CHAPTER 3

SOLUTIONS AND REAGENTS
Section (i): Preparation of reagents and solutions

AR grade chemical are used for preparation of reagents and solutions in the present investigations.

Buffer solution (pH 3.5)

Buffer solution was obtained by diluting a mixture of 50 ml of 0.2M potassium acid phthalate and 8.4 ml of 0.2M HCl to 200 ml with distilled water and the pH is adjusted to 3.5.

Bromocresol green: (0.5% w/v)

Bromocresol green solution is prepared by dissolving 500 mg of bromocresol green (Loba) in 100 ml of distilled water.

DDQ Vanillin solution (1% w/v)

DDQ (2, 3-dichloro 5, 6-dicyano-p-benzoquinone) (Loba Chem., India) solution is prepared by dissolving 100 mg in 100 ml of distilled water.

Hydrochloric acid (0.1 N)

Hydrochloric acid solution (0.1N) is prepared by diluting the requisite volume of concentrated AR hydrochloric acid with distilled water and standardized by usual procedure.

Sodium nitrite (0.1N)

0.69 g of Sodium nitrite is dissolved in distilled water and the resulting solution is made upto the mark in 100 ml standard flask with distilled water. This solution is standardized by the usual analytical procedure.
**Resorcinol (1%)**

Accurately weighed 1.0 g of resorcinol solution is dissolved in methanol and the volume adjusted to 100 ml with methanol.

**Urea solution (1%)**

Accurately weighed 1 gm of Urea is dissolved in double distilled water and the volume made up to 100 ml with double distilled water.

**Tenofovir difoproxil fumarate solution**

About 50 mg of pure tenofovir difoproxil fumarate solution (1mg/ml) is accurately weighed and dissolved in methanol and diluted to 50 ml with methanol. The stock solution is further diluted to get the desired working concentration.

**Ramipril solution**

The stock solution (1mg ml⁻¹) of ramipril is prepared by dissolving 50 mg of drug in 50 ml of methanol. A portion of this stock solution is diluted stepwise with the methanol to obtain working standard solutions of 100 µg/mL.

**Esomeprazole magnesium**

An accurately weighed 50 mg of esomeprazole magnesium is dissolved in methanol and the volume is adjusted to 50 ml with methanol. Further dilution is made to obtain the working concentration of 100 µg/mL.

**Alfuzosin hydrochloride solution**

Fifty mg of pure alfuzosin hydrochloride is dissolved in 50 ml methanol and further diluted to obtain the working concentration of 100 µg/mL.
Mosapride solution

An accurately weighed 50 mg of mosapride is dissolved in methanol. The volume is adjusted to 50 ml with methanol in 50 ml standard flask. One ml of this solution contains 1 mg/mL. The stock solution is further diluted to get desired concentration of 200 μg/mL.

Mesalamine

50 mg of pure mesalamine is dissolved in methanol and the volume is adjusted to 50 ml with methanol. The stock solution is further diluted to get working concentration of 200 μg/mL.

Lamotrigine solution

Pure lamotrigine (50 mg) is dissolved in 50 ml methanol to obtain a stock solution of 1 mg/mL. The final concentration of lamotrigine is brought to 100 μg/mL with methanol.

Dapsone solution

50 mg of dapsone is dissolved in 50 ml methanol. 1.0 ml of the above stock solution is further diluted to 50 ml with methanol to get working concentration of 200 μg/mL.

Amiloride solution

50 mg of amiloride is dissolved in 50 ml methanol. 1.0 ml of the above stock solution is further diluted to 50 ml with methanol to get working concentration of 100 μg/mL.
Pramipexole solution

Pure pramipexole (50 mg) is dissolved in 50 ml methanol to obtain a stock solution of 1 mg/mL. The final concentration of pramipexole is diluted to 100 μg/mL with methanol.
Section (ii): Brief profile of selected drugs

(a) Ramipril

Ramipril, 2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S, 3S, 5S)-2-azabicyclo [3.3.0] octane carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor. Ramipril is a 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative. It is a white, crystalline substance soluble in polar organic solvents and buffered aqueous solutions. Ramipril melts between 105°C and 112°C. Ramiprilat, the diacid metabolite of ramipril, is a non-sulfhydryl angiotensin converting enzyme inhibitor. Ramipril is converted to ramiprilat by hepatic cleavage of the ester group. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduces the degradation of bradykinin1. The structural formula of ramipril is given in Fig. 3.2.1.

![Structural formula of ramipril](image)

Fig. 3.2.1: Ramipril

Uses: It is used in the treatment of hypertensive patients.

Doses

Initially 1.25 mg once daily given at bedtime. 2.5-5 mg daily as a single dose up to 10 mg daily as needed. Ramipril are supplied as tablets for oral administration containing 1.25 mg, 2.5 mg, 5 mg and 10 mg of ramipril. The inactive ingredients
present are calcium sulphate dihydrate, pregelatinized starch, sodium bicarbonate, and sodium stearyl fumarate.

Adverse effects

Nausea, vomiting, diarrhea, dizziness, fatigue, headache, abdominal pain, cough. Rarely symptomatic hypotension, renal impairment, hypersensitivity reactions etc., are the adverse effect of ramipril.

(b) Tenofovir disoproxil fumarate (TDF)

Chemically, TDF is \(\text{[(2/R)-1-(6-amino-9H-purin-9-yl) propan-2-yl]oxy} \text{methyl})\text{phosphonic acid. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI). Tenofovir disoproxil fumarate (a pro drug of Tenofovir), which is a fumaric acid salt of bis isopropoxycarbonyloxymethyl ester derivative of Tenofovir.}

In vivo Tenofovir disoproxil fumarate is converted to Tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate. Tenofovir exhibits activity against HIV-1 reverse transcriptase. The structural formula of tenofovir disoproxil fumarate is shown in Fig. 3.2.2.
Fig. 3.2.2: Tenofovir disoproxil fumarate

**Uses:** It is used for the treatment of HIV patients.

**Doses**

Tenofovir disoproxil fumarate is a prodrug form of Tenofovir. Tenofovir is also available in a fixed-dose combination with emtricitabine in a product with the brand name Truvada for once-a-day dosing. (Emtricitabine is marketed as a single-compound product called Emtriva, also by Gilead.) Atripla, a fixed-dose triple combination of tenofovir, emtricitabine and efavirenz, was approved by the FDA on 12 July 2006 and is now available, providing a single daily dose for the treatment of HIV are available in pharmaceutical markets.

**Adverse effects**

The most common side effects associated with tenofovir include nausea, vomiting, diarrhea, and asthenia. Less frequent side effects include hepatotoxicity, abdominal pain, and flatulence. Tenofovir has also been implicated in causing renal toxicity, particularly at elevated concentrations. Tenofovir can cause acute renal failure, Fanconi syndrome, proteinuria or tubular necrosis. These side effects are due to accumulation of the drug in proximal tubules. Tenofovir can interact with
didanosine by increasing didanosine's concentration. It also decreases the concentration of atazanavir sulfate.

(c) Esomeprazole magnesium

Esomeprazole magnesium chemically, bis (5-methoxy-2-[(S)-[(4-methoxy-3,5- dimethyl- 2-pyridinyl) methyl] sulfinyl]- 1H-benzimidazole-1yl) magnesium trihydrate. Esomeprazole is the S-isomer of omeprazole, which is a mixture of the S- and R- isomers. The magnesium salt is a white to slightly colored crystalline powder. It contains 3 moles of water of solvation and is slightly soluble in water. The stability of esomeprazole magnesium is a function of pH; it rapidly degrades in acidic media, but it has acceptable stability under alkaline conditions. At pH 6.8 (buffer), the half-life of the magnesium salt is about 19 hours at 25°C and about 8 hours at 37°C. Its molecular formula is \((\text{C}_{17}\text{H}_{18}\text{N}_{3}\text{O}_{3}\text{S})\ 2\text{Mg} \cdot 3\text{H}_2\text{O}\) with molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. The structure of esomeprazole magnesium is shown in Fig. 3.2.3.

Fig. 3.2.3: Esomeprazole magnesium

Uses

It is used in the treatment of intravenous gastro-oesophageal reflux disease.

Doses

Esomeprazole tablets 10 mg, 20 mg, or 40 mg are available in different trade names and in the form of the same enteric-coated granules used in capsules, and also
inactive granules. The inactive granules are composed of the following ingredients: dextrose, xanthan gum, crospovidone, citric acid, iron oxide, and hydroxyl propyl cellulose. The esomeprazole granules and inactive granules are constituted with water to form a suspension and are given by oral, nasogastric, or gastric administration.

Adverse effects

Headache, diarrohea, abdominal pain, nausea, flatulence, dry mouth, constipation, hyponatraemia, anaphylaxis are the adverse effects of esomeprazole.

(d) Amiloride

Amiloride, chemically, is 3,5-diamino-6-chloro-N-(diaminomethylene) pyrazine-2-carboxamide mechanism of action. Amiloride works by directly blocking the epithelial sodium channel there by inhibiting sodium reabsorption in the distal convoluted tubules and collecting ducts in the kidneys (this mechanism is the same for triamterene). This promotes the loss of sodium and water from the body, but without depleting potassium. The drug is often used in conjunction with thiazide (e.g. co-amilozide) or loop diuretics (e.g. co-amilofruse). Due to its potassium-sparing capacities, hyperkalemia (high blood potassium levels) are occasionally observed in patients taking amiloride. The risk is high in concurrent use of ACE inhibitors or spironolactonet is soluble in methanol. The structure of amiloride is as follows in Fig. 3.2.4.
Amiloride

Uses

It is used in the management of hypertension and congestive heart failure.

Doses

Amiloride is available in the markets as combination drugs with furosemide.

Adverse effects

Nausea, diarrhea, dizziness, and photosensitivity hypotension bone marrow depression, fatigue muscle cramps raised CPK level.

(e) Lamotrigine

Lamotrigine chemically, 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine. It is a novel anticonvulsant drug. Molecular formula of lamotrigine is $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5$. It is freely soluble in water and in methanol, sparingly soluble in ethanol. The structure of lamotrigine is shown in Fig. 3.2.5.
Uses

Lamotrigine is also used in the treatment of depression and bipolar disorder.

Doses

Lamotrigine tablets 25mg, 100mg, 150mg and 200mg are available in different trade names.

Adverse effect

Permanent staining of teeth, nausea, rash, dysphagia, photosensitivity, hypersensitivity, haemolytic anaemia, raised blood urea, liver enzymes and bilirubin.

(f) Dapsone

Chemically, Dapsone (C_{12}H_{12}N_{2}O_{2}S M.W. 248.3) is bis(4-aminophenyl) sulphone. It is white or creamy-white, crystalline powder, freely soluble in ethanol (95%) and in acetone but very slightly soluble in water. It is soluble in dilute mineral acids. The structural formula of dapsone is shown in Fig. 3.2.6.

\[ \text{H}_2\text{N} \quad \text{SO}_2 \quad \text{NH}_2 \]

Fig. 3.2.6: Dapsone

Uses

Dapsone is used in the treatment of Leprosy caused by Mycobacterium leprae. It is diaminodiphenyl sulphone (DDS) the simplest, oldest, cheapest, most active, and most commonly used member of its class.
All other sulfones are converted in the body to diamino diphenyl sulfone; many have been used, but none is superior. It is leprostatic at low concentrations and arrests the growth of many other bacteria sensitive to sulfonamides. Specificity for *M. leprae* may be due to difference in the affinity of its folate synthesise. Doses of dapsone needed for the treatment of acute infections are too toxic, so not prescribed.

**Doses**

Initial dose, 25 to 50 mg twice weekly, increasing by 50 to 100 mg, every month to a maximum of 0.2 to 0.4 g twice weekly. Dapsone 100 mg tablets are available in pharmaceutical markets.

**Adverse effects**

These are uncommon at doses of 100 mg/day or less. Haemolysis is the most important dose related toxicity—reflects oxidizing property. Patients with G-6-PD deficiency are more susceptible; dose>50 mg/day produce haemolysis in them.

Gastric intolerance-nausea and anorexia are frequent in the beginning decrease later. Other side effects are methemoglobinemia, headache, parenthesis, mental symptoms and drug fever. Hepatitis and agranulocytosis are other rare complications.

**(g) Mosapride citrate**

Mosapride citrate chemically, (±)-4-amino-5-chloro-2-ethoxy-N[4-(4-fluorobenyl)-2-morpholinylmethyl]benzamide citrate dehydrate. It is soluble in methanol. The structure of mosapride is as follows in the given Fig. 3.2.7.
Uses

It is used in the gastrointestinal symptoms associated with chronic gastritis.

Doses

2.5 to 10 mg two to three times a day, with metals. Mosapride is available in the market by different trade names by different manufactures REMOT 5-mg tablet, MOZA 5-mg tablet, MIC Tab 5-mg and REMO 5-mg tablet.

Adverse effect

Diarrhoea, constipation, headache, abdominal pain, irritated feeling and dizziness.

(h) Alfuzosin hydrochloride

Chemically alfuzosin hydrochloride (AFZ) is a (R,S)-N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide hydrochloride. Alfuzosin hydrochloride is an alpha1-adrenoreceptor blocker. It is used in the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia and has been tried in the treatment of hypertension. The empirical formula and molecular weight of alfuzosin hydrochloride are C_{19}H_{27}N_{5}O_{4}.HCl and
425.9 respectively. AFZ is a white to off-white crystalline powder that melts at approximately 240°C. It is freely soluble in water, sparingly soluble in alcohol, and practically insoluble in dichloromethane. Alfuzosin is a basic compound with a pKₐ value of 8.13 and is stable under normal conditions of temperature and light. Its structure is as shown in Fig 3.2.8.

![Fig 3.2.8: Alfuzosin hydrochloride](image)

**Uses**

It is used in the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia and has been tried in the treatment of hypertension. AFZ is a selective antagonist of post-synaptic alpha₁-adrenoreceptors, showing some myorelaxant effects. It is given orally for the treatment of the signs and symptoms of benign prostatic hyperplasia.

**Doses**

The recommended dosage is one contains 10 mg (alfuzosin hydro chloride extended-release tablets) tablet daily to be taken immediately after the same metal each day. AFZ is available commercially as 10 mg tablets. The USFDA approved AFZ (UROXATRAL tablets, Sanofi-Synthelabo) for the treatment of benign prostatic hyperplasia in June 2003.
**Adverse effects**

The most commonly observed adverse experiences seen in association with AFZ are dizziness, hypotension or postural hypotension and syncope. The tablet also contains the following inactive ingredients: colloidal silicon dioxide, ethyl cellulose, hydrogenated castor oil, hydroxypropyl methylcellulose, magnesium stearate, mannitol, microcrystalline cellulose, povidone, and yellow ferric oxide.

(i) Mesalamine

Mesalamine also known as Mesalazine, chemically known as 5-aminosalicylic acid is used for its local effects in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn’s disease. It is very slightly soluble in water, practically insoluble in alcohol, dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid. The structure of mesalamine is as follows in the given Fig. 3.2.9.

![Fig. 3.2.9: Mesalamine](image)

**Uses**

It is used for its local effects in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn’s disease.
Doses

Each 250 mg capsule contains 250 mg of mesalamine. It also contains the following inactive ingredients: acetylated monoglyceride, castor oil, colloidal silicon dioxide, ethylcellulose, hydroxypropyl methylcellulose, starch, stearic acid, sugar, talc, and white wax. The capsule shell contains D&C Yellow #10, FD&C Blue #1, FD&C Green #3, gelatin, titanium dioxide, and other ingredients.

Each 500 mg capsule contains 500 mg of mesalamine. It also contains the following inactive ingredients: acetylated monoglyceride, castor oil, colloidal silicon dioxide, ethylcellulose, hydroxypropyl methylcellulose, starch, stearic acid, sugar, talc, and white wax. The capsule shell contains FD&C Blue #1, gelatin, titanium dioxide, and other ingredients.

Adverse effects

severe stomach pain, cramping, fever, headache, and bloody diarrhea. Less serious side effects may include: mild nausea, vomiting, stomach cramps, diarrhea, and gas.

(j) Pramipexole

The chemical name of pramipexole dihydrochloride is (S)-2-amino-4,5,6,7-tetrahydro-6-(propylamino) benzothiazole dihydrochloride monohydrate. Its empirical formula is C_{10}H_{17}N_{3}S \cdot 2\text{HCl} \cdot \text{H}_2\text{O}, and its molecular weight is 302.27. Pramipexole dihydrochloride is a white to off-white powder substance. Melting occurs in the range of 296°C to 301°C, with decomposition. Pramipexole dihydrochloride is more than 20% soluble in water, about 8% in methanol, about 0.5% in ethanol, and practically
insoluble in dichloromethane. The structural formula of pramipexole is as shown in Fig. 3.2.10.

![Pramipexole structural formula](image)

**Fig. 3.2.10: Pramipexole**

**Uses**

It is used in the treatment of Parkinson's disease and restless legs syndrome (RLS). It is also sometimes used off-label as a treatment for cluster headache or to counteract the problems with low libido experienced by some users of SSRI antidepressant drugs.

**Doses**

MIRAPEX tablets, for oral administration, contain 0.125 mg, 0.25 mg, 0.5 mg, 1 mg, or 1.5 mg of pramipexole dihydrochloride monohydrate. Inactive ingredients consist of mannitol, corn starch, colloidal silicon dioxide, povidone, and magnesium stearate.

**Adverse effects**

Extreme drowsiness, falling asleep suddenly, even after feeling alert; hallucinations, fever, stiff muscles, confusion, sweating, fast or uneven heartbeats, nausea, sweating and feeling light-headed.