INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.2.2022.12(28)

ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF RAMIPRILIN ITS BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ramipril in Tablet dosage form. Chromatogram was run through xterra (4.6 x 150mm, 5µm). Mobile phase containing Methanol and phosphate buffer in the ratio of 20:80 was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength for ramipril was 222nm. Retention time of Ramipril were found to be 100.26 %RSD of the Ramipril were found to be 0.9 %Recover was Obtained for Ramipril. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

Keywords: Ramipril, RP-HPLC, Chromatography and retention time.

INTRODUCTION

1. Importance of drug analysis

'Health is wealth'. It is vital fact that a healthy body is desire of every human being. Good health is first condition to enjoy the life and all other things which mankind is having. Nowadays peoples are more concentrating towards health. Even governmental bodies of different countries and World health organization (WHO) are also focusing for health of human being. Health care is prevention, treatment and management of illness and preservation of mental and physical well being. Health care embraces all the goods and services designed to promote health including preventive, curative and palliative in interventions. The Health care industry is considered an industry or profession which includes people's exercise of skill or judgment or providing of a service related to the prevention or improvement of the health of the individuals or the treatment or care of individuals who are injured, sick, disabled or infirm. The delivery of modern health care depends on an Interdisciplinary Team.

The medical model of health focuses on the eradication of illness through diagnosis and effective treatment. A traditional view is that improvement in health results from advancements in medical science. Advancements in medical science bring varieties of medicines. Medicines are key part of the health care system. The numerous medicines are introducing into the world-market and also, that is increasing every year. These medicines are being either new entities or partial structural modification of the existing one. So, to evaluate quality and efficacy of these medicines is also important factor. Right from the beginning of discovery of any medicine quality and efficacy of the same are checked by quantification means. Quality and efficacy are checked by either observing effect of drug on various animal models or analytical means. The option of animal models is not practically suitable for every batch of medicine as it's require long time, high cost and more man-power. Later option of analytical way is more suitable, highly precise, safe and selective.

The analytical way deals with quality standards which are assigned for products to have desirable efficacy of the medicines. Sample representing any batch are analyzed for these standards and it is assumed that drug/medicine which is having such standards are having desire effect on use. Quality control is a concept, which strives to produce a perfect product by series.

HPLC Pumps

Because of the small particles used in modern HPLC, modern LC pumps need to operate reliably and precisely at pressures of 10,000 psi or at least 6,000 psi. To operate at these pressures and remain sensibly inert to the wide variety of solvents used HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats. For analytical purposes HPLC pumps should have flow rates that range from 0 to 10 ml/min., but for preparative HPLC, flow rates in excess of 100 ml/min may be required. It is extremely difficult to provide a very constant flow rate at very low flow rates. If 1% is considered acceptable then for 1ml/min a flow variation of less than 10µl/min is required. This level of constancy is required because most HPLC detectors are flow sensitive and errors in quantization will result from change in flow rate.

HPLC Sample Valves

Since sample valves come between the pump and the column it follows that HPLC sample valves must also tolerate pressures up to 10,000 psi. For analytical HPLC, the sample volume should be selectable from sub micro liter to a few micro liters, whereas in preparative HPLC the sample volume may be even greater than 10 ml. To maintain system efficiency the sample valve must be designed to have very low dispersion characteristics, this is true not only for flow dispersion but also for the less obvious problems of dispersion caused by sample adsorption/desorption on valve surfaces and diffusion of sample into and out of the mating surfaces between valve moving parts. It goes without saying that the valves must deliver a very constant sample size but this is usually attained by the use of a constant size sample loop.

HPLC Columns

HPLC columns are packed with very fine particles (usually a few microns in diameter). The very fine particles are required to attain the low dispersion that give the high plate counts expected of modern HPLC. Plate counts in excess of 25,000 plates per column are possible with modern columns, however, these very high efficiencies are very rarely found with real samples because of the dispersion associated with injection valves, detectors, data acquisition systems and the dispersion due to the higher molecular weight of real samples as opposed to
the common test samples. Packing these small particles into the column is a difficult technical

HPLC Detectors

UV/Vis spectrophotometers, including diode array detectors, are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, light scattering detectors, radioactivity detectors or other special detectors may also be used.

Detector consists of a flow-through cell mounted at the end of the column. 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. Fixed (mercury lamp), variable (deuterium or high pressure xenon lamp), and multi-wavelength detectors are widely available. Modern variable wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi-wavelength detectors measure absorbance at two or more wavelengths simultaneously. In diode array multi-wavelength detectors, continuous radiation is passed through the sample cell, and then resolved into its constituent wavelengths, which are individually detected by the photodiode array. These detectors acquire absorbance data over the entire UV-visible range, thus providing the analyst with chromatograms at multiple, selectable wavelengths, spectra of the eluting peaks and also peak purity.

Differential refractometer detectors measure the difference between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographic compounds as it emerges from the column. Refractive index detectors are used to detect non-UV absorbing compounds.

Fluorometric detectors are sensitive to compounds that are inherently fluorescent or that can be converted to fluorescent derivatives either by chemical transformation of the compound or by coupling with fluorescent reagents at specific functional groups.

Potentiometric, voltametric, or polarographic electrochemical detectors are useful for the quantitation of species that can be oxidized or reduced at a working electrode. These detectors are selective, sensitive, and reliable, but require conducting mobile phases free of dissolved oxygen and reducible

metal ions. Electrochemical detectors with carbon-paste electrodes may be used advantageously to measure nanogram quantities of easily oxidized compounds, notably phenols and catechols.
In order to give an accurate chromatographic profile the detector sampling (cell) volume must be a small fraction of the solute elution volume. If the detector volume were larger than the elution volume then you would have peaks that appeared with flat tops as the whole peak would be resident in the detector at the same time. This means that as column volumes decrease and system efficiencies increase the volume of the detector cell volume must also decrease. It is odds for the requirement for detector to maintain high sensitivity as this is usually dependant on having a larger cell volume. Again, this requires the very careful design of modern detectors. Many types of detectors can use with HPLC system like UV-Visible or PDA (Photo Diode Array), RI (Refractive Index), Fluorescence, ECD (Electro Chemical Detector), ELSD (Evaporative Light Scattering detector) and many others hyphenated techniques like MS, MS/MS and NMR as well as evaporative IR.

HPLC Data acquisition
In HPLC data acquisition system the higher sampling rate needed for the rapidly eluting narrow peaks of the HPLC chromatogram. Although the theoretical number of samples needed for good quantization are actually quite small, for real systems a hundred samples or more per peak is recommended; thus, for a 4 sec wide peak, a rate of 25 samples per second may be required. The same data analysis and reporting software can be used as in ordinary LC.

CONCLUSION
HPLC is probably the most universal type of analytical procedure; its application areas include quality control, process control, forensic analysis, environmental monitoring and clinical testing. In addition HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures. It has achieved this position as a result of the constant evolution of the equipment used in LC to provide higher and higher efficiencies at faster and faster analysis times with a constant incorporation of new highly selective column packings.

Introduction to HPLC Methods of Analysis for Drugs
Most of the drugs in single/multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures. Some of the advantages are

- Speed (analysis can be accomplished in 20 minutes or less).
- Greater sensitivity (various detectors can be employed).
- Improved resolution (wide variety of stationary phases).
- Reusable columns (expensive columns but can be used for many analysis).
- Ideal for the substances of low volatility.
- Easy sample recovery, handling and maintenance.
- Instrumentation tends itself to automation and quantitation (less time and less labour).
- Precise and reproducible.
- Calculations are done by integrator itself.
- Suitable for preparative liquid chromatography on a much larger scale.

Separation Mode of HPLC
Various modes of HPLC utilized to separate compounds are classified as follows:
1) Adsorption chromatography
2) Normal-phase chromatography
3) Reversed-phase chromatography
4) Ion-pair chromatography
5) Ion-exchange chromatography
6) Size exclusion chromatography

1. Adsorption chromatography
Adsorption chromatography uses polar stationary phases with relatively non-polar mobile phases. Separations in adsorption chromatography result to a great extent from the interaction of sample polar functional groups with discrete adsorption sites on the stationary phase. Adsorption chromatography is usually considered appropriate for the separation of nonionic molecules that are soluble in organic solvents.

2. Normal-phase chromatography

In HPLC, if stationary phase is more polar than the mobile phase, it is termed as normalphase liquid chromatography. Polar bonded phases that have a diol, cyano, diethylamino, amino, or diamino functional groups are used as stationary phase in normal-phase chromatography. Due to lower affinity of non-polar compounds to the stationary phases used, non-polar compounds are elute first while polar compounds are retained for longer time. Normal-phase chromatography is widely applied for chiral separations.

3. Reversed-phase chromatography

In HPLC, if stationary phase is less polar than the mobile phase, it is termed as reversedphase liquid chromatography. In this technique, C18, C8, Phenyl, and cyano-propyl functional groups that chemically bonded to micro porous silica particles are used as stationary phase. Retention in reversed phase chromatography occurs by nonspecific hydrophobic interactions of the solute with stationary phase. The ubiquitous application of reversed-phase chromatography arise from the fact that practically all organic molecules have hydrophobic regions in their structures and effectively interact with the stationary phase. It is estimated that over 65% (possibly as high as 90%) of all HPLC separations are executed in the reversed-phase mode. The rationale for this includes the simplicity, versatility, and scope of the reversed-phase method[14].

4. Ion-pair chromatography

Ionic or partially ionic compounds can be chromatographed on reversed phase columns by using ion-pairing reagents. These reagents are typically long chain alkyl anions or cations that, when used in dilute concentrations, can increase the retention of analyte ions. C-5 to C-10 alkyl sulfonates are commonly used for cationic compounds while C-5 to C-8 alkyl ammonium salts are generally used in the cases of anionic solutes.

5. Ion-exchange chromatography

Ion-exchange chromatography is an adaptable technique used primarily for the separation of ionic or easily ionizable species. The stationary phase is characterized by the presence of charged centers having exchangeable counter ions. Both anions and cations can be separated by choosing the suitable ion-exchange medium. Ion-exchange chromatography employs the

DRUG PROFILE

RAMIPRIL

Description

Ramipril is an ACE inhibitor used for the management of hypertension and the reduction of cardiovascular mortality following myocardial infarction in Themodynamically stable patients with clinical signs of congestive heart failure

Structure

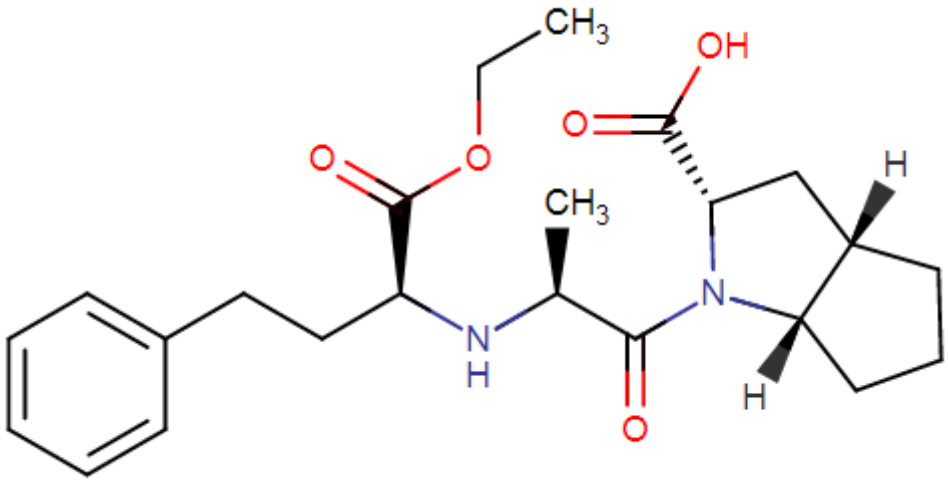


Fig. 4: Structure of Ramipril

Chemical Name
(2*S*,3*aS*,6*aS*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3*a*,4,5,6,6*a*-hexahydro-2*H*-cyclopenta[b]pyrrole-2-carboxylic acid.

Molecular Formula
$C_{23}H_{32}N_2O_5$

Molecular Weight
416.5g/mol

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Xterra (4.6 x 150mm, 5μm)
Buffer	:	Phosphate buffer
pH	:	4.5
Mobile phase	:	Methanol: Phosphate buffer (20:80)
Flow rate	:	1 ml per min
Wavelength	:	222nm
Injection volume	:	20 μL
Run time	:	10 min.

EXPERIMENTAL METHOD

Table 1: Instruments used

SL. NO	INSTRUMENT	MODEL
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

SL. No	Chemical	Company Name
1	Ramipril	PHARMATRAIN
2	Water for HPLC	FINER chemical LTD
3	Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	MOLYCHEM
5	KH_2PO_4	MERCK
6	NaOH	FINER chemical LTD

Method Development and Design of Separation Method
Methods for analyzing drugs in single or multi component dosage forms can be developed, provided one has knowledge about the nature of the sample, namely, its molecular weight, polarity, ionic character and the solubility parameter. An exact recipe for HPLC, however, cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. In general one begins with reversed phase chromatography, when the compounds are hydrophilic in nature with many polar groups and are water soluble.
The organic phase concentration required for the mobile phase can be estimated by gradient elution method. For aqueous sample mixtures, the best way to start is with gradient reversed phase chromatography. Gradient can be started with 5-10 % organic phase in the mobile phase and the organic phase concentration (methanol or acetonitrile) can be increased up to 100 % within 30-45 min. Separation can then be optimized by changing the initial mobile phase composition and the slope of the gradient according to the chromatogram obtained from the preliminary run. The initial mobile phase composition can be estimated on the basis of where the compounds of interest were eluted, namely at what mobile phase composition.
Changing the polarity of mobile phase can alter elution of drug molecules. The elution strength of a mobile phase depends upon its polarity, the stronger the polarity, higher is the elution. Ionic samples

(acidic or basic) can be separated, if they are present in undissociated form. Dissociation of ionic samples may be suppressed by the proper selection of pH.
The pH of the mobile phase has to be selected in such a way that the compounds are not ionized. If the retention times are too short, the decrease of the organic phase concentration in the mobile phase can be in steps of 5%. If the retention times are too long, an increase of the organic phase concentration is needed.
In UV detection, good analytical results are obtained only when the wavelength is selected carefully. This requires knowledge of the UV spectra of the individual components present in the sample. If analyte standards are available, their UV spectra can be measured prior to HPLC method development.
The molar absorbance at the detection wavelength is also an important parameter. When peaks are not detected in the chromatograms, it is possible that the sample quantity is not enough for the detection. An injection of volume of 20 µL from a solution of 1mg/mL concentration normally provides good signals for UV active compounds around 220 nm. Even if the compounds exhibit higher λmax, they absorb strongly at lower wavelength.
It is not always necessary to detect compounds at their maximum absorbance. It is, however, advantageous to avoid the detection at the sloppy part of the UV spectrum for precise quantitation. When acceptable peaks are detected on the chromatogram, the investigation of the peak shapes can help further method development.
The addition of peak modifiers to the mobile phase can affect the separation of ionic samples. For examples, the retention of the basic compounds can be influenced by the addition of small amounts of triethylamine (a peak modifier) to the mobile phase. Similarly for acidic compounds small amounts of acids such as acetic acid can be used. This can lead to useful changes in selectivity.

The parameters that are affected by the changes in chromatographic conditions are:
1. Resolution (RS).
2. Capacity factor (k').
3. Selectivity (α).
4. Plate number (N).
5. Asymmetry factor (T).

1. Resolution (RS)
Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture.

$$R = \frac{2(tR,2 - tR,1)}{Wb,1 + \mathrm{Wb},2} = 1.\frac{177(tR,2 - tR,1)}{W0.5,1 + W0.5,2}$$

If the peak base widths wb,1 and wb,2 are approximately the same, the resolution R signifies the number of times the peak width wb can be fitted into the distance between the peak maxima. At a resolution of R=0.5, two maxima can still be perceived separately. For quantitative analysis, a resolution of up to R=1.5 is desirable; greater values of the resolution lead only to unnecessarily long analysis times.
The resolution R is dependent on the parameters k2' (capacity factor of the later eluted substance), selectivity α and plate number N of the column:

$$R = \frac{\sqrt{N}}{4} * \frac{\alpha - 1}{\alpha} * \frac{k2'}{1 + k2'}$$

2. Capacity factor (k')
The retention time tR is the qualitative information of a chromatogram. it is constant for a given component provided the chromatographic conditions remain unchanged (column, mobile phase, temperature etc.). for the characterization of substance, it is more convenient to quote the capacity factor k' since, in contrast to the retention times, this is dependent neither on the flow of the eluent nor on the column length

$$k' = \frac{tR'}{t0} = \frac{tR - t0}{t0} = \frac{tR}{t0} - 1$$

3. Selectivity (α)

The selectivity (or separation factor) is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components. This parameter is independent of the column efficiency, it only depends on the nature of the components, eluent type, eluent composition and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency.

The ideal value of α is 2. It can be calculated by using formula,

$$\alpha = \frac{k2'}{k1'} = \frac{tR,2 - t0}{tR,1 - t0} \left(k2' > k1'\right)$$

4. **Plate number (N)**

An additional useful quantity to characterize a separation system is the plate number N (number of theoretical plates). A theoretical plate is defined as that zone of separation system within which a thermodynamic equilibrium is established between the mean concentration of a component in the stationary phase and its mean concentration in the mobile phase. Efficiency is calculated by using the formula,

N = 16 (tR / wb)2

Where, tR is the retention time.
wb is the peak width.

5. **Asymmetry factor (T)**

The elution of chromatographic signals as Gaussian peaks is often not achieved in practice. An asymmetric peak shape, known as tailing, is often found. The peak asymmetry is quantified by the asymmetry factor (tailing factor) T with a and b being determined at 10 % peak height:

$$\mathbf{T} = \frac{\mathbf{b}}{\mathbf{a}}$$

For the trouble-free evaluation of the area of a peak, T must be < 2.5, above this, the end of the peak can be recognized only with difficulty. For a well-packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

HPLC METHOD DEVELOPMENT

Wave length selection

UV spectrum of 10 µg/ml Ramipril in diluent (mobile phase composition) was record by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 222 nm. At this wavelength both the drugs show good absorbance.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Methanol: 0.1% OPA, Acetonitrile: Phosphate buffer and Methanol: Phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate buffer (pH 4.5) in proportion 20: 80 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column Phenomenex column, Inertsil ODS column and YMC column. Xterra (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Phosphate buffer

Take 3.4 gms of potassium di hydrogen ortho phosphate in 1000 ml of HPLC water, pH was adjusted with NaOH up to 4.5. Final solution was filtered through 0.45 µ Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 200 ml of Methanol(20%) and 800 ml of above buffer (80%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Standard Solution Preparation

Accurately weigh and transfer 100 mg of Ramipril working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (30 ppm Ramipril)

Sample Solution Preparation

Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 100 mg (750 mg of tablet power) of Ramipril sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution)

Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (30 ppm Ramipril)

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Ramipril peaks and calculate the %Assay by using the formulae.

VALIDATION PARAMETERS

1. LINEARITY:

Preparation of stock solution

Accurately weigh and transfer 100 mg of Ramipril working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – I (10 ppm of Ramipril**)**

1 ml of above stock solutions has taken in 10 ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II (20 ppm of Ramipril**)**

2 ml of above stock solutions has taken in 10 ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – III (30 ppm of Ramipril**)**

3 ml of above stock solutions has taken in 10 ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – IV (40 ppm of Ramipril**)**

4 ml of above stock solutions has taken in 10 ml of volumetric flask, dilute up to the mark with diluent

Preparation of Level – V (50 ppm of Ramipril**)**

5 ml of above stock solutions has taken in 10 ml of volumetric flask, dilute up to the mark with diluent

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance Criteria
Correlation coefficient should be not less than 0.99.

2. PRECISION
Preparation of stock solution
Accurately weigh and transfer 100 mg of Ramipril working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (30 ppm Ramipril)

Procedure
The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Acceptance Criteria
The % RSD for the area of six standard injections results should not be more than 2%
.
3. INTERMEDIATE PRECISION/RUGGEDNESS
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution
Accurately weigh and transfer 100 mg of Ramipril working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (30 ppm Ramipril)

Procedure
The standard solutions prepared in the precision were injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

4. ACCURACY
Preparation of Standard stock solution
Accurately weigh and transfer 100 mg of Ramipril working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (30 ppm Ramipril)

Preparation Sample solutions
For preparation of 50% solution (With respect to target Assay concentration)
Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 50 mg (375 mg of tablet power) of Ramipril sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (15 ppm Ramipril)
For preparation of 100% solution (With respect to target Assay concentration)
Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 100 mg (750 mg of tablet power) of Ramipril sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent

and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (30 ppm Ramipril)

For preparation of 150% solution (With respect to target Assay concentration)
Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 150 mg (1125 mg of tablet power) of Ramipril sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (45 ppm Ramipril)

Procedure
Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.
Calculate the Amount found and Amount added for Ramipriland calculate the individual recovery and mean recovery values.

Acceptance Criteria
The % Recovery for each level should be between 98.0 to 102.0%.

5. ROBUSTNESS
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.
A. The flow rate was varied at 0.8 ml/min to 1.2 ml/min.
Standard solution 30 ppm of Ramipril was prepared and analysed using the varied flow rates along with method flow rate.
On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.
***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.
The organic composition in the Mobile phase was varied from 18% to 22%.
Standard solution 30 ppm of Ramipril was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.
On evaluation of the above results, it can be concluded that the variation in 10%.
Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10%
***** Results for actual Mobile phase composition (20:80 Methanol: Phosphate buffer pH 4.5) has been considered from Accuracy standard.

RESULTS AND DISCUSSION
SYSTEM SUITABILITY

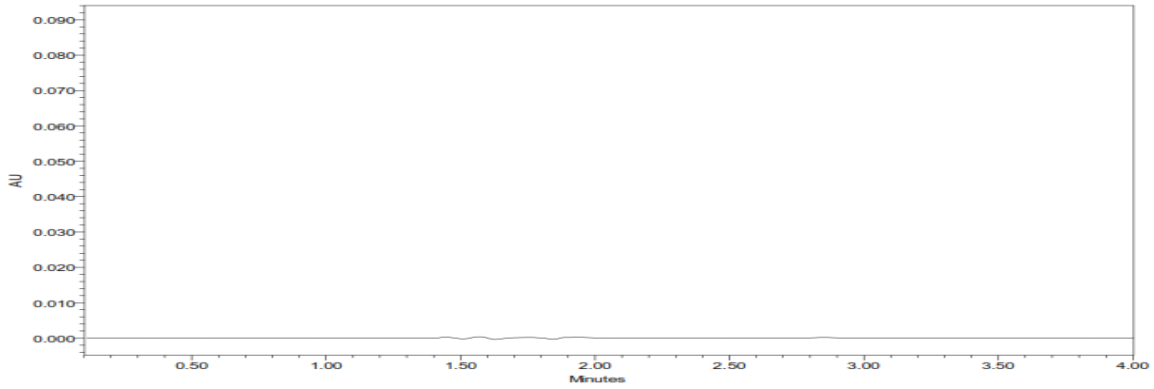


Fig. 1: Chromatogram for Blank

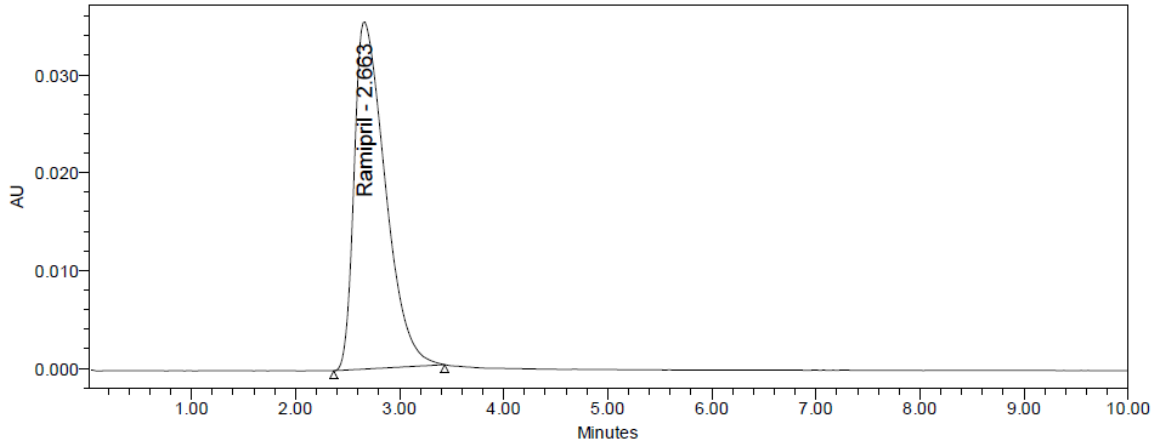


Fig. 2: Chromatogram for system suitability

Table 3: Results of system suitability parameters

S. No.	Name	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Ramipril	2.663	227215	34184	1.27	4083.63

Acceptance criteria

- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION PARAMETERS

1. LINEARITY

The linearity range was found to lie from 10µg/ml to 50µg/ml of Ramipril and chromatograms are shown below.

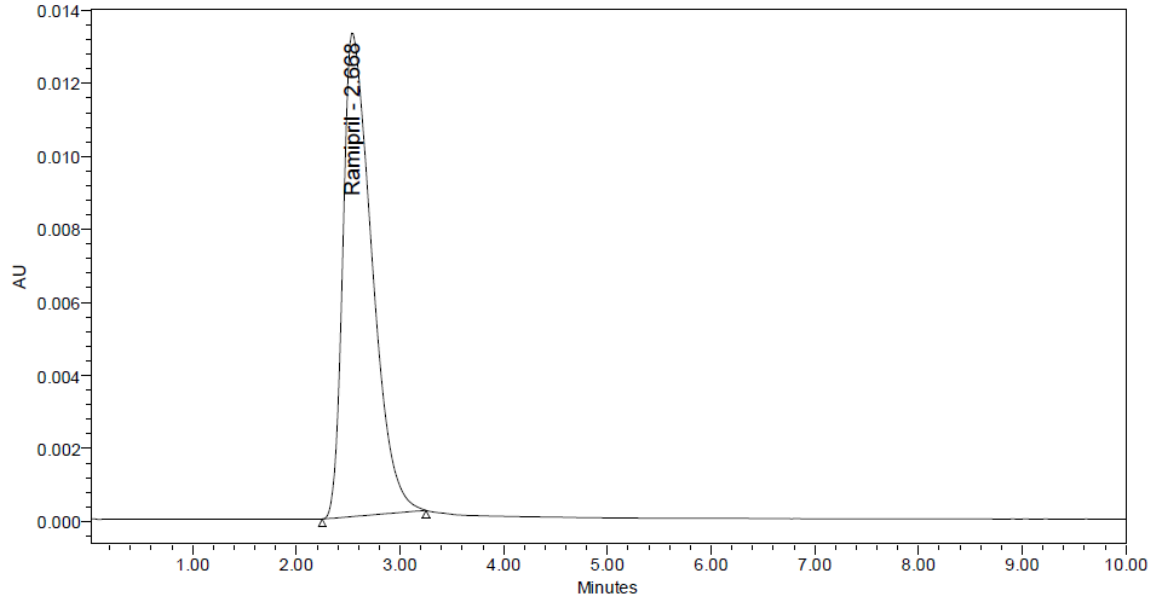


Fig. 3: Chromatogram for linearity-1

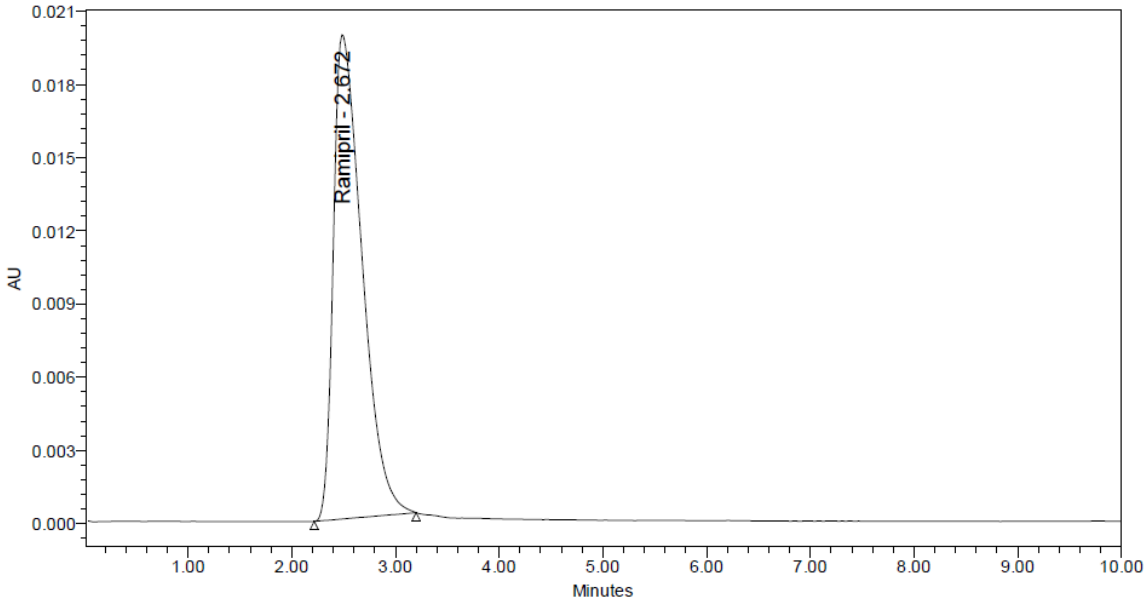


Fig. 4: Chromatogram for linearity-2

2. PRECISION

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

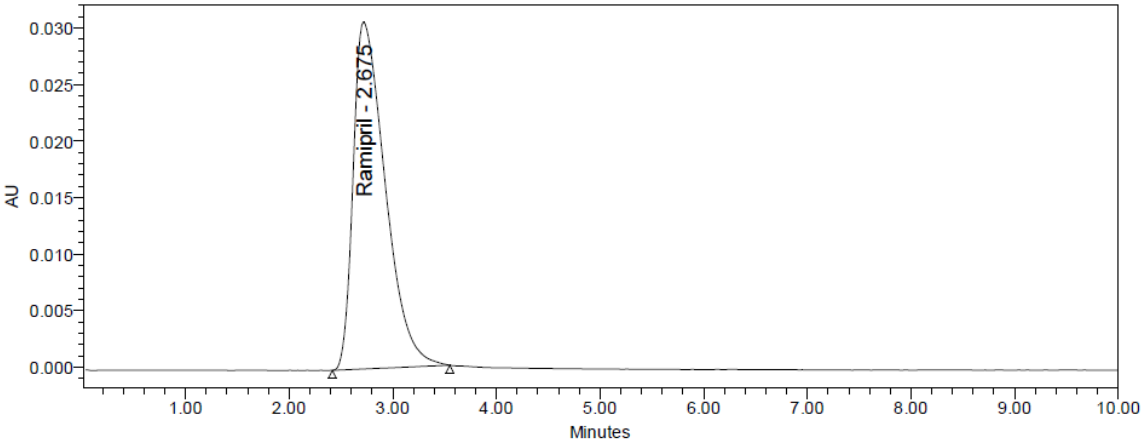


Fig. 5: Chromatogram for Precision -1

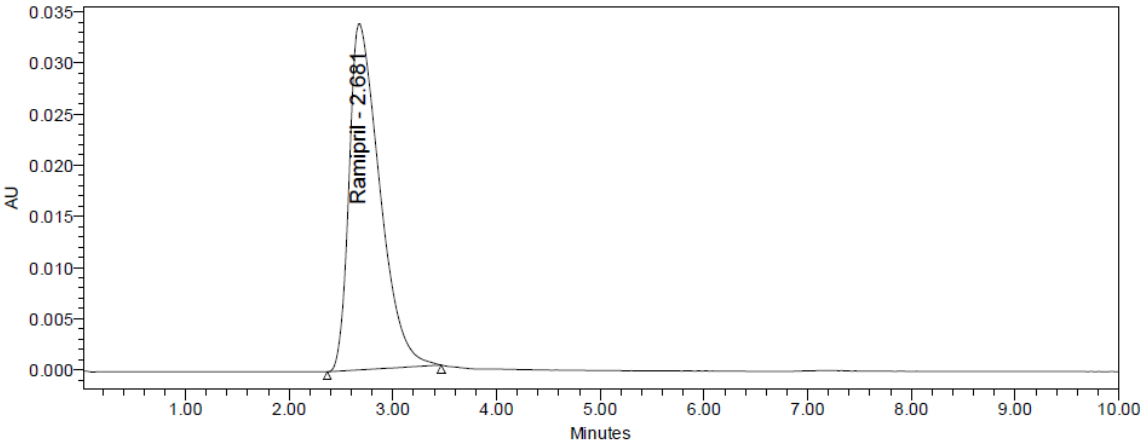


Fig. 6: Chromatogram for Precision -2

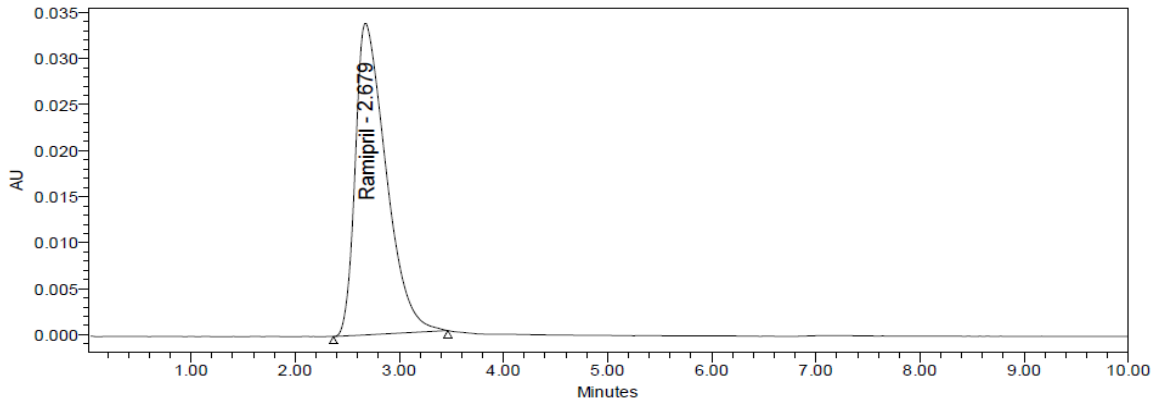


Fig. 7: Chromatogram for Precision -3

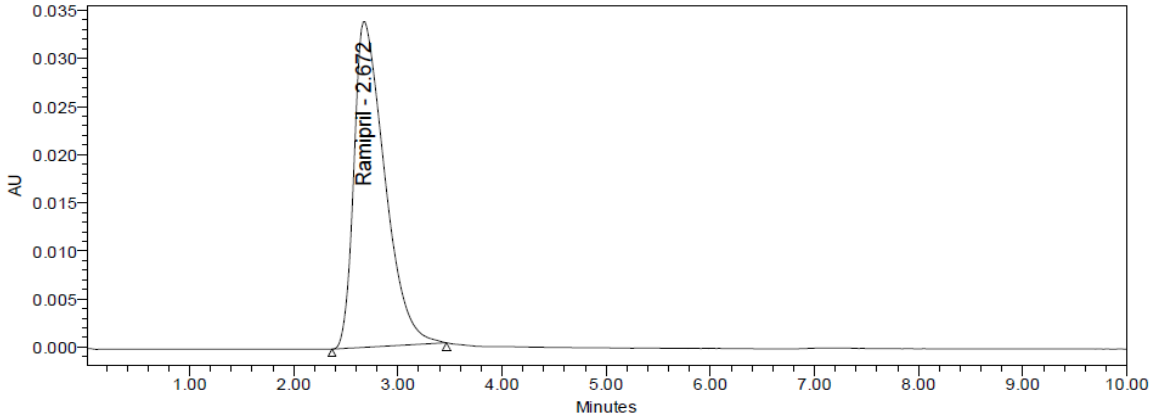


Fig. 8: Chromatogram for Precision -4

Table 4: Results of Precision for Ramipril

Injection	Area
Injection-1	227934
Injection-2	229378
Injection-3	224385
Injection-4	228979
Injection-5	226498
Injection-6	224984
Average	227026.3
Standard Deviation	2078.2

Table 5: Results for variation in flow for Ramipril

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4186.97	1.28
2	1.0	4083.63	1.27
3	1.1	4231.65	1.25

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

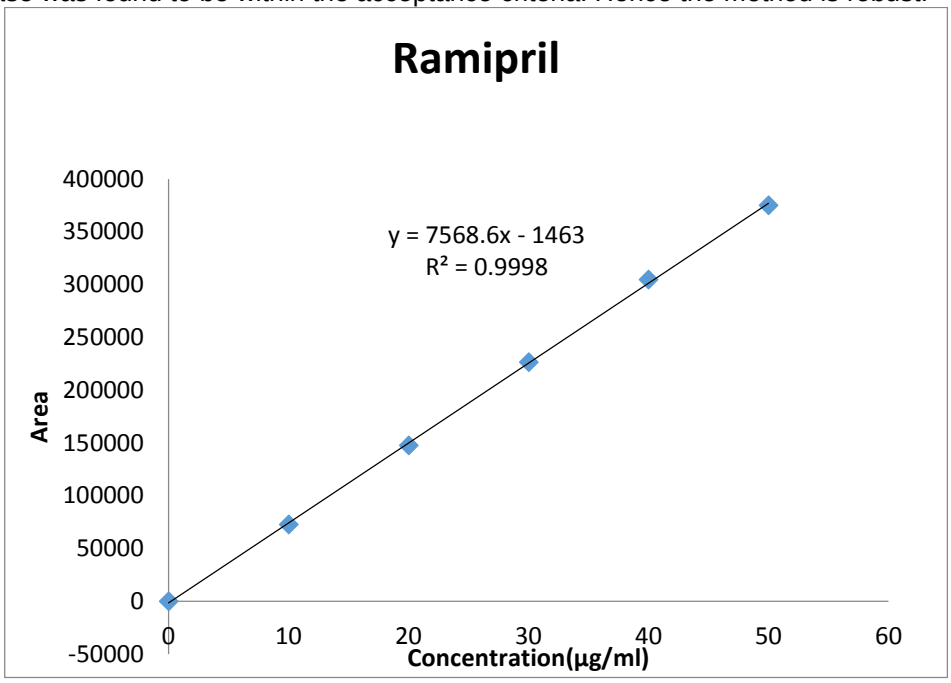
Table 6: Results for variation in mobile phase composition for Ramipril

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3963.85	1.29
2	*Actual	4083.63	1.27
3	10% more	4205.88	1.27

***** Results for actual Mobile phase composition have been considered from Accuracy standard.

Acceptance criteria

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.



SUMMARY AND CONCLUSION

The estimation of Ramipril was done by RP-HPLC.

The assay of Ramipril was performed with tablets and the % assay was found to be 100.26 which show that the method is useful for routine analysis.

The linearity of Ramipril was found to be linear with a correlation coefficient of 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.9 for Ramipril which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 1.0 for Ramipril which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 100.71% for Ramipril. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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