HPTLC METHODSFOR SIMULTANEOUS ESTIMATION OF RILPIVIRIN

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Abstract : A chromatographic method based on the HPTLC with low standard deviation and % RSD with high degree of accuracy and precision, for the estimation of Rilpivirin has been developed. Recovery studies of the Rilpivirin drug have been performed on samples with an extra 80, 100, and 120% of the drugs from standard solutions of Rilpivirin. Acid, base and hydrogen peroxide-induced degradation studies have been carried out on Rilpivirin to ensure the stability of the proposed chromatographic method. It has been demonstrated that the proposed method is accurate, precise and selective and can be employed successfully for the estimation of Rilpivirin.

Keywords: Rilpivirin, HPTLC andDensitogram.

I. INTRODUCTION

Chromatography is a laboratory method for analysis of chemicals to determine purity, impurities, similarity, identification etc. Chromatography today is performed with the help of different instruments, materials, techniques, as would be required to analyse hundreds of thousands of chemicals. The prominent chromatography methods are High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Gas Chromatography (GC) etc.

High Performance Thin Layer Chromatography (HPTLC) is a powerful method equally suitable for qualitative and quantitative analytical tasks. Applications of HPTLC, such as identification and quantitation of constituents, impurities, active substances, process development and optimization, process monitoring, and cleaning validation have been demonstrated. HPTLC has been reported to provide excellent separation, qualitative and quantitative analysis of a wide range of compounds, such as herbal and botanical dietary supplements, nutraceuticals, traditional western medicines, traditional Chinese medicines and Ayurvedic (Indian) medicines and determination of radiolabeled substances in chemical, biochemical, biological, pharmaceutical, and medicinal samples. HPTLC is superior to other analytical techniques in terms of total cost and time for analysis. It is an offline process in which various stages are carried out independently. Important features of HPTLC include the ability to analyze crude samples containing multi-components, application of large number of sample and a series of standards using the spray-on technique, choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step, processing of standards and samples identically on the same plate leading to better accuracy and precision of quantification, different and universal selective detection methods, and in situ spectra recording in sequence to obtain positive identification of fractions, storage of total sample on layer without time constrains[1-3].

HPTLC-Methodology:

Set the analytical objective first that may be quantification or qualitative identification or separation of two components/multi-component mixtures or optimization of analysis time before starting HPTLC. Method for analyzing drugs in multi-component dosage forms by HPTLC demands primary knowledge about the nature of the sample, namely, structure, polarity, volatility, stability, and the solubility parameter. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, with what kind of mobile phase. Selection of stationary phase is quite easy, that is, to start with silica gel which is reasonable and nearly suits all kind of drugs. Mobile phase optimization is carried out by using three level techniques. First level involves use of neat solvents and then by finding some such solvents which can have average separation power for the desired drugs. Second level involves decreasing or increasing solvent strength using hexane or water for respective purposes. Third level involves trying of mixtures instead of neat solvents from the selected solvents of first and second level which can further be optimized by the use of modifier like acids or bases.

Analytes are detected using fluorescence mode or absorbance mode. But, if the analytes are not detected perfectly than it needs change of stationary phase or mobile phase or need the help of pre or post chromatographic derivatization. Optimization can be started only after a reasonable chromatogram which can be done by slight change in mobile-phase composition. This leads to a reasonable chromatogram which has all the desired peaks in symmetry and well separated. Procedure for HPTLC method development is outlined as follow (Figure 1).[3]

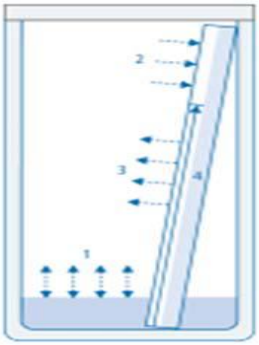


Figure.1. process in developing chamber

Stationary Phase:

HPTLC is the most advanced form of modern TLC. It uses HPTLC plates featuring small particles with a narrow size distribution which results in homogenous layers with a smooth surface to be obtained. HPTLC uses smaller plates (10 × 10 or 10 × 20 cm). HPTLC plates provide improved resolution, higher detection sensitivity, and improved in situ quantification and are used for industrial pharmaceutical densitometric quantitative analysis. Normal phase adsorption TLC on silica gel with a less polar mobile phase, such as chloroform– methanol, has been used for more than 90% of reported analysis of pharmaceuticals and drugs [4-11].

Mobile Phase:

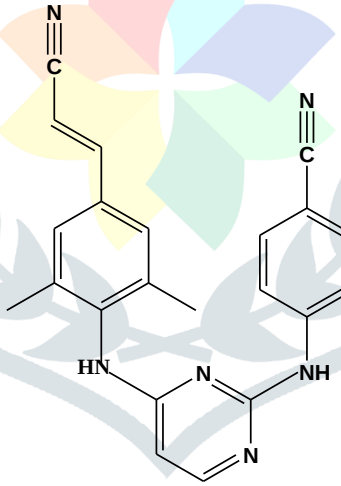
The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte. The mobile-phase systems are used based on their diverse selectivity properties are diethyl ether, methylene chloride, and chloroform combined individually or together with hexane as the strength adjusting solvent for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed-phase TLC. Separations by ion pairing on C-18 layers are done with a mobile phase such as methanol–0.1 M acetate buffer (pH 3.5) containing 25 mM sodium pentanesulfonate (15.5:4.5) [13-21].

Preparation of plate:

TLC plates can be made with suitable apparatus. Such layers do not adhere well to the glass support. Pre-coated plates use small quantities of very high molecular weight polymer as binder overcomes most limitations of a homemade layer.Precoated layers are reasonably abrasion resistant, very uniform in layer thickness, reproducible, pre-activated, and ready to use. They are available with glass or aluminum or polyester support. Aluminum foil plates are less expensive to buy, cheaper, can be cut, and therefore easy to carry around or transport or mail. Glass plates are the best for highest quality of results. Most often, layers containing a fluorescent indicator F 254 are used. This enables the visualization of samples in a UV cabinet very simply, instantly, and in a nondestructive manner.Commonly used size of plates in TLC is 20×20 cm and in HPTLC 20 × 10 cm or 10 × 10 cm is widespread.

Simultaneous estimation of Rilpivirin, Emtricitabine and Tenofovir

Rilpivirinischemically4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl]-2,6dimethylphenyl}amino)pyrimidin-2-yl]amino}benzonitrile. Rilpivirin is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infected patients. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance of Rilpivirin is less likely to develop than other NNRTI's Literature review revealed that UV[21-25],HPLC[26-34]and HPTLC[29-33]methods have been reported for analysis of Rilpivirin as a single form and in combination with other drugs. To date there have been no published reports on simultaneous quantitation of Rilpivirin by HPTLC in bulk drug and in tablet dosage form. This present study reports for the first time the simultaneous quantitation of Rilpivirin by HPTLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH Guidelines [39].



Structure of Rilpivirin

II. MATERIAL AND METHODS

MATERIAL

Pure drugs Rilpivirin were obtained as gift sample from EmcurePune,India. Methanol and Ethyl acetate was obtained from Qualigens Fine Chemicals Ltd. All chemical used were of analytical grade. HPTLC aluminum plates pre-coated with silica gel 60F254 (10 cm X 10 cm) were from Merck. Densitometry was carried out using Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) fitted with win-CATS software version 1.4.3.6336. Samples were applied as band on the HPTLC plates using the spray-on technique of CamagLinomat V under nitrogen gas flow, and developed in a Camag 10 cm X 10 cm twin trough chamber.

METHOD

Method development:

Standard stock solutions 20 µg/ml of Rilpivirinwas prepared in methanol as solvent. Solutions of 2 µl were applied on the HPTLC plates as spot bands of 6 mm using Linomat V. Application positions were at least 15 mm from the sides and 10 mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was saturated with mobile phase vapours for 20 min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 8 cm. Then the plates were dried on a hot plate. Room temperature and relative humidity were always maintained at

25°C ± 2 and 60 % ± 5. Densitometric scanning was done in absorbance mode at 272 for Rilpivirinusing a deuterium lamp. The slit dimensions were 5 mm X 0.45 mm and the scanning speed was 20 mm/s and the data resolution at 100µm/step.

METHOD VALIDATION

Linearity and range: From the mixed standard stock solution 20µg/ml of Rilpivirin, 2µl to 7µl solution was spotted on HPTLC plate to obtain finalconcentration 40-140µg/spot for Rilpivirin.Each concentration was applied six times to the HPTLC plate. The plate was then developed as per procedure described above.

Precision: The intra-day precision (RSD, %) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter- day precision (RSD %) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Limits of Detection and Quantitation: To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × *N*/*B* and LOQ = 10 × *N*/*B*, where *N* is the standard deviation of the peak areas of the drugs (*n* = 3), taken as a measure of noise, and *B* is the slope of the corresponding calibration plot.

Specificity: The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved the drugs very efficiently, as shown in Figure 2.

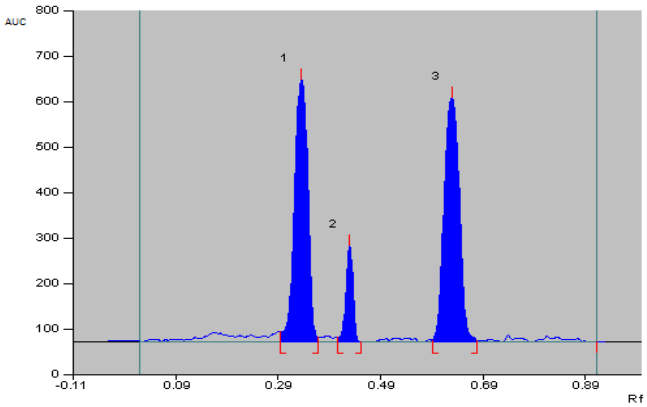


Figure 2: Densitogram of Rilpivirin

Accuracy: Analysed samples were overapplied with an extra 80, 100, and 120% of the drugs from standard solutions ofRilpivirin the mixtures were reanalyzed by use of the method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

Robustness: Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

Analysis of a marketed formulation

To determine the content of Rilpivirin in conventional tablet compressed on tablet mini(Rimek) press in institute with reference to (Brand name: Complera, Label claim: 25 mg of Rilpivirin per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 5 mg of Rilpivirin were transferred into a 25 mL volumetric flask containing 15-20 mL methanol, sonicated for 30 min with occasional shaking and diluted to 25 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (200µg/mL for Rilpivirin).Pipette out 1ml of supernatant solution and dilute to 10ml with methanol. Then 3 µL of the spot was applied which gave final concentration of 100 ng/spot for Rilpivirin, 800 ng/spot. The HPTLC plate was then developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

Forced Degradation Studies: To ensure that the analytical method was stability indicating, stress studies were performed.

a) Acid Degradation Studies: 2ml of 0.1N hydrochloric acid was added to 5mg of Rilpivirin. This solution was allowed to stand for 3hour at 100°C after that make dilutions to obtain 20µg/ml solution of Rilpivirin.

b) Alkali Degradation Studies:2ml of 0.1N Sodium Hydroxide was added to 5mg of Rilpivirin. This solution was allowed to stand for 3hour at 100°C after that make dilutions to obtain 20µg/ml solution of Rilpivirin.

c) Oxidation studies: 2ml of 3% Hydrogen Peroxide was added to 5mg of Rilpivirin. This solution was allowed to stand for 3hour at 100°C after that make dilutions to obtain 20µg/ml solution of Rilpivirin.

Method Development:

The HPTLC procedure was optimized for simultaneous determination of Rilpivirin. The mobile phase Methanol: Toluene: Ethyl acetate: Ammonia (1.5:5.5:1.5:0.1 v/v/v/v) resulted in good resolution, and sharp and symmetrical peaks were obtained at Rf 0.59 ± 0.02 for Rilpivirin. It was observed that prewashing of HPTLC plates with methanol (followed by drying and activation) and pre saturation of HPTLC chamber with mobile phase for 20 min (optimum chamber saturation time) ensured good reproducibility and peak shape of three drugs.

VALIDATION OF THE METHOD

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response andconcentration over the ranges 40-140 µgper spot for Rilpivirin. Eachconcentration was applied in triplicate on the HPTLC plate (Table I).

Table I: Linear regression data for drugs

Parameter	Rilpivirin
Linearity range	40-140µg/spot
correlation coefficient (r^2)	0.998
Slope	38.60
Intercept	102.5

LOD and LOQ

The LOD & LOQ were determined from slope of the lowest part of the calibration plot.LOD and LOQ of respected drug shown in table (II)

Table II: LOD &LOQ for drugs

Parameter	Rilpivirin
LOD	3.67
LOQ	11.14

Precision: The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table (III)reveal the high precision of the method.

Table III: Statistical evaluation of precision of developed method (n=3)

Drug	Conc. (µg/band)	Intra day			Inter day		
		*%mean	*SD	*%RSD	*%mean	*SD	*%RSD
Rilpivirin	100	98.86	0.63	0.63	99.16	1.13	1.14

*Mean of three determinations, SD: Standard Deviation, R.S.D: Relative Standard Deviation

Recovery Studies: When the method was used for extraction and subsequent analysis of three drugs from the pharmaceutical dosage forms, and the extract was over applied with 80, 100, and 120% of additional drug. As shown in the Table (IV) good recoveries of the Rilpivirin in the range from 98.00 to 102.00 % were obtained at various added concentrations. The average recoveries of three levels (nine determinations) were 99.10± 0.50 % for Rilpivirin.

Table IV: Recovery study Data

Drug	Level of % recovery	*%mean	*SD	*%RSD
Rilpivirin	80%	98.25	0.11	0.11
	100%	99.53	0.59	0.59
	120%	99.52	1.14	1.14

*Mean of three determinations, SD: Standard Deviation, R.S.D: Relative Standard Deviation

Robustness: The standard deviations of peak areas were calculated for the aaforementioned four parameters (variation in composition of the mobile phase, amount of mobile phase, Time from spotting to chromatography, Time from chromatography to scanning) and coefficients of variation were found to be less than 2% in all cases as shown in Table (V).

Table V: Results of Robustness

Parameters	%RSD for Rilpivirin*
Mobile phase composition (0.1 ml)	98.68
Amount of mobile phase	98.97
Time from spotting to chromatography	98.67
Time from chromatography to scanning	98.96

*Mean of three determinations, R.S.D: Relative Standard Deviation

Forced Degradation Studies

HPTLC studies of the samples obtained during the stress testing of Rilpivirinunder different conditions. Different degradations peak as shown in figures 3-5.The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error. The amount of drug recovered after degradation studies and the Rf of the degradation products are given in table (VI).

a) Acid induced degradation

The drugs were degraded in the acidic condition and shows different degradation products at Rf 0.01 for Rilpivirin and 0.15, 0.24 for Emtricitabine and 0.14, 0.29, 0.79 for Tenofovir as shows in the figures 3-5.

b) Base induced degradation

The drugs were degraded in the alkaline condition and shows different degradation products at Rf0.14, 0.51 for Rilpivirin and 0.25 for Emtricitabine and 0.02 for Tenofovir as shows in the figures 3-5.

c) Hydrogen peroxide-induced degradation

The drugs were degraded in hydrogen peroxide (3%) at room temperature shows different degradation products at Rf 0.23, 0.35, 0.45, 0.46 for Rilpivirin and 0.57, 0.37 for Emtricitabine and 0.58 for Tenofovir as shows in the figure8-10.

Table VI: Results of Forced Degradation studies

Stress condition	Drug	Mass balance (%assay of recovered + %impurities + % degradents)	Rf values of degradation Products
Acid hydrolysis (0.1N HCl)	Rilpivirin	100.20	0.01
Alkali hydrolysis (0.1N NaOH)	Rilpivirin	99.96	0.14,0.51
Oxidation(3%H_2O_2)	Rilpivirin	99.06	0.23,0.35,0.45,0.46

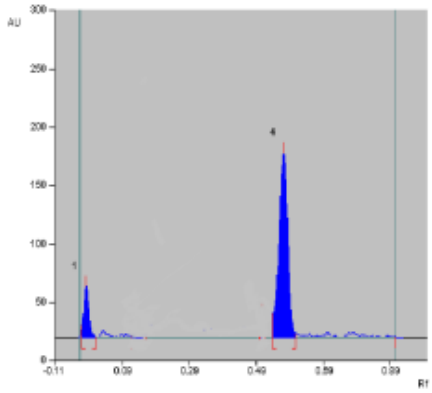


Figure3: Densitogram of acid hydrolysis ofRilpivirin

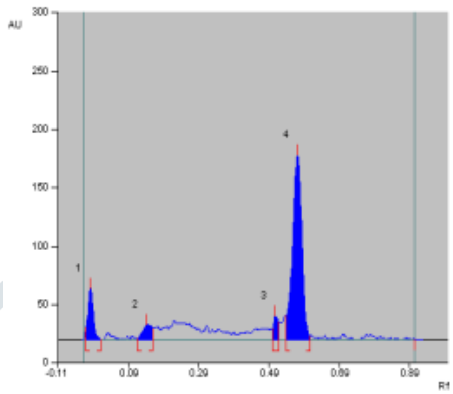


Figure4:Densitogram of alkali hydrolysisof Rilpivirin

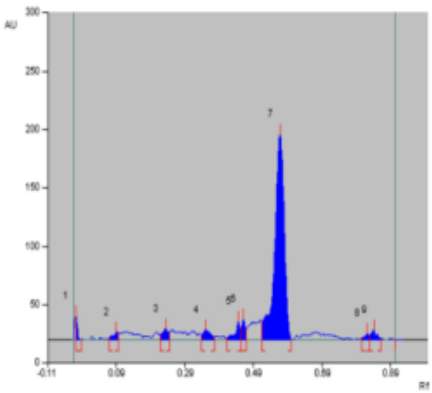


Figure 5: Densitogram of oxidative degradation of Rilpivirin

III. CONCLUSION

The proposed method based on the HPTLC was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy for the proposed method. Hence, it can be concluded that the developed chromatographic method is accurate, precise and selective and can be employed successfullyfor the estimation ofRilpivirin in bulk and formulation.

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