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USE OF REDOX REACTION FOR THE ESTIMATION OF BENZIMIDAZOLE DERIVATIVE (LEDIPASVIR) BY SPECTROPHOTOMETRIC QUANTIFICATION

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ABSTRACT

A simple spectrophotometric methodhas been developed for the significant measurements of ledipasvir in the pure form and pharmaceutical formulations. In this method the solutions of ledipasvir were scanned at two different wavelengths. These methods are based on redox reaction between ledipasvir and ferric chloride which upon complexation with 1, 10 phenanthroline to form a reddish orange colour complex which shows absorption maximum at 520.0nm.In the method 2 the ferric chloride complexes with potassium ferric cyanide to form bluish orange colour complex which show absorption maximum at 713.6nm.These methods accept the beer's law. The concentrations of solutions in the range of 10-50µg/ml and 20-100µg/ml respectively. These methods are also validated as per International council for Harmonization Guidelines and the results were within the acceptance limits. The assay percentage was found to be 99.02% for method 1and 115% for method 2. The developed methods were based on redox reaction used for the analysis of ledipasvir present in various pharmaceutical dosage forms.

Keywords: Spectrophotometry, ledipasvir, ferric chloride, 1, 10 phenanthroline, potassium ferric cyanide.

INTRODUCTION

Ledipasvir is a direct acting antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). It is an inhibitor of the Hepatitis C Virus (HCV) NS5A protein required for viral RNA replication and assembly of HCV virions. It is effective against genotypes 1a, 1b, 4a, and 5a and with a lesser activity against genotypes 2a and 3a of HCV.

Ledipasvir is chemically named as methyl N-[(2S)-1-[(6S)-6-[5-[9,9-difluoro-7-[2-[(1R,3S,4S)-2-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]-2-azabicyclo [2.2.1]heptan-3-yl]-3H-benzimidazol-5-yl]fluoren-2-yl]-1H-imidazol-2-yl]-5-azaspiro [2.4] heptan-5-yl]-3-methyl-1-oxobutan-2-yl] carbamate.

It has a Molecular Formula $C_{49}H_{54}F_2N_8O_6$ and Molecular Weight 889 g/mol.

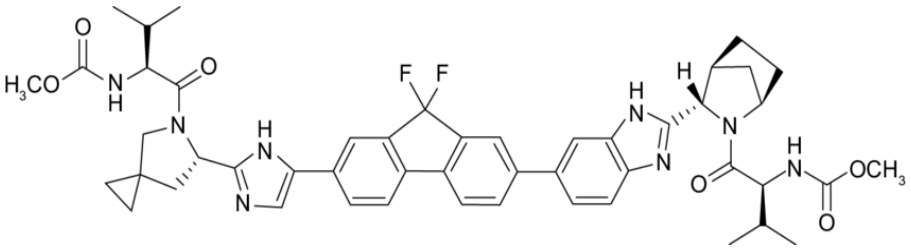

Fig: 1 structure of ledipasvir

Literature review onLedipasvir revealed several analytical methods for its qualification either alone or in combination with other drugs.

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The method was developed by using UV- VIS spectrophotometer. UV-VIS spectrophotometer is an analytical technique which concerns with the measurement of absorption of electromagnetic radiation which ranges from 200-400 for UV and 400-800 for Visible.
Simple and sensitive spectrophotometric methods were developed for the significant measurements of ledipasvir in the pure form and pharmaceutical formulations. These methods are based on redox reaction between ledipasvir and ferric chloride which upon complexation with 1, 10 phenanthroline to form a reddish orange colour complex which shows absorption maximum at 520.0nm in method 1.
In the method 2 the ferric chloride complexes with potassium ferric cyanide to form bluish orange colour complex which show absorption maximum at 713.6nm. By utilizing grade chemicals and reagents these methods were established.

MATERIALS& METHODS

Instrument: Double - beam shimadzu ultra violet – visible spectrophotometer(systronics) is used for the absorbance of analytical solutions. Spectral band width 0.1nm, wavelength accuracy ± 0.1 nm and a pair of 1 cm path length matched quartz cells were included in it.

Chemicals and reagents:

1, 10-phenanthroline reagent (0.1M):
The reagent, 1, 10-phenanthroline (2 g) was accurately weighed and dissolved in sufficient methanol (in a volumetric flask) to produce 100ml.

Ferric chloride reagent (0.3% w/v):
Ferric chloride (0.3 g) was weighed accurately and dissolved in sufficient distilled water (in a volumetric flask) to produce 100 ml.
Potassium Ferric cyanide (0.2% w/v):
Potassium ferric cyanide (0.3g) was weighed accurately and dissolved in sufficient distilled water in a volumetric flask to produce 100ml.
Preparation of Standard stock solution:
The stock solution of ledipasvir (0.01mg) was made by solubilizing10ml of methanol. The required concentrations of Ledipasvir for the λ_{max} determination and for further analysis.
OPTIMIZATION OF THE METHOD
In this the methods were optimised by using two different reagents at two different wavelengths.
METHOD-1
In thismethod the solutions of ledipasvir were prepared and scanned in the visible spectrum mode from 400-800nm. In this 1-10 phenanthroline used as a reagent.
Calibration Procedure of ledipasvir for method 1
A series of 0.1, 0.2, 0.3, 0.4 and 0.5ml of Ledipasvir standard solution (10µg/ml) was taken in 10ml volumetric flask. To this add ferric chloride solution (2ml, 0.1M) were added and allowed to stand in water bath at 70°c for 15 minutes. Then the test tubes were cooled to room temperature & the solutions were made up to 10ml with Methanol, to ensure the colour development through redox -coupling reaction against blank solution and read the absorbance at 520nm.
Assay for method 1:
Take tablets of ledipasvir were weighed accurately and make it to a fine powder. Take 10mg of ledipasvir powder was dissolved in methanol; the contents were shaken thoroughly for 5 minutes. Take 0.4ml of above solution and add 1ml of $fecl_3$ and 2ml of 1-10 phenanthroline. Test tubes are allowed to stand in water bath for 15 minutes. Then they are allowed to cool at room temperature. The solution was made up to 10ml with methanol and the coloured chromogen was spectrophotometrically measured at 520 nm against the blank solution.
METHOD -2
In this method solution of Ledipasvir standard stock solutionwas prepared and scanned in the visible spectrum mode from 400 nm to 800 nm by using potassium ferric cyanide as a reagent.
Calibration Procedure of ledipasvir for method 2
A series of 0.2, 0.4, 0.6, 0.8 and 1.0ml of Ledipasvir standard solution (10µg/ml) were taken in 10ml volumetric flask. To this add Ferric chloride solution (1ml, 0.3%w/v) were added and shaken vigorously and kept aside for 5 minutes. The volume of volumetric flask was made up to mark with methanol. The colour development of Redox- coupling reaction against blank solution and read the absorbance at 713.6nm.

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Assay for method 2:

Take tablets of ledipasvir were weighed accurately and make it to a fine powder. Take 10mg of ledipasvir powder was dissolved in methanol; the contents were shaken thoroughly for 5 minutes. Take 0.8ml of above solution and add 1ml of $fecl_3$ and 0.5ml potassium ferric cyanide and shaken thoroughly and wait for 5minutes. The solution was made up to 10ml with methanol and the coloured chromogen was spectrophotometrically measured at 713.6 nm against the blank solution.

Results and discussion:

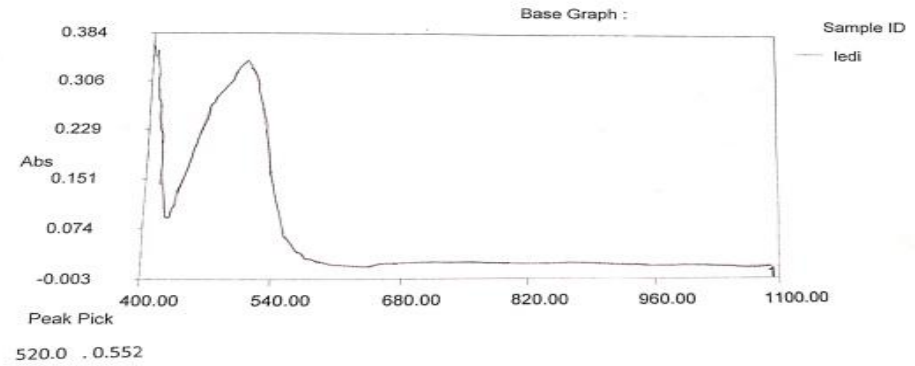

Fig:2Method 1 Ledipasvir absorption maximum at 520.0nm.

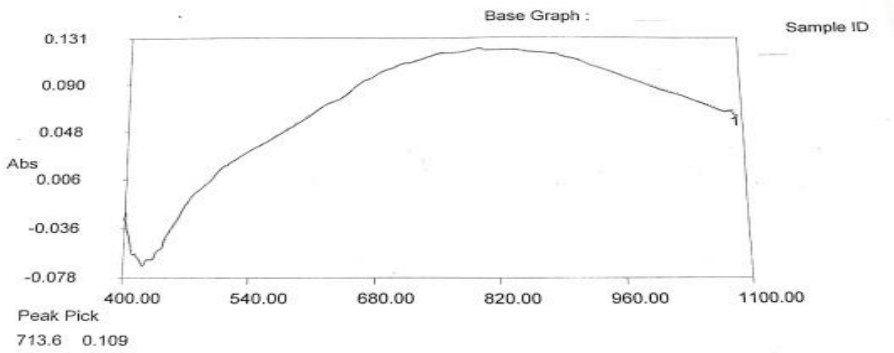

Fig:3Method 2 Ledipasvir absorption maximum at 713.6nm.

Linearity:

For the prepared concentration of solutions, the results were plotted graph against concentration vs absorbance. The method was found to be linear over the prepared concentration range with standard deviation of y=0.377x+0.5455 and regression value was fond to be 0.9975 for method 1 and standard deviation of y=0.593x-0.0258 and regression value was found to be 0.9942 for method 2.

s.no	Concentration (µg/ml) method 1	Absorbance	Concentration (µg/ml) method 2	Absorbance
1	0.1	0.582	0.2	0.096
2	0.2	0.619	0.4	0.195
3	0.3	0.663	0.6	0.340
4	0.4	0.698	0.8	0.465
5	0.5	0.731	1.0	0.554

Table:1 method 1 & method 2 Calibration curve data of Ledipasvir

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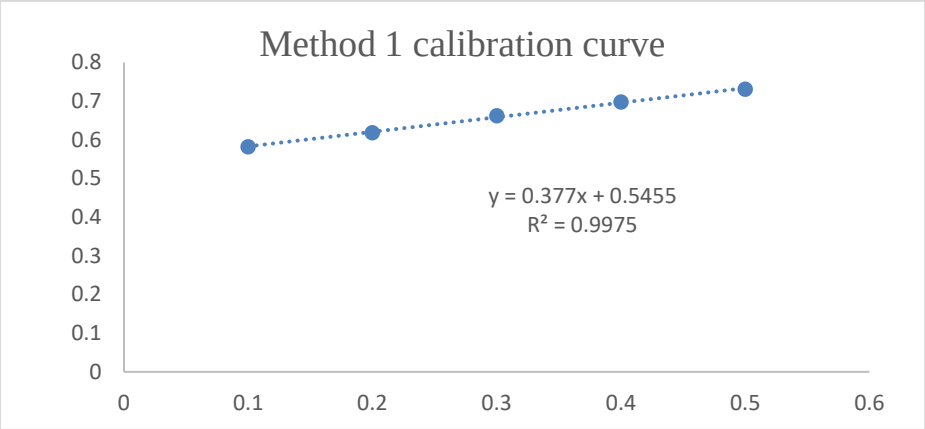

Fig:4 Method1 calibration curve of Ledipasvir

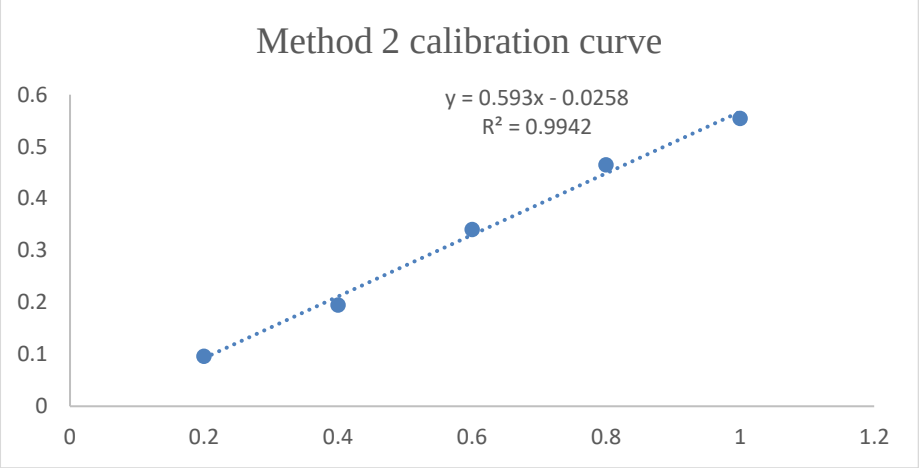

Fig:5 Method 2 calibration curve of Ledipasvir

Precision:

S. No	Parameters	Intra-day precision		Inter day precision				
		Method 1	Method 2	Method 1	Method 2			
1	Average (Mean)	0.00588	0.0002304	0.014508	0.000192			
2	Standard deviation	0.00753	0.00020845	0.01692	0.000186			
3				% RSD	1.2804	0.9047	1.1662	0.9698

Table:2Intra-day & inter day precision data of Ledipasvir

Accuracy:

Drug Name	% Recovery	Amount Present (µg/ml)	Amount of drug taken (µg/ml)	Total amount of drug found(µg/ml)	% of amount recovered	Mean
Ledipasvir	80	2	0.2	0.18	90	90
	100	2	0.4	0.50	125	113.6
	120	2	0.6	0.68	113.3	126.6

Table:3 Accuracy data of Ledipasvir for Method 1

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Table:4 Accuracy data of Ledipasvir for Method 2

Limit Of Detection (LOD) And Limit Of Quantification (LOQ):

The limit of detection and limit if quantification is the lowest concentrations of analyte that can be reliably detected and quantified respectively. The LOD and LOQ are found to be 0.0106 and 0.0322µg/ml for method 1 and 0.03446 and 0.10442µg/ml for method 2 respectively.

VALIDATION PARAMETERS

Parameters	**Results for method 1**	**Results for method 2**
ƛ maximum	520.0nm	713.6nm
Linearity range	1-5µg/ml	2-10µg/ml
Regression equation	Y=0.377x+0.5455	Y=0.593x-0.0258
Correlation coefficient	0.9975	0.9942
Slope(m)	0.377	0.6005
y-Intercept(c)	0.5454	0.6782
LOD	0.010626	0.03446
LOQ	0.032201	0.10442
Accuracy (%mean recovery)	113.18	109.98
Inter day precision	0.01762	0.000436
Intraday precision	0.0237	0.000405
Sandal's sensitivity	0.0004524	0.002051
Molar absorptivity	0.1078×10^4	0.5077×10^4

Table:5 validation parameter data for Ledipasvir

CONCLUSION

The method 1is based on redox reaction titrations. Fe^{3+} ion undergoes oxidation and gives Fe^{2+}ion which complexes with 1,10- phenanthroline to form reddish orange colored complex and shows absorption maximum at 520.0nm.

The method 2 is based on redox reaction titrations. Fe^{3+} ion undergoes oxidation and gives Fe^{2+}ion which complexes with potassium ferric cyanide form bluish green colored complex and shows absorption maximum at 713.6nm.

These validated methods were accepted for the assay of ledipasvir in formulation and results are 99.02% and 115% respectively.

According to International council for harmonization guidelines these methods were validated for accuracy, precision, linearity, selectivity and robustness.

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Drug name	**%Recovery**	**Amount present (µg/ml)**	**Amount of drug Taken (µg/ml)**	**Total amount of drug found (µg/ml)**	**% of amount recovered**	**Mean**
Ledipasvir	80	2	0.2	0.23	115	106.6
	100	2	0.4	0.48	120	117.33
	120	2	0.6	0.67	116	115.6

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