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A New Validated LCMS/MS Method for the Determination of Rilpivirine	Dr. Raj Kumar Bolledula	**Pharm.Analysis**	International Journal of Pharmaceutical Sciences Review and Research	**2020**	ISSN 0976 – 044X	https://globalresearchonline.net/journalcontents/v62-1/10.pdf	https://globalresearchonline.net/journalcontents/v62-1/10.pdf	yes

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Research Article

A New Validated LCMS/MS Method for the Determination of Rilpivirine

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Received: 16-02-2020; **Revised:** 20-04-2020; **Accepted:** 28-04-2020.

ABSTRACT

Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). A novel validated LCMS method has been developed for the quantitative determination of Rilpivirine in active pharmaceutical ingredients and in its Pharmaceutical dosage. Using a column Kinetex C18, 100x4.6mm, 2.6µ with a mobile phase containing a mixture of 0.1% Formic acid and Acetonitrile: Methanol: Isopropanol 65:15:25% v/v in ratio of (5:95) %v/v. The flow rate was 0.6 ml/min and effluent were monitored at 282 nm and a peak eluted at 2.06 min and column oven temperature was maintained ambient. The optimized method was validated for specificity by performing forced degradation and it was found that the main peak was pure in all degradation conditions proving that the method was a stability indicating one. The method was linear in the range of 0.03 µg/ml to 0.14µg/ml and the correlation coefficient was found to be 0.9997. The method was also found to be accurate in 50 to 150% of test concentration. The Limit of Quantification of the method was found to be 10.7836 pg/ml. A mass compatible Liquid chromatographic method was optimized and validated as per 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 method was found to be repeatable, precise, linear and accurate.

Keywords: Rilpivirine, analytical validation, LCMS, NNRTI, ODS, ICH, LOD, LOQ.

INTRODUCTION

EDURANT (rilpivirine) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance of Rilpivirine is less likely to develop than other NNRTI's. FDA approved on May 20, 2011. Treatment of HIV-1 infections in treatment-naive patients with HIV-1 RNA ≤100,000 copies/mL in combination with at least 2 other antiretroviral agents. Each tablet contains 27.5 mg of rilpivirine hydrochloride, which is equivalent to 25 mg of rilpivirine. The chemical name for rilpivirine hydrochloride is 4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6- dimethylphenyl] amino]2-pyrimidinyl] amino] benzonitrile mono hydrochloride. Its molecular formula is $C_{22}H_{18}N_6$ • HCl and its molecular weight is 402.88. Rilpivirine hydrochloride has the following structural formula [1-3].

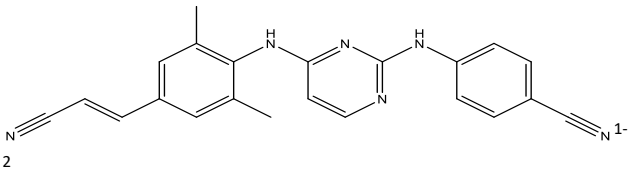

Figure 1: Showing the structure of Rilpivirine hydrochloride

Rilpivirine hydrochloride is a white to almost white powder. Rilpivirine hydrochloride is practically insoluble in water over a wide pH range. Each EDURANT tablet also contains the inactive ingredients croscarmellose sodium, lactose monohydrate, magnesium stearate, polysorbate 20, povidone K30 and silicified microcrystalline cellulose. The tablet coating contains hypromellose 2910 6 mPa.s, lactose monohydrate, PEG 3000, titanium dioxide and triacetin.[3-4]

MATERIALS AND METHODS [5-7]

Preliminary step in method development is to select a diluent. Once an optimized method is selected, mobile phase is selected as diluent based on recovery studies. In selection of diluent, solubility study of drug in different aqueous and organic solvents were performed. Aqueous solvents like water, 1N NaOH, 1N HCl were considered; Organic solvents like methanol, ethanol, acetonitrile and water/acetonitrile (50/50)%v/v were considered.

The selected API was tested for solubility in all the above solvents by traditional shake flask method. According to this method, the selected API were added in excess in each solvents and shaken at a predetermined time which was about 24hours. Then saturation was preliminarily confirmed by physical observation of presence of un-dissolved material. Then the solutions were filtered, and the filtrate were taken to analysis under same temperature. The amount of API solubilized was determined by Ultraviolet spectroscopy and observing the intensity of λmax for each APIs in individual solvents. According to USP General Notice and Requirements, the compendial substance was indicated by one of the

following term based on the solubility. Similar terms were indicated to depict the solubility of the selected API [8-12].

Table 1: Showing solubility compilation of selected solvents

Solvents	Rilpivirine
Water	Insoluble
1N HCl	Soluble
1N NaOH	Insoluble
Methanol	Soluble
Ethanol	Soluble
Acetonitrile	Soluble
Water/Acetonitrile (50:50) %v/v	Soluble

Selection of appropriate wavelength for quantifying the main analyte is a prime prerequisite for initiating method development. The wavelength at which maximum absorbance of analyte is exhibited is selected as its working wavelength [13-14]. To attain it, suitable concertation of drug solution was scanned in UV spectra. The UV spectrum has been for individual API for the diluted solution are recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450 in the scanning range of 400nm-200nm. It was scanned with the concentration of 10ug/ml Shown λ max at 282nm.

Optimization of Chromatographic parameters

The important steps for optimization of chromatographic parameters like selection of HPLC column, Mobile phase composition, flow rate and column oven temperature. As the main intention of development of LCMS method is to detect and quantify Rilpivirine at lower concentration, Rilpivirine standard of 0.1PPM were prepared from standard stock solution. As the peak shape also depends on diluents, different diluents were screened for optimizing the method.

Initial trials started with the selection of regularly used mobile 0.1% formic acid and acetonitrile with a flow rate of 0.6mL/min. Kromasil C18, 250*4.6mm, 5µ and column oven temperature of 60°C.

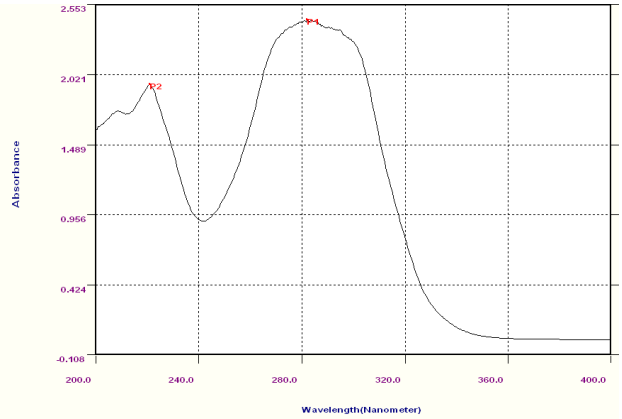

Figure 2: Showing UV-Spectrum for Rilpivirine at 282nm

Table 2: Showing Optimized chromatographic conditions

Parameters	Optimized Conditions
HPLC Column	Kinetex C18, 100*4.6mm,2.6µ
Mobile Phase	Mobile Phase A: 0.1% Formic acid,
	Mobile Phase B: Acetonitrile: Methanol: Isopropanol 65:15:25 %v/v of (5:95) %v/v
Diluents	Acetonitrile: Methanol: Isopropanol 65:15:25%v/v
Flow rate	0.6 ml/min
Concentration of sample	0.1 ppm
Injection Volume	10µL
Detector	UV-282nm
Retention Time (About)	2.06 Minutes

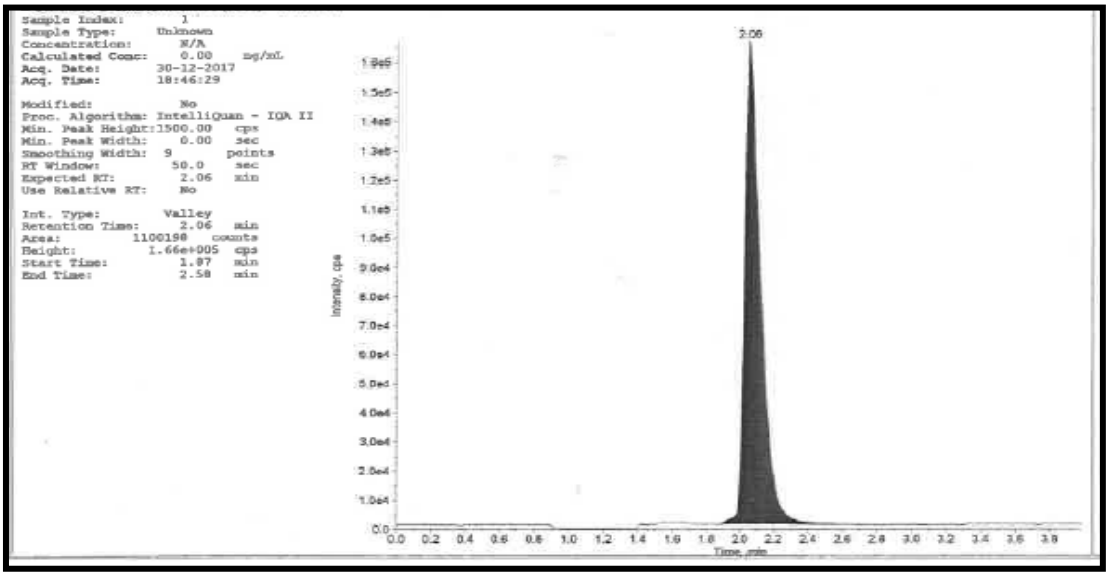

Figure 3: Showing optimized MRM Chromatogram

Optimized LCMS Method for Rilpivirine estimation

The optimised source and Mass spectra parameters are:

Source Parameters

MRM Conditions

Table 3: MRM Conditions

Q1Mass	Q3Mass	Dwell time	DP	EP	CE	CXP
429.2	411.2	200	80	8	17	12
429.2	129	200	80	8	22	5

Table 4: Mass Source and Compound Parameter

Parameter	Value
CUR	25
IS	5500
TEM	550
GS1	45.00 psi
GS2	50.00 psi
CAD	Medium

Instrument Parameters Mass:

Scan type : MRM

Polarity : Positive

Resolution : Unit (Q1&Q3)

Table 5: Valco valve program

Total time(min)	Position
0	A
0.9	B
1.4	A

Note: A=Source, B=Waste

REULTS AND DISCUSSIONS

Determination of Assay of Rilpivirine in Edurant 25mg tablets using the optimized method

Preparation of Standard Solution

Weighed about 25mg of Rilpivirine into 25mL volumetric flask, dissolved & make up to volume with acetonitrile. Further dilution was done by transferring appropriate volume to obtain a concentration of 0.1µg/ml in diluent.

Preparation of Sample

20 tablets of Edurant 25mg tablets were weighed and powdered. The tablet powder equivalent to 25mg of Rilpivirine was weighed accurately and transferred into 50mL volumetric flask. 30ml of acetonitrile was added to the flask, sonicated for 10min and made up to the mark with water. Later, it was filtered through 0.45µ filter to produce a concentration of 500µg/ml. Further dilution was done by transferring appropriate volume to obtain a concentration of 0.1µg/mL in diluent. The results of the assay were shown in table No.3

Table 6: Assay Results of Edurant 25mg tablets

Formulation	Component	Label Claim (mg)	Amount Found (mg)	% Assay
Edurant 25mg tablets	Rilpivirine	25	25.8	103.2

Method Validation Results

Table 7: Showing Method development conditions of Rilpivirine

Parameter		Acceptance Criteria	Results
Assay (%)	-	95.0% to 105.0%	103.2
LOQ (µg/ml	-	NA	10.78pg/ml
Linearity	Correlation Coefficient	>0.990	0.9997
Accuracy	Recovery at 50%	95.0% to 105.0%	100.6
	Recovery at 100%		100.2
	Recovery at 150%		100.7
Precision	Repeatability	%RSD>2.0%	0.02
	Intermediate Precision Day-1		0.02
	Intermediate Precision Day-2		0.03
Robustness	Flow (0.5 ml/min)	%RSD>2.0%	0.05
	Flow (0.7 ml/min)		0.03
	Temperature (55°C)		0.11
	Temperature (65°C)		0.08

Linearity

The linearity of the method was established in the concentration range of 0.03µg/ml to 0.14µg/ml for Rilpivirine. The straight-line equation obtained was y=4E+06 x-3963.3 and the regression coefficient was found to be 0.9997. The Linearity data was tabulated in table 6.18 and linearity plot in Figure 4.

Table 8: Showing Linearity data for Rilpivirine

Rilpivirine Linearity Plot		
Concentration Level (%)	**Concentration in µg/ml**	**Peak Area**
30	0.027836	94733
50	0.046394	160717
80	0.07423	258652
100	0.092788	329613
120	0.111344	384549
150	0.139182	492049
Correlation Coefficient (r)		**0.9994**
Slope		**3546532.56**
Intercept		**-3963.2507**

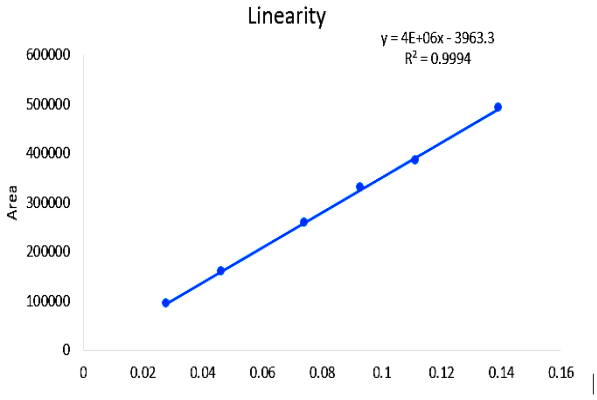

Figure 4: Linearity plot for Rilpivirine

Accuracy

Accuracy of the optimized method was determined by recovery studies. The recovery studies were carried out at three replicates at each level (50%, 100% and 150%), the % recovery was in between 95.0-105.0% and %RSD was found to be less than 2.0. The results were tabulated in Table 9.

Table 9: Recovery data for Rilpivirine

% Level	Amount added (pg/ml)	Peak Area	Amount Found (pg/ml)	% Recovery	Mean Recovery	%RSD
50%	50.15	156347	50.2362	100.17	99.95	1.2
		157427	50.5832	100.72		
		154375	49.6025	98.95		
100%	100.2	310099	99.6385	99.44	99.7	
		311253	100.0093	99.41		
		312320	100.3522	100.25		
150%	150.8	483493	155.3521	102.88	101.09	
		474565	152.4834	101.05		
		467422	150.1883	99.33		

Specificity

Specificity of the method was established injecting Blank, Standard and sample into the optimised method. The results were tabulated in table 10.

Table 10: Showing the results of specificity

Name of the solution	Retention Time	Area
Blank (Diluent)	Not Detected	Not Detected
Standard solution	2.06	274002
Sample	2.05	267327

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (±0.1ml/min) and Temperature (±5°C) are studied to determine the robustness of the method are performed. The %RSD of retention time and area under the curve of Rilpivirine for n=6 in each condition was found to be less than 2.0%. The Results are tabulated in table 11.

Table 11: Robustness parameter for Rilpivirine

Change in parameter	% RSD
Flow (0.5 ml/min)	0.05
Flow (0.7 ml/min)	0.03
Temperature (55^{0}C)	0.11
Temperature (650C)	0.08

CONCLUSION

The developed LCMS analytical method meets the validation criteria made by ICH. The proposed LCMS method is rapid, sensitive, precise and accurate for the determination of Rilpivirine and can be reliably adopted for routine quality control analysis of Rilpivirine in Bulk and its pharmaceutical formulations.

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Source of Support: Nil, **Conflict of Interest:** None.