ANALYSIS OF SECONDARY AMINO GROUP CONTAINING DRUGS IN BULK POWDER AND THEIR DOSAGE FORM (PHARMACEUTICAL FORMULATIONS)

A THESIS

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Certificate

This is to certify that the research work embodied in this thesis entitle, ***"Analysis of secondary amino group containing drugs in bulk powder and their dosage form (Pharmaceutical formulations)"*** was carried out by Mr. Nurudin Pyaralibhai Jivani at B.K. Mody Government Pharmacy College, affiliated with Saurashtra University, Rajkot under my guidance and supervision. This work is up to my satisfaction. The work embodied in this thesis is original and no part of the thesis has been submitted previously to this university or to any other university for the award of Ph. D. or any other degree or diploma.

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DECLARATION

I, hereby declare that the thesis entitled ***"Analysis of secondary amino group containing drugs in bulk powder and their dosage form (Pharmaceutical formulations)"*** is genuine record of research work carried out by me under the guidance and supervision of **Dr. B.N. Suhagia** Professor & Head, Department of Pharmaceutical Chemistry, L.M. College of Pharmacy, Ahemedabad – 380 009. No part of thesis has been submitted to any University or Institution for the award of any degree or diploma.

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Date:

Place:

Nurudin Pyaralibhai Jivani

Dedicated
to
Almighty God,
My Parents,
My Wife
and
My Children

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1. INTRODUCTION

1.1. ANTIDEPRESSANTS

Depression is the most common mental disorder that affects a large number of individuals in all countries. However, depression is under diagnosed and frequently under treated. [Audrius Sveikata et al., 2002]. Recent evidence suggests that depressive episodes, if left untreated, may heighten severity of subsequent episodes and may increase need for more health care resources.

The importance of depression as a major public health problem is emphasized by finding its place in the range of global burden of diseases. Depression was the fourth largest cause of disease worldwide in 1990, and by 2020 it is expected to be the second largest cause of disease. [Lopcz AD et al., 1996]. This problem can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his/her everyday responsibility. At its worst, depression can lead to suicide, a tragic fatality associated with the loss of about 850 000 lives every year [WHO, 2005]

As per World Health Organization,

- Depression is common, affecting about 121 million people worldwide.
- Depression occurs in persons of all genders and ages.
- Depression is among the leading causes of disability worldwide.
- Depression can be reliably diagnosed and treated in primary care.
- Fewer than 25% of those affected have access to effective treatments.

Antidepressant medications and brief forms of psychotherapy are effective for 60 –80 % of those affected and can be delivered in primary care. However, fewer than 25% of those affected (in some countries fewer than 10%) received such treatment.

Over 10% of the population will have depression within their lifetime. The range of lifetime risk for major depressive disorder in community samples is from 10 to 25% for women and 5 to 12% for men. Some individuals have only a single episode with full return to premorbid functioning; however, 50 to 85 % have recurrence of depression.

Antidepressants are the drugs which relieve the symptoms of major depressive disorders and may results in an increased output of behaviour. Antidepressants are as a class of psychotropic medication has the following broad range of indications [Janicak PG et al., 2001].

- Mood disorders:
- Major depressive disorder, bipolar disorder, cyclothymic disorder, dysthymic disorder.
- Psychotic disorders:
- Substance-induced mood disorders:
- Sleep disorders
- Anxiety disorders
- Eating disorders
- Substance related disorders
- Others: Pain syndromes, irritable bowel syndrome, enuresis, arrhythmia and some immune dysfunction.

1.1.1. Classification of Antidepressants:

Contemporary antidepressant classification system is based on the mechanism of action, which is presumed to be responsible for their antidepressant effects. A pharmacodynamic system of classification has advantages because it incorporates the current theories of disease pathophysiology. This system can easily accommodate new agents as they become available because it is based on the established pharmacology of drugs. According to this the currently available antidepressant drugs are classified into the following categories. [Beers MH et al., 1999; Kent JM, 2000; Davis KL et al., 2002; Stahl SM 1998].

[1] Non- selective antidepressants

(a) Serotonin and noradrenaline reuptake inhibition with effects on multiple receptors system and sodium conductance.
E.g. Tricyclic antidepressants [TCAs]
(b) Monoamine oxidase inhibitors. [MAOIs]

[2] Selective reuptake inhibitors

(a) Selective serotonin reuptake inhibitors. [SSRIs]
(b) Selective noradrenaline reuptake inhibitors. [NARIs]
(c) Serotonin and noradrenaline reuptake inhibitors. [SNRIs]
(d) Noradrenaline and dopamine reuptake inhibitors. [NDRIs]

[3] Receptor blockers

(a) Serotonin [5-HT2A, 5HT2C and 5HT3] receptor blockade with noradrenalin (alpha-2) receptor blockade [Noradrenergic and specific serotonergic antidepressant] (NaSSA)
(b) Serotonin (5HT2A) receptor blockade with serotonin reuptake inhibitors.

[4] Others

Clinically it seems that inhibitors of noradrenaline reuptake and 5-HT (5-Hydroxy Tryptamine, Serotonin) reuptake are equally effective as an antidepressant. From the pharmacological studies of reserpine, it was postulated that depression must be associated with decreased functional amine dependant synaptic transmission. This idea provided the basis for what become know as amine hypothesis of depression. While the amine hypothesis is undoubtedly too simplistic, it has provided the major experimental models for the discovery of new antidepressant drugs. As a result all currently available antidepressant drugs except bupropion are having

their primary action on the metabolism, reuptake, or selective receptor antagonism of noradrenaline, serotonin or both.

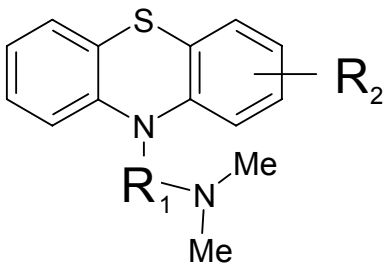
[1] Non selective antidepressants

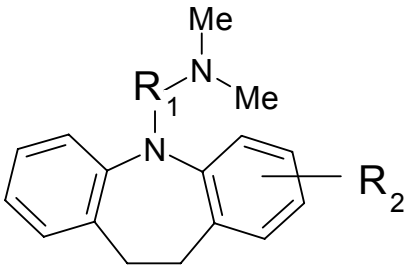
The history of antidepressant pharmacotherapy begins with the tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs). Both of these classes of antidepressants were successful and were discovered by chance. [Janicak PG et al., 2001]. The first antidepressant tricyclic and monoamine oxidase inhibitors became available in the late 1950s.

(a) Tricyclic antidepressants (TCAs)

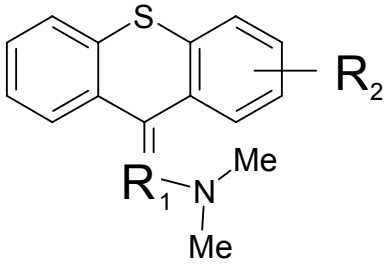
Tricyclic antidepressants are an important group of antidepressants in clinical use. They are, however, far from ideal in practice. Most of the tricyclic antidepressants are derivatives of dibenzazepine or structurally related compounds. Structurally they are closely related to phenothiazine derivatives, which are one of the potential antipsychotic agents. Sulphur in aromatic linkages has been known to be isosteric to –CH=CH- . Replacement of sulphur of phenothiazine with –CH=CH- could give dibenzazepine system, with seven membered central ring and saturation of –CH=CH- to $–CH_2CH_2$- could lead to the 10, 11- dihydro derivatives. Imipramine and clomipramine were found to be no use in schizophrenia but effective against depression. Similar changes to the structure of thioxanthene type antipsychotic drugs resulted in drugs such as amitriptyline. They differ from phenothiazine and thioxanthene principally in the incorporation of an extra atom in the central ring which twists the structure, so that molecule in no longer planner as in phenothiazine and thioxanthere. [Kadam SS et al., 2006]. All these compounds are tertiary amines, with two methyl groups attached to the basic nitrogen atom. They are quite rapidly demethylated in vivo, to corresponding secondary amines (desipramine, nortriptyline) which are themselve active other tricyclic derivatives with slightly modified bridge structures include doxapine, amoxapine and dithiapine.

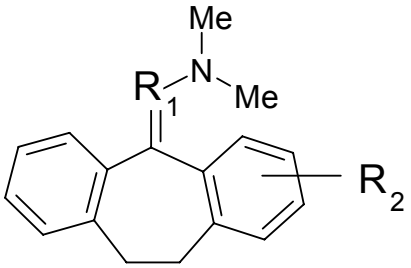
(i) Phenothiazine (Antipsychotics) ⟶ Dibenzazepine (Antidepressants)





(ii) Thioxanthene (Antipsychotics) ⟶ Dibenzocycloheptane (Antidepressants)





Mechanism of action:

Tricyclic antidepressants potentiate central noradrenergic and serotonergic function. They inhibit the reuptake of norepinephrine and serotonin by neuron terminals by competition for the binding site of transport protein. Synthesis of amines, storage in synaptic vesicles and release are not directly affected, though some TCAs appears to increase transmitter release indirectly by blocking alpha-2 adreno receptors. Most TCAs inhibit the reuptake of norepinephrine and 5-HT but much less effect on dopamine reuptake. It improves emotional symptoms mainly an enhancement of 5HT mediated transmission where as relief to biological symptoms results from facilitation of norepinephrine transmission.

The choice of initial antidepressant legitimately varies considerably among clinicians and countries [Gelder MG et al., 2000].

(b) Monoamine oxidase inhibitors:

The potential of monoamine oxidase inhibitors as an antidepressant was discovered accidentally. In 1951, isoniazid came to be recognized as an effective tuberculostatic agent. A year later its N-isopropyl derivative, iproniazid, was made available for treating tuberculosis. Amazingly, it was observed that iproniazid had mood elevating effects in patients. Soon after many hydrazine derivatives were found to be active as such. The action and use are very similar to tricyclic antidrepressants, i.e., they inhibit the reuptake of nor epinephrine and serotonin by neuron terminals. Monoamine oxidase inhibitors have been employed less often than the tricyclic antidepressants, probably because of the possibility of drug interaction and dietary precautions that are required to be observed.

[2] Selective reuptake inhibitors

(a) Selective serotonin reuptake inhibitors [SSRIs]

Selective serotonin reuptake inhibitors are so called, because they selectively inhibit the reuptake of serotonin [5-HT] from the synaptic gap. The simplified concept of selective serotonin reuptake inhibitors is usually used to describe how these agents work but it does not describe important features of the group as a whole and of the individual agents. An important consequence of increasing serotonin is the stimulation of post synaptic serotonergic receptors. There are many subtypes of serotonergic receptors. There is an opinion that stimulation of serotonin 2C [5HT-2C] receptors in the CNS is responsible for the increase of anxiety that is often associated with early stages of SSRIs therapy [Audrius sveikata et al., 2002]. Stimulation of $5HT_3$ and $5HT_4$ receptors in the gut is thought to account for the nausea and gastrointestinal upset that may occur in the first few days or weeks.

1.1.2. Fluoxetine hydrochloride

Introduction:

Fluoxetine hydrochloride is a selective serotonin reuptake inhibitor which is clinically effective for the treatment of depression [Stark P et al., 1985]. Fluoxetine and its major metabolite norfluoxetine act as neuronal inhibitors of serotonin reuptake and result in both increased serotonin concentration at the synaptic cleft and autoreceptor stimulation [Schmidt MJ et at., 1988; Wong DT et al., 1975]. Fluoxetine hydrochloride has been shown to have comparable efficacy to tricyclic antidepressants but with fewer anticholinergic side effect [Chouinard G, 1985; Lader M, 1988]. Fluoxetine hydrochloride has been primarily studied for the treatment of depression, but more recently has been studied for the treatment of bulimia and severe obesity [Ferguson JM et al., 1987; Levina LR et al., 1987].

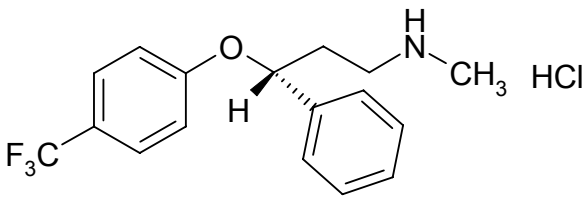
Chemical Name:

d,1-N-methyl-3-phenyl-3-[(α, α, α-trifluoro-p-tolyl)oxy]polyamine hydrochloride

OR

[(3RS)-N-Methyl-3-phenyl-3-(4-trifluoromethyl phenoxy) propan-1-amine] hydrochloride

Structural formula:



Emperical formula: $C_{17}H_{18}F_3NO$ HCl

Molecular Weight: 345.79

Appearance, color and odour:

Fluoxetine hydrochloride is a white to off white, crystalline, odorless powder.

Melting Point: 158.4 to 158.9 ^{0}C

Solubility:

Fluoxetine hydrochloride is freely soluble in methanol and ethanol; soluble in acetonitrile, chloroform and acetone; slightly soluble in ethyl acetate, dichloromethane and water (With sonication at pH: 1.2, 4.5 and 7.0). The maximum solubility of fluoxetine obtained in water is 14 mg/ml. Fluoxetine is essentially insoluble in toluene, cyclohexane and hexane.

Stability:

Fluoxetine hydrochloride is a very stable molecule under normal storage conditions. The only known degradation products are α-[2(methyl amino) ethyl] benzene methanol and p-trifluoromethyl phenol. They are formed under acidic stress conditions (3mg fluoxetine/1ml 0.1 N HCl refluxed for 48 hrs) [Donald SR et al., 1990] or when irradiated for five hrs with a mercury arc lamp [Souter RW et al., 1976]. Fluoxetine hydrochloride is stable as the bulk drug stored at 25 ^{0}C for five years and at 50 ^{0}C for two years.

Metabolism:

The main metabolite of fluoxetine is norfluoxetine, an active metabolite with similar physiological activity as its parent compound. Metabolism occurs in the liver by N-demethylation [Lemberger L et al., 1985; Aronoff GR et al., 1984]. Studies in animals have shown that fluoxetine is also

metabolized into p-trifluoromethylphenol by O-dealkylation. Other known metabolites include glucuronides of both fluoxetine and norfluoxetine.

Mechanism of Action:

Fluoxetine and its major metabolite norfluoxetine act as neuronal inhibitors of serotonin reuptake and result in both increased serotonin concentration at the synaptic cleft. Fluoxetine has significant activity at serotonin 2C [5HT-2C] receptors, and serotonin 2C receptors are presumed to be involved in the regulation of appetite and food intake. The affinity for serotonin 2C receptors might explain why fluoxetine is also the SSRI that gained approval for eating disorders. Blockade of the 5HT-2C receptors may be involved in the alleviation of psychosis [Boet JA et al., 2001]. Fluoxetine has some activity with noradrenaline reuptake inhibition as well [Shilash R. et al., 2000]. Fluoxetine also binds to cytochrome P_{450} 3AG and 206, this is important for drug interaction.

1.1.3. Sertraline hydrochloride

Introduction:

Sertraline hydrochloride is an antidepressant for oral administration. It is chemically unrelated to trycyclic, tetracyclic, or other available antidepressants. It is a novel inhibitor of serotonin reuptake in the brain [Koe BK et al., 1983].

Chemical Name:

(1S-Cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine hydrochloride [CAS-79559-97-0]
Preferred use name.

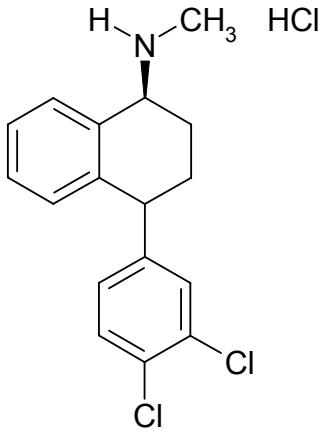
OR

Cis-(1S,4S)-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride [J. Med. Chem. 1984, 27(11), 1508]

OR

Cis-(1S)-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride [US Patent 4,536,518]

Structural Formula:



- **Empirical Formula:** $C_{17}H_{18}NCl_3$
- **Molecular Weight:** 342.7
- **Appearance, Color, Odour:**

Sertraline hydrochloride is a white to off-white, crystalline powder having no odour. It is an irritant, contact with skin and eyes should be avoided.

- **Melting Point:** 245-250 ^{0}C

- **Solubitity:**

Sertraline hydrochloride is freely soluble in ethanol, chloroform, methanol, dimethyl sulfoxide and N,N-dimethyl formamide. Soluble in acetone, acetonitrile, isopropyl alcohol. The solubility of a saturated solution of sertraline hydrochloride in distilled water at room temperature is 3 mg/ml. The pH of this saturated solution is 5.3. The solubility in water is pH dependent; in acidic pH (i.e. 0 to 7 pH) it is freely soluble. As the pH of solution increase from 7 to 12, the solubility decreases and above12 pH, it is almost insoluble [Bruce M et al., 1996]

- **Stability:**

Sertraline hydrochloride is a very stable molecule under normal storage condition as well as a variety of challenge conditions. The only trace degradation product identified was the ketone, i.e. [4-5-(3,4-dichlorophenyl)-3,4-dihydro-1(2H)-naphthalenone] which was obtained under extreme conditions only (i.e. refluxing with 5 N HCl or with 5 N NaOH for 3 hrs]. The molecule is stable at 30 ^{0}C for 60 months.

- **Metabolism:**

Sertraline undergoes extensive first pass metabolism. The principal initial pathway of metabolism for setraline is N-demethylation. N-demethyl setraline has a plasma terminal elimination half-life of 62 to 104 hrs. The N-demethyl setraline is substantially less active than parent molecule. Both sertraline and N-demethyl setraline undergo oxidative deamination and subsequent reduction, hydroxylation and glucuronide conjugation. [Tremaine LM et al., 1989]

- **Mechanism of action:**

The mechanism of action of setraline is presumed to be linked to its inhibition of CNS neuronal uptake of serotonin [5HT]. In vitro studies have shown that sertraline has no significant affinity for adrenergic (alpha 1, alpha 2, beta), chlolinergic, GABA, dopaminergic, histaminergic and serotonergic (5HT 1A, 5HT 1B, 5HT 2) or benzodiazepine receptors. Sertraline does not inhibits monoamine oxidase.

(b) Selective noradrenaline reuptake inhibitors [NARIs]

Some TCAs (e.g. desipramine, nortriptyline) block noradrenaline reuptake more potently than serotonin reuptake. These TCAs are not really selective noradrenaline reuptake blockers since they block other receptors like H_1, acetylcholine – M and noradrenaline α-1 receptors as well. The first really selective noradrenergic reuptake inhibitor (NARI) is reboxetine. Reboxetine does not inhibit electrically excitable membranes and for this reason overdose of reboxetine should not couse significant risk of cardiotoxicity or seizures. [Janicak PG et al., 2001; Mucci M, 1997]. The efficacy of reboxetine in anxiety and panic, not predicted from the known psychopharmacology of noradrenaline suggests a role for the noradrenaline pathway in anxiety and panic [Bruncllo N et al., 2002].

(c) Serotonin and nordrenaline reuptake inhibitors [SNRIs]

These drugs block the reuptake of serotonin and noradrenaline (like TCAs such as amitriptyline) but differ from TCAs by their lack of receptor blocking activity at H_1, acetylcholine – M and noradrinaline α-1 and α-2 receptors. They should also lack membrane stabilizing activity. They should therefore not cause sedation or postural hypotension or fatality in overdose..

1.1.4. Duloxetine hydrochloride

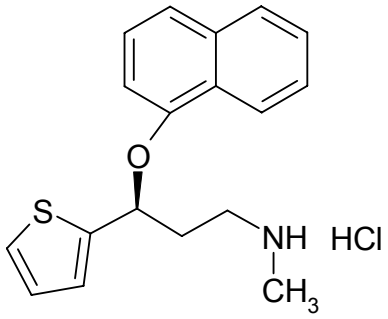
- **Introduction:**

Duloxetine and venlafaxine are the new drugs in this group, which inhibit first serotonin reuptake and later they inhibit noradrenaline reuptake as well [Gabbard GO, 2001]. Duloxetine is official as hydrochloride salt.

- **Chemical Name:**

(+) – (S)- N-methyl-γ-(1-naphthyloxy)-2-thiophene propylamine hydrochloride

- **Structural Formula:**



- **Empirical Formula:** $C_{18}H_{19}NOS\ HCl$

- **Molecular Formula:** 333.88

- **Appearance, Color, Odour:**

  Duloxetine hydrochloride is a white to slightly brownish white solid, and odourless.

- **Melting Point:** 163-167 ^{o}C

- **Solubility:**

Duloxetine hydrochloride is freely soluble in methanol, ethanol, chloroform and dimethyl sulphoxide. Soluble in acetonitrile, and acetone. Slightly soluble in water.

- **Stability:**

Duloxetine hydrochloride degraded in acidic media. So it is available as enteric coated pellets which prevent degradation of drug in acidic environment of the stomach while overall stable compound.

- **Metabolism:**

The major biotransformation pathways for duloxetine involve oxidation of the naphthyl ring followed by conjugation and further oxidation. Both CYP2D6 and CYP1A2 catalyse the oxidation of the naphthyl ring in vitro. Metabolites found in plasma include 4-hydroxy duloxetine glucuronide and 5-hydroxy, 6-hydroxy duloxetine sulphate. Many additional metabolites have been identified in urine. Most of (more than 70%) the duloxetine dose appears in the urine as metabolites of duloxetine, about 20% is excreted in the feces and only trace (< 1% of dose) amounts of unchanged duloxetine are present in the urine.

- **Mechanism of Action:**

The antidepressant and pain inhibitory action of duloxetine is believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS. Preclinical studies have shown that duloxetine is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a less potent inhibitor of dopamine reuptake. Duloxetine has no significant affinity for dopaminergic, adrenergic, cholinergic, histaminergic, opioid, glutamate and GABA receptors in vitro. Duloxetine does not inhibit monoamine oxidase (MAO). Duloxetine

undergoes extensinve metabolism, but the major circulating metabolites have not been shown to contribute significantly to the pharmacologic activity of duloxetine [Cymbalta, Eli Lilly and Company, 2004].

(d) Noradrenaline and dopamine reuptake inhibitors [NDRIs]

Bupropion is a relatively week inhibitor of dopamine reuptake, with modest effects on noradrenaline reuptake and no effects on serotonin reuptake. It does not appear to be associated with down regulation of postsynaptic beta adrenergic receptors. However, presumed mechanism of action of bupropion is based on inhibition of reuptake of dopamine and noradrenaline. Its weak affinity for these reuptake pumps has raised questions whether these mechanisms are relevant to its antidepressant activity. The combined plasma concentration of bupropion and its three active metabolites, hydroxybupropion, threohydrobupropion, erythrohydrobupropion are responsible for the inhibition of both these pumps [Schatzberg AI; 2001]

[3] Receptor Blockers

(a) Noradrenergic and specific serotonergic antidepressant [NaSSA]

The novel antidepressant mirtazapine has dual mode of action, it is a noradrenergic and specific serotonergic antidepressants that acts by antagonizing central alpha-2 adrenergic auto and hetero receptors, as well as by blocking 5-HT2 and 5-HT3 receptors. Mirtazapine enhances noradrenergic and serotonergic neurotransmission. Bioavailability of mirtazapine is approximately 50% peak plasma concentration is reached within 2 hours. The elimination half-life ranges from 20 to 40 hours. Mirtazapine has linear pharmacokinetics. It is approximately 85% bound to protein in plasma. Blockade of presynaptic central alpha-2 adrenergic autoreceptors leads to enhanced noradrenergic neurotransmission via increased noradrenergic cell firing and noradrenaline release. The overall effect of these pharmacological action is increased noradrenergic and 5HT1A activity [Sami AK; 2001]

(b) Serotonin (5-HT2A) receptor blockade with serotonin reuptake inhibitors

Mefazodone has selective and unique effects on the serotonin system. Mefazodone blocks serotonin reuptake, while functioning as a 5-HT2A receptor antagonist. Mefazodone has some unusual pharmacological properties. Mefazodone is metabolized by CYP303/4 to form the active metabolite m-chlorophenylpiperazine, which is a potent serotonin agonist. This metabolite is a 5-HT 2C agonist and could paradoxically cause anxiety and stimulation instead of anxiety reduction and sedation. Mefazodone has nonlinear kinetics, which results in greater than proportional mean plasma concentration with higher doses. The elimination half-live is 2-4 hours for the parent compound. Mefazodone is extensively (about 99%) but loosely bound to proteins [Schatzberg AI; 2001]

[4] Others:

Tianeptine the drug that has been found to be an effective antidepressant. Tianeptine actually increases the uptake of serotonin into nerve endings, an effect that is opposite to the standard selective serotonin inhibitors. Tianeptine decreases both serotonin transporter mRNA and that of binding site [Schatzberg AI; 2001]

1.2 Antihypertensive drugs and angiotention – II antagonist

Hypertension is a common disorder, which, if not effectively treated, results in a greatly increased probability of coronary thrombosis, strokes and renal failure. Until about 1950, there was no effective treatment, and the development of antihypertensive drugs, which greatly increase life expectancy, has been a major, but largely unsung, therapeutic success story [Cohen JN; 1996].

There are a few recognizable and surgically treatable causes of hypertension, such as phaeochromocytoma, steroid secreting tumours of the adrenal cortex, renal artery stenosis and so on, but the great majority of cases involves no obvious causative factor, and is grouped as essential hypertension. The pathophysiology is intimately related to the kidneys and leads to narrowing of the lumen of systemic arterioles. The raised peripheral vascular resistance calls into play various physiological responses ivolving the cardiovascular system, nervous system and kidney. The following figure summarises major physiological mechanisms that control arterial blood pressure, and shows the sites at which some antihypertensive drugs act.

In hypertension, the small arterioles used for transferring blood from the arteries to the tissue capillaries and venus circulation get under excessive stimulation of sympathetic nervous system. This may result arteriole contraction and increase peripheral resistance to the flow of blood. As a result blood pressure increases. The agents used to reduce blood pressure are known as antihypertensive agents.

The World Health Organization (WHO) has defined “Hypertension” as a state in which systolic pressure is 150 mm of Hg or more and diastolic pressure is 95 mm of Hg or more. As per the statistical data given by WHO in 1990, every year approximately 15 million people have died due to heart disease. The renal and cardiovascular complications resulting from this prolong elevation of blood pressure. The drug therapy reduce cardiac output / or and reduce total peripheral resistance without correcting the cause.

For practical guidance, most physicians classified.

- ❖ Diastolic pressure if 90 to 110 mm of Hg is consider as mild hypertension.
- ❖ Diastolic pressure if 110 to 130 mm of Hg is consider as moderate hypertension.
- ❖ Diastolic pressure if more than 130 mm of Hg is consider as severe hypertension.

1.2.1. Classification of antihypertensive agents:

All currently available antihypertensive drugs act mainly by interfering with normal hemostatic mechanisms and this provides a useful basis for the classification of these drugs. They are classified as [Rang HP et al., 2003; Bruntoni LL et al., 2005; Satoskar RS et al., 2003].

(i) Drugs acting centrally:
E.g. Clonidine hydrochloride, Guanabenz, Guanfacine, Methyldopa, Methyl dopate

(ii) Ganglion blocking agents:
E.g. Mecamylamine hydrochloride, Pentolinium tartarate, Trimethaphan camsylate

(iii) Drugs acting on the post ganglionic sympathetic nerve ending:
(a) Adrenergic neurone blockers: e.g. Gaunethidine, Bethanidine, Bretylium
(b) Catecholamine depletors: e.g. Reserpine, Deserpine, Rescinnamine

(iv) Drugs acting on adrenergic receptors
(a) α–adrenergic blocking agents: e.g. Phentolamine, Prazosin, Phenoxybenzamine
(b) β – adrenergic blocking agents: e. g. Propanolol, Atenolol
(c) Mixed blockers: e.g. Labetalol
(d) Imidazole receptor antagonist: e.g. Ritmanidine

(v) Drugs acting directly on the vascular smooth muscles (Vasodilators)

(a) Arterial vasodilators:
e.g. Hydrallazine, Diazoxide, Minoxidil, Sodium nitropruside

(b) Calcium Channel Blockers:
e.g. Nifedipine, Amlodipine, Verapamil, Diltiazem

(vi) Drugs acting on Renin Angiotensin System

(a) Drug blocks rennin release: e.g. Propranolol, Clonidine

(b) Angiotensin Converting Enzyme inhibitors: e.g. Captopril, Lisinopril,
Ramipril

(c) Angiotensin – II antagonists: e.g. Saralasin

(d) Angiotensin – II (Specific AT_1) receptor blockers: e.g. Losartan, Valsartan, Candesartan, Olmesartan, Irbesartan

(e) Aldosterone antagonist: e.g. Spironolactone

(vii) Diuretics:

(a) Thiazides: e.g. Hydrochlorothiazide

(b) Loop diuretics: e.g. Furosemide

(c) Potassium sparing diuretics: e.g Triamterene, Amiloride

(viii) Drug act by reflex mechanism: e.g. Veratrum alkaloid

1.2.2. Drugs acting on renin angiotensin system

Renin is a proteolytic enzyme having molecular weight 42000 and stored in the granules of the juxtaglomerular cell in the walls of the afferent arterioles of the kidney. Upon release into the renal arterial blood stream, rennin catalyses the conversion of angiotensinogen as per following chart.

Angiotensinogen (Plasma α_2 – globulin)
(An inactive precursor)

↓ Renin

Angiotensin – I (Decapeptide synthesize in liver)

↓ Angiotensin Coverting Enzyme (ACE)

Angiotensin – II (Octapeptide most potent vasoconstrictors)

↓ Aminopeptidase enzyme (Hydrolysis which lead to loss of one Amino acid)

Angiotensin – III (Heptapeptide active intermediate)

↓ Aminopeptidase carboxypeptidase & andopeptidase

Inactive peptide fragmets

The rennin angiotensin system is an important part of homeostatic mechanism in the body. It works to maintain the blood pressure at the normal level. It also regulates the electrolyte balance by controlling aldosterone biosynthesis and release from adrenal cortex. Similarly the juxtaglomerular cells are directly innerved by central sympathetic nerves. Hence under the conditions of strain and stress, the sympathetic stimulation may lead to hypertension due to the activation of rennin angiotensin system [Kadam SS et al., 2006]

The rennin angiotensin system plays a determinant role in the regulation of blood pressure. Angiotensins are potent vasoconstrictors. They tend to increase the peripheral vascular resistance. The octapeptide hormone angiotensin – II is the active product of rennin angiotensin system which

causes vasoconstriction when binds to AT_1 receptor. The angiotensin induced release of aldosterone increases the sodium ion retention in plasma, resulting into an increase in plasma volume. The overall result of all these effects is hypertension. Hence one can expect that angiotensin antagonists would be effective antihypertensive agents. This expectation was proved to be correct by the development of saralasin (1971) and captopril (1977), each being the member of two distinct classes [Goodfriend TL et al., 1996].

AT_1 antagonists constitute the most recent class of them. They block the receptor in a competitive or insurmountable way so that angiotensin – II cannot bind at the active site. The discovery by Dufont group of a series of (biphenylmethyl) imidazoles as nonpeptidic potent and orally active angiotensin – II receptor (AT_1 subtype) antagonists has opened up a completely new field in angiotensin – II antagonist research. Losartan is parent molecule of this class. A number of studies have appeared in which the imidazole moiety of losartan is successively replaced by other heterocycles indicating the AT_1 receptor is quite permissive in accepting this region of the nonpeptide antagonist. This leads to the generation of more potent drugs valsartan, irbesartan, candesartan, olmesartan and eprosartan [Dzau VJ et al., 1993; Timmermans PB et al., 1993].

1.2.3. Valsartan

- **Introduction:**

Valsartan, a specific angiotensin – II antagonist, is used alone or with other antihypertensive agents to treat hypertension. It is competitively blocks the binding of angiotensin – II to type I angiotensin (AT_1) receptor and therefore block the effects of angiotensin more selectively than do the ACE inhibitors. It reduces the angiotensin induced vasoconstriction, sodium reabsorption and aldosterone release. It does not inhibit the breakdown of bradykinin.

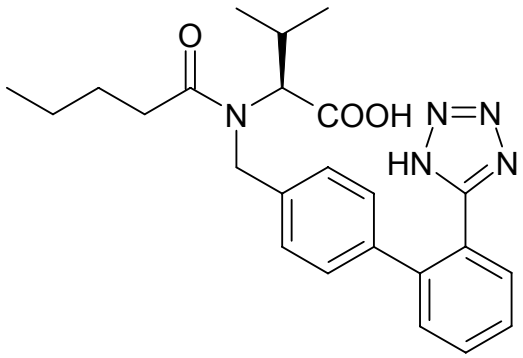
- **Chemical Name:**

N-(1-oxopentyl)-N-[{2-(1H-tetrazol-5-yl)- (1,1-biphenyl) -4-yl}methyl]-L-valine

OR

N-[p-(o-1H-Tetrazol-5-ylphenyl) benzyl]-N-valeryl-1-valine

Structural formula:



- **Empirical Formula:** $C_{24}H_{29}N_5O_3$
- **Molecular weight:** 435.5
- **Appearance, Color and Odour:**

  A white or almost white practically odourless amorphous powder.

- **Melting Point:** 108 – 110 ^{0}C
- **Solubility:**

Valsartan is freely soluble in methanol, ethanol and chloroform. Soluble in water, phosphate buffer at pH 7.4 and in acid solution.

- **Stability:**

Valsartan is a very stable molecule under normal storage conditions.

- **Mechanism of Action:**

Valsartan compete with angiotensin – II for binding at the AT_1 receptor subtype. As angiotensin – II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in a decrease in systemic vascular resistance. AT_1 receptor antagonists could be more effective than ACE inhibitors (e.g. captopril, enalepril, lisinopril etc.). AT_1 receptor antagonist does not cause the dry cough that is sometimes caused by ACE inhibitors.

- **Metabolism and Elimination:**

Excretion of valsartan is rapid, with complete elimination demonstrable at 48 hrs. Valsartan is predominantly excreted unchanged in the bile and, to a lesser extent, in urine. The hepatic route constitutes the major route of excretion 94 – 96 % of the delivered dose may be recovered in the feces. Oxidation biotransformation of valsartan in the liver is slow and constitutes only a very small fraction of total drug elimination. Metabolites in the excreta include the tetra – N – glucose conjugate, the tetrazole biphenyl methanolic acid derivative in mice and the valeryl – 4 – hydroxyl valsartan derivative. Small amounts of other metabolites may also be detected in the liver, including an acylglucoride conjugate. Additional racemization does not occur in vivo when valsartan is administered orally [Hein L et al., 1995].

1.3. ION PAIR COMPLEXES:

Many amines and quaternary ammonium compounds can be determined in aqueous solution by forming a salt or ion pair between the positively charged or negatively charged nitrogenous compound and, an opposite charged dye or indicator molecule. This ion-pair formed is extracted into an organic solvent and concentration of extracted dye is measured spectrophotometrically. This technique is called ion pair extraction technique (Sethi PD; 1997).

1.3.1. Types:

There are two types of ion-pair extraction technique.

1. Acid dye method

Here drug is positively charged and dye is acidic in nature and negatively charged as anionic form at given pH.

2. Basic dye method

Here drug is negatively charged and dye is basic in nature and positively charged as cationic form at given pH.

The acidic and basic dye techniques are important analytical application of phase transfer principle to the colorimetric determination of various amines (Kenneth A; 1982).

1.3.2. Principle:

Colorimetric analysis also can be based on molecular complex formation. Recall that charge-transfer complexation often is accompanied by the development of an intense charge-transfer absorption band and this can be put to analytical use for example, tertiary amines can be determined

spectrophotometrically by complexation with tetracyanoethylene. Many complex formation reactions are used in conjunction with or as the basis for a separation either by liquid-liquid extraction or chromatography (Alfonso R. Gennaro, 2000).

The *acid-dye technique* is an important analytical application of phase transfer principle for the colorimetric determination of amines (Higuchi, T. 1961).

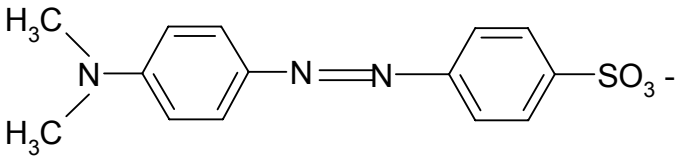
A weakly acidic acid-base indicator (the acid or basic dye) that ionizes to produce an anion is added to an aqueous solution of an amine, the pH being adjusted so the dye is predominantly in the ionized state. If, at this pH the amine is protonated, an ion pair is formed and this ion pair is extracted into an organic solvent, where the concentration of the extracted dye (which is equal to the concentration of the amine) is measured spectrophotometrically. The technique is also applicable to the determination of quaternary ammonium compounds. If the amine is not protonated, evidently an ion pair cannot be formed, but an extractable association complex may be formed from the anionic dye and the neutral amine (Kenneth A Connors, 1982).

The equilibrium expression for ion-pair formation can be expressed as.

$$A^+ \text{ aq.} + D^- \text{ aq.} \rightarrow \text{AD org.}$$

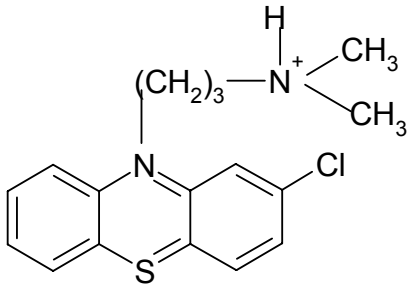
A^+ aq. is the protonated amine in aqueous phase. D^- aq. is the anionic pairing dye in aqueous phase and AD org. is the final ion-pair complex extractable into organic phase. Extracting the complex with aqueous acid or alkali depending on the anion pairing dye readily decomposes these complexes. (Sethi, P.D., 1997)

Basic drug chlorpromazine makes ion-pair complex with acidic dye methyl orange (Zhou Z. et al., 1996).

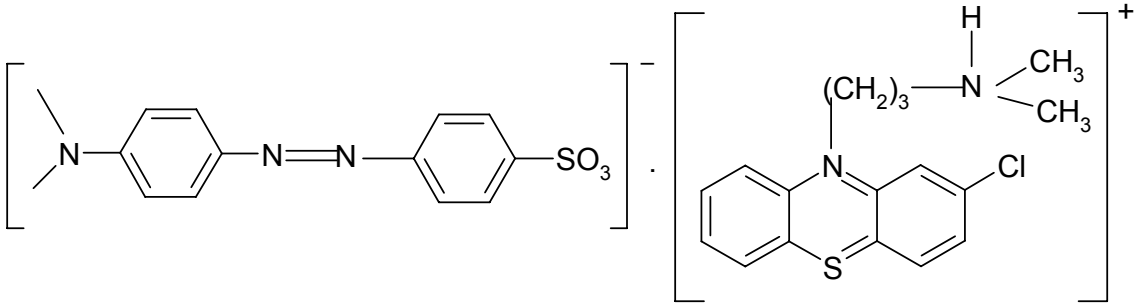


Methyl orange (anionic dye)

+



Chlorpromazine (cationic drug)



Methyl orange **Chlorpromazine**

(Neutral lipophilic ion-pair complex)

The principle of the acid dye technique can be applied in a basic-dye method in which the charge types are reversed.

Amines in their anionic or protonated state form a complex with a cationic or anionic dye species respectively to form complexes, which are distinguished by their solubility in organic solvents such as chloroform, benzene, dichloromethane. Thus by using a pairing reagent, a colored complex is produced and the amines can thus be measured colorimetrically.
The equilibrium expression for ion-pair formation can be expressed as

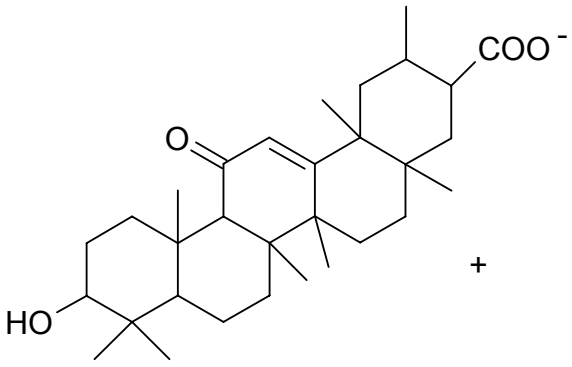
A^-_{aq} + D^+_{aq} ⟷ AD_{org}

A^-_{aq} = anionic drug in aq. Phase

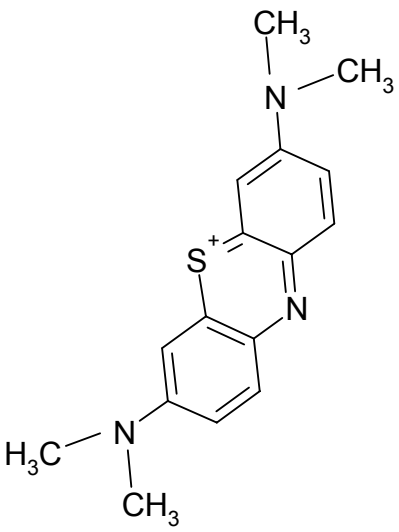
D^+_{aq} = cationic dye in aq. Phase

AD_{org} = ion-pair complex extractable into organic phase.

For example the acidic component of glycyrrhiza (Glycyrrhetinic acid) was determined by extraction of its ion pair with the basic dye methylene blue (Habib, A.A.M., 1979 and Pesez, M., 1974)

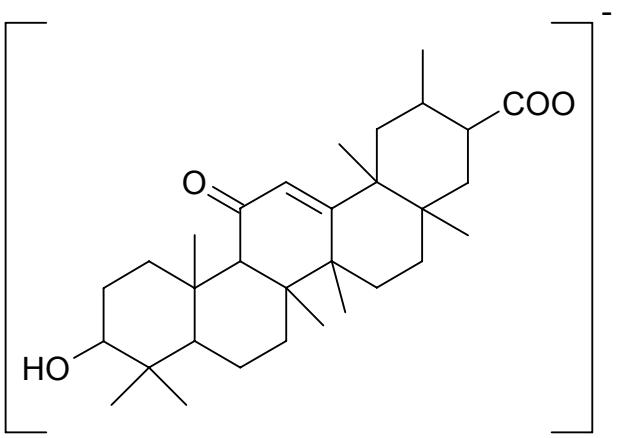


Glycyrrhetinic acid (anionic drug)

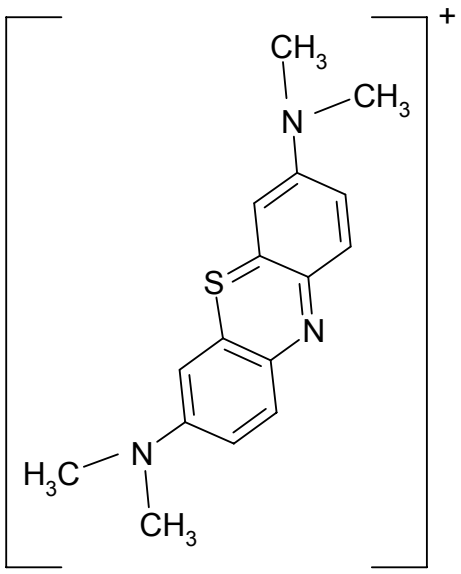


Methylene blue (cationic dye)

↓



.



Glycyrrhetinic acid **Methylene blue**

(Neutral lipophilic ion-pair complex)

The pH of aqueous phase is critical to the success of method. The pH should be usually being near or below pKa of the basic dye (Higuchi T. and Bodin J.I.1961).

1.3.3: Theory:

The relevant experimental conditions (pH of the buffer, extracting solvent and absorption maxima) for several reported compounds are given in Table 1.1.

The success of the method is based on the condition that only the complexed form of the dye is extractable. So each molecule of amine results in the complexation of one molecule of dye and this is extracted into the organic phase. Whereas concentration of dye is an indirect measure of the amount of amine. In order to ensure the non extractability of the excess uncomplexed dye, a dye is used that is a neutral weak acid and the aqueous pH is controlled at a level above the pK of the dye thus converting it to its anionic form (Higuchi T. and Bodin J.I.1961).

Among the dyes that have been commonly used are bromocresol purple, methyl orange, thymol blue, etc (acidic dyes), safranin O, methylene blue, etc (basic dyes) and organic solvents generally used for extraction of drug-dye complex from aqueous phase are chloroform, dichloromethane and benzene. The indicator dye is added in excess and the pH of the aqueous solution is adjusted if necessary to a value where both the amine and dye are in the ionized forms. The ion-pair is separated from the excess indicator by extraction into the organic solvent, and the absorbance is measured at the λ_{max}. The molar absorptivity of the ion-pairs formed between quaternary ammonium compounds and dyes are typically 0.9×10^4 to 4×10^4 in acid and base dye technique. So both the dye techniques provide more sensitive techniques for certain nitrogen containing compounds. The acid-dye technique is used officially in European Pharmacopoeia for the assay of formulations containing certain quaternary ammonium salts or amines, i.e., biperidine Lactate injection, clonidine hydrochloride injection and tablets, neostigmine methylsulphate injection, and benzhexol hydrochloride Tablets (Backett, A.H., 2002).

Some selectivity can be achieved by control of pH; for example the alkaloid berberine was determined in the presence of hydrastine and canadine by acid dye extraction (with bromocresol purple) at pH 7.2 (El-Masry, S. and Korany, M.A. 1980).

Theoretical condition which helps in choosing a rational system for a particular determination involves the knowledge of the dissociation constant of

acidic dye and the basic compound, or the basic dye and the acidic compound and the pH dependence of the partition characteristics of the two substances and their addition product between aqueous and organic solvent. Since dyes are used in excess one of the basic requirements of this technique is that unreacted dye must be wholly retained in aqueous phase at the pH employed. If the initial partition coefficient of the dye (in acid form) is high, the pH of the organic phase, pH of the buffer may not have much role. As pH has no significant influence on partition characteristics of the dye above its pT (The pH at which titration in presence of a given indicator is ended is sometimes known as its titration exponent, denoted by pT). (Alexeyev, V., 1994), it is always advisable to explore the area between pK and pT (Table 1.2.) to find out the most critical pH for a system i.e. the lowest pH at which the dye is wholly retained in the aqueous phase on shaking with an organic solvent. Most nitrogenous bases of pharmaceutical interest have pK between 4 and 8. Thus at a particular pH, the extent to which any base will exist in its free form will depend on its pKa value. The condition required is that the medium may not be too acidic so as to retain the base in the aqueous phase. It is sometimes necessary to operate at a pH somewhat higher than the pT of the indicator (dye). General guideline is that pH of the buffer chosen should be some what above the pKa of the base to be estimated and then select the dye for which this pH is optimum. The sensitivity of this method depends primarily on molar extinction coefficient of the dye in the medium in which the absorbance is measured (Sethi, P.D., 1997).

1.3.4: Basic requirements for ion-pair technique:

1. Drug and dye both are required to be soluble in the same solvent and ion-pair complex must be least soluble in aqueous solvent as compare to organic solvent used for extraction of complex.
2. Stoichiometric ion pair must be formed between positively or negatively charged nitrogenous compound and corresponding acidic and basic dye respectively.
3. This ion pair is quantitatively extractable in organic phase.

4. The unreacted dye molecules must not be extracted into the organic phase as they are insoluble in organic phase (Sethi PD; 1997).

1.3.5: Important factors:

There are three major factors that significantly influence the success of method.

a) Dye selection and its concentration
b) pH of aqueous phase
c) Solvent for extraction of ion-pair complex (Das Gupta. 1973 & Kenneth A. 1982)

1.3.6: Advantages and Limitations:

Advantages

1. Ion-pair technique increase sensitivity of drugs that poorly absorbs in UV region.
2. Method is highly specific in nature because complex between particular drug and dye only form if specific pH is provided.
3. In most cases it has been proven that, no any excipient in the dosage form interferes with complex formation. So method can be used for routine analysis. (Sethi, P.D., 1997).

Limitations:

1. Absorption maximum is not sharp and difficult to locate.
2. Reagent blanks sometimes very high due to impurities in dye.
3. Formation of complex is pH dependent and presence of other substance can affect the pH (Sethi, P.D., 1997).

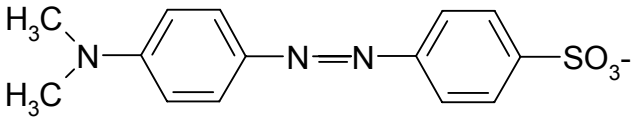
1.3.7 Chemistry of dyes used as complexing agents

1.3.7.1 Methyl orange (tropaeolin d) (Jeffery, G.H., 1989)

- **Chemical Name:**

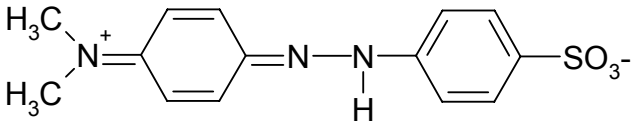
  Dimethylamino- phenyl azobenzene- sulphonic acid sodium salt.

- **Structural formula:**



(Base form, pH > 4.4, yellow)

NaOH ⇅ HCl



(Acid form, pH < 2.9, red)

- **Molecular Formula:** $C_{14}H_{14}N_3Na0_3S$

- **Molecular Weight:** 327.34

- **Appearance:**

  Orange- Yellow powder or crystalline scales.

- **Solubility:**

  Soluble in 500 parts water; more soluble in hot water, practically insoluble in alcohol.

- **Dissociation constant** (pK_{ind}): 3.7

- **pH range**

  (transition interval) and color change : 2.9 - 4.4

  Red at pH< 2.9, pH> 4.4 yellow

1.3.7.2 Safranin o (Jeffery, G.H., 1989)

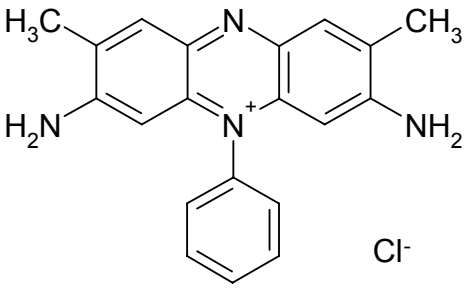
- **Chemical name:**

  3,7-diamino-2, 8-dimethyl-5 phenylphenazinium chloride

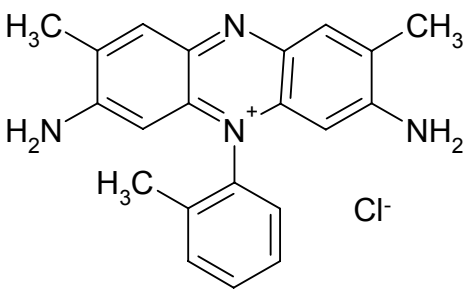
  or

  3,7-diamino-2, 8, 16-trimethyl-5 phenylphenazinium chloride

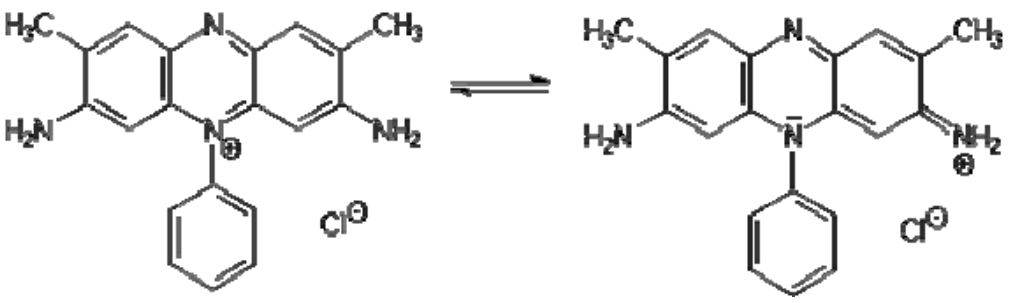
- **Structure :**



Dimethyl safranin



Trimehtyl safranin



Dimethyl Safranin

- **Molecular Formula:** $C_{20}H_{19}N_4Cl$ (dimethyl)
  $C_{21}H_{21}N_4Cl$ (trimethyl)

- **Molecular Weight:** 350.8 (dimethyl)
  364.9 (trimethyl)

- **Appearance:**
  Maroon red crystalline powder

- **Solubility:**
  5.45% in Water; 3.5% in Alcohol; 3.5% in Glycol and 0.0% in xylene

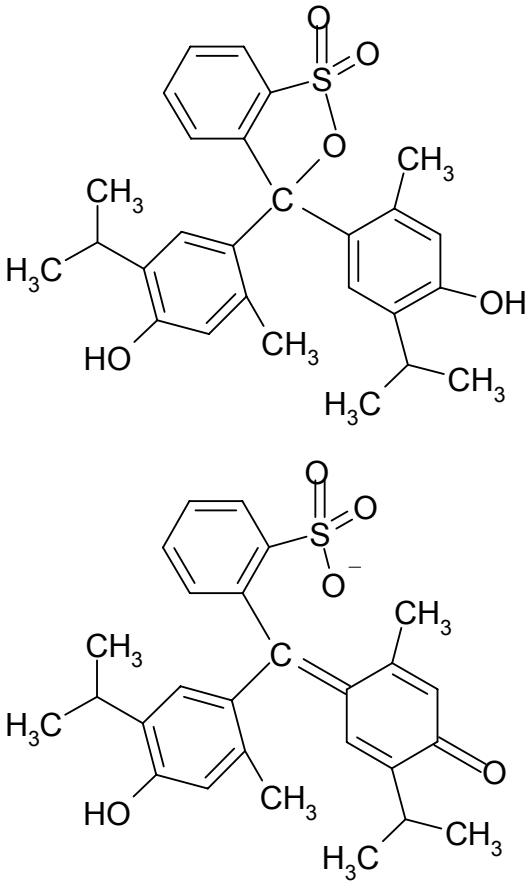
- **Dissociation constant** (pK_{ind}): 7.9

- **pH range:** 3.2 – 5.6

1.3.7.3 Thymol blue (Jeffery, G.H. *et.al*., 1989)

- **Chemical Name:** Thymolsulphophthalein

- **Structural formula:**



(Acid form, pH < 1.2, red) (Base form, pH > 2.8, yellow)

- **Molecular Formula:** $C_{27}H_{30}O_5$
- **Molecular Weight :** 466.60
- **Appearance:**

  Brownish-Green crystalline powder; characteristic odor

- **Solubility:**

Insoluble in water, soluble in alcohol and dilute alkali solutions.

- **Dissociation constant** (pK_{ind}): 1.7

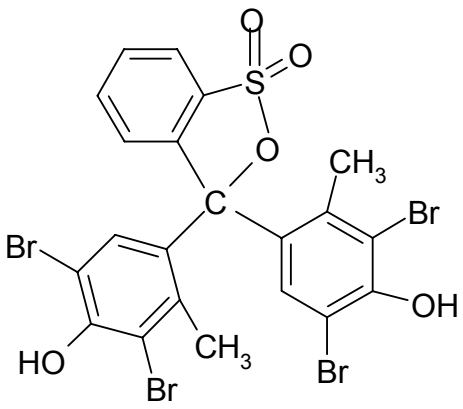
- **pH range**

(transition interval) and color change: 1.2 - 2.8
Red at pH< 1.2, pH> 2.8 yellow

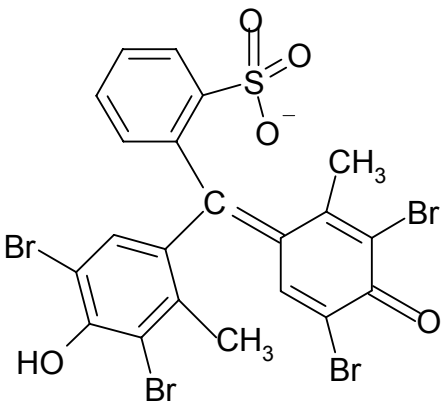
1.3.7.4 Bromo cresol green (Jeffery, G.H. *et.al*., 1989)

- **Chemical Name:** Tetrabromo-m-cresol sulphophthalein

- **Structural formula:**



(Acid form, pH < 3.8, yellow)



(Base form, pH > 5.4, blue-green)

- **Molecular Formula :** $C_{21}H_{14}Br_4O_5S$

- **Molecular Weight:** 698.02

- **Appearance:**

Slightly yellow crystals from acetic acid

- **Solubility:**

Sparingly soluble in water, readily soluble in alcohol, ether, ethyl acetate, fairly soluble in benzene

- **Dissociation constant** (pK_{ind}): 4.7

- **pH range**

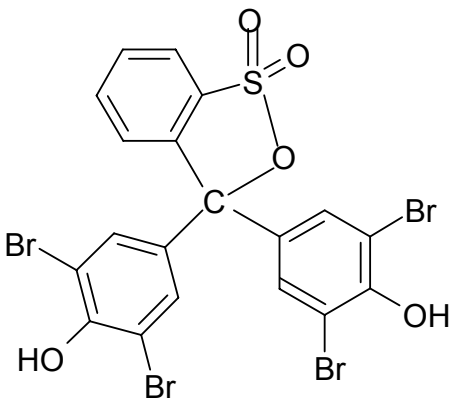
(transition interval) and color change : 3.8 - 5.4
Yellow at pH< 3.8, pH> 5.4 Blue-Green

1.3.7.5 Bromophenol blue (Jeffery, G.H. *et.al*., 1989)

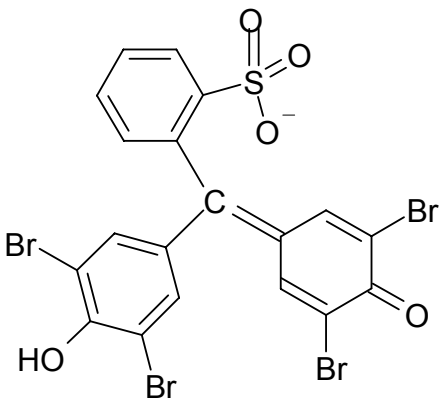
- **Chemical Name:**

Tetrabromophenol sulphophthalein

- **Structural formula:**



(Acid form, pH < 3.0, yellow)



(Base form, pH > 4.6, purple)

- **Molecular Formula:** $C_{19}H_{10}Br_4O_5S$

- **Molecular Weight:** 669.97
- **Appearance:**

  Orange- Yellow powder or crystalline scales.

- **Solubility:**

  Soluble in water (about 0.4 gm/ 100ml), more soluble in methyl alcohol, ethyl alcohol and benzene. Freely soluble in NaOH solutions with the formation of a water-soluble sodium salt

- **Dissociation constant** (pK_{ind}): 4.1

- **pH range**

  (transition interval) and color change : 3.0 - 4.6
  Yellow at pH< 3.0, pH> 4.6 purple

Table 1.1. pH of the buffer, extracting solvent and λ_{max} for analysis of several nitrogenous drugs

Sr. No	Drug	Dye	pH (Buffer)	Extracting Solvent	λ max (nm)	Reference
1	Fluoxetine HCl	MO TB	4.0 8.0	Chloroform	433 410	Prabhakar A.H. *et al.*1999
2	Trazodone HCl	BPB	3.4	Chloroform	414	El-Gindy A. *et al.,*, 2001
3	Chlorpromazine	MO	6.0	Chloroform	424	Zhou Z. *et al.,*, 1996
4	Strychnine and brucine	MO	5.0	Chloroform	424	El-Masry S. *et al.* 1978

5	Meclizine HCl	MO	2.8 (Mc Il vaine)	Chloroform	440	Hom FS. *et al.,*, 1977
6	Ipeca alkaloids	MO	5.0	Chloroform	460 283	Saleh MR. *et al.,*, 1979
7	3-dimethyl amino methyl khellin	MO	5.0	Chloroform	420	Abu-Shady H.,1978
8	Protein	BPB	1.0	Chloroform	595	Lin LY. et. al., 1989
9	Enrofloxacin Pefloxacin	BPB MO	2.3-2.5 3.6	Chloroform Chloroform	420 424	Mostafa S. *et al.,*, 2002
10	Sulfur dioxide	TB I_2 + TB	2.4 3.0	Chloroform Chloroform	545 430	Gayathri N. *et al.,*, 2001
11	Metho trimeprazine	BPB	3.0	Chloroform	409	Kedor-Hackmann ER. *et al.* 2000
12	Tilidine	BCG BPB	3.5	Chloroform	415 411	Dobrila ZS. *et al.,*, 1990
13	Ceterizine HCl	BCP BPB	2.64 Walpole	Chloroform	409 414	Gowda BG. *et al.,*, 2001
14	pH of fresh water	BCP PR	4.5-8.5	Chloroform	432,589 433,558	Yao w. *et al.,*, 2001
15	Guanidino drugs	BCP	3.8	Chloroform	415	Wahbi AA. *et al.,*, 1993
16	Urinary albumin	BPB	5.0	Chloroform	610	Schosinsky KH. *et al.,*, 1987
17	Pilocarpine	BCP	6.0	Chloroform	580	El-Masry S. *et al.* 1980

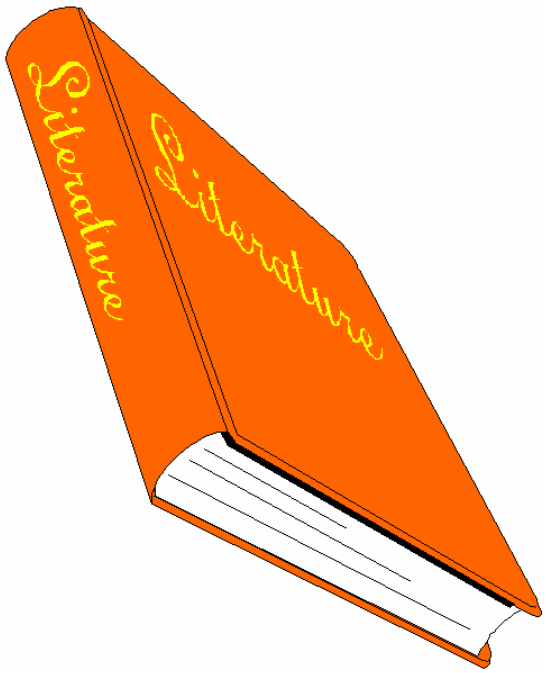
18	Sparfloxacin	BTB	3.5	Chloroform	385	Marona HR. *et al.,*, 2001
19	Bismuth	MTB	5.5	Chloroform	548	Themellis DG. *et al.,*, 2001
20	Ioperamide HCl	TB BPB NBB	2.7 2.8 6.0	Chloroform	414 415 627	El-sherif ZA. *et al.,* 2000
21	Clonidine	BCG	3.0	Chloroform	415	Zivanov-Stakic D., *et al.*
22	Methenamine	BCG	5.0	Chloroform	412	Strom JG. *et al.,*, 1986
23	Promethazine HCl	BCG	2.7-2.8	Chloroform	415	Emami Khoi AA., 1983
24	Amino qunioline	BPB	1.5	Chloroform	415-420	El-Ashry SM. *et al.,*, 1994
25	Piperazine HCl	BPB	1.5	Chloroform	410	Abdel-Gawad FM., 1997
26	Total steroid Bases in solanum species	MO	4.7	Chloroform	420	Jan Birner, 1969
27	Hydrastis Alkloids	BCP	7.2	Chloroform	590	Savsan El-Masry, *et al*., 1980
28	Berberine	BCP	5.6	Chloroform	580	
29	Hyoscyamine	BCP	6.6	Chloroform	420	Savsan El-Masry, Saleh A.H.K. 1973

Table 1.2. pK, pT, Appropriate extracting solvent and pH of buffer for several dyes

Dye	pK	pT	pH	Organic Solvent
Bromocresol purple	6.3	6.8	5.4	Benzene
Bromothymol blue	7.0	7.6	5.0	Toluene
Bromocresol green	4.7	5.4	6.6	Chloroform
Bromo phenol blue	4.0	4.6	5.6	Chloroform
Bromochlorophenol blue	4.2	4.8	4.0	Benzene
Chlorophenol red	6.0	6.6	6.5	Benzene
Methyl orange	3.8	4.4	5.0	Ethylene dichloride, chloroform, benzene

2.

REVIEW OF LITERATURE…



2. LITERATURE REVIEW

2.1 Antidepressants

Numerous analytical methods have been designed and developed for the determination of Fluoxetine, sertraline and Duloxetine in pharmaceutical dosage form and in biological fluids [Eap CB et al; 1996] are reviewed as follows:

2.1.1 Fluoxetine hydrochloride

Thin Layer Chromatography

A thin layer chromatographic technique can be used to determine the identity and purity of Fluoxetine hydrochloride. Precoated 20 x 20 cm silica gel 60F254 TLC plates are used in combination with binary solvent system consisting of 90% methanol and 10% concentrated ammonium hydroxide. Visualization is performed by exposing the plate to iodine vapours prior to viewing under short UV light (254 nm) [Donald SR et al; 1990].

A simple, rapid and accurate thin layer chromatographic method has been developed for determination of Fluoxetine and Paroxetine in dosage forms. The drugs were chromatographed on silica gel 60 F 254 plates in horizontal chambers with benzene-acetone-ethanol-25% aqueous ammonia [9+7+2+1 v/v] as a mobile phase. Densitometric detection was performed at 218 nm and 293 nm for Fluoxetine and Paroxetine, respectively, video densitometric detection was performed at 254 nm for both drugs. The range of linearity was 2-10 µg per spot for Fluoxetine and 0.5 – 8 µg per spot for Paroxetine [Robert S, et al; 2003]

Table 2.1. Estimation of Fluoxetine hydrochloride by Thin Layer Chromatography

Sr. No.	Specifications	Reference
1	The identity and purity of Fluoxetine was determined by Thin Layer Chromatography Method	Donald SR et al; 1990
2	Determination of Fluoxetine and Paroxetine in pharmaceutical formulations by densitometric and videodensitometric Thin Layer Chromatography method	Robert S et al; 2003

High Performance Liquid Chromatography

A High Performance Liquid Chromatographic method with fluorescence detector has been developed for the determination of Fluoxetine and nor Fluoxetine in human plasma. Sample was used after liq – liq extraction on a C_8 column with a mixture of perchlorate buffer and acetonitrile as mobile phase. Fluoxetine, nor Fluoxetine and internal standard (Paroxetine) were eluted in less than 9 minutes. Response for both analytes was linearly obtained in a range 2.5 – 500 ng/ml [Raggi MA et al; 1999].

Analysis by reversed phase liquid chromatography on a xterra MA C_{18} column using a fast gradient. Fluoxetine, norFluoxetine and fluvoxamine (internal standard) were ionized using the turbolon spray interface operating in positive ion mode. The method is linear over the range 0.5 – 50 µg/ml [Green R et al; 2002].

Simultaneous determination of Fluoxetine, Citalopram, Paroxetine, Venlafaxine in plasma by high performance liquid chromatography. The separation of the analytes was performed on a macherey – nagel C_{18} column, using water (Formic acid 0.6%, ammonium acetate 30 mmol/L)-acetonitrile

[35:65 v/v] as a mobile phase, with a flow rate of 0.85 ml/min. The compounds were ionized in the electrospray ionization mass spectrometry. The calibration curves were linear in the range of 5.0 – 1000 ng/ml for all compounds. [He J. et al; 2005]

A rapid and sensitive high performance liquid chromatographic method has been developed for determination of Fluoxetine and nor Fluoxetine in human plasma using Paroxetine as internal standard. The compounds were separated on a C_{18} column using acetonitrile-40 mM potassium di hydrogen phosphate buffer (pH 2.3) in the ratio of 31:69 (v/v) as the mobile phase. The quantification of both were made by fluorescence detector. The assay for each analyte was linear over the range of 1 – 39 and 0.9 – 36 µg/ml respectively. [Laurian V. et al; 2005].

The High Performance Liquid Chromatographic method is described for determination of Fluoxetine and nor Fluoxetine in plasma via reversed phase column and UV detector. The limit of quantification was 14 nmol/L for both compounds [Ilerena A. et al; 2003].

Selective liquid chromatographic method for determination of Fluoxetine in plasma was performed on novapak C_{18} column with an isocratic mobile phase consisting of phosphate buffer-acetonitrile – methanol – triethyl amine (58:30:10:2 v/v) adjusted to pH 7 using UV detector. The calibration curve was linear over the concentration range of 10 – 200 µg/ml. [Afshin Z et al; 2001]

Separation of Fluoxetine enantiomers on five chiral stationary phase [chiralcel OD-H, chiralcel OJ-H, chiralpak AD-H, cyclobond I 2000 DM and kromasil CHI-TBB] was investigated using hexane-isopropanol-diethyl amine as proportion of mobile phase. [Jie Z et al; 2007]

A simple, accurate and sensitive high performance liquid chromatographic technique was described for the determination of Fluoxetine in the capsule dosage form, human plasma and biological fluid. The

determination was performed with a reversed phase C_{18} column with UV detector. The isocratic mobile phase consists of acetonitrile and triethylamine buffer (48:52 v/v). The calibration curve was linear over the concentration range of 10-300 µg/L. [Mohamed AE et al; 2002]

A sensitive and selective liquid chromatographic – tandem mass spectrometric method was developed and validated for Paroxetine and Fluoxetine on a C_{18} analytical column. The retention time was 1.6 and 1.7 for Paroxetine and Fluoxetine respectively. [Massaroti P et al; 2005]

An official method for determination of Fluoxetine hydrochloride capsule by liquid chromatographic separation followed by UV detection was mention in British Pharmacopoeia – 2000 and United State Pharmacopoeia, XXIV edition – 2000

A reversed phase high performance liquid chromatographic method has been developed to determine Fluoxetine in pharmaceutical dosage forms. The mobile phase consists of acetonitrile-water-triethylamine (50:49:1 v/v). Water and triethylamine adusted to pH 6 with phosphoric acid, using DuPont Zorbax RX column. The flow rate is 1.0 ml/min and UV detection is at 260 nm. The method is linear over the range of 25-800 µg/ml and it is also applicable for determination of drugs and its active metabolite from serum. [Orsulak PJ et al; 1988]

Table 2.2. Estimation of Fluoxetine hydrochloride by Liquid Chromatography

Sr. No.	Specification	Reference
01	Determination of Fluoxetine and norFluoxetine in human plasma using fluorescene detector. Linearily: 25 – 500 ng/ml	Raggi MA et al; 1999

02	Determination of Fluoxetine and norFluoxetine in human plasma by liquid spectrometry-tandem mass spectrometry. Linearity : 0.5 – 50 µg/ml	Green R et al; 2002
03	Simultaneous determination of Fluoxetine, Citalopram, Paroxetine, Venlafaxine in plasma by High Performance Liquid Chromatography – electrospray ionization mass spectrometry. Linearity for all : 5.0 – 1000 ng/ml	He J. et al; 2005
04	Determination of Fluoxetine and nor Fluoxetine in human plasma by High Performance Liquid Chromatography – fluorescene detector. Linearity for Fluoxetine : 1.0 – 39.0 µg/ml, nor Fluoxetine : 0.9 – 36 µg/ml	Laurian V. et al; 2005
05	Determination of Fluoxetine and nor Fluoxetine in plasma by High Performance Liquid Chromatography, using UV detector. The limit of quantification was 14 nmol/L	Ilerena Adrian et al; 2003
06	Selective liquid chromatographic method was developed using UV detector for Fluoxetine in plasma. Linearity : 10 – 200 µg/ml	Afshin Z et al; 2001
07	Comparision of performance of chiral stationary phase for separation of Fluoxetine enantiomers.	Jie Z et al: 2007
08	Sensitive HPLC technique is described for determination of Fluoxetine in capsule dosage form and in plasma using UV detector. Linearity : 10 – 300 µg/L	Mohamed AE et al; 2002
09	Determination of Paroxetine and Fluoxetine by liquid chromatographic – tandem mass spectrophotometric method was developed and validated.	Massaroti P et al; 2005

10	Determination of Fluoxetine hydrochloride capsule by High Performance Liquid Chromatography using UV detector.	British Pharmacopoeia – 2000 Page No. 1961-1962
11	Determination of Fluoxetine hydrochloride capsule by liquid chromatographic method using UV detector.	United State Pharmacopoeia, XXIV, edition-2000 page no. 739-740
12	Determination of Fluoxetine in pharmaceutical dosage form and serum by reversed phase High Performance Liquid Chromatography using UV detector.	Orsulak PJ et al; 1988

Gas Chromatography

Due to the reasonable thermal stability and the volatility of Fluoxetine, a gas chromatographic technique has been used to determine Fluoxetine.

A gas chromatographic method has been developed for determination of Fluoxetine in dosage forms, plasma and urine by using an HP Model 5713 GC with electron capture detector. [Nash JF et al; 1982; and Lopez C et al; 1989]

An electron capture gas chromatographic procedure was developed for the simultaneous analysis of the enantiomers of Fluoxetine and norFluoxetine. The assay involves basic extraction of these enantiomers from biological sample, followed by their conversion to diastereomers using the chiral derivatizing reagent (S)-(-)-N-trifluoroacetylpropyl chloride. [Torok-Both GA et al; 1992]

A simple and fast capillary gas chromatographic method using flame ionization detector was developed column head pressure (80 Kpa), injector

and detector temp (260 ^{0}C and 250 ^{0}C), time and temperature for the splitless step (0.75 min. and 60 ^{0}C), size and sample (2 µL) and oven temperature program, providing analysis time shorter than 10 min., recoveries between 97.5 to 102.5 %. [Berzas Nevado JJ et al; 2000]

Table 2.3. Estimation of Fluoxetine hydrochloride by Gas Chromatography

Sr. No.	Specifications	Reference
01	Determination of Fluoxetine in dosage form, plasma and urine by gas chromatographic method using electron capture detector.	Nash JF et al; 1982 and Lopes C et al; 1989
02	Simultaneous determination of Fluoxetine and norFluoxetine enantiomers in biological sample by gas chromatographic method using electron capture detector.	Torok-Both GA et al; 1992
03	Determination of Fluoxetine, fluvoxamine and clomipramine in pharmaceutical dosage form by capillary gas chromatography using flame ionization detector.	Berzas Nevado JJ et al; 2000

Capillary Electrophoresis

A simple, rapid and sensitive procedure using non aqueous capillary electrophoresis to measure Fluoxetine and active metabolite norFluoxetine has been developed and validated optimum separation of Fluoxetine and norFluoxetine by measuring at 230 nm was obtained on a 60 cm X 75 µm capillary using a non aqueous solution system of 7:3 methanol – acetonitrile containing 15 mM ammonium acetate. Detection limits of 10 µg/ml were obtained. [Rogriguez FJ et al; 2005]

A simple and rapid method for determination of Fluoxetine in pharmaceutical formulation by capillary electrophoresis method has been developed. A different capillary was used; the high sensitivity cell and the cyclodextrins in the BGE were not necessary. The detection wavelength was changed from 195 to 205 nm to obtain less noisy base line. Good linearity was found in the concentration range 0.25 – 50 µg/ml. [Mandrioli R et al; 2002]

Table 2.4. Estimation of Fluoxetine hydrochloride by Capillary Electrophoresis

Sr. No.	Specifications	Reference
01	Determination of Fluoxetine and nor Fluoxetine by non aqueous capillary electrophoresis in human urine.	Rogriguez FJ et al; 2005
02	Determination of Fluoxetine in pharmaceutical formulation by capillary electrophoresis method.	Mandrioli R et al; 2002

Voltametric Method

The determination of Fluoxetine in pharmaceutical formulation using adsorptive square wave cathodic stripping voltametric method was developed. Linear calibration graphs were obtained in the range 0.52 – 5.2 µM. [Roque DS et al; 1999]

The oxidative behavior of Fluoxetine was studied at a glassy carbon electrode in various buffer systems and at different pH using differential pulse and square wave voltammetry. A new square wave voltametric method was developed for determination of Fluoxetine in pharmaceutical formulations using borate (pH 9) buffer solution as supporting electrolyte. Under the optimized conditions, a linear response was obtained in the range 10 to 16 µM with a detection limit of 1.0 µM. [Rui PL et al; 2006]

Electroanalytical methods based on square wave adsorptive-stripping voltammetry and flow injection analysis was developed for Fluoxetine. The method was based on the reduction of Fluoxetine at a mercury drop electrode at 1.2 V versus Ag/AgCl, in a phosphate buffer at pH 12. This method was successfully applied in the quantification of Fluoxetine in pharmaceutical formulation, serum and in drug dissolution studies. The presence of dissolved oxygen did not interfere significantly with the analysis. [Henri PA et al; 2007]

Table 2.5 Estimation of Fluoxetine hydrochloride by Voltammetry

Sr. No.	Specifications	Reference
01	Determination of Fluoxetine in pharmaceutical formulation using square wave adsorptive cathodic stripping voltametry.	Roque DS et al; 1999
02	Voltametric quantification of Fluoxetine in pharmaceutical formulation using differential pulse and square wave voltametry.	Rui PL et al; 2006
03	Electroanalytical method based on square wave adsorptive stripping voltametry and flow injection analysis for Fluoxetine.	Harri PA et al; 2007

Spectrophotometric method

Spectrophotometric technique was developed for determination of Fluoxetine and sertraline in bulk and pharmaceutical formulations. The methods are based mainly on charge transfer complexation reaction of drugs with either π acceptor [Chloranil and 2,3-dichloro-5,6-dicyanobenzoquinone] or σ acceptor [Iodine]. The colour produced is quantified spectrophotometrically at 550, 450 and 263 nm for Fluoxetine and 450, 455 and 290 nm for sertraline respectively. The method determined the cited drugs in concentration ranges of 80 – 640, 16 – 112 and 7.5 – 60 µg/ml with

mean percentage recoveries of 99.83, 99.76 and 100 %. [Bebawy LI et al; 1999]

A rapid, sensitive and economical spectrophotometric method was developed for the determination of Fluoxetine in bulk and its dosage form. The method was based on the formation of yellow ion pair complex due to the action of methyl orange and thymol blue in acidic (pH 4) and basic (pH 8) medium respectively under optimum condition it shows an absorption maxima at 433 nm (MO) and 410 nm (TB). In both cases Beer's law is obeyed at 1 – 20 µg/ml with MO and 4 – 24 µg/ml with TB. [Prabhakar AH et at; 1999]

A rapid, simple and accurate spectrophotometric method was presented for the determination of Fluoxetine by batch and flow injection method. The method is based on complexation of drug with phenolphthalein-β-cyclodextrin (PHP-β-CD) inclusion complex and measured at 554 nm. The linearity was observed between 7.0×10^{-6} – 24×10^{-4} mol/L. [Abbas A et al; 2006]

Fluvoxamine and Fluoxetine react with eriochrom cyanine R to form pink-ion association compound. These compounds are insoluble in water but fairly soluble in organic solvents. Addition of methyl cellulose affects the solubility of compounds and increase the absorbance of the solution. It gives good linearity in the concentration ranges 0.6-15 and 0.3-5 µg/ml respectively. [Barbara S.; 2001]

Simple and sensitive spectrophotometric methods were developed and validated for the determination of Fluoxetine, sertraline and Paroxetine in their pharmaceutical dosage forms. These methods were based on the reaction of the N-alkylvinylamine formed from the interaction of the free secondary amino group in the drugs and acetaldehyde with each of three haloquinone, i.e. chloranil, bromanil and 2,3-dichloronaphtho quinone, to give coloured vinyl amino substituted quinone, which measured at 665, 655 and 580 nm respectively. The linearity was observed in the range 4 – 120 µg/ml. [Ibrahim AD; 2005]

A new simple and sensitive spectrophotometric method was developed for determination of Fluoxetine in bulk and its dosage form in UV region and measured at 225 nm. The Beer's Law obeyed in the concentration range of 2.5 – 25 µg/ml. [Sujatha K et al; 2004]

A simple, fast and precise extractive spectrophotometric method was developed for the determination of Fluoxetine in pharmaceutical formulations. The method is based on formation of coloured complex with bromothymol blue and measured at 412 nm. The method obeyed Beer's Law in the range of 1.5 – 20 µg/ml. [Naik MT et al; 1999]

Spectrophotometric method for the determination of Fluoxetine in bulk and dosage form was developed. The method is based on the formation of yellow ion-pair complex with methyl orange in phthalate buffer pH 4.0 and thymol blue in phosphate buffer pH 8.0. [Anandkumari HP et al; 1999]

Table 2.6. Estimation of Fluoxetine hydrochloride by Spectrophotometry

Sr No.	Specifications	Reference
01	Spectrophotometric determination of Fluoxetine and sertraline using chloranil, 2,3-dichloro-5,6-dicyanobenzoquinone and iodine.	Bebawy LI et al; 1999
02	Spectrophotometric determination of Fluoxetine in bulk and pharmaceutical formulation using methyl orange and thymol blue.	Prabhakar AH et al; 1999
03	Spectrophotometric determination of Fluoxetine by batch and flow injection method using PHP-β-CD inclusion complex.	Abbas A et al; 2006
04	Spectrophotometric determination of fluvoxamine and Fluoxetine using eriochrom cyanine R to form pink-ion association compound.	Barbara S.; 2001

05	Development and validation of spectrophotometric methods for determination of Fluoxetine, sertraline and Paroxetine using various haloquinone.	Ibrahim AD; 2005
06	Spectrophotometric method for determination of Fluoxetine in UV region.	Sujatha K et at; 2004
07	Spectrophotometric method for determination of Fluoxetine by formation of coloured complex with bromothymol blue.	Naik MT et al; 1999
08	Spectrophotometric determination of Fluoxetine using methyl orange and thymol blue to form ion pair complex.	Anandkumari HP et al; 1999

Spectrofluorimetric method

A simple, rapid and sensitive method for determination of Fluoxetine in pharmaceutical dosage form has been developed. Fluorescene emission intensity was measured at 293 nm, while exciting at 230 nm. The linearity was observed in the concentration range 0.25 – 5.0 µg/ml. [Mandrioli R et al; 2002]

Table 2.7. Estimation of Fluoxetine hydrochloride by Spectrofluorimetry

Sr No.	Specifications	Reference
01	Spectrofluorimetric method for determination of Fluoxetine in bulk and pharmaceutical dosage form.	Mandrioli R et al; 2002

2.1.2 Sertraline hydrochloride

High Performance Liquid Chromatography

A sensitive stereo specific high performance liquid chromatographic method for the determination of Sertraline in bulk, tablets and capsules was developed. The composition of mobile phase was aqueous 170 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid) containing 18 mM hydroxypropyl-β-cyclodextrine-acetonitrile (68:32 v/v) at a flow rate of 1.0 ml/min. The UV detection was set at 225 nm. Calibration curve was linear in the range of 1 – 120 µg/ml. [Chen D et al; 2004]

Micellar electrokinetic chromatographic method has been developed for separation of cis-trans isomers and enantiomers of sertraline. An optimum separation was achieved using a buffer (pH 11.5) of 35 mM sodium borate containing 30 mM sodium deoxycholate and 20 mM hydroxy-β-cyclodextrine. The measurement was carried out at 210 nm by UV detector. This method was successfully applied for the detection of sertraline in bulk and various dosage forms. [Deving C et al; 2004]

A high performance liquid chromatographic method was developed and validated for the determination of sertraline & nefazodone in bulk and pharmaceutical formulations. The samples were chromatographed on a supercosil RP-18 column using methanol-phosphate buffer pH 4.5 (20:80 v/v) & methanol-acetonitrile-phosphate buffer at pH 5.5 (10:50:40 v/v) respectively measured at 270 nm (sertraline) and 265 nm (nefazodone). [Nevin ERK; 2003]

A fully automated turbulent flow liquid chromatography – tandem mass spectrometry technique was developed for monitoring 13 different antidepressants in human serum. The calibration curves for all antidepressant were found in the range 10 to 500 ng/ml. [Sauvage FL et al; 2006]

A sensitive, simple and rapid method was developed for determination of paroxatine, sertraline and fluvoxamine using LC/MS and LC/MS/MS, where dextro methorphan was used as internal standard. The samples of biological fluid were mixed with formic acid and directly subjected to LC/MS. The calibration curves were linear in the range 5 – 80 ng/ml. [Hattori H et al; 2005]

A simple and sensitive method was developed for the determination of sertraline in bulk and formulation using isocratic reversed phase high performance liquid chromatography. Wales Nova Pak C_{18} column and acetonitrite-methanol-0.05 M acetic acid and 0.02 M triethylamine buffer (45:15:40 v/v) mobile phase was used. The UV detection was made at 254 nm. [Bruce MJ et al; 1996]

High performance liquid chromatographic method was developed to measure sertraline and its metabolites in biological fluid using radiolabeled drug and radioactivity detector and /or a variable wavelength UV detector. Waters C_{18} µBondapak column and acetonitrile-50 mM sodium phosphate (pH 4.5)-acetonitrile-50 mM ammonium acetate (pH 5.0) was used as the mobile phase. [Tremain LM et al; 1989]

Estimation of high performance liquid chromatographic technique has been reported for measuring sertraline and desmethyl sertraline in mouse cerebral cortex using versapack C_{18} column and acetonitrile-0.25 potassium phosphate (pH 2.7) 30:70 v/v mobile phase. The detection was carried out at 235 nm in UV detector. [Weiner HL et al; 1990]

Determination of sertraline in bulk and pharmaceutical formulation using high performance liquid chromatography technique has officially been reported.

The method of determination of sertraline and desmethyl sertraline in human serum was developed. A new solid phase extraction method employing the dual functionality clean screen cartidge followed by reverse

phase liquid chromatographic analysis. The response was linear over the concentration range 0.01 – 25 mg/l for both. [Rogowsky D et al; 1994]

A new rapid and sensitive high performance liquid chromatographic method has been developed for determination of 11 most commonly prescribed antidepressants [Fluoxetine, sertraline, Paroxetine, citalopram, fluvoxamine, meclobemide, mirtazapine, milnacipram, toloxatone, venlafaxine, viloxazine] and two active metabolite norFluoxetine and desmethyl venlafaxine. It involves liquid – liquid extraction followed by liquid chromatography coupled to photodiode array UV detection. [Duvemeuil et al; 2003]

Sertraline hydrochloride was determined by high performance liquid chromatography – UV detection technique at 270 nm. The sample was chromatographed on a supercosil RP – 18 columns. The mobile phase were methanol:phosphate buffer pH 4.5. [Erk N; 2003]

Table 2.8. Estimation of Sertraline hydrochloride by High Performance Liquid Chromatography

Sr. No.	Specification	Reference
01	Determination of sertraline in bulk and formulation by high performance liquid chromatography with UV detector.	Chen D et al; 2004
02	Optimized separation of Cis-trans isomers and enantiomers of sertraline by micellar electrokinetic chromatography.	Deving C et al; 2004
03	Determination of sertraline and nefazodone in pharmaceutical formulations by high performance liquid chromatography with UV detector.	Nevin ERK; 2003
04	Liquid chromatography-tandem mass spectrophotometric technique is developed for monitoring antidepressant in human serum.	Sauvage FL at at; 2006

05	Rapid, sensitive and simple determination of selective serotonin inhibitor in human serum by liquid chromatography-MS and LC/MS/MS.	Hattori H et al; 2005
06	Determination of sertraline in bulk and formulation using isocratic reverse phase high performance liquid chromatography – UV detection system.	Bruce MJ et al; 1996
07	Determination of sertraline in biological fluid using High Performance Liquid Chromatography variable wavelength UV detector.	Tremain LM et al; 1989
08	Determination of sertraline in mouse cerebral cortex using high performance liquid chromatography – UV detector.	Weiner HL et al; 1990
09	Determination of sertraline in pharmaceutical dosage form using high performance liquid chromatography – UV detection technique.	B. P. – 2000 & USP – XXIV 2000.
10	Determination of sertraline and desmethyl sertraline from human serum using reverse phase liquid chromatography.	Rogowsky D et al; 1994
11	A high performance liquid chromatographic – photodiode array UV detection for therapeutic drug monitoring of 11 most commonly prescribed antidepressants in human plasma.	Duvemeuil et al; 2003
12	Sertraline was determined by high performance liquid chromatography – UV detection method.	Erk N.; 2003

Gas Chromatography

Various analytical methods using gas chromatographic technique has been developed. The determination of sertraline in biological fluid in which sertraline is extracted from plasma and derivatized with trifluoroaceticanhydride. The derivative is chromatographed using either a mass spectrometric detector [Fouda HG et al; 1987] or a 63 Ni electron capture detector. [Tremaine LM et al; 1989]

The determination of sertraline and desmethyl sertraline was developed using gas chromatographic method and mass spectrometric detector. [Rogowsky D et al; 1994]

A gas chromatographic method was developed for estimation of plasma concentration of citalopram, Paroxetine, sertraline and their pharmacologically active N-desmethyl metabolites using mass spectrometric detector. [Eap CB et al; 1998]

A rapid and sensitive gas chromatography-mass spectrometry, with the selected ion monitoring mode for the determination of sertraline in human serum was developed. The linear response was obtained in the range of 0.2 – 10.0 ng/ml. [Kim KM et al; 2002]

Rapid and sensitive determination of sertraline in human plasma was developed using gas chromatography-mass spectrophotomeric method. [Mee KK et al; 2002]

Table 2.9. Estimation of Sertraline hydrochloride by Gas Chromatography

Sr. No.	Specification	Reference
01	Determination of sertraline in biological fluid by Gas Chromatography using mass spectrometric detector or electron capture detector.	Fouda HG et al; 1987 & Tremaine LM et al; 1989
02	Determination of sertraline and desmethyl sertraline by gas chromatographic – mass spectrometric system.	Rogowsky D et al; 1994
03	Simultaneous determination of human plasma levels of citalopram, Paroxetine, sertraline and their metabolite by gas chromatography – mass spectrometry.	Eap CB et al; 1998

04	Rapid and sensitive determination of sertraline in human plasma using GC – MS.	Kim KM et al; 2002
05	Rapid and sensitive determination of sertraline in human plasma using GC – MS.	Mee KK et al; 2002

Spectrophotometric method

Rapid and simple method for quantitative analysis of sertraline hydrochloride was carried out with first derivative spectrophotometry and measured its first derivative signal at 271.6 – 275.5 nm. The linear response was observed in the range of 8.0 – 46 µg/ml. [Erk N.; 2003]

Spectrophotometric method was developed and validated for determination of sertraline, Fluoxetine and Paroxetine in pharmaceutical dosage forms. The method was based on reaction of the N-alkylvinyl amine [Formed by interaction of drugs and aldehyde] with haloquinone (chloranil, bromanil and 2,3-dichloro naphthoquinone) to give coloured vinyl amino substituted quinones and measured at 665, 655 and 580 nm with chloranil, bromanil and 2,3-dichloronaphthoquinone respectively and observed good linearity. [Darwish IA; 2005]

A spectrophotometric procedure was reported for determination of Fluoxetine and sertraline. The method is based on charge transfer complexation reaction of drugs with either π acceptor (chloranil and 2,3-dichloro-5,6 dicyano benzoquinone] or σ acceptor (Iodine). The colour produced was measured at 450, 455 and 290 nm for sertraline with chloranil, DDQ and iodine respectively. [Bebawy LI et al; 1999]

A simple and accurate first derivative spectrophotometric method was developed for nafazodone and sertraline in pharmaceutical dosage form. The measurement was carried out of their first derivative signals at 241.8 – 256.7 nm and 271.6 – 275.5 nm (Peak to peak amplitude) respectively. The

calibration graphs were established for 10 – 42.0 µg/ml and 8 – 46 µg/ml respectively. [Nevin ERK; 2003]

A simple and reproducible spectrophotometric method has been developed for determination of sertraline, Fluoxetine and venlafaxine in pharmaceutical formulation. The method is based on reaction of drugs and ion pair agents (bromothymol blue, bromocresol green or bromophenol blue) to produce yellow coloured ion pair complexes in acidic buffers and extracted in chloroform and measured at optimum wavelength. Beer's Law was obeyed within the concentration range from 1 – 15 µg/ml. [Armagan D et al; 2006]

Spectrophotometric determination of sertraline in bulk and pharmaceutical dosage form was developed. Solution of drug was prepared in methanolic hydrochloric acid and was measured at 275 nm. [Bruce MJ et al; 1996]

Table 2.10. Estimation of Sertraline hydrochloride by Spectrophotometry

Sr. No.	Specification	Reference
01	Rapid and simple spectrophotometric method was developed for determination of sertraline hydrochloride.	Erk N.; 2003
02	Development and validation of spectrophotometic method for determination of sertraline, Fluoxetine and Paroxetine in pharmaceutical dosage form.	Darwish IA; 2005
03	Spectrophotometric determination of Fluoxetine and sertraline using chloranil, 2,3-dichloro-5,6-dicyano benzoquinone and iodine.	Bebawy LI et al; 1999
04	Determination of nafazodone and sertraline in pharmaceutical formulation using first derivative spectrophotometic method.	Nevin ERK; 2002

05	Spectrophotometric determination of certain antidepressants using ion pair reagents to form ion pair complexes.	Armagan D et al; 2006
06	U. V. spectrophotometric determination of sertraline hydrochloride was carried out in methanolic hydrochloric acid solution.	Bruce MJ et al; 1996

Voltametric method

An electro analytical method for determination of sertraline was developed. A flow injection wave cathodic stripping voltametric method has been used for determination of sertraline in bulk and pharmaceutical dosage forms. The method shows linearity between peak current intensity and sertraline concentration. [Henri PA et al; 2005]

Table 2.11. Estimation of Sertraline hydrochloride by Voltammetry

Sr. No.	Specification	Reference
01	Determination of sertraline in bulk and pharmaceutical dosage forms using a flow injection wave cathodic stripping voltametric method.	Henri PA et al; 2005

Capillary Electrophoresis

A method for the analysis of sertraline and its main metabolite N-desmethyl sertraline in human plasma was developed using capillary electrophoretic method with LIF detection (λ = 488nm). After pretreatment of biological fluid, followed by derivatization step in which reboxetine was used as a internal standard. The electrophoretic run is completed in 7.5 min.

Linearity was observed in the plasma concentration range of 3.0 to 500 µg/ml. [Alessandro M. et al; 2007]

Table 2.12. Estimation of Sertraline hydrochloride by Capillary Electrophoresis

Sr. No.	Specification	Reference
01	Capillary Electrophoretic determination of sertraline and N-desmethyl sertraline in human plasma by CE with LIF detection.	Alessandro M. et al; 2007

2.1.3 Duloxetine hydrochloride

High Performance Liquid Chromatographic Method

A rapid and sensitive liquid chromatography – mass spectrometric (LC/MS) method for determination of Duloxetine in human plasma using flupentinol as an internal standard was developed and validated. Sample preparation of the plasma involved deprotination with acetonitrile followed by high performance liquid chromatography using thermo hypersil – hypurity C_{18} column. A single quadrupole mass spectrometer in the selected ion – monitoring mode to detect the $[M + H]^+$ ions at 298 m/z for Duloxetine and at 435 m/z for internal standard. [Ma N et al; 2007]

A determination of Duloxetine in pharmaceutical dosage form by high performance liquid chromatography – spectrophotometric technique using inertsil CN_3 column was developed. 1% triethylamine solution (adjusted to pH 6 by phosphate buffer) – acetonitrile (30:70 v/v) was used as mobile phase. The detection was carried out at 290 nm. The range of linearity was 0.06 – 0.96 µg/ml. [Chang G et al; 2007]

A sensitive bioanalytical method for the determination of two major metabolites of Duloxetine (4-hydroxy Duloxetine glucuronide and 5-hydroxy-4-methoxy Duloxetine sulphate) in plasma was reported using liquid chromatography – tandem mass spectrometric method. [Satonin DK et al; 2007]

A high performance liquid chromatographic method was developed and validated for Duloxetine and desmethyl Duloxetine in human plasma. The human plasma was adjusted to pH 10 with 1.0 M sodium carbonate and extracted with hexane which contain 2% isopropylalcohol. The concentrated extract was derivatized with dansyl chloride (500 µg/ml) and was separated using phenomenon primesphere $5C_{18}$HC column followed by fluorescene detection with excitation and emission wavelength at 285 nm and 525 nm respectively. The linearity was observed between 2 to 64 ng/ml for both. [Jason TJ et al; 1996]

High performance liquid chromatography – tandem mass spectroscopy was developed for the determination of Duloxetine in pharmaceutical dosage form. Good result was obtained in the range of 5 – 100 ng/ml. [Michael HS et al; 2004]

A rapid, sensitive and accurate liquid chromatographic–tandem mass spectrometry (LC–MS–MS) method is described for the determination of Duloxetine in human plasma. Duloxetine was extracted from plasma using methanol and separated on a C18 column. The mobile phase consisting of a mixture of acetonitrile and 5 mM ammonium acetate (45:55, v/v, pH 3.5) was delivered at a flow rate of 0.3 ml/min. Atmospheric pressure ionization (API) source was operated in positive ion mode. Multiple reaction monitoring (MRM) mode using the transitions of *m*/*z* 298.1 → *m*/*z* 44.0 and *m*/*z* 376.2 → *m*/*z* 123.2 were used to quantify Duloxetine and internal standard (I.S.), respectively. The linearity was obtained over the concentration range of 0.1–50.0 ng/ml and the lower limit of quantitation (LLOQ) was 0.1 ng/ml. [Selvan PS et al; 2007]

A rapid and sensitive high-performance liquid chromatographic method was developed for Duloxetine analysis in human plasma. The assays were carried out using a C8 reversed-phase column and a mobile phase composed of 60% aqueous phosphate buffer containing triethylamine at pH 3.0 and 40% acetonitrile. The UV detector was set at 230 nm and loxapine was used as the internal standard. An original pre-treatment of plasma samples was developed, based on solid-phase extraction (SPE) with mixed-mode reversed phase—strong cation exchange cartridges (30 mg, 1 mL). The extraction yields values were higher than 90%. Linearity was found in the 2–200 ng mL^{-1} Duloxetine concentration range; the limit of quantitation was 2.0 ng mL^{-1} and the limit of detection was 0.7 ng mL^{-1}. [Raggi MA et al; 2007]

A new high-performance liquid chromatographic method is presented for the determination of 10 frequently prescribed tricyclic and nontricyclic antidepressants: imipramine, amitriptyline, clomipramine, Fluoxetine, sertraline, Paroxetine, citalopram, mirtazapine, moclobemide and Duloxetine. The simple and accurate sample preparation step, consisted of liquid:liquid extraction with recoveries ranging between 72% and 86%, except for moclobemide (59%). Separation was obtained using a reverse phase Select B column under isocratic conditions with UV detection (230 nm). The mobile phase consisted of 35% of a mixture of acetonitrile/methanol (92:8, v/v) and 65% of 0.25 mol L^{-1} sodium acetate buffer, pH 4.5. The standard curves were linear over a working range of 2.5–1000 ng mL^{-1} for moclobemide, 5–2000 ng mL^{-1} for citalopram, Duloxetine, Fluoxetine, 10–2000 ng mL^{-1} for sertraline, imipramine, Paroxetine, mirtazapine and clomipramine. The intra-assay and inter-assay precision and accuracy were studied at three concentrations (50, 200, and 500 ng mL^{-1}). The intra-assay coefficients of variation (CVs) for all compounds were less than 8.8%, and all inter-CVs were less than 10%. Limits of quantification were 2.5 ng mL^{-1} for moclobemide, 5 ng mL^{-1} for citalopram, Duloxetine and amitriptyline, and 10 ng mL^{-1} for mirtazapine, Paroxetine, imipramine, Fluoxetine, sertraline, and clomipramine. No interference of the drugs normally associated with antidepressants was observed. The method has been successfully applied to the analysis of real

samples, for the drug monitoring of ten frequently prescribed tricyclic and non-tricyclic antidepressant drugs. [Regina HCQ et al; 2007]

A simple reversed-phase high-performance liquid chromatographic method employing C-18 column has been developed for simultaneous analysis of three intermediates in the synthesis of S-Duloxetine, the antidepressant drug, viz., 2-acetyl thiophene (AT), N,N-dimethyl-3-keto-(2-thienyl)-propanamine (DKTP) and (S)-N,N-dimethyl-3-hydroxy-(2-thienyl)-propanamine (DHTP). Good separations were achieved by employing an isocratic system using acetonitrile and 0.05 M phosphate buffer (pH 7.0) containing 0.02% diethylamine. The detection was carried out at 241 nm. The method was validated for linearity, range, accuracy and precision. The developed method was applied for monitoring the progress of chemical synthesis of DKTP from AT followed by the biocatalytic reduction of DKTP to DHTP as the disappearance of the substrate and formation of the product can be monitored simultaneously by the present method. [Pankaj S et al; 2005]

Table 2.13. Estimation of Sertraline hydrochloride by High Performance Liquid Chromatography

Sr. No.	Specification	Reference
01	Determination of Duloxetine in human plasma using liquid chromatography – mass spectrometric method was developed.	Ma N et al; 2007
02	High Performance Liquid Chromatography – UV Spectrophotometric determination of Duloxetine in pharmaceutical formulation was developed.	Chang G et al; 2007
03	Development and validation of liquid chromatography – tandem mass spectrometric method for determination of Duloxetine in human plasma.	Satonin DK et al; 2007

04	High Performance Liquid Chromatographic method was developed for determination of Duloxetine and desmethyl Duloxetine in human plasma.	Jason TJ et al; 1996
05	High Performance Liquid Chromatography – tandem mass spectroscopy was developed for determination of Duloxetine.	Michael HS et al; 2004
06	Determination of Duloxetine in human plasma by liquid chromatography with atmospheric pressure ionization–tandem mass spectrometry and its application to pharmacokinetic study	Selvan PS et al; 2007
07	HPLC analysis of the novel antidepressant Duloxetine in human plasma after an original solid-phase extraction procedure	Raggi MA et al; 2007
08	Reliable HPLC method for therapeutic drug monitoring of frequently prescribed tricyclic and nontricyclic antidepressants	Regina HCQ et al; 2007
09	High-performance liquid chromatographic method for the simultaneous estimation of the key intermediates of Duloxetine	Pankaj S et al; 2005

Spectrophotometric Method

A simple, sensitive and accurate ultra violet spectrophotometric method was developed for the determination of Duloxetine in raw material and capsules validation of the method yielded good results concerning range, linearity, precision and accuracy. The absorbance was measured at 290 nm. The linearity range was found to be 5 – 50 µg/ml. [Kamila MH et al; 2007]

Table 2.14. Estimation of Sertraline hydrochloride by Spectrophotometry

Sr. No.	Specification	Reference
01	A simple, sensitive and accurate UV Spectrophotometric method was developed for Duloxetine in pharmaceutical formulation.	Kamila MH et al; 2007

Capillary Gas Chromatographic Method

A capillary gas chromatographic method for the determination of residual organic solvents in Duloxetine was developed. [Jian OL et al; 2006]

Table 2.15. Estimation of Sertraline hydrochloride by Capillary Gas Chromatography

Sr. No.	Specification	Reference
01	A residual organic solvent was determined from Duloxetine by capillary gas chromatographic technique.	Jian OL et al; 2006

2.2 Antihypertensive agents

2.2.1 Valsartan

High Performance Liquid Chromatographic Method

A high performance liquid chromatographic method for the determination of Valsartan in human plasma was developed. The assay is based on protein precipitation with methanol and reversed phase liquid chromatography with fluorometric detection. The liquid chromatography was performed on an octadecyl silica column using acetonitrile – 15mM dihydrogen potassium phosphate, pH 2 (45:55 v/v) as the mobile phase. The fluorimetric detection was operated at 234/374 nm (excitation / emission wavelength). The limit of quantification was 98 ng/ml. [Macek J et al; 2006]

A sensitive liquid chromatography – tandem mass spectrometry method for the determination of Valsartan in human plasma was developed and validated. [Nozomu K et al; 2007]

A simple and fast method for the simultaneous determination of Valsartan and its metabolite in human plasma using high performance liquid chromatographic – UV and fluorimetric methods. The separation was performed on an RP C_{18} Atlantis column using acetonitrile – trifluoroacetic acid and phosphate buffer (5 mM, pH=2.5) [50:50 v/v] as the mobile phase. The eluent was monitored with fluorescence detector at 234 and 378 nm excitation and emission wavelength respectively and at 254 nm using photometric detector. [Gorka I et al; 2007]

A rapid and specific high performance liquid chromatographic method was developed and validated for the simultaneous determination of ketoprofen, Valsartan and pantoprazol in human plasma. The separation was performed using chromasil C_{18} column and 0.02 M sodium dihydrogen phosphate buffer (pH 3.15) – acetonitrile (58:42 v/v) used as the mobile

phase. The detection was carried out using diode array detector. [Kocyigit B et al; 2006]

A rapid high performance liquid chromatographic method was developed for the determination of Valsartan in pharmaceutical dosage form. The separation was performed on C_{18} column using acetonitrile – phosphate buffer as mobile phase, where losartan is used as an internal standard. The detection was carried out at 265 nm with a UV detector. The assay was linear over the concentration range 1.0 – 5.0 µg/ml. [Tatar S et al; 2002]

A high performance liquid chromatographic – UV spectrometric method was developed for the determination of Valsartan and hydrochlorthiazide simultaneously in combined dosage forms. The separation was carried out on a reversed phase column using 0.02 M phosphate buffer (pH 3.2) – acetonitrile (55:45 v/v) mobile phase for the separation of Valsartan and hydrochlorthiazide. The linearity were observed in concentration range 0.06 – 1.8 µg/ml and 0.07 – 0.5 µg/ml respectively. [Satana E et al; 2001]

Several angiotensin II receptor antagonists were analysed from human plasma and urine. Under optimized extraction condition, the protein component of the biological sample was flushed through the monolithic capillary, while the analytes were successfully trapped coupled to HPLC with fluorescence detection. This on line in tube solid – phase micro extraction (SPME) method was successfully applied for the determination of candesartan, losartan, irbesartan, valsartan, telmisartan, and their detection limit were found to be 0.1 – 15.3 ng/ml and 0.1 – 15.2 ng/ml in human plasma and urine respectively. The method was linear over the range of 0.5 – 200 ng/ml for telmisartan, 5 – 2000 ng/ml for candensartan and irbesartan, 10 – 2000 ng/ml for Valsartan and 50 – 5000 ng/ml for losartan with correlation co-efficient being above 0.9985 in plasma and above 0.9994 in urine sample. [Jing H et al; 2005]

A method for the simultaneous determination of the β-blockers (Atenolol, Sotalol, metoprolol, Bisoprolol, Propranolol and Carvedilol), the

calcium channel antagonists (diltiazem, amlodipine and verapamil), the angiotensin – II antagonists (Losartan, Irbesartan, Valsartan, Telmisartan) and the antiarrythmic drug (Flecainide), in whole blood samples was developed. Sample clean – up was achieved by precipitation and solid phase extraction with mixed mode column. Quantification was performed by reversed phase high performance liquid chromatography with positive electrospray ionization mass spectrometric detection. The method has been developed and robustness tested by systematically searching for satisfactory conditions using good linearity was observed. [Lena K et al; 2007]

A rapid, sensitive and accurate liquid chromatographic –tandem mass spectrometric method is described for the simultaneous determination of nebivolol and Valsartan in human plasma. Nebivolol and Valsartan were extracted from plasma using acetonitrile and separated on a C_{18} column. The mobile phase consisting of a mixture of acetonitrile and 0.05mM formic acid (50:50 v/v, pH 3.5) was delivered at a flow rate of 0.25 ml/min. Atmospheric pressure ionization (API) source was operated in both positive and negative ion mode for nebivolol and valsartan, respectively. Selected reaction monitoring mode (SRM) using the transitions of m/z 406.1 ----> m/z 150.9; m/z 434.2 ----> m/z 179.0 and m/z 409.4 ----> m/z 228.1 were used to quantify nebivolol, Valsartan and internal standard (IS), respectively. The linearity was obtained over the concentration range of 0.01 – 50.0 ng/ml and 1.0 – 2000.0 ng/ml and the lower limits of quantitation were 0.01 ng/ml and 1.0 mg/ml for nebivolol and valsartan, respectively. This method was successfully applied to the pharmacokinetic study of fixed dose combination (FDC) of nebivolol and Valsartan formulation product after an oral administration to healthy human subjects. [Selvan PS et al; 2007]

A selective, accurate and precise high-performance liquid chromatographic assay coupled to fluorescence detection was developed for the detection of some angiotensin II receptor antagonists (ARA II): Losartan, Irbesartan, Valsartan, Candesartan cilexetil and its metabolite Candesartan M1. The analytes and the internal standard (bumetanide, a high-ceiling diuretic) were extracted from plasma under acidic conditions by means of

solid-phase extraction using C_8 cartridges. This procedure allowed recoveries close to 80% for all these drugs excluding Candesartan cilexetil (70%) which presented adsorption processes on glass and plastic walls. The analytes and potential interferences were separated on a reversed-phase column, μBondapak C_{18}, at room temperature. A gradient elution mode was used to carry out the separation, the optimal mobile phase being composed of acetonitrile–5 m*M* acetate buffer, pH 4, at variable flow-rates (from 1.0 to 1.2 ml/min). Fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 375 nm. Intra- and inter-day relative standard deviations for all the compounds were lower than 8% except for Losartan (12%) and the method assesses a quite good accuracy (percentage of relative error ~6% in most of the cases). The limit of quantitation for these compounds was 3 ng/ml for Candesartan cilexetil and M1, 16 ng/ml for Losartan and 50 ng/ml for Irbesartan and Valsartan, which allows their determination at expected plasma concentration levels. This assay method has been successfully applied to plasma samples obtained from hypertensive patients under clinical studies after oral administration of a therapeutic dose of some of these ARA II compounds. [Alonso RM et al; 2002]

A stereospecific HPLC method for the quantitation of CGP 49309 in samples of its corresponding enantiomer Valsartan has been developed and validated. The enantiomeric separation was achieved on a 5 μm silica-bonded α_1-acid glycoprotein column (Chiral AGP) with a phosphate buffer, pH 7, containing 2% (v/v) 2-propanol as a mobile phase. The linearity was established in the range 0.1–4% (*r*>;0.999). The limit of quantitation was 0.1% and the limit of detection was 0.04%. The accuracy of the method was found to be 96.7% (average). For the precision (repeatability), a relative standard deviation value of 2.4% was found. Similarly, a stereoselective HPLC method was also developed and validated for the quantitation of the enantiomer of the starting material used for the synthesis of valsartan, namely (*R*)- valinebenzyl ester tosylate. Baseline resolution of the enantiomers of valinebenzyl ester tosylate could be achieved on the chiral crown ether column Crownpak CR (Daicel) at 50°C using water-methanol-trifluoroacetic acid (850:150:1, v/v) as a mobile phase. The linearity was established in the range 0.5-5% (*r*>;0.999).

The accuracy of the method was found to be 100.5% (average). For the precision (repeatability), a relative standard deviation value of 3.4% was found. Both methods were found to be suitable for the analysis of the respective analytes. [Eric F et al; 1996]

Table 2.16. Estimation of Valsartan by High Performance Liquid Chromatography

Sr. No.	Specification	Reference
01	Rapid determination of Valsartan in human plasma by protein precipitation and high performance liquid chromatographic method was developed.	Macek J et al; 2006
02	Development and validation of a method for quantitative determination of Valsartan in human plasma was developed by liquid chromatography – tandem mass spectrometry.	Nozomu K et al; 2007
03	Biovalidation of high performance liquid chromatography – UV – fluorescence method was developed for Valsartan and its metabolite in human plasma.	Gorka I et al; 2007
04	Determination and validation of Ketoprofen, Pantoprazole and Valsartan together in human plasma was developed using HPLC.	Kocyigit B et al; 2006
05	Determination of Valsartan in pharmaceutical formulation using HPLC – UV Spectrophotometric method.	Tatar S et al; 2002
06	Determination of Valsartan in pharmaceutical formulation using HPLC – UV Spectrophotometric method.	Satana E et al; 2001
07	Biocompatible in tube solid – phase micro extraction coupled to HPLC for the determination of angiotensin II receptor antagonists in human plasma and urine was developed.	Jing N et al; 2005

08	Simultaneous determination of 6 β-blockers, 3 calcium channel blockers, 4 angiotensin – II antagonists and one antiarrhythmic drug in post mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry was developed.	Lena K et al; 2007
09	Simultaneous determination of fixed dose combination of Nebivolol and Valsartan in human plasma by liquid chromatographic – tandem mass spectrometric method was developed.	Selvan PS et al; 2007
10	Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection	Alonso RM et al; 2002
11	Development and validation of chiral high-performance liquid chromatographic methods for the quantitation of Valsartan and of the tosylate of valinebenzyl ester.	Eric F et al; 1996

High Performance Thin Layer Chromatographic Method

A new, simple, accurate, and precise high-performance thin-layer chromatographic (HPTLC) method has been established for simultaneous analysis of Valsartan and hydrochlorothiazide in tablet formulations. Standard and sample solutions of Valsartan and hydrochlorothiazide were applied to precoated silica gel G 60 F_{254} HPTLC plates and the plates were developed with chloroform-ethyl acetate-acetic acid, 5:5:0.2 v/v, as mobile phase. UV detection was performed densitometrically at 248 nm. The retention factors of Valsartan and hydrochlorothiazide were 0.27 and 0.56, respectively. The linear range was 800 – 5600 ng per spot for Valsartan and 125 – 875 ng per spot for hydrochlorothiazide; the correlation coefficients, r, were 0.9998 and 0.9988, respectively. The method was validated in accordance with the requirements of ICH guidelines and was shown to be suitable for purpose.

The method was successfully used for determination of the drugs in tablets. Tablets excipients did not interfere with the chromatography. [Kadam BR et at; 2007]

Table 2.17. Estimation of Valsartan by High Performance Thin Layer Chromatography

Sr. No.	Specification	Reference
01	Determination of Valsartan and hydrochlorothiazide in tablets was developed using high performance thin layer chromatography with ultraviolet absorption densitometry.	Kadam BR et al; 2007

Spectrophotometric Method

A simple, sensitive and rapid method for the determination of Valsartan was developed using ultra violet spectrophotometric method. The measurement was carried out at 205.6 nm. The linearity was observed in the range of 2.0 – 10 µg/ml while in second method, the distance between two extreemum value (peak to peak amplitude) 221.6 and 231.2 nm were measured in the second order derivative spectra of standard solution. [Tatar S et al; 2002]

First derivative UV Spectrophotometric method was developed for simultaneous determination of Valsartan and hydrochlorothiazide in combined dosage forms. The derivative procedure was based on the linear relationship between the drug concentration and the first derivative amplitude at 270.6 and 335 nm for Valsartan and hydrochlorothiazide respectively. The calibration graph were linear in the range of 12 – 36.1 µg/ml and 4 – 12.1 µg/ml for Valsartan and hydrochlorothiazide respectively. [Satana E et al; 2001]

The two new method for the simultaneous determination of Valsartan and hydrochlorothiazide in pharmaceutical dosage forms have been developed. The first method, based on compensation technique is presented for derivative Spectrophotometric determination of binary mixtures with overlapping spectra by using ratio of derivative maxima or the derivative minimum, the exact compensation of either component in the mixture can be achieved followed by its determination. The second method, differential derivative spectrophotometry, comprised of measurement of the difference absorptivities derivatised in the first order of a tablet extract in 0.1 N NaOH relative to that of an equimolar solution in methanol at wavelength of 227.8 and 276.5 nm respectively. [Nevin E; 2002]

Two new spectral approach (graphical and chemometric techniques) were applied for resolution of a binary mixture of Valsartan (VST) and hydrochlorothiazide (HCT) in tablets without a separation procedure. In the ratio derivative spectrophotometry, analytical derivative amplitudes were measured at 231.5 and 260.5 nm for VST and at 270.6 nm for HCT in the first derivative of the ratio spectra. The calibration graphs follow Beer's law in the ranges of 8 – 24 µg/ml for VST and 2 -10 µg/ml for HCT. The prepared calibrations for the first technique were tested for the synthetic binary mixtures and 1.76 % for VST, 102.5 % and 2.41 % for HCT, respectively. In the inverse least square technique, absorbances matrix were produced by measurements in the spectral range from 225 to 280 nm of the interval of $\Delta\lambda$ = 5 nm at 12 wavelengths in zero order spectra of the various binary mixtures. The prepared calibration set for the absorbance and concentration values were used to predict the concentration of VST and HCT in their binary mixtures and tablets. The "Maple V" software was used for the numerical calculations. For this technique, mean recoveries and relative standard derivations were found as 101.2 % and 1.58 % for VST and 96.2 % and 2.35% for HCT. Both techniques were successfully applied for the determination of these two drugs in tablets. [Erdal D et al: 2005]

Table 2.18. Estimation of Valsartan by Spectrophotometry

Sr. No.	Specification	Reference
01	UV and second derivative spectrophotometric method have been developed for Valsartan in pharmaceutical formulations.	Tatar S et al; 2002
02	Simultaneous determination of Valsartan and hydrochlorothiazide using first derivative UV spectrophotometric method was developed.	Satana E et al; 2001
03	Spectrophotometric determination of Valsartan and hydrochlorothiazide in pharmaceutical dosage forms was developed.	Nevin E; 2002
04	Spectral resolution of binary mixture containing Valsartan and hydrochlorothiazide in tablets by ratio spectra derivative and inverse least square techniques were developed.	Erdal D et al; 2005

Spectrofluorimetric Method

A spectrofluorimetric method has been developed for the determination of two angiotensin II receptor antagonists (ARA II): Losartan and Valsartan. A fractional factorial design and a central composite design were used. The key factors considered in the optimization process were pH, temperature and emission slit width. Maximum fluorescent intensity was established as response for each experiment. The response surfaces confirmed the robustness of the method. A clean-up procedure was used for urine samples that consisted of a solid-phase extraction using C_8 cartridges. The total analysis time was lower than 30 min. This method proved to be accurate (RE, 8%), precise (intra- and inter-day coefficients of variation were lower than 8% and sensitive enough (LOQ c.a. 0.5 μg ml^{-1}) to be applied to the determination of Losartan and Valsartan in urine samples. [Alonso RM et al; 2001]

Table 2.19. Estimation of Valsartan by Spectrofluorimetry

Sr. No.	Specification	Reference
01	Experimental design methodologies to optimise the spectrofluorimetric determination of Losartan and Valsartan in human urine	Alonso RM et al; 2001

Micellar Electrokinetic Capillary Chromatographic Method

Micellar electrokinetic capillary chromatographic method was developed for the separation of six angiotensin-II-receptor antagonists (ARA-IIs): candesartan, eprosartan mesylate, irbesartan, losartan potassium, telmisartan, and valsartan. A face-centred central composite design was applied to study the effect of the pH, the molarity of the running buffer, and the concentration of the micelle-forming agent on the separation properties. A combination of the studied parameters permitted the separation of the six ARA-IIs, which was best carried out using a 55-m*M* sodium phosphate buffer solution (pH 6.5) containing 15 m*M* of sodium dodecyl sulfate. The same system can also be applied for the quantitative determination of these compounds, but only for the more stable ARA-IIs (candesartan, eprosartan mesylate, losartan potassium, and valsartan). Some system parameters (linearity, precision, and accuracy) were validated. [Hillaert S et al; 2003]

Table 2.20. Estimation of Valsartan by Micellar Electrokinetic Capillary Chromatography

Sr. No.	Specification	Reference
01	Optimization and validation of a micellar electrokinetic chromatographic method for the analysis of several angiotensin-II-receptor antagonists	Hillaert S et al; 2003

Capillary Zone Electrophoretic Method

A capillary zone electrophoretic method was developed for separation of six angiotensin-II-receptor antagonists (ARA-IIs): candesartan, eprosartan, irbesartan, losartan potassium, telmisartan, and valsartan. A three-level, full-factorial design was applied to study the effect of the pH and molarity of the running buffer on separation. Combination of the studied parameters permitted the separation of the six ARA-IIs, which was best carried out using 60 m*M* sodium phosphate buffer (pH 2.5). The same system can also be applied for the quantitative determination of these compounds, but only for the more soluble ones. Some parameters (linearity, precision and accuracy) were validated. [Hillaert S et al; 2002]

Table 2.21. Estimation of Valsartan by Capillary Zone Electrophoresis

Sr. No.	Specification	Reference
01	Optimization and validation of a capillary zone electrophoretic method for the analysis of several angiotensin-II-receptor antagonists	Hillaert S et al; 2002

2.3 Ion pair complexes

A spectrophotometric procedure for the determination of terfenadine and a number of its pharmaceutical preparations has been developed that offers advantages of simplicity, rapidity, sensitivity and stability indication over the official USP (1995) method. The proposed method is based on the formation of ion-pairs by the reaction of terfenadine with some chromotropic acid mono- and bis-azo dyes. Different variables affecting the ion-pair formation were studied and optimized. At the maximum absorption of 557, 521, 592 and 543 nm, Beer's law is obeyed in the range 0.2–18.6, 0.2–16.4, 0.2–25.0 and 0.2–22.2 μg ml^{-1} on using reagents I, II, III and IV, respectively. The stoichiometric ratio and stability of each ion-pair were estimated and the mechanism of the reaction is discussed. The molar absorptivity and Sandell sensitivity of the produced ion-pairs were calculated in addition to Ringbom optimum concentration ranges. Statistical treatment of the experimental results indicates that the procedures are precise and accurate. Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures. The reliability of the methods was established by parallel determination against the official USP method. The procedures described were successfully applied to the determination of the bulk drug and its pharmaceutical formulations by applying the standard addition technique. [Amin AS et al; 1999]

Simple, sensitive, and accurate visible spectrophotometric methods are described for the determination of Paroxetine hydrochloride (PA) in tablets. Among them, the first 3 methods are based on the ion-pair complexes of PA formed with bromothymol blue (BTB), bromophenol blue (BPB), and bromocresol green (BCG) in aqueous acidic buffers. The complex species extracted into chloroform were quantitatively measured at 414 nm with BTB and BCG and at 412 nm with BPB. Beer's law was obeyed over the concentration ranges of 2–20, 2–16, and 2–16 μg/mL, respectively. The fourth method described is based on a coupling reaction between PA and 7-chloro-4-nitrobenzofurazon (NBD-Cl) in borate buffer, pH 8.5, in which a yellow reaction product that was measured at 478 nm was formed. The Beer's law

range for this method was 2–10 μg/mL. The last method developed describes the interaction of PA base, as an n-electron donor, with 7,7,8,8-tetracyanoquinodimethane (TCNQ), as a π-acceptor, in acetonitrile to give blue-colored $TCNQ^{-}$ radical anion with absorption maxima at 750 and 845 nm. Measured at 845 nm, the absorbance–concentration plot was rectilinear over the range of 1.5–15 μg/mL. The new methods developed were successfully applied to the determination of PA in tablets without any interference from common tablet excipients. The results of the methods were in good agreement with those obtained with an official liquid chromatographic method. This report describes first colorimetric methods for the determination of PA. [Armagan O et al; 2005]

Extractive spectrophotometric methods are described for the determination of lercanidipine (LER) either in pure form or in pharmaceutical formulations. The methods involve formation of coloured chloroform extractable ion-pair complexes with bromothymol blue (BTB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 417 and 416 nm for BTB and BCG, respectively. The optimization of the reaction conditions was investigated. Beer's law is obeyed in the concentration ranges 6.0-42.0 μg/ml(-1) or 7.1-43.8 μg/ml(-1) with BTB or BCG, respectively. The composition of the ion-pairs was found to be 1:1 by Job's method. The specific absorptivities, molar absorptivities, Sandell sensitivities, standard deviations and percent recoveries were evaluated. Also, LER was determined by measurement of its first derivative signals at 245 nm. Calibration graph was established for 4.2-58.0 μg/ml(-1) of LER. The methods have been applied to the determination of drug in commercial tablets. Results of analysis were validated. No interferences were observed from common pharmaceutical adjuvants. [Erk N; 2003]

A simple, extraction-free spectrophotometric method is proposed for the analysis of some β-blockers, namely atenolol, timolol and nadolol. The method is based on the interaction of the drugs in chloroform with 0.1% chloroformic solutions of acidic sulphophthalein dyes to form stable, yellow-coloured, ion-pair complexes peaking at 415nm. The dyes used were

bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP). Under the optimum conditions, the three drugs could be assayed in the concentration range 1-10 µg/ with correlation coefficient (n=5) more than 0.999 in all cases. The stoichiometry of the reaction was found to be 1:1 in all cases and the conditional stability constant (K"F) of the complexes have been calculated. The free energy changes were determined for all complexes formed. The interference likely to be introduced from co-formulated drugs was studied and their tolerance limits were determined. [Al-Ghannam SM; 2006]

A spectrophotometric method is described for the determination of oxiconazole in raw material and in topical lotion. This method is based on the reaction of the oxiconazole with methyl orange in buffered aqueous solution of citric acid at pH 2.3. The chromogen, being extractable with dichloromethane, could be measured quantitatively with maximum absorption at 427 nm. The Lambert-Beer law was obeyed in the concentration range of 4.0-14.0 µg/ml. A prospective validation of the method showed that the method was linear (r=0.9995), precise (intra-day: CV=1.57% and inter-day: CV=1.50%) and accurate (mean recoveries: 99.69%). [Milano J. et al; 2005]

A sensitive method for determining ethambutol (20-100 µg) in aqueous solutions and tablets is described, using bromthymol blue as a complex-forming agent. Extraction of the complex in chloroform as well as in methylene chloride or ethylene dichloride is accomplished readily at an optimum pH 7. A stoichiometric relationship of 1:2 between ethambutol and the acid dye is proved. Cycloserine, isoniazid, and sodium aminosalicylate do not interfere with the assay. The reineckate precipitation method for ethambutol determination is compared and its direineckate derivative is identified. [Girgis EH et al; 1974]

Three simple, sensitive and accurate spectrophotometric methods have been developed for the determination of oxomemazine hydrochloride in bulk and pharmaceutical formulations. These methods are based on the formation of yellow ion-pair complexes between the examined drug and bromocresol green (BCG), bromocresol purple (BCP), and bromophenol blue

(BPB) as sulphophthalein dyes in acetate-HCl buffer of pH 3.6, 3.4, and 4.0, respectively. The formed complexes were extracted with dichloromethane and measured at 405 nm for all three systems. The best conditions of the reactions were studied and optimized. Beer's law was obeyed in the concentration ranges 2.0-12, 2.0-13, and 2.0-14 μg mL^{-1} with molar absorptivities of 3.2×10^4, 3.7×10^4, and 3.1×10^4 L mol^{-1} cm^{-1} for the BCG, BCP, and BPB methods, respectively. Sandell's sensitivity, correlation coefficient, detection and quantification limits are also calculated. The proposed methods have been applied successfully for the analysis of the drug in pure form and in its dosage forms. No interference was observed from common pharmaceutical excipients. Statistical comparison of the results with those obtained by HPLC method shows excellent agreement and indicates no significant difference in accuracy and precision. [Akram ME; 2005]

Two simple, quick and sensitive spectrophotometric methods are described for the determination of enrofloxacin and Pefloxacin. The methods are based on the reaction of these drugs with bromophenol blue (BPB) and methyl orange (MO) in buffered aqueous solution at pH 2.3–2.5 in case of bromophenol blue and at pH 3.6 with MO to give highly coloured complex species, extractable with chloroform. The coloured products are quantitated spectrophotometrically at 420 and 424 nm for BPB and MO, respectively. Optimisation of the different experimental conditions is described. Beer's law is obeyed in the concentration ranges 2–12 and 2–18 μg ml^{-1} with BPB and in the ranges 1–12 and 4–40 μg $ml^{-1}$with MO for enrofloxacin and pefloxacin, respectively. The proposed methods are applied for determination of Enroxil oral solution, Peflacine tablets and Peflacine ampoules with mean percentage accuracies 99.5±0.99, 99.39±1.05 and 100.02±0.895, respectively, with BPB and 100.30±0.89, 100.25±0.98 and 100.20±0.72, respectively, with MO. [Mostafa S et al; 2002]

It has been established that the antibiotic pefloxacin (Abaktal) methane-sulphonate reacts with Fe(III) at pH 1.00–8.00 to form a water-soluble complex with maximum absorbance at 360 nm. The composition of the complex, determined spectrophotometrically by the application of Job's,

molar-ratio and Bent—French's methods, was pefloxacin: Fe(III) = 1:1 (pH = 2.50; λ = 360 nm; μ = 0.1 M). The relative stability constant, obtained by the methods of Sommer and Asmus was $10^{5.02}$ (pH = 2.50; λ = 360 nm; μ = 0.1 M). The molar absorptivity of the complex at 360 nm was found to be 4.8 × 10^3 l mol^{-1} cm^{-1}, Beer's law was followed for pefloxacin concentrations of 2.15–85.88 μg ml^{-1}. The lower sensitivity limit of the method was 2.15 μg ml^{-1}. The relative standard deviation (*n* = 10) was 0.57–1.07%. The method can be applied to the rapid and simple determination of pefloxacin in aqueous solutions and tablets. [Jelikic SM et al; 1989]

A direct, extraction-free spectrophotometric method has been developed for the determination of cinnarizine in pharmaceutical preparations. The method is based on ion-pair formation between the drug and three acidic (sulphonphthalein) dyes; namely bromocresol green (BCG), bromocresol purple (BCP) and bromophenol blue (BPB) which induces an instantaneous bathochromic shift of the maximum in the drug spectrum. Conformity to Beer's law enabled the assay of dosage forms of the drug. Compared with a reference method, the results obtained were of equal accuracy and precision. A more detailed investigation of the cinnarizine-BCG ion pair complex was made with respect to its composition, association constant and free energy change. In addition, this method was also found to be specific for the analysis of cinnarizine in the presence of some of the co-formulated drugs, such as pyridoxine hydrochloride and digoxin. [Abdine H et al; 2002]

Two simple, rapid and sensitive extractive spectrophotometric methods have been developed for the assay of trazodone hydrochloride (TRH) in pure and pharmaceutical formulations. These methods are based on the formation of chloroform soluble ion-association complexes of TRH with bromothymol blue (BTB) and with bromocresol purple (BCP) in KCl–HCl buffer of pH 2.0 (for BTB) and in NaOAc–AcOH buffer of pH of 3.6 (for BCP) with absorption maximum at 423 nm and at 408 nm for BTB and BCP, respectively. Reaction conditions were optimized to obtain the maximum color intensity. The absorbance was found to increase linearly with increase in concentration of TRH, which was corroborated by the calculated correlation coefficient values

(0.9996, 0.9945). The systems obeyed Beer's law in the range of 0.2—14.5 and 0.2—14.1 µg/ml for BTB and BCP, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. No interference was observed from common excipients present in pharmaceutical formulations. The proposed methods are simple, accurate and suitable for quality control applications. [Ramanaboyina SK et al; 2006]

A simple extractive spectrophotometric method has been developed for the estimation of sildenafil citrate in both pure and pharmaceutical dosage forms. The method is based on the formation of ion-pair complexes of the drug with three acid dyes namely bromothymol blue (BTB), bromocresol green (BCG), bromophenol blue (BPB) in acidic buffer solutions followed by their extraction in organic solvent (chloroform). The absorbance of the organic layer was measured at their respective wavelength of maximum absorbance against the corresponding reagent blank. The method has been statistically evaluated and is found to be precise and accurate. [Reddy MN et al; 2002]

Two new rapid, sensitive and economical spectrophotometric methods are described for the determination of Fluoxetine hydrochloride in bulk and in pharmaceutical formulations. Both methods are based on the formation of a yellow ion-pair complex due to the action of methyl orange (MO) and thymol blue (TM) on Fluoxetine in acidic (pH 4.0) and basic (pH 8.0) medium, respectively. Under optimised conditions they show an absorption maxima at 433 nm (MO) and 410 nm (TB), with molar absorptivities of 2.12×10^{-4} and 4.207×10^{-3} l mol^{-1} cm^{-1} and Sandell's Sensitivities of 1.64×10^{-2} and 0.082 µg cm^{-2} per 0.001 absorbance unit for MO and TB, respectively. The colour is stable for 5 min after extraction. In both cases Beer's Law is obeyed at 1–20 µg mol^{-1} with MO and 4–24 µg mol^{-1} with TB. The proposal method was successfully extended to pharmaceutical preparations—capsules. The results obtained by both the agreement and E.P. (3rd edition) were in good agreement and statistical comparison by Student's *t*-test and variance ratio *F*-test showed no significant difference in the three methods. [Prabhakar AH et al; 1999]

Three methods are described for the determination of trazodone hydrochloride in pharmaceutical tablets. The spectrophotometric method was based on the formation of yellow ion pair complex between the basic nitrogen of the drug and bromophenol blue at pH 3.4. The formed complex was extracted with chloroform and measured at 414 nm. The spectrofluorimetric method was based on measurement of the native fluorescence of the drug in 50% acetic acid upon excitation at a maximum of 320 nm and the emission wavelength is 435 nm. The third method was based on the high performance liquid chromatographic determination of trazodone hydrochloride using a reversed phase, ODS column, with a mobile phase of acetonitrile--phosphate buffer at pH 4.5 (60:40, v/v). Quantization was achieved with UV detection at 250 nm based on peak area. The three methods were simple, accurate and suitable for quality control application. [El-Gindy A. et al; 2001]

A rapid method is presented for determining strychnine and brucine in liquid galenicals. At pH 5.0, both strychnine and brucine are complexed with methyl orange. After treatment with 0.1N NaOH, the liberated alkaloids are determined spectrophotometrically, using the 2-wavelength method of analysis. The method has been successfully applied to the analysis of 4 batches of nux vomica tincture, nux vomica acid, and nux vomica alkaline mixtures. The method has a relative standard deviation of 0.52%. [El-Massy S. et al; 1978]

An acid-dye colormetric method was described for the determination of chlorpromazine hydrochloride in the compound preparation. The method was based on the reaction of chlorpromazine hydrochloride with methyl orange, to form a yellow complex, which then was extracted by chloroform and exhibited a maximum absorption at 424 nm. The optimal conditions for determination were selected by orthogonal design test. The linear range of this method was 20-120 μg/ml (r = 0.9997). The average recovery of the three sample solutions of different concentrations was 99.72% +/- 0.46% (n = 6). The other ingredients of preparation do not interfere with chlorpromazine determination. This method is more sensitive and accurate and can be used for quality control of this compound preparation. [Zhou Z. et al; 1996]

A method, based on ion-pair extraction, is described for the quantification of meclizine hydrochloride in various pharmaceutical dosage forms, for content uniformity determination, and for concentration monitoring in dissolution and bioavailability studies. Methyl orange, dissolved in pH 2.8 McIlvaine buffer, gave excellent recovery of meclizine after its isolation from aqueous solutions of gelatin, urine, and blood serum. The chloroform-extracted molecular species appeared to be a 1:1 ion-pair. Beer's law was obeyed for a wide concentration range. Because the extracted species seemed well defined and stable and since a surface or an interphase adsorption phenomenon was not a problem, the reported method is considered sensitive, accurate, precise, rapid, and simple. [Hom FS et al; 1977]

A simple method, requiring no chromatographic separation, is presented for the determination of the total and non-phenolic alkaloids in ipeca and its preparations. The complex formed between the alkaloid and methyl orange at pH 5.0 is extracted with chloroform and treated with 0.1N NaOH. The liberated dye, determined at 460 nm, is a measure of the total alkaloids. The chloroform phase remaining is treated with 0.1N H_2SO_4, and the acid extract is measured at 283 nm for the non-phenolic alkaloids, calculated as emetine. The proposed method was successfully applied to samples of ipeca powder, ipeca tincture, and 3 British Pharmaceutical Codex mixtures containing ipeca tincture, namely, ipecacuanha mixture, pediatric; ipecacuanha and ammonia mixture, pediatric; and belladonna and ipecacuanha mixture, pediatric. The proposed method compares favorably with the Egyptian Pharmacopoeia, British Pharmacopoeia, and USP methods and has a relative standard deviation of 1.54%. The present procedure is less time-consuming and requires about 45 and 90 min for the assay of ipeca tincture and powder, respectively. Only a small sample (0.2 mL tincture of 1.0 g powder) is required. [Saleh MR et al;1980]

Seven colorimetric methods (Biuret, Lowry, a modified Lowry technique using bicinchoninic acid, Coomassie brilliant blue G (CBBG) dye-binding in phosphoric acid, perchloric acid or hydrochloric acid, and bromophenol blue

dye-binding) were evaluated for determination of protein concentration in human whole saliva. Using bovine serum albumin (BSA), mucin, and thyroglobulin as protein standards to calculate salivary protein concentration gave different results among the assays used and even with the different standards of the same method. Among the seven methods used, the CBBG dye-binding assay in hydrochloric acid appeared to provide the most accurate estimation of protein concentration in human saliva. Using BSA as a standard, the mean values of human salivary proteins from 39 healthy individuals ranged from 0.72 to 2.45 mg/ml. The optimum conditions for the CBBG dye-binding assay in hydrochloric acid are: (1) the absorbance at 595 nm was measured between 15 to 30 minutes after addition of reagent. (2) the optimum concentration of dye in hydrochloric acid was 0.06-0.12% (w/v) for the assay. (3) the dye reagent was stable within one month. [Lin LY et al; 1989]

A simple and sensitive spectrophotometric method was developed for the determination of trace amounts of sulfur dioxide. The method is based on the reaction of SO_2 with a known excess of ICl as the oxidant. The unreacted ICl iodinates thymol blue under acidic conditions. The λmax of thymol blue is at 545 nm under acidic conditions, and on lodination λmax shifts to 430 nm. This shift results in a decrease in the absorbance at 545 nm. The amount of uniodinated thymol blue present depends on the concentration of unreacted ICl, which in turn depends on the SO_2 concentration. The system obeys Beer's law in the range 0-30 microg SO_2 in a final volume of 25 mL, having a molar absorptivity of 3.2 x 10(4) L/mol cm with a relative standard deviation (RSD) of 2% at 24 µg SO2 (n = 10). The uniodinated dye can be extracted into 5 mL isoamyl alcohol under acidic conditions for measurement of absorbance. The extraction method obeys Beer's law in the range 0-5 µg SO_2, having a molar absorpitivity of 4.16 x 10(4) L/mol x cm with an RSD of 1.9% at 4 µg SO_2 (n = 10). The method has been successfully applied to the determination of atmospheric SO_2. [Gayathri N. et al; 2001]

A spectrophotometric method is described for determination of methotrimeprazine (levomepromazine). The aim of this work was to develop a simple, rapid, precise, and accurate visible spectrophotometric method for

determination of methotrimeprazine in tablet, oral solution, and injection. The method is based on methotrimeprazine reaction with bromophenol blue, resulting in a stable, light yellow-green ion-pair complex that, after extraction with chloroform, presented maximum absorption at 409 nm. Beer's law was obeyed in the concentration range from 5.0 to 25.0 µg/ml. The proposed standardized method was applied to commercially available dosage forms. The accuracy of the method was confirmed by recovery tests. [Kedor-Hackmann ERM et al; 2000]

A spectrophotometric method is described for the determination of tilidine in its dosage forms (injection, drops, suppositories). The method is based on ion-pair extraction with chloroform at pH 3.5 using bromocresol green or bromophenol blue as the ion-pairing reagents. The spectrophotometric measurements are carried out at the absorption maxima at 415 and 411 nm, respectively. [Dobrila ZS et al; 1990]

Two simple, rapid and sensitive extractive spectrophotometric methods have been developed for the assay of ceterizine hydrochloride (CTZH) in bulk drug and in pharmaceutical preparations. These methods are based on the formation of chloroform soluble complexes between CTZH with bromocresol purple (BCP) or bromophenol blue (BPB) in Walpole buffer of pH 2.64 with an absorption maximum at 409 nm and at 414 nm for BCP and BPB, respectively. Reaction conditions were optimised to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of CTZH, which was corroborated by the calculated correlation coefficient value (0.9991-0.9995). The system obeyed Beer's law in the range of 1-16 and 1.5-21 µg/ml(-1) for BCP and BPB, respectively. The various analytical parameters have been evaluated. The results obtained by the proposed methods were statistically compared by means of students t-test and by the variance ratio, F-test with those of the reported method and have shown to be in excellent agreement with the reported method. [Gowda BG et al; 2001]

The dissociation constants (KI = [H+][I2-]/[HI-]) of two sulfonephthalein indicators (bromocresol purple and phenol red) were determined as function of temperature (10-30 degrees C) at zero ionic strength. Freshwater pH, on the free hydrogen ion concentration scale (molal units), can be precisely calculated from measurements of indicator absorbance ratios (lambda2A/lambda1A) using the following equations: pH = pKI + log((R - e1)/(e2 - Re3)) and pKI = pKI(degrees) - AdeltaZ2(mu1/2 /(1 + mu1/2) - 0.3 mu), where R = lambda2A/lambda1A, pKI = -log KI, mu is the ionic strength, deltaZ2 = 4, and values of A for 283 less than or = T less than or = 303 can be estimated from the equation: A = 0.5092 + (T-298.15) x 8.5 x 10(-4). For bromocresol purple (lambda1 = 432 nm, lambda2 = 589 nm), pKI(degrees) = 5.226 + 378.1/T, e1 = 0.00387, e2 = 2.858, and e3 = 0.0181. For phenol red (lambda1 = 433 nm, lambda2 = 558 nm), pKI(degrees) = 5.798 + 666.7/T, e1 = 0.00244, e2= 2.734, and e3 = 0.1075. These two indicators can be used to make accurate pH measurements of freshwaters (river water, lake water, groundwater, rainwater, etc) within the range 4.5 less than or =pH less than or =8.5. The precision of pH measurements using phenol red in well-buffered freshwaters is on the order of +/-0.001 or better. [Yao W., et al; 2001]

Two simple, sensitive and accurate spectrophotometric methods for the determination of loperamide hydrochloride (lop. HCl) are described. The first method is based on the formation of ion-pair association complex (1:1) with bromothymol blue (BTB), bromophenol blue (BPB) and naphthol blue black B (NBB). The coloured products are extracted into chloroform, and measured spectrophotometrically at 414 (BTB), 415 (BPB) and 627 nm (NBB). Beer's law was obeyed in the ranges of 5–35, 5–30 and 0.8–11.2 μg ml^{-1} for BTB, BPB and NBB methods, respectively. The method was found to be specific for the analysis of the drug in presence of its degradation products which can be detected by HPLC procedure. The second method is based on the reaction of the basic loperamide with iodine in chloroform to give molecular charge-transfer complex with intense bands at 295 and 363 nm. Beer's law was obeyed in the ranges 2.5–17.5 and 2.5–22.5 μg ml^{-1} for the method at 295 and 363 nm, respectively. Optimization of the different experimental conditions are described for both methods. The proposed methods have been

applied successfully for the analysis of the drug in pure form and in its dosage forms. The methods have the advantage of being highly sensitive and simple for the determination of a small dose drug, which is also a weak UV-absorbing compound. [El-sherif ZA et al; 2000]

A simple and sensitive method for the determination of clonidine in dosage forms is presented. The method is based on colour reaction of clonidine with bromocresol green, whereby a yellow coloured ion pair is formed. [Zivanov SD et al; 1990]

An ion-pair extraction technique is described for separating methenamine, a urinary tract antibacterial agent, from formaldehyde in human urine samples. Separation conditions are developed from extraction constants for the methenamine-bromocresol green ion-pair. The technique involves adsorption of the ion-pair on to a silica cartridge and elution with methylene chloride:1-pentanol (95:5). Methenamine is freed from the ion-pair by the addition of excess tetrabutylammonium iodide and converted to formaldehyde (determined spectrophotometrically) by reaction with ammonia and acetylacetone. Linear standard plots were obtained from urine containing methenamine which was diluted to 10-160 µg/ml. The lower limit of detection was 6 µg/ml of methenamine. Absolute recovery from urine was greater than or equal to 94.5%. The precision (CV) of detection of methenamine in the presence of formaldehyde was less than 2%, and less than or equal to 4.5% for the detection of formaldehyde in the presence of methenamine. No interferences were noted. The applicability of the method was demonstrated by analysis of human urine levels of both methenamine and formaldehyde following oral administration of a methenamine salt to a volunteer. [Strom JG Jr et al; 1986]

A spectrophotometric method was developed for determining promethazine hydrochloride (I) complexed with bromcresol green and then extracted with chloroform. The complex in chloroform showed maximum absorption at 415 nm and obeyed Beer's law over 1.2-8.5 µg/ml. The complex molar absorptivity was 1.93 X 10(4) M. Complex formation and extraction

were complete and quantitative over pH 2.7-2.8. The promethazine hydrochloride-bromcresol green molar ratio was 1:1. Excipients, coloring matter, flavoring agents, and other substances likely to be present in promethazine preparations did not interfere in the determination. Direct determination in tablet, syrup, and injection preparations were carried out satisfactorily. [Emami Khoi AA; 1983]

Simple and sensitive spectrophotometric methods are described for the assay of three piperazine derivatives; ketoconazole, piribedil and prazosin hydrochloride based on charge-transfer and ion-pair complexation reactions. The first method is based on the reaction of the basic drug with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) in acetonitrile. The orange-red colour formed due to the formation of charge-transfer complex showed maximum absorbance at 460 nm. The second method is based upon the interaction of the basic drug in dry chloroform with bromophenol blue (BPB) in the same solvent to produce a stable yellow ion-pair complex which absorbs at 410 nm. Beer's law was obeyed for both methods and the relative standard deviations were found to be less than 1%. The two methods can be applied to the analysis of tablets, with no evidence of interference from excipients. A more detailed investigation of the complex was made with respect to its composition, association constant and free energy change. [Abdel-Gawad FM; 1997]

A simple and rapid colorimetric method for the assay of amodiaquine hydrochloride, chloroquine phosphate and primaquine phosphate is described. The method is based on the interaction of the drug base with bromophenol blue to give a stable ion-pair complex. The spectra of the complex shows a maxima at 415-420 nm with high apparent molar absorptivities. Beer's law was obeyed in the concentration range 1-8, 2-10 and 2-12 µg/ml for amodiaquine hydrochloride, primaquine phosphate and chloroquine phosphate respectively. The proposed method was applied to the determination of these drugs in certain formulations and the results were favourably comparable to the official methods. [El-Ashry SM et al; 1994]

Hydrastine, canadine, and berberine were determined by the acid-dye technique. At pH 5.6, both the tertiary and the quaternary hydrastis alkaloids formed ion-pairs with bromcresol purple. The liberated hydrastine and canadine from the alkaloid-dye complexes were determined spectrophotometrically in the presence of berberine by the three-wavelength method of analysis. However, berberine could not be assayed successfully by this method due to significant partitioning in the aqueous phase during extraction. At pH 7.2, only berberine could ion-pair with bromcresol purple to form a chloroform-extractable complex. Consequently, the berberine content was analyzed selectively through the colorimetric determination of the combined dye at 580 nm. The suitability of the proposed methods was examined through the analyses of synthetic mixtures of hydrastis alkaloids and samples of hydrastis tincture and liquid extract. Percentage recoveries were 98.2-101.4 for the synthetic mixtures and 98.4-101.7 for the tincture and liquid extract spiked with berberine. [El-Masry S et al; 1980]

Two spectrophotometric methods are described for the determination of guanethidine sulphate (I), guanfacine hydrochloride (II), guanoclor sulphate (III), guanoxan sulphate (IV) and debrisoquine sulphate (V). The first method involves ion-pair formation of the selected compounds (I–V) with bromocresol purple at pH 3.8. The yellow ion pair is extracted with chloroform and the absorbance is measured at about 415 nm. The second method is based on the reaction of the basic guanidino compounds (I, III–V) with iodine in chloroform to give molecular charge-transfer complexes with maximum absorbance at 292 and 345 nm. Beer's law was obeyed for both methods and the relative standard deviations were found to be less than 2%. The apparent molar absorptivities were found to be 2.1×10^4 to 6.9×10^4 l mol^{-1} cm^{-1} using bromocresol purple and 0.7×10^4 to 2.4×10^4 l mol^{-1} cm^{-1} using iodine. The investigated drugs were assayed in tablets. The mean percentage recoveries were found to be 99.8–100.8% by the acid-dye method and around 100.4% by the charge-transfer complexation method. [Wahbi AAM et al; 1993]

This procedure for routine quantification of albumin in urine is based on the dye-binding properties of albumin with bromphenol blue. The absorbance

of 100 microL of urine mixed with 3 mL of color reagent is measured against blank reagent at 610 nm after 30 s. Results vary linearly with albumin concentration up to 6 g/L. The reaction is pH independent in the physiological range. It is not subject to substantial interference by uric acid, creatinine, calcium, sodium chloride, or bilirubin. The presence of globulins produces a small positive error. Within-run precision (CV) was 4.8, 1.5, and 0.9%, and day-to-day precision was 11.2, 2.0, and 1.9%, for samples containing albumin at about 0.1, 1.0, and 6.0 g/L, respectively. Results by a radial-immunodiffusion method (x) correlated well with those by the proposed method (y): r = 0.986; y = 0.98x + 0.096; n = 64. The method can also be used to detect globulins, such as Bence Jones protein, by measuring the ratio of the absorbance at 30 min to that at 30 s. [Schosinsky KH et al;1987]

A quick method for the determination of pilocarpine in eye drops in the presence of decomposition products is described. The method involves complexation of the alkaloid with bromocresol purple at pH 6. After treatment with 0.1N NaOH, the liberated dye is measured at 580 nm. The method has a relative standard deviation of 1.99%, and has been successfully applied to the analysis of 2 batches of pilocarpine eye drops. The recommended method was also used to monitor the stability of a pilocarpine nitrate solution in 0.05N NaOH at 65 degrees C. The BPC method failed to detect any significant decomposition after 2 h incubation, but the recommended method revealed 87.5% decomposition. [El-Masry S. et al; 1980]

A visible light spectrophotometric method is described for the determination of sparfloxacin in tablets. The procedure is based on the complexation of bromothymol blue 0.5% and sparfloxacin to form a compound of yellow colour with maximum absorption at 385 nm. The Lambert–Beer law was obeyed in the concentration range of 2–12 mg/l. The present study describes a sensitive and accurate method for the determination of the concentration of sparfloxacin in tablets. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay. [Marona HRN et al; 2001]

A new simple and rapid flow injection method is reported for the direct determination of bismuth in pharmaceutical products. Methylthymol Blue (MTB) was used as a chromogenic reagent and the absorbance of the colored Bi(III)-MTB complex produced was monitored at 548 nm. The various chemical and physical variables were optimized and a study of interfering ions was also carried out. Linear calibration graphs were obtained from 0 to 100 mg l-1 Bi(m) (120 injections per hour). The precision was very good (sr = 1.3%) and the limit of detection was cL = 0.150 mg l-1. The average accuracy was also very good (er = 0.75%) and was evaluated by comparison of the results obtained with those claimed by the manufacturers. The method was found to be adequately selective, considering the ions that the samples contain. [Themelis D G et al; 2001]

Table 2.22. Estimation of drugs using ion pair complex methods

Sr. No.	Specification	Reference
01	Utility of the ion-pair formation for spectrophotometric determination of Terfenadine in pure form and in some pharmaceutical formulations	Amin AS et al; 1999
02	Spectrophotometric Methods for the Determination of the Antidepressant Drug Paroxetine Hydrochloride in Tablets	Armagan O et al; 2005
03	Extractive spectrophotometric methods for determination of Lercanidipine.	Erk N;2003
04	A simple spectrophotometric method for the determination of α, β-blockers in dosage forms	Al-Ghannam SM; 2006
05	Spectrophotometric determination of Oxiconazole in topical lotion using methyl orange.	Milano J. et al; 2005
06	Ion-pair extraction and precipitation methods for Ethambutol determination	Girgis EH et al; 1974

07	Extractive Spectrophotometric Methods for the Determination of Oxomemazine Hydrochloride in Bulk and Pharmaceutical Formulations Using Bromocresol Green, Bromocresol Purple and Bromophenol Blue	Akram ME; 2005
08	Spectrophotometric determination of enrofloxacin and pefloxacin through ion-pair complex formation	Mostafa S et al; 2002
09	Spectrophotometric determination of pefloxacin in pharmaceutical preparations	Jelikic SM et al; 1989
10	Simple spectrophotometric determination of cinnarizine in its dosage forms.	Abdine H et al; 2002
11	Sensitive Extractive Spectrophotometric Methods for the Determination of Trazodone Hydrochloride in Pharmaceutical Formulations	Ramanaboyina SK et al; 2006
12	Extractive spectrophotometric determination of sildenafil citrate in pharmaceutical dosage forms	Reddy MN et al; 2002
13	Spectrophotometric determination of Fluoxetine hydrochloride in bulk and in pharmaceutical formulations	Prabhakar AH et al; 1999
14	Spectrophotometric, spectrofluorimetric and LC determination of trazodone hydrochloride.	El-Gindy A. et al; 2001
15	Spectrophotometric determination of strychnine and brucine in liquid galenicals.	El-Massy S. et al; 1978
16	Determination of content of chlorpromazine hydrochloride in compound metamizole sodium microenema by acid-dye colorimetric method	Zhou Z. et al; 1996
17	Determination of meclizine hydrochloride by ion-pair extraction with methyl orange.	Hom FS et al; 1977
18	Determination of meclizine hydrochloride by ion-pair extraction with methyl orange.	Saleh MR et al; 1980
19	Determination of protein concentration in human saliva	Lin LY et al; 1989

20	Spectrophotometric determination of sulfur dioxide in air, using thymol blue.	Gayathri N. et al; 2001
21	Determination of methotrimeprazine in pharmaceutical preparations by visible spectrophotometry	Kedor-Hackmann ERM et al; 2000
22	Spectrophotometric determination of tilidine using bromocresol green and bromophenol blue.	Dobrila ZS et al; 1990
23	Extractive spectrophotometric determination of ceterizine HCl in pharmaceutical preparations.	Gowda BG et al; 2001
24	Spectrophotometric determination of freshwater pH using bromocresol purple and phenol red.	Yao W., et al; 2001
25	Spectrophotometric determination of loperamide hydrochloride by acid-dye and charge-transfer complexation methods in the presence of its degradation products	El-sherif ZA et al; 2000
26	Spectrophotometric determination of clonidine in dosage forms using bromocresol green.	Zivanov SD et al; 1990
27	Separation and quantitation of methenamine in urine by ion-pair extraction.	Strom JG Jr et al; 1986
28	Spectrophotometric promethazine hydrochloride determination using bromcresol green.	Emami Khoi AA; 1983
29	Spectrophotometric determination of some pharmaceutical piperazine derivatives through charge-transfer and ion-pair complexation reactions.	Abdel-Gawad FM; 1997
30	Studies of complex formation between the bromophenol blue and some important aminoquinoline antimalarials.	El-Ashry SM et al; 1994
31	Colorimetric and spectrophotometric determinations of hydrastis alkaloids in pharmaceutical preparations.	El-Masry S et al; 1980

32	Spectrophotometric analysis of some guanidino drugs by acid-dye and charge-transfer complexation methods	Wahbi AAM et al; 1993
33	Simple spectrophotometric determination of urinary albumin by dye-binding with use of bromphenol blue.	Schosinsky KH et al;1987
34	New spectrophotometric assay for pilocarpine.	El-Masry S. et al; 1980
35	Spectrophotometric determination of sparfloxacin in pharmaceutical formulations using bromothymol blue	Marona HRN et al; 2001
36	Flow injection manifold for the direct spectrophotometric determination of bismuth in pharmaceutical products using Methylthymol Blue as a chromogenic reagent.	Themelis D G et al; 2001

3.

AIM OF THE PRESENT WORK…

3. AIM OF PRESENT WORK

Rapid strides have been made in the area of analysis of pharmacologically active molecules. Recent advancements in the area of modern analytical techniques include HPLC, LC – MS, GLC, GC – MS, HPTLC, and Ion pair chromatography. These methods are time consuming and require sophisticated instruments and chemicals, which are too costly to afford especially by small – scale industries.

Selective serotonin reuptake inhibitors and angiotensin II receptor antagonists are prime drugs in the field of antidepressants and antihypertensive therapy. A very few analytical methods have been reported. Therefore it was thought of interest to develop spectrophotometric method for the estimation of these drugs.

The proposed research project is aimed at developing accurate, precise, and reliable methods for the estimation of selective serotonin reuptake inhibitors and angiotensin II receptor antagonists in their pharmaceutical dosage forms. The major approach taken into consideration was to develop chromophore using acid – dye and basic – dye technique and planned to develop simple UV – visible spectrophotometric methods for the estimation of pharmaceutical dosage forms.

The project work was planned considering following steps:

1. A simple colorimetric acid dye technique was developed and validated.
2. The proposed method was applied for the analysis of market formulations.
3. A simple colorimetric basic dye technique was developed and validated.
4. The developed method was applied for analysis of valsartan tablets and its combined dosage forms.
5. Dissolution profile study of pharmaceutical formulations (Fluoxetine hydrochloride and Sertraline hydrochloride) was carried out using developed acid dye technique.
6. The developed basic dye technique was applied to study the dissolution profile of valsartan.

GLIMPESES OF THE RESEARCH PROJECT WORK

1. Fluoxetine hydrochloride

(a) A simple colorimetric acid dye technique for the estimation of fluoxetine hydrochloride in pharmaceutical dosage forms.

(b) Dissolution profile and in – vitro evaluation of dosage form containing fluoxetine hydrochloride.

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2. Sertraline hydrochloride

(a) A colorimetric acid dye technique for the estimation of sertraline hydrochloride in pharmaceutical dosage forms.

(b) Dissolution profile and in – vitro evaluation of dosage form containing sertraline hydrochloride.

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3. Duloxetine hydrochloride

(a) A colorimetric acid dye technique for the estimation of duloxetine hydrochloride in pharmaceutical dosage forms.

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4. Valsartan

(a) A colorimetric basic dye technique for the estimation of valsartan.

(b) Determination of valsartan in combined dosage form with hydrochlorthiazide using basic dye technique.

(c) Determination of valsartan in combined dosage form with S-Amlodipine using basic dye technique.

(a) Dissolution profile and in – vitro evaluation of valsartan in various pharmaceutical dosage form.

4.

EXPERIMENTAL…

4. EXPERIMENTAL

4.1. Apparatus.

- Double beam spectrophotometer (Shimadzu, model UV – 1601 with 1.0 cm matched quartz cells was employed for spectral measurements.)
- pH meter (Systronic)
- Monopan balance (Sartorius)
- Sonicator (Frontline, model FS – 4)
- Cyclomixer (Remi)
- Distillation assembly (Janaki Impex Pvt. Ltd.)
- Constant temperature water bath (Cintex)

4.2. Reagents and Materials.

- Fluoxetine hydrochloride (Gift sample from Torrent Pharmaceuticals Limited, Ahmedabad)
- Sertraline hydrochloride (Gift sample from Restac Pharmaceuticals, Ahmedabad)
- Duloxetine hydrochloride (Gift sample from Sun Pharma, Baroda)
- Valsartan (Gift sample from Torrent Pharmaceuticals Limited, Ahmedabad)
- S – Amlodepine besylate (Gift sample from Cadila Pharmaceuticals, Ahmedabad)
- Hydrochlorothiazide (Gift sample from Astron pharmaceuticals, Ahmedabad)
- Ethanol (Baroda chemical Ind. Ltd. Dabhoi)
- Safranin O (BDH chemicals Ltd., England)
- Methyl orange, Anhydrous sodium sulphate, Chloroform, Sodium hydroxide, Potassium di hydrogen phosphate, Potassium hydrogen phthalate, Potassium chloride, Methanol (SD's Fine Chemicals Pvt. Ltd.)

- Whatman filter paper no. 42
- Double Distilled water.
- The dosage forms of all the drugs were procured from local market.

4.3. Spectrophotometric determination of Fluoxetine hydrochloride.

4.3.1. Preparation of 0.1N Hydrochloric acid.

Hydrochloric acid (8.5 ml) was diluted to 1000 ml with distilled water.

4.3.2. Preparation of standard solution of Fluoxetine hydrochloride.

Accurately weighed Fluoxetine hydrochloride (50.0 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (10.0 ml) and diluted to 100.0 ml with 0.1N Hydrochloric acid. The solution (25.0 ml) was diluted further to 100 ml with 0.1N Hydrochloric acid to give a solution having strength of 125.0 µg/ml.

4.3.3. Preparation of methyl orange solution.

Accurately weighed methyl orange (50.0 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (40.0 ml) and diluted to 100 ml with distilled water to give 0.05 % w/v solution of methyl orange.

Washing of the methyl orange solution

The above dye solution was washed by 3 X 10 ml of chloroform to remove chloroform soluble impurities and minimize absorption value of reagent blank.

4.3.4. Preparation of buffer solution.

The various buffer solutions (pH 1.0 – 8.0) were prepared (Table 4.1)

Table 4.1. Preparation of buffer solutions (pH 1.0 – 8.0) [http://delloyd]

pH	Method of preparation
1.0	0.2M potassium chloride (50.0 ml) + 0.2M HCl (134.0 ml)
2.0	0.2M potassium chloride (50.0 ml) + 0.2M HCl (13.0 ml)
3.0	0.1M potassium hydrogen phthalate (100.0 ml) + 0.1M HCl (44.6 ml)
4.0	0.1M potassium hydrogen phthalate (100.0 ml) + 0.1M HCl (0.2 ml)
5.0	0.1M potassium hydrogen phthalate (100.0 ml) + 0.1M NaOH (45.2 ml)
6.0	0.1M potassium di hydrogen phosphate (100.0 ml) + 0.1M NaOH (11.2 ml)
7.0	0.1M potassium di hydrogen phosphate (100.0 ml) + 0.1M NaOH (58.2 ml)
8.0	0.1M potassium di hydrogen phosphate (100.0 ml) + 0.1M NaOH (93.4 ml)

4.3.5. Determination of wavelength of maximum absorbance of Fluoxetine hydrochloride.

Standard solution of Fluoxetine hydrochloride (0.5 ml) was transferred into a glass stoppered tube containing 2.0 ml of methyl orange dye solution. The volume was adjusted to 5.0 ml with 0.1N Hydrochloric acid. Chloroform (5.0 ml) was added and Mixed well for one minute using cyclomixer. The drug – dye ion pair complex was extracted in chloroform. The chloroform layer was separated out, dried over anhydrous sodium sulphate (1.0 gm). The absorbance of the colored solution was scanned on spectrophotometer in the range of 400 – 800 nm against reagent blank. The blank was prepared similarly in which volume of standard solution of fluoxetine hydrochloride was replaced by an equal volume of 0.1N hydrochloric acid.

Maximum absorbance was obtained at 426.0 nm (Fig. 5.1).

4.3.6. Calibration curve for Fluoxetine hydrochloride.

Standard solutions of Fluoxetine hydrochloride (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml) were transferred into a series of glass stoppered tubes, each containing 2.0 ml of methyl orange solution. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.3.5. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank.

The Lambert – Beer's law was obeyed in the concentration range of 5.0 – 30.0 µg/ml (Table 5.1, Fig. 5.4).

4.3.7. Factors affecting the development of color.

4.3.7.1. Effect of pH in buffer and 0.1 N hydrochloric acid.

Standard solutions of Fluoxetine hydrochloride (0.5 ml) was transferred in to a series of glass stoppered tubes containing 2.0 ml of methyl orange solution. The volumes were adjusted with buffer solution (5.0 ml) having pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 8.0 and 0.1N hydrochloric acid respectively and analysed as described under 4.3.5. The absorbance of the reaction mixtures were measured at 426.0 nm against reagent blank.

Maximum absorbance was obtained in presence of 0.1N hydrochloric acid and 1 to 4 pH buffer. (Table 5.4, Fig. 5.7)

4.3.7.2. Effect of Concentration of methyl orange solution.

Standard solution of Fluoxetine hydrochloride (0.5 ml) was transferred into a series of glass stoppered tube containing (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml) of methyl orange solution. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.3.5. The absorbance of the reaction mixtures were measured at 426.0 nm against reagent blank.

Maximum absorbance was obtained in presence of 2.0 ml of methyl orange solution (Table 5.5, Fig. 5.8).

4.3.7.3. Color Stability.

Standard solution of Fluoxetine hydrochloride (0.5 ml) was transferred into a glass stoppered tube containing 2.0 ml of methyl orange solutions. The volume was adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.3.5. The absorbance of the colored solution was measured at 426.0 nm against reagent blank at (1, 2, 3, 4, 8, 12, 24, 36, and 48 hours) time interval.

The color remained stable for 48 hours (Table 5.6, Fig. 5.9).

4.3.8. Validation of Proposed method

4.3.8.1. Linearity

It is the ability of the method within a given range to obtain best result, which is directly proportional to the concentration of analyte in the sample.

The absorbance value obtained at 426.0 nm of different concentration of fluoxetine hydrochloride was used to plot a calibration curve. The linearity range for fluoxetine hydrochloride was found to be 5.0 – 30.0 µg/ml and correlation coefficient was found to be 0.9991 (Table 5.1, Fig. 5.4).

4.3.8.2. Accuracy

The accuracy of the analytical method is closeness of the result obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amount of analyte. Accuracy is a measure of the exactness of the analytical method.

Accuracy data of Fluoxetine hydrochloride

Pre analyzed sample solution (A) = 125 μg/ml
Standard drug solution (B) = 125 μg/ml
Sample solutions were prepared from above solutions.

1) 0.2ml of solution A + 0.0ml of solution B
2) 0.2ml of solution A + 0.2ml of solution B
3) 0.2ml of solution A + 0.4ml of solution B
4) 0.2ml of solution A + 0.6ml of solution B
5) 0.2ml of solution A + 0.8ml of solution B

The above series of solutions (1 to 5) were transferred in to glass stoperred tubes. Methyl orange (2.0 ml) was added and the volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.3.5. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank.

The % recovery experiment revealed good accuracy. The % recovery of fluoxetine hydrochloride was found to be 99.68 % – 101.88 % (Table 5.7).

4.3.8.3. Precision

Precision is the measure of either the degree of reproducibility or repeatability of the analytical method. It is expressed as the standard deviation or relative standard deviation (co-efficient of variance).

4.3.8.3.1. Repeatability of measurement of absorbance

Standard solutions of Fluoxetine hydrochloride (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml) were transferred into a series of glass stoppered tubes, each containing 2.0 ml of methyl orange solution. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid. The drug – dye ion pair complex were

extracted similarly as described under 4.3.5. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank. The absorbance of the same solutions was measured seven times and % CV was calculated (Table 5.10).

4.3.8.3.2. Intra day and Inter day precision

Variation of results within the same day is called intra day variation, and variation of results within days is called inter day variation. Intra day precision was determined by analyzing drug as per the procedure described in 4.3.5 for three times in the same day. The inter day precision was determined by analyzing the fluoxetine hydrochloride as per the procedure described in 4.3.5 daily for three days (Table 5.13).

4.3.8.4. Limit of detection (LOD)

It is the lowest concentration of analyte in a sample that can be detected, but not necessarily be quantified under the stated experimental condition.

Different concentrations of standard drug solution were used and minimum detectable limit was found. LOD value was calculated from the calibration curve using equation LOD = 3.3 SD / b. (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOD was found to be 0.9803 (Table 5.20)

4.3.8.5. Limit of quantitation (LOQ)

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy under stated experimental condition. LOQ value was calculated from the calibration graph using equation LOQ = 10 SD / b (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOQ was found to be 2.9707 (Table 5.20).

4.3.8.6. Specificity

It is the ability of the developed analytical method to detect analyte quantitatively in the presence of other component, which are expected to be present in the sample matrix or other related substances.

4.3.8.7. Job's method for determination of Stoichiometry

Job's method, also called the method of continuous variation, is a simple and effective approach to the determination of chemical reaction at stoichiometry. In Job's continuous method equimolar solutions of drug and dye are used.

Preparation of working standard drug solution

The working standard solution of Fluoxetine hydrochloride 125 μg/ml was used. Its concentration was equal to 3.615 X 10^{-7} M/ml.

Preparation of Methyl orange solution: 3.615 × 10^{-7} M/ml

Accurately weighed quantity of methyl orange (11.833 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (40.0 ml) and diluted to 100 ml with distilled water.

Procedure

Standard solutions Fluoxetine hydrochloride (0.0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0ml) were transferred in a series of glass stoppered tube. The volume of each glass stoppered tube was adjusted up to 1.0 ml with methyl orange solution to get mole fraction of Fluoxetine hydrochloride as 0.0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.3.5.

It was found that Fluoxetine hydrochloride formed 1:1 complex with methyl orange (Table 5.16, Fig. 5.10).

4.3.8.8. Analysis of marketed formulations

For tablets: Twenty tablets were accurately weighed and powdered.

For capsules: Twenty capsules were weighed and removed powder from shell. Then shell were weighed and the weight of the powder obtained by deducting weight of the shell from weight of capsules.

A quantity of the powder equivalent to 50.0 mg of Fluoxetine hydrochloride was transferred into 100 ml volumetric flask containing ethanol (10.0 ml) and sonicated for five minutes. Volume was adjusted to 100 ml with 0.1N hydrochloric acid and filtered through Whatman filter paper no. 42.

The solutions (0.5 and 0.8 ml) were transferred into glass stoppered tubes containing 2.0 ml methyl orange solution. The volume was adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.3.5. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank.

The amount of Fluoxetine hydrochloride was determined by referring to calibration curve (Table 5.17).

4.4. Spectrophotometric determination of Sertraline hydrochloride.

4.4.1. Preparation of 0.1N Hydrochloric acid.

As described under 4.3.1.

4.4.2. Preparation of standard solution of Sertraline hydrochloride.

Accurately weighed Sertraline hydrochloride (50.0 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (10.0 ml) and diluted to 100.0 ml with 0.1N Hydrochloric acid. The solution (10.0 ml) was diluted further to 100 ml with 0.1N hydrochloric acid to give a solution having strength of 50.0 µg/ml.

4.4.3. Preparation of methyl orange solution.

As described under 4.3.3.

4.4.4. Preparation of buffer solution.

As described under 4.3.4.

4.4.5. Determination of wavelength of maximum absorbance of Sertraline hydrochloride.

Standard solution of Sertraline hydrochloride (0.5 ml) was transferred into a glass stoppered tube containing 2.0 ml of methyl orange dye solution. The volume was adjusted to 5.0 ml with 0.1N Hydrochloric acid. Chloroform (5.0 ml) was added and Mixed well for one minute using cyclomixer. The drug – dye ion pair complex was extracted in chloroform. The chloroform layer was separated out, dried over anhydrous sodium sulphate (1.0 gm). The absorbance of the colored solution was scanned on spectrophotometer in the range of 400.0 – 800.0 nm against reagent blank The blank was prepared similarly in which volume of standard solution of sertraline hydrochloride was replaced by 0.1 N hydrochloric acid.

Maximum absorbance was obtained at 425.0 nm (Fig. 5.2).

4.4.6. Calibration curve for Sertraline hydrochloride

Standard solutions of Sertraline hydrochloride (0.2, 0.4, 0.6, 0.8, 1.0 1.2 and 1.4ml) were transferred into a series of glass stoppered tubes, each containing 2.0 ml of methyl orange solution. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.4.5. The absorbance of the colored solutions was measured at 425.0 nm against reagent blank.

The Lambert – Beer's law was obeyed in the concentration range of 2.0 – 14.0 µg/ml (Table 5.2, Fig. 5.5).

4.4.7. Factors affecting the development of color.

4.4.7.1. Effect of pH in buffer and 0.1 N hydrochloric acid.

Standard solutions of Sertraline hydrochloride (0.5 ml) was transferred in to a series of glass stoppered tubes containing 2.0 ml of methyl orange solution. The volumes were adjusted with buffer solution (5.0 ml) having pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 8.0 and 0.1N hydrochloric acid respectively and analysed as described under 4.4.5. The absorbance of the reaction mixtures were measured at 425.0 nm against reagent blank.

Maximum absorbance was obtained in presence of 0.1N hydrochloric acid and at 1.0 pH buffer (Table 5.4, Fig. 5.7).

4.4.7.2. Effect of concentration of methyl orange solution

Standard solution of Sertraline hydrochloride (0.5 ml) was transferred in to a series of glass stoppered tube containing (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5ml) of methyl orange solutions. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.4.5. The absorbance of the reaction mixtures were measured at 425.0 nm against reagent blank.

Maximum absorbance was obtained in presence of 2.0 ml of methyl orange solution (Table. 5.5, Fig. 5.8).

4.4.7.3. Color Stability.

Standard solution of Sertraline hydrochloride (0.5 ml) was transferred into a glass stoppered tube containing 2.0 ml of methyl orange solutions. The volume was adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.4.5. The absorbance of the colored solution was measured at 425.0 nm against reagent blank at interval of (1, 2, 3, 4, 8, 12, 24, 36, and 48 hours).

The color intensity remained constant for 48.0 hours (Table. 5.6, Fig. 5.9).

4.4.8. Validation of Proposed method.

4.4.8.1. Linearity.

It is the ability of the method within a given range to obtain best result, which is directly proportional to the concentration of analyte in the sample.

The absorbance value obtained at 425.0 nm of different concentration of Sertraline hydrochloride was used to plot a calibration curve. The linearity range for Sertraline hydrochloride was found to be 2.0 – 14.0 µg/ml and correlation coefficient was found to be 0.9985 (Table. 5.2, Fig. 5.5).

4.4.8.2. Accuracy

The accuracy of the analytical method is closeness of the result obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amount of analyte. Accuracy is a measure of the exactness of the analytical method.

Accuracy data of Sertraline hydrochloride

Pre analyzed sample solution (A) = 50 μg/ml
Standard drug solution (B) = 50 μg/ml
Sample solutions were prepared from above solutions.

1) 0.2ml of solution A + 0.0ml of solution B
2) 0.2ml of solution A + 0.2ml of solution B
3) 0.2ml of solution A + 0.4ml of solution B
4) 0.2ml of solution A + 0.6ml of solution B
5) 0.2ml of solution A + 0.8ml of solution B

The above series of solutions (1 to 5) were transferred in to glass stoperred tubes. Methyl orange (2.0 ml) was added and the volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.4.5. The absorbance of the colored solutions was measured at 425.0 nm against reagent blank.

The % recovery experiment revealed good accuracy. The % recovery of Sertraline hydrochloride was found to be **98.48 %** to **101.78 %** (Table 5.8).

4.4.8.3. Precision.

Precision is the measure of either the degree of reproducibility or repeatability of the analytical method. It is expressed as the standard deviation or relative standard deviation (co-efficient of variance).

4.4.8.3.1. Repeatability of measurement of absorbance

Standard solutions of Sertraline hydrochloride (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4ml) were transferred into a series of glass stoppered tubes, each containing 2.0 ml of methyl orange solution. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid. The drug – dye ion pair complex were

extracted similarly as described under 4.4.5. The absorbance of the colored solutions was measured at 425.0 nm against reagent blank. The absorbance of the same solutions was measured seven times and % CV was calculated (Table 5.11).

4.4.8.3.2. Intra day and Inter day precision

Variation of results within the same day is called intra day variation, and variation of results within days is called inter day variation. Intra day precision was determined by analyzing drug as per the procedure described in 4.1.4.4. For three time in the same day. The inter day precision was determined by analyzing the Sertraline hydrochloride as per the procedure described in 4.1.4.4. daily for three days (Table 5.14).

4.4.8.4. Limit of detection (LOD)

It is the lowest concentration of analyte in a sample that can be detected, but not necessarily be quantified under the stated experimental condition.

Different concentrations of standard drug solution were used and minimum detectable limit was found. LOD value was calculated from the calibration curve using equation LOD = 3.3 SD / b. (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOD was found to be **0.9803** (Table 5.20)

4.4.8.5. Limit of quantification (LOQ)

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy under stated experimental condition. LOQ value was calculated from the calibration curve using equation LOQ = 10 SD / b (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOQ was found to be **2.9707** (Table 5.20).

4.4.8.6. Specificity.

It is the ability of the developed analytical method to detect analyte quantitatively in the presence of other component, which are expected to be present in the sample matrix or other related substances.

4.4.9. Job's method for determination of Stoichiometry.

Job's method, also called the method of continuous variation, is a simple and effective approach to the determination of chemical reaction at stoichiometry. In Job's continuous method equimolar solutions of drug and dye are used.

Preparation of working standard drug solution

The working standard solution of Sertraline hydrochloride 50 μg/ml was used. Its concentration was equal to 1.459 X 10^{-7} M/ml.

Preparation of Methyl orange solution: 1.459 × 10^{-7} M/ml

Accurately weighed methyl orange (5.324 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (40.0 ml) and diluted to 100 ml with distilled water.

Procedure

Standard solutions Sertraline hydrochloride (0.0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0ml) were transferred in a series of glass stoppered tube. The volume of each glass stoppered tube was adjusted up to 1.0 ml with methyl orange solution to get mole fraction of Sertraline hydrochloride as 0.0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.4.5.

It was found that Sertraline hydrochloride form 1:1 complex with methyl orange (Table. 5.16, Fig. 5.10).

4.4.10. Analysis of marketed formulation

Twenty tablets were accurately weighed and powdered. A quantity of the powder equivalent to 50.0 mg of Sertraline hydrochloride was transferred into 100 ml volumetric flask containing ethanol (10.0 ml) and sonicated for five minutes. Volume was adjusted to 100 ml with 0.1N hydrochloric acid and filtered through Whatman filter paper no.42. The solution (10.0 ml) was diluted further to 100 ml with 0.1N Hydrochloric acid. Aliquots (0.5 and 0.8 ml) were transferred into glass stoppered tubes containing 2.0 ml methyl orange solution. The volumes were adjusted to 5.0 ml with 0.1N Hydrochloric acid and analysed as described under 4.4.5. The absorbance of the colored solutions was measured at 425.0 nm against reagent blank.

The amount of Sertraline hydrochloride was determined by referring to the calibration curve (Table. 5.18).

4.5. Spectrophotometric determination of Duloxetine hydrochloride

4.5.1. Preparation of Phthalate Buffer (pH 2.2 - 4.0)

Potassium hydrogen phthalate, 0.2M (50 ml) was transferred into 200 ml volumetric flask, specified volume of 0.2M hydrochloric acid was added as per following table. The final volume was adjusted up to the mark with distilled water (IP 1996: A-144).

Table 4.2. Preparation of phthalate buffer (pH 2.2 – 4.0)

pH	Hydrochloric acid. 0.2M (ml)
2.2	49.5
2.4	42.2
3.0	22.3
3.2	15.7
3.6	6.3
4.0	0.1

4.5.2. Preparation of standard solution of Duloxetine hydrochloride

Accurately weighed Duloxetine hydrochloride (50.0 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (10.0 ml) and diluted to 100.0 ml with Phthalate buffer (pH 3.0). The solution (10.0 ml) was diluted further to 100.0 ml with the same solvent to give a stock solution having strength of 50.0 µg / ml.

4.5.3. Preparation of methyl orange solution.

As described under 4.3.3.

4.5.4. Determination of wavelength of maximum absorbance of Duloxetine hydrochloride

Standard solution of Duloxetine hydrochloride (0.5 ml) was transferred into a glass stoppered tube containing 2.0 ml of methyl orange dye solution. The volume was adjusted to 5.0 ml with Phthalate buffer (pH 3.0). Chloroform (5.0 ml) was added and Mixed well for one minute using cyclomixer. The drug – dye ion pair complex was extracted in chloroform. The chloroform layer was separated out, dried over anhydrous sodium sulphate (1.0 gm). The absorbance of the colored solution was scanned on spectrophotometer in the range of 400 – 800 nm against reagent blank. The blank was prepared similarly in which volume of standard solution of duloxetine hydrochloride was replaced by an equal volume of phthalate buffer (pH 3.0).

Maximum absorbance was obtained at 426.0 nm (Fig. 5.3).

4.5.5. Calibration curve for Duloxetine hydrochloride

Standard solutions of Duloxetine hydrochloride (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) were transferred into a series of glass stoppered tubes, each containing 2.0 ml of methyl orange solution. The volumes were adjusted to 5.0 ml with Phthalate buffer (pH 3.0) and analysed as described under 4.5.4. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank.

The Lambert – Beer's law was obeyed in the concentration range of 2.0 – 12.0 µg/ml (Table. 5.3, Fig. 5.6).

4.5.6. Factors affecting the development of color

4.5.6.1. Effect of pH of Buffer

Standard solutions of Duloxetine hydrochloride (0.5 ml) was transferred in to a series of glass stoppered tubes containing 2.0 ml of methyl orange

solution. The volumes were adjusted to 5.0 ml with phthalate buffer having pH (2.2, 2.6, 3, 3.2, 3.6 and 4.0) and analysed as described under 4.5.4. The absorbance of the reaction mixtures were measured at 426.0 nm against reagent blank.

Maximum absorbance was obtained in presence of phthalate buffer (pH 3.0) (Table. 5.4, Fig. 5.7).

4.5.6.2. Effect of Concentration of methyl orange solution

Standard solution of Duloxetine hydrochloride (0.5 ml) was transferred into a series of glass stoppered tube containing (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5ml) of methyl orange solutions. The volumes were adjusted to 5.0 ml with Phthalate buffer (pH 3.0) and analysed as described under 4.5.4. The absorbance of the reaction mixtures were measured at 426.0 nm against reagent blank.

Maximum absorbance was obtained in presence of 2.0 ml of methyl orange solution (Table. 5.8, Fig. 5.5).

4.5.6.3. Color Stability.

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Standard solution of Duloxetine hydrochloride (0.5 ml) was transferred into a glass stoppered tube containing 2.0 ml of methyl orange solutions. The volume was adjusted to 5.0 ml with Phthalate buffer (pH 3.0) and analysed as described under 4.5.4. The absorbance of the colored solution was measured at 426.0 nm against reagent blank at interval of (1, 2, 3, 4, 8, 12, 24, 36, and 48 hours).

The color intensity remained constant for 48 hours (Table. 5.6, Fig. 5.9).

4.5.7. Validation of proposed method.

4.5.7.1. Linearity.

It is the ability of the method within a given range to obtain best result, which is directly proportional to the concentration of analyte in the sample.

The absorbance value obtained at 426.0 nm of different concentration of Duloxetine hydrochloride was used to plot a calibration curve. The linearity range for Duloxetine hydrochloride was found to be 2.0 – 12.0 µg/ml and correlation coefficient was found to be 0.9988 (Table. 5.3, Fig. 5.6).

4.5.7.2. Accuracy

The accuracy of the analytical method is closeness of the result obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amount of analyte. Accuracy is a measure of the exactness of the analytical method.

Accuracy data of Duloxetine hydrochloride

Pre analyzed sample solution (A) = 50 µg/ml
Standard drug solution (B) = 50 µg/ml
Sample solutions were prepared from above solutions.

1) 0.2ml of solution A + 0.0ml of solution B
2) 0.2ml of solution A + 0.2ml of solution B
3) 0.2ml of solution A + 0.4ml of solution B
4) 0.2ml of solution A + 0.6ml of solution B
5) 0.2ml of solution A + 0.8ml of solution B

The above series of solutions (1 to 5) were transferred in to glass stoperred tubes. Methyl orange (2.0 ml) was added and the volumes were

adjusted to 5.0 ml with phthalate buffer (pH 3.0) and analysed as described under 4.5.4. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank.

The % recovery experiment revealed good accuracy. The % recovery of Duloxetine hydrochloride was found to be 99.08 % to 101.89 % (Table 5.9).

4.5.7.3. Precision

Precision is the measure of either the degree of reproducibility or repeatability of the analytical method. It is expressed as the standard deviation or relative standard deviation (co-efficient of variance).

4.5.7.3.1. Repeatability of measurement of absorbance

Standard solutions of Duloxetine hydrochloride (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) were transferred into a series of glass stoppered tubes, each containing 2.0 ml of methyl orange solution. The volumes were adjusted to 5.0 ml with Phthalate buffer (pH 3.0). The drug – dye ion pair complex were extracted similarly as described under 4.5.4. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank. The absorbance of the same solutions was measured seven times and % CV was calculated (Table 5.12).

4.5.7.3.2. Intra day and Inter day precision

Variation of results within the same day is called intra day variation, and variation of results within days is called inter day variation. Intra day precision was determined by analyzing drug as per the procedure described in 4.5.4. For three time in the same day. The inter day precision was determined by analyzing the Duloxetine hydrochloride as per the procedure described in 4.5.4. daily for three days (Table 5.15).

4.5.7.4. Limit of detection (LOD)

It is the lowest concentration of analyte in a sample that can be detected, but not necessarily be quantified under the stated experimental condition.

Different concentrations of standard drug solution were used and minimum detectable limit was found. LOD values was calculated from the calibration graph using equation LOD = 3.3 SD / b. (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOD was found to be 0.4902 (Table 5.21)

4.5.7.5. Limit of quantification (LOQ)

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy under stated experimental condition. LOQ value was calculated from the calibration graph using equation LOQ = 10 SD / b (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOQ was found to be 1.4854 (Table 5.21).

4.5.7.6. Specificity

It is the ability of the developed analytical method to detect analyte quantitatively in the presence of other component, which are expected to be present in the sample matrix or other related substances.

4.5.8. Job's method for determination of Stoichiometry

Job's method, also called the method of continuous variation, is a simple and effective approach to the determination of chemical reaction at stoichiometry. In Job's continuous method equimolar solutions of drug and dye are used.

Preparation of working standard drug solution

The working standard solution of Duloxetine hydrochloride (50.0 μg/ml) was used. Its concentration was equal to 1.498 X 10^{-7} M/ml.

Preparation of Methyl orange solution: 1.498 × 10^{-7} M/ml

Accurately weighed methyl orange (4.902 mg) was transferred into 100 ml volumetric flask, dissolved in ethanol (40.0 ml) and diluted to 100 ml with distilled water.

Procedure

Standard solutions of Duloxetine hydrochloride (0.0, 0.2, 0.4, 0.5. 0.6, 0.8, and 1.0ml) were transferred in a series of glass stoppered tube. The volume of each glass stoppered tube was adjusted up to 1.0 ml with methyl orange solution to get mole fraction of Duloxetine hydrochloride as 0.0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0. The volumes were adjusted to 5.0 ml with Phthalate buffer (pH 3.0) and analysed as described under 4.5.4.

It was found that Duloxetine hydrochloride form 1:1 complex with methyl orange (Table 5.16, Fig. 5.10).

4.5.9. Analysis of marketed formulations

For tablets: Twenty tablets were accurately weighed and powdered.

For capsules: Twenty capsules were weighed and removed powder from shell. Then shell were weighed and the weight of the powder obtained by deducting weight of the shell from weight of capsules.

A quantity of the powder equivalent to 50.0 mg of Duloxetine hydrochloride was transferred into 100 ml volumetric flask containing ethanol (10.0 ml) and sonicated for five minutes. Volume was adjusted to 100 ml with Phthalate buffer (pH 3.0) and filtered through Whatman filter paper no.42. The

solution (10.0 ml) was diluted further to 100 ml with Phthalate buffer (pH 3.0) to give a solution having strength of 50.0 µg / ml.

Aliquots (0.5 and 0.8 ml) were transferred into glass stoppered tubes containing 2.0 ml methyl orange solution. The volume was adjusted to 5.0 ml with Phthalate buffer (pH 3.0) and analysed as described under 4.5.4. The absorbance of the colored solutions was measured at 426.0 nm against reagent blank.

The amount of Duloxetine hydrochloride was determined by referring to calibration curve (Table 5.19).

4.6. Spectrophotometric determination of Valsartan (Basic dye method)

4.6.1. Apparatus and Instruments

As described under 4.1.1

4.6.2. Reagents and Materials

As described under 4.1.2

4.6.3. Preparation of buffer solutions (pH 4.0 – 8.0)

Various buffer solutions were prepared as per following.
(IP 1996: A-144, A-145).

Table 4.3. Preparation of buffer solutions (pH 4.0 – 8.0)

pH	Types of buffer	Preparation
4.0	Acid phthalate buffer	Potassium hydrogen phthalate, 0.2M (50.0 ml) + Hydrochloric acid, 0.2M (0.1 ml), the volume was adjusted up to 200 ml with distilled water.
5.0	Neutralized phthalate buffer	Potassium hydrogen phthalate, 0.2M (50.0 ml) + Sodium hydroxide, 0.2M (22.6 ml), the volume was adjusted up to 200 ml with distilled water.
6.0	Phosphate buffer	Potassium dihydrogen phosphate, 0.2M (50.0 ml) + Sodium hydroxide, 0.2M (5.6 ml), the volume was adjusted up to 200 ml with distilled water.
6.4	Phosphate buffer	Potassium dihydrogen phosphate, 0.2M (50.0 ml) + Sodium hydroxide, 0.2M (11.6 ml), the volume was adjusted up to 200 ml with distilled water.

6.8	Phosphate buffer	Potassium dihydrogen phosphate, 0.2M (50.0 ml) + Sodium hydroxide, 0.2M (22.4 ml), the volume was adjusted up to 200 ml with distilled water.
7.0	Phosphate buffer	Potassium dihydrogen phosphate, 0.2M (50.0 ml) + Sodium hydroxide, 0.2M (29.1 ml), the volume was adjusted up to 200 ml with distilled water.
8.0	Phosphate buffer	Potassium dihydrogen phosphate, 0.2M (50.0 ml) + Sodium hydroxide, 0.2M (46.1 ml), the volume was adjusted up to 200 ml with distilled water.

4.6.4. Preparation of safranin O solution

Accurately weighed safranin O (50.0 mg) was transferred into 100 ml volumetric flask, dissolved in phosphate buffer (pH 6.8) and diluted to 100 ml with same solvent to give 0.05 % w/v solution of safranin O.

Washing of safranin O solution

The above dye solution was washed by 3 X 10 ml of chloroform to remove chloroform soluble impurities and minimize absorption value of reagent blank.

4.6.5. Preparation of standard solution of Valsartan

Accurately weighed valsartan (100.0 mg) was transferred into 100 ml volumetric flask, dissolved in methanol (10.0 ml) and diluted by distilled water up to the mark. The solution (10.0 ml) was diluted further to 100.0 ml with distilled water. The final solution contained 100.0 μg/ml of Valsartan.

4.6.6 Preparation of standard solution of Hydrochlorothiazide

Accurately weighed hydrochlorothiazide (100.0 mg) was transferred into 100 ml volumetric flask, dissolved in methanol (10.0 ml) and diluted by distilled water up to the mark. The solution (10.0 ml) was diluted further to 100.0 ml with distilled water. The final solution contained 100.0 µg/ml of hydrochlorothiazide.

4.6.7 Preparation of standard solution of s- Amlodipine besylate

Accurately weighed s- Amlodipine besylate (100.0 mg) was transferred into 100ml volumetric flask, dissolved in methanol (10.0 ml) and diluted with distilled water up to mark. The solution (10.0 ml) was diluted further to 100 ml with distilled water. The final solution contained 100.0 µg/ml of s- amlodipine besylate.

4.6.8. Determination of wavelength of maximum absorbance of Valsartan

Standard solution of Valsartan (1.0 ml) was pipetted out into glass-stoppered tube containing Sefranin O solution (2.5 ml) and mixed thoroughly. The volume was adjusted with phosphate buffer (5.0 ml; pH 6.8). Chloroform (5.0 ml) was added and mixed well for one minute using cyclomixer. The drug – dye ion pair complex was extracted in chloroform. The chloroform layer was separated out, dried over anhydrous sodium sulphate (1.0 gm). The absorbance of the colored solution was scanned on spectrophotometer in the range of 400-800 nm against reagent blank. The blank was prepared similarly in which volume of standard solution of valsartan was replaced by an equal volume of phosphate buffer (pH 6.8).

Maximum absorbance was obtained at 518.8 nm. (Fig. 5.17).

4.6.9. Calibration curve of Valsartan

Standard solutions of Valsartan (0.25, 0.50, 0.75, 1.0, 1.25 ml) were transferred into a series of glass-stoppered tubes and volumes were adjusted to 2.5 ml with phosphate buffer (pH 6.8). Sefranin O solution (2.5 ml) was added and mixed thoroughly and analysed as described under 4.6.8. The absorbance of the colored solution was measured at 518.8 nm against reagent blank.

The Lambert – Beer's law was obeyed in the concentration range of 5.0 – 25.0 µg/ml (Table. 5.31, Fig. 5.18).

4.6.10. Factor affecting the development of color

4.6.10.1. Effect of pH of buffer

Standard solution of Valsartan (0.5 ml) was transferred in to five different glass-stoppered tubes, each containing (2.5 ml) safranin O solution. The volumes were adjusted to 5.0 ml with phosphate buffer (pH 4.0, 5.0, 6.0, 6.4, 6.8, 7.0 and 8.0) and mixed thoroughly and analysed as described under 4.6.8.

The absorbance of the colored solutions were measured at 518.8 nm against reagent blank. The optimum pH was found to be 6.8 for the reaction (Table 5.23, Fig. 5.13).

4.6.10.2 Effect of concentration of safranin O solution

Standard solution of Valsartan (0.5 ml) was transferred in to a series of glass-stoppered tubes, containing (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ml) safranin O solutions. The final volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as as described under 4.6.8. The absorbance of the reaction mixtures was measured at 518.8 nm against reagent blank.

Maximum absorbance was obtained in presence of 2.5 ml of safranin O solution (Table. 5.33, Fig. 5.20).

4.6.10.3. Color Stability.

Standard solution of Valsartan (0.5 ml) was transferred into a glass stoppered tube containing safranin O solution (2.5 ml). The volume was adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solution was measured at 518.8 nm against reagent blank at (1, 2, 3, 4, 8, 12, 24, 36, and 48 hours) time interval.

The color intensity remained constant for 48.0 hours (Table 5.25, Fig. 5.15).

4.6.11. Validation of proposed method

4.6.11.1. Linearity

It is the ability of the method within a given range to obtain best result, which is directly proportional to the concentration of analyte in the sample.

The absorbance value obtained at 518.8 nm of different concentration of valsartan was used to plot a calibration curve. The linearity range for valsartan was found to be 5.0 – 25.0 µg/ml and correlation coefficient was found to be 0.9994 (Table. 5.22, Fig. 5.12).

4.6.11.2. Accuracy

The accuracy of the analytical method is closeness of the result obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amount of analyte. Accuracy is a measure of the exactness of the analytical method.

Accuracy data of Valsartan

Pre analyzed sample solution (A) = 100 μg/ml
Standard drug solution (B) = 100 μg/ml
Sample solutions were prepared from above solutions.

1) 0.25ml of solution A + 0.0ml of solution B
2) 0.25ml of solution A + 0.25ml of solution B
3) 0.25ml of solution A + 0.5ml of solution B
4) 0.25ml of solution A + 0.75ml of solution B
5) 0.25ml of solution A + 1.0ml of solution B

The above series of solutions (1 to 5) were transferred in to glass stoperred tubes. Safranin O solution (2.5 ml) was added and the volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solutions was measured at 518.8 nm against reagent blank.

The % recovery experiment revealed good accuracy. The % recovery of Valsartan was found to be 99.59 % to 101.35 % (Table 5.26).

4.6.11.3. Precision

Precision is the measure of either the degree of reproducibility or repeatability of the analytical method. It is expressed as the standard deviation or relative standard deviation (co-efficient of variance).

4.6.11.3.1. Repeatability of measurement of absorbance

Standard solutions of Valsartan (0.25, 0.50, 0.75, 1.0, 1.25 ml) were transferred into a series of glass-stoppered tubes and volumes were adjusted to 2.5 ml with phosphate buffer (pH 6.8). Sefranin O solution (2.5 ml) was added into each and analysed as described under 4.6.8. The absorbance of

the colored solutions was measured at 518.8 nm against reagent blank. The absorbance of the same solutions was measured seven times and % CV was calculated (Table 5.27).

4.6.11.3.2. Intra day and Inter day precision

Variation of results within the same day is called intra day variation, and variation of results within days is called inter day variation. Intra day precision was determined by analyzing drug as per the procedure described in 4.6.8. For three time in the same day. The inter day precision was determined by analyzing Valsartan as per the procedure described in 4.6.8. daily for three days (Table. 5.28).

4.6.11.4. Limit of detection (LOD)

It is the lowest concentration of analyte in a sample that can be detected, but not necessarily be quantified under the stated experimental condition.

Different concentrations of standard drug solution were used and minimum detectable limit was found. LOD values was calculated from the calibration graph using equation LOD = 3.3 SD / b. (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOD was found to be 1.0603 (Table. 5.38)

4.6.11.5. Limit of quantification (LOQ)

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy under stated experimental condition. LOQ value was calculated from the calibration graph using equation LOQ = 10 SD / b (Where, SD = Standard Deviation and b = Slop of corresponding calibration curve). The LOQ was found to be 3.2129 (Table. 5.38).

4.6.11.6. Specificity

It is the ability of the developed analytical method to detect analyte quantitatively in the presence of other component, which are expected to be present in the sample matrix or other related substances.

4.6.12. Job's method for determination of Stoichiometry

Job's method, also called the method of continuous variation, is a simple and effective approach to the determination of chemical reaction at stoichiometry. In Job's continuous method equimolar solutions of drug and dye are used.

Preparation of working standard drug solution

The working standard solution of Valsartan 100 μg/ml was used. Its concentration was equal to 2.296 X 10^{-7} M/ml.

Preparation of Safranin O solution: 2.296 × 10^{-7} M/ml

Accurately weighed safranin O (83.78 mg) was transferred into 100 ml volumetric flask, dissolved in phosphate buffer (pH 6.8) and diluted to 100 ml with the same solvent.

Procedure

Standard solutions Valsartan (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 ml) were transferred in a series of glass stoppered tubes. The volumes of each glass stoppered tube were adjusted up to 2.0 ml with safranin O solution to get mole fraction of Valsartan as 0.0, 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875 and 1.0. The volumes were adjusted (5.0 ml) with phosphate buffer (pH 6.8). The drug – dye ion pair complex were extracted similarly as described under 4.6.8.

It was found that Valsartan form 1:1 complex with safranin O (Table 5.29, Fig. 5.16).

4.6.13. Analysis of marketed formulations

For Tablets: Twenty tablets were accurately weighed and powered.

For Capsules: Twenty Capsules were weighed and powder removed from shell. Then shells were weighed and the weights of the powder obtained by deducting weight of the shell from weight of capsule.

A quantity of the powder equivalent to 100.0 mg of Valsartan was transferred into 100 ml volumetric flask; methanol (10.0 ml) was added and sonicated for 5 minutes. The volume was adjusted up to the mark with distilled water to obtain Valsartan concentration 1mg/ml. The solution was filtered through whatman filter paper no.42. Solution (10.0 ml) was diluted further to 100ml with distilled water. The final solution of Valsartan was contained 100µg/ml.

The solutions (0.5 and 1.0 ml) were transferred into glass stoppered tubes containing safranin O solution (2.5 ml). The volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solutions was measured at 518.8 nm against reagent blank.

The amount of Valsartan present in pharmaceutical dosage forms were determined by referring to the standard calibration curve of Valsartan (Table. 5.40, Fig. 5.18)

4.7. Analysis of Valsartan in combined dosage forms (One component analysis)

4.7.1. Analysis of Valsartan in presence of Hydrochlorothiazide

4.7.1.1. Preparation of standard solution of Hydrochlorothiazide

As described under 4.6.6.

4.7.1.2. Preparation of standard solution of Valsartan

As described under 4.6.5.

4.7.1.3. Interference of Hydrochlorothiazide on absorbance of Valsartan – safranin O solution complex

The standard solutions of Hydrochlorothiazide (0.25, 0.5, 0.75, 1.0 and 1.25ml) were transferred into series of glass stopper tubes, each containing standard solution of Valsartan (0.5 ml). Safranin O solution (2.5 ml) was added to each tubes. The final volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solutions were measured at 518.8 nm against reagent blank (Table. 5.31).

4.7.1.4. Analysis of marketed formulations Containing Valsartan and Hydrochlorothiazide

For Tablets: Twenty tablets were accurately weighed and powered.

For Capsules: Twenty Capsules were weighed and powder removed from shell. Then shells were weighed and the weights of the powder obtained by deducting weight of the shell from weight of capsule.

A quantity of the powder equivalent to 100.0 mg of Valsartan was transferred into 100 ml volumetric flask; methanol (10.0 ml) was added and sonicated for 5 minutes. The volume was adjusted up to the mark with distilled water to obtain Valsartan concentration 1mg/ml. The solution was filtered

through whatman filter paper no.42. Solution (10.0 ml) was diluted to 100ml with distilled water. The final solution of Valsartan was contained 100 µg/ml.

The solutions (0.5 and 1.0 ml) were transferred into glass stoppered tubes containing safranin O solution (2.5 ml). The volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solutions was measured at 518.8 nm against reagent blank.

The amount of Valsartan present in pharmaceutical dosage forms were determined by referring to the standard calibration curve (Table. 5.33).

4.7.2. Analysis of Valsartan in presence of s- Amlodipine besylate

4.7.2.1. Preparation of standard solution of s- Amlodipine besylate

As described under 4.6.7.

4.7.2.2. Preparation of standard solution of Valsartan

As described under 4.6.5.

4.7.2.3. Interference of s- Amlodipine besylate on absorbance of Valsartan – safranin O dye complex

The standard solutions of s- Amlodipine besylate (0.25, 0.5, 0.75, 1.0 and 1.25ml) were transferred into series of glass stopper tubes, each containing standard solution of Valsartan (0.5 ml). Safranin O solution (2.5 ml) was added to each tubes. The final volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solutions was measured at 518.8 nm against reagent blank (Table. 5.32).

4.7.2.4. Analysis of marketed formulations containing Valsartan and s- Amlodipine besylate.

Twenty capsules were weighed and powder removed from shell. Then shell were weighed and the weights of the powder obtained by deducting weight of the shell from weight of capsule.

A quantity of the powder equivalent to 100.0 mg of Valsartan was transferred into 100 ml volumetric flask; methanol (10.0 ml) was added and sonicated for 5 minutes. The volume was adjusted up to the mark with distilled water to obtain Valsartan concentration 1.0 mg/ml. The solution was filtered through whatman filter paper no.42. Solution (10.0 ml) was diluted further to 100ml with distilled water. The final solution of Valsartan was contained 100 µg/ml.

The solutions (0.5 and 1.0 ml) were transferred into glass stoppered tubes containing safranin O solution (2.5 ml). The volumes were adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solutions was measured at 518.8 nm against reagent blank.

The amount of Valsartan present in pharmaceutical dosage forms were determined by referring to the standard calibration curve (Table 5.33).

5.

RESULTS & DISCUSSION…

5. RESULTS AND DISCUSSION

Selective serotonin reuptake inhibitors are one of the important class of antidepressant drugs. This work mainly reports the applicability of acid dye technique for the routine analysis of nitrogenous drug molecules. A wide array of dyes was used and efforts were made to apply this method to most of the antidepressants that are in vogue in the contemporary therapy today.

The study brought to light certain important aspect of acid dye complexation. Literature survey revealed that to increase the sensitivity of the method, the ion-pair complex that is formed has to be extracted into organic layer, and brought back to aqueous layer (acidic or basic) by shaking the organic layer with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide depending on the dye initially used for complex formation. The dye used in the complex formation is quantitatively extracted with aqueous layer and the absorbance of the resultant aqueous solution is measured. However, in actual practice, this process is tedious and time consuming due to formation of strong emulsions which interferes with extraction.

This method, by itself, is not a novel one. Sufficient amount of data is available where ion-pair complexing was utilized for the generation of chromophores with a number of drug molecules (table 1.1). In spite of that, the study brought certain unfocussed areas of the method.

The secondary nitrogen containing drugs having capability to form ion pair complexes with dyes. This method was so far reported but not been explored for applicability in combined dosage form. Therefore it was thought of interest to modify the method in which drug was liberated itself in organic phase to avoid further extraction step.

The role of ethanol/methanol employed in the preparation of drug or dye solutions has not been discussed anywhere in the literature. After

meticulous experimentation, it was conclude that methanol or ethanol does not interfere in the intensity of the color.

As per the theory of acid dye technique, free dye remains insoluble in organic phase viz. chloroform, it then extracted in an alkaline aqueous phase (Sethi, P.D, 1997). In our modification of the method, the drugs (Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride) were directly forms complexes with methyl orange in 0.1N hydrochloric acid and it was found that relatively basic compounds readily form complex with methyl orange and was easily extracted in chloroform.

To obliterate the possibility of interference from chloroform soluble fraction of the dye, washing of dye with chloroform was done prior to complexation. This gives clear colorless blank and rules out possibility of the presence of any uncomplexed chemical in the dye, if any, being extracted with chloroform along with the drug-dye complex.

The affinity/specificity towards buffers, their pH and dyes were also different for different drugs undertaken for study. This phenomenon was observed even for compounds of the same pharmacological category. This is because the dye and pH requirements are mainly based on the environment of nitrogen and overall acidic /basic nature of the molecule. In our drugs (Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride) methyl orange gives ion pair complex directly with 0.1N hydrochloric acid.

This combination of drug-dye was selected after extensive experimentation with different buffers and dyes for all the drugs and the optimum combination was selected.

Mechanism of proposed method

In the proposed method for the estimation like Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride, (secondary aliphatic

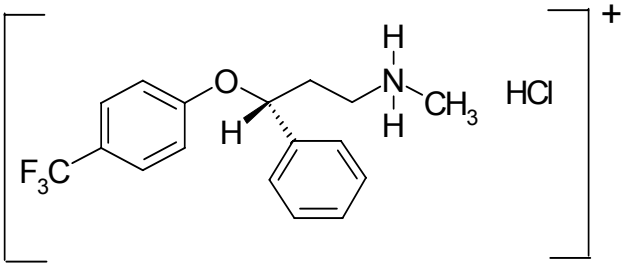
nitrogen containing basic drugs) formed ion pair complex with acidic dye methyl orange as mentioned below.

The equilibrium for ion-pair formation can be expressed as;

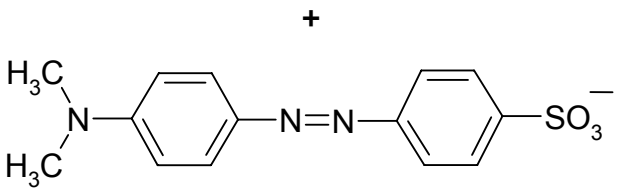
$$A^+ \text{ aq.} + D^- \text{ aq.} \longrightarrow AD \text{ org.}$$

A^+ aq. is the protonated amine in aqueous phase. D^- aq. is the anionic pairing dye in aqueous phase and AD org. is the final ion-pair complex extractable into organic phase.

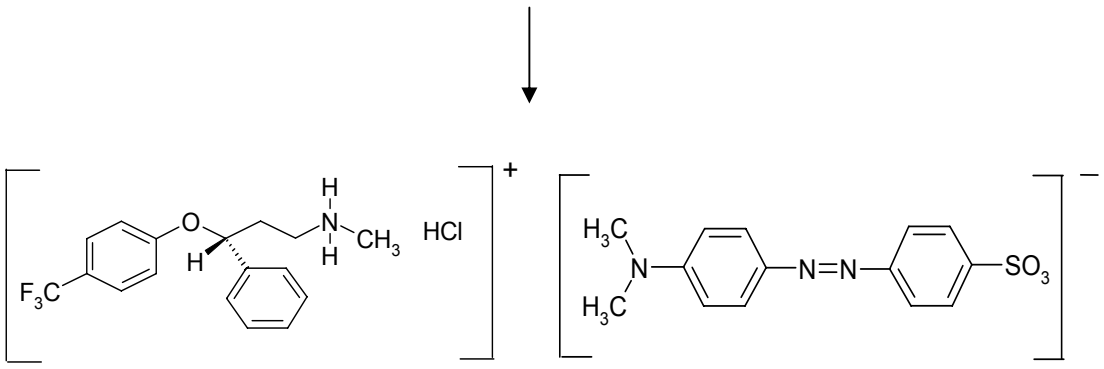
1. Ion – pair Complex with Fluoxetine Hydrochloride



Fluoxetine hydrochloride (cationic drug)

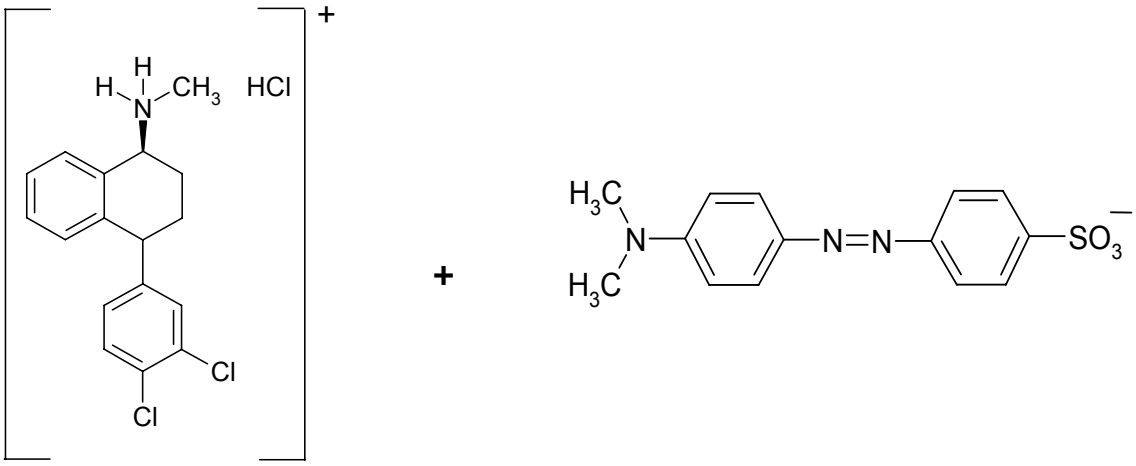


Methyl orange (anionic dye)

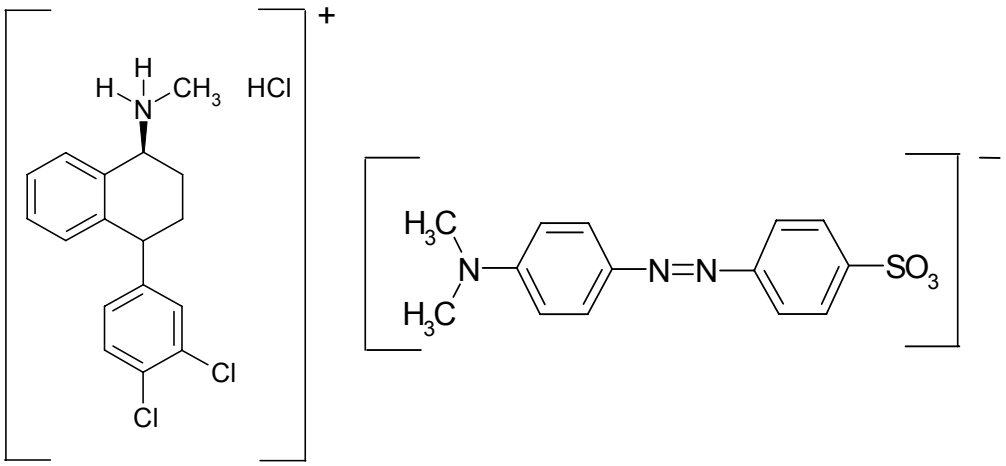


Fluoxetine hydrochloride – Methyl orange Ion Pair Complex

2. Ion – pair Complex with Sertraline Hydrochloride

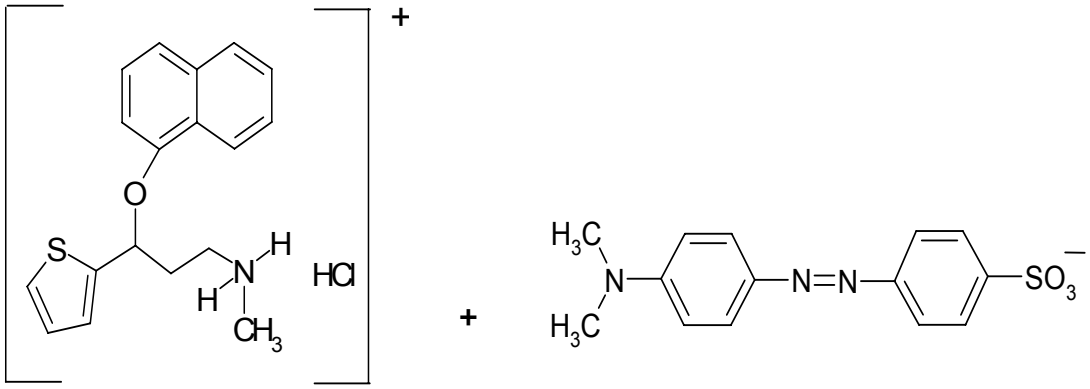


Sertraline hydrochloride (cationic drug) **Methyl orange (anionic dye)**

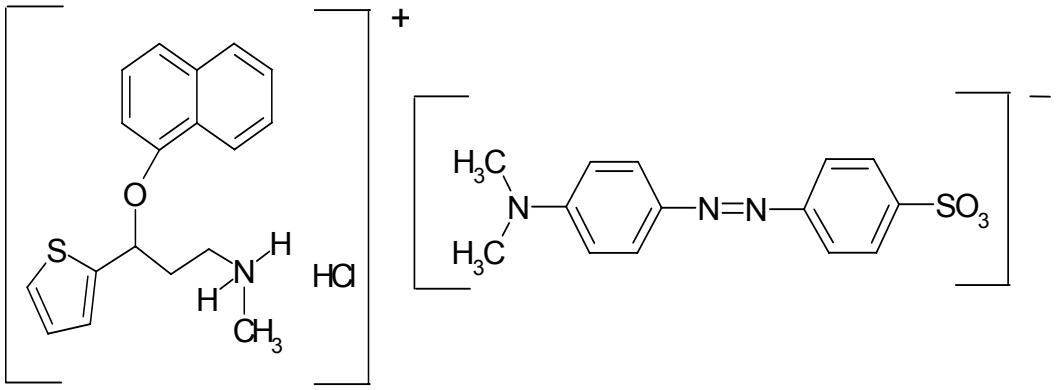


Sertraline hydrochloride – methyl orange ion pair complex

3. Ion – pair Complex with Duloxetine Hydrochloride



Duloxetine hydrochloride (cationic drug) **Methyl orange (anionic dye)**



Duloxetine hydrochloride – methyl orange ion pair complex

Factors affecting the development of color

A chemical reaction is affected by many of experimental parameters. Various experimental conditions such as concentration of dye, effect of pH and effect of time have been studied and optimized.

Effect of concentration of dye

Yellow colored chromophore was formed as a result of the reaction between the drugs (Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride) and Methyl orange. The intensity of ion pair complex in chloroform was measured. Optimum concentration of dye was found to be 2.0 ml of 0.05 % w/v for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride (Table 5.5, Fig. 5.8).

Effect of pH of buffer

Various buffer solutions having pH ranges (1.0 - 8.0) and 0.1N hydrochloric acid were used. Maximum absorbance was obtained in presence of 0.1N hydrochloric acid (pH 1.0 - 4.0) for fluoxetine hydrochloride while maximum absorbance was obtained in presence of 0.1N hydrochloric acid (pH 1.0) for sertraline hydrochloride. Duloxetine hydrochloride shown maximum absorbance with phthalate buffer (pH 3.0) and acetate buffer (pH 3.0) (Table 5.4, Fig. 5.7).

Color Stability

The stability of colored solution was studied at room temperature. The drug – dye complex was stabled for more than 48 hours for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride (Table 5.6, Fig. 5.9).

Validation of the proposed method

Linearity

The absorbance values obtained at respective λ_{max} of different concentration of drugs were used to plot a calibration curve. The linearity range for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride was found to be 5.0 – 30.0 µg/ml, 2.0 – 14.0 µg/ml and 2.0 – 12.0 µg/ml respectively.

The data with graph along with Standard deviation and % CV given for Fluoxetine hydrochloride (Table 5.1, Fig. 5.4), Sertraline hydrochloride (Table 5.2, Fig. 5.5) and Duloxetine hydrochloride (Table 5.3, Fig. 5.6) respectively.

Accuracy

The % recovery experiment revealed good accuracy. The average % recovery of Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were found to be 99.68 – 101.88 %, 98.48 – 101.78 % and 99.08 – 101.89 % respectively (Table 5.7, 5.8, 5.9).

Precision

The %CV obtained for repeatability of measurements of absorbance for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were found to be 0.24 – 0.68 %, 0.19 – 0.53 %, and 0.23 – 0.95 % respectively (Table 5.10, 5.11, 5.12).

The %CV obtained for intra day precision for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were found to be 0.89 % – 1.66 %, 0.75 % – 2.04 %, and 0.56 % – 1.24 % respectively (Table 5.13, 5.14, 5.15).

The %CV obtained for inter day precision for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were found to be 1.59 – 2.78 %, 1.01 – 2.51 %, and 0.98 – 2.54 % respectively (Table 5.13, 5.14, 5.15).

Limit of detection (LOD)

Limit of detection for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were found to be 0.98 µg/ml, 0.21 µg/ml and 0.49 µg/ml respectively (Table 5.20).

Limit of quantification (LOQ)

Limit of quantitation for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were found to be 2.97 µg/ml, 0.63 µg/ml and 1.49 µg/ml respectively (Table 5.20).

Validation parameters are summarized in Table 5.20

Optical and regression characteristics are summarized in Table 5.21

Job's continuous variation method

Job's continuous variation was conducted for Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride which formed 1:1 complex with methyl orange (Table 5.16, Fig. 5.10).

Analysis of marketed formulations

The marketed formulation of Fluoxetine hydrochloride, Sertraline hydrochloride and Duloxetine hydrochloride were analyzed with the proposed method (Table 5.17, 5.18, 5.19). The result obtained are in good agreement with labeled value of dosage forms.

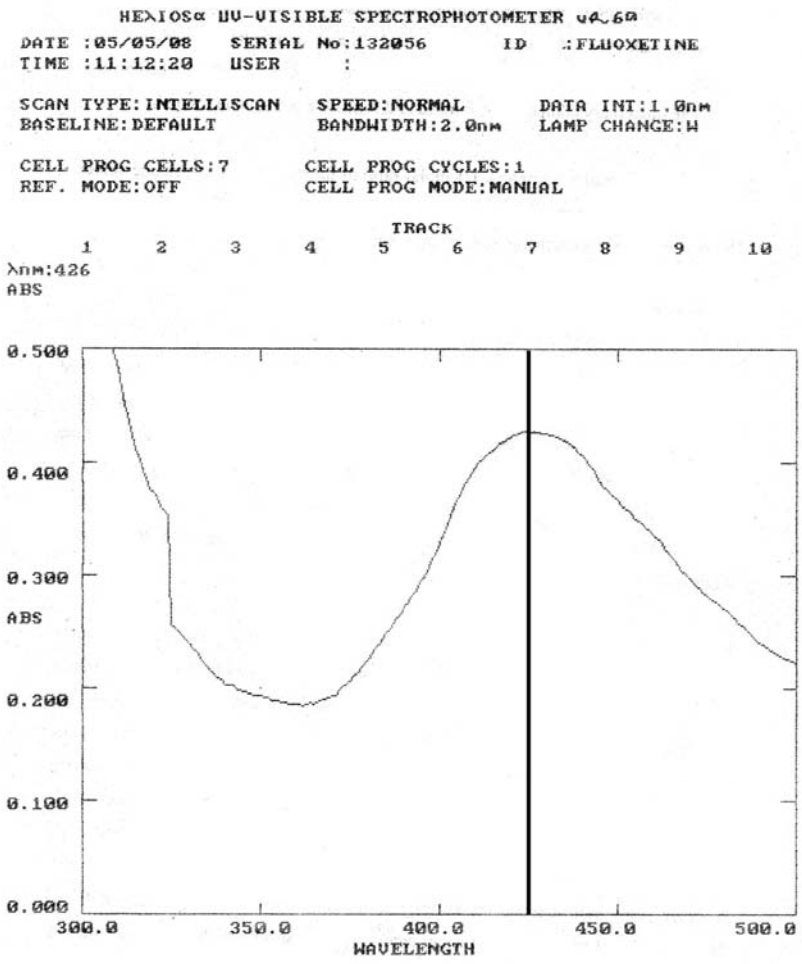


Fig. 5.1: A Visible spectrum of Fluoxetine hydrochloride at 426.0 nm

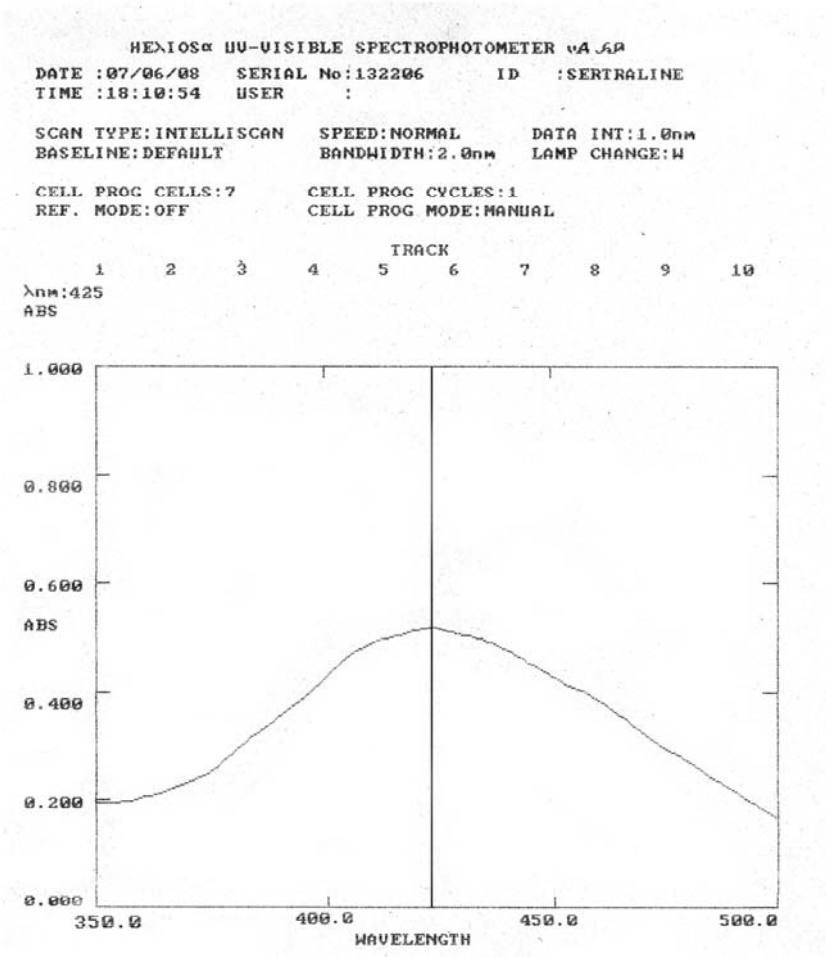


Fig. 5.2: A Visible spectrum of Sertraline hydrochloride at 425.0 nm

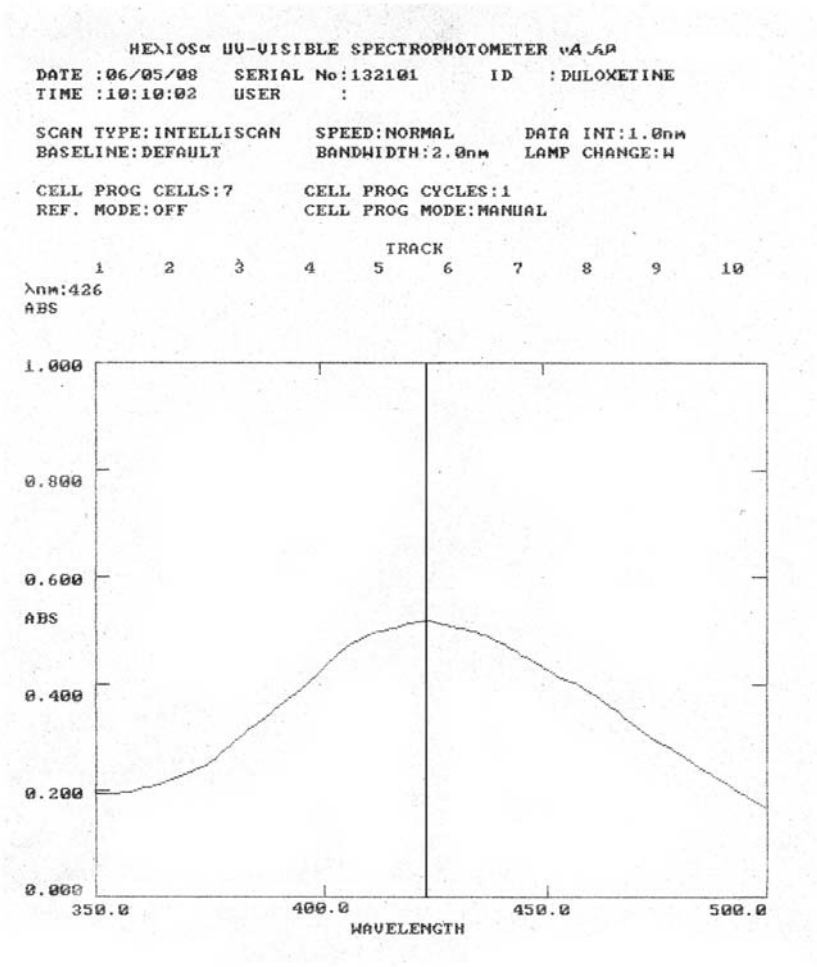


Fig. 5.3: A Visible spectrum of Duloxetine hydrochloride at 426.0 nm

Table 5.1. Calibration data for Fluoxetine hydrochloride in 0.1N HCl

Concentration of Fluoxetine HCl (µg/ml)	Absorbance at 426.0 nm Mean ± S.D. (n = 5)	% CV
5.0	0.211± 0.0032	1.50
10.0	0.362 ± 0.0045	1.25
15.0	0.482 ± 0.0029	0.61
20.0	0.624 ± 0.0050	0.80
25.0	0.774 ± 0.0019	0.95
30.0	0.922 ± 0.0035	1.29

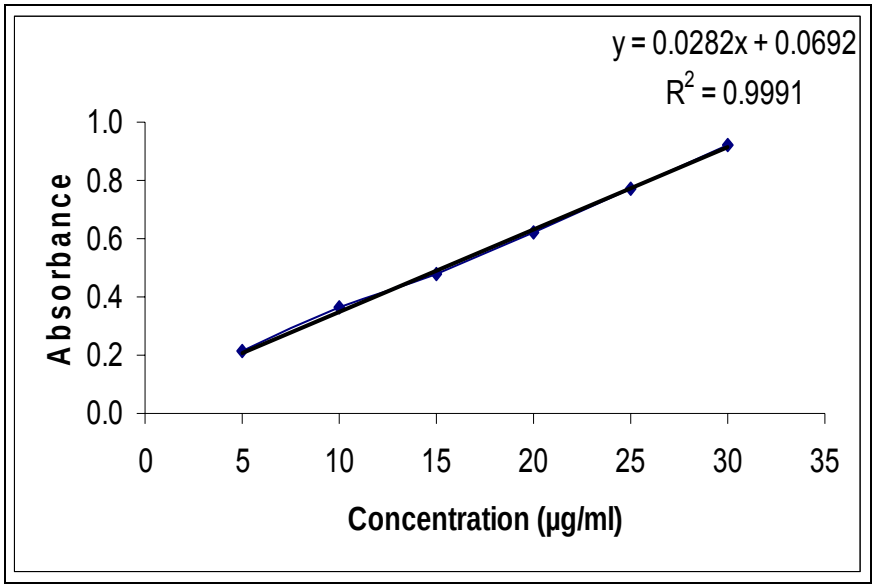


Fig. 5.4. Calibration curve for Fluoxetine hydrochloride at 426.0 nm

Table 5.2. Calibration data for Sertraline hydrochloride in 0.1N HCl

Concentration of Sertraline HCl (µg/ml)	Absorbance at 425.0 nm Mean ± S.D. (n = 5)	% CV
2.0	0.164 ± 0.0030	1.85
4.0	0.336 ± 0.0022	0.66
6.0	0.476 ± 0.0058	1.21
8.0	0.606 ± 0.0025	0.42
10.0	0.787 ± 0.0037	0.48
12.0	0.914 ± 0.0036	0.40
14.0	1.087 ± 0.0088	0.81

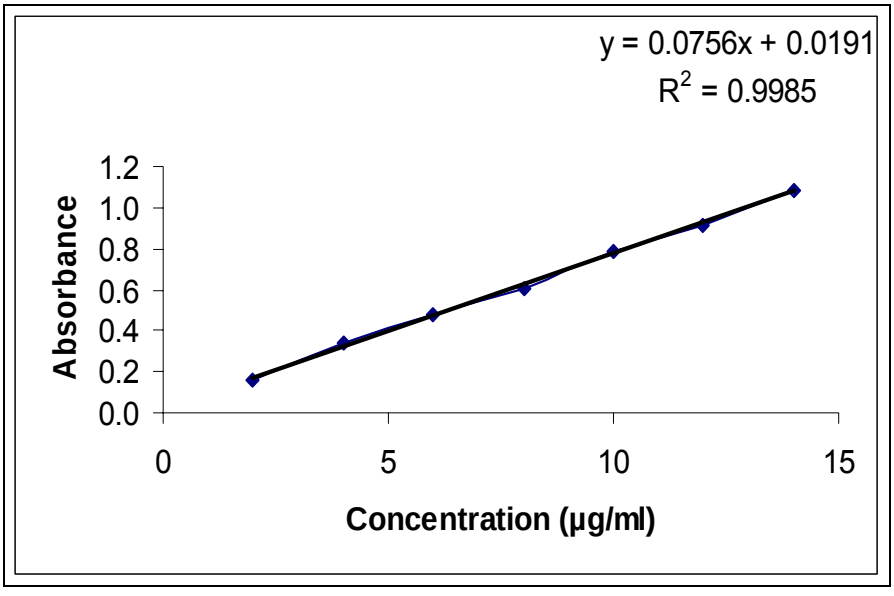


Fig. 5.5: Calibration curve for Sertraline hydrochloride at 425.0 nm

Table 5.3. Calibration data for Duloxetine hydrochloride in Phthalate buffer pH 3.0

Concentration of Duloxetine HCl (µg/ml)	Absorbance at 426.0 nm Mean ± S.D. (n = 5)	% CV
2.0	0.212 ± 0.0065	1.55
4.0	0.408 ± 0.0150	1.42
6.0	0.589 ± 0.0080	1.35
8.0	0.764 ± 0.0054	0.70
10.0	0.918 ± 0.0102	1.11
12.0	1.083 ± 0.0090	0.82

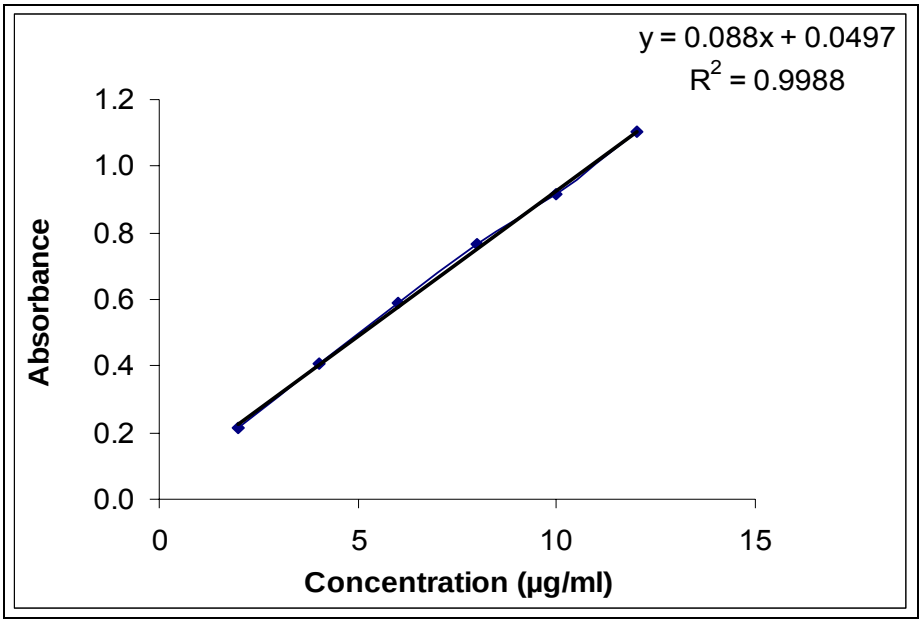


Fig. 5.6: Calibration curve for Duloxetine hydrochloride at 426.0 nm

Table 5.4. Effect of pH of buffer solutions.

Concentration of drug (µg/mL)	pH of buffer solution	Absorbance		
		Fluoxetine hydrochloride	Sertraline hydrochloride	Duloxetine hydrochloride
5.0	1.0	0.212	0.408	0.166
5.0	2.0	0.211	0.381	0.205
5.0	3.0	0.209	0.352	0.511
5.0	4.0	0.211	0.342	0.509
5.0	5.0	0.184	0.311	0.455
5.0	6.0	0.151	0.289	0.312
5.0	7.0	0.132	0.251	0.296
5.0	8.0	0.062	0.121	0.148
5.0	0.1 N HCl	0.212	0.409	0.155

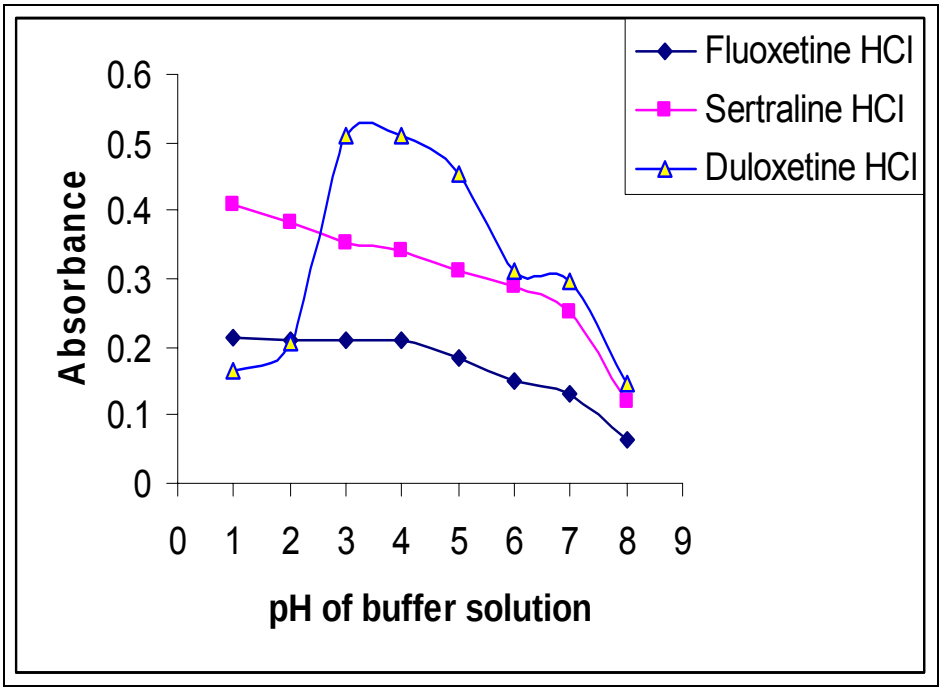


Fig. 5.7. Effect of pH of buffer

Table 5.5. Effect of concentration of methyl orange

Concentration of drug (µg/ml)	Methyl orange solution (ml)	Absorbance		
		Fluoxetine hydrochloride	Sertraline hydrochloride	Duloxetine hydrochloride
5.0	0.5	0.060	0.110	0.101
5.0	1.0	0.110	0.213	0.253
5.0	1.5	0.192	0.305	0.365
5.0	2.0	0.211	0.411	0.502
5.0	2.5	0.210	0.409	0.500
5.0	3.0	0.208	0.410	0.499
5.0	3.5	0.209	0.409	0.501

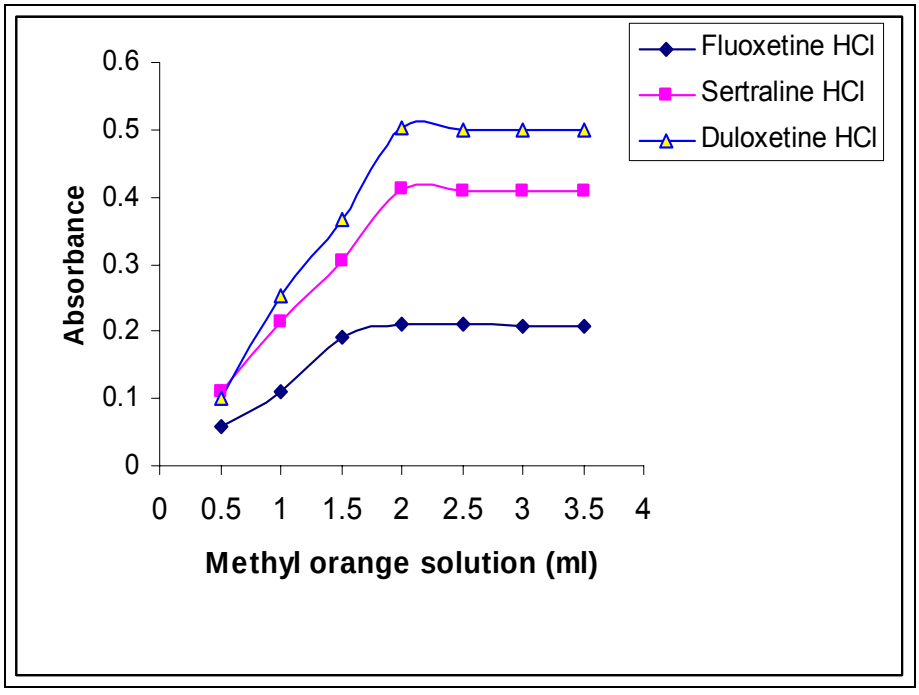


Fig. 5.8. Effect of concentration of methyl orange

Table 5.6. Effect of time on stability of color complex

Concentration of drug (µg/ml)	Time (hours)	Absorbance		
		Fluoxetine hydrochloride	Sertraline hydrochloride	Duloxetine hydrochloride
5.0	0.0	0.212	0.412	0.509
5.0	1.0	0.211	0.411	0.510
5.0	2.0	0.210	0.410	0.507
5.0	6.0	0.213	0.412	0.508
5.0	12.0	0.211	0.412	0.510
5.0	18.0	0.210	0.409	0.509
5.0	24.0	0.209	0.410	0.511
5.0	36.0	0.211	0.413	0.508
5.0	48.0	0.212	0.410	0.510

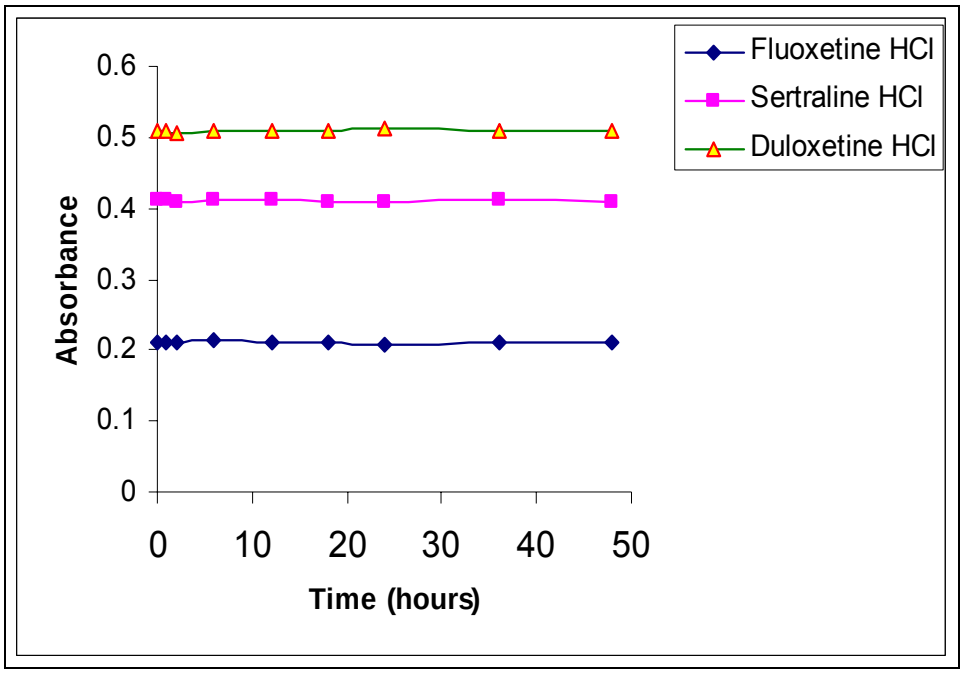


Fig. 5.9. Effect of time on stability of color complex

Table 5.7. Recovery data for Fluoxetine hydrochloride

Amount of sample drug taken (µg/mL)	Amount of standard drug added (µg/mL)	Amount of drug found (µg/mL) Mean ± SD	% Recovery ± % CV (n=5)
5.0	0.0	5.01 ± 0.013	100.15 ± 0.85
5.0	5.0	10.07 ± 0.062	101.35 ± 1.22
5.0	10.0	15.03 ± 0.056	100.61 ± 1.02
5.0	15.0	19.96 ± 0.051	99.68 ± 1.34
5.0	20.0	25.12 ± 0.061	100.78 ± 1.14
5.0	25.0	30.28 ± 0.072	101.88 ± 1.96

Table 5.8. Recovery data for Sertraline hydrochloride

Amount of sample drug taken (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug found (µg/ml) Mean ± SD	% Recovery ± % CV (n=5)
4.0	0.0	3.92 ± 0.023	98.48 ± 1.05
4.0	2.0	6.07 ± 0.078	100.64 ± 1.45
4.0	4.0	8.03 ± 0.051	100.13 ± 1.23
4.0	6.0	9.96 ± 0.065	99.45 ± 1.77
4.0	8.0	12.12 ± 0.023	101.78 ± 1.28
4.0	10.0	14.09 ± 0.012	100.78 ± 0.96

Table 5.9. Recovery data for Duloxetine hydrochloride

Amount of sample drug taken (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug found (µg/ml) Mean ± SD	% Recovery ± % CV (n=5)
4.0	0.0	3.95 ± 0.013	99.08 ± 1.09
4.0	2.0	6.07 ± 0.062	100.72 ± 1.58
4.0	4.0	8.03 ± 0.056	100.14 ± 1.43
4.0	6.0	9.96 ± 0.051	99.45 ± 1.83
4.0	8.0	12.12 ± 0.072	101.89 ± 1.20

Table 5.10. Results of repeatability of measurements of absorbance for Fluoxetine hydrochloride

Concentration (µg/ml)	Absorbance Mean ± SD (n=7)	% CV
5.0	0.211 ± 0.0019	0.68
10.0	0.362 ± 0.0021	0.36
15.0	0.481 ± 0.0025	0.24
20.0	0.614 ± 0.0022	0.42
25.0	0.772 ± 0.0031	0.38
30.0	0.918 ± 0.0023	0.59

Table 5.11. Results of repeatability of measurements of absorbance for Sertraline hydrochloride

Concentration (µg/mL)	Absorbance Mean ± SD (n=7)	% CV
2.0	0.165 ± 0.0066	0.53
4.0	0.339 ± 0.0014	0.19
6.0	0.480 ± 0.0085	0.28
8.0	0.605 ± 0.0056	0.47
10.0	0.793 ± 0.0041	0.37
12.0	0.916 ± 0.0048	0.52
14.0	1.081 ± 0.0048	0.41

Table 5.12. Results of repeatability of measurements of absorbance for Duloxetine hydrochloride

Concentration (µg/ml)	Absorbance Mean ± SD (n=7)	% CV
2.0	0.221 ± 0.0043	0.56
4.0	0.414 ± 0.0081	0.95
6.0	0.598 ± 0.0035	0.26
8.0	0.779 ± 0.0065	0.24
10.0	0.932 ± 0.0048	0.23
12.0	1.102 ± 0.0029	0.43

Table5.13. Precision data for Fluoxetine hydrochloride

Concentration (µg/ml)	Intra day precision		Inter day precision	
	Absorbance Mean ± SD (n=3)	% CV	Absorbance Mean ± SD (n=3)	% CV
5.0	0.209 ± 0.0045	1.66	0.211 ± 0.0052	2.18
10.0	0.359 ± 0.0087	1.02	0.361 ± 0.0092	2.78
15.0	0.480 ± 0.0109	1.56	0.484 ± 0.0048	2.20
20.0	0.620 ± 0.0092	1.12	0.614 ± 0.0102	1.62
25.0	0.773 ± 0.0083	0.89	0.770 ± 0.0073	1.59
30.0	0.922 ± 0.0065	1.23	0.928 ± 0.0059	2.23

Table5.14. Precision data for Sertraline hydrochloride

Concentration (µg/ml)	Intra day precision		Inter day precision	
	Absorbance Mean ± SD (n=3)	% CV	Absorbance Mean ± SD (n=3)	% CV
2.0	0.160 ± 0.0026	0.93	0.167 ± 0.0106	2.35
4.0	0.336 ± 0.0068	1.75	0.334 ± 0.0044	1.01
6.0	0.471 ± 0.0078	1.55	0.471 ± 0.0023	2.46
8.0	0.606 ± 0.0099	2.04	0.603 ± 0.0075	2.51
10.0	0.785 ± 0.0053	1.22	0.786 ± 0.0046	1.18
12.0	0.911 ± 0.0042	0.93	0.918 ± 0.0088	2.05
14.0	1.087 ± 0.0105	0.75	1.099 ± 0.0091	1.09

Table 5.15. Precision data for Duloxetine hydrochloride

Concentration (µg/ml)	Intra day precision		Inter day precision	
	Absorbance Mean ± SD (n=3)	% CV	Absorbance Mean ± SD (n=3)	% CV
2.0	0.206 ± 0.0048	0.56	0.209 ± 0.0053	1.36
4.0	0.422 ± 0.0062	1.05	0.387 ± 0.0091	2.04
6.0	0.579 ± 0.0095	0.99	0.588 ± 0.0108	1.85
8.0	0.758 ± 0.0120	1.24	0.761 ± 0.0086	0.98
10.0	0.917 ± 0.0078	0.87	0.912 ± 0.0091	1.50
12.0	1.110 ± 0.0036	1.21	1.091 ± 0.0097	2.54

Table 5.16. Data for Job's continuous variation method

Mole fraction of drug	Mole fraction of methyl orange	Absorbance		
		Fluoxetine HCl	Sertraline HCl	Duloxetine HCl
0.0	1.0	0.000	0.000	0.000
0.2	0.8	0.064	0.092	0.165
0.4	0.6	0.208	0.228	0.371
0.5	0.5	0.215	0.391	0.492
0.6	0.4	0.207	0.239	0.369
0.8	0.2	0.049	0.089	0.172
1.0	0.0	0.000	0.000	0.000

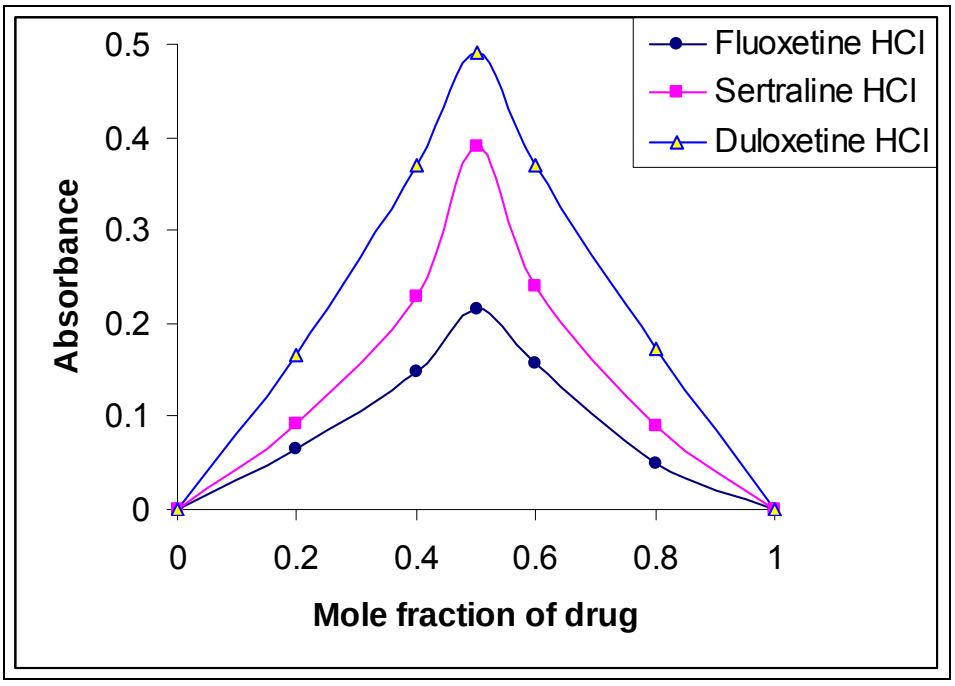


Fig. 5.10. Job's continuous variation method

Table 5.17. Analysis of Fluoxetine hydrochloride in dosage forms

Formulation	Label claim (mg)	% found Mean ± SD (n = 5)	% CV
Tablet - 1	10.0	99.35 ± 0.104	1.05
Tablet - 2	20.0	100.70 ± 0.630	0.62
Capsule - 1	20.0	98.93 ± 0.882	1.25
Capsule - 2	60.0	101.05 ± 0.451	1.06

Table 5.18. Analysis of Sertraline hydrochloride in dosage forms

Formulation	Label claim (mg)	% found Mean ± SD (n = 5)	% CV
Tablet - 1	25.0	101.53 ± 0.857	1.44
Tablet - 2	50.0	98.960 ± 0.632	2.43
Tablet - 3	100.0	99.16 ± 1.077	1.68

Table 5.19. Analysis of Duloxetine hydrochloride in dosage forms

Formulation	Label claim (mg)	% found Mean ± SD (n = 5)	% CV
Tablet - 1	20.0	99.50 ± 0.225	1.54
Tablet - 2	40.0	100.67 ± 0.247	1.62
Capsule - 1	20.0	98.67 ± 0.494	2.25
Capsule - 2	40.0	102.00 ± 0.816	2.06

Table 5.20. Summary of validation parameters

Parameter	Fluoxetine HCl	Sertraline HCl	Duloxetine HCl
Linearity range (μg/ml)	5.0 – 30.0	2.0 – 14.0	2.0 – 12.0
Limit of detection (LOD) (μg/ml)	0.98	0.21	0.49
Limit of quantification (LOQ) (μg/ml)	2.97	0.63	1.49
Precision (% CV)			
• Repeatability	0.24 – 0.68	0.19 – 0.53	0.23 – 0.95
• Intraday	0.89 – 1.66	0.75 – 2.04	0.56 – 1.24
• Interday	1.59 – 2.78	1.01 – 2.51	0.98 – 2.54
% Recovery	99.68 – 101.88	98.48 – 101.78	99.08 – 101.89
Specificity	Specific	Specific	Specific

Table 5.21. Optical and regression characteristics

Parameters	Fluoxetine HCl	Sertraline HCl	Duloxetine HCl
λ_{max} (nm)	426.0	425.0	426.0
Regression equation (Y = mx + c)	Y = 0.0282x + 0.0692	Y = 0.0756x + 0.0191	Y = 0.0880x + 0.0497
Slope (b)	0.0282	0.0756	0.0880
Intercept (a)	0.0692	0.0191	0.0497
Correlation coefficient (r^2)	0.9991	0.9985	0.9988
Sandell's sensitivity (μg/ml/cm^2)	0.0237	0.0094	0.0122
Molar absorptivity (L/mol.cm)	7.13 x 10^3	2.69 x 10^4	3.27 x 10^4

AT_1 Receptors blocker

AT_1 receptors blockers are one of the well- known class of antihypertensive drugs. Losartan is parent molecule of this class. Losartan can be estimated by both acid and basic dye. Losartan posses' different functional moieties such as N alkyl substituted imidazole (basic) and tetrazole (acidic) of varied reactivity. Alkyl substituted nitrogen in imidazole reacts with acidic dye bromocresol green and tetrazole with basic dye safranine O. Therefore it was thought of interest to develop basic dye method for other drugs belongs to the same class and having tetrazole moiety in the structure.

It was found that tetrazole ring system was responsible for the ion-pair complex formation. As tetrazole ring system it self contain four nitrogen, but ring resonance make it acidic and this property was utilized in development of ion pair technique with basic dye. Valsartan contain tetrazole ring system in its structure and this method was proven successfully.

Mechanism of proposed method

In the proposed basic dye method for the estimation of Valsartan, which containing tetrazole ring system makes ion pair complex with basic dye safranin O as mentioned below.

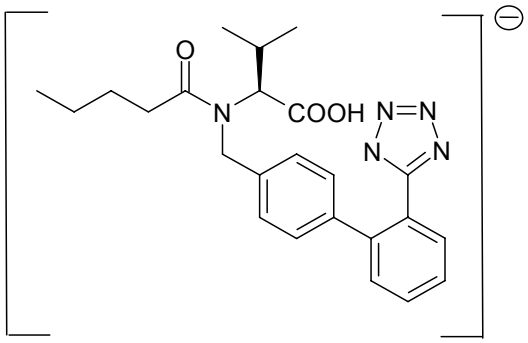
The equilibrium expression for ion-pair formation can be expressed as

A^-_{aq} = anionic drug in aq. Phase

D^+_{aq} = cationic dye in aq. Phase

AD_{org} = ion-pair complex extractable into organic phase

Ion – pair Complex with Valsartan



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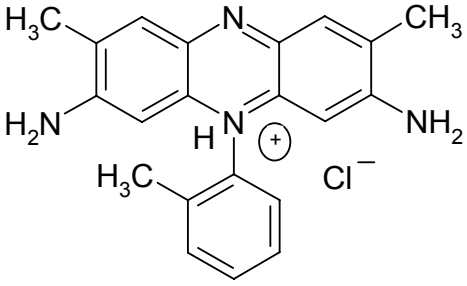
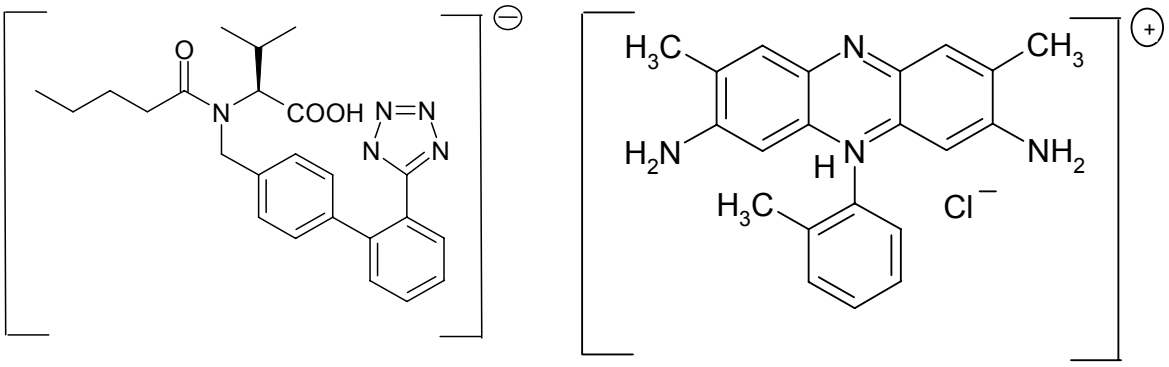


Valsartan (Anionic Drug) **Safranin O (Cationic Dye)**



Valsartan – Safranin O Ion Pair Complex

Factors affecting the development of color:

A chemical reaction is affected by many of experimental parameters. Various experimental conditions such as concentration of dye, effect of time for stability of color and effect of pH of buffer have been studied and optimized to maximize the sensitivity and reproducibility of the method.

Effect of concentration of dye

Red colored chromophore was formed as a result of the reaction between the drug and Safranin O. The intensity of ion pair complex in chloroform was measured. Optimum concentration of dye was found to be 2.5 ml of 0.05 % w/v (Table 5.24, Fig. 5.14).

Color Stability.

The stability of colored solution was studied at room temperature and shows that drug – dye complex was stable for 48 hours. (Table 5.25, Fig 5.15).

Effect of pH of buffer

Initially citrate buffer was used for pH optimization. Then it was counterchecked by phosphate buffer USP. Similar results were found through both buffers. Citrate buffer and glycine- NaCl buffer ranging from 3 to 9.5 were utilized for optimization. Optimum pH, where ion- associated complex showed maximum absorbance, was found to be pH 6.8 (Table 5.23, Fig. 5.13).

Validation of the proposed method

Linearity

The absorbance value obtain at the respective λ_{max} of different concentration of drug was used to plot a calibration curve. The linearity range for valsartan 5 – 25 µg/ml (Table 5.22, Fig. 5.12).

Accuracy

The % recovery experiment revealed good accuracy. The average % recovery of valsartan was found to be 99.59 - 101.35 % (Table 5.26).

Precision

The %CV obtained for repeatability of measurements of absorbance for Valsartan was found to be 0.21 - 0.88 % (Table 5.27).

The %CV obtained for intra day precision for Valsartan was found to be 1.02 - 1.96 % (Table 5.28).

The %CV obtained for inter day precision for was found to be 1.51 - 2.18 % (Table 5.28).

Limit of detection (LOD)

Limit of detection for valsartan was found to be 0.5 (Table 5.34)

Limit of quantification (LOQ)

Limit of quantitation for valsartan was found to be 5.0 (Table 5.34)

Validation parameters are summarized in Table 5.34

Optical and regression characteristics are summarized in Table 5.35

Job's continuous variation method

Job's continuous variation was conducted for Valsartan which form 1:1 complex with safranin O dye (Table 5.29, Fig. 5.16).

Analysis of market formulations:

The market formulation of Valsartan was analyzed with the proposed method (Table 5.30).

Analysis of Valsartan in Combined dosage form (One Component Analysis)

The basic dye method was used for analysis of the drug in combined dosage form.

Hydrochlorothiazide as well as s-amlodipine did not form an ion pair complex with the safranin O solution. Hence, combined dosage forms like valsartan - hydrochlorothiazide and valsartan – s-amlodipine were taken into analysis. There was absolutely no interference of hydrochlorothiazide, s-amlodipine or usual excipients in the analysis of the drug under study. Hydrochlorothaizide and s-amlodipine were added in increasing order to the drug and it was observed that irrespective of the quantities present, hydrochlorothiazide and s-amlodipine did not interfere in the analysis. (Table no. 5.31, 5.32, 5.33)

All other experimental conditions applied to the bulk drugs and their formulations, were found to be suitable for combined dosage forms. The results obtained are very convincing.

Thus in present study, the basic dye technique was successfully employed for analysis of one drug in presence of another non-ion –pair forming drug in combined dosage forms.

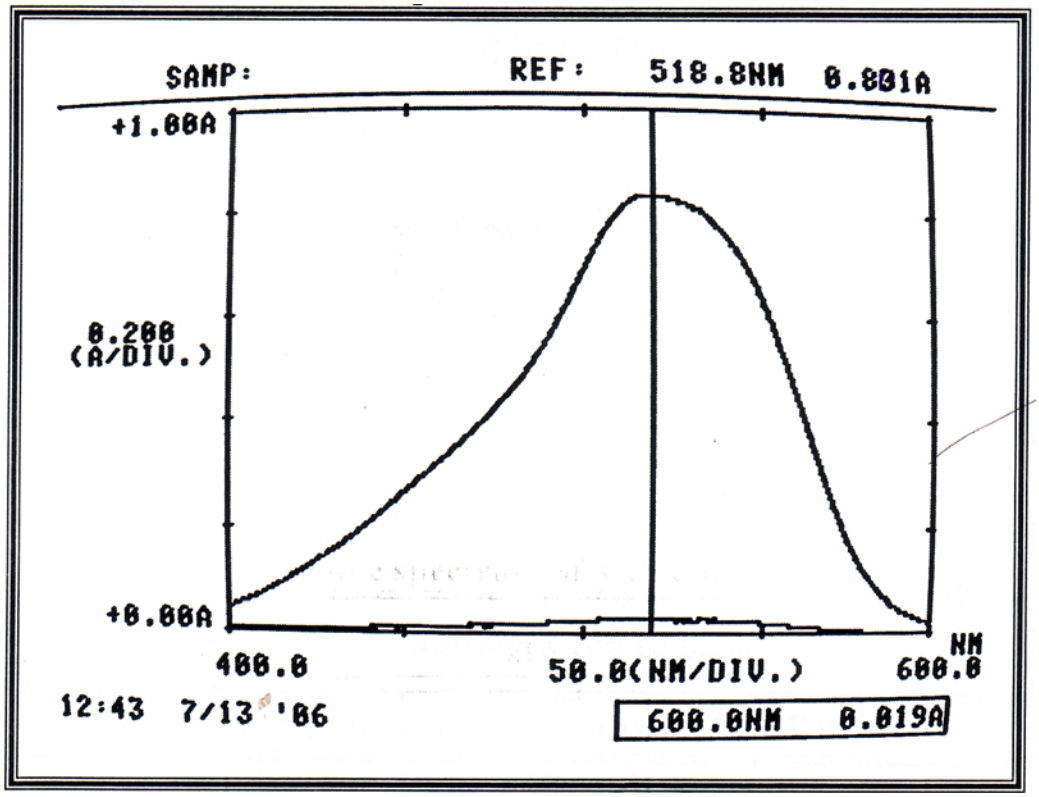


Fig. 5.11 A visible Spectrum of Valsartan at 518.8 nm

Table 5.22. Calibration data for Valsartan in phosphate buffer pH 6.8

Concentration (µg/ml)	Absorbance Mean ± SD (n=5)	% CV
5.0	0.157 ± 0.0057	1.65
10.0	0.319 ± 0.0067	2.10
15.0	0.470 ± 0.0069	1.48
20.0	0.634 ± 0.0058	0.90
25.0	0.809 ± 0.0075	0.92

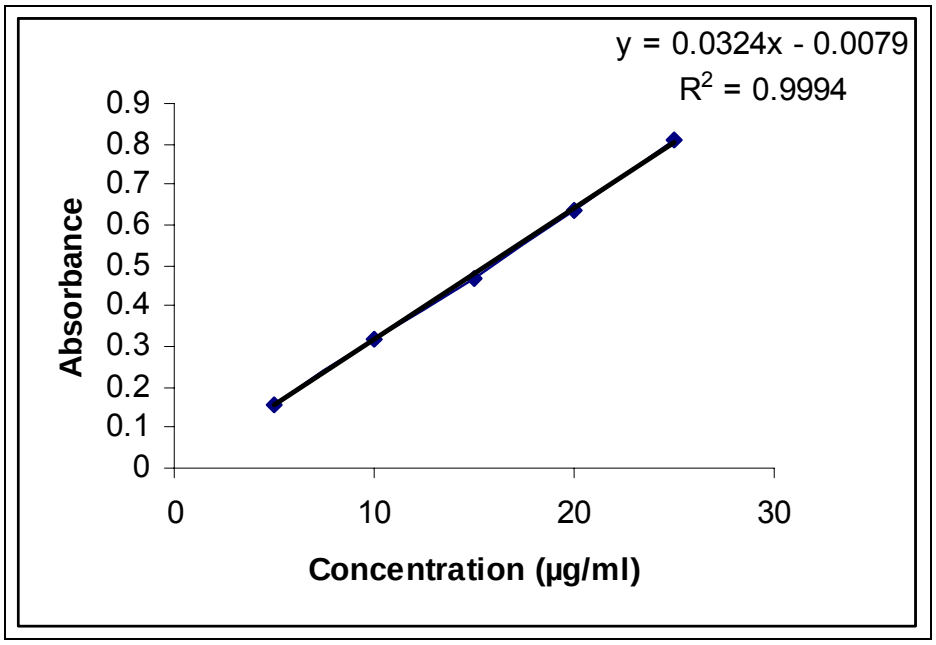


Fig. 5.12. Calibration curve for Valsartan at 518.8 nm

Table 5.23. Effect of pH of buffer solutions

Concentration of drug (µg/ml)	pH of buffer solution	Absorbance
10.0	4.0	0.091
10.0	5.0	0.132
10.0	6.0	0.164
10.0	6.4	0.299
10.0	6.8	0.32
10.0	7.0	0.306
10.0	8.0	0.187

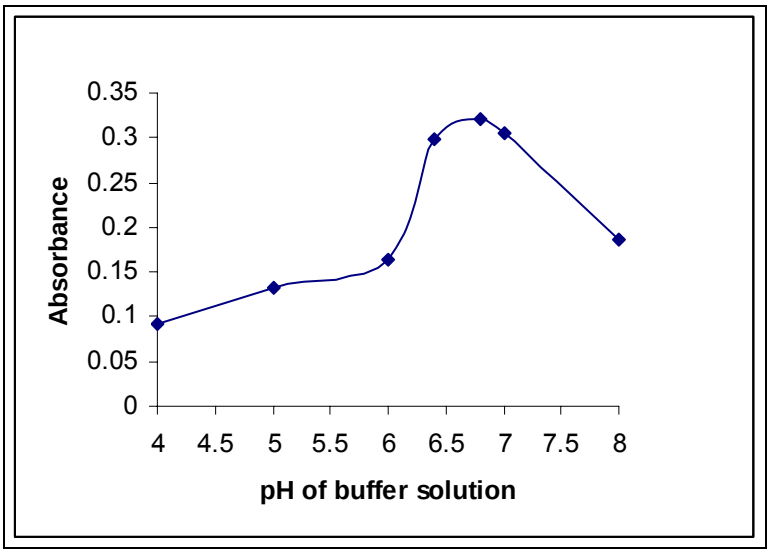


Fig. 5.13. Effect of pH of phosphate buffer

Table 5.24. Effect of concentration of safranin O

Concentration of drug (µg/ml)	Safranin O solution (ml)	Absorbance
10.0	0.5	0.185
10.0	1.0	0.205
10.0	1.5	0.235
10.0	2.0	0.281
10.0	2.5	0.321
10.0	3.0	0.318
10.0	3.5	0.320

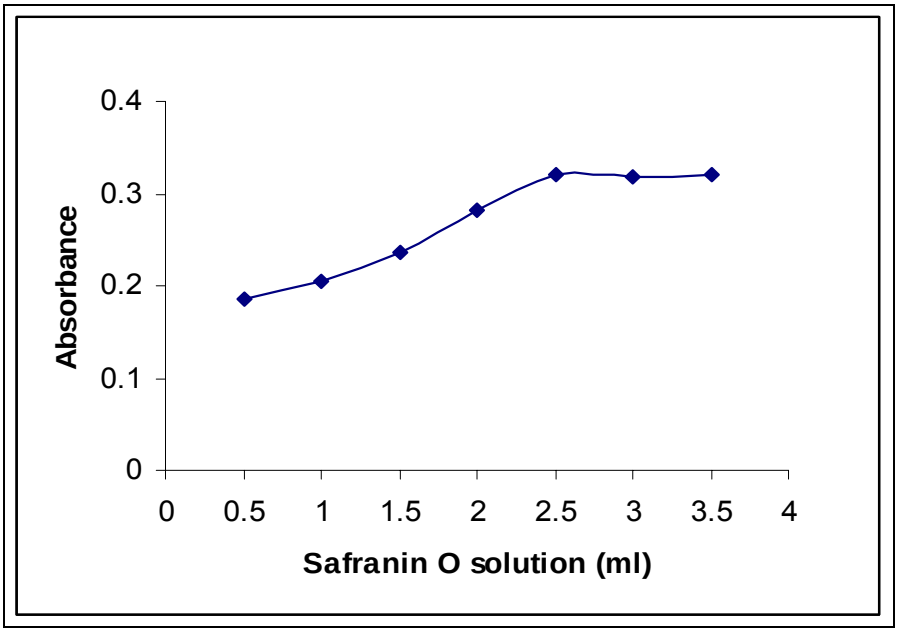


Fig. 5.14. Effect of concentration of safranin O

Table 5.25. Effect of time for stability of color

Concentration of drug (µg/ml)	Time (hours)	Absorbance
10.0	0.0	0.320
10.0	1.0	0.321
10.0	2.0	0.318
10.0	6.0	0.320
10.0	12.0	0.317
10.0	18.0	0.319
10.0	24.0	0.321
10.0	36.0	0.320
10.0	48.0	0.318

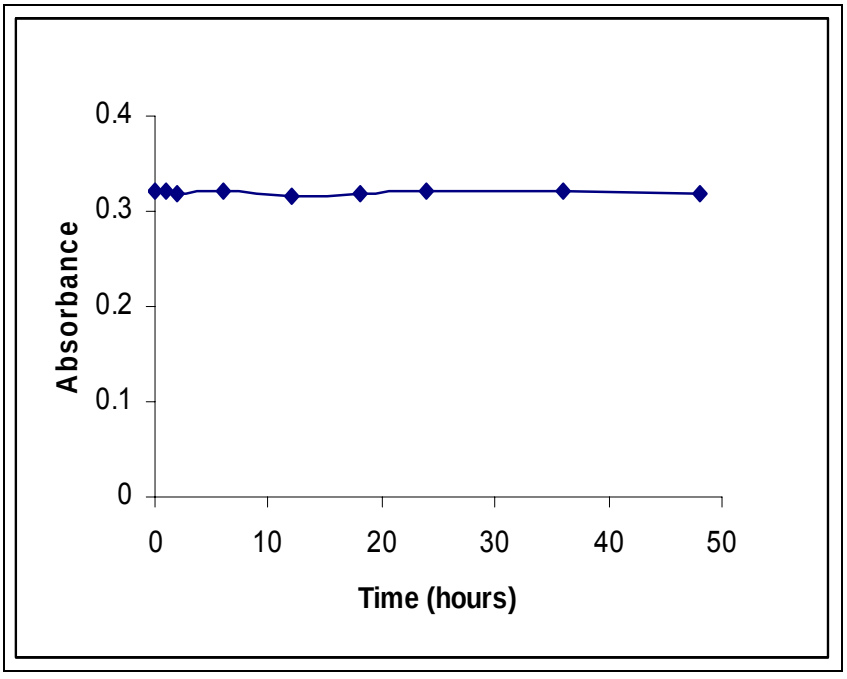


Fig. 5.15. Effect of time for stability of color

Table 5.26. Recovery data for Valsartan in phosphate buffer pH 6.8

Amount of sample taken (µg/ml)	Amount of standard added (µg/ml)	Amount found (µg/ml) Mean ± SD	% Recovery ± % CV (n=5)
5.0	0.0	5.01 ± 0.013	100.15 ± 0.85
5.0	5.0	10.07 ± 0.062	101.35 ± 1.22
5.0	10.0	14.96 ± 0.051	99.68 ± 1.34
5.0	15.0	19.99 ± 0.020	99.59 ± 1.37
5.0	20.0	25.03 ± 0.056	100.61 ± 1.02

5.27. Results of repeatability of measurements of absorbance of Valsartan at 518.8 nm

Concentration (µg/ml)	Absorbance Mean ± SD (n=7)	% CV
5.0	0.158 ± 0.0035	0.61
10.0	0.321 ± 0.0062	0.32
15.0	0.472 ± 0.0029	0.21
20.0	0.633 ± 0.0054	0.40
25.0	0.811 ± 0.0039	0.88

Table 5.28. Precision data for Valsartan in phosphate buffer pH 6.8

Concentration (µg/ml)	Intra day precision		Inter day precision	
	Absorbance Mean ± SD (n=3)	% CV	Absorbance Mean ± SD (n=3)	% CV
5.0	0.152 ± 0.0045	1.96	0.161 ± 0.0051	2.18
10.0	0.299 ± 0.0060	1.02	0.330 ± 0.0070	2.12
15.0	0.468 ± 0.0085	1.82	0.466 ± 0.0100	2.15
20.0	0.641 ± 0.0075	1.18	0.651 ± 0.0098	1.51
25.0	0.788 ± 0.0071	0.90	0.803 ± 0.0103	1.28

Table 5.29. Data for Job's continuous variation method

Mole fraction of Valsartan	Mole fraction of safranin O	Absorbance of Valsartan
0.000	1.000	0.000
0.125	0.875	0.155
0.250	0.750	0.210
0.375	0.625	0.275
0.500	0.500	0.318
0.625	0.375	0.261
0.750	0.250	0.189
0.875	0.125	0.129
1.000	0.000	0.000

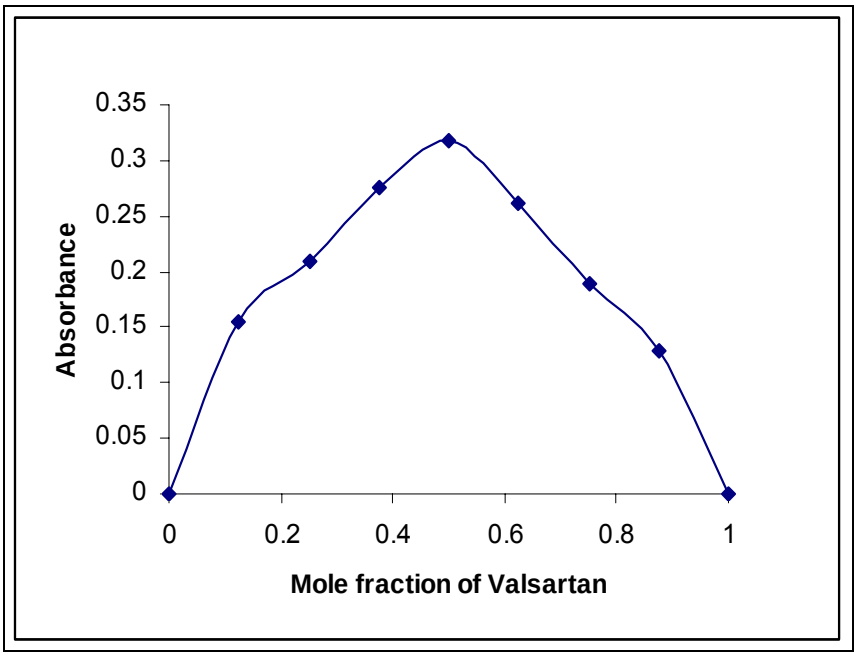


Fig. 5.16. Job's continuous variation method

Table 5.30. Analysis of market formulations of Valsartan

Formulation	Label claim (mg)	% I found Mean ± SD (n = 5)	% CV
Tablet - 1	80.0	99.64 ± 0.021	2.28
Tablet - 2	160.0	100.07 ± 0.038	2.81
Capsule - 1	40.0	99.91 ± 0.045	1.89
Capsule - 2	80.0	98.88 ± 0.081	2.06

Table5.31. Interference of Hydrochlorothiazide on absorbance of Valsartan-safranin O complex

Concentration of Valsartan (µg/ml)	Concentration of Hydrochlorothiazide (µg/ml)	Absorbance ± SD (n=5)	% CV
10.0	5.0	0.318 ± 0.0033	1.23
10.0	10.0	0.319 ± 0.0059	0.92
10.0	15.0	0.317 ± 0.0068	0.97
10.0	20.0	0.321 ± 0.0031	1.05
10.0	25.0	0.319 ± 0.0034	1.53

Table 5.32. Interference of s-Amlodepine besylate on absorbance of Valsartan-safranin O complex

Concentration of Valsartan (µg/ml)	Concentration of s-Amlodepine besylate (µg/ml)	Absorbance ± SD (n=5)	% CV
10.0	5.0	0.320 ± 0.0031	1.73
10.0	10.0	0.321 ± 0.0024	1.25
10.0	15.0	0.318 ± 0.0032	0.94
10.0	20.0	0.319 ± 0.0050	2.05
10.0	25.0	0.322 ± 0.0039	1.33

Table 5.33. Analysis of marketed formulations of Valsartan in combination with Hydrochlorothiazide and s-Amlodipine besylate individually.

Formulation	Label claim (mg)	% found Mean ± SD (n = 5)	% CV
Tablet - 1	Valsartan 80.0 Hydrochlorothiazide 12.5	99.92 ± 0.028	2.18
Tablet - 2	Valsartan 80.0 Hydrochlorothiazide 12.5	98.04 ± 0.075	2.04
Capsule - 1	Valsartan 80.0 s-Amlodipine besylate 2.5	100.59 ± 0.071	1.24

Table 5.34. Summary of validation parameters of Valsartan

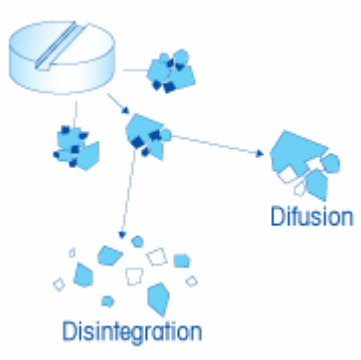
Parameter	Valsartan
Linearity range (μg/ml)	5.0 – 25.0
Limit of detection (LOD) (μg/ml)	0.50
Limit of quantification (LOQ) (μg/ml)	5.00
Precision (% CV) • Repeatability • Intraday • Interday	 0.21 – 0.88 0.90 – 1.96 1.28 – 2.18
% Recovery	99.59 – 101.35
Specificity	Specific

Table 5.35. Optical and regression characteristics of Valsartan

Parameters	Results of Valsartan
λ_{max} (nm)	518.8
Regression equation (Y = mx + c)	Y= 0.0321x – 0.0079
Slope (b)	0.0321
Intercept (a)	0.0079
Correlation coefficient (r^2)	0.9994
Sandell's sensitivity ($\mu g/ml/cm^2$)	0.0314
Molar absorptivity (L/mol.cm)	1.39×10^4

6.

DISSOLUTION PROFILE STUDY…



6. DISSOLUTION PROFILE STUDY

6.1. Introduction

Dissolution testing has been emerged as a single most important test to ensure quality of the product when carried out appropriately. Knowledge of theoretical as well as practical aspects of dissolution testing proved to be very important for pharmaceutical scientist engaged in product development and quality control. Since the dissolution rate of a drug from its dosage form can often become the rate limiting process in the bio-availability, efforts have been laid down in the development of reliable in vitro test methods that can mimic the in vivo conditions.

Objectives of dissolution test

1. As quality control tool
2. In the development of new drug products
3. To assists in the determination of bioavailability and bioequivalence.
4. For characterizing the biopharmaceutical quality of a product at Different stages of life cycle.
5. For choosing between different alternative formulation candidates.
6. As a supportive in the evaluation and interpretation of possible risks.

Official apparatuses

USP App- I

It can be used for the dissolution testing of capsules. It consists of the following:

- A covered vessel made up of glass or other inert transparent material
- A motor assembly
- A metallic drive shaft

- A cylindrical basket – 40 mesh (USP)
- A suitable water bath in which vessel can be partially immersed and have capacity to maintain specified temp inside the vessel

USP App- II

It is used for the dissolution testing of tablets. It consists of the following:

- Paddle and shaft as a stirring element
- A small, loose piece of non – reactive material (few turns of wire helix) may be attached to dosage units that would otherwise float
- All others points, (from (1) to (3)) are same as in App 1

USP App- III

It is used for the dissolution testing of extended release dosage forms. The assembly consists of;

- A set of cylindrical flat bottom glass vessels
- A set of glass reciprocating cylinders
- Stainless steel fittings and polypropylene screens to fit tops and bottoms of the reciprocating cylinders.
- A motor and a drive assembly
- A water bath
- Distance traveled by the reciprocating cylinder during the upward and downward stroke 9.9 – 10.1 cm

USP App- IV

It is used for dissolution testing of delayed release dosage forms. It consists of

- A reservoir
- A pump for dissolution medium

- A flow through cell
- A water bath
- Delivery range of the pump is 240 – 960 ml/hr. Flow rate is 4.8 and 16 ml/min
- Bottom cone filled with small glass beads of 1mm diameter of which one bead is of about 5 mm positioned at apex to protect the fluid entry tube
- A tablet holder for positioning of special dosage forms e.g. inlay tablets

USP App- V

It is used for dissolution testing for transdermal dosage forms.

- It consists of stainless steel disc assembly in addition to paddle apparatus for holding transdermal dosage forms.

USP App- VI

It is used for dissolution testing for transdermal dosage forms.

- Basket is replaced and a shaft with a stainless steel cylinder stirring element and a water bath to maintain specified temp during test. Dosage form is spreaded on the cylinder in form of flat film.

USP App- VII

It is used for dissolution testing of variety of dosage forms such as transdermal dosage forms and solid dosage forms. It consists of:

- A set of reciprocating disc shaped sample holders

Testing conditions

(1) Dissolution medium

Composition : Generally water is used as solvent for the dissolution medium but pH and surface tension of pure water varies depending upon source of water pH may change due to absorption of CO_2 from air. Additions of organic solvents are generally avoided.

pH: It should be in between 1- 6.8. For low pH 0.1 N HCl is used. For high pH (4.5 – 8.0 pH) – USP buffers are used.

Volume: USP specifies it in between 50 ml – 1000ml. Generally 900 ml is used. For poorly soluble drugs 4000 ml can be used but it is considered as modification.

Agitation: It should be in between 50 -100 rpm but should not be more than 150. In cell flow rate should be 4, 8 or 16 ml/min.

Temperature: It should be 37±0.2 ^{o}C for transdermal dosage forms.

Deaeration: It should be done by suitable validated method.

(2) Qualification and validation

Apparatus suitability test (AST) with calibrators is a further important aspect of qualification and validation. The use of USP calibrator tablets (disintegrating and non-disintegrating) is recommended. System suitability test of USP App-3 has to be performed with both multi particulate and a mono particulate standard formulation. AST are recommended to be performed not less than twice per year per equipment and after any occasion of equipment change, significant repair or movement.

Validation of dissolution tests for solid dosage forms

- Specificity
- Accuracy
- Filter bios calculations
- Precision and ruggedness
- Geometrical and dimensional accuracy

(3) Dissolution specifications

They are set to ensure batch to batch consistency with the range, which guarantees acceptable biopharmaceutical performances in vivo. For immediate and very fast drug releasing dosage form in a single point test at least 80% of the drug substance is dissolved in 15 min under reasonable justified test conditions.

For controlled release – At least three points are required.

1st specification point – To prevent dose dumping it should be set after a testing interval of one or two hours or corresponding to a dissolved amount 20 – 30 % of labeled drug substance.

2nd specification point – It should define the dissolution pattern and thus be around 50 % release of labeled drug substance.

3rd specification point – It should release quantitative drug release generally > 80 %

The dissolution run in quality control should be extended for the time interval at least 80 % of drug substance is dissolved.

Shorter time test intervals can be acceptable in special cases.

(4) Factors affecting dissolution time

Factors related to the physicochemical property of drug

- Solid phase characteristics
- Polymorphism
- Amorphous / crystal state
- Free or salt form
- Complexation, eutectics
- Partical size
- Surfactants

Excipients and additives

- Granulating agents and binders
- Disintegrating agents
- Lubricants
- Surfactants

Factors related to dosage forms

- Granule size
- Drug excipients interactions
- Compression force
- Degradation
- Storage of dosage forms

Factors related to the dissolution testing devices

- Eccentricity of agitating element
- Vibration
- Agitation intensity
- Stirring element alignment

- Flow pattern disturbances
- Sampling probes position and filter
- Dosage form position
- Type of device

Factor related to dissolution test parameter

- Temperature
- Dissolution medium (composition, viscosity, dissolution)

Miscellaneous factors

- Adsorption
- Sorption

6.2 Dissolution study

6.2. 1. Apparatus

- Dissolution test apparatus [Electro lab]
- Double beam spectrophotometric [shimadzu model UV- 1601]
- Sartorious monopan balance
- Digital pH meter (cystronic)
- Sonicator
- Glass stoppered tubes

6.2. 2 Reagents and materials

- Dye solution
- 0.1 N hydrochloric acid

6.2. 3 Preparation of solution (As per 4.1.2)

6.2.4. Dissolution profile study (Fluoxetine hydrochloride, Sertraline hydrochloride)

The dissolution profile study was carried out using dissolution apparatus USP – II (Electrolab, India) containing 900 ml of 0.1N hydrochloric acid used as a dissolution medium at 37±2 ^{0}C at 50 rpm. Carefully transferred tablet/capsule of same brand (Fluoxetine hydrochloride, Sertraline hydrochloride) into six different baskets. Samples were withdrawn (5.0 ml) at interval of 10, 20, 30, 45, 60 & 90 minutes from each basket and simultaneously same amount was replaced by 0.1N hydrochloric acid into each basket.

An aliquot (1.0 ml) was pipetted out in to a glass stoppered tube containing methyl orange solution (2.0 ml). The final volume was adjusted to 5.0 ml with 0.1N hydrochloric acid and analysed as described under 4.3.5 and

4.4.5 for fluoxetine hydrochloride and sertraline hydrochloride respectively. The absorbance of the colored solution was measured at 426.0 nm and 425.0 nm for fluoxetine hydrochloride and sertraline hydrochloride respectively against reagent blank. The concentrations of drugs (Fluoxetine hydrochloride, Sertraline hydrochloride) were determined. The dissolution profile study for five different branded tablet/capsule of fluoxetine hydrochloride (Table no. 6.1 to 6.5 and Fig. 6.1 to 6.6) and sertraline hydrochloride (Table no. 6.6 to 6.10 and Fig. no. 6.7 to 6.12) were carried out.

6.2.5. Dissolution profile study (Valsartan).

The dissolution profile study was carried out using dissolution apparatus USP – II (Electrolab, India) containing 900 ml of phosphate buffer (pH 6.8) used as a dissolution medium at 37±2 ^{0}C at 50 rpm. Carefully transferred tablet/capsule of same brand of valsartan (single as well as combined dosage forms) into six different baskets. Samples were withdrawn (5.0 ml) at interval of 5, 10, 15, 20, 25 and 30 minutes from each basket and simultaneously same amount was replaced by phosphate buffer into each basket.

An aliquot (1.0 ml) was pipetted out in to a glass stoppered tube containing safranin O solution (2.5 ml). The final volume was adjusted to 5.0 ml with phosphate buffer (pH 6.8) and analysed as described under 4.6.8. The absorbance of the colored solution was measured at 518.8 nm against reagent blank. Five different branded tablets/capsules of Valsartan (single as well as combined dosage forms) were taken for dissolution profile study. The concentration of Valsartan was determined. (Table no. 6.11 to 6.15, Fig. no. 6.13 to 6.18)

6.2.6 Results and Discussion

Dissolution test is an important quality control tool for routine analysis. Dissolution test for fluoxetine hydrochloride is official in IP, BP and USP and they specify Q limit at 80 %, while for sertraline hydrochloride and valsartan the dissolution test in not prescribed by any pharmacopeias.

US-FDA described single point dissolution test for the highly soluble and rapidly dissolving drug product with specification of not less than 85 % (Q = 80 %) in 60 minutes or less is sufficient as a routine quality.

The dissolution profile study for fluoxetine hydrochloride and sertraline hydrochloride were carried out using 0.1N hydrochloric acid as a dissolution medium at 37±2 ^{0}C and analysed by proposed acid dye technique. Five different branded tablet/capsule were used. It was found that almost 80 % dissolution was completed within 30 minutes, in all the analysed dosage forms. (Table no. 6.1 to 6.10, Fig. no. 6.1 to 6.12)

Same way dissolution profile study for valsartan in single as well as combined dosage form was carried out using phosphate buffer (pH 6.8) as a dissolution medium at 37±2 ^{0}C and analysed by proposed basic dye technique. It was found that almost 90 to 95 % dissolution was completed within 30 minutes, in all the analysed dosage forms (Table no. 6.11 to 6.15, Fig. no. 6.13 to 6.18).

Table 6.1. Dissolution profile of Afzot tablet (20.0 mg Fluoxetine HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	61.22	59.91	65.18	63.81	65.72	62.62	63.08 ± 3.01
20	72.81	78.88	74.29	73.92	77.79	76.66	75.72 ± 2.26
30	81.32	86.85	84.43	79.45	78.78	83.56	82.40 ± 2.40
45	86.62	88.33	84.72	89.92	83.59	87.45	86.77 ± 2.33
60	91.41	94.36	93.46	92.96	91.37	94.73	93.05 ± 1.43
90	97.32	98.47	94.59	99.1	97.43	97.76	97.45 ± 1.55

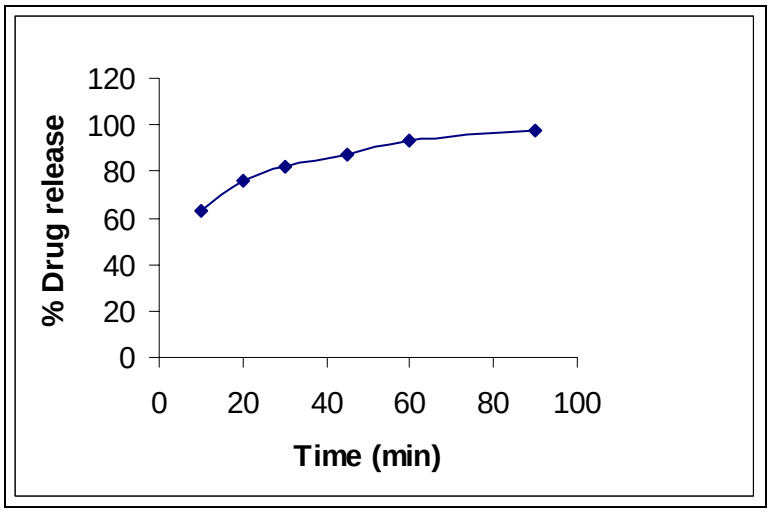


Fig. 6.1. Dissolution profile of Afzot tablet (20.0 mg Fluoxetine HCl)

Table 6.2. Dissolution profile of Exiten tablet (20.0 mg Fluoxetine HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	56.91	59.61	61.6	64.52	58.62	56.94	59.70 ± 2.95
20	70.82	64.64	69.45	68.64	71.43	72.48	69.58 ± 2.78
30	78.54	79.52	82.72	81.62	77.95	75.92	79.38 ± 2.49
45	88.62	83.83	84.34	84.92	87.39	85.56	85.78 ± 1.86
60	94.56	91.44	96.47	91.91	93.52	94.59	93.75 ± 1.87
90	97.16	98.39	94.52	97.59	99.22	98.23	97.52 ± 1.63

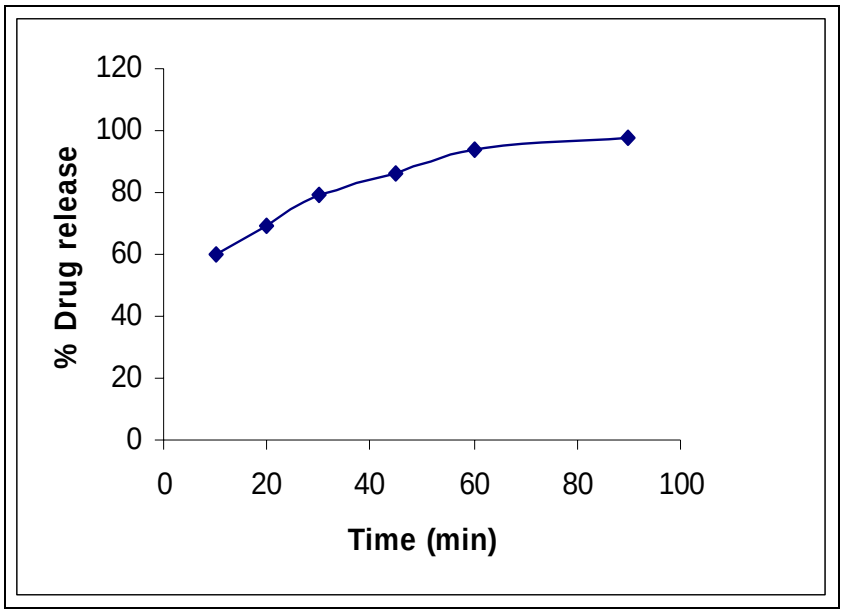


Fig 6.2. Dissolution profile of Exiten tablet (20.0 mg Fluoxetine HCl)

Table 6.3. Dissolution profile of Flunat tablet (20.0 mg Fluoxetine HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	59.10	52.16	60.92	56.47	57.16	58.84	57.44 ± 3.02
20	66.24	69.13	62.73	65.42	69.48	64.92	66.32 ± 2.59
30	79.29	81.92	78.62	83.67	81.91	82.28	81.28 ± 1.93
45	83.71	89.34	84.46	90.83	91.27	84.32	87.32 ± 3.53
60	93.62	91.57	97.40	93.76	97.73	93.96	94.67 ± 2.40
90	99.30	94.43	99.32	97.48	96.24	97.95	97.45 ± 1.88

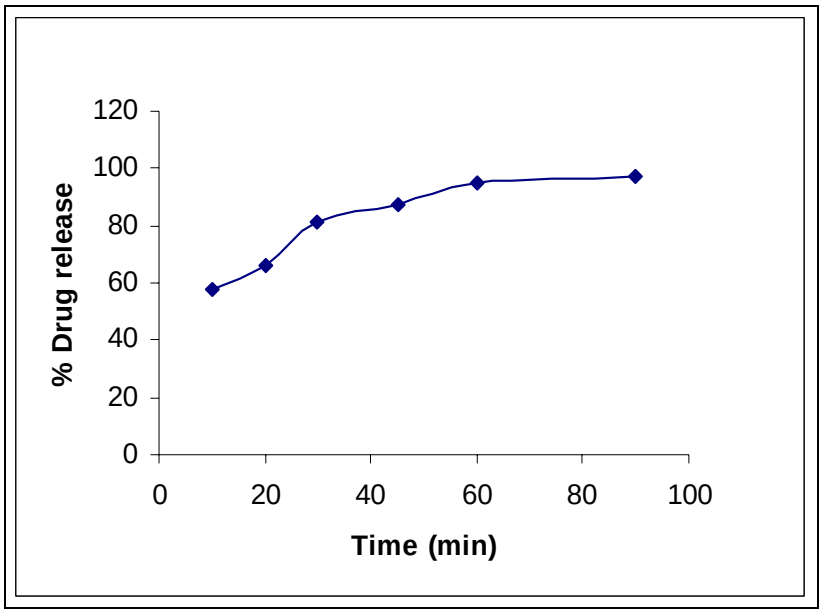


Fig 6.3. Dissolution profile of Flunat tablet (20.0 mg Fluoxetine HCl)

Table 6.4. Dissolution profile of Flunil capsule (20.0 mg Fluoxetine HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	62.65	58.12	61.94	59.11	61.52	56.66	60.00 ± 2.39
20	68.73	71.43	73.83	71.27	77.42	69.25	71.99 ± 3.22
30	81.28	82.91	79.22	77.38	83.34	78.62	80.46 ± 2.42
45	85.24	86.88	83.29	91.46	87.92	87.67	87.08 ± 2.76
60	92.52	91.23	94.39	93.82	89.59	91.48	92.17 ± 1.78
90	96.48	94.42	96.91	96.83	93.28	99.26	96.20 ± 2.10

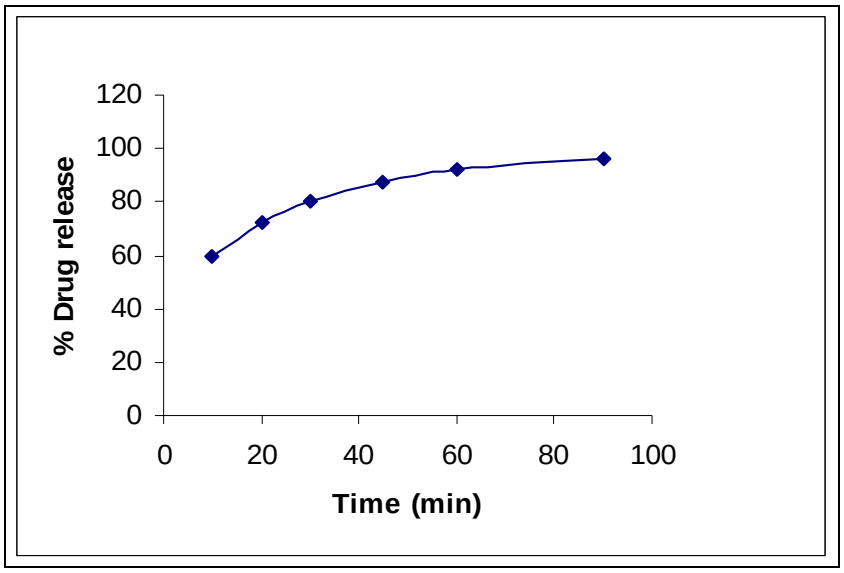


Fig. 6.4. Dissolution profile of Flunil capsule (20.0 mg Fluoxetine HCl

Table 6.5. Dissolution profile of Flutinol capsule (20.0 mg Fluoxetine HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	54.93	59.56	53.82	61.83	58.92	59.58	58.11 ± 3.07
20	66.75	69.48	64.78	69.54	72.58	67.63	68.46 ± 2.70
30	82.47	78.91	79.53	76.56	71.79	79.73	78.17 ± 3.65
45	86.62	84.73	81.49	89.72	85.48	86.71	85.79 ± 2.71
60	93.82	91.66	88.7	89.81	96.57	92.82	92.23 ± 2.84
90	98.44	94.52	91.35	98.77	93.53	96.43	95.51 ± 2.91

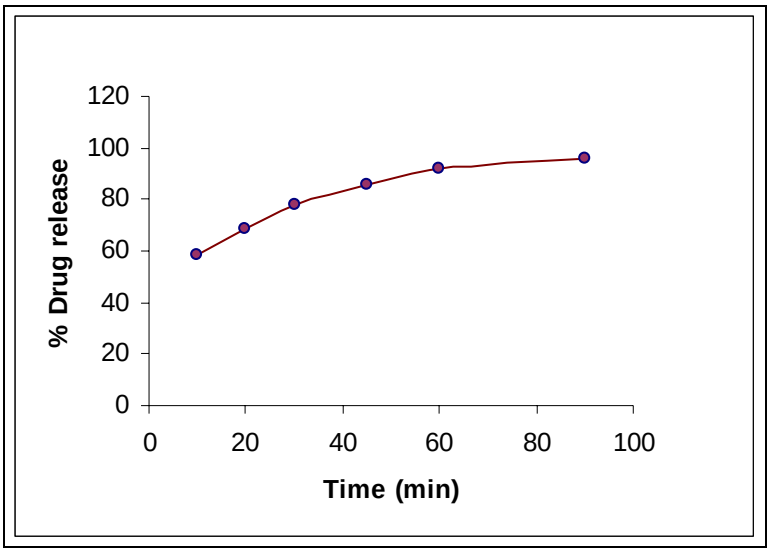


Fig. 6.5. Dissolution profile of Flutinol capsule (20.0 mg Fluoxetine HCl)

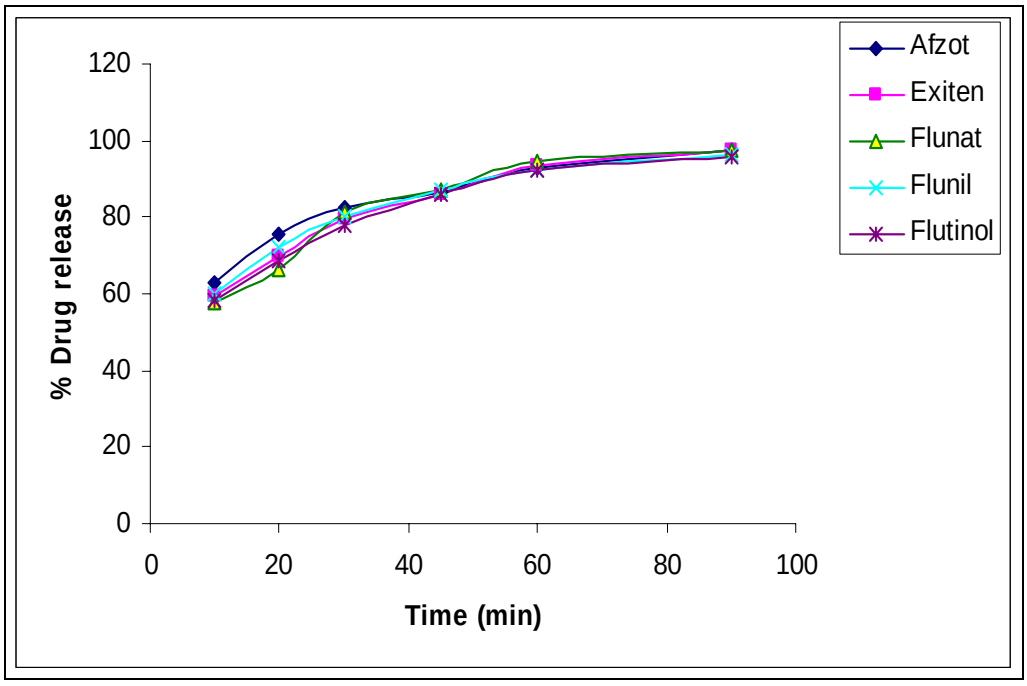


Fig. 6.6. Overlay of dissolution profile curve (Fluoxetine hydrochloride)

Table 6.6. Dissolution profile of Serenata tablet (50.0 mg Sertraline HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	63.15	61.92	62.55	64.52	59.92	62.47	62.42 ± 1.51
20	71.72	74.8	75.27	74.78	77.48	78.57	75.44 ± 2.39
30	82.42	79.35	77.48	81.4	78.34	82.92	80.32 ± 2.25
45	86.25	85.28	88.61	84.68	87.77	88.81	86.90 ± 1.75
60	91.6	89.46	92.73	88.2	92.45	93.65	91.35 ± 2.10
90	95.52	96.56	94.94	96.49	97.23	98.14	96.48 ± 1.15

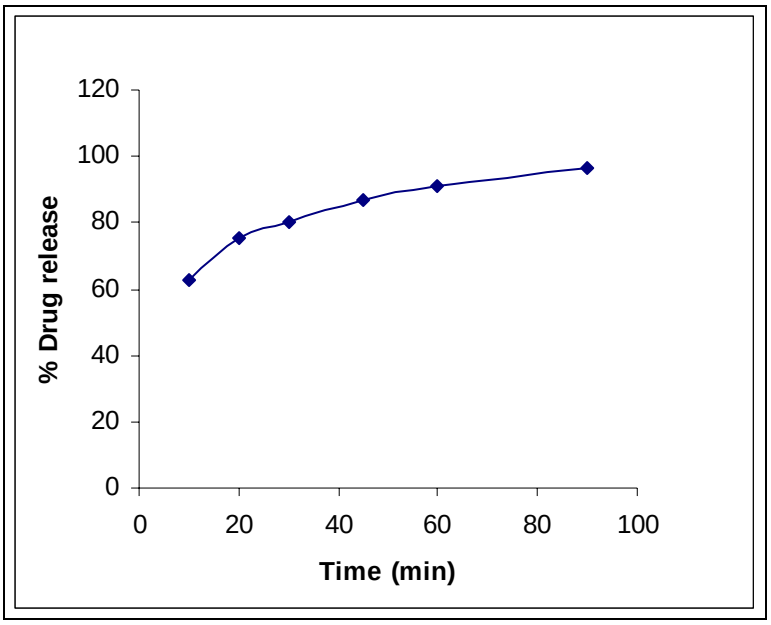


Fig. 6.7. Dissolution profile of Serenata tablet (50.0 mg Sertraline HCl)

Table 6.7. Dissolution profile of Serlin tablet (50.0 mg Sertraline HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	58.10	63.42	59.62	62.44	64.95	61.67	61.70 ± 2.50
20	77.34	74.47	71.42	79.63	77.10	75.83	75.97 ± 2.81
30	79.44	82.43	83.95	80.56	78.38	81.43	81.03 ± 2.02
45	89.90	84.13	87.24	89.73	90.62	91.46	88.85 ± 2.71
60	95.33	91.16	93.62	92.88	91.86	93.42	93.05 ± 1.46
90	98.12	94.42	96.63	93.58	95.73	99.15	96.27 ± 2.14

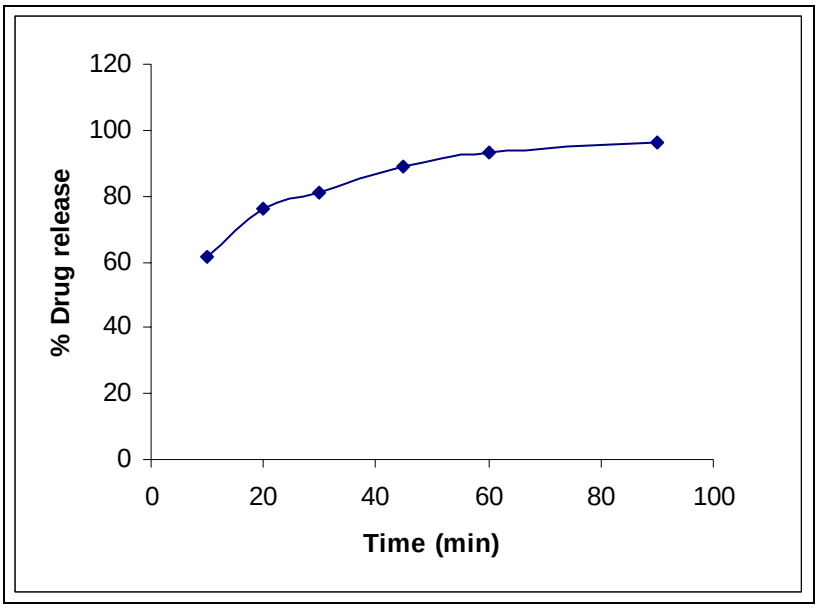


Table 6.8. Dissolution profile of Serlin tablet (50.0 mg Sertraline HCl)

Table 6.8. Dissolution profile of Inosert tablet (50.0 mg Sertraline HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	57.43	61.94	63.92	60.23	59.2	63.96	61.11 ± 2.63
20	73.86	69.58	72.96	77.41	71.62	74.72	73.36 ±2.68
30	88.47	81.42	84.84	83.52	87.43	83.75	84.91 ± 2.63
45	91.57	89.44	87.33	92.43	88.48	81.63	88.48 ± 3.86
60	93.59	92.16	89.17	93.82	94.27	95.47	93.08 ± 2.19
90	95.48	92.22	96.25	98.62	95.52	98.55	96.11 ± 2.37

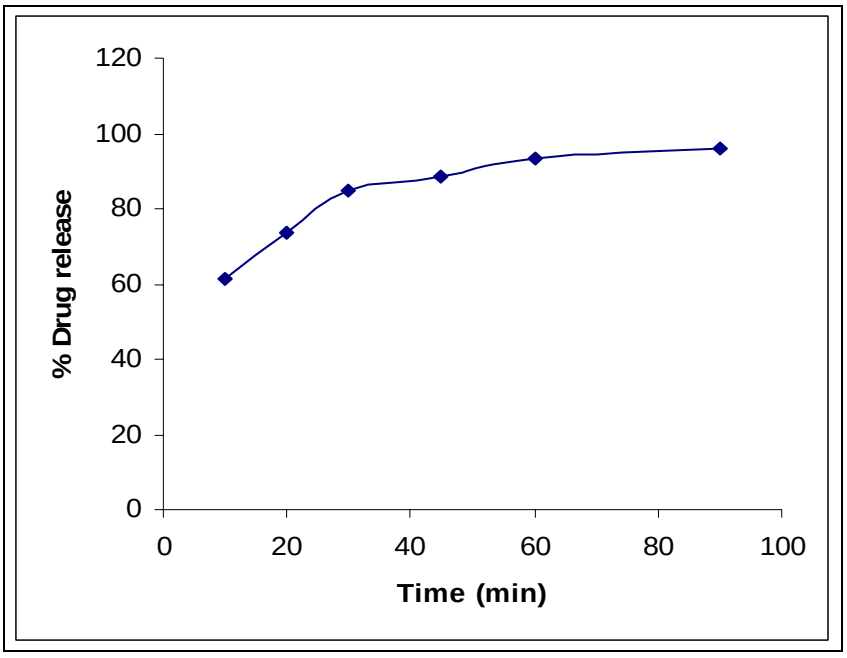


Fig. 6.9. Dissolution profile of Inosert tablet (50.0 mg Sertraline HCl)

Table 6.9. Dissolution profile of Daxid tablet (50.0 mg Sertraline HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	60.32	63.91	61.94	65.2	61.96	59.57	62.15 ± 2.12
20	68.42	66.43	73.78	65.82	66.74	76.45	69.61 ± 4.43
30	74.67	79.73	81.42	75.88	73.72	81.75	77.86 ± 354
45	77.37	81.62	83.65	82.73	79.52	83.82	81.45 ± 2.55
60	79.48	88.48	86.48	89.25	81.67	91.42	86.13 ± 4.64
90	94.57	96.43	97.18	95.66	98.41	95.77	96.34 ± 1.34

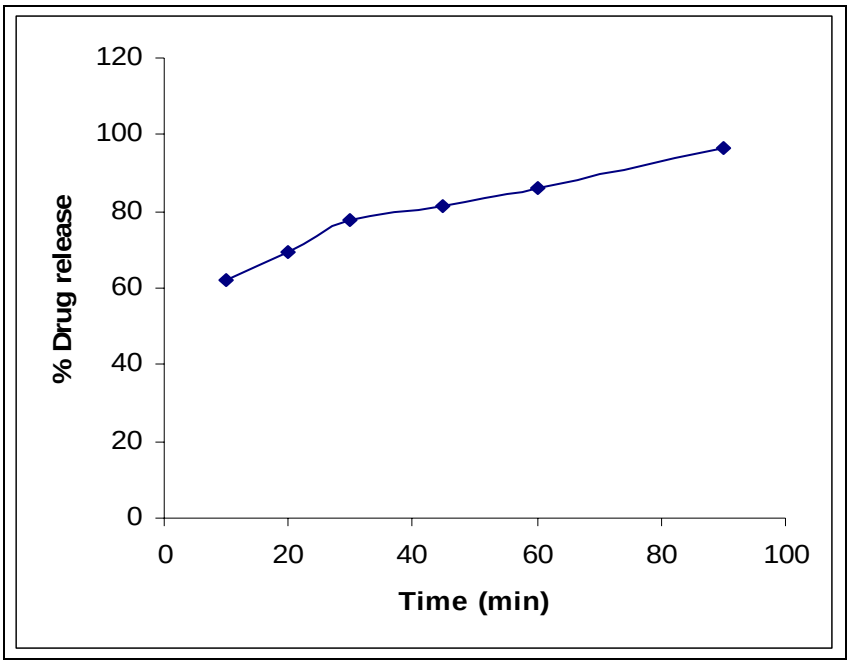


Table 6.10. Dissolution profile of Daxid tablet (50.0 mg Sertraline HCl)

Table 6.10. Dissolution profile of Serdep tablet (50.0 mg Sertraline HCl)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
10	58.60	64.72	61.79	59.84	65.42	67.16	62.92 ± 3.37
20	71.15	79.91	69.56	74.47	78.72	72.83	74.44 ± 4.13
30	85.82	83.52	84.83	81.53	78.49	79.22	82.24 ± 2.99
45	81.79	89.67	90.97	88.58	82.77	83.35	86.19 ± 3.99
60	85.81	92.40	92.42	91.66	94.49	90.18	91.16 ± 2.97
90	96.27	94.32	97.16	94.69	94.62	97.28	95.72 ± 1.34

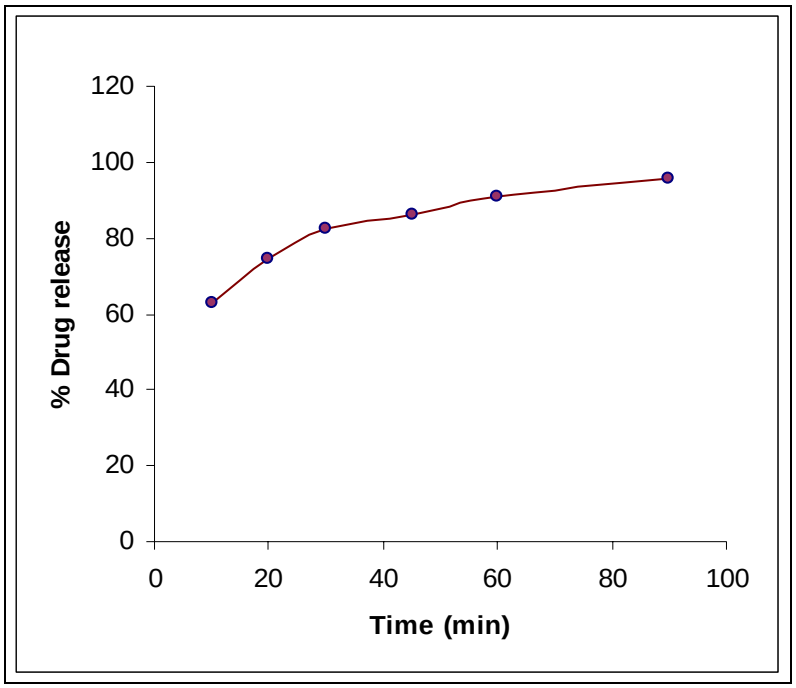


Fig. 6.11. Dissolution profile of Serdep tablet (50.0 mg Sertraline HCl)

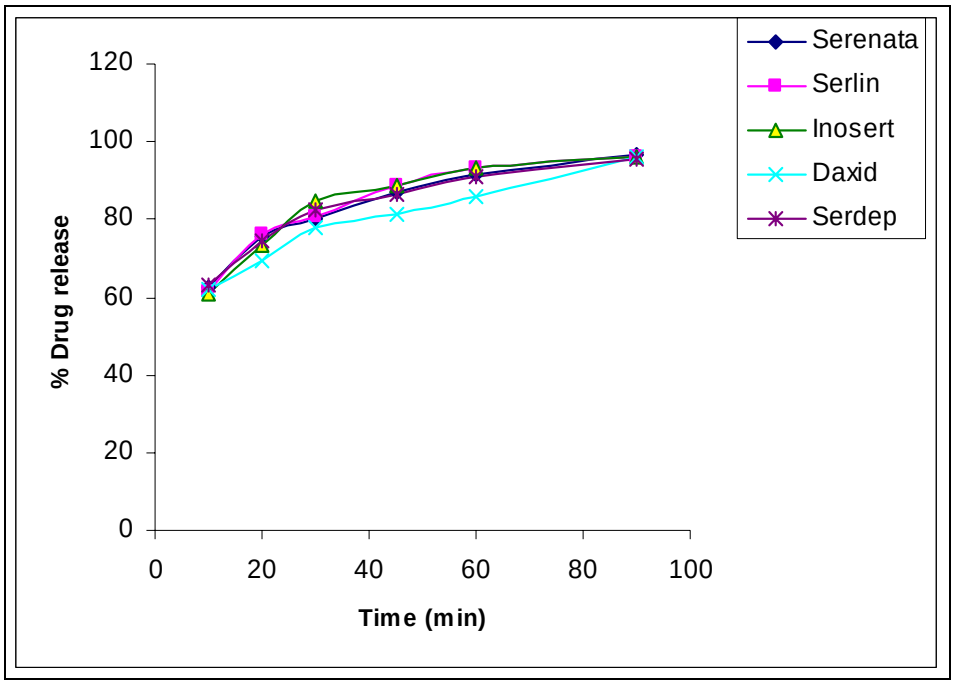


Fig. 6.12. Overlay of dissolution profile curve (Sertraline HCl)

Table 6.11. Dissolution profile of Valzar tablet (80.0 mg Valsartan)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
5	32.24	42.41	40.11	36.44	33.25	31.14	35.93±4.55
10	61.23	70.44	71.65	65.04	60.11	62.25	65.12±4.89
15	88.25	94.07	96.23	93.93	91.24	89.52	92.21±3.05
20	94.66	98.07	100.32	97.04	95.23	94.45	96.63±2.30
25	96.44	98.98	101.11	98.37	98.22	98.52	98.61±1.50
30	97.25	101.58	101.54	99.41	98.52	100.74	99.84±1.75

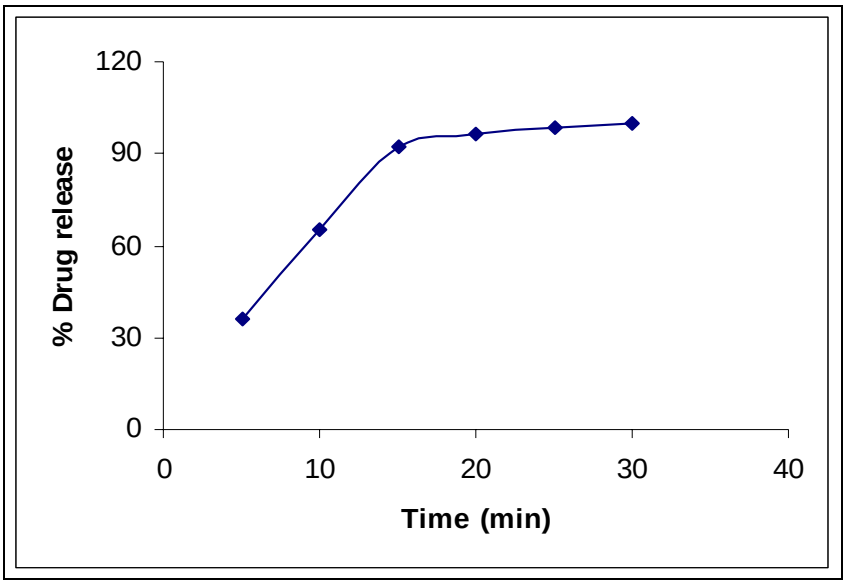


Fig. 6.13. Dissolution profile of Valzar tablet (80.0 mg Valsartan)

Table 6.12. Dissolution profile of Valzar H tablet (80.0 mg Valsartan)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
5	31.21	38.25	35.85	34.69	41.08	40.55	36.94±3.77
10	65.24	70.31	68.31	70.92	66.62	68.23	68.27±2.15
15	91.98	97.23	96.25	100.62	97.54	99.08	97.12±2.95
20	94.22	97.69	99.08	101.23	98.15	99.85	98.37±2.39
25	97.98	98.46	100.00	101.08	98.62	100.15	99.38±1.20
30	99.23	98.62	100.62	101.69	101.08	100.77	100.34±1.17

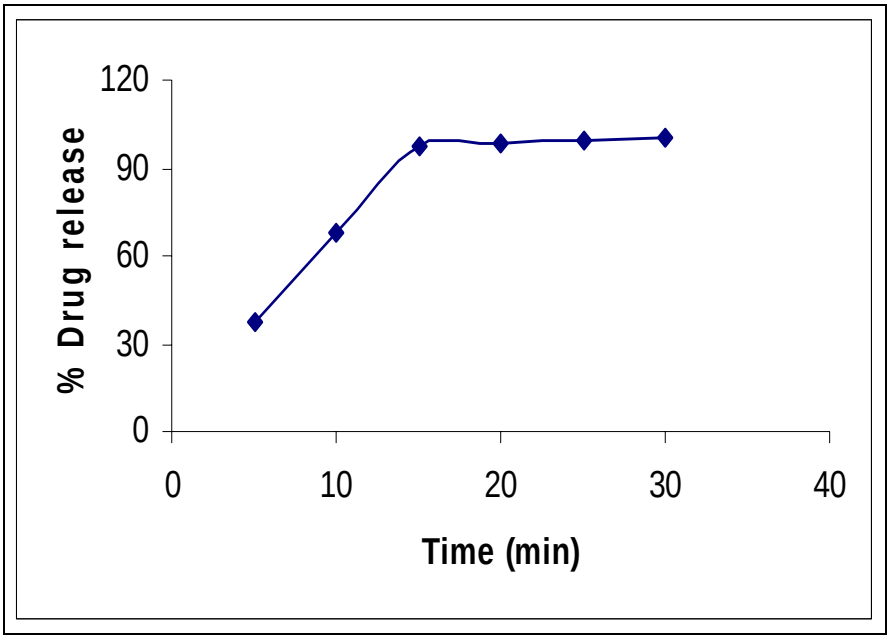


Fig 6.14. Dissolution profile of Valzar H tablet (80.0 mg Valsartan)

Table 6.13. Dissolution profile of Valent H tablet (80.0 mg Valsartan)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
5	42.11	48.03	44.25	48.33	48.79	42.42	45.655±3.086
10	58.64	61.06	64.65	57.22	59.39	55.89	59.475±3.096
15	88.95	90.61	82.23	92.73	90.91	91.97	89.567±3.818
20	95.23	96.82	92.36	99.7	96.67	96.5	96.213±2.393
25	98.33	97.12	97.42	100.24	96.97	98.94	98.170±1.266
30	98.64	97.72	97.27	100.15	99.09	99.55	98.737±1.093

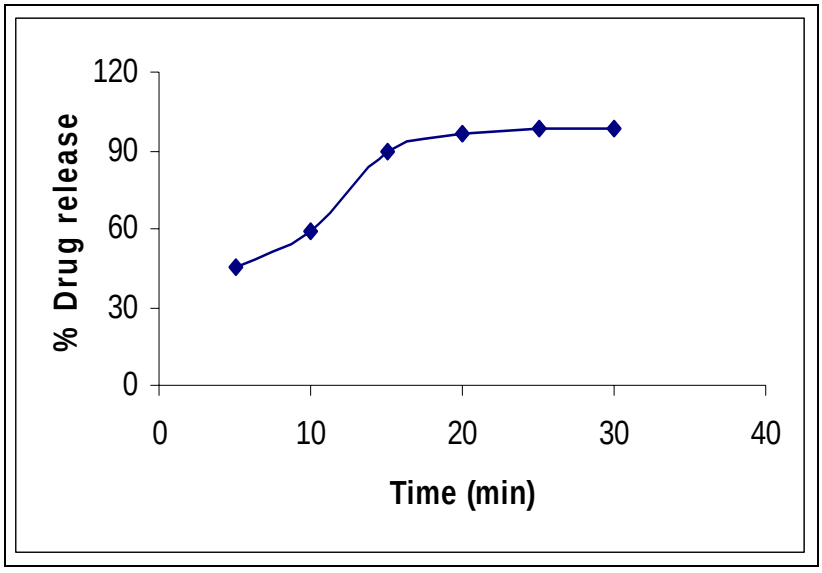


Fig 6.15. Dissolution profile of Valent- H tablet (80.0 mg Valsartan)

Table 6.14. Dissolution profile of Starval-80 capsule (80.0 mg Valsartan)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
5	32.87	21.82	20	22.42	29.35	20.15	24.43±5.37
10	60.12	58.79	52.23	51.44	58.59	56.21	56.23±3.639
15	75.23	67.14	68.23	71.06	74.45	69.09	70.87±3.34
20	90.23	86.67	86.21	87.42	89.97	96.5	89.50±3.82
25	99.98	98.48	94.23	96.64	98.87	98.94	97.86±2.08
30	100.22	98.79	99.39	98.64	99.91	98.48	99.24±0.72

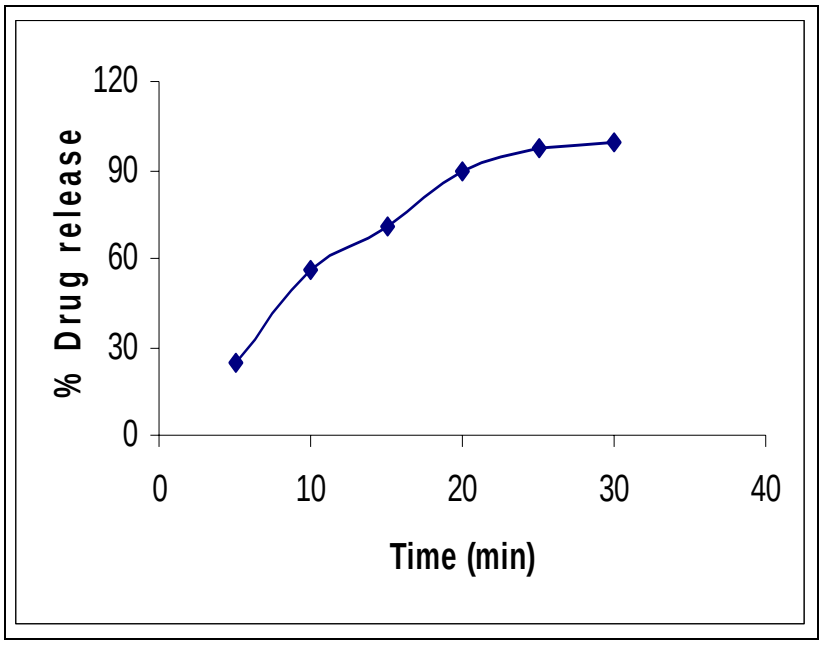


Fig 6.16. Dissolution profile of Starval-80 capsule (80.0 mg Valsartan)

Table 6.15. Dissolution profile of Valsar-SM capsule (80.0 mg Valsartan)

Time (minute)	Cumulative % drug release						% drug release mean ± SD
	1	2	3	4	5	6	
5	22.24	16.82	17.13	15.12	25.24	16.36	18.82±3.99
10	64.58	57.87	55.71	57.25	66.78	55.4	59.60±4.85
15	82.87	80.4	78.55	77.93	84.47	74.84	79.84±3.50
20	89.2	82.89	88.27	89.97	92.45	84.27	87.84±3.60
25	98.61	94.87	100.46	100.15	98.46	100	98.76±2.08
30	100.27	99.69	100.62	100.93	99.14	100.62	100.21±0.64

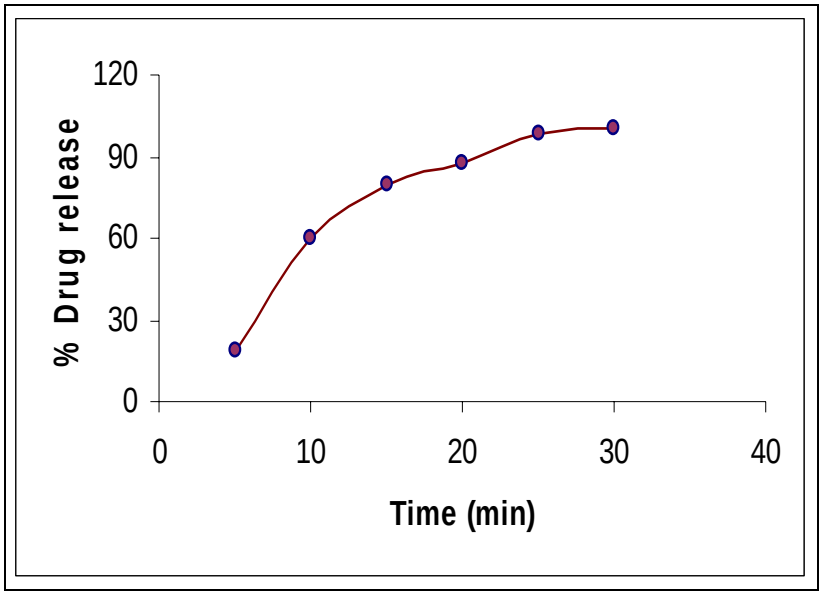


Fig 6.17. Dissolution profile of Valsar-SM capsule (80.0 mg Valsartan)

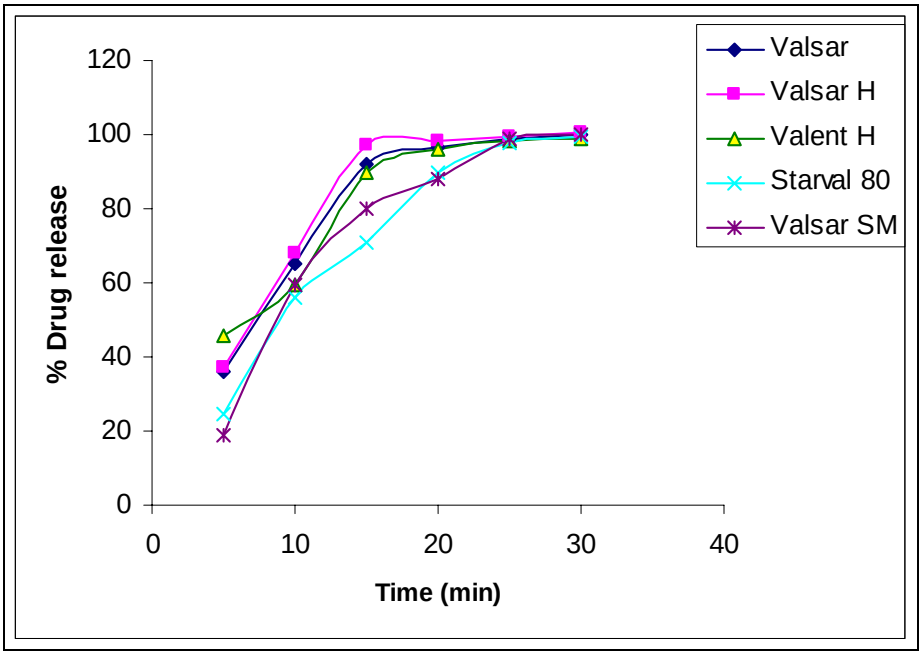


Fig. 6.18. Overlay of dissolution profile curve (Valsartan)

7.

SUMMARY

7. SUMMARY

1. The present investigation is study on the utility of ion – pair complex of acid dye as well as basic dye with different nitrogen containing molecules, which are widely used as an antidepressants and specific AT_1 receptor antagonists.

2. The proposed methods were validated and found to be very simple, accurate, sensitive and cost effective for determination of fluoxetine, Sertraline, duloxetine (acid dye method) and valsartan (basic dye method) in bulk and dosage forms.

3a. Valsartan was analyzed in combined dosage forms (valsartan + hydrochlorthiazide, valsartan + s-amlodipine) by basic dye method. Hydrochlorthiazide and s-amlodipine does not form ion – pair complex with safranin O solution, and therefore did not interfere in the analysis.

3b. Excipients and other diluents used in the tablets and capsules did not interfere in analysis carried out either by acid dye technique or basic dye technique.

4. The composition of ion – pair was determined by Job's continuous variation method using equimolar solution. It was found that 1:1 stoichiometric complex was formed for valsartan with safranin O solution.

5. The ion pair composition of fluoxetine hydrochloride, sertraline hydrochloride and duloxetine hydrochloride were also determined by job's continuous variation method using equimolar solution. It was found that 1:1 stochiometric complex was formed for fluoxetine hydrochloride, sertraline hydrochloride and duloxetine hydrochloride with methyl orange.

6. The proposed methods are equally applicable for dissolution profile study for fluoxetine hydrochloride, sertraline hydrochloride (acid dye method) in dosage forms and valsartan (basic dye method) in single as well as combined dosage forms.

7. In dissolution profile study of fluoxetine hydrochloride and sertraline hydrochloride, six marketed formulations were evaluated and all of them show more than 85% release in thirty minutes.

8. In dissolution profile study of valsartan, six marketed formulations including single and combined dosage forms were evaluated and all of them show more than 90% release in thirty minutes.

9. This study suggests that ion – pair complexation method can be employed for routine analysis of fluoxetine, sertraline, duloxetine (acid dye complexation) and valsartan (basic dye complexation) in dosage forms.

8.

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