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

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ABSTRACT:-
Rapidandsensitivereversephasehighperformancechromatography(RP-HPLC)methodforsimultaneousestimationoflevosalbutamoland ipratropiumbromideusingthemobilephaseconsistingofmethanoland potassiumhydroxidepHmaintain3.Withphosphoricacid.Chromatography isaphysicalmethodorseperationofcomplexmixturewasdiscovered adsorptionbasedseperationofmixturecalledchromatography.Determination oflevosalbutamolsulphateandIpratropiumbromidecombinationForm. Reversephase–HPLCmethodwasdevelopedfordeterminationorestimation ofdrug.IpratropiumBromidepressurisednumuliserdoseinhater.Salbutamol isbronchiodilatorandIpratropiumBromideisCOPD(chronicobstructive pulmonarydisease). Theexcipientspresentintheformulationswerenotinterfarewithassay.The methodissuitableforapplicationinquality-maintaininlaboratoriesFor accuracyorsafetyandbioavailabilitythatdrug.Levosalbutamolsulphate relaxesthesmoothmuscleofallairwaysFromthetracheatotheterminal Bronchioles.Salbutamolsulphateisalsousedinthechronicobstructive pulmonary disease (COPD).

INTRODUCTION:-

Relaxesthesmoothmuscleofallairwaysfromtracheatotheterminal bronchiales.IncreasethecyclicAMPcontractionisalso associatedwithcells inairways.Levosalbutamolactsasafunctionalagonistthatrelaxesthe airwaytherebyprotectingagainstallbronchiocinstrictorchallengeswhileitis thepridominantrecognisedthatbetaadrenargicadrenargicreceptorareon brochiolessmoothmuscle.thebetareceptorintheheart.10-50%.ofwhich arebetaadrenargicreceptor.Itischemically4(1R-2-(tertbutylamino)-1- hydroxyethyl)-2-hydroxymethyl)phenol. Salbutamolsulphateisalsousedinthechronicobstructivepulmanory disease.(COPD)refferstochronicbronchitisandemphysema,whichisapair oftwocommonlyco-existingdiseaseofthelungs.Inwhichtheairways becomesnarrowed.COPDalsoknownaschronicobstructivelungsdisease (COLD),chronicobstructiveairwaydisease(COAD). Chronicairway limitations.(CAL)andchronicobstructiverespiratorydisease(CORD). Importantmanagementstrategiesaresmockingcessation,vaccinations, rehabilitationandtherapyobtainedtheintakethecombinationipratropium bromideandlevosalbutamolsulphatewillhelpintargettingdifferentaspects ofCOPDbronchiodilationthroughdifferentmechanismsandimmflamation withinhaledsteroids.SulphuricacidisashortactingBeta2adrenargic receptoragonistusedforreliefofbronchospamsinconditionsuchasasthma COPD. Itisalsoindicatethemanagementofacuteattackofbronchiospasm. salbutamolsulphateactsbystimulatingtheadenylcyclaseenzymewhich catalysestheformationofcyclic3-5AdinosinemonophosphatecyclicAMP theseformsmediatethecellularresponses.Relaxationofbronchiolesand smoothmuscle.

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Salbutamolsulphateiseffectivebyoralandinhalationalroute of administration.Salbutamolsulphatehasusedintablet,syrup,meterddose inhalerandnebulizedinhalationsolution.IpratropiumBromide(Figure2) antagonizestheactionofacetylcholinebyblockingmuscarinicCholinergic receptorsresultinginbronchodilationanddryingofrespiratorytract secretions.Ipratropiumblocksmuscarinicacetylcholinereceptors,without specificityforsubtypes.Thereforepromotesthedegradationof cyclicGuanosinemonophosphate(cGMP),resultingindecreased intracellularconcentrationofcGMP.Mostlikelyduetoactionsofc GMPon intracellularcalcium,thisresultsindecreasecontractilityofsmoothmuscle inthelung,inhibitingbronchoconstrictionandmucussecretion. ItIsanonselectiveMuscarinicantagonist,anddoesnotdiffuseintotheblood, whichpreventssystemicsideeffects.Ipratropiumisaderivativeof atropinebutisaQuaternaryamineandthereforedoesnotcrossbloodbrain barrier.Whichpreventscentralsideeffectsanticholinergicsyndrome.

- Chemicallyitis8(methyl-8(-1-methylethyl)-8-azoniabicyclo)oct-3- hydroxy-2-phenylpropanate
- Bronchiolesdisorders,inrhinitis,andasanantiarrhythmic.Itblocks muscarinicccholinergicreceptors,withoutspecificityforsubtypes, resulting in a decrease in the formation of cyclicguanosine monophosphate(cGMP).Itisfreelysolubleinwaterandmethanol, sparinglysoluble in ethanol, and insoluble inlipophilicsolventssuch asether,chloroformandfluorocarbons.Thecombinationpreparation ipratropiumbromide/salbutamol isa formulation containing ipratropium bromide and salbutamolsulphate used in the managementofchronicobstructivepulmonarydisease(COPD)and asthma.

PressurisedmeterdoseinhalercontainingLevosalbutamolsulphateand Ipratropiumbromideischemically4-[(1R)-2-(tertbutylamino)-1-hydroxyethyl]- 2-(hydroxymethyl)phenol, and [8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]oct-3-yl]3-hydroxy-2-phenyl-propanoate.Andhas empirical formula of C13H21NO3 and C20H30BrNO3 Levosalbutamol-Itisindicatedforpatientswithchronicobstructive pulmonarydiseases(COPD)onregularaerosolbronchospasmandwho requireasecondbronchodilator.6-9LevosalbutamolSulphateiswhiteto almostwhitecrystallinepowderfreelysolubleinwaterand Ipratropium bromideisalsowhiteoralmostwhitecrystallinepowder,solubleinwater, freelysolubleinmethanol,slightlysolubleinethanol(95 %) Indicatedforpatientswithchronicobstructivepulmonary diseases(COPD)on regularaerosolbronchospasmandwhorequireasecondbronchodilatordrug whichisusedintheCOPDandantihypertensivedrugs.Levosalbutamol sulphateactsasaexpectorantandipratropiumbromideactsas anticholinergicactivity.

- Themethodsofestimationandvalidationoflevosalbutamolsulphate andipratropiumbromideareasfollows:-

1).Liniarity(analyticalmethodforvalidation).Ofdrug
2).Accuracy(tofindmeanvalueortruevalue)
Othertechniquesarealsousedtodevelopmentandestimationofdrug.
RP-HPLCMethod(Reversephase–High-performanceliquid chromatography).
Acombinationofipratropiumbromide(IB)andsalbutamoliscommonlyused totreatasthmainchildrenandadolescents.
ToevaluatetheefficacyandsafetyofIB+salbutamolinthetreatmentof asthmainchildrenandadolescents.
IB+salbutamolmaybemoreeffectivethansalbutamolaloneforthe treatmentofasthmainchildrenandadolescents,especiallyinthosewith severeandmoderatetosevereasthmaexacerbation.Theverylowtohigh qualityofevidenceindicatedthatfuturewell-designeddouble-blindRCTswith largesampleareneededforresearchonevaluatingtheeffectivenessofIB+ salbutamoltreatmentforasthmainchildrenandadolescen.
Asthmaisthemostcommonchronicdiseaseamongchildrenandis estimatedtoaffect300millionindividualsworldwideInChina,asthma affects3%ofchildren≤14yearsofageandtheprevalence ofchildhood

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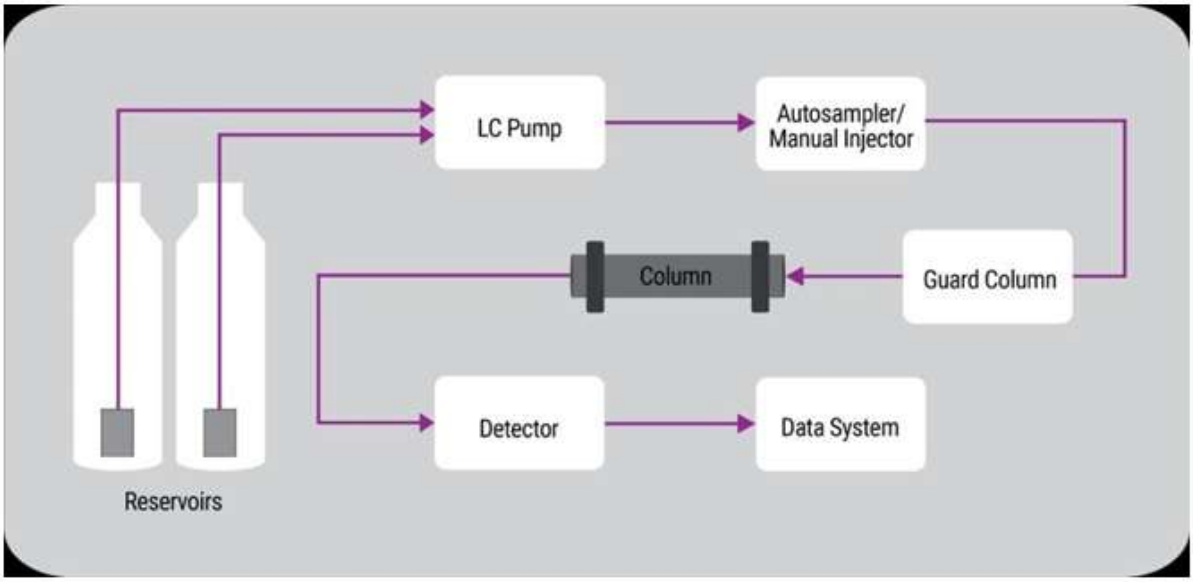
asthmahasincreasedby50%overthepast10years.Asthma-related hospitalizationcannegativelyaffectthequalityoflifeofchildrenandtheir caregivers.Additionally,healthcareexpendituresforasthma-related conditionsimposeconsiderableeconomicburdenonsociety.Almostall availableguidelinesrecommendthattherepeatedadministrationofinhaled short-actingβ2-agonists(SABAs,upto4–10puffsevery20minutesforthe firsthour)isaneffectiveandefficientwaytoachieverapidreversalofairflow limitationinpatientswithmild-to-moderateasthmaexacerbation.Inthe latestguideline.SABA-onlytreatmentisnolongerrecommendedforasthma inadultsoradolescentsduetoitsriskofasthma-relateddeathandurgent asthma-relatedhealthcare.Currently,severalavailableguidelineshave recommendedtheadditionofipratropiumbromide(IB), ashort-acting muscarinicacetylcholinereceptorantagonist,toSABAasanoptional treatmentforchildrenandadolescentswithacuteasthma exacerbation. AlthoughIBdoesnotseemtobeveryefficientincontrolling asthma,several studieshavedemonstratedthatacombinationofIBandsalbutamol sulphate.isassociatedwithfewerhospitalizationsandgreaterimprovementin peakexpiratoryflow(PEF)andforcedexpiratoryvolumeinonesecond(FEV1)



comparedwithSABAaloneinchildrenandadolescents withmoderate-to- severeasthmaexacerbation[10–15].TheadditionofIBtoSABAhasbeen recommendedinthefirsthouroftreatmentforchildrenwith moderate-severe exacerbationsHowever,theserecommendationslackuniformitywithrespect totheoptimalage,severityofasthma,andco-interventionwithotherasthma controllersforsuchtherapy.

Instrumentation:-
ofRP-HPCLmethod

- **MethodusedinRP-HPLCareasfollows:-**

1).Thesampleisfirstdissolvedinaliquidorthemobilephase. Thissolutionistheninjectedbymeansofamanualinjectororanautosamplerintoa continuousflowofmobilephase,beingdeliveredbyapump,andcarriedontotheLC columnwhichcontainsa stationaryphase.

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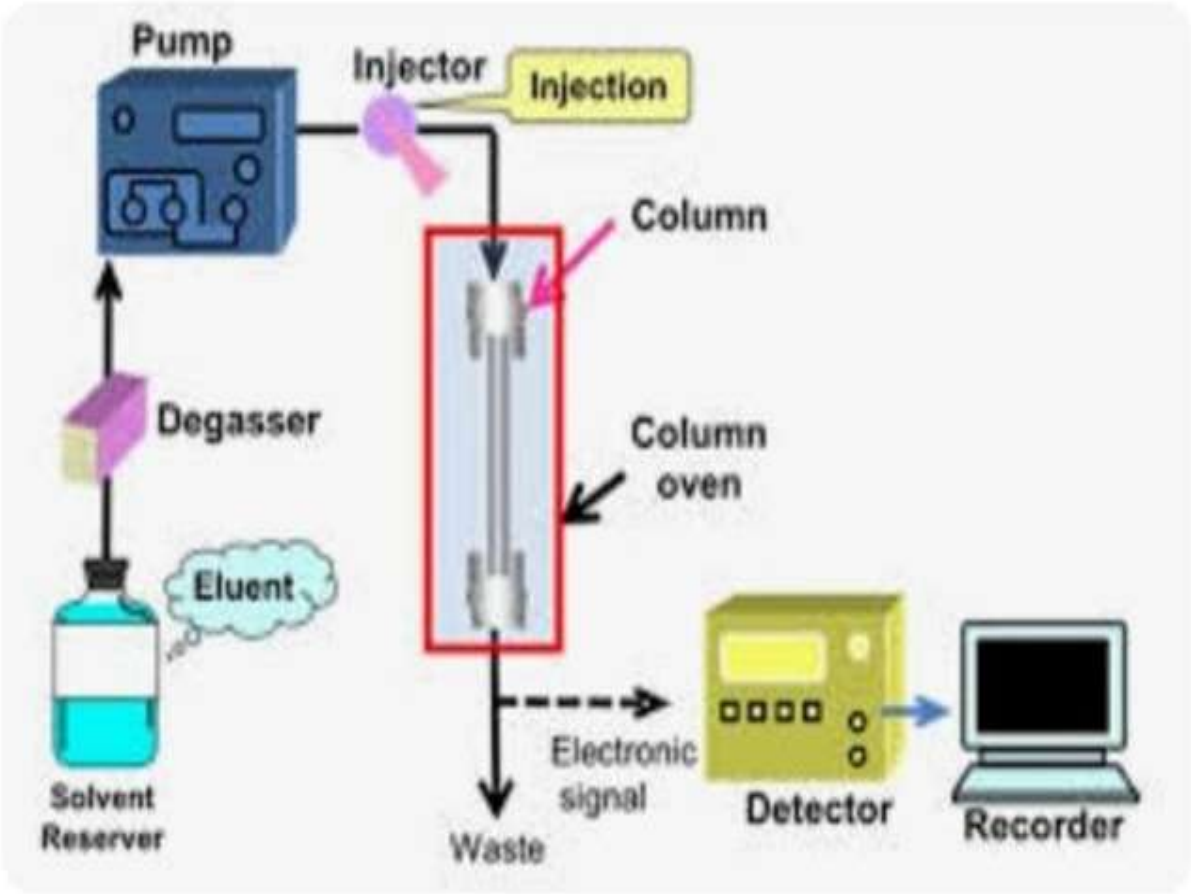
2).Thevariouscomponentsofthesampletravelthroughth ecolumnatdifferentspeeds duetotheirinteractionsbetweenthemobileandstationar yphases,resultinginthe componentsseparatingfromoneanother.Thedifferenttrav eltimesarereferredtoasthe components'
3).Whencomponentsemergefromthecolumn,theyarecarr iedtoadetectorwherea physicalpropertyofthecompoundsismeasured,sucha sabsorptionoflightforUV detection.
It'simportanttonotethattherearemanydifferentdetectorsav ailable.Someofthemost commondetectorsareultraviolet/visible(UV/Vis),photod iodearray(PDA),fluorescence (FL),andrefractiveindex(RI).Eachresponseplottedtime, resultinginachromatogram
PrincipleofRP-HPLC
Onthebasisoftheabsorptionofsolvent
ComponentsofRP-HPLCchromatographytechniques.

1) Samplecell(stationaryphase+mobilephase).
2) Automiser
3) Monochromator
4) Detector
5) RecorderOramplifier.



ChemicalstructureofdrugusedinCOPDareasfollows:-

1) Levosalbutamolsulphate
2) Ipratropiumbromide

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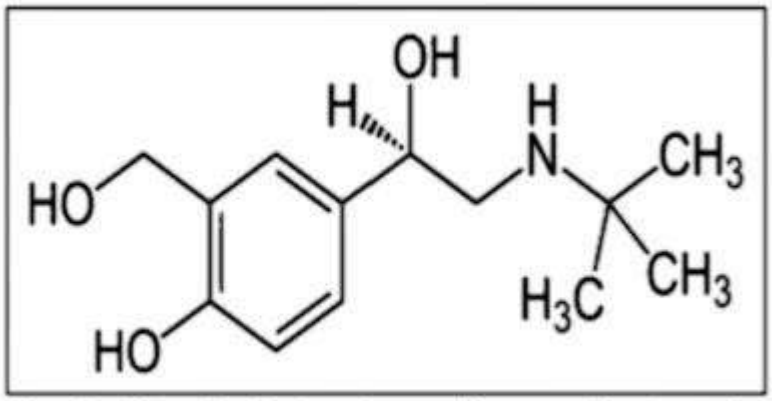

Fig. 1: Chemical structure of Levosalbutamol

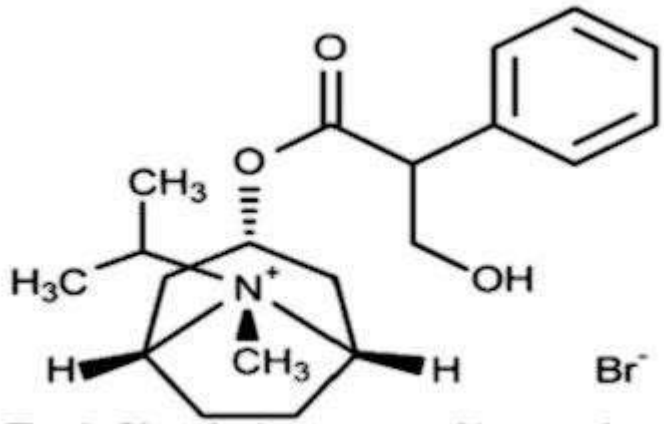

Fig. 2: Chemical structure of Ipratropium bromide

Detectionofdifferentgroupofdrugbyusingdifferenttechniquesof chromatographymethodareasfollows:-

Rgroup chromatographymethod
1).Alkyl Reversephase
2) cyano. Normal Creversephase
3) Amide. Reversephase
4) Amino. NormalCreversephase
5) Dimethylamine. Weakionexchanger
6) Quaternaryamine. Stronganionexchanger
Ii7)carboxylicacid. Weakcationexchanger
8)phenyl. Reversephase
9)Sulfonicacid strongcationexchanger

MOBILEPHASE:-
ThemobilephaseusedinHPLCdependsonthecomponentstobeseparated andthetechniqueusedwhethernormalphase,reversephaseorionexchange chromatographyetc.

(i) Foraspirin,ibuprofen,thesolventsystemusedis water-acetonitrile-methanolindefiniteproportionandpHadjustedintheacidicrange with phosphoric acid.

(ii) Forparacetamol,indomethacin,thesolventsystemusedis methanol-water-dioxaneinadefiniteproportion.

Aim:-TodevelopmentandvalidationofRP-HPLCmethodforsimultaneous estimationoflevosalbutamolsulphateandipratropiumbromidebulkand numuliser dosage form .
TovalidatethemethodaccordingtoICHguidelines.
Objective:-

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1) New,easy,delicate,preciseandeconomicalanalytic altechniquesfor RPHPLCtestingofthetitleingredients.
2) Validatetheproposedmethodfortheintendedanalytic alapplicationin accordance with USPandICH guidelines.
3) Applytheproposedmethodfortheanalysisofdosage form.

A).Stepsindevelopingthemethodandoptimizationofchro matographic condition.

- Literaturesurvey
- Selectionofdrugs.
- Selectionofdetectionwavelength
- Selectionofchromatographicconditions
- SelectionofMobilePhase(SelectionofOrganicsol ventandaqueous solvent).
- SelectionofsuitablepH.
- SelectionofColumnandColumntemperature.
- OptimizationofMobilephase,ColumnandSolvents ystem.

B).Stabilityindicatinganalyticalmethodvalidationusing RP-HPLCasperICH guidelines.

- Specificity
- Linearity
- Precision

Planofwork:-
Theexperimentalworkhasbeenplannedasfollows:
Reviewoftheliteratureforlevosalbutamolsulphateregardi ngphysicaland
chemicalproperties,variousAnalyticalmethodsthatwe reconductedfor
levosalbutamolsulphateformsthebasisfordevelopme ntofnew
AnalyticalRP-
HPLCmethodforlevosalbutamolsulphate.

DEVELOPMENTOFTHEMETHODBYRP-HPLC
1).Selectionofthesolventtobeusedasdiluentsandmobile phase.
Choosingthesuitablesolventinwhichthedrugissoluble andstable.
Theymustbeeasilyavailable,economicalandoftheHP LCgrade
2).SelectionofMobilephase:
Forthemobilephase,thefirstvariabletobedecidediswhethe ranorganicor aqueouseluents
Shouldbeused.WiththeRP-
HPLCanalysis,eitheranaqueouseluentora very polar organic
SolventsuchasWaterandMethanolshouldbefixed.Ifthe K'valuesaretoo large with an
Aqueoussolvent,organicsolventshouldbetried.IftheK'va luearetoolow with organic solvent
Theseparationshouldbeattemptedusingamixtureoftwoso lventswith variousproperties.

- K'-

capacityfactorisameasurementofthedegreewherethepea kofthe interestis
Locatedwithrespecttovoidvolume,i.e.Elutiontimeofnon-retained components.

GenerallythevalueofK'is>2.
Ifabufferisused,thepHaswellasionicstrengthofthebufferc anbetried
1).Inordertoselectthewavelengthtocarryouttheanalysis,c ritical examinationofthe
Ultravioletabsorbancespectraofthedrugshouldbedone.
2).Aperfectstudyofthestructureofdruganditsphysicoche micalproperties;
toselecttheChromatographicparameters.
3).Methodselectionforquantitativechromatographicass essment.Workingin rangedetermination.
4).Validationofthemethodestablishedbyfollowingtherules oftheICH.

VALIDATIONOFMETHOD
Validationisaprocessofestablishingdocumentedeviden ce,which
providesahigh.Degreeofassurancethataspecificactivit ywill
consistentlyproduceadesiredresultorproductmeeting its
predeterminedspecificationsandqualitycharacteristics.

Methodvalidationistheprocessofdemonstratingthepro ceduresare
suitablefortheirintendeduseandthattheysupportthe identity ,quality,
purity,andpotencyandbioavailabilityofthedrugsubstanc esanddrug.

Simply,methodvalidationisfortheimprovethequalityofthat product.
Thismethodanddeterminelimitsofallowedvariabilityfort heconditions neededtoduringtheprocess.

Differentvalidationmethods:- 1).Identificationtest.
2).Quantitativetestforimpuritiescontent.
3).Limittestforthecontrolofimpurities.
4).Quantitativetestoftheactivemoietyinsamplesofdrugsu bstanceon

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drugproductonotherselectedcomponentsinthedrugproduct.
Assayproceduresareintendedtomeasuretheanalystpresentingiven sample,assayrepresentaquantitativemeasurementofthemajor

Chemicalordrugs:-

SR No.	Ingredients	Company	Brand /Batch no.
1	Levosalbutamol Sulphate eq. to Levosalbutamol 2.5 mg+ Water for Injections	CIPLA	Salbair A3003AP
2	Levosalbutamol Sulphate eq. to Levosalbutamol 1.25 mg +Ipratropium bromide 0.50mg + Water for Injections	CIPLA	Salbair-I A2607JAP

ExperimentalChemicalsandReagents:-
Ipratropiumbromideof99%(MolecularWeight:412.37 g/mol)and Levosalbutamolof99%(MolecularWeight:239.31g/mol)purityare acquiredfromCiplaPharmaceuticalsMumbai,India. AcetonitrileHPLCGradefromRankemFinechemicalsof HPLCGrade PotassiumPhosphate(DibasicKHPO)(0.03MfromRankemFine Chemicals AR grade Ortho-PhosphoricAcid,85%,QuligensFinechemicalsandHPLCGrade water.

- PreparationofthePrimaryStandardDrugsolution

Astandardstocksolutionofthedrugswaspreparedbydissolving20mgof Ipratropiumbromideand50mgofLevosalbutamolin10. Mlvolumetric flaskcontaining5mlofdiluent(50:50v/vAcetonitrile:Water),sonicated forabout15minandthenmadeupto10mlwithdiluenttogetheprimary standardstocksolutioncontaining2mg/mlofIpratropium bromideand5 mgimlofLevosalbutamol. ForanalysisofIpratropiumbromideinrotacaps.asimple easilyavailable andreliableRP-HPLCmethodwithUV-detectionhasbeendevelopedand validatedforthesimultaneousdeterminationofIpratropiumbromideand Levosalbutamolconcentrationsinmetereddoseinhalers.

- PreparationofWorkingStandardDrugSolution

mloftheabovestocksolutionwastakenin100mlvolumetricflaskand thereaftermadeupto100mlwithdiluent(50-

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50vivAcetonitrileWater)toget theworkingstandardsolutioncontaining20µg/mlofIpratropiumBromide 0.1and50µg/mlofLevosalbutamol.Fromtheabovework ingstandard1.0ml 1.5ml2.0ml2.5mlC3.0midilutionsweremadeandtransferredin10mi volumetricflaskandthereaftermadeupto10mlwithdiluentsIpratropium bromideand5-15µg/mlofLevosalbutamolrespectively. AnalysisofPharmaceuticalMetered InhalersRemovethepressurizedcontainer(Duolin InhalerMDI,Cipla,EachachpuffpuncontainsIpratropiumbromide20µg /mlandLevosalbutamol50µg/mlaresuspendedinpropellantHFA227-qs innetweightofcontentsequivalentto21µgofIpratropiumBromideand60 µgofLevosalbutamol)fromtheactuatorandremoveallthelabelsand markingswithsuitablesolvent.Drythecontainer,replaceinitsactuator, shakeforabout30secondsandprimethemeteredvalveasfollows. Dischargeonceforwaste:waitfornotlessthan5secondsanddischarge againtowaste.Removethepressurizedcontainerfromitsactuator,clean thevalvestem(internallyandexternally)andthevalveferrulebywashing withasuitablesolvent.Drythecompletevalveassembly,usinganairline fittedwithanappropriatenarrowjettoensurethatallsolventisremoved fromtheinsideofthevalvestem.Placeatripodstainlesssteelbaseplate withacentralcircularindentationof1.5mmindiameterinasmallvessel suitableforshakingandadd15mlofdiluent.Thesizeofthevesselissuch thatwhenthepressurizedInhalationisdischargedinto15mlofdiluent dischargetakesplacenotlessthan25mmbelowthesurfaceofthe solvent.Shakethepressurizedcontainerforabout30secondsandplacein invertedpositioninthevessel.Discharge120deliveriesbelowthesurface ofthesolventactuatingthevalveatintervalsofnotlessthan5seconds, maintainingthe

RESULT AND CONCLUSION:-

ConsidertheefficiencyofthedrugofRPHPLCmethod development simple,accurate,rapidforsimultaneousestimationoflevosalbutamol sulphateandipratropiumbromideinbulkandnumuliserdosageform.The proposedmethodwassimple,specificandsensitiveandcanbeusedfor simultaneousestimationofLevosalbutamolandIpratropium.Bromidein bulkandnumuliserdosageforms.Theresultofthestudyfollowsthe protocolofICHguidelinesanditcanbesuccessfullyappliedforthe simultaneousestimationofthemarketedproductsofLevosalbutamoland Ipratropiumbromideinbulkandnumuliserdosageform

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