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DEVELOPMENT OF NEW ANALYTICAL TECHNIQUES FOR THE QUALITY OF ANTI-AIDS DRUGS

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Abstract

A simple, accurate, sensitive and rugged analytical method has been developed and validated for related estimation of Emticitabine in capsule dosage form. Chromatographic separation was achieved on a Hypersil BDS C18 (100 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature) by a mobile phase consisted of buffer, acetonitrile and Methanol (85:10:5 v/v) with apparent pH of 2.2 and a flow rate of 1.5 ml/min. The detection wavelength was set at 280 nm. The different analytical parameters such as linearity, accuracy, precision, robustness were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The method was linear (r = 0.9990) at a concentration range of 50 - 149 µg/ml. The proposed method is highly sensitive, precise, accurate and easy to perform hence was successfully applied for the quantification bulk and capsule dosage form.

INTRODUCTION

Emitracitabine

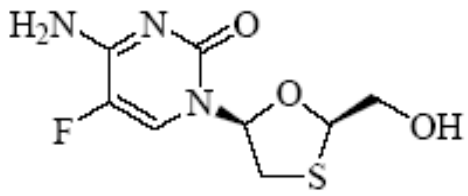
Emticitabine is indicated for the treatment of human immunodeficiency virus type 1 (HIV-1) infection.

C.A.S. No **:** 143491-57-0

Trade name **:** EMTRIVA.

Chemical name: 4-amino-5-Fluoro-1-[(2*R*,5*S)]-2*(Hydroxymethyl)-[1,3]-Oxathiolan-5-yl]-2-(1H)-Pyrimidinone

Structural formula:



Chemical formula **:** $C_8H_{10}FN_3O_3S$

Emticitabine is a white to off-white powder with a molecular mass of 247.24. It is freely soluble in methanol and water.[1,2,]

Literature review reveals that very few analytical methods are available for the determination of Emticitabine and the official monograph is not available in any pharmacopoeia.[3,4,5,6]

MATERIALS AND METHODS

Experimental

Instrumentation:

High Performance Liquid Chromatograph system with Waters 2695 separations module, 2696 Photo diode array detector (Waters alliance) and a stainless steel column 100 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 µm diameter was employed for the study. Detection of the drug was done by using a 2696 Photo diode array detector (Waters alliance) All the weighings in the experiments were done with Digisum Electronic analytical balance (model DI 707).

Chemicals and reagents

The reference sample of Emticitabine is obtained from Aurobindo Pharma Limited, Hyderabad, India.

Purified water was prepared by using 0.45 Millipore Milli-Q water purification systems. HPLC grade acetonitrile and methanol (Merck, Mumbai) were used for preparing the mobile phase and the diluent. Potassium dihydrogen orthophosphate, 1-Octane sulfonic Acid sodium salt and

orthophosphoric acid are of analytical grade obtained from Aurobindo Pharma Limited, Hyderabad, India.

Preparation of Standard Solution:

Accurately weigh and transfer about 50 mg of Emtricitabine working standard into 50ml clean, dry volumetric flask, add 30 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Further dilute 5 mL of this solution to 50 ml with diluent and mix. Filter through 0.45 µ filter. (Millipore PVDF)

Preparation of Sample Solution:

Transfer 5 intact capsules in to 1000 mL clean, dry volumetric flask, add 100 ml of water and sonicate for 15 minutes with intermittent vigorous shaking or until the capsule shells get disperse completely. Add about 600 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Centrifuge the solution at 10000 RPM for 10 minutes. Further dilute 5 mL of the supernatant solution to 50 mL with diluent and mix. Filter through 0.45 µ filter. (Millipore PVDF)

RESULTS AND DISCUSSIONS:

Method development

A number of eluting systems were examined for optimization of the mobile phase for separation of the drug. We tried phosphate buffer of same pH range but the preparation of water containing orthophosphoric acid is easy to prepare. Mixtures containing buffer, acetonitrile and methanol were used as eluting systems in different proportions. A mixture of buffer, acetonitrile and methanol in the ratio of 85:10:5 v/v provided an efficient separation of the drug with good peak shapes and retention times. A flow rate of 1.5 ml/min was found to be optimum which gave retention time of 5.884. The column temperature was kept constant at 30^{o}C. Under these chromatographic conditions the run time of the sample was 15 min.

Specificity and selectivity of the proposed method

Specificity is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific. This

parameter was performed to know the retention time of sample and standard. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and excipients) showed almost no interfering peaks within retention time ranges. Fig.1 and 2 show the representative chromatograms for standard and the sample.

Quantitative aspects

Linearity

A series of solutions were prepared using Emtricitabine working standard at concentration levels from 50% to 150% of test concentration and each solution was injected in to HPLC as per methodology.

The linearity of the method was demonstrated over the concentration range of 50%-150%. Aliquots of 50, 70, 89.99, 99.99. 109.99, 129.99 and 149.99 µg /ml were prepared and each solutions were injected in to HPLC system as per test procedure. A calibration curve was plotted for concentration v/s peak area and was given in the **Fig: 3.**

From the Linearity data it was observed that the method was showing linearity in the concentration range from 50 to 150 of test concentration. Correlation coefficient was found to be 0.999. The data of linearity was illustrated in **Table 1**. The linearity curve was plotted and given in **Figure 3.**

System Suitability

Standard solution of Emtricitabine standard was injected five times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections.

From the system suitability studies it was observed that % RSD for the peak areas of five replicate injections of the standard was found to be 0.1. Theoretical plates are found to be 5226. USP tailing factor was found to be 1.0. All the parameters were within the limit. The results of system suitability studies were given in **Table: 2**. and the standard Chromatograms can be seen in **Fig: 4, 5, 6, 7 and 8**.

Accuracy

Assay was performed in triplicate for various concentrations of Emtricitabine equivalent to 50%, 75%, 100%, 125% and 150 % of test concentration using Emtricitabine drug substance and

Emtricitabine capsules placebo as per the test method and injected each solution into the HPLC system as per methodology. The average % recovery of Emtricitabine was calculated and the results were summarized in **Table: 3.**

The recovery results indicating that the test method has an acceptable level of accuracy for the assay of Emtricitabine in Emtricitabine Capsules from 50% to 150% of test concentration.

Precision

System Precision: Standard solution was prepared as per test method and injected six times in to the chromatogram

The results were given in **Table: 4.**

Method Precision: It is very important that the methoddeveloped should be precise. Six replicates of the sampleprepared from the commercial tablets were injected andAssay was calculated to measure the repeatability of retentiontimes and peak area of standard and sample.

The results were given in **Table: 5.**

Ruggedness (Intermediate precision)

Six sample solutions were prepared individually using same batch of Emtricitabine capsules (used for method precision) as per test method and injected each solution using different column, system and analyst on different day. These results (Set-I) were compared with method precision results (Set-II)

The results were given in **Table: 6.**

Robustness

The robustness of the proposed method was determined by deliberately varying method parameters such as flow (10%), wavelength (5 nm), % organic in mobile phase (2% absolute), Column oven temperature (5^{o}C) and pH (0.2 Units). Analyzed the test sample in replicate at each variable condition to evaluate the system suitability.

The results were given in **Table: 7.**

ACKNOWLEDGEMENTS

The author is thankful to Aurobindo Pharma Ltd, Hyderabad, India for providing the sample of Emticitabine as a gift and for providing necessary facilities to carry out the research work and also Dr.M.V.V. Nageswara reddy who guided during the experiment.

Specificity:

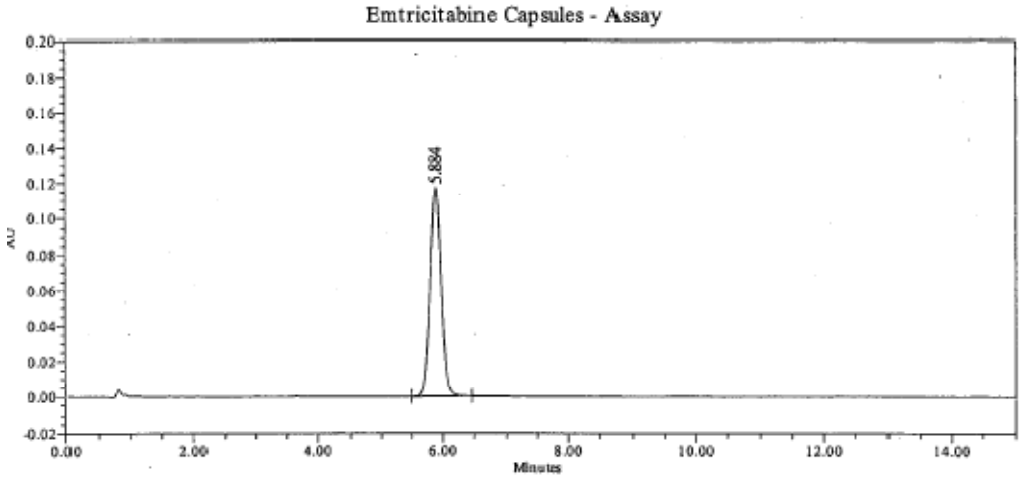


Figure 1 Standard Chromatogram for Emtricitabine Identification

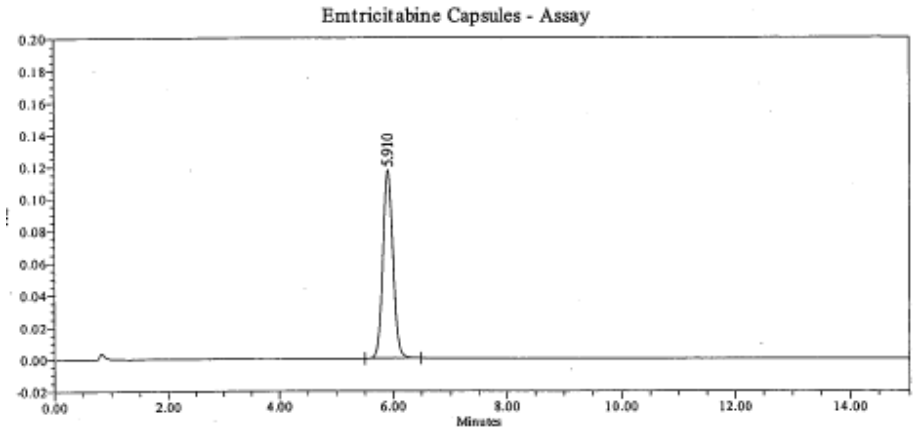


Figure 2 Sample Chromatogram for Emtricitabine Identification

LINEARITY:

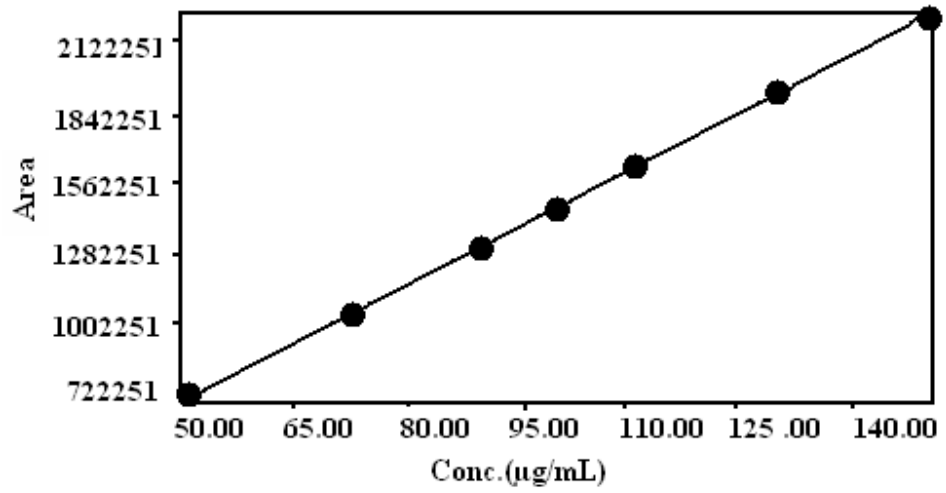


Figure 3 Linearity Plot (Concentration Vs Response)

Table 1

Linearity Results

% Concentration (Approx.)	Concentration (µg/mL)	Average area	Statistical Analysis	
50	50.00	722251	Slope	14550
70	70.00	1012000	Intercept	-7486
90	89.99	1299280	% Y-Intercept	0.5
100	99.99	1442986		
110	109.99	1595819	Residual Sum of squares	3401
130	129.99	1881278		
150	149.99	2178114	Correlation Coefficient	0.9999

System suitability:

A. Chromatogram of System Suitability

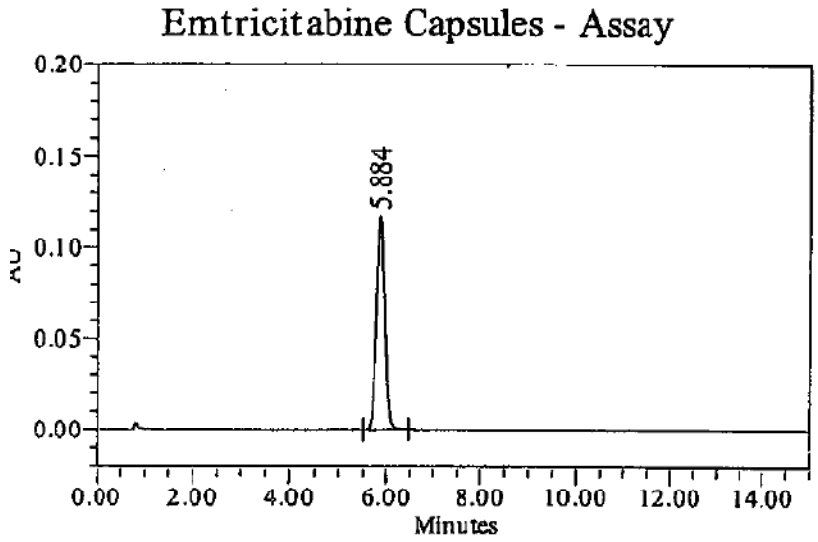


Figure 4

	RT	Area (μV* sec)	USP Plate Count	USP Tailing	Name
1	**5.887**	**1450970**	**5226**	**1.0**	**Emtricitabine**

B. Chromatogram of System Suitability-Replicate injections of Standard

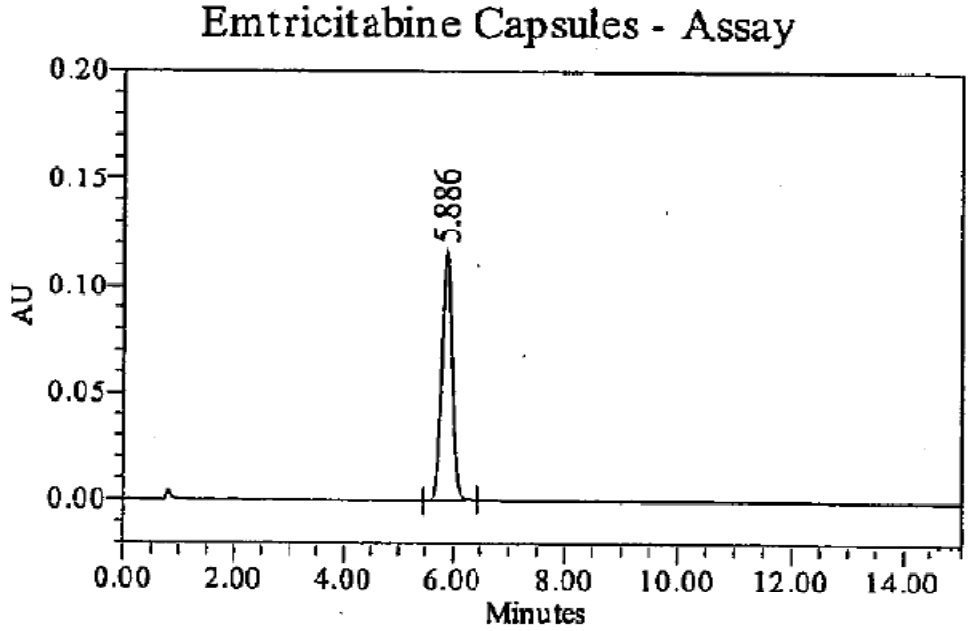


Figure 5

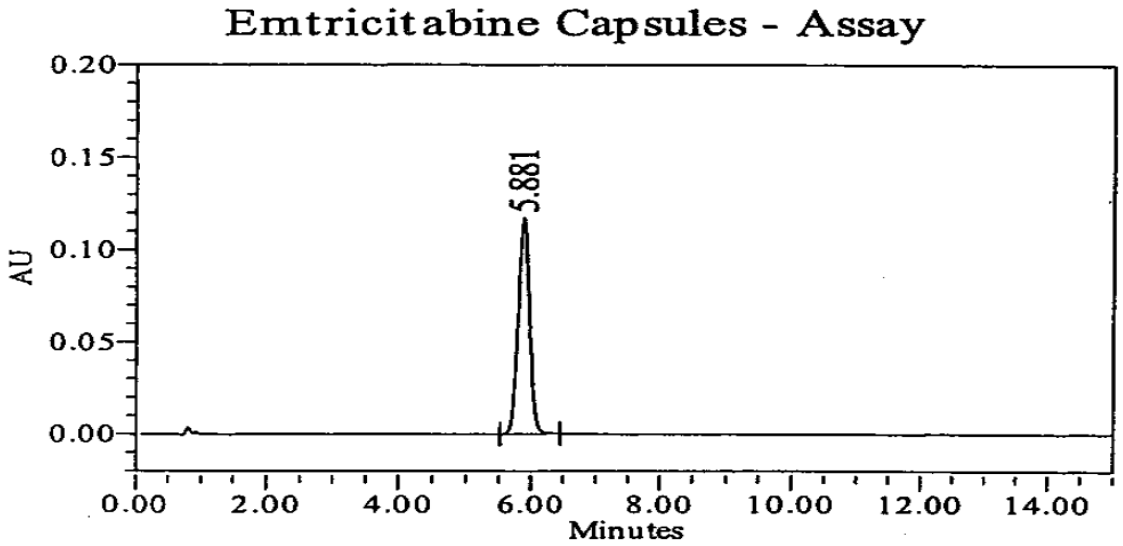


Figure 6

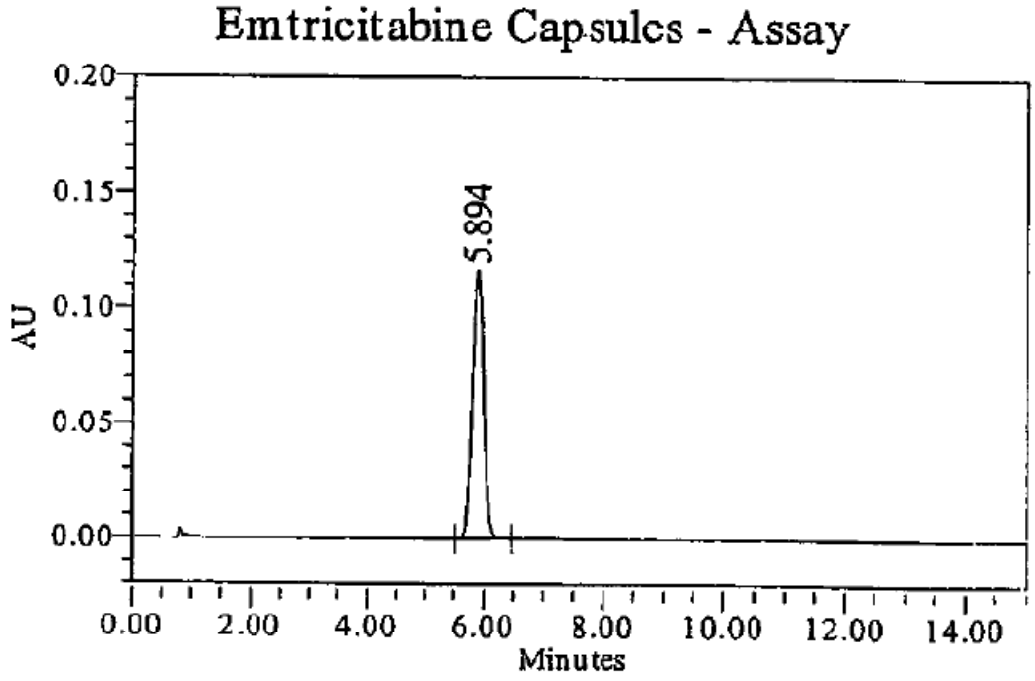


Figure 7

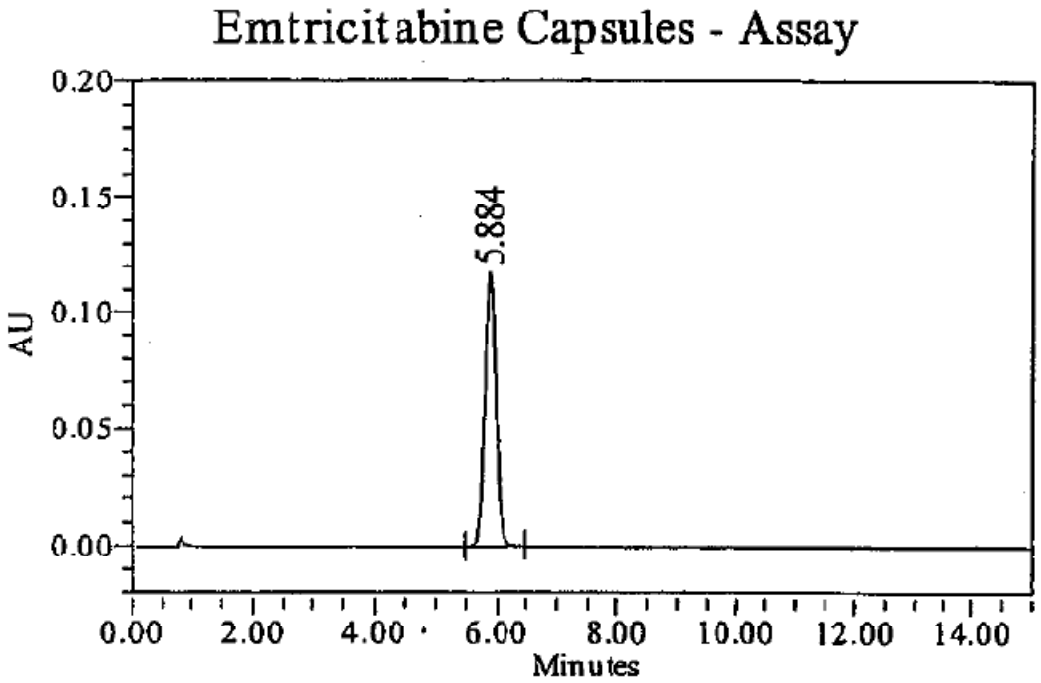


Figure 8

Table 2

Standard Chromatogram values for System suitability

Injection	RT	Peak Area
1	5.884	1455171
2	5.886	1453815
3	5.881	1452842
4	5.894	1453973
5	5.884	1457018
Statistical Analysis		
Mean	5.89	1454564
SD	0.005	1602
% RSD	0.0003	0.1

Accuracy

Table 3

% Recovery Results for Emtricitabine

Concentration/Sample ID	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis	
50% Level Sample 1	501.2	501.0	100.0	**Mean**	**100.0**
50% Level Sample 2	500.6	500.0	100.1	**SD**	**0.06**
`50% Level Sample 3	500.2	500.0	100.0	**% RSD**	**0.1**
75% Level Sample 1	750.4	750.3	100.0	**Mean**	**100.1**
75% Level Sample 2	749.0	751.6	100.3	**SD**	0.15
75% Level Sample 3	750.8	751.6	100.1	**% RSD**	**0.1**
100% Level Sample 1	1000.9	1008.5	100.8	**Mean**	**100.7**

100% Level Sample 2	999.4	1008.8	100.9	**SD**	0.32
100% Level Sample 3	998.7	1001.2	100.3	**% RSD**	**0.3**
125% Level Sample 1	1250.1	1251.1	100.1	**Mean**	**100.1**
125% Level Sample 2	1249.5	1252.2	100.2	**SD**	**0.06**
125% Level Sample 3	1250.5	1251.6	100.1	**% RSD**	**0.1**
150% Level Sample 1	1500.2	1499.2	99.9	**Mean**	**100.1**
150% Level Sample 2	1498.20	1501.8	100.2	**SD**	**0.15**
150% Level Sample 3	1499.1	1500.7	100.1	**% RSD**	**0.1**

Overall Statistical Analysis

Mean	**100.2**	**SD**	**0.28**	**%RSD**	**0.3**	**95% Confidence Interval**	**±0.2**

Precision

A) System Precision:

Table 4

Standard Chromatogram values for Repeatability

Injection No	Peak Area	Statistical Analysis	
1	1402429	Mean	1401246
2	1401564	SD	3324
3	1405468	%RSD	0.2
4	1400191		
5	1402366	95% Confidence Interval	±3489
6	1395455		

Test results are showing that the results are precise

B) Method Precision:

Table 5

Sample Chromatogram values for Repeatability

Injection No	Assay (mg/Capsule)	Statistical Analysis	
1	200.9	Mean	201.2
2	201.1	SD	0.31
3	200.8	%RSD	0.2
4	201.4		
5	201.6	95% Confidence Interval	±0.3
6	201.3		

Test results are showing that the results are precise.

Ruggedness (Intermediate precision)

Table 6

Results for intermediate Precision

Sample	Assay (mg/Capsule)	
	Set-I	**Set-II**
1	200.9	201.9
2	201.1	201.7
3	200.8	201.9
4	201.4	200.7
5	201.6	200.4
6	201.3	200.5
Mean	201.2	201.2
SD	0.31	0.72
%RSD	0.2	0.4

95% Confidence Interval	±0.3	
Set	**I**	**II**
Analyst	**Analyst-I**	**Analyst-II**
Equipment ID	**ARE-212**	**ARE-207**
Column ID	**LCC-1444**	**LCC-1377**
Day	**07-05-2011**	**15-5-2011**

Robustness

Table 7

Results for System suitability

Parameter	Variation	System Suitability		
		USP plate count	**USP Tailing**	**%RSD**
STP	---	4550	0.98	0.4
Flow Rate	- 10%	4941	0.99	0.4
	+ 10%	4340	0.99	0.3
Wavelength	- 5 nm	4548	0.99	0.4
	+ 5 nm	4551	0.98	0.4
% Organic in Mobile phase	- 2% absolute	4348	0.97	0.1
	+ 2% absolute	3822	0.97	0.0
Column oven Temperature	- 5°C	4482	0.99	0.4
	+ 5°C	4820	0.99	0.2
pH	- 0.2 units	3790	0.97	0.1
	+ 0.2 units	3606	0.96	0.0

The above results indicating that the test is robust for all variable conditions outlined in the above table.

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