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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Cefotaxime Sodium and Paracetamol in Synthetic Mixture

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Abstract

The cefotaxime sodium (CFT) and paracetamol (PCM) havebeen used as broad-spectrumantibiotics and antipyretics, respectively. Our goal was to develop a fast and highly sensitive simultaneous method of CFT and PCM. Itwas detected by a UV detector using a MerckC18column.The optimized and developed method found that the mobile phase was optimal with a flow rate of 0.8 mLmin1 of 1% formic acid in methanol.We found that the limits of detection and quantitationare in ppm. It was found that the retention of both drugs was less than 4 minutes. Accuracy and precision were at their limits. The proposed method has been validated for its synthetic mixtures, and it hasbeen observed that this method can be used for routine analysis of CFT and PCM at one dosage from.

Keywords: Dual drug detection, RP-HPLC, Method development, Optimized method, Formic acid.

1. Introduction

The injectable cefotaxime sodium (CFT) is a broad-spectrum bactericidal cephalosporin antibiotic. It is chemically7-[2-amino-4-thiazolyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo ovt-2-ene-2-carboxylate (Fig. 1a). The drug is highly active in vitro against first- or second-generation cephalosporin-sensitive or resistant Gram-negative bacteria. The activity against gram-positive bacteria is similar to that of other cephalosporins [1].

Pseudomonas and Bacteroides species although some strains of Bacteroides fragilis are resistant. Paracetamol(PCM) is chemically it is N-(4-hydroxyphenyl) Acetamide. It is widely used as an analgesic (analgesic) and antipyretic (antipyretic) that can be purchased at pharmacies. It is commonly used to relieve headaches and other minor pains and pains and is a major ingredient in many cold and flu remedies (Fig. 1b). Since no method has been reported to estimate the selected combinations simultaneously, this combination is very useful and leaves room for the development of new methods by liquid chromatography [2].

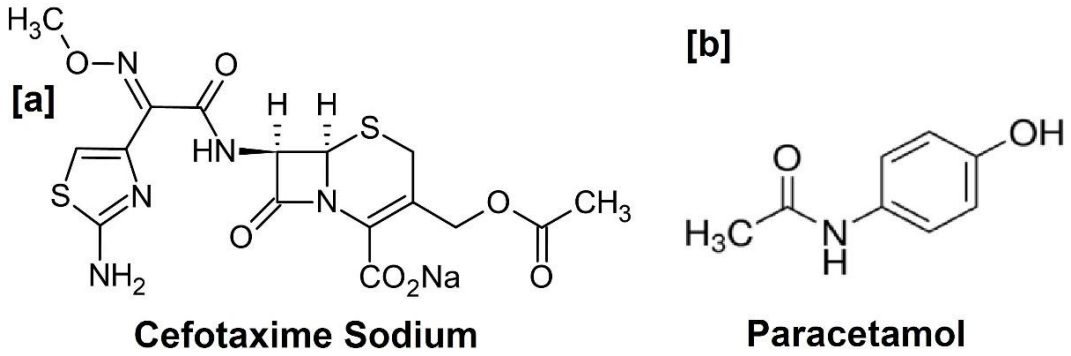

Fig. 1. Chemical structure of (a) CFT, and (b) PCM

2. Methods and Materials

2.1. Instruments, reagents and chemicals

A HPLC (LC-20AD, Shimadzu, Japan) connected to computer loaded with spinchrom chromatographic software. System was coupled with SPD-20A prominence UV/Vis detector. All weights were taken on semi microbalance (Shimadzu -AX -200 electronic balances). Cefotaxime sodium(CFT) and Paracetamol(PCM) were obtained as gift sample from Sisco Research laboratories Pvt. Ltd. Maharashtra, India.Formic acid Acetonitrile and MilliQ water were HPLC grade supplied by Merck chemicals Mumbai, India**.**

2.2. Solubility studies

The solubility of CFT and PCM were checked in different solvents and the data is given in Table 1.

Table 1.Solubility study of CFT and PCM

Solvent	CFT	PCM
Methanol	Soluble	Soluble
Acetonitrile	Soluble	Sparingly soluble
Water	Soluble	Sparingly soluble

2.3. Selection of wavelength

Standard stock solution of 100 µg.mL-1 of CFT and PCM were prepared separately using methanol as a solvent. Dilutions of both CFT and PCM (10 µg.mL-1) were prepared from the stock. They were scanned under UV Range and overlain spectra obtained as in Fig.2 and the isosbestic point of 230 nm was selected for the study [2].

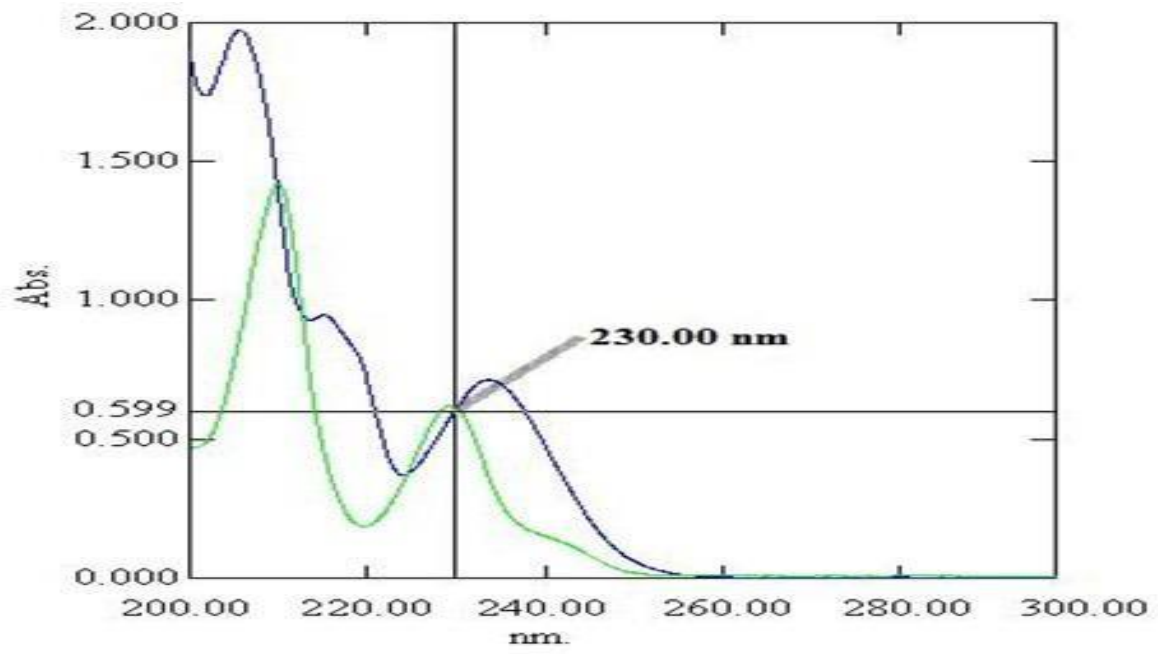

Fig.2. Overlain spectra of CFTandPCM

2.4. Preparation of mobile phase

The mobile phase was prepared by mixing different solvents like acetonitrile, methanol and formic acid at different ratio then the solution was filtered and degassed. The different chromatographic trials were done for judgment for best optimal method and mobile phase for development [3]. The various trails are detailed below Table 2. The different chromatograms are given below in Fig.3.

Table 2. Different chromatographic trials

S.No	Trial-1	Trial-2	Trial-3	Trial-4
Flow-	0.8	0.8	0.8	0.8
Detector	230	230	230	230
Mobile phase	Methanol ACN Water	Methanol ACN	1% v/v Formic acid in Methanol ACN	1% v/v Formic acid in Methanol
Ratio	(50:10:40)	(90:10)	(90:10)	100
Flow	Isocratic	Isocratic	Isocratic	Isocratic
Run time	10 min	10 min	10 min	10 min

* ACN:-Acetonitrile;mL:-milliliter;min:-minute;v/v:-volumeby volume

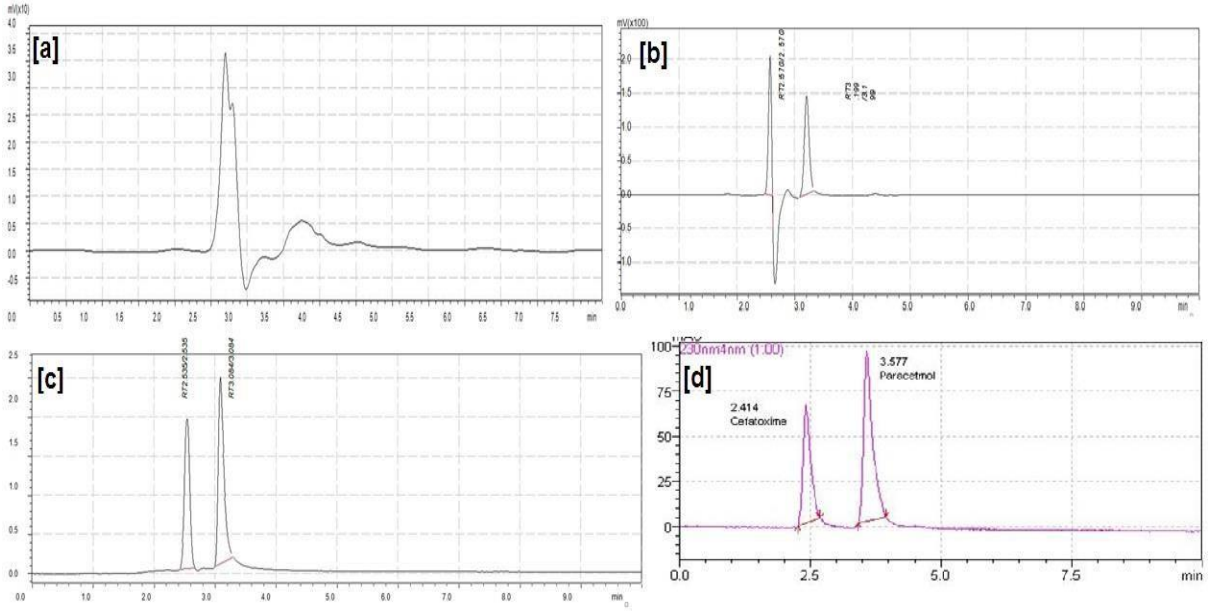

Fig. 3. Chromatogram of trails (a) Trial 1, (b) Trial 2, (c) Trial 3, and (d) Trial 4

2.5. Preparation of optimum mobile phase and parameters

It was prepared after several trails as per best chromatograms.The solubility of CFT and PCM were determined and was found to be soluble in methanol. As per the overlain spectra isobestic point was observed at 230 nm and satisfactory chromatograms were obtained at this wavelength. So, the analysis wavelength was chosen as 230 nm. In RP-HPLC method, the conditions were optimized to obtain an adequate elution of compounds. Various optimized mobile phase compositions were selected to elute titled drugs [4]. Mobile phase and flow rate selection was based on peak parameters (height, tailing factor, theoretical plates, capacity or symmetry factor) and run time. The system with 1% Formic acid in methanol and flow rate of 0.8 mL min^{-1} was found to be satisfactory. Blank chromatogram is shown in Fig.4.

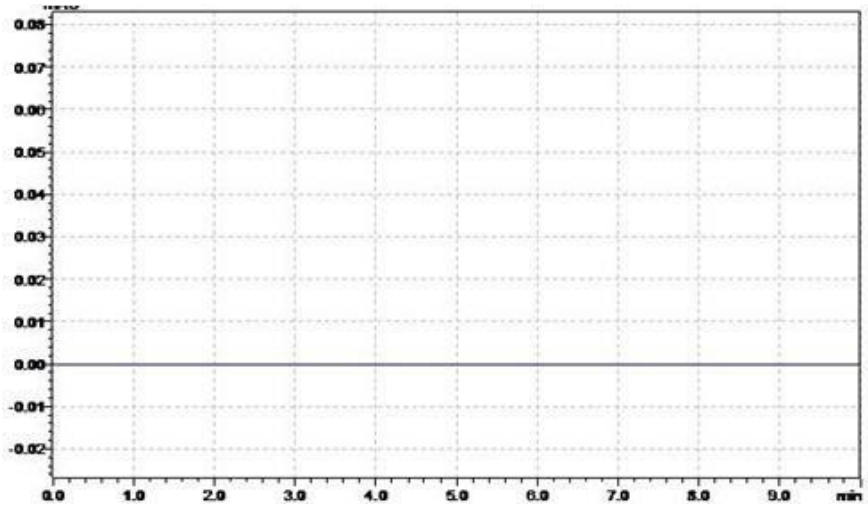

Fig.4. Blank Chromatogram of optimized mobile phase

2.6. Preparation of Standard Solutions

The pharmaceutical grade of CFT and PCM were procured from Micro Labs Pvt. Ltd. Stock solution of CFT and PCM were prepared by dissolving 10 mg of CFT and PCM in 5 mL of methanol in 10 mL volumetric flask separately and volume was made up to 10 mL using the methanol to get a standard stock solution of concentration 1 $mg.mL^{-1}$. The solutions were filtered using 0.2 µm syringe filter and this solution was used for analysis.

2.7. Preparation of calibration curve

From the stock solution (1000 $\mu g.mL^{-1}$), aliquots of CFT and PCM were pipette out into a series of 10 mL volumetric flasks and methanol was added to get a final concentration of 1- 100 $\mu g.mL^{-1}$. The volume was made up to the mark. The solutions were filtered through 0.2 µ syringe filter and 10 µL of this solution was injected to the column and peak areas were measured. The calibration curve was established. Linear correlations were found between peak area and concentration and are described by regression equation. The Beer's law is obeyed in the concentration range of 1 – 40 $\mu g.mL^{-1}$.

2.8. Preparation of Sample Solution

Marketed injection (H-mol) containing 70 mg of PCM was taken and diluted with methanol to get a dilution of 1000$\mu g.mL^{-1}$ solution and filtered through a 0.2 µ membrane filter. The CFT marketed injection (Taxime) containing 100 mg of CFT was taken and diluted to get a concentration of 1000 $\mu g.mL^{-1}$ solution with methanol and was filtered through 0.2 µ membrane filter. Filtrates were used to prepare the desired concentrations [5].The solutions and mobile phase used were degassed and filtered. The sample and standard solutions used were filtered using 0.2 µ syringe filters before

injecting to the column. Various trails were performed changing the mobile phase, column, flow rate, injection temperature to obtain good chromatogram with high separation and resolution.

2.8. Analytical method validation

2.8.1. System suitability

Five replicate injections of standard solution (5µg.mL^{-1}) were injected and the chromatograms were recorded. The system is suitable for analysis ifthe percentage relative standard deviation (%RSD) of area counts in five replicate injections should be not more than 2.0%. The tailing factor, theoretical plate count and % RSD of the peak area of CFT and PCM recorded.

2.8.2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analytes in samples within a given range. Various concentration of 1-100 µg.mL^{-1} of CFT and PCM were prepared. A graph of concentration against chromatographic area was plotted for CFT and PCM respectively. The regression line obtained was linear [6]. From the data obtained, co-relation coefficient, slope and Y-intercept were calculated.

2.8.3. Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated using the mathematical equations.

$$DL = 3.3\ \sigma/S$$

$$QL = 10\ \sigma/S$$

Where, σ =Standard deviation of the response

S = Slope of the calibration curve

2.8.4. Accuracy

Accuracy is performed in three different levels for CFT and PCM spiked known quantity of marketed sample, at 80%, 100 and 120%level into the bulk sample. Samples are analyzed in triplicate for each level. From the results, % recovery was calculated. T should lie in range of 98 to 102.0 %.

$$\%\ \text{Recovery} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}}\ \text{X}\ 100$$

Accurately weighed about 1.44, 1.8 and 2.16 mg of mg of both the standard API each were transferred into separate 100 mL volumetric flask. To the three different vessels 1.8 mg each of marketed CFT and PCM was added. To it added 10mL of methanol and sonicated for 10 min with intermittent shaking. Solution was allowed to cool at room temperature, made up to mark with

methanol and mixed well, filtered through 0.2 µ syringe filter. Further 1mL of this was diluted up to 10 mL with solvent phase and mixed well.

2.8.5. Precision

The System Precisionchecked by injecting six sample injections and checking the reproducibility in the retention time and area. The % RSD calculated must be less than 2 %.

Method Precision

Intraday Precision

The intraday precision is checked by using standard CFT and PCM to ensure that the analytical system is precise. The retention time and area of nine determinations was measured and RSD was calculated. % RSD of the assay value for nine determinations shall not be more than 2.0%.

Interday precision(IP)

The IP is checked by using same standard CFT and PCM sample analysed for intraday precision on alternate day to ensure that the analytical system is precise. The retention time and area of nine determinations was measured and RSD was calculated. % RSD of the assay value for nine determinations shall not be more than 2.0%.

2.5.6. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was done by changing the column temperature (± 5 °C), flow rate (± 0.1 mL), wavelength (± 2 nm). All the system suitability parameters must be met as per the method.

2.6.7. Ruggedness

This is to prove the lack of influence of operational and environmental variablesof the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst [7, 8]. All the system suitability parameters shall be met as per the method.

2.7. Solution stability

The standard and test solution were prepared and stored at room temperature for 18 hour. The % difference of the area response with respect to initial shall not be morethan 2.0% [9].

2.8. Assay

The column was equilibrated for at least 30 min, using mobile phase with aflow rate of 0.8 mL/min. Detector was set at a wavelength of 230 nm. Five sets of the drug solutions having

concentration 5 µg.mL^{-1} of CFT and PCM in methanol were prepared, filtered and injected to the column. The retention times of CFT and PCM in bulk drug in five injections were found to be 2.464 min and 3.589 min respectively and the retention time were found 2.468 min and 3.59 min respectively in formulation [10]. Using peak areas of the chromatograms the drug concentration was calculated from calibration curve.

3. Results and Discussion

3.1. System suitability

It was observed that the method complies with the system suitability parameters as follows.

Table 3.Results of System Suitability Parameters

S. No	System Suitability in standard solution	Observations		Proposed Criteria
		Cefotaxime	PCM	
1	% RSD of analyte peak	0.89	1.04	NMT 2.0%
2	Tailing factor for analyte peak	1.001	0.998	NMT 2.0
3	Plate count for analyte peak	3689.45	4314.592	Should be NLT 2000
4	Resolution	12.489		Should be NLT 2.0

3.2. Linearity and range

The linearity for CFT and PCM was checked in the concentration range of 1 – 100 µg.mL^{-1} and the range of 1 – 40 µg.mL^{-1} was found obeying Beer's range [11]. The results of regression parameters are shown in Table 4 and Fig. 5a and bdepict the calibration graphs of CFT and PCM.

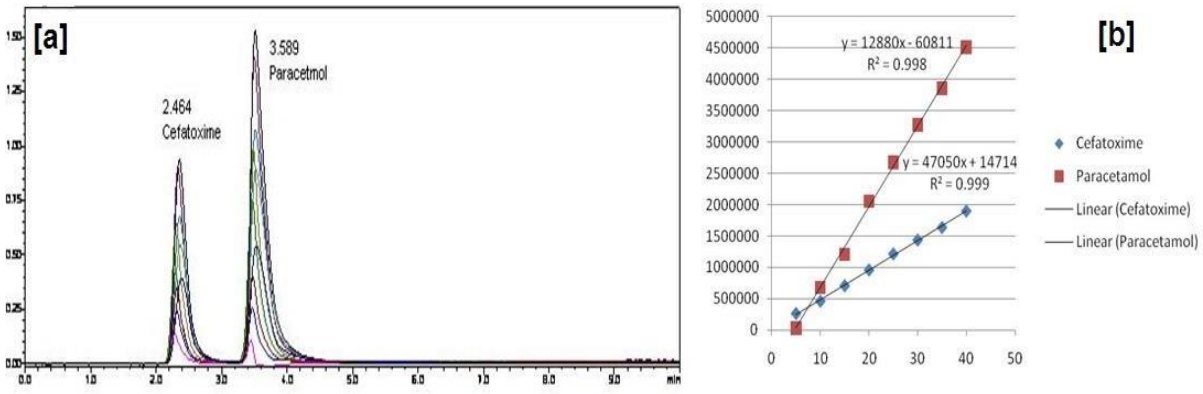

Fig. 5. (a) Linear Chromatogram of standard mixture at 230 nm, (b) linearity graph of CFT and PCM

Table 4. Results of Regression Parameters

Parameter	CFT	PCM
Range (µg.mL^{-1})	1-40	1-40
Regression Equation	y= 47050x+14714	y= 12880x-60811
Regression coefficient (r^2)	0.999	0.998
Slope	47050	12880
Intercept	14714	-60811

3.3. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Recovery was calculated at 80, 100 and 120%. It was found to be 100.07, 98.83 and 98.46 % for CFT. The recovery for PCM at 80, 100, and 120% was found to be 99.81, 99.40 and 100.12 % respectively and found within the limits. The results are shown in Table 5and6.

Table 5: Accuracy for CFT

S. No	Amount of pure drug (µg.mL^{-1})	Amount of formulation added (µg.mL^{-1})	Total amount of drug (µg.mL^{-1})	Percent Recovery*	Percent RSD
1	14.4	18	32.4	100.073	0.334
2	18	18	36	98.83	0.441
3	21.6	18	39.6	98.46	0.349

Table 6.Accuracy for PCM

S. No	Amount of pure drug (µg.mL^{-1})	Amount of formulation added (µg.mL^{-1})	Total amount of drug (µg.mL^{-1})	Percent Recovery*	Percent RSD
1	14.4	18	32.4	99.81	0.348
2	18	18	36	99.40	0.337
3	21.6	18	39.6	100.12	0.464

* Mean of six readings.

3.4. Precision

3.4.1. System Precision

The system precision is to ensure that the analytical system is working properly towards the selected

method [12]. It is done by injecting the 6 samples and comparing the area of the samples. The results of system precision are given in the Table 7. The method was found to be precise with % RSD of CFT and PCM as 1.39 and 1.25 respectively.

Table 7.System Precision of CFT and PCM

Injection No.	CFT	PCM
1	1742718	2051299
2	1718528	2039270
3	1708312	2054829
4	1779070	2035638
5	1740615	2112614
6	1712022	2047818
Average	1733544.17	2056911.33
% RSD	1.399	1.25

3.4.2. Method Precision

Intraday and inter day precision done for LQC (1 µg.mL^{-1}), MQC (20 µg.mL^{-1}), HQC (40 µg.mL^{-1}). The results were given in Table 8. The method was found to be precise with % RSD for intraday and IP of CFT for LQC, MQC and HQC as 0.436, 0.394, 0.442 and 0.221, 0.421, 0.416 respectively and for PCM as 0.394, 0.380, 0.584 respectively for intraday and 0.203, 0.481and 0.333 respectively for intraday.

Table 8.Method Precision of CFT and PCM

CFT				PCM			
Intraday		Interday		Intraday		Interday	
Conc. (µg.mL^{-1})	%RSD*	Conc. (µg.mL^{-1})	%RSD*	Conc. (µg.mL^{-1})	%RSD*	Conc. (µg.mL^{-1})	%RSD*
1	0.436	1	0.221	1	0.394	1	0.203
20	0.394	20	0.421	20	0.380	20	0.481
40	0.442	40	0.416	40	0.584	40	0.333

* Mean of nine readings

3.5. Ruggedness and robustness

The robustness and durability of an analysisprogram is a measure of its ability to remain unaffected

by small but intentional changes in method parameters. Stability and durabilityare verified by different parameters (suchas mobile phase composition, detection wavelength, column temperature) and different analysts on different days [13]. The method turnedout to be robust and durable. The robustness and robustness values are given in Table 9.

Table 9. Ruggedness and Robustness data of CFM and PCM

S. No	Parameters	Variations	System suitability results		
			%RSD	Tailing	Plate Count
1	% Formic acid	0.9%	0.51	0.984	3694.23
		1.1%	0.46	0.875	3984.26
2	Wavelength	228 nm	0.51	0.945	4369.8
		229 nm	0.34	0.871	3951.78
		231 nm	0.28	0.786	3981.31
		232 nm	0.42	0.829	4162.50
3	Column temperature (± 5^0C)	20^0C	0.56	0.981	4176.98
		30^0C	0.49	0.863	4031.56
4	Different Analyst	Analyst-I	0.51	0.817	3862.71
		Analyst-II	0.58	0.848	4501.81
	Different day	Day 1	0.46	0.932	3985.63
		Day 2	0.52	0.901	3709.41

6. Limit of Detection & Limit of Quantitation

The detection limit is the lowest amount of analyte in a sample that can be detected but not necessarily quantified under established experimental conditions. The limit of quantification is the lowest amount of analyte in a sample that can be quantified with acceptable precision and accuracy under established experimental conditions [14]. The results of LOD and LOQ are shown in Table. 10.

Table 10. LOD and LOQ data of CFT and PCM

Drug	LOD	LOQ
CFT	0.316 µg	0.959 µg
PCM	0.248 µg	0.7515 µg

4. Conclusion

The method is simple, specific and easy to perform, and the time required to analyze the sample is very short. The low limit of quantification and the limit of detection make this method suitable for quality control. Due to the good separation and resolution of the chromatographic peaks, this method can simultaneously measure CFT and PCM. The method turned out to be linear, precise, exact, robust and durable.

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