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FOOD AND DRUG ADMINISTRATION

CERTIFICATE

This is to certify that work described in this thesis entitled "ANALYSIS OF VARIOUS FOOD AND DRUGS SAMPLE BY USING UV-VISIBLE SPECTROMETRIC, RP-HPLC, GC AND HPTLC METHOD" has been carried out by (Ms. Honey Sanjay Dumralia) under my supervision. I certify that this is her bonafide work. The work described is original and has not been submitted for any degree to this or any other University.

Date : 12th May 2021

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This is to certify that, **Miss. Honey Dumralia** of S.H.P.T. College, S.N.D.T. University – M.Sc. Part-II has successfully completed internship project in Analytical chemistry at "**FOOD AND DRUG ADMINISTRATION MAHARASHTRA**" under the guidance of **HOD PROF. JAYA GADE AND PROF. SULEKHA GOTMARE** in partial fulfilment for M.Sc. Analytical chemistry Program during the year 2020-2021.

This project is absolutely genuine and does not indulge in plagiarism of any kind.

TEACHAER IN-CHARGE HEAD OF THE DEPARTMENT (ANALYTICAL CHEMISTRY)

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CONTENTS

Sr.No	Text abbreviations	
1	PTC	Pharmaceutical Technology Centre
2	RP-HPLC	Reversed Phase High Performance Liquid Chromatography
3	HPLC	High Performance Liquid Chromatography
4	HPTLC	High Performance Thin Layer Chromatography
5	TLC	Thin Layer Chromatography
6	SOP	Standard Operating Procedure
7	STP	Standard Testing Protocol
8	USP	United States Pharmacopoeia
9	BP	British Pharmacopoeia
10	WS	Working Standard
11	RT	Retention Time
12	MP	Mobile Phase
13	RSD	Relative Standard Deviation
14	SD	Standard Deviation
15	AVG	Average
16	ND	Not Detected
17	SPL	Sample
18	STD	Standard
19	NMT	Not More Than
20	NLT	Not Less Than
21	LOD	Limit of Detection
22	LOQ	Limit of Quantitation
23	GLP	Good Lab Practice
24	GMP	Good Manufacturing Practice
25	FDA	Food and Drug Administration
26	API	Active Pharmaceutical Ingredient
27	FSSAI	Food Safety and Standards Authority of India

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ABSTRACT

An accurate and precise versatile spectrophotometric instrument was used for identification of food and drugs products for that various method were follow. As instrument was used as UV-Spectrophotometric, RP-HPLC, HPTLC, GC, Dissolution, Disintegration, are type for instrument was used for Drug analysis method. Drugs were study of Luliconazole, Tinidazole and more in UV-Visible Spectroscopy for to identify the content or percentage should present guideline under given Pharmacopoeia. For RH-HPLC Drugs are were used to calculated present of content and percentage of tablet, syrup and more. GC Chromatography was carried out for analyzed for mostly volatile component as hand sanitizer which content ethyl alcohol 70% and other impurities such like methanol and acetone were content. In HPTLC method was to identify present of impurities or adulteration in ayuvedic cough syrup in such as steroids. Food analysis are used in identify the present of fat and moisture content in paneer/channa milk products. Dissolution of drugs molecule were carried out for disintegration of liquid medium under the experimental condition.

Introduction

1.1 Importance of Analytical Methods:

Quality is significant in every product or service, but it is important in medicine as it involves life. Unlike other consumer goods, there can't be no subsequent quality. Therefore analytical methods which are a degree of quality of the drugs play a very vital role in drug development and routine analysis to assure that a drug product meets the established standard and that it is stable and will continue to meet the required quality throughout its shelf life.
These methods should be specific and subtle to monitor the known and unknown impurities. The method has to be documented in such a format that they can be produced over a period of time from laboratory to laboratory, i.e. these methods should be validated.

1.2 Introduction to Analytical Methods:

Analytical methods are defined as the set of techniques that allow us to know qualitatively and /or quantitatively the composition of any material and chemical state in which it is located. Analytical methods are required to characterize drug substance and drug product composition during all phases of pharmaceutical development. Early phase methods must support changes in synthetic routes and dosage form and interpret the structures and levels of impurities. In later phases, the goals change to the development of rugged and robust methods for release and stability estimation.
Analysis includes a wide range of simple and instrumental analytical methods, but the most widely used analytical methods for quality assurance are spectroscopy and chromatography based. Most quantitative analysis require measuring analytes, impurities and/or preservatives in the presence of drug product and /or drug substances. Therefore, separation or resolution of the components are required prior quantitative analysis. In such cases chromatographic techniques are used for quantitative analysis.
In cases where interference of active pharmaceutical drug and matrix is not observed, quantitative measurements methods are directly developed using spectroscopic or classical titration. For analytical methods based on UV-Visible Spectroscopy, Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Gas Chromatography (GC), HPTLC and have been developed.

1.3 Ultraviolet and Visible Absorption Spectrophotometry:

Ultraviolet and visible absorption spectrophotometry is the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of 185 nm to 380 nm, and 380 nm to 780 nm of the spectrum, respectively.
The magnitude of the absorption of a solution is expressed in terms of the absorbance, A, defined as the logarithm to base 10 of the reciprocal of transmittance (T) for monochromatic radiation:

$$A = \log_{10} (I_0/I)$$

where 10 is the intensity of the incident radiation I is the intensity of the transmitted radiation.
The absorbance depends on the concentration of the absorbing substance in the solution and the thickness ·of the absorbing layer taken for measurement.
For convenience of reference and for ease in calculations, the specific absorbance of a 1 per cent w/v solution is adopted in this Pharmacopoeia for several substances unless otherwise indicated, and it refers to the absorbance of a 1 per cent w/v solution in a 1 cm cell and measured at a defined wavelength. It is evaluated by the expression

$$A (1 \text{ percent}, 1 \text{ cm}) = A/cl,$$

where c is the concentration of the absorbing substance expressed as percentage w/v and I is the thickness of the absorbing layer in cm. The value of A (l per cent, 1 cm) at a particular wavelength in a given solvent is a property of the absorbing substance.
Unless otherwise stated, measure the absorbance at the prescribed wavelength using a path length of 1 cm and at 240 to 26°. Unless otherwise stated, the measurements are carried out with reference to the same solvent or the same mixture of solvents.

Apparatus:

A spectrophotometer, suitable for measuring in the ultraviolet and visible ranges of the spectrum consists of an optical system capable of producing monochromatic light in the range of 200 nm to 800 nm and a device suitable for measuring the absorbance.
The two empty cells used for the solutions under examination and the reference liquid must have the same spectral characteristics. Where double-beam-recording instruments are used, the solvent cell is placed in the reference beam.

Control of wavelengths:

Verify the wavelength scale using the absorption maxima of holmium perchlorate solution, the line of hydrogen or deuterium discharge lamp or the lines of a mercury vapour are shown below. The permitted tolerance is ± 1 nm for the range 200 nm to 400 nm and ± 3 nm for the range 400 nm to 600 nm

Control of absorbance:

Check the absorbance using suitable filters or a solution of potassium dichromate UV at the wavelengths indicated in Table 1, which gives for each wavelength the exact values and permitted limits of the specific absorbance. The tolerance for the absorbance is ± 0.01.Use solutions of potassium dichromate UV which has been previously dried to constant weight at 130°. For the control of absorbance at 235 urn, 257 nm, 313 urn and 350 urn, dissolve 57.0-63.0 mg of potassium dichromate UV in 0.005 M sulphuric acid and dilute to 1000.0 rn1 with the same acid. For the control of absorbance at 430 urn, dissolve 57.0-63.0 mg of potassium dichromate UV in 0.005 M sulphuric acid and dilute to 100.0 rn1 with the same acid.

Limit of stray light:

Stray light may be detected at a given wavelength with suitable filters or solutions; for example, absorbance of a 1.2 per cent w/v solution of potassium chloride in a 1 cm cell should be greater than 2.0 at about 200 nm when compared with water as reference liquid.

Resolution power:

When stated in a monograph, record the spectrum of 0.02 per cent v/v solution of toluene in hexane UV. The ratio of the absorbance at the minimum at about 269 nm to that at the maximum at about 266 nm is not less than 1.5 unless otherwise specified in the monograph.

Spectral slit width:

When measuring the absorbance at an absorption maximum the spectral slit width must be small compared with the halfwidth of the absorption band otherwise erroneously low absorbance will be measured. Particular care is needed for certain substances and the instrumental slit width used should always be such that further reduction does not result in an increased absorbance reading.

Cells:

The absorbances of the cells intended to contain the solution under examination and the reference liquid, when filled with the same solvent should be identical. If this is not the case, an appropriate correction must be applied. The tolerance on the path length of the cells used is ± 0.005 cm. Cells should be cleaned and handled with great care.

Solvents:

In measuring the absorbance of a solution at a given wavelength, the absorbance of the reference cell and its contents should not exceed 0.4 and should preferably be less than 0.2 when measured the reference to air at the same wavelength. The solvent in the reference cell should be of the same lot as that used to prepare the solution and must be free from fluorescence at the wavelength of measurement. Ethanol (95 per cent), ethanol, methanol, and cyclohexane, used as solvents, should have an absorbance measured in a 1 cm cell at about 254 nm with reference to water not exceeding 0.10.

Determination of absorbance:

Unless otherwise directed, measure the absorbance at the prescribed wavelength using a path length of 1 cm at 24° to 26°·If necessary, the path length may be varied provided that compliance with Beer's Law has been shown over the range in question. A statement in a assay or test of the wavelength at which maximum absorption occurs implies that the maximum occurs either precisely at or within ± 2 run of the given wavelength. Likewise, a statement in a test of the absorbance, A, at a given wavelength or at the maximum at about a specified wavelength implies that the measured absorbance is within ± 3 per cent of the stated value.
When an assay or test prescribes the use of a Reference Substance, make the spectrophotometric measurements with the solution prepared from the Reference Substance by the official directions and then with the corresponding solution, prepared from the substance under examination. Carry out the second measurement as quickly as possible after the first, using the same cell and same experimental conditions.
Unless otherwise specified, the requirements in the monographs for light absorption in the tests and assays apply to the dried or anhydrous material, where a standard is given for loss on drying of content of water, respectively. Similar considerations apply where standards are given for solvent content. In calculating the result, the loss on drying or contents of water or solvent, determined by the method specified in the monograph, are taken in to account.

Derivative spectrophotometry

Derivative spectrophotometry involves the transformation of absorption spectra (zero-order) into first-, second- or higher-order derivative spectra.
A first order-derivative spectrum is a plot of the gradient of the absorption curve (rate of change of the absorbance with wavelength, $dA/d\lambda$) against wavelength.
A second order-derivative spectrum is a plot of the curvature of the absorption spectrum ($d^2A/d\lambda^2$) against wavelength. If the absorbance follows the Beer- Lambert relationship, the second order-derivative at any wavelength λ is related to the concentration by the following expression.

$$d^2A/d\lambda^2 = d^2A\ (1\ \text{per cent}, 1\text{cm}) \times cd/d\lambda^2$$

where, A = the absorbance at wavelength λ,
A (l per cent, 1 cm) = the specific absorbance at wavelength λ
c = the concentration of the absorbing solute express as a percentage w/v,
d = the thickness of the absorbing layer in cm.

Apparatus:

A spectrophotometer complying with the requirements for Control of wavelengths and Control of absorbances described above and-equipped-with-an-analog resistance-capacitance differentiation module or a digital differentiator or another means of producing second order-derivative spectra should be used in accordance with the manufacturer's instructions. Some methods of producing second order-derivative spectra lead to a wavelength shift relative to the zero-order spectrum and this should be considered when necessary. Unless otherwise stated in the monograph, the spectral slit width of the spectrophotometer, where variable, should be set as described under Spectral slit width above. The cells and solvents used should comply with the statements given under Cells and Solvents respectively.

Reagents.

The temperatures of all solutions used in the test should not differ by more than OS.

Resolution.

When stated in a monograph, record the second order-derivative spectrum in the range 255 nm to 275 nm of a0.020 per cent v/v solution of toluene and in methanol using methanol in the reference cell. A small negative extremum (or trough) located between two large negative extrema (or troughs) at about 261 nm and 268 nm should be clearly visible.

Procedure.

Prepare the solution of the substance under examination, adjust the various instrument settings as stated in the manufacturer's instructions and calculate the amount of the substance under examination as stated in the monograph.

1.4. Disintegration Test

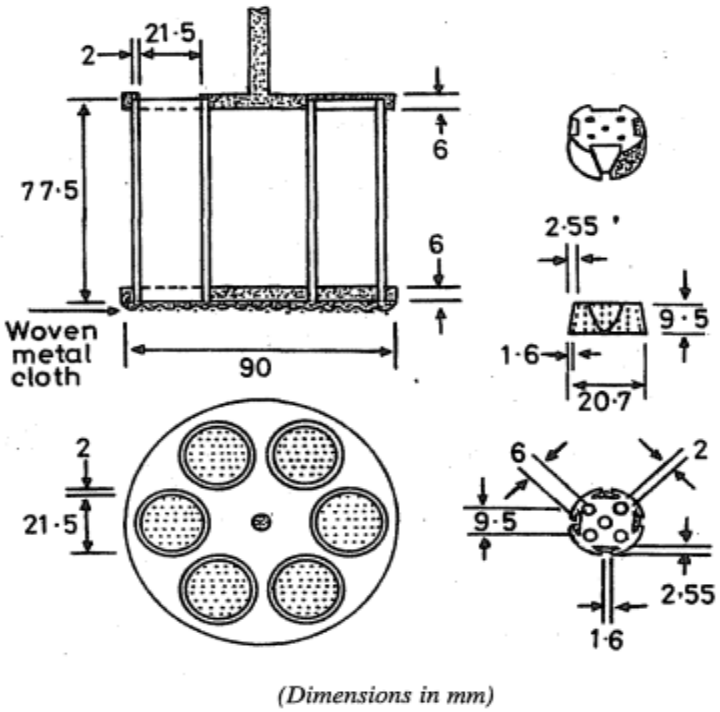
This test determines whether dosage forms such as tablets, capsules, boluses pessaries and suppositories disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental conditions.

For the purpose of this test, disintegration does not imply complete solution of the dosage unit or even of its active constituent. Disintegration is defined as that state in which no residue of the unit under test remains on the screen of the apparatus or, if a residue remains, it consists of fragments of disintegrated parts of tablets component parts such as insoluble coating of the tablets or of capsule shells, or of any melted fatty substance from the pessary or suppository or is a soft mass with no palpable core. If discs have been used with capsules, any residue remaining on the lower surfaces of the discs consists only of fragments of shells.

For tablets and capsules

Apparatus

The apparatus consists of a basket-rack assembly, a 1-litre beaker, a thermostatic arrangement for heating the fluid and a mechanical device for raising and lowering the basket in the immersion fluid at a constant frequency rate.



(Dimensions in mm)

Fig 1.17. Apparatus for Disintegration of Tablets and Capsules

Basket-rack assembly. The basket-rack assembly is rigid and supports six cylindrical glass tubes, 77.5 ± 2.5 mm long, 21.5 mm in internal diameter and with a wall thickness of about 2 mm (Fig. 2.5.1-1). The tubes are held vertically by two superimposed transparent plastic plates, 90 ± 2 mm in diameter and 6.75 ± 1.75 mm thick perforated by six holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to the underside of the lower plate is a woven stainless-steel wire cloth with a plain square weave with 2.0 ± 0.2 mm mesh apertures and with a wire diameter of 0.615 ± 0.045 mm. The upper plate is covered with a stainless-steel disc perforated by six holes, each about 24 ± 2 mm in diameter, which fits over the tubes and holds them between the plastic plates. The holes coincide with those of the upper plastic plate and the upper open ends of the glass tubes. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis.

The plates are held rigidly in position and 77.5 mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to the device for raising and lowering it smoothly at a constant frequency of between 28 and 32

cycles per minute through a distance of 50 to 60 trim. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction should be smooth and not abrupt. There should be no appreciable horizontal
motion or movement of the axis from the vertical.
The design of the basket-rack assembly may be somewhat different provided specifications for the glass tubes and the screen mesh size are unchanged.

Discs. A cylindrical disc for each tube, each 20.7 ± 0.15 mm thick in diameter and 9.5 ± 0.15 mm thick, made of transparent. plastic with a relative density of 1.18 to 1.20, and pierced with five holes, each 2 mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6 mm from the centre of the disc. Four equally-spaced grooves are cut in the
lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5 mm wide and 2.55 mm deep and at the lower surface 1.6 mm square.

Medium. The assembly is suspended in the liquid medium in a suitable vessel, preferably a I-litre beaker. The volume of liquid is such that the wire mesh at its highest point is at least
25 mm below the surface of the liquid, and at its lower point is at least 25 mm above the bottom of the beaker. At no time should the top of the basket-rack assembly become submerged. There is a thermostatic arrangement for heating the liquid and maintaining the temperature at 37° ± 2°.

Method. Unless otherwise stated in the individual monograph, introduce one tablet or capsule into each tube and, if directed in the appropriate general monograph, add a disc to each tube. Suspend the assembly in the beaker containing the specified liquid and operate the apparatus for the specified time. Remove the assembly from the liquid. The tablets or capsules pass the test if all of them have disintegrated.

If 1 or 2 tablets or capsules fail to disintegrate, repeat the test on 12 additional tablets or capsules; not less than 16 of the totals of 18 tablets or capsules tested disintegrate.
If the tablets or capsules adhere to the disc and the preparation under examination fails to comply, repeat the test omitting the disc. The preparation complies with the test if all the tablets or capsules in the repeat test disintegrate.

Uncoated Tablets

Uncoated tablets may be single-layer tablets resulting from a single compression of particles or multi-layer tablets consisting of parallel layers obtained by successive compression of particles of different compositions. No treatment is applied to such tablets after compression. Any added substances are not specifically intended to modify the release of their active ingredient(s) in the digestive fluids.
uncoated tablet is examined under a lens, either a relatively
uniform texture (single-layer tablets) or a stratified structure
(multi-layer tablets) is seen; there are no signs of coating.
Tests
Disintegration. Use water as the liquid. Add a disc to each tube. Operate the apparatus for 15 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets omitting the discs, The tablets comply with the test if all 6

tablets have disintegrated.

Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as resins, gums, inactive and insoluble filters, sugars, plasticisers, polyhydric alcohols waxes, etc. The coating may also contain medicaments. In compression coated tablets the coating is applied by compressing around the tablets granules prepared from tablet excipients such as lactose, calcium phosphate, etc. Substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is thin, the tablets are described as film-coated.

Use water as the liquid. Add a disc to each tube. Operate the apparatus for 60 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets, If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing water with 0.1.M hydrochloric acid. The tablets. comply with the test if all 6 tablets have disintegrated in the acid medium.

Film-coated Tablet

Carry out the test described above but operate the apparatus the for 30 minutes, unless otherwise stated m the individual monograph.

If coated tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets omitting the discs. The tablets comply with the test if all 6 tablets have disintegrated.

Dispersible Tablets

Dispersible tablets are uncoated or film-coated tablets that produce a uniform dispersion in water and may contain permitted flavouring and sweetening agents. However, if saccharin, including its sodium and potassium salts, is used a sweetening agent, its concentration in dispersible tablets meant for paediatric use should be restricted so as to limit its intake to 5 mg/kg of bodyweight.

1.5. Dissolution Test

This test is designed to determine compliance with the dissolution requirements for solid dosage forms administered orally. The test is intended for a capsule or tablet.

Use Apparatus 1 unless otherwise directed. All parts of the apparatus that may come into contact with the preparation under examination or with the dissolution medium are chemically inert and do not adsorb, react or interfere with the preparation under examination. All metal parts of the apparatus that may come into contact with the preparation or the dissolution medium must be made from stainless steel, type 316 or equivalent or coated with a suitable material to ensure that such parts do not react or interfere with the preparation under examination or the dissolution medium.

No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation or vibration beyond that due to the smoothly rotating element.

An apparatus that permits observation of the preparation under examination and the stirrer during the test is preferable.

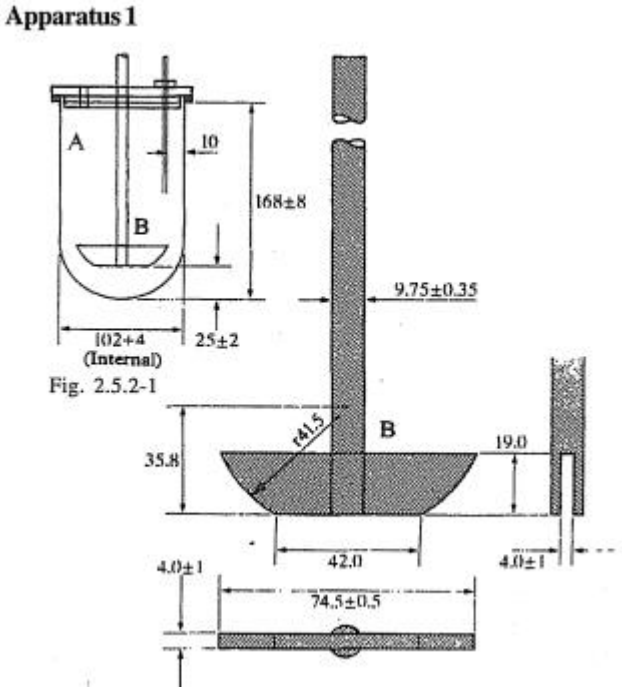


Fig 1.18. Apparatus for Paddle blade

An assembly consisting of the following:

a. A cylindrical vessel, A, made of borosilicate glass or any other suitable transparent material, with a hemispherical bottom and with a nominal capacity of 1000ml and an inside diameter of 98-106 mm (Fig.2.5.2-1). The vessel has a flanged upper rim and is fitted with a lid that has a number of openings, one of which is central.

b. A motor with a speed regulator capable of maintaining the speed of rotation of the paddle within 4 per cent of that specified in the individual monograph. The motor is fitted with a stirring element which consists of a drive shaft and blade forming a paddle, B (Fig.2.5.2-2).

The blade passes through the diameter of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The shaft is positioned so that its axis is within 2 mm of the axis of the vessel and the lower edge of the blade is 23 to 27 mm from the inside bottom of the vessel. The apparatus operates in such a way that the paddle rotates smoothly and without significant wobble.

c. A water-bath set to maintain the dissolution medium at 36.5° to 37.5°. The bath liquid is kept in constant and smooth motion during the test. The vessel is securely clamped in the water- bath in such a way that the displacement vibration from other equipment, including the water circulation device, is minimised.

The assembly is the same as in Apparatus 1 except that in the stirring element, the paddle is replaced by a basket, D (see Figs. 2.5.2-3 and 2.5.2-4). The metallic shaft rotates smoothly and without significant wobble. The basket consists of two components. The top part, with a

vent, is attached to the shaft C, it is fitted with three spring clips, or other suitable means, that allow removal of the lower part for introduction of the preparation under examination and that firmly hold the lower part of the basket concentric with the axis of the vessel during rotation. The lower detachable part of the basket is made of welded-steam cloth, with a wire thickness of0.254 mm diameter and with 0.381mm square openings, formed into a cylinder with narrow rim of sheet metal around the top and the bottom. The basket may be plated with a 2.5 mm layer of gold for use with acidic media. The distance between the inside bottom of the vessel and the basket is maintained at 23 to 27 mm during the test.

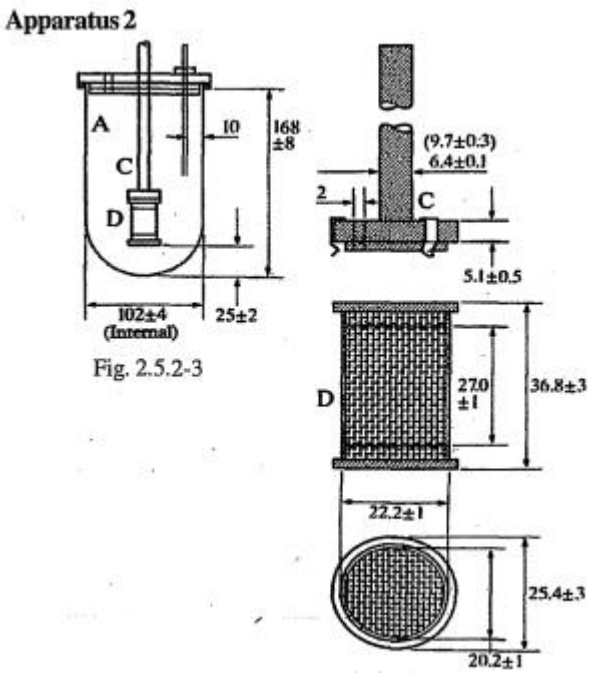


Fig. 2.5.2-3

Figure 1.19. Apparatus for Basket

Dissolution medium.

Use the dissolution medium specified in the individual monograph.
If the medium is a buffered solution, adjust the solution so that its pH is within 0.05 units of the pH specified in the monograph. The dissolution medium should be deaerated prior to testing.

Time.

Where a single time specification is given in the monograph, the test may be concluded in a shorter period if the requirement for the minimum amount dissolved is met. If two or more times are specified, specimen are to be withdrawn only at the stated times, within a tolerance of ± 2 per cent.

Method

Conventional and prolonged-release solid dosage forms

Place the stated volume of the dissolution medium, free from dissolved air, into the vessel of the apparatus. Assemble the apparatus and warm the dissolution medium to 36.5° to 37.so. Unless otherwise stated, place one dosage unit in the apparatus; taking care to exclude air bubbles from the surface of the dosage unit. When Apparatus I is used, allow the tablet or capsule to sink to the bottom of the vessel prior to the rotation of the paddle. A suitable device such as a wire of glass helix may be used to keep horizontal at the bottom of the vessel tablets or capsules that would otherwise float. When Apparatus 2 is used, place the tablet or capsule in a dry basket at the beginning of each test. Lower the basket into position before rotation.
Operate the apparatus immediately at the speed of rotation specified in the individual monograph. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the dissolution medium and the top of the rotating blade or basket, not less than 10 mm from the wall of the vessel. Except in the case of single sampling, add a volume of dissolution medium equal to the volume of the samples

withdrawn. Filter the sample solution promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Discard the first few ml of the filtrate. Perform the analysis as directed in the individual monograph. Repeat the whole operation five times. Where two or more tablets or capsules are directed to be placed together in the apparatus, carry out six replicate tests.
For each of the tablet or capsule tested, calculate the amount of dissolved active ingredient in solution as a percentage of the stated amount where two or more tablets or capsules are placed together, determine for each test the amount of active ingredient in solution per tablet or capsules and calculate as a percentage of the stated amount.

Acceptance criteria

Conventional-release dosage forms

Unless otherwise specified, the requirements are met if the quantities of active substance dissolved from the dosage units conform to Table. If the results do not conform to the requirements at stage S_1 given in the table, continue testing with additional dosage units through stages S_2 and S_3 unless the results conform at stage S_2.

Where capsule shells interfere with the analysis, remove the contents of not less than 6 capsules as completely as possible, and dissolve the empty capsule shells in the specified volume of the dissolution medium. Perform the analysis as directed in the individual monograph. Make any necessary correction. Correction factors should not be greater than 25 per cent of the stated amount.

Table 1.5 of Acceptance criteria for dissolution

Prolonged-release dosage forms
Unless otherwise specified, the requirements are met if the quantities of active substance dissolved from the dosage units conform to Table 2. If the results do not conform to the requirements at stage L_1 given in the table, continue testing with additional dosage units through stages L_2 and L_3 unless the results conform at stage L_2. The limits embrace each value of D, the amount dissolved at each specified dosing interval.
Where more than one range is specified, the acceptance criteria apply to each range.

Table 1

Level	Number tested	Acceptance criteria
S_1	6	Each unit is not less than D* + 5 per cent**.
S_2	6	Average of 12 units (S_1 +S_2) is equal to or greater than D, and no unit is less than D – 15 per cent**.
S_3	12	Average of 24 units (S_1+S_2+S_3) is equal to or greater than D, not, More than 2 units are less than D – 15 per cent** and no unit is less than D – 25 per cent**.

*D is the amount of dissolved active ingredient specified in the individual monograph, expressed as a percentage of the labelled content.

**Percentages of the labelled content.

1.6. Gas Chromatography

Gas Chromatography (GC), also known as Gas Liquid Chromatography (GLC), is a technique for separation of mixtures into components by a process which depends on the redistribution of the components between a stationary phase or support material in the form of a liquid, solid or combination of both and a gaseous mobile phase. It is applicable to substances or their derivatives which are volatilized under the temperatures employed. GC is based on mechanisms of adsorption, mass distribution or size exclusion.

Apparatus

The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and a data acquisition system. The carrier gas flows through the column at a controlled rate or pressure and then through the detector.

Injectors.

Direct injections of solutions are the usual mode of sample introduction unless otherwise prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporization chamber which may be equipped with a stream splitter.
Injections of vapour phase may also be effected by static or dynamic head space injection systems~ Dynamic head space (purge and trap) injection system include a sparging device by which volatile substances from a solution are swept into an adsorbent column maintained a low temperature. Retained substances are then desorbed into the mobile phase by rapid heating of the adsorbent column.
Static head space injection systems include thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow the volatile components of the sample to reach equilibrium between the non-gaseous phase and the vapour phase. After equilibrium has been established, a pre- determined quantity of the head space vapour from the vial is automatically introduced into the gas chromatograph either by a heated syringe or a transfer line and sample loop.

Stationary Phases.

Stationary phases are contained inside the column. A GC column can be:
A) a fused silica capillary column whose wall is coated with the stationary phase.
B) a metallic or glass column packed with inert particles impregnated with the stationary phase
C) a metallic or glass column packed with solid stationary phase.
A wide range of chemical substances are used as stationary phase in GC. These include polyethylene glycols, high molecular weight esters and amides, hydrocarbons, silicon gums and fluids (polysiloxanes substituted with methyl, phenyl, cyano, vinyl or fluroalkyl groups or mixtures of these) and solid adsorbents like micro porous cross-linked polyaromatic beads, molecular sieves etc.
Capillary columns can be of 0.1 mm to 0.53 mm in internal diameter and 10 meter to 100 meter in length. The liquid or solid stationary phase may be chemically bonded to the inner surface of the tubing with a coating thickness of 0.1 micron to 5.0 microns.

Packed columns, made of glass or metal, are usually 1 meter to 3 meter in length with an internal diameter of2 mm to 4 mm. Support materials must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanising prior to coating with liquid phase. Acid washed, flux-calcinated diatomaceous earth is often used as support material. The support materials are available in various particle sizes, the most commonly used ones in the range of 100 to 120 mesh.

Mobile phases.

Mobile phases that are employed in GC are inert gases. The commonly used gases are Nitrogen, Hydrogen and Helium. The source of carrier gas can be a pressurized cylinder or a gas generator which can provide a continuous flow of the gas. The purity of the gas should be minimum 99.99 per cent. The gas should pass through a purification panel having suitable filters for the removal of residual moisture, oxygen and hydrocarbons before entering the GC. The pressure and flow rate of. the carrier gas should be adjusted to get optimum separation of sample components.

Detectors.

Flame ionization detectors are used, unless otherwise mentioned. Additional detectors which may be used include: thermal conductivity, electron capture, nitrogen- phosphorus, flame photometric and mass spectrometric depending upon the purpose of the analysis.

Method.

Equilibrate the column, the injector and the detector (flame ionisation, unless otherwise stated in the individual monograph) at the specified temperatures and flow rates until a stable base line is obtained. Prepare the test and reference solutions as prescribed in the monograph. The solutions must be free from solid particles. Using the solution of the reference substance determine experimentally suitable instrument settings and volumes of solutions to be injected to produce an adequate response. Inject the selected volumes of the solutions prescribed in the monograph and record the resulting chromatograms. Repeat the determinations to ensure a consistent response. Determine the peak areas or peak heights corresponding to the peaks of interest. In determinations requiring temperature programming, peak areas should be considered. From the values obtained, calculate the content of the components being determined.

Normalization.

Where reference is made to normalization for the estimation of one or more components, the total area of the peak or peaks due to the components is expressed as a percentage of the sum of the areas of all the peaks derived from the substance being examined.

Internal Standard.

Where reference is made to internal standard method for the estimation of one or more components, a suitable internal standard should be selected for the purpose. The selected internal standard should not contain any impurity that is likely to interfere in the determination described in the monograph.

Performance Resolution.

Unless otherwise stated in the monograph, the Resolution factor, Rs, between measured peaks on the chromatogram must be greater than 1.0 and is defined by the expression:

$$Rs = 1.18(t_{r2} - t_{r1}) / W_{h1} + W_{h2}$$

t_{r1} and t_{r2} = retention times or distances, along the baseline from the point of injection to the perpendiculars dropped from the maxima of 2 adjacent peaks. $t_{r2} > t_{r1}$

W_{hl} and W_{h2} = peak widths at half height.

A resolution greater than 1.5 corresponds to baseline separation. The values of $t_{r1} > t_{r2}$, W_{hl} and W_{h2} must be expressed in the same unit of measurement.

Symmetry factor. The symmetry factor or tailing factor of a peak is calculated from the expression:

$$As = w_{0.05} / 2d$$

where, $W_{0.05}$ = width of the peak at one-twentieth of the peak height,

d = distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

A value of 1.0 signifies complete or ideal symmetry. Column efficiency. When column efficiency is stated in the monograph, it is defined in terms of the number of theoretical plates, N, by the expression:

$$N=5.54(t_r/W_h)^2$$

where, tr = retention time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,

Wh = width of the peak at half height. The apparent number of theoretical plates varies with the sample component as well as with the column.

Capacity factor or mass distribution ratio, The capacity factor, k' also known as mass distribution ratio, Dm is defined as:

$$D_m = \frac{\text{amount of solute in stationary phase}}{\text{amount of solute in mobile phase}} = Kc\ (V_S/V_M)$$

where, Kc = equilibrium distribution coefficient,

Vs = volume of stationary phase,

Vm = volume of mobile phase.

The capacity factor of a component may be calculated from the chromatogram using the equation:

$$K' = \frac{t_R - t_M}{t_M}$$

where, t_R= retention time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,

t_M = time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to an unretained peak.

Relative retention.

Absolute retention time of a compound may vary depending upon column and analysis conditions. Comparisons are normally made in terms of relative retention, a, which is calculated from the expression:

$$a = \frac{t_{R2} - t_M}{t_{R1} - t_M}$$

where, t_{R2} = retention time of the peak of interest,
t_{R1} = retention time of the reference peak,
t_M = hold-up time: time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to an unretained peak.

Where the value of t_M is small, the relative retention time, Rr, may be estimated from the expression:

$$Rr = t_{R2}/t_{R1}$$

Signal to noise ratio. The signal-to- noise ratio is determined from the expression:

$$S/N = 2H/h$$

where, H = height of the peak corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half- height.

h = range of the background noise in a chromatogram obtained after injection of a blank, observed over a distance equal to 20 times the width at half-height of the peak in the chromatogram obtained with. The prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

System suitability. Unless otherwise stated in the monograph, the maximum permitted relative standard deviation for replicate injections of the prescribed reference solution does not exceed 2 per cent. This requirement applies only to assays.

Adjustment to chromatographic conditions

Adjustments may be made to the parameters of the test as given below in order to satisfy the system suitability criteria without fundamentally modifying the method.
Stationary phase,

- column length: ± 70 per cent,
- column internal diameter: ± 50 per cent,
- particle size: reduction of not more than 50 per cent, no increase,

- film thickness: - 50 per cent to + 100 per cent.

Flow rate. ± 50 per cent.
Temperature. ± 10 per cent.
Injection volume. May be decreased provided detection and repeatability are satisfactory.

Static head - space gas chromatography

Static head-space gas chromatography is a technique suitable for separating and determining volatile compounds present in non-volatile solid or liquid samples. The method is based on the analysis of the vapour phase in equilibrium with the solid or liquid phase.

Apparatus

The apparatus consists of a gas chromatograph connected to a head space sampler intended to introduce the vapour in the GC column. The sample to be analyzed is introduced into a glass container fitted with a suitable stopper. The container is placed in a thermostatically controlled chamber at a temperature set according to the substance under examination. The sample is held at this temperature long enough to allow equilibrium to be established between the solid or liquid phase and the vapour phase. After the equilibration period, a portion of the vapour phase can be transferred to the GC column either by a heated syringe or with the help of a transfer line and fixed volume loop. Using the reference preparations, determine suitable instrument settings to produce an adequate response.

1.7. Liquid Chromatography

Liquid chromatography (LC) is a separation technique based on the difference in the distribution of components between two non-miscible phases in which liquid mobile phase elutes through a stationary phase in a column. The three forms of high-performance liquid chromatography most often used are based on mechanism of partition, adsorption and ion exchange.
Ion exchange chromatography, also referred to as ion chromatography, is an analytical technique for the separation and determination of ionic solutes i.e. inorganic cations,
inorganic anions, low molecular weight (water soluble) organic acids and bases etc. The separation of ionic solutes takes place on the basis of ion exchange on stationary phases with charged functional groups. The functional groups typically are quaternary ammonium groups for anion exchange and negatively charged groups like sulphonates for cation exchange. The corresponding counter ions are located in vicinity of the functional groups and can be exchanged with other ions of the same charge in the mobile phase. Thus, various ionic components of the sample can be separated based on their differential affinities towards the immobilized stationary and the liquid mobile phase.
The combination of ion exchange columns with conductivity detection with or without chemical suppression represents the most important and popular type of ion chromatography. In the technique with chemical suppression, the conductivity of the ionic mobile phase is suppressed both chemically and electronically. In case of technique without chemical
suppression, the conductivity of mobile phase is suppressed only electronically.
In addition, size exclusion and stereochemical interaction phenomena are also used' for separation.

Apparatus

A pumping system, an injector, a chromatographic column with or without a column temperature controller, a detector and a data acquisition system (a computer, an integrator or a chart recorder) are the essential components of the equipment. For ion exchange chromatography a suppressor column is installed between main column and detector. The mobile phase is supplied from one or several reservoirs and flows through the column, usually at a constant rate, and then through the detector. Any part of the system that is in contact with the mobile phase should be constructed of materials inert to corrosive components of the mobile phase. The entire system dead volume has to be kept at the minimum. The tubing length and diameter of plumbing between the injector, column and
detector has to be kept at the minimum. Higher volumes in these connections lead to increased dispersion and tailing of peaks.

Pumping systems

The pumping systems deliver metered amounts of the mobile phase from the solvent reservoirs to the column through high- pressure tubing and fittings. Modem systems consist of one or more computer-controlled metering pumps that can be programmed to vary the ratio of mobile phase components, as is required for gradient elution chromatography, or to make an isocratic mobile phase (i.e., mobile phases having a fixed ratio of solvents). The system should be capable of delivering the mobile phase at a constant rate with minimal fluctuations over extended periods of time. Pumps may be provided with a mechanism for 'bleeding' the system of any entrapped air.

Injectors

After dissolution in the mobile phase or other suitable solvent, samples that are to be chromatographed are injected, either manually. by a syringe or by fixed-loop injectors, or automatically by autosamplers. An autosampler consists of a carousel or rack to hold sample vials with tops that have a pierceable septum or stopper and an injection device to transfer samples from the vials to a loop from which it is loaded into the chromatograph. Some autosamplers can be programmed to control sample volume, the number of injections and loop rinse cycles, the interval between injections, and other operating variables like temperature. Manual partial filling of loops is not recommended because of poorer injection volume precision.

Stationary phases and columns

The different types of stationary phases employed in liquid chromatography are:

- silica, alumina or porous graphite, used in normal-phase chromatography, wherein separation is based on the principle of adsorption and/or mass distribution,
- chemically modified silica, polymers or porous graphite, used in reversed-phase chromatography, where in separation is based principally on partition of the molecules between the mobile phase and the stationary phase,
- resins or polymers with acid or basic groups, used in ion exchange chromatography, wherein separation is based on competition of the ions to be separated with those in the mobile phase,
- porous silica or polymers, used in size-exclusion chromatography, wherein separation is based on differences between the volumes of the molecules to be separated and the corresponding steric exclusion,
- stationary phases like cellulose or amylose derivatives, proteins or peptides, cyclodextrins, etc., chemically modified, specially for chiral chromatography for separation-of enantiomers.

For most pharmaceutical analysis, separation is achieved by partitioning of compounds in the test solution between the mobile and stationary phases. Systems consisting of polar stationary phases and non-polar mobile phases are described as normal phases while the opposite arrangement (i.e., polar mobile phases and non-polar stationary phases) are called
reversed-phases. In reversed-phase chromatography utilising chemically modified silica, the silanol groups of silica on the surface of the support react with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase influences the separation properties of the chromatographic system. Commonly used bonded phases are octyl (Cs), octadecyl (CIS), phenyl (C6Hs), cyanopropyl (CN), aminopropyl (N'B:2) and diol.

Unless otherwise stated by the manufacturer, silica-based reversed-phase columns are considered to be stable in a mobile phase having an -apparent pH in the range 2.0 to 8.0. It is advisable to use a pre-column before the analytical column while using mobile phases of high pH with a silica-based column. Columns composed of porous graphite or particles of polymeric materials like styrene-divinylbenzene copolymer are stable over a wider range of pH. Making the mobile phase more or less polar controls the affinity of a compound for the stationary phase, and thus its retention time on the column.
Mobile phase polarity can be varied by the addition of a second, and sometimes a third or even a fourth, component.

For analytical separations, the particle size of the most commonly used stationary phases varies between 3 µm and 10 µm. The particles may be spherical or irregular, of varying porosity and specific surface area. These parameters contribute to the chromatographic behaviour of a particular stationary phase. In the case of reversed phases, the nature of the stationary phase, the extent of bonding, e.g., expressed as the carbon loading, and whether the stationary phase is end capped (i.e., residual silanol groups are silylated) or base deactivated are additional determining factors. When residual silanol groups are present, tailing of peaks particularly of basic substances can occur.

Base deactivation of the stationary phases is carried out by removal of basic components by careful washing and hydrolysing most of the superficial siloxane bridges.
Columns are usually made of stainless steel unless otherwise specified in the monograph. For analytical chromatography, they may be of varying length and internal diameter. Columns with internal diameters of less than 2 mm are often referred to as microbore columns.

In ion exchange chromatography, the column is made of a rigid material, usually plastic. It is generally 5 cm to 30 cm long with an internal diameter of 2 mm to 10 mm. The stationary phase is usually in the form of small diameter particles, 5 µm to 10 µm, uniformly packed into the column. The cation exchangers are usually obtained by sulphonation and anion exchangers by attaching quaternary ammonium groups to the polymeric resins.

The temperature of the mobile phase and the column must be kept constant during an analysis. Most separations are performed at ambient conditions, but columns may be heated to give higher efficiency; In order to reduce the possibility of degradation of the stationary phase or of occurrence of changes in the composition of the mobile phase, heating of the columns above 60° is not recommended. In some cases, a particular commercial brand of column that has been found suitable is mentioned, but such statements do not imply that a different but equivalent commercial brand cannot be used. It may be necessary with a particular chromatograph to modify the conditions specified in the monograph but it should be ensured that comparable results are obtained under the modified conditions.

Detectors

A detector consists of a flow through cell mounted at the end of the column and capable of detecting various types of components in the eluate. The recommended volume of the detector flow cell is 3 µl to 20 µl. Ultraviolet/visible (UV/Vis) spectrophotometers, including diode array detectors, are the most commonly employed detectors. A beam of UV radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes. The wavelength setting is specified in the individual monograph.

Fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, light scattering detectors, radioactivity detectors or other special detectors may also be used. Fluorimetric detectors are sensitive to compounds that are fluorescent or that can be converted to fluorescent derivatives. Differential refractometer detectors measure the difference between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographed compound as it emerges from the column.

Electrochemical detectors are suitable for measuring nanogram quantities of easily oxidisable compounds like phenols and catechols.
Conductivity detector is the detector of choice in ion exchange chromatography. UV-visible detectors for absorbing species, indirect UV-visible detectors, Amperometric detectors and fluorescence detectors are also employed for specific applications.

Mobile phases

In case of normal-phase chromatography, less polar solvents (e.g. hexane, dichloromethane) are employed. The presence of water or polar solvents in the mobile phase is to be strictly controlled to obtain reproducible results. In reversed-phase chromatography, aqueous mobile phases or polar solvents with or without organic modifiers are employed. Components of the mobile phase are usually filtered to remove particles greater than 0.45 µm. Multicomponent mobile phases are prepared by measuring the required volumes (unless masses are specified) of the individual components, followed by mixing. Alternatively, individual pumps controlled by proportioning valves, which mix the solvents in the desired proportion, may deliver the solvents. It is advisable to have the mobile phase solvents or solvent mixtures degassed using a vacuum pump or other suitable means that will not affect the composition of the mixture. For accurate quantitative analysis, high purity reagents and HPLC grade organic solvents must be used. Adjustment of the pH, if necessary, is effected using the aqueous component of the mobile phase. The system is flushed with a mixture of water and the organic modifier of the mobile phase (in a suitable composition) after the completion of chromatography when buffer solutions are used. On completion of the analysis, it is necessary to wash the column with appropriate solvent followed by storage in recommended solvent. During storage, both the ends of column need to be plugged properly to prevent drying of the column bed.
A counter-ion for ion-pair chromatography or a chiral selector for chromatography using an achiral stationary phase may also, be used to modify mobile phases.

Data acquisition systems

Modem data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas as well as sample identification and method variables.
Data may be collected on simple recorders for manual measurement or on stand alone integrators, which range in complexity from those providing a printout of peak areas to those providing chromatograms with peak areas and peak heights calculated and data stored for possible subsequent reprocessing.

Method

Equilibrate the column with the prescribed mobile phase and flow rate, at room temperature or at a temperature specified in the monograph, until a stable baseline is achieved. Prepare the test and standard solutions as described in the individual monograph. The solutions should be free from solid particles.
A blank injection of the mobile phase and the sample diluent should be carried out and monitored during the test to detect any interference.

Determinations

General

Normalisation procedure — The-percentage-content-of-one or more components of the substance under examination is calculated by determining the area of the peak or peaks as a percentage of the total area of all the peaks, excluding those due to solvents or any added reagents and those below the 'ignore' limit.
Internal standard procedure — Equal amounts of a component that gets resolved from the substance under examination (the internal standard) are added to the test solution and a reference solution. The Internal standard shall be one that does not react with the substance under examination, shall be stable and shall not contain impurities with a retention time similar to that of the substance under examination. The concentration of the substance under examination is determined by comparing the ratio of the peak areas or peak heights due to the substance under examination and the internal standard in the test solution with the ratio of the peak areas or peak heights due to the reference substance and the internal standard in the reference solution.
External standard procedure — The concentration of the component(s) to be analysed is determined by comparing the response(s) obtained with the test solution to the response(s) obtained with a reference solution.
Response factor — The sensitivity of the detector is the signal output per unit concentration or unit mass of a substance in the mobile phase entering the detector. The sensitivity of a detector relative to a standard substance is expressed as response factor.
Correction factor — This is the reciprocal of the response factor.
Secondary peaks — A secondary peak is a peak in the chromatogram other than the principal peak and any peaks due to internal standard, solvent or derivatising agents.

Quantitative methods

For assays and for quantitative estimations of components, the internal standard procedure or the external standard procedure may be adopted. The normalisation procedure is not normally applied for such determinations. In tests for related substances, either the external standard procedure with a single reference solution or the normalisation procedure may be used. However, with both these methods, when a dilution of the test solution is used for comparison, the responses of the related substances should be similar to the substance itself (response factor of 0.8 to 1.2); otherwise correction factors may have to be applied.
Peak areas and peak heights are usually proportional to the quantity of compounds eluting. Peak areas are generally used but may be less accurate if peak interference occurs. For accurate quantitative work, the components to be measured should be separated from any interfering components. Peak tailing and fronting and the measurement of peaks on solvent tails are to be avoided.

Related substances tests are sometimes based on the determination of peaks due to impurities, expressed as a percentage of the area due to the drug peak. However, it is preferable to compare impurity peaks to the chromatogram of a standard at a similar concentration. The standard may be the drug itself at a level corresponding to say, 0.5 per cent impurity, or in the case of toxic or signal impurities, a standard of the impurity itself.
When the test for related substances prescribes the summation of impurities or there is a

quantitative determination of an impurity, an appropriate threshold setting and appropriate conditions for the integration of the peak areas should be chosen. In such tests the 'ignore' limit, e.g. the areas of peaks whose areas are below the limit are not taken into account, is generally, 0.05 per cent. Thus, the threshold setting of the data collection system corresponds to, at least, half of the ignore limit
Peaks due to the solvent(s) used to dissolve the sample are also to be ignored.

System suitability

This is an integral part of liquid chromatographic method for assuring adequate performance of the system. Because of normal variations in equipment, supplies and techniques, a system suitability test is required to ensure that a given operating system may be generally applicable. System suitability also verifies that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done.
Replicate injections of a standard solution used in the assay or other standard solution are compared to determine whether the requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation if the requirement is 2.0 per cent or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0 per cent.
Replicate injections of the standard solution may be made before the injection of samples or may be interspersed among sample injections. System suitability must be demonstrated throughout the run by injection of an appropriate control preparation at appropriate intervals, including at the end of the analysis. The control preparation can be the standard solution used in the test or a solution containing a known amount of analyte.
No sample analysis is acceptable unless the requirements of system suitability have been met. Test results obtained while the system fails system suitability requirements are unacceptable.

Adjustment of chromatographic conditions

Some adjustments of chromatographic conditions may be done for obtaining the required system suitability requirements.
These include:

Mobile phase. Minor solvent component of a mixture: ± 30 sper cent relative or ± 2 per cent absolute, whichever is the larger; no other component altered by more than 10 per cent absolute;
Concentration of salts. In the buffer component of the mobile phase; ± 10 per cent;

pH of the aqueous component of the mobile phase ± 0.2 pH, unless otherwise stated in the monograph, or ± pH when neutral substances are to be examined;

Detector wavelength. No adjustment;

Stationary phase.

- column length: ± 70 per cent,
- column internal diameter: ± 25 per cent,
- particle size: reduction of not more than 50 per cent, no increase;

Flow rate. ± 50 per cent. If in the monograph, the retention time of the principal peak is indicated, the flow rate may be adjusted if the column internal diameter has been changed. No decrease in the flow rate if the monograph uses apparent number of theoretical plates in the qualification statement;

Temperature. ± 10 percent, to amaxirnumof60°;

Injection volume~ May be increased if detection and repeatability of the peak(s) to be determined are satisfactory.
Multiple adjustments should be avoided as they may have a cumulative effect on the performance of the system.
Sometimes, particularly in reversed-phase chromatographic methods, it may be advisable to change the column with another of the same type (e.g. Cl8 silica gel) from another manufacturer.
Figure.1.7 is a graphical representation of the common events during chromatography and assists in understanding the various terms more commonly employed and discussed below.

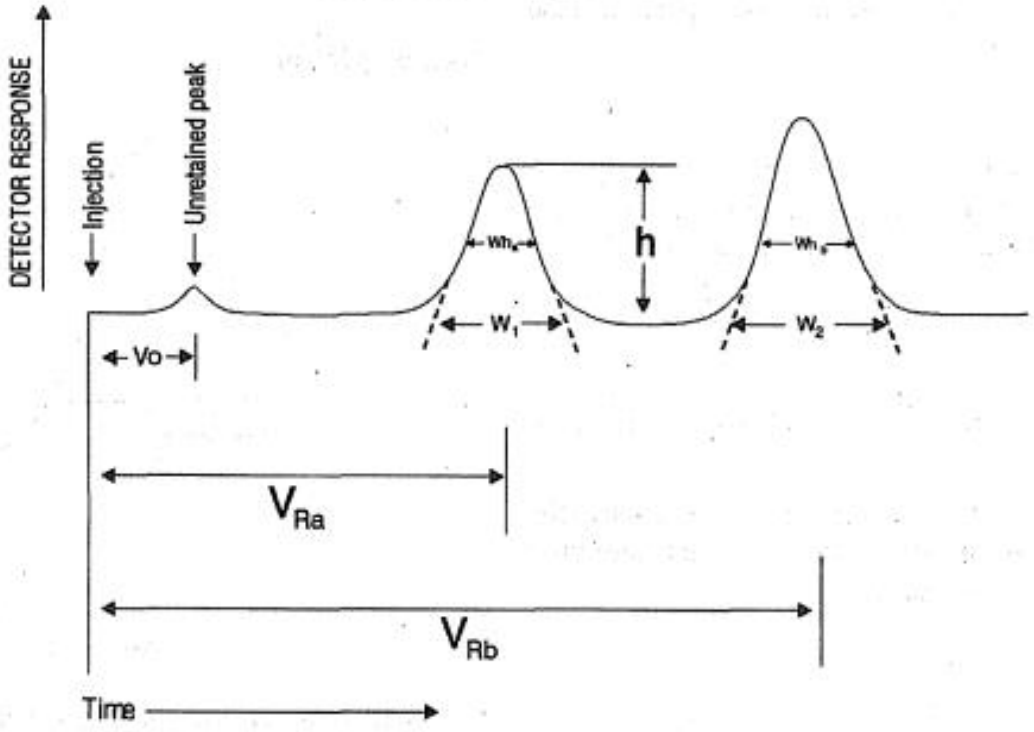


Figure 1.7.

Resolution

The resolution or resolution factor, R, is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug.
Resolution between peaks of similar height of two components may be defined by the expression:

$$R = \frac{2\,(V_{Rb} - V_{Ra})}{W_2 + W_1}$$

Where, V_{Rb} and V_{Ra} = retention times or distances along the baseline between the point of injection and perpendiculars dropped from the maxima of two adjacent peaks.

W_2 and W_1 = corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the base line.

Where electronic integrators are used, it may be convenient to determine the resolution, R, by the equation:

$$R = \frac{1.18\,(V_{Rb} - V_{Ra})}{W_{ha} + W_{hb}}$$

Where, V_{Rb} and V_{Ra} = retention times or distances along the baseline-between the-point of injection and perpendiculars dropped from the maxima of two adjacent peaks,

W_{hb} and W_{ha} = the respective peak widths measured at half-peak height.

The values of W_{ha}, W_{hb}, V_{Ra} and V_{Rb} must be expressed in the same unit of measurement.

Peak-to-valley ratio

The peak-to-valley ratio (p/v) may be used as a system suitability requirement in a test for related substances when baseline separation between two peaks is not reached.

$$(p/v) = \frac{H_p}{H_v}$$

where, H_p = height above the extrapolated baseline of the minor peak,

H_v = height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks.

Capacity factor

The capacity factor, also called mass distribution ratio, K', is stated in the monograph. It is defined by the expression

$$K' = (V_{Rb} - V_o)/ V_o$$

where, V_{Rb} = retention time or distance along the baseline between the point of injection and perpendicular dropped from the maximum of the peak of interest.

V_o = the distance along the baseline between the point of injection and perpendicular dropped from the maximum of the peak of an unretained component.

The values of V_{Rb} and V_o must be expressed in the same unit of measurement.

Column efficiency

Column efficiency can also be used as a system suitability requirement. It is a measure of peak sharpness, which is important for the detection of trace components. It is defined in terms of the number of theoretical plates N, by the expression

$$N = 5.54\ V_R^2 / W_h^2$$

where, V_R = retention time or distance along the baseline between the point of injection and a perpendicular dropped from the maximum of the peak of interest.

W_h = the width of the peak of interest at half-peak height.

The values of V_R and W_h must be expressed in the same unit of measurement.

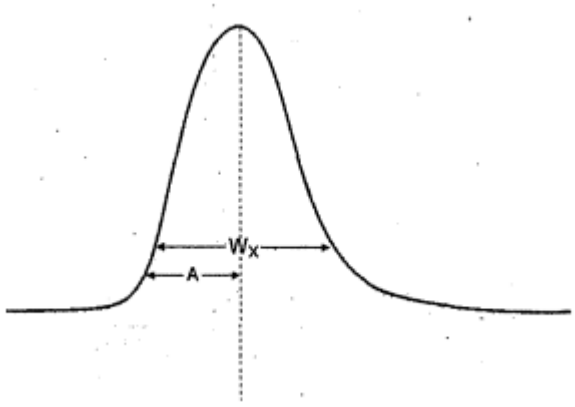


Fig. 1.8. Symmetry factor

Symmetry factor or tailing factor, S, of a peak Fig. 2 is a measure of peak symmetry. Itis unity for perfectly symmetrical peaks and its value increase as tailing become more pronounced. It is calculated from the expression

$$S = W_x / 2A$$

where, W_x = the width of the peak at 5.0 per cent of the peak height,

A = the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5.0 per cent of the peak height.

Relative retention

Relative retention, $r_{a/b}$, is calculated as an estimate from the expression

$$r_{a/b} = \frac{t_{r,b}}{t_{r,a}}$$

Where, $t_{r,b}$ = retention time of the peak of interest,

$t_{r,a}$ = retention time of the reference peak (usually the peak corresponding to the substance under examination).

Signal to noise ratio

The signal-to- noise ratio is determined from the expression:

$$S/N=2H/h$$

where, H = height of the peak corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the. extrapolated baseline of the signal observed over a distance equal to 20 times the width at half-height.

h = range of the background noise in a chromatogram obtained after injection of a blank, observed over a distance equal to 20 times the width at half-height of the peak in the chromatogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

2 **Review of literature:**

General review on methods of simultaneous determination of hypertensive drugs.

Still present many analytical methods are available on traditional or classical methods and these are not assuring more accuracy for drug analysis. As well HPLC assay methods are available for single hypertensive dosage form and combined of 2 molecules dosage forms. Very less assay method available for combined of 3 molecules dosage forms. No HPLC method available for combined of <3 molecules dosage forms from literature.

The regulatory guidelines mandated the need for establishing stability-indicating assay33

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The practical steps for establishing the stability indicating method are elusive in the regulatory guidelines and pharmacopoeias. Hence a literature was undertaken replete with the publications on the development of methods of drug substance and drug products.

Recent progress in methods development has been largely a result of improvements in analytical instrumentation. This is especially true for chromatographs and detectors. Isocratic and gradient reverse-phase HPLC have evolved as the primary techniques for the analysis of drugs. The HPLC detector of choice for many types of methods development is the photodiode array (PDA) detector because it can be used for both quantitative and qualitative analysis. The use of a PDA detector to determine peak purity of the active ingredient in stressed samples greatly facilitates the development of stability-indicating assays.

The ultraviolet (UV) absorbance detector remains the most common HPLC detector for potency and impurity analysis. Once specificity has been demonstrated, the PDA detector is replaced with a variable wavelength detector and the HPLC effluent is monitored at fixed wavelengths. Stability-indicating and impurity methods often are required to measure analytes within a wide concentration range.

The review of literature began with factual collection of large of number of methods reported over the past decades under the nomenclature.

AIM AND OBJECTIVES OF THE STUDY

To develop an analytical method of drug molecule Phenylephrine Hydrochloride, Chlorpheniramine Maleate formulation for assay and preservative content using RP-HPLC

- Selection of column and flow rate.
- Selection of mobile phase.
- Preparation of sample & standard solutions.
- Selection of chromatographic conditions.
- Optimization of method.
- To check system suitability.
- Choosing proper gradient mode separation of impurities.

DRUG PROFILE

Name of sample: COUGH SYRUP

Name of ingredient: PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENIRAMINE MELATE, SODIUM CITRATE.

Composition: EACH 5 ML CONTAIN: -

PARACETAMOL …. 125 MG
CHLORPHENIRAMINE MALEATE ….. 1 MG
SODIUM CITRATE …… 60 MG
PHENYLEPHRINE HYDROCHLORIDE ……. 5 MG

Therapeutic classification: COUGH COLD PREPARATION

Description: RED COLOUR SYRUP

Colour: CARMOISINE AND TARTRAZINE.

Dosage from: SYRUP

Approved therapeutic use: Cough Syrup is used to temporarily treat cough, chest congestion, and stuffy nose symptoms caused by the common cold, flu, allergies, hay fever, or other breathing illnesses (e.g., sinusitis, bronchitis).

Side effects: Dizziness, headache, nausea, nervousness, or trouble sleeping may occur. If any of these effects persist or worsen, contact your doctor or pharmacist promptly.

Over dosage: Agitation, Confusion, Hallucinations, Seizures.

INSTRUMENT AND REAGENTS

Instruments
HPLC System-Agilent 1200 RRLC series
HPLC System-Shimadzu LC2010C HT
Nexis GC-2030TOGAS1
CAMAG® Automatic TLC Sampler 4 (ATS 4)
CAMAG® TLC Visualizer 2
Mettler Toledo® - HE Series Moisture Analyzers
Lab-India Disso 2000 dissolution tester model

Table .Instrument used for the method development

Reagents	Grade	Manufacturer
Water	Milli-Q	Millipore
Acetonitrile	HPLC Grade	Merck
Methanol	HPLC Grade	Merck
Ortho phosphoric acid (OPA)	N/A	Merck
Ethanol 99,9%	HPLC Grade	Merck
Isopropanol p.a	HPLC Grade	Merck
Potassium dihydrogen phosphate	N/A	Merck
Toluene	HPLC Grade	Merck
Ethyl acetate	HPLC Grade	Merck
Diethyl Acetate	HPLC Grade	Merck
Petroleum Ether	HPLC Grade	Merck

Table . Reagents used for method development

Aim: - To develop analytical method for assay for Chlorpheniramine Maleate and Phenylephrine Hydrochloride by RP-HPLC

Description:

Chlorpheniramine maleate is chemically 2- [p-Chloro - a - [2-(dimethyl amino) ethyl] benzyl] pyridine maleate, is a histamine H1 antagonist used in allergic reactions, hay fever, rhinitis, urticaria, and asthma.

Phenylephrine Hydrochloride is (R)-I-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride is α-adrenoreceptor agonist, decreases nasal congestion, and increases drainage of sinus cavities

Materials and Methods:

Chlorpheniramine maleate and Phenylephrine Hydrochloride were obtained from FDA Organization lab. Cough syrup were taken for study which contain 1mg/5ml each Chlorpheniramine maleate. Acetonitrile, Ortho phosphoric acid (OPA) and HPLC grade water were used of analytical grade from Merck.

Buffer Preparation:

Adjust the pH of the purified water to 2.5 ± 0.05 with diluted ortho-phosphoric acid (OPA) and mix well and filter.

Diluent:

Water: Acetonitrile has taken in the ratio 50:50 %v/v.

Labelled amount: 1 mg/ 5 ml and 5mg/ 5ml each respectively.

Standard stock solution preparation:

24.8mg of Chlorpheniramine maleate and 25.9 mg Phenylephrine Hydrochloride standards were accurately weighed and transferred into a 25ml clean dry volumetric flask, 15 ml of diluent was added, sonicated for 5 minutes and made up to the final volume with diluent. Further 1ml from the above stock solution was taken into a 25ml volumetric flask and made up to 25ml with diluent.

Sample solution preparation:

Take Syrup in test-tube and Pipette out approximately 2.5ml of Chlorpheniramine maleate and Phenylephrine Hydrochloride was taken and then transferred into a 25 ml clean dry volumetric flask, 15 ml of diluent was added, sonicated for 5 minutes and made up to the final volume with diluent.

Chromatographic Conditions:

The criteria employed for selecting the mobile phase for the analysis of the drugs were cost involved, time required for the analysis and better separation of drugs. Chromatographic separation was performed on reverse phase Waters Discovery C18 (250 x 4.6 mm, 5m) column. The mobile phase consisted of water (pH 2 adjusted with OPA): acetonitrile (60:40v/v) with UV-Vis detection at 254 nm. The flow rate was set at 20µml/min for simultaneous determination of Chlorpheniramine maleate and Phenylephrine Hydrochloride.

Method Development:

Different chromatographic conditions were tried for separation and resolution. Waters symmetry Discovery column was found satisfactory. Peak purity of Chlorpheniramine maleate and Dextromethorphan was checked using UV-Vis detector and 218 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of Chlorpheniramine maleate and Phenylephrine Hydrochloride from standard preparation and from pharmaceutical formulation was shown in Fig. 1 and 2.

GRAPH

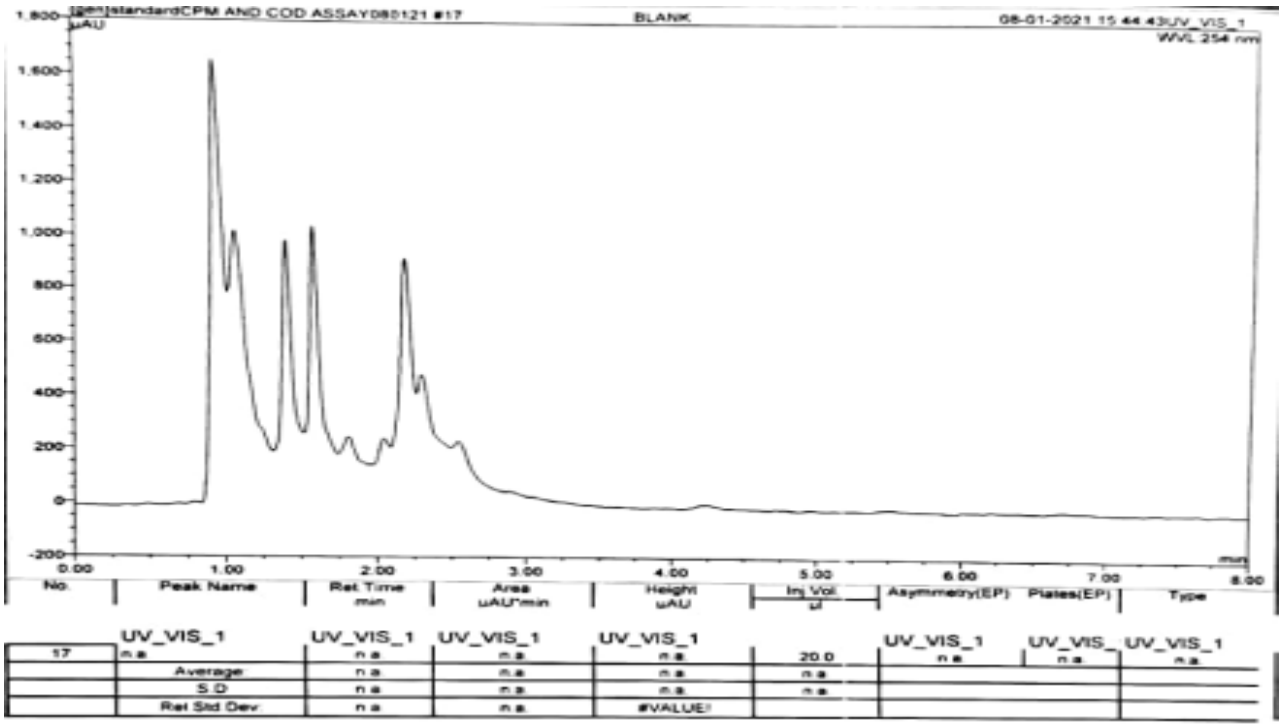


Fig 1.11. Chromatogram of Blank

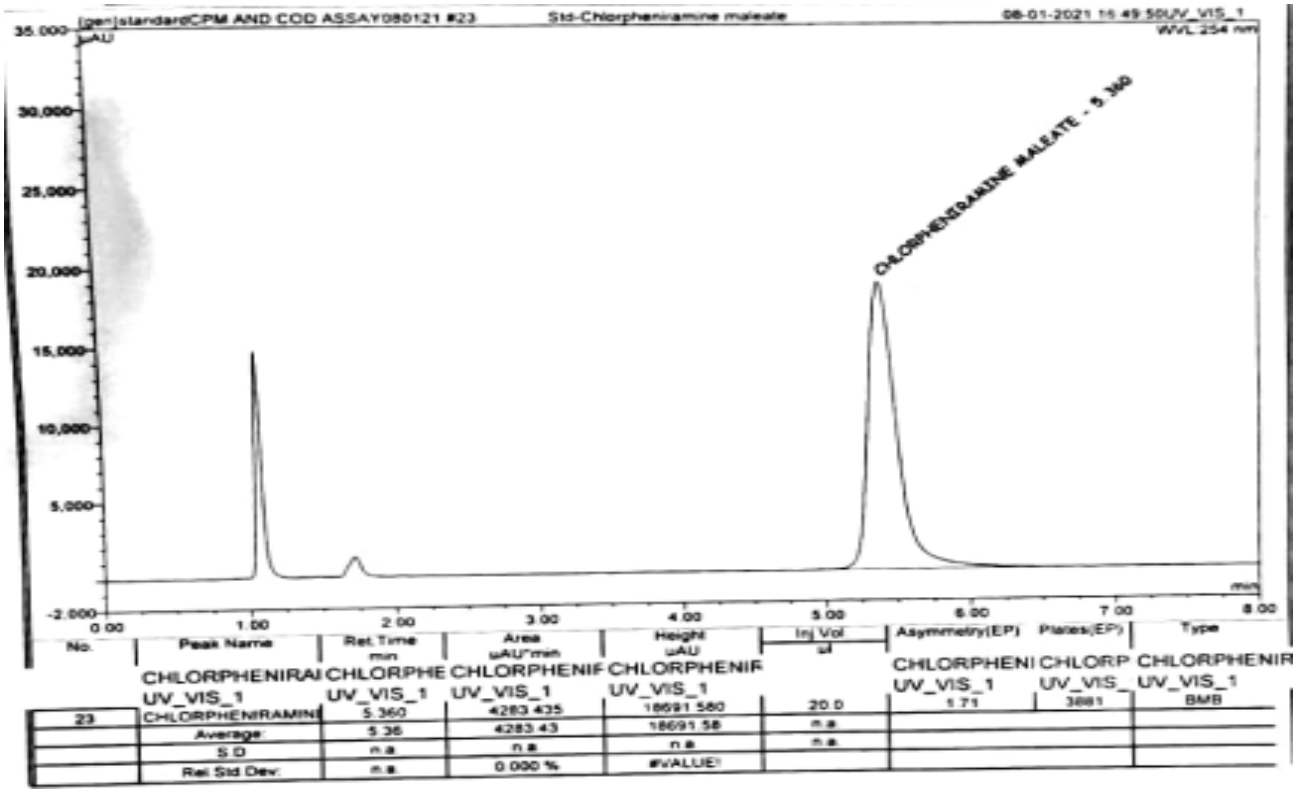
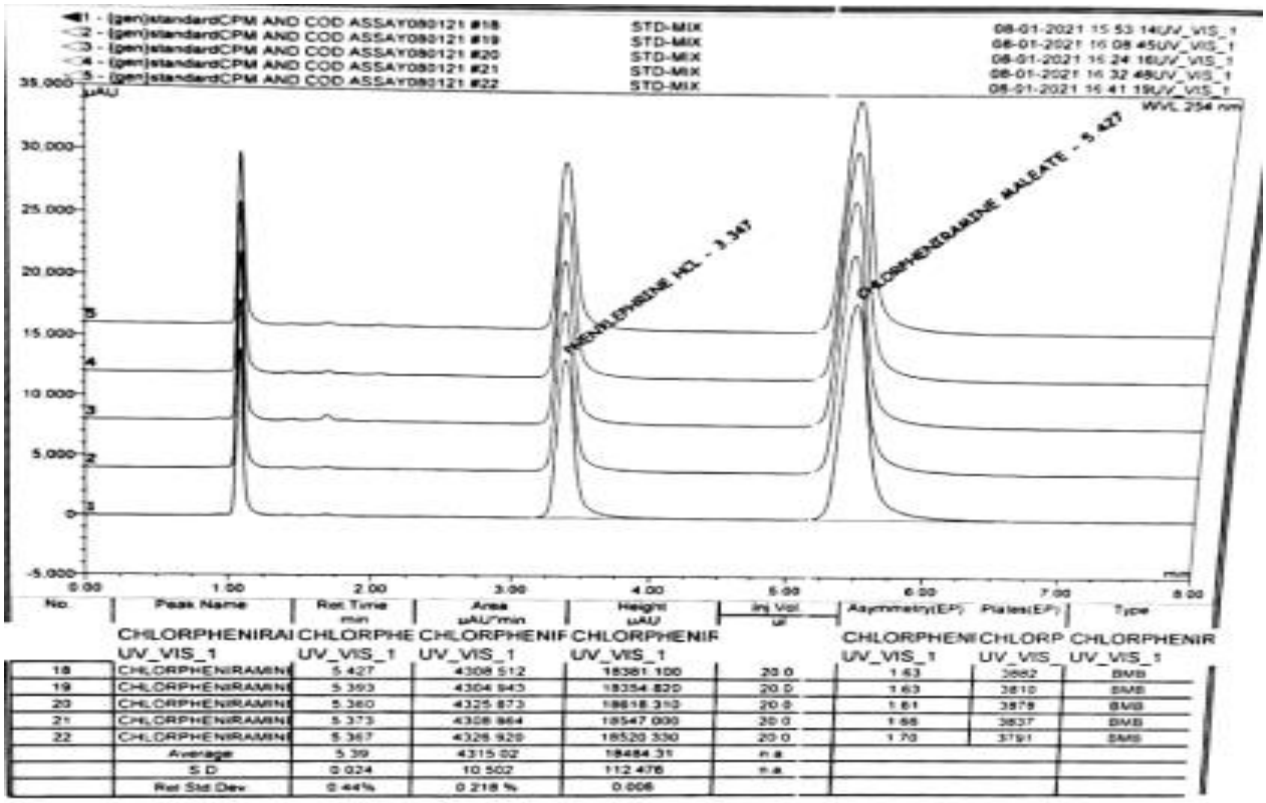
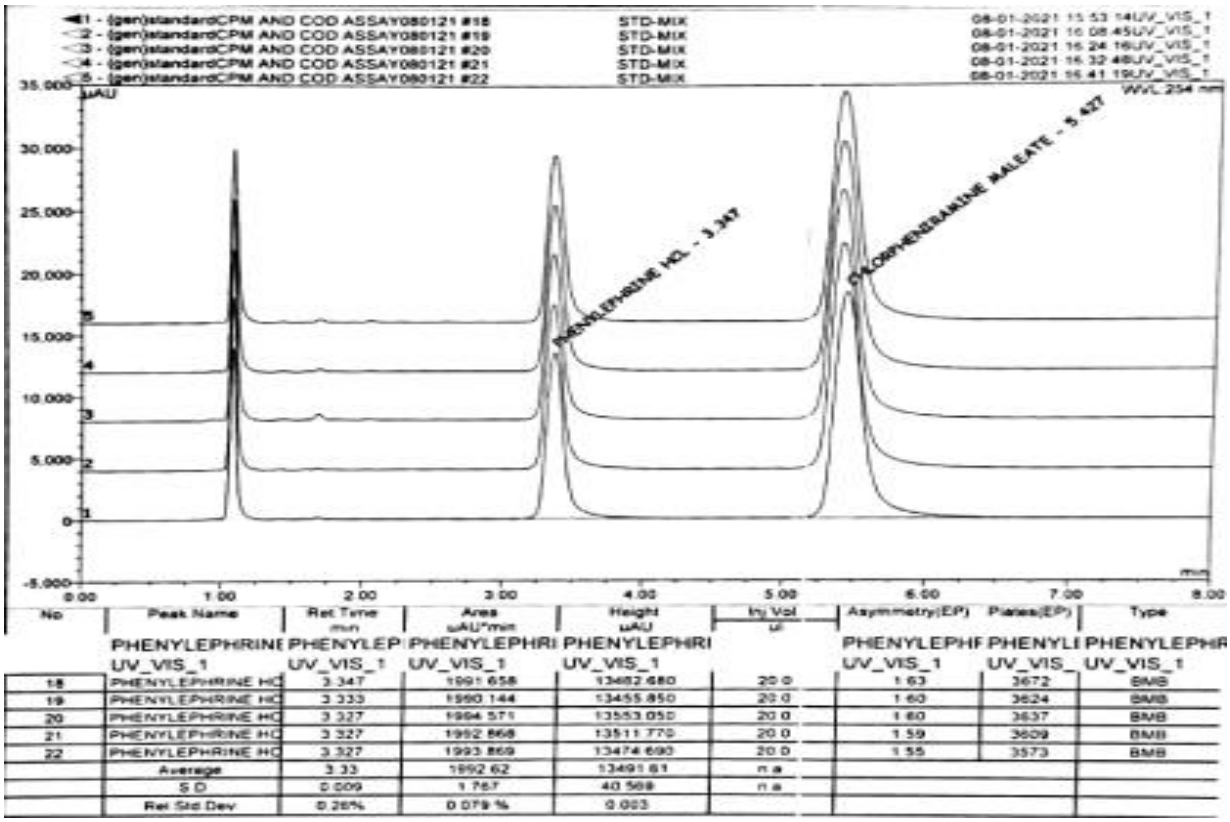


Fig 1.12. Chromatogram of Standard of Chlorpheniramine Maleate.



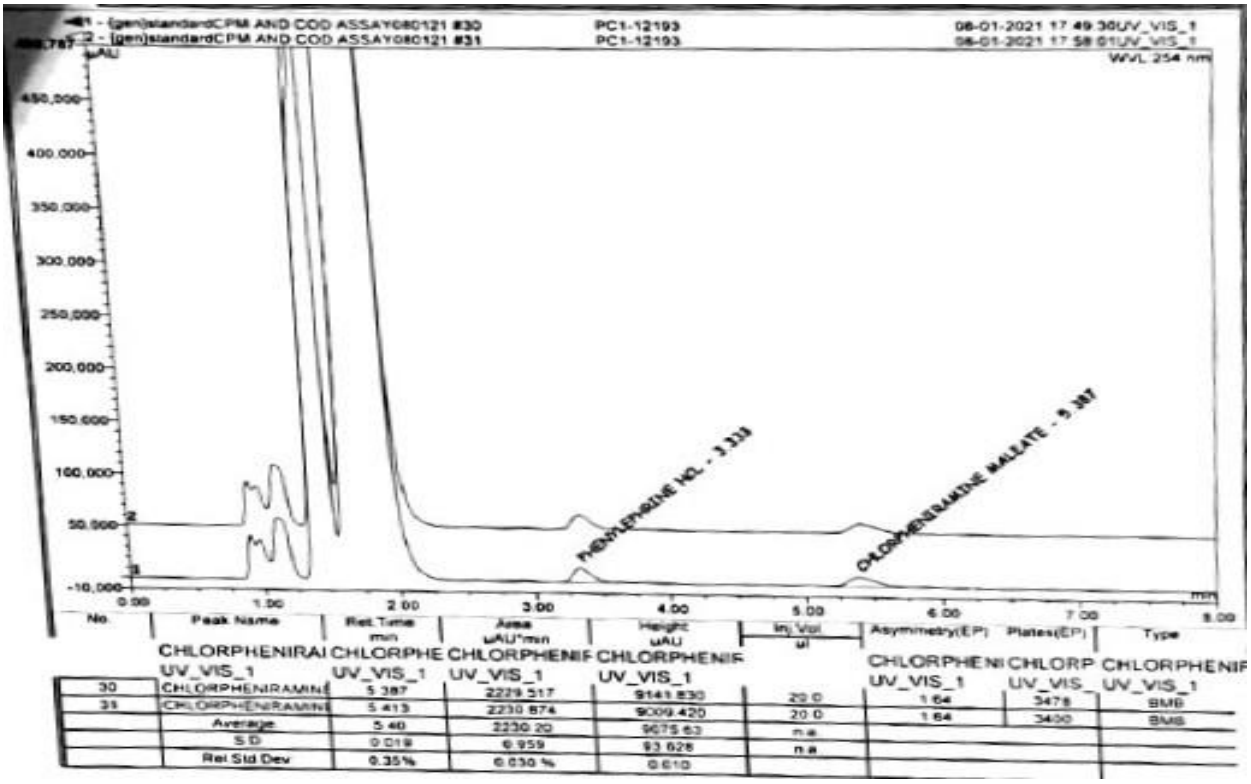
No.	Peak Name	Ret Time min	Area µAU*min	Height µAU	Inj Vol ul	Asymmetry(EP)	Plates(EP)	Type
	CHLORPHENIRAI	CHLORPHE	CHLORPHENIR	CHLORPHENIR		CHLORPHENI	CHLORP	CHLORPHENIR
	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1	UV_VIS_	UV_VIS_1
18	CHLORPHENIRAMIN	5.427	4308.512	18381.100	20.0	1.63	3662	BMB
19	CHLORPHENIRAMIN	5.393	4304.943	18354.820	20.0	1.63	3610	BMB
20	CHLORPHENIRAMIN	5.360	4325.873	18618.310	20.0	1.61	3678	BMB
21	CHLORPHENIRAMIN	5.373	4308.864	18547.000	20.0	1.68	3637	BMB
22	CHLORPHENIRAMIN	5.367	4328.920	18520.330	20.0	1.70	3791	BMB
	Average	5.39	4315.02	18484.31	n.a			
	S.D	0.024	10.502	112.476	n.a			
	Rel Std Dev	0.44%	0.218 %	0.608				

Fig1.13. Chromatogram of Mixture Standard of Chlorpheniramine Maleate



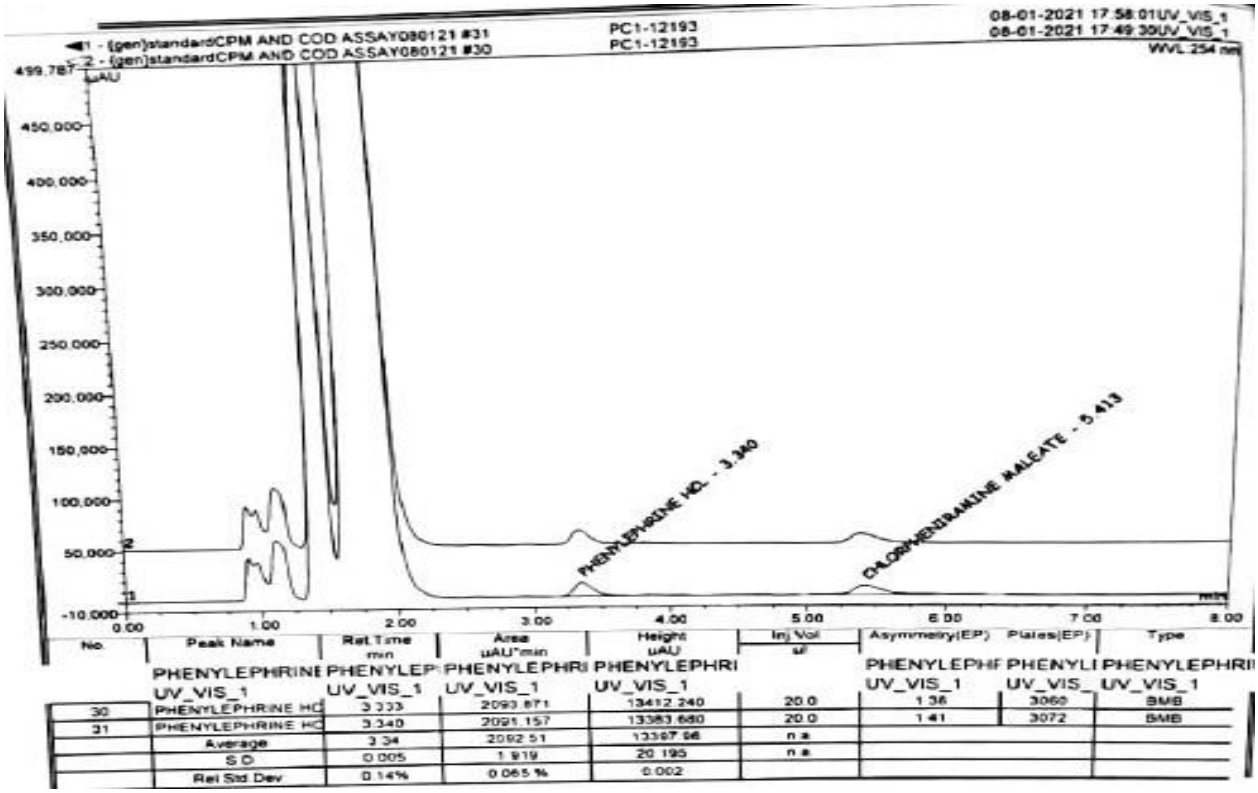
No	Peak Name	Ret Time min	Area µAU*min	Height µAU	Inj Vol ul	Asymmetry(EP)	Plates(EP)	Type
	PHENYLEPHRINE	PHENYLEP	PHENYLEPHRI	PHENYLEPHRI		PHENYLEPHF	PHENYLI	PHENYLEPHR
	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1	UV_VIS_	UV_VIS_1
18	PHENYLEPHRINE HC	3.347	1991.658	13482.680	20.0	1.63	3672	BMB
19	PHENYLEPHRINE HC	3.333	1990.144	13455.850	20.0	1.60	3624	BMB
20	PHENYLEPHRINE HC	3.327	1994.571	13553.050	20.0	1.60	3637	BMB
21	PHENYLEPHRINE HC	3.327	1992.868	13511.770	20.0	1.59	3609	BMB
22	PHENYLEPHRINE HC	3.327	1993.869	13474.690	20.0	1.55	3573	BMB
	Average	3.33	1992.62	13491.61	n.a			
	S.D	0.009	1.767	40.569	n.a			
	Rel Std Dev	0.26%	0.079 %	0.003				

Fig1.14. Chromatogram of Mixture Standard of Phenylephrine Hydrochlorid



No.	Peak Name	Ret.Time min	Area uAU*min	Height uAU	Inj Vol ul	Asymmetry(EP)	Plates(EP)	Type
	CHLORPHENIRAI	CHLORPHE	CHLORPHENIF	CHLORPHENIF		CHLORPHENI	CHLORP	CHLORPHENIF
	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1	UV_VIS_	UV_VIS_1
30	CHLORPHENIRAMINE	5.387	2229.517	9141.830	20.0	1.64	3478	BMB
31	CHLORPHENIRAMINE	5.413	2230.874	9009.420	20.0	1.64	3430	BMB
	Average	5.40	2230.20	9075.63	n.a			
	S.D	0.018	0.959	93.628	n.a			
	Rel.Std.Dev	0.35%	0.030 %	0.010				

Fig1.15. Chromatogram of Sample of Chlorpheniramine Maleate



No.	Peak Name	Ret.Time min	Area uAU*min	Height uAU	Inj Vol ul	Asymmetry(EP)	Plates(EP)	Type
	PHENYLEPHRINE	PHENYLEP	PHENYLEPHRI	PHENYLEPHRI		PHENYLEPHF	PHENYLI	PHENYLEPHRI
	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1		UV_VIS_1	UV_VIS_	UV_VIS_1
30	PHENYLEPHRINE HC	3.333	2093.871	13412.240	20.0	1.36	3060	BMB
31	PHENYLEPHRINE HC	3.340	2091.157	13383.680	20.0	1.41	3072	BMB
	Average	3.34	2092.51	13397.96	n.a			
	S.D	0.005	1.919	20.195	n.a			
	Rel.Std.Dev	0.14%	0.065 %	0.002				

Fig1.16. Chromatogram of Sample of Phenylephrine Hydrochloride

Table 1.3 of Chlorpheniramine Maleate

SR.NO	STANDARD		SAMPLE	
	RT	AREA/HEIGHT	RT	AREA/HEIGHT
1	5.39	4308.512	5.40	2229.517
2		4304.943		2230.874
3		4325.873		
4		4308.864		
5		4326.920		
6				
AVERAGE		4315.02		2230.20

CALCULATIONS:

$$\%\text{Assay} = \frac{AREA\ OF\ SAMPLE}{AREA\ OF\ STANDARD} \times \frac{CONCENTRATION\ OF\ STANDARD}{CONCENTRATION\ OF\ SAMPLE} \times \frac{PURITY\ OF\ STANDARD}{100} \times \frac{100}{1000}$$

$$= \frac{2230.20}{4315.02} \times \frac{24.8}{25} \times \frac{1}{25} \times \frac{25}{2.5} \times \frac{99.28}{100} \times 5$$

= 1.018 mg/ 5ml

Which is **101.8%** of labelled amount

Result: The given sample contain 1.018 mg / 5ml of chlorpheniramine which is

1.018 mg / 5ml of chlorpheniramine which is 101.8% of labelled amount.

Limit: 90% to 110% of the labelled amount

Table 1.4 of Phenylephrine Hydrochloride

SR.NO	STANDARD		SAMPLE	
	RT	AREA/HEIGHT	RT	AREA/HEIGHT
1	3.33	1991.658	3.34	2093.871
2		1990.144		2091.157
3		1994.571		
4		1992.868		
5		1993.869		
6				
AVERAGE		1992.62		2092.51

CALCULATIONS:

$$\%\text{Assay} = \frac{AREA\ OF\ SAMPLE}{AREA\ OF\ STANDARD} \times \frac{CONCENTRATION\ OF\ STANDARD}{CONCENTRATION\ OF\ SAMPLE} \times \frac{PURITY\ OF\ STANDARD}{100}$$

$$= \frac{2092.51}{1992.62} \times \frac{25.9}{25} \times \frac{2.5}{25} \times \frac{25}{2.5} \times \frac{99.22}{100} \times$$

= 1.079 mg/ 5ml

Which is **107.9%** of labelled amount

Result: The given sample contain 1.079 mg / 5ml of chlorpheniramine which is 1.018 mg / 5ml of chlorpheniramine which is 107.9% of labelled amount.

Limit: 90% to 110% of the labelled amount

Accuracy:

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalysed by the proposed method and the percent recovery was reported.

Limit of Detection and Limit of Quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Chlorpheniramine maleate and

Dextromethorphan were experimentally determined by injecting six injections of each drug. The LOD of Chlorpheniramine maleate and Dextromethorphan was found to be 0.04 µg/ml and 0.11 µg/ml respectively. The LOQ of Chlorpheniramine maleate and Dextromethorphan was found to be 0.14 µg/ml and 0.43 µg/ml respectively.

Robustness:

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, wavelength detection, flow rate, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ±2^{o} C in the column temperature and ±0.2ml/min in the flow rate, were tried individually. A solution of 100 % test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate.

Conclusion:

The proposed RP-HPLC method was found to be simple, accurate, precise, robust, rapid and economical. This method gives good resolution between two compounds with a short analysis time. Hence this method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Chlorpheniramine maleate and Phenylephrine Hydrochloride.

Aim: To develop analytical method for assay for Cough Syrup each 5 ml by UV-Visible

Spectrophotometry

Material and Method:

Labelled amount: 125 MG / 5 ML
Name of standard: Paracetamol
Purity: - 99.37%
Diluent: Dilute a volume containing of 25 mg of Paracetamol to 10 ml of with methanol and filter if necessary.
Reference solution: A 0.25 per cent w/v solution of paracetamol RS in methanol
Reading at **257 nm** in UV Spectrophotometry
For standard: - 0.363
For sample: - 0.408

Standard Stock Preparation:

Weigh accurately about 24.2 mg for working standard in 50 ml volumetric flask, add 30 ml of diluent and sonicate to dissolve. Then make volume up to the mark with diluent and mix well. Take 1 ml of standard form that 50 ml volumetric flask, and transfer in to 100 ml volumetric flask. Then make volume up to the mark with diluent and mix well.

24.2 mg → 50 ml
1 ml → 100 ml

Sample Stock Preparation:

Weigh accurately about 1.1605 mg of sample in 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Then make volume up to the mark with diluent mix well.
Take 1 ml of standard form that 100 ml volumetric flask, and transfer in to 50 ml volumetric flask. Then make volume up to the mark with diluent and mix well.

1.1605 gm → 100 ml
1 ml → 50 ml

<u>UV-Visible Spectrophotometry:</u>

Standard Paracetamol:

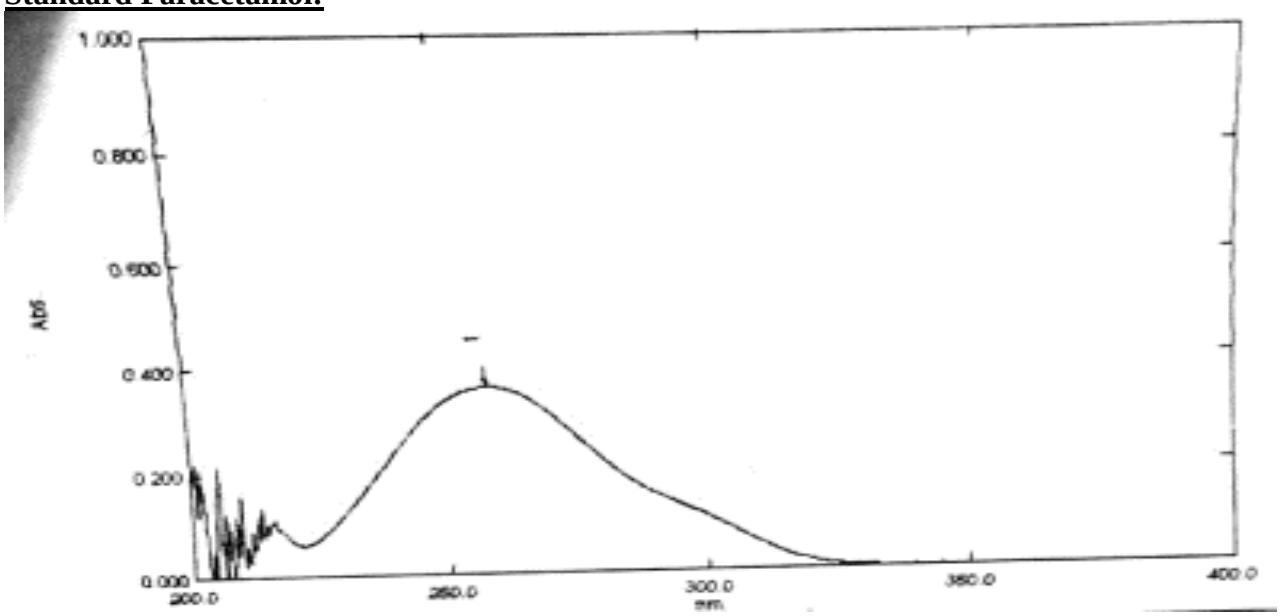


Fig1.9. UV Spectrum of Standard Paracetamol

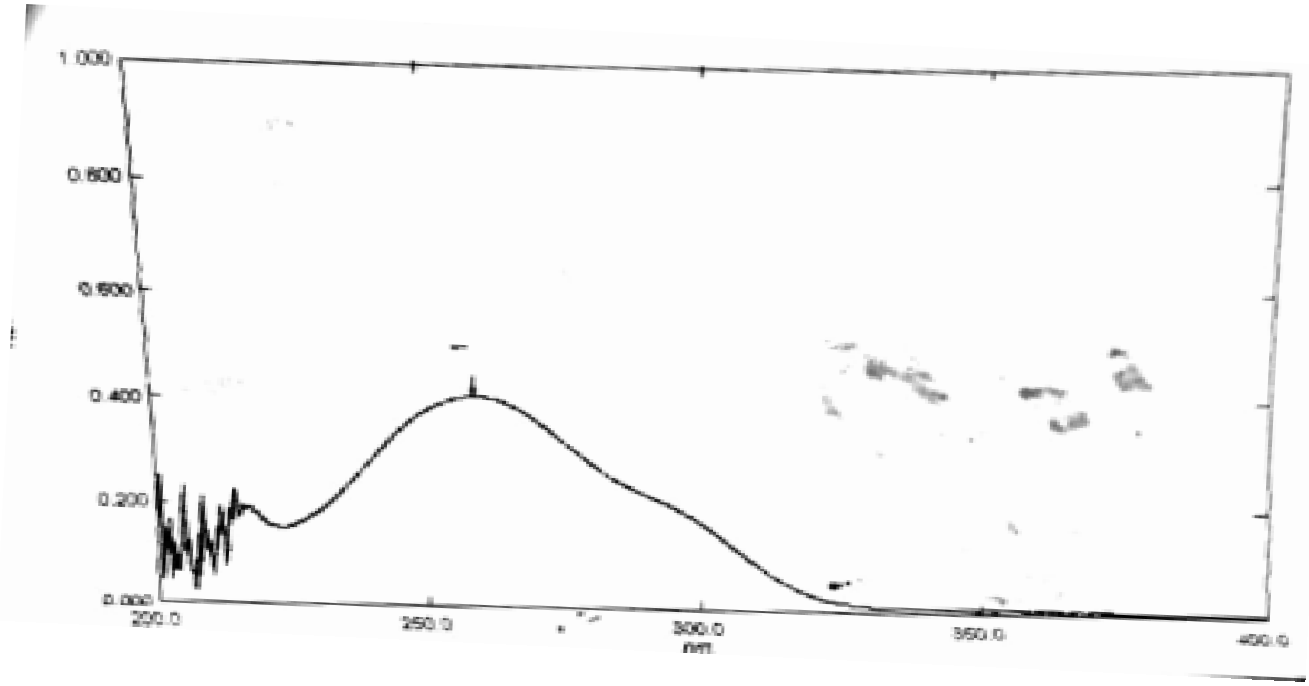


Fig1.10. UV Spectrum of Sample Paracetamol

Calculation: Content of Paracetamol:

$$\%\text{Assay} = \frac{AREA\ OF\ SAMPLE}{AREA\ OF\ STANDARD} \times \frac{CONCENTRATION\ OF\ STANDARD}{CONCENTRATION\ OF\ SAMPLE} \times \frac{PURITY\ OF\ STANDARD}{100} \times \frac{100}{1000}$$

$$= \frac{0.408}{0.363} \times \frac{24.2}{50} \times \frac{1}{100} \times \frac{100}{1.1605} \times \frac{50}{1} \times 5$$

= 136 mg/5 ml W/W

Result: The sample content of 136 mg/5 ml W/W of Paracetamol which is 108.8% of the labelled amount.

Limit: 90% to 110% of the labelled amount.

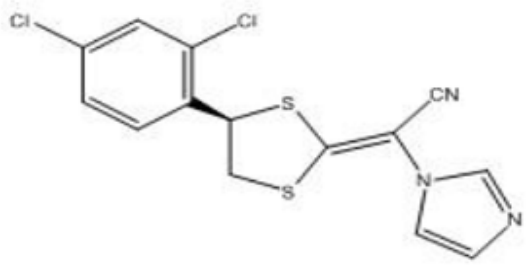
AIM: To develop analytical method for assay for Luliconazole Cream by UV-Visible Spectrophotometer

Identification: -

Luliconazole is a prescription medicine used to treat the symptoms of Ringworm (Tinea Corporis), Jock Itch (Tinea Cruris) and Athlete's Foot (Tinea Pedis). Luliconazole may be used alone or with other medications. Luliconazole belongs to a class of drugs called Antifungals, Topical. It is not known if Luliconazole is safe and effective in children younger than 12 years of age.

Description: -

Luliconazole Cream, 1% contains 1% luliconazole, an azole antifungal agent, in a white cream for topical application.
Luliconazole is (2E)-2-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile. Its structural formula is:



Luliconazole Cream, 1% for topical use Structural Formula - Illustration
The molecular formula is C14H9Cl2N3S2 with a molecular weight of 354.28. Luliconazole is the R enantiomer and contains one chiral centre. The double bond adjacent to the dithiolane group is in the E configuration.
Luliconazole Cream, 1% contains 10 mg of luliconazole per gram of cream in a vehicle consisting of benzyl alcohol, butylated hydroxytoluene, cetostearyl alcohol, isopropyl myristate, medium-chain triglycerides, methylparaben, polysorbate 60, propylene glycol, purified water, and sorbitan monostearate.
DOSAGE
When treating interdigital tinea pedis, a thin layer of Luliconazole Cream, 1% should be applied to the affected area and approximately 1 inch of the immediate surrounding area once daily for 2 weeks.
When treating tinea cruris or tinea corporis, Luliconazole Cream, 1% should be applied to the affected area and approximately 1 inch of the immediate surrounding area once daily for 1 week.

Material and Method:

Name of ingredient: - Luliconazole
Labelled amount: - 1% W/W
Name of standard: - Luliconazole

Purity: - 99.5%

Standard preparation:

Weigh accurately about 26.3 mg for working standard in 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Then make volume up to the mark with diluent and mix well. Take 2 ml of standard form that 100 ml volumetric flask, and transfer in to 50 ml volumetric flask. Then make volume up to the mark with diluent and mix well.

26.3 MG → 100 ML

2 ML → 50 ML

Sample preparation:

Weigh accurately about 2.5036 mg of sample in 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Then make volume up to the mark with diluent mix well.
Take 2 ml of standard form that 100 ml volumetric flask, and transfer in to 50 ml volumetric flask. Then make volume up to the mark with diluent and mix well.

2.5036 MG → 100 ML

2 ML → 50 ML

Reading at **296 nm** in UV Spectrophotometry

For Standard: - **0.615**

For Sample: - **0.537**

GRAPH

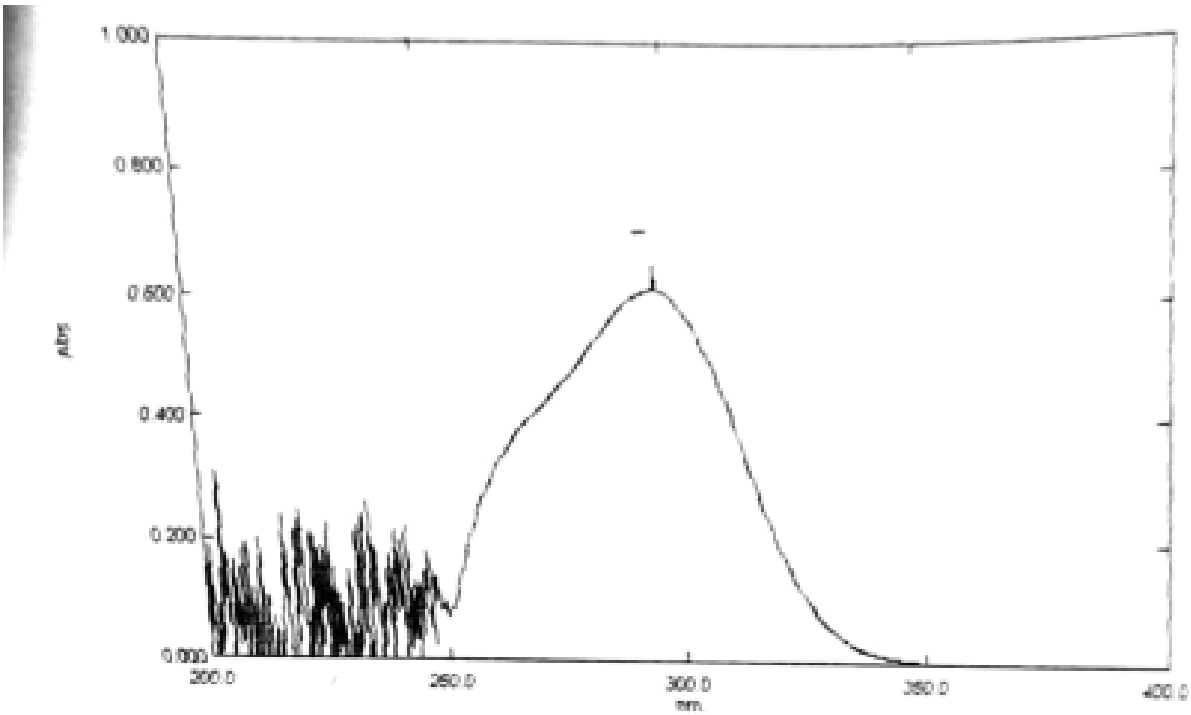


Figure1.1:- UV Spectrum of Standard of Lulicoanzole

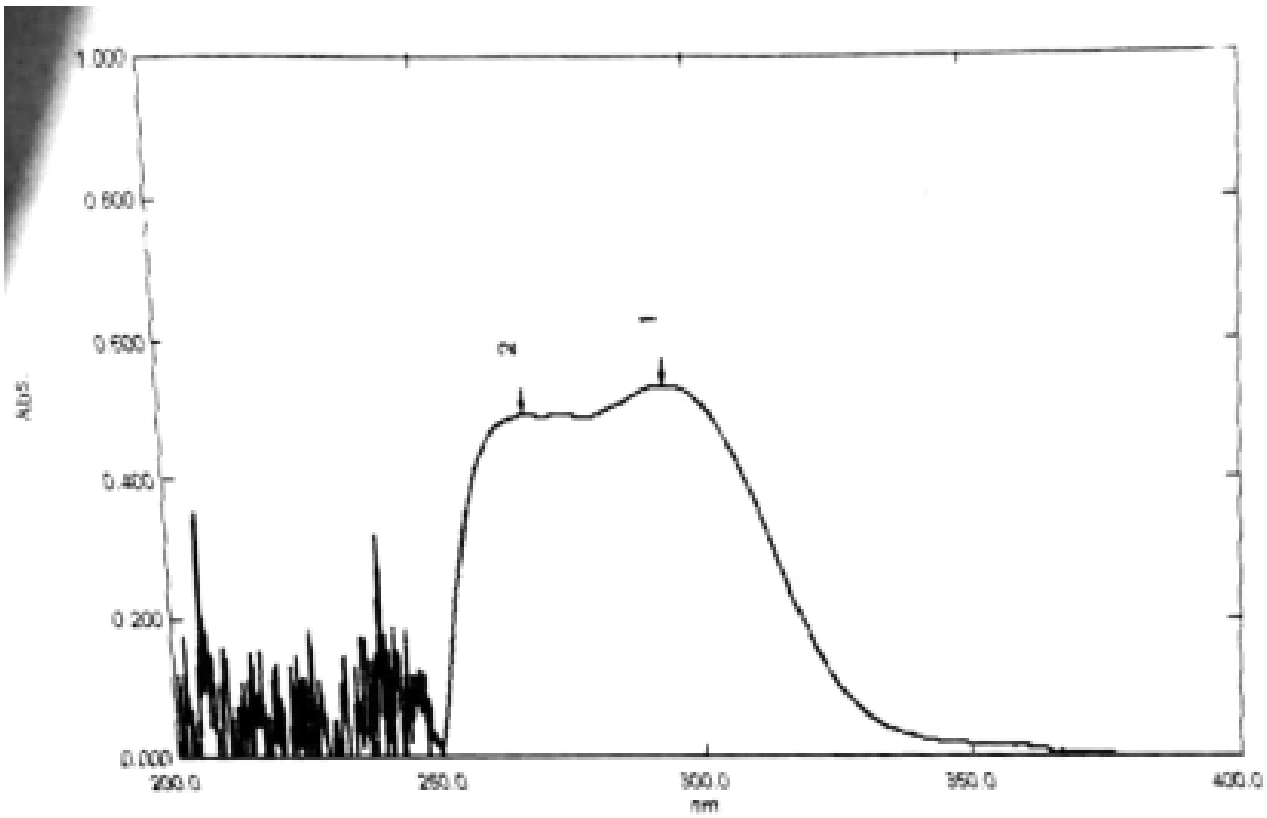


Figure1.2:- UV Spectrum of Sample of Luliconazole

Calculation: Content of Luliconazole:

$$\%\text{Assay} = \frac{AREA\ OF\ SAMPLE}{AREA\ OF\ STANDARD} \text{ x } \frac{CONCENTRATION\ \ OF\ STANDARD}{CONCENTRATION\ OF\ SAMPLE} \text{ x } \frac{PURITY\ OF\ STANDARD}{100} \text{ x } \frac{100}{1000}$$

$$= \frac{0.537}{0.615} \times \frac{26.3}{100} \times \frac{2}{50} \times \frac{100}{2.5036} \times \frac{50}{2} \times \frac{99.5}{100} \times \frac{100}{1000}$$

= 0.913 % W/W

Result: The sample content of 0.913% W/W of Luliconazole which is 91.3% of the labelled amount.

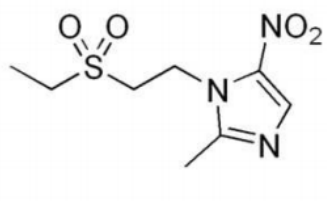
Limit: 90% to 110% of the labelled amount.

AIM: To develop analytical method for assay for Tinidazole by UV-Visible

Spectrophotometer.

Description: -

Tinidazole (TZ), [1-(2-(ethylsulfonyl) ethyl)-2-methyl-5-nitroimidazole], is an effective antiprotozoal and antibacterial agent. It is used for treatment of amoebiasis, giardiasis and trichomoniasis.



TZ

Name of ingredient: - Tinidazole
Labelled amount: - 1% W/W
Name of standard: - Tinidazole
Purity: - 99.5%
Standard preparation:

Weigh accurately about 26.3 mg for working standard in 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Then make volume up to the mark with diluent and mix well. Take 2 ml of standard form that 100 ml volumetric flask, and transfer in to 50 ml volumetric flask. Then make volume up to the mark with diluent and mix well.

26.3 MG → 100 ML

2 ML → 50 ML

Sample preparation:

Weigh accurately about 2.5036 mg of sample in 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Then make volume up to the mark with diluent mix well.
Take 2 ml of standard form that 100 ml volumetric flask, and transfer in to 50 ml volumetric flask. Then make volume up to the mark with diluent and mix well.

2.5036 MG → 100 ML

2 ML → 50 ML

Reading at **319 nm** in UV Spectrophotometry
For Standard: - **0.486**
For Sample: - **0.537**

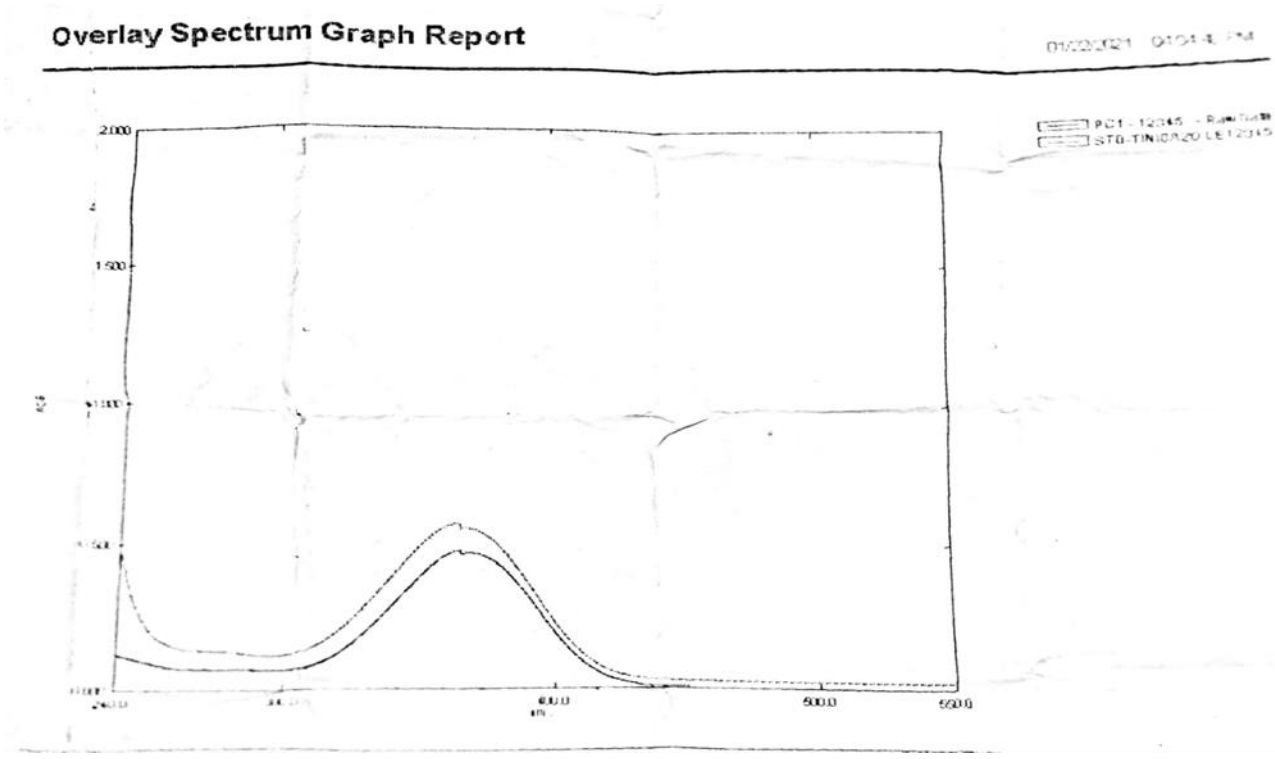


Figure 1.3 UV Spectrum of Standard and Sample Tinidazole

Calculation: Content of Tinidazole:

$$\%\text{Assay} = \frac{AREA\ OF\ SAMPLE}{AREA\ OF\ STANDARD} \times \frac{CONCENTRATION\ OF\ STANDARD}{CONCENTRATION\ OF\ SAMPLE} \times \frac{PURITY\ OF\ STANDARD}{100} \times \frac{100}{1000}$$

$$= \frac{0.486}{0.537} \times \frac{26.3}{100} \times \frac{2}{50} \times \frac{100}{2.5036} \times \frac{50}{2} \times \frac{99.5}{100} \times \frac{100}{1000}$$

$$= 0.946\ \%\ W/W$$

Result: The sample content of 0.913% W/W of Luliconazole which is 94.6% of the labelled amount.

Limit: 90% to 110% of the labelled amount

AIM: To develop analytical method for assay for Ethanol Hand Sanitizer by GC

Description:

Ethanol is an organic chemical compound. It is a simple alcohol with the chemical formula C_2H_6O. Its formula can be also written as $CH_3{-}CH_2{-}OH$ or C_2H_5OH (an ethyl group linked to a hydroxyl group), and is often abbreviated as EtOH. Ethanol is a volatile, flammable, colourless liquid with a slight characteristic odour. It is a psychoactive substance, recreational drug, and the active ingredient in alcoholic drinks.

Materials and Methods:

Ethanol Hand Sanitizer were obtained from FDA Organization lab. Hand Sanitizer were taken for study which contain 70% of Ethanol. Aqua demineralisation, ethanol 99,9% p.a., isopropanol p.a. (Merck) and HPLC grade water were used of analytical grade from Merck.

Labelled amount: 70%

Equipment/Instrument

- Agilent 7010B GC-TQ equipped with a 7693 AutoSampler and an 8890 GC
- DB-624 30m x 0.25mm x 1.4µm
- Volumetric Glassware: Class A
- Pipets
- Airtight gas syringe (500µL)
- 2mL GC vials

Diluent and Blank: Aqua double distilled water

Table 1.1 of Mixed Stock Standard preparation.

Prepare a mixed stock standard solution in acetonitrile with the following concentrations.

Impurity/Analyte	Density (µg/µL)	Volume of Impurity (µL)	Dilution Volume (mL)	Concentration (µg/mL)	Concentration (ppm)
Methanol	791	200	100	1582	62039
Benzene	874	10		4.4	171
Acetaldehyde	785	15		117.8	4618
Acetal	831	15		124.5	4888
Ethanol	789	500		3945	154706
Isopropanol	785	500		3925	153922
Acetone	790	200		1580	61961
1-Propanol	804			1608	63059
Ethyl Acetate	902			1804	70745
2-Butanol	808			1616	63373
Isobutanol	803			1606	62980
1-Butanol	810			1620	63529
3-Methyl-1-Butanol	809			1618	63451
Amyl Alcohol	811			1622	63608

Note: Benzene is first diluted in 10mL and then 0.5mL of that solution is diluted in the stock 100mL standard. Concentration (ppm) is total concentration in a bottle of hand sanitizer and a sampling volume of 0.3mL. Density of hand sanitizer compounded as described in the FDA Guidances is ~0.85g/mL.

Standard Preparation:

Transfer 5mL of the mixed stock standard via Class A volumetric pipette to a 25mL volumetric flask containing approximately 8mL of acetonitrile. Dilute to volume with acetonitrile and mix well. Prepare fresh daily.

Spiked Recovery Standard Preparation

Transfer 1mL of the mixed stock standard via a Class A volumetric pipette to a 100mL volumetric flask containing approximately 80mL of acetonitrile. Dilute to volume with acetonitrile and mix.

Hand Sanitizer Sample Preparation

Transfer 0.3mL of hand sanitizer sample via air displacement pipette to a 10mL volumetric flask containing approximately 8mL of acetonitrile. Dilute to volume with acetonitrile and mix.

Spiked Recovery Hand Sanitizer Sample Preparation

Transfer 0.3mL of hand sanitizer sample via air displacement pipette to a 15mL conical centrifuge tube and add 10mL of Spiked Recovery Standard via Class A pipette. Cap and mix well.

Unspiked Sample Preparation

Transfer 0.3mL of hand sanitizer sample via air displacement pipette to a 15mL conical centrifuge tube and add 10mL of acetonitrile via Class A pipette. Cap and mix well.

Spiking Solution Preparation

Transfer 0.3mL of acetonitrile via air displacement pipette to a 15mL conical centrifuge tube and add 10mL of Spiked Recovery Standard via Class A pipette. Cap and mix well.

Hand Sanitizer Density Determination

Density of the hand sanitizer sample needs to be determined. This may be done utilizing a pycnometer or utilizing a graduated cylinder and an analytical balance to measure out and weigh 1mL of hand sanitizer.

Chromatographic Conditions

- Carrier Gas: Helium
- Run Time: 15.667min
- Flow Rate: 1.0mL/min
- Injector Temp: 250oC
- Injection Vol.: 1.0µL
- Injection Type: Pulsed Split (50:1 split w/ 25psi pulse for 0.5min)
- Oven Temp Gradient: 40oC(5min) □ 240oC at 30oC/min (4min)

%Assay for Labelled Alcohol (Ethanol or Isopropanol)

Assay Sample Preparation:

If the ethanol or isopropanol peak for the impurity sample is more than 5x greater than the ethanol or isopropanol peak in the standard, dilute the impurity sample so that the resulting peak area should be approximately 0.5x the standard's peak area for those alcohols. If the peak area is less than 5x greater, then utilize the values from the impurity testing. Prepare fresh daily.

Injection Order

- Inject Blank (use diluent) at least once at the beginning of a sequence. A blank injection

can be done between standards and samples or different samples if carryover is observed.

• If sample is being run the same day as the impurity sample, inject standard solution once before the sample. If it is within 10% of the average calculated for the impurity study system suitability, then run the sample. If not, inject for three consecutive times before the injection of the first sample. If run on a different day, prepare a new standard and follow Injection order for the Impurity Study.

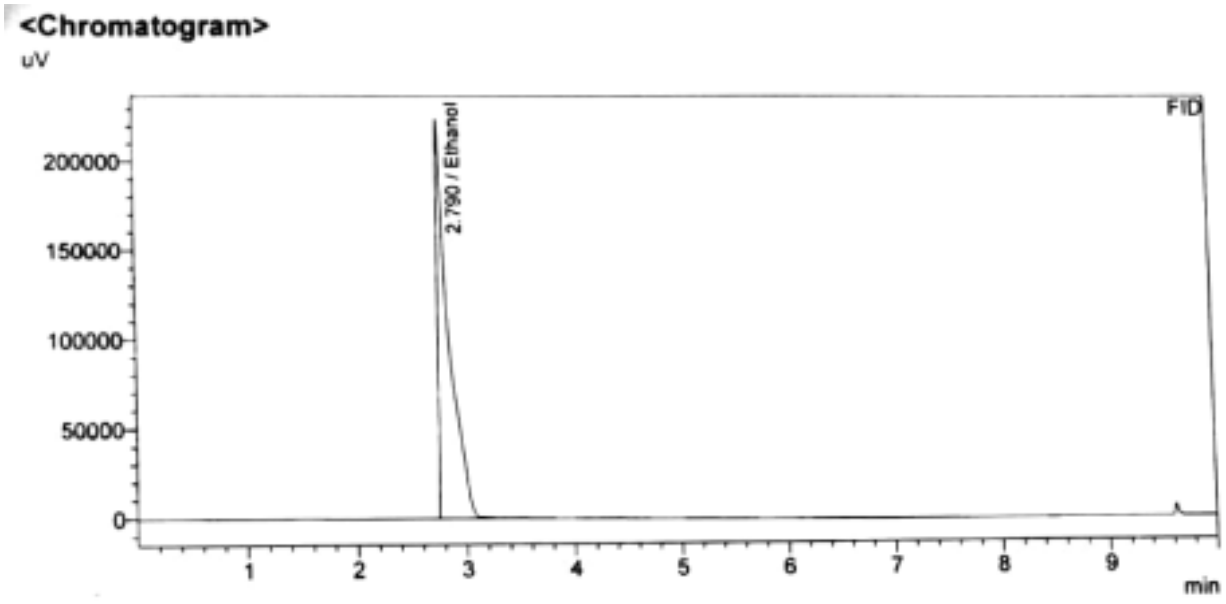
• Inject Standard solution once every six injections of samples and at the end of a sequence. If run on the same day as the impurity study, as long as system suitability below is met, just bracket the samples with two standard injections. If run the following day, the total number of standard solution injections needs to be at least six.

System Suitability Criteria

• For same day runs, the % RSD of the peak area for ethanol or isopropanol for all injections of standard solution when compared to the % RSD in the impurity study standard should be no more than 10%.

• For runs on different days, the % RSD of the peak area for ethanol or isopropanol for all injections of standard solution that day ($n \geq 6$) should be no more than 10%.

Graph

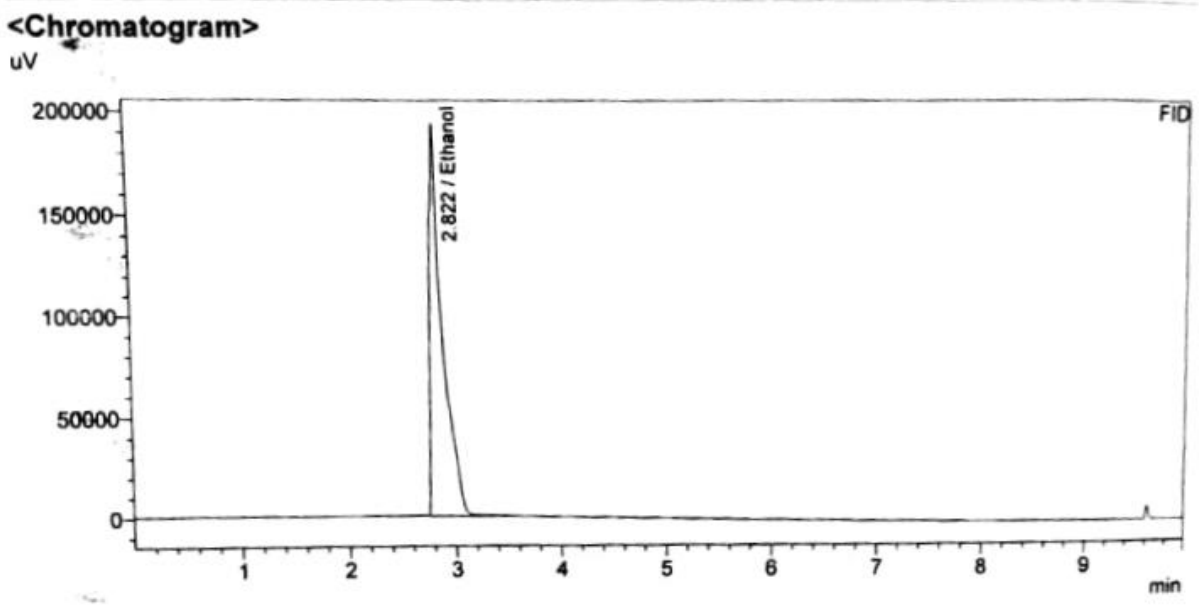


<Peak Table>

FID

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.790	1548038	224415	0.000	%	S	Ethanol
Total		1548038	224415				

Fig1.4. Chromatogram of Sample of Ethyl Alcohol



<Peak Table>

FID

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.822	1542802	193862	0.000	%	SV	Ethanol
Total		1542802	193862				

Fig1.5. Chromatogram of Sample of Ethyl Alcohol

Table 1.2 of Ethyl Alcohol

Sr. No	Standard		Sample	
	RT	Area/Height	RT	Area/Height
1	2.78	1392722	2.8	1542802
2		1381551	2.79	1548033
3		1415235		
4		1374337		
5		1379023		
6				
Average		1392573.6		1545417.5

CALCULATIONS:

$$\%\text{Assay} = \frac{AREA\ OF\ SAMPLE}{AREA\ OF\ STANDARD} \times \frac{CONCENTRATION\ OF\ STANDARD}{CONCENTRATION\ OF\ SAMPLE} \times \frac{PURITY\ OF\ STANDARD}{100}$$

$$= \frac{1545417.5}{1392573.6} \times \frac{5}{25} \times \frac{2}{25} \times \frac{100}{2.5} \times \frac{99.90}{100} \times 100$$

$$= 70.93\%$$

Which is **101.32%** of labelled amount

Result: The given sample contain 70.93% of Ethyl Alcohol which is 101.32% of labelled amount.

Limit: 90% to 110% of the labelled amount.

Aim: Dissolution study and method validation of alprazolam by high performance liquid chromatography method in pharmaceutical dosage form.

Introduction:-

The 8-chloro-1-methyl-6-phenyl-4H-(1,2,4) triazolo (4,3a)1,4 benzodiazepine is a chemical name of alprazolam. The alprazolam is used treat short acting anxiolytic disorder.

The drug also used for sedative, hypnotic, anticonvulsant, amnesic and skeletal muscle relaxant property. It is a long-term use drug and abuse causes a physical dependence. Some side effects of alprazolam were occurs in patients. If signs of an allergic reaction occur - such as hives; difficulty breathing; swelling of face, lips, tongue, or throat. In the literature the liquid chromatography1,2 and spectrophotometric and HPTLC methods were suggested for the assay of alprazolam. The aim of present work was to develop, simple, accurate and precise method for the determination of alprazolam. For the analysis of alprazolam, a new developed method was more useful. In the suggested method validated of was carried out as per ICH guideline.

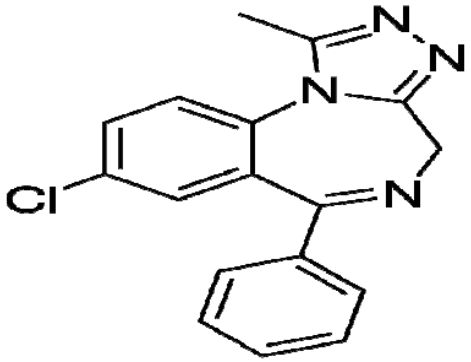


Fig 1.20. Structure of Alprazolam

Materials and methods

Instrument and reagents:

i. HPLC system was used equipped with separation module and UV detector [Merck]

ii. The dissolution study Lab-India Disso 2000 dissolution tester model was used at a temperature of 37°C, iii. Analytical balance of made Shimadzu was used.

Reagents and materials:

i. Alprazolam reference standard was obtained from reputed firm with certificate analysis.

ii. Millipore water was used for preparing dissolution media. The chemical used was analytical reagent grade and HPLC grade. Alprazolam tablets containing Alprazolam (0.25 mg) where procured from reputed firm.

Dissolution study: Method development:

Individual six tablets where weighed containing 0.25 mg alprazolam and transferred into separate dissolution apparatus bowls containing 500 ml of potassium dihydrogen phosphate buffer

The determination solubility and amount of drug release was determined in 500 ml of potassium dihydrogen phosphate buffer pH 6.8. The USP paddle method (USP apparatus II) at 100rpm was used for determination of percentage drug release tests. The bowl temperature was stabilized at 37°C. After 2, 4, 8, 10 and 12 hrs, the aliquots were taken from bowl. Filtered the aliquots with 0.45µ nylon filter and injected. The formulated results are
presented in Table-1.

The amount and percentage of drug release was calculated using HPLC method.

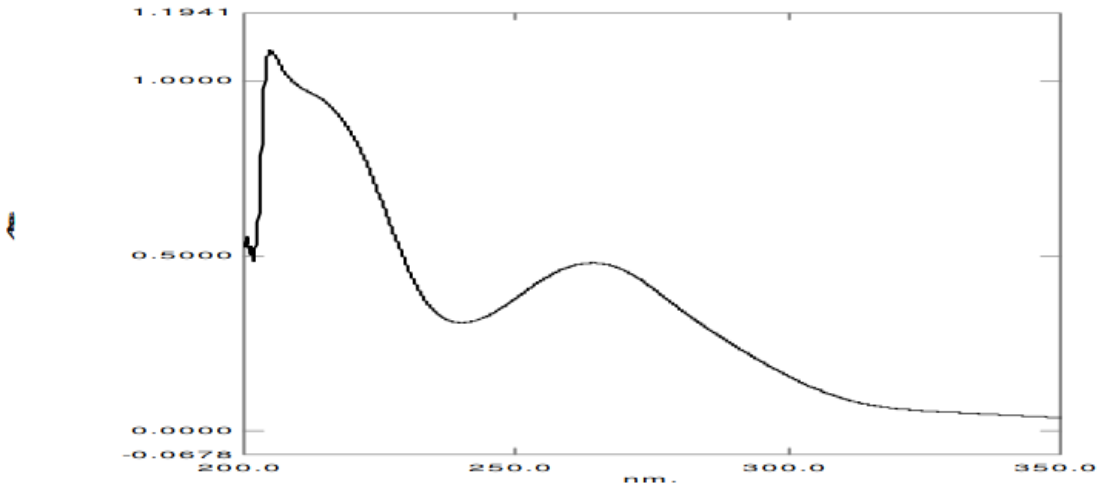


Fig 1.21. UV Spectrum of Alprazolam

HPLC Method:

Standard solution: Weighed and transferred 5 mg of alprazolam standard into 100ml volumetric flask, added about 50 ml of diluent. This solution was sonicate 1 minute and adjusted the volume up to the mark with diluents to obtained concentration as 50 µg / ml. Dilute 1 ml of 50 µg/ ml solution to 10 ml volumetric flask with diluents to get concentration 5 µg/ml. Further dilute 1 ml of above solution to 10 ml and diluted with diluent. Final concentration of solution was obtained 0.5 µg / ml.

Sample solution: By Accurate weighing average weight of twenty tablets was determined. From the tablet of alprazolam powder equivalent to 0.25 mg was weighed, to this added 50ml of diluents and sonicated for 1 minute and adjusted volume up to 100 ml with diluents to give

concentration as 50 µg/ ml. Filter this solution with 0.45µ nylon filter. A 0.1 ml of 50 µg/ ml of filtered solution were further diluted to 10 ml with diluent to get concentration with diluents to get concentration 0.5 µg/ ml.

Chromatographic conditions:

The separation of alprazolam was confirmed at room temperature on symmetry shield RP8 (150 x 3.9 mm i.d.5µ) column. A mixture of (60:40 % v/v) of buffer and acetonitrile was used as mobile phase. 0.1 % orthophosphoric acid adjusted the pH 3.0 with tri-ethylamine was used as buffer. The 225 nm was set as detector wavelength and the 30 µl was an injection volume.

Table 1.6: - Results of accuracy of alprazolam for dissolution study

Stage	No. of replicate	Weight in mg	Pek-area	Quantity recovered (%)	% recovery	Mean recovery
50%	1	0.125	335817	50.74	101.48	100.30
	2	0.125	331946	50.16	100.31	
	3	0.125	327919	49.55	99.09	
100%	1	0.251	661343	99.93	99.93	99.27
	2	0.252	654064	98.83	98.83	
	3	0.251	655689	99.07	99.07	
150%	1	0.374	989320	149.48	99.65	99.05
	2	0.375	975586	147.41	98.27	
	3	0.375	985059	148.84	99.23	
				Mean recover of all level		99.54

GRAPH

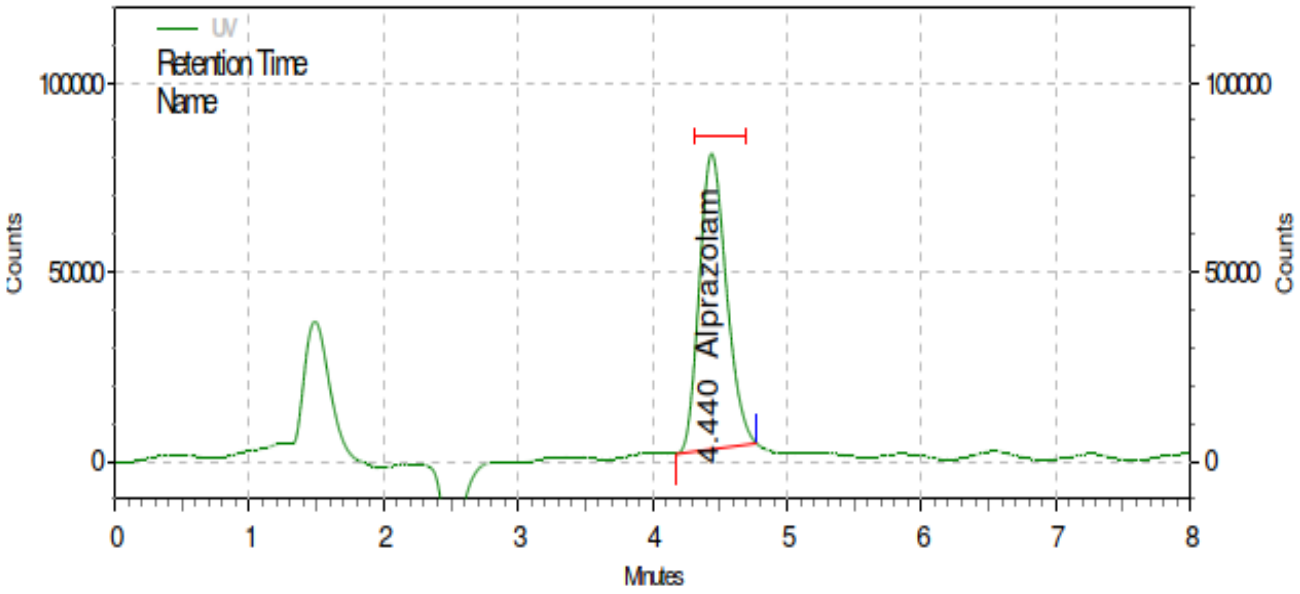


Figure 1.22. Chromatogram of alprazolam (standard).

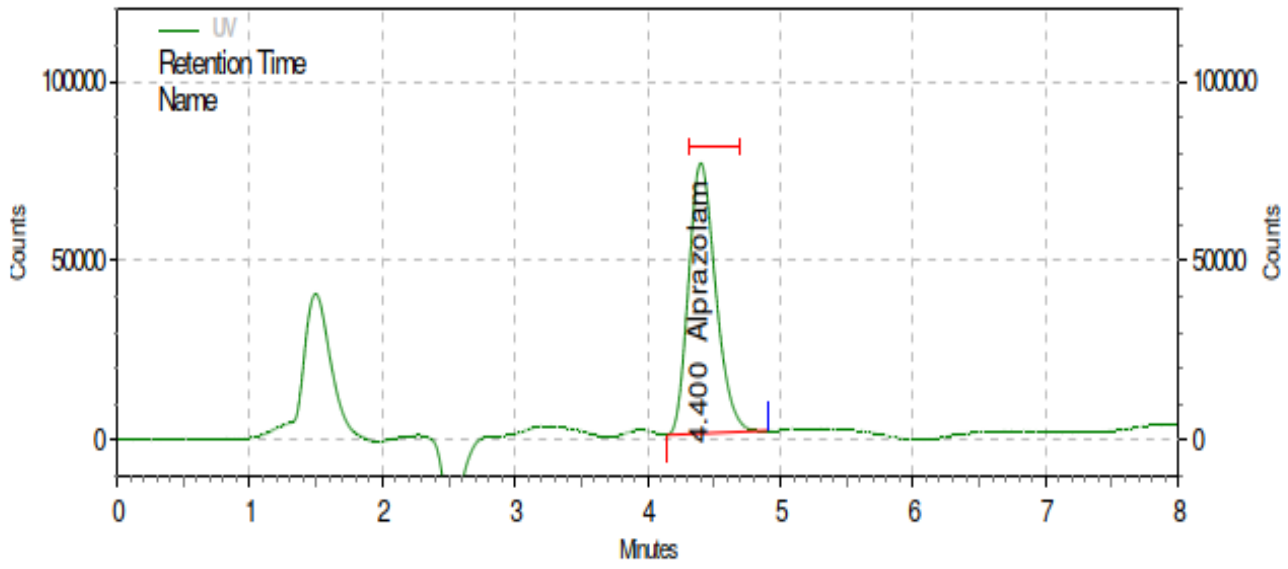


Figure 1.23. Chromatogram of alprazolam (sample).

Results and discussion

Dissolution study: The solubility of alprazolam, dose criteria was checked and the dissolution

medium was selected. Potassium dihydrogen phosphate buffer was selected for the dissolution medium and study was done. The drug was feely soluble in phosphate buffer medium as it is insoluble in acidic medium. At 100 rpm, % drug release was better potassium phosphate pH 6.8 buffer. About 96% of drug was released at 12 hrs in potassium dihydrogen phosphate buffer pH 6.8 at 100 rpm. Hence phosphate buffer pH 6.8 was used as dissolution medium for further study.

After 12 hrs at 100 rpm alprazolam drug was released completely. The cumulative percentages of drug released.

Linearity was determined by injecting standard solution in the range of 50–150 µg/ml and the calibration curve was plotted by area against concentration levels. The representative linear equation was y = 1000000x - 13910 and correlation coefficient 0.996 for alprazolam. From this linearity data the method is linear.

The method was found to be accurate with % recovery of 99.05%–100.30% using standard addition method. For the precision of dissolution method, the % RSD for repeatability study was observed 1.85. The % RSD values indicating method if precise.

HPLC method:

The revered phase HPLC method developed and validated as per ICH guidelines for the determination of alprazolam. The results which are shows limit value of standard deviation and percent relative standard deviation. The accuracy study reveals that mean recovery after spiking experiment were between 99.78 to 99.87, an indicative of accurate method. Accordingly, it can be concluded that the developed reverse phase HPLC method is meets validation criteria and it is strongly useful for study of alprazolam in regular analysis. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies.

Conclusion

The dissolution conditions were 500 ml of potassium dihydrogen phosphate buffer pH 6.8 at 37°C, paddle apparatus at stirring speed 100 rpm for 12 hrs. Thus, proposed dissolution method and HPLC method can be applied successfully for quality control analysis of alprazolam in tablet formulation.

Aim: The aim of this study was to find out a simple, accurate and sensitive HPTLC method for the detection and quantification of marker molecule, churna (alkaliods) on these Ayurvedic formulations for standardization.

Materials and methods:

Chemicals and reagents

TLC plates coated with silica gel 60F254 for HPTLC were purchased from Merck, and sample of cough syrup were given for FDA Organization. All other chemicals, reagents and solvents were used are of AR grade.

Preparation of test samples

1 g of each raw material and churna sample was taken separately, refluxed with 20 ml of methanol for 30 min, filtered through Whatmann filter paper no. 41 and this procedure was repeated thrice. The pooled filtrate was concentrated and volume was adjusted to 10 ml with methanol in a volumetric flask. Aliquot of each extract was further diluted 50% for quantification by HPTLC.

Standard solution

Standard stock solution was prepared by dissolving 10 mg of Chlorpheniramine Maleate, Dextromethasone HBr, Phenylephrine Hydrochloride, Diphenylephrine Hydrochloride, Salbutamol Sulphate in 10 ml methanol and sonicated, which yields a solution of concentration 1 mg/ml. Working standard was prepared from this stock solution at concentration 10 µg/ml.

Instrumentation and chromatographic condition

A Camag HPTLC system comprising of Linomate V automatic sample applicator with Camag TLC Scanner 3 and Camag WinCAT software were used for detection and quantification of churna in the single herbs and Ayurvedic formulations. The standard solutions and test samples were spotted in the form of bands (8 mm bandwidth) with 100 µl Hamilton syringe on pre-coated silica gel plates (Merck, 60F254, 10 × 10 cm) using Camag Linomate V applicator. The plates developed upto 80 mm with a solvent system (toluene: ethyl acetate = 7:3, v/v) in Camag glass twin-trough chamber previously saturated with mobile phase vapor for 30 min at 25 °C. The densitometric scanning was performed on Camag TLC Scanner 3 at absorbance 342 nm (deuterium lamp, slit dimension 5.0 × 0.45 µm) and operated by multilevel WinCATS planar chromatography manager software. Spots were well resolved in the chromatogram of extracts of samples from single herbs or Ayurvedic powder formulations, and the spot of standard Chlorpheniramine Maleate, Dextromethasone HBr, Phenylephrine Hydrochloride, Diphenylephrine Hydrochloride, Salbutamol Sulphate was at Rf value in Figure given below.

GRAPH

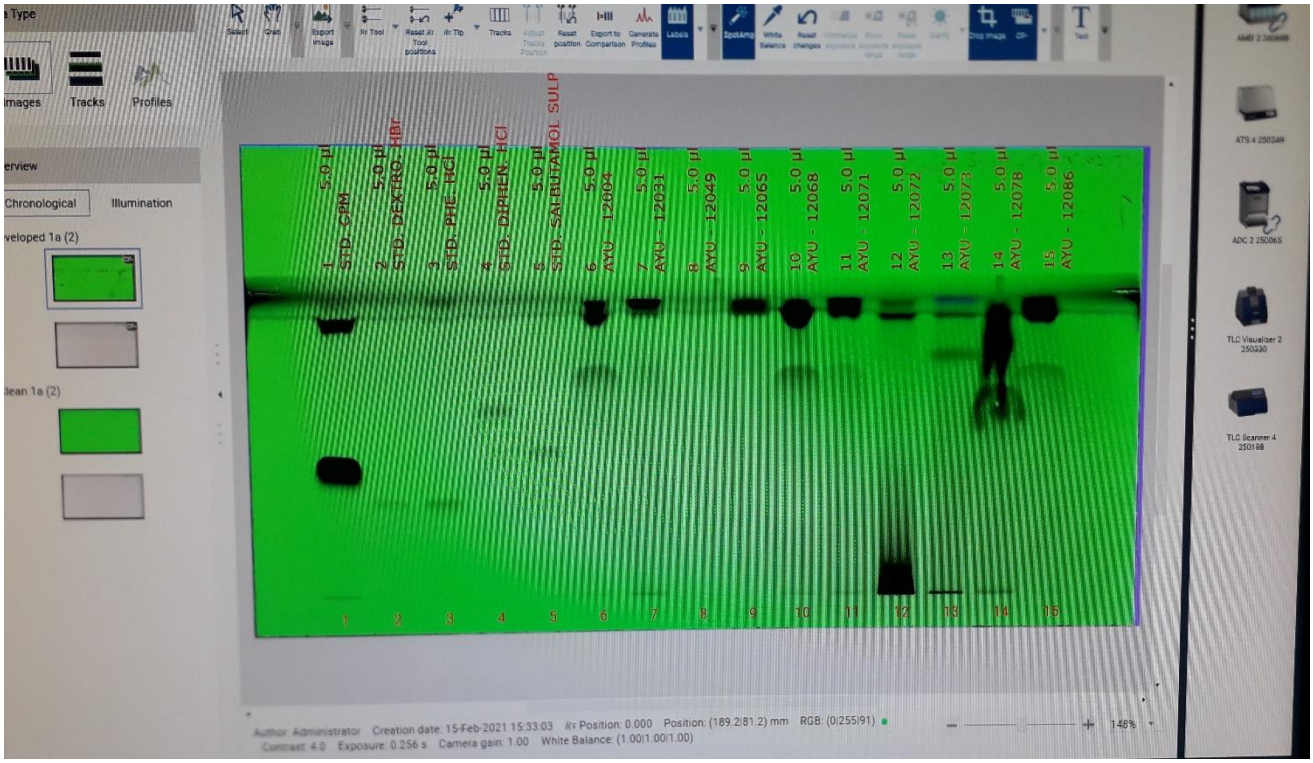


Fig 1.25. Chromatogram of Standard and Sample (HPTLC)

Results and discussion

Churna are used for identify the steroids adulterant are not present in none of the Ayurvedic sample which are certified for Medicinal used. Sample of Churna is a common marker compound present in several species of Piperaceae family such as P. longum, P. nigrum, P. chaba etc. This alkaloid is tasteless, but its stereoisomer, chavicine, is the active ingredient in black pepper that provides its characteristic taste. Loss of pungency during storage of black pepper is attributed to the slow isomerization of chavicine into piperine. Piperine is considered as a known marker compound that is usually assayed to authenticate pipul, marich etc. abundantly used in compound formulation of Ayurvedic drugs from ancient times. The physicochemical properties of the test samples were permitted within the limit values

The HPTLC procedure was optimized with a view to develop a stability indicating assay method. The solvent system of the mobile phase having toluene: ethyl acetate (7: 3, v/v) gave dense, compact and well separated spots of the single herbal ingredients as also Ayurvedic formulation at 342 nm.

Conclusion

This HPTLC method can be successfully employed for standardization and quantitative analysis of churna in Ayurvedic formulations as well as raw materials and also be helpful to clinicians and pharmacists.

Aim: To Determination of moisture, fat and starch in Channa/Paneer

Preparation of Sample of Channa/Paneer

Pass channa sample through 8 mesh sieve three times or grate sample and mix thoroughly or grind to a uniform mass in a glass pestle and mortar. Grate the paneer sample quickly through a suitable grater. Mix the grated sample thoroughly. Transfer

the grated sample to an air-tight container to await analysis, which should be carried as soon as possible after grinding. Keep sample in an airtight container until the time of analysis. If delay is unavoidable, take all precautions to ensure proper preservation of the sample, and to prevent condensation of moisture on the inside surface of the container. The storage temperature should be below 10°C.

Table 1.7. of Sample of Channa/Paneer.

Code Sample of Paneer/Channa by FDA	Characteristic of Paneer/Channa
M-2060/20	Malai Paneer
M-2137/20	Paneer
M-1914/20	Channa
M-1137/20	Paneer
M-2160/20	Channa
M-1457/20	Channa
M-2140/20	Malai Paneer
M-18/20	Channa
M-1194/20	Paneer

Determination of Moisture in Channa/Paneer

The moisture content of channa is the loss in mass, expressed as a percentage by mass when the product is heated in an air oven at 102 ± 2°C to constant mass.

Apparatus

A. Flat-bottom dishes with lid: Dishes of nickel, aluminium or of other suitable metal not affected by boiling water, 70 to 80 mm in diameter and not more than 25 mm deep, provided with short glass stirring rod having a widened flat end. The dishes shall have lids which fit well and can readily be removed.

B. Hot air oven: Maintained at 102 ± 1°C.

C. Desiccator: Containing an efficient desiccant.

D. Sand: Which passes through 500 μ sieve and is retained by 180 μ sieve. It shall be prepared by digestion with concentrated HCl, followed by thorough washing with water. It shall then be dried and ignited till it is dull red.

Procedure

A. Heat the flat-bottomed metal dish containing 20 g of prepared sand and a stirring rod, in hot air oven for about 1 h. Allow to cool in an efficient desiccator for 30 to 40 min. Weigh accurately 3 g of the prepared sample of channa into a flat-bottomed dish (with a cover) previously dried and weighed containing about 20 g of prepared sand and a stirring rod.

B. Saturate the sand by careful addition of a few drops of distilled water, and thoroughly mix the wet sand with the channa sample by stirring with the glass rod, smoothing out lumps and spreading the mixture over the bottom of the dish.

C. Place the dish on a boiling water-bath for 20 to 30 min, then wipe the bottom of the dish. Transfer the dish containing the material, along with glass rod after uncovering in an oven maintained at 102 □ 1°C for about 4 h.

D. After 4 h replace the lid, transfer the covered dish to the desiccator, allow it to cool to room temperature and weigh it accurately and quickly to the nearest 0.1 mg.

E. Heat the uncovered dish and lid in the oven at 102 ± 1°C for further 1 h, replace the lid, allow the covered dish to cool to room temperature in the desiccator and weigh it. Repeat the process of drying, cooling and weighing, until the successive weighing do not differ by more than 0.5 mg. Record the weight.

Calculation

$$Moisture\ \%\ by\ mass = \frac{M1 - M2}{M1 - M} \times 100$$

Where,

M = mass in g, of the empty dish with containing glass rod;

M1 = Initial mass in g of the dish, lid, glass rod along with the material taken for

analysis;

M2 = the final mass in g of the dish, lid, glass rod along with the material after drying.

Table 1.8. of Moisture content of Paneer/Channa Sample

Sr. No	Weight of Sample	Moisture content in Sample
1	10.4758g	61.2%
2	10.5574g	47.08%
3	10.5984g	60.21%
4	10.6548g	59.25%
5	10.7643g	45.39%
6	10.9235g	44.67%
7	10.3521g	33.78%
8	10.0261g	43.45%
9	10.1653g	38.54%

Determination of Fat (by Acid Digestion Method) in Channa/Paneer

Weigh accurately 1-2 g of prepared sample in a 100 ml beaker. Add 10 ml of conc. hydrochloric acid. Heat on a Bunsen burner, stirring continuously with a glass rod, or on a boiling water bath until all solid particles are dissolved. Cool to room temperature. Add 10 ml of ethyl alcohol first to the beaker and later transfer the contents to the Mojonnier fat extraction flask or the Rohrig tube Transfer to the Mojonnier fat extraction flask. Mix well. Then, add 25 ml of ethyl ether to the beaker and from the beaker to the

Mojonnier flask or the Rohrig tube. Stopper with cork or a stopper of synthetic rubber

resistant to usual fat solvents. Shake vigorously for one minute. Add 25 ml of petroleum ether and repeat vigorous shaking for one minute. Centrifuge Mojonnier flask at about 600 rpm. If Rohrig tube is used, let it stand until upper liquid is practically clear. Decant the ether solution into a suitable flask, metal dish or glass bowl. Wash the tip and the stopper of the extraction flask or tube with a mixture of equal parts of the two solvents and add the washings to the weighing flask or tube and repeat extraction of liquid remaining in the flask or tube successively using 15 ml of each solvent. Add, if necessary, water to bring its level in tube to original mark. This may be necessary in the case of milk powder. Repeat extraction using 15 ml of each solvent. Evaporate the solvent completely on water bath at a temperature that does not cause sputtering or bumping. Dry the fat in oven at 102 ± 2°C to a constant weight. Weigh the cooled flask or metal dish or glass bowl. Remove the fat completely from the container with warm petroleum ether and weigh as before.

Calculation

$$\text{Fat, \% (w/w)} = \frac{100\ (W1 - W2)}{W3}$$

Where,

W1 = Weight in g of contents in the flask or metal dish or glass bowl before removal of fat.

W2 = Weight in g of contents in the flask or metal dish or glass bowl after removal of fat and

W3 = Weight in g of material taken for the test.

Table 1.9. of Fat content in Sample

Sr. No	Weight of beaker (W1)	Weight of ghee + beaker (W2)	Weight of Sample (W3)	Fat%	B.R. reading
1	146.1212	147.0273	10.4758g	28.65%	41.37%
2	149.8228	150.8831	10.5574g	30.10%	42.83%
3	139.9920	141.2483	10.5984g	31.80%	40.49%
4	148.5624	149.5462	10.6548g	35.65%	41.65%

5	143.3636	150.5305	10.7643g	66.58%	42.36%
6	141.4266	142.8615	10.9235g	13.14%	40.06%
7	142.7240	144.8604	10.3521g	20.63%	41.63%
8	149.7151	151.1647	10.0261g	14.58%	43.38%
9	148.7791	150.3860	10.1653g	15.80%	47.26%

Result:

The product shall conform to the compositional specifications provided in the table below:−

[62][Parameter	*Chhana or Paneer*	*Medium fat Chhana or Paneer*	*Low fat Chhana or Paneer*
Moisture, maximum, %, (m/m)	65.0 (for *Chhana)* 60.0 (for *Panner)*	65.0 (for *Chhana)* 60.0 (for *Panner)*	70.0 (for *Chhana)* 70.0 (for *Panner)*
Milk fat, %, (m/m), dry matter basis	50.0 (minimum)	More than 20.0 and less than 50.0	20.0(maximum)]

Butyro Refractometer (BR) Reading is the index of the purity of foods like ghee, sweets, fats and oils which can be accurately measured with the help of Butyro Refractometer Meter or BR meter. There are several BR meters that are especially designed for accurate reading of composition of butter, ghee and edible oil to check for purity.

The BR value for ghee is supposed to be at 40o C. An increase in the reading above this indicates that the ghee could be adulterated with vegetable oil or animal body fat.

BR meter give highly accurate readings so as to determine if the ghee sample is within the BR range as provided in the standards. The standards of quality of ghee produced in India vary according to the State or Union Territory and therefore the BR reading can be between **40.0 to 45.0** depending on the state and region.

Conclusion:

The method of Butyro Refractometer (BR) Reading, Moisture and Fat content test are specified for all milk and milk product test are helpful for identify the adulterant present in given sample which legal for FSSAI Organization.

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13. Appendix

APPENDIX