CHAPTER 2

Theory and Instrumentation of Infrared, Raman, UV-Visible Spectroscopy and HPLC technique

2.1 Introduction

The vibrational spectrum of a molecule is considered to be a unique physical property and is characteristic of that particular molecule. As such, the infrared spectrum can be used as a finger print for identification of all types of organic and many types of inorganic compounds. The functional groups of inorganic materials and the molecular composition of surfaces can be determined using the spectrum. It enables the quantitative determination of compounds in mixtures. Infrared spectroscopy is successfully employed in the determination of molecular conformation of structural isomers, stereochemistry of geometrical isomers and the molecular orientation in polymers and solutions. Raman spectroscopy when used in conjunction with IR data is very powerful in determining the ground state structure. Hence it is a very important tool in drug design, formulation and quality analysis. Apart from this spectroscopy finds wide application in the study of biomolecules, thus rendering itself useful for diagnostic purpose. Chromatographic techniques play a vital role in measuring the levels of active drugs, synthetic byproducts, or degradation products in pharmaceutical dosage forms. It is an accurate tool for measuring compounds such as amino acids, nucleic acids and proteins in physiological samples.
Hence it is widely used in pharmaceutical research, development, manufacturing and quality control. This chapter presents a detailed view of the theory and instrumentation of Infrared, Raman, UV-Visible spectroscopy and HPLC technique.

2.2 Infrared Spectroscopy

Infrared spectroscopy is an important and popular tool for structural elucidation and compound identification, employed by physicists and chemists all over the world (1-3). The infrared radiation spans a section of the electromagnetic spectrum having wavenumbers ranging from about 13000cm\(^{-1}\) to 33cm\(^{-1}\). It is bound by the red end of the visible region at high frequencies and the microwave region at low frequencies. The most frequently used region is 4000-400 cm\(^{-1}\) which is the mid IR region. The far IR region is used for analysis of organic, inorganic, and organometallic compounds involving heavy atom. The near IR spectroscopy offers high-speed quantitative analysis without consumption or destruction of the sample. Infrared spectrum is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids and solids. They give accurate results irrespective of the nature of the chosen sample.
A polyatomic non-linear molecule has $3n-6$ normal modes of vibration and linear molecules have $3n-5$ fundamental vibrational modes. Among these normal modes of vibration, those that produce a net change in the dipole moment may result in IR activity and those that give polarizability changes may give rise to Raman activity. Some vibration may be both IR and Raman active. The total number of observed absorption bands is generally different from the total number of fundamental vibration. It is reduced because some modes are not IR active and a single frequency can cause more than one mode of motion to occur. Conversely, additional bands are generated by the appearance of overtones, combinations of fundamental frequencies, differences of fundamental frequencies, coupling interaction of two fundamental absorption frequencies and coupling interactions between fundamental vibrations and overtones. The intensities of overtone, combination, and difference bands are less than those of the fundamental bands. The combination and blending of all the factors thus create a unique IR spectrum for each compound (4).

The major types of molecular vibrations are stretching and bending. The stretching vibrations consist of symmetrical and asymmetrical stretching. The bending vibrations are composed of scissoring, wagging, twisting and rocking motions. The infrared radiation is absorbed and the associated energy is converted into different types of stretching and bending motions. The
absorption involves discrete, quantized energy levels and the individual vibrational motion is usually accompanied by other rotation motions. These combinations lead to the absorption bands in the mid IR region (5). The frequency of these absorptions depends upon the relative masses of the atoms, the force constants and the geometry of the atoms. The frequency of vibration is directly proportional to the strength of the bond or the force constant and is inversely related to the masses of the atoms bonded to one another. IR absorption information is generally present in the form of spectrum with wavelength or wavenumbers as the x-axis and absorption intensity or percentage transmittance as the y-axis.

2.2.1 Fourier Transform Infrared Spectroscopy

The dispersive spectrometers have been replaced by Fourier transform spectrometers due to their superior speed and sensitivity. They have greatly extended the capabilities of infrared spectroscopy and have been applied to many areas that are very difficult or nearly impossible to analyze by dispersive instruments. Conventional spectroscopy is a frequency domain spectroscopy in which radiant power data are recorded as a function of radiant frequency. Time domain spectroscopy, in contrast, is concerned with changes in radiant power with time. In Fourier transform spectrometers, an interferogram that provides information over the entire IR region to which the detector is responsive is created. A mathematical operation known as Fourier transformation converts
Fig. 2.1 Model of Perkin Elmer Spectrum one FTIR spectrometer

Fig. 2.2 Optical path diagram of FTIR spectrophotometer
the interferogram which is a time domain spectrum to a much familiar frequency domain spectrum showing intensity versus frequency. Also, in Fourier transform spectroscopy the resolution element for a spectrum are measured simultaneously (multiplex method) unlike in conventional spectroscopy (6).

2.2.2 Instrumentation

There are three basic spectrometer components in a Fourier Transform system. They are the radiation source, interferometer and the detector. A simplified optical layout of a typical FTIR spectrometer is illustrated in Fig. 2.2. The popular radiation sources are Nernst glower, Globar and Nichrome coil. They are often water-cooled in FTIR instruments to provide better power and stability (7, 8).

The interferometer divides radiant beams, generates an optical path difference between the beams, and then recombines them in order to produce repetitive interference signals measured as a function of optical path difference by a detector. Thus the interferometer produces the interference signals, which contain infrared spectral information generated after passing through a sample. The most commonly used interferometer is a Michelson interferometer. It consists of three active components: a moving mirror, a fixed mirror, and a beam splitter. The two mirrors are perpendicular to each other. The beamsplitter is a semi reflecting device and is often made by depositing a thin
film of germanium onto a flat KBr substrate. Radiation from the broadband IR source is collimated and directed into the interferometer, and impinges on the beamsplitter. At the beamsplitter, half the IR beam is transmitted to the fixed mirror and the remaining half is reflected to the moving mirror. After the divided beams are reflected from the two mirrors, they are recombined at the beamsplitter. Due to changes in the relative position of the moving mirror to the fixed mirror, an interference pattern is generated. The resulting beam then passes through the sample and is eventually focused on the detector. The two most popular detectors for a FTIR spectrometer are deuterated triglycine sulfate (DTGS) and mercury cadmium telluride (MCT).

2.2.3 Sample handling techniques

It is possible to obtain the IR spectrum of samples in different forms such as liquid, solid, and gas. But those materials that are opaque to IR radiation must be dissolved or diluted in transparent matrix in order to obtain the spectra (9, 10).

Pressed-Pellet Technique

Pellets are used for solid samples that are difficult to melt or dissolve in any suitable IR-transparent solvents. The samples (0.5 to 1.0mg) is finely ground and intimately mixed with approximately 100 mg of dry KBr or other alkali powder. The mixture is then pressed into transparent disc in a die at sufficiently high pressure. To minimize band distortion due to scattering of
radiation, the sample should be ground to particles of 2μm or less in size. As KBr does not absorb in the region 4000-400 cm⁻¹, a complete spectrum of the solid sample is obtained in this range.

*Mulling Technique*

The sample (1 to 5mg) is ground with a mulling agent (1 to 2 drops) to give a two phase mixture and this mull is pressed between two infrared transmitting plates to form a thin film whose spectrum is recorded. The common mulling agents include mineral oil or Nujol, Fluorolube and Hexachlorobutadiene.

*Liquid Samples*

Liquid cells are used for dilute solutions of solid and liquid samples that are dissolved in relatively IR-transparent solvent. Since no single solvent is transparent through the entire mid IR region, suitable solvents are chosen in the region of interest. Infrared spectra of aqueous sample are recorded by the special types of liquid cells such as thin cells of BaF₂, AgCl or KRS-5.

Sodium chloride discs are the most popular choice for non-aqueous liquids. A drop of neat sample is squeezed between two salt plates to form a film of approximately .01mm in thickness. It is also possible to place a film of sample on salt plates by melting a relatively low-melting solid and squeezing it between two plates. This smear technique is one of the simplest ways to obtain infrared spectra. Thin films of non volatile liquids or solids can be deposited on
an IR-transmitting salt plate by solvent evaporation. The sample is first dissolved in a reasonably volatile solvent. A few drops of the resulting solution are placed on the plate. After evaporating the solvent, a thin film of sample is obtained for subsequent spectra acquisition.

_Gaseous Samples_

Gas cells are made of glass or metal body. They consist of two IR transparent end windows and valves for filling gas from external sources. They provide vacuum-tight light paths from a few centimeters to 120m. Longer path lengths are obtained by using internal mirrors at the ends of the cell. It is a common practice to record infrared spectrum along with polystyrene band at 1601 cm\(^{-1}\) marked on as a check of frequency accuracy.

In the present work, most of the FTIR spectral measurements have been made using Perkin Elemer Spectrum One spectrometer and a few with Bruker IFS 66V spectrophotometer over the range 4000-400 cm\(^{-1}\). A model of the Perkin Elmer spectrometer is shown in Fig. 2.2. It has a SiC source module, a deuterated triglycine sulfate (DTGS) detector and provided with ZnSe optics. The entire operation is controlled by a PC with windows based program for acquisition, display and processing of spectral data. The frequencies of all the sharp bands are accurate to ±1 cm\(^{-1}\). The pressed pellet technique was adopted for solid samples and blood plasma was smeared on thallium bromide pellet and was air dried before recording the spectrum.
2.3 Raman Spectroscopy

Raman effect is a scattering phenomenon where the energy of the incident photons is much larger than vibrational transition energies. When the high energy photons are scattered without change then it is termed as Rayleigh scattering. However, a small fraction are scattered from molecular centers with less energy than they had before the interaction. These photons give rise to Raman-Strokes lines. Another series of scattered photons has greater energy than those of the exciting radiation. These are called anti-Stokes photons. The differences in the energies of the scattered and incident photons correspond to vibrational transitions and from this it is concluded that molecules are promoted to an excited vibrational state, just as they are in IR. Stokes radiation is generally more intense than anti-Stokes radiation and for this reason, only the Stokes part of a spectrum is generally used. This is because of the fact that the relative populations of higher energy states are lesser, thus favouring Stokes emission over the anti-Stokes. The ratio of the anti-Stokes to Stokes intensities will increase with temperature because a larger fraction of molecules will be in excited state under this circumstance. It is important that the magnitude of Raman shifts is independent of the wavelength of excitation. The intensity or power of a normal Raman peak depends in a complex way upon the polarizability of the molecule, the intensity of the source and the concentration of the active group and as well as other factors.
The information obtained in a Raman experiment is quite different from that in an IR experiment as the quantum mechanical selection rules are different. In IR, absorption can take place only if there is change in dipole moment during the vibration, on the other hand, a change in polarizability is required in order for Raman scattering to occur. Thus homonuclear diatomic compounds are IR-inactive (since they do not possess a permanent dipole moment) but show Raman spectra since their vibration is accompanied by change in polarizability of molecules. According to the 'mutual exclusion rule', for centrosymmetric molecules the vibration which is active in IR is inactive in Raman and vice versa. Using IR and Raman together allows one to establish the existence of center of symmetry quite easily (11, 12).

Raman scattering information is presented as an intensity-versus-wavelength shift in cm\(^{-1}\). The position of a given IR band in cm\(^{-1}\) is identical to the wavelength shift of the Raman band because the same transition is being excited by both processes. Thus, fundamental group frequencies from IR tables can be conveniently used in Raman studies to obtain structural information.

Raman depolarization ratio is an important parameter in Raman spectroscopy which has no counter part in infrared spectroscopy. The Raman depolarization ratio may be defined as \(\rho = \frac{I_\parallel}{I_{11}}\) where \(I_\parallel\) is the intensity of the scattered radiation whose plane of polarization is perpendicular to that of the incident radiation and \(I_{11}\) is the intensity of scattered radiation whose plane of
polarization is parallel to that of the incident radiation. If the vibrational motion preserves the symmetry of the molecule, then the value of \( \rho \) will range from 0 to just less than \( \frac{3}{4} \). If the symmetry of the molecule is reduced by the vibration, the ratio will be \( \frac{3}{4} \) within experimental error. The depolarization ratio is dependent upon the symmetry of the vibrations responsible for scattering and is thus useful in correlating Raman lines with modes of vibration.

2.3.1 Instrumentation

The modern Raman instrumentation consists of four basic units: a laser source, sample optics, monochromator and detector cum recorder. The schematic diagram of the Fourier transform Raman spectrometer is given in Fig. 2.4. The laser radiation 1064nm from Nd:YAG laser if filtered to perfect its monochromaticity and is then focused on the sample. The light reflected and back scattered are then filtered. By using holographic diffraction gratings as monochromators and with multiple dispersion stages, a high degree of stray laser rejection is achieved and only the Raman scattering is allowed to pass through a Michelson interferometer. The interferogram is then collected and detected on a NIR detector. The germanium photodetector operating at the liquid nitrogen temperature or indium doped gallium arsenide that operates at room temperature are employed. The detector signal is digitized and Fourier transformed to generate a spectrogram. A spectrum is plotted as intensity of scattering versus frequency shift using a software.
Fig. 2.3 Model of Bruker IFS 66V FTRaman spectrometer

Fig. 2.4 Optical path diagram of FTRaman Spectrometer
The Raman scattering from samples is directly proportion to the intensity of the incident radiation of the sample, to the fourth power of the frequency of the incident radiation and to the concentration of the sample. For this reason, the development of high intensity laser systems has revolutionized Raman spectroscopic techniques. Some of the common lasers used as source are Ar+, Kr+, He/Ne, diode and ND:YAG lasers. The last two sources are finding more and more use as they can be operated at much higher power without causing photodecomposition of the sample and fluorescence is much less intense or non-existent with these lasers (13).

2.3.2 Sample handling techniques

Solid samples

Powders, microcrystalline and amorphous materials may be packed into melting-point capillaries or NMR tubes. Translucent solids may be mounted as it is at the focal point of the laser beam thus simplifying the sample handling procedure.

Liquid Samples

Liquid samples may be contained in glass capillary tubes, NMR tubes, cuvettes and even beakers. Raman scattering due to water is quite weak and so aqueous solutions may be easily examined by Raman spectroscopy and not by infrared. Thus the analysis of biological fluids and tissue samples using Raman spectroscopy finds widespread applications.
Gas Samples

In case of gases the multiple reflection technique is adopted where the laser beam is reflected several times back and forth through the sample, in order to enhance the signals.

In the present work the FTRaman spectrum was recorded using 1064 nm line of Nd : YAG laser as excitation wavelength in the region 3500–100 cm\(^{-1}\) on Bruker IFS 66V spectrometer equipped with FRA 106 FTRaman module accessory. The sample compartment was equipped with a reference source and all necessary optics for collecting radiation scattered at 90° and at 180°. The interferometer consists of a CaF\(_2\) beam splitter and liquid nitrogen pre-cooled germanium diode was used as the detector. The successive improvement in experimental techniques and measurement systems make Raman spectroscopy one of the most versatile and powerful tool for investigation of matter (14).

2.4 UV-Visible Spectroscopy

UV-Visible spectrophotometry has considerable importance as one of the spectroscopic methods supporting drug research, quality assessment and trace elemental interaction studies (15). The UV-Visible spectra of compounds are associated with transitions between electronic energy levels. The transitions are generally between a bonding or lone pair orbital and an empty non-bonding or antibonding orbital. Absorption of energy is quantized and results in the
promotion of electrons (σ, π, n* transitions) from low-energy orbitals in the ground state to high energy orbitals in an excited state. The highest energy separation is observed when electrons in sigma bonds are excited, giving rise to absorption in the 120-200nm region. Above 200nm, however, excitations of electrons from p- and d- orbitals and particularly π conjugated systems give rise to readily measurable and informative spectra (16). The region extending from 200 to 380nm is called near ultraviolet region. The region below 200nm is called the far or vacuum ultraviolet region.

The transition moment, or dipole moment of transition, is proportional to the change in the electronic charge distribution occurring during excitation. Intense absorption occurs when a transition is accompanied by a large change in the transition moment. The intensity of absorption may be expressed as transmittance give by \( T = I/I_0 \) where \( I \) is the intensity of radiation emerging from the sample and \( I_0 \) is the intensity of radiation energy striking the sample. The expression of absorption intensity can also be derived from the Beer-Lambert law that states a relationship between transmittance, sample thickness and concentration of the absorbing species. The concentration of the absorbing species is quantitatively determined using the Beer-Lambert law (17).

2.4.1 Instrumentation

The basic parts of a spectrophotometer are a light source of incandescent bulb (for the visible wavelengths), or a deuterium arc lamp, a sample holder, a
diffraction grating or monochromator to separate the different wavelengths of light and a detector. The detector is typically a photo diode or a CCD. Photodiodes are used with monochromators which filter the light so that only light of a single wavelength reaches the detector. Diffraction gratings are used with CCDs, which collects light of different wavelengths on different pixels.

A UV-Visible spectrophotometer can be either single beam or double beam. In a single beam instrument all of the light passes through the sample cell. It must be measured by removing the sample. In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference and the other beam passes through the sample. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beams are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam. The optical path diagram is shown in Fig. 2.6.

The samples for UV-Visible spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1cm which is treated as the path length in Beer-Lambert procedure. The best cuvettes are made of high quality quartz and have windows that are perfectly
Fig. 2.5 Model of SHIMADZU UV-Visible spectrophotometer

Fig. 2.6 Optical path diagram of UV-Visible spectrophotometer
normal to the direction of the beam inorder to minimize reflection loss. The entrance and exit sides of the cells are made plane parallel surfaces. A photomultiplier tube receives the radiation from the sample and the reference cells and produces photo-current proportional to the incident radiation. For this purpose, certain solid state detectors are used. The entire sequence is controlled and monitored by interfacing the device with a microprocessor or a computer (18).

In the present investigation the UV-Visible spectral measurement was made using Shimadzu UV-1601 double beam UV-Visible spectrometer shown in Fig 2.5. This instrument has two lamps, a tungsten lamp that covers the region from 350nm to 1100nm and a deuterium lamp that covers the region from 190nm to 350nm. The monochromator is mounted onto a highly stable optical bench to ensure integrity and trouble-free operation. A silicon photodiode is used as detector and the spectrum can be used in scanning spectrum mode or kinetics mode or quantitative analysis mode. Data acquisition and processing is controlled through the Shimadzu UV-Probe software. With the advance in automatic recording and many improvements in instrumentation, a large number of excellent UV spectrophotometers are available, that are capable of meeting all requirements of an analyst. Hence it has enormous utility in the study of drug molecules and at times, even in the analysis of biomolecules.
2.5 Chromatography

Chromatography is a technique in which solutes are resolved by differential rate of elution as they pass through a chromatographic column. Their separation is governed by their distribution between the mobile and the stationary phases. The successful use of liquid chromatography for a given problem requires the right combination of a variety of operating conditions such as the type of column packing and the mobile phase, column length and diameter, mobile phase flow rate, column temperature, and sample size. Chromatography is now the premiere method for the separation of closely related chemical species. In addition, it is used for qualitative identification and quantitative determination of separated species (19).

2.5.1 High performance Liquid Chromatography

High performance liquid chromatography is one of the most widely used analytical separation techniques. It is a widely accepted separation technique for both sample analysis and purification in a variety of areas including the pharmaceutical, biotechnological, environmental, polymer, and food industries. In this method the stationary phase is contained in a column, one end of which is attached to source of pressurized liquid eluent termed as mobile phase. The sample to be analyzed is introduced in a small volume to the stream of mobile phase and is retarded by the stationary phase as it traverses the length of the column. This method termed isocratic high-performance liquid chromatography
has undergone a further refinement by varying the mobile phase composition during the analysis and is known a gradient elution. HPLC is enjoying a steady increase in numbers of both instrumental sales and publications that describe new and innovative applications (20). The reasons for the popularity of the method are its sensitivity, ready adaptability to accurate quantitative determination, solubility for separating nonvolatile species or thermally fragile ones and its applicability to substances that are of prime interest to industry and many fields of science. Some recent growth areas include miniaturization of HPLC systems, analysis of nucleic acids, intact proteins and protein digests, analysis of carbohydrates and chiral analysis.

2.5.2 Reverse-Phase High Performance Liquid Chromatography

The reverse-phase HPLC (RP-HPLC) uses a hydrophobic packing, usually with an octadecyl(C-18) or octyl (C-8) functional group for the stationary phase. In RP-HPLC the driving force for retention is not the favorable interaction of solute with the stationary phase unlike normal-phase chromatography, but the effect of the mobile phase solvent in forcing the solute into hydrocarbonaceous stationary phase. Non-polar solutes are squeezed out of the mobile phase but bind with the hydrocarbon moieties of the stationary phase. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Rather, the
elution order of the classes of compounds in the normal-phase is reversed and hence the name reverse-phase chromatography (21).

2.5.3 Instrumentation

HPLC instrumentation is made up of eight basic components: mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing, and a computer or integrator. Fig 2.8 is a schematic diagram showing the important components of a typical high performance liquid chromatograph.

Mobile Phase Reservoir

The mobile phase reservoir can be any clean, inert container such as an empty solvent jug, a laboratory flask or a commercial reservoir. It contains 1 or 2 L of solvent and has a cap that allows the tubing inlet line to pass through. It is important to degas solvent before use to prevent small gas bubbles present in the mobile phase collecting in other components. When preparing the mobile phase it is important to use highly purified buffer salts and reagents, preferably HPLC grade.

Solvent Delivery Systems

The purpose of the pump or solvent delivery system is to ensure the delivery of a precise, reproducible, constant, and pulse-free flow of mobile phase. There are two classes of HPLC pumps: constant pressure pumps and constant flow pumps, with the latter being by far the most common. Most
Fig 2.7 Model of SHIMADZU VP Series HPLC

Fig 2.8 Schematic diagram of High Performance Liquid Chromatogram
separations can be done using isocratic elution, which is the use of single-solvent system that does not change during the analysis. For more complex analyses, gradient elution is required. Gradient elution is done by gradually strengthening the mobile phase composition thought-out the separation. The highly retained compounds are eluted more quickly and the compounds that are eluted earlier remain well resolved.

*Column*

The column is the heart of the HPLC instrument because the separation occurs here. It is generally made of 316-grade stainless steel, which is relatively inert to chemical corrosion and is packaged with the desired stationary phase. Common dimensions for analytical scale columns are in the range of 10 to 25m long and 3 to 9mm inner diameter.

*Detectors*

The important role of the HPLC detector is to monitor the solutes as they are eluted from the column. The detector generates an electrical signal that is proportional to the level of some property of the mobile phase or solutes. A detector that measures a property of both the solute and mobile phase, such as a refractive index detector, is a bulk property detector. Likewise, a detector that measures a property of the solute only, such as a UV detector, is a solute property detector and is significantly more sensitive. Some characteristic features pertaining to a good HPLC detector are sensitivity, linearity,
predictability in response, reliability, nondestructiveness, ease of use and low dead volume.

*Connective tubing*

It should be made of a material that is inert to the solvents in the mobile phase. It is made of stainless steel or inert plastic. The connections between the tubing and the different components in the system are fitted with unions that are designed to minimize dead volume. The inner diameter of the tubing should be kept to a minimum in order to reduce the effect of band broadening.

*Computer or Integrator*

It is a data collection device connected to the detector which takes the electronic signal produced by the detector and plots it as a chromatogram, which can be evaluated by the user. Both integrators and computers can integrate the peaks in the chromatograms, and computers have the further advantage that they electronically save chromatograms for later evaluation (22).

Reverse phase high performance liquid chromatographic methods for the assay of the various chosen pharmaceutical formulations in this study have been carried out with Shimadzu HPLC VP series system shown in Fig.2.8. This is equipped with single LC-20AT pump and uses a Phenomenex C-18 110A column of dimension (250 x 4.6 mm with particle size 5μm) as the stationary phase. The mobile phase which depends on the nature of the drug, is filtered through a 0.45μm membrane filter and degassed. The solvent delivery method
uses a series-type double plunger than can withstand maximum discharge pressure of 40MPa. A variable programmable UV-Visible detector SPD-20A with photo diode array detector detects the output signal. It operates in the wavelength region 190 to 700nm, with wavelength accuracy of 1nm. The HPLC system is equipped with the spinchrome software to acquire, store and analyze the data.
References


