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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF MARAVIROC IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: To develop and validate simple, sensitive, precise, rapid and cost effective method for determination of Maraviroc in bulk and pharmaceutical formulations as per ICH Guidelines.

Methods: A simple double beam UV Spectrophotometric method has been developed and validated with different parameters such as Linearity, Precision, Repeatability, Limit of Detection (LOD), Limit of Quantification (LOQ), Accuracy and Ruggedness**.**

Results: Maraviroc in 0.1N HCl shows maximum absorbance at 210 nm. Beer's law was obeyed in the concentration range of 5-25 mcg mL-1, The LOD and LOQ were found to be 0.389 mcg/ml and 1.178 mcg/ml respectively. A recovery of Maraviroc in tablet formulation was observed in the range of 99.39-100.20%. Percentages assay of Maraviroc in tablet was more than 99%.

Conclusion: The proposed method is precise, accurate and reproducible and can be used for routine analysis of Maraviroc in bulk and pharmaceutical formulations.

Keywords: Maraviroc, UV spectrophotometer, Analysis, Dosage form, Method validation.

INTRODUCTION

Maraviroc is the first CCR5 antagonist and only oral entry inhibitor approved for the treatment of HIV-1infection. These acts as a human immunodeficiency virus type 1 (HIV-1) co receptor. Binding of Maraviroc to this receptor prevents the interaction of HIV-1 gp 120 with CCR5-tropic HIV-1 and thereby inhibits the virus from entering the cell [1,2, 3]. Maraviroc is described as 4,4 difluoro N {(1S) 3 [exo 3 (3 isopropyl 5 methyl 4H 1,2,4 triazol 4 yl) 8 azabicyclo [3.2.1] oct 8 yl] 1 phenyl propyl} cyclohexane carboxamid [4]. Maraviroc is a white to yellowish or brownish powder with a molecular weight of 514.The molecular formula of Maraviroc is $C_{29}H_{41}F_2N_5O$ and Its Chemical structure is given below (Figure 1).Maraviroc is practically insoluble in water, slightly soluble in ethanol, Soluble in Methanol, Dimethyl sulfoxide and PEG 400 [5, 6].

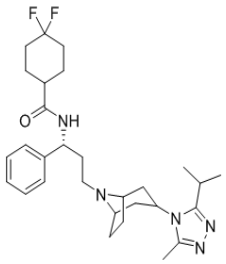

Fig. 1: Maraviroc chemical structure

Maraviroc belongs to BCS class III drug. Along with it's poor water solubility it also have only slight solubility in most of organic solvents, but it have good solubility in 0.1N HCl.[7] Because of its poor solubility profile there is no UV method to estimate the Maraviroc in their routine analysis and from their formulation. Available literature states only HPLC method of estimation of Maraviroc at 210nm [8,9].Though HPLC method is highly sensitive and accurate but cost of analysis is too high. But there is no work in the literature reported about the UV Spectrophotometric method for the analysis of drugMaraviroc in pharmaceutical formulations. Thus there is need to develop simple rapid and economical method for routine analysis of Maraviroc. The objective of present study was to develop and validate simple, sensitive, accurate, precise, rapid and economical method for estimation of Maraviroc in bulk and pharmaceutical formulations as per ICH Guidelines [10,11].

METHOD AND MATERIALS [12, 13]

Instruments and reagents

An analytically pure sample of Maraviroc was obtained from Hetro Lab, Hyderabad as a gift sample. All the chemicals used were of analytical grade. Triple distilled water was used to prepare Solutions. UV-Visible Spectro-photometer (LABINDIA UV-3092 PC and UV 3000+), with wavelength accuracy is 0.5 nm, spectral band width 1 nm and 1 cm matched quartz cells. Electric balance (Shimadzu Uniblog).

Selection of Wavelength

Maraviroc is very soluble in 0.1NHCL so 0.1NHCL solvent was selected for through out the study. Maraviroc 15µg/ml of working standard solution was scanned in between 200nm to 400 nm; Maraviroc shows maximum absorption in 210nm by UV sepectrophotometer (Figure 2).

Preparation of working standard drug solution

The standard Maraviroc 100mg was weighed accurately and transferred to 100ml volumetric flask. Drug was dissolved and diluted up to the mark with 0.1N HCl solution to obtain final concentration of 1000 mcg/ml, then the solution was ultrasonicated for few minutes and the resulting Solution was used as working standard solution.

Preparation of calibration curve

From this stock solution, appropriate dilution was made to get final concentration of 5, 10, 15, 20, and 25 mcg/ml were prepared and absorbance was taken at λmax 210 nm. Averages of such 5 sets of values were taken for standard calibration curve, and the calibration curve was plotted.

Precision

The precision was determined by repeatability (intraday) and intermediate precision (interday). Intra day precision study was carried out by preparing drug (Maraviroc) solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine inter day precision. Precision (intraday and intermediate precision) was expressed as % relative standard deviation (Table 2).

Accuracy

Accuracy was established at 50%, 100% and 150% levels by additio n of standard drug of Maraviroc to placebo.

Ruggedness

Ruggedness was determined by performing the same proposed met hod on different instrument,method was carried out by two different analysts and by performing the method on different days to check the reproducibility.

LOQ and LOD

The limit of detection (LOD) and limit of quantification (LOQ) were c alculated based on the standarddeviation of the response (y intercep ts of regression lines) and the slope using 3 independent analytical curves, as defined by ICH. Maraviroc LOD and LOQ were calculated a s 3.3σ/S and 10σ/S,respectively, where σ is the standard deviation of Y intercept (ICH guidelines) and S is the slope of the Maraviroc calibration curve.

Analysis of marketed formulations

For the estimation of Maraviroc in tablets formulations by this method 20 tablets of marketed brand of Maraviroc were weighed and triturate to fine powder. Amount of powder equivalent to 150 mg drug was taken and extracted with 50 ml of 0.1N HCl solution under sonication for 30 min. The Volume was made up to 100 ml with 0.1 N HCl and mixed; above solution was filtered through Whatman filter paper No. 41. The solution was suitably diluted so as to obtain a concentration in the linearity range. Then the absorbance of these solutions was measured at 210 nm against blank. The results of analysis are shown in (Table 9).

RESULT AND DISCUSSION

In the start of the method development for Maraviroc, different solvents were tested such as ethanol, water, 0.1N HCl, 0.1N NaOH and Phosphate buffer (pH7.4). Due to greater solubility an d reproducible readings of maximum absorbance, 0.1 N HCl was selected for further work. The absorption spectrum of Maraviroc was measured in the range 200 400 nm against the blank solution 0.1N HCL similarly prepared. From the drug scan it was found that the maximum Maraviroc UV absorbance occurs at 210 nm (Figure 2) which was used as λmax for the method development and the method was validated by studying the following parameters.

Linearity

A linear relationship was found between the absorbance and the concentration of Maraviroc in the range of 5 to 25µg mL-1. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis. The correlation coefficient was 0.998 indicating excellent linearity ($r2 > 0.999$).

Precision

Theprecision of the method was expressed in terms of % relative sta ndard deviation (%RSD). The % RSD values found to be less than 2 f or intra day and interday precision, the precision result showed a good reproducibility. The result is expressed in Table 2.

Repeatability

Repeatability was determined by analyzing 15µg/ml concentration of Maraviroc solution for six times and %RSD was found to be 0.714, which is less than 2. The result is expressed in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of Maraviroc were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ were found 0.389 µg/ml and 1.178 µg/ml respectively.

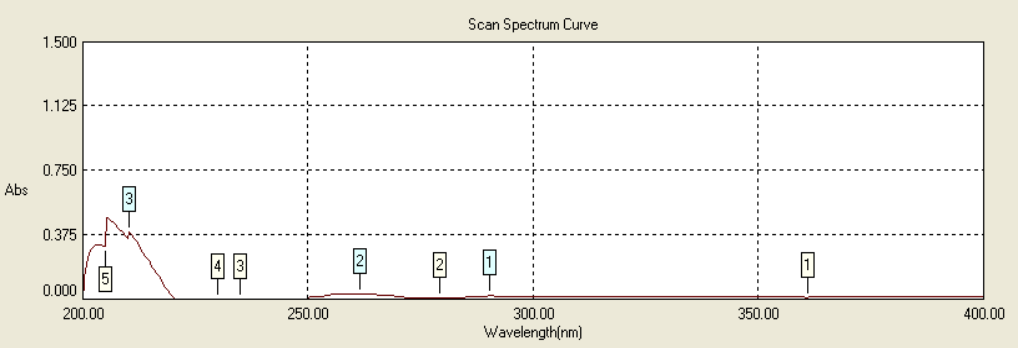

Fig. 2: UV spectrum of the standard Maraviroc

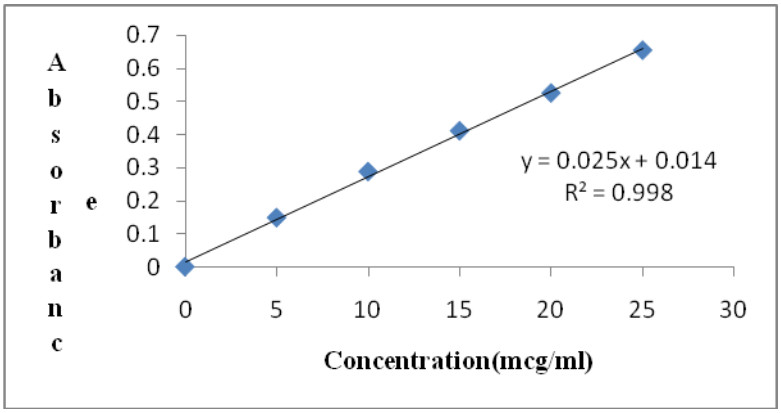

Fig. 3: Calibration graph

Table 2: Intermediate & Interday precision

Concentration (µg/ml)	%RSD		Average %RSD	%RSD			Average %RSD
	Instrument I	Instrument II		Day1	Day2	Day3	
15	0.598	0.482	0.54	0.598	0.6027	0.6362	0.6123

Table 3: Data for repeatability

Sample concentration (µg/ml)	No. of Measurement	Absorbance	Statistical Analysis
15µg/ml	1	0.411	
	2	0.416	Mean-0.4123
	3	0.408	SD-0.002944
	4	0.413	%RSD-0.714
	5	0.411	
	6	0.415	

Accuracy (Recovery Test)

As an additional check on the accuracy of the method was studied by recovery experiments. The recovery assay values for Maraviroc ranged from 99.39 ± 0.9603 to 100.20 ± 0.6977 with SD value not more than 1.5 which indicates good recovery at 50 % to 150% estimation of Maraviroc (Table 4). No organic solvent is required for the extraction of Maraviroc from formulation which reduces the cost of estimation.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD (Table 5).

Robustness

Robustness analysis was carried out at two different temperatures, room temperature and at 29^{0}C to determine the robustness of the method and the respective absorbance was measured. The results were indicated as %RSD (Table 6).

Determination of Maraviroc in Tablets

The validated method was applied to the determination of Maraviroc in Tablets. Twenty tablets were assayed and the results are shown in (Table 7) indicating that the amount of drug in tablet samples meet with requirements (99–102% of the label claim).

Table 4: Data for Accuracy Test

Concentration level	Sample No	Amount added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Statistical Analysis
50%	1	15	15.05	100.36%	Mean -99.39
	2	15	14.9	99.39%	SD-0.9603
	3	15	14.76	98.44%	%RSD-0.9661
100%	1	30	30.04	100.12%	Mean -99.48
	2	30	29.82	99.39%	SD-0.6042
	3	30	29.67	98.91%	%RSD-0.6073
150%	1	45	45.3	100.68%	Mean -100.20
	2	45	44.73	99.39%	SD-0.6977
	3	45	45.23	100.52%	%RSD-0.696

Table 5: Results showing Ruggedness

Analyst 1		
Concentration (µg/ml)	**Absorbance**	**Statistical analysis**
15	0.414	
15	0.416	Mean -0.4158
15	0.415	SD-0.00147
15	0.418	%RSD-0.35
15	0.417	
15	0.415	
Analyst 2		
15	0.414	
15	0.417	Mean -0.4156
15	0.413	SD-0.00216
15	0.415	%RSD-0.519
15	0.416	
15	0.419	

Table 6: Results showing Robustness

Room temperature		
Concentration (µg/ml)	**Absorbance**	**Statistical analysis**
15	0.414	Mean -0.414
15	0.413	SD-0.001
15	0.415	%RSD-0.241
Temperature 29^{0}C		
15	0.412	Mean -0.4123
15	0.413	SD-0.518
15	0.412	%RSD-0.110

Table 7: Results of analysis of formulations

Drug	Labeled amount (mg/tab)	Amt present	SD
Maraviroc	150	149.78	99.85 ± 0.513

CONCLUSIONS

It could be concluded that the developed method for estimation of Maraviroc in pharmaceutical dosage forms and in bulk is simple sensitive, accurate, precise, reproducible, and economical. The proposed method can be used for routine quality control analysis of Maraviroc in bulk and pharmaceutical formulation.

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