

Research Article

RP-HPLC Method Development and Validation for Simultaneous Estimation of Clarithromycin and Paracetamol

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The present work describes a simple, rapid, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of clarithromycin (CLA) and paracetamol (PCM). C18 column (Kromasil ODS, 5 μ m, 250 \times 4.6 mm) and a mobile phase containing phosphate buffer (0.05 M) along with 1-octane sulphonic acid sodium salt monohydrate (0.005 M) adjusted to pH 3.2: acetonitrile (50 : 50 v/v) mixture was used for the separation and quantification. The flow rate was 1.0 mL/min and the eluents were detected by UV detector at 205 nm. The retention times were found to be 2.21 and 3.73 mins, respectively. The developed method was validated according to ICH guidelines Q2 (R1) and found to be linear within the range of 75–175 μ g/mL for both drugs. The developed method was applied successfully for assay of clarithromycin and paracetamol in their combined in-house developed dosage forms and *in vitro* dissolution studies.

1. Introduction

Clarithromycin is a second generation macrolide with broad spectrum of antibiotic activity. It is active against the organisms which are responsible for bacterial exacerbations of lower respiratory tract infections [1]. Clarithromycin (6-O-methyl erythromycin) is synthesized by substituting a methoxy group for the C-6 hydroxyl group of erythromycin. This substitution creates a more acid stable antimicrobial and prevents the degradation of the erythromycin base to the hemiketal intermediate. The increased acid stability of clarithromycin results in improved oral bioavailability and reduced gastrointestinal intolerance [2]. Clarithromycin is characterized by favorable oral bioavailability that achieves 52% to 60%, whereas its antimicrobial activity involves a concentration and time dependent mode of bacteriostatic action [3]. Clarithromycin is metabolized in the liver and in the stomach. Approximately 22% of an oral dose is recovered as parent compound, 18% in the urine and 4% in the faeces. Clearance of clarithromycin decreases with increasing dose, probably because of saturable hepatic metabolism [4] (see Figure 1).

Paracetamol is a common analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains [5]. Paracetamol is chemically 4-hydroxyacetanilide is a centrally and peripherally acting non-opioid analgesic and antipyretic [6]. Paracetamol lacks many of the side effects of aspirin, unlike other common analgesics such as aspirin and ibuprofen, and has no anti-inflammatory properties, and so it is not a member of the class of drugs known as *nonsteroidal anti-inflammatory drugs* or NSAIDs. Paracetamol does not irritate the lining of the stomach or affect blood coagulation as compared to aspirin. At normal therapeutic doses, paracetamol is metabolized very fast and completely by undergoing glucuronidation and sulphonation to inactive metabolites that are eliminated in the urine [5, 7] (see Figure 2).

The present study was designed to develop a simple, precise, and rapid analytical RP-HPLC procedure, which can be used for the analysis of assay method for simultaneous estimation of clarithromycin and paracetamol as there was only individual methods reported for both drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for

TABLE 1: Specificity data.

	Average standard area* (mV)	Avg sample area* (mV)	Standard deviation	RSD%	Inference
CLA	361.82	359.06	1.95	0.54	RSD% found to be <2
PCM	4839.05	4824.29	10.43	0.21	

*n = 6.

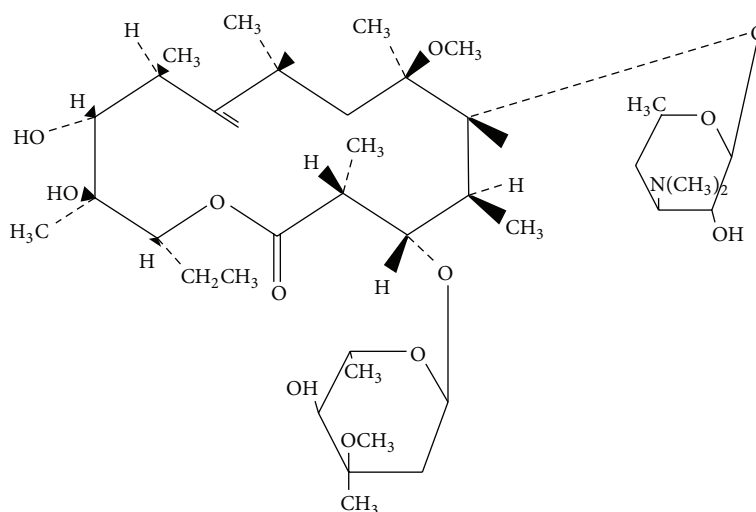


FIGURE 1: Structure of clarithromycin.

TABLE 2: Repeatability and intermediate precision.

	CLA	PCM	Inference
Repeatability (RSD%)	1.40	0.36	
Intermediate precision			% RSD was found to be < 2
Analyst 1 (RSD%)	0.99	0.15	
Analyst 2 (RSD%)	0.93	0.11	

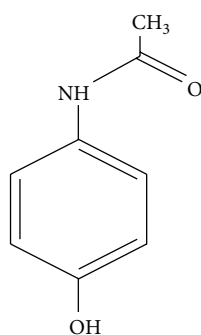


FIGURE 2: Structure of paracetamol.

the simultaneous estimation of these two drugs in their combined dosage forms. Literature survey of clarithromycin and paracetamol revealed several methods for detecting these drugs individually but there is no method for their simultaneous estimation using RP-HPLC. These drugs were given as regimen for the treatment of upper respiratory tract infections and tonsillitis.

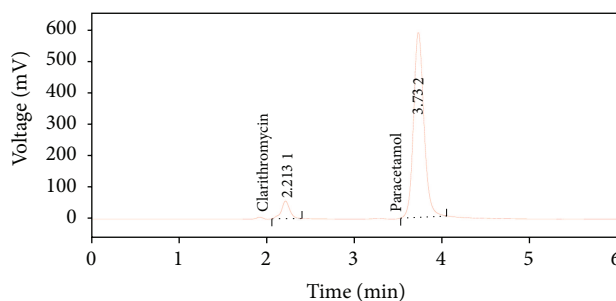


FIGURE 3: Optimized chromatogram of clarithromycin and paracetamol.

The developed method was validated as per ICH guidelines and its updated international convention. The linearity of response, precision, ruggedness and robustness of the described method has been checked.

2. Experimental

2.1. Chemicals and Reagents. Clarithromycin standard and API were procured by Jackson Laboratories, Amritsar, India. Paracetamol standard and API were gifted by Shandong yinfied pharmaceutical Co., Ltd., China. Acetonitrile was purchased from Standard Reagents Pvt. Ltd., Hyderabad, India. 1-Octane sulphonic acid sodium salt monohydrate and orthophosphoric acid were purchased from Rankem Ltd., New Delhi, India. All the other reagents used were of analytical grade.

TABLE 3: Results of LOD and LOQ of clarithromycin and paracetamol.

Method	Range ($\mu\text{g/mL}$)	Linear regression equation	R^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
RP-HPLC-CLA	75–175	$2.8086x + 12.432$	0.9952	5.230	15.849
RP-HPLC-PCM	75–175	$33.423x + 80.568$	0.9971	5.232	15.855

TABLE 4: Recovery data for clarithromycin.

Std conc ($\mu\text{g/mL}$)	Mean Std area* (mV)	Spl conc (std conc + spiked amount) ($\mu\text{g/mL}$)	Mean Spl area* (mV)	Concentration obtained ($\mu\text{g/mL}$)	Recovery %
100	303.43	106.25	326.21	107.51	101.18
125	365.61	131.25	387.04	132.42	100.89
150	425.25	156.25	447.75	157.93	101.07

* $n = 3$.

2.2. HPLC Instrumentation and Conditions. The analysis was carried out on a HPLC system (Shimadzu-LC 20AT) equipped with UV detector, pressure controlled by prominence pump and operated by Spinchrom Software. C18 column (250 mm \times 4.6 mm i.d., particle size 5 μm) was used for separation. Mobile phase used for separation was mixture containing monobasic phosphate buffer (0.05 M) along with 1-octane sulphonic acid sodium salt monohydrate (ion-pair reagent) adjusted to pH 3.2 with orthophosphoric acid and acetonitrile (50 : 50 v/v). The flow rate was kept at 1.0 mL/min, column temperature was ambient (25°C), eluents were detected by UV detector at 205 nm, and the injection volume was 20 μL .

2.2.1. Preparation of Phosphate Buffer pH 3.2. The monobasic potassium phosphate was accurately weighed about 6.8 gm and dissolved in distilled water and to this 1.175 gm of 1-octane sulphonic acid sodium salt monohydrate was added and dissolved and volume was made up to mark in 1000 mL volumetric flask.

2.2.2. Preparation of Mobile Phase. Mobile phase was prepared by mixing 50 volumes of acetonitrile and 50 volumes of 0.05 M phosphate buffer along with 0.005 M 1-octane sulphonic acid sodium salt monohydrate and was adjusted to pH 3.2 with orthophosphoric acid. The mobile phase was ultrasonicated, filtered through 0.45 μm membrane filter, and degassed.

2.2.3. Preparation of Standard Stock Solution. Standard stock solution was prepared by weighing accurately 125 mg each of clarithromycin and paracetamol individually and dissolved in the 5 mL of mobile phase in 100 mL volumetric flask and made up volume with mobile phase. From the above stock solution 5 mL was taken separately and diluted to 50 mL to obtain a final concentration 125 $\mu\text{g/mL}$.

2.2.4. Preparation of Working Standard Stock Solution. Working standard stock solution was prepared by weighing accurately 125 mg each of clarithromycin and paracetamol individually and dissolved in 5 mL of methanol and volume made

up with mobile phase in 100 mL volumetric flask. From the above stock solution 5 mL was taken separately and diluted to 50 mL to obtain a final concentration 125 $\mu\text{g/mL}$.

2.3. Method Validation

2.3.1. Specificity. Specificity of the method was determined by comparison between standard drug and sample. Fixed concentrations of 125 $\mu\text{g/mL}$ of standard and working test solutions were injected to the HPLC system for six times and were analyzed. Percentage of RSD was calculated from their peak areas.

2.3.2. Precision

Repeatability. Precision of the method was studied by making repeated injections of the mixture of drugs on the same day for intraday precision. The coefficient of variation (CV) after five determinations was determined at 125 $\mu\text{g/mL}$ for both drugs.

Intermediate Precision. Intermediate precision was carried out by injecting three replicates of standard concentration (125 $\mu\text{g/mL}$) by different analysts. The % RSD was calculated.

2.3.3. Linearity. The linearity of measurement was evaluated by analyzing standard solutions of CLA and PCM in the range of 75–175 $\mu\text{g/mL}$ for both drugs and calibration plot was constructed.

2.3.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ of CLA and PCM were determined by calibration curve method. Solutions of clarithromycin and paracetamol were prepared in the range of 75–175 $\mu\text{g/mL}$ and injected in triplicate.

2.3.5. Accuracy. Accuracy of the method was calculated by recovery studies at three levels by standard addition method, that is, spiking about 6.5 $\mu\text{g/mL}$ of each of CLA and PCM to the standard solutions containing 100, 125, and 150 $\mu\text{g/mL}$.

TABLE 5: Recovery data for paracetamol.

Std. concentration ($\mu\text{g/mL}$)	Mean Std area* (mV)	Spl. concentration (std conc + spiked amount) ($\mu\text{g/mL}$)	Mean Spl area* (mV)	Concentration obtained ($\mu\text{g/mL}$)	Recovery %
100	3776.59	106.25	3966.03	107.51	98.84
125	4802.27	131.25	5093.54	132.58	101.01
150	5416.54	156.25	5704.80	157.98	101.11

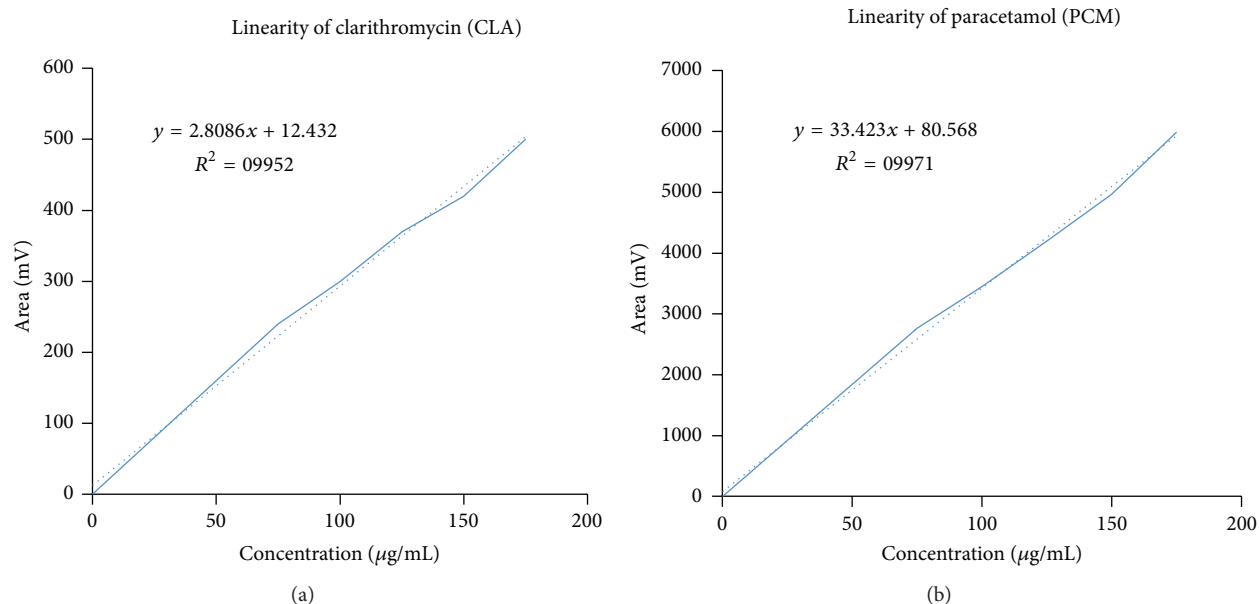
* $n = 3$.FIGURE 4: Linearity plot of CLA (a) and PCM (b) (75–175 $\mu\text{g/mL}$) standard solution.

TABLE 6: Results of robustness.

Parameter	CLA (% RSD)	PCM (% RSD)	Inference
Flow rate (± 0.2 mL/min)			
0.8 mL/min	0.31	0.12	RSD % was found to be <2
1.2 mL/min	0.59	0.25	
Wavelength (± 2 nm)			
203 nm	0.42	0.23	
207 nm	0.30	0.25	

TABLE 7: System suitability parameters.

Parameters	Proposed method	
	PCM	CLA
Retention times	2.21	3.73
Theoretical plates	29044	48040
Tailing factor	0.833	1.242
Resolution	7.992	

The samples were injected in triplicate and their % recovery was determined.

2.3.6. Robustness. Influence of small changes in chromatographic conditions such as change in flow rate, that is, ± 0.2 mL/mins and wavelength of detection ± 2 nm, was studied to determine the robustness of the method for the development of RP-HPLC method for the simultaneous estimation of CLA and PCM and their %RSD was determined.

2.3.7. System Suitability. The stock solution containing 125 $\mu\text{g/mL}$ was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit.

3. Results and Discussion

3.1. Optimization of Chromatographic Conditions. To develop suitable RP-HPLC method for simultaneous estimation of clarithromycin and paracetamol, different chromatographic conditions were applied and optimized chromatographic conditions were developed (see Figure 3).

Optimized chromatographic conditions are as follows:

instrument: HPLC Shimadzu separation module LC-20AT Prominence liquid chromatograph,

mobile phase: acetonitrile: phosphate buffer along with 1-octane sulphonic acid sodium monohydrate pH 3.2 (50 : 50 v/v),

column: Kromasil C18 (250 mm × 4.6 mm, 5 µm),

injection volume: 20 µL,

flow rate: 1.0 mL/min,

detection wavelength: 205 nm,

run time: 8 min,

temperature: Ambient (25°C).

3.2. Validation

3.2.1. Specificity. (See Table 1).

3.2.2. Precision and Intermediate Precision. Precision of the method was studied by making repeated injections of the mixture of drugs. The Coefficient of variation (CV) after five determinations was 1.4% at 125 µg/mL for CLA and 0.36% at 125 µg/mL for PCM (see Table 2).

3.2.3. Linearity. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. LOD and LOQ of CLA and PCM were determined by calibration curve method. Solutions of clarithromycin and paracetamol were prepared in the range of 75–175 µg/mL and injected in triplicate (see Figure 4).

3.2.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ of clarithromycin and paracetamol were determined by calibration curve method. Solutions of clarithromycin and paracetamol were prepared in the range of 75–175 µg/mL and injected in triplicate (see Table 3).

3.2.5. Accuracy. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for clarithromycin and paracetamol were 101.04% and 99.76%, respectively (see Tables 4 and 5).

3.2.6. Robustness. The method for the development of RP-HPLC method for the simultaneous estimation of CLA and PCM was found to be robust as the % RSD was found to be less than 2 (see Table 6).

3.2.7. System Suitability. The resolution, number of theoretical plates, and peak asymmetry were calculated for the standard solutions. The stock solution containing 125 µg/mL was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit (see Table 7).

4. Conclusion

The proposed RP-HPLC method was used for the simultaneous estimation of clarithromycin and paracetamol was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, *in vitro* dissolution study of combinational dosage formulations containing clarithromycin and paracetamol.

Conflict of Interests

The authors do not have a direct financial relation with the commercial identity mentioned in paper that might lead to a conflict of interests for any of the authors.

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