METHOD DEVELOPMENT AND VALIDATION OF ALBUTEROL AND IPTATROPIUM BROMIDE IN TABLET DOSAGE FORM BY USING RP-HPLC

SYED GHOUSE
Assistant Professor
Department of Pharmaceutical Analysis and Quality Assurance
VIJAYA college of Pharmacy
Hyderabad
Contact No: 9700248964
syedghouse27@gmail.com

DR.R.SUTHAKARAN
Professor and Principal
Department of Pharm. Chemistry and Analysis
Vijaya College of Pharmacy,Hyderabad
9440394053
drsutharaj@gmail.com

INTRODUCTION

An additional suitable expression for a pharmaceutical is active pharmaceutical ingredient (API) / active ingredient to differentiate it from a devised product / drug product is ready by designing a drug sub with inert ingredient (excipient) to prepare a drug product that is appropriate for admin to patients.

The process is usually residential in an AR&D & transferred to QC / other dept. as needed. At times they are transported to other divisions.

1. Recognized uniqueness and clarity.

2. Recognized bio ccessibility/dissolution.

DIFFERENT TYPES OF CHROMATOGRAPHY

- Adsorption Chromatography
- Partition Chromatography
- Ion switch Chromatography
- Molecular barring Chromatography
- Affinity Chromatography

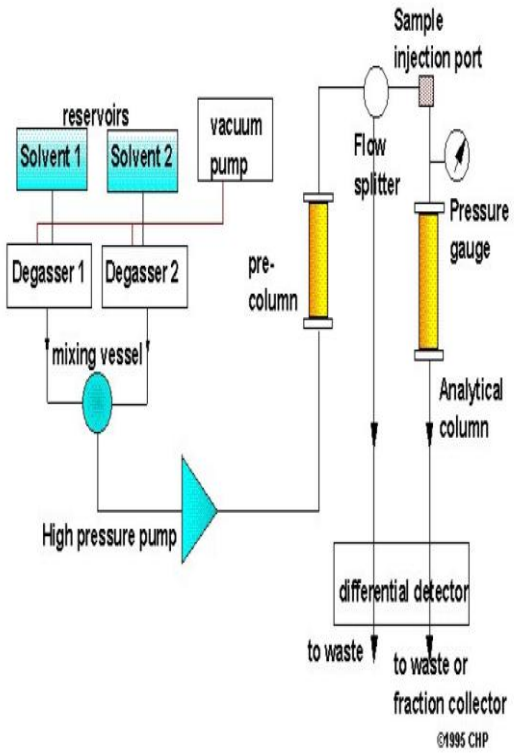
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

HPLC is able to separate macromolecules and ionic species, labile natural products, polymeric materials and a wide variety of other high-molecular weight poly functional groups.

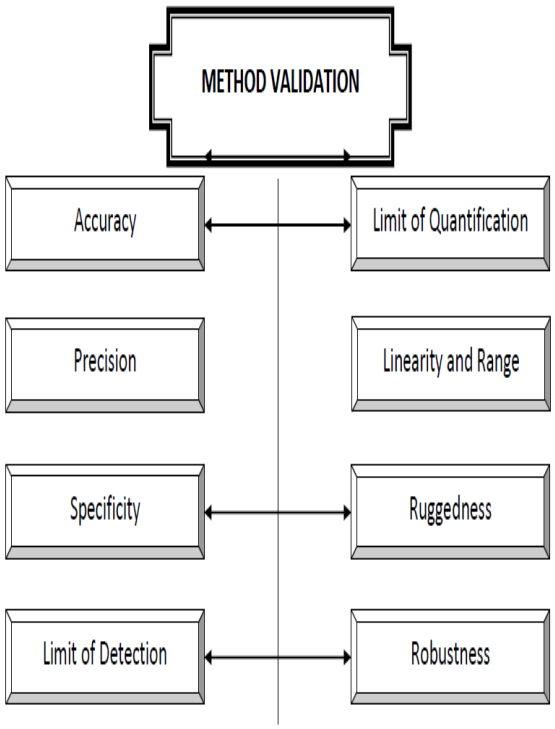
Basic principle of HPLC

High performance liquid chromatography (HPLC) is a separation technique utilizing differences in distribution of compounds to two phases; called stationary phase & mobile phase. The stationary phase a thin layer created on the surface of fine particles and the mobile phase designates the liquid flowing over the particles. Under a certain dynamic conditions, each component in a sample has difference distribution equilibrium depending on solubility in the phases and or molecular size.

Schematic diagram for HPLC instrument



METHOD VALIDATION



LITERATURE REVIEW

Gajanan B. Kasawar, Mazahar N. Farooqui Development and validation of a stability indicating RP-HPLC method for the simultaneous determination of related substances of albuterol sulfate and ipratropium bromide in nasal solution. A new RP-HPLC method was developed and validated for the simultaneous assay of albuterol sulphate and Ipratropium bromide in nasal inhalations. a non-polar peerless basic C8 column using a mixture of anhydrous potassium dihydrogen orthophosphate,1-pentane sulphonic acid sodium salt monohydrate (pH-4.0) and acetonitrile (95:5 v/v) along with a mixture of anhydrous potassium dihydrogen orthophosphate, 1-pentane sulphonic acid sodium salt monohydrate (pH 4.0) and acetonitrile (70:30 v/v) as mobile phase in gradient elution mode. The retention time for albuterol sulphate and ipratropium bromide was at 2.927 ± 0.25 min and 10.479 ± 0.76 min. and the analyte peaks were analysed at 276 nm. and 220 nm. respectively over a run time of 22 minutes. The method obeyed range of 0.0100 – 0.2080 mg/mL and 0.0023 – 0.0468 mg/mL for albuterol sulphate and ipratropium bromide respectively and the low coefficients of variation obtained in the intraday (0.8 % – 1.0 %) and inter day precision (1.1 % – 1.4 %) study are indicative of the precision of the method. High recovery of albuterol sulphate (98.0 – 102.0 %) and ipratropium bromide (98.1 – 101.9 %) indicate the accuracy of the method.

GUMMADI SOWJANYA, Jillella V L N Seshagiri Rao, DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ALBUTEROL SULPHATE AND IPRATROPIUM BROMIDE IN NASAL INHALATIONS A new RP-HPLC method was developed and validated for the simultaneous assay of albuterol sulphate and Ipratropium bromide in nasal inhalations. a non-polar peerless basic C8 column using a mixture of anhydrous potassium dihydrogen orthophosphate,1-pentane sulphonic acid sodium salt monohydrate (pH-4.0) and acetonitrile (95:5 v/v) along with a mixture of anhydrous potassium dihydrogen orthophosphate, 1-pentane sulphonic acid sodium salt monohydrate (pH 4.0) and acetonitrile (70:30 v/v) as mobile phase in gradient elution mode. The retention time for albuterol sulphate and ipratropium bromide was at 2.927 ± 0.25 min and 10.479 ± 0.76 min. and the analyte peaks were analysed at 276 nm. and 220 nm. respectively over a run time of 22 minutes. The method obeyed range of 0.0100 –

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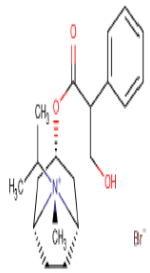
Drug Profile

Ipratropium

IUPAC Name	(1R,3R,5S,8R)-3-[(3-hydroxy-2-phenylpropanoyl)oxy]-8-methyl-8-(propan-2-yl)-8-azabicyclo[3.2.1]octan-8-ium bromide
Structure	
Molecular formula	$C_{20}H_{30}BrNO_3$
Molecular weight	412.361gm/mol
Half life	2-4 hours
Category	• Bronchodilator Agents • Muscarinic Antagonists

MATERIALS AND METHODS

: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu(LC 20 AT VP)
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(250x4.6mm) 5µm

Table : Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Phosphate	AR Grade
Acetonitrile	HPLC Grade
Potassium dihydrogen phosphate	AR Grade

RESULTS AND DISCUSSION

Solubility Studies

ALBUTEROL:

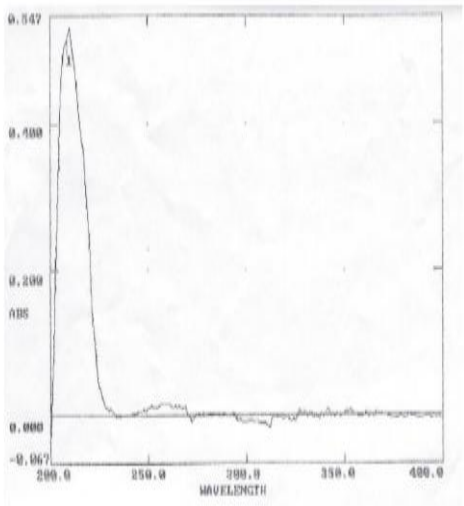
Soluble in methanol. Insoluble in water.

IPRATROPIUM BROMIDE:

very slightly soluble in water, soluble in methanol & ACN.

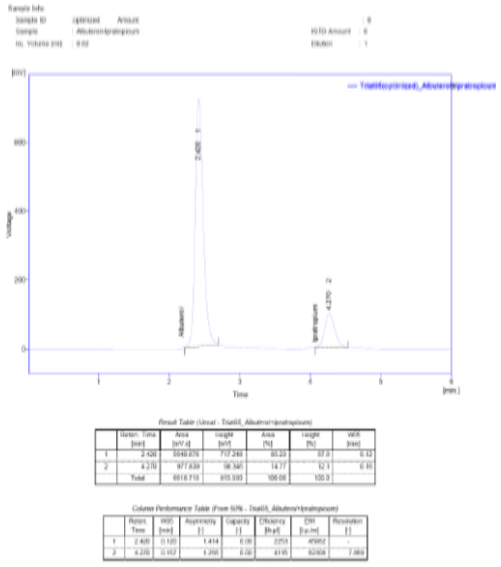
Determination of ISOBESTIC POINT(λmax)

The lambda max of maximum absorption (λmax) of the drug, 10 µg/ml sol of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 223 nm for ALBUTEROL, 207 nm for IPRATROPIUM BROMIDE and 239 nm for the combination.



UV-VIS spectrum of IPRATROPIUM BROMIDE

METHOD DEVELOPMENT OF ALBUTEROLand IPRATROPIUM BROMIDE



Chromatogram of IPRATROPIUM BROMIDE and ALBUTEROLby using mobile phase

Observation:

- All the system suitability requirements were met.
- The peak irregularity factor was <2 for both IPRATROPIUM BROMIDE & ALBUTEROL.
- The efficiency was ≥ 2000 ALBUTEROL &. IPRATROPIUM BROMIDE
- Resolution b/w 2 peaks >1.5.
- Hence this method was for optimized.

Assay

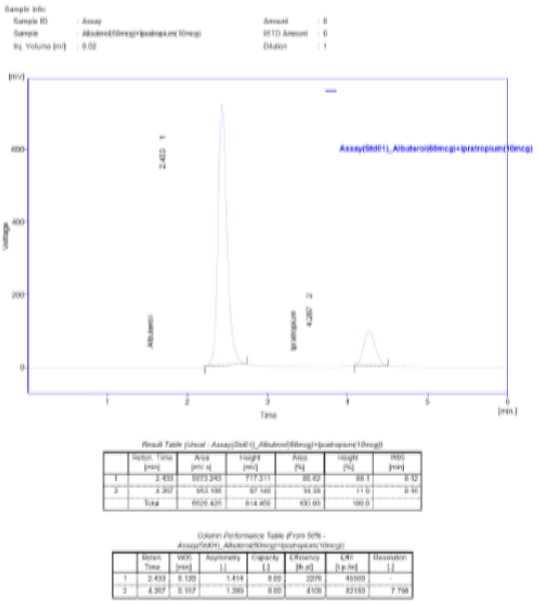
Preparation of mixed standard solution

Weigh 60 mg of ALBUTEROL and 40 mg of IPRATROPIUM BROMIDE in 100 ml of Vf & dissolve in 10ml of M.P & make up the vol with mobile phase. From above SS 60 µg/ml of ALBUTEROL and 40 µg/ml of IPRATROPIUM BROMIDE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

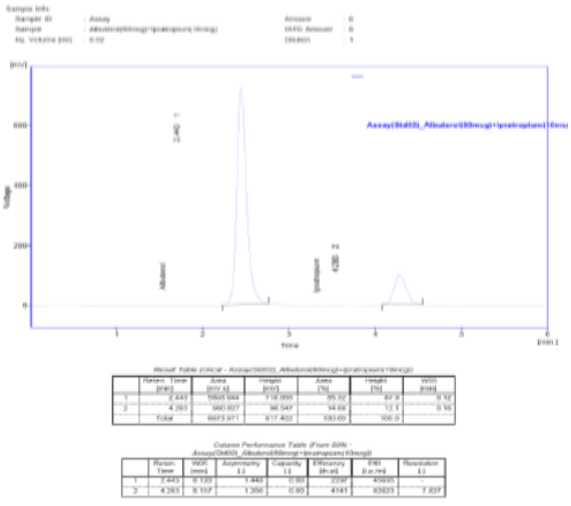
Calculation

The amount of IPRATROPIUM BROMIDE & ALBUTEROLpresent in the formulation by using the formula given below, and results shown in above table:

$$\%\,\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$



Chromatogram of Assay standard preparation-1



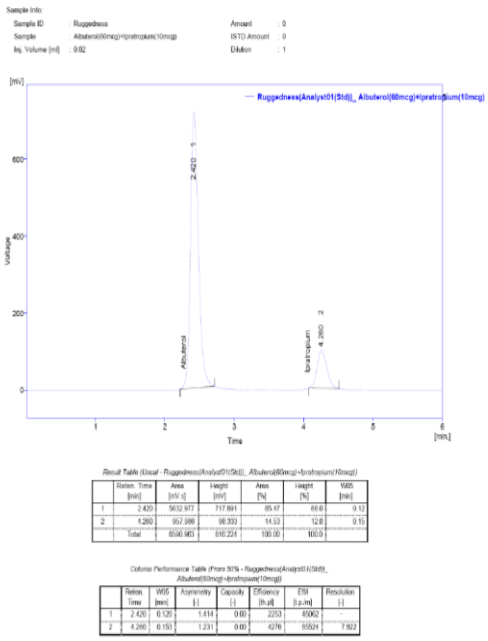
Chromatogram of Assay standard preparation-2

Trial-5: (Optimized): Chromatographic conditions

Mobile phase : Methanol:Water
pH : -
Ratio : 80:20
Column : Inertsil ODS 3V column, C18(250x4.6 ID) 5µm
Wavelength : 239 nm
Flow rate : 1.0ml/min

Ruggedness

Acceptance criteria: The % RSD of Assay values between two analysts should be NMT 2.0%.



Chromatogram of Analyst 01 standard preparation

Results for Ruggedness

ALBUTEROL	%Assay	IPRATROPIUM BROMIDE	%Assay
Analyst 01	100.53	Analyst 01	98.65
Anaylst 02	100.40	Anaylst 02	100.41

Conclusions

Analytical Method Development and validation for simultaneous estimation of Albuterol and Ipratropium Bromide in Pharmaceutical dosage forms". From the results & parameters it was concluded that, this recently developed process for the estimation of Ipratropium Bromide & Albuterol was found to be simple, accurate ,precise, and high resolution & shorter RT makes this method more satisfactory & cost efficient and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Reference:

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6. GUMMADI SOWJANYA, Jillella V L N Seshagiri Rao, Development and validation of a new rp-hplc method for the simultaneous determination of albuterol sulphate and ipratropium bromide in nasal inhalations
7. Gajanan B. Kasawar, Mazahar N. Farooqui Development and validation of a stability indicating RP-HPLC method for the simultaneous determination of related substances of albuterol sulfate and ipratropium bromide in nasal solution.