Novel analytical method development for estimation of Remdesivir in bulk byusing Bromocresol purple- Acid dye

R. Karthikeyan[1], VijaykumarSayeli[2],V.Vijayan[1], BinoyVargheseCheriyan[3], M.Sumithra[4],Semmal Syed Meerasa[5],M. Sakthiganapathi[1], G. Haema[1], V.Abishek[6]

[1]School of Pharmacy, Sri Balaji Vidyapeeth, SBV Campus, Pillayarkuppam, Puducherry -607 402, India

[2]Department of pharmacology, Mamata Medical College, Khammam, Telangana State, India.

[3]Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences,Chennai – 602 105.

[4]Department of pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, VISTAS, Chennai.

[5]Department of Physiology, College of Medicine, Shaqra University, Saudi Arabia

[6]Chettinad School of Pharmaceutical Sciences, Chettinad Academy of Research and Education, Chengalpattu, Tamil Nadu, India

Prof. R. Karthikeyan,
School of Pharmacy, Sri Balaji Vidyapeeth,
SBV Campus, Pillayarkuppam,
Puducherry, India
Email: professorrkn@gmail.com,
Mobile : 91-8056267514

Abstract:

A simple UV spectrophotometric method has been developed for the quantitative estimation of remdesivir (REM) by complex formation with an acid dye bromocresol purple (BCP). This method involves the formation of a yellow-coloured ion-pair complex ofbromocresol purplereagent with remdesivir using chloroform at pH 2.The resultant complex was measured at itsλ_{max} of 420 nm. Beer Lambert's range was found to be 4 to 12 µg/ml with a good correlation coefficient (R^2= 0.9977).The proposed method was validated as per ICH guidelines.The developed method has been used for the determination of remdesivir in bulk and can be adaptedfor pharmaceutical formulations.

Keywords: Ion-pair complex, Bromocresol purple, Remdesivir, Chloroform, ICH

Introduction:

Remdesivir is an antiviral drug, it was widely used throughout the world during the pandemic to treat COVID-19.Remdesivir (Figure 1) is a prodrug of 1'-cyano-substituted adenosine

nucleotide analogue that actively competes with ATP for incorporation into newly synthesized viral RNA by the corresponding RdRp complex. [1-4]

A literature survey revealed that several methods including UV, HPLC, and LC-MS have been reported for the estimation of Remdesivir.[5-8] In our present study we intended to develop a novel and simple extractive spectroscopic technique using bromocresol purple as an acid-dye reagent.

Materials and methods:

All the chemicals (methanol, HCl, KCl, BCP, chloroform) used in the study were of analytical grade. The API (remdesivir) was obtained as a gift sample from Hetero drugs Ltd. Shimadzu 1800 UV visible spectrophotometer and borosilicate glass wares were used throughout our study.

Preparation of standard and working stock solution:

Accurately weighed and transferred 10 mg of pure remdesivir drug into a 10 ml standard flask then made up the volume with methanol and labelled it as standard stock solution. About 10 ml of standard stock solution was pipetted and transferred intoa 100 ml standard flask and made up the volume with distilled water and labelled as working stock solution. [9,10]

Determination of absorption maxima:

The lambda max (or) absorption maxima of remdesivir wereobtained by scanning an 8mcg/ml solution of REM-BCP complex in the visible region (380 – 800 nm) using a UV-Vis spectrophotometer.

Validation parameters:

Construction of calibration curve/ Linearity

Suitable aliquots (0.4, 0.6, 0.8, 1, 1.2 ml) of working stock solutions were taken in five different separating funnels, 5 ml of chloroform, 2 ml of KCl – HCl buffer (pH 2), 2 ml of 0.05% w/w BCP were added in each separating funnel. The solution was allowed to stand for 20 minutes until the phases get separated. The chloroform layerswere collectedand scanned from 380 to 800 nm.The calibration curve was plotted using the absorbance of these solutions.

Limit of detection and Limit of quantitation

Using the calibration curve, the LOD and LOQ for remdesivir can be determined. The formulas

employed were:

LOD = 3.3 x Standard deviation / Slope

LOQ = 10 x Standard deviation / Slope

Accuracy and Precision

The accuracy and Precision of the proposed method were carried out as per ICH guidelines. Accuracy was carried out by taking the absorbance value of 3 solutions of varying concentrations and precision was carried out by taking the absorbance value of 6 solutions of the same concentration. [11,12] The results were tabulated.

Results and discussion:

Determination of absorption maxima:

The absorption maxima of the REM-BCP (yellow-coloured ion-pair) complex was found to be 420 nm. The proposed mechanism of the complex formation has been depicted in Figure 3

Validation parameters:

Calibration curve / Linearity

The overlain spectra of allfive solutions of varying concentration of REM-BCP complex has been given in Figure 4. The calibration curve of absorbance vs concentration was plotted (Figure 5) and a good correlation coefficient of 0.9977 was obtained.

LOD and LOQ:

The slope was determined, and LOD and LOQ were calculated using the formula as depicted in the methodology. The LOD and LOQ were found to be 0.006 and 0.013 mcg/ml respectively.

Accuracy and Precision

The absorbance values of all the samples were tabulated in Tables 1 and 2. The developed method was found to be accurate and preciseas the % purity was between 98-103% and the % RSD was found to be less than 2% respectively.

Conclusion:

A novel, simple, accurate and precise extractive spectrophotometric method for remdesivirhas been developed using an acid dye reagent and it can be successfully adopted in the quantitative estimation of remdesivir in bulk. This method can also be extended for routine analysis of pharmaceutical dosage forms of remdesivir.

Acknowledgement:

The authors are thankful to Mr. Y.V. Rao, Assistant Professor, VFSTR, Guntur. Andhra Pradesh for helped us by giving free sample of Remdesivir for this research work.

Conflict of interest:

No conflict of interest

References:

1. Eastman RT, Roth JS, Brimacombe KR, et al. Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19 [published correction appears in ACS Cent Sci. 2020 Jun 24;6(6):1009]. ACS Cent Sci. 2020;6(5):672-683. doi:10.1021/acscentsci.0c00489
2. Remdesivir [Internet]. Uses, Interactions, Mechanism of Action | DrugBank Online. [cited 2022 Dec 24]. Available from: https://go.drugbank.com/drugs/DB14761
3. Gordon CJ, Tchesnokov EP, Woolner E, Perry JK, Feng JY, Porter DP, et al. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. Journal of Biological Chemistry. 2020;295(20):6785–97.
4. P. Sandeep, K. Ilango. A review of the pharmaceutical analytical profile of an antiviral drug: Remdesivir. Journal of Pharmaceutical Negative Results. 2022;:217–25.
5. Hamdy MMA, Abdel Moneim MM, Kamal MF. Accelerated stability study of the ester prodrug remdesivir: Recently FDA-approved Covid-19 antiviral using reversed-phase-HPLC with fluorimetric and diode array detection. Biomed Chromatogr. 2021;35(12):e5212. doi:10.1002/bmc.5212
6. Ibrahim Bulduk ; Erten Akbel. A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations [Internet]. Taylor &Francis. [cited 2022Dec 24]. Available from: https://www.tandfonline.com/doi/full/10.1080/16583655.2021.1991737
7. Pashaei Y. Analytical methods for the determination of remdesivir as a promising antiviral candidate drug for the COVID-19 pandemic. Drug Discoveries & Therapeutics. 2020;14(6):273–81.

8. Xiao D, John Ling KH, Tarnowski T, Humeniuk R, German P, Mathias A, et al. Validation of LC-MS/MS methods for determination of remdesivir and its metabolites GS-441524 and GS-704277 in acidified human plasma and their application in COVID-19-related clinical studies. Analytical Biochemistry. 2021;617:114118.
9. Souri E, Abbasi A, Amanlou M, Tehrani MB. Spectrophotometric determination of Aprepitant in bulk and pharmaceutical dosage forms using Bromocresol Green as a chromogenic reagent. Asian Journal of Chemistry. 2018;30(6):1331–4.
10. Shrivastava A, Saxena P, Gupta VB. Spectrophotometric estimation of tamsulosin hydrochloride by acid-dye method. Pharmaceutical Methods. 2011;2(1):53–60.
11. Abdelazim AH, Ramzy S. Spectrophotometric quantitative analysis of remdesivir using acid dye reagent selected by computational calculations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2022;276:121188.
12. Sadlapurkar AV, Barache UB, Shaikh AB, Dhale PC, Gaikwad SH, Lokhande TN. Statistically designed extractive spectrophotometric determination scheme for bismuth(iii) with 2-chlorobenzaldehyde thiocarbohydrazone: Analysis of environmental and real resources. Chemical Data Collections. 2022;37:100798.

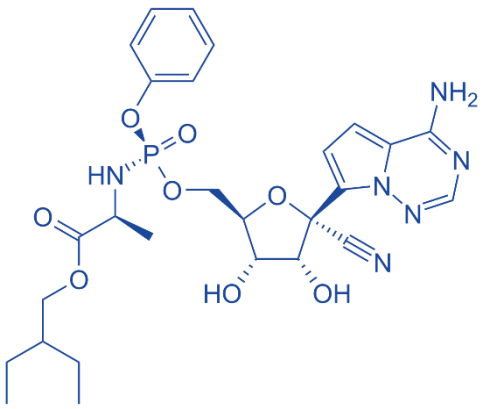

Figure 1. Structure of Remdesivir

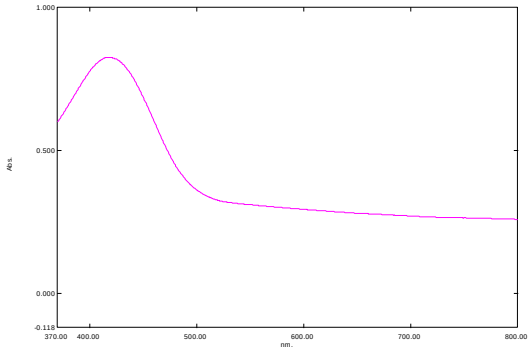

Figure 2. Spectra showing lambda max of REM-BCP complex

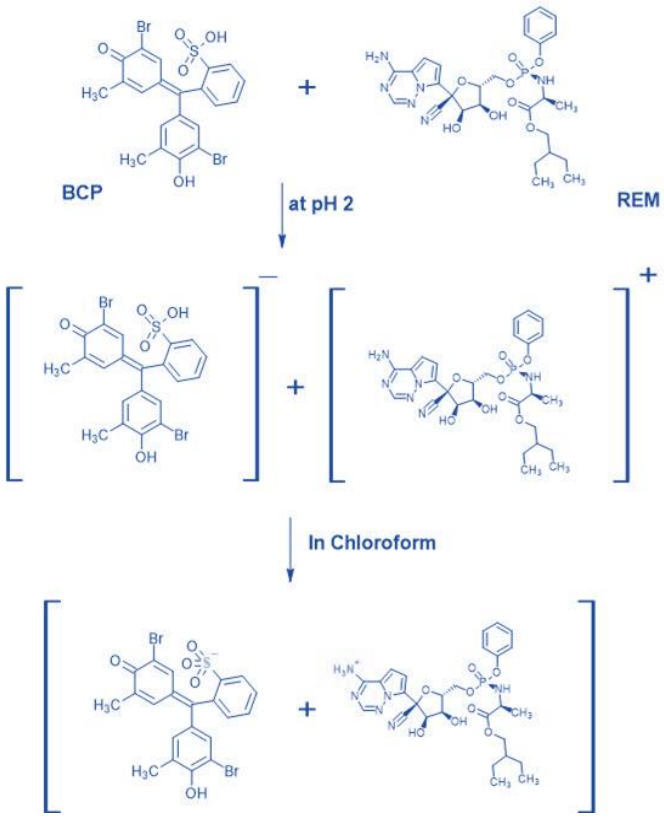

Figure 5. Proposed mechanism for REM-BCP complex

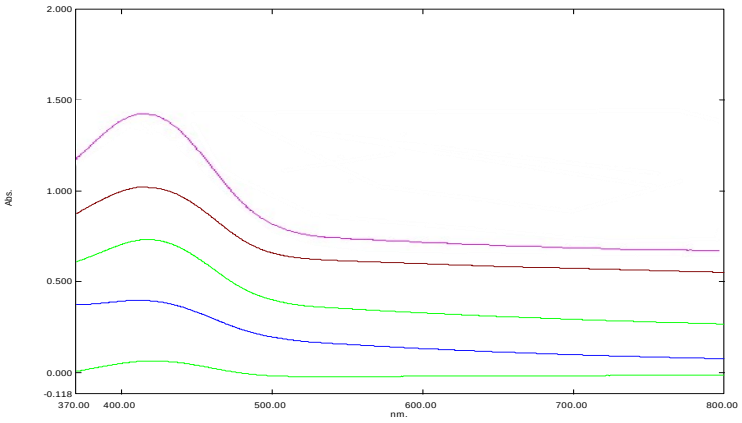

Figure 4.Overlain spectra of REM-BCP complex

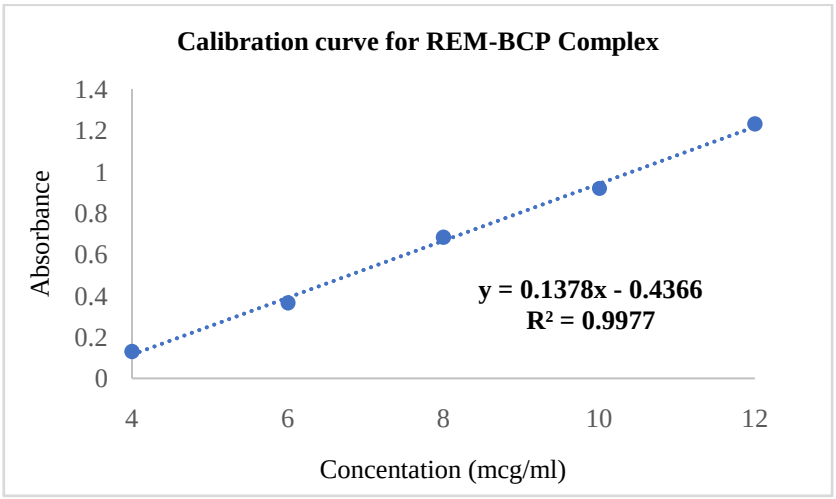

Figure 5.Calibration curve of REM-BCP complex

Table 1. Accuracy data for REM-BCP complex

Concentration	Absorbance	% purity (w/w)
4 mcg/ml	**0.132**	**101.82**
6 mcg/ml	**0.380**	**98.62**
8 mcg/ml	**0.675**	**99.86**

Table 2. Precision data for REM-BCP complex

Concentration	Absorbance	% purity (w/w)	%RSD
10 mcg/ml	**0.924**	**99.32**	**0.470**
	0.932	**100.51**	
	0.928	**99.86**	
	0.926	**99.24**	
	0.931	**100.22**	
	0.936	**101.47**	