# "DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM OXALATE AND ETIZOLAM"

**A THESIS SUBMITTED TO**

## NIRMA UNIVERSITY

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR**

**THE DEGREE OF**

## MASTER OF PHARMACY

**IN**

## PHARMACEUTICAL ANALYSIS

**BY**

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**UNDER THE GUIDANCE OF**

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# *DECLARATION*

*I declare that the thesis "Development and Validation of RP-HPLC Method For Simultaneous Estimation of Escitalopram Oxalate and Etizolam" has been prepared by me under the guidance of Parthiv Panchal, Research Scientist, Astron Research limited and Dr. Priti J. Mehta, Professor and Head, Department of Pharmaceutical Analysis Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.*

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# *CERTIFICATE*

*This is to certify that* ***Mr. SHALAV P. KELA*** *have prepared the thesis entitled "DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM OXALATE AND ETIZOLAM", in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under my guidance. They have carried out the work at the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University.*

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# ACKNOWLEDGEMENT

It gives me immense pleasure today when I take an opportunity to acknowledge all those personalities who contributed directly or indirectly to my project. This research would not have been possible without the whole hearted encouragement, guidance, support, and cooperation of my beloved family, teachers, friends, well wishers and relatives. Probably I would have never achieved this without their support and blessings. With profound appreciation, I acknowledge to one and all.

I wish to express my sincere thanks, with a deep sense of gratitude, to my respected guide **Dr.Priti J. Mehta,** Senior Associate Professor, Dept. of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University for initiating and suggesting the theme of work, for her valuable guidance, supervision, creative suggestions and meticulous attention, sustained interest, immense guidance, dedicated support she has bestowed upon me for the timely completion of this work. I am extremely indebted to her for her motivational inspiration, kind expertise during the writing up of my thesis and the scientific attitude she has nurtured in me which will definitely stand in all my future endeavours.

I am extremely grateful to **Hardik sir** and **Nrupesh sir**, Dept., of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University for their continuous encouragement and everlasting support throughout the course of this dissertation work.

I am grateful to **Dr. Manjunath Ghate**, I/c Director, Institute of Pharmacy, Nirma University for providing all necessary help and facility for my work and also for her constant support and encouragement.

My heartest thanks to **Deepaksir and Omkarsir** for constantly helping out in my work and solving my queries.

I would also like to thank **ASTRON RESEARCH LIMITED** for providing me sophisticated analytical instrumentation facility. I would like to thanks Mr. Parthiv Panchal, Rahul Chaudhary, Natwat Solanki, Mr. Maulin Parikh, Mr. Vishal Modi, Kiran Patel and all the other members of the Astron Team.

My friends are like ropes which have pulled me up from my lows and held me down firmly in my highs. A special word of gratitude to my friends **Deval, Gargi, Harsh, Nishadh,Pankti, Ravi, Tushar and Ujjval** who were always there besides me with the hand of support and encouragement to make his effort a successful task.

I would also like to acknowledge my colleagues for their help throughout my work.

I sincerely thanks to **Mrs Geeta Christain,** for library facilities and constant encouragement during my work. I owe special thanks to **Nitinbhai** for helping me in maximum utilization of computer lab. I also wish to acknowledge **Satejbhai, Shreyasbhai, Bipinbhai, Vikrambhai and Devraj** for providing me all the materials required in my work.

Last but not least I am indebted infinitely to love, care and trust being showered on me by my **Family** without their consistent prayers, affectionate blessings, selfless care and endless confidence in me, I would have never come to the stage of writing this acknowledge.

Date: Shalav Kela

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# ABSTRACT

# 1. ABSTRACT

The combination of Escitalopram and Etizolam is mainly used for anti-psychotic therapy. The aim was to develop a UV and HPLC method for simultaneous estimation of both the drugs in the combined dosage form. In UV spectrophotmetric first order derivative spectroscopy was developed. First order spectra of ESC and ETI showed zero absorbance at 260.40 and at 243.80 nm respectively. So both ESC and ETI were estimated at wavelength 243.80 nm and 260.40 nm respectively. The calibration curve were linear in the range of 80 - 220 µg/ml for ESC ($R^2$= 0.9969) and 8 – 50 µg/ml for ETI ($R^2$=0.9939). In HPLC method Inertsil ODS-2 column (150 mm x 4.6mm x 5µm) with mobile phase containing Potassium dihydrogen phosphate (pH: 4.5): Acetonitrile: Methanol (65:30:5) was used and were detected at 240 nm. The linearity for ESC and ETI was in the range of150 – 650 µg/ml and 15 – 65 µg/ml respectively. All the other parameters were validated according to the ICH guidelines. High percentage recovery shows that there is no interference of excipients in the dosage form. The method was applied on pharmaceutical dosage form. The proposed methods was accurate, precise, sensitive and cost-effective that can be successfully applied to bulk drugs and pharmaceutical dosage forms.

# CHAPTER 2

# INTRODUCTION

# CHAPTER 2

# INTRODUCTION

## CONTENTS

# 1.1 INTRODUCTION TO ANXIETY

Anxiety is a term used to describe uncomfortable feelings of nervousness, worry and tension. It is a generalized mood condition that can often occur without an identifiable triggering stimulus [1]. As such, it is distinguished from fear, which is an emotional response to a perceived threat. Additionally, fear is related to the specific behaviors of escape and avoidance, whereas anxiety is related to situations perceived as uncontrollable or unavoidable. The word is derived from the Latin, *angere*, which means to choke or strangle [2].

Physical effects of anxiety may include heart palpitations, muscle weakness and tension, fatigue, nausea, chest pain, shortness of breath, stomach aches, or headaches. The body prepares to deal with a threat: blood pressure and heart rate are increased, sweating is increased, blood flow to the major muscle groups is increased and immune and digestive system functions are inhibited. Panic attacks usually come without warning and although the fear is generally irrational, the perception of danger is very real.

Anxiety does not only consist of physical effects; there are many emotional ones as well. They include feelings of apprehension or dread, trouble concentrating, feeling tense or jumpy, anticipating the worst, irritability, restlessness, watching (and waiting) for signs (and occurrences) of danger and feeling like your mind's gone blank as well as nightmares/bad dreams [3].

## 1.1.1 TYPES OF ANXIETY [4]

Anxiety disorders have been classified according to the severity and duration of their symptoms and specific behavioral characteristics.

Categories include:

- Generalized Anxiety Disorder (GAD), which is long lasting and low-grade
- Panic disorder, which has more dramatic symptoms
- Phobias
- Obsessive-Compulsive Disorder (OCD)

- Post-Traumatic Stress Disorder (PTSD)

GAD and panic disorder are the most common.

## 1.1.2 COMMON SYMPTOMS OF ANXIETY [3]

- Feeling shaky, jittery, or nervous
- Feeling tense, fearful, or apprehensive
- Avoiding certain places or activities because of fear
- A pounding or racing heart
- Trouble catching your breath when nervous
- Unjustified sweating or trembling
- A knot in your stomach
- A lump in your throat
- Finding yourself pacing
- Being afraid to close your eyes at night for fear that you may die in your sleep
- Fear of losing control or going crazy
- Fear of dying.

## 1.1.3 PREVENTION AND TREATMENT OF ANXIETY [5]

Prevention of anxiety essentially involves an awareness of life's stresses and your own ability to cope with them.

In essence, you might develop coping mechanisms for all of life's stresses. Strategies might include these:

- Physical well-being through exercise, healthy eating habits, and adequate rest
- Relaxation exercises including deep breathing
- Interpersonal skills in dealing with difficult people and situations or parenting skills training in dealing with your children

- **Psychotherapy:** There are several types of psycho therapies used to treat anxiety disorders. Behavioral therapy focuses on changing specific actions and uses such techniques as diaphragmatic breathing (to combat hyperventilation which is common when panicking). During exposure therapy the patient is gradually exposed to what frightens them to help them cope with their fears. Cognitive behavioral therapy teaches the patient to react differently to situations that trigger attacks and focuses on helping them change their thinking patterns. Group therapy and self-help groups can also be helpful.
- **Watch Your Diet:** Eat food high in calcium, magnesium, phosphorus, and potassium since these nutrients are depleted by stress. Limit meats and other animal proteins and eat lots of fruits, grains, and vegetables instead.
- **Avoid Caffeine:** Caffeine can trigger panic attacks so avoid coffee, chocolate, some sodas and some teas, and other products containing caffeine.
- **Avoid Refined Sugars And Simple Carbohydrates:** Cut simple sugars, carbonated soft drinks and alcohol out of the diet.

### 1.1.4 MEDICATION [6, 7, 8, 9]

- **Antidepressants:** Selective Serotonin Reuptake Inhibitors are slow acting and may not take effect for several weeks after beginning use. Tricyclic Antidepressants can cause weight gain, sleepiness, constipation, and headache. Monoamine Oxidase Inhibitors can cause severe high blood pressure if taken with red wine, cheese, beer or other foods containing tyramine and may cause dangerous interactions when taken with certain non-prescription drugs such as certain cold remedies.

- **Anti-anxiety drugs**: Benzodiazepines are addictive and the patient can develop a tolerance for the drug so that it eventually stops working for them entirely. Azapirones are less effective than benzodiazepines but do not induce tolerance. Beta-blockers do not cause depression or sleepiness.

# 1.2 INTRODUCTION TO DEPRESSION

Depression is one of the most common and debilitating psychiatric disorders and is a leading cause of suicide.. Persons with bipolar disorder will also have manic or hypomanic episodes [2]. Depression is one of the most common psychiatric disorders. Symptoms of depression are often subtle and unrecognized both by patients and physicians. The brain contains a network of interconnected nerve cells called neurons. The junction between the neurons is called the synaptic junction. The transmission of impulses from one neuron to another is facilitated by chemicals called neuro-transmitters [4]. The impulse triggers the release of neurotransmitters from one neuron, which cross the synaptic junction and attach themselves to the receptors in the adjacent neuron sending the message through. Later the neurotransmitter returns to the initial neuron via the reuptake channel. One of the causes of depression is believed to be the depletion of the amine neurotransmitters, serotonin and norepinephrine [5].

## 1.2.1 TYPES OF DEPRESSION [4]

Depressive disorders come in different forms, just as is the case with other illnesses such as heart disease. Some of the more common types of depressive illness are discussed here.

- Major Depression
- Dysthymia
- Manic Depression
- Postnatal Depression
- Seasonal Affective Disorder

## 1.2.2 SYMPTOMS OF DEPRESSION

Listed below are a number of symptoms associated with depression. Not everyone who is depressed will experience every symptom. Some people experience a few symptoms, some many. Severity of symptoms varies with individuals and also varies over time.

- feelings of helplessness and hopelessness
- feeling useless, inadequate, bad
- self hatred, constant questioning of thoughts and actions, an overwhelming need for reassurance
- being vulnerable and "over-sensitive"
- feeling guilty
- a loss of energy and motivation, that makes even the simplest tasks or decision seem difficult
- self harm
- loss or gain in weight
- difficulty with getting off to sleep, or (less frequently) and excessive desire to sleep
- agitation and restlessness
- loss of sex drive
- Finding it impossible to concentrate for any length of time, forgetfulness. A sense of unreality.
- Physical aches and pains, sometimes with the fear that you are seriously ill.

## 1.2.3 PREVENTION AND TREATMENT OF DEPRESSION [5, 10]

Treatment of depression usually involves medication, psychotherapy or a combination of both. Other treatments may include Electro Convulsive Therapy (ECT), light therapy and alternative treatments.

- **Psychotherapy**: Psychotherapy involves talking to family doctor, counselor, psychiatrist or therapist about things that are occurring in a person's life. The aim of psychotherapy is to remove all symptoms of depression and return a person to a normal life. There are three psychotherapies commonly used to treat depression: behavioral therapy, cognitive therapy or interpersonal therapy.

- **Electro Convulsive Therapy (ECT)**: ECT, also called electroshock treatment, is used for severely depressed patients and/or those who have not responded to antidepressant medication and/or psychotherapy. During this therapy, an electric

current travels through electrodes placed on the temples, causing a generalized shock that produces biochemical changes in the brain

## 1.2.4 MEDICATION [6, 7, 8]

There are more than 20 antidepressant drugs currently available. Antidepressants correct the chemical imbalance in the brain. Because a variety of drugs target different neurotransmitters and imbalances of these neurotransmitters can vary from patient to patient, some drugs may be more effective than others for any individual. Sometimes a combination of drugs is best.

There are three (3) groups of antidepressant medications most commonly used to treat depression:

- **Tri Cyclic Antidepressants (TCAs), which include:**

  Amitriptyline, Imipramine, Nortryptyline, Despiramine.

- **Monoamine Oxidase Inhibitors (MAOIs)** include Phenelzine and Tranylcypromine.

- **Selective Serotonin Reuptake Inhibitors (SSRIs) include:**

  Fluoxetine, Fluvoxamine, Paroxetine, Sertraline, Citalopram, Escitalopram Oxalate.

# 2.Introduction to Class of Drugs

## 2.1 Anti-Psychotic Drugs

An **Antipsychotic** (or **neuroleptic**) is a tranquilizing psychiatric medication primarily used to manage psychosis (including delusions or hallucinations, as well as disordered thought), particularly in schizophrenia and bipolar disorder. First and second generations of medication tend to block receptors in the brain's dopamine pathways but antipsychotic drugs encompass a wide range of receptor targets [6,7].

A number of harmful and undesired (adverse) effects have been observed, including lowered life expectancy, weight gain, enlarged breasts and milk discharge in men and women (hyperprolactinaemia), lowered white blood cell count (agranulocytosis), involuntary repetitive body movements (tardive dyskinesia), diabetes, an inability to sit still or remain motionless (akathisia), sexual dysfunction, a return of psychosis requiring increasing the dosage due to cells producing more neurochemicals to compensate for the drugs (tardive psychosis) and a potential for permanent chemical dependence leading to psychosis much worse than before treatment began if the drug dosage is ever lowered or stopped (tardive dysphrenia) [8].

### 2.1.1 Common antipsychotics [8]

Commonly used antipsychotic medications are listed below by drug group.

#### I. First generation anti-psychotics (Typical Anti-psychotics drugs)

**1. Butyrophenones**

- Haloperidol
- Droperidol

**2. Phenothiazines**

- Chlorpromazine
- Fluphenazine
- Perphenazine

3. **Thioxanthenes**

- Chlorprothixene
- Clopenthixol
- Flupenthixol

## *II.* Second generation antipsychotics (Atypical antipsychotic)

- Clozapine .
- Olanzapine
- Risperidone

## III. Third generation antipsychotics

- Aripiprazole

## 2.1.2 Drug Action [9, 10]

All antipsychotic drugs tend to block $D_2$ receptors in the dopamine pathways of the brain. This means that dopamine released in these pathways has less effect. Excess release of dopamine in the mesolimbic pathway has been linked to psychotic experiences. It is the blockade of dopamine receptors in this pathway that is thought to control psychotic experiences.

Typical antipsychotics are not particularly selective and also block dopamine receptors in the mesocortical pathway, tuberoinfundibular pathway and the nigrostriatal pathway.. High-potency antipsychotics such as haloperidol, in general, have doses of a few milligrams and cause less sleepiness and calming effects than low-potency antipsychotics such as chlorpromazine and thioridazine, which have dosages of several hundred milligrams. The latter have a greater degree of anticholinergic and antihistaminergic activity, which can counteract dopamine-related side effects.

Atypical antipsychotic drugs have a similar blocking effect on $D_2$ receptors. Some also block or partially block serotonin receptors (particularly $5HT_{2A,\ C}$ and $5HT_{1A}$ receptors) ranging from risperidone, which acts overwhelmingly on serotonin receptors, to amisulpride, which has no serotonergic activity. The additional effects on serotonin receptors may be why some of them can benefit the "negative symptoms" of schizophrenia.

## 3. RATIONALE OF COMBINATION:

- Escitalopram increases intrasynaptic levels of the neurotransmitter serotonin by blocking there uptake of the neurotransmitter into the neuron. Of the SSRIs currently on the market escitalopram has the highest affinity for the human serotonin transporter (SERT).
- Escitalopram enhances its own binding via an additional interaction with another allosteric site on the transporter. *R*-citalopram also enhances binding of escitalopram, and therefore the allosteric interaction cannot explain the observed counteracting effect. But still, the allosteric binding of escitalopram to the serotonin transporter is of unquestionable research interest, its clinical relevance is unclear since the binding of escitalopram to the allosteric site is at least 1000 times weaker than to the primary binding site.

- Etizolam acts by **increasing the efficiency of GABA receptor** to decrease the excitability of neurons. Basically at first Etizolam binds with $GABA_A$ receptors and then this complex binds with GABA which increases the conduction of chlorine ions. This increased conductance raises the membrane potential of the neuron, resulting in inhibition of neuronal firing.

- Etizolam and Escitalopram are mainly used as anti-psychotic drug. Etizolam induce sleep if it is given in high concentration. Research has showed that Etizolam is very less effective in low concentration. Escitalopram shows late effect (around 10 days). So as the effect of Etizolam is decreased, Escitalopram starts showing its effect.

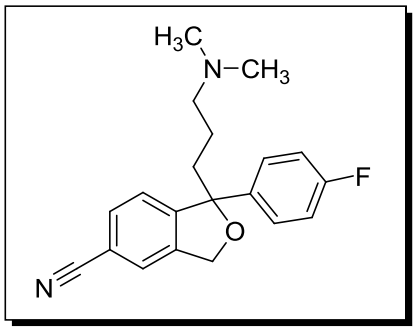
- And so to get the **synergistic effect** while treating anxiety, the combination of this two drugs is preferred.

# 4. INTRODUCTION TO DRUGS:

## 4.1 Drug Profile of Escitalopram Oxalate

**IUPAC Name:** (*S*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile

**Structural Formula:**




**Molecular Formula:** $C_{20}H_{21}FN_2O \cdot C_2H_2O_4$

**Molecular Weight:** 324.392 g/mol, (414.43 g/mol as oxalate) [11]

**CAS No:** 128196-01-0

**Official Status:** Official in USP Pending monograph

**Category:** Antidepressant of Selective Serotonin Reuptake Inhibitor (SSRI) Class [8, 9]

## Physicochemical Properties:

- **Appearance:** Fine, white to slightly-yellow powder
- **Solubility:** Sparingly soluble in water, ethanol; Slightly soluble in ethyl acetate; Insoluble in heptanes; Freely soluble in methanol, chloroform, DMSO, acetonitrile; Soluble in isotonic saline solution, ,
- **Melting point:** 150-152°C
- **Dissociation Constant:** $pK_a$ : 9.0-9.3
- **Storage:** Store in a cool and dry place [12]

## Major Use of the Escitalopram Oxalate:

- **Major depressive disorder:**

For the acute and maintenance treatment of major depressive disorder in adults and in adolescents 12 to 17 years of age. A major depressive episode (DSM-IV) implies a prominent and relatively persistent depressed mood that usually interferes with daily functioning, and includes at least five of the following nine symptoms: depressed mood, loss of interest in usual activities, significant change in weight and/or appetite, insomnia or hypersomnia, increased fatigue, feelings of guilt or worthlessness, a suicide attempt or suicidal ideation.

- **Generalized anxiety disorder:**

Generalized Anxiety Disorder (DSM-IV) is characterized by excessive anxiety and worry (apprehensive expectation) that is persistent for at least 6 months and which the person finds difficult to control [10].

## Pharmacological Actions and Clinical Pharmacology:

- **Mechanism of action [5, 6]:**

Escitalopram increases intrasynaptic levels of the neurotransmitter serotonin by blocking thereuptake of the neurotransmitter into the neuron. Of the SSRIs currently on the market escitalopram has the highest affinity for the human serotonin transporter (SERT).

The enantiomer of escitalopram (*R*-citalopram) counteracts to a certain degree the serotonin-enhancing action of escitalopram. As a result, escitalopram is a **more potent** antidepressant than citalopram, which is a mixture of escitalopram and *R*-citalopram.

Escitalopram enhances its own binding via an additional interaction with another allosteric site on the transporter. *R*-citalopram also enhances binding of escitalopram, and therefore the allosteric interaction cannot explain the observed

counteracting effect. But still, the allosteric binding of escitalopram to the serotonin transporter is of unquestionable research interest, its clinical relevance is unclear since the binding of escitalopram to the allosteric site is at least 1000 times weaker than to the primary binding site.

In vitro studies using human liver microsomes indicated that CYP3A4 and CYP2C19 are the primary isozymes involved in the N-demethylation of Escitalopram. The resulting metabolites, demethylescitalopram and didemethylescitalopram are significantly less active and their contribution to overall action of escitalopram is negligible.

- **Pharmacokinetics [8, 9]:**

Escitalopram is well absorbed following the oral administration, with plasma concentration usually attained within 5 hours. The bioavailability of the Escitalopram was found to be 80% with the protein binding of 56%. The onset of action usually occurs within 1-4 weeks. Food does not affect the absorption. It metabolizes in liver, specifically the enzymes CYP3A4 and CYP2C19. In geriatric patients, AUC is increased up to 50%. The drug is having the half-life of around 27-32 hours. It crosses the placental barrier and distributes in the breast milk. The route of the elimination is via urine. The hepatic impairment decrease the oral clearance by 37% and doubles its half life. Mild to moderate renal impairment decreases racemic citalopram oral clearance by 17%.

The single- and multiple-dose pharmacokinetics of escitalopram are linear and dose-proportional in a dose range of 10 to 30 mg/day. Biotransformation of escitalopram is mainly hepatic, with a mean terminal half-life of about 27-32 hours. With once-daily dosing, steady state plasma concentrations are achieved within approximately one week. At steady state, the extent of accumulation of escitalopram in plasma in young healthy subjects was 2.2-2.5 times the plasma concentrations observed after a single dose. The tablet and the oral solution dosage forms of escitalopram oxalate are bioequivalent.

- **Pharmacodynamics:**

In vitro and in vivo studies in animals suggest that Escitalopram is a highly Selective Serotonin Reuptake Inhibitor (SSRI) with minimal effects on norepinephrine and dopamine neuronal reuptake. Escitalopram is at least 100-fold more potent than the R-enantiomer with respect to inhibition of 5-HT reuptake and inhibition of 5-HT neuronal firing rate. Tolerance to a model of antidepressant effect in rats was not induced by long-term (up to 5 weeks) treatment with escitalopram. Escitalopram has no or very low affinity for serotonergic ($5\text{-}HT_{1\text{-}7}$) or other receptors including alpha- and beta-adrenergic, dopamine ($D_{1\text{-}5}$), histamine ($H_{1\text{-}3}$), muscarinic ($M_{1\text{-}5}$), and benzodiazepine receptors. Escitalopram also does not bind to, or has low affinity for, various ion channels including $Na^+$, $K^+$, $Cl^-$, and $Ca^{++}$ channels. Antagonism of muscarinic, histaminergic, and adrenergic receptors has been hypothesized to be associated with various anticholinergic, sedative, and cardiovascular side effects of other psychotropic drugs.

- **Contraindications:**

Monoamine oxidase inhibitors (MAOIs): Concomitant use in patients taking monoamine oxidase inhibitors (MAOIs) is contraindicated with Escitalopram oxalate.

Pimozide: Concomitant use in patients taking pimozide is also contraindicated.

- **Therapeutic Dosage:**

**Major Depressive Disorder [9]**

***Adolescents***

The recommended dose of 10 mg once daily. A flexible-dose trial (10 to 20 mg/day) demonstrated the effectiveness. If the dose is increased to 20 mg, this should occur after a minimum of three weeks.

***Adults***

The recommended dose is 10 mg once daily. A fixed-dose trial demonstrated the effectiveness of both 10 mg and 20 mg, but failed to demonstrate a greater benefit of 20 mg over 10 mg. If the dose is increased to 20 mg, this should occur after a minimum of one week.

***Maintenance Treatment***

It is generally agreed that acute episodes of major depressive disorder require several months or longer of sustained pharmacological therapy beyond response to the acute episode. Systematic evaluation of continuing 10 or 20 mg/day in adults patients with major depressive disorder who responded while taking Escitalopram oxalate during an 8-week, acute-treatment phase demonstrated a benefit of such maintenance treatment.

**Generalized Anxiety Disorder**

***Adults***

The recommended starting dose of 10 mg once daily. If the dose is increased to 20 mg, this should occur after a minimum of one week.

***Maintenance Treatment***

Generalized anxiety disorder is recognized as a chronic condition. The efficacy in the treatment of GAD beyond 8 weeks has not been systematically studied.

- **Discontinuation of Treatment with the drug:**

Symptoms associated with discontinuation of Escitalopram oxalate and other SSRIs and SNRIs have been reported. Patients should be monitored for these symptoms when discontinuing treatment.

A gradual reduction in the dose rather than abrupt cessation is recommended whenever possible. If intolerable symptoms occur following a decrease in the dose or upon discontinuation of treatment, then resuming the previously prescribed dose may be considered.

- **Adverse Effects [8, 9]:**

There are many adverse effects related to Escitalopram oxalate which include dryness in mouth, increase sweating. It also affects Central nervous system and may cause dizziness, insomnia, appetite decreased headache. Hypertension may build up due to Escitalopram oxalate. It affects musculoskeletal system due to which stiffness of the muscles is experienced. Respiratory related problems like rhinitis, sinusitis, and bronchitis may get develop. Sexual dysfunction and menstrual disorder is also observed.

- **WARNING:**

Antidepressant medications are used to treat a variety of conditions, including depression and other mental/mood disorders. These medications can help prevent suicidal thoughts/attempts and provide other important benefits.

However, studies have shown that a small number of people (especially people younger than 25) who take antidepressants for any condition may experience worsening depression, other mental/mood symptoms, or suicidal thoughts/attempts. Therefore, it is very important to talk with the doctor about the risks and benefits of antidepressant medication (especially for people younger than 25), even if treatment is not for a mental/mood condition.

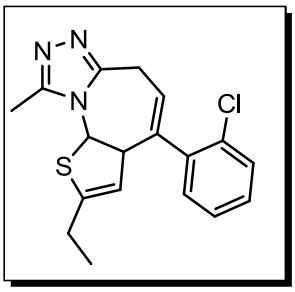
Report immediately if you notice worsening depression/other psychiatric conditions, unusual behavior changes (including possible suicidal thoughts/attempts), or other mental/mood changes (including new/worsening anxiety, panic attacks, trouble sleeping, irritability, hostile/angry feelings, impulsive actions, severe restlessness, very rapid speech).

Be especially watchful for these symptoms when a new antidepressant is started or when the dose is changed.

## 4.2 Drug Profile of Etizolam

**IUPAC Name:** 6-(o-chlorophenyl)-8-ethyl-1-methyl-1-methyl-4H-s-triazolo[3,4-c]-thieno[2,3-e]-[1,4]diazepines

**Structural Formula:**




**Molecular Formula:** $C_{17}H_{15}ClN_4S$

**Molecular Weight:** 342.9

**CAS No:** 40054-69-1

**Official Status:** Japanese Pharmacopeia [13]

**Category:** Anti-psycotic Psycholeptics, Anxiolytics, Benzodiazepine derivatives [8, 9].

### Physicochemical Properties:

- **Appearance:** For white or slightly yellow crystalline powder, odorless, tasteless
- **Solubility:** Almost insoluble in water; Soluble in methanol, chloroform; soluble in ethanol; Sparingly soluble in acetonitrile and in acetic anhydride
- **Melting Point**: 146-149° C
- **Partition Coefficient**: 3.1
- **Storage:** Light resistant [12]

## Major Use of the Etizolam

**Anxiety:**

Anxiety is a term used to describe uncomfortable feelings of nervousness, worry, and tension. It is a generalized mood condition that can often occur without an identifiable triggering stimulus [1]. As such, it is distinguished from fear, which is an emotional response to a perceived threat.

Physical effects of anxiety may include heart palpitations, muscle weakness and tension, fatigue, nausea, chest pain, shortness of breath, stomach aches, or headaches. External signs of anxiety may include pale skin, sweating, trembling, and pupillary dilation. Although panic attacks are not experienced by every person who has anxiety, they are a common symptom. Panic attacks usually come without warning, and although the fear is generally irrational, the perception of danger is very real [3].

**Pharmacological Actions and Clinical Pharmacology:**

- **Mechanism of action:**

Etizolam a thienodiazepine benzodiazepine derivative, is absorbed fairly rapidly with peak plasma levels achieved between 30 minutes and 2 hours and has a mean elimination half life of about 3 and a half hours. Etizolam possesses potent hypnotic properties. Etizolam acts as a full agonist at the benzodiazepine receptor to produce its range of therapeutic as well as adverse effects. Similar to other benzodiazepines etizolam binds unselectively to benzodiazepine receptor subtypes [1].

In addition etizolam unlike most other benzodiazepines has prolactogenic effects leading to an increase in prolactin blood levels [2].

Etizolam belongs to a new class of diazepines, thienotriazolodiazepines. This new class is easily oxidized, rapidly metabolized, and has a lower risk of accumulation, even after prolonged treatment. Etizolam has an anxiolytic action about 6 times greater than that of diazepam [3]. Etizolam produces, especially at higher dosages, a reduction in time

taken to fall asleep, an increase in total sleep time and a reduction in the number of awakenings. During tests there were not substantial changes in deep sleep [4]. There is a reduction of REM sleep. In EEG tests of healthy volunteers Etizolam showed some characteristics of tricyclic antidepressants [5].

- **Contraindications [7, 8]:**

Etizolam may cause respiratory depression in susceptible individuals. For that reason, they are contraindicated in people with myasthenia gravis, sleep apnea, bronchitis, and COPD. Caution is required when etizolam are used in people with personality disorders or mental retardation because of frequent paradoxical reactions. In major depression, they may precipitate suicidal tendencies and are sometimes used for suicidal overdoses. Individuals with a history of alcohol, opioid and barbiturate abuse should avoid benzodiazepines, as there is a risk of life-threatening interactions with these drugs.

***Pregnancy***

Exposure to etizolam during pregnancy has been associated with a slightly increased (from 0.06 to 0.07%) risk of cleft palate in newborns. Cases of neonatal withdrawal syndrome have been described in infants chronically exposed to benzodiazepines in utero. This syndrome may be hard to recognize, as it starts several days after delivery, for example, as late as 21 day for chlordiazepoxide. The symptoms include tremors, hypertonia, hyperreflexia, hyperactivity, and vomiting and may last for up to three to six months. Tapering down the dose during pregnancy may lessen its severity. Using the lowest effective dose for the shortest period of time minimizes the risks to the unborn child.

***Elderly***

The benefits of etizolam are least and the risks are greatest in the elderly. The elderly are at an increased risk of dependence and are more sensitive to the adverse effects such as memory problems, daytime sedation, impaired motor coordination, and increased risk of motor vehicle accidents and falls. Adverse effects on cognition can be mistaken for the effects of old age. The benefits of withdrawal include improved cognition,

alertness, mobility, reduced risk incontinance, and a reduced risk of falls and fractures. Short to intermediate-acting etizolam are preferred in the elderly.

However, like antidepressants, they have little evidence of effectiveness, although antipsychotics have shown some benefit.

- **Therapeutic Dosage:**

**Anxiety :** 0.51mg BID or TID,

**Panic disorders with agoraphobia :** 0.5mg BID,

**Neurosis or depression :** 1 mg TID,

**Psychosomatic disease, cervical vertebra disease, low back pain or Tension headache :** 0.5mg TID,

**Sleep disorder :** 13mg at bedtime.

**Maximum dose :** Adults: 3 mg/day, Elderly 1.5mg/day.

Treatment should be as short as possible; duratïon of treatment should not exceed 8-12 weeks

- **Adverse Effects:**

**Oversedation**

Oversedation is a dose-related extension of the sedative/hypnotic effects of etizolam. Symptoms include drowsiness, poor concentration, incoordination, muscle weakness, dizziness and mental confusion. When etizolam are taken at night as sleeping pills, sedation may persist the next day as "hangover" effects, particularly with slowly eliminated preparations. However, tolerance to the sedative effects usually develops over a week or two and anxious patients taking etizolam during the day rarely complain of sleepiness although fine judgement and some memory functions may still be impaired.

Oversedation persists longer and is more marked in the elderly and may contribute to falls and fractures.

Oversedation from etizolam contributes to accidents at home and at work and studies from many countries has shown a significant association between the use of etizolam and the risk of serious traffic accidents.

**Memory impairment**

Etizolam have long been known to cause amnesia, an effect which is utilised when the drugs are used as premedication before major surgery or for minor surgical procedures. Loss of memory for unpleasant events is a welcome effect in these circumstances. For this purpose, fairly large single doses are employed.

Oral doses of etizolam in the dosage range used for insomnia or anxiety can also cause memory impairment. Acquisition of new information is deficient, partly because of lack of concentration and attention. In addition, the drugs cause a specific deficit in "episodic" memory, the remembering of recent events, the circumstances in which they occurred, and their sequence in time. By contrast, other memory functions (memory for words, ability to remember a telephone number for a few seconds, and recall of long-term memories) are not impaired. Impairment of episodic memory may occasionally lead to memory lapses or "blackouts". It is claimed that in some instances such memory lapses may be responsible for uncharacteristic behaviours such as shop-lifting.

Etizolam are often prescribed for acute stress-related reactions. At the time they may afford relief from the distress of catastrophic disasters, but if used for more than a few days they may prevent the normal psychological adjustment to such trauma.

**Bereavement, psychotherapy**

In the case of loss or bereavement they may inhibit the grieving process which may remain unresolved for many years.

# 5.INTRODUCTION TO METHOD OF ANALYSIS

## 5.1 UV METHOD FOR ANALYSIS OF DRUG COMPONENTS [14, 15]

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) involves the spectroscopy of photons in the UV-visible region. This means it uses light in the visible and adjacent (near ultraviolet (UV) and near infrared (NIR)) ranges. The absorption in the visible ranges directly affects the color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

There are many methods available for the simultaneous UV estimation of the drug combinations like

(a)Assay as a single-component sample

(b) Assay using absorbance corrected for interference

(c) Simultaneous equation method

(d) Absorbance ratio method

(e) Geometric correction method

(f) Orthogonal polynomial method

(g) Difference Spectrophotometry

(h) Dual Wavelength Spectrophotometry

(i) Least square approximation

(j) Derivative Spectroscopy

A first-order derivative is the rate of change of absorbance with respect to wavelength. A first order derivative starts and finishes at zero. It also passes through zero at the same wavelength as _max of the absorbance band. Either side of this point are positive and negative bands with maximum and minimum at the same wavelengths as the inflection points in the absorbance band. This bipolar function is characteristic of all odd-order derivatives.

## 5.2 RP-HPLC METHOD FOR ANALYSIS OF DRUG COMPONENTS

### 5.2.1 Mechanism [16, 17]:

Retention by interaction of stationary phases non polar hydrocarbon chain with non polar parts of the sample molecules.

HPLC instrument consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column.

The solvent [18] used in RP-HPLC are methanol, acetonitrile, water, various types of buffer and tetrahydrofuran. It is necessary to degas the solvent so that no air bubbles can interfere during the practical. This degassing is done by various methods like Sparging, Heat, Vacuum, Sonication [19, 20, 21] etc. Pumps like Syringe pump, Reciprocating pump and Pheumatic pump are mainly used in RP-HPLC system.

Sometimes it gets necessary to fix a pre-column before the column. This precolumn is mainly fixed when the operating condition causes the acute dissolution of the silica. This acute dissolution leads to column bed subsidence resulting in formation of void at top of the column which can lead to broadening of peak and increase pressure. Thus the use of precolumn shall reduce adverse effects of low or high pH mobile phase. It extends life of column.

HPLC columns [22, 23] are the heart of the system. Stable and high performance column is essential requisite for rugged, reproducible method.

**Sample Injection System:** Injection systems, includes manual injector, standard auto sampler, high-performance auto sampler, high-performance auto sampler SL plus, micro well-plate auto sampler, preparative auto sampler and dual-loop auto sampler as well as the thermostat.

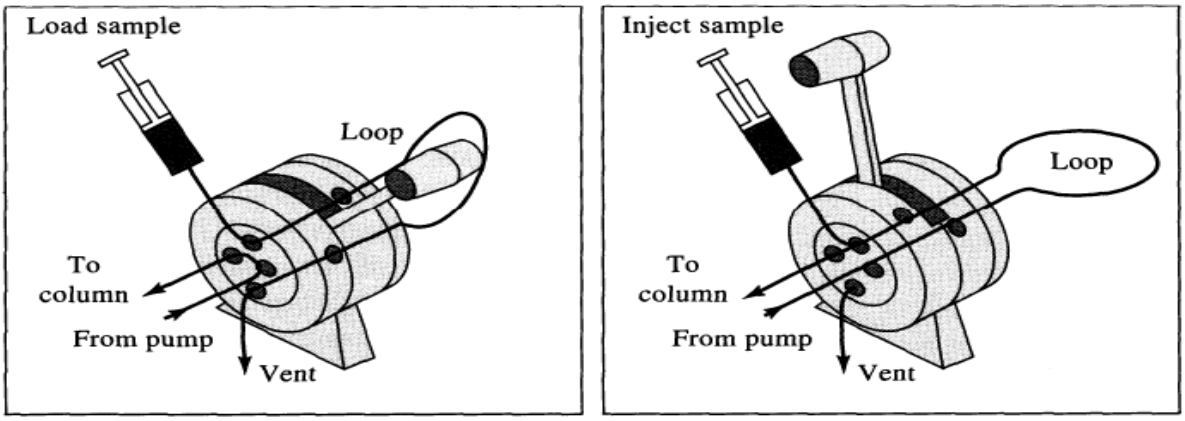



**Figure 1: Sample Injector System**

**Guard column**:

The purpose of the guard column is to protect expensive of analytical column by removing partical garbage and strongly irreversible retained sample component which decrease the life time of analytical column.

**Detectors** [24]

- **Two types of detectors:**

1. **Bulk property detectors**: They are based on some bulk properties of eluent, such as RI and are not suitable for gradient elution and are usually less sensitive than solute property.
2. **Solute property detectors:** Performed by measuring some types of physical or chemical property that is specific to solute only. So can be used with gradient elution.

# 6. VALIDATION OF ANALYTICAL METHODS [25 , 26, 27]

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness etc. When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by some different persons, in same or different laboratories, using different reagents, different equipments, etc.

**Advantage of analytical method validation**

The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user. Although the validation exercise may appear costly and time consuming, it results are inexpensive and eliminates frustrating repetitions, leads to better time management in the end. Minor change in the conditions such as reagents supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics that are evaluated. Typical validation characteristics that may be considered are listed below:

1. Accuracy
2. Precision
3. Linearity
4. Range
5. Limit of Detection (LOD) and Limit of Quantification (LOQ)
6. Specificity

7. Selectivity
8. Robustness

### 5.3.1 Accuracy

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. As per ICH guideline, accuracy is defined as "the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found". Accuracy is a measure of exactness of the analytical method.

**Accuracy can be measured by several methods:**

The true value can be obtained from an established reference method. In this approach assumes that the uncertainty of the reference method is known.

Accuracy can be assessed by analyzing a sample with known concentration, for example, a certified reference material, and comparing measured value with the true value as supplied with the material.

Recovery is found from the following formula

$$\% \text{ Recovery} = (C_{\text{fortified}} - C_{\text{unfortified}}) \times 100 / C_{\text{std added}} \text{ (QAAC)}$$

Where,

$C_{\text{fortified}}$ is concentration of drug from matrix with standard addition of drug

$C_{\text{unfortified}}$ is concentration of drug without addition

$C_{\text{std added}}$ is standard added drug in solution of drug

### 5.3.2 Precision

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. As per ICH guideline, precision of a method is "express the closeness of agreement (degree of scatter) between a series of successive measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions".

The measured standard deviation can be subdivided into 3 categories:

- Repeatability
- Intermediate precision
- Reproducibility

Repeatability gives the degree of precision obtained when the method is replicated in the same laboratory within short intervals and in the same conditions. Reproducibility represents precision obtained under variations in conditions of assays such as different analyst, equipment and reagents, laboratory and days.

The precision of an analytical method is usually expressed as the standard deviation (SD) or relative standard deviation (RSD).

The standard deviation is calculated from the following formula

$$SD = \sqrt{\frac{\sum (X_i - X)^2}{N-1}}$$

Where,

Xi = Individual measurement in the set

X = Arithmetic mean of the set

N = Number of replicates taken in the set

$$RSD = \frac{SD}{X}$$

%RSD or coefficient of variance (CV) is expressed as

$$\%RSD = CV = \frac{SD}{X} * 100$$

### 5.3.3 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighings of synthetic mixtures of the test product components, using the proposed procedure.

The relationship between the sample concentration and its signal is first order type. This line, known as the calibration line, is expressed by an estimated first order equation.

**y = mx + c**

where, y = measured signal

x = concentration of sample

c = intercept

m = slope of line

### 5.3.4 Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method. [63]

### 5.3.5 Specificity and Selectivity

Specificity of an analytical method is the ability to measure accurately and specifically the analyte in presence of components that may be expected to be present in the sample matrix.

Specificity is expressed as degree of bias of test results obtained by analysis of sample containing added impurities, degradation product, related chemical compounds or placebo ingredients when compared with test results from samples without added samples. Bias may be expressed as difference in assay results between two group samples. Thus specificity is a measure of the degree of interference in the analysis of complex sample mixture.

Selectivity is ability of analytical method to differentiate various substances in the sample. One basic difference in selectivity and specificity is that, selectivity is restricted to qualitative detection of the components from the sample matrix whereas specificity means quantitative measurement of one or more analytes. Selectivity generally applies to a separative method whereas specificity is applicable to a non-separative method. The titration methods are good examples of specificity and chromatographic methods are both selective and specific.

**Procedure for establishment of selectivity of a method**

1. Analyze sample and reference materials by the candidate and other independent methods.
2. Assess the ability of the methods to confirm free identity analyte and their ability to measure the analyte in solution from the interference present
3. Choose the most appropriate method
4. Analyze sample containing various suspected interference in the presence of the analyte of present
5. Examine the effect of interferences and whether further development is required.

### 5.3.6 Sensitivity

Sensitivity of the method expressed in terms of Sandell's sensitivity. Sandell's sensitivity refers to the number of milligrams of the drug determined converted to the colored product, which in a column solution of cross section 1 $cm^2$ shows an absorbance of 0.001 (expressed as log $cm^2$, 0.001 absorbance $unit^{-1}$).

$$S = N\frac{M}{\varepsilon}$$

Where, M = Molecular weight,

ε = Molar absorptivity of colored species

N = Number of atoms in molecule

### 5.3.7 Limit of detection (LOD) and Limit of Quantification (LOQ)

**Limit of detection (LOD)**

It is a quantitative parameter. LOD is the lowest concentration of the analyte in sample that can be detected, but not necessarily quantities precisely and accurately. It is expressed in terms of concentration units. Limit of Detection values are always specific for a particular set of experimental conditions.

Limit of Detection by definition encompasses

1. Instrumental detection limit (IDL) is the lowest limit that the instrument can detect and is based on the samples that have not gone any sample preparative steps.
2. Method detection limit (MDL) is similar to IDL but is based on samples that have gone through entire sequence of sample preparation prior to analysis.

In chromatography, the detection limit is the injected amount that results in a peak with a height at least two or three times as high as the baseline noise level. Besides this signal/noise method, the ICH describes three more methods:

1. **Visual inspection:** The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
2. **Standard deviation of the response based on the standard deviation of the blank:** Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.
3. **Standard deviation of the response based on the slope of the calibration curve:** A specific calibration curve is studied using samples containing an analyte in the range of the limit of detection. The residual standard deviation of a regression line, or the standard deviation of y-intercepts of regression lines, may be used as the standard deviation.

**Limit of Quantification (LOQ)**

It is the lowest concentration of analyte in a sample that may be measured in a sample matrix such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. The value of LOQ is almost 3-10 times higher than LOD. The LOQ also varies with the type of method employed and nature of samples.

**Table 1: Approaches for Determining the LOD and LOQ**

| APPROACH | LIMIT OF DETECTION | LIMIT OF QUANTIFICATION |
|---|---|---|
| Signal-to-noise | 3:1 or 2:1 | 10:01 |
| Standard deviation of the response and the slope (S) | 3.3 x σ/S | 10 x σ/S |

S = The slope of calibration curve

σ = The standard deviation of the response

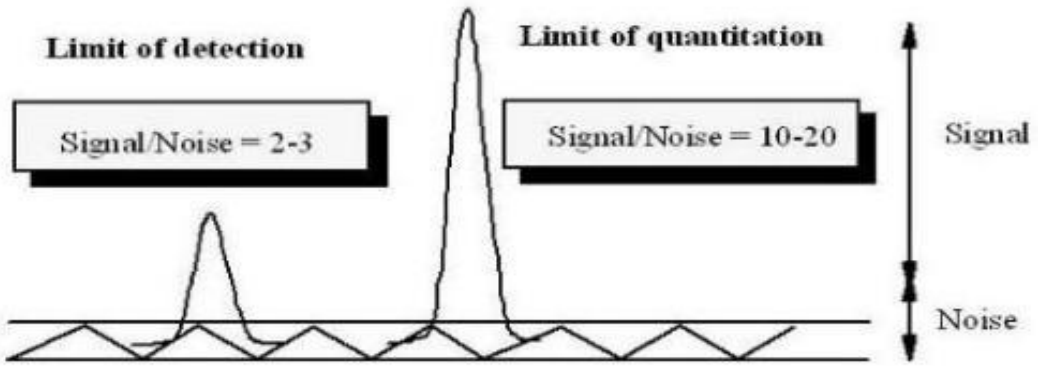
The slope S may be estimated from the calibration curve of the analyte. The estimation of σ may be carried out in a variety of ways, for example:

1. **Based on the standard deviation of the blank**

   Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

2. **Based on calibration curve**

   A specific calibration curve should be studied using samples containing an analyte in the range of detection limit. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression line may be used as the standard deviation.




### 5.3.8 Ruggedness

Ruggedness is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test conditions such as different laboratories, different analysts, different instruments and different batches or brands of reagents, different elapsed assay times and different assay temperature etc. This method is also called as Intermediate Precision.

### 5.3.9 Robustness

Robustness is the measurement of capability of analytical method to remain unaffected by small but deliberate deviation in the method parameters. Robustness testing is normally restricted to methods that are to be used repetitively in the same laboratory. It means that the method repeatable when intentional variations such as changes in concentration, use of different analyte, wavelength of detection, use of different dilutions, change of column of the same type, small changes in the mobile phase etc are introduced in the method.

# CHAPTER 3

# LITERATURE REVIEW

# 3.1 LITERATURE REVIEW OF ESCITALOPRAM

## A. REPORTED METHODS OF ESCITALOPRAM (HPLC METHODS)

**Table 2: Reported HPLC Method for Estimation of Escitalopram**

| NO | MATRIX | COLUMN | MOBILE PHASE | CONDITIONS | REMARKS | Ref No |
|---|---|---|---|---|---|---|
| 1 | Tablets (Nexito, Lexapro) | Hypersil BDS C8, 5µ column, 250 x 4.6 mm I.D | Methanol: Disodium HydrogenPhosphate: Acetonitrile (28:44:28v/v/v, pH 7.0±0.05) | **Flow rate** : 1.5 ml/min (20 µl), **Detector** : UV detection at 226 nm | **Retention Time:** 8.45 min **Linear Range:** 0.5-1.5 mg/mL | 28 |
| 2 | Tablets (With Clonazepam) | HiQ SiL C18 column, 250 x 4.6 mm I.D | Acetonitrile- 0.005 M Tetrabutyl ammonium hydrogen sulfate (55 + 45, v/v) | **Flow rate** :1.0 ml/min **Detector** : UV detection at 287 nm | **Linear range** : 10-60 µg/ml for ESC and 0.5-3.0 µg/ml for CLO | 29 |
| 3 | Human Plasma | LiChrospher CN 20 µm | Phosphate buffer 8 mmol/pH 6.4/acetonitrile (50/50, v/v) | **Flow rate** : 1.5 ml/min (20 µl), **Detector** : UV detection at 210 nm | **Linear Range:** 0.5-1.5 mg/ml **LOD:** 6 ng/ml | 30 |

## B. REPORTED METHODS OF ESCITALOPRAM (HPTLC METHODS)

**Table 3: Reported HPTLC Method for Estimation of Escitalopram**

| NO | MATRIX | COLUMN | SOLVENT SYSTEM | CONDITION | REMARK | Ref No |
|---|---|---|---|---|---|---|
| 1 | Tablets (Cpram-S 10 mg) | TLC Al- plates precoated with silica gel 60F-254 | Toluene: Acetone: Ethanol: Ammonia (5:1:1:0.2 v/v/v/v) | **Detector:** UV detection at 239 nm | Rf value: 0.50 ± 0.02<br>**Linear range** :200-1200 ng/spot<br>**LOD:** 20 ng/spot<br>**LOQ:** 50 ng/spot | 31 |
| 2 | Tablets (With Colnaze-pam) | TLC Al- plates precoated with silica gel 60F-254 | Toluene:Ethyl acetate:Triethyl-amine (7:3.5:3, *v/v/v)* | **Detector:** 258 nm by Densito-metric Scanning | **Linear range** :250-2500 ng/band for ESC, 50-500 ng/band for CLO | 32 |
| 3 | Tablets (With Clonaze-pam) | TLC Al- plates precoated with silica gel 60F-254 | Methano: Toluene: Triethylamine (1:3.5:0.1, v/v/v) | **Detector:** 253 nm by densitometric scanning | Rf value: 0.36 for ESC and 0.49 CLO<br>**Linear range** : 50-150 ng/spot for ESC and 5-15 ng/spot for CLO<br>**LOD:** 20 ng/spot<br>**LOQ:** 50 ng/spot | 33 |

## C. REPORTED METHODS OF ESCITALOPRAM (UV VISIBLE SPECTROPHOTOMETRY)

**Table 4: Reported UV Visible Spectrophotometry Method for Estimation of Escitalopram**

| NO | MATRIX | METHOD | CONDITIONS | REMARKS | Ref No |
|---|---|---|---|---|---|
| 1 | Tablets | zero order spectrophotometric method | zero order values (absorbance) measured at 238 nm | Calibration graph was constructed at 238 nm and found linear in concentration range of 2-20 µg/ml with correlation coefficient 0.9999 | 34 |
| 2 | Tablets | UV spectrophotometric method | Absorption maxima was measured at 284 nm | Linear concentration range of 20-120 µg/ml with correlation coefficient 0.9999 was found with molarabsorptivity of 2.249 $\times 10^{3}$ L/mol. cm | 35 |
| 3 | Tablets (With Clonaze-pam) | UV spectrophotometric method | Absorption Maxima: 238 nm for ESC and 273 nm for CLO | Linearity Range: 5-100 µg/ml for ESC and 5-50 µg/ml for CLO | 36 |
| 4 | Tablets (With Clonaze-pam) | Simultaneous estimation by UV Spectroscopy, using multi-component mode of analysis. | Absorption Maxima: 238 nm for ESC and 308 nm for CLO | Linearity Range: 8 - 44 µg/ml for ESC and 0.4-2.2 µg/ml for CLO | 37 |
| 5 | Tablets (With ESC and Nefopam) | simultaneous estimation by UV Spectrophotometer | Absorption Maxima: 266 for ESC and 284 for NEF | Linearity Range: 50-400 µg/ml for NEF and 25-200 µg/ml for ESC | 38 |

## D. REPORTED METHODS OF ESCITALOPRAM (LC-MS METHODS)

**Table 5: Reported LC-MS Methods for Estimation of Escitalopram**

| NO | MATRIX | COLUMN | MOBILE PHASE | CONDITIONS | REMARKS | Ref No |
|---|---|---|---|---|---|---|
| 1 | Human Plasma (LC/ESI/MS Method) | ODS YMC™ AQ 150 mm × 4.6 mm | 2.0 mM ammonium acetate (pH 5.0)–acetonitrile (54:46, v/v) | Liquid–liquid extraction of the analyte from human plasma with a mixture of diethyl ether and dichloromethane (70:30, v/v) | **Flow rate** : 1.0 ml/min Ion signals for ESC: *m/z* 325.0 **Linear Range:** 1.0 – 200 ng/ml | 39 |
| 2 | Bulk Drug (LC/ESI/MS and LC/NMR) | | | Ion trap and Q-TOF mass analyzer were employed to carry out MS/MS | | 40 |

## E. REPORTED METHODS OF ESCITALOPRAM (MISCELLANEOUS METHODS)

**Table 6: Reported Miscellaneous Methods for Estimation of Escitalopram**

| NO | METHOD | CONDITIONS | REMARKS | Ref No |
|---|---|---|---|---|
| 1 | Capillary Electro-phoresis | 50 µm, 47/40 cm fused-silica capillary, 20 mM phosphate buffer, pH 2.5, containing 0.5 mg/ml β-cyclodextrin and 22 mg/ml sulfated β-cyclodextrin as background electrolyte | **LOD:** 0.02% for all compounds, **LOQ:** 0.05%, relative to a concentration of ESC of 5 mg/ml | 41 |
| 2 | Colorimetric method (in pure form and in tablets) | Based on the formation of chloroform soluble ion associates with bromocresol green acidic dye, The extract of ion associates exhibited absorption maxima at 417 nm obeying Beer's law in the range of 2-10 µg/ml. | Recovery of drug was found 98-102% | 42 |

## 3.2 LITERATURE REVIEW OF ETIZOLAM

### A. REPORTED METHOD OF ETIZOLAM (HPLC)

**Table 7: Reported HPLC Methods for Estimation of Etizolam**

| NO | MATRIX | COLUMN | MOBILE PHASE | CONDITIONS | REMARKS | Ref No |
|---|---|---|---|---|---|---|
| 1 | Human Serum | Condition 1: C 18 reversed phase column, TSK gel 2µ column, 100 x 4.6 mm I.D. **Condition 2:** Hypersil ODS-C18 5µ column, 100 x 4.6 mm I.D. | Methhanol-5mM and $NaH_2PO_4$ (pH 6) (45:55, v/v) | **Flow rate** : 0.65 ml/min (20 µl), **Detector** : UV detection at 254 nm | **Retention times** under condition 1 were shorter. **Sensitivity and limits of quantification** were about four to ten times better under condition 1 than under condition 2. | 43 |

## B. REPORTED METHOD OF ETIZOLAM (HPTLC)

**Table 8: Reported HPTLC Methods for Estimation of Escitalopram**

| NO | MATRIX | COLUMN | SOLVENT SYSTEM | CONDITION | REMARK | Ref No |
|---|---|---|---|---|---|---|
| 1 | Tablets (with triazolam and their metabolities) | RP-18 F-254S HPTLC plate | chloroform/acetone/ ammonia(80:20:0.5 v/v) | **Detector:** UV detection at 254 nm | **LOD:** 0.1-0.2 micro gram | 44 |

## C. REPORTED METHOD OF ETIZOLAM (BIOANALYTICAL)

**Table 9: Reported Bioanalytical Methods for Estimation of Escitalopram**

| NO | MATRIX | METHOD | CONDITION / REMARKS | Ref No |
|---|---|---|---|---|
| 1 | Human Plasma and urine samples | LC/MS | **Calibration range:** 10-500 ng/ml<br>**LOD:** 2 ng/ml | 45 |

## 3.3 LITERATURE REVIEW OF ESCITALOPRAM AND ETIZOLAM COMBINATION:

**Table 10: Reported UV Visible Spectrophotometry Method for Estimation of Escitalopram and Etizolam in combination**

| NO | MATRIX | METHOD | CONDITIONS | REMARKS | Ref No |
|---|---|---|---|---|---|
| 1 | Tablets | Simultaneous Equation by UV Spectrophotometer | Absorbance Maxima: 238 nm for ESC and 242 nm for ETI | **Linearity Range:** 5 – 35 µg/ml for ESC and 0.5 – 15 µg/ml for ETI | 46 |

# CHAPTER 4

# AIM OF PRESENT WORK

# CHAPTER 4

# AIM OF PRESENT WORK

Quantitative analysis of any drug is an important tool in an industry, it is important to determine that the raw material, intermediate products as well as final products meet its specification and are of required quality. The numbers of drugs and drug formulation introduced into market has been increasing at an alarming rate. These drugs or formulation may be either new entities or partial structural modification of existing ones or novel dosage forms.

Pharmaceutical analytical procedures may be used for identification tests and quantitative tests of the active moiety in samples of drug substance or drug product. In past few decades number of new chemical entities and newer formulation of known entities have been introduced in to the market.

Multi component dosage forms are to be effective due to their combined mode of action in the body. The development of assay procedures for such dosage forms poses considerable challenges to analytical chemist owing to complexity of these dosage forms as they contain multiple drug entities and a variety of drug excipients. The estimation of the individual drugs in these multi component dosage forms becomes difficult due to cumbersome extraction or isolation procedures.

The literature review revealed that Chromatographic, Spectroscopic, Electrochemical methods are reported for the determination of Escitalopram Oxalate while very few methods are available for determination of Etizolam. There was only one method i.e. simultaneous equation method reported for determination of Escitalopram Oxalate and Etizolam in their combined dosage forms. So, it was thought of interest to develop a spetrophotometric and RP-HPLC method for determination of both drugs in their combined dosage forms.

## Objective of present work:

1. To develop and validate First Order Derivative Method for simultaneous estimation of Escitalopram and Etizolam by in their combined dosage form.
2. To develop and validate RP-HPLC method for simultaneous estimation of Escitalopram and Etizolam in their combined dosage form.

# CHAPTER 5

# IDENTIFICATION OF DRUGS

# 5. Identification of Drugs

## ➢ Instrumentation

- **U.V. Visible Spectrophotometer**

  Model-UV-1700PC Series; Version 2.2 with spectral slit width of 2 nm; Wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells. (Shimadzu, Japan)

- **Melting Point Apparatus**

  Model- VEEGO Corporation Melting Point Apparatus

- **FT-IR**

  Model- Shimadzu 8400 Series, (Shimadzu Japan)

## 5.1 Melting point Determination

Melting point of Escitalopram and Etizolam has been determined using melting point apparatus. The melting point of the compounds were taken by open capillary method.

**Table 11: Melting Point of Escitalopram and Etizolam**

| Drug | Reported Melting Point(°C) [11] | Observed Melting Point(°C) |
|---|---|---|
| Escitalopram | 150 - 152 °C | 149 – 153 °C |
| Etizolam | 146 - 149 °C | 145 – 148 °C |

## 5.2 Identification by UV Visible Spectrophotometer.

### 5.1.1 UV spectra of Escitalopram and Etizolam

UV-spectrum of Escitalopram (10 µg/ml) and Etizolam (10µg/ml) in methanol was taken.

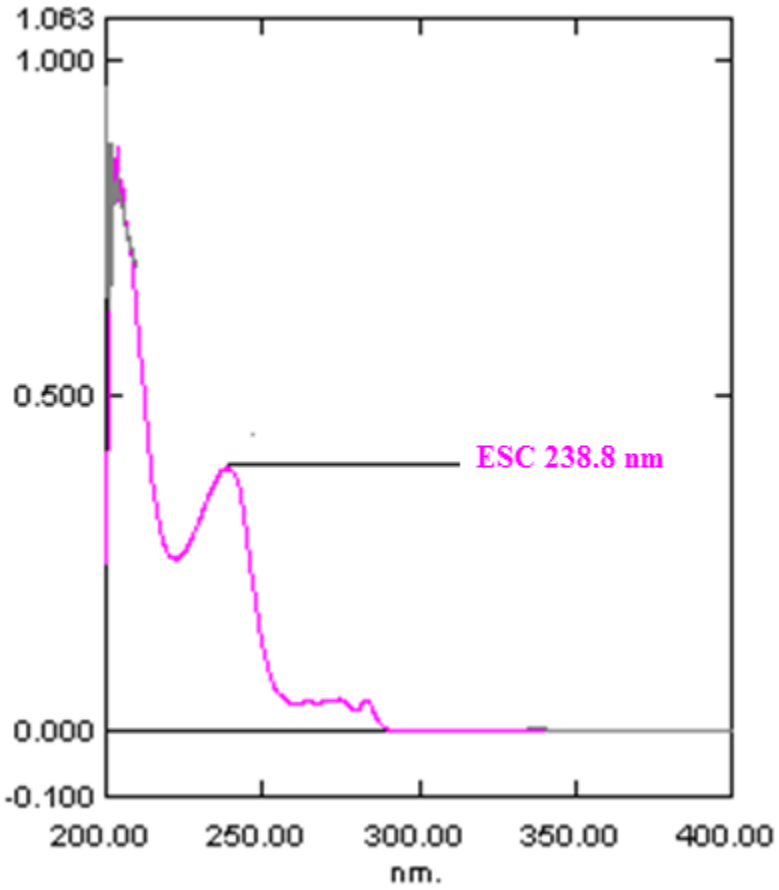



**Figure 2.**UV spectrum of Escitaloprma (10 µg/ml) in methanol

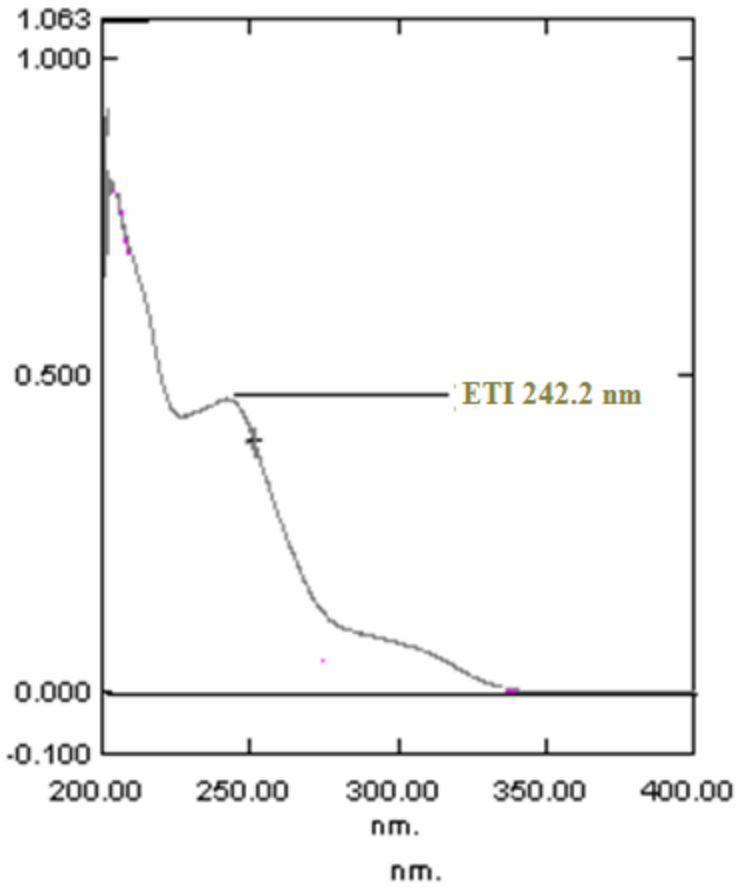



**Figure 3:** UV spectrum of Etizolam (10 µg/ml) in methanol

**Table 12: U.V. Absorbance (Wavelength Maxima) for Escitalopram and Etizolam**

| Drug Name | Reported Peak (nm) | Peak Obtained (nm) |
|---|---|---|
| **Escitalopram** | 238 [34] | 238.8 |
| **Etizolam** | 242 [46] | 242.2 |

## 5.3 Identification by F.T.I.R.

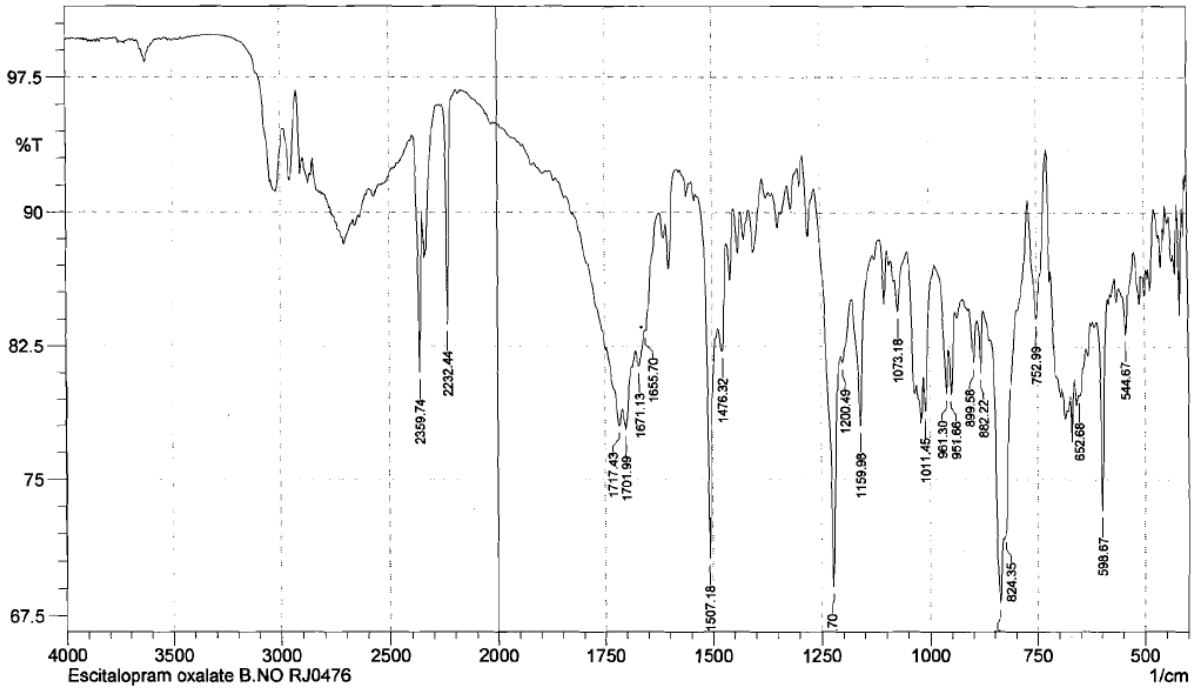



**Figure 4.**IR spectra Escitalopram

**Table 13: IR interpretation of Escitalopram:**

| No | Peak ($cm^{-1}$) | Intensity | Functional group |
|---|---|---|---|
| 1 | 2359.74 | 80.99 | Nitrile |
| 2 | 2232.44 | 83.71 | Nitrile |
| 3 | 1710.99 | 78.82 | Keone |
| 4 | 1476.32 | 82.21 | Aromatic C=C |
| 5 | 1200.49 | 81.59 | C-O in COOH |
| 6 | 598.67, 837.86 | 73.30, 68.21 | Aromatic Ring Stretch |

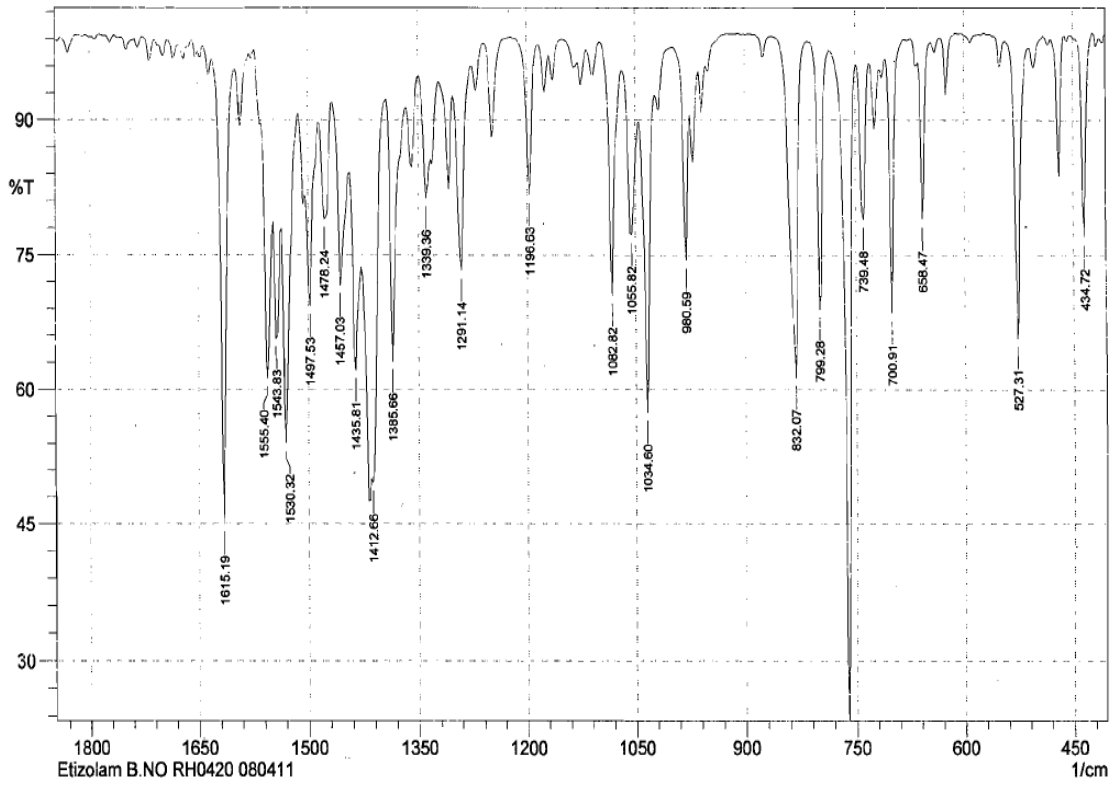



**Figure 5.**IR spectra Etizolam

**Table 14: IR interpretation of Etizolam:**

| No | Peak ($cm^{-1}$) | Intensity | Functional group |
|---|---|---|---|
| 1 | 760.7 | 23.57 | Strong C-Cl |
| 2 | 980.59 | 74.41 | C-S |
| 3 | 1555.4 | 61.20 | C=N |
| 4 | 1530.32 | 54.01 | C=C Aromatic |
| 5 | 1082.82 | 70.475 | C-N Strech |

# CHAPTER 8

# COMPARISON

# CHAPTER 8

## COMPARISON OF U.V AND RP-HPLC METHODS FOR ESTIMATION OF ESCITALOPRMA OXALATE AND ETIZOLAM IN PHARMACEUTICAL DOSAGE FORMS

In comparative study both the methods (i.e. UV visible Spectroscopy and RP-HPLC) for assay of Escitalopram and Etizolam were compared for the significant difference between them.

**Table 41: Comparison of UV and RP-HPLC Data**

| Brand name | Drugs | % Assay results | |
|---|---|---|---|
| | | U.V | RP-HPLC |
| Etilaam - S | Escitalopram Oxalate | 99.73 | 100.39 |
| | | 99.57 | 100.20 |
| | | 99.49 | 100.25 |
| | Etizolam | 98.43 | 99.90 |
| | | 98.90 | 99.78 |
| | | 98.21 | 99.68 |

### 7.3.1 Paired Two Sample T-Test For Analytical Method Comparison:

**Degree of Freedom:** 4

**Null Hypothesis**: There is no significant difference between two assay results by two analytical assay methods.

**Alternate Hypothesis:** There is significant difference between two assay results by two analytical assay methods.

**Table 42: Paired Two Sample T-Test Results for Method Comparison**

| Sr. No. | Parameter | Market Formulations [ETILAAM-S] (n=3) | | | |
|---|---|---|---|---|---|
| | | Escitalopram | | Etizolam | |
| | | UV | HPLC | UV | HPLC |
| 1. | Mean | 99.59 | 99.78 | 99.51 | 100.27 |
| 2. | Variance | 0.42 | 0.08 | 0.19 | 0.09 |
| 3. | T stat (t cal) | 0.948 | | 1.984 | |
| 4. | t Critical two tail (t crit) | 2.776 | | 2.776 | |
| 5. | t cal < t crit | Yes | | Yes | |
| 6. | Null Hypothesis | Pass | | Pass | |
| 7. | Alternate Hypothesis | Fail | | Fail | |

**Conclusion:** Null Hypothesis passes; hence there is no significant difference between two analytical methods employed. Both the methods can be used successfully for the assay of Escitalopram and Etizolam.

# CHAPTER 9

# SUMMARY

# CHAPTER 9

# SUMMARY

Spectrophotometric and Reverse Phase - High Performance Liquid Chromatography (RP-HPLC) methods were developed for estimation of Escitalopram Oxalate and Etizolam in pharmaceutical dosage forms. The methods were validated statistically and by recovery study.

- The simultaneous equation method for estimation of Escitalopram and Etizolam was found most sensitive as it obeys Beer's law in the range of 5-40 µg/ml for both Escitalopram Oxalate and Etizolam. 1st order derivative spectroscopic was performed and the absorbance were noted at 265.6 nm for Etizolam and at 241.2 for Escitalopram Oxalate. Molecular weight of Escitalopram Oxalate and Etizolam is 414.43 and 342.9 respectively. The lowest quantifiable amount of Escitalopram Oxalate and Etizolam was found to be 1.808 µg/ml and 1.382 µg/ml.

- For estimation of Escitalopram Oxalate and Etizolam by RP-HPLC, Inertsil ODS-2 column (150 mm* 4.6mm i.d., 5µm) with mobile phase containing 30 mM potassium dihydrogen phosphate: Aectonitrile: Methanol (65:30:5 v/v; pH 4.5) was used, and U.V detection at 240 nm. The retention time of Escitalopram Oxalate and Etizolam was found to be 3.631 ± 0.015 min and 10.127 ± 0.012 min respectively. The method was found most sensitive as it obeys Beer's law in the range of 150-500 µg/ml for Escitalopram Oxalate and 15-50 µg/ml for Etizolam. The lowest quantifiable amount of Escitalopram Oxalate and Etizolam was found to be 0.1373 µg/ml and 0.1912 µg/ml respectively.

- High % recoveries 98-101 % for both the methods show that the methods are free from the interference of excipients used in the formulation.

- The methods were compared statistically by t-test. The results show that there is no significant statistical difference between the results obtained by above mentioned methods.

# CHAPTER 10

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