Development Validation of RP-HPLC Method for Simultaneously Estimation of Levosalbut Amolsulphate and IpratropiumbromIde in bulk and numuliser Dosage Form.

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The importantmanagementstrategiesaresmokingcessatio n,vaccinations, rehabilitationandtherapyobtainedtheintakethecombinationpratropium bromideandlevosulbutamolsulphatewillhelpparentettigedifferentaspectsofCOPD bronchodilationthroughdifferentmechanismsandmimmflamation withinhalersteroids.SulphuricacidisashortactingBet a2adrenargic receptoragonistusedforreliefofbronchospiamsincondit ionsuchasaasthma COPD.

Itisalsoindicatethemanagementofacuteattackofbronchospasm. Salbutamolsulphateactsbystimulatingtheadenylyclase enzymewhich catalysestheformationofcyclic3-5AdinosinemonophosphatecyclicAMP thesiformsmediatethecellularresponses.Relaxationof bronchiolesand smoothmuscle.
Salbutamolsulphateiseffectivebyoralandinhalationalrout eof administration.Salbutamolsulphatehasusedintablet,syr up,metereddose inhalerandnebulizedinhalaionsolution.IpratropiumB romide(Figure2) antagonizetheactionofacetylcholinebyblockingmusc arinicCholinergic receptorsresultinginbronchodilationanddryingofrespir atorytract. Ipratropiumblocksmuscarinicacetylcholinergic receptors,without specificityforsubtypes.Thereforepromotethedegradati on of cyclicGuanosinemonophosphate(cGMP),resultingi ncreased intracellularconcentrationofcGMP.Mostlikelydue-to cGMPOn intracellularcalcium,thisresultsindecreasecontractilityo f smoothmuscle inthelung,inhibitingbronchoconstrictionandmucussecretion.

ItIsanonselectiveMuscarinicantagonist,anddoesnotdiff useintotheblood,whichpreventionstheintracardiac effects.Ipratropiumisaderivativeof atropinebutisaQuaternaryamineandthereforedoesnotcr ossbloodbrain barrier.Whichpreventssentralsideeffectsanticholinerg icsyndrome.

- Chemicallyitis[(methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]oct-3-yl)hydroxy-2-phenyl]propanoate. Andhas empirical formula of C13H21NO3 and C20H30BrNO3
- Levosalbutamol- Itsisindicatedforpatientswithchronicobstructivelpulmonarydiseases(COPD)onregularaerosolbronchospasmmandwho requireasecondbronchodilator.6-9LevosalbutamolSulphateiswhiteoalmostwhitethesolidosclusterspiroofwaterand Leviprotium bromidealsowhitealmostwhitethesolidosclusterspiroofwater, sol ubeinwater, freelysolubleinmethanol,slightlysolubleinethanol(95 %)

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Toevaluateitheefficacyandsafetysalbutamolares areusesince1960 fortreatasthmainchildrenandadolescents.

ToevaluatetheefficacyandsafetyofI+B+salbutamolintreatmentof asthmachildrenandadolescents. I+B+salbutamolmaybemoreeffectivespulmonarysalbutamol oneforthe treatmentofasthmachildrenandadolescents,especially inthosewith severeandmoderatetosevereasthma exacerbation.Theverylowqualityoffevidencedicatedthatfuturewell-designeddouble-blindRCTswith largesamplereareneede toperformonevaluatingtheeffectivenessofI+B+ salbutamolintreatmentofasthmachildrenandadolescents. Asthmaisthemostcommonchronicdiseaseamongchild renandisestimatedtoaffect300millionindividualsworldwide.InC hina,asthma affects3%ofchildren≤14yearsofageandthe prevalenceofchildhood
Asthma has increased by 50% over the past 10 years. Asthma-related hospitalization can negatively affect the quality of life of children and their caregivers. Additionally, healthcare expenditures for asthma-related conditions impose considerable economic burden on society. Almost all available guidelines recommend that the repeated administration of inhaled short-acting β2-agonists (SABAs, up to 4–10 puffs every 20 minutes for the first hour) is an effective and efficient way to achieve rapid relief of airflow limitation in patients with mild-to-moderate asthma exacerbation. In the latest guideline, SABA-only treatment is no longer recommended for asthma in adults or adolescents due to the risk of asthma-related death and urgent asthma-related healthcare. Currently, several available guidelines recommend the addition of ipratropium bromide (IB), a short-acting muscarinic acetylcholine receptor antagonist, to SABA as an optional treatment for children and adolescents with acute asthma exacerbation. Although IB does not seem to be very efficient in controlling asthma, several studies have demonstrated that a combination of IB and albuterol sulphate is associated with fewer hospitalizations and greater improvement in peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1).

**Instrumentation:**
- **RP-HPLC method**
- **Method used in RP-HPLC (Careas follows):**
  1. The sample is first dissolved in liquid or the mobile phase.
  2. The sample is then injected into a continuous flow of mobile phase, being delivered by a pump and carried onto the LC column which contains a stationary phase.

Compared with SABA alone in children and adolescents with moderate-to-severe asthma exacerbation [10–15], the addition of IB to SABA has been recommended in the first hour of treatment for children with moderate-to-severe exacerbations. However, these recommendations lack uniformity with respect to the optimum age, severity of asthma, and co-intervention with other asthma controllers for such therapy.
2). The various components of the sample travel through the column at different speeds due to their interactions between the mobile and stationary phases, resulting in the components separating from one another. The different travel times are referred to as the components' peak times.

3). When components emerge from the column, they are carried to a detector where a physical property of the compounds is measured, such as absorption of light for UV detection. It's important to note that there are many different detectors available. Some of them include ultraviolet/visible (UV/Vis), photodiode array (PDA), fluorescence (FL), and refractive index (RI). Each response plotted over time results in a chromatogram.

Principle of RP-HPLC

On the basis of the absorption of the solvent component, the principle of RP-HPLC chromatography techniques includes:

1) Sample cell (stationary phase + mobile phase).
2) Automiser
3) Monochromator
4) Detector
5) Recorder or amplifier.

Chemical structure of drug used in COPD areas follows:

1) Levosalbutamol sulphate
2) Ipratropium bromide
Detection of different group of drugs by using different techniques of chromatography method as follows:

R group chromatography method

1) Alkyl Reversephase
2) cyano. Normal Creversephase
3) Amide. Reversephase
4) Amino. Normal Creversephase
5) Dimethylamine. Weak ion exchanger
6) Quaternary amine. Strong ion exchanger
7) Carboxylic acid. Weak cation exchanger
8) Phenyl. Reversephase
9) Sulfonic acid. Strong cation exchanger

MOBILE PHASE:
The mobile phase used in HPLC depends on the components to be separated and the technique used whether normal phase, reverse phase, or ion exchange chromatography etc.

(i) For aspirin, ibuprofen, the solvent system used is water-acetonitrile-methanol in definite proportion and pH adjusted in the acidic range with phosphoric acid.

(ii) For paracetamol, indomethacin, the solvent system used is methanol-water-dioxane in definite proportion.

Aim: To development and validation of RP-HPLC method for simultaneous estimation of levosalbutamol sulfate and ipratropium bromide bulk and numuliser dosage form. To validate the method according to ICH guidelines.

Objective: -
1) New, easy, delicate, precise and economical analytical techniques for RPHPLC testing of the title ingredients.

2) Validate the proposed method for the intended analytical application in accordance with USP and ICH guidelines.

3) Apply the proposed method for the analysis of dosage form.

A). Steps in developing the method and optimization of chromatographic condition.
   - Literature survey
   - Selection of drugs
   - Selection of detection wavelength
   - Selection of chromatographic conditions
   - Selection of Mobile Phase (Selection of Organic solvent and aqueous solvent).
   - Selection of suitable pH
   - Selection of Column and Column temperature
   - Optimization of Mobile phase, Column and Solvents system.

B). Stability indicating analytical method validation using RP-HPLC as per ICH guidelines.
   - Specificity
   - Linearity
   - Precision

Plan of work:
The experimental work has been planned as follows:
Review of the literature for levosalbutamol sulphate regarding physical and chemical properties, various analytical methods that we re-conducted; for levosalbutamol sulphate form the basis for development of new analytical RP-HPLC method for levosalbutamol sulphate.

DEVELOPMENT OF THE METHOD BY RP-HPLC
1). Selection of the solvent to be used as diluents and mobile phase.
   Choosing the suitable solvent in which the drug is soluble and stable. They must be easily available, economical and of the HP LC grade.
2). Selection of Mobile phase:
   For the mobile phase, the first variable to be decided is whether an organic or aqueous eluent should be used. With the RP-HPLC analysis, either an aqueous eluent or a very polar organic solvent such as Water and Methanol should be fixed. If the K’ value is too large with an aqueous solvent, organic solvents should be tried. If the K’ value is too low with an organic solvent, the separations should be attempted using a mixture of two solvents with various properties.
   - K’-capacity factor is a measurement of the degree where the peak of interest is located with respect to void volume, i.e., elution time of non-retained components.

Generally, the value of K’ is > 2. If a buffer is used, the pH and ionic strength of the buffer can be critical.
1). In order to select the wavelength to carry out the analysis, a critical examination of the Ultraviolet absorbance spectra of the drug should be done.
2). A perfect study of the structure of the drug and its physicochemical properties; to select the Chromatographic parameters.
4). Validation of the method established by following the rules of the ICH.

VALIDATION OF METHOD
Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce desired results or product meeting its predetermined specifications and quality characteristics.

Method validation is the process of demonstrating the procedure is suitable for the intended use and that it supports the identity, quality, purity and potency and bioavailability of the drug substance and drug.

Simply, method validation is for the improvement of the quality of the product. This method and determine if the method is followed variability for the conditions needed to be determined during the process.

Different validation methods:
1). Identification test.
2). Quantitative test for impurities content.
3). Limit test for the control of impurities.
4). Quantitative test of the active moiety in samples of drug substances.

Chemicalordrugs:

<table>
<thead>
<tr>
<th>SR No.</th>
<th>Ingredients</th>
<th>Company</th>
<th>Brand/Batch no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Levosalbutamol Sulphate eq. to Levosalbutamol 2.5 mg + Water for Injections</td>
<td>CIPLA</td>
<td>Salbair A5003AP</td>
</tr>
<tr>
<td>2</td>
<td>Levosalbutamol Sulphate eq. to Levosalbutamol 1.25 mg + Ipratropium Bromide 0.50 mg + Water for Injections</td>
<td>CIPLA</td>
<td>Salbair I A2607JAP</td>
</tr>
</tbody>
</table>

ExperimentalChemicals and Reagents:

- Ipratropium bromide 99% (Molecular Weight: 412.37 g/mol) and Levosalbutamol 99% (Molecular Weight: 239.31 g/mol) purity acquired from Cipla Pharmaceuticals Mumbai, India.
- Acetonitrile HPLC Grade from Rankem Fine Chemicals and HPLC Grade water.
- Potassium Phosphate (Dibasic KHPO) 0.03 M from Ran kem Fine Chemicals AR grade.
- Ortho-Phosphoric Acid, 85%, Quligens Fine Chemicals and HPLC Grade water.

Ml volumetric flask containing 5 ml of diluent (50:50 v/v Acetonitrile: Water), sonicated for about 15 min and then made up to 10 ml with diluent to get the primary standard stock solution containing 2 mg/ml Ipratropium bromide and 5 mg/ml Levosalbutamol.

For analysis of Ipratropium bromide in rotacaps, a simple, easily available and reliable RP-HPLC method with UV-detection has been developed and validated for the simultaneous determination of Ipratropium bromide and Levosalbutamol concentrations in metered-dose inhalers.

Preparation of the Primary Standard Drug Solution:

A standard stock solution of the drug was prepared by dissolving 20 mg of Ipratropium bromide and 50 mg of Levosalbutamol in 10 mL of diluent.

Preparation of Working Standard Drug Solution:

Ml of the above stock solution was taken in 100 ml volumetric flask and thereafter made up to 100 ml with diluent (50-
50% Acetonitrile: Water) to get the working standard solution containing 20 µg/mL of Ipratropium Bromide, 0.1 and 50 µg/mL of Levosalbutamol. From the above work, 1.5 ml 2% 5 ml C30, 0.1 and 50 µg/mL of Ipratropium Bromide and 5-15 µg/mL of Levosalbutamol respectively. Analysis of Pharmaceutical Metered Inhalers: Remove the pressurized container (Duolin Inhaler MDI, Cipla, Each puff contains Ipratropium bromide 20 µg /mL and Levosalbutamol 50 µg/mL as suspended in propellant HFA 227-qs in net weight of content. The equivalent to 21 µg of Ipratropium Bromide and 60 µg of Levosalbutamol from the actuator and remove all the labels and markings with suitable solvent. Dry the container, replace the actuator, shake for about 30 seconds and prime the metered valve assembly. Discharge once for waste: wait for not less than 5 seconds and discharge again to waste. Remove the pressurized container from the actuator, clean the valve stem (internally and externally) and the valve ferrule. Use the isopropyl alcohol as the solvent. Dry the complete valve assembly using a clean air line fitted with an appropriate narrow jet to ensure that all solvent is removed from the inside of the valve stem. Place a tripped stainless steel base plate with a central circular indentation of 1.5 mm diameter in the small vessel suitable for shaking and add 15 ml of the diluent. The valve stem is soaked in the solvent. Shake the pressurized container for about 30 seconds and place in an inverted position in the vessel. Discharge 120 deliveries from the valve stem at a rate of not less than 5 seconds, maintaining the pressure.

RESULT AND CONCLUSION:
Consider the efficiency of the drug of RPHPLC method development simple, accurate, rapid for simultaneous estimation of Levosalbutamol, sulphate and Ipratropium bromide in bulk and numuliser dosage forms. The proposed method was simple, specific and sensitive and can be used for simultaneous estimation of Levosalbutamol and Ipratropium bromide in bulk and numuliser dosage forms. The result of the study followed the protocol of ICH guidelines and can be successfully applied for the simultaneous estimation of the marketed products of Levosalbutamol and Ipratropium bromide in bulk and numuliser dosage forms.

REFERENCE:
[2]. Jeyabalan, Garima Yadav, Rajesh Yadav, Subash Gupta and Habibullah Khalilullah, rapid and accurate RP-HPLC method was developed for the determination of levosalbutamol in pure and tablet dosage forms. Journal of Applied Pharmaceutical Sciences, 02(06); 2012: 155-158
[3]. Gajanan B. Kasawar, Mazahar. Farooqui, RP-HPLC method was developed for the quantification of related impurities of albuterol sulfate (AS) and ipratropium bromide (IB) in liquisolid pharmaceutical dosage form. Journal of Pharmaceutical and Biomedical Analysis, Volume 52 Issue 1, May 2010, page 19-29
[4]. Dr. Pai Nandini R. Patil, Swapnali Suhas, The high performance liquid chromatographic method was developed for the determination of Levosalbutamol sulphate and Ipratropium bromide pressurized metered dose inhaler forms. Research Journal of Pharmacy and Technology, 2013, volume 6, issue: 7, First page (774) Last page (779)
[5]. Sohan S. Chitlange, Kaushalendra K. Chaturvedi and Sagar B. Wankhede, developed and validated
HPLC method for simultaneous estimation of salbutamol and prednisolone respectively in tablet dosage form. 

V. Sri Kalyani, D. Meena Bhathini, M. Anusha, B. Chandra Priyanka and B. Chandra, developed and validated RP-HPLC method was developed for the simultaneous estimation of Salbutamol Sulphate and Ambroxol Hydrochloride in bulk and pharmaceutical dosage forms. 

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Deepak Kumar Jain, Pratibha Patell Abhay Kushwaha, Ram Sneh Raghuvanshi and Nishank Jain, reverse phase liquid chromatographic method has been developed and validated for the simultaneous determination of Salbutamol Sulphate and Doxiphylline in present tablets dosage forms. Der Pharmaicia Lettre, 2011:3(4):56-6243. 


Sensitive rapid oil-in-water (O/W) microemulsion high performance liquid chromatography method has been developed. Bioimpacts, 2013;3(1):37-42.


[13]. Laurence M. Harwood, Christopher J.


