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Research

Rp-Hplc Method Development And Validation Of Telbivudine In Bulk And Marketed Pharmaceutical Dosage Form

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Abstract

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method forthe estimation of Telbivudine in bulk form and marketed pharmaceutical dosage form. Separation of Telbivudine was successfully achieved on a Symmetry ODS C18 (4.6 x 250mm, 5µm particle size) column in an isocratic mode of separation utilizing Methanol: Phosphate Buffer (35:65% v/v) (pH-3.2 adjusted with orthophosphoric acid) at a flow rate of 1.0mL/min and the detection was carried out at 278nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 30-70mcg/mL for Telbivudine.The correlation coefficient was found to be 0.999 for Telbivudine. The LOD and LOQ for Telbivudine were found to be 0.9µg/mL and 2.7µg/mL respectively. The proposed method was found to be good percentage recovery for Telbivudine, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: Telbivudine, RP-HPLC, Accuracy, Precision, ICH Guidelines.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery,development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and food drug administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite

similar instrumentation .It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (high performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods. The developed chromatographic methods further validated as per ICH or USFDA guidelines for all the critical parameters. To access the precision and to evaluate the results of analysis the analyst must use statistical methods. These methods include confidence limit, regression analysis to establish calibration curves. In each analysis the critical response parameters must be optimized and recognized if possible.

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches like chemistry, physics and microbiology etc. Pharmaceutical analytical techniques are applied mainly in two areas, quantitative analysis and qualitative analysis, although there are several other applications.Drugs and pharmaceuticals are chemicals or like substances, which or of organic inorganic or other origin. Whatever may be the origin, we some property of the medicinal agent to measure them quantitatively or qualitatively. In recent years, several analytical techniques have been evolved thatcombine two or more methods into one called “hyphenated” technique e.g. GC/MS, LC/MS etc. The complete analysis of a substance consists of four main steps.

The concept of analytical chemistry lies in the simple, precise and accurate measurements. These determinations require highly sophisticated instruments and methods like mass spectroscopy, gas chromatography, high performance thin layer chromatography, high performance liquid chromatography etc. The HPLC method is sensitive, accurate, precise and desirable for routine estimation of drugs in formulations.

Thereby it is advantageous than volumetric methods. Many HPLC methods has been developed and validated for the quantitative determination of various marketed drugs. Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure theidentity, purity, potency and performance of drug products majority of analytical developmenteffort goes into validating a stability indicating method. So it is a quantitative analytical methodbased on the structure and chemical properties of each active ingredient of the drug formulation. Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.

On the literature survey, it was found that most of the analytical methodavailable for the above mentioned drug is applicable for quantification in plasma samples, the most widely used method being liquid chromatography-mass chromatography. So it is felt that there is a need to develop accurate, precise analytical methods for the estimation of the drug in solid dosage formulation.

Newer analytical methods are developed for these drugs or drug combinations of the below reasons

- There may not be suitable method for a particular analyte in the specific matrix.
- Existing method may be too error prone or unreliable (have poor accuracy and precision).
- Existing method may be expensive, time consuming, energy intensive and may not be provide sensitive or analyte selectivity, and not easy for automation.
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods.
- There may be need for an alternate method to confirm, for legal and scientific reasons.

The newly developed analytical methods having their importance in different fields that include, research and development centre (R&D), quality control department (QC), approved testing laboratories, chemical analysis laboratories etc. For analysis of these drugs different analytical methods are routinely being used.

Chromatography

Techniques related to chromatography have been used for centuries to separate materials such as dyes extracted from plants. Russian botanist Tswett is credited with the discovery of chromatography. In 1903 he succeeded in separating leaf pigments using a solid polar stationary phase, It was not until 1930s that this technique was followed by Kuhn and Lederer as well as Reichstein and van Euw for the separation of natural products. Martin and Synge were awarded the Nobile prize for their work in 1941 in which they described liquid-liquid chromatography. Martin and Synge applied the concept of theoretical plates as a measure of chromatographic efficiency. The term “chromatography” (Color-writing derived from the Greek for Color-chroma and Write-Graphing).

Chromatography in the pharmaceutical world

In the modern pharmaceutical industry, chromatography is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The development of new chemical entities (NCEs) is comprised of two major activities drugdiscovery anddevelopment. The goal of the drug discovered is to investigate a plethora of compounds employing fast screening approaches, leading to generation of lead compounds and then narrowing the selection through targeted synthesis and selective screening (lead optimization). The main functions of drug development are to completely characterize candidate compounds by performing drug metabolism, preclinical and clinical screening, and clinical trials. Throughout this drug discovery and development paradigm, rugged analytical HPLC separation methods are developed, at each phase of development to analyses of a myriad of samples are performed to adequately control and monitor the quality of the prospective drug candidates, excipients and final products. Effective and fast method development is of paramount importance throughout this drug development life cycle. This requires a thorough understanding of HPLC principles and theory which have solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization.

High performance liquid chromatography

Brief Historical prospective of chromatography

The historical development of liquid chromatography has been extensively reviewed and can be traced as far back as they early 1900, where the Russian botanist Zwett used a variant of liquid chromatography to separate some colored plant substances. The focus was on modern development in HPLC, a term that was coined in late 1960s with the advent of more sophisticated instrumentation, better engineered separation columns, and reliable and highly efficient stationary phases and packaging materials. These technological advances have been, In part, fuelled, by the need to separate an increasinglylarge variety of differing compounds classes encountered as API s, e.g. Antibiotic, sulphonamides nucleosides, fat soluble vitamins neutral and non polar compounds. Additional challenges include developing faster and more consistent HPLC methods requiring higher flow rates, while maintaining peak shape, peak symmetry and efficiencies. Another important analytical challenge is the desire to detect and accurately quantify low levels of impurities at level present in API materials. High-pressure liquid chromatography quickly improved with the development of column packing materials. Additional convenience of on-line detectors became rapidly a powerful separation technique and is today called as high-performance liquid chromatography (HPLC)

- One of the early problems with liquid state chromatography was the slow rate at which analysis took place. Early methods use gravity feed, and it was not uncommon diffusion and soon.
- This problem was largely overcome by the advent high-performance liquid chromatography (HPLC). In this system the pressure is applied to the column forcing the mobile phase through at much higher rate.
- For an analysis to take several days to complete. This led not only to great delay but also the excessive time on the column and thus inevitably led to loss of resolution.

HPLC

Inhigh performance liquid chromatography, mobile as well as the stationary phase compete for the distribution of the sample components. In case of HPLC, separation is based on adsorption and partition.Adsorption chromatography employs high-surface area particles that adsorb the solute molecules. Usually a polar solid such as silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptanes, octane or chloroform are used in adsorption chromatography. In partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can either polar or non-polar. If the stationary phase is non-polar, it is called normal phase partition chromatography. If the opposite case holds, it is called reversed-phase partition chromatography. In normal phase mode, the polar molecule partition preferentially in to the stationary phase and are retained longer than non-polar compounds. In reverse phase partition chromatography, the opposite behavior is observed.

Types of HPLC techniques

Based on modes of chromatography:

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation:

- ❖ Adsorption chromatography
- ❖ Ion exchange chromatography
- ❖ Size exclusion chromatography

- ❖ Affinity chromatography

Based on elution technique:

- o Isocratic separation
- o Gradient separation

Based on the scale of operation:

- Analytical HPLC
- Preparative HPLC

Ion exchange chromatography: Due to differences in the affinity of ions for the in exchange.
Size exclusion chromatography: Due to differences in molecular weight and size of the molecules to be separated.
Affinity chromatography: Separation is based on a chemical interaction specific tothe target species. The more popular revered phase mode uses a buffer and added counter-ion of opposite charge to the sample with separation being influencedby pH, ionic strength, temperature, concentration and type of organic co-solvents.
Chiral chromatography: Separation of the enantiomers can be achieved on chiral stationary phases by the formation of diastereomers.
Analytical HPLC: Only analysis of the samples is done. Recovery of the samples for reusing is normally not done.

Most commonly used methods in HPLC
Normal phase chromatography

For a polar stationary bed like silica we need to choose a relatively non-polar Mobile phase. This mode of operation is termed as normal phase chromatography. Here the least polar component elutes first, and increasing the mobile phase polarity leads to decrease in elution time. Non-polar solvents like pentane, Hexane, isooctane, cyclohexane etc. are more popular. It is mainly used for separation of nonionic, non-polar to medium polar substances.

Reverse phase chromatography

In 1960s, chromatographers started modifying the polar nature of the silanol group by chemically reacting silicon with organic silanes. The object was to make silica less polar or non-polar so that polar solvents can be used to separate water-soluble polar compounds. Since the ionic nature of the reverted, the chromatographic separation carried out with such silica is preferred to as reverse- phase chromatography. Here the most post components elutes first. Increasing mobile phase polarity leads to decrease in elution time. Common solvents used in this mode include methanol /acetonitrile /isopropanal etc. Mostly used for separation of ionic and polar substances. The parameters that govern the retention in reversed phase system are the following:

a. The chemical nature of the stationary phase surface.
b. The type of solvents that compose the mobile phase.
c. pH and ionic strength of the mobile phase.

Isocratic elution: A separation in which the mobile phase composition remains constant throughout the procedure is termed as a isocratic (meaning constant composition).
Gradient elution: The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution.

Instrumentation of HPLC
The mobile phase components HPLC instrument and their working functions are described below.

- Mobile phase and reservoir
- Solvent degassing system
- Pump
- Injector
- Colum
- Detector
- Data system

MATERIALS AND METHODS

Telbivudine (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Telbivudine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.5ml of the above Telbivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer (35:65% v/v) (pH-3.2)respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, Zodiac and Xterra. Symmetry ODS C18 (4.6 x 250mm, 5µm particle size)Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and PDA996 detector model.
Temperature	:	Ambient
Column	:	Symmetry ODS C18 (4.6 x 250mm, 5µm particle size)
Mobile phase	:	Methanol: Phosphate Buffer (35:65% v/v) (pH-3.2)
Flow rate	:	1.0mL/min
Wavelength	:	278 nm
Injection volume	:	20 µl
Run time	:	8 minutes

Method validation

Preparation of buffer and mobile phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.2)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH-3.2 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Column	:	Symmetry ODS C18 (4.6 x 250mm, 5µm particle size)
Column temperature	:	Ambient
Wavelength	:	278 nm
Mobile phase ratio	:	Methanol: Phosphate Buffer (35:65% v/v) (pH-3.2)
Flow rate	:	1.0mL/min
Injection volume	:	20 µl
Run time	:	8 minutes

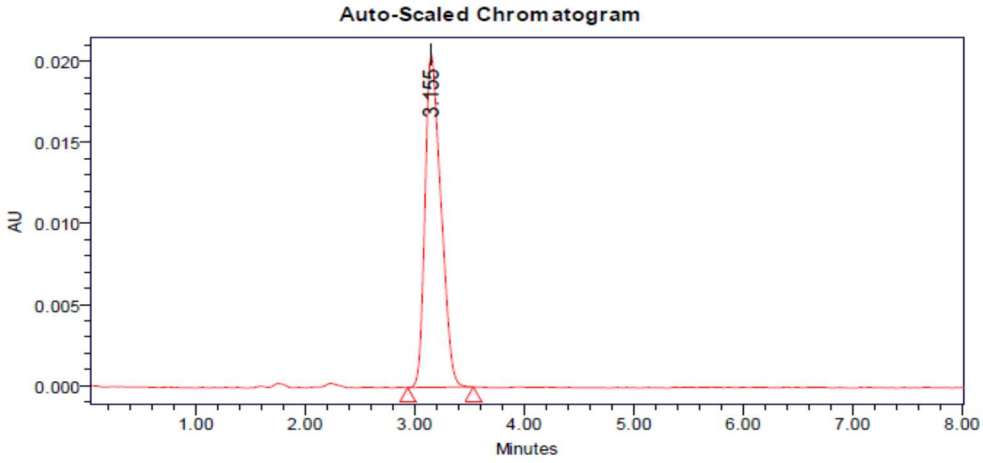

Fig 1: Optimized Chromatogram (Standard)

Table 1: Peak results for Optimized Chromatogram

S.No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Telbivudine	3.155	365985	26532	1.46	6452

This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

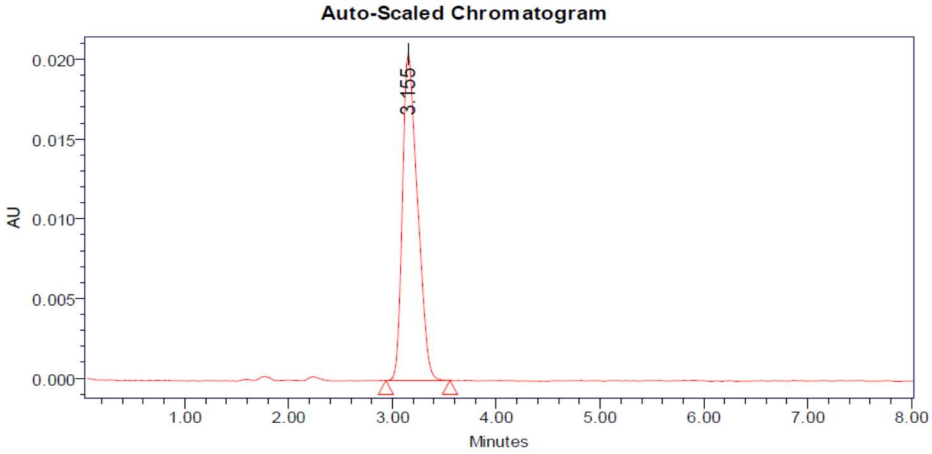

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Telbivudine	3.155	366586	26985	1.49	6546

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Results of Assay (Standard)for Telbivudine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telbivudine	3.146	365987	26658	6524	1.46
2	Telbivudine	3.123	365874	26584	6465	1.45
3	Telbivudine	3.192	365897	26536	6542	1.46
4	Telbivudine	3.164	365876	26535	6547	1.46
5	Telbivudine	3.181	365847	26498	6542	1.45
Mean			365896.2			
Std.Dev.			53.77453			
%RSD			0.014697			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Sample)

Table 4: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Telbivudine	3.170	365985	26587	1.46	6453	1
2	Telbivudine	3.174	366898	26659	1.45	6532	2
3	Telbivudine	3.170	365487	26548	1.46	6485	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Telbivudine in pharmaceutical dosage form was found to be 99.79%.

Linearity
Chromatographic data for linearity study

Table 5: Data for Linearity

Concentration µg/ml	Average Peak Area
30	219897
40	292568
50	361986
60	431487
70	499859

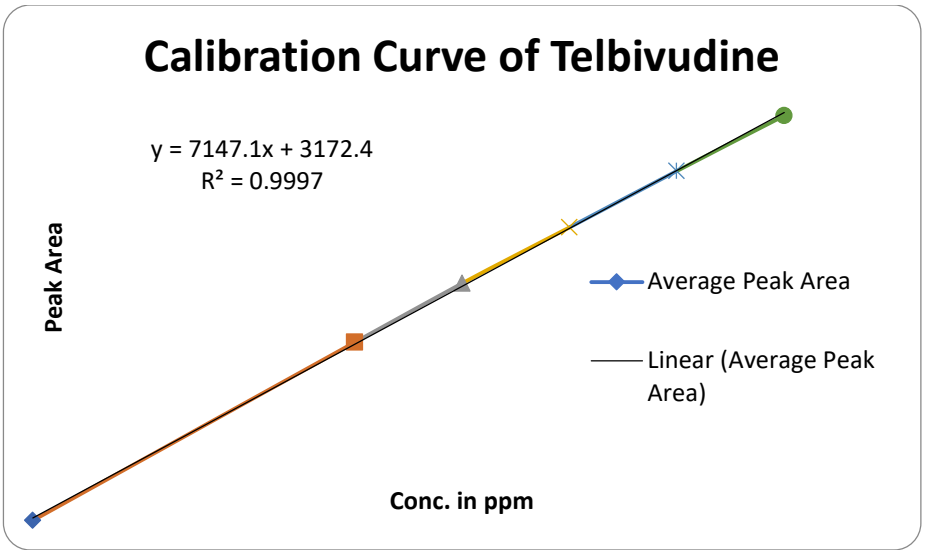

Fig 3: Calibration Curve of Telbivudine

Repeatability

Table 6: Results of method precision for Telbivudine:

S. No.	Peak name	Retention time	Area(µV* sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telbivudine	3.165	366598	26589	6532	1.45
2	Telbivudine	3.163	365895	26598	6652	1.46
3	Telbivudine	3.158	366587	26658	6589	1.45
4	Telbivudine	3.167	366589	26547	6541	1.46
5	Telbivudine	3.171	365879	26659	6589	1.46
Mean			366309.6			
Std.dev			385.843			
%RSD			0.105332			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day 1

Table 7: Results of ruggedness for Telbivudine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Telbivudine	3.165	366985	26785	6587	1.48
2	Telbivudine	3.163	365895	26587	6598	1.47
3	Telbivudine	30158	366584	26658	6584	1.49
4	Telbivudine	3.167	365985	26587	6498	1.46
5	Telbivudine	3.171	366857	26659	6536	1.46
6	Telbivudine	3.171	365984	26857	6698	1.48
Mean			366381.7			
Std.Dev.			486.4605			
%RSD			0.132774			

- *%RSD of Six different sample solutions should not more than 2.*

Table 8: Results of Intermediate precision Analyst 2 for Telbivudine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Telbivudine	3.173	367854	27854	6523	1.48
2	Telbivudine	3.134	368547	27547	6458	1.47
3	Telbivudine	3.161	367895	27865	6598	1.49
4	Telbivudine	3.174	368542	27854	6521	1.48
5	Telbivudine	3.199	368954	27698	6354	1.49
6	Telbivudine	3.199	367856	27865	6458	1.48
Mean			368274.7			
Std.Dev.			469.7904			
%RSD			0.127565			

- *%RSD of Six different sample solutions should not more than 2.*

Accuracy

Table 9: The accuracy results for Telbivudine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	183019.7	25	25.164	100.656	100.467%
100%	361946	50	50.199	100.398	
150%	541075	75	75.262	100.349	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 10: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	365985	3.155	6452	1.46
Less Flow rate of 0.9 mL/min	378547	3.488	6526	1.44
More Flow rate of 1.1 mL/min	356242	2.877	6452	1.45
Less organic phase	345625	4.705	6359	1.45
More organic phase	356289	2.090	6542	1.42

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present research, a fast, simple, accurate, precise, and linear HPLC method has been developed and validated for Telbivudine, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Telbivudine. In addition, the main features of the developed method are short run time and retention time around 8 min. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions. Hence the proposed RP-HPLC method proved to be simple, accurate and reproducible for the determination of Telbivudine in a reasonable run time. The method was validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used by quality control laboratories.

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