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Method Development and Validation for Simultaneous Estimation of Rilpivirine and Doult Gravier In Bulk and Pharmaceutical Tablet Dosage Form by Rp-Hplc And Uvspectroscopy

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ABSTRACT
A simple, accurate, precise method was developed for the simultaneous estimation of theRilpivirine and doultigravir in Tablet dosage from. The upside of UV technique over HPLCstrategy is that the proposed UV technique does not require the detailed treatment and strategiesgenerally connected with chromatographic technique. It is less tedious and temperate. A factualcorrelation of the quantitative assurance of Rilpivirine and Dolutegravir demonstrates that HPLCstrategy asmoreexactandexactthanUVtechnique.Theoutcom esshowHPLCandUVspectrotometry techniques are sufficient strategies to evaluate Rilpivirine and Dolutegravir inunadulterated frame and its measurements shape. A basic, Accurate, exact strategy was created.RetentiontimeofRilpivirineandDolutegravir wereobservedtobe2.201minand2.925min.The%RS DoftheRilpivirineandDolutegravirwereandobserved tobe0.2and0.2separately.andthen%Recoverywasgot tenas99.37%and99.70%forRilpivirineandDolutegra vir separately. LOD, LOQ esteems acquired from relapse conditions of Rilpivirine andDolutegravirwere0.31,0.93and0.23,0.70separate ly.RelapseconditionofRilpivirineisy =29227x+4046, and y = 34463x+4061 of Dolutegravir. Maintenance times were diminished andthat run time was diminished, so the technique created was basic and efficient that can beembracedin standard Qualitycontrol test inIndustries.

Keywords: rilpivirine and doultgravierrp-hplc and uvspectroscopy

I. INTRODUCTION

Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) whichisusedforthetreatmentofHIV-1infectionsintreatment-naivepatients.[5]Itisadiarylpyrimidine derivative, a class of molecules that resemble pyrimidine nucleotidesfound in DNA.[6] The Sinternal conformational flexibility of rilpivirine and the plasticityof it interacting binding site gives it a very high potency and an unlikely generation ofresistance compared toother NNRTI's.Rilpivirine,incombination with dolutegravir, wasapprovedas part of the first complete treatmentregimenwith onlytwodrugs for thetreatmentof adults withHIV-1 namedJuluca.Rilpivirineisanon-competitiveNNRTIthatbindstoreversetranscriptase. Its binding results in the blockage of RNA and DNA- dependent DNA polymeraseactivities, like HIV-1 replication.It does not present activity against human DNA polymerases α,β and γ. Rilpivirine binds to the HIV-1 reverse transcriptase (RT) and its flexible structurearound the aromatic rings allows the adaptation to changes in the non-nucleoside RT bindingpocket. mutationsor rilpivirineresistanceMainly hepatically metabolized by CYP3A. Because it is highly protein bound, itsfree plasma concentration is very small thus is unlikely to inhibit cytochrome proteins to aclinicallyrelevant degree despitebeingan inhibitor ofCYP3A4, CYP2C19,and CYP2B6.[1]

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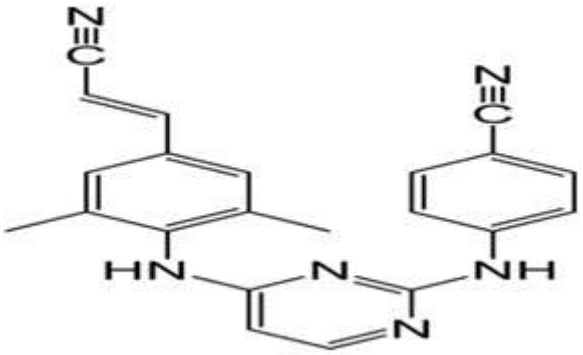
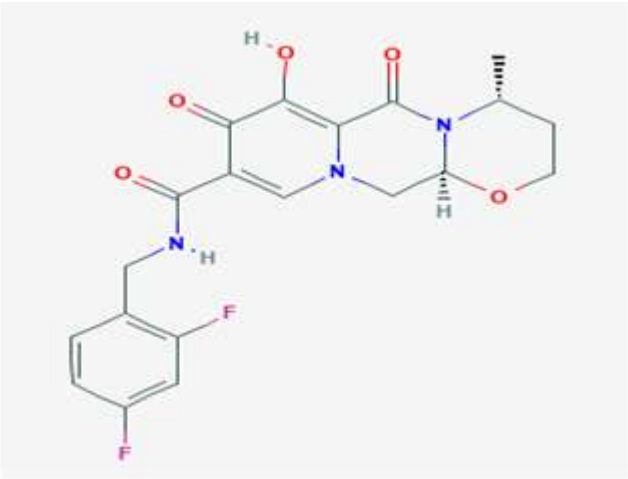


Structure of Rilpivirine

Dolutegravir is a HIV-1 intergrase inhibitor that blocks the strand transferstep of the integration of the viral genome into the host cell (INSTI).[1] The effect of thisdrug has no homology in human host cells which gives it an excellent tolerability andminimaltoxicity. dolutegravir, in combinationwith rilpivirine, was approved as part of the first complete treatment regimen with only twodrugsforthe treatment of adults with HIV-1 named Juluca. Dolutegravir is an HIV-1 antiviral agent. It inhibits HIV integrase bybindingtotheactive site andblockingthestrandtransferstepofretroviralDNAintegrationinthe host cell. The
strand transfer step is essential in the HIV r retroviralDNAintegrationinthe host cell. The
strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity. Dolutegravir has a mean EC50value of 0.5 nm (0.21 mg/ml) to 2.1 nm(0.85 mg/ml) inperipheral blood mononuclear cells (PBMCs) andMT-4cells.[4]



Structure of Dolutegravir

II. MATERIALS AND METHOD

Materials:

Rilpivirine and Dolutegravir pure drugs (API), Combination Rilpivirine and Dolutegravir tablets (Juluca®), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium
dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solventsarefromRankem

Instruments:

ElectronicsBalance-Denverp^{H}meter-BVK enterprises,IndiaUltrasonicator-BVKenterprisesWATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo DiodeArraydetector and Autosampler integrated with Empower 2Software.UV-VISspectrophotometerPGInstrumentsT60withspeci

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albandwidthof2mmand 10mm and matched quartz cells integrated with UV win 6 Software was used formeasuringabsorbances of Rilpivirineand Dolutegravirsolutions.

Preparation of Standard stock solutions:
Accurately weighed 12.5mg of Rilpivirine, 25mg ofDolutegravir and transferred to 50ml volumetric flask and 3/4th of diluents was added to theseflask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standardstocksolution. (250µg/ml of Rilpivirineand 500µg/ml ofDolutegravir)

Preparation of Sample stock solutions:
5 tablets were weighed and the average weight of eachtablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100mlvolumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume wasmade up with diluent and filtered by HPLC filters (250µg/ml of Rilpivirine and 500µg/ml ofDolutegravir)

Methoddevelopment:

PREPARATIONOF STOCKSOLUTIONANDSELECTIONOFWAVELENGTHFORANALYSIS:
StandardstocksolutionsofRilpivirineandDolutegravirhydrochloridewerepreparedseparately by adding 10mgof drug to methanol taken in 10ml volumetric flasks and thensonicatedforfiveminutesandthevolumewasmade upwithmethanol.Theresultingsolutions contain 1mg/ml of the drug. The stock solutions of Rilpivirine and Dolutegravirwerefurtherdilutedwithwatertoobtainth econcentrationof30µg/ml.Theresultingsolutionswerethenscannedin UVspectrophotometerfrom400to200 nm.Fromtheresulting spectra λmax for Rilpivirine and Dolutegravir were calculated separately.Theoverlay spectra of Rilpivirine and Dolutegravir was also recorded. From the overlay spectraisoabsorptivepoint of RilpivirineandDolutegravirwascalculated.

Parameters	Rilpivirine	Dolutegravir	**Acceptancecriteria**
Tailing	**1.18**	**1.14**	**NMT2.0**
Platecount	**5119**	**7212**	**NLT2000**
%RSD ofpeakarea	**0.54**	**0.55**	**NMT2.0**
Retentiontime	**2.196**	**2.925**	**---**

Method optimization: A simple RP-HPLCwasdevelopedforestimationof Rilpivirine and Dolutegravirinpharmaceutical dosage form using WATERS HPLC. The mobile phase is the ratioof60%0.01NkH_2po_4($4P^H$): 50%Acetonitrile mobile phase chosen after several trials.The flow rate is 1 ml/min. The retentiontimesis2.201minand2.925minfor Rilpivirine and Dolutegravirrespectively.

Systemsuitabilityparameters:
The system suitability parameters were determined by preparing standard solutions of Rilpivirine(25ppm) and Dolutegravir (50ppm) and the solutions were injected six times and the parameterslikepeak tailing,resolution andUSP platecount weredetermined.
The%RSDfor thearea of sixstandardinjectionsresults shouldnotbe morethan2%. method. We should not find interfering peaks inblank and placebo at retention times of these drugs in this method. So this method was said to bespecific

LODsamplePreparation:
0.25ml each from two standard stock solutions was pipetted out and transferred to two separate10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each ofRilpivirine, Dolutegravir, solutions respectively were transferred to 10ml volumetric flasks andmadeup with thesame diluents

LOQsamplePreparation:
0.25ml each from two standard stock solutions was pipetted out and transferred to two separate10mlvolumetric flaskandmade upwithdiluent.Fromtheabovesolutions0.3mleachof Rilpivirine, Dolutegravir, solutions respectively were transferred to 10ml volumetric flasks andmadeupwith thesame diluent.

Pression: It is studied or evaluated by therepeatabilitystudieswhichisdeterminedby the injecting the same concentration ofrun six times.
Linearity: Thelinearityforhydrochlorothiazideandirbesartanwasevaluated by relation between peak areasandconcentrationofeachdrugwithacorrelationc

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oefficientof0.999forbothdrug

Accuracy: Itisanalyzedbyconductingthreedifferentconcentrationsoftheworking standards. With the percentage of50%,100%and150%injecteachconcentration three times into HPLC andcalculate the average percentage recovery.Themeanpercentagerecoveryof Rilpivirine andDolutegravir 99.37%and 99.70%

Robustness: Robustnessshouldbeconsidered during development phase andalsodependsonthetypeofprocedureunder study. The robustness of a method isthe ability toremain unaffected by smallchanges in parameters such as pH of themobilephase,temperature,%organicsolvent strength and buffer concentrations,etc.todeterminetherobustnessofthemethodexperimentalconditionswerepurposelyaltered, andchromatographiccharacters were evaluated. In this work wealter the ratio of mobile phase and flow rateofthe mobile phase.

Degradationstudies:

Oxidation:

To 1 ml of stock solutionof Rilpivirine and Dolutegravir, 1 ml of 20% hydrogenperoxide(H2O2) was added separately. The solutionswere keptfor 30 minat 60^0c.For HPLCstudy,theresultantsolutionwasdilutedtoobtain 25µg/ml&50µg/mlsolutionand10µlwereinjectedintothesystemandthechromatogramswererecordedtoassesstheStability of sample

AlkaliDegradationStudies:

To 1 mlof stock solutionRilpivirine and Dolutegravir, 1 mlof 2N sodiumhydroxidewasaddedandrefluxedfor30minsat 60^0c.Theresultantsolutionwasdilutedtoobtain25µg/ml&50µg/ml solutionand10 µl were injected into the system and the chromatogramswererecorded to assess thestabilityof sample.

DryHeatDegradationStudies:

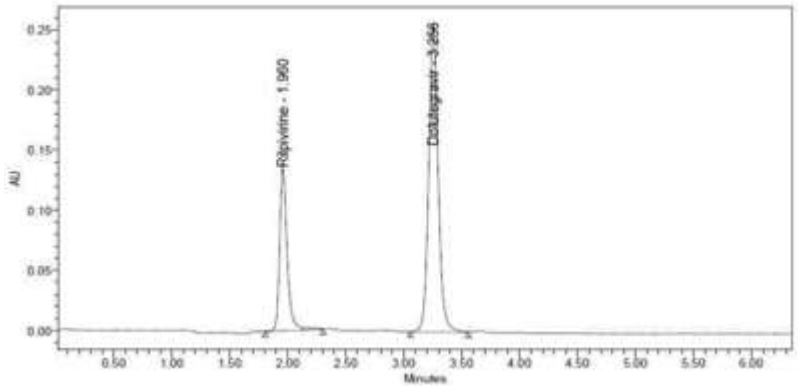
The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 25µg/ml & 50µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

PhotoStabilitystudies:

Thephotochemicalstabilityofthedrugwasalsostudied byexposingthe250µg/ml&500µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was dilutedtoobtain25µg/ml&50µg/mlsolutionsand10µl wereinjectedintothesystemandthechromatograms were recorded to assess thestabilityofsample.

NeutralDegradationStudies:

Stresstestingunderneutralconditionswasstudiedbyrefluxingthedruginwaterfor1hr temperature of 60º. For HPLC study, the resultant solution was dilutedto25µg/ml&50µg/mlsolution and 10 µl were injected into the system and the chromatogramswererecordedtoassessthe stabilityof thesample**.**



Standard chromatogram

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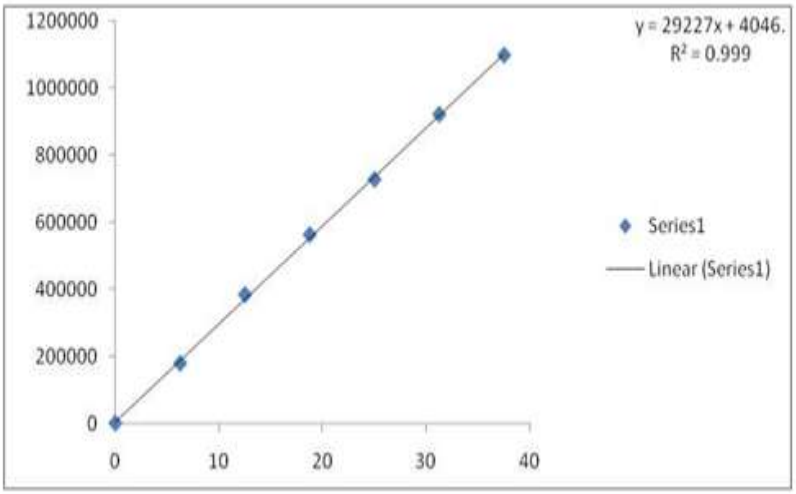
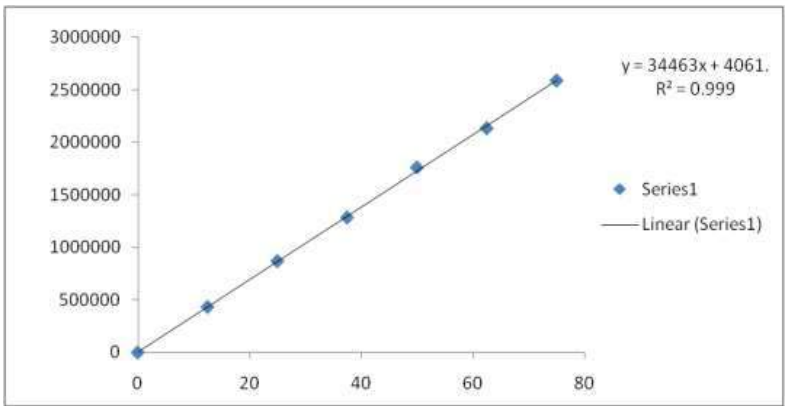
Rilpivirine		Dolutegravir	
Conc (µg/mL)	**Peakarea**	**Conc (µg/mL)**	**Peakarea**
0	0	0	0
6.25	178375	12.5	434004
12.5	382746	25	869493
18.75	561256	37.5	1285274
25	725937	50	1761823
31.25	919783	62.5	2135401
37.5	1096213	75	2588975

Calibration of Rilpivirine





Calibration of Dolutegravir

Accuracy: Accuracy table of Rilpivirine

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%Level	Amount Spiked(µg/mL)	Amountrecovered (µg/mL)	%Recovery	Mean %Recovery
50%	12.5	12.411811	99.29	99.37%
	12.5	12.408116	99.26	
	12.5	12.391145	99.13	
100%	25	24.807849	99.23	
	25	24.988435	99.95	
	25	24.847367	99.39	
150%	37.5	37.43624	99.83	
	37.5	37.170185	99.12	
	37.5	37.174051	99.13	

Systemprecisiontableof RilpivirineandDolutegravir

S.No	Areaof Rilpivirine	AreaofDolutegravir
1.	720158	1767937
2.	724280	1760728
3.	724098	1764283
4.	728175	1768199
5.	722901	1767927
6.	724613	1762830
Mean	724038	1765317
S.D	2602.1	3171.6
%RSD	0.4	0.2

Accuracytableof Dolutegravir

%Level	Amount Spiked(µg/mL)	Amountrecovered (µg/mL)	%Recovery	Mean %Recovery
50%	25	24.992833	99.97	
	25	24.987349	99.95	
	25	24.956678	99.83	
100%	50	49.62554	99.25	
	50	49.789891	99.58	
	50	49.697037	99.39	

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150%	75	74.828222	99.77	99.70%
	75	74.843165	99.79	
	75	74.842121	99.79	

RobustnessdataforRilpivirineandDolutegravir.

S.no	Condition	%RSD ofRilpivirine	%RSD ofDolutegravir
1	Flowrate (-)1.1ml/min	1.4	1.5
2	Flowrate (+)1.3ml/min	0.9	0.8
3	Mobilephase(-)65B:35A	0.3	0.5
4	Mobilephase(+)55B:45A	0.2	0.4
5	Temperature(-)25°C	0.7	0.9
6	Temperature(+)35°C	0.5	0.8

III. SUMMARY AND CONCLUSION

HPLC				
Parameters		**Rilpivirine**	**Dolutegravir**	**LIMIT**
SLinearityRange(µg/ml)		6.25-37.5µg/ml	12.5-75µg/ml	
Regression co-efficient		0.999	0.999	R<1
Assay(%meanassay)		99.52%	99.41%	90-110%
Specificity		Specific	Specific	Nointerferenceof anypeak
Systemprecision %RSD		0.4	0.2	NMT2.0%
Methodprecision %RSD		0.2	0.2	NMT2.0%
Accuracy%recovery		99.37%	99.70%	98-102%
LOD		0.31	0.23	NMT3
LOQ		0.23	0.70	NMT10
Robustness	**FM**	1.4	1.5	%RSDNMT2.0
	FP	0.9	0.8	
	MM	0.3	0.5	
	MP	0.2	0.4	
	TM	0.7	0.9	
	TP	0.5	0.8	

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UV				
Correlation co-efficient	-	0.999	0.999	R<1
Linearityrange	-	1.25µg/ml-7.5µg/ml	2.5 µg/ml - 1.5µg/ml	R<1
Specificity	-	Specific	Specific	Nointerferenceof anypeak
Absoptionmaximum(nm)	-	259 nm	257 nm	
Assay of %RSD	-	0.21	0.72	NMT2.0%
Repeatability(n=6)	-	1.08	0.12	NMT2.0%
%Recovery	-	99.89%	99.18%	99-101%

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of theRilpivirine and doultigravir in Tablet dosage from. The upside of UV technique over HPLCstrategy is that the proposed UV technique does not require the detailed treatment and strategiesgenerally connected with chromatographic technique. It is less tedious and temperate. A factualcorrelation of the quantitative assurance of Rilpivirine and Dolutegravir demonstrates that HPLCstrategy asmoreexactandexactthanUVtechnique.TheoutcomesshowHPLCandUVspectrotometry techniques are sufficient strategies to evaluate Rilpivirine and Dolutegravir inunadulterated frame and its measurements shape. A basic, Accurate, exact strategy was created.RetentiontimeofRilpivirineandDolutegravirwereobservedtobe2.201minand2.925min.The%RSDoftheRilpivirineandDolutegravirwereandobservedtobe0.2and0.2separately.andthen%Recoverywasgottenas99.37%and99.70%forRilpivirineandDolutegravir separately. LOD, LOQ esteems acquired from relapse conditions of Rilpivirine andDolutegravirwere0.31,0.93and0.23,0.70separately.RelapseconditionofRilpivirineisy =29227x+4046, and y = 34463x+4061 of Dolutegravir. Maintenance times were diminished andthat run time was diminished, so the technique created was basic and efficient that can beembracedin standard Qualitycontrol test inIndustries.

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