World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

HPLC method development and validation for the estimation of methyl prednisolone in marketed formulation

Shivani Jaiswal [1,*], BK Dubey [1], Deepak Basedia [1], Sunil Shah [2], Vivek Singh Thakur [2], Ashok Kumar Budhwani [1] and Ishika Mishra [1]

[1] *Technocrats Institute of Technology Pharmacy, BHEL Bhopal Madhya Pradesh, India.*
[2] *TIT College of Pharmacy, BHEL Bhopal Madhya Pradesh, India.*

World Journal of Biology Pharmacy and Health Sciences, 2024, 20(01), 276–288

Publication history: Received on 01 June 2024; revised on 12 July 2024; accepted on 15 July 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.20.1.0410

Abstract

In the present research work, a successful attempt was made for "Method, development and validation for the estimation of drug in marketed formulation" which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation. HPLC method was found to be linearin the range of1- 5 µg/ml methyl prednisolone with the correlation coefficient near to one (0.999) respectively. The validation and the reliability of proposed method were as sessed by recovery study. The recovery of added standards (80%, 100% 120%) was ranging near to one for atorvastatin and clopidogrel respectively. Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for constant flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing the data generated were used. The is ocratic mobile phase consisted of Methanol: Acetonitrile in the ratio of 50:50v/vat a flow rate of 1.0ml min^{-1}. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 254.0 nm was selected as the detection wave length for UV-vis. Detector.

Keywords: HPLC; Methyl prednisolone; Acetonitrile; Atorvastatin; Reproducibility

1. Introduction

1.1. Chromatographical method

Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phase and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase. As a consequence, solutes are eluted from the system as local concentrations in the mobile phase in the order of their increasing distribution coefficients with respect to the stationary phase; a separation is achieved.

Due to several advances made in the chromatographic techniques, it can be used for separation, identification and quantification purposes. Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients, to a number of inert materials like diluents, disintegrants, colors and flavors. Inorderto ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components prior to quantitative analysis.

*Corresponding author: Shivani Jaiswal

1.2. High performance liquid chromatography

Liquid chromatography is based upon the phenomenon that, under the same conditions, each component in a mixture interacts with its environment differently from other components. Since HPLC is basically a separating technique, it is always used in conjunction with another analytical tool for quantitative and qualitative analysis.

Advances in column technology, are high pressure pumping systems and sensitive detectors which have transformed liquid column chromatography into a high speed, high efficiency method of separation. This advanced technology is based up on the use of small bore (2.5 mm – internal diameter) columns and small particle size (3-50µm) that allow fast equilibrium between stationary and mobile phases. This small particle column technology requires high pressure pumping system capable of delivering the mobile phase at high pressure, as much as 300atmospheres,toachieve flow rates of several ml per minute. Since it is often necessary to use small amounts of analyte (usually less than 20 µg) with the column packing, sensitive detectors are needed.

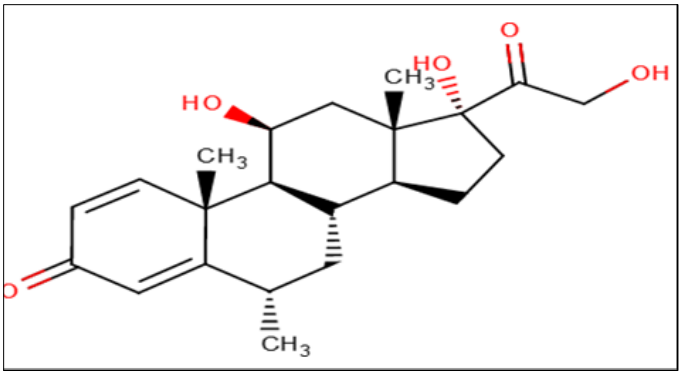

Figure 1 Structure of Methyl prednisolone

2. Material and method

2.1. Standards and Reagents

2.1.1. Standard

Table 1 standard name and source

Standard	Source
MPS (Methylprednisolone)	Bio plus life science, Bangalore

2.1.2. Sample

Table 2 Sample name and source

Sample	Dose
GEMDROL-4tab	Methylprednisolone4mg

2.1.3. Reagents used in experiment

Table 3 Reagent used in experiment and source

Chemical/ Reagent	Grade	Manufacturer
Methanol	HPLC Grade	Merck specialties Pvt, Ltd. Mumbai
Water	HPLC Grade	Merck specialties Pvt, Ltd. Mumbai

Acetonitrile	HPLC Grade	Merck specialties Pvt, Ltd. Mumbai

2.1.4. Apparatus/Equipment's used in experiment

Table 4 Types of Apparatus/Equipment's used in experiment

Components	Volume	Type
Volumetric flasks	10 ml,2 5 ml, 50 ml, 100 ml	Boro silicate glass type I
Pipettes	1 ml,2 ml,5 ml,10 ml	Boro silicate glass type I
Measuring cylinder	100 ml	Boro silicate glasstype I
Beaker	100 ml,250 ml,500 ml	Boro silicate glass type I
Whatman Filter	-	Filter Paper No.41

2.1.5. Instrument at Melting Range

Table 5 Instrument at Melting Range

Component	Brand/Model/ Software	Manufacturer/ Supplier
Melting point Apparatus	Chemiline	CL-725

2.1.6. HPLC complete setup supplier name and model

Table 6 HPLC complete setup supplier name and model

Component	Brand/Model/ Software	Manufacturer/Supplier
HPLC	Waters	Waters
HPLC Column	C18(25cm x0.46cm)Hypersil BDS	Agilent Technologies
Pump	515	Waters
Ultrasonic WaterBath	Fast Clean	Ultrasonic cleaner
pH meter	-	Electroquip's Digital pH meter
Analytical Balance	AUX-200	Shimadzu

2.1.7. UV Visible Spectrophotometer setup supplier name and model

Table 7 UV Visible Spectrophotometer setup supplier name and model

Component	Rand/Model/Software	Manufacturer/Supplier
UV Visible spectrophotometer	Labindia3000Plus	Lab India
Cuvette	Quartz cuvette	Shimadzu Corporation, Kyoto, Japan
Analytical Balance	AUX-200	Shimadzu

2.2. Identification and characterization of drugs

2.2.1. Physical characterization of drug

The drugs MPS was physically characterized on the beginning of appearance, color and odor. All these parameters were recorded and compared with the literature.

2.2.2. Melting point determination

The melting point determined used for the strength of mind of melting point of MPS by the open capillary methods. The melting point of drug was recorded and compared with literature values. The Melting point of MPS was found 230-2320C respectively.

Identification byIR:

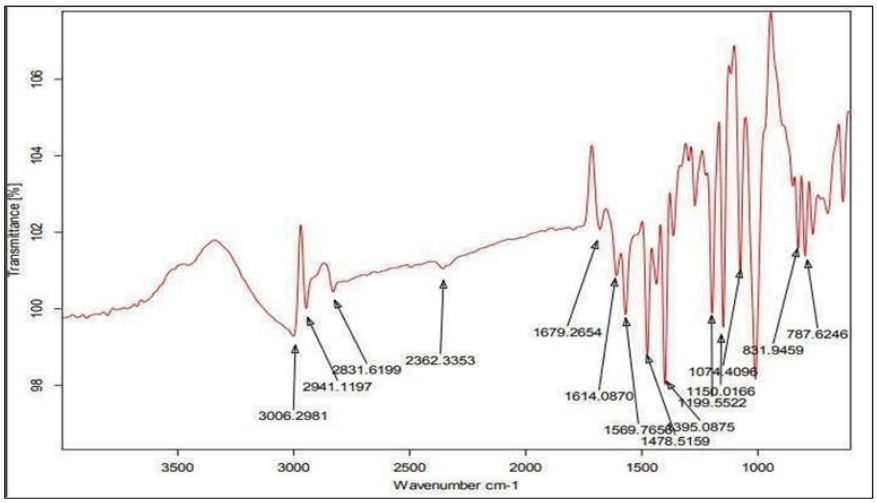

Figure 2 IR spectra of Sample MPS

Table 8 Interpretation of Methyl prednisolone

S.No.	Observed (wave number)	Functional Group
1.	3006.2981	O-H stretching
2.	2831.6199	C-H stretching
3.	1614.0870	C=O stretching

3. Results

3.1. Solubility

Solubility of MPS was determined at 25±1°C. Accurately weighed 10 mg MPS was added indifferent 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No.41.The filtrates were diluted suitably and analyzed visually.

Table 9 Solubility of drugs indifferent solvents

Solvent	Solubility
	Methyl prednisolone
Water	Slightly Soluble
0.1NHCl	Slightly Soluble
0.1NNaoH	Slightly Insoluble
Methanol	Freely Soluble
Ethanol	Soluble
Acetonitrile	Soluble

3.1.1. Selection of MobilePhase

Table 10 Mobile Phase Selection

Mobile Phase	**Ratio**	**Retention Time**
	Remark	**MPS**
Water: Methanol	50:50 v/v	Poor Resolution
Water: Acetonitrile	50:50v/v	Poor Resolution
ACN: Methanol	50:50 v/v	Most suitable
		2.072

3.1.2. Chromatograms of mobile phase trial

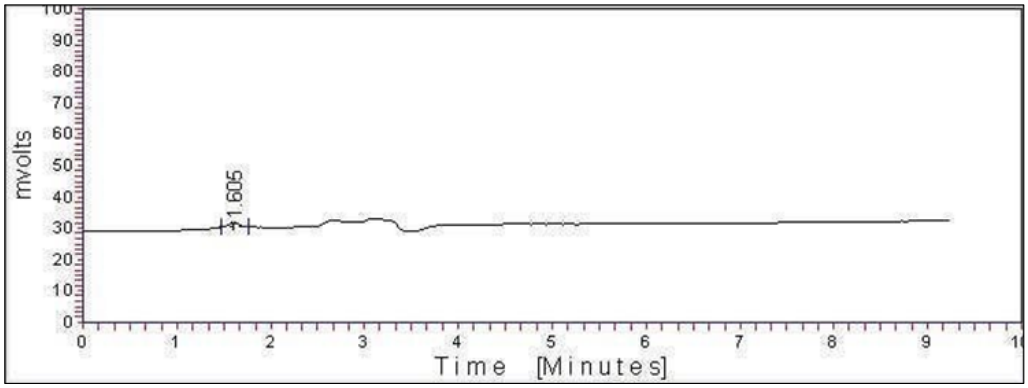

Figure 3 Trail graph of Methyl prednisolone in Water: Acetonitrile(50:50 v/v)

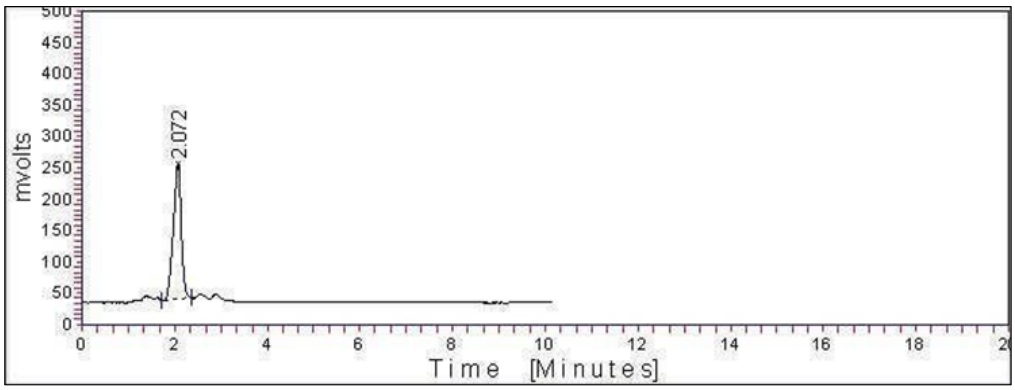

Figure 4 Suitable graph of Methyl prednisolone in Acetonitrile: Methanol(50:50 v/v)

3.1.3. Selection of Diluent

Diluents used for preparation of sample were compatible with mobile phase and no any significant affectretention and resolution of analyte. After various trials methanol was used as diluents.

3.1.4. Selection of separation variable

Table 11 Separation Variable

Variable	**Condition**
Column	
Dimension.	250mmx4.60mm
ParticleSize	5µm
BondedPhase	Octadecylsilane(C18)
MobilePhase	
Methanol	50
Acetonitrile	50
Diluent	**Methanol**
Flowrate	1.0 ml/min
Temperature	Ambient
Sample Size	20 l
Detection wavelength	254nm
Retention time	
Methylprednisolone	2.072±0.2min.

Table 12 Linearity of MPS

Standard Concentration µg/ml	**Area under Curve(AUC)**						**Mean**
	Rep-1	**Rep-2**	**Rep-3**	**Rep-4**	**Rep-5**	**Rep-6**	
1	215.56	225.658	210.478	220.789	232.745	236.658	23.649
2	436.65	445.658	449.987	425.658	432.145	434.789	37.483
3	645.85	650.569	635.587	642.478	631.145	638.145	40.630
4	845.65	855.658	835.658	835.785	825.745	835.745	39.042
5	1054.5	1045.58	1025.65	1035.22	1045.58	1036.65	040.54
CorrelCoeff (r2)							0.999
Slope(m)							207.2
Intercept(c)							12.22

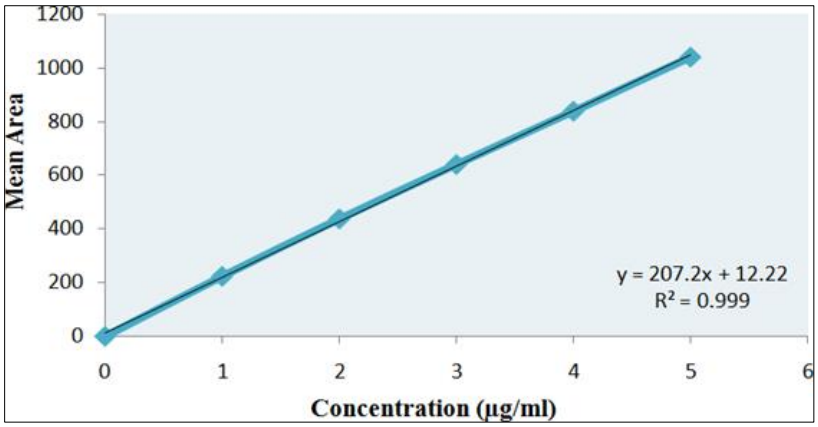

Figure 5 Calibration Curve of MPS

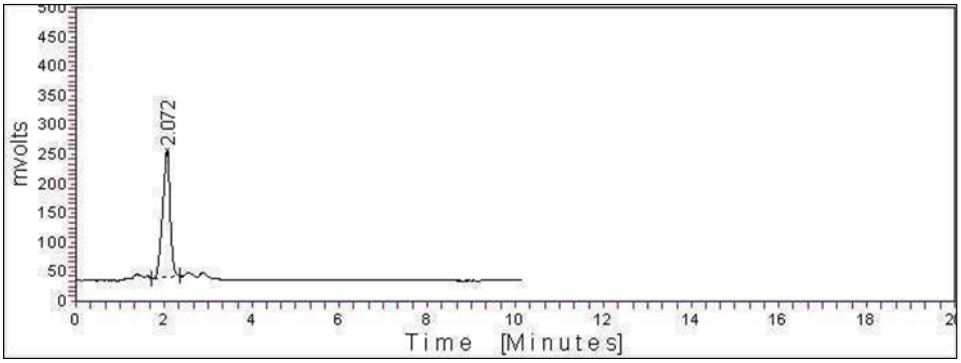

Figure 6 Chromatogram of MPS

3.2. System Suitability Parameters

Table 13 System Suitability Parameters of MPS

System suitability Parameter	RT	AUC	Theoretical plates	Trailing factor
Rep-1	2.072	215.568	3025	2
Rep-2	2.075	225.658	3036	8
Rep-3	2.074	210.478	3045	5
Rep-4	2.078	220.789	3058	1
Rep-5	2.073	232.745	3065	5
Rep-6	2.071	236.658	3041	4
Mean	2.074	223.649	3045.000	08
S.D.	0.002	9.156	13.329	34

3.3. Laboratory Sample Analysis

Table 14 Laboratory Sample Analyses

Standard Number	Concentration of MPS (µg/ml)
1.	1
2.	2
3.	3
4.	4
5.	5

3.4. Validation of developed Method

3.4.1. Linearity

Table 15 Response Ratio Data for Linearity of MPS

Concentration (µg/ml)	Mean AUC	Response Ratio
1	223.64	223.649
2	437.48	218.741
3	640.62	213.543
4	839.04	209.760
5	1040.54	208.109
Mean		214.761
SD		6.434
%RSD		2.996

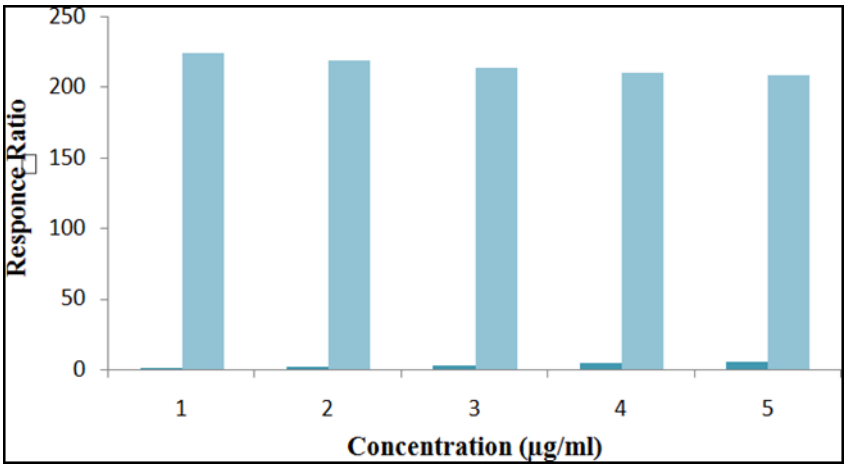

Figure 7 Response Ratio Curve of MPS

3.4.2. Specificity

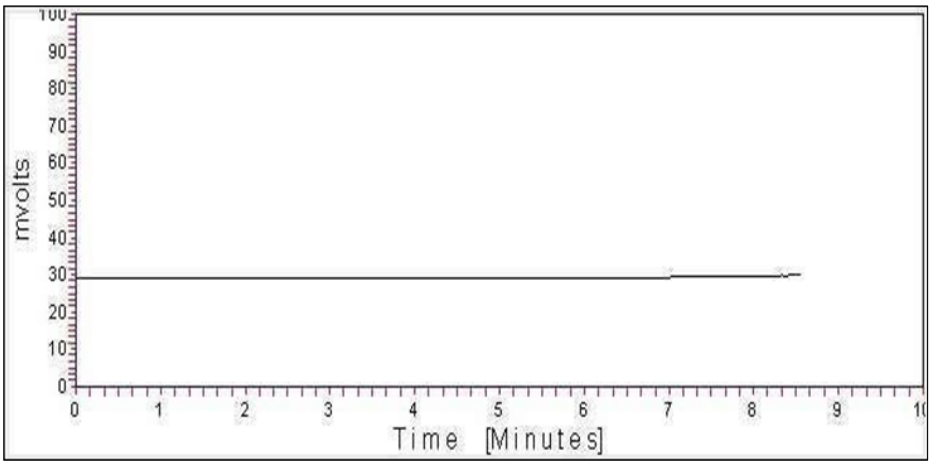

Figure 8 Chromatogram of Blank

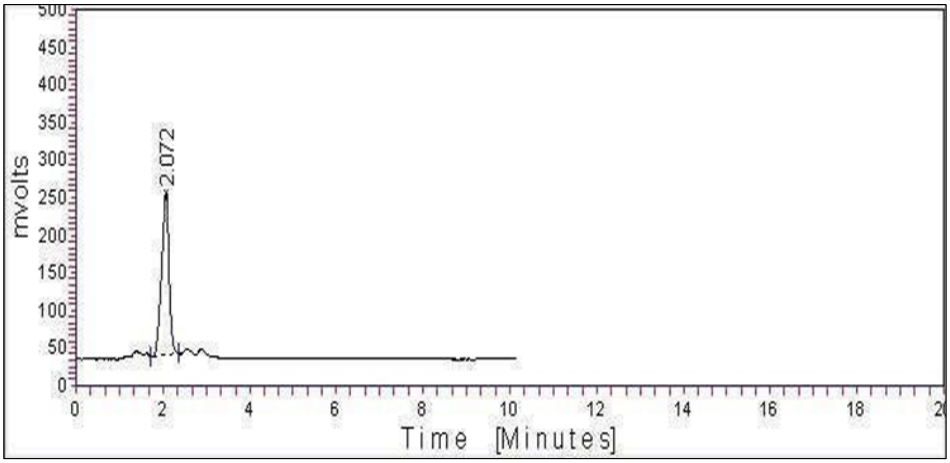

Figure 9 Chromatogram of the drug

3.4.3. Accuracy

Table 16 Recovery Study of MPS (80% Level)

Conc.of sample (µg/ml)	**Amt. Added (µg/ml)**	**Conc.Found.(µ g/ml)**			**conc.Found**			**Mea n % con c.**
		Rep- 1	**Rep- 2**	**Rep- 3**	**Rep-1**	**Rep-2**	**Rep-3**	
1	0.8	0.78	0.77	0.79	97.50	25	98.75	97.50
2	1.6	1.59	1.59	0.6	99.38	75	100.00	99.38
3	2.4	2.39	2.41	0.38	99.58	0.42	99.17	99.72
							MEAN	98.87
							SD	0.976
							%RSD	0.987

Table 17 Recovery study of MPS (120%Level)

Conc.of sample (µg/ml)	Amt. Added (µg/ml)	Conc.Found.(µ g/ml)			conc.Found			Mea n % con c.
		Rep- 1	Rep- 2	Rep- 3	Rep-1	Rep-2	Rep-3	
1	1.2	1.19	1.18	1.19	99.17	98.33	99.17	98.89
2	2.4	2.39	2.4	2.38	99.58	00.00	99.17	99.58
3	3.6	3.58	3.41	3.38	99.44	94.72	93.89	96.02
							MEAN	98.16
							SD	1.543
							%RSD	1.572

3.4.4. Precision

The stock solution was prepared. The precision is established in three differences:

- Intermediate Precision
- Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for MPS reported in table.

Table 18 Day-to-Day variation of MPS

Conc. Rep.	Concentration found(g/ml)					
	1	2	3	4	5	
Day1	1	1.98	2.98	3.95	4.98	
Day2	8	1.95	2.95	3.96	4.95	
Day3	5	1.92	2.85	3.98	4.85	
MEAN	9	1.96	2.95	3.97	4.95	
%MEAN	50	98.13	98.17	99.31	98.90	98.60
SD	30	0.030	0.068	0.015	0.068	0.04
%RSD	30	0.031	0.069	0.015	0.069	0.04

3.4.5. Analyst to Analyst:

Table 19 Analyst to analyst variation of MPS

Concrep.	Concentration found(g/ml)				
	1	2	3	4	5
Analyst 1	0.99	1.98	2.98	3.95	5.01
Analyst 2	0.95	1.96	2.96	3.89	4.98
MEAN	0.98	1.98	2.98	3.95	5.00
%MEAN	00	00	99.33	98.67	99.93
SD	28	14	0.014	0.042	0.021

%RSD	29	14	0.014	0.043	0.021

3.4.6. Repeatability

Table 20 Repeatability of MPS

Conc. Rep.	**Concentration found(µg/ml)**					
	1	**2**	**3**	**4**	**5**	
Replicate- 1	0.99	1.98	2.98	3.95	4.98	
Replicate- 2	0.98	1.95	2.95	3.96	4.95	
Replicate- 3	0.95	1.92	2.85	3.98	4.85	
Replicate- 4	0.95	1.89	2.78	3.92	4.95	
Replicate- 5	0.96	1.99	2.99	3.89	4.88	
MEAN	0.97	1.96	2.93	3.95	4.94	
% MEAN	97.17	97.75	97.50	98.75	98.70	98.204
S D	0.018	0.042	0.091	0.035	0.054	0.049
%RSD	0.019	0.043	0.094	0.036	0.055	0.05

3.4.7. Robustness

Table 21 Robustness of MPS

Conc. Rep.	**Concentration found(g/ml)**					**Mean**
	1	**2**	**3**	**4**	**5**	
Replicate-1	0.99	1.95	2.96	3.98	4.99	
Replicate-2	0.95	1.99	2.85	3.99	4.89	
Replicate-3	0.96	1.98	2.78	3.95	4.88	
Replicate-4	0.98	1.87	2.98	3.89	4.96	
Replicate-5	0.97	1.89	2.96	3.99	4.98	
MEAN	0.975	1.947	2.922	3.967	4.950	
%MEAN	97.500	97.333	97.389	99.167	99.000	
SD	0.016	0.054	0.087	0.042	0.051	
%RSD	0.016	0.055	0.089	0.043	0.052	

3.4.8. Detection Limit and Quantitation Limit

Table 22 LOD and LOQ of MPS

Name	**LOD(g/ml)**	**LOQ(g/ml)**
MPS	0.15	0.48

3.5. Analysis of both the drug in Tablet Sample

Table 23 Result of assay of tablet formulation

MPS*

Label Claim(mg)	4mg
%Found(mg)	3.99
%Assay	99.75
%RSD	0.032

*Average of three determination

4. Conclusion

The proposed methods were found to be linear in the range of 1-5µg/ml with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] B.Ramu,N.RamakrishnaandB.Shivashanker.Formulationoflamotrigineorodispersibletablets by using new generation superdisintegrants.World journal of pharmacy and pharmaceutical sciences.2015:4(06):631-643.

[2] Prakash goudanavar, shivam H shah, doddayyahiremath. Development and characterization of lamotrigine orodispersible tablets: inclusion complex with hydroxypropyl β cyclodextrin. International Journal of Pharmacy and Pharmaceutical Sciences.2011;3(3):208-214.

[3] MagdaTargaMartins,ClésioSoldatelliPaim, MartinSteppe.Developmentofadissolution test forlamotrigine in tablet form using an ultraviolet method. Brazilian Journal of Pharmaceutical Sciences.2010;46(2):179-186.

[4] R. R. Poonuru and C. S. R. Gonugunta. Bimodal Gastroretentive Drug Delivery Systems of Lamotrigine.FormulationandEvaluation.IndianJournalofPharmaceutical Sciences2014:476- 482.

[5] Adriana Ruiz, Fanny Cuesta , Sergio Parra, Blanca Montoya, Margarita Restrepo, RosendoArchbold, Lina Peña and Gloria Holguín.Bioequivalence Evaluation of Two Formulations of Lamotrigine Tablets in Healthy Volunteers. Journal of J Bioequivalence &Bioavailability.2012;4(3):030-034.

[6] ArtiMohanr and S.K.Ghosh.Development and Validation of UV/Visible Spectrophotometric Method forthe Estimation of Lamotrigine in Bulk and Pharmaceutical Formulations.American Journal of Phytomedicine and Clinical Therapeutics.2013;2(11):1246-1251.

[7] P.K.Lakshmi, Swetha Reddy, C. Kishore, B. Satish Reddy.Formulation and Evaluation of Oral Disintegrating Tablets of Lamotrigine Solid Dispersions. Iranian Journal of Pharmaceutical Sciences 2013: 9 (1):1-12.

[8] Geeta v yadav, sushma r singh. Gastroretentive drug delivery system of lamotrigine: in vivo evaluation. International Journal of Pharmacy and Pharmaceutical Sciences. 2014. 6(3): 279- 285.

[9] Anusha.M, S.Duraivel, DebjitBhowmik, PravinKhirwadkar.Formulation and evaluation of mouth dissolving tablets of lamotrigine. Indian Journal of Research in Pharmacy and Biotechnology.2015;3(2):120-123.

[10] Jatinderpal Singh, Rajeev Garg, and Ghanshyam Das Gupta. Enhancement of Solubility of Lamotrigine by Solid Dispersion and Development of Orally Disintegrating Tablets Using 32 Full Factorial Design. Journal of Pharmaceutics.2015:1-8.

[11] Ahmed Abdel Bary, Omaima N. El-Gazayerly and Mahmoud M. Alburyhi. Formulation of immediate release lamotrigine tablets and bioequivalence study. Journal of Chemical and Pharmaceutical Research.2013;5(10):266-271.

[12] Navdeep Saini and KoyalSaini.Quantitative determination of lamotrigi1ne in bulk and dosage formbyUVSpectrophotometry.JournalofAppliedPharmaceuticalScience.201101(03):113- 116.

[13] Rajesh Kumar Maheshwari, Ravish Jain and PriyaGeorge.Formulation Development And Evaluation Of Controlled Release Tablets Of Lamotrigine Using Mixed Solvency Concept. Bulletin of PharmaceuticalResearch 2015;5(1):14-9.

[14] Bibaswan Mishra, Prasanta Kumar Biswal, Prasanna Kumar Dixit and ManasMahapatra. Formulationdevelopmentandevaluationoftastemaskedrapidlydissolvingfilmsoflamotrigine. European Journal ofPharmaceutical and Medical Research. 2017;4(1):447-