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Original Article

STABILITY INDICATING UPLC METHOD FOR QUANTIFICATION OF TOLPERISONE HCL AND PARACETAMOL FROM MUSCLE RELAXANT COMBINATION TABLET

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ABSTRACT

Objective: In the present work, rapid and sensitive isocratic RP-UPLC method was established and comprehensive validation study for the estimation of Tolperisone HCl and Paracetamol was carried out according to international conference on harmonization (ICH) guidelines.

Methods: Simultaneous estimation was chromatographed using 0.1% o-phosphoric acid in water and acetonitrile (70: 30 v/v) as a mobile phase at a flow rate of 0.20 ml/min with 35 ºC column temperature. Chromatographic separation accomplished isocratically on Acquity UPLC BEH C_{18} (50 mm ×2.1 mm, particle size 1.7 µm) and detection utilizing photodiode array detector at 254 nm. Injection volume was 2.0 µl.

Results: The calibration curve was linear over the wide concentration range of 6.0µg/ml to 54.0µg/ml and 20.0µg/ml to 180.0µg/ml for Tolperisone HCl and Paracetamol, respectively. The retention time of Tolperisone HCl and Paracetamol was 1.396 and 2.625 min, respectively and the total analysis time was 5.0 min. Based on the results, the validated method was effectively applied for the estimation of Tolperisone HCl and Paracetamol in combined dosage form and in single pharmaceutical formulations with a new generation instrument, ultra performance liquid chromatography (UPLC). Moreover, the method helps to get better quality control and to pledge therapeutic efficacy.

Conclusion: The method is simple, less time consuming and comparatively cost effective than existing methods.

Keywords: Simultaneous, Isocratic, Stability indicating, Ultra performance liquid chromatography, Tolperisone HCl, Paracetamol.

INTRODUCTION

Tolperisone HCl, 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one is a piperidine derivative. The drug acting as a muscle relaxant for spastic paralysis and another encephalopathy are manifested with muscular dystopia and other muscle related pathological problem caused by neurological diseases [1]. Paracetamol, N-(4-hydroxyphenyl) ethanamide acting as a mild analgesic as well as an antipyretic agent to reduce fever and pain, Paracetamol is an active metabolite of Phenacetin [2]. The chemical structures are given in fig. 1.

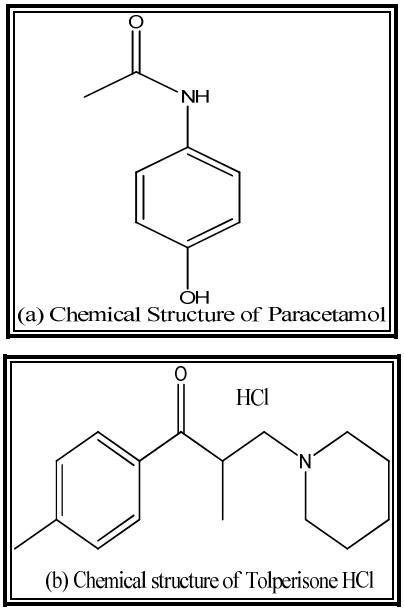


Fig. 1: Chemical structure of (a) Paracetamol (b) Tolperisone HCl

According to the review of the literature, there are many methods available for the estimation of Tolperisone HCl or Paracetamol either in single or combination pharmaceutical dosage form or in bulk drug by HPLC and HPTLC [3-5]. Some analytical methods are reported for the analysis of both the drugs from different sample matrix in single dosage form or bulk [6-9]. A spectrometric method for the simultaneous quantitative analysis of Tolperisone HCl and Paracetamol in the tablet has been reported [10, 11]. There is no any method reported for the simultaneous determination of Tolperisone HCl and Paracetamol by UPLC. The objective of this work was to develop a simple, buffer free, rapid and robust stability indicating assay method. The content uniformity study was carried out to show the applicability of the method for the drug product. All the validation parameter performed as per ICH Q2 (R1) guideline [12].

MATERIALS AND METHODS

Reagents and chemicals

The Tolperisone HCl and Paracetamol reference standards were gifted by Hetero labs limited, Visakhapatnam, India. The commercially available combination and individual pharmaceutical formulations labeled 150 mg Tolperisone HCl and 500 mg Paracetamol content were purchased from the market. HPLC grade acetonitrile and o-phosphoric acid purchased from Merck India Limited (Mumbai, India). High purity water was prepared using Milli-Q, Millipore (Milford, USA) water purification system. The other chemicals like hydrochloric acid, sodium hydroxide pellets and hydrogen peroxide solution 30% (v/v) were analytical grade, purchased from Ranbaxy Fine Chemicals (New Delhi, India) and 0.45 µm and 0.22µm membrane filters were obtained from Pall Life Sciences (Mumbai, India).

Equipment

The Waters Acquity™ UPLC chromatographic system used to perform development and validation (Waters, Milford, MA, USA). This system consists of a binary solvent manager (BMS), the photodiode array detector, sample manager (SM) and column oven

connected to a multi-instrument data acquisition and processing system Empower 2.1 version. A Sartorius CPA2P analytical microbalance (Gottingen, Germany), an ultrasonic bath SONICA used for degassing purpose from Spincotech Pvt. Ltd. (Mumbai, India). Milli-Q, Elix-3 water purification system (Millipore, Milford, USA) used as a HPLC grade water source.

Chromatographic condition

Chromatographic analysis was performed on waters Acquity BEH C18, 50 mm x2.1 mm and 1.7 µm columns. The mobile phase consisted mixture of 0.1% ortho-phosphoric acid in water: acetonitrile (70:30 v/v) the mixture was degassed in an ultrasonic bath. The flow rate of the mobile phase was adjusted at 0.20 ml/min and the injection volume was 2.0 µl. Elution was monitored using PDA detector at a wavelength of 254 nm at a column temperature 35 °C.

Stock solutions for Standard

The stock solution for the standard was prepared from the reference standard to furnish the final concentration of Tolperisone HCl 150 µg/ml and Paracetamol 500µg/ml for standard stock solutions preparation.

Stock solutions for test

The stock solution for the sample was prepared from Pharmaceutical formulation (twenty combination tablets were crushed and powdered) to obtain a final concentration of Tolperisone HCl 150µg/ml and Paracetamol 500µg/ml (equivalent weight) for test stock solutions preparation.

Standard and test solution

Standard and test solutions were prepared (30µg/ml of Tolperisone HCl solution and 100µg/ml of Paracetamol) from respective stock solution by taking 5 ml stock solution in 25 ml volumetric flask and dilute with mobile phase up to 25 ml.

RESULTS

Method development and optimization

A selection of the method depends upon the nature of the sample, solubility, and molecular weight. Tolperisone HCl and Paracetamol can be easily soluble in water acetonitrile. Column and mobile phase selection made to avoid the all inexcusable aspects like high column backpressure, a poor resolution between two peaks, less theoretical plates, peak tailing and shape.

The Paracetamol and Tolperisone HCl were eluting at all most same retention time, using different column chemistry as well as column length like HSS T3 (1.8 µm particle size), BEH C 8 (1.7 µm particle size), BEH Shield RP 18 (1.7 µm particle size) with different mobile phase combinations such as 0.02M phosphate buffer, 0.02M acetate buffer with acetonitrile and methanol. The result was found satisfactory with 0.1% o-phosphoric acid in water: acetonitrile as an isocratic elution. Method optimization made with different mobile phase composition starts from 50:50 to 70:30, 0.1% OPA in water: acetonitrile (table 1). The satisfactory results found with 70:30 ratios, at 35 °C temperature with adopted all chromatographic condition.

Table 1: Summary of mobile phase optimization

Mobile Phase-A	Mobile Phase-B	Observation from chromatogram
Water	Methanol	Very broad peaks with peak splitting and less resolved peaks
Water	Acetonitrile	Broad, less resolved peaks with tailing
5 mM Ammonium acetate	Methanol	Merged peaks, very broad and symmetry factor is not satisfactory
5 mM Ammonium acetate	Acetonitrile	Broad, less resolved peaks
5 mM Potassium dihydrogen phosphate(pH= 2.5 by OPA)	Methanol	Peak eluted early with peak tailing, Poor resolution.
0.1% o-phosphoric acid in water	Methanol	Peaks are broad but resolved
0.1% o-phosphoric acid in water	Acetonitrile	Good, sharp peak shape with good System Suitability results.

The standard solution was screened over the range 190-400 nm by use of photodiode array detector. On the basis of peak absorption maxima of the analyte and the degradation products, the 254 nm was decided as the detection wavelength. This gives the maximum chromatographic compatibility to the method. The chromatographs of standard preparation (a) and test preparation (b) are given in fig. 2.

Stress degradation-specificity study

Forced degradation studies were carried out for the applicability of the method as a stability-indicating method for the estimation of Tolperisone HCl and Paracetamol. The study was performed on Tolperisone HCl and Paracetamol by applying stress conditions like exposure of daylight (72h), acid and alkali hydrolysis (1 N HCl and NaOH), oxidation (3% H_2O_2) and heat (60 °C, 72h). Acid, alkali at 40°C and oxidation degradation was carried out at room temperature for 2h in a water bath. The peak purity of the principal peaks of the chromatogram of Tolperisone HCl and Paracetamol (combination tablet) degradation sample was checked with the help of a PDA detector.

The peaks of degradation products could be observed from the chromatographs of oxidation and acidic stress conditions given in fig. 3(a) and (b) respectively. The outcome of the study shows degradation under all stress condition in more or less percent (table 2). The principle peaks were observed pure and well resolved from degradation product, that confirmed by PDA peak purity spectrum. The result indicates that proposed method is specific and stability indicating.

Validation parameters

Method validation characteristics deal with solution stability study, system suitability, linearity, limit of quantification, limit of detection, accuracy, precision and robustness.

The method for the simultaneous estimation of Tolperisone HCl and Paracetamol was validated as per ICH guidelines.

Solution stability

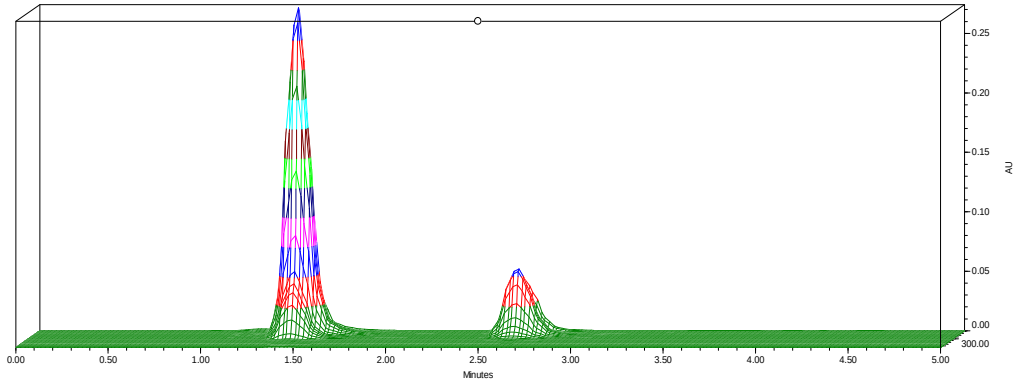
Solution stability period for the solutions of the standard preparation and test preparation was evaluated. The solutions were stored at 3-5 °C and ambient temperature without protection against light and tested at the interval of 6, 12, 24, 36 and 48h. The responses for the aged solution were evaluated using a freshly prepared standard solution after each interval.

The solution stability was evaluated by the means of retention time, area and degradation occurred during the study.

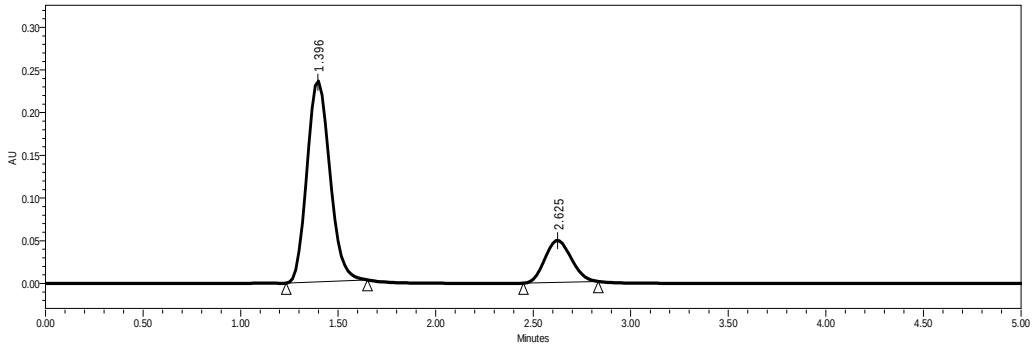
However, the results show that not much variation in the peak area and retention time for test solution against the standard solution and no degradation happened during the study. The percentage assay for both the drugs was obtained 98.71% to 101.30%.

Accuracy study

Accuracy was assessed by the recovery study at three level 50%, 100% and 150% of sample concentrations, for each level three different sets were prepared and injected in duplicate. The % recovery was found between 99.46-100.54% and 99.55-100.13% for Tolperisone HCl and Paracetamol, respectively. The results are under the acceptance criteria to ICH guideline Q2 (A) (table 3).

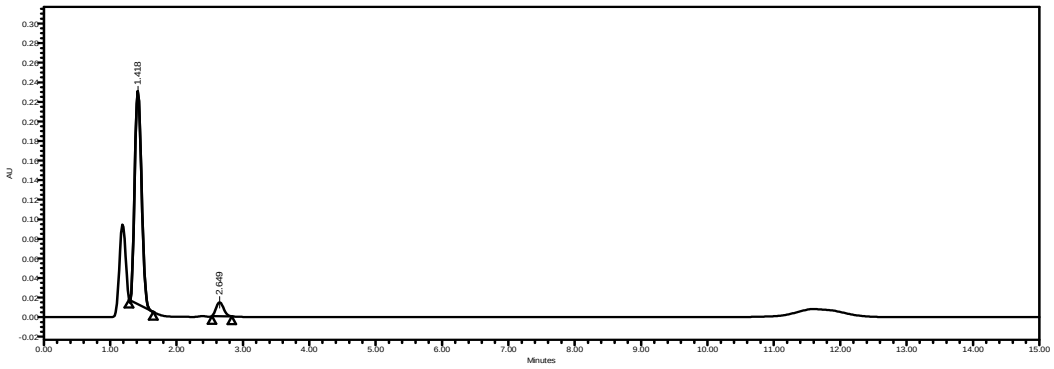


(a) Chromatogram of Paracetamol and Tolperisone HCl reference standard

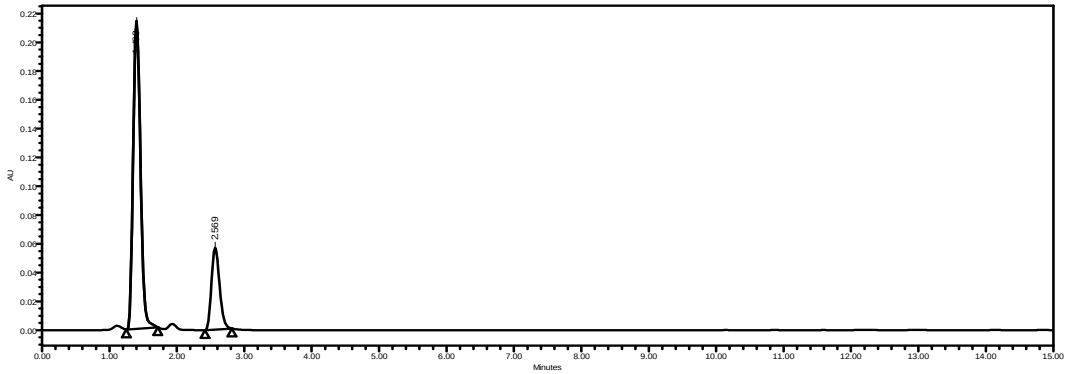


(b) Chromatogram of Paracetamol and Tolperisone HCl combination tablet

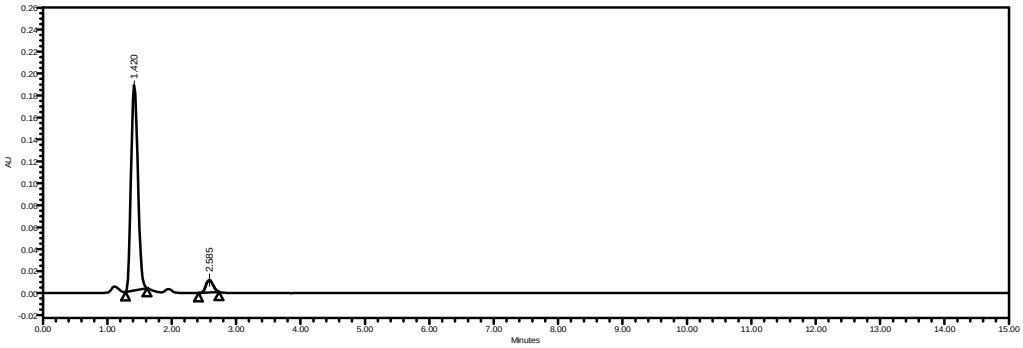
Fig. 2: Chromatographs of standard (a) and test preparation (b)



(a) Oxidation stress degradation



(b) Acidic stress degradation



(c) Alkali stress degradation

Fig. 3: Chromatographs of stress degradation study

Table 2: Data table for force degradation results

Name of drug	Sample type	Mean Area[a]	Sample Weight (mg)	% Recovery	% Degradation
Tolperisone HCl	Standard	489653	15.0	-----	-----
	Acid	451319	14.9	96.8	3.2
	Alkali	444010	15.2	94.4	5.6
	Oxidation	410119	15.1	92.8	7.2
	Thermal	473921	15.0	89.5	10.5
	Photolytic	456012	14.8	83.2	16.8
Paracetamol	Standard	1941020	50.0	-----	-----
	Acid	1749104	49.9	95.5	4.5
	Alkali	1700309	50.0	93.1	6.9
	Oxidation	1652913	49.8	90.3	9.7
	Thermal	1853621	50.1	87.6	12.4
	Photolytic	1799822	49.8	85.5	14.5

a: Mean area of five injections of standard and two injections of sample treated with degradation conditions

Table 3: Accuracy study of Tolperisone HCl and paracetamol

Drug substance	Accuracy level %	Prepared concentration (µg/ml)[a]	Observed Concentration (µg/ml)[a]	Mean recovery (%)	RSD (%)
TOL	50%	15.70	15.79	100.54	1.43
	100%	30.12	29.96	99.46	0.59
	150%	44.86	45.04	100.41	1.00
PAR	50%	50.49	50.50	100.03	1.09
	100%	99.74	99.54	100.13	0.82
	150%	149.02	148.35	99.55	0.25

a: Mean of three replicates for each accuracy level

Limit of detection and limit of quantification

The measurement of LOD and LOQ were performed by preparing the serial dilution of stock solution until the signal to noise ratios were 3:1 and 10:1 for Limit of detection and Limit of quantification, respectively. LOD for Paracetamol and Tolperisone HCl were 0.2 µg/ml and 0.06 µg/ml and LOQ values were 0.5µg/ml and 0.15 µg/ml, respectively.

Linearity study

The peak area of Tolperisone HCl and Paracetamol showed linear calibration curve with respect to concentrations over the range of 6-54µg/ml and 20-180µg/ml for Tolperisone HCl and Paracetamol respectively. The linear regression equations were y = 96814x-88376, correlation coefficient 0.9999 for Tolperisone HCl and y = 47275x-47746, correlation coefficient 0.9998 for Paracetamol, where x is the concentration in µg/ml and y is the peak absorbance in units. The result showed an excellent correlation exists between the peak area and concentration of the drugs solution within the selected wide range of concentration, indicated previously. The "Linear Regression Least Squares Fit" was the data analysis tool for linearity.

Precision study

The precision study was carried out in terms of method precision and intermediate precision. Method precision was assessed by analyzing three times, multiple preparations of the same drug sample. Six different sample sets were injected to evaluate method precision on the same day.

Moreover, freshly prepared sample solutions were analyzed on each of two successive days for intermediate precision study. The % assay values calculated against peak area of the standard solution for drug samples. The content uniformity test also performed for different ten sets and % RSD calculated for both study were well within the acceptance criteria, less than 2% RSD of peak area.

Robustness study

In robustness study, influences of different (k) chromatographic parameters were evaluated by assaying test solution after small but deliberate changes in the analytical conditions. The factor examined were the amount of acetonitrile, an amount of OPA, column

temperature, detection wavelength, column lot and flow rate. There was no significance influence observed on retention time and % assay during this study, which suggests that method, is highly robust. The results are given in (table 4).

Table 4: Summary data of robustness study of Tolperisone HCl and Paracetamol

Robustness Conditions	% Assay[a]	RT[b] (min)	% RSD[d] of STD	System Suitability Parameters	
				Theoretical Plates[e]	USP Tailing[e]
Summary of paracetamol					
Flow rate 0.18 ml/min	100.82	1.407	0.54	6655	1.137
Flow rate 0.22 ml/min	101.06	1.212	0.47	6475	1.140
0.1% OPA: acetonitrile (68:32)	100.62	1.312	0.56	6525	1.135
0.1% OPA: acetonitrile (72:28)	102.00	1.360	1.22	6550	1.137
Column temperature 28 ° C	99.97	1.332	1.08	6545	1.137
Column temperature 32 ° C	101.34	1.326	0.47	6522	1.136
Detection wavelength 251 nm	99.15	1.329	0.96	6534	1.136
Detection wavelength 257 nm	101.39	1.329	0.45	6535	1.137
Summary of tolperisone HCl					
Flow rate 0.18 ml/min	99.96	2.636	0.47	2708	1.136
Flow rate 0.22 ml/min	99.95	2.274	0.78	2598	1.137
0.1% OPA: acetonitrile (68:32)	99.41	2.856	1.02	2678	1.134
0.1% OPA: acetonitrile (72:28)	99.94	2.847	1.12	2657	1.136
Column temperature 28 °C	99.94	2.479	0.43	2631	1.135
Column temperature 32 °C	101.45	2.451	0.35	2611	1.134
Detection wavelength 251 nm	98.39	2.463	0.41	2621	1.135
Detection wavelength 257 nm	101.41	2.462	0.83	2618	1.135

a: Mean of % assay of five replicate, b: Mean retention time of five replicate injections, c: Mean of the percentage relative standard deviation of standard sample, d: Mean theoretical plates and USP tailing factor obtain from five replicate samples

System suitability studies

The standard solution of 100µg/ml of Tolperisone HCl solution and 30µg/ml of Paracetamol was prepared and injected before all validation parameter. System suitability parameters like theoretical plates, peak asymmetry, resolution, % RSD for peak area and retention time were calculated from the chromatogram of standard solution (table 5).

Table 5: Result summary of system suitability study

System Suitability Parameter[e] In-house Limits	% RSD[a] NMT[b] 2.0	Theoretical Plates NLT[c] 6000	Tailing Factor NMT[b] 2.0	% RSD[a] NMT[b] 2.0	Theoretical Plates NLT[c] 2500	Tailing Factor NMT[b] 2.0
Validation parameters	**Paracetamol**			**Tolperisone HCl**		
Solution stability	0.02	6676	1.136	0.47	2669	1.134
Specificity	0.69	6679	1.136	0.23	2659	1.134
Linearity	0.52	6681	1.137	0.83	2657	1.135
LOQ[d]	0.18	6672	1.136	0.98	2662	1.136
Method precision	0.45	6671	1.137	0.44	2661	1.135
Inter[f]. precision	0.18	6675	1.136	0.25	2667	1.134
Accuracy	0.89	6684	1.136	0.85	2679	1.134
Robustness	0.71	6552	1.137	0.69	2648	1.135
a: Relative standard deviation				b: Not more than		
c: Not less than				d: Limit of Quantification		
e: Results of all parameters obtain from five replicate injection of standard.				f: Intermediate		

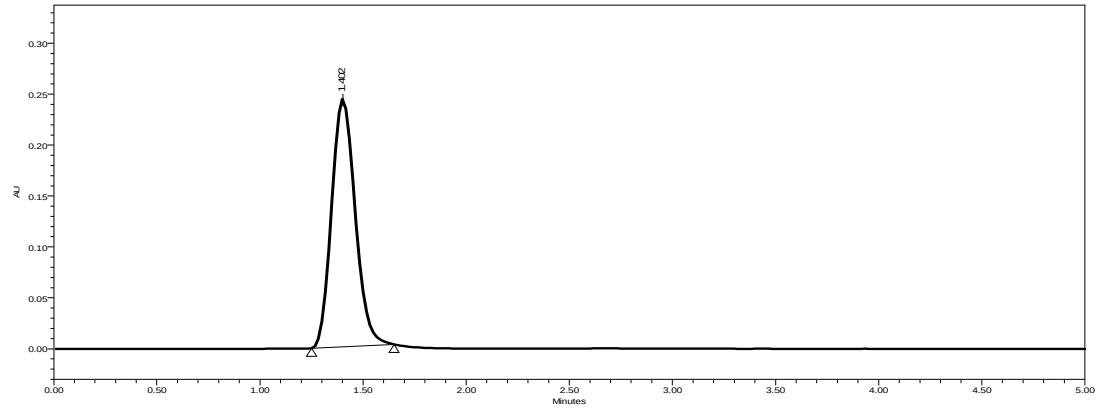
Results were verified by measurement of resolution (RS>2.0), % RSD for peak area and retention time (%RSD<2.0), tailing factor (T<2.0), peak asymmetry (A<2.0) and theoretical plates (N>4000) from chromatogram of standard solution.

Application of method

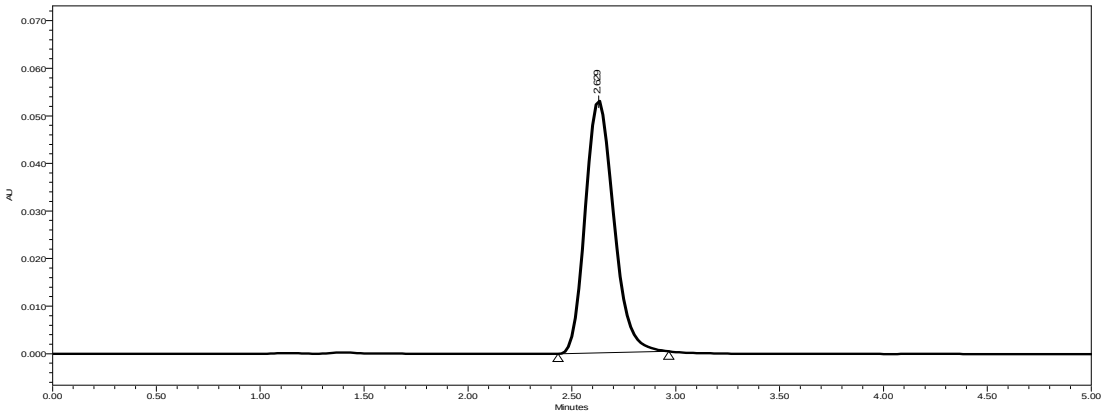
The work presented here is applicable for in-process quality control as well as the label claim study and content uniformity test. It is also useful for the determination of Tolperisone HCL and Paracetamol as a combination dosage form and in individual drug substance form. The label claim study carried out using different marketed tablets. The individual tablets labeled with Paracetamol, 500 mg (TAMOL, Apex Pharmaceuticals Ltd, India) and Tolperisone HCl, 150 mg (Tolpidol FC, Themis Medicare Ltd, India) and combination tablet (MYO-MR PLUS, Amanath Pharmaceuticals, India) were taken for test. The percentage RSD (<2%) and assay (98-102%) of six individual injections was measured. The results for both the formulations found within the limit. The chromatogram of individual marketed formulation preparations are given in fig. 4 (a) and (b) for Paracetamol and Tolperisone HCl.

DISCUSSION

The reported methods were developed and validated using HPLC instrument, whereas proposed research work deals with the column packing material with sub 2 µm particle size with a new generation instrument UPLC. The importance of the presented method over other reported methods includes; stability indicating methods with the shorter run-time of both the drugs which elute within 3 minutes. The flow rate is very low, thus the consumption of organic solvent is very less. The method is also applicable for the individual marketed formulations are discussed. The peak purity of both the drugs was checked using a PDA detector was found satisfactory and not reported by others.



(a) Paracetamol (TAMOL 500 mg, Apex Pharmaceuticals Ltd, India)



(b) Tolperisone HCl (Tolpidol FC 150 mg, Themis Medicare Ltd, India)

Fig. 4: Chromatographs of individual marked formulations

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CONFLICT OF INTERESTS

Declared None.

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