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Micellar Liquid Chromatographic Method Development for Determination and Stability Indicating of Nelfinavir Mesylate in Pharmaceutical Formulation

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Abstract: A rapid, simple and sensitive liquid chromatographic procedure that use micellar mobile phase containing only Tween-20 and n-butanol, is reported for the determination of Nelfinavir Mesylate. The determination of Nelfinavir Mesylate could be achieved with a micellar mobile phase of 2% n-butanol in 0.5 molL^{-1} Tween-20, with retention time below 9 minute. The working standard curve was linear (R=0.9990) over the concentration range of 5 ppm to 100ppm with detection limit. The method was environment friendly and economical in term of time taken and amount of solvent used.

Keywords: Miceller liquid chromatography, Surfactant, Nelfinavir Mesylate, Tween-20, Stability studies.

Introduction

Nelfinavir Mesylate [1] is a novel HIV-1 protease inhibitor; with a chemical name (3S, 4aS,8aS)-N- (1,1-Dimethylethyl) decahydro-2- [(2R, 3R)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl) amino]–4-(phenylthio) butyl]-3-isoquinolinecarboxamide methanesulfonate .It is an antiretroviral drug that acts by binding reversibly to HIV protease thereby preventing cleavage of the viral precursor polyproteins. Literature survey reveals many Chromatographic methods [3-10] for the determination of Nelfinavir in biological fluids and in combination with other antiviral and few Spectrophotometric methods [11-14] Miceller liquid chromatography has been reported as a suitable technique for pharmaceuticals and intermediate for drug and cosmetics interest [15]. Miceller solution can replace conventional aqueous organic mobile phase with good results. Miceller liquid chromatography (MLC) is a reversed phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant (Ionic or Non ionic) above its critical concentration (CMC) [16]. In these conditions the stationary phase is modified with an approximately constant amount of surfactants monomers, and solubilizing capability of mobile phase is altered by the presence of micelles, giving rise to diverse interactions (Hydrophobic, ionic and satiric) with major implications and selectivity. This technique has evolved up to becoming a real alternative in some internsace to classical RPLC with hydro-organic mixtures, owing to its peculiar features and unique advantages. The idea of using pure micellar solution as mobile phase is very attractive owing to the lower cost and toxicity, and the reduced environmental impact. In practice, however, the addition of small amount of organic to the micellar solution is needed to achieve retention in particular time window. Miceller mobile phases have been used with different bonded stationary phases (mostly C8, C18 and cyanopropyle). The most common surfactant are the anionic sodium dodecyl

sulphate (SDS) cationic cetytrimethylammonium bromide (CTAB), and non-ionic Tween-20, several organic solvents have been used as modifiers, short/medium chain alcohols and acetonitrile being the most suitable. The presence of micellar contributes well above their solubility in water. Also, the risk of evaporation is diminished. The development of meaningful dissolution procedure for compounds with limited water solubility has been a great challenge. It has been seen that surfactant play very important role in solubilizing organic and in-organic salt by reducing interfacial tension and contract angle between solid particles and aqueous media. Thus improving compounds adaptability and increasing surface availability for compounds dissolutions [17-20].

2. Experimental:

2.1 Reagents & standards

Tween-20, n-butanol and water were obtained from Merck. All reagents were of HPLC grade unless otherwise specified.

Chromatographic condition of method

The Licrosphere C_{18} column was used 25°C temperature. The mobile phase considered 2% n-Butanol in 0.5 $molL^{-1}$ Tween-20 pH adjusted to 4.2 $\pm$ 0.01 with o-phosphoric acid. It was pumped at flow rate of 1ml /min. the mobile phase was passed through nylon 0.45 μm membrane filters and degassed before use. The UV detection wavelength was 249 nm. Mobile phase flow rate was 1.5 ml/min. twenty micro liters of sample were injected into the HPLC for each analysis. A Waters column heater module was used to maintain a constant column temperature of 25^0C. Peak purity analysis was carried out over a wavelength range 200-400 nm through the use of the software. The stability chamber utilized during forced degradation studies was a controlled by temperature controller. All measurements were carried out at room temperature (25 ± 0.1^0C).

Preparation of standard stock solution

The equivalent of 625 mg Nelfinavir Mesylate were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of 2% n-Butanol in 0.5 $molL^{-1}$ Tween-20 pH adjusted to 4.2 $\pm$ 0.01 with o-phosphoric acid.. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 625 μg/ml of Nelfinavir Mesylate. The two main advantages of micellar procedure are the elimination of organic solvents and simplification of sample preparation step. The correlation coefficient was found 0.9981. According to International Conference on Harmonization (ICH) guidelines the following expression is used to evaluate LOD and LOQ.

Preparation of sample solution

Twenty tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 625mg of Nelfinavir Mesylate was taken in 25ml volumetric flask and dissolved in 75ml of n-Butanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through whattman no 41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent.

Result and Discussion

Method Development

Optimal separation of related substances from each other and from Nelfinavir Mesylate was achieved with an Isocratic mobile phase. A mobile phase temperature of 25^0 C was employed for the separation. No significant degradation of Nelfinavir Mesylate was observed at 25^0C temperature during its elution time. Typical chromatogram with retention time and elution order observed for Nelfinavir Mesylate is presented in fig 1. In this study after many experiments a new mobile phase with a higher eluting strength 2% n-butanol in 0.5 $molL^{-1}$ Tween-20 was found satisfactory. In this work, it is demonstrated that mobile phase based on Tween-20 with n-Butanol are suitable for the analysis of Nelfinavir Mesylate. The two main advantages of micellar procedure are the elimination of organic solvents and simplification of sample preparation step. The seven point's calibration graphs were constructed covering a concentration range. 0.5 to 15 mg/ml. linear relationship was obtained between the peak area ratios of Nelfinavir Mesylate in the concentration range 10 ppm to 100 ppm. The correlation coefficient was found 0.9990. According to International Conference on Harmonization (ICH) guidelines the following expression is used to evaluate LOD and LOQ.

Accuracy

The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to pre-analyzed sample. Each determination was performed in triplicate.

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of Nelfinavir Mesylate.The precision of the assay was determined by repeatability (intra-day) and

intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days. Five sample solutions were prepared and assayed.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of Nelfinavir Mesylate at concentration 0.5µg/ml and 15µg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation.

Sensitivity-detection limit:

The detection limit was calculated by the equation

LOD = 3.3S.D./b, where S.D.

is the standard deviation of the intercept and b is the slope of the regression line. The calculated detection limit for the standard solution was 0.652µg mL^{-1}

Quantification limit:

The quantitation limit was examined by the equation LOQ = 10 S.D./b. The lower limit of quantitation for the standard solution was found to be 0. 0.892µg/ml

Specificity:

Specificity is the ability of the method to measure the analytical response in the presence of all potential impurities. For the specificity test, chromatogram of the standard solution of Nelfinavir Mesylate were recorded under selected conditions. The response of the analyze in this mixture was compared with the response of pure Nelfinavir Mesylate. It was found that assay results were not changed.

Stability:

In this study, Nelfinavir Mesylate stock solution were kept in the -dark at $+4^0$C for 15 days and were analyzed at different times (every day). It has been seen that repeatable peak currents of Nelfinavir Mesylate stock solution occurred up to 15 days and after that the peak current decreased significantly. So the solutions were found to be stable for 15 days.

Table 1.System suitability test parameter for Nelfinavir Mesylate

Property (n*=6)	**Nelfinavir Mesylate**
Retention time(min)	6.23
Tailing factor	4.56
Capacity factor	1.073
Theoretical plates number	26541
Resolution	1.98

* n = Number of determination

Table 2. Recovery Studies Nelfinavir Mesylate

Nelfinavir Mesylate			
Label claimed	%Amount added	Found in(µg/ml)	%recovery
625	50	625.11	100.11
	150	624.96	99.97
	150	625.01	100.01

Table 3.Regression Analysis of Calibration Graph for Nelfinavir Mesylate

Parameter	**Nelfinavir Mesylate**
Concentration range	0.5-15 µg/ml
Slope	32843
SD[$] of the slope	21.03
Intercept	67451
SD[a] of the intercept	16.29
Correlation coefficient	0.9990

Table 4. Summary of validation parameter Nelfinavir Mesylate

Parameter	Nelfinavir Mesylate
LOD[a]	0.652µg/ml
LOQ[b]	0.892µg/ml
Accuracy, %	100.06 ± 1.32
Repeatability(RSD[c], %, n =6)	3.084
Precision (RSD, %)	
Intraday(n =3)	0.886
Interday(n = 3)	1.213

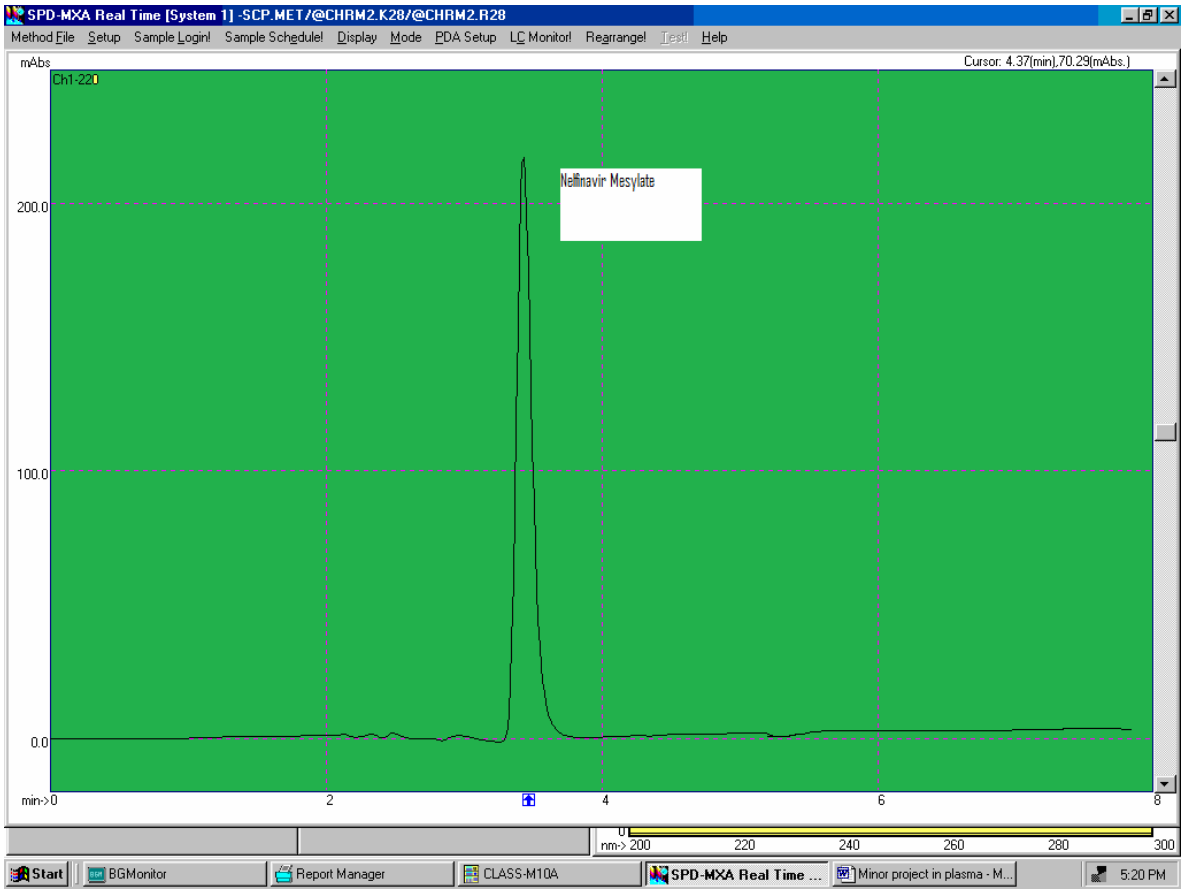

Fig-01-Chromatogram of Nelfinavir Mesylate obtained using miceller mobile phase 2% n-Butanol in 0.05 mol L^{-1} Tween-20

Conclusion

The proposed miceller chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be convenient and effective for the quality control of Nelfinavir Mesylate. There are certain advantages associated with this method such as dissolution, high selectivity, sensitivity, low cost, less time consuming, less hazardous and low limit of detection. Moreover, the lower solvent consumption along with the short analytical run time of 6.23 minutes leads to a cost effective and environment friendly chromatographic procedure. Consequently the proposed method has a high potential of good analytical alternative for determining quality of Nelfinavir Mesylate.

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