World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Method Development and Validation for Simultaneous Estimation of telithromycin and Ketoprofen by RP-HPLC

More Kishore*, Nakka Sandhyarani, Shaik Shajana Begum

Department of Pharmaceutical Analysis, Sana College of Pharmacy, Kodad-508206, Telangana, India

Received: 12-03-2018 / Revised Accepted: 28-04-2018 / Published: 01-05-2018

ABSTRACT

The present work describes a simple, rapid, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of telithromycin (TLM) and ketoprofen (KP). C18 column (chromosil ODS, 4 μm, 200× 4.0mm) 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 (45 : 55 v/v) mixture was used for the separation and quantification. The flow rate was0.8 mL/min and the eluents were detected by UV detector at 225 nm. The retention times were found to be 3.48 and 5.42 mins, respectively. The developed method was validated according to ICH guidelines Q2 (R1) and found to be linear within the range of 75–175 μg/mL for both drugs. The developed method was applied successfully for assay of telithromycin and ketoprofen in their combined in-house developed dosage forms and *in vitro* dissolution studies.

Keywords: Telithromycin, Ketoprofen, Chromosil

Address for Correspondence: Dr M. Kishore, HOD, Department of Pharmaceutical Analysis, Sana College of Pharmacy, Kodad-508206; Email: morekishore.pharma@gmail.com

How to Cite this Article: M. Kishore. Method Development and Validation for Simultaneous Estimation of Telithromycin and Ketoprofen by RP-HPLC. World J Pharm Sci 2018; 6(5): 74-79.

INTRODUCTION

Telithromycin 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]. The increased acid stability of telithromycin results in improved oral bioavailability and reduced gastrointestinal intolerance [2]. Telithromycin 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]. Telithromycin is metabolized in the liver and in the stomach. Approximately 25% of an oral dose is recovered as parent compound, 19% in the urine and 5% in the faeces. Clearance of telithromycin decreases with increasing dose, probably because of saturable hepatic metabolism [4] . Ketoprofen is a common analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains [5]. Ketoprofen 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 TLMs of drugs known as *nonsteroidal anti-inflammatory drugs* or NSAIDs. Ketoprofen does not irritate the lining of the stomach or affect blood coagulation as compared to aspirin. At normal therapeutic doses, ketoprofen is metabolized very fast and completely by undergoing glucuronidation and sulphonation to inactive metabolites that are eliminated in the urine [5, 7]

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 telithromycin and ketoprofen 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 the simultaneous estimation of these two drugs in their combined dosage forms. Literature survey of telithromycin and ketoprofen 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.

EXPERIMENTAL DESIGN

Chemicals and Reagents: Telithromycin standard and API were procured by Johnson Laboratories, Amritsar, India. Ketoprofen standard and API were gifted by Shandong granules india pharmaceuticals Hyderabad. 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.

HPLC Instrumentation and Conditions: The analysis was carried out on a HPLC system (Shimadzu-LC 20AT) equipped with UV detector, pressure controlled by promi column (200 mm x 4.0 mm i.d., particle size 4 m) 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 (45: 55 v/v). The flow rate was kept at 0.8 mL/min, column temperature was ambient (25^0 C), eluents were detected by UV detector at 225 nm, and the injection volume was 20 L.

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.

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

Preparation of Standard Stock Solution: Standard stock solution was prepared by weighing accurately 125 mg each of telithromycin and ketoprofen 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 125µg/mL.

Preparation of Working Standard Stock Solution: Working standard stock solution was prepared by weighing accurately 125 mg each of telithromycin and ketoprofen 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 125µg/mL.

Method validation

Specificity: Specificity of method was determined by comparison between standard drug and sample. Fixed concentations of 125 µg/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.

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 µg/mL for both drugs.

Intermediate Precision: Intermediate precision was carried out by injecting three replicates of standard concentration (125 µg/mL) by different analysts. The % RSD was calculated.

Linearity: The linearity of measurement was evaluated by analyzing standard solutions of TLM and KP in the range of 75–175 μg/mL for both drugs and calibration plot was constructed.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ of TLM and KP were determined by calibration curve method. Solutions of telithromycin and ketoprofen were prepared in the range of 75–175 μg/mL and injected in triplicate.

Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method, that is, spiking about 6.5 μg/mL of each of TLM and KP to the standard solutions containing 100, 125, and 150 μg/mL.

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

System Suitability: The stock solution containing125 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.

Figure 1: linearity of telithromycin

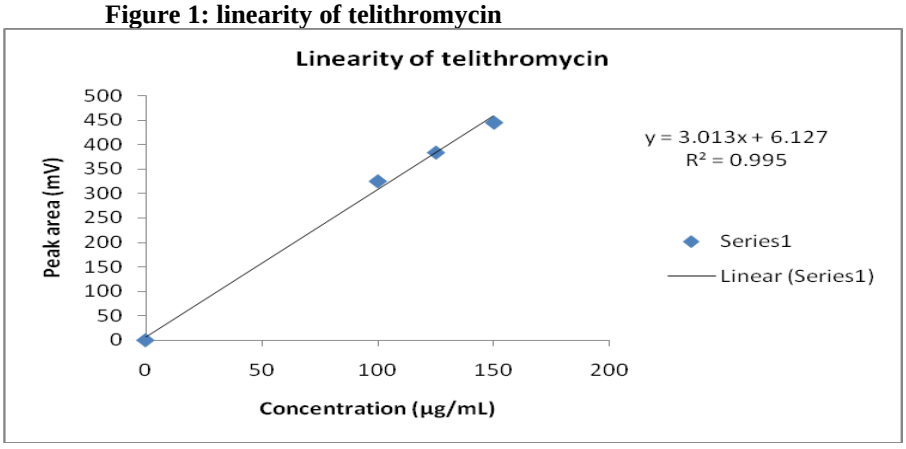

Figure 2: Linearity of ketoprofen

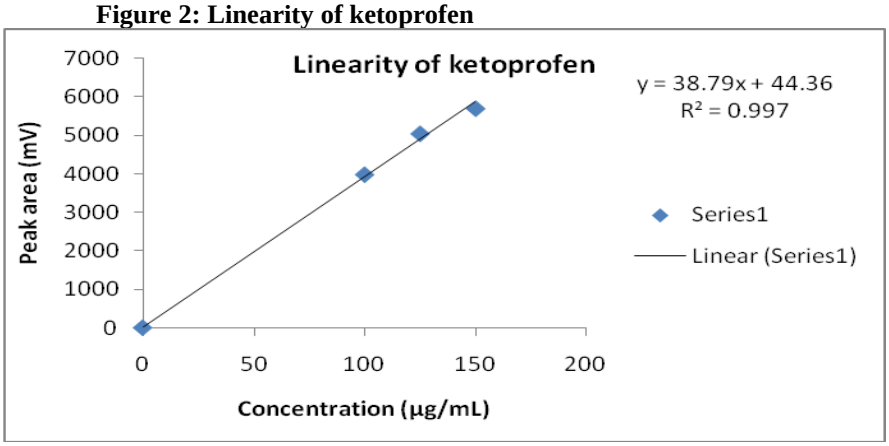

	Table1: Specificity data				
	Average standard area (mv)	Average sample area (mv)	Standard deviation	RSD%	Inference
TLM	358.25	357.09	1.25	0.49	RSD% found tobe < 2
KP	4789.02	4685.55	9.83	0.19	

	Table 2: Repeatability and intermediate precision			
	TLM	KP	Inference	
Repeatability	1.38	0.34	% RSD was found to be < 2	
Intermediate precision				
Analyst 1 (RSD %)	0.98	0.16		
Analyst 2 (RSD %)	0.92	0.12		

	Table 3: Results of LOD and LOQ of telithromycin and ketoprofen				
Method	Range (µg/mL)	Linear regression equation	Regression value	LOD (µg/mL)	LOQ (µg/mL)
RP-HPLC-TLM	75-175	2.7852x+11.756	0.9951	5.138	14.587
RP-HPLC-KP	75-175	33.423x+80.568	0.9971	5.123	14.86

	Table 4: Recovery data for telithromycin				
Std conc (µg/mL)	Mean std area (mV)	Spl conc (std conc+spiked amount) (µg/mL)	Mean Spl area (mV)	Conc obtained (µg/mL)	Recovery %
100	302.65	105.35	325.24	106.25	100.85
120	368.51	130.55	384.06	131.14	100.45
150	424.52	155.65	445.28	156.38	100.46
n=3					

	Table 5: Recovery data for ketoprofen				
Std conc (µg/mL)	Mean std area (mV)	Spl conc (std conc+spiked amount) (µg/mL)	Mean Spl area (mV)	Conc obtained (µg/mL)	Recovery %
100	3658.69	105.65	3981.06	106.59	100.88
120	4789.26	133.45	5044.68	134.44	100.74
150	5418.36	157.36	5698.85	159.25	101.2
n=3					

Table 6: Results of robustness			
Parameter	TLM (% RSD)	KP (%RSD)	Inference
Flow rate			RSD % was found to be < 2
0.8mL/min	0.29	0.11	
1.2mL/min	0.58	0.24	

Figure 3 : Optimized chromatogram of telithromycin and ketoprofen

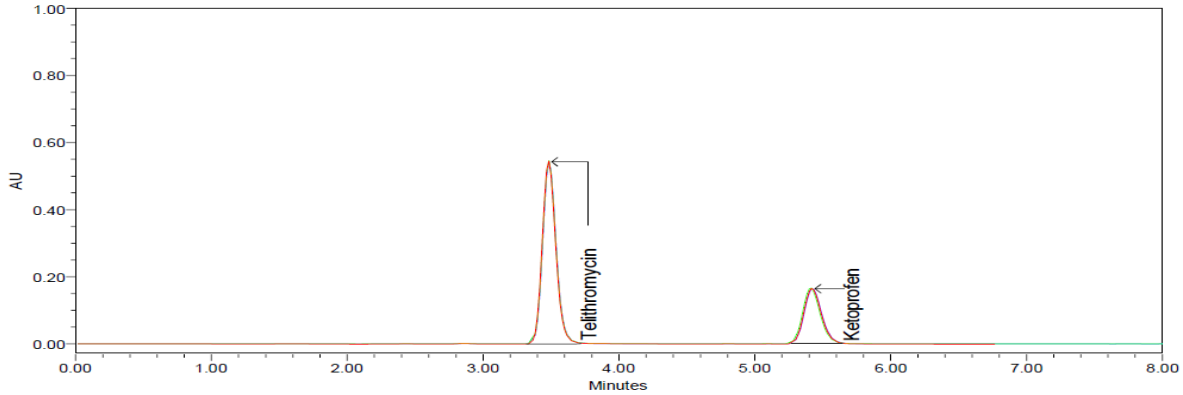

Table 7 A) Peak Name: telithromycin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	telithromycin	3.482	5841.552692	5841. 552692	1.129968
2	telithromycin	3.485	5854.699191	5854. 699191	1.137179
3	telithromycin	3.483	5893.311214	5893. 311214	1.132695
Mean			3766852.3		
Std. Dev			12013.7		
% RSD			0.3		

Table 7 B) Peak Name: ketoprofen

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	ketoprofen	5.42	1449723	8353. 483760	1.149951
2	ketoprofen	5.423	1439041	8358. 143277	1.148473
3	ketoprofen	5.412	1456499	8420. 155914	1.148562
Mean			1448421		
Std. Dev			8801.5		
% RSD			0.6		

RESULTS AND DISCUSSION

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

Optimized chromatographic conditions are as follows:

mobile phase: acetonitrile: phosphate buffer along with 1-octane sulphonic acid sodium monohydrate pH 3.2 (45 : 55 v/v),
column: chromosil C18 (200mm × 4.0 mm, 4 μm),
injection volume: 20 μL,
flow rate: 0.8 mL/min,
detection wavelength: 225 nm,
run time: 8min,
temperature: Ambient (25^0C).

Validation

Specificity. **(See Table 1)**

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 TLM and 0.36% at 125 μg/mL for KP (see Table 2).

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 TLM and KP were determined by calibration curve method. Solutions of telithromycin and ketoprofen were prepared in the range of 75–175 μg/mL and injected in triplicate (see Figure 1&2).

Limit of Detection (LOD) and Limit of Quantization: *(LOQ).* LOD and LOQ of telithromycin and ketopofen were determined by calibration curve method. Solutions of telithromycin and ketoprofen were prepared in the range of 75–175 μg/mL and injected in triplicate (see Table 3).

Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for telithromycin and ketoprofen were 100.58% and 100.94%, respectively (see Tables 4 and 5).

Robustness: The method for the development of RPHPLC method for the simultaneous estimation of TLM and KP was found to be robust as the % RSD was found to be less than 2 (see Table 6).

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 7A & 7B).

Conclusion

The proposed RP-HPLC method was used for the simultaneous estimation of telithromycin and ketoprofen 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 telithromycin and ketoprofen.

REFERENCES

1. J. M. Zuckerman, “Macrolides and ketolides: azithromycin, clarithromycin, telithromycin,” *Infectious Disease Clinics of North America*, vol. 18, no. 3, pp. 621–649, 2004.
2. D. Adam, E. Glaser-Caldow, J. Wachter et al., “Comparative efficacy of clarithromycin modified-release and telithromycin immediate-release formulations in the treatment of lower respiratory tract infection,” *Clinical Therapeutics*, vol. 23, no. 4, pp. 585–595, 2001.
3. A. Giannopoulos, G. Koratzanis, E. J. Giamarellos-Bourboulis,C. Panou, I. Adamakis, andH. Giamarellou, “Pharmacokinetics of clarithromycin in the prostate: implications for the treatment of chronic abacterial prostatitis,” *Journal of Urology*, vol. 165, no.1, pp. 97–99, 2001.
4. P. G. Davey, “The pharmacokinetics of telithromycin and its 14-OH metabolite,” *Journal of Hospital Infection*, vol. 19, pp. 29–37, 1991.
5. M. E. Bosch, A. J. R. S´anchez, F. S. Rojas, and C. B. Ojeda, “Determination of paracetamol: historical evolution,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 42, no. 3, pp. 291–321, 2006.
6. M. Khanmohammadi, M. Soleimani, F. Morovvat, A. B. Garmarudi, M. Khalafbeigi, and K. Ghasemi, “Simultaneous determination of ketoprofen and codeine phosphate in tablets by TGA and chemometrics,” *Thermochimica Acta*, vol. 530, pp. 128–132, 2012.
7. Y. Ozkan, A. Savaser, Y. Ozalp, and A. Isimer, “Dissolution properties of different designed and formulated salbutamol tablet dosage forms,” *Acta Poloniae Pharmaceutica*, vol. 57, no.4, pp. 33–41, 2000.