RESEARCH ARTICLE

RP-HPLC Method Development and Validation For Simultaneous Estimation of Paracetamol and Mefenamic Acid in Pharmaceutical Suspension

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Received: 07th October, 19; Revised: 13th November, 19, Accepted: 15th December, 19; Available Online: 25th December, 2019

ABSTRACT

Paracetamol (PCT) and mefenamic acid (MFA), in combination, is recommended widely for the treatment of antipyretic and anti-inflammatory conditions. The present works carry a new simple, rapid, precise, accurate, and sensitized method of high-performance liquid chromatography with UV detection for simultaneous quantification of PCT and MFA. The samples are eluted in isocratic mode using a Phenomenex ODS 3V C_{18} (4.6mm × 250 mm i.d, with a particle size of 5µm) with the mobile composition of methanol: phosphate buffer p^H 7.1 (70:30) delivered at a flow rate of 1ml/min with the detection wavelength of 254 nm. It shows good linearity response in the concentration range of 15-35 µg/mL and 6-14 µg/ml for PCT and MFA with the retention times of 3.0 min and 4.8 min, respectively. The quantities of PCT and MFA in pharmaceutical suspension were found to be 99.01% and 101.02%, respectively. The method was quantitatively evaluated according to intracerebral hemorrhage (ICH) guidelines taking into consideration the required parameters, and the results obtained are within acceptable limits. So, the proposed method can be employed in the routine analysis and evaluation of MFA and PCT in both bulk and suspension dosage form.

Keywords: Paracetamol, Mefenamic acid, RP-HPLC, Validation.

International Journal of Pharmaceutical Quality Assurance (2019); DOI: 10.25258/ijpqa.10.4.4

How to cite this article: Kopparapu, V.N.K., Kasula, P., Ankinapalli, A.K.R. and Venkateswarlu, M (2019). RP-HPLC Method Development and Validation For Simultaneous Estimation of Paracetamol and Mefenamic Acid in Pharmaceutical Suspension. International Journal of Pharmaceutical Quality Assurance 10(4): 583-587.

Source of support: Nil

Conflict of interest: None.

INTRODUCTION

Paracetamol (PCT) (Figure 1) is chemically N - (4-hydroxy-phenyl) acetamide and is used as an analgesic and anti-pyretic agent.[1] It has a narrow therapeutic index–the therapeutic dose is close to the toxic dose.[2] Mefenamic acid (MFA) (Figure 2) is 2-[(2, 3-dimethyl phenyl) amino] benzoic acid, an anthranilic acid derivative, is a member of the fenamate group of nonsteroidal anti-inflammatory drugs (NSAIDs)[3]. It exhibits anti-inflammatory, analgesic, and antipyretic activities. Similar to other NSAIDs, MFA inhibits prostaglandin synthetase.[4] Both the drugs have been co-formulated and widely used to reduce pyretic and inflammatory effects in combination. These drugs are official in Indian Pharmacopeia,[5] British Pharmacopeia,[6] and United States Pharmacopeia.[7] Literature review reveals that various analytical techniques viz, UV spectrophotometry,[8-11] High-performance liquid chromatography (HPLC),[12-13] and High-performance thin layer chromatography[14] were reported for the

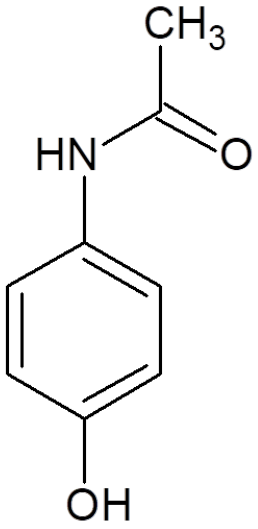

Figure 1: Chemical structure of PCT

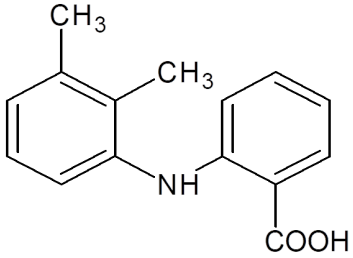

Figure 2: Chemical structure of MFA

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analysis of PCT and MFA in single and their combination in pharmaceuticals.

The reported methods are reviewed, and there is no single method reported for analysis of these two drugs in oral suspensions. Preparation of sample for analysis of suspension is inexpensive and rapid, hence the objective of present work is to develop a reliable method for simultaneous estimation of PCT and MFA in oral suspension and to validate the method as per ICH guidelines.[15-16]

MATERIALS AND METHODS

Instrumentation

The analysis was performed on a chromatographic system of Shimadzu prominence consisting of LC 20 AD liquid pump equipped with a manual 20 µL sample injection loop. Chromatographic separation was achieved on Phenomenex ODS 3V C_{18} (4.6mm × 250 mm i.d, with a particle size of 5µm) analytical column with a UV detector. Data acquisition was made with LC solution software.

Chemicals and reagents

Methanol of HPLC grade and the chemicals potassium dihydrogen phosphate, sodium hydroxide of analytical grade, was procured from Merck India Ltd, Mumbai. Reference standards of PCT and MFA were procured from Hetero drugs Ltd, Hyderabad. Suspension dosage form Meftagesic-P with a label claim of 125 mg PCT and 50 mg MFA for every 5mL, manufactured by Blue cross laboratories Ltd was purchased from the market and used for the study.

Preparation of phosphate buffer pH (7.1)

Place 50 mL of 0.02 M potassium dihydrogen phosphate in 200 mL of volumetric flask add the sufficient volume of 0.1M sodium hydroxide for adjusting the pH to 7.1 and then add water to volume.[17]

Preparation of the mobile phase

Filtered and degassed mixture of methanol and phosphate buffer (pH-7.1) in the ratio of 70:30 and filter through a 0.45µ membrane filter, and it is employed as a solvent for analysis.

Standard preparation and calibration graph

To a 50 mL volumetric flask accurately weighed 62.5 mg of PCT and 25 mg of MFA was added, dissolved in a minimum quantity of solvent and finally made up to the mark making a concentration of 1250 µg/mL for PCT and 500 µg/mL for MFA taken as Standard stock-I. From the above-prepared stock solution, 1 mL is diluted to 10 mL to give a concentration of 125 µg/mL for PCT and 50 µg/mL for MFA, taken as Standard stock-II. All the required aliquots for the study was prepared from Stock-II. 1.2 mL, 1.6 mL, 2 mL, 2.4 mL, 2.8 mL of Stock-II was pipetted and made up to mark in 10 mL volumetric flasks to make concentrations of 15-35 µg/mL and 6-14 µg/mL for PCT and MFA respectively.

Sample preparation

For suspension, obtaining uniformity prior to sampling is necessary by handshaking mechanical shaking, vortexing, or sonication to ensure uniformity. Samples may then be taken volumetrically or gravimetrically.[18-20]

After vortexing the sample is transferred volumetrically, accurately 25 mL of suspension (each 5mL consists of 125 mg of PCT and 50 mg of MFA) into 250 mL of volumetric flask. Add 125 mL of the mobile phase, then warm it for 10 minutes. Filter the solution through vacuum suction, then make up the filtrate up to 250 mL with mobile phase solution (1000 µg/mL). Transfer 10 mL from the above stock solution to 100 mL volumetric flask and make up to 100 mL with mobile phase (100 µg/mL). Transfer 25 mL of above solution into 100 mL volumetric flask and make up to 100 mL with mobile phase to make a concentration of 25 µg/mL and is used for the analysis.

Method validation

The method was validated as per ICH guidelines for specificity, linearity, accuracy, precision, assay, and robustness.

System suitability parameters

System suitability parameters retention time (R_t), USP theoretical plate count (TP), USP tailing factor (T) and resolution were assessed from 6 injections of PCT and MFA standards of 15 µg/mL and 6 µg/mL concentrations respectively.

Specificity

Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the endogenous material at the retention times of PCT and MFA.

Linearity

Appropriate aliquots of standard stock solution of PCT and MFA were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 15-35 µg/mL for PCT, and 6–14 µg/mL for MFA and the solutions were injected into the system and chromatograms were recorded.

Accuracy

The accuracy of the method was determined by recovery experiments. A standard addition method was employed for this experiment. A known quantity of each drug substance (PCT and MFA) corresponding to 80%, 100% and 120% of the label claim of each drug was added. Each set of the addition was repeated three times. The accuracy was expressed as a percentage of analytes recovered.

Precision

The method was checked for both intra-day and inter-day precision.

Intra-day precision

Repeatability of the developed method was assessed by 9 determinations covering three concentrations each of three replicates. The % RSD was calculated for the results obtained.

Inter-day precision

Variation in the results for the developed method was assessed in 3 different days (n = 6). The % RSD was calculated for the results obtained.

Robustness

Typical variations, including change in flow rate (± 0.8mL of optimized flow rate), change in the organic phase composition of mobile phase (± 10 mL), and change in wavelength (± 1nm) were assessed in triplicate injections, and the %RSD was calculated.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In order to develop an isocratic reversed-phase HPLC method for the simultaneous determination of PCT and MFA in combined dosage form, the chromatographic conditions were optimized. The different compositions of the mobile phase were changed for getting better separation of these analytes. Thus, the mobile phase composed of the mixture of methanol and phosphate buffer in the ratio of (70:30 v/v) was finalized. The better separation, peak symmetry, and reproducibility were obtained with phenomenex C_{18}, 250mm x 4.6mm, 5 µm column. Both these analytes gave better responses at 254 nm wavelength using UV detector. The flow rate kept was 1.0 mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention times of PCT and MFA were found to be 3.0 min and 4.8 min, respectively. The optimized chromatogram is represented in Figure 3.

Validation

The proposed method was shown short elution time and good separation between PCT and MFA. The system suitability test was performed as per the ICH guidelines to confirm the suitability and the reproducibility of the method. Six consecutive injections of the standard solution were performed and evaluated for repeatability, tailing factor, theoretical plates and resolution. The tailing factor and theoretical plates were found to be within the acceptable limits as shown in Table 1.

The specificity of the method was checked by injecting blank and placebo. No chromatographic interference was observed from endogenous material.

The method was linear over the range 15–35 µg/mL and 6-14 µg/mL for PCT and MFA, respectively. The calibration curve was constructed by plotting the response factor against a concentration of drugs (Figure 4, Figure 5). The slope and intercept value for calibration curve was Y = 34161x+17957, R^2 = 0.9994 for PCT and Y = 41894x-4876.6, R^2 = 0.9993 for MFA. The results show an excellent correlation between the response factor and the concentration of drugs.

The accuracy of the method was determined by the standard addition method at three different levels, with each determination was performed in triplicate. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for PCT and MFA from the combination formulation are shown in Table 2. The results are well within the acceptable limits, and hence the method is accurate.

The developed method was validated for intra-day and inter-day precision. Six injections of mixed standards of 15 µg/

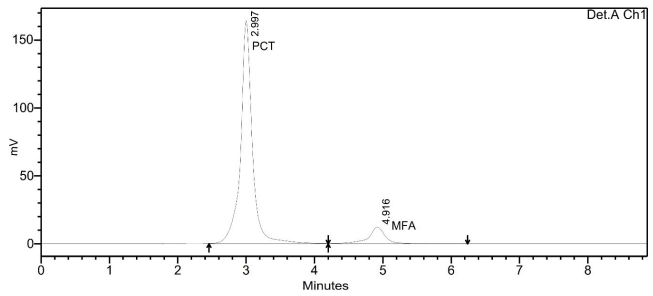

Figure 3: Optimised chromatogram of PCT & MFA

Table 1: System suitability parameters of PCT & MFA

Parameters	*PCT*	*MFA*	*Acceptable limits*
R_t (min)	3.0 ± 0.03	4.8 ± 0.10	≥ 2
TP	2485 ± 6	2800 ± 34	≥ 2000
T	1.1 ± 0.02	1.1 ± 0.02	≤ 2

Table 2: Accuracy data of the proposed method

	*% Recovery**	
Recovery Range	*PCT*	*MFA*
80%	100.64	99.43
100%	103.27	99.63
120%	100.94	99.26
Mean % Recovery	101.62	99.44

*Mean of % recovery of triplicate injections

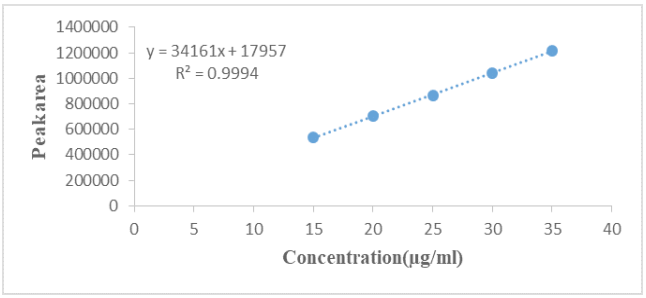

Figure 4: Calibration graph of PCT

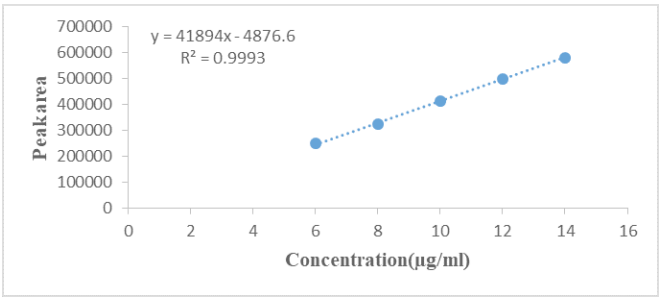

Figure 5: Calibration graph of MFA

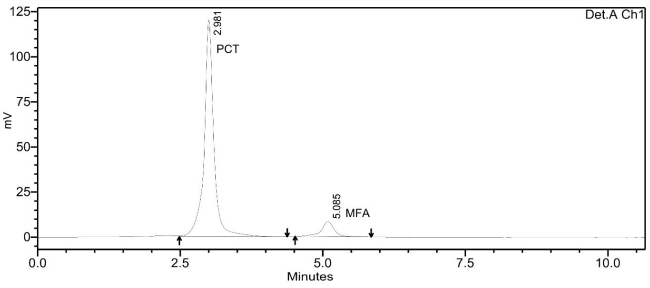

Figure 6: Assay Chromatogram of suspension dosage form

Table 3: Precision data of the proposed method

	% RSD	
Precision type	*PCT*	*MFA*
Intra-day*	1.3659	0.0003
Inter-day**	1.9659	0.0003

*Mean of %RSD for three different concentrations injected in triplicate
**Mean of %RSD for six injections in 3 days

Table 4: Robustness data of the proposed method

		% RSD	
Parameter	*Condition*	*PCT*	*MFA*
Change in flow rate (± 0.2 ml/ min)	1.2 mL/ min	0.7	0.4
	0.8 mL/ min	0.3	0.4
Change in organic phase composition Methanol: Buffer (± 10 mL)	75:25	0.3	0.5
	65:35	0.4	0.9
Change in detector wavelength (± 2 nm)	254 nm	0.5	0.5
	272 nm	1.1	0.6

mL of PCT and 6 µg/mL of MFA were injected, and %RSD calculated for injection repeatability (Table 3).

For assay sample prepared was injected in sextuplicate (Figure 6), and the average assay of replicate analysis was found to be 99.01% for PCT and 101.02% for MFA with a relative standard deviation of 0.48% and 0.52% respectively.

The stability of both the standard and the sample was determined by monitoring the peak area responses of the standard solution and the sample solution of PCT and MFA at 2, 12 and 24 hours at room temperature. The robustness of the method was performed by deliberately changing the chromatographic conditions i.e, flow rate, organic strength of the mobile phase and detector wavelength which showed the results within acceptable limits as shown. The standard solution and three different sample preparations were injected in each varied condition and the assay was checked. Under all varied conditions, it has been found that the %RSD for the assay values for PCT and MFA were found to be well within the acceptance limit of 2%.

CONCLUSION

A new RP-HPLC method was developed for the simultaneous estimation of PCT and MFA in bulk and suspension dosage form with the developed method successfully eluting the analytes within 5 minutes of injection. The developed method was validated according to ICH guidelines for parameters specificity, linearity, accuracy, precision, assay, and robustness, which all were within the acceptable limits depicting that the method proposed is fast, linear, accurate, and precise. Therefore, the developed method can be effectively used for the routine analysis in the simultaneous estimation of PCT and MFA in bulk and suspension dosage form.

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