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Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Tolperisone HCl and Paracetamol in bulk and its pharmaceutical formulations

M. V. Satyanarayana[1]*, T. N. V. S. S. Satyadev[2], V. Anuradha[3] and Ch. Rama Krishna[4]

[1]*Dept. of Freshman Engineering, PVP Siddhartha Institute of Technology, Kanuru, Vijayawada, Andhra Pradesh, India*
[2]*PG Centre, P.B. Siddhartha College of Arts and Science, Vijayawada, Andhra Pradesh, India*
[3]*Department of Chemistry, Vignan PG College, Pedapalakaluru, Guntur, India*
[4]*Department of Chemistry, RVR & JC College of Engineering, Guntur, India*

ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed and validated for simultaneous estimation of Tolperisone and Paracetamol in tablet formulations. The separation was achieved by using C-18 column (Hypersil BDS, 150 x 4.6mm i.d.); in mobile phase pH 3.6 Phosphate Buffer +0.1% Tetra ethyl amine and acetonitrile in the ratio of 700:300 v/v. The flow rate was 1.0 mL.min^{-1} and the separated drugs were detected using UV detector at the wavelength of 267 nm. The retention times of Tolperisone and Paracetamol were 4.65 and 2.39 minutes respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The developed method was validated for specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per ICH guidelines. Linearity for Tolperisone and Paracetamol were found in the range of 500-1500 µg/ml and 150-450µg/ml, respectively. The percentage recoveries for Tolperisone and Paracetamol ranged from 98.6-100.7 % and 98.6-100.1%, respectively. The proposed method could be used for routine analysis of Tolperisone and Paracetamol in their combined dosage forms. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography; Tolperisone, Paracetamol, Combined dosage forms; Simultaneous estimation, Validation

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INTRODUCTION

Tolperisone Hydrochloride (TOL), chemically (R, S) 2-methyl-1-(4 - methyl phenyl)-3- (1-piperidyl) propane -1 one is a piperidine derivative[1] [Fig. 1]. It is a centrally acting muscle relaxant which is used in the treatment of different pathological conditions like acute and chronic muscle spasm, electroconvulsive therapy, neurological conditions and orthopedic manipulation - multiocular sclerosis, myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, Arthrosis of the large joints obliterating artherosclerosis of the extremity vessels, Diabetical angiopathy, thromboangitis obliterans, raynauds syndrome[2, 3]. Tolperisone hydrochloride is official in Japanese pharmacopoeia[1]. Paracetamol is N- (4 - hydroxyphenyl) acetamide, a Para-aminophenol derivative [Fig. 2], has analgesic and antipyretic properties and weak anti-inflammatory activity. Paracetamol is official in Indian Pharmacopoeia[4], British Pharmacopoeia [5], United States Pharmacopoeia[6] and European Pharmacopoeia[7]. A combination of Tolperisone HCl with Paracetamol has been approved in India in the proportion of 150:500 mg proportion respectively. The literature survey revealed that there are some analytical methods reported for Tolperiosone Hydrochloride like Spectrophotometric [8-11], HPTLC [12], RP-HPLC [13-17] either individually or in

combination with other drug and also reported on biological fluids. Many methods have been reported in literature for determination of paracetamol with other drugs[15-17]. TOL and PCM combination is not official in any pharmacopoeia. Hence, aim of present work is to develop simple, feasible, effective and economic validated analytical techniques for quantification of Tolperisone HCl & Paracetamol simultaneously in Pharmaceutical tablet formulation.

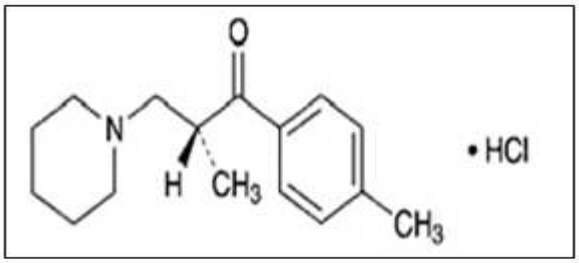


Fig. 1: Structure of Tolperisone Hydrochloride

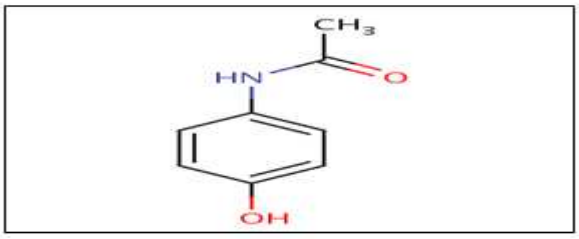


Fig. 2: Structure of Paracetamol

Literature survey reveals that tolperisone can be estimated by spectrophotometry [3] ,HPLC [4,5,6]and by HPTLC[7] methods individually or in combination with other drugs. Paracetamol is reported to be estimated by spectrophotometry [8] and HPLC[9,10] .The reported methods are applicable for the estimation of either for TPS or PTC individually or in combination with other drugs from pharmaceutical dosage forms or biological fluids. But all those methods are not reported any degradation studies to prove that the method is stability indicating method. The present work describes the development of a validated stability indicating analytical RP-HPLC method, which can quantify these Components simultaneously from a combined dosage form.

MATERIALS AND METHODS

2.1. Chemicals and Reagents

Milli-Q Water, Methanol (HPLC Grade), Orthophosphoric acid (GR Grade), Potassium dihydrogen phosphate monohydrate (GR Grade) were obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

2.2. Instrumentation and Chromatographic Conditions

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Hypersil BDS ((Make: Thermo); 150 mmx4.6 mm I.D; particle size 5 µm)) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Buffer preparation

Dissolved 2.72g of Potassium dihydrogen Phosphate in 1000mL of Milli-Q Water, adjusted pH to 3.6 with dilute ortho phosphoric acid and filtered the solution through 0.45µm membrane filter.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer and Methanol in the ratio of 700:300 v/v respectively.

Diluent preparation

Mobile Phase was used as diluent.

Standard preparation:
Accurately weighed and transferred about 100mg of Paracetamol and 30.0mg of Tolperisone into a 100 mL volumetric flask, added 30 mL of diluent and sonicated to dissolve. The solution was cooled to room temperature and diluted to volume with diluent.

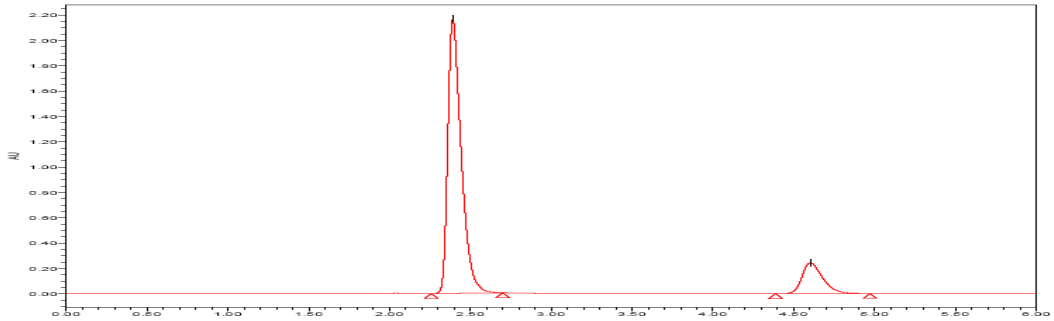
Sample preparation:
Weighed and finely powdered not fewer than 20 Tablets. Accurately weighed and transferred equivalent to 500g of Paracetamol and 150mg of Tolperisone into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mixed. Filtered the solution through 0.45 µm membrane filter. Transfered 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent.

Chromatographic conditions
An Hypersil ODS ((Make: Thermo; 150mm x 4.6 mm I.D; particle size 5µm)) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10µL. The photodiode array detector was set to a wavelength of 267nm for the detection and Chromatographic runtime was 6 minutes.

RESULTS AND DISCUSSION

Method development [1-5]
Spectroscopic analysis of compounds showed that (I) and (II) have maximum UV absorbance (λmax) at 267 nm(For Tolperisone), 242 nm(For Paracetamol) respectively. Therefore, the chromatographic detection was performed at 267nm using a Photo diode array detector as paracetamol also exhibits good response at Tolperisone λmax . To develop a suitable and robust LC method for the determination of Tolperisone and Paracetamol, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Hypersil BDS ((Make: Thermo; 150 mmx4.6 mm I.D; particle size 5 µm)) with the following mobile phase. Accurately transferred about pH 6.8 Phosphate buffer. Filtered the solution through 0.45µm membrane filter. Prepared a filtered and degassed mixture of Buffer and Methanol in the ratio of 500:500 v/v respectively. It was observed that when a combination of two drugs was injected, Paracetamol and Tolperisone together gave a single split M shape peak.

Figure 2: A typical HPLC Chromatogram showing the peak of Paracetamol and Tolperisone



For next trial the mobile phase composition was changed slightly. The mobile phase composition was Buffer and Methanol in the ratio of 600:400 v/v. The trail resulted in broad and well separated peaks. Again the mobile phase composition changed slightly to Buffer and Methanol in the ratio of 700:300 v/v respectively as eluent at flow rate 1.0 mL/min. UV detection was performed at 267nm. The retention time of Paracetamol is 2.39 minutes and Tolperisone is about 4.65 (refer Fig-2.) and the peak shape for these two was good.

Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Different experiments were performed to optimize the mobile phase and adequate separation of drugs achieved. The optimized mobile phase was determined as a mixture of Buffer and Methanol (700:300) at a flow rate of 1.0 mL.min-1. Under these conditions (I) and (II) were eluted at 2.39 and 4.65, minutes respectively with a run time of 6 min.

The chromatogram of Paracetamol and Tolperisone standard using the proposed method is shown in (Fig-2.) System suitability results of the method are presented in Table-1.

A typical chromatogram for simultaneous estimation of (I) and (II) obtained by using the aforementioned mobile phase of 10 µL of the assay preparation is illustrated in Fig. 2.

Fig. 2. HPLC chromatogram obtained during simultaneous determination of Paracetamol (I), and Tolperisone (II).

Method validation [13-15]

The developed RP-LC method extensively validated for assay of Paracetamol (I), and Tolperisone (II) using the following Parameters.

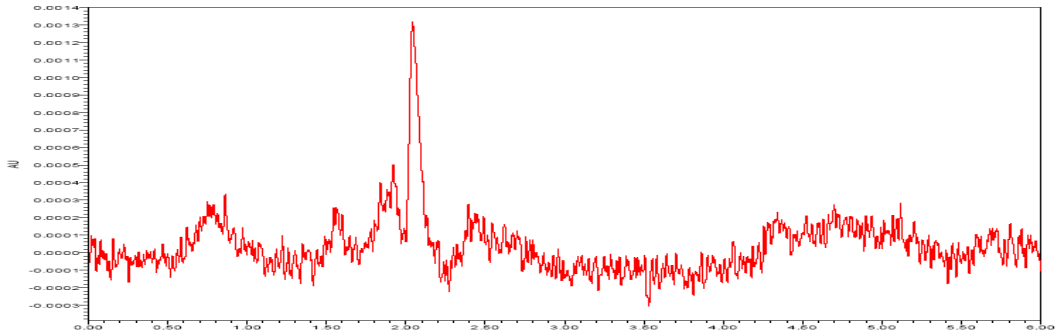
Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Fig. no.-3)** showed no peaks at the retention time of Paracetamol and Tolperisone peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Paracetamol and Tolperisone in Paracetamol and Tolperisone tablets. Similarly Chromatogram of Placebo solution **(Fig. no.-4)** showed no peaks at the retention time of Paracetamol and Tolperisone peak. This indicates that the Placebo used in sample preparation do not interfere in estimation of Paracetamol and Tolperisone in Paracetamol and Tolperisone tablets.

The chromatogram of Paracetamol and Tolperisone Blank using the proposed method is shown in **Fig- 3**.

Figure 3: A typical HPLC Chromatogram showing the no interference of diluent for Paracetamol and Tolperisone



The chromatogram of Paracetamol and Tolperisone Placebo using the proposed method is shown in **Fig-4**.

Figure 4: A typical HPLC Chromatogram showing the no interference of placebo for Paracetamol and Tolperisone

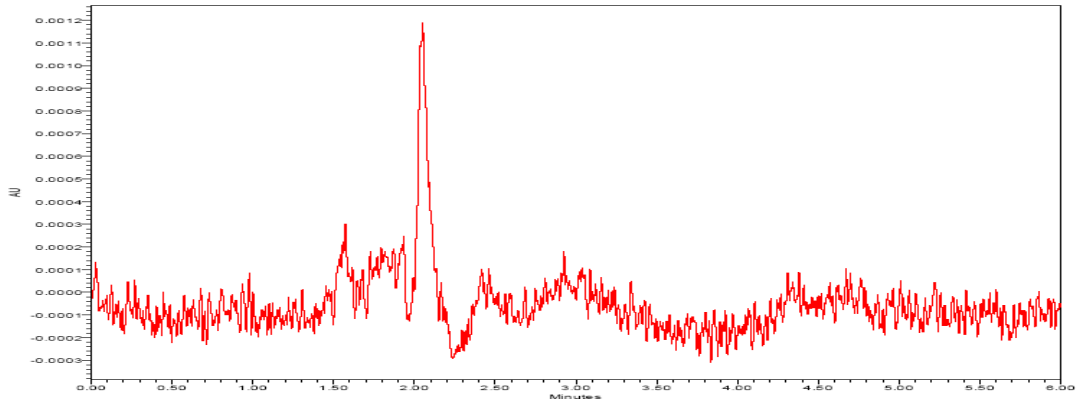


Table 1: System suitability parameters for Paracetamol and Tolperisone by proposed method

Name of the Compound	Retention Time	Theoretical plate	Tailing factor	USP Resolution
Paracetamol	2.39	4025	1.54	-
Tolperisone	4.65	7353	1.36	4.14

Forced Degradation:
Control Sample:
Weighed and finely powdered not fewer than 20 Tablets. Accurately weighed and transferred equivalent to 500mg of Paracetamol and 150mg of Tolperisone into a 100 mL volumetric flask, added 70 mL of diluents, sonicated for 15 minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mixed. Filtered the solution through 0.45 µm membrane filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent.

Acid Degradation Sample:
In the preparation of acid degradation sample the procedure adopted was same as mentioned for controlled sample up to sonication and before filtering the sonicated solution added 5mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralized with 1N NaOH and diluted to volume with diluent and mixed. Filtered the solution through 0.45 µm membrane Filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent. Refer **(Fig. no.-5A)**

Base Degradation Sample:
As mentioned at acid degradation, before sonication instead of adding acid, added 5 mL of 1N NaOH. The same procedure was repeated and excess of acid was neutralised with 1N acid. Refer **(Fig. no.-5B)**

Peroxide Degradation Sample : +
Refluxed for 30min at 60°C after adding 5mL of Hydrogen Peroxide before filtration as mentioned above, then cooled to room temperature and diluted to volume with diluent and mixed. Filtered the solution through 0.45 µm membrane Filter. Transferred 5.0 mL of the above solution into a 25 mL volumetric flask and diluted to volume with diluent. Refer **(Fig. no.-5C)**

Thermal Degradation Sample:
Powder collected from 20 tablets was exposed to heat at 105°C for about 5days. The same powder was weighed and solution was prepared for thermal degradation using the same procedure adopted for the preparation of controlled sample. Refer **(Fig. no.-5D)**

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

Figure 5A: A typical HPLC Chromatogram showing the degradation profile of Tolperisone and Paracetamol in Acidic hydrolysis by proposed method

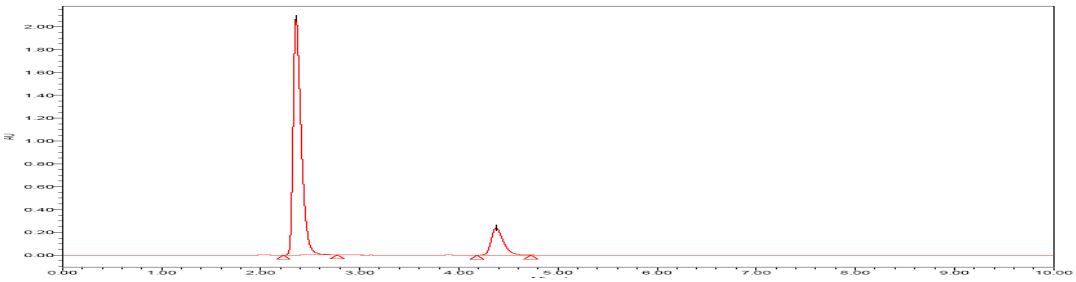


Figure 5B: A typical HPLC Chromatogram showing the degradation profile of Tolperisone and Paracetamol in Basic hydrolysis by proposed method

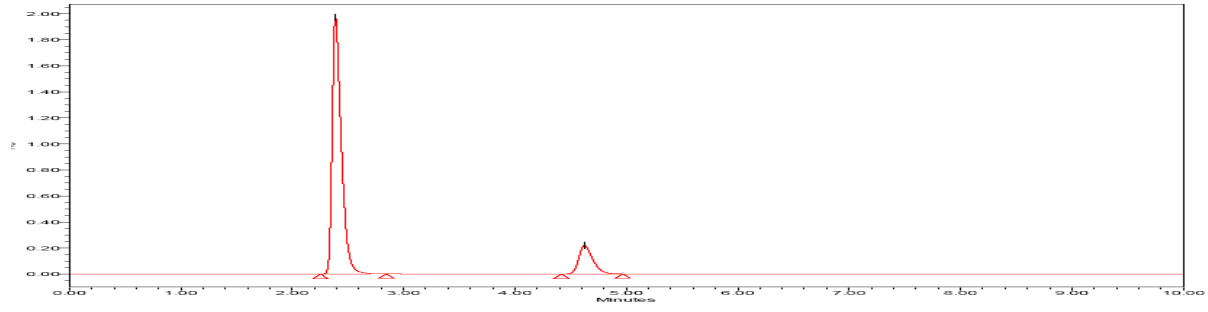


Figure 5C: A typical HPLC Chromatogram showing the degradation profile of Tolperisone and Paracetamol in Peroxide hydrolysis by proposed method

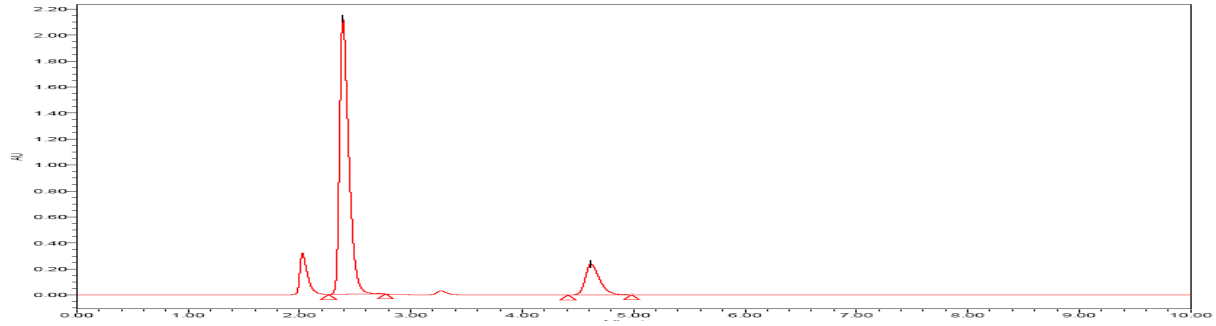
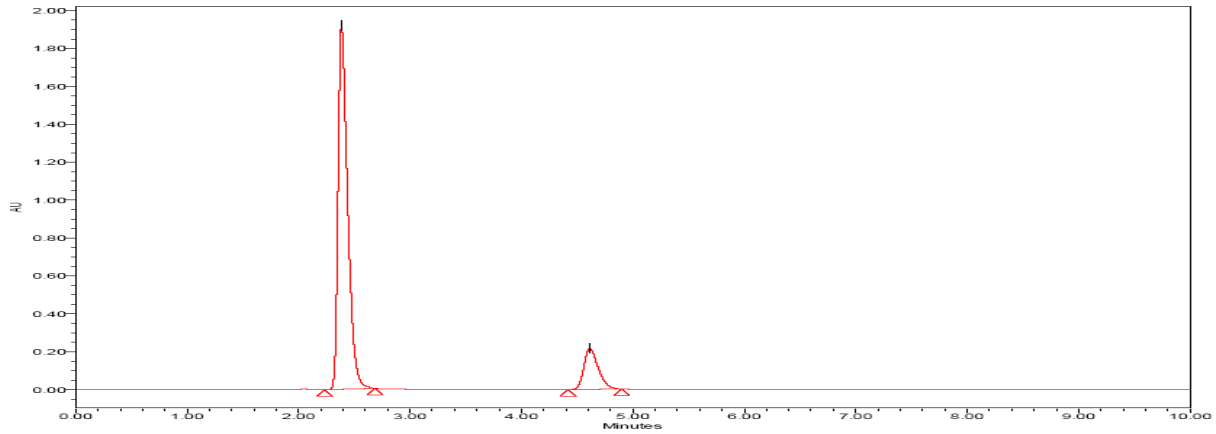


Figure 5D: A typical HPLC Chromatogram showing the degradation profile of Tolperisone and Paracetamol in Thermal degradation by proposed method



Precision

In the study of the instrumental system precision where, a RSD of 0.21% was obtained for the standard area of Paracetamol and 0.1 for Tolperisone obtained corresponding to the first day, Similarly being 0.3% for Paracetamol and 0.2% for Tolperisone for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 0.5% and the 95% confidence interval of 0.5 with the assay range of 98.6-99.9 for Paaracetamol. Similarly The method precision study for six sample preparations in marketed samples showed a RSD of 0.7% and the 95% confidence interval of 0.7 with the assay range of 99.2-100.7 for Tolperisone.

Table 2: Method Precision (Inter and Intraday) studies for Paracetamol and Tolperisone HCl by proposed method

Summary showing Method Precision by Proposed Method			
For Tolperisone		*For Paracetamol*	
Method Precision (Inter &Intra Day)		Method Precision (Inter &Intra Day)	
100.1	100.20	99.4	99.9
100.7	100.4	99.7	100.1
99.4	99.6	98.7	98.8
99.3	99.7	98.7	99.0
99.2	99.5	98.6	99.0
100.5	99.6	99.2	99.2
Overall Avg.	99.9		99.2
Overage Std Dev.	0.51		0.49
Over all %RSD	0.50		0.50

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 0.3 %(For Standard of Paracetamol). And 0.2% (For Standard of Tolperisone). The same study was carried out for different analysts (n = 6 number of samples per analyst) obtaining a RSD of 0.5 %(Intermediate Precision) and 95% confidence interval of 0.6 with the assay range of 98.8-100.1 for Paracetamol. Similarly, obtaining a RSD of 0.4 %(Intermediate Precision) and 95% confidence interval of 0.4 with the assay range of 99.5-100.4 for Tolperisone. The Overall %RSD for n=12 is 0.5. for Paracetamol and 0.5 for

Tolperisone. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Tolperisone and Paracetamol, analyzed as per the proposed method. The percentage recoveries were found in the range of 99.3 to 100.9 with an overall %RSD of 0.5 for Paracetamol and the percentage recoveries were found in the range of 100.1 to 100.6 with an overall %RSD of 0.2 for Tolperisone. From the data obtained, given in Table-3A and Table-3B, the method was found to be accurate.

Table 3A: Recovery studies for Paracetamol by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	100.6-100.9	0.1	0.5
100	99.3-100.0	0.4	
150	99.7-99.8	0.1	

Table 3B: Recovery studies for Tolperisone by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	100.2-100.5	0.1	0.2
100	100.1-100.6	0.3	
150	100.3-100.5	0.1	

Linearity of detector response

The standard curve was obtained in the concentration range of 500-1500µg/ml for Paracetamol and 150-450µg/mL for Tolperisone. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure-6A(For Paracetamol) and Figure-6B(For Tolperisone) to demonstrate the linearity of the proposed method.

From the data obtained, given in Table-4A (For Paracetamol) and Table-4B (For Tolperisone), the method was found to be linear within the proposed range.

Table 4A: Linearity studies for Paracetamol by proposed method

Linearity Study for Paracetamol		
% Level	**Conc. µg/mL**	**Area**
50	498.32	6202543
75	747.48	9373723
100	996.64	12522835
125	1245.80	15626825
150	1494.96	18744544
Slope		12535.0
Intercept		-40748.0
% Y-Intercept		-325.1
Residual Sum of Squares		25001.0
CC(r)		0.99999
RSQ(r2)		0.99998
LLD		6.58
LLQ		19.94

Figure 6A: Calibration curve for Paracetamol

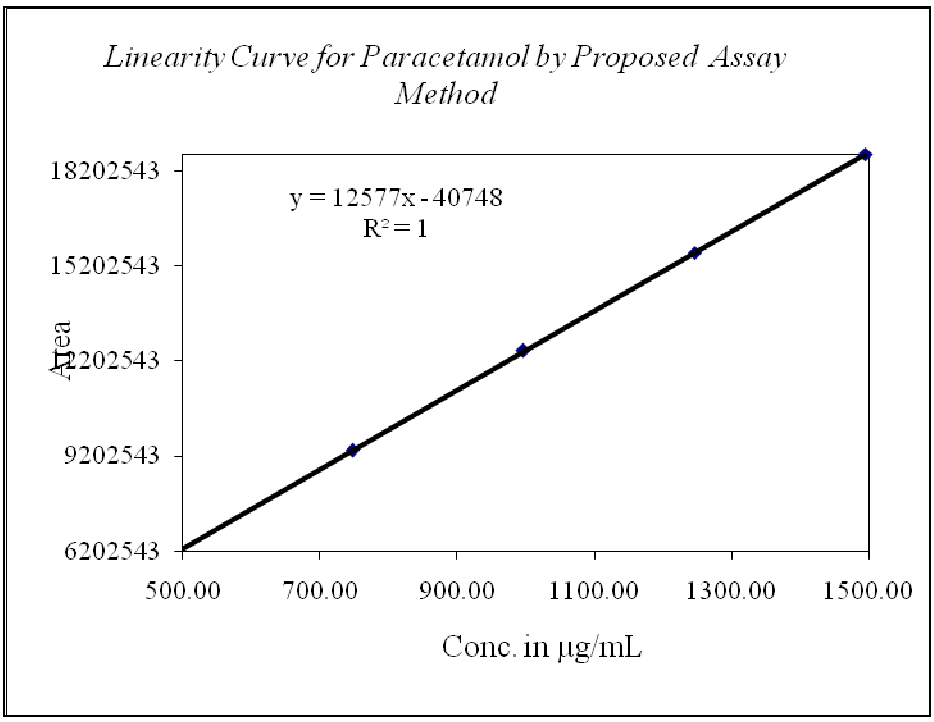
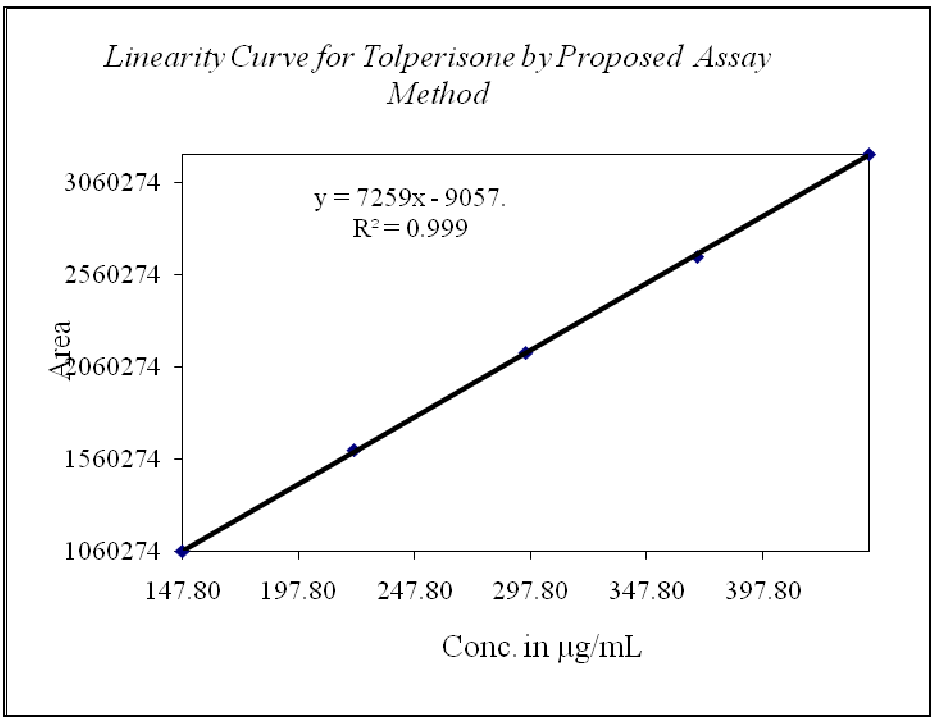


Table 4B: Linearity studies for Tolperisone by proposed method

Linearity Study for Tolperisone		
% Level	**Conc. µg/mL**	**Area**
50	147.80	1060274
75	221.70	1607774
100	295.60	2139750
125	369.50	2658662
150	443.40	3217022
Slope		7259.0
Intercept		-9057.0
% Y-Intercept		-124.8
Residual Sum of Squares		10698.0
CC(r)		0.99994
RSQ(r2)		0.99988
LLD		4.86
LLQ		14.74

Figure 6B: Calibration curve for Tolperisone



CONCLUSION

An RP-HPLC method for simultaneous estimation of Tolperisone and Paracetamol was developed and validated as per ICH guidelines.

The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 500-1500µg/mL for Paracetamol and 150-450µg/mL for Tolperisone. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed.

We have developed a fast, simple and reliable analytical method for determination of Tolperisone and Paracetamol in pharmaceutical preparation using RP-HPLC. As there is no interference of blank and placebo at the retention time of Paracetamol and Tolperisone. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Tolperisone and Paracetamol in bulk, its different pharmaceutical dosage forms.

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