6. PRELIMINARY WORK

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Six drugs were selected from antihyperlipidemic class, EZE from bile acid sequestering agent, SIMVA, LOVA, ROSU and PRAVA from HMG-CoA reductase inhibitor and nicotinic acid.

Before starting any experimental work it was necessary to check some physicochemical parameters like solubility, melting point, etc. and conform by compression with given standard data.

6.1 Drug substance

Pharmaceutical grade of ezetimibe (EZE), simvastatin (SIMVA), lovastatin (LOVA), Rosuvastatin calcium (ROSU) and Pravastatin sodium (PRAVA) reference standards were kindly supplied as gift samples by Torrent Research Center, Ahmadabad, India. Nicotinic acid (NICO) was purchased from

6.2 Physicochemical parameters

Drug substances obtained form the sources were characterized by studying some physicochemical parameters.

6.2.1. Melting point

Melting points of all five drugs were determined on melting point apparatus from Thermo Electronic Corporation. Melting points of drugs are reported in table 6.1.

Table 6.1: Melting point of drugs reported and taken.

Sr. No.	Drug	**Reported Melting point (°C)**	**Melting point (°C) (mean*)**
1	EZE	164 -166°C	166
2	SIMVA	135-138°C	134
3	LOVA	174.5°C	173
4	ROSU	Not reported	163
5	PRAVA	138-142°C	140
6	NICO	236.6 °C	236

* Mean of three determinations.

6.2.2. IR

IR spectrum of all five drugs were taken on FTIR 8400 S, of Shimadzu. IR spectra of EZE, SIMVA, LOVA, ROSUVA and PRAVA are reported in figure 6.1 to 6.5,

respectively. Spectra were compared with standard spectra[1]. Functional group peaks are reported in table 6.2.

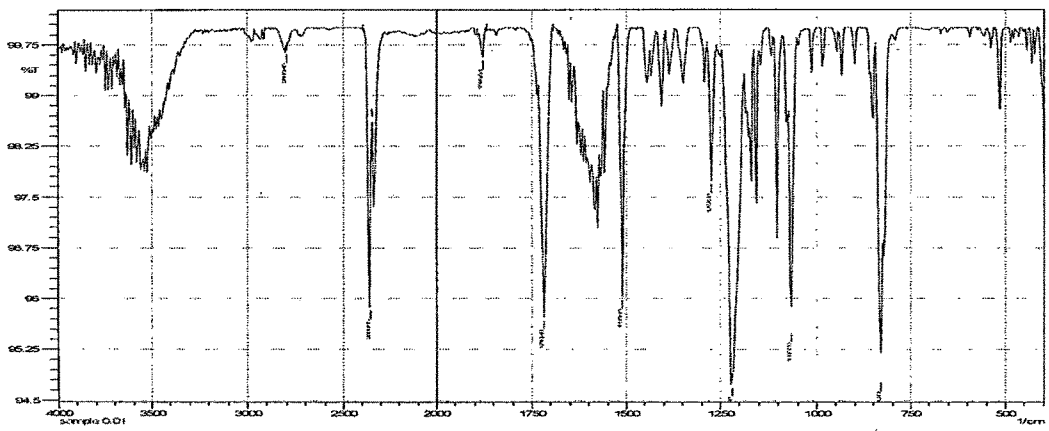

Fig. 6.1: IR spectra of EZE

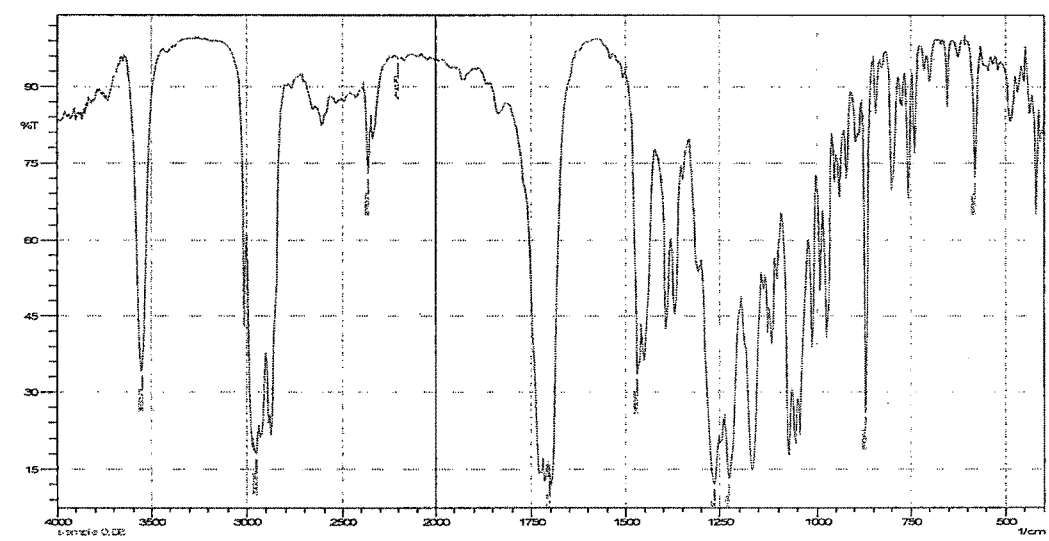

Figure 6.2: IR spectra of SIMVA

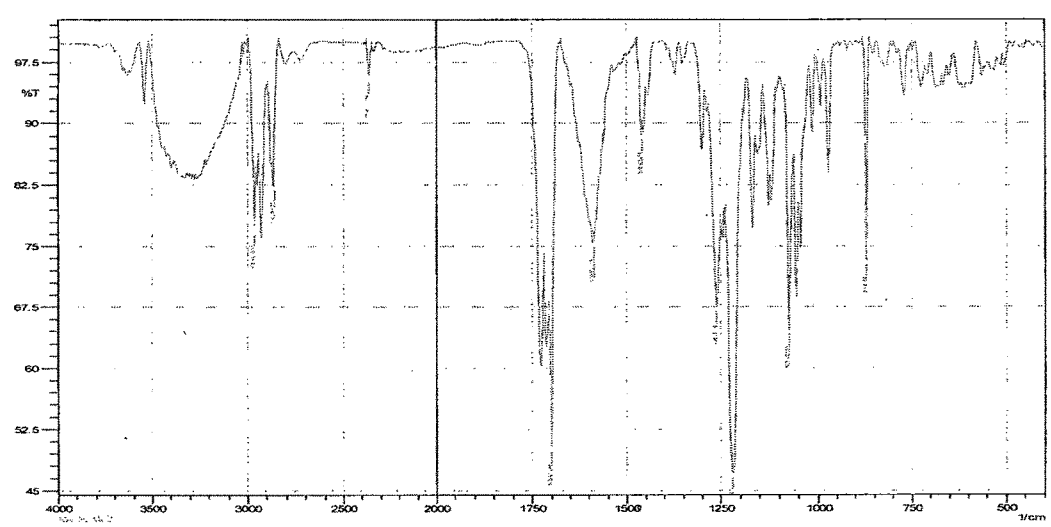

Figure 6.3: IR spectra of LOVA

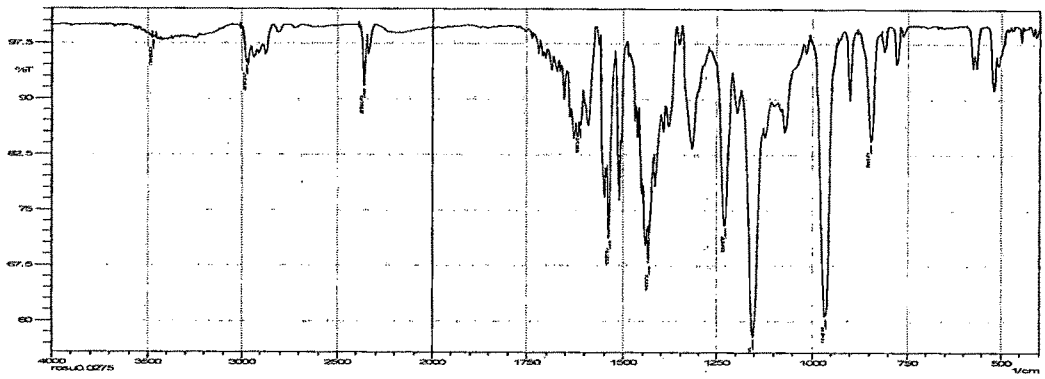

Figure 6.4: IR spectra of ROSU

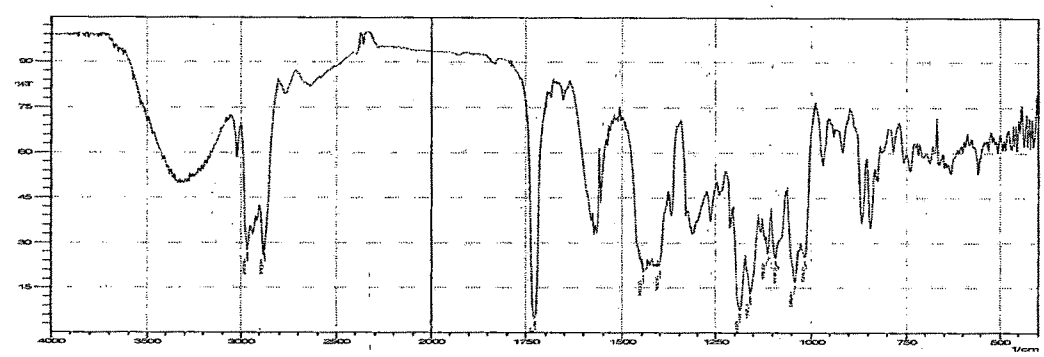

Figure 6.5: IR spectra of PRAVA

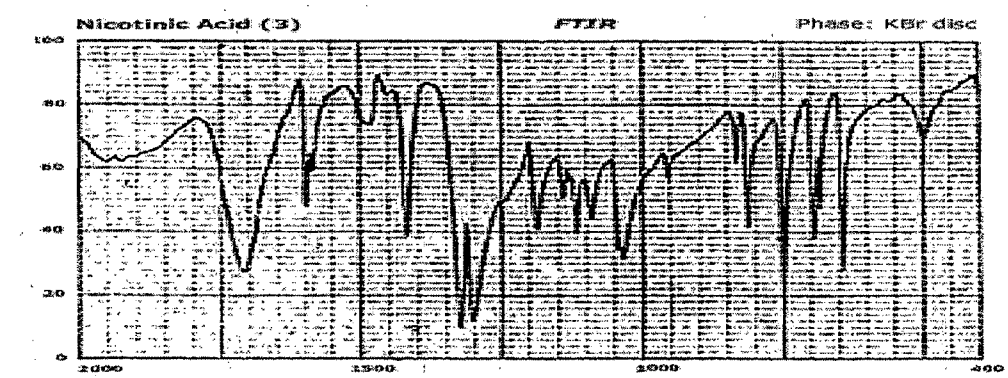

Figure 6.6: IR spectra of NICO

Table 6.2: Functional group peak of drugs

Sr. No.	Drugs	Reported Functional group peak (1/cm)	Functional group peak (1/cm)
1	EZE	Not reported	2800,2356, 1878, 1510, 1243
2	SIMVA	1718, 1459, 1389, 1267	1729.23, 1469, 1269, 3550
3	LOVA	Not reported	1695, 1593, 1243
4	ROSU	Not reported	3469, 2970, 2358, 1538, 844
5	PRAVA	1727, 1579, 1187	2362, 1730, 1580, 1145

6	NICO	1752, 1432, 1305, 1305	1750, 1423, 1300, 1350

6.2.3. Solubility

Solubility of drugs was checked in different solvents and the data along with the reported data is shown in table 6.3.

Table 6.3: Solubility of drug in different solvent

Sr. No.	Drug/solvent	EZE	SIMVA	LOVA	ROSU	PRAVA	NICO
1	Water	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)	100mg /1.5ml	100mg/0.5ml (Freely soluble)	100 mg/5ml (Sparingly soluble)
2	Methanol	100mg/1ml (Freely to very soluble)	100mg/1ml (Freely to very soluble)	100mg/1ml (Freely to very soluble)	100mg /0.5ml	100mg/0.5ml (Freely soluble)	5mg/100ml (Insoluble)
3	Chloroform	100mg/1.5ml (Soluble)	100mg/1.5ml (Soluble)	100mg/1.5ml (Soluble)	100mg /0.5ml	5mg/100ml (Insoluble)	(Slightly soluble)
4	Acetonitrile	100mg/1.5ml (Soluble)	100mg/1.5ml (Soluble)	100mg/1.5ml (Soluble)	100mg /0.5ml	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)
5	0.1 N NaOH	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)	100mg /0.5ml	100mg/0.5ml (Freely soluble)	100mg/5ml (Dissolve)
6	0.1 N HCl	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)	5mg/100ml (Insoluble)	100mg /0.5ml	100mg/0.5ml (Freely soluble)	100mg/5ml (Dissolve)

The values in parentheses are the reported values.

6.2.4. UV spectra in different solvent

UV spectrum of the drugs solutions in different solvents are reported in fig. 6.6 to 6.10.

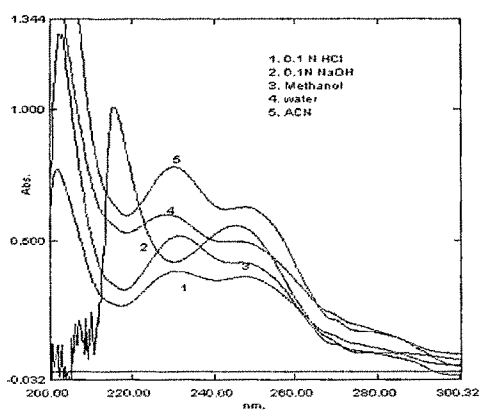

Figure 6.6: UV spectra of EZE in different solvents

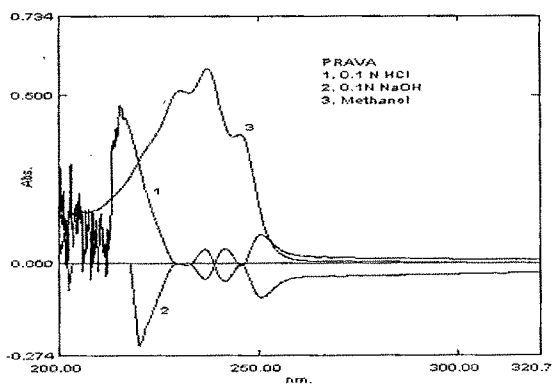

Figure 6.7: UV spectra of PRAVA in different solvents

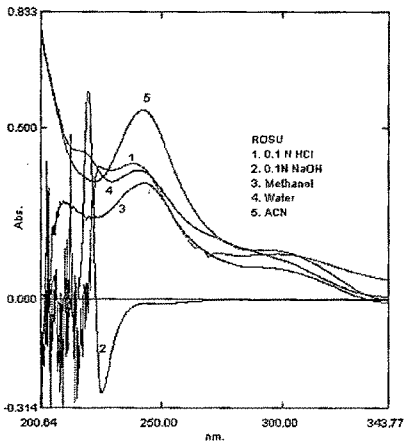

Figure 6.8: UV spectra of ROSU in different solvents

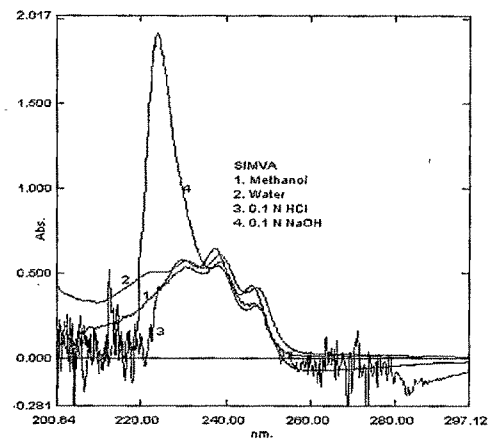

Figure 6.9: UV spectra of SIMVA in different solvents

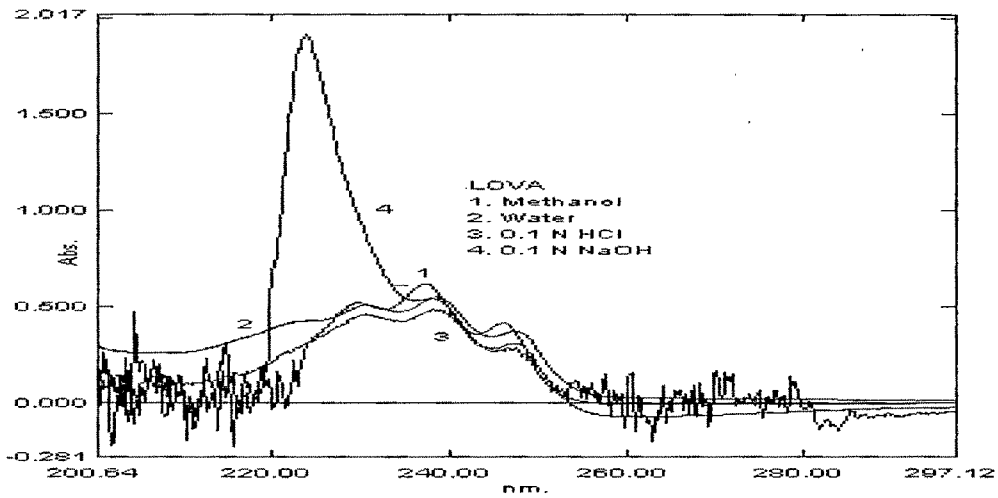

Figure 6.10: UV spectra of LOVA in different solvents

6.3 Instrumentation and apparatus

The instruments and apparatus used while developing new analytical methods are described in the following section.

- Balance, Model ALC 210.4 (Acculab)
- Ultra Sonicator (Fast Clean Ultrasonic Cleaner)

6.3.1. Spectroscopy

- UV-visible spectrophotometer, Double beam, Model 1700(Shimadzu) with matched Quartz cuvettes and loaded with UV Probe software.

6.3.2. FTIR

- Fourier Transform Infrared Spectrophotometer, DRIFT, Model FTIR – 8400S (shimadzu)

6.3.3. Chemometric

- Shimadzu- 1700 UV/Vis spectrophotometer as describe under section 6.3.1.
- The numerical calculations were performed using MATLAB 6.1 software and Excel

6.3.4. HPLC

- HPLC, Model: LC-10ATvp (Shimadzu) with rheodyne injector, UV-Visible detector, Model: SPD-10 AVP (Shimadzu) and class VP software.
- HPLC Column, C_{18} (size-250 x 4.60 mm, I.D-5 µ) (Phenomenex)
- Nylon filter 0.45 µm
- PH meter (Thermo electro corporation)
- Centrifuge (ELTEX, Research centrifuge TC 4100D, Ahembadabad)
- Vortex shaker (SPINIX, Mumbai)

6.3.5. HPTLC

- Pre-coated silica gel aluminum Plate 60F–254 (20 × 10 cm with 250 µm thickness) (E. Merck)
- Desaga – 25 µl Dosing syringe (Hamilton Co., Reno, Nevada)
- Desaga – 10 µl Applicator syringe, GASTIGHT, Model 1701 (Hamilton, Bonaduz, Schweiz)
- Desaga Applicator, AS30win
- Desaga – Twin trough chamber (100 × 100) with stainless steel Lid
- Desaga TLC scanner, Proquant
- Desaga Photo chamber, Providoc with Canon power shot G_5 digital camera
- UV cabinet with dual wavelength UV lamp (254 nm and 366 nm)
- pH meter (Thermo electron Corporation)

Apparatus: Calibrated Borosil pipettes and volumetric flasks were used throughout.

6.4. Chemicals and Reagents

6.4.1. Spectroscopy and chemometric

- Methanol AR (S. D. fine) was used throughout the study
- NaOH (S. D. fine) was of AR grade. 0.1M NaOH solution was prepared and standardized as per IP 96 procedure.
- HCl (S. D. fine) was of AR grade. 0.1M HCl solution was prepared and standardized as per IP 96 procedure.

6.4.2. FTIR

- KBr (S. D. fine) of Analytical grade was used throughout the study

6.4.3. HPLC

- Acetonitrile, HPLC grade (S.D. Fine Chemicals Ltd., Mumbai)
- Methanol, HPLC grade (Spectro) was used throughout the study
- Formic acid AR (S.D. Fine Chemicals Ltd., Mumbai)
- Triple Distilled Water (laboratory prepared)
- Sodium hydroxide, hydrochloric acid and 30 % Hydrogen peroxide were purchased from Qualigens Fine Chemicals (Glaxo Ltd.).
- Nylone 0.45µm, 47 mm membrane filters (Gelman Laboratory, Mumbai).
- Plasma for laboratory

6.4.4. HPTLC

- Methanol (S. D. fine Chemicals Ltd., Mumbai)
- Benzene (Finar chemicals, Ahmedabad)
- Acetonitrile (ACS chemicals, Ahmedabad)
- Toluene(S. D. fine Chemicals Ltd., Mumbai)
- Ethlylacetate(S. D. fine Chemicals Ltd., Mumbai)
- Hexane(ACS chemicals, Ahmebadad)
- Chloroform(S. D. fine Chemicals Ltd., Mumbai)
- Formic acid(S.D. Fine Chemicals Ltd., Mumbai)

6.5 Marketed formulations selected for analyzed by developed methods.

All tablets selected were of 10 mg dose per tablet. Table 6.5 shows the trade name of drug, company of manufacturing and also the method for which tablet was used.

Table 6.5: Marketed formulation selected for single component analysis

Sr. No.	Drug	Tread Name	Method	Company
1	EZE	ZETICA	UV method only	Torrent
		EZETIB	UV and chromatographic methods	Unisearch
		EZEDOC	IR and chromatographic methods	Lupin
2	SIMVA	IFISTATIN	UV method only	JB chemicals
		STATIN	For all methods	Unisearch
3	LOVA	AZTATIN	UV method only	Sun
		LOVACARD	For all methods	Cipla
4	ROSU	NOVASTAT	For all methods	Lupin
		FORTIUS	UV method only	Nic.Piramal
5	PRAVA	PRAVATOR	For all methods	solus)

All tablets selected were of 10 mg dose per tablets. Table 6.6 shows detailed information about the combination formulations analyzed by newly developed methods. Here only EZE and SIMVA and SIMVA and NICO combination marketed formulation available. For other combination synthetic mixture was prepared by combination of ten tablets of the two drugs. Combination for study was selected as per clinical data for best used of hyperlipidemia instead of monotherapy. The combination laboratory mixture was also prepared from the stock solution by mixing the components in ratio of 1: 1.

Table 6.6: Marketed formulation selected for combination analysis

Sr. No.	Drug	Tread Name
1	EZE and SIMVA	**SIMVAS-EZ**
2	EZE and PRAVA	**EZEDOC +PRAVATOR**
3	EZE and LOVA	**EZEDOC + AZTATIN**
4	EZE and ROSU	**EZEDOC + NOVASTAT**
5	SIMVA and NICO	**SIMVOTIN**

6.6. Standard steps for IR spectroscopic methods.

Performs quantitative analysis of a sample that is composed of multiple components using the multiple linear regression (MLR) method. A new calibration curve was created in the [Calibration graph] sub-tab. The calibration curve can be evaluated in the [Prediagnosis] tab. After the calibration curve has been completed, a quantitative analysis calculation of an unknown sample can be started in the [Quant] tab.

6.7. Standard conditions for HPTLC method:

Following standard conditions were used while developing HPTLC methods for selected drugs-

- **Stationary phase:** Pre-coated silica gel aluminum Plate 60F–254 (20 × 10 cm with 250 µm thickness) (E. Merck) pre-washed with Methanol then dried for 30 minute at 50°C.
- **Mobile phase:** Varies as per methods
- **Chamber saturation:** 30 min

- **Plate width:** 50 mm (Varies as per requirements)
- **Band width:** 3 mm
- **Distance between spots:** 5 mm
- **Rate of spotting:** 10 sec/µl
- **Distance run:** Start: 15 mm
- **Scanning Wave length:** different for each method
- **Slit width:** 4 mm
- **Slit height:** 0.02 mm
- **Evaluation mode:** Excitation
- **Lamp:** Deuterium/Tungsten
- **Number of spot varies as per requriment**

Pre-treatment of pre-coated plates:

TLC plate was placed in twin trough glass chamber containing methanol as mobile phase. Methanol was allowed to run up to upper edge of plate (ascending method). Plate was removed and allowed to dry in oven at 50^0C for 30 min. For the actual experiment the plate was allowed to come to room temperature and used immediately.

6.8. Methodology of developed methods:

The new analytical methods described later for drugs in single or binary component analysis are heavily summarized to confine the bulk of this thesis in a limit. But the general methodology adopted while developing these methods included the following steps-

- Preparation of standard solutions: single and mixed standards when required were prepared from a stock solution after necessary dilutions.
    - Selection of analytical wavelengths
    - Calibration curve: a series of standards, single or mixed were prepared in the required concentration range. The absorbance of these solutions was measured.
    - Validation of analytical method.
    - Result and discussion
    - Preparation of sample solution form marketed formulation.
    - Conclusion