6mm) Linearity Range: 0.25-1.5 mg/ml Retention Time: 8.45 min Flow Rate: 1.5 ml/min %Recovery: 99.05% LOD: 0.023 µg/ml LOQ: 0.072 µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>Escitalopram in Tablet Dosage Forms</td>
<td>UV Spectrophotometric Method</td>
<td>Zero Order Derivative Wavelength:238 nm Solvent: Methanol : Water (8:2v/v) Linearity Range: 2-20 µg/ml Correlation Coefficient (R²): 0.9999 LOD:0.160 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Escitalopram in Tablet Dosage Forms</td>
<td>HPLC Method</td>
<td>Detection Wavelength: 238 nm  Mobile Phase: Acetonitrile : Methanol : 5 mM ammonium acetate buffer pH: 3 (30:20:50 v/v/v)  Stationary phase: Kromosil 5 µ column (250 x 4.6 mm)  Linearity Range: 5.09 - 15.27 μg/ml  Correlation Coefficient ($R^2$): 0.9997  Retention Time: 5.36 min  Flow Rate: 1.0 ml/min  % Recovery: 101.86%</td>
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<tr>
<td>5</td>
<td>Escitalopram oxalate in Tablet Dosage Forms</td>
<td>Spectrophotometric Method</td>
<td>Wavelength: 507 nm  Solvent: Methanol  Linearity Range: 2 - 14 μg/ml  % Recovery: 100.5%  Correlation Coefficient ($R^2$): 0.9983  LOD: 0.5 μg/ml  LOQ: 2 μg/ml</td>
</tr>
<tr>
<td>6</td>
<td>Escitalopram oxalate and clonazepam in combined dosage form</td>
<td>UV Spectrometry Method</td>
<td>First Order Derivative Wavelength  Escitalopram oxalate: 238 nm  Clonazepam: 273 nm  Solvent: Methanol  Linearity Range: 5 - 100 μg/ml  % Recovery: 99.07  Clonazepam: 98.56</td>
</tr>
<tr>
<td>7</td>
<td>Escitalopram oxalate and Etizolam in combined dosage form</td>
<td>RP-HPLC Method</td>
<td>Detection Wavelength: 254 nm  Mobile Phase: Acetonitrile:0.005 M Hexane Sulfonic Acid pH 3.0 (40 : 60 v/v/v)  Stationary phase: Kromasil 100 C18, 5 µ(150 x 4.6 mm)  Linearity Range:  Escitalopram : 20 - 160 μg/ml  Etizolam : 2 – 16 μg/ml  Correlation coefficient:  Escitalopram: 0.9994  Etizolam: 0.9993  % Recovery:  Escitalopram: 98.14-101.72%  Etizolam: 98.83-101.12%  Retention Time:  Escitalopram: 3.66 min  Etizolam: 8.07 min</td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 1 ml/min</td>
<td>Detection Wavelength:</td>
<td>First derivative spectroscopy</td>
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<tr>
<td>9</td>
<td>Escitalopram oxalate and Flupentixol dihydrochloride in combined dosage form</td>
<td>Escitalopram: 237 nm, Flupentixol dihydrochloride: 226 nm</td>
<td></td>
</tr>
</tbody>
</table>
Correlation coefficient: Escitalopram: 0.9995, Flupentixol dihydrochloride: 0.9991  
LOD: Escitalopram: 0.82 µg/ml, Flupentixol dihydrochloride: 0.04 µg/ml  
LOQ: Escitalopram: 2.5 µg/ml, Flupentixol dihydrochloride: 0.15 µg/ml |
| 10 | Escitalopram oxalate and clonazepam in combined dosage form | Waveslength: Escitalopram oxalate: 238 nm, Clonazepam: 222 nm  
Solvent: Methanol  
Linearity Range: Escitalopram oxalate: 10 - 24 µg/ml, Clonazepam: 2 - 14 µg/ml  
LOD: Escitalopram oxalate: 0.44 µg/ml, Clonazepam: 0.53 µg/ml  
LOQ: Escitalopram oxalate: 1.33 µg/ml, Clonazepam: 1.61 µg/ml  
Correlation coefficient: Escitalopram: 0.9992, Clonazepam: 0.9992s |
| 11 | Escitalopram oxalate and clonazepam in combined dosage form | Detection Wavelength: 240 nm  
Mobile Phase: buffer: acetonitrile (50:50 v/v)  
Stationary phase: Hypersil ODS C18 column (250mm X 4.6mm; 5µ)  
Linearity Range: Escitalopram: 20 - 120 µg/ml, Clonazepam: 1 - 6 µg/ml  
Correlation coefficient: Escitalopram: 0.9992, Clonazepam: 0.9991  
LOD: Escitalopram: 2.39 µg/ml, Clonazepam: 0.064 µg/ml  
LOQ: Escitalopram: 7.27 µg/ml, Clonazepam: 0.194 µg/ml  
Retention Time: Escitalopram: 2.840 ± 0.007 min, Clonazepam: 4.007 ± 0.006 min  
Flow rate: 1 ml/min |
<table>
<thead>
<tr>
<th>Page</th>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td><strong>RP – HPLC Method</strong></td>
<td><strong>Detection Wavelength:</strong> 239 nm&lt;br&gt;<strong>Mobile Phase:</strong> buffer: Methanol: Phosphate buffer (pH 6.0) (80:20 v/v)&lt;br&gt;<strong>Stationary phase:</strong> Agilent C18, 250 × 4.6 mm, 5μ particle size column&lt;br&gt;<strong>Linearity Range:</strong> 2-20 ppm&lt;br&gt;<strong>Correlation coefficient:</strong> Escitalopram: 0.995&lt;br&gt;Fenofibrate: 0.996&lt;br&gt;<strong>LOD:</strong> Escitalopram: 50ng/ml&lt;br&gt;Fenofibrate: 100ng/ml&lt;br&gt;<strong>LOQ:</strong> Escitalopram: 100ng/ml&lt;br&gt;Fenofibrate: 200ng/ml&lt;br&gt;<strong>Retention Time:</strong>&lt;br&gt;Escitalopram: 2.7min&lt;br&gt;Fenofibrate: 8.3 min&lt;br&gt;<strong>Flow rate:</strong> 1ml/min</td>
</tr>
<tr>
<td>13</td>
<td><strong>Spectrophotometric Method</strong></td>
<td><strong>First Method:</strong> Simultaneous Equation Method:&lt;br&gt;<strong>Wavelength:</strong>&lt;br&gt;Escitalopram: 238.2 nm&lt;br&gt;Etizolam: 251.6 nm&lt;br&gt;<strong>LOD:</strong>&lt;br&gt;Escitalopram: 1.13&lt;br&gt;Etizolam: 0.60&lt;br&gt;<strong>LOQ:</strong>&lt;br&gt;Escitalopram: 3.42&lt;br&gt;Etizolam: 1.83s&lt;br&gt;<strong>Second Method:</strong>&lt;br&gt;<strong>Q-Absorbance Ratio:</strong>&lt;br&gt;Isosorbptive Point:&lt;br&gt;Escitalopram: 238.2 nm&lt;br&gt;Etizolam: 248.8 nm&lt;br&gt;<strong>LOD:</strong>&lt;br&gt;Escitalopram: 1.13&lt;br&gt;Etizolam: 0.57&lt;br&gt;<strong>LOQ:</strong>&lt;br&gt;Escitalopram: 3.42&lt;br&gt;Etizolam: 1.72&lt;br&gt;<strong>Third Method:</strong>&lt;br&gt;<strong>Absorbance correction method</strong>&lt;br&gt;Escitalopram: 238.2 nm&lt;br&gt;Etizolam: 292.8 nm&lt;br&gt;<strong>LOD:</strong>&lt;br&gt;Escitalopram: 1.13&lt;br&gt;Etizolam: 0.57&lt;br&gt;<strong>LOQ:</strong>&lt;br&gt;Escitalopram: 3.42&lt;br&gt;Etizolam: 1.72&lt;br&gt;<strong>Solvent:</strong> 0.1 N NaOH&lt;br&gt;<strong>Linearity Range:</strong>&lt;br&gt;Escitalopram: 10-60 μg/ml&lt;br&gt;Etizolam: 5-30 μg/ml&lt;br&gt;<strong>Correlation Coefficient (R²):</strong>&lt;br&gt;Escitalopram: 0.9989&lt;br&gt;Etizolam: 0.9998</td>
</tr>
</tbody>
</table>
14  Escitalopram oxalate in tablet dosage form  Stability indicating HPTLC METHOD  

**Chromatographic Development:**
- **Detection Wavelength:** 239 nm
- **Stationary phase:** TLC aluminum plates precoated with silica gel 60F-254
- **Mobile Phase:** toluene: acetone: ethanol: ammonia (5:1:1:0.2 v/v/v/v)
- **Linearity Range:** 100-1000 ng/spot-1.
- **Correlation Coefficient (R²):** 0.9987
- **LOD:** 20 ng/spot-1.
- **LOQ:** 50 ng/spot-1.
- **%Recovery:** 98.72

![Image](image.png)

15  Escitalopram in bulk and pharmaceutical dosage form  Extractive spectrometric method  

**Method- A**
- **Detection Wavelength:** 415 nm
- **Linearity range:** 2-10µg/ml
- **Correlation coefficient:** 0.9986
- **%RSD:** 1.98
- **%Range of error:** 1.656

**Method- B**
- **Detection wavelength:** 426 nm
- **Linearity range:** 2-10µg/ml
- **Correlation coefficient:** 0.9998
- **%RSD:** 1.97
- **%Range of error:** 1.647

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Eszopiclone is official in United State pharmacopoeia (USP NF 2016).

**TABLE 2.1: OFFICIAL METHODS FOR ESTIMATION OF ESZOPICLONE**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>DRUG</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
</tr>
</thead>
</table>
| 1       | Eszopiclone      | Liquid chromatography                    | **Detection Wavelength:** 303 nm  
                      |                                | **Mobile Phase:** Buffer: Acetonitrile (62:38 v/v)  
                      |                                | **Stationary Phase:** 4.6-mm 25-cm; 5-µm packing L1  
                      |                                | **Flow Rate:** 1.5 ml/min       |

**TABLE 2.2 REPORTED METHOD OF ESZOPICLONE**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>DRUG</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
</tr>
</thead>
</table>
| 1       | Eszopiclone in Bulk and Tablet Dosage Forms | Spectrometric Method | **Wavelength:** 308 nm  
                      |                                | **Solvent:** Methanol  
                      |                                | **Linearity Range:** 4-24 µg/ml  
                      |                                | **Correlation Coefficient (R²):** 0.9995  
                      |                                | **LOD:** 0.624 µg/ml  
                      |                                | **LOQ:** 1.130 µg/ml       |
| 2       | Eszopiclone in Bulk and Tablet Dosage Forms | RP-HPLC Method | **Detection Wavelength:** 305 nm  
                      |                                | **Mobile Phase:** Methanol: Water (80:20 v/v)  
                      |                                | **Stationary phase:** Phenomenex Gemini C18 column(250 mm x 4.6.0 mm, 5 µ)  
                      |                                | **Linearity Range:** 5-30µg/ml  
                      |                                | **Retention Time:** 5.38 min  
                      |                                | **Flow Rate:** 1.0 ml/min  
                      |                                | **%Recovery:** 99.90-100.09%  
                      |                                | **LOD:** 0.310/ml  
                      |                                | **LOQ:** 0.572µg/ml       |
| 3       | Eszopiclone in Bulk and Pharmaceutical Dosage Forms | UV Spectrophotometric Method | **Zero Order Derivative**  
                      |                                | **Wavelength:** 250 nm  
                      |                                | **Second Order Derivative**  
                      |                                | **Wavelength:** 241 nm  
                      |                                | **Solvent:** Methanol  
                      |                                | **Linearity Range:** 10-50 µg/ml  
<pre><code>                  |                                | **Correlation Coefficient (R²):** 0.999    |
</code></pre>
<table>
<thead>
<tr>
<th>No.</th>
<th>Sample Description</th>
<th>Method Type</th>
<th>Detection Wavelength</th>
<th>Mobile Phase</th>
<th>Stationary phase</th>
<th>Linearity Range</th>
<th>Correlation Coefficient ($R^2$)</th>
<th>Retention Time</th>
<th>Flow Rate</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Eszopiclone in Rabbit Plasma</td>
<td>RP-HPLC-MS Method</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Detection Wavelength:</td>
<td>15 mM Ammonium format:</td>
<td>mobile phase:</td>
<td>Linearity Range:</td>
<td>Correlation Coefficient ($R^2$):</td>
<td>Retention Time:</td>
<td>Flow Rate:</td>
<td>% Recovery:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>methanol (15:85 v/v)</td>
<td>Ascentis express CN (50X4.6 mm, 2.7 μm column)</td>
<td>mobile phase:</td>
<td>0.05 - 210.0 mg/ml</td>
<td>9850</td>
<td>0.6 mL/min</td>
<td></td>
<td>77.46%</td>
</tr>
<tr>
<td>5</td>
<td>Eszopiclone and its Degradation Products</td>
<td>HPLC Method</td>
<td>Detection Wavelength:</td>
<td>304 nm</td>
<td>Mobile Phase: Methanol: Water(40:60) pH-2.5</td>
<td>Linerarity Range: 4-24 μg/mL</td>
<td>Correlation Coefficient ($R^2$): 0.9982</td>
<td>Acid Hydrolysis Stability-Indicating Assay: (70:30 v/v, pH 7.2)</td>
<td>Stationary phase: Thermo Hypersil BDS-C18 (250 mm x 4.6 mm, 5.0 μ)</td>
<td>Relative Retention Time (RRT): Acid Hydrolysis Stability-Indicating Assay: 2.95 min</td>
</tr>
<tr>
<td>6</td>
<td>Eszopiclone in Pure Form and Pharmaceutical Dosage Forms</td>
<td>UPLC Method</td>
<td>Wavelength: 305 nm</td>
<td>Mobile Phase: Sodium phosphate buffer : Acetonitrile (85:25 v/v)</td>
<td>stationary phase: HSS C18, 100 mm x 2.1 mm, column with 1.7μm particles</td>
<td>Linerarity Range: 0.05-20 μg/ml</td>
<td>Correlation Coefficient ($R^2$): 0.996</td>
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<tr>
<td>7</td>
<td>Eszopiclone in Pharmaceutical Tablet Forms</td>
<td>HPLC Method</td>
<td>Detection wavelength:</td>
<td>303</td>
<td>Mobile Phase: Phosphate Buffer (3.5 pH) : Acetonitrile (50:50 v/v)</td>
<td>Stationary Phase: Purospher® Star RP18e,(150 x 4.6 mm; 5μ)</td>
<td>Retention Time: 4.762</td>
<td>Flow Rate: 1.5 mL/min</td>
<td>LOD: 0.054μg/ml</td>
<td>LOQ: 0.132μg/ml</td>
</tr>
<tr>
<td>8</td>
<td>Eszopiclone combined with escitalopram oxalate</td>
<td>UV Spectrophotometric method</td>
<td>Detection wavelength:</td>
<td>304nm</td>
<td>Mobile Phase: Escopionc1e,238nm</td>
<td>Escitalopram oxalate: 238nm</td>
<td>Linearity range: 5-25μg/ml</td>
<td>LOD: Escitalopram oxalate: 2.5</td>
<td>LOD: Escitalopram oxalate: 5</td>
<td>LOD: 1.5</td>
</tr>
</tbody>
</table>
CONCLUSION
This review represents the reported chromatographic methods: developed and validated for determination of Escitalopram oxalate and Eszopiclone. All the reported method was simple, precise and accurate those mostly emphasize separation techniques like liquid and gas chromatography. The analysis is done on individual and several combinations of Escitalopram oxalate and Eszopiclone with other drugs. Comparing various validation parameters of already reported methods, it can be concluded that different analytical methods like spectrophotometric, HPTLC and HPLC can be developed for escitalopram oxalate and eszopiclone showing its simplicity, sensitivity (low LOD and LOQ values) linearity and accuracy. Most of the researchers have used the reversed-phase HPLC and UV absorbance detection because this provided with best available reliability, repeatability, analysis time and sensitivity.

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