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A NEW VALIDATED STABILITY-INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF TELBIVUDINE

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ABSTRACT

Telbivudine 5'–triphosphate inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, thymidine 5'–triphosphate. This leads to the chain termination of DNA synthesis, thereby inhibiting viral replication. Incorporation of telbivudine 5'–triphosphate into viral DNA also causes DNA chain termination, resulting in inhibition of HBV replication. Telbivudine inhibits anti compliment or second-strand DNA. A novel, stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Telbivudine in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using develosil C_{18}, 5µm, 150 x 4.6 mm i.d. column with a mobile phase containing a mixture of acetonitrile: phosphate buffer (pH 3.0) (40:60v/v) and conditions optimized were flow rate (1.0 ml/minute), wavelength (273 nm), Run time was 10 min and a peak eluted at 3.52 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 0-40µg/ml. Stress degradation conditions were established for Telbivudine by subjecting it to acid, base, oxidation and thermal stress. The stress samples were assayed against a qualified reference standard and the mass balance was close to 99.35%.The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of in bulk drug and in its pharmaceutical dosage form.

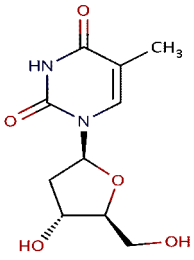
Keywords: Tebivudine, RP-HPLC, ODS, ICH, LOD, LOQ

INTRODUCTION:

TELBIVUDINE[1-6]

Telbivudine (Sebivo) is a synthetic thymidine nucleoside analog with specific activity against the hepatitis B virus. Telbivudine is orally administered, with good tolerance, lack of toxicity and no dose-limiting side effects.

STRUCTURE:



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Nomenclature: 1-[(2S, 4R, 5S)-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-1, 2, 3, 4-tetrahydropyrimidine-2, 4-Dione
Molecular formula: $C_{10}H_{14}N_2O_5$
Molecular weight: 242.2286
Category: .Antiviral drug
Solubility: Sparingly soluble in water (>20 mg/ml) DMSO 48 mg/ml (198 mM);
PKa: 9.96
Mechanism of Action:
Telbivudine 5'–triphosphate inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, thymidine 5'–triphosphate. This leads to the chain termination of DNA synthesis, thereby inhibiting viral replication. Incorporation of telbivudine 5'–triphosphate into viral DNA also causes DNA chain termination, resulting in inhibition of HBV replication. Telbivudine inhibits anticompliment or second-strand DNA.

EXPERIMENTAL METHODS [7-19]

CHARACTERIZATION OF TELBIVUDINE

Physiochemical **characteristics**

Description of **Telbivudine:** slightly yellowish powder

Solubility **of Telbivudine:** The solubility of drug sample was determined according to I.P. 1996.

Two 10 ml and one 250 ml volumetric flasks were taken.

Flask 1: 10 mg of Telbivudine was accurately weighed and transferred to 10 ml volumetric flask. 0.1 ml of water was added into it. The contents were mixed for one minute. The drug was slightly dissolved. Again 0.1 ml of water was added into the volumetric flask. The contents were mixed for one minute. The solubility state was noted.

Flask 2: 10mg of Telbivudine was accurately weighed and transferred to 10 ml volumetric flask. 0.1 ml of methanol was added into it. The contents were mixed for one minute. The drug was slightly dissolved. Again 0.1 ml of methanol was added into the volumetric flask. The contents were mixed for one minute. The solubility state was noted.

Flask 3: Accurately weighed Telbivudine (10 mg) was transferred to 250 ml volumetric flask. Added 10 ml of acetonitrile to it. Mixed the solution for one minute. The drug could not dissolve. Added more 90 ml of acetonitrile to the volumetric flask. Mixed the solution for two minutes. The drug could not dissolve. Added more 100 ml of water to the volumetric flask. Mixed the solution for two minutes. The solubility state was noted.

METHOD DEVELOPMENT AND ITS VALIDATION FOR TELBIVUDINE BY RP-HPLC-

Selection of wavelength

HPLC Instrumentation & Conditions:

The HPLC system employed was **HITACHI L2130** with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400)**.**

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Telbivudine, so that the same wave number can be utilized in HPLC UV detector for estimating the Telbivudine. While scanning the Telbivudine solution we observed the absorption maxima was 273 nm. The UV spectrum has been recorded on ELICO, corp. make UV – VIS spectrophotometer model UV-2450. The scanned UV spectrum is attached in

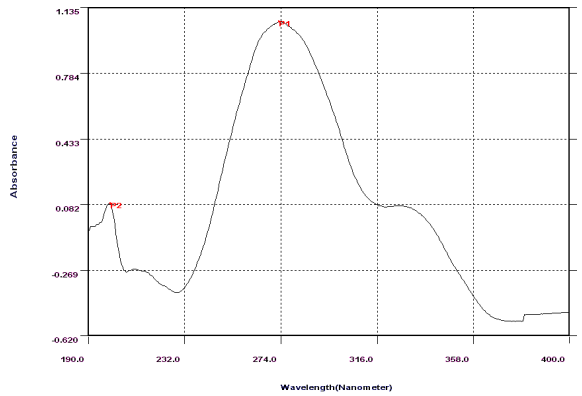

Fig 1: Showing the UV spectrum of Telbivudine

Preparation of standard solution of Telbivudine

25 mg of Telbivudine was weighed accurately and transferred into 25 ml volumetric flask. About 10 ml of mobile phase was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 1000 µg/ml of Telbivudine. From the stock solution again 0.2 ml was taken in a 10 ml volumetric flask & volume was make up to the mark by mobile phase. This solution contains 20 µg/ml of Telbivudine which has been injected to HPLC.

Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

MOBILE PHASE PREPARATION

The mobile phase used in this analysis consists of a mixture of Buffer (0.05 M potassium dihydrogen phosphate & pH adjusted to 3.4 with orthophosphoric acid) and Acetronitrile in a ratio of 60:40. To the 600 ml of this buffer solution was added and properly mixed with 400 ml of acetronitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment

Sample & Standard Preparation for the Analysis

10 mg of Tebivudine standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The sample was analysed by HPLC by using the above method and a very nicely resolved peak has been obtained at a Retention Time of about 3.52 min. The respective chromatogram is attached in the following page.

Optimization of Chromatographic Conditions:

The chromatographic conditions were optimized by different trails. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc. The Optimum conditions obtained from experiments can be summarized as Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm I.D was used for analysis at column temperature 45°C. The mobile phase was pumped through the column at a flow rate of 1.0 mL/ min. The sample injection volume was 20 µL and the sample temperature was maintained at Ambient. The wavelength of UV-273 nm was set for Tebivudine and Chromatographic Gradient programme runtime was 10 minutes.

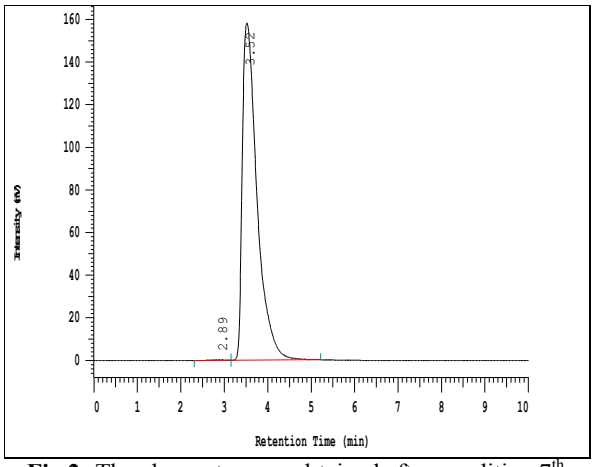

Fig 2: The chromatogram obtained after condition 7th trail, Typical chromatogram of Telbivudine (Rt 3.52)

Here the peaks were separated and shown better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.

METHOD VALIDATION

Linearity and Range

Linearity range was found to be 0-40 µg/ml for Telbivudine. The correlation coefficient was found to be 0.995, the slope was found to be 14357 and intercept was found to be 24166 for Telbivudine.

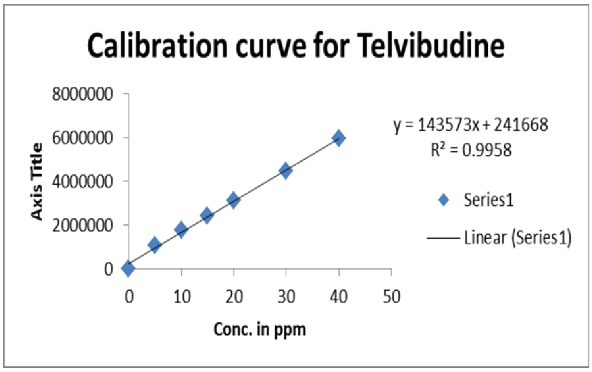

Fig 3: Standard curve for Telbivudine

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
5	1097425
10	1804634
15	2441499
20	3148965
30	4463929
40	5963978

Table 1: Standard curve for Telbivudine

Accuracy

In nine different 10 ml volumetric flasks, 1 ml of the pre-analyzed capsule solution (100 µg/ml) was taken and added 1, 2, 3 ml of standard solution of bulk (API) mixture (100µg/ml) and the volume was made up to 10 ml with mobile phase.

The solutions were then injected into the HPLC system and the peak areas were recorded. The data are shown in Table 02.

Table 2: Data of recovery studies

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
	Pure drug	Formulation	Pure drug	
S_1 : 80 %	16	20	99.63	Mean= 99.67667%
S_2 : 80 %	16	20	99.92	S.D. = 0.223681
S_3 : 80 %	16	20	99.48	% R.S.D.= 0.224407
S_4 : 100 %	20	20	99.19	Mean= 99.19%
S_5 : 100 %	20	20	99.25	S.D. = 0.06
S_6 : 100 %	20	20	99.13	% R.S.D.= 0.06049
S_7 : 120 %	24	20	99.25	Mean= 99.49%
S_8 : 120 %	24	20	99.54	S.D. = 0.219317
S_9 : 120 %	24	20	99.68	% R.S.D. = 0.220441

The mean recovery was found to be 99.882% for Telbivudine. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

PRECISION- Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Telbivudine. The percent relative standard deviations were calculated for Telbivudine are presented in the table-3.

HPLC Injection Replicates of Telbivudine	Retention Time	Area
Replicate – 1	3.52	3147654
Replicate – 2	3.53	3125482
Replicate – 3	3.53	3125860
Replicate – 4	3.53	3146975
Replicate – 5	3.53	3135862
Average	3.528	3136367
Standard Deviation	0.004472	10828.91
% RSD	0.126761	0.345269

Table 3: Data showing repeatability analysis

INTERMEDIATE PRECISION

For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and RSD. The data are shown in **Table 4**

Conc. of Telbivudine (API) (µg/ml)	Observed Conc. Of Telbivudine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.01	1.05	10.06	0.24
20	20.003	0.55	20.084	0.41
30	29.84	0.18	29.95	0.18

Table 4: Data for Telbivudine analysis

Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The LOD was found to be 0.452 µg/ml and LOQ was found to be 1.356 µg/ml for Telbivudine which represents that sensitivity of the method is high.

SYSTEM SUITABILITY PARAMETER

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 5.

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	Asymmetry	T ≤ 2	Telbivudine=0.148
3	Theoretical plate	N > 2000	Telbivudine=6246

Table 5: Data of System Suitability Parameter

FORCED DEGRADATION STUDIES:

Following protocol was strictly adhered to for forced degradation of Telbivudine Active Pharmaceutical Ingredient (API). The API (Telbivudine) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after a long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation

ACID HYDROLYSIS:

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCL (after all optimized conditions)

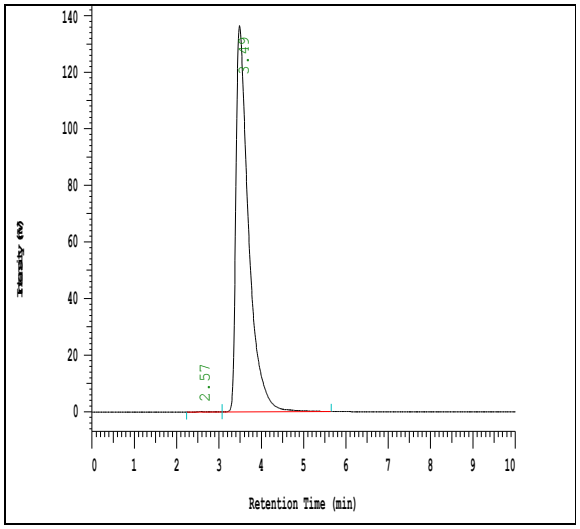

Fig 4: Chromatogram showing degradation for Telbivudine in 0.1 N HCL

Peak results

Sl. No	RT	PEAK AREA	PEAK CONCENTRATION
1	3.49	3102589	99.887

BASIC HYDROLYSIS

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of . NaOH (after all optimized conditions)

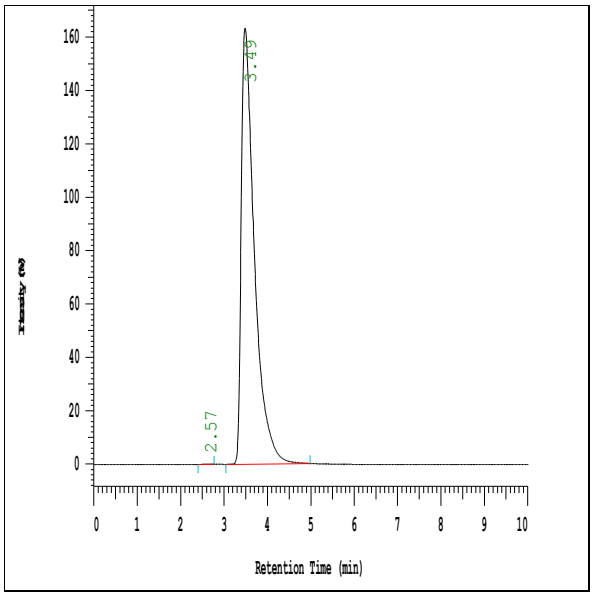

Fig 5: Chromatogram showing degradation related impurity in 0.1 N NaOH

Peak results

Sl. no	Name	RT	Area	PEAK CONCENTRATION
1	Telbivudine	3.49	2917874	99.887

THERMAL DEGRADATION

An accurately weighed 1 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 ^{0}C for 24 hrs. Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions)

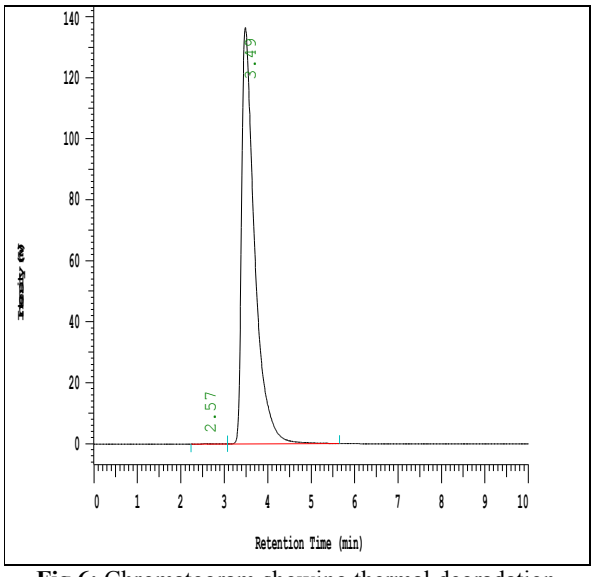

Fig 6: Chromatogram showing thermal degradation studies

Peak results

Sl. no	Name	RT	Area
1	Telbivudine	3.49	3129815

Oxidation with (3%) H_2O_2:

Accurately weighed 1 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H2O2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 ppm solution. The above sample was injected into the HPLC system.

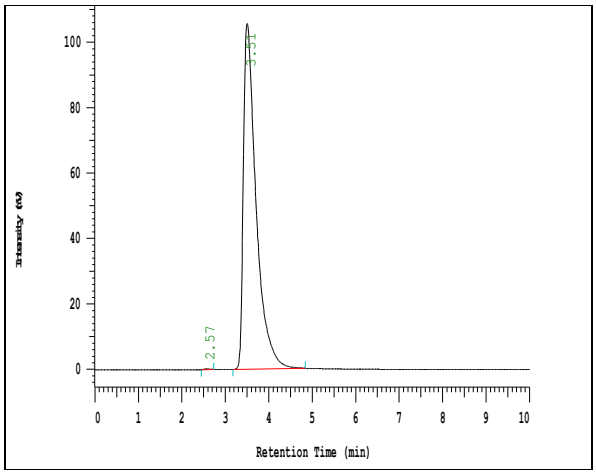

Fig 7: Chromatogram showing oxidative degradation.

Peak results

Sl. No	Name	RT	Area
1	Telbivudine	3.51	3158422

Results of degradation studies:

The results of the stress studies indicated the specificity of the method that has been developed. Telbivudine was stable in photolytic & temperature stress conditions. The result of forced degradation studies are given in the following table.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	95.49	5.05	100.54
Basic Hydrolysis (0.I M NaOH)	24Hrs.	90.03	10.37	100.40
Thermal Degradation (50 ^{0}C)	24Hrs.	99.35	----------	99.35
UV (254nm)	24Hrs.	98.31	---------	99.31
3 % Hydrogen peroxide	24Hrs.	91.37	08.46	99.83

Table 7: Results of force degradation studies of Telbivudine API.

RESULT and DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Telbivudine, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetronitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be soluble in Acetronitrile & dichloromethane and partially soluble in methanol. Drug was insoluble in water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Telbivudine it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 ul were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Telbivudine in different formulations

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Telbivudine API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Telbivudine in API.

REERENCES

1. http://www.nlm.nih.gov/medlineplus/druginfo/meds/a605028.html
2. http://en.wikipedia.org/wiki/Telbuvidine
3. http://www.rxlist.com/sebivo-drug.htm
4. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202022Orig1s000TOC.cfm
5. http://www.sebivo.com/index.aspx
6. http://www.drugbank.ca/drugs/DB00338#pharmacology
7. Scholars Research Library Der Pharmacia Lettre, 2012, 4 (1):76-86 ISSN 0974-248X USA CODEN: DPLEB4
8. Journal of Pharmaceutical and Biomedical Analysis Volume 21, Issue 2, 1 November 1999, Pages 371–382
9. Tropical Journal of Pharmaceutical Research, October 2009; 8 (5): 449-454 © Pharmacotherapy Group
10. Instrumental Method of Analysis by Rabi Sankar, P-18-6, P-18-3
11. Practical HPLC Method Development by Lloyd R. Snyder *et al*; 2nd edition, P-503
12. Guidance for industry, Analytical Procedure and Method Validation, U.S. Department of Health and Human Services FDA, August 2000 (www.fda.gov/guidance/index.htm)
13. The United State Pharmacopeia 25/National Formulary 20, ch. 1225, pg. 2256-2259 (The United State Pharmacopeia Convention, Inc., Rockville, Maryland, 2002)
14. ICH Q2B: Validation of Analytical Procedure; Methodology (International Conferences on Harmonization of Technical requirements for the registration of Drugs for Human use, Geneva, Switzerland, May 1997)
15. ICH Q2B: Validation of Analytical Procedure; Methodology (International Conferences on Harmonization of Technical requirements for the registration of Drugs for Human use, Geneva, Switzerland, Nov 2003)
16. Journal of Chromatography .B, Analytical Technologies in the Biomedical and life Sciences. 2008 March 1; 863(2): 258-265. published on 2008 Jan 18
17. Kassen Mussen nicht fur "Acomplia" zahlen. Tagesschau.de (2006-10-17). Retrieved on 2007-06-13.
18. Matheson A.J., Noble S., Drugs, Volume 59, Number 4, April 2000, pp. 829-835(7).
19. Dawei Zhou, Xuntian Jiang, and Xinping Fang, A Rapid LC-MS/MS Method for the Determination of Telbivudine in Human Plasma, XenoBiotic Laboratories, Inc., 107 Morgan Lane, Plainsboro, NJ 08536

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