North Asian International Research Journal of Sciences, Engineering & I.T.

ISSN: 2454-7514 Vol. 6, Issue-2 February-2020

IRJIF. I.F. : 3.821

Index Copernicus Value: 52.88 Thomson Reuters ID: S-8304-2016

A Peer Reviewed Refereed Journal

UV VISCIBLE SPECTROPHOTOMETRIC ESTIMATION OF ENTECAVIR IN BULK AND ITS PHARMACEUTICAL FORMULATION BY DIAZOTISATION METHOD USING 2-NAPHTHOL

D. VENKATESWARLU[1*], G. SREEDEVI[1], I.E. CHAKRAVARTHY[1], N. RAMI REDDY[2] AND K. PRABHAVATHI[2]

[1]Department of Chemistry, Rayalaseema University, Kurnool, AP-518004, India
[2]Department of Chemistry, S.B.S.Y.M. Degree College, Kurnool, A.P-518004, India

ABSTRACT:

A simple, sensitive, rapid and accurate spectrophotometric method has been developed for the estimation of entecavir in pharmaceutical formulations. The proposed method was based on the formation of azodye with 2-naphthol which form brownred colour complex of entecavir with 2-naphthol. The absorbance of the extractable azodye is measured at the wavelength of maximum absorbance 373 nm against the reagent blank. Results obtained are statistically validated and found to be reproducible.

Key words: ***Spectrophotometry, entecavir, 2-naphthol.***

MATERIALS AND METHOD:

Instrument:

All measurement were done on Milton Roy 1001spectrophotometer by using 10 mm matched quartz cuvettes.

Materials and reagents:

Preparation of reagents and solutions:

Sodium nitrite (2.0 N):

13.78 g of Sodium nitrite (Merck) is dissolved in distilled water and the resulting solution is made up to the mark in 100 ml standard flask with distilled water.

Sodium Hydroxide Solution (10%): 10 g of Sodium Hydroxide (Merck) dissolved in 100 ml of distilled ater.

2- Naphthol (3.0 M): 43.23 f of 2- Naphthol (Merck) dissolved in 100 ml of 10% Sodium Hydroxide (Merck) Solution.

Concentrated Hydrochloric Acid: 36 % (Merck) is directly used at necessary analytical region.

INTRODUCTION:

Entecavir chemically designation as 2-amino-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentyl]-1H-purin-6-one. Entecavir is a guanosine nucleoside analogue used in the treatment of chronic hepatitis B virus (HBV) infection. Entecavir therapy can be associated with flares of the underlying hepatitis B during or after therapy, but has not been linked to cases of clinically apparent liver injury. molecular formula is $C_{12}H_{15}N_5O_3$. Molecular weight is 277.279 g/mol, Entecavir is a white to off-white powder. It is slightly soluble in water (2.4 mg/mL), and the pH of the saturated solution in water is 7.9 at 25° ± 0.5° C. Entecavir has the following fig.1.

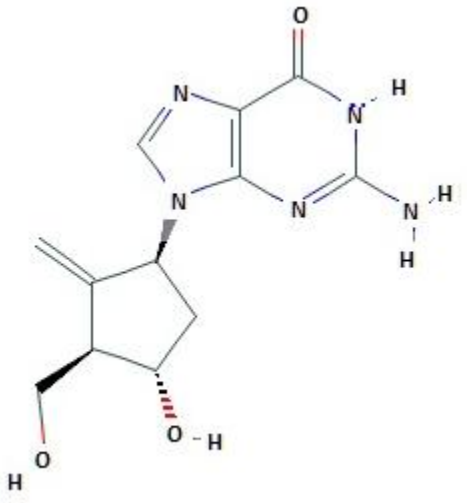

Fig. 1: Structure of entecavir

Diazotisation and coupling reaction of entecavir with 2-naphthol:

Entecavir is a novel nucleoside analogue reverse transctipase inhibitor drug that has selective anti haptitis B virus (HBV) activity. It is a deoxy guanine nucleoside analogue, inhibits hepatitis B-virus (HBV) DNA polymerase.

The amino group in entecavir is diazotised with sodium nitrite and hydrochloric acid at 0°C temperature. After diazotisation, the diazonium salt is coupled with 2-naphthol. The orange red coloured chromogen formed in the method is stable for more than 24 hours. The orange red coloured chromogen is used to determine the entecavir spectrophotometrically.

Entecavir could be readily diazotized in acid medium and the resultant diazonium cation would then react with coupling reagent 2-naphthol by electrophilic substitution at the position ortho to the phenolic hydroxyl group 2-naphthol and results in the formation of the coloured product. The reaction sequence can be shown in the fig.2.

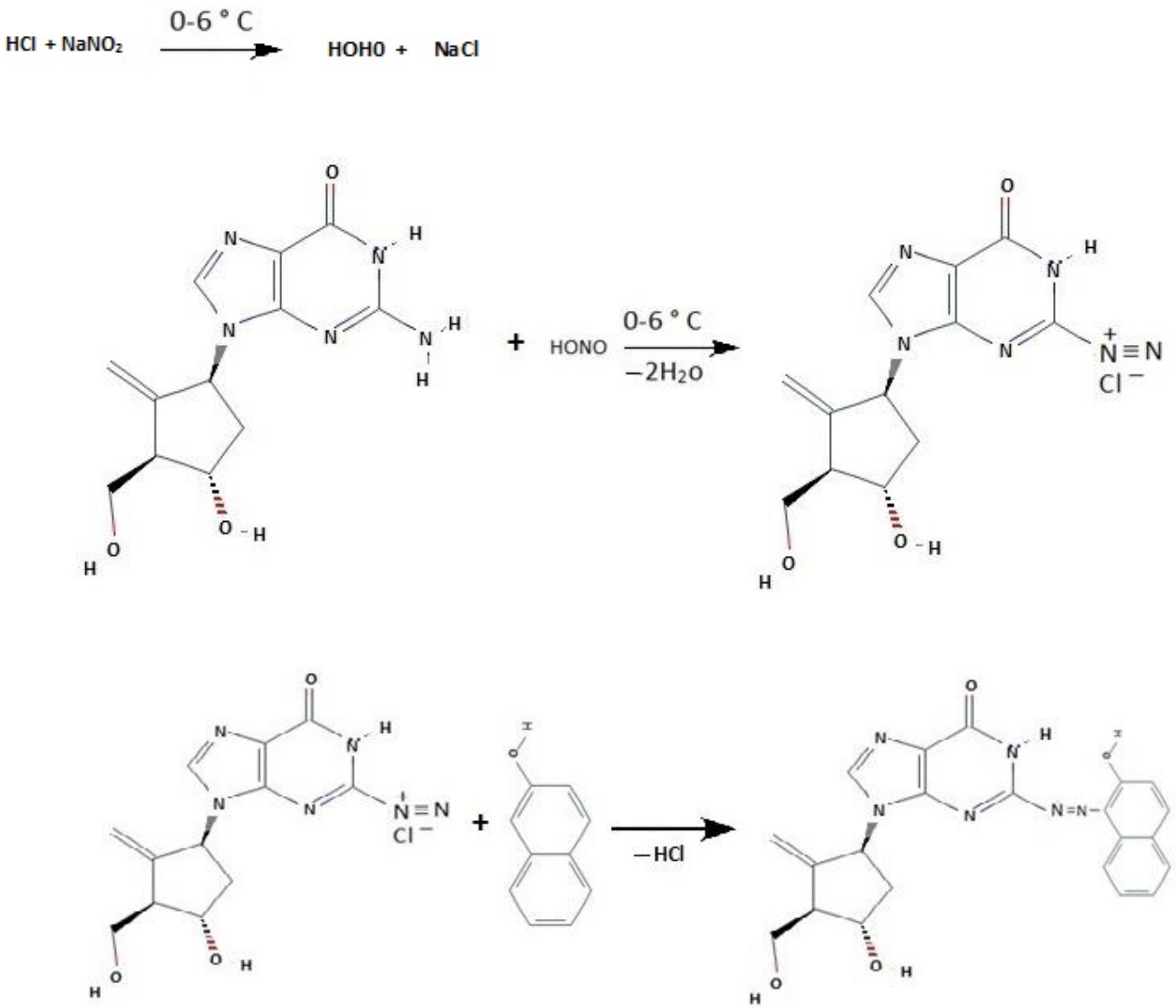

Fig. 2: The reaction sequence showing the formation of azodye between entecavir and 2-naphthol:

Spectrum of diazotized entecavir treated with 2-naphthol:

The wavelength of maximum absorbance of the diazotised drug treated with 2-naphthol solution is ascertained by the following procedure.

1 ml of entecavir solution (50 µg/ml) is transferred into a 10 ml volumetric flask. To this, 2.0 ml of 0.1N hydrochloric acid and 1.5 ml of cold 0.1N sodium nitrite solution are added. The resultant solution is well mixed, and then allowed to stand for five minutes at 0-5^{o}C temperature for diazotization. To this solution 1.0 ml of 1% urea solution is added and shaken frequently for nitrogen gas to escape. Then 1.0 ml of 0.5N sodium hydroxide and 1.0 ml of 2-naphthol solution are added and the volume is made to 10 ml with methanol. The absorbance of the orange red colour formed is measured in the wavelength range of 300 to 500 nm, against the reagent blank. The spectrum is given in fig.3.

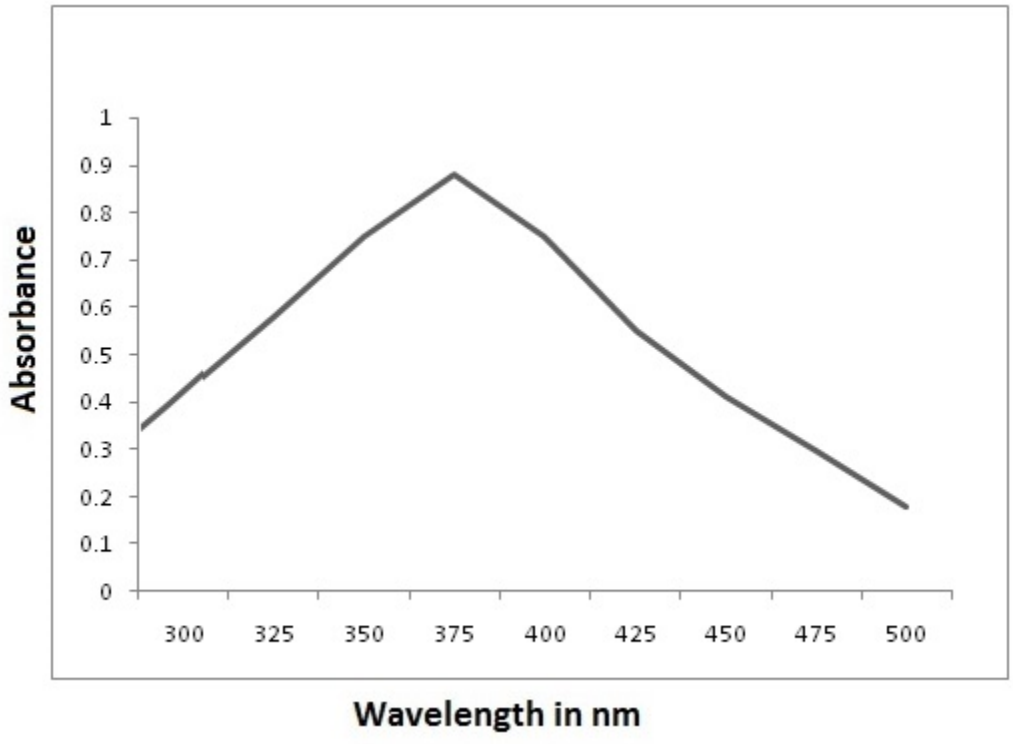

Fig.3. Absorption spectrum of entecavir-2-naphthol azo dye

From fig.3, it is clear that the diazotised drug treated with 2-naphthol solution has maximum absorbance at 373 nm. Hence, all further studies are made at 373 nm.

The optimal conditions for the determination of entecavir are arrived at by the following steps.

Effect of concentration of hydrochloric acid on the diazotization and coupling reaction:

The stability of the colour species depends on the concentration of hydrochloric acid. The effect of hydrochloric acid on the absorbance is studied by varying the volume of hydrochloric acid (0.1N) and measuring the absorbance at 373 nm. The data is presented in table.1.

Table.1.
Effect of concentration of hydrochloric acid solution on absorbance

Volume of HCl (ml)	Absorbance at 373 nm.
1.0	0.383
1.5	0.651
2.0	0.890
2.5	0.887
3.0	0.884

The data in table.1 show that 2.0 ml of hydrochloric produces maximum absorbance and hence the same concentration is maintained throughout the experimental work.

Effect of concentration of sodium nitrite on the absorbance of coupling reaction is studied by the following procedure:

In a series of 10 ml volumetric flasks containing 1.0 ml of (50 µg/ml) entecavir, 2.0 ml of 0.1N hydrochloric acid, 1.0 ml of (3.0 M) 2-naphthol solution, 1.0 ml of 1% urea solution, 1.0 ml of 0.1N sodium hydroxide solution are taken and varying amounts of sodium nitrite are added. The contents are made up to the mark and set aside for 5 minutes for completion of the reaction. The absorbance of the resultant solution is measured at 373 nm and the data are presented in table.2.

Table.2:
Effect of concentration of sodium nitrite

Volume of Sodium nitrite (ml)	Absorbance at 373 nm.
0.5	0.405
1.0	0.618
1.5	0.878
2.0	0.870
2.5	0.872

The data in table.2 indicate that 1.5 ml of sodium nitrite is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

Effect of concentration of resorcinol on the coupling reaction is studied by the following procedure:

In a series of 10 ml volumetric flasks containing 1.0 ml of (50 µg/ml) entecavir, 2.0 ml of 0.1N hydrochloric acid, 1.5 ml of 0.1N sodium nitrite solution, 1.0 ml of 1% urea solution, 1.0 ml of 0.1N sodium hydroxide solution are taken and varying amounts of 2-naphthol are added. The contents are made upto the mark and set aside for 5 minutes for completion of the reaction. The absorbance of the resultant solutions is measured at 373 nm and the data are presented in table.3.

Table.3:
Effect of concentration of 2-naphthol

Volume of 2-naphthol (ml)	Absorbance at 373 nm.
0.5	0.552
1.0	0.892
1.5	0.890
2.0	0.887

The data in table.3 indicate that 1.0 ml of 2-naphthol is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies

Construction of Calibration Curve:

To study the effect of drug concentration on the absorbance of the coupling reaction under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of entecavir.

Various aliquots of the standard entecavir solution ranging from 0.2-1.0 ml are transferred into a series of 10 ml volumetric flasks. To each flask, 2.0 ml of 0.1N hydrochloric acid solution and 1.5 ml of cold 0.1N sodium nitrite solution are added. The resultant solution in each flask is well shaken and allowed to stand for five minutes at

0-5^{0}C temperature for diazotization to complete. 1.0 ml of 1% urea solution is added to each flask and the solution is shaken frequently to allow nitrogen gas to escape. Then 1.0 ml of 0.1N sodium hydroxide solution and 1.0 ml of 2-naphthol solution are added and the volume in each flask is made upto 10 ml with methanol. A orange red colour is formed. The maximum absorbance of the orange red colour solution is measured at 362 nm against the reagent blank. Calibration graph is obtained by plotting absorbance values against the concentration of entecavir solution. The calibration curve is found to be linear over a concentration range of 20 to 100 µg of entecavir. The amount of entecavir present in the sample is estimated from the calibration graph. The results are presented in fig.4.

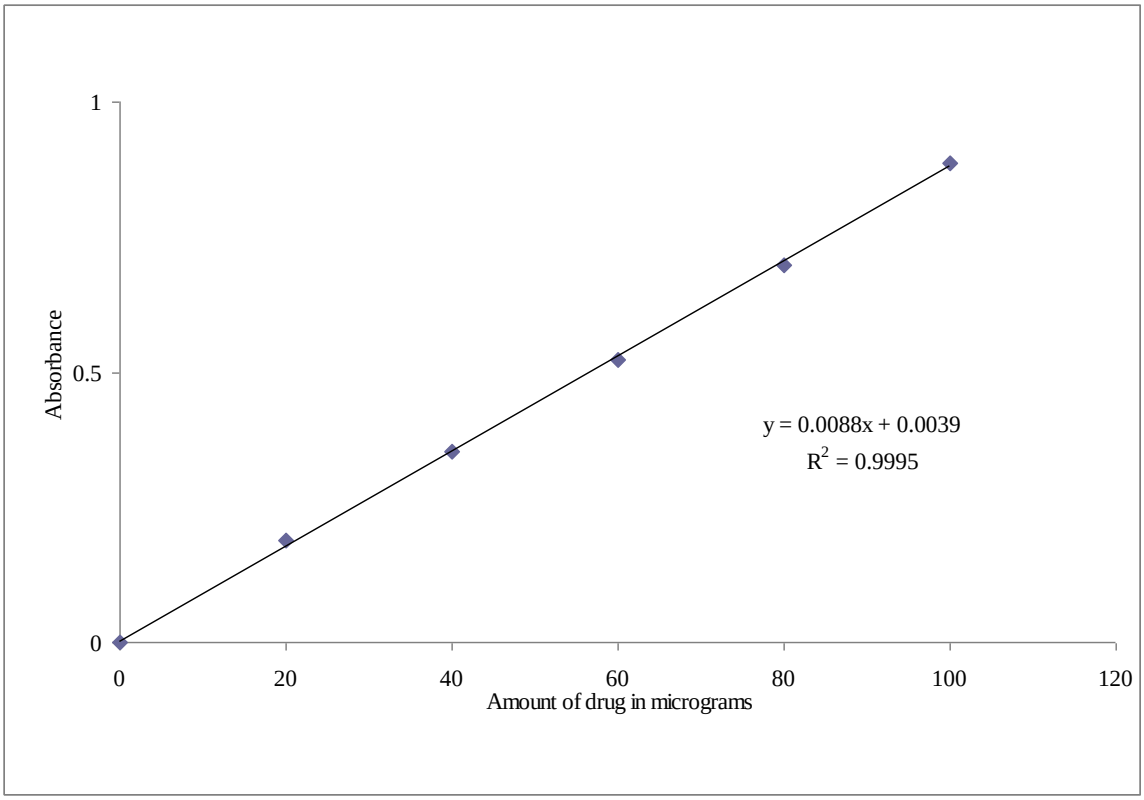

Fig.4: Calibration curve of entecavir

Assay of entecavir in pharmaceutical formulations:

The proposed procedure for the assay of entecavir is applied for its determination in commercial tablets.

Preparation of the sample solution:

Powdered tablet equivalent to 50 mg of the drug is weighed accurately and transferred into a 50 ml beaker and mixed well with 30 ml of methanol. The solution is filtered and transferred into a 50 ml volumetric flask and the volume is made up to 50 ml with methanol. The concentration of the drug solutions is now 1mg/ml. This stock

solution is further diluted to obtain the working concentration of 50 µg/ml.

The pharmaceutical preparation as prepared above is analysed by the following procedure.

Assay Procedure: Known volumes of the drug formulation prepare as above are transferred into a series of 10 ml volumetric flasks and 2 ml of 0.1N hydrochloric acid solution, 1.5 ml of 0.1N sodium nitrite solution are added. The resultant solution in each flask is shaken well and allowed to stand for five minutes at 0-5^{0}C temperature for diazotization. Then 1.0 ml of 1% urea solution, 1 ml of 0.5N sodium hydroxide and 1.0 ml of 2-naphthol solution is added. The absorbance of the resultant solution is measured at 373 nm. The amount of entecavir in the pharmaceutical formulation is evaluated from the predetermined calibration plot. The results are present in table.5.

RESULTS AND DISCUSSION:

Entecavir undergoes diazotisation when treated with sodium nitrite and hydrochloric acid. The excess nitrous acid during the diazotisation is removed by the addition of urea solution. The solution was shaken frequently to allow the nitrogen gas to escape. The diazonium cation reacts with the coupling reagent, 2-naphthol by electrophilic substitution at the o-position of the coupling agent to produce a orange red azo product. This orange red colour product shows maximum absorbance at 373 nm. The colour of the product is stable for more than 24 hours. The calibration curve (concentration vs. absorbance) is linear over the range of 20-100 µg of entecavir. The optical characteristics of the proposed method such as absorption maxima, Beer´s law limits, molar absorptivity and Sandell´s sensitivity are presented in Table.4. The molar absorptivity and Sandell´s sensitivity values shows sensitivity of the method. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized in the Table.4. The value of correlation coefficient was 0.999, which indicated the good linearity of calibration lines. The values of standard deviation are low, indicates high accuracy and reproducibility of the method. The‘t’ calculated values are compares well with the theoretical value of 2.78 there by indicating that the precision of the method is good. There no effect of additives and excipients such starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of entecavir in bulk drugs samples and pharmaceutical formulations.

Table.4:
Optical characteristics of proposed method

parameters	Proposed method
λmax (nm)	373
Beer's law limit (µg/ml)	20-100
Molar absorptivity (l mole-^{1} cm-^{1})	0.977x10^{5}
Sandell's sensitivity (µg cm-^{2} / 0.001 absorbance unit)	0.00302
Regression equation (Y = a + bx)	0.0088x+0.0039
Slope (b)	0.0088
Intercept (a)	0.0039
Correlation coefficient (r)	0.9995

*Y = a+bx, where Y is the absorbance and X concentration in µg / ml

Table.5:
Assay of entecavir in tablets

S.No	Sample (mg)	*Amount Found(mg) ±S.D*	% label claim	*t_{cal}
1	1	1.01±0.02	101	0.8695
2	1	1.02±0.05	102	0.80

***Average of five determination based on the label claim**

REFERENCES:

1. Malipatil, S. M.; Athanikar, Bharath S.; Dipali, Mogal, UV-Sectrophotometric Estimation of Entecavir in Tablet Dosage Form, Pharma Science Monitor ., 3(3), 56-67, 2012.
2. N. Raghavendra Babu, Y. Padmavathi , P. Ravi Kumar , R. Swethan Babu , D. Venkat Vijaya and Anjali Polker, Development of New Spectrometric Method for Estimation of Entecavir Monohydrate in Formulation Using 3-Amino Phenol as Chromogenic Reagent, J. Pharm. Sci. & Res., 11(6), 2452-2457, 2019.
3. V. Kiran Kumar and N.Appala Raju, Spectrophotometric estimation of Entecavir in pharmaceutical formulations, Biomedical & Pharmacology Journal , 1(2), 417-420, 2008.

4. Asmaa A.Elzaher, Marwa A.Fouad, Ola M.Elhoussini, Yasmine Essam-EldinBehery, Validated spectrometric determination of penciclovir and Entecavir in bulk and in pharmaceutical preparations, Bulletin of Faculty of Pharmacy, 54(2), 175-179, 2016.
5. M. Rajeswari , P. Subrahmanyam , G. Devala Rao and G. Sudakar Sai Babu, Noves Spectrophotometric Method for the Determination of Entecavir in Pharmaceutical Dosage Form, International Journal of Pharma and Bio Sciences, 2(3), 210-213, 2011.
6. B. Raj Kumar , A New Validated Stability-Indicating RP-HPLC Method for the Determination of Entecavir, JGTPS, 5(3), 1833 –1838, 2014.
7. Ashraf Muhammad, Hafiz Muhammad Nauman Shabbir, Hayat, Muhammad Munawar, Rahman, Jameel, Ejaz, Samina, Iqbal, Muhammad and Nasim, Faiz-ul-Hassan, HPLC Determination of Entecavir in Pure, Tablet Dosage Form and Spiked Plasma, Journal of the Chemical Society of Pakistan , 39(1), 1-5, 2017.
8. Hamza Altaf, Muhammad Ashraf, Muhammad M. Hayat, Abid Hussain, Nadeem Shahzad, Muhammad B. Ahmad and Jameel Rahman, HPLC Method for Simultaneous Determination of Entecavir and Tenofovir in Human Spiked Plasma and Pharmaceutical Dosage Forms, Latin American Journal of Pharmacy, 34(3),1-5, 2015.