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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RITONAVIR, OMBITASVIR & PARITAPREVIR BY RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ritonavir, Ombitasvir and Paritaprevir in tablet dosage form. Chromatogram was run through Ascentis c18 150 x 4.6 mm, 5µ. Mobile phase containing Acetonitrile and Water in the proportion of 60:40 was pumped through column at a flow rate of 0.8ml/min. Temperature was maintained at 30°C. Optimized wavelength for Ritonavir, Ombitasvir and Paritaprevir was 265nm. Retention time of Ritonavir, Ombitasvir and Paritaprevir Were found to be 2.147 min; 2.732 min and 3.790 min. %RSD of system precision for Ritonavir, Ombitasvir and Paritaprevir were and found to be 0.4, 1.0 and 1.0 respectively. %RSD of method precision for Ritonavir, Ombitasvir and Paritaprevir were and found to be 0.5, 0.4 and 0.8 respectively. % recovery was obtained as 100.30%, 100.19% and 100.15% for Ritonavir, Ombitasvir and Paritaprevir respectively. LOD, LOQ values are obtained from regression equations of Ritonavir, Ombitasvir and Paritaprevir were 0.14ppm, 0.44ppm, 0.06ppm, 0.19ppm and 0.42ppm, 1.28ppm respectively. Regression equation of Ritonavir was y = 28427x + 10360, Ombitasvir was y = 8648.x + 422.2 and of Paritaprevir was y = 39168x + 17464. Retention times are not as much as different techniques so the method developed were basic and conservative that can be received in standard Quality control test in Industries. Retention times are decreased so the method developed basic and conservative embraced in general Quality control test in Industries.

KEYWORDS: Ritonavir. Ombitasvir and Paritaprevir, RP-HPLC.

INTRODUCTION

Chemically Ritonavir (RTN) represses the HIV viral proteinase compound that typically divides the auxiliary and replicative proteins that emerge from significant HIV qualities, for example, muffle and pol. Stifler encodes proteins associated with the center and the nucleocapsid, while pol encodes the HIV invert transcriptase, ribonuclease H, integrase, and protease. The pol-encoded proteins are at first interpreted as a bigger precursor polypeptide, choke pol, and should be severed by HIV protease to shape other supplement proteins. Ritonavir keeps the cleavage of the stifler pol polyprotein, which brings about noninfectious, juvenile viral particles. Ritonavir is a strong inhibitor of cytochrome P450 CYP3A4 isoenzyme display both in the intestinal tract and liver. It is a sort II ligand that superbly fits into the CYP3A4 dynamic site depression and irreversibly ties to the heme press by means of the thiazole nitrogen, which diminishes the redox capability of the protein and blocks its lessening with the redox accomplice, cytochrome P450 reductase. Ritonavir may likewise assume a part in constraining cell transport and efflux of other protease inhibitors through the P-glycoprotein and MRP efflux channels. Structure of the RTN was shown in figure 1 (A).[1]

Chemically Ombitasvir (OMB) is an inhibitor of the HCV non-basic protein 5A. While the exact part of this protein is obscure, it is basic to viral replication and virion get together [FDA Label]. Potential methods of activity of NS5A inhibitors like Elbasvir incorporate blocking flagging associations, redistribution of NS5A from the endoplasmic reticulum to the surface of lipid beads, and alteration of the HCV replication complex. Structure of the OMB was shown in figure 1 (B).[2]

Chemically Paritaprevir (PRP) is a powerful inhibitor of the NS3/4A serine protease of Hepatitis C Virus (HCV) [FDA Label]. Following viral replication of HCV hereditary material and interpretation into a solitary polypeptide, Nonstructural Protein 3 (NS3) and its actuating cofactor Nonstructural Protein 4A (NS4A) are in charge of dividing it into the accompanying auxiliary and nonstructural proteins required for get together into develop infection: NS3, NS4A, NS4B, NS5A, and NS5B. By hindering viral protease NS3/4A, paritaprevir in this way counteracts viral replication and capacity. Structure of the PRP was shown in figure 1 (C).[3]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two

or three drugs.[5-9] But there is only very few HPLC methods are available for simultaneous estimation of RTN, OMB and PRP, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

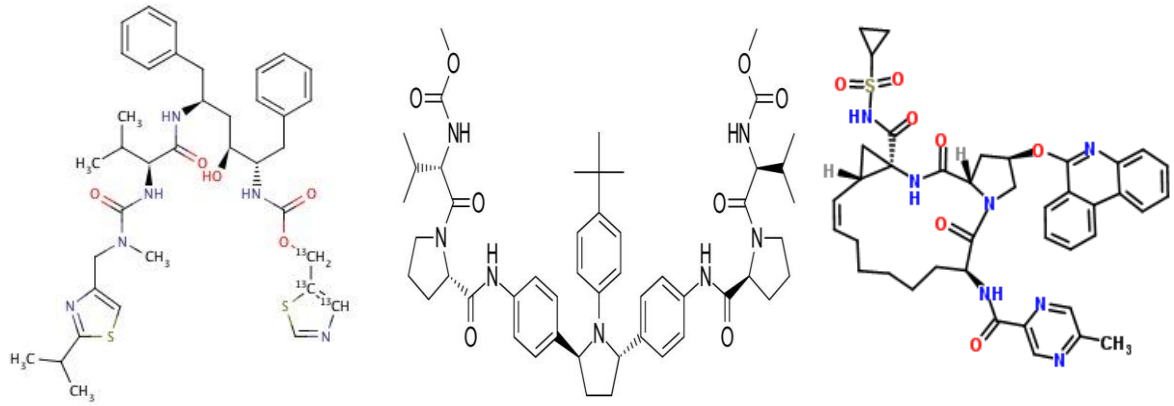

Figure 1: Structure of (A)Ritonavir (B) Ombitasvir (C) Paritaprevir.

MATERIALS AND METHODS

Reagents and Chemicals: Ritonavir, Ombitasvir & Paritaprevir pure drugs (API), Combination Ritonavir, Ombitasvir & Paritaprevir, Distilled water, Acetonitrile, Tri ethyl amine, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Ascentis c18 150 x 4.6 mm, 5µ. As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Ascentis c18 150 x 4.6 mm, 5µ column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of Acetonitrile and water was taken in the ratio of (60:40%v/v) mobile phase for the investigation with a flow rate of a 0.8ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 265nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Buffer : Water - in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water

Preparation of mixture Standard stock solution: Precisely measured 25mg of Ritonavir, 6.25mg of Ombitasvir and 37.5mg of Paritaprevir and transferred to three 50ml volumetric flasks independently. 10ml of methanol was added and sonicated for 15mins. Volume were made up with water and acetonitrile (50:50) and marked as Standard stock arrangement 1, 2 and 3.

Preparation of Sample (Tablet) stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 6mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.

Optimized chromatographic conditions

Column Used	:	Ascentis c18 150 x 4.6 mm, 5µ
Mobile phase	:	Acetonitrile : Water (60:40v/v)
Flow rate	:	0.8ml/min
Wavelength	:	265.0 nm
Temperature	:	30◦C
Injection Volume	:	10.0µl

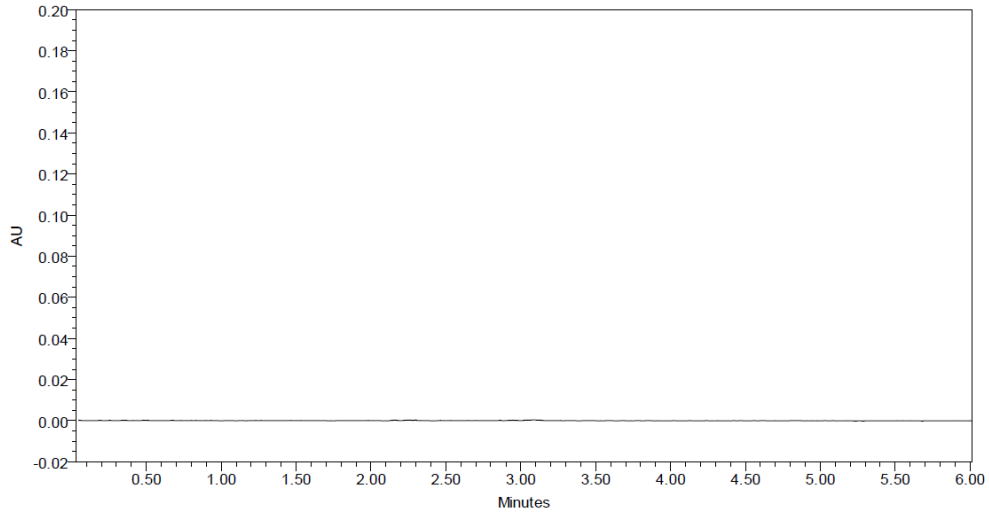

Figure 2: Blank chromatogram.

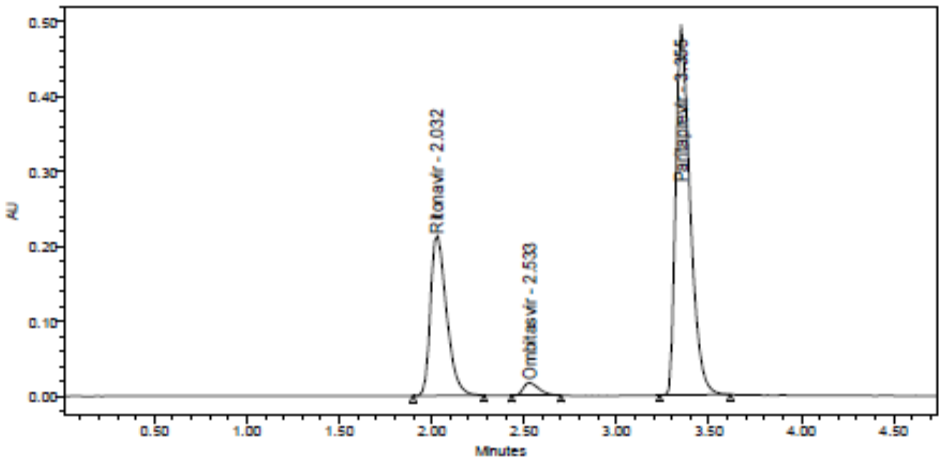

Figure 3: Chromatogram of standard mixture of RTN, OMB & PRP.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Ritonavir	2.032	1394994	1.34	5	5447
2	Ombitasvir	2.533	107049	1.40	4.9	5691
3	Paritaprevir	3.355	2886583	1.42	4.6	9797

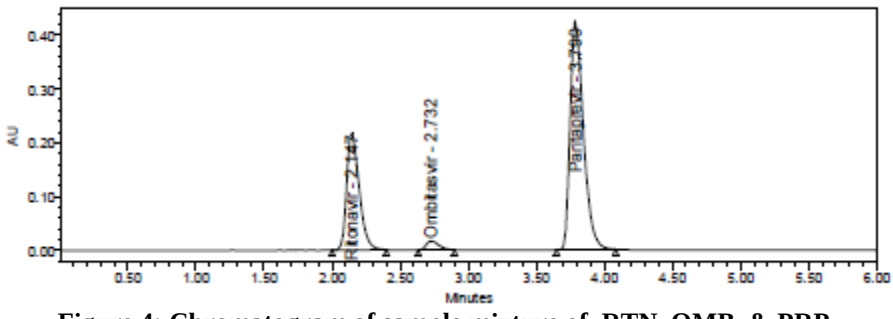

Figure 4: Chromatogram of sample mixture of RTN, OMB & PRP.

VALIDATION

The above optimized chromatographic method has been validated for the assay of RTN, OMB & PRP using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Ritonavir, Ombitasvir and Paritaprevir (RTN, OMB & PRP)drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml)

and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Ritonavir (25mg)+ Ombitasvir (6.25mg)+ Paritaprevir (37.5mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Ritonavir (25mg)+ Ombitasvir (6.25mg)+ Paritaprevir (37.5mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Ritonavir, Ombitasvir and Paritaprevir. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for RTN was found to be y = 28427x + 10360 (slope, intercept and correlation coefficient were found to be 28427, 10360 and 0.999 respectively) and linear over beer's range of 12.5-75 µg/ml. The regression equation for OMB was found to be y = 8648x + 422.2 (slope, intercept and correlation coefficient were found to be 8648, 422.2 and 0.999 respectively) and linear over beer's range of 3.125-18.75 µg/ml. The regression equation for PRP was found to be y= 39168x + 17464 (slope, intercept and correlation coefficient were found to be 39168, 17464 and 0.999 respectively) and linear over beer's range of 18.75-112.5µg/ml. Linearity graph of RTN, OMB & PRP were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Ritonavir, Ombitasvir and Paritaprevir were and found to be 0.4, 1.0 and 1.0 respectively. %RSD of method precision for Ritonavir, Ombitasvir and Paritaprevir were and found to be 0.5, 0.4 and 0.8 respectively. % recovery was obtained as 100.30%, 100.19% and 100.15% for Ritonavir, Ombitasvir and Paritaprevir respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug RTN, OMB & PRP and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 100.30%, 100.19% and 100.15% w/w for 50%, 100% and 150% respectively.. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for RTN, OMB & PRP was found to be 0.14µg/ml, 0.06 µg/ml and 0.42 µg/ml respectively. LOQ for RTN, OMB & PRP was found to be 0.44µg/ml, 0.19 µg/ml and 1.28 µg/ml respectively. Summary of all the validation parameter shown in table 6.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 1: Linearity table for RTN, OMB & PRP.

Ritonavir		Ombitasvir		Paritaprevir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
12.5	364310	3.125	27529	18.75	759229
25	736497	6.25	53338	37.5	1505660
37.5	1067078	9.375	81078	56.25	2205779
50	1452292	12.5	113783	75	2985677
62.5	1780944	15.625	133046	93.75	3663455
75	2133444	18.75	161767	112.5	4424962

Table 2: System precision table of RTN, OMB & PRP.

S. No	Area of Ritonavir	Area of Ombitasvir	Area of Paritaprevir
1.	1387812	107487	2876087
2.	1399330	106425	2865723
3.	1388338	108249	2851861
4.	1395570	106891	2922528
5.	1398995	107833	2879601
6.	1392739	105410	2923697
Mean	1394994	107049	2886583
S.D	5050.1	1034.1	29897.0
%RSD	0.4	1.0	1.0

Table 3: degradation data of RTN.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.07	0.206	0.261
2	Alkali	5.40	0.218	0.272
3	Oxidation	4.28	0.206	0.267
4	Thermal	2.61	0.225	0.271
5	UV	1.49	0.212	0.259
6	Water	0.83	0.217	0.279

Table 4: degradation data of OMB

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.89	0.261	0.334
2	Alkali	4.98	0.209	0.334
3	Oxidation	4.33	0.306	0.455
4	Thermal	2.28	0.578	0.983
5	UV	1.66	0.575	0.763
6	Water	0.98	0.237	0.406

Table 5: degradation data of PRP.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	7.30	0.219	0.264
2	Alkali	5.55	0.195	0.261
3	Oxidation	4.74	0.106	0.269
4	Thermal	2.07	0.098	0.256
5	UV	1.81	0.102	0.264
6	Water	1.03	0.101	0.255

Table 6: summary of validation data of RTN, OMB & PRP.

Parameters		Ritonavir.	Ombitasvir	Paritaprevir	LIMIT
Linearity Range (µg/ml)		12.5-75 µg/ml	3.125-18.75 µg/ml	18.75-112.5µg/ml	R< 1
Regression coefficient		0.999	0.999	0.999	
Slope(m)		28427	8648	39168	
Intercept(c)		10360	422.2	17464	
Regression equation (Y=mx+c)		y= 28427x + 10360	y=8648x + 422.2	y=39168x + 17464	
Assay (% mean assay)		99.89%	99.95%	100.12%	90-110%
Specificity		Specific	Specific	Specific	No interference of any peak
System precision %RSD		0.4	1.0	1.0	NMT 2.0%
Method precision%RSD		0.5	0.4	0.8	NMT 2.0%
Accuracy% recovery		100.30%	100.19%	100.15%	98-102%
LOD		0.14µg/ml	0.06 µg/ml	0.42 µg/ml	NMT 3 µg/ml
LOQ		0.44µg/ml	0.19 µg/ml	1.28 µg/ml	NMT 10µg/ml
Robustness	**FM**	0.8	1.1	0.6	%RSD NMT 2.0
	FP	0.3	0.8	0.4	
	MM	0.3	1.0	0.9	
	MP	1.3	0.2	0.6	
	TM	0.8	0.9	0.8	
	TP	0.7	0.8	1.0	

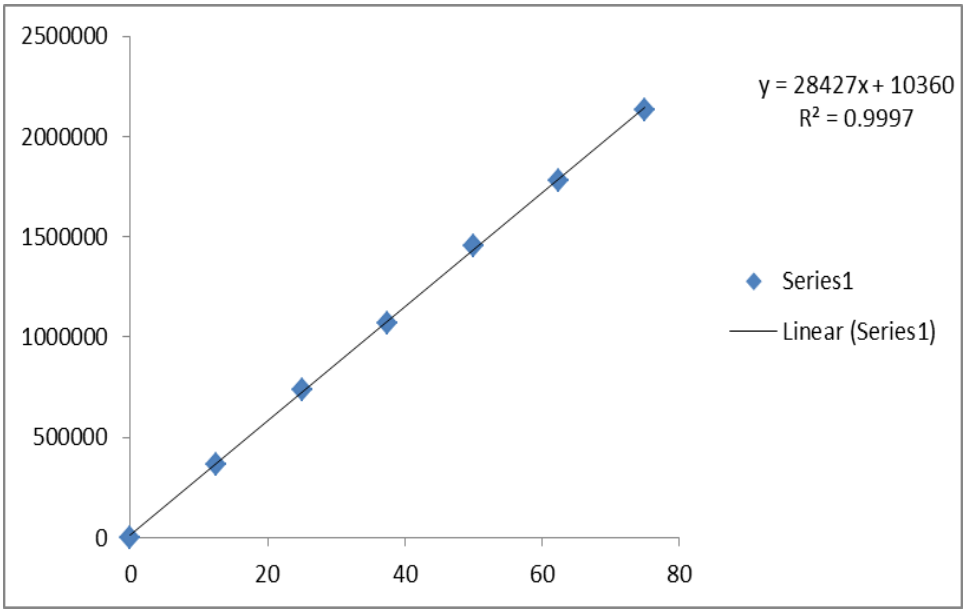

Fig No 7 Linearity curve of Ritonavir.

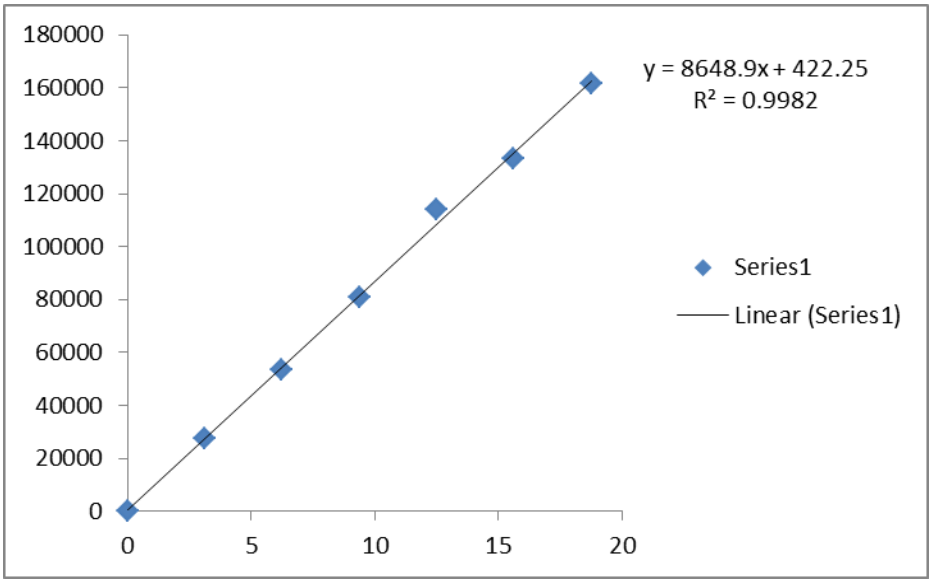

Fig No 8 Linearity curve of Ombitasvir.

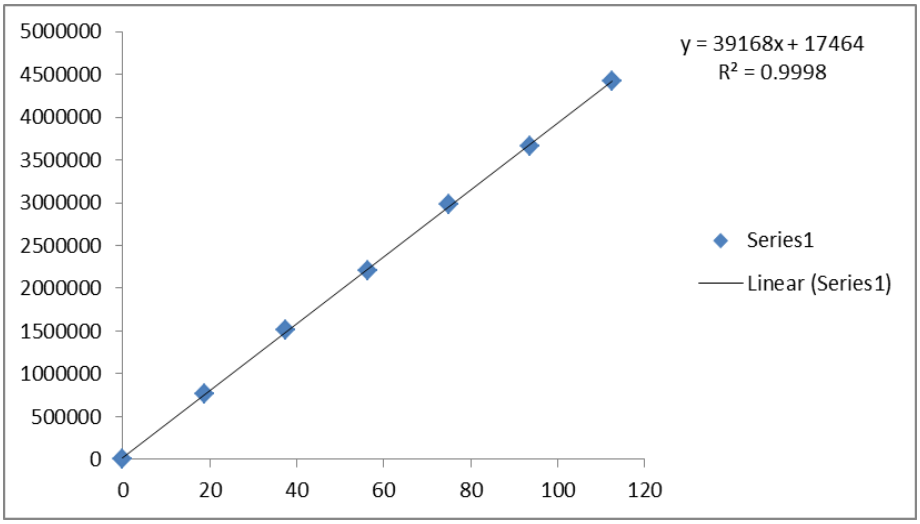

Fig No 9: Linearity curve of Paritaprevir.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Ritonavir, Ombitasvir and Paritaprevir in Tablet dosage form was developed and the proposed method as suitable for routine analysis of RTN, OMB & PRP.

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