K. K. Jhankal et al /J. Pharm. Sci. & Res. Vol. 7(1), 2015, 10-13

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Quantification of Antiviral Drug Entecavir in Pharmaceutical Formulation by Voltammetric Techniques

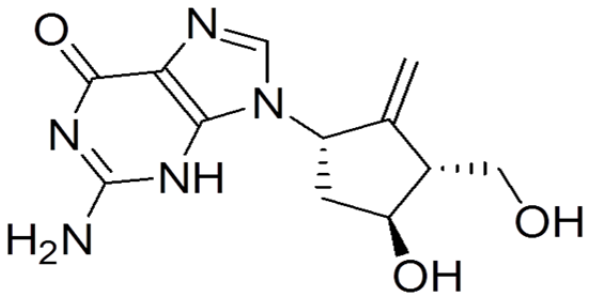
K. K. Jhankal, A. Sharma, Ramswaroop and D. K. Sharma
Electrochemical Research Laboratory,
Department of chemistry,
University of Rajasthan, Jaipur
Rajasthan, India-302004

Abstract
The electrochemical reduction behaviour of antiviral drug entecavir was studied at glassy carbon electrode in 0.02 M Britton-Robinson (BR) buffer solution using cyclic voltammetry and square wave cathodic adsorptive stripping voltammetry (SWCAdSV). Voltammetric results showed one well defined reduction peak in the potential range -0.3 to -0.4 V (vs Ag/AgCl). A linear calibration curve for SWCAdS Voltammetry analysis was constructed in the entecavir concentration range $3x10^{-6}$ to $1.5x10^{-5}$mol L^{-1}. The value of limit of detection (LOD) and limit of quantification (LOQ) were $2.27x10^{-6}$ and $5.57x10^{-6}$mol L^{-1} respectively.

Keywords: Entecavir, Voltammetry, Pharmaceutical formulation, glassy carbon electrode.

INTRODUCTION

Entecavir (**Scheme**), 2-amino-9-[(1S, 3R, 4S)-4-hydroxy-3-(hydroxyl methyl)-2-methylidenecyclopentyl]-3H-purin-6-one, belongs to a group of antiviral drugs used in the treatment of the hepatitis B infection. Entecavir is a selective competitive inhibitor of HBV polymerase, which blocks both priming and elongation steps in the replication process. Entecavir as a novel guanine analogue provides fast effect and high efficiency, minimal side effects or mitochondrial toxicity and less resistance in a chronic therapy [1–3].Several methods have been reported for determination of entecavir which include reverse phase extraction, chromatography, LC-MS, high performance liquid chromatography (HPLC), solid phase extraction (SPE) and salting out liquid extraction (SALLE) [4-6].



Scheme: Chemical Structure of Entecavir

Although spectrophotometry and chromatography are most commonly used techniques, but these involve many derivatization steps and extraction purification approaches prior to final analysis. These techniques are very time consuming and the demand of expensive, sophisticated instrumentation and highly skilled personnel restrict their use in routine analysis. Since the last decade, electro-analytical techniques have been widely used in the field of pharmaceuticals analysis, as these techniques are inexpensive, rapid and do not require derivatization or the time consuming purification steps after extraction, when compared with other analytical techniques. However, no electro-analytical methods have yet been reported for the quantification of Entecavir in pharmaceutical formulations. The purpose of the present study is to develop fast and sensitive voltammetric method for the detection and quantification of Entecavir in pharmaceutical formulations using square-wave cathodicadsorptive stripping voltammetry and cyclic voltammetry due to higher sensitivity, simplicity and lower limit of detection

MATERIALS AND METHODS

Entecavir was obtained from local pharmacy under the trade name Entavir (Cipla)and was used without further purification. A stock standard solution of bulk Entecavir (1×10^{-3}mol L^{-1}) was prepared in water and stored at 4°C until assay. The Working solutions ($1\times10^{-6}$to 1×10^{-4}mol L^{-1}) were prepared daily by appropriate dilution of the standard solution of bulk Entecavir with water just before use. A series of BR buffer of pH values 2.8 to 12 was prepared and used as a supporting electrolyte.

Apparatus

Model 1230A (SR 400) electrochemical analyzer (CHI Instrument, USA) was employed for electrochemical techniques, with a totally automated attached to a PC with proper CHI 100W version 2.3 software for total control of the experiments and data acquisition and treatment. Athree-electrodecellsystemwasused with activated glassy carbon electrode as working electrode, Ag/AgCl (3 M KCl) as the reference electrode and a platinum wire as the auxiliary electrode. A digital pH-meter (CHINO- DB-1011) was used for measuring the pH values of the solutions investigated.

Analytical Procedure

10 mL of the total solution containing BR buffer of pH 7.0 and the appropriate concentration of the Entecavir were transferred into the electrochemical cell, through which a pure deoxygenated nitrogen stream was passed for 10 minutes to remove the oxygen gas before measurements. Electrochemical pre-treatment was always performed in the same solution in which the measurement was subsequently carried out. The accumulation of Entecavir at the working

electrode was carried out for a selected time while the solution was stirred at 2000 rpm. The square wave cathodic adsorptive stripping and cyclic voltammograms were recorded after optimization of operational parameters.

RESULTS AND DISCUSSION

Electrochemical reduction of Entecavir was investigated at glassy carbon electrode using Square Wave Cathodic Adsorptive Strippins Voltammetry (SWCAdSV) and Cyclic Voltammetry (CV). In all the electro-analytical techniques well defined reduction peak were obtained at the potential -0.39 V (vs Ag/AgCl) which was used for the analytical measurement.

Effect of pH

The effect of pH on cathodic peak current of $1x10^{-4}$mol L^{-1}Entecavir was investigated by CV. The pH range was studied from 3 to 12. A well-defined reduction peak and a high peak current was obtained at pH 7, as a result further studies were carried out at this pH. **Figure 1** shows the effect of pH of supporting electrolyte on peak current. A shift of the peak potential towards more negative direction value was observed as the pH increased, indicate the existence of a protonation reaction coupled with the Entecavir reduction process.

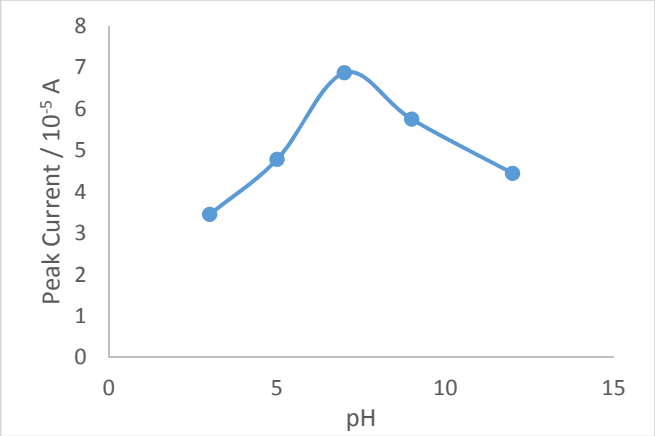


Figure 1. Effect of pH of medium on cathodic peak current

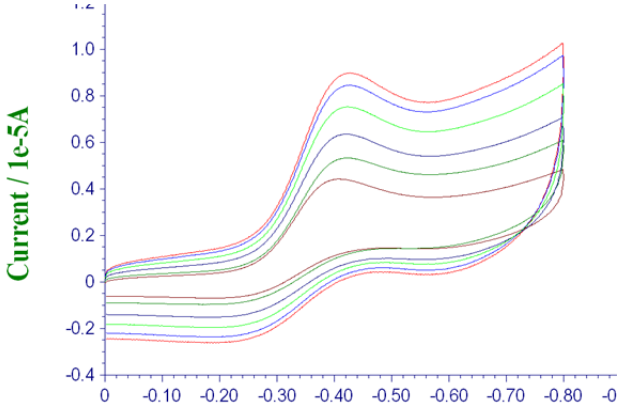


Figure 2. Cyclic voltammograms of entecavir ($2x10^{-5}$ M) at different scan rates (50, 100, 150, 200, 250 and 300 mVs^{-1})

Effect of Scan Rate

The whole procedure for cyclic voltammetry was repeated for entecavir with different scan rate value from 50 to 250 mVs^{-1} while other parameter kept constant.(**Figure 2**) Change in peak current (i_p) and peak potential (E_p) of reduction peak of entecavir due to the changing in scan rate values were observed. Graph of E_p versus log υ, i_p versus $υ^{1/2}$ were plotted.

The peak potential shifted towards more negative values with increase in scan rate, confirms the irreversible nature of reduction process. A straight line was observed when peak potential (E_p/V) was plotted against log υ (mVs^{-1}) at a particular concentration and pH value of 7 can be expressed by the equation.(**Figure 3**)

$$Ep = 0.0327 \log υ + 0.3385 \qquad R^2 = 0.9908 \quad (i)$$

The value of α, calculated from the slop of the plot is 0.453. Generally α is assumed to be 0.5 in a totally irreversible electrode process, so reduction of entecavir at glassy carbon electrode is irreversible. The effect of scan rate ($υ^{1/2}$) on peak current (i_p) was examined under the above experimental condition. As the scan rate was increased from 50 to 250 mVs^{-1} at a fixed concentration of entecavir, (i) the peak potential shifted cathodically, (ii) the peak current increased steadily, and (iii) the peak current function (i_p/AC $υ^{1/2}$) exhibited nearly constancy.A straight line is obtained when i_p(μA) is plotted $υ^{1/2}$(mV/s), which may be expressed by the equation (**Figure 4**)

$$I_p(μA) = 0.2061\ υ^{1/2} + 1.2567 \qquad R^2 = 0.9942 \quad (ii)$$

The linear relationship existing between peak current (i_p) and square root of the scan rate ($υ^{1/2}$) with a slope 0.2061, showed that the reduction process is predominantly diffusion controlled in the whole scan rate range studied.

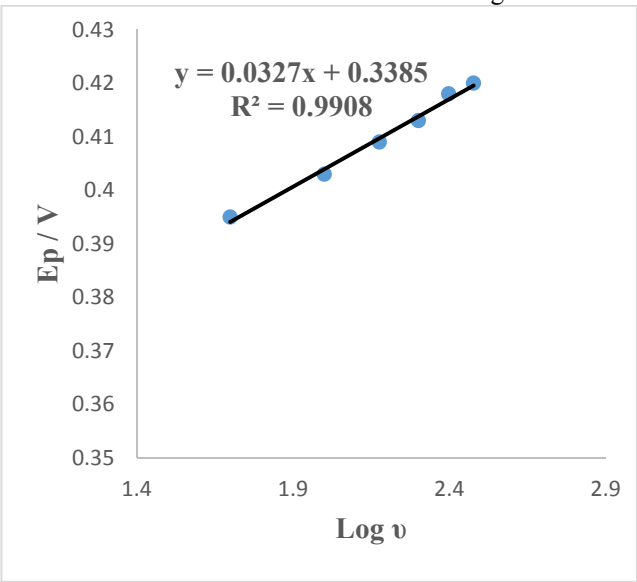


Figure 3. Influence of scan rate [log υ (mV/s)] on peak potential (E_p/V) of Cyclic Voltammograms of entecavir

Square Wave Cathodic Adsorptive Stripping Voltammetric Studies

The best result with respect to signal enhancement and peak shape accompanied by sharper response was obtained with BR buffer at pH 7 (**Figure 4**). This supporting

electrolyte was chosen for the subsequent experiments. In order to develop a voltammetric method for the determination of the entecavir in pharmaceuticals Square Wave Cathodic Adsorptive Stripping Voltammetry (SWCAdSV) was selected. SWCAdSVis effective and rapid electro-analytical technique with well-established advantages, including good discrimination against background current and low detection and determination limits.

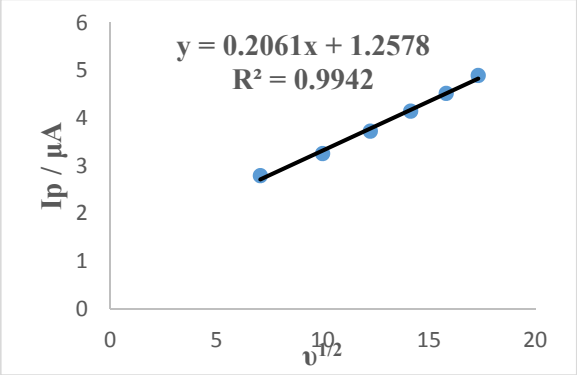


Figure 4. Influence of root of scan rate [$\upsilon^{1/2}$ $(mV/s)^{1/2}$] on peak current (I_p/µA) of Cyclic Voltammograms of entecavir

Validation of the method

Validation of the proposed SWCAdSVtechnique for the assay of entecavir in pharmaceutical dosages was carries out via estimation of the range of linearity, the limit of detection (LOD), the limit of quantification (LOQ) and selectivity [18-22].The linear relationship observed between peak current and concentration of the entecavir. The applicability of the proposed SWCAdSVprocedure as analytical methods for the determination of entecavir was examined by measuring the stripping peak current as a function of concentration of bulk drug at least three times under the optimized operational parameter. The linear regression equation is expressed as (**Figure 5**)

For SWCAdSV

$I_p(\mu A) = 0.3901C(\mu M) + 1.2567$ $R^2 = 0.9943$ (iii)

The regression plot showed that there is a linear dependence of the current intensity on concentration in SWCAdSVtechniques over the above said range.

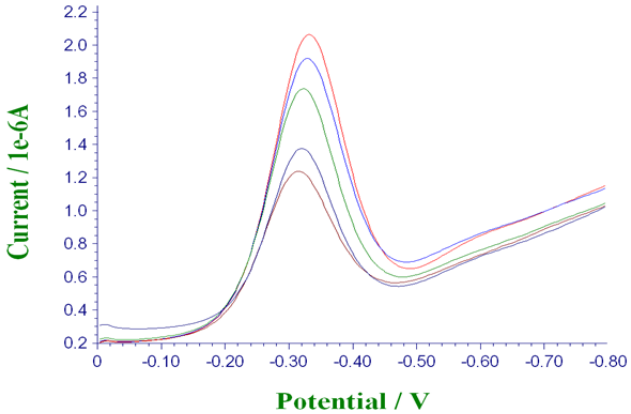


Figure 5. DPCAdSVoltammograms of entecavir at different concentrations

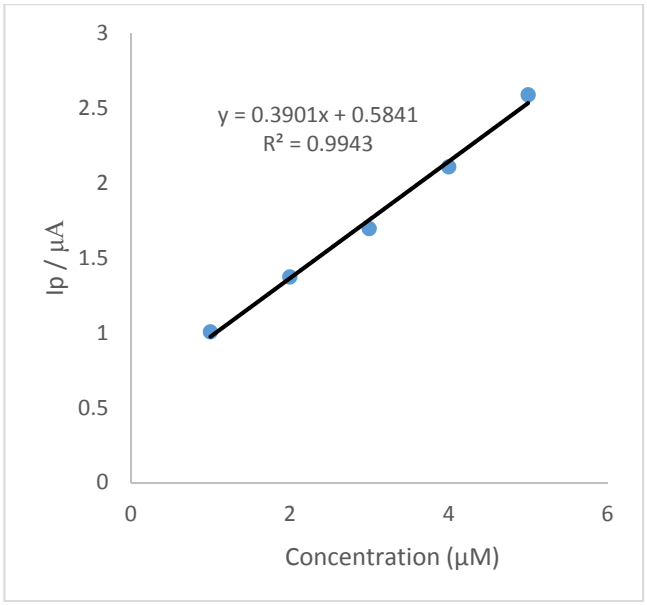


Figure 6. Plot of peak current vs. concentration from SWCAdSvoltammograms

Specificity

Specificity is the ability of method to measure the analyte's response in the presence of all the potential impurities. The specificity of the proposed procedure for the assay of entecavir was identified by studying the effect of excipients that often accompany entecavir in its tablets. An attractive feature of an analytical procedure is its relative freedom from interference by the excipients. The effect of additives like starch, lactose, magnesium stearate, gelatin and sugar granules was studied by analysing a sample solution containing $4x10^{-5}$ M Entecavir mixed with these excipients under the optimized condition. The result indicate excellent percentage recoveries of entecavir as 99.19% and 98.78% for bulk drug and mixed with excipients respectively. This means that the inactive excipients present in the pharmaceutical should not interfere with the Entecavir drug during its assay using the proposed procedure.

Sensitivity / Detection Limit

The limit of detection (LOD) is an important quantity in in chemical analysis. The LOD is the smallest concentration or amount that can be detected with reasonable certainty for a given analytical procedure. The limit of quantification (LOQ) is the lower limit of concentration for precise quantitative measurements. *LOD* and *LOQ* were calculated from the electro-reduction peak current using the following equations [23, 24]:

$LOD = 3\ S/b$

$LOQ = 10S/b$

Here *S* is the standard deviation of the peak currents (five runs), *b* is the slope of the calibration curve.

The calibration plot of the peak current versus the concentration was found to be linear over the range given in the **Table 1**.

Application to Analysis of pharmaceutical dosage form

The developed procedure was applied successfully for the determination of entecavir in pharmaceutical formulation (**Table 2**). There was no need for any precipitation,

evaporation or extraction step prior to the drug assay. The accuracy of the method was determined by its recovery during experiments. In order to detect the interaction excipients in this method, the standard addition techniques was applied to same preparation, which were analysed by calibration curve. The result demonstrate the validity of the proposed method for the determination of Entecavir in tablets. The mean percentage recovery showed no significance excipients interference, thus the procedure was able to detect assays of Entecavir in the presence of excipients and hence it can be considered specific.

Table 1. Characterisation data of entecavir calibration plots in BR buffer of pH value 7.0

Parameters	**SWCAdSV**
Concentration Range (M)	2 x 10^{-7} to 5.6x1o^{-5}
Slope	0.3901
Intercept	0.5841
Correlation Coefficient	0.9943
LOQ(M)	2.27x10^{-6} M
LOD (M)	5.57x10^{-6} M

Table 2. Assay results from entecavir tablets (Entavir) and mean recoveries in Entavirtablets

Parameters	**Bulk Entecavir Drug**	**Entecavir mixed with Excipients**
Amount Added (M)	4x10^{-5}	4x10^{-5}
AmountFound (M)	3.97x10^{-5}	3.991x10^{-5}
Average Recovery (%)	99.19	99.78
RSD (%)	1.02	0.97

CONCLUSION

We were able to examine the voltammetric behaviour of entecavir in aqueous media. The electrochemical reduction of entecavir under the conditions described in this work is irreversible process controlled by diffusion. A validated differential pulse and square wave stripping voltammetric procedure was developed and successfully applied to the estimation of entecavir in pharmaceutical formulation. These methods are quick and relatively cheap to operate compared with alternative HPCL methods. They are suitable for routine analysis in quality control laboratories, to be applied for the analysis of entecavir in pure form and in tablet. In these methods, the high percentage of recovery shows that the compound are almost completely extracted from tablet formulation, and the result indicate that the developed method can be used to quantify entecavir without interference from other ingradients.

ACKNOWLEDGMENT.

Author (K. K. Jhankal) thankful to C.S.I.R, New Delhi for providing financial assistance as SRF.

REFERENCES

[1] Rivkin, *Curr. Med. Res. Opin.*2005, 21, 1845-1851.
[2] Rivkin, *Drugs Today*, 2007, 43, 201-210.
[3] Zhao,F. J., Tang, H., Zhang,Q. H., Yang,J.,Davey,A. K., Wang,J. P.,*J. Chromatogr. B*, 2012, 119, 881-882.
[4] Zhang,D., Fu,Y., Gale,J. P., Furby,A. F.,Arnold, M. E., *J. Pharm. Biomed. Anal.*, 2009, 49, 1027-1033.
[5] Challa,B. R., Awen,B. Z.,Chandu, B. R., Rihanaparveen,S., *J. Chromatogr. B,*2011, 879, 769-775.
[6] L. Nováková, T. Gottvald, H. Vlcková, F. Trejtnar, J. Mandíková, P. Solich, *J. Chromatogr. A* 1259 (2012) 237-244.
[7] Wang, J. H., *J. Chromatogr. A*, 2001, 918, 435-441.
[8] Viddesh, R. B., Dhorda, U. J.,Sundaresan, M., *Anal. Chim. Acta*, 1998, 376, 221-227.
[9] Zuman, P., Fijalek Z., *J. Electroanal. Chem.,*1990, 296, 583-587.
[10] Nevin, E., Levent, M., Altun, M., *J. Pharm. Biomed. Anal.*2001, 25, 115-121.
[11] Mishal, A., Sober, D., *J. Pharm. Biomed. Anal.*, 2005, 39, 819-824.
[12] Al-Shaalan, N. H., *Am. J. Appl. Sci.*, 2007, 4, 66-70.
[13] E. Vega, N. Sola, *J. Pharm. Biomed. Anal.*, 2001,25, 523-528.
[14] Galal, S. M., Bedair, M., El-Sayed, M. A., *J. Pharm. Belg.*, 1991, 45, 315-319.
[15] Nagaraja,P., Sunitha,K. R., Vasantha,R. A., Yathirajan,H. S., *J. Pharm. Biomed. Anal.*, 2002, 28, 527-531.
[16] Kumari,M., Sharma,D. K.,*J. Korean Chem. Soc.*, 2011, 55, 50-56.
[17] Mourya,G. L., Jhankal,K. K., Jones,L. A., Bhargava,S. K., Sharma,D. K., *Der Pharm. Lett.,* 2012, 4 , 1599-1606.
[18] Ozkan,S. A., Uslu,B., *Anal. Bioanal. Chem.*,2002, 372, 582-586.
[19] Nicholson,R. S., Shain, *Anal.Chem.*, 1964, 36, 706-709.
[20] A. J. Bard, L. Faulkner, *Electrochemical Methods, Fundamentals and Applications,* John Wiley and Sons, New York, 1980.
[21] Bond, A. M., *ModernPolarographicMethods inAnalytical Chemistry,* MarcelDekker, NewYork, 1980.
[22] Brett,C. M. A., Brett,A. M. O., *Electrochemistry: Principles Methods and Applications,* Oxford University Press, 1993.
[23] Kissinger,P. T., Heineman,W. R., *Laboratory Techniques in Electroanalytical Chemistry,* Marcel Dekker Inc, New York, 1996.
[24] Riley,C. M., Rosanske,T. W., *Development and validation of Analytical Methods,* Elsevier Science Ltd, New York, 1996.
[25] Swartz,M. E., Krull,I. S., *Analytical Method Development and Validation*. Marcel Dekker, New York, 1997.