International Journal of
Medicinal Chemistry & Analysis
e ISSN 2249 – 7587
Print ISSN 2249 - 7595
www.ijmca.com
Research Article

DEVELOPMENT AND VALIDATION OF NEW STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF SELECTED COMBINATIONAL ANTIVIRAL DRUGS IN BULK AND PHARMACEUTICAL DOSSAGE FORMS

K. Kranthi Kiran*[1], A.Srinivasa Rao[2], D.Gowri Sankar[3]

[1]Research Scholar, [2]Research Guide, Pharmaceutical Analysis & Quality Assurance Division, Sri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India.
[3]Joint Research Guide, Pharmaceutical Analysis & Quality Assurance Division, A.U College of Pharmaceutical Sciences, Andhra Pradesh, India.

ABSTRACT

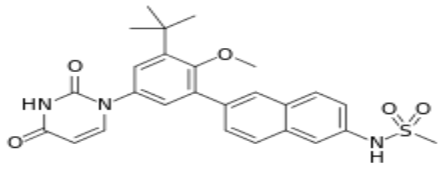
An isocratic Simultaneous estimation by RP-HPLC Method were developed and validated for the quantification of Dasabuvir, Ombitasvir, Paritaprevir and Ritonavir in tablet dosage form. Quantification was achieved by using a reversed-phase C18 column (INERTSIL Column, 5µ, 150 mm × 4.6 mm) at ambient temperature with mobile phase consisting of Mixed Ammonium acetate Buffer: Acetonitrile (40: 60). The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 262nm. The average retention time for Dasabuvir, Ombitasvir, Paritaprevir and Ritonavir were found to be 2.13 min, 2.82min, 3.90min and 5.75. The proposed method was validated for selectivity, precision, linearity and accuracy. The developed method was successfully applied to estimate the amount of Dasabuvir, Ombitasvir, Paritaprevir and Ritonavir in tablet dosage form.

Keywords: Dasabuvir, Ombitasvir, Paritaprevir, Ritonavir RP-HPLC method, Inertsil Column, Methanol, Acetonitrile, Ammonium acetate and Validation.

Corresponding Author: - **K. Kranthi Kiran** Email: Kothapallikranthikiran@gmail.com

INTRODUCTION

Dasabuvir:



Structure of dasabuvir

IUPAC Name	N-{6-[3-tert-butyl-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-methoxyphenyl]naphthalen-2-yl}methanesulfonamide
Molecular formula	$C_{26}H_{27}N_3O_5S$
Molecular weight	493.58
Half-life	7hrs
Category	Antivirals for Systemic Use

Dasabuvir is an antiviral drug used for the treatment of hepatitis C virus (HCV) infection. As a Non-Nucleoside NS5B Polymerase Inhibitor, dasabuvir

Access this article online

Home page: http://ijmca.com/

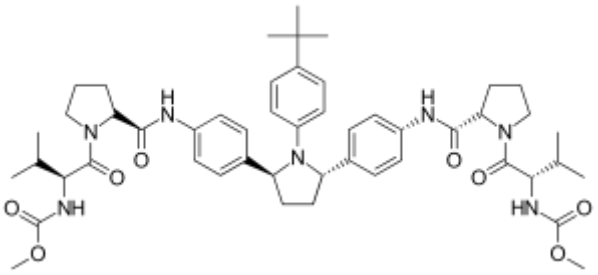
DOI: http://dx.doi.org/10.21276/ijmca.2017.7.2.4

Quick Response code

Received:10.06.17 **Revised**:12.06.17 **Accepted**:25.06.17

functions by inhibiting Nonstructural Protein 5B (NS5B), an RNA-dependent RNA polymerase required for viral replication of Hepatitis C Virus. By binding to NS5b outside of the active site of the enzyme, dasabuvir induces a conformational change thereby preventing further elongation of the nascent viral genome. A limitation of binding outside of the active site is that these binding sites are poorly preserved across the viral genotypes. This results in a limited potential for cross-genotypic activity and increased potential for development of resistance. Dasabuvir is therefore limited to treating genotypes 1a and 1b, and must be used in combination with other antiviral products. Dasabuvir is currently approved for use in the management of Hepatitis C infection in combination with ombitasvir, paritaprevir, and ritonavir as the combination product Viekira Pak.

Ombitasvir:

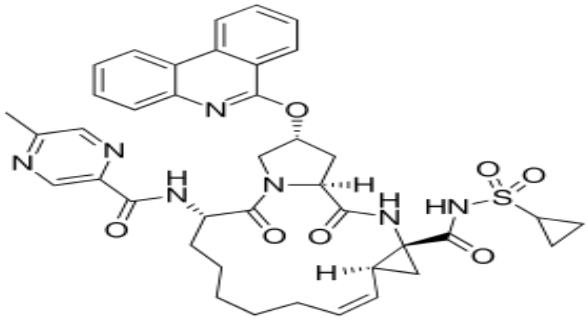


Structure of ombitasvir

Ombitasvir is a direct acting inhibitor of Nonstructural Protein 5A (NS5A) of Hepatitis C Virus (HCV) and is currently approved for use in combination with other antivirals for the treatment of chronic HCV infection. Despite lacking enzymatic activity, NS5A is necessary for viral replication and virion assembly through interaction with other nonstructural proteins. By combining ombitasvir with other antiretroviral medications into fixed dose products, the viral lifecycle can be targeted at multiple stages while simultaneously reducing the risk of developing resistant viral strains. Within Canada and the United States, Ombitasvir is currently available in three fixed dose products: Viekira Pak (FDA), Technivie (FDA and Health Canada), and Holkira Pak (Health Canada). When used within the fixed dose combination product of paritaprevir/ombitasvir/ritonavir (Technivie), ombitasvir is indicated in combination with ribavirin for the treatment of patients with genotype 4 chronic hepatitis C virus (HCV) infection without cirrhosis. When used within the fixed dose combination product of paritaprevir/ombitasvir/dasabuvir/ritonavir (Viekira Pak), ombitasvir is indicated for the treatment of HCV genotype 1b without cirrhosis or with compensated cirrhosis, and when combined with ribavirin for the treatment of HCV genotype 1a without cirrhosis or with compensated cirrhosis.

IUPAC Name	Methyl N-[(2S)-1-[(2S)-2-({4-[(2S,5S)-1-(4-tert-butylphenyl)-5-{4-[(2S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]pyrrolidine-2-amido]phenyl}pyrrolidin-2-yl]phenyl}carbamoyl)pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate.
Molecular formula	$C_{50}H_{67}N_7O_8$
Molecular weight	894.127
Category	Antivirals for Systemic Use

Paritaprevir



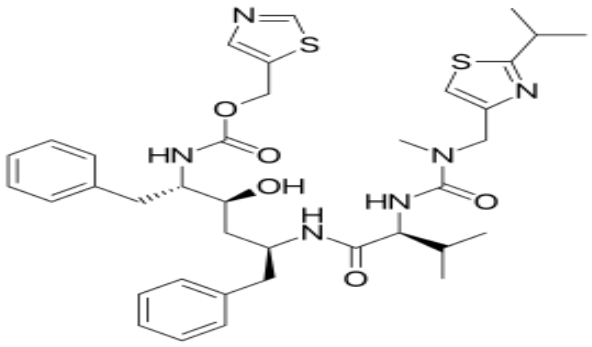
Structure of paritaprevir

Paritaprevir is a potent inhibitor of the NS3/4A serine protease of Hepatitis C Virus (HCV). Following viral replication of HCV genetic material and translation into a single polypeptide, Nonstructural Protein 3 (NS3) and its activating cofactor Nonstructural Protein 4A (NS4A) are responsible for cleaving it into the following structural and nonstructural proteins required for assembly into mature virus: NS3, NS4A, NS4B, NS5A, and NS5B. By inhibiting viral protease NS3/4A, paritaprevir therefore prevents viral replication and function. As a newer generation and directly acting HCV antiviral, paritaprevir products have better Sustained Virological Response (SVR) rates, higher barriers to resistance, fewer side effects, and a reduced pill burden compared to older agents such as bocepravir, telaprevir, peginterferon, and ribavirin. By combining multiple antiretroviral medications into fixed dose products, the viral lifecycle can be targeted at multiple stages while simultaneously reducing the risk of developing resistant viral strains. Within Canada and the United States, paritaprevir is currently available in three fixed dose products: Viekira Pak (FDA), Technivie (FDA and Health Canada), and Holkira Pak (Health Canada). When used within the fixed dose combination product of paritaprevir/ombitasvir/ritonavir (Technivie), paritaprevir is indicated in combination with ribavirin for the treatment of patients with genotype 4 chronic hepatitis C virus (HCV) infection without cirrhosis. When used

within the fixed dose combination product of paritaprevir/ombitasvir/dasabuvir/ritonavir (Viekira Pak), paritaprevir is indicated for the treatment of HCV genotype 1b without cirrhosis or with compensated cirrhosis, and when combined with ribavirin for the treatment of HCV genotype 1a without cirrhosis or with compensated cirrhosis.

IUPAC Name	(1S,4R,6R,7Z,14S,18R)-N-(cyclopropanesulfonyl)-14-(5-methylpyrazine-2-amido)-2,15-dioxo-18-(phenanthridin-6-yloxy)-3,16-diazatricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxamide
Molecular formula	$C_{40}H_{43}N_7O_7S$
Molecular weight	765.89
Category	• NS3/4A Protease Inhibitors

Ritonavir:



Structure of ritonavir

Ritonavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles.

Ritonavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Ritonavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs.

IUPAC Name	1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate
Molecular formula	$C_{37}H_{48}N_6O_5S_2$
Molecular weight	720.944
Category	• Direct Acting Antivirals

EXPERIMENTAL METHODS:

Equipments: The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software , reversed phase C18 column (Inertsil 5µ, 150 mm × 4.6 mm) as stationary phase. Thermo electron corporation double beam UV-visible spectrophotometer (vision pro-software) ,Ultrasonic cleaner, Shimadzu analytical balance AY-220,Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials: Pharmaceutically pure sample of dasabuvir, ombitasvir, paritaprevir and ritonavir were obtained as gift samples from Chandra Labs, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification.
HPLC-grade Acetonitrile and Methanol ware from standard reagents pvt ltd. Ammonium Acetate (AR grade) was from Merck.

Brand Name: **Viekirapak** This tablet formulation contains Dasabuvir-250 mg and Ombitasvir-12.5mg, Paritaprevir 75mg, Ritonavir 50mg label claims were procured from local market.

Chromatographic conditions: The sample separation was achieved on a C18 (5 µ, 15 cm X 4.6 mm i.d.) Inertsil column, aided by mobile phase mixture of Ammonium acetate Buffer : Acetonitrile (40:60)), that was filtered and degassed prior to use, at a flow rate of 1ml/min. Injection volume is 20 µl and detected at 262 nm at ambient temperatures.

Preparation of mobile phase:

Buffer Preparation: Weigh accurately about 0.77gms of Ammonium acetate and dissolve with 1000ml of HPLC Grade water

Mobile phase: Then add 40 volumes of buffer, 60volumes of Acetonitrile and sonicated for 15 min and filtered through a 0.45 µ membrane filter.

Analysis of formulation

Preparation of standard solution:

Weigh accurately 500mg of DASABUVIR, 2.5mg of OMBITASVIR, 10mg of PARITAPREVIR and RITONAVIR 1mg in 100 ml of volumetric flask and

dissolve in 70ml of mobile phase and make up the volume with mobile phase from above stock solution 160µg/ml of DASABUVIR and 0.8µg/ml of OMBITASVIR and 3.2µg/ml of PARITAPREVIR 0.3µg/ml RITONAVIR is prepared by diluting 1.6ml to 50ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution (Tablet Formulation): 20 tablets (each tablet contains 500mg of dasabuvir and 2.5mg of ombitasvir and 10mg of paritaprevir and 1mg of ritonavir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. weight equivalent to 513.5mg of dasabuvir, ombitasvir, paritaprevir and ritonavir were dissolved in sufficient mobile phase. after that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. further dilutions are prepared in 5 replicates of 160µg/ml of dasabuvir and 0.8µg/ml of ombitasvir and 3.2µg/ml of paritaprevir and 0.3 µg/ml ritonavir was made by adding 1.6 ml of stock solution to 50 ml of mobile phase.

RESULTS AND DISCUSSION:

Determination of working wavelength(λmax): The λmax was found to be 262n

After several initial trails with mixtures of Methanol, Acetonitrile and Different buffer in various combinations and proportions, a trail with a mobile phase mixture of Ammonium acetate Buffer : Acetonitrile (40:60) brought sharp and well resolved peaks. The chromatogram was shown in Figure-2**.**

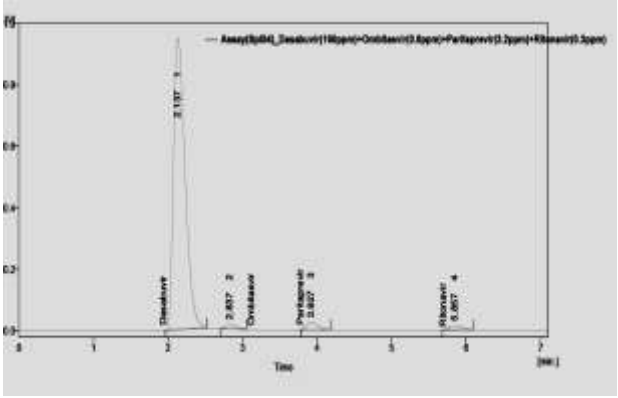


Figure: 2 Chromatogram of dasabuvir, ombitasvir, paritaprevir and ritonavir

METHOD VALIDATION:

Linearity:

Preparation of Standard solution

Weigh accurately 1000mg of dasabuvir and 5mg of ombitasvir and 20mg of paritaprevir and 2mg of ritonavir in 200 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. Then diluted 50ml by taking 0.8ml, 1.2ml, 1.6ml, 2ml, 2.4ml from the standard stock solution. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 3160µg/ml of dasabuvir and 0.8µg/ml of ombitasvir and 3.2µg/ml of paritaprevir and 0.3 µg/ml ritonavir without changing the parameter of the proposed chromatographic method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 different days over a period of 1 week for 160µg/ml of DASABUVIR and 0.8µg/ml of ombitasvir and 3.2µg/ml of paritaprevir and 0.3 µg/ml ritonavir. the result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

LOD = 3.3 δ/S ……. (3)

LOQ =10 δ/S …….. (4)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

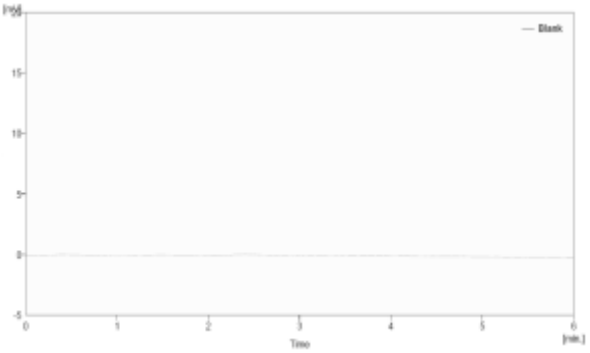
The slope S may be estimated from the calibration curve of the analyte.

Accuracy (recovery study):

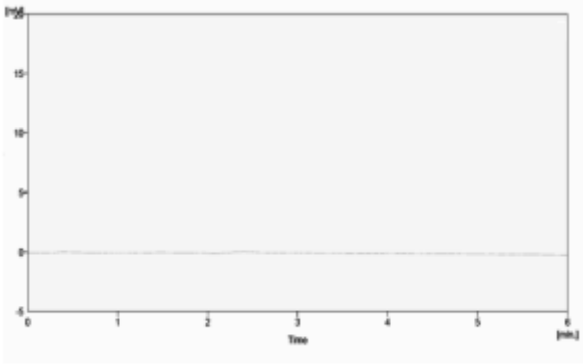
Tthe accuracy of the method was determined by calculating the recoveries of dasabuvirombitasvir and paritaprevir and ritonavir by the standard addition method. known amounts of standard solutions of dasabuvir, ombitasvir and paritaprevir and ritonavir were added at 10% concentration to pre quantified sample solutions (figure no.5.1 and 5.2). the amount of dasabuvir and ombitasvir and paritaprevir and ritonavir recovered was estimated by using the following formulas.

Specificity: In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should

be no interference of the diluents, placebo at retention time of drug substances.



Typical Chromatogram for Blank



Typical Chromatogram for Placebo

Robustness:

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table no.4)

DISCUSSION:

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods. . the iso bestic point of dasabuvir, ombitasvir, paritaprevir and ritonavir were found to be 262nm by scanning in uv region. the chromatographic method was optimised with mobile phase consisting of ammonium acetete buffer: acetonitrile: (30: 40:60) and inertsil column(c18 150* 4.6mm & 5um). all the validation parameters were studied at a the wavelength 262nm. accuracy was determined by calculating the recovery (table no.3) and the results were in acceptable range (limit 98-102%). the method was successfully used to determine the amount of dasabuvir,ombitasvir,paritaprevir and ritonavir present in the tablet. the results obtained were in good agreement with the corresponding labeled amount (table no.3). the method was linear in the concentration range of 80 to 240µg/ml for dasabuvir, 0.4 to 1.2µg/ml for ombitasvir, paritaprevir 1.6 to 4.8µg/ml &ritonavir 0.16 to 0.48µg/ (figure no.1&1.1, table no.1&1.1). precision was calculated as repeatability and intra and inter day variations (% rsd) for the drug (table no.7 &8). robustness and ruggedness results were in acceptable range (table no.4 and table no.5). summary of all validation parameters for method is given in table no.9. by observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. hence the method can be employed for the routine analysis dasabuvir,ombitasvir,paritaprevir and ritonavir in tablet dosage form.

Table 1. Linearity of dasabuvir

S. No.	Concentration (µg/mL)	Area
1	80	6731.496
2	120	8330.181
3	160	10216.88
4	200	11286.567
5	240	12672.62

Table 2. Linearity of ombitasvir

S. No.	Concentration (µg/mL)	Area
1	0.4	74.024
2	0.6	108.134
3	0.8	146.43
4	1	169.786
5	1.2	193.51

Table 3. Linearity of paritaprevir

S. No.	Concentration (µg/mL)	Area
1	1.6	159.726
2	2.4	194.062
3	3.2	238.175
4	4	276.206
5	4.8	297.744

Table 4. Linearity of ritonavir

S. No.	Concentration (µg/mL)	Area
1	0.16	105.987
2	0.23	142.069
3	0.32	175.169
4	0.36	195.172
5	0.48	225.956

Table 6. LOD and LOQ values Calculated from calibration curve:

Dasabuvir			Ombitasvir	
	mcg	Area	Mcg	Area
LOD	2.70	31.33	1.27	41.75
LOQ	8.19	94.94	3.84	126.52

Table 7. Recovery results

S. No	Name of Sample	Preparation	%Recovery	Average
1	Dasabuvir	Preparation-01	99.83	100.4
2		Preparation-02	101.66	
3		Preparation-03	99.72	
4	Ombitasvir	Preparation-01	101.8	101.5
5		Preparation-02	99.34	
6		Preparation-03	100.49	
7	Paritaprevir	Preparation-01	100.89	100.5
8		Preparation-02	101.1	
9		Preparation-03	99.65	
10	Ritonavir	Preparation-01	100.6	100.1
11		Preparation-02	100.38	
12		Preparation-03	99.48	

Table 8. Method Precision Results

S.No	Name of Sample	%Assay
1	Dasabuvir	100.11
4	Ombitasvir	100.28
7	Paritaprevir	100.77
10	Ritonavir	100.89

Table 9. Results of Ruggedness

S.No	Name of Sample	Analyst-01	Analyst-02	%RSD
1	Dasabuvir	100.11	99.83	0.20
4	Ombitasvir	100.28	100.84	0.39
7	Paritaprevir	100.77	100.45	0.22
10	Ritonavir	100.89	100.63	0.18

Table 11. Results of Robustness (Flow rate)

S.No	Name of Sample	Theoretical plates	Asymmetry	Resolution
1	Dasabuvir	3524	1.01	-
4	Ombitasvir	4696	0.98	2.1
7	Paritaprevir	5874	1.21	3.5
10	Ritonavir	4585	1.15	4.1

Results of Robustness (Wavelength)

S.No	Name of Sample	Theoretical plates	Asymmetry	Resolution
1	Dasabuvir	4582	1.04	-
4	Ombitasvir	4585	0.99	2.2
7	Paritaprevir	5455	1.18	3.4
10	Ritonavir	4125	1.09	4.1

Table 12. Validation parameters of evaluated method

Parameter	Limit	Value obtained
Accuracy (%Recovery)	98-102%	99.89 to 101.66% (Dasabuvir) 99.34to101.80% (Ombitasvir) 99.65 To 100.89% (Paritaprevir) 99.48 To 100.6(Ritonavir)
Regression coefficient (R^2 value)	NLT 0.99%	0.998 for Dasabuvir , 0.997 for Ombitasvir, 0.997 for Paritaprevir, 0.998 for Ritonavir
precision(Repeatability) (%RSD, n = 6)	NMT 1% (For RT) NMT 2% (For Area)	Dasabuvir 0.77,Ombitasvir 1.21 Paritaprevir 0.87, Ritonavir 1.04
Intermediate Precision (%RSD)		Dasabuvir 0.20,Ombitasvir 0.39, Paritaprevir 0.22,Ritonavir 0.18
Robustness (%Assay)	System suitability criteria within the limit	Complies

SD=Standard deviation, %RSD = Relative standard deviation

Fig. 1. Linearity graph of dasabuvir

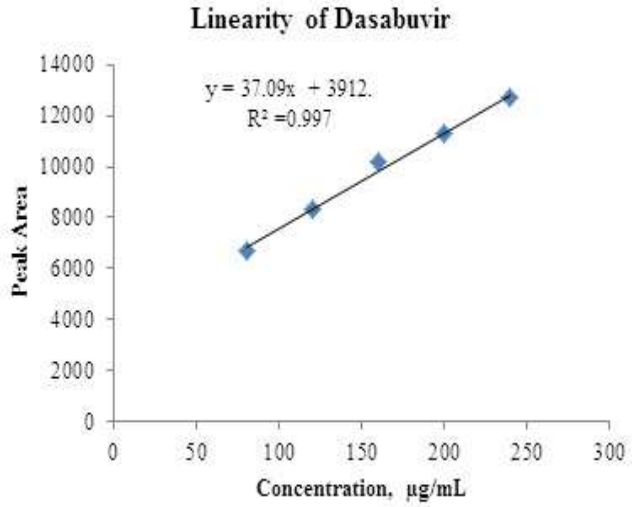


Fig. 2. Linearity graph of ombitasvir

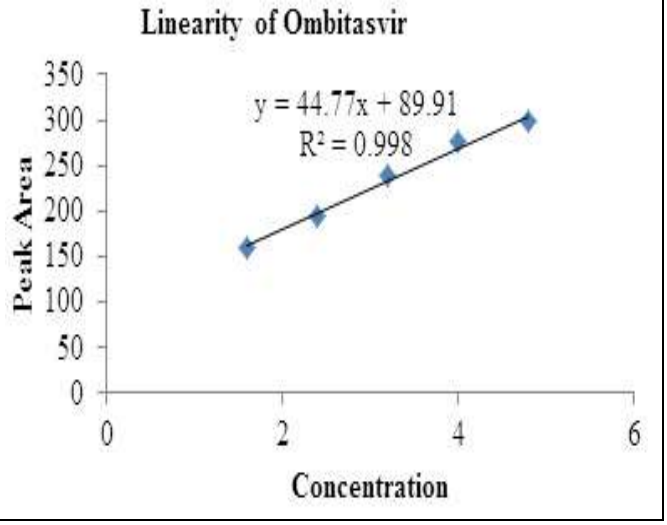


Fig. 3. Linearity graph of paritaprevir

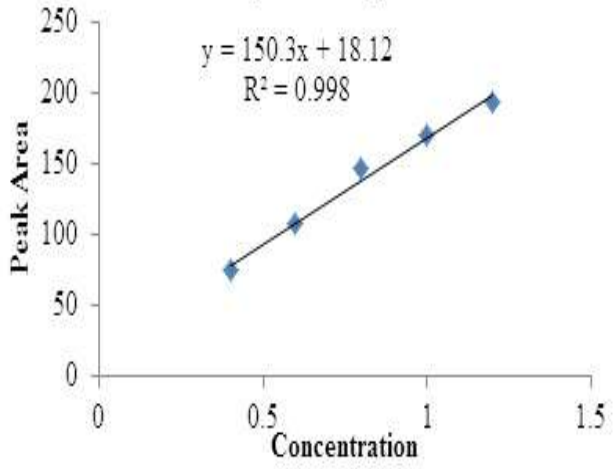
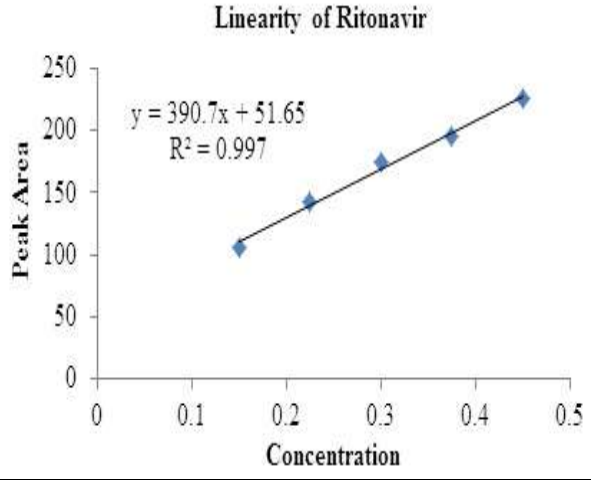


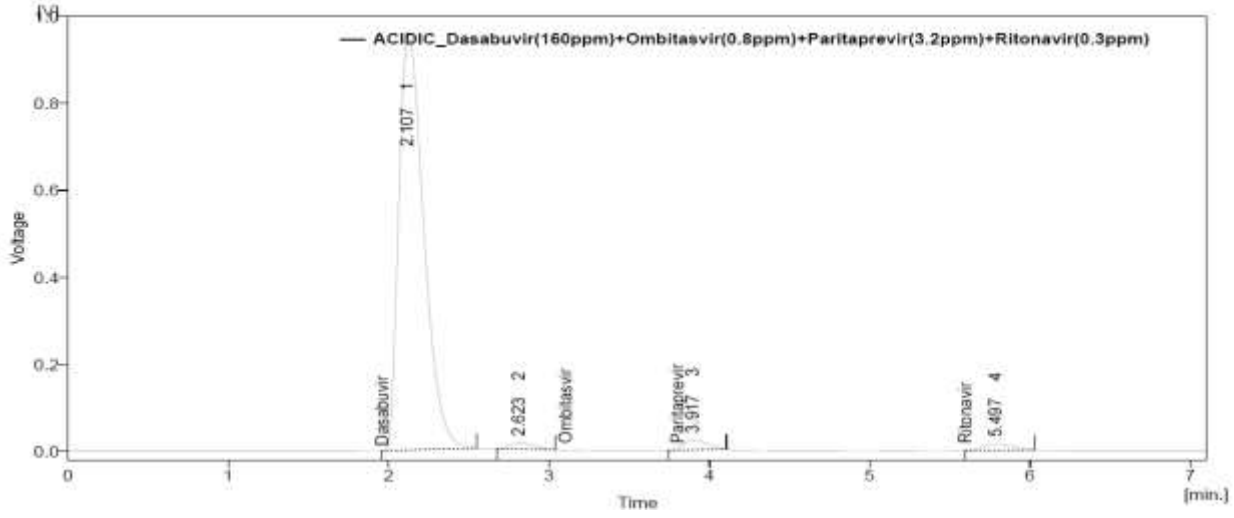
Figure 4. Linearity (calibration) curve of dasabuvir, ombitasvir, paritaprevir and ritonavir



STABILITY STUDIES:

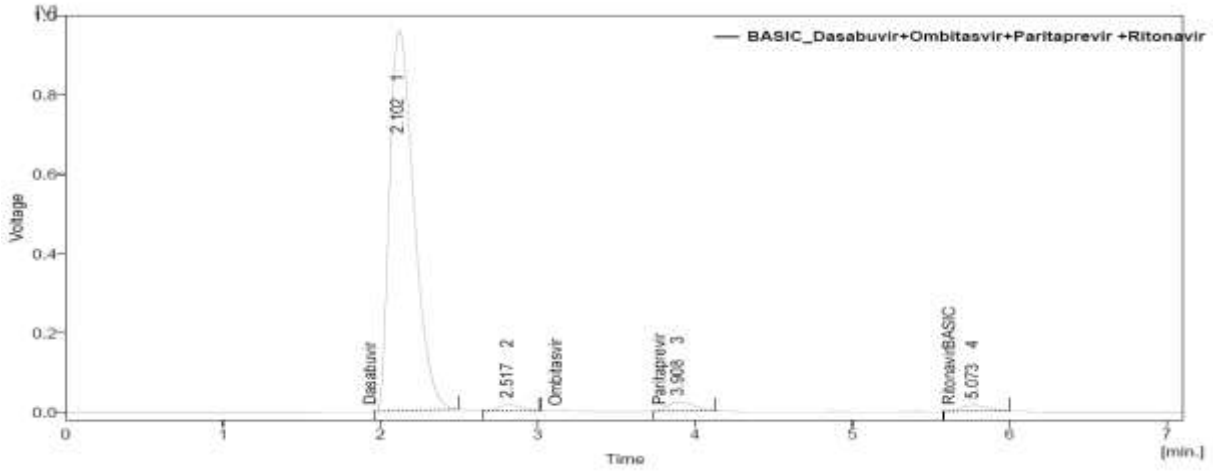
ACIDIC:

The hydrolytic degradation of a New drug (API) at a known concentration in acidic and alkaline conditions can be studied by refluxing the drug in 0.1 N HCL/NaOH for 8 hrs and subsequently withdraw the samples at different time periods for each reaction condition and neutralize it.



BASIC:

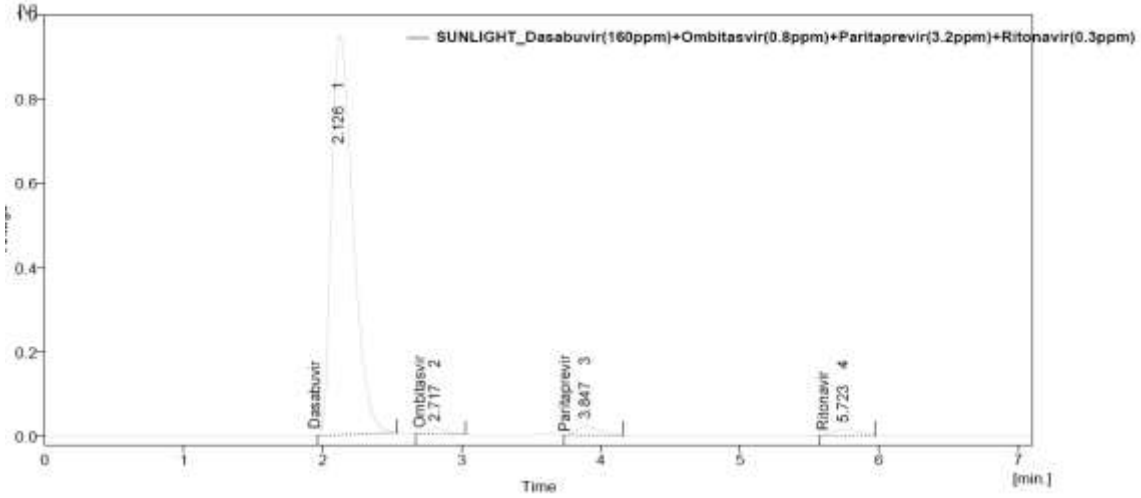
The hydrolytic degradation of a New drug (API) at a known concentration in acidic and alkaline conditions can be studied by refluxing the drug in 0.1 N NAOH/HCL for 8 hrs and subsequently withdraw the samples at different time periods for each reaction condition and neutralize it.



Chromatogram for BASIC

SUNLIGHT:

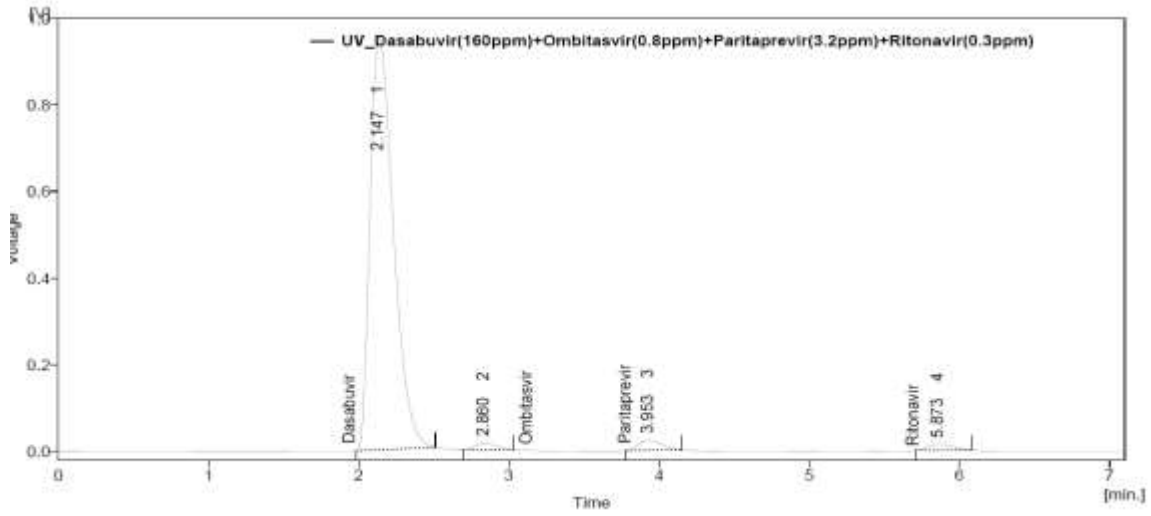
Expose the drug to sunlight for 3 days or 7 days after 3 days check the degradation



Chromatogram for Sunlight

UV:

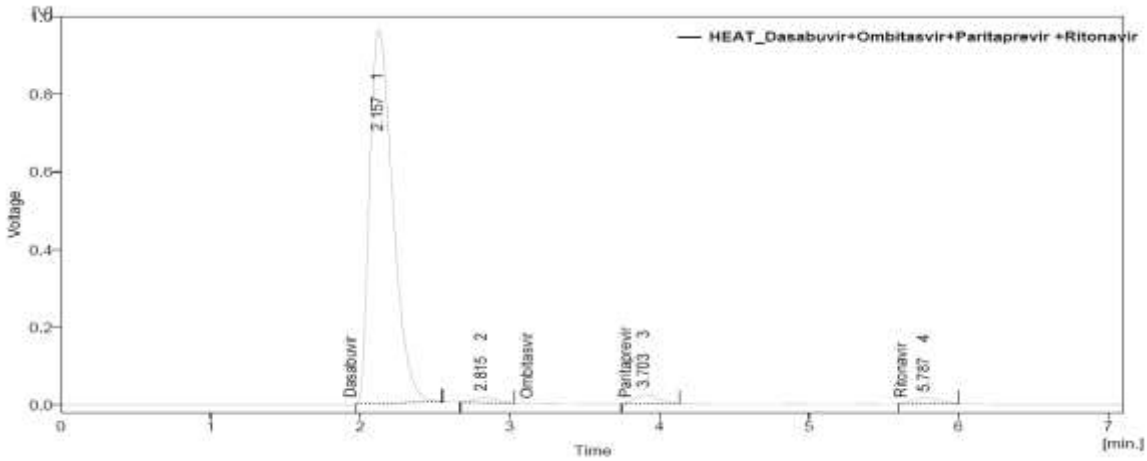
Photo degradation is a surface-mediated phenomenon. The photolytic studies should be carried out by exposure to light, using either a combination of cool white and ultraviolet fluorescent lamps, or one among the xenon and metal halide lamps. Exposure energy should be minimum of 1.2 millon Lux-h fluorescent light and 200Wh/m^2 UV and if decomposition is not seen, the drug can be declared photostable. Total exposure of drug substance to light measured with the help of Lux meter/Watt meter.The light sources described below may be used for photo stability testing.



Chromatogram for UV

TEMPURATURE:

Stress testing is likely to be carried out on single batch of the drug substance (API). Thermolytic degradation may lead to hydrolysis / dehydration / isomerization / epimerization / decarboxylation / rearrangements and some kinds of polymerization reactions. ICH guidelines suggest that thermolytic degradation study should be carried out at temperatures (in 10 increments e.g. 50ºC, 60ºC, etc.) above that for accelerated testing and withdraw the sample at different time intervals during reaction condition. If reasonable degradation (i.e. 5-20%) has seen, testing can be stopped at this point.



CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of dasabuvir, ombitasvir, paritaprevir and ritonavir was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries.

ACKNOWLEDGEMENT: The author highly thankful to Chandra Labs, Kukatpally, Hyderabad, India for providing all the facilities to carry out this work.

REFERENCES

1. Viekira Pak. The American Society of Health-System Pharmacists. Retrieved 8 December 2016.
2. Ombitasvir-Paritaprevir-Ritonavir and Dasabuvir (Viekira Pak) - Treatment - Hepatitis C Online". www.hepatitisc.uw.edu. Retrieved 2016-11-09.

European Medicines Agency - Find medicine - Exviera. www.ema.europa.eu. 24 May 2016. Retrieved 2016-11-09.
3. Časar, Zdenko (2016). Synthesis of Heterocycles in Contemporary Medicinal Chemistry. Springer. p. 92. ISBN 9783319399171.
4. WHO Model List of Essential Medicines (19th List) (PDF). World Health Organization. April 2015. Retrieved 8 December 2016.
5. FDA approves Technivie for treatment of chronic hepatitis C genotype 4. Food and Drug Administration. July 24, 2015.
6. Jordan J. Feld; Kris V. Kowdley; Eoin Coakley; Samuel Sigal; David R. Nelson; Darrell Crawford; Ola Weiland; Humberto Aguilar; Junyuan Xiong; Tami Pilot-Matias; Barbara DaSilva-Tillmann; Lois Larsen; Thomas Podsadecki & Barry Bernstein (2014). Treatment of HCV with ABT-450/r–Ombitasvir and Dasabuvir with Ribavirin". N Engl J Med. 370, 1594–1603.
7. Seng-Lai Tan; Yupeng He, eds. (2011). Hepatitis C: antiviral drug discovery and development. Norfolk: Caister academic press. p. 210. ISBN 9781904455783. Retrieved 28 April 2014.
8. Donald Jensen; Nancy Reau, eds. (2013). Hepatitis C. New York: Oxford University Press. p. 144. ISBN 9780199844296. Retrieved 28 April 2014.
9. Abbott Announces Phase 3 Hepatitis C Program Details. Abbott company website. Abbott Laboratories. Retrieved 28 April 2014.
10. Kowdley, Kris V.; Lawitz, Eric; Poordad, Fred; Cohen, Daniel E.; Nelson, David R.; Zeuzem, Stefan; Everson, Gregory T.; Kwo, Paul; Foster, Graham R.; Sulkowski, Mark S.; Xie, Wangang; Pilot-Matias, Tami; Liossis, George; Larsen, Lois; Khatri, Amit; Podsadecki, Thomas; Bernstein, Barry (2014). Phase 2b Trial of Interferon-free Therapy for Hepatitis C Virus Genotype 1. New England Journal of Medicine. **370** (3): 222–232.
11. Ritonavir Pregnancy and Breastfeeding Warnings". drugs.com. Retrieved 23 October 2015.
12. British National Formulary 69 (69 ed.). Pharmaceutical Pr. March 31, 2015. p. 426. ISBN 9780857111562.
13. Hacker, Miles (2009). Pharmacology principles and practice. Amsterdam: Academic Press/Elsevier. p. 550. ISBN 9780080919225.
14. WHO Model List of Essential Medicines (19th List) (PDF). World Health Organization. April 2015. Retrieved 8 December 2016.
15. Ritonavir. International Drug Price Indicator Guide. Retrieved 23 October 2015.

Cite this article:
Kranthi Kiran K. Development and Validation of New Stability Indicating RP-HPLC method for the determination of selected combinational Anti-Viral drugs in bulk and pharmaceutical dosage forms. *International Journal of Medicinal Chemistry & Analysis*,2017;7(2):1-8. DOI: http://dx.doi.org/10.21276/ijmca.2017.7.2.4