CODEN: WJAPAC Impact Factor: 5.48 ISSN: 3049-3013

World Journal of Advance Pharmaceutical Sciences

Volume 3, Issue 8, Page: 8-15 Research Article www.wjaps.com

NOVEL, SIMPLE ROBUST ISOCRATIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF MAROPITANT CITRATE AND METACRESOL TABLET AND LIQUID PHARMACEUTICAL DOSAGE FORM BY USING RP HPLC WITH UV/PDA DETECTOR

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How to cite this Article: Amol S. Gaikwad, Manohar V. Lokhande*. (2026). NOVEL, SIMPLE ROBUST ISOCRATIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF MAROPITANT CITRATE AND METACRESOL TABLET AND LIQUID PHARMACEUTICAL DOSAGE FORM BY USING RP HPLC WITH UV/PDA DETECTOR. World Journal of Advance Pharmaceutical Sciences, 3(8), 8-15.

Article Info

Article Received: 02 June 2026,

Article Revised: 22 June 2026,

Article Accepted: 12 July 2026.

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DOI: https://doi.org/10.5281/zenodo.21706372

ABSTRACT

Maropitant citrate and Metacresol are widely used in veterinary and pharmaceutical formulations. Reliable analytical methods are essential for simultaneous estimation across diverse dosage forms to ensure quality, safety, and efficacy. To develop and validate a novel, simple, and robust isocratic RP-HPLC method for the simultaneous quantification of Maropitant citrate and Metacresol in injectable, gel, syrup, and tablet formulations. Chromatographic separation was achieved using a Baker Bond C8 column (250 × 4.6 mm, 5 µm) at 40 °C with an isocratic mobile phase of monobasic phosphate buffer and acetonitrile (70:30 %v/v), at a flow rate of 1.0 mL/min. Detection was performed at 220 nm using a UV/PDA detector. Metacresol eluted at ~3.8 minutes and Maropitant at ~6 minutes, with no interference at analyte retention times. The calibration curve showed excellent linearity (r^2 = 0.999) across 50–150% concentration range. Recovery values (97–103%) confirmed accuracy. Precision, intermediate precision, and robustness studies demonstrated reproducibility, with system suitability parameters unaffected by minor variations. The developed RP-HPLC method is novel, accurate, precise, and robust. It is suitable for routine quality control and regulatory analysis of Maropitant citrate and Metacresol in multiple pharmaceutical dosage forms.

KEYWORDS: Maropitant, Metacresol, RP-HPLC, method development, Validation, ICH.

INTRODUCTION

Maropitant (*Figure 1*) is a well-established antiemetic medication, available in tablet, injectable solutions, and syrup dosage forms. It is widely used in feline practice for the management of both acute and chronic vomiting.[1] The injectable formulation contains 10 mg/mL of maropitant citrate along with 6.3 mg/mL of meta-cresol, which serves as a preservative.[2] The IUPAC name is (2S,3S)-N-[(5-tert-butyl-2-methoxyphenyl) methyl]-2-(diphenyl methyl)-1-azabicyclo [2.2.2] octan-3-amine 2-hydroxypropane-1,2,3-tricarboxylic acid hydrate and molecular formula is $C_{38}H_{50}N_2O_9$.[3-4] Only one reported HPLC method reported for Maropitant and its related degradants in bulk drug substances.[5] Metacresol (*Figure 2*) is a colorless viscous liquid and chemical name is 3-methylphenol or 3-hydroxytoluene. It is commonly used as a bactericide in biotechnical processing and as a antimicrobial (preservative) content in pharmaceutical preparation like injection & syrup.[6] Several analytical quantitation methods are reported for estimation of M- cresol individually as well as in combination of other analytes.[7-11]

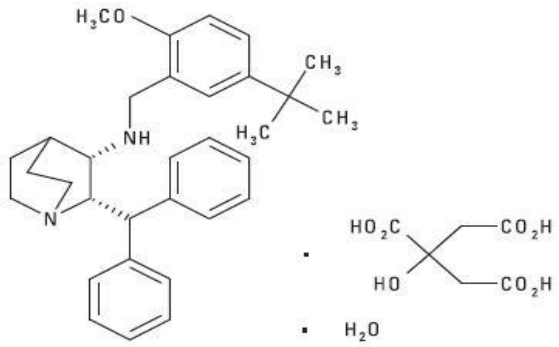

Figure 1: Molecular structure of Maropitant.

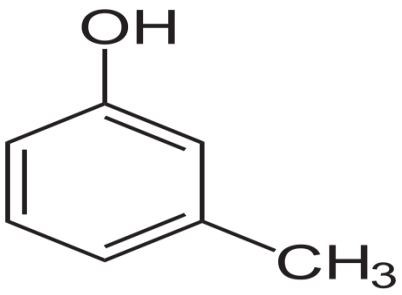

Figure 2: Molecular structure of Metacresol.

A comprehensive review of the available literature revealed that, to date; only a single HPLC-based analytical method has been reported for the detection of Maropitant citrate in its active pharmaceutical ingredient (API) form. However, no validated HPLC methods have been documented for the quantitative determination of Maropitant citrate across different pharmaceutical dosage forms such as tablets, injectables, and syrups. In response to this gap, we developed and validated a novel RP-HPLC method capable of simultaneously quantifying Maropitant citrate and its preservative content in a variety of commercial formulations.

MATERIALS AND METHODS

All reagents and buffers utilized in the method development and validation were of HPLC grade. Acetonitrile, sodium dihydrogen orthophosphate, and orthophosphoric acid (all supplied by Rankem) were employed, along with Milli-Q grade purified water. Analytical instrumentation included an HPLC system equipped with a PDA/UV detector (Waters), an analytical balance (Mettler Toledo), and a calibrated pH meter (Mettler Toledo). A reference standard of Maropitant citrate with a purity of 99.8% was obtained from Synzeal Research Pvt. Ltd and 99 % purity of metacresol procured from Vijrachem. Commercial pharmaceutical formulations tablets, injectables, and syrups were procured from the market for development and analysis.

Method optimization: The method development process involved experimenting with various proportions of phosphate buffer (pH 3.0) and acetonitrile on several C18 columns (250 × 4.6 mm, 5 µm), these conditions did not yield satisfactory peak symmetry and column efficiency. The optimal chromatographic performance was achieved using a Baker bond C18 column (250 × 4.6 mm, 5 µm), with an isocratic mobile phase consisting of 20 mM sodium phosphate buffer and acetonitrile in a 30:70 %v/v ratio. The column temperature was maintained at 45 °C, and the sample compartment was kept at 15 °C. All injections were performed using a 10 µL injection volume, and detection was carried out at a wavelength of 220 nm.

Mobile phase preparation: A 10 Mm buffer solution prepared, by dissolving sodium dihydrogen phosphate dihydrate in 800 mL of HPLC-grade water, followed by sonication to ensure complete dissolution. The pH of the solution adjusted to 3.1 using dilute orthophosphoric acid, then filtered through a 0.45 µm membrane filter and degassed prior to use. The mobile phase prepared by mixing the buffer with acetonitrile in a 30:70 %v/v ratio.

Standard preparation: About 50 mg of Maropitant standard was dissolved and diluted in 50 ml with diluent and labeled as Maropitant standard stock solution. About 40 mg of Metacresol standard was dissolved and diluted in 50 ml with diluent and labeled as Metacresol standard stock solution. Diluted 5 ml of Maropitant standard stock solution and 5 ml of metacresol standard stock solution with diluent.

Sample preparation for Tablet: A minimum of 20 tablets were accurately weighed to calculate the average tablet weight, finely ground using a mortar and pestle to achieve a uniform powder. A portion of this powder, corresponding to 50 mg of Maropitant, was transferred to a 50 mL volumetric flask. Approximately 35 mL of diluent was added, and the mixture was subjected to sonication for 20 minutes, with gentle mixing at 2-minute intervals to facilitate complete extraction. After sonication, the solution was allowed to cool to ambient temperature and diluted to the mark with the same diluent. The final solution was filtered through a 0.45 µm membrane filter, discarding the initial 5 mL of the filtrate. Subsequently, 5 mL of the filtrate was further diluted to 50 mL with diluent, mixed thoroughly, and analyzed by HPLC.

Sample preparation for Injectable/syrup: A quantity of the sample containing an equivalent of 50 mg of Maropitant was precisely weighed and transferred into a 50 mL volumetric flask. To this, 35 mL of the diluent was added, and the mixture was subjected to sonication for 20 minutes, with intermittent swirling at 2-minute intervals to ensure complete dissolution. Following sonication, the solution was brought to room temperature and the volume was adjusted to 50 mL using the same diluent. The resulting solution was filtered through a 0.45 µm membrane filter, with the initial 5 mL of the filtrate discarded. Subsequently, 5 mL of the clear filtrate was further diluted to 50 mL with diluent, mixed uniformly, and analyzed using the HPLC system.

System suitability parameters: In accordance with established chromatographic guidelines, key system suitability parameters were evaluated, including tailing factor (symmetry factor), column efficiency (theoretical plate count), retention time, and the percentage relative standard deviation (%RSD) of peak areas from five replicate injections of the standard solution. These parameters were assessed to ensure method consistency and compliance with the acceptance criteria outlined in USP General Chapter <621>. Specifically, the %RSD for peak areas was required to be not more than 2.0%, the tailing factor not more than 2.0, and the theoretical plate count not less than 2000.

Analytical Method Validation: The developed methodology validated as per ICH/USP/IP/BP validation guidelines in term of specificity, linearity, accuracy, precision, robustness.

Specificity: The specificity of the developed method was evaluated by injecting blank, standard, and sample solutions. The objective was to assess any potential interference at the retention time of the principal analyte. No co-eluting peaks were observed at the retention time of both the analytes in the blank chromatograms, confirming the method's selectivity. Additionally, peak purity was assessed using a photodiode array (PDA) detector to ensure that the analytes peaks are spectrally homogeneous and free from co-eluting impurities.[12]

Precision: Method precision was comprehensively assessed by evaluating three key parameters: system precision, method precision, and intermediate precision.

System precision was determined by injecting five replicates of the standard solution into the HPLC system under identical conditions. The percentage relative standard deviation (%RSD) of the resulting peak areas was calculated to ensure the repeatability of the injection process and to confirm the stable performance of the chromatographic system. Method precision involved preparing six individual sample solutions following the described analytical procedure and analyzing them under identical conditions. The assay results for these six preparations were used to compute the %RSD.[13] Intermediate precision was evaluated to determine the method's reproducibility under different conditions. Another set of six sample preparations was analyzed by a different analyst, using a different HPLC instrument and column, on a separate day. The combined assay results of twelve samples were used to calculate the %RSD, and the findings are summarized.

Accuracy: Accuracy in term of recovery was performed by preparing the samples from 50%, to 150% of working concentrations. All the preparations injected in triplicate in the HPLC and calculated the % drug recovered at each level.

Linearity: Linearity of the developed methodology performed by preparing different concentrations of the standard solutions from 50 to 150% level of working concentration and injected triplicate each level. The linearity graph plotted for area under the curve vs the concentrations and linear regression curve (r2) calculated.[14]

Robustness: Robustness refers to the ability of an analytical method to remain unaffected by small, deliberate variations in method parameters, thereby indicating its reliability during routine use. To evaluate the robustness of the developed method, intentional changes were made to critical experimental conditions, including column temperature, flow rate, detection wavelength, organic composition of the mobile phase, and pH of the buffer. Under each modified condition, the complete injection sequence was performed, and system suitability parameters such as retention time, tailing factor, theoretical plates, and %RSD were monitored to ensure consistent method performance.[15]

Solution stability and filter study: The solution stability of the all the preparations monitored at different time interval, like, 0, 8, 12, 24, and 48 hrs. The solution were stored at benchtop at room temperature and analyzed against fresh preparations at selected time points. In the filtration study, the sample filtered through the different makes of filter by discarding the first 0 ml, 2 ml, 5 ml filtrate and analyzed. The % assay of different filter VS mdi 0.45µ filter calculated and interpreted.

RESULTS AND DISCUSSION

The developed methodology validated as per ICH guideline and findings are reported as below.

Selectivity: No interference was observed at the retention time of the principal analyte in chromatograms of the blank and placebo solutions, confirming the method's selectivity. Furthermore, the peak purity assessment, as determined by the PDA detector[16], showed that the purity angle was less than the purity threshold, indicating that the Maropitant peak was spectrally pure and free from co-eluting impurities. Representative chromatograms for the blank, standard, and sample solutions are presented in *Figures 3 to 5, respectively.*

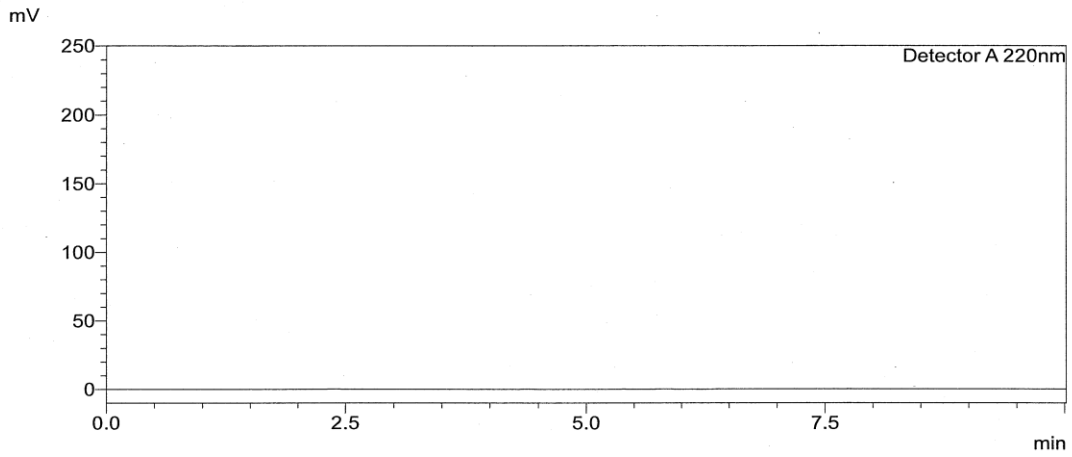

Figure 3: Tentative chromatogram of blank.

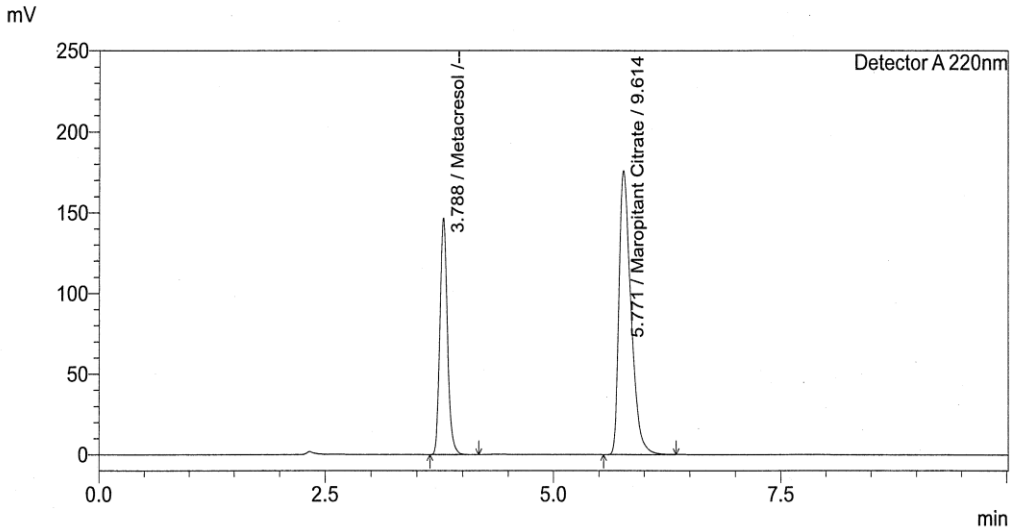

Figure 4: Tentative chromatogram of standard preparation.

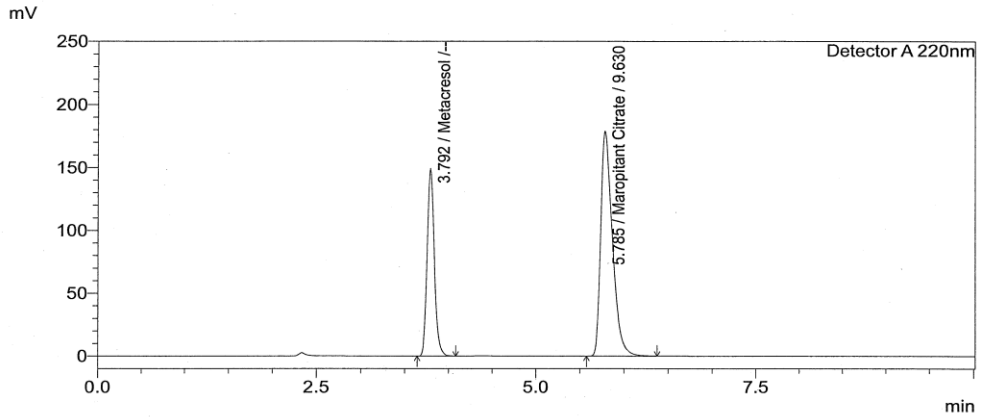

Figure 5: Tentative chromatogram of sample preparation.

Precision: The precision of the method was confirmed by analyzing five replicate injections of the standard solution, with the resulting %RSD found to be within the acceptable limit of not more than 2.0%, indicating reliable system performance.[17] Additionally, the assay results from six sample preparations analyzed during the method precision study, as well as six more from the intermediate precision study, demonstrated consistent results. The detailed assay values and corresponding statistical analysis are summarized in *Table 1and Table 2.*

Table 1: Method precision results Maropitant and metacresol.

Sr. No	Sample No.	% Assay of Maropitant.	% Assay of Metacresol.
1	Sample-1	99.6	102.3
2	Sample-2	98.6	100.8
3	Sample-3	99.7	99.8
4	Sample-4	100.5	100.8
5	Sample-5	102.1	100.4
6	Sample-6	98.5	101.4
Average		**98.8**	
Standard deviation		**1.33**	
% RSD		**1.34**	

Table 2: Intermediate method precision results Maropitant and metacresol.

Sr. No	Sample No.	% Assay of Maropitant.	% Assay of Metacresol.
1	Sample-1	98.8	97.9
2	Sample-2	100.3	101.2
3	Sample-3	101.4	100.3
4	Sample-4	99.2	98.6
5	Sample-5	99.6	99.4
6	Sample-6	97.9	100.7
Average		**99.5**	
Standard deviation		**1.21**	
% RSD		**1.22**	

Linearity: Linearity of the method was established over the specified concentration range, with the linear regression analysis for the principal peak yielding a correlation coefficient (r^2) of 0.9996, indicating excellent linearity. The corresponding linearity plots are illustrated in *Figures 6 and 7***,** area response in *Table 3 and 4*.

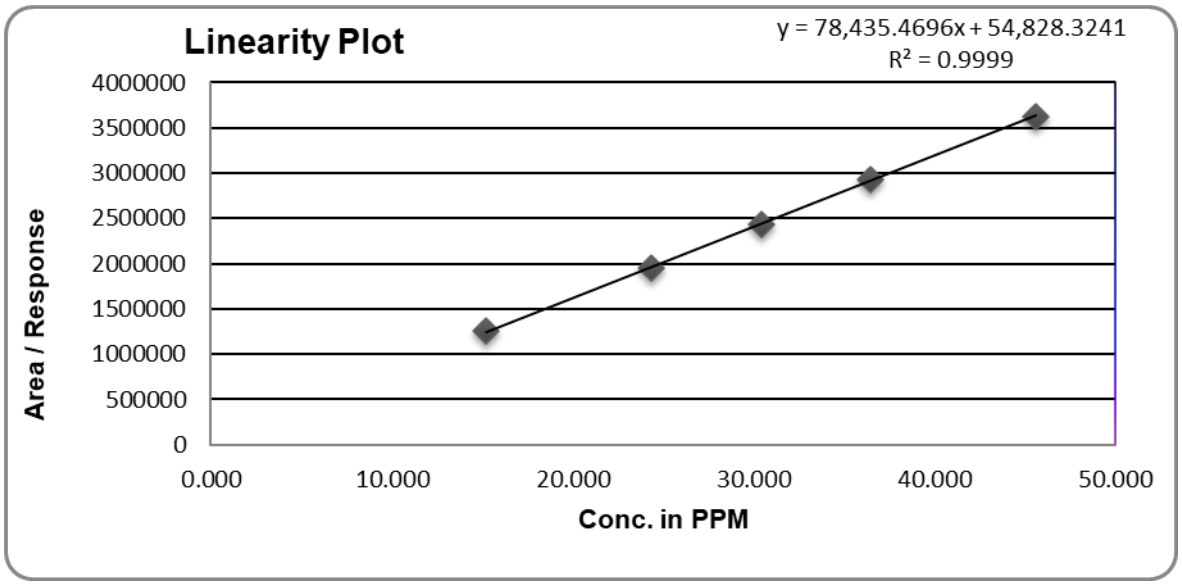

Figure 6: Linearity plots of Maropitant.

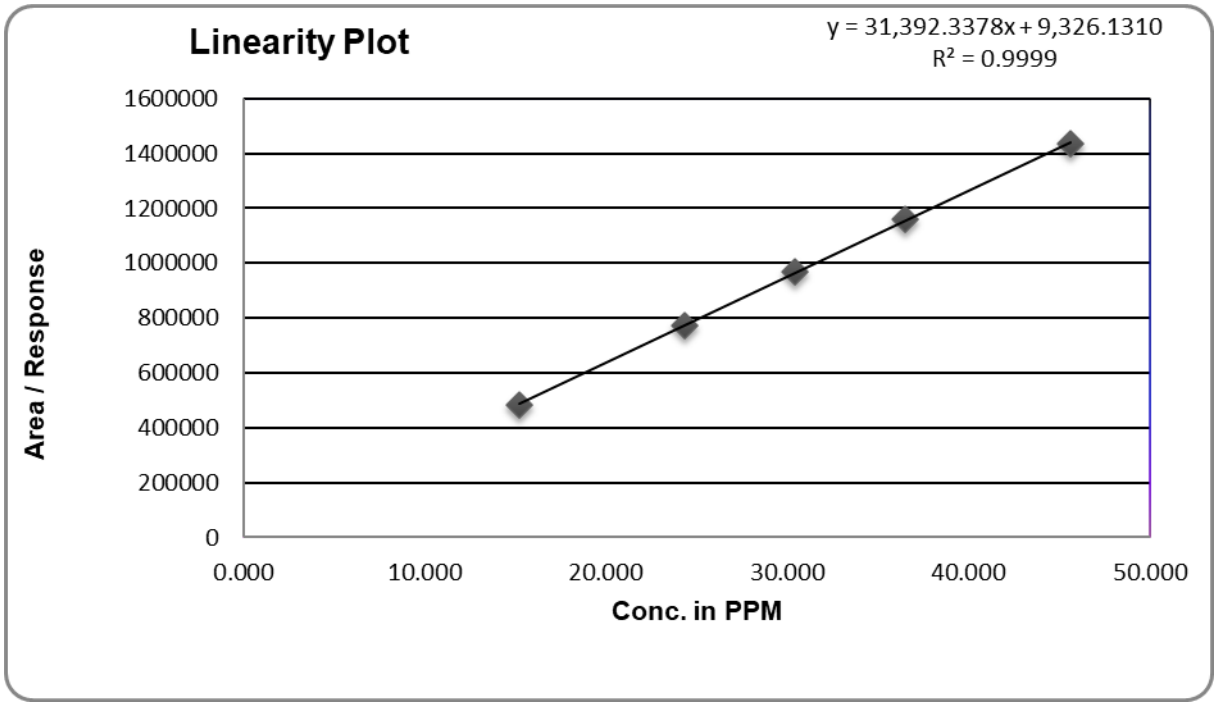

Figure 7: Linearity plots of Metacresol.

Table 3: Linearity response of Maropitant.

Conc. in PPM	Area / Response
15.200	1250169
24.320	1952126
30.400	2440158
36.480	2928187
45.600	3625693
Slope	**78435.4696**
Intercept	**54828.3241**
Correlation Coefficient [R]	**1.0000**
R^2	**0.9999**

Table 4: Linearity response of Metacresol.

Conc. in PPM	Area / Response
15.200	483126
24.320	773625
30.400	966930
36.480	1158316
45.600	1436269
Slope	**31392.3378**
Intercept	**9326.1310**
Correlation Coefficient [R]	**0.9999**
R^2	**0.9999**

Robustness: No significant changes observed in the robustness study. The % RSD and % assay at each deliberate change found well within the limits (2%). The theoretical plates are found more than 2000 and tailing factor less than 2 at all changes.

Solution stability and filter study: The standard preparation and sample preparation are stable up to 48 hrs. at room temperature. There should be minimum 5 ml discard sample preparation during the filtration study. It was observed that, below 2 ml discard, absorption of molecule in the filter and hence lowering in the assay value.

CONCLUSION

A straightforward and robust reverse-phase HPLC (RP-HPLC) method was successfully developed and validated for the accurate quantification of Maropitant and metacresol across various pharmaceutical dosage forms. All validation parameters including specificity,

precision, accuracy, linearity, robustness, and system suitability met the acceptable criteria outlined in regulatory guidelines. The method demonstrated no interference from blank, confirming its selectivity. Notably, the approach employs a simple isocratic elution, has a short runtime, and avoids the use of hazardous solvents, making it both cost-effective and environmentally friendly. To the best of our knowledge, this is the first reported RP-HPLC method capable of quantifying Maropitant and metacresol in multiple dosage forms. The developed method offers significant advantages in terms of time and cost savings, and is well-suited for routine quality control applications in the pharmaceutical industry.

ACKNOWLEDGMENTS

Grateful thanks to PTVA Management, Principal, and Head of Chemistry, Sathaye College, for their valuable support and guidance in facilitating my registration for the Ph.D. degree.

Conflicts of interest: No conflicts of interest.

Funding source: No funding.

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