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Development of New Spectrometric Method for Estimation of Entecavir Monohydrate in Formulation Using 3-Amino Phenol as Chromogenic Reagent

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Abstract

Aim: This study was aimed to demonstrate a new visible spectrophotometric method for the determination of entecavir monohydrate in pure and dosage forms using chromogenic reagents.
Method: This Method is based on reaction of drug with 3-amino phenol in acidic media to yield yellow colored chromogen exhibiting absorption maximum at 437nm.
Results: Beer's law is obeyed in the concentration range of 400-2000ng/mL with coefficient of determination (r^2) as 0.996. The Limit of detection and Limit of Quantitation were found to be 151.8ng/mL and 460ng/mL respectively.
Conclusion: The developed methods have been validated as per the ICH Q2 (R1) guidelines. The results demonstrate that the method is linear, precise and accurate. The proposed method were successfully applied for determination of Entecavir monohydrate in pharmaceutical dosage forms (tablets) with good recovery and reproducibility.

Keywords: 3-amino phenol, Entecavir monohydrate, spectrophotometric determination, ICH guidelines.

INTRODUCTION

[1,2]Entecavir chemically 2-amino-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentyl -6,9-dihydro-3H-purin-6-one monohydrate (Figure-1) is an oral antiviral drug used in the treatment of hepatitis B infection. Entecavir is a guanine analogue that inhibits all three steps in the viral replication process. Functional group present in organic drugs determine the way of analyzing then because they are responsible for the properties of substance and determine the identification reactions and the methods of quantitative determine of drugs. Knowing the functional group one can easily analyze any organic drug with a complicated structure. In the present investigation visible spectrophotometric methods have been developed for the drug by developing colour in each case with appropriate reagent. [3]3- amino phenol is an organic compound with formula $C_6H_4(NH_2)(OH)$. It is an aromatic amine and aromatic alcohol. It is the meta isomer of 2-aminophenol and 4-aminophenol. Literature review [4-27] reveals that many HPLC and LC/MS/MS spectrophotometric methods have been developed for the estimation of Entecavir monohydrate in bulk dosage forms.

Although spectrophotometric methods are the instrumental method of choice commonly used in industrial laboratories, no Chemometric method with the reagent using 3-amino phenol for the determination of Entecavir monohydrate in bulk dosage form.

Therefore the need for a fast, low cost, sensitive and selective method is obvious especially for routine quality control analysis of pharmaceutical products containing Entecavir monohydrate.

Now a days no one are using simple conventional reagent such as 3-amino phenol as chromogenic reagent because chemometric methods have lack of sensitivity. Hence we planned to develop a sensitive method using chromogenic reagent.

MATERIALS AND METHODS

Chemicals and Reagents

3-amino phenol (Sd Fine Chemicals Ltd (SDFCL), Mumbai), Conc. HCl (Finar (FC) Chemicals Ltd), Sodium nitrite (Sd Fine Chemicals Ltd (SDFCL), Mumbai), Ammonium sulphamate (Sd Fine Chemicals Ltd (SDFCL), Mumbai), Entecavir monohydrate (Pure drug) (Gift Sample from Aurobindo laboratories, Hyderabad, India), Entecavir monohydrate Tablets (Wocrkhardt Ltd Alentos Entecavir Tablets IP 0.5mg).

Instruments

A double beam UV-Visible spectrophotometer (Shimadzu , model-1800) connected to computer loaded with Schimadzu UV probe 2.41 Software was used for all spectrophotometric measurements. A Digital Balance (Shimadzu BL-220H), PH-meter (ELICO, LI 127), Ultra Sonic Bath Sonicator (PCI Analysis 6.5 li200H), Refrigerated Centrifuge (Eltek RC4100F), Hot Air Oven (Tempo Equipment Private Limited).

Method development and method optimization

Selection of wavelength (λ_{max})

The drug was scanned for its absorbance in visible range of 400-800nm. Absorption maximum was found to be at 437nm with 3-amino phenol. Refer figure 3

3-amino phenol Reagent (ETV with 3-amino phenol)

In this study, when 3-amino phenol reacts with sodium nitrite at 0-5°C, it forms diazonium salt which is highly reactive and readily attacked by 3-amino phenol. 3-amino phenol couples with this highly reactive diazotized compound and leads to the formation for a colored complex which is measured at 437nm. Reaction scheme of Entecavir monohydrate with 3-amino phenol is given in figure 2.

Solution preparation

Preparation of 3-amino phenol (1.5%) : Dissolve 150mg of 3-amino phenol in distilled water.

Preparation of 0.1% sodium nitrite: Dissolve 100mg of sodium nitrite in 100ml of distilled water.

Preparation of 0.1% ammonium sulphamate: Dissolve 100mg of ammonium sulphamate in 100ml of distilled water.

Preparation of 0.1N HCl: Dissolve 0.85ml of conc. HCl in 100ml of distilled water.

Preparation of stock solution: Standard Entecavir monohydrate, 10mg was weighed and transferred to 10ml volumetric flask and dissolved in 0.1N HCl. The flask was shaken and was made upto the mark with 0.1 N HCl to give a solution of 1000µg/ml. From this stock solution 1ml was pipette out into another 10ml volumetric flask and the volume was made upto 10ml with 0.1N HCl to give 100µg/ml. From this, 1ml was pipette out into another 10ml volumetric flask and the volume was made upto 10ml with 0.1N HCl to give 10µg/ml.

Calibration curve for Entecavir monohydrate with 3-amino phenol (400-2000ng/ml)

From 10µg/ml stock, aliquots of 0.4, 0.8, 1.2, 1.6, 2ml was taken in 10ml test tubes to which 1ml of sodium nitrite (0.1%w/v) was added. This reaction was carried out by keeping the flask in an ice tray so as to maintain (0-5°) temperature for 5 mins. Later, to this add 1ml of ammonium suplhamate (0.1%w/v) and wait for 5 mins and then add 1ml of 3-amino phenol (1.5%w/v) and wait till 5mins for color development i.e. yellow color (whole procedure was carried out in an ice bath). Then the volume was made upto 10ml with distilled water to give a solution of 400, 800, 1200, 1600 and 2000ng/ml. The absorbance of the resulting coloured solution was measured against respective blank solution (i.e. without drug) in visible region i.e., 300-800 nm which shows a maximum absorbance at 437nm and the spectrum in shown in figure 3

Estimation of entecavir in dosage forms using 3-amino phenol Reagent

Twenty tablets of entecavir (Alentos - Entecavir tablets IP 0.5mg, Manufactured by Hetero labs Limited) were weighed and finely powdered. The powder equivalent to 0.5mg was weighed and transferred to 10ml volumetric flask. The flask was shaken and volume was made up to mark with distilled water to obtain a solution of 10µg/ml.

From the above solution (100µg/ml) of standard drug solution, 3.5ml was pipetted out and required amount of sample solution was spiked into 10ml graduated tube followed by addition of 1ml of sodium nitrite (0.1%w/v) and 1ml of ammonium sulphamate (0.1%w/v) and 1ml of 3-amino phenol (1.5%w/v) solution and was made to 10ml using distilled water. The absorbance of resulting yellow colored solution was measured at 437nm against appropriate reagent blank. The obtained results are given in table 12.

Validation of Method:

The developed method was validated by determining the parameters defined by the ICH Guidelines.

Linearity

The linearity of the method was established by performing linear regression analysis for the calibration curve constructed between concentration and absorbance.

The linearity values for drug Entecavir with 3-amino phenol reagent are given below in table - 1 and graph is shown below graph -1.

Limit of Detection and Limit of Quantitation

The sensitivity of proposed method for measurement of Entecavir monohydrate was estimated in terms of LOD & LOQ.

The LOD and LOQ values are calculated as given in table - 2.

The Optimum conditions and Spectral data is tabulated in table - 3.

Precision: The precision of the developed analytical method was assessed by checking repeatability, intra-day precision and inter-day precision for Entecavir monohydrate drug

using 3-amino phenol as reagent.

Repeatability

Repeatability results obtained for six replicates of standard solutions of Entecavir were shown in table 4.

Intermediate Precision

The intra-day and inter day precision results are obtained for three replicates of three concentrations of Entecavir monohydrate. The absorbance values of each sample solutions was used for calculation of % RSD and the results are tabulated in table - 5 and table - 6 respectively.

Accuracy

The analytical accuracy is the nearness of the results obtained against the real values at each level of Entecavir concentration. The results obtained for accuracy studies for the drug substance and drug product were reported in terms of % RSD and % recovery respectively.

- **For drug substance:** Accuracy data of Entecavir monohydrate (pure drug) at 437nm using 3-amino phenol is shown in table -7 .
- **For drug product (Recovery study):** Recovery data of Entecavir monohydrate (drug product) at 437nm 3-amino phenol Reagent is shown in table - 8.

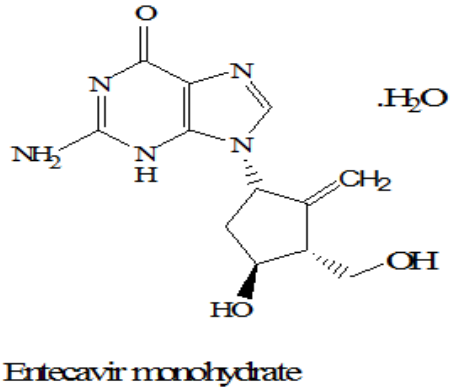
Assay

The proposed method was then applied for the determination of entecavir monohydrate in marketed formulations (tablets) Alentos Entecavir Tablets manufactured by (Wocrkhardt Pharmaceutical Ltd. India), contains 0.5mg of entecavir monohydrate. The %purity of the drug was presented in table - 9**.**

Color stability

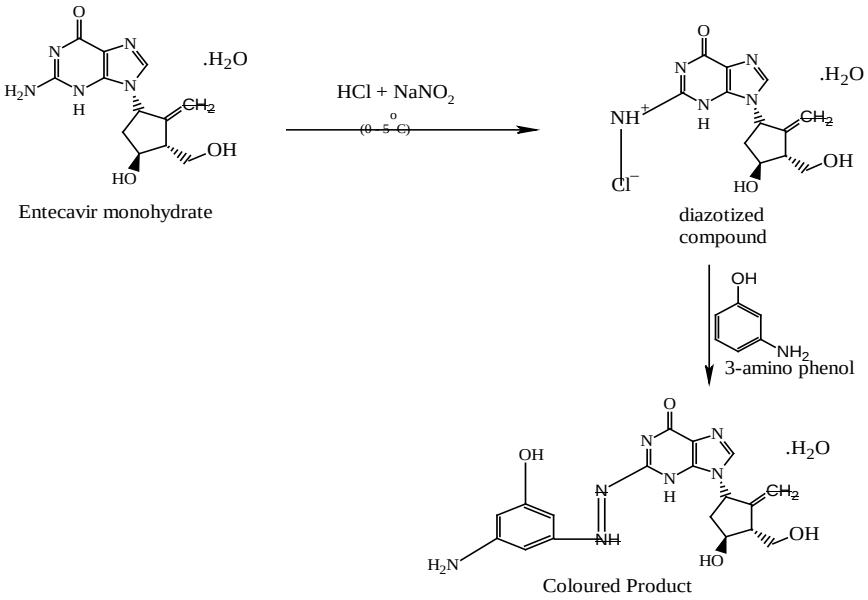
The stability of the color of the drug substance as well as the drug product was checked for different sample concentration and reported. The color for drug product and standard were found to be stable for 24 hrs.

RESULTS AND DISCUSSION:



Entecavir monohydrate

Figure 1: Structure of Entecavir monohydrate



Entecavir monohydrate

diazotized compound

Coloured Product

Figure 2: Reaction scheme of Entecavir monohydrate with 3-amino phenol

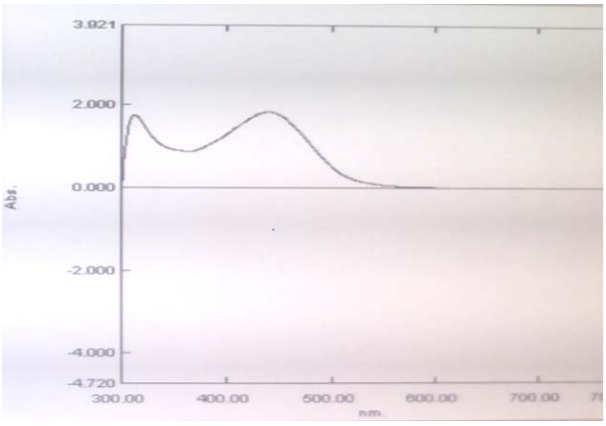


Figure 3: Spectra of ETV with 3-amino phenol (1mcg/ml)

Table - 1: Calibration Curve Data

Concentration(ng/ml)	Absorbance
400	0.099
800	0.426
1200	0.823
1600	1.264
2000	1.715

Table - 2

S.No.	Drug with Reagent	LOD	LOQ
1	Entecavir with 3-amino phenol	151.8µg/ml	460µg/ml

Table - 3: Optimum conditions and Spectral data

CONDITIONS	ETV with 3 – amino phenol
λ_{max}	437nm
Beer's Law Range	400-2000ng/ml
Sandell's sensitivity(µg/cm^2/0.001 absorbance units)	4.301ng/mg
Limit of detection (ng/ml or µg/ml)	151.8ng/ml
Limit of Quantitation (ng/ml or µg/ml)	460ng/ml
Regression equation	y=0.001x-0.3556
Slope	0.001
Intercept	0.3556
Correlation coefficient	0.996

Table - 4 : Repeatability data of Entecavir at 437nm using 3-amino phenol

S.No.	Concentration (ng/ml)	Absorbance	Mean*±Standard deviation	%RSD
1	800	0.830	0.851±0.02	2
2	800	0.837		
3	800	0.852		
4	800	0.856		
5	800	0.861		
6	800	0.873		

*Average of six determinations

Table - 5 : Intra-day precision data of entecavir at 437nm using 3-amino phenol Reagent

Concentration(ng/ml)	Mean absorbance values*			Mean±SD	%RSD
	Morning	Afternoon	Evening		
640	0.987	0.972	0.984	0.981±0.0079	0.805
800	1.904	1.898	1.902	1.901±0.003	0.157
960	2.134	2.141	2.135	2.139±0.004	0.187

*Average of 3 determinations

Table - 6 : Inter-day precision data of entecavir at 437nm using 3-amino phenol Reagent

Concentration(ng/ml)	Mean absorbance values*			Mean±SD	%RSD
	Day-1	Day-2	Day-3		
640	1.216	1.262	1.281	1.253±0.03	2
800	2.314	2.376	2.373	2.354±0.04	1.7
960	3.106	3.147	3.113	3.122±0.02	0.64

*Average of 3 determinations

Table - 7 : Accuracy data of Entecavir monohydrate (pure drug) at 437nm using 3-amino phenol

S.No	Level	Concentration (ng/ml))	Mean* ±Standard deviation	%RSD
1	80%	640	0.402 ± 0.0007	0.174
2	100%	800	0.426 ± 0.0007	0.164
3	120%	960	0.532 ± 0.0003	0.056

*Average of three replicates

Table - 8 : Recovery data of Entecavir monohydrate (drug product) at 437nm 3-amino phenol Reagent

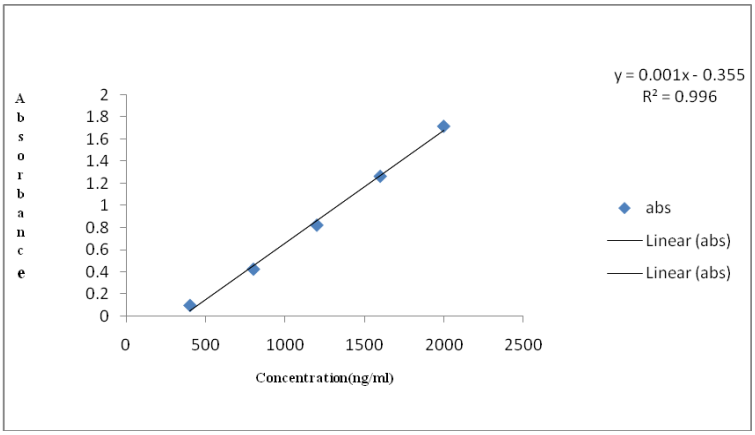
Tablet used	Levels	Amount of sample added (mL)	Amount of standard added (ng/mL)	Amount recovered (ng/mL)	%Recovery± Standard Deviation*
Entecavir	80%	0.64	10	757	118.2 ±0.0007
	100%	0.8	10	781	97.6±0.0007
	120%	0.96	10	891	92.8±0.0003

*Average of three replicates

Table - 9 : Assay results of entecavir monohyrate at 437nm using 3-amino phenol Reagent

Tablet used	Label Claim (mg)	Amount found	% Purity
ETV with 3-amino phenol	0.5	0.884ng	110.5%

***Acceptance Criteria*:** 89.8 to 111.3%.



Graph -1: Calibration curve

CONCLUSION

A simple visible spectrophotometric method was developed using chromogenic reagent and validated for estimation of Entecavir monohydrate in dosage forms.

The method was developed by using 3-amino phenol as chromogenic reagent and the method was found to be linear in the range of 400-2000ng/ml in acidic media. Analysis was carried out at λmax at 437nm.

The developed method was validated as per ICH guidelines and the validation parameters were found to be well within the acceptance criteria. The proposed method were found to be linear, accurate and precise. Developed method was simple, sensitive, economic and reproducible which can be used for routine quality control of Entecavir monohydrate dosage forms.

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