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ROBUST RP-HPLC METHOD DEVELOPED AND VALIDATED FOR SIMULTANEOUS ESTIMATION OF ANTI-HIV DRUGS IN PHARMACEUTICAL SUBSTANCE AND FORMULATION

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ABSTRACT

The present research work was carried out using a simple RP-HPLC method for simultaneous estimation of Lamivudine and Rilpivirinein bulk and pharmaceutical formulation. The separation was carried out by Phenomenox C18 (4.6 ×250mm, 5µ ID) used as a stationary phase and MeCN: MeOH and 0.1 % of DEA (pH 3.0 was adjusted with 10 % OPA) used as a mobile phase in the ratio of 30:15:55 at a 0.9ml^{-1} flow rate and the peak detection was carried out 245nm (isobestic point). Overall run time was 10.0 min and the retention time was 2.49, 7.01 min for Lamivudine and Rilpivirine respectively. The proposed method was validated as perthe ICH guidelines and found to be specific, linear, selective, and precise. Linearity range is 2-10µg/ml of both Lamivudine and Rilpivirine and the LOD is 2.32ng/ml, 3.24ng/ml and LOQ is 8.43ng/ml, 10.35ng/ml of Lamivudine and Rilpivirine respectively. This method can be applied on bulk and commercially available individual and combined pharmaceutical dosage forms. This method can be utilized in marketed formulation to identify the purity, degradation and assay value.

KEYWORDS

Lamivudine, Rilpivirine, ICH guidelines, RP-HPLC and Method development.

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INTRODUCTION

The fixed dose combination is highly efficient in eradicating human immune deficiency virus (HIV), since single drug therapy is rapidly ineffective for antiretroviral treatment because of its drug resistance; and now a day's new paradigm is to combine more than two combinations are preferred for antiretroviral therapy. Rilpivirine and

Lamivudine are a nucleoside reverse transcriptase inhibitors (NRTI) were officially approved for treating against antiretroviral therapy LMV was approved for combination with other retroviral drugs and its special socioeconomic importance because of their widespread frequency in humans[1-5]. LMV is a synthetic dideoxy- nucleoside derivative that is active against HIV and hepatitis B virus (HBV). The chemical structure of Rilpivirine and Lamivudine was given in Figure No.1.

Rilpivirine is chemically known as 4-[[4-[4-[(E)-2-cyanoethenyl]-2, 6-dimethylanilino] pyrimidin-2-yl] amino] benzonitrile. It's non-nucleoside reverse transcriptase inhibitor andused in combination with other antiretrovirals to specifically treat HIV-1.It has a long duration of action as the oral tablet is given daily and the intramuscular suspension is given monthly. Patients should be counselled regarding the risk of hypersensitivity reactions, hepatotoxicity, depressive disorders, and the redistribution or accumulation of body fat[6-9].

Although there are number of methods reported for the analysis of Rilpivirine and Lamivudine individually and in combination with other drugs, no method is found available for the simultaneous estimation of all these drugs with single mobile phase system[10-14].

EXPERIMENTAL

MATERIALS AND METHODS

Working standards of Rilpivirine and Lamivudine were gifts from Hetro Labs, Hydrabad, India. Themarketed formulation was purchased from whole saler (LAZID-N tab). Acetonitrile (MeCN) and Methanol (MeOH) of HPLC grade and Triethylamine (TEA), Diethylamine (DEA) and other reagents of analytical-reagent grade were from SD Fine Chemicals (Mumbai, India). The HPLCgrade water was prepared by using Milli-Q Academic, Millipore, Bangalore, India.

HPLC instrumentation and conditions

Chromatographic separations of Rilpivirine and Lamivudine were carried out, with the ternary mobile phase consisted of a mixture of MeCN: MeOH and 0.25% of DEA (pH 2.50 was adjusted with 10% ortho phosphoric acid) and Phenomenox C18, (4.6 ×250mm, 5µ ID) was used as a stationary phase then detection were made at 245nm at ambient temprature.

Stock and working standard solutions

Stock and working standard solutions of Rilpivirine and Lamivudine were prepared using mobile phase as a diluting solvent. Standard solutions employed for the optimization procedure constituted a mixture of Rilpivirine and Lamivudine at 10.0µgmL^{-1} were prepared respectively. For quantification of analytes in markets formulation samples, individual calibration curves peak area ratios of Rilpivirine and Lamivudine versus drug concentrations were established at five levels; 2.0-10µg mL^{-1} for LMV and RPV.

Formulation sample preparation

Twenty tablets were weighed and finely powdered. An amount of pharmaceutical products powder equivalent to 50mg of Rilpivirine and Lamivudine was accurately weighed and transferred in a 50ml volumetric flask. This mixture was sonicated for 15 min for complete extraction of drugs and the solution was made up to the mark with the mobile phase. Then further diluted, to obtain a concentration of 10µg/ml for Rilpivirine and Lamivudine respectively. The solution was centrifuged at 5000RPM for 15 min; the clear supernatant was collected and filtered through a 0.2µm membrane filter (Gelman Science, India) and 20µl of this solution was injected into the HPLC system. Final chromatogram shown in Figure No.2.

RESULTS AND DISCUSSION

Preliminary experiments

Based on literature review by trial and error method to initiated method development of liquid chromatographic such as (i) Different type of stationary phase (C18, C8 and C6), (ii) Different range of pH, (iii) Different flow rate (iv) Different type of mobile phase additives (OPA, Diethylamine, Triethylamine, THF), based on the studies. To obtain an acceptable analytical retention time, good quality of separation (resolution, capacity factor, symmetric factor, good peak shape), further need to optimize the chromatographic separation. For the

optimization purpose we further research work conducted.

Various types of stationary phase available for the RP-HPLC and we tried phenyl, C18, C8 and C6 columns. Well resolved peak separation and excesses of asymmetric factor, less peak resolution were observed on C8 and C6 columns. Moreover phenyl and amino columns are not suitable for this analyte. Among these C18 gave good peak separation and satisfactory retention time, resolution and capacity factor.

For mobile phase initially acetonitrile was selected as the organic phase and HPLC water was selected as an aqueous phase, then various ranges of pH (pH was adjusted with 10% orthophosphoric acid) were tried. In the above combination of mobile phase were tested in different proportion (50:50, 40:60, 60:40, 70:30) and at 50: 50 (MeCN: water (pH 3.5) ratio only we observed valuable retention time but poor resolution, capacity factor and poor peak separation, then introduced methanol to overcome this problem.

From the selected above mobile phase we added 0.05 to 1.0% triethylamine and there are no significant changes in resolution and the peak overlapping. Then we tried acetic acid (0.1- 0.5 %) in aqoues phase small variation in resolution, so then tried with 0.05 - 1.0% of diethylamine, it produced significant improvements in resolution and good peak shape.

Method validation

The proposed liquid chromatographic method was validated by following ICH guidelines. Validation parameters like selectivity, specificity, linearity, limit of detection and quantification, accuracy, precision, stability and robustness were addressed.

Specificity

The specificity of the method was evaluated by assessing the chromatograms of most commonly used excipients (starch, lactose monohydrate, methyl cellulose, titanium dioxide and magnesiumstearate) with that of the standard drugs. There were no excipient speaks co-eluted with the analytes, indicating that the optimized assay method is selective and specific in relation to the excipients used in this study.

Linearity

The linearity of the method was established at five levels over the concentration ranges of 2.0-10µg mL^{-1} for LMV and RPV approximately from 20 to 200% of nominal range of analyte[15-18]. Peak areas (y) of LMV and RPV were plotted versus their respective concentrations (χ) and linear regression analysis performed on the resultant calibration curves (n=6). The correlation coefficients (R2) were found to be more than 0.999 and the details given in Table No.1 and Figure No.3.

Limits of Detection (LOD) and Limits of Quantitation (LOQ)

In accordance with ICH recommendations, the approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantification limits. LOD and LOQ values were estimated as *[(standard deviation of repeatability)/(slope of the regression equation)]* by multiplying with 3.3 and 10 respectively. Using the above equations, the LOD were estimated at 2.32ng/ml, 3.24ng/ml and LOQ were estimated at 8.43ng/ml, 10.35ng/ml of Lamivudine and Rilpivirine respectively.

Accuracy

The accuracy of the method was determined by analyzing Quality Control (QC) standards prepared at three levels of 80, 100 and 120% of the expected assay value or label claim of the analytes in the commercial formulation. QC samples were prepared as three replicates at each concentration level by spiking the standard drugs with the placebo excipients, which were left overnight to allow matrix-analyte interactions to occur. The % recovery of the analytes at each level (n = 3) and mean % recovery (n = 9) were determined and %accuracy was expressed as [(calculated amount/predicted amount) × 100]. Accuracy, assessed by spike recovery, in which the % recovery of both enantiomers it is at each level (n = 3) and mean % recovery (n = 9) were found to be 98.65 and 98.75% for LMV and RPV respectively. The recoveries of each drugs at each level were found well within the acceptable criteria of bias, ±2.0%. The mean % recovery (n = 9) for each enantiomer was also tested for significance by using

Student t-test. Since the tCalc is less than the theoretical t value (tCrit = 2.314), at 5% significance level, the null hypothesis (the recovery is unity or 100%) was accepted.

Precision

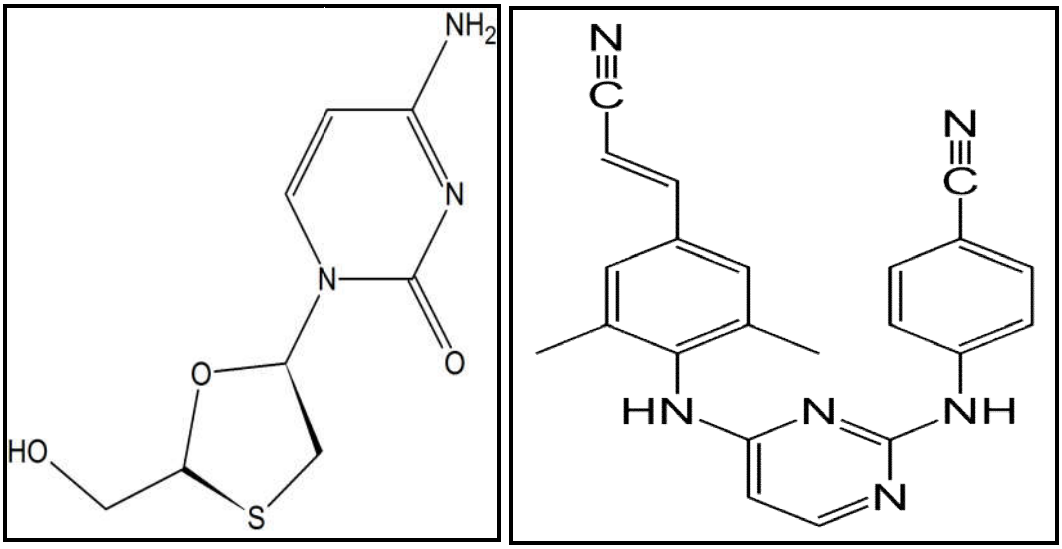
The precision was established by injecting three different concentrations of each analyte for LMV and RPV each in six replicates, for intraday precision (repeatability) and on three consecutive days for the intermediate precision (reproducibility). Precision was expressed by the %RSD of the analyte peak area. Results for all studied compounds met the proposed requirement %RSD ≤ 2%. The intra and inter-day precision (n = 6.0) was confirmed since, the % CV were well within the target criterion of ≤ 2.0.

Robustness

The robustness of the proposed method was assessed to provide an indication of its reliability during normal usage with respect to small, but deliberate variations in experimental parameters such as variations in MeCN concentration (30% ± 0.5), the flow rate (0.9 ± 0.05) and the pH (3.0 ± 0.5%) did not alter the assay values more than 1.0% and therefore it would be concluded that the method conditions are robust.

Table No.1: Validation parameters of rilpivirine and lamivudine

S.No	Parameters	Lamivudine	Rilpivirine
1	Linearity range (µg/ml)	2-10µg/ml	2-10µg/ml
2	Slope	423416x	846832x
3	Correlation coefficient R2	0.999	0.999
4	Rt	2.49 min	7.09 min
5	Tailing factor	0.9	0.79
6	LOD	2.32ng/ml	3.21ng/ml
7	LOQ	8.43ng/ml	10.35ng/ml
8	Theoretical plates (USP)	5540	7322



[Lamivudine] [Rilpivirine]

Figure No.1: Chemical structure of rilpivirine and lamivudine

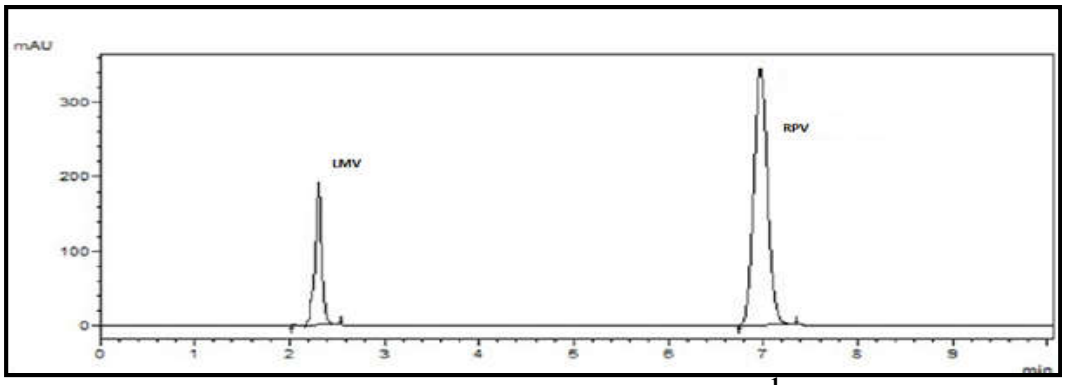


Figure No.2: Respective chromatograms of Rilpivirine (10.0 µgmL^{-1}) and Lamivudine (10.0µgmL^{-1}) optimal conditions

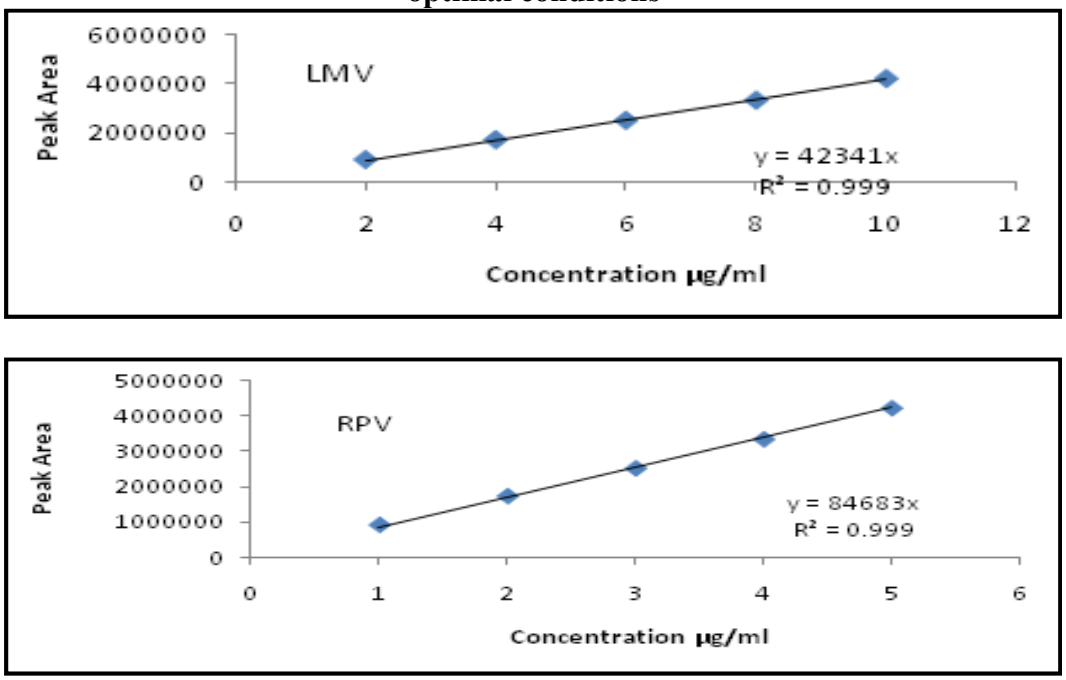


Figure No.3: Linearity graph of rilpivirine and lamividine

CONCLUSION

A rapid, simple, robust and efficient isocratic reversed-phase high-performance liquid chromatography method was developed, optimized and validated for the simultaneous determination of the Rilpivirine and Lamividine in API and pharmaceutical formulations.

The analytical results obtained lead to the conclusion that the developed method performs well with regard to precision, accuracy, rapidity, sensitivity and robustness, with single mobile phase allows quantifying the Rilpivirine and Lamividine. Therefore, it could be successfully employed for the analysis of these antiretroviral drugs in formulations samples.

This optimized method has to be utilized for the simultaneous quantitative analysis of Rilpivirine and Lamividine in Bulk and pharmaceutical formulations. The method can be applied for the marketed (commercial) formulation samples such as Edurant tab containing (RPV= 25mg) Epivir tab containing (LMV= 150mg). The mean, % SD recoveries values achieved were within the parenthesis being the % CV of the six replicates and the % CV of the assay results were < 2.0, indicating the precision of the analytical methodology.

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CONFLICT OF INTEREST

All authors' declared no conflict of interests.

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