CHAPTER 2

RAW MATERIALS, INSTRUMENTS, PRODUCT INFORMATION AND ANALYTICAL METHODS

I. RAW MATERIALS

Sr. no.	Raw Material	Source
1.	Venlafaxine HCl	Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India
2.	Glipizide	Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India
3.	Metoprolol tartrate	IPCA Laboratories Limited, Kandivli, Mumbai, India
4.	Nifedipine	Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India
5.	Oxybutynin chloride	Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India
6.	Atenolol	Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India
7.	Lactose	Pharmatose DCL 11, DMV International, Veghel, The Netherlands
8.	Microcrystalline cellulose	Avicel PH-101, FMC, Ireland
9.	Mannitol	Pearlitol 200 SD, Roquette, France
10.	Sodium chloride	Qualigens Fine Chemicals, Mumbai, India
11.	Potassium chloride	Qualigens Fine Chemicals, Mumbai, India
12.	Tartaric acid	Qualigens Fine Chemicals, Mumbai, India
13.	Meglumine	Qualigens Fine Chemicals, Mumbai, India
14.	Povidone K-30	Kollidon30, BASF, Ludwigshafen, Germany
15.	Magnesium stearate	Signet Chemical Corporation, Mumbai, India
16.	Colloidal silicon dioxide	Aerosil 200, Degussa, Frankfurt, Germany
17.	Sorbitol	Qualigens Fine Chemicals, Mumbai, India
18.	Talc	Qualigens Fine Chemicals, Mumbai, India
19.	Cellulose acetate	CA-398-10 NF, Eastman Chemical Inc, Kingsport, TN, USA
20.	PEG-400	S.D. Fine Chemicals Ltd, Mumbai, India
21.	PEG-6000	S.D. Fine Chemicals Ltd, Mumbai, India

22.	HPMC K4M	Methocel K4M, Colorcon Asia Pvt. Ltd, Goa, India
23.	HPMC K15M	Methocel K15M, Colorcon Asia Pvt. Ltd, Goa, India
24.	HPMC K100M	Methocel K100M, Colorcon Asia Pvt. Ltd, Goa, India
25.	HPMC K100LV	Methocel K100LV, Colorcon Asia Pvt. Ltd, Goa, India
26.	Poloxomer-188	Lutrol F-68, BASF, Ludwigshafen, Germany
27.	Polyethylene oxide	Polyox WSR N750 and WSR N10, The Dow Chemical Company, MI, USA
28.	Starch	Pure-dent B700, Grain Processing Corporation, IA, USA
29.	Dichloromethane	Merck Limited, Mumbai, India
30.	Methanol	Merck Limited, Mumbai, India
31.	Acetone	Merck Limited, Mumbai, India
32.	Effexor XR (Venlafaxine)	Wyeth Inc. USA
33.	Glucotrol XL (Glipizide)	Pfizer Inc. / Alza Inc. USA
34.	Toprol XL (Metoprolol tartrate)	Astra Zeneca Inc. USA
35.	Procardia XL (Nifedipine)	Pfizer Inc. / Alza Inc. USA
36.	Ditropan XL (Oxybutynin chloride)	Alza Inc. USA
37.	Ethanol (99.5%V/V)	Baroda Chem. Ind. Ltd., Baroda, India
38.	Glacial acetic acid	S. D. Finechem Limited, Mumbai, India
39.	Hydrochloric acid	S. D. Finechem Limited, Mumbai, India
40.	Isopropyl alcohol	Merck Limited, Mumbai, India
41.	Potassium dihydrogen phosphate	S. D. Finechem Limited, Mumbai, India
42.	Sodium hydroxide	S. D. Finechem Limited, Mumbai, India
43.	Sodium lauryl sulphate	Qualigens Fine Chemicals, Mumbai, India

II. INSTRUMENTS

Sr. no.	Instruments	Make
1.	Compression	8-station compression machine, KMP-8, Cadmach Engg, Ahmedabad, India.
2.	Coating	Perforated pan, GAC – 205, Gansons Ltd., Mumbai, India.
3.	Digital weighing balance	AG-64, Mettler Toledo, Switzerland
4.	Tap density tester	ETD-1020, Electrolab, Mumbai, India.
5.	Moisture analyzer	LJ-16 Mettler Toledo, Switzerland
6.	Hardness tester	6-D, Dr Schleuniger Pharmatron, Manchester, NH, USA
7.	pH meter	Mettler Toledo, Switzerland
8.	Tray dryer	BO-6, Bombay Eng. Works, Mumbai, India
9.	Friability tester	EF-2, Electrolab, Mumbai, India
10.	Thickness gauge	Digimatic Caliper, Mitutoyo, Japan
11.	Water bath	SW 23, Julabo Labortechnik, Germany
12.	Stability chamber	Thermolab, Mumbai, India
13.	Differential scanning calorimeter	PerkinElmer, Pyris 1- DSC, Waltham, MA, USA
14.	PXRD	X-ray diffractometer, Multiflex 3KW, Rigaku, USA
15.	Dissolution apparatus	USP type I, 2100C, Distek Inc, NJ, USA
16.	UV spectrophotometer	8453, Agilent Technologies, Singapore.
17.	Scanning electron microscope (SEM)	Philips ESEM-TMP, Philips, Netherlands.
18.	Rota evaporator	Buchi, Germany
19.	HPLC	Shimadzu LC-2010, Kyoto, Japan
20.	HSMG	Rotamix HSMG-10, Kevin Engg, Ahmedabad, India
21.	Digital Microscore	Leica DMLB, Leica Microsystems GmbH, Nussloch, Germany

III. PRODUCT INFORMATION

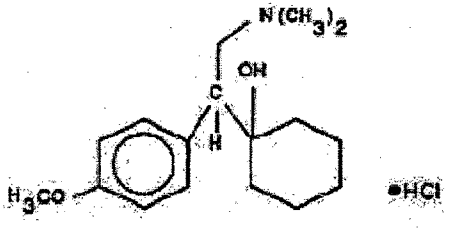
1. VENLAFAXINE HCl

CAS Registry number : [99300-78-4]

Molecular Formula : $C_{17}H_{27}NO_2$ HCl.

Molecular Weight : 313.87

Chemical Structure :



Chemical Name : (R/S)-1-[2-(dimethylamino)-1- (4-methoxyphenyl)ethyl] cyclohexanol hydrochloride or (±)-1-[.- [(dimethylamino) methyl] -pmethoxybenzyl] cyclohexanol hydrochloride.

Category : Antidepressant

Description : White to off-white crystalline solid

Melting Point : 215 – 217 °C

Solubility : 572 mg/mL in water
(adjusted to ionic strength of 0.2 M with sodium chloride).

Trade name : Effexor, Effexor-XR (Wyeth-Ayerst)

– Pharmacokinetic Summary

Bioavailability : 45%

Protein binding : 27%

Metabolism : Hepatic

Half life : 5 ± 2 hours (parent compound); 11 ± 2 hours (active metabolite)

Excretion : Renal

– **Clinical Pharmacology**

Pharmacological Actions

The mechanism of the antidepressant action of VH in humans is believed to be associated with its potentiation of neurotransmitter activity in the CNS. Preclinical studies have shown that VH and its active metabolite, O-desmethylvenlafaxine (ODV), are potent inhibitors of neuronal serotonin and norepinephrine reuptake and weak inhibitors of dopamine reuptake. VH and ODV have no significant affinity for muscarinic cholinergic, H1-histaminergic, or α1-adrenergic receptors in vitro. Pharmacologic activity at these receptors is hypothesized to be associated with the various anticholinergic, sedative, and cardiovascular effects seen with other psychotropic drugs. VH and ODV do not possess monoamine oxidase (MAO) inhibitory activity.

Pharmacokinetics

VH is well absorbed and extensively metabolized in the liver. O-desmethylvenlafaxine (ODV) is the only major active metabolite. On the basis of mass balance studies, at least 92% of a single dose of VH is absorbed. Approximately 87% of a VH dose is recovered in the urine within 48 hours as either unchanged VH (5%), unconjugated ODV (29%), conjugated ODV (26%), or other minor inactive metabolites (27%). Renal elimination of VH and its metabolites is the primary route of excretion. The relative bioavailability of VH from a tablet was 100% when compared to an oral solution. Food has no significant effect on the absorption of VH or on the formation of ODV. The degree of binding of VH to human plasma is 27% ± 2% at concentrations ranging from 2.5 to 2215 ng/mL. The degree of ODV binding to human plasma is 30% ± 12% at concentrations ranging from 100 to 500 ng/mL. Protein-binding-induced drug interactions with VH are not expected. Steady-state concentrations of both VH and ODV in plasma were attained within 3 days of multiple-dose therapy. VH and ODV exhibited linear kinetics over the dose range of 75 to 450 mg total dose per day (administered on a q8h schedule). Plasma clearance, elimination half-life and steady-state volume of distribution were unaltered for both VH and ODV after multiple-dosing. Mean ± SD steady-state plasma clearance of VH and ODV is 1.3 ± 0.6 and 0.4 ± 0.2 L/h/kg, respectively; elimination half-life is 5 ± 2 and 11 ± 2 hours, respectively; and steady-state volume of distribution is 7.5 ± 3.7 L/kg and 5.7 ± 1.8 L/kg, respectively. When equal daily doses of VH were administered as either b.i.d.

or t.i.d. regimens, the drug exposure (AUC) and fluctuation in plasma levels of VH and ODV were comparable following both regimens.

- **Indications**

VH is used primarily for the treatment of depression, generalized anxiety disorder, social anxiety disorder, and panic disorder in adults.

For most patients, the recommended starting dose for VH extended capsule is 75 mg/day, administered in a single dose.

Panic Disorder

It is recommended that initial single doses of 37.5 mg/day of VH extended capsule be used for 7 days.

- **Dosage and Administration**

VH extended capsule should be administered in a single dose with food either in the morning or in the evening at approximately the same time each day. Each capsule should be swallowed whole with fluid and not divided, crushed, chewed, or placed in water, or it may be administered by carefully opening the capsule and sprinkling the entire contents on a spoonful of applesauce. This drug/food mixture should be swallowed immediately without chewing and followed with a glass of water to ensure complete swallowing of the pellets.

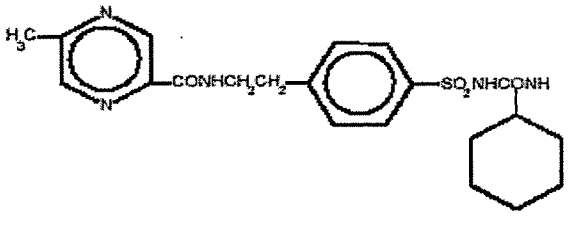
2. GLIPIZIDE

CAS Registry number : [29094-61-9]

Molecular Formula : $C_{21}H_{27}N_5O_4S$

Molecular Weight : 445.55

Chemical Structure :



Chemical Name : 1-cyclohexyl-3-[[p-[2-(5-methylpyrazinecarboxamido) ethyl] phenyl]sulfonyl]urea

Category	:	Oral blood-glucose-lowering drug of the sulfonylurea class
Description	:	Whitish, odorless powder
Melting Point	:	208 – 209 °C
Solubility	:	It is insoluble in water and alcohols, but soluble in 0.1 N NaOH
Trade name	:	Glibenese (Pfizer) ; Glucotrol (Roerig) ; Mindiab (Farmitalia) ; Minidiab (Erba) ; Ozidia (Pfizer)

– **Pharmacokinetic Summary**

Bioavailability	:	100% (regular formulation), 90% (extended release)
Protein binding	:	98 to 99%
Metabolism	:	Hepatic hydroxylation
Half life	:	2 to 5 hours
Excretion	:	Renal and fecal

– **Clinical Pharmacology**

Pharmacological Actions

The primary mode of action of GZ in experimental animals appears to be the stimulation of insulin secretion from the beta cells of pancreatic islet tissue and is thus dependent on functioning beta cells in the pancreatic islets. In man, stimulation of insulin secretion by GZ in response to a meal is undoubtedly of major importance. Fasting insulin levels are not elevated even on long-term GZ administration, but the postprandial insulin response continues to be enhanced after at least 6 months of treatment. The insulinotropic response to a meal occurs within 30 minutes after an oral dose of GZ in diabetic patients, but elevated insulin levels do not persist beyond the time of the meal challenge. Extrapancreatic effects may play a part in the mechanism of action of oral sulfonylurea hypoglycemic drugs.

Pharmacokinetics

Gastrointestinal absorption of GZ in man is uniform, rapid, and essentially complete. Peak plasma concentrations occur 1–3 hours after a single oral dose. The half-life of

elimination ranges from 2–4 hours in normal subjects, whether given intravenously or orally. The metabolic and excretory patterns are similar with the two routes of administration, indicating that first-pass metabolism is not significant. GZ does not accumulate in plasma on repeated oral administration. Total absorption and disposition of an oral dose was unaffected by food in normal volunteers, but absorption was delayed by about 40 minutes. Thus GZ was more effective when administered about 30 minutes before, rather than with, a test meal in diabetic patients. Protein binding was studied in serum from volunteers who received either oral or intravenous GZ and found to be 98–99% one hour after either route of administration. The apparent volume of distribution of GZ after intravenous administration was 11 liters, indicative of localization within the extracellular fluid compartment.

The metabolism of GZ is extensive and occurs mainly in the liver. The primary metabolites are inactive hydroxylation products and polar conjugates and are excreted mainly in the urine. Less than 10% unchanged GZ is found in the urine.

- **Indications**

GZ tablets are indicated as an adjunct to diet for the control of hyperglycemia and its associated symptomatology in patients with type 2 diabetes formerly known as non-insulin-dependent diabetes mellitus (NIDDM) or maturity-onset diabetes, after an adequate trial of dietary therapy has proved unsatisfactory. GZ extended-release tablets are indicated when diet alone has been unsuccessful in correcting hyperglycemia, but even after the introduction of the drug in the patient's regimen, dietary measures should continue to be considered as important. In 12 week, well-controlled studies there was a maximal average net reduction in hemoglobin A1C of 1.7% in absolute units between placebo-treated and GZ-extended-release tablet-treated patients.

- **Dosage and Administration**

There is no fixed dosage regimen for the management of diabetes mellitus with GZ or any other hypoglycemic agent. In general, GZ should be given approximately 30 minutes before a meal to achieve the greatest reduction in postprandial hyperglycemia.

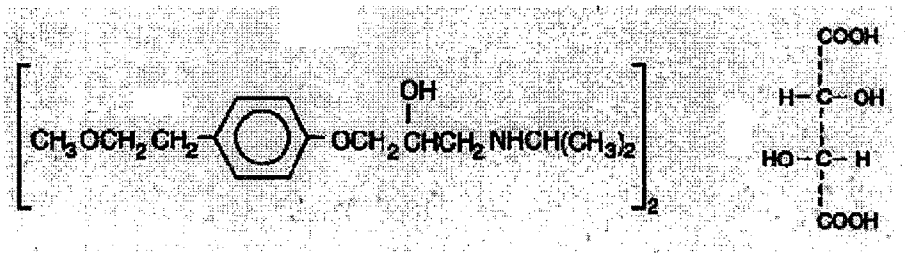
3. METOPROLOL TARTRATE

CAS Registry number : [56392-17-7]

Molecular Formula : $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$

Molecular Weight : 684.82

Chemical Structure :



Chemical Name : Metoprolol tartrate USP is (±)-1-(Isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-propanol L-(+)-tartrate (2:1) salt

Category : MT, is a selective beta1-adrenoreceptor blocking agent

Description : White, practically odorless, crystalline powder

Melting Point : 208 – 209 °C

Solubility : It is freely soluble in water, methanol and chloroform

Trade names : Beloc (Astra) , Betaloc (Astra) , Lopressor (Ciba-Geigy) , Lopresor (Ciba-Geigy) , Prelis (Ciba-Geigy) , Seloken Haumlssle), Selopral (Haumlssle) , Selo-Zok (Haumlssle) .

– **Pharmacokinetic Summary**

Bioavailability : 12%

Protein binding : 98 to 99%

Metabolism : Hepatic

Half life : 3 to 7 hours

Excretion : Renal

– **Clinical Pharmacology**

Pharmacological Actions

MT is a beta-adrenergic receptor blocking agent. In vitro and in vivo animal studies have shown that it has a preferential effect on beta1 adrenoreceptors, chiefly located in cardiac muscle. This preferential effect is not absolute, however, and at higher doses, metoprolol also inhibits beta2 adrenoreceptors, chiefly located in the bronchial and vascular musculature.

Clinical pharmacology studies have confirmed the beta-blocking activity of metoprolol in man, as shown by (1) reduction in heart rate and cardiac output at rest and upon exercise, (2) reduction of systolic blood pressure upon exercise, (3) inhibition of isoproterenol-induced tachycardia, and (4) reduction of reflex orthostatic tachycardia.

Pharmacokinetics

In man, absorption of metoprolol is rapid and complete. Plasma levels following oral administration, however, approximate 50% of levels following intravenous administration, indicating about 50% first-pass metabolism. Plasma levels achieved are highly variable after oral administration. Only a small fraction of the drug (about 12%) is bound to human serum albumin. Elimination is mainly by biotransformation in the liver, and the plasma half-life ranges from approximately 3 to 7 hours. Equivalent maximal beta-blocking effect is achieved with oral and intravenous doses in the ratio of approximately 2.5:1.

In patients with angina pectoris, plasma concentration measured at 1 hour is linearly related to the oral dose within the range of 50 mg to 400 mg. Exercise heart rate and systolic blood pressure are reduced in relation to the logarithm of the oral dose of metoprolol. The increase in exercise capacity and the reduction in left ventricular ischemia are also significantly related to the logarithm of the oral dose.

– **Indications and Usage**

Hypertension:

MT tablets are indicated for the treatment of hypertension. They may be used alone or in combination with other antihypertensive agents.

Angina Pectoris:

MT tablets are indicated in the long-term treatment of angina pectoris.

Myocardial Infarction:

MT tablets and injection are indicated in the treatment of hemodynamically stable patients with definite or suspected acute myocardial infarction to reduce cardiovascular mortality. Treatment with intravenous MT can be initiated as soon as the patient's clinical condition allows

- **Dosage and Administration**

Hypertension:

The dosage of MT should be individualized. MT should be taken with or immediately following meals.

The usual initial dosage is 100 mg daily in single or divided doses, whether used alone or added to a diuretic. While once-daily dosing is effective and can maintain a reduction in blood pressure throughout the day, lower doses (especially 100 mg) may not maintain a full effect at the end of the 24-hour period, and larger or more frequent daily doses may be required.

Angina Pectoris:

The dosage of MT should be individualized. MT should be taken with or immediately following meals.

The usual initial dosage is 100 mg daily, given in two divided doses. The dosage may be gradually increased at weekly intervals until optimum clinical response has been obtained or there is pronounced slowing of the heart rate.

Myocardial Infarction:

Early Treatment

During the early phase of definite or suspected acute myocardial infarction, treatment with metoprolol can be initiated as soon as possible after the patient's arrival in the hospital. Such treatment should be initiated in a coronary care or similar unit immediately after the patient's hemodynamic condition has stabilized.

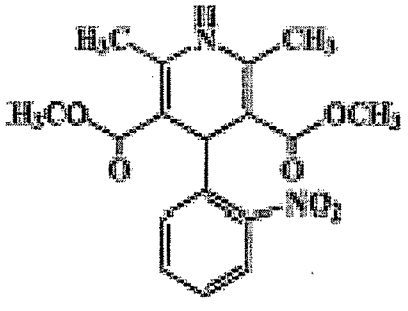
4. NIFEDIPINE

CAS Registry number : [21829-25-4]

Molecular Formula : $C_{17}H_{18}N_2O_6$

Molecular Weight : 346.34

Chemical Structure :



Chemical Name : 1,4-Dihydro-2,6-dimethyl-4-(2-nitro phenyl)-3,5-pyridinedicarboxylic acid dimethyl ester

Category : Antianginal; antihypertensive.

Description : Yellow crystals

Melting Point : 172 – 174 °C

Solubility : At 20° (g/L): acetone 250, methylene chloride 160, chloroform 140, ethyl acetate 50, methanol 26, ethanol 17.

Trade names : Adalat (Bayer), Bonacid (Lilly), Chronadalate (Bayer), Cordicant (Mundipharma), Cordilan (Andreu), Procardia (Pfizer) Betaloc (Astra), Lopressor (Ciba-Geigy).

– **Pharmacokinetic Summary**

Bioavailability : 45-56%

Protein binding : 92-98%

Metabolism : Gastrointestinal, Hepatic

Half life : 2 hours

Excretion : Renal: >50%, Biliary: 5-15%

- **Clinical Pharmacology**

Pharmacological Actions

NP is a calcium ion influx inhibitor (slow-channel blocker or calcium ion antagonist) and inhibits the transmembrane influx of calcium ions into cardiac muscle and smooth muscle. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. NP selectively inhibits calcium ion influx across the cell membrane of cardiac muscle and vascular smooth muscle without altering serum calcium concentrations.

Pharmacokinetics

NP is rapidly and fully absorbed after oral administration. The drug is detectable in serum 10 minutes after oral administration, and peak blood levels occur in approximately 30 minutes. Bioavailability is proportional to dose from 10 to 30 mg; half-life does not change significantly with dose. There is little difference in relative bioavailability when NP capsules are given orally and either swallowed whole, bitten and swallowed, or, bitten and held sublingually. However, biting through the capsule prior to swallowing does result in slightly earlier plasma concentrations (27 ng/ml 10 minutes after 10 mg) than if capsules are swallowed intact. It is highly bound by serum proteins. NP is extensively converted to inactive metabolites and approximately 80% of NP and metabolites are eliminated via the kidneys. The half-life of NP in plasma is approximately 2 hours. Since hepatic biotransformation is the predominant route for the disposition of NP, the pharmacokinetics may be altered in patients with chronic liver disease. Patients with hepatic impairment (liver cirrhosis) have a longer disposition half-life and higher bioavailability of NP than healthy volunteers. The degree of serum protein binding of NP is high (92-98%). Protein binding may be greatly reduced in patients with renal or hepatic impairment.

- **Indications**

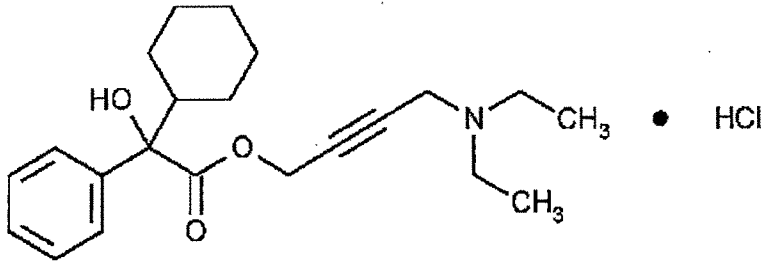
Vasospastic Angina and Hypertension

– **Dosage and Administration**

Therapy should be initiated with the 10 mg capsule. The starting dose is one 10 mg capsule, swallowed whole, 3 times/day. The usual effective dose range is 10-20 mg 3 times daily. Some patients, especially those with evidence of coronary artery spasm, respond only to higher doses, more frequent administration, or both. In such patients, doses of 20-30 mg 3 or 4 times daily may be effective. Doses above 120 mg daily are rarely necessary. More than 180 mg per day is not recommended.

5. OXYBUTYNIN CHLORIDE

CAS Registry number	:	[1508-65-2]
Molecular Formula	:	$C_{22}H_{31}NO_3.HCl$
Molecular Weight	:	393.9
Chemical Structure	:	



Chemical Name	:	Alpha-Cyclohexyl-alpha-hydroxybenzene acetic acid 4-(diethylamino)-2-butynyl ester; alpha-phenylcyclo hexaneglycolic acid 4-(diethylamino)-2-butynyl ester;
Category	:	Antispasmodic and anticholinergic agent
Description	:	Whitish, odorless powder
Melting Point	:	129 – 130 °C
Solubility	:	Soluble in water, acids. Practically insoluble in alkali.
Trade names	:	Cystrin (Pharmacia and Upjohn) , Ditropan (HMR) , Dridase

(Pharmacia and Upjohn) , Pollakisu (Kodama) , Tropax (Bristol-Myers Squibb) .

- **Pharmacokinetic Summary**

Protein binding	:	91%-93%
Half life	:	12.4 - 13.2 hours

- **Clinical Pharmacology**

Pharmacological Actions

OC exerts a direct antispasmodic effect on smooth muscle and inhibits the muscarinic action of acetylcholine on smooth muscle. OC exhibits only one-fifth of the anticholinergic activity of atropine on the rabbit detrusor muscle, but four to ten times the antispasmodic activity. No blocking effects occur at skeletal neuromuscular junctions or autonomic ganglia (antinicotinic effects).

OC relaxes bladder smooth muscle. In patients with conditions characterized by involuntary bladder contractions, cystometric studies have demonstrated that oxybutynin increases bladder (vesical) capacity, diminishes the frequency of uninhibited contractions of the detrusor muscle, and delays the initial desire to void. Oxybutynin thus decreases urgency and the frequency of both incontinent episodes and voluntary urination.

Antimuscarinic activity resides predominantly in the R-isomer. A metabolite, desethyloxybutynin, has pharmacological activity similar to that of oxybutynin in in vitro studies.

Pharmacokinetics

Absorption

Following the first dose of oxybutynin, plasma concentrations rise for 4 to 6 hours; thereafter steady concentrations are maintained for up to 24 hours, minimizing fluctuations between peak and trough concentrations associated with Oxybutynin.

The relative bioavailabilities of R- and S- oxybutynin from oxybutynin are 156% and 187%, respectively, compared with oxybutynin.

Distribution

Plasma concentrations of oxybutynin decline biexponentially following intravenous or oral administration. The volume of distribution is 193 L after intravenous administration of 5 mg OC.

Metabolism

Oxybutynin is metabolized primarily by the cytochrome P450 enzyme systems, particularly C.P.A. found mostly in the liver and gut wall. Its metabolic products include phenylcyclohexylglycolic acid, which is pharmacologically inactive, and desethyl oxybutynin, which is pharmacologically active. Following oxybutynin administration, plasma concentrations of R- and S-desethyl oxybutynin are 73% and 92%, respectively, of concentrations observed with oxybutynin.

Excretion

Oxybutynin is extensively metabolized by the liver, with less than 0.1% of the administered dose excreted unchanged in the urine. Also, less than 0.1% of the administered dose is excreted as the metabolite desethylOxybutynin.

- **Dosage and Administration**

Oxybutynin must be swallowed whole with the aid of liquids, and must not be chewed, divided, or crushed.

Oxybutynin may be administered with or without food.

The recommended starting dose of oxybutynin is 5 mg once daily. Dosage may be adjusted in 5-mg increments to achieve a balance of efficacy and tolerability (up to a maximum of 30 mg/day). In general, dosage adjustment may proceed at approximately weekly intervals

- **Indications**

oxybutynin is indicaed for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and frequency.

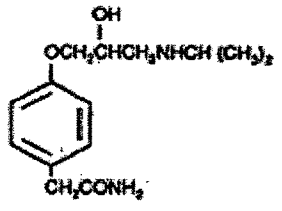
6. ATENOLOL

CAS Registry number : [29122-68-7]

Molecular Formula : $C_{14}H_{22}N_2O_3$

Molecular Weight : 266.34

Chemical Structure :



Chemical Name : 4-[2-Hydroxy-3-[(1- methylethyl)amino]propoxy] benzeneacetamide; 2-[p- [2-hydroxy-3-(isopropyl amino)propoxy]phenyl]acetamide;

Category : Antihypertensive, antianginal, antiarrhythmic (class II).

Description : Whitish, odorless powder

Melting Point : 146 – 148 °C

Solubility : Freely soluble in methanol; sparingly soluble in 96% ethanol;

slightly sol in water, isopropanol.

Trade names : Atehex al (Hexal) ; Ateno basan (Sagitta) ; Atenol (CT) ; Cuxanorm (TAD) ; Ibinolo (IBI) ; Myocord (Elvetium-Rhodia) ; Prenormine (Zeneca) ; Seles Beta (Schwarz) ; Tenoblock (Leiras) ; Tenormin (Zeneca) ; Uniloc (Nycomed) .

– **Pharmacokinetic Summary**

Bioavailability : 40-50%

Protein binding : 6-16%

Metabolism : Hepatic <10%

Half life : 6-7hours

Excretion : Renal

– **Clinical Pharmacology**

Pharmacological Actions

AT is a beta1-selective (cardioselective) beta-adrenergic receptor blocking agent without membrane stabilizing or intrinsic sympathomimetic (partial agonist) activities. This preferential effect is not absolute, however, and at higher doses, AT inhibits beta2-adrenoreceptors, chiefly located in the bronchial and vascular musculature.

Pharmacokinetics

In man, absorption of an oral dose is rapid and consistent but incomplete. Approximately 50% of an oral dose is absorbed from the gastrointestinal tract, the remainder being excreted unchanged in the feces. Peak blood levels are reached between two (2) and four (4) hours after ingestion. Unlike propranolol or metoprolol, but like nadolol, AT undergoes little or no metabolism by the liver, and the absorbed portion is eliminated primarily by renal excretion. Over 85% of an intravenous dose is excreted in urine within 24 hours compared with approximately 50% for an oral dose. AT also differs from propranolol in that only a small amount (6%-16%) is bound to proteins in the plasma. This kinetic profile results in relatively consistent plasma drug levels with about a fourfold interpatient variation.

The elimination half-life of oral AT is approximately 6 to 7 hours, and there is no alteration of the kinetic profile of the drug by chronic administration. Following intravenous administration, peak plasma levels are reached within 5 minutes. Declines from peak levels are rapid (5- to 10-fold) during the first 7 hours; thereafter, plasma levels decay with a half-life similar to that of orally administered drug. Following oral doses of 50 mg or 100 mg, both beta-blocking and antihypertensive effects persist for at least 24 hours. When renal function is impaired, elimination of AT is closely related to the glomerular filtration rate; significant accumulation occurs when the creatinine clearance falls below 35 mL/min/1.73m2

- **Indications**

Hypertension:

AT is indicated in the management of hypertension. It may be used alone or concomitantly with other antihypertensive agents, particularly with a thiazide-type diuretic.

Angina Pectoris Due to Coronary Atherosclerosis:

AT is indicated for the long-term management of patients with angina pectoris.

Acute Myocardial Infarction:

AT is indicated in the management of hemodynamically stable patients with definite or suspected acute myocardial infarction to reduce cardiovascular mortality. Treatment can be initiated as soon as the patient's clinical condition allows.

- **Dosage and Administration**

Hypertension:

The initial dose of AT is 50 mg given as one tablet a day either alone or added to diuretic therapy. The full effect of this dose will usually be seen within one to two weeks. If an optimal response is not achieved, the dosage should be increased to AT 100 mg given as one tablet a day.

Angina Pectoris:

The initial dose of AT is 50 mg given as one tablet a day. If an optimal response is not achieved within one week, the dosage should be increased to AT 100 mg given as one tablet a day.

Acute Myocardial Infarction:

In patients with definite or suspected acute myocardial infarction, treatment with AT I.V. Injection should be initiated as soon as possible after the patient's arrival in the hospital and after eligibility is established. Such treatment should be initiated in a coronary care or similar unit immediately after the patient's hemodynamic condition has stabilized. Treatment should begin with the intravenous administration of 5 mg AT over 5 minutes followed by another 5 mg intravenous injection 10 minutes later. AT I.V. Injection should be administered under carefully controlled conditions including monitoring of blood pressure, heart rate, and electrocardiogram.

IV. ANALYTICAL METHODS

PREPARATION OF BUFFERS

- **Preparation of Simulated Gastric Fluid -SGF (pH 1.2)**

2 gm of sodium chloride and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 8.5 ml of concentrated hydrochloric acid was added and the volume was adjusted with distilled water upto 1000 ml. pH was adjusted to 1.2. (USP 30, 2007).

- **Preparation of Acetate Buffer (pH 4.5)**

2.99 gm of sodium acetate trihydrate and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 14 ml of acetic acid solution was added and the volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

- **Preparation of Simulated Intestinal Fluid -SIF (pH 6.8)**

250 ml of 0.2 M potassium phosphate monobasic was placed in a 1000 ml volumetric flask, 112 ml of 0.2 M sodium hydroxide was added and volume was adjusted with distilled water upto 1000 ml. pH was adjusted to 6.8 (USP 30, 2007).

1. VENLAFAXINE HCL

Venlafaxine HCl (DMF grade) was obtained from Cadila Healthcare Ltd., Moraiya, Ahmedabad, India.

- **Determination of Assay by HPLC**

Buffer Solution: 1.74 g of dipotassium hydrogen phosphate was dissolved in 1000 mL of Milli Q water and the pH was adjusted to 7.0 with 10 % orthophosphoric acid.

Mobile phase: A filtered and degassed mixture of buffer solution and acetonitrile was prepared in the ratio of 35:65.

Diluent: Mobile phase was used as diluent.

Standard preparation: An accurately weighed quantity of 56 mg of VH working standard was transferred to a 50-mL volumetric flask. About 25 mL of diluent was added and dissolved by sonication. Volume was made up to the mark with diluent and mixed. 5.0 mL of this solution was diluted to 50.0 mL with diluent and mixed.

Sample preparation: Accurately 20 tablets were weighed and the average weight was calculated. The tablets were crushed into a fine powder. An accurately weighed quantity of tablets powder equivalent to about 50 mg of VH was transferred to a 50-mL volumetric flask. About 25 mL of diluent was added and sonicated with occasional shaking for about 20 minutes. The volume was made up to the mark with diluent and mixed. 5.0 mL of this solution was diluted to 50.0 mL with diluent and mixed. The solution was filtered through 0.45 µm Millipore HVLP filter, the filtrate was collected by discarding first few mL of the filtrate.

- **Chromatographic system**

Column	: Kromasil C18, (25 cm x 4.6 mm), 5 µm
Detector (UV)	: 227 nm
Flow rate	: 1.0 mL/min.
Injection volume	: 10 µL
Column temperature	: 45°C

- **Determination of Percent Drug Release (In-Vitro dissolution) by UV**

Preparation of Calibration Curves of Venlafaxine HCl

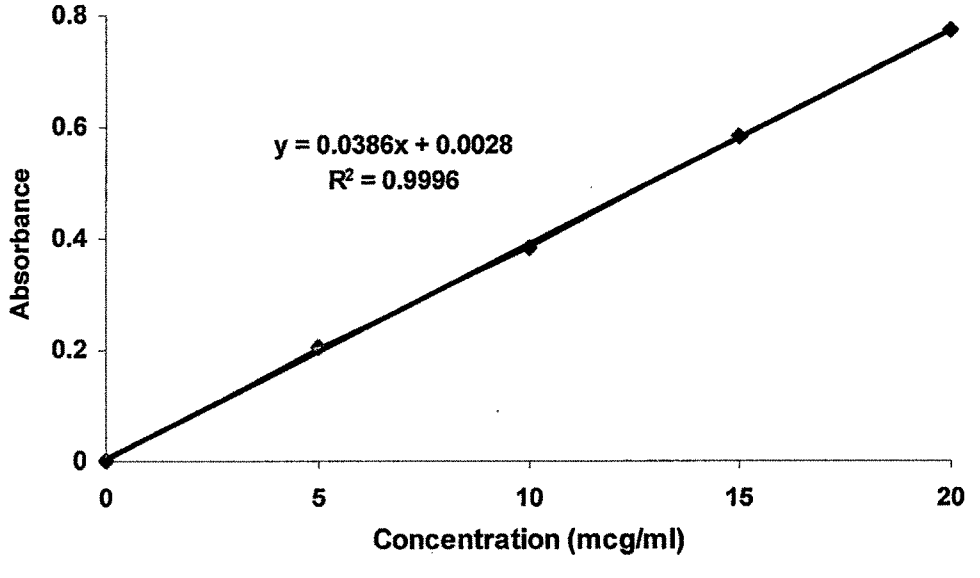
An accurately weighed quantity of 100 mg of sample was transferred to a 100-mL volumetric flask. About 50 mL of dissolution media was added and sonicated to dissolve. Volume was made up to the mark with dissolution media and mixed. 10 ml of this solution was taken in another 100 ml volumetric flask and diluted up to 100 ml with dissolution media to get a stock solution of 100 µg/ml concentration. From the stock solution, aliquots of 5, 10, 15 and 20 ml were pipetted out in to a series of 100 ml volumetric flasks. The volume of the aliquots were made up to 100 ml with distilled water, thus a range of concentration between 5 to 20 µg/ml were obtained. The

absorbance of these solutions were measured in a UV spectrophotometer at λ max 225 nm against blank reference. The absorbance data obtained are represented in Table 2.1 and plotted in the form of standard curve in Fig. 2.1.

Table 2.1 Absorbance data of Venlafaxine HCl in distilled water at 225 nm.

S.No.	Concentration (µg/ml)	Absorbance	Statistical Parameters
1.	0	0.0000	
2.	5	0.2043	Correlation Coefficient = 0.9996
3.	10	0.3814	
4.	15	0.5816	Standard error = 0.0039
5.	20	0.7760	

Fig 2.1 Calibration Curve of Venlafaxine HCl in distilled water



Similar procedure was followed for the preparation of calibration curves of VH in SGF (pH 1.2), Acetate buffer (pH 4.5) and SIF (pH 6.8). The absorbance was measured at 225 nm against blank reference.

– **Determination of Interference of Excipients in the estimation of Percent Drug Release**

50 mg of each excipient were added separately to a series of 10 ml volumetric flasks containing 1 ml of 100 µg/ml drug solution in distilled water. The flasks were kept for 6 hours with occasional shaking and volume made up to 10 ml with distilled water. The solutions were shacked, filtered and scanned for λmax and the absorbance of these solution were measured at 225 nm against blank reference. The absorbance obtained was compared with the absorbance of plain drug solution of same concentration. The observations are shown in Table 2.2.

Table 2.2 Absorbance data of Venlafaxine HCl in the presence of excipients.

S.No.	Additives	Absorbance	Interference
1.	Plain drug solution	0.3812	NIL
2.	Sodium Chloride	0.3815	NIL
3.	Mannitol	0.3821	NIL
4.	Povidone	0.3824	NIL
5.	Hydroxypropyl methyl cellulose	0.3819	NIL
6.	PEG-400	0.3825	NIL

2. GLIPIZIDE

Glipizide USP (DMF grade) was obtained from Cadila Healthcare Ltd., Moraiya, Ahmedabad, India.

– **Determination of Assay by HPLC**

Buffer solution : 1.78 g disodium hydrogen phosphate dihydrate and 1.44 g of sodium lauryl sulpate was dissolved in 1000 mL of water and filtered through 0.45 µm filter.

Mobile phase: A filtered and degassed mixture of buffer solution and acetonitrile was prepared in a ratio of 60:40. The pH was adjusted to 4.0 ± 0.05 with phosphoric acid.

Diluent : A mixture of acetonitrile and water was prepared in the ratio of 80: 20.

Standard preparation: An accurately weighed quantity of about 25 mg of GZ USPRS/working standard was transferred to a 200 mL volumetric flask. About 100 mL of acetonitrile was added and sonicated to dissolve. Volume was made upto the mark with acetonitrile and mixed.

Sample preparation: Accurately 20 tablets were weighed and the average weight was calculated. The tablets were crushed into a fine powder. An accurately weighed quantity of tablets powder equivalent to about 5 mg of GZ was transferred to a 100 mL volumetric flask. About 75 mL of diluent was added and sonicated with occasional shaking for 30 minutes. Volume was made up to the mark with diluent and mixed. 5.0 mL of this solution was diluted to 50.0 mL with diluent and mixed. The solution was filtered through 0.45 µm Millipore PVDF filter and the filtrate was collected by discarding first few mL of the filtrate.

- **Chromatographic system**

Column	: Hypersil BDS, C8, (150 mm x 4.6 mm), 5 µm
Detector (UV)	: 225 nm
Flow rate	: 1.5 mL/minute
Injection volume	: 10 µL
Column temperature	: 40°C

- **Determination of Percent Drug Release (In-Vitro dissolution) by HPLC**

The dissolution samples were analysed using HPLC assay method as mentioned above.

3. METOPROLOL TARTRATE

Metoprolol Tartrate IP was obtained from IPCA Laboratories Ltd., Mumbai, India.

- **Determination of Assay by HPLC**

Buffer Solution: 0.01 M potassium dihydrogen phosphate was used as buffer solution.

Mobile phase: A filtered and degassed mixture of buffer solution, acetonitrile, and methanol was prepared in the ratio of 55:22.5:22.5

Diluent: Mobile phase was used as diluent.

Standard preparation: An accurately weighed quantity of 100 mg of MT working standard was transferred to a 100-mL volumetric flask. About 25 mL of diluent was added and sonicated to dissolve. Volume was made up to the mark with diluent and mixed. 5.0 mL of this solution was dilute to 50.0 mL with diluent and mixed.

Sample preparation: Accurately 20 tablets were weighed and the average weight was calculated. The tablets were crushed into a fine powder. An accurately weighed quantity of tablets powder equivalent to about 100 mg of MT was transferred to a 100-mL volumetric flask. About 25 mL of diluent was added and sonicated with occasional shaking for about 20 minutes. The volume was made up to the mark with diluent and mixed. 5.0 mL of this solution was diluted to 50.0 mL with diluent and mixed. The solution was filtered through 0.45 µm Millipore HVLP filter, the filtrate was collected by discarding first few mL of the filtrate.

- **Chromatographic system**

Column	: RP C18, (25 cm x 4.6 mm), 5 µm
Detector (UV)	: 274 nm
Flow rate	: 1.2 mL/min.
Injection volume	: 10 µL
Column temperature	: 40°C

- **Determination of Percent Drug Release (In-Vitro dissolution) by UV**

Preparation of Calibration Curves of MT

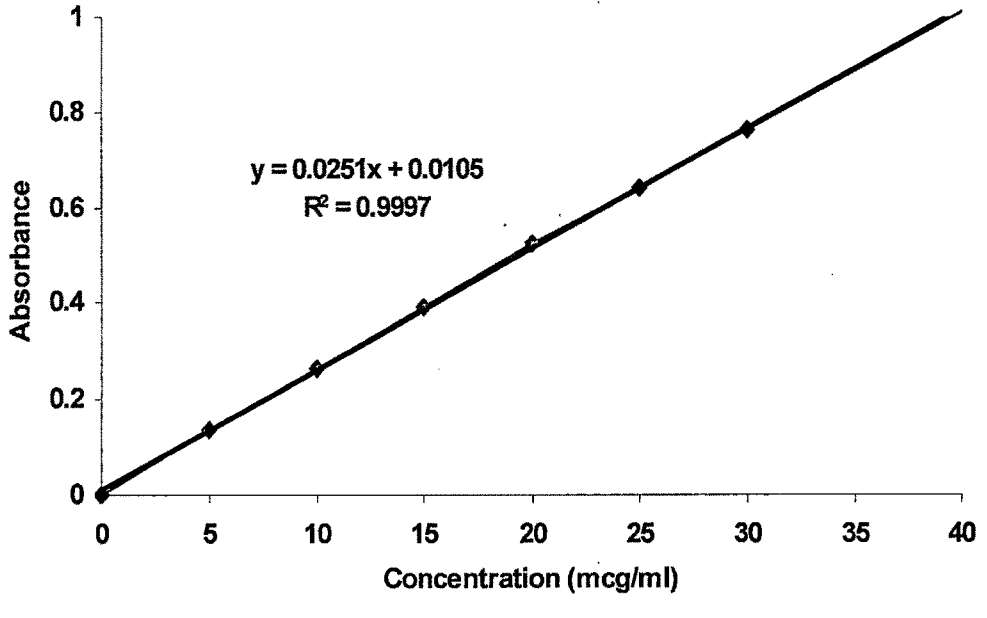
An accurately weighed quantity of 100 mg of sample was transferred to a 100-mL volumetric flask. About 50 mL of dissolution media was added and sonicated to dissolve. The volume was made up to the mark with dissolution media and mixed. 10 ml of this solution was taken in another 100 ml volumetric flask and diluted up to 100 ml with dissolution media to get a stock solution of 100 µg/ml concentration. From the stock solution, aliquots of 5, 10, 15 ...upto 40 ml were pipetted out in to a series of 100 ml volumetric flasks. The volume of the aliquots were made up to 100 ml with distilled

water, thus a range of concentration between 5 to 40 µg/ml were obtained. The absorbance of these solutions was measured in a UV spectrophotometer at λ max 223 nm against blank reference. The absorbance data obtained were linearly regressed and are represented in Table 2.3 and plotted in the form of standard curve in Fig. 2.2.

Table 2.3 Absorbance data of Metoprolol tartrate in distilled water at 223 nm.

S.No.	Concentration (µg/ml)	Absorbance	Statistical Parameters
1.	0	0.0000	Correlation Coefficient = 0.9997 Standard error = 0.0027
2.	5	0.1374	
3.	10	0.2665	
4.	15	0.3917	
5.	20	0.5220	
6.	25	0.6380	
7.	30	0.7616	
8.	40	1.0114	

Fig 2.2 Calibration Curve of Metoprolol tartrate in distilled water



Similar procedure was followed for the preparation of Calibration Curves of VH in SGF (pH 1.2), Acetate buffer (pH 4.5) and SIF (pH 6.8). The absorbance was measured at 223 nm against blank reference.

- **Determination of Interference of Excipients in the estimation of Percent Drug Release**

50 mg of each excipient were added separately to a series of 10 ml volumetric flasks containing 1 ml of 100 μg/ml drug solution in distilled water. The flasks were kept for 6 hours with occasional shaking and volume made up to 10 ml with distilled water. The solutions were shacked, filtered and scanned for λmax and the absorbance of these solution were measured at 225 nm against blank reference. The absorbance obtained was compared with the absorbance of plain drug solution of same concentration. The observations are shown in Table 2.4.

Table 2.4 Absorbance data of Venlafaxine HCl in the presence of excipients.

S.No.	Additives	Absorbance	Interference
1.	Plain drug solution	0.2664	NIL
2.	Polyethylene oxide	0.2625	NIL
3.	Sodium chloride	0.2633	NIL
4.	Starch	0.2648	NIL
5.	PEG-400	0.26289	NIL

4. NIFEDIPINE

Nifedipine USP (DMF grade) was obtained from Cadila Healthcare Ltd., Moraiya, Ahmedabad, India.

- **Determination of Assay by HPLC**

Mobile phase : A suitable mixture of water, acetonitrile and methanol (50 : 25 : 25), was prepared and degassed.

Standard preparation : An accurately weighed quantity of about 25 mg of NP working standard was transferred to a 25 ml volumetric flask. About 15 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol and mixed. Further 5 ml of the above solution was diluted to 50 ml with mobile phase and mixed.

Sample preparation : Tablets equivalent to about 420 mg of NP was weighed . The tablets were powdered and the powder was transferred to a 250 ml volumetric flask containing 130 ml of water. A mixture of acetonitrile and methanol (1 : 1) was added to volume and stirred for 30 minutes. The resulting suspension was centrifuged to obtain a clear supernatant stock solution. 3 ml of the stock solution was transferred to a 50 ml volumetric flask, diluted with mobile phase to volume, mixed and filtered to obtain a solution having a concentration of about 0.1 mg of NP per ml.

- **Chromatographic system**

Column	: C18 (L1), 4.6 mm x 25 cm and 2.1 mm x 3 cm guard column.
Detector (UV)	: 265 nm
Flow rate	: 1.0 ml/min
Inj. Volume	: 25 µl

- **Determination of Percent Drug Release (In-Vitro dissolution) by HPLC**

The dissolution samples were analysed using HPLC assay method as mentioned above.

5. OXYBUTYNIN CHLORIDE

Oxybutynin chloride USP (DMF grade) was obtained from Harman Finochem Ltd., Chikalthana, Aurangabad, India.

- **Determination of Assay by HPLC**

Mobile phase: Mobile phase used was mobile phase-A [water:methanol{800:200} + 0.2 mL triethylamine, with pH 3.5, which was adjusted by Ortho phosphoric acid] and mobile phase-B [acetonitrile] in the ratio of 70:30 at a flow rate 1.5 mL/min. Temperature of the column was maintained at 30 °C.

– **Chromatographic system**

Column	: YMC-Pack-CN, 4.6 mm x 25 cm x 5µm particle size.
Detector (UV)	: 203 nm
Flow rate	: 1.5 ml/min
Inj. Volume	: 25 µl

– **Determination of Percent Drug Release (In-Vitro dissolution) by HPLC**

The dissolution samples were analysed using HPLC assay method as mentioned above.

6. ATENOLOL

Atenolol USP (DMF grade) was obtained from Cadila Healthcare Ltd., Moraiya, Ahmedabad, India.

– **Determination of Assay by HPLC**

Buffer solution: 1.1 g of sodium 1-heptanesulfonatea and 0.71 g of anhydrous disodium hydrogen phosphate was dissolved in 700 mL of Milli Q water and 2.0 mL of dibutylamine was added. The pH was adjusted to 3.0 with 0.8 M orthophosphoric acid. The solution was filtered through 0.45 µm Millipore HVLP filter.

Mobile phase: A degassed mixture of buffer solution and methanol was prepared in the ratio of 70 : 30.

Diluent: Mobile phase was used as diluent

Standard preparation: An accurately weighed quantity of about 100 mg of AT working standard was transferred to a 100-mL volumetric flask. About 60 mL of mobile phase was added and sonicated to dissolve for 5 minutes. The volume was made up to the mark with mobile phase and mixed. 5.0 mL of this solution was diluted to 50.0 mL with mobile phase and mixed. Further 5.0 mL of this solution was diluted to 50.0 mL with mobile

phase and mixed. The solution was filtered through 0.45 µm Millipore HVLP filter, the filtrate was collected by discarding first few mL of the filtrate.

Sample preparation: 20 tablets were weighed and the average weight was calculated. From the 20 tablets, 10 tablets were weighed and were transferred to a 1000-mL volumetric flask. About 100 mL of mobile phase was added and sonciated for about 15 minutes to disintegrate completely. Further 400 mL of mobile phase was added and sonicated for 15 minutes. The volume was made up to the mark with mobile phase and mixed and was allowed to settle. 5.0 mL of the clear supernatant solution was diluted to 50.0 mL with mobile phase and mixed. Further 5.0 mL of this solution was diluted to 50.0 mL with mobile phase and mixed. The solution was filtered through 0.45 µm Millipore HVLP filter, the filtrate was collected by discarding first few mL of the filtrate.

- **Chromatographic system**

Column	: Kromasil C18, (30 cm x 4.0 mm), 5 µm
Detector (UV)	: 226 nm
Flow rate	: 0.6 mL/min
Injection volume	: 20 µL
Column temperature	: Ambient

- **Determination of Percent Drug Release (In-Vitro dissolution) by UV**

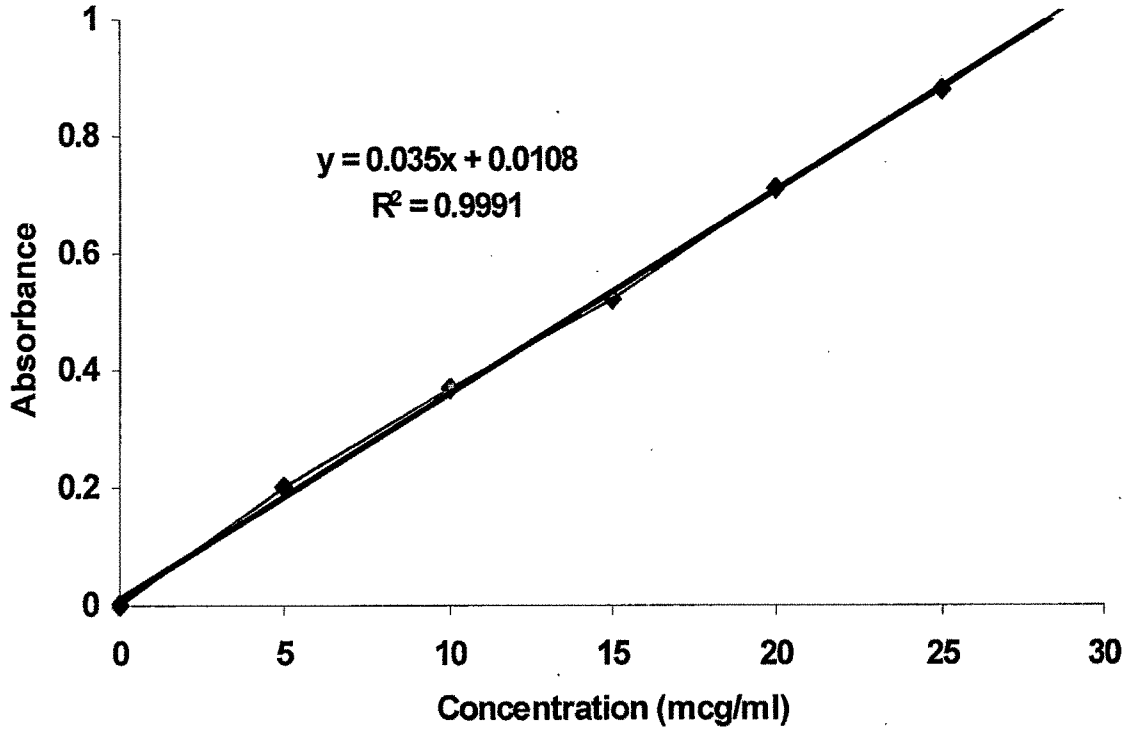
Preparation of Calibration Curves of AT

An accurately weighed quantity of 100 mg of sample was transferred to a 100-mL volumetric flask. About 50 mL of dissolution media was added and sonicated to dissolve. The volume was made up to the mark with dissolution media and mixed. 10 ml of this solution was taken in another 100 ml volumetric flask and diluted up to 100 ml with dissolution media to get a stock solution of 100 µg/ml concentration. From the stock solution, aliquots of 5, 10, 15....upto 30 ml were pipetted out in to a series of 100 ml volumetric flasks. The volume of the aliquots were made up to 100 ml with distilled water, thus a range of concentration between 5 to 30 µg/ml were obtained. The absorbance of these solutions was measured in a UV spectrophotometer at λ max 225 nm against blank reference. The absorbance data obtained are represented in Table 2.5 and plotted in the form of standard curve in Fig. 2.3.

Table 2.5 Absorbance data of Atenolol in distilled water at 225 nm.

S.No.	Concentration (µg/ml)	Absorbance	Statistical Parameters
1.	0	0.0000	Correlation Coefficient = 0.9997 Standard error = 0.0075
2.	5	0.2056	
3.	10	0.3710	
4.	15	0.5176	
5.	20	0.7124	
6.	25	0.8813	
7.	30	1.0638	

Fig 2.3 Calibration Curve of Atenolol in distilled water



Similar procedure was followed for the preparation of Calibration Curves of AT in SGF (pH 1.2), Acetate buffer (pH 4.5) and SIF (pH 6.8). The absorbance was measured at 225 nm against blank reference.

Determination of Interference of Excipients in the estimation of Percent Drug Release

50 mg of each excipient were added separately to a series of 10 ml volumetric flasks containing 1 ml of 100 μg/ml drug solution in distilled water. The flasks were kept for 6 hours with occasional shaking and volume made up to 10 ml with distilled water. The solutions were shacked, filtered and scanned for λmax and the absorbance of these solution were measured at 225 nm against blank reference. The absorbance obtained was compared with the absorbance of plain drug solution of same concentration. The observations are shown in Table 2.6.

Table 2.6 Absorbance data of Atenolol in the presence of excipients.

S.No.	Additives	Absorbance	Interference
1.	Plain drug solution	0.3715	NIL
2.	Tartaric acid	0.3721	NIL
3.	Mannitol	0.3724	NIL
4.	Potassium chloride	0.3764	NIL
5.	Starch	0.3776	NIL
6.	Povidone	0.3714	NIL
7.	PEG-400	0.3719	NIL
8.	Sorbitol	0.3746	NIL
9.	Hydroxypropyl methyl cellulose	0.3745	NIL
10.	PEG-6000	0.3744	NIL