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Simultaneous Estiamtion of *Dolutegravir* and *Rilpivirine* and Their Impurities using RP-HPLC

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ABSTRACT

A very simple, more accurate, and highly precise process is refined to development of two combination drugs *Dolutegravir* (DUA), *Rilpivirine* (RPV) in the tablet dosage form. For this development agilent 100-5c_{18} column (250mmx4.6Mm). Acetonitrile as 40v/v and phosphate buffer which is maintained at ph 4.0 As 60v/vis passed with the help of column. Flow rate was measure as 0.80Ml/minute. Column temperature is accompanied as ambient. Upgraded wavelength is 235 nm. Run time is identified as 10 min and the volume injected for this analysis is 20 µL. Retention time of dua and rpv were found to be 2.6 & 3.9 Minute. % Rsd of the dua and rpv were and found to be 0.87, 0.40 As intraday and 0.75 And 0.56 As inter day precision. % Recovery was obtained as 98.59%, 100.7 % For dua and rpv respectively. The Rt values are minimized as well as run time was decreased. Finally, concluded that this proposed and developed process is more simple as well as economical which should be prevalent in regular QC labs and present in the plasma samples.

Keywords: DUA, RPV, RP-HPLC, Retention time, Precision.

INTRODUCTION

Dolutegravir (DUA), is available as Tivicay. It is acting as an antiretroviral medication. This can be used along with drugs for treating HIV/AIDS.[1] It also utilized a part of post exposure prophylaxis, for the prevention of infection caused by HIV follows potential exposure.[2] This drug is utilized for the treatment for adults those who are infected with HIV-those who are not taken any treatment or therapy. This drug may also be using by children with age 12 years with body weight 40 kg.[3] The agency named as FDA approved the drugs named as Tivicay and Tivicay PD in healthcare ViiV.[4] Another

drug *Rilpivirine*(RPV), is available as Edurant and Rekambys. This is the drug developed by Tibotec. This drug is also used to treat HIV/AIDS.[5,6] It is a second-generation NNRTI consists of larger than capability, long thermo luminescence and lesser other effects whenever those are correlated by NNRTIs.[7,8] As per the author for these drugs[9] they are used UPLC-MS/MS bio analytical method. Authors are used simple protein precipitation by using methanol is process for preparing plasma samples of 50.00 µL. This method is validated in the range as 9.7–9700, 52–10,470, 9.7–9730, 73–14,680 and 15–3010 ng/mL. Recovery is ≥76%. The samples of plasma are examined in the Long-term stability of patients which is identified in a time interval of 365 days at a temperature as–40°C at 4 months for etravirine. Run time is 10 minute. Chromatographic separation of *Dolutegravir* and *Rilpivirine*[10] is achieved performed by using INERTSIL ODS C18 (250×4.6 mm, 5µm) column. 0.1% OPA and acetonitrile are used as a mobile phase with a composition as 60v/v and 40 v/v. 10 min is the run time. 1.0 mL min^{-1} is the flow rate which is observed at a temperature of 250°C. Wavelength is 230 nm. Retention time is 3.4 min and 4.3 min. Linearity limit are 0.999 and 0.999. As per the authors[11] Phenomenex C18 (150x4.6mm, 5µm) is used for this measurement. Mobile phase is 0.1% OPA and acetonitrile as 60:40 v/v. 1.0 mL/min is the rate of flow. 262nm is the wavelength identified 4.35 min, and 7.73 min are the retention times. Linearity is 10 µg/mL to 150 µg/mL for the drug named as DTG and 5 µg/mL to 75 µg/mL to the drug named as RPV. 98 and 102% are the precision and recoveries. Both the drugs DTG as well as RPV drugs were release 98% in a time interval of 120 min which is observed in the body of rat. The parameters are calculated by considering the rules and regulations given by an international agency named as ICH. This process is applied to plasma samples to study in the areas of bioanalytical. INERTSIL ODS[12] column (250×4.6mm, 5µm) is used in this analysis. At pH 6.8 phosphate Buffer as well as acetonitrile are used in the ratio of 35v/v and 65v/v) as mobile phase. 1.0 mL/min is rate of flow. 3.285 min, and 4.635 min are retention times. 99.97% and 100.63% is the purity percentage. 3209 is the system suitability, 1.13 is the theoretical plates, and 5210 is the tailing factor. RP-HPLC-PDA with a BBD[13] is used Kromasil 150 mm× 4.6 mm, 5 µm C 18. Assay results were 99.19% and 99.09%. The forced degradation values are 5.96, 4.79, 3.27, 2.36, 0.99, and 4.35 and for DTGR is 5.67, 4.44, 4.09, 1.81, 0.43, and 4. For these two drugs[14] standard solution consists of strength as 100 µg/mL and 50 µg/mL are noted at 2.427 min and 4.436 minute. The purity percentage is 99.22% w/v and 99.81% w/v. Calibration plots were linear at 80 µg/mL to 120 µg/mL and 30 µg/mL to 70 µg/mL. Recovery percent is 98–102%. Both the values of LODas well are LOQ are 0.0440 µg/mL, 0.060 µg/mL and 0.134 µg/mL, 0.183 µg/mL. The absorption maxima[15] of drugs are found at 259.20nm and 305.80nm. Both drugs followed the beer lamberts law in the range of 4 µg/mL to 24.00 µg/mL and 1.00 µg/mL to 8.00 µg/mL to the drugs named as DOL and RIL.

MATERIALS AND METHODS

Analytical/HPLC-grade tertiary butyl alcohol from Alfa Aesar. The monobasic Sodium Hydrogen phosphate, Acetonitrile, potassium hydrogen phosphate, acetic acid as analytical reagent (AR) grade and procured from Spectrum Chemicals, Mumbai, India. Both the DOLT and RIL are taken as gift samples. HPLC-grade methanol and acetonitrile were procured from J. T. Baker. Di-potassium hydrogen phosphate and orthophosphoric acid from Merck Life Sciences. Used high-quality purified water (Milli-Q) for all experimental analyses. The chromatographic analysis was conducted using a Waters Alliance 2695 Module from Waters Corporation.

The standard solutions consist of DUA and RPV are passed into chromatographic system as well as run in various systems of solvent. After reviewing literature, various mobile phases in contrasting configuration also various pH values are tried to identify best circumstances to the separation. Finally, it is concluded as both the solutions like acetonitrile and phosphate buffer gives satisfactory results as correlated to remaining phases. Described composition is tested by disparate ratios as well as by the utilization of various rates of flow. Optimal content for mobile phase is utilized as acetonitrile as well as buffer of phosphate which is maintained a pH at a 4.0. Rate of flow is identified at 0.8 mL/minute. For this analysis authors are used the column as Agilent 100-5C 18 column [250mmx4.6mm].UV-Detector is utilized to this analysis.

Optimization of Chromatographic Conditions

For this analysis acetonitrile, pH 4 maintained phosphate buffer used as a mobile phase in a volumetric ratio as 40v/v and 60v/v. The flow rate is considered in between 0.5 mL/min to 1.5 mL/minute. Finally the rate of flow is measured at

1.0 mL/minute. Upgraded Optimal wavelength is noted at 235 nm. Run time is identified as 10 min and the volume injected for this analysis is 20 µL. Retention time of DUA and RPV were found to be 2.6 & 3.9 minute.

Preparation of solutions

Mobile phase is prepared by using pH as 4 maintained for phosphate buffer is prepared by dissolving 0.5040 g of Na_2HPO_4 along with 0.301 g of KH_2PO_4 in water of grade HPLC after that pH is adjusted as 4 by utilizing glacial CH_3COOH. For this content required amount of water is added and after that marked to the volume 100.00 mL. Finally the contents are thoroughly mixed at a time interval 10 minute.

Stock solutions is prepared by taking Exactly weighed 25.000 mg of DOLT and RIL prevail taken in 2 standard flasks with volume 25.00 mL. The contents are thoroughly mixed with the help of mobile phase after that the volume is marked by using the same solvent to achieve stock solutions A as DOLT also B as RIL. The strength of each solution is identified as 1000 g/mL of each and every drug. By using these primary stock solutions, 2.50 mL of each one is taken as A as well as B. The contents are taken into 10.00 mL standard flask also volume is marked with mobile phase for gettingstrength as 250.00 µg/mL of each is DUA and *Rilpivirine* (RPV).

Pharma Dosage Sample is prepared by using available tablets in the marketare weighed after that subjected to the crushing process. The power which is obtained is equivalent to 80.00 mg of DUAas well as 12.50 mg of RPV are subjected to weighing process after that taken into to a 25.00 mL standard flask. The obtained contents are subjected to dissolution in a 10.00 mL of mobile phase at a time interval of 15 minute. Finally, the contents are filtered with0.45 membrane filter. Sonication was performed at a time interval of 20 min with the help of defined stock solution working stock solution is prepared.

EXPERIMENTAL

This process is optimized by keeping the aim to develop systematic procedure to both the drugs DOLT and RIL. Acceptable retention times, theoretical plates, as well as very good resolution are noted by acetonitrile 40v/v, pH 4 contained phosphate buffer 60v/v. Agilent100-5 C18 column, P134 [250×4.5 mm] is used for this analysis. 235nm is the wavelength noted.

RESULTS AND DISCUSSION

System suitability

This parameter is performed with the help of total five injections which contains the solution of 100% strengthfor 250.00 µg/mL of DUA and RPV. The total number of theoretical plates (N) which are identified after that computed tailing factors as T are expressed in Table 1 and 2. And the related chromatograms are represented in the Figure 1.

Table 1: System Suitability Parameters

Parameters	DUA	RPV
Rtin min	2.5	3.8
Theoretical plates as N	12672	10499
Tailing factor as T	1.29	1.14
Resolution as Rs	1.8	

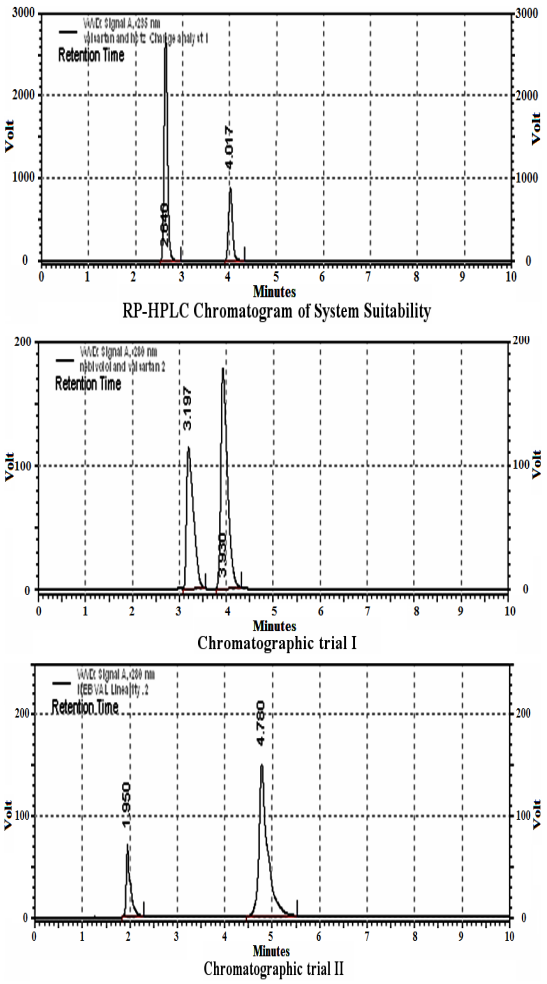


Fig. 1. Chromatogram of System Suitability, Chromatogram for trial–I and II

Table 2: Chromatographic trials

Trials	Arrangement of mobile phase	Rate of flow in mL/min	Drug	Retention Time in min
1	Methanol : Water	1 mL/min	DOLT	3.19
			RIL	3.93
2	Acetonitrile : Phosphate buffer pH (3.0)	1 mL/min	DOLT	1.9
			RIL	4.7

Accuracy

To this parameter various studies are performed with the help of standard accession process. A well-knownamount of pure drug is combined for pre-analyzed sample as well as the contents by this process after that percent recovery is reported. Final results so obtained are given in Table 3 and the related chromatograms are represented in the Figure 2.

Table 3: At n=3 Accuracy results

Recovery level	DUA Quantity Added as µg/mL Std.,	test	DUA Quantity Found in µg/mL	%Recovery	RPV Quantity Added as µg/m) Std.,	Test	RPV QuantityFound in µg/mL	% Recovery
50%	100	100	197.5	98.75	100	100	199.2	99.6
100%	100	150	245.3	98.13	100	150	257.7	103
150%	100	200	296.6	98.89	100	200	299.3	99.76
Mean recovery			98.59				100.7	

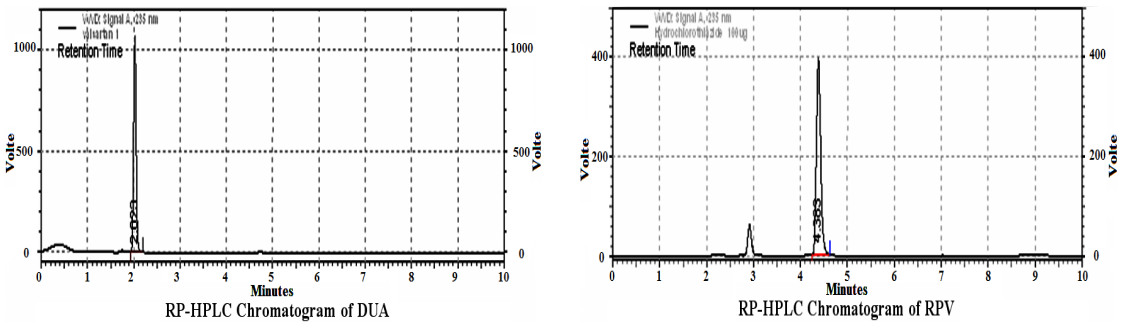


Fig. 2. Chromatograms for DUA and RPV

Precision

For this parameter we are documented by calculating %RSD for total 6duplicate injections containing 100% strength as 250.00 g/mL for DUA and RPV. On the same day also to intermediate precision. %RSD is computed by studies of repeated over various days. The values so obtained are tabulated in the below Table 4. And the intra and inter day precision of these two drugs are represented in the Figure 3.

Table 4: At n=6 precision results

Drug	Intraday precision as %RSD	Interday precision as %RSD
DUA	0.79	0.88
RPV	0.39	0.48

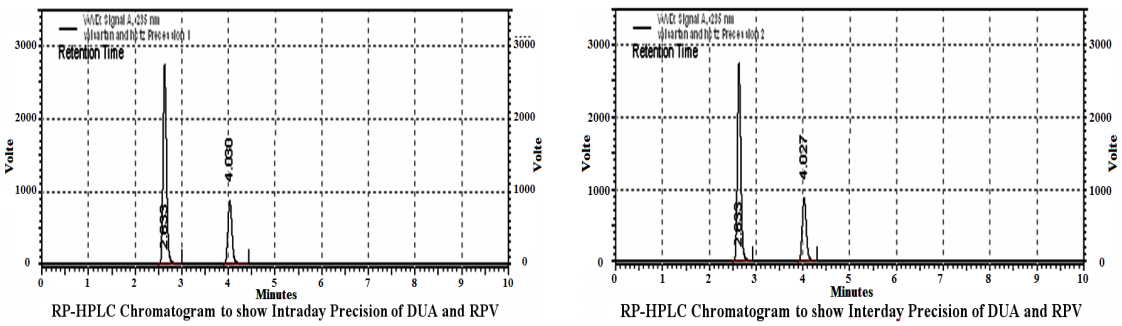


Fig. 3. Intraday and Interday Precision of DUA and RPV

Specificity

This parameter is measured by testing standard materials opposite to the potential intervention. By the experimental analysis it

is identified that this process is more specific by utilization of test solution, and there are other interferences are identified. This is mainly because of excipients. The values so obtained are represented as the Table 5. The chromatograms for both the drugs named as DUA as well as RPV which are obtained without other interference are shown in the Figure 4.

Table 5: Specificity Parameters (n=5)

Parameters	DUA	RPV
Retention time calculated in min	2.9	3.8
Theoretical plates as N	11796	10499
Tailing factor as T	1.29	1.17
Resolution as Rs	1.9	

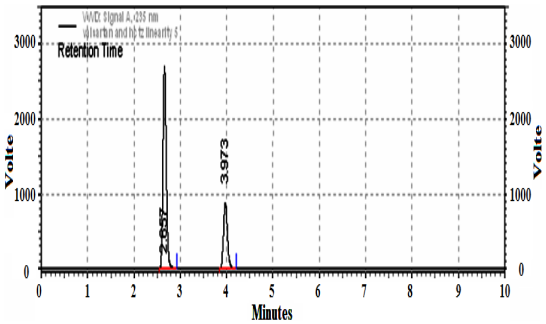


Fig. 4. RP-HPLC Chromatogram to show Specificity of Sample Solution

Calibration and Linearity

In order to determine strength of the linearity which is ranged from 50 g/mL to 250 g/mL for both the drugs DUA as well as RPV. For this experimental analysis we are used them, exact aliquots are pipette by working stock solution which is named as A in a series of 10.00 mL standard flask. The volume is marked by using suitable solvent. Every solution is passed as n triplicate. curves of calibration are drawn by using the observed peak areas against strength. The calibration curves to both the drugs named as DUA as well as RPV are represented in Fig. 5 and 6 and their related parameters of linearity are given in the Table 2.

Robustness

This parameter for this experimental process is documented as altering chromatographic circumstances includes the composition of mobile phase, detection of the wavelength, as well as rate of flow for its authenticity also to know cramped changes in this process circumstances. Solution of high strength by specified variations in operational circumstances are passed to instrument in triplicate. Values so identified for these drugs named as DUA and RPV are reported in the Table 7. Chromatogram of Robustness Study for DUA and RPVat a defined Flow rate, wavelength and analyst were represented in the Figure 7.

Table 6: At n=3Linearity results

Parameters	DUA	RPV
Slope	184489	68818
y intercept	111759	24921
Correlation coefficient r^2	0.9399	0.9098
Regression Equation	y=183599x+111597	y = 69917x+25513
Linearity range	50 µg/mL to 50 g/mL	50 µg/mlto 250 µg/mL

Table 7: Results for Robustness

Parameters as n=3	%RSD	
	DUA	RPV
233nm wavelength detection	0.68	0.67
237nm wavelength detection	0.16	0.86
0.60ml/min as flow rate	0.28	0.97
1.00ml/min as flow rate	0.51	0.39
Analyst I	0.69	0.72
Analyst II	0.95	0.85

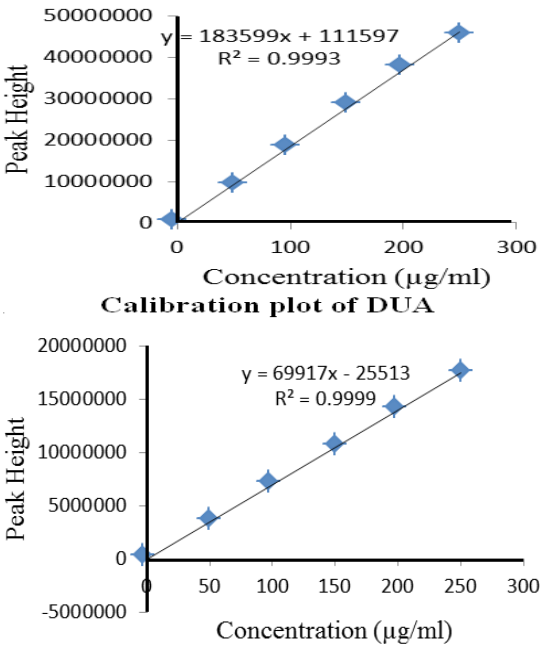
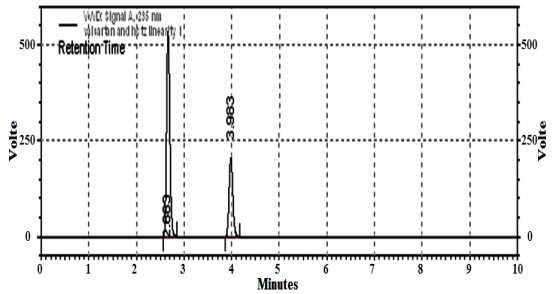
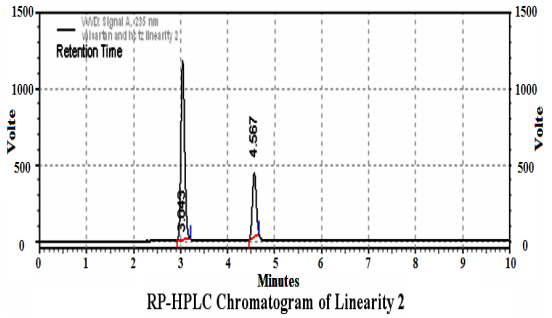


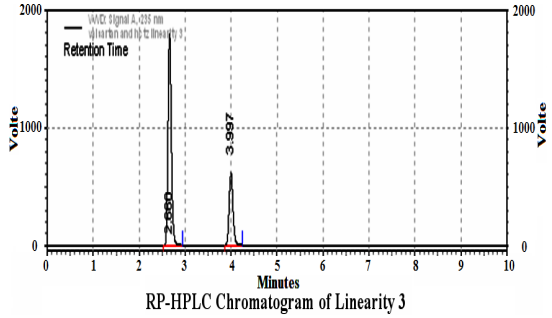
Fig. 5. Calibration curves for DUA and RPV



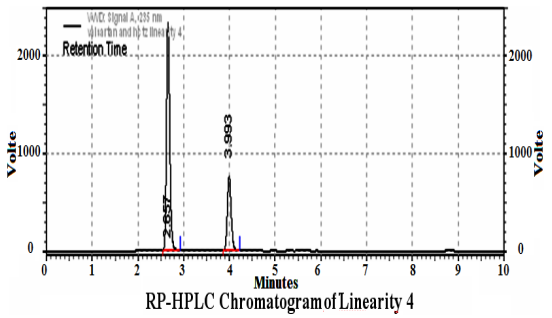
RP-HPLC Chromatogram of Linearity 1



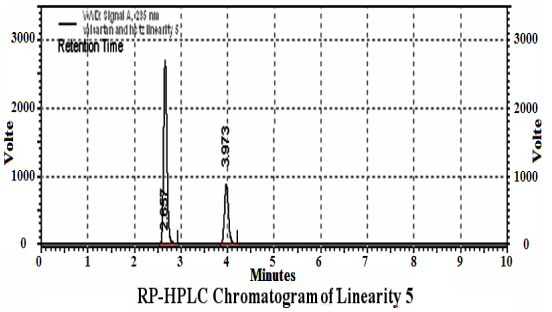
RP-HPLC Chromatogram of Linearity 2



RP-HPLC Chromatogram of Linearity 3

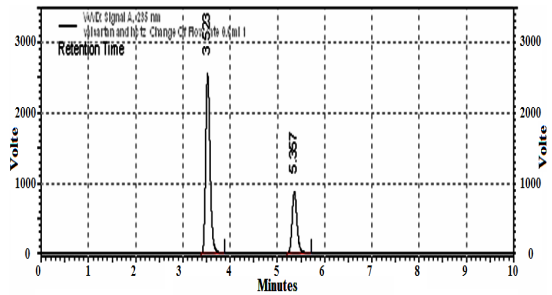


RP-HPLC Chromatogram of Linearity 4

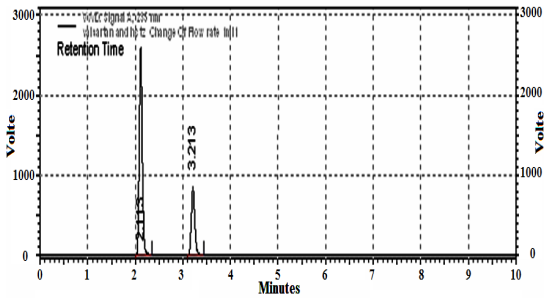


RP-HPLC Chromatogram of Linearity 5

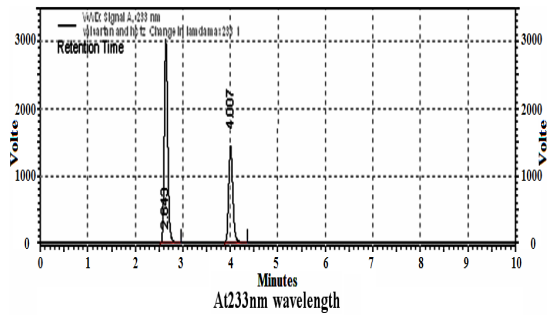
Fig. 6. Linearity curves for DUA and RPV



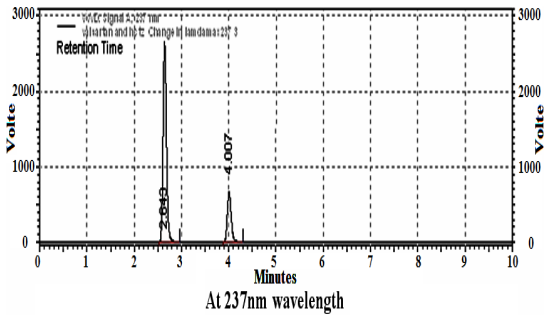
Flow rate At0.60ml/min flow rate



At 1.00ml/min flow rate



At233nm wavelength



At 237nm wavelength

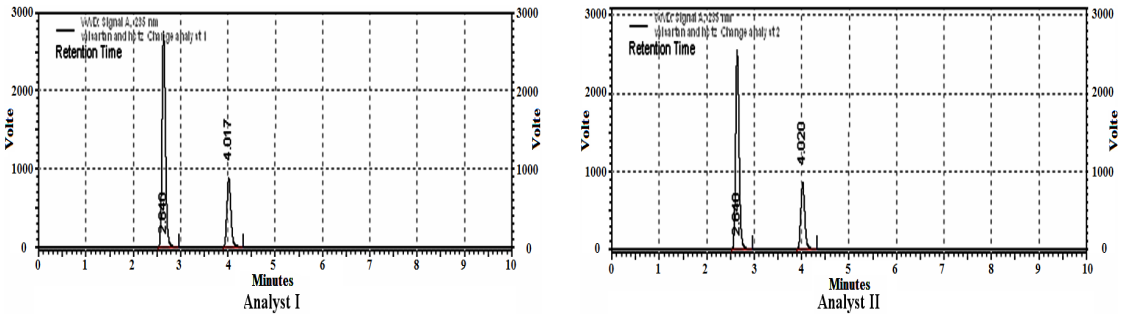


Fig. 7. RP-HPLC Chromatogram of Robustness Study for DUA and RPVat a defined Flow rate, wavelength and analyst

Detection and Quantitation Limits

These parameters are computed by slopes for plots of calibration as well as the standard deviation noted as SD for areas of the peak. The values are represented in the 8. And recovery levels are represented in the Figure 8.

Table 8: Detection limit for DUA and RPV

Parameters	DUA	RPV
LOD	2.00 µg/mL	1.19 µg/mL
LOQ	6.60 µg/mL	3.92 µg/mL

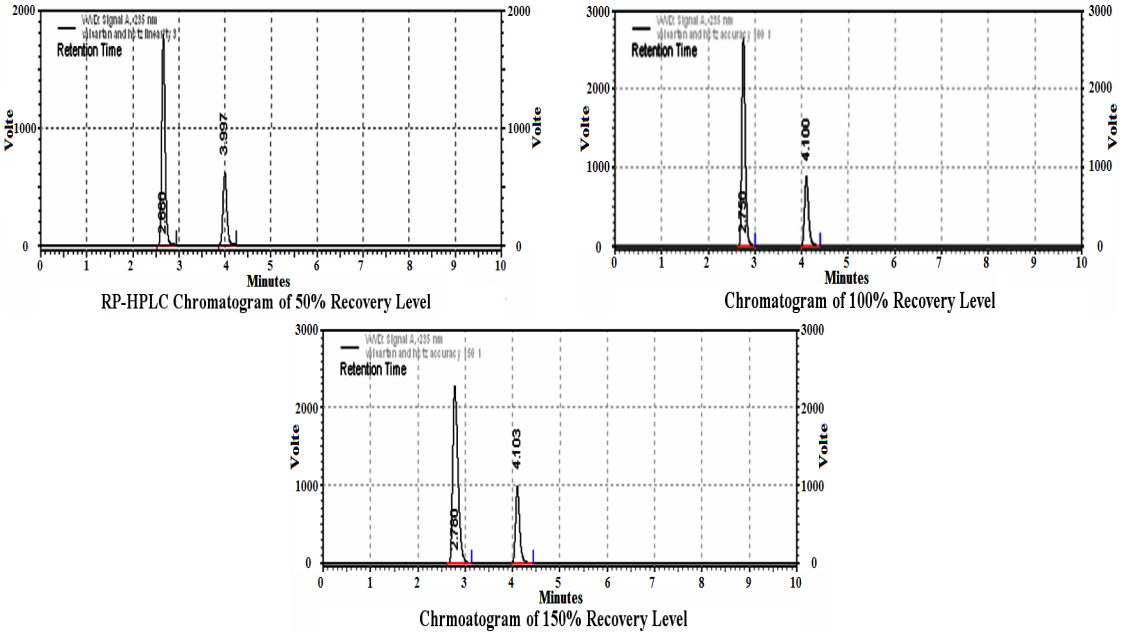


Fig. 8. RP-HPLCChrmoatogram of 50%, 100% and 150% Recovery Level

Analysis of Marketed Formulations

20.00 µl solution of sample with strength as 250 µg/mL for DUA µg/mL and 250 µg/mL of RPV is passed into system of chromatography and responses of the peak are noted. Solution is passed in total three times into column. The quantity of drug which is present as well as the purity percentage is demonstrated by comparing areas of peak standards by samples of testing solution. Aemblematic chromatogram to the assay of marketed formulation are represented in the Fig. 5 also identified DUA are represented in the Table 9. The assayed marketed formulation is represented in the Figure 9.

Table 9: At n=3 results for assay in marketed formulation

Drug	Label claim as mg/tablet	Recovered quantity	%quantity found in drug
DUA	50	49.23	98.46%
RPV	25	24.8	99.2%

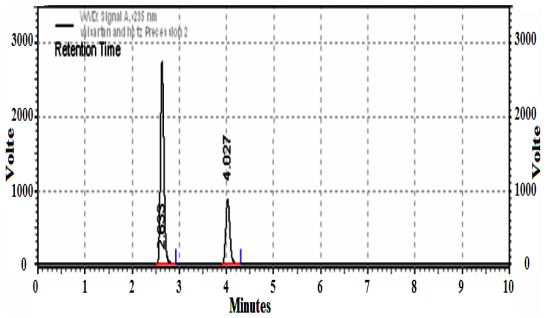


Fig. 9. RP-HPLC Chromatogram of Assay of Marketed formulation

CONCLUSION

Throughout the analytical process analytes are stable. For this development Agilent 100-5C18 column (250mmx4.6mm). Acetonitrile as 40v/v and Phosphate buffer which is maintained at pH 4.0as 60v/v is passed with the help of column. The mobile phase is used for this analysis is so simple for the preparation and economical. Flow rate was measure as 0.80 mL/minute. Column temperature is accompanied as ambient. Upgraded wavelength is 235nm. Run time is identified as 10 min and the volume injected for this analysis is 20 µL. Retention time of DUA and RPV were found to be 2.6 & 3.9 minute. %RSD of the DUA and RPV were and found to be 0.87, 0.40 as intraday and 0.75 and 0.56 as inter day precision. %Recovery was obtained as 98.59%, 100.7% for DUA and RPV respectively. The Rt values are minimized as well as run time was decreased. By using the experimental values as well as the parameters, it is finalized as both the drugs DUA as well as RPV are found as ecofriendly, reliable, reproducible simple, precise, accurate along with the high resolution also less retention time which produce this proposed process is more acceptable as well as cost-effective. This method is not a time consuming also this should be utilized clinical studies to analysis of bioavailability as well as bioequivalence.

This proposed method is profitably applied to assess in vitro dissolution of DUA as well as RPV which is loaded as a fixed dose consolidation of the tablets. Same method by using equal mobile phase is enforced over plasma of rat plasma and authors are not observed any other interference.

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Conflicts of Interest

There are no conflicts of interest among the authors to perform this work.

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