METHOD DEVELOPMENT AND ANALYTICAL METHOD VALIDATION OF SOFOSBUVIR ANTI HEPATITIS-C PHARMACEUTICAL PRODUCT AS PER ICH GUIDELINE

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ABSTRACT

Now a day in the world approximately 170 million people are suffering from this disease. This (Sofosbuvir) is a unique research discovered molecule of the present. Research reports explain due to this drug 80% patients are being recovered in the world. This drug is very costly because it is being imported from abroad. Now it is trying to manufacture this drug product in our homeland Pharma Industry in which my company is one of them. So to analyze this costly material (Sofosbuvir) for quality, purity and integrity it was needed to have a comprehensive and validated test method because its test method is not still published in any international pharmacopeia (e.g. USP, BP, JP and EU). By following the ICH guideline, I developed a less costly, effective,simple and reliable HPLC method by using easily available solvents(Methanol,Acetronitrile and 0.1% Phosphoric acid). Due to my development, not only our Pharma industry could get benefit from this but also world wide Pharma can gain benefit too.This method has been validated for Linearity,Accuracy,Precission,Specificity and Robustness.The developed method could be employed for the routine analysis of Sofosbuvir in tablet dosage form.

Key Words: RH-HPLC.Method Validation,Sofosbuvir.

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Presented to

Hamdard Institute of Education and Social Sciences, HAMDARD UNIVERSITY KARACHI 2015

CHAPTER I

Introduction

Vibrenta (sofosbuvir) tablets are formulated as white film coated tablet plain on both side . The tablets are to be marketed in a single dosage strenght.InactIive ingridients micro crystal- in cellulose(102),mannitol,AC-DI-SAL,Talcum and magnesium stearate.

Sofasbuvir tablets are also indicated in combination with other agents for th treatment of chronic hepatitis C in adults.The recommended dose is one tablet daily with or without food.

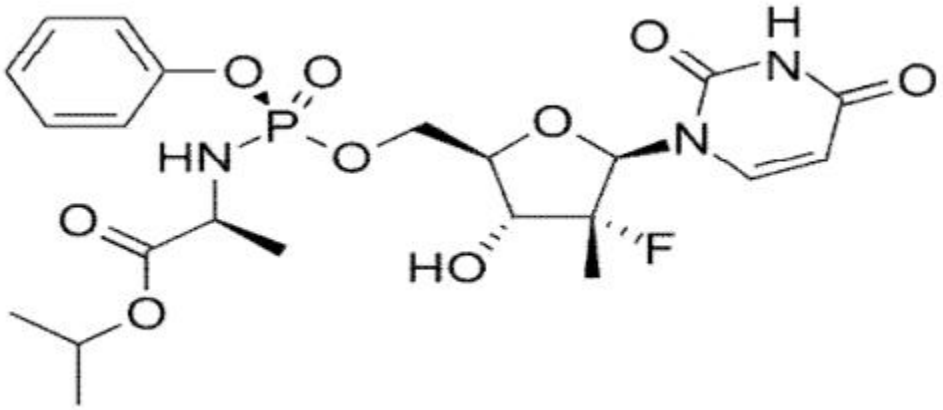
Reviews

Drug substance (sofasbuvir)

chemical name:-

(s)-isoprphyl2-((s))-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(IIH)-yl)-4-fluoro-3-hydroxy-4-melthyltetrahydrofuran-2-yl)methoxy)(phenoxy,(phasphorlamino)propanoate.

Chemical structure:-



Molecularformula (C22H29F N3 O9 P)

Molecular weight (529.458/mol)

*Sofasbuvir is a new molecular entity.Sofosbuvir is relatively stable, no extraordinary storage precautious are required.The proposed retest period of months when stored in the recommanded container closure system and under the proposed storage conditions is granted.The proposed expiry for the product is II4 months when stored in the commercial packing at the recomended storage condition of *store below 30C°* based on the stability data provided and in accordance*

with ICH (QIE),the Agency grants the proposed expiry. ChronicHCV (CHC) infection is a global public health problem,with approximately 170 million persons chronically infected who are at an increased risk of morbidity and mortality due to liver cirrhosis. Specfication for sofasbuvir drug substance are adequate and include tests for apperance, identification,clarity of solution, assay ,impurity content,organic volatile impurities/residual solvents,elemental impurities and partical size.

No degradation products have bean observed over time in any of the batches of drug substance stored at long term and accelerated data,or retest period months is assinged for sofasbuvir when stored at therecommended

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storage condition.Based on stress studies, temperature excurisions and shipping and handling of the DS at temperature 30 C ° may be permitted.

In general the manufacturing process is conventional and appears quite robust.Changes in the processing parameters had little effect on the finished product.Dug product testing will be carried out by HPLC,FTIR in Tabros Pharma pvt.ltd.

All drug product manufacturing and testing facilites were found acceptable by the office of compliance.

Reasonable drug product specifications with tests for appreance, identity ,assay ,degradants, dose uniformity and dissolution are propoed.The analytical methods are descibed in reasonable detail and they have been validated .Satisfactory batch analyses are prodvided for 3 batches manufactured using the proposed commercial process from drug substance/Product. No out of specification results are seen and no degrandants are observed.The primary stability batches and the annual stability batches will also be tested for microbial limit.The drug prouduct is extremely stable.Satisfactory stability data covering 1II months at 30C°/65%RH and 6 months at 40C°/75%RH are provided for three scale batches manufacture at tabros pharma.Satisfactory stability data covering 6 months at 30C°/65%RH and 40C°75%RH are performed at Tabros pharma.There are no out specification result and no obvious trends.No degradants what so ever are observed.

Sofosbuvir(Vibrenta) is a nucleotide prodrug of II-deoxy-II-fluoro-II-C-metylurindine mono phasphate that is converted to the active uridine triphasphate form(GS-461II03)with in the hepatocyte and is a HCV NS5B-directed inhibitor that has displayed patent inhibition of HCV

Development of validated analytical method for SOF.

Sofosbuvir tablet: *sofasbuvir is an nucleotide analog used in single or in combination with other drugs for the treatment of hepatitis C virus (HCV) infection.Sofosbuvir based regimens provide a higher cure rate,with fewer side effects. Sofosbuvir(Vibrenta) allow most patients to be treated succesfully without use of peginteferon.*

Sofosbuvir(Vibrenta) inhibits the RNA polmerase that the hepatitis C virus used to replicate its RNA.

Sofasbuvir is used for the treatment of chronic hepititis c ,genotypes 1,2,3 and 4,in combination with pegylated interferon.

CHAPTER VI-MATTERIAL AND METHOD

Material and Method

Equipment :*HPLC,Water(15II5)*

Material: *The Vibrenta coated tablet 400mg from Tabros pharmaceutical Laboratory and has the follow composition. InactIive ingridients micro crystal- in cellulose(10II), mannitol, AC-DI-SAL,Talcum and magnesium stearate.*

OBJECTIVE*:*

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below.

1- *Specificity*
2- *Precession*
3- *Accuracy*
4- *Linearity*
5- *Range*
6- *Robustness*

Analytical Procedure:

The analytical procedure refer to the way of performing the analysis. It should describe in detail the step necessary to perform each analytical test.This may include but is not limited to the Sample.the reference Standard and the reagents preparations,use of the apparatus,generation of the the calibration curve,use of the formulae for the calculation.

Method development:

Different columns,mobile phase, flow and column temperature were tested in the development of analytical method.C8,C18 columns of the same parameters and condition(1ml/min,flow,mobilephase,injection valume of 20 ul ,temperature 30°C.For the mobilephase,methanol/water,Accetonitrile/waterand Accetonitrile/methanol /0.1%phosphoric acid mixtures were tested,with the other parameter kept constant. The mobilephase holdup time,resolution,Sofosbuvir peak asymmetry and quantity of fractions defined by the reading of an integrations from the chromatograms were assesed.The concentration of the test samples was 20ug/ml throughout method development.

VIBRENTA (SOFOSBUVIR)TABLET

Assay by H.P.L.C Method

CHROMATOGRAPHIC SYSTEM:-

EQUIPMENTS:

SHIMADZU(LC-6A)WATERS 1525/2489/UV 116

MOBILE PHASE:-

Acetonitrile	*300ml*
Methanol	*100ml*
0.1%Phosphoric Acid	*600ml*

PARAMETERS:-

Column	*4.6 mm x 150 mm C18*
Wavelenght	*210nm*
Flow rate	*1.5 ml/min*
Inject Volume	*20 ul*
Temprature	*30°C*

STANDARD PREPRATION:-

Weigh accurately 50 mg sofasbuvir standard in a 50 ml volumetric flask,Add about II0 ml of methanol,sonicate for 5 minutes.dissolve and make up the volume with methanol.

SAMPLE PREPARATION:-

Weigh and grind to powder 20 Tablets.Take accurately a quantity of the powder Equivalent to 50 mg of sofasbuvir in 50 ml volumetric and filter through whatman paper.

PROCEDURE:-

Filter and separtely inject equal volume(about 20 ul)of the standard and the sample prepration into the chromatograph in triplicate,record the chromatogram and measure the responses of the major peaks.calculate the quantity in mg oif sofosbuvir by the formula given below:

CALCULATION FOR SOFOSBUVIR:-

$$\text{Sofasbuvir} = \frac{SpT \times C2 \times 400}{S2T \times C1}$$

Mg/tab

$$\frac{Mg/tab}{400} \times 100 = \%\ \text{of sofosbuvir}$$

Where

SpT is the major peak response of sofosbuvir in sample preparation.

St T is the major peak response of sofosbuvir in standard prepration.

C2 is the concntretion of sofosbuvir in mcg/ml in standard prepration.

C1 is the concntretion of sofosbuvir in mcg/ml in sample prepration.

REQUIRMENT:

Sofosbuvir : 400 mg/tab (90.0%--110.0%)

Application of validation

OBJECTIVE:

Vibrenta Tablet 400mg Tablet is selected to validate the analytical test method for assay of Sofosbuvir.

VALIDATION PARAMETERS:

1. ***LINEARITY & RANGE***
2. ***ACCURACY***
3. ***PRECISION***
   a) *Repeatability Precision*
   b) *Intermediate Precision*
4. ***SPECIFICITY:***

a)Blank Placebo Sample (without active substances)

b)Double Quantity of Excipients (with active substances)

5. ROBUSTNESS:

a) HPLC Column(Different Brands)

b) Mobile Phase (Change of Phosphoric Acid)

VALIDATION OF ASSAY OF SOFOSBUVIR:

GLASSWARE REQUIRED:

1. *Volumetric Flask*
2. *Pipette*
3. *Beaker*
4. *Measuring Cylinder*

Pyrex

REAGENTS USED:

1. *Acetonitrile (HPLC Grade)*
2. *Phosphoric Acid*
3. *Distilled Water Purified Grade*
4. *Methanol (HPLC Grade)*

Merck

MOBILE PHASE PREPARATION:

Acetonitrile [HPLC grade]	*300 ml*
Methanol [HPLC grade]	*100 ml*
0.1% Phosphoric Acid	*600 ml*

INSTRUMENTS USED:

1.	*HPLC [Waters 15II5 (Auto-Sampler)]*	*USA*
2.	*HPLC Column (C-18)*	*Meck*
3.	*Whatman Filter Papers*	*USA*
4.	*Electronic Balance*	*France*
5.	*pH Meter*	*Germany*
6.	*Unltra Sonic Bath*	*Germany*
7.	*Waters's Software (Breeze) for Chromatograms*	*USA*

CHROMATOGRAPHIC SYSTEM: -

Column	*MERCK-C18 (4.6 x 150 cm)*
Temperature	*Ambient*
Wavelength	*210 nm*
Flow rate	*1.5 ml / min.*
Inject Volume	*2I0 µl*

BLANK PREPARATION (PLACEBO SAMPLE):

Mix following quantities of Excipient calculated for 100 tablets.

S. No.	***Inactive Material (Excipients)***	***Quantity (gm)***
1	*Microcrystalline Cellulose 10II*	*11.00*
II	*Mannitol*	*5.00*
3	*Ac-Di-Sol*	*1.50*
4	*Talcum*	*1.50*
5	*Magnesium Stearate*	*1.00*

VALIDATION SCOPE*:*

This validation of analytical testing method covers the following strength of Vibrenta Tablets.

1. *Vibrenta 400mg Tablet*

1. *LINEARITY & RANGE:*

DEFINITION:

Linearity of an analytical procedure is its ability, within a given a range, to test results which are directly proportional to the concentration of analyte in the sample.

STANDARD OF SOFOSBUVIR:

Weight of Sofosbuvir = 50.0 mg : Lot No. : YF20141008

Potency of Sofosbuvir = 99.90%

SOLUTION PREPARATION OF SOFOSBUVIR:

40.2, 45.3, 50.1, 55.4, and 60.0 mg of Sofosbuvir standard (representing 80, 90, 100, 110, and 1II0% respectively) were taken in five separate 50 ml volumetric flasks,

dissolved in and make up to volume with Methanol and mixed well. Filtered in HPLC vials through 0.45 µm filter paper using swinage. Dilutions from 80 % to 1II0 % were applied on HPLC as per method and results were found as under.

INJECTION SEQUENCE:

Inject 20µl of each solution once.

THEORETICAL VALUES OF SOFOSBUVIR:

80% solution = 40mg/50x1000 = 800mcg/ml

90% solution = 45mg/50x1000 = 900mcg/ml

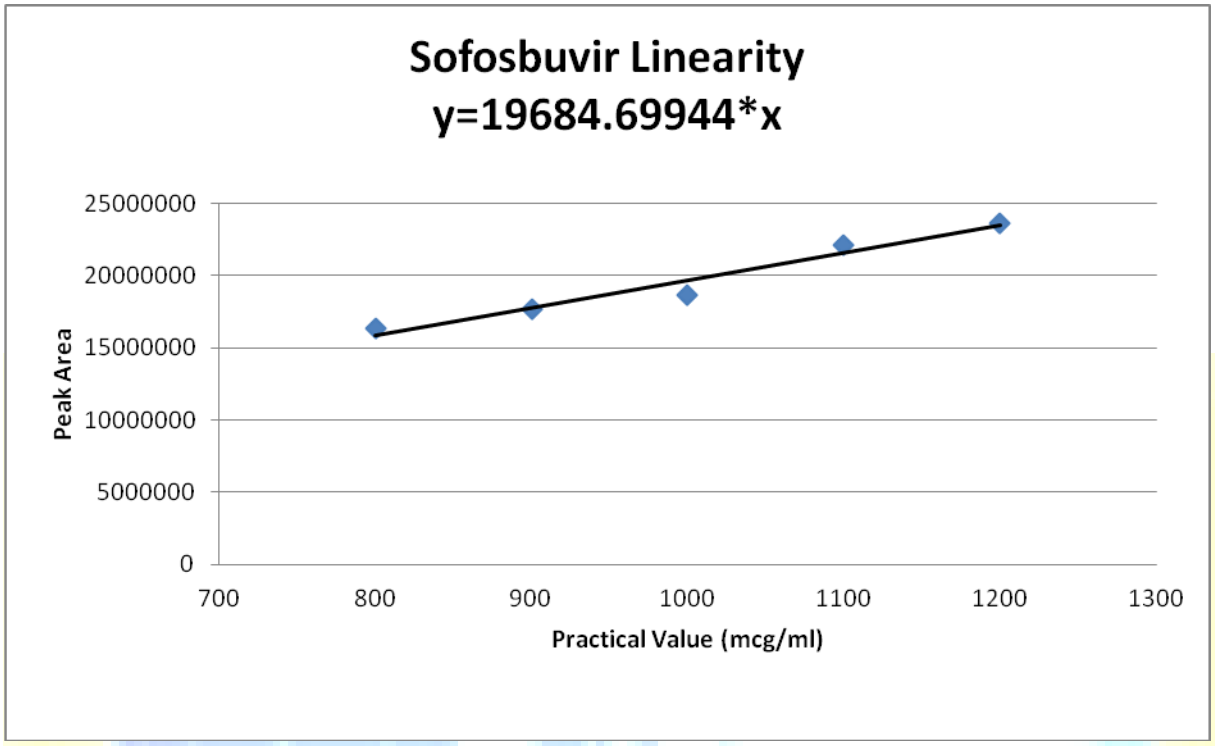
100% solution = 50mg/50x1000 = 1000mcg/ml

110% solution = 55mg/50x1000 = 1100mcg/ml

1II0% solution = 60mg/50x1000 = 1II00mcg/ml

LINEARITY & RANGE FOR SOFOSBUVIR

S. No	***Theoretical Concentration***	***Peak Area Run 1***	***Peak Area Run II***	***Mean Peak Area (1+II) / II***	***Practical Concentration***
1	*800mcg/ml (80%)*	*16383II34*	*16355690*	*1636946II.00*	*804 mcg / ml*
II	*900mcg/ml (90%)*	*1770630II*	*17633355*	*176698II8.50*	*906 mcg / ml*
3	*1000 mcg/ml (100%)*	*18635406*	*18669681*	*1865II543.50*	*1002 mcg / ml*
4	*1100 mcg/ml (110%)*	*21991560*	*2223708*	*22117634.00*	*1108 mcg /ml*
5	*1200 mcg/ml (1II0%)*	*23715809*	*23636876*	*23676342.50*	*1200 mcg / ml*



CONCLUSION:*The plotted graph between Practical concentration and peak area shows linear line, hence comply the requirement of test of linearity. The coefficient of correlation is calculated to be 0.9909413 Linearity found between the range 80%---120%.*

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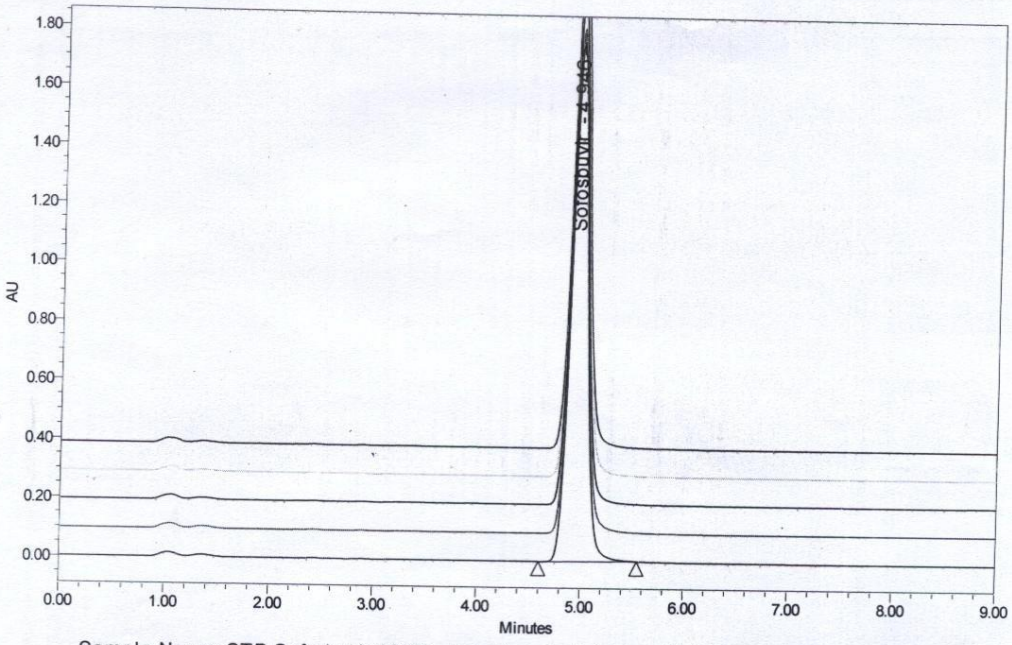
QUALITY CONTROL
Project Name Vibrenta Tablet
Reported by User: irfan

TABROS PHARMA (PVT)LTD.
L/20 / B, Sector 22, F.B. Industrial Area,
Karachi - 75950, Pakistan.

Breeze 2
HPLC System



Sample Name: STD Sofosbuvir 100%; Date Acquired: 14-Apr-15 11:37:26 AM PKT; Vial: 1:A,1; Injection: 1
Sample Name: STD Sofosbuvir 100%; Date Acquired: 14-Apr-15 11:46:48 AM PKT; Vial: 1:A,1; Injection: 2
Sample Name: STD Sofosbuvir 100%; Date Acquired: 14-Apr-15 11:56:11 AM PKT; Vial: 1:A,1; Injection: 3
Sample Name: STD Sofosbuvir 100%; Date Acquired: 14-Apr-15 12:05:35 PM PKT; Vial: 1:A,1; Injection: 4
Sample Name: STD Sofosbuvir 100%; Date Acquired: 14-Apr-15 12:14:56 PM PKT; Vial: 1:A,1; Injection: 5

Peak Summary with Statistics
Peak Name: Sofosbuvir.

	Sample Name	Vial	Inj.	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	Amount	Units
1	STD Sofosbuvir 100%	1:A,1	1	Sofosbuvir.	4.946	19492760	100.00	1770220	10000.000	mcg/ml
2	STD Sofosbuvir 100%	1:A,1	2	Sofosbuvir.	4.947	18926483	100.00	1719623	10000.000	mcg/ml
3	STD Sofosbuvir 100%	1:A,1	5	Sofosbuvir.	4.950	19210893	100.00	1741390	10000.000	mcg/ml
4	STD Sofosbuvir 100%	1:A,1	4	Sofosbuvir.	4.949	19091292	100.00	1729079	10000.000	mcg/ml
5	STD Sofosbuvir 100%	1:A,1	3	Sofosbuvir.	4.946	19175728	100.00	1740212	10000.000	mcg/ml

Report Method: Peak Summary Report
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QUALITY CONTROL Ltd

TABROS PHARMA (PVT)LTD.

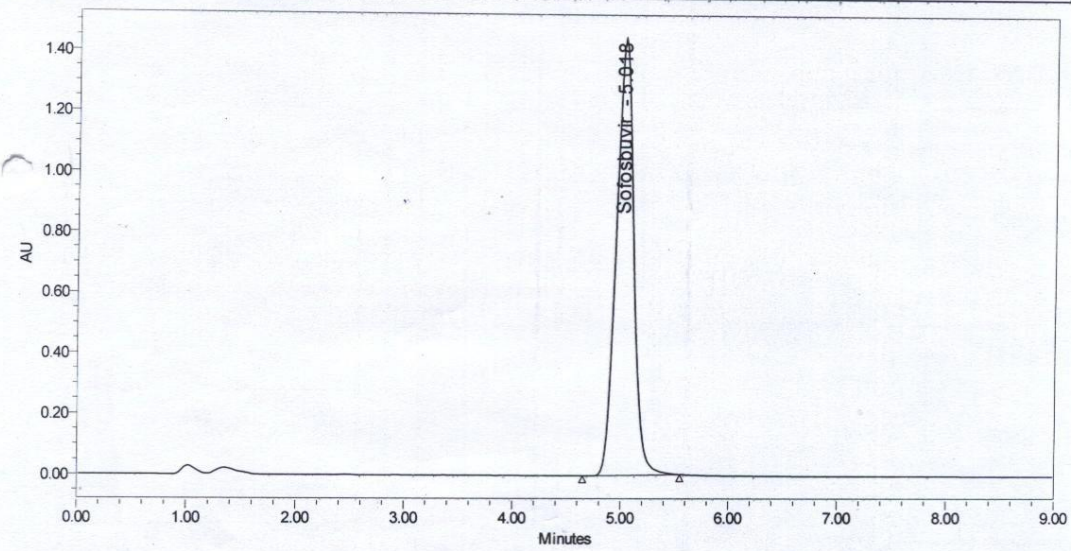
L/20 / B, Sector 22, F.B. Industrial Area,
Karachi - 75950, Pakistan.

Breeze 2 HPLC System

Project Name: Vibrenta Tablet
Reported by User: irfan

SAMPLE INFORMATION

Sample Name:	STD Sofosbuvir 80%	Acquired By:	irfan
Sample Type:	Standard	Date Acquired:	14-Apr-15 10:00:52 AM PKT
Vial:	1:A,1	Acq. Method:	Vibrenta Tablet MS
Injection #:	1	Date Processed:	14-Apr-15 10:16:22 AM PKT
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	9.00 Minutes	Channel Desc.:	W2489 ChA 210nm
Column Type:	C18	Sample Set Name	Linearity 140415



	Peak Name	RT (min)	Amount	Units	Area (µV*sec)	% Area	Height (µV)
1	Sofosbuvir.	5.018	10000.000	mcg/ml	16383234	100.00	1452847

	% Height
1	100.00

Report Method: Individual Control Report
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2. ACCURACY:

DEFINITION:

It express the closeness of agreement between the value that is either as a conventional true value or an accepted reference value and the value found.

RECOVERY OF THE SOFOSBUVIR IN PRODUCT:

PREPARATION OF SOFOSBUVIR STANDARD SOLUTION:

Weigh and transfer approximately 50.0 mg of Sofosbuvir reference standard into a 50 ml volumetric flask, dissolve and make up to mark with Methanol, mix well. Filter in a HPLC vial through 0.45 µm filter paper using swinage.

STANDARD DILUTION:

50 mg / 50 ml x 1000 = 1000 mcg / ml

PREPARATION OF SAMPLE SOLUTION:

Take accurately respective quantities of Sofosbuvir standard each in 50ml volumetric flask respectively, to prepare 80%, 90%, 100%, 110% and 1II0% test solution in accordance to 400mg Sofosbuvir per tablet. Add 40ml of Methanol in each flask, sonicate for 5 minutes and stir for 30 minutes. Then make up to volume with Methanol and mixed well. Filtered in a HPLC vials through 0.45 µm filter.

S.No.	Test Solution	Standard Amount (mg)	Placebo Excipient Amount (mg)	Solvent Quantity (Methanol)	Conc. of Test Solution
01	Test Solution # 01	40.1	II0.II	50ml	80.0%
0II	Test Solution # 0II	45.II	IIII.7	50ml	90.0%
03	Test Solution # 03	50.4	II5.5	50ml	100.0%
04	Test Solution # 04	55.5	II7.8	50ml	110.0%
05	Test Solution # 05	60.3	30.4	50ml	1II0.0%

ACCURACY FOR SOFOSBUVIR:

% Solution	*Theoretical Conc. (mcg/ml) True value*	*Peak Area1*	*Peak AreaII*	*Peak Area Mean*	*Recovered Conc. (mcg/ml) Recovered Value*	*% Recovered*
80% of Test Conc.	*800*	*16259672*	*16485052*	*16372362*	*855.349*	*101.21%*
90% of Test Conc.	*900*	*18196021*	*18116012*	*18156016.5*	*948.533*	*99.70%*
100% of Test Conc.	*1000*	*19122256*	*19368250*	*19245253*	*1005.439*	*100.86%*
110% of Test Conc.	*1100*	*21645909*	*21557739*	*21601824*	*1128.554*	*101.62%*
120% of Test Conc.	*1200*	*23276953*	*23371353*	*23324153*	*1218.535*	*100.96%*
STD	*100II*	*1*	*19492760*	*19179431.25*	—	——
		II	*18626483*			
		3	*19175728*			
		4	*19091292*			
		5	*19210893*			
Mean =						*100.87%*
%RSD =						*0.01*

CALCULATION*:*

Recovered Value of Sofosbuvir (mcg/ml) = $\frac{\text{Area of sample x Conc. of Std. (mcg/ml)}}{\text{Area of Std.}}$

% of Content (Recovered) = $\frac{\text{Mean Peak Area of Sample x Conc. of Std (mcg/ml) x 100}}{\text{Mean Peak Area of Std. x Conc. of Sample (mcg/ml)}}$

CONCLUSION:*The results of recovered values of Sofosbuvir are closed to the true values mean = 100.87% with RSD 0.01%.*

1. ***PRECISION***

DEFINITION:

Precision express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be a measure of either the degree of repeatability or reproducibility of the analytical method under normal operating conditions.

3.1 REPEATABILITY PRECISION:

DEFINITION:

It expresses the precision under the same operating conditions over a short interval of time.

PROCEDURE:

PREPARATION OF SAMPLE SOLUTION:

Weigh and transfer approximately 50.0 mg of Sofosbuvir reference standard into a 50 ml volumetric flask, dissolve and dilute with Methanol to volume, mix well. Filter in a HPLC vial through 0.45 µm filter.

SAMPLE SOLUTION:

50 mg / 50 ml x 1000 = 1000 mcg / ml

INJECTION SEQUENCE:

Inject Six times 20µl of sample solution.

Sample Solution Conc.	***Peak Area of Sample***		***Mean Peak Area***	***SDT. DEV***	***%RSD***
1000.0mcg/ml (100%)	*1*	*19492760*	*19184977.506*	*185392.879*	*0.97%*
	2	*18926483*			
	3	*19212709*			
	4	*19091292*			
	5	*19210893*			
	6	*19175728*			

$$\%RSD = \frac{SD \times 100}{Mean\ Area}$$

***CONCLUSION**:*

The repeatability of sample solution of Sofosbuvir were tested and found very precised with a precision of RSD 0.97%

3.II INTERMEDIATE PRECISION:

***DEFINITION**:*

Intermediate Precision express within Laboratory variation, as on different days with different analyst or equipment within Laboratory.

***PROCEDURE**:*

To check reproducibility of testing method by two analysts using same model of HPLC [Waters 1535 (Auto Sampler)] on different days to perform assay of Rosuvastatin.

S.No.	***Analyst***	***Test Performed on***	***Result***	***%RSD***
1	*Analyst - I*	*14-04-2015*	*101.80%*	*0.04%*
2	*Analyst – 2*	*15-04-2015*	*101.86%*	

CONCLUSION:

The repeatability of the Analytical Testing Method was found very precise when tested by two Analysts on different working days with a precision (RSD = 0.04%)

Tabros Pharma (Pvt) Ltd

QUALITY CONTROL

TABROS PHARMA (PVT) LTD.

L/20 / B, Sector 22, F.B. Industrial Area, Karachi - 75950, Pakistan.

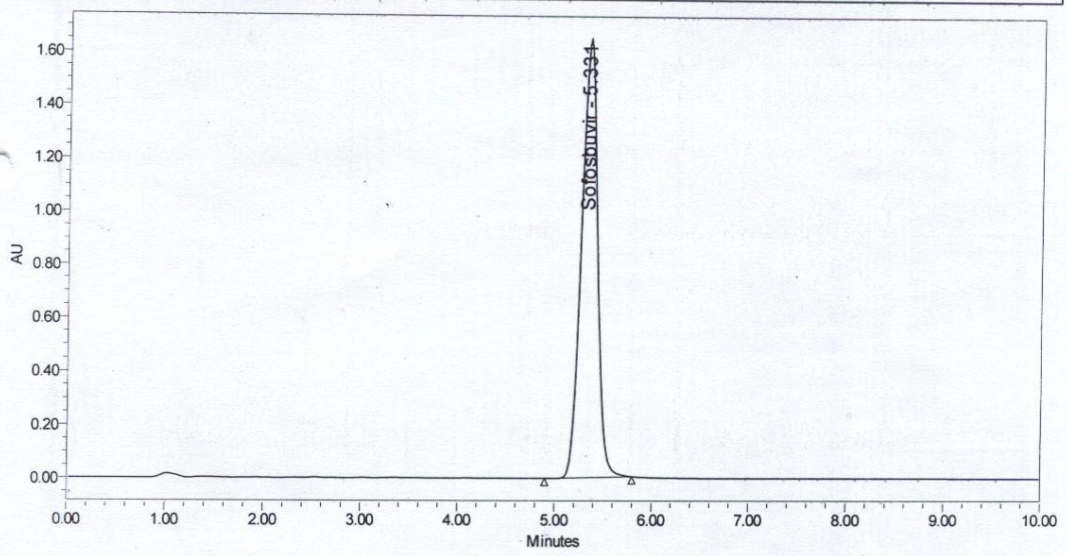
Project Name: Vibrenta Tablet

Reported by User: irfan

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	SDT (Sofosbuvir)	Acquired By:	sadia
Sample Type:	Standard	Date Acquired:	15-Apr-15 9:46:14 AM PKT
Vial:	1:A,1	Acq. Method:	Vibrenta Tablet MS
Injection #:	1	Date Processed:	15-Apr-15 10:58:43 AM PKT
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.00 Minutes	Channel Desc.:	W2489 ChA 210nm
Column Type:	C18	Sample Set Name	Analyst II 150415



	Peak Name	RT (min)	Amount	Units	Area (µV*sec)	% Area	Height (µV)
1	Sofosbuvir	5.331	1000.000	mcg/ml	19237502	100.00	1656884

	% Height
1	100.00

Report Method: Individual Control Report

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2. *SPECIFICITY*

DEFINITION:*It is the ability to assess unequivocally the analyte in the pressence of components which may be expected to be present.Our goal is to distinguish and quantify the response of the target compounds from theresponses of all the compounds. Analytical techniques that can measure the analyte response in the pressence of all potential sample components should be used for specificity validation. A frequently used techique in pharmaceutical laboratories*

To check the method validity following parameters were changed.

100 ***Page***

a) Blank Placebo Sample (without active substances)

b) Double Quantity of Excipients (with active substances)

PROCEDURE: ***PREPARATION OF STANDARD SOLUTION:***

Preparation of Working Standard :

Weigh accurately and transfer 50.0 mg of Sofosbuvir reference standard into 50 ml volumetric flask, add 30 ml of Methanol, sonicate for 5 minutes, dissolve and make up the volume with Methanol. Filter in a HPLC vial through 0.45 micron filter paper using swinage.

Sofosbuvir: *50 mg / 50 ml x 1000 = 1000 mcg/ml*

INJECT SEQUENCE:

Inject 20µl of standard solution.

SAMPLE PREPARATION:

a) BLANK PLACEBO SAMPLE (WITHOUT ACTIVE SUBSTANCES)

Weigh 200.0 mg of blank (Placebo Sample) and mix for 15 minutes in a 50ml Methanol, and make up the volume with Methanol, filter the solution through 0.45 micron filter paper using swinage.

INJECT SEQUENCE:

Inject 20µl of sample solution once.

b) DOUBLE QUANTITY OF EXCIPIENTS (WITH ACTIVE SUBSTANCES)

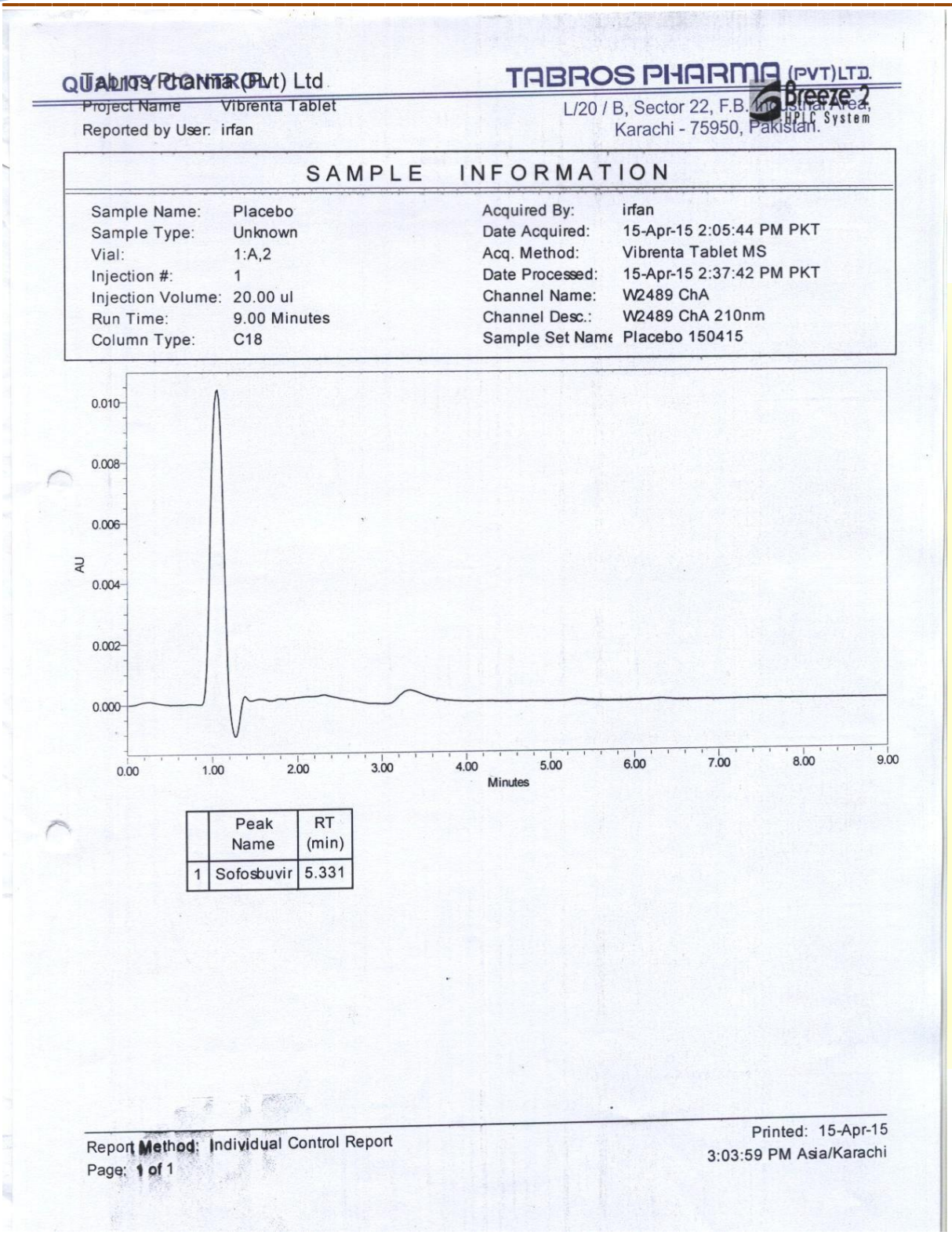
Single	*Normal Excipients*	*Sofosbuvir 400 mg*

	200mg	
Double	*Double Excipients 400.0mg*	*Sofosbuvir 400 mg*

Take 50.0 mg of Excipients (Placebo Sample) powder equivalent to II tablet of Vibrenta Tablet 400mg and add accurately 50mg Sofosbuvir, in 50ml volumetric flask and make up with Methanol.

INJECT SEQUENCE:

Inject II0µl of sample solution once.



Tabros Pharma (Pvt) Ltd

QUALITY CONTROL

TABROS PHARMA (PVT) LTD.

L/20 / B, Sector 22, F.B. Industrial Area, Karachi - 75950, Pakistan.

Project Name: Vibrenta Tablet

Reported by User: irfan

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Placebo	Acquired By:	irfan
Sample Type:	Unknown	Date Acquired:	15-Apr-15 2:05:44 PM PKT
Vial:	1:A,2	Acq. Method:	Vibrenta Tablet MS
Injection #:	1	Date Processed:	15-Apr-15 2:37:42 PM PKT
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	9.00 Minutes	Channel Desc.:	W2489 ChA 210nm
Column Type:	C18	Sample Set Name	Placebo 150415

	Peak Name	RT (min)
1	Sofosbuvir	5.331

Report Method: Individual Control Report

Page: 1 of 1

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3. ***ROBUSTNESS***:

DEFINITION:

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal use.

c) HPLC Column(Different Brands)

d) Mobile Phase (Change of Phosphoric Acid)

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To check the method validity following parameters were changed

c) HPLC COLUMN (DIFFERENT BRANDS)

Test analysis was performed by changing the HPLC Column from Merck to Waters (replaced column).

1. *C18 4.6mm x 150mm, 5µ MERCK (Germany)*
2. *C18 4.6mm x 150mm, 5µ WATERS (USA)*

d) MOBILE PHASE (CHANGE OF PHOSPHORIC ACID)

Prepare a mixture of Acetonitrile, Methanol and Water 3000ml. Separately, add 0.5ml, 1.0ml & 1.5ml Phosphoric Acid in each 1000ml mixture of Acetonitrile, Methanol and Water to make three different mobile phase (I, II, III).

MOBILE PHASE PREPARATION:

Mobile Phase	***Acetonitrile [HPLC grade]***	***Methanol [HPLC grade]***	***Phosphoric Acid***	***Distilled Water***
Mobile Phase I	*300ml*	*100ml*	*0.5ml*	*600 ml*
Mobile Phase II	*300ml*	*100ml*	*1.0ml*	*600ml*
Mobile Phase III	*300ml*	*100ml*	*1.5ml*	*600ml*

CHROMATOGRAPHIC CONDITION:

Column *MERCK C18 (4.6 x 150 mm)*

Temperature *Ambient*

Wavelength	*210 nm*
Flow rate	*1.5 ml / min.*
Inject Volume	*20 µl*

RESULTS: ***SOFOSBUVIR***

Conditions		***Results(%)***
Blank	*Blank without active (Excipients only)*	*Nil*
Double Excipients	*Assay with double amount of Excipients*	*101.46%*
Column	*MERCK C18 (4.6 x 150 cm)*	*101.II7%*
	C18 4.6mm x 150mm, 5µ ,WATERS (USA)	*100.II6%*
Mobile Phase	*(I) Phosphoric Acid 0.5ml*	*101.II8%*
	(II) Phosphoric Acid 1.0ml	*101.80%*
	(III) Phosphoric Acid 1.5ml	*100.87%*

CALCULATION*:*

$$\% \text{ of content} = \frac{\text{Area of Sample X Conc. of Std. (mcg/ml) X 100}}{\text{Area of Std. X Conc. of Sample (mcg/ml)}}$$

CONCLUSION*:he analytical test method found robust which did not affected by the (a) Blank Placebo Sample (without active substances), (b) Double Quantity of Excipients (with active substances), (c) HPLC Column (Different Brands) and (d) Mobile Phase (Change of Phosphoric Acid).*

The assay results of Sofosbuvir found without significant difference (RSD = 0.527%)

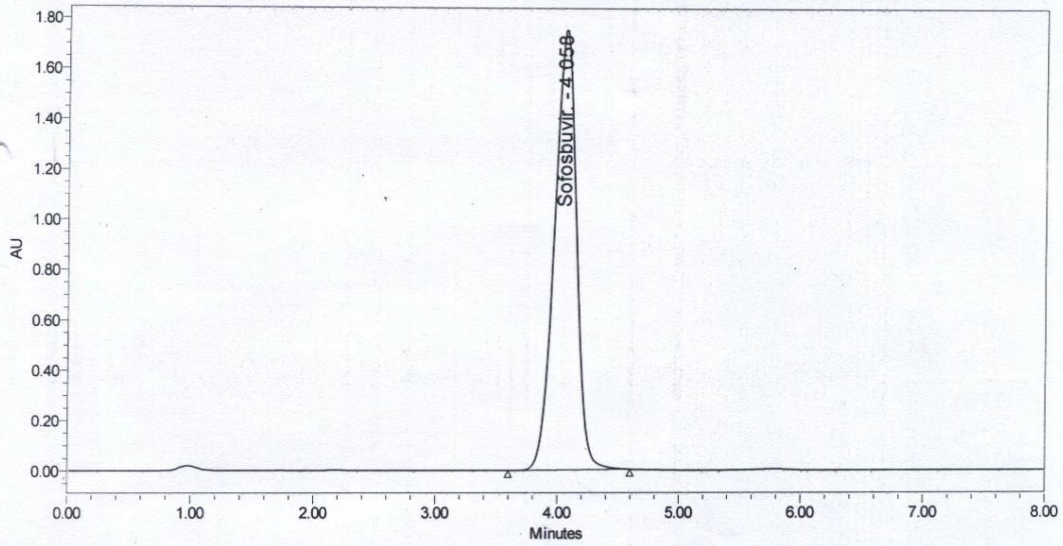
Tabros Pharma (Pvt) Ltd
QUALITY CONTROL
Project Name: Vibrenta Tablet
Reported by User: irfan

TABROS PHARMA (PVT) LTD.
L/20 / B, Sector 22, F.B. Industrial Area,
Karachi - 75950, Pakistan.

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Waters column Test B#Exp–001	Acquired By:	irfan
Sample Type:	Unknown	Date Acquired:	16-Apr-15 1:19:52 PM PKT
Vial:	1:A,2	Acq. Method:	Vibrenta Tablet MS
Injection #:	2	Date Processed:	16-Apr-15 1:38:32 PM PKT
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	8.00 Minutes	Channel Desc.:	W2489 ChA 210nm
Column Type:	C18	Sample Set Name	waters column 160415



	Peak Name	RT (min)	Amount	Units	Area (µV*sec)	% Area	Height (µV)
1	Sofosbuvir.	4.058	1115.769	mcg/ml	22213215	100.00	1761498

	% Height
1	100.00

Report Method: Individual Control Report
Page: 1 of 1

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CHAPTER 6-RESULT AND DISCUSSION

Results and discussion:

The present work involves estimation of Vibrenta(Sofosbuvir) tablet using reverse phase high pressure liquid chromatography(HPLC) .The current trend followed by the industries is developing a methodology which can save sophisticated instrument and chemist's valuable time which the product analysis can be done very fast ,thereby solving the time.The developed method was validated with a halistic approch according to ICH guidelines and detials of findings are expressed in what follows.

1-Specificity of the method detail:

The Retention times of the standard drugs was measured and it was found to be5.0 minute for Sofosbuvir.The drug was mixed and injected for taking the chromatogram.Similary ,a placebo sample was injected and found no peak.This indicate that there is no chromatographic interference between analyte and placebo.The pharmaceutical dosage form(tablet)was obtained. The retention time of the drug in dosage form was found tobe 5.0minutes.There is no specific change in retention of the standard and drug which indicates that there is no drug-excipients interference.

2-System Suitability Test:

Five injections of standard solution were injected for this purpose. The retention time, areas, resolution,theoretical plates values and peak asymmetry were calculated for standard.Percentage RSD value was calculated.

3-Linearity:

The correlation coefficeint (r) obtained was calculated it was found to be greater than 0.99 for SOFO.The concentration was found to be proportional to the area and response of the detector was determined to be linear over the rangeof 0.II to 0.6ug/ml.

4-Accuracy:

The results indicat that the recoveries are well within the acceptance range of 80---1II0%,Therefore method is accurate and it can be used for the estimation of SOFO.

5-Robustness:

Due to deliberate change in the chromatographic conditions of the method like flow rate,PH,wavelength and column temperature,excellent performance of the method was observed. This indicate that the method is Robust.

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