Journal of Current Chemical & Pharmaceutical Sciences

J. Curr. Chem. Pharm. Sc.: 3(4), 2013, 243-254
ISSN 2277-2871

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINING IMPURITY PROFILING FORTOLPERISONE HYDROCHLORIDE IN TABLET DOSAGE FORM

NANDINI R. PAI and SEEMA S. SAWANT*

Department of Chemistry, D. G. Ruparel College of Arts, Science & Commerce Senapati Bapat Marg, Mahim (West), MUMBAI – 400016 (M.S.) INDIA

(*Received : 11.09.2013; Revised : 18.09.2013; Accepted : 20.09.2013*)

ABSTRACT

A simple, rapid, sensitive, accurate, precise and reproducible high performance liquid chromatographic method was developed to estimate impurity[1,2] profile for Tolperisone hydrochloride[3] in tablet[4,5] dosage[2] form. The HPLC[2] analysis used a reversed phase Oyster ODS (300 x 4.6 mm, 5 µm) column and a mobile phase constituted of buffer and acetonitrile (45 : 55 % v/v). The buffer was composed of 95.0 mL 0.1 M citric acid, and 5.0 mL of 0.2 M sodium dihydrogen phosphate in 1000 mL of water, mix and adjusted the pH to 2.5 with orthophosphoric acid and with addition of 10 g of sodium lauryl sulfate. The wavelength of the detection was 260 nm. The validation[6,7] data showed that the method is sensitive, specific and reproducible for the impurity determination of Tolperisone hydrochloride in the tablet dosage form. The method was linear from 0.5 µg/mL to 3.5 µg/mL for Tolperisone hydrochloride, impurity E[2,3] (vinyl ketone) and 2-methylhydroxy impurity and from 0.2 µg/mL to 1.4 µg/mL for impurity 4-MMP.[2,3] The accuracy of the method was found to be 99.39% for 4-MMP impurity, 99.38% for impurity E (vinyl ketone) and 100.22% for 2-methylhydroxy impurity.[8,9] Inter and intraday assay relative standard deviation (RSD) was found 0.32% for 4-MMP impurity, 0.32% for impurity E (vinyl ketone) and 0.47% for 2-methylhydroxy impurity in tablet dosage form. The proposed method provided an accurate and precise analysis of 4-MMP impurity, impurity E (vinyl ketone) and 2-methylhydroxy impurity for Tolperisonehydrochloride in pharmaceutical dosage form.

Key words: 4-MMP, Vinyl ketone, 2-methyl hydroxy, Validation.

INTRODUCTION

Impurity[1] profiling of active pharmaceutical ingredients and formulations is one of the most challenging tasks of analytical chemists in the pharmaceutical industry. Tolperisone hydrochlorideis chemically 2-methyl-1-(4-methyl phenyl)-3-(1-piperidyl)propane-1-one is a piperidine derivative and the structure was shown in Fig. 1.

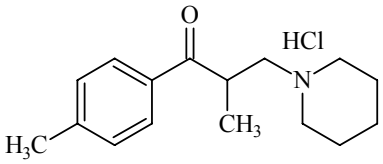

Fig. 1: Chemical structure of Tolperisone hydrochloride

Available online at www.sadgurupublications.com
*Author for correspondence; E-mail: ssawant_s@yahoo.com

Tolperisonea central muscle[10-12] relaxant suitable for cerebral arteriosclerosis and for treating extra piramidal movement disorders. It is a centrally acting muscle relaxant,[13,14] which is used in the treatment of different pathological[5,15,16] conditions like multiocular sclerosis, myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbarsyndrome, Arthrosis of the large joint sobliterating artherosclerosis of the extremityvessels, diabeticalangiopathy, thromboangitisobliterans, raynaudssyndrome. Tolperisone hydrochloride is official in Japanese pharmacopoeia.[3] Tolperisone has the unique property of mediating[17,18] muscle relaxation without concomitant sedation and it does not cause in coordination, weakness and mental confusion or withdrawal phenomena, in contrast to other muscle relaxants.

During the synthesis[8,13,14] of Tolperisone hydrochloride, some possible degradation impurities or organic[1] impurities or due to mannich[9] condensation reaction some trace impurities get generated. The literature survey revealed that there are some analytical methods reported for Tolperisone hydrochloride either individually like visible spectrophotometric method,[5,16] HPTLC[15] or in combination[11,17] with other drugs by RP-HPLC,[2,12,18] and also reported on biological fluids. In biological fluids such as human plasma[12] and urine by LC-MS and LC-MS-MS were reported previously. However, the method that identified some process related or degradation[14,19] impurities by RP-HPLC method has been reported. This method described the analysis and identification of some process[14,19] related impurities 1-(4-methylphenyl)propan-1-one (4-MMP), 2-methyl-1-(4-methylphenyl)prop-2-en-1-one (vinyl ketone) and trace impurity generated due to mannich condensation reaction i.e. 2-methyl analog of Tolperisone (2-methyl hydroxy impurity) in Tolperisone hydrochloride in it's tablet dosage form and by complementary use of the HPLC techniques. In the present study, we aimed to develop and validate a RP-HPLC-DAD impurity study method thatallowed resolution, detection and quantitation of Tolperisone hydrochloride and it's impurities 4-MMP, vinyl ketone, 2-methyl hydroxyl impurity in it's tablet dosage form.

We report the development and validation[6,7] of a simple HPLC impurity determination with UV detection for the quantitative determination of4-MMP, vinyl ketone, 2-methyl hydroxy impurities in tablet dosage form.

EXPERIMENTAL

Materials and methods

Chemicals and reagents

All the reagents were of analytical-reagent grade. De-ionized water (Millipore), HPLC-grade acetonitrile, Sodium dihydrogen phosphate AR grade, citric acid AR grade, sodium lauryl sulphate AR grade, orthophosphoric acid AR grade were used.

Instrumentation

The HPLC system was composed of LC 2010 Shimadzu system fitted with Prominence PDA detector with LC Solution software. Analytical column used for this method was Oyster ODS (300 mm x 4.6 mm) 5 µm.

Buffer preparation

Add 95.0 mL of 0.1 M citric acid, and 5.0 mL of 0.2 M sodium dihydrogen phosphate in 1000 mL of water, mix and adjust the pH to 2.5 with orthophosphoric acid. Add 10 g of sodium lauryl sulfate.

Mobile phase preparation

Acetonitrile and buffer in the ratio of 55 : 45

Mobile phase used as a diluent.

Standard preparation

Tolperisone hydrochloride reference substance was accurately weighed (25 mg) and dissolved in 15 mL quantity of diluent in a 50 mL volumetric flask and diluted upto the mark with diluent and it was further diluted to generate a concentration of 2.5 µg/mL

Impurity standard preparation

4-MMP impurity: 4-MMP impurity was accurately weighed (5 mg) and dissolved in 150 mL of diluent in 200 mL volumetric flask and diluted upto the mark with diluent and it was further diluted to generate a concentration of 1.0 µg/mL**.** Vinyl ketone impurity: Vinyl ketone impurity was accurately weighed (5 mg) and dissolved in 150 mL diluent in 200 mL volumetric flask and diluted upto the mark with diluent and it was further diluted to generate a concentration of 2.5 µg/mL 2-methyl hydroxy impurity: 2-methyl hydroxy impurity was accurately weighed (5 mg) and dissolved in 150 mL diluent in 200 mL volumetric flask and diluted upto the mark with diluent and it was further diluted to generate a concentration of 2.5 µg/mL.

System suitability solution preparation

Tolperisone hydrochloride reference substance was accurately weighed (25 mg) and dissolved in 15 mL diluent in a 50 mL volumetric flask. Added 2 mL of 25 µg/mL of 4-MMP impurity solution, 5 mL of 25 µg/mL of vinyl ketone impurity solution and 5 mL 25 µg/mL of 2-methyl hydroxy impurity solution and diluted upto the mark with diluent to get the concentration of 500 µg/mL for Tolperisone hydrochloride, 1.0 µg/mL of 4-MMP impurity and 2.5 µg/mL of each of vinyl ketone and 2-methylhydroxy impurity.

Sample preparation

Twenty tablets of Tolperisone hydrochloride (150 mg of Tolperisone hydrochloride) were separately weighed and grounded to fine powder. An amount of tablet powder equivalent to 25 mg of Tolperisone hydrochloride was transferred into a 50 mL volumetric flask and added about 15 mL of diluent; sonicated for 10 minutes to dissolve and made up the volume to 50 mL with diluent to generate a concentration of 500 µg/mL. Filtered through 0.45 µ filter paper.

Chromatographic conditions

Before the mobile phase was delivered into the system, buffer and acetonitrile were filtered through 0.2 µm, PVDF membrane filter and degassed using vacuum. The chromatographic conditions which were used for the analysis are reproduced below -

Column: Oyster ODS (300 mm x 4.6 mm) 5 µm

Wavelength: 260 nm

Injection volume: 10 µL

Flow rate: 1.2 mL/min

Column temperature: 30ºC

Run time: 40 min

Method development

Detection wavelength for the HPLC study was selected as 260 nm after recording the UV spectrum from 190 to 800 nm of the drug and representative sample from standard, impurity standard solution and

sample solution by using PDA detector HPLC. The suitable area and peak selectivity of Tolperisone hydrochloride and it's impurities were observed at this wavelength. The chromatographic conditions were optimized for resolution of the peak of the drug and it's impurity under each condition by varying the stationary phase, proportion of methanol/acetonitrile/water in the mobile phase and the flow rate using representative samples. Several trials using various proportions of acetonitrile and water as mobile phase were carried out. However, to attain the selective resolution of Tolperisone hydrochloride and it's impurities, acetonitrile and citric acid and sodium dihydrogen phosphate mixture buffer was introduced as the third proportion; apparent pH 2.5 was adjusted by orthophosphoric acid, sufficient amount of sodium lauryl sulphate was added to improve the dissolution of these impurities and better elution. Subsequently, a mixture of different mobile phase composition was used to optimize the chromatographic conditions for resolving Tolperisone hydrochloride and it's impurities in a single run. An appropriate blank was injected before the analysis of all the samples. Such an optimized method was then used to study the impurity study of Tolperisone hydrochloride in it's tablet dosage form.

Method validation

Method validation was conducted according to published guidelines. Impurity profiling was evaluated by intraday and inter day (two different days) precision and determined from replicate analysis of samples (500 µg/mL). Analysis of six different sample solutions was performed in the same day for intraday precision. The precision were expressed in terms of RSD from mean intra and inter day sample analysis accuracy of the method was tested by adding a known amount of 4-MMP impurity standard (0.25, 1 and 1.25 µg/mL), vinyl ketone and 2-methyl hydroxy impurity standard (both of 0.625, 2.5 and 3.125 µg/mL) in three sample solutions. Calculated the percent recovery from the peak areas obtained for diluted solutions.

Signal-to-noise ratios were employed to estimate limits of detection (3 : 1) and limits of quantitation (10 : 1) for 4-MMP, vinyl ketone and 2-methyl hydroxy impurity. The specificity of a method is its suitability for analysis of a substance in presence of impurities. Specificity of the method was established through the study of the resolution (Rs) of Tolperisone hydrochloride samples. Overall selectivity was established through determination of drug purity and R speak area RSD each time. Various system suitability parameters were also evaluated on a mixture sample on different days using freshly prepared mobile phase each time.

Robustness was tested by analysis of variations in analytical condition. Influence of mobile phase composition and pH were evaluated. The chromatographic parameters monitored were peak retention time, tailing factor and theoretical plate number.

RESULTS AND DISCUSSION

Method development and optimization

Using a mobile phase consisting of different buffers with methanol and acetonitrile at different concentrations and at different mobile phase pH values were attempted. Changes in the analytical procedure were tested. Different mobile phases with different proportions of organic modifier (acetonitrile) were tried. The pH value of the mobile phase was checked over a wide range (2.5-4.0). The pH of the aqueous phase was adjusted with orthophosphoric acid. It was observed that the peak shape and retention time of Tolperisone was broad compared to the buffer-acetonitrile composition as mobile phase. After various trials of different buffer and acetonitrile ratios as mobile phase, sodium dihydrogen phosphate with citric acid was selected as buffer, pH was adjusted to 2.5 with orthophosphoric acid, with addition of sodium lauryl sulfate and buffer-acetonitrile ratio was chosen to be 45 : 55. Chromatographic run was evaluated using Oyster ODS column. After selecting the best conditions based on peak performance, the run time of the proposed

method was 40 min with isocratic elution. During injection of a standard and sample solution, the retention times found were about 16.6 minute for Tolperisone hydrochloride, about 9.0 minute for 4-MMP impurity, about 11.0 minute for vinyl ketone impurity and about 13.0 minute for 2-methylhydroxy impurity. It shows good resolution of chromatogram with symmetrical peak. The proposed chromatographic conditions were found to be appropriate for quantitative determination. System suitability tests were carried out as per ICH guidelines and the parameters are summarized in Table 2 referred to in Specificity validation parameter. Figs. 2, 3 and 4 are shown for system suitability, standard and sample solution graph.

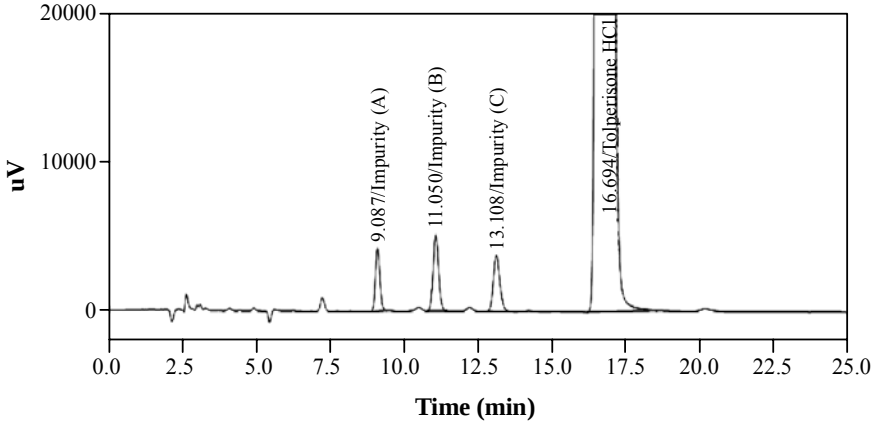

Fig. 2: Chromatogram of the system suitability solution

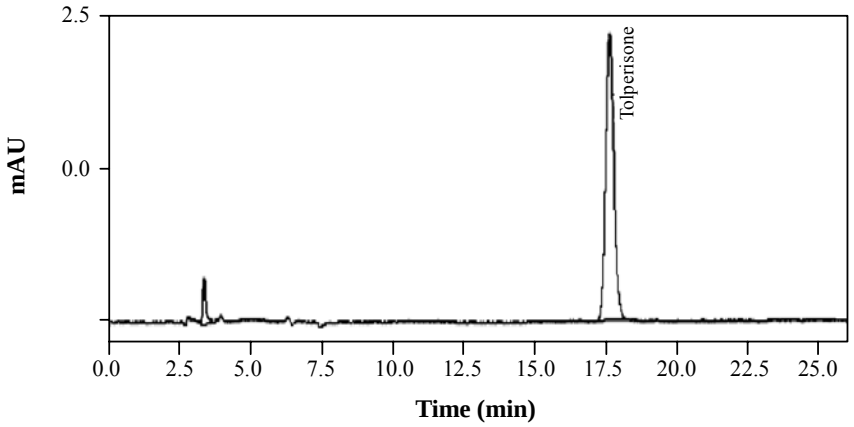

Fig. 3: Chromatogram of the standard solution

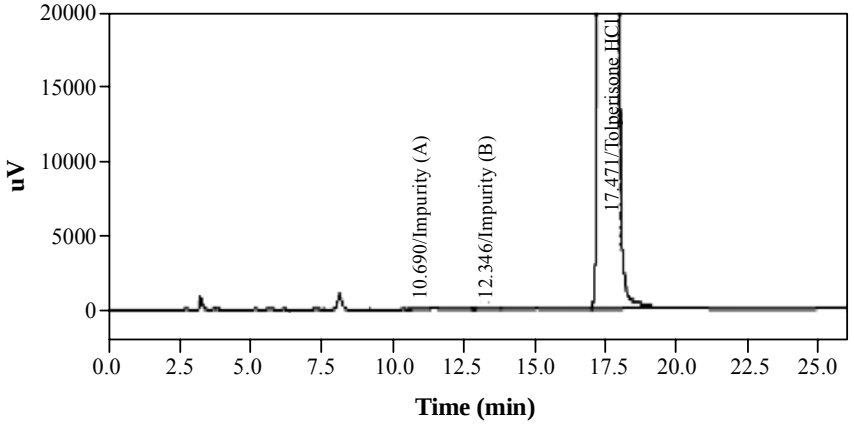

Fig. 4: Chromatogram of the sample solution

Response factor

The measurement of the response factor for each impurity determination is important when the calculations are being made on a relative percent basis. Hence, authentic samples of the known impurities and Tolperisone hydrochloride were dissolved in the diluent and injected, and then responses were calculated.

Method validation

Linearity: Linearity was studied by preparing standard solutions at different concentration levels for Tolperisone hydrochlorideand it's impurities. The linearity range was found to be 0.5-3.5 µg/mL for Tolperisone hydrochloride, 0.2-1.4 µg/mL for 4-MMP impurity and 0.5-3.5 µg/mL for vinyl ketone and 2-methyl hydroxy impurities.

Table 1 shows linearity values observed for Tolperisone hydrochloride and it's impurities. Figs. 5, 6, 7 and 8 are given for linearity graph of Tolperisone hydrochloride, 4-MMP, vinyl ketone and 2-methyl hydroxy impurity, respectively.

Table 1: Linearity values observed for Tolperisone hydrochloride and it's impurities

Linearity parameter	Tolperisone hydrochloride	4-MMP impurity	Vinyl ketone impurity	2-methyl hydroxy impurity
Concentration range	0.5-3.5 µg/mL	0.2-1.4 µg/mL	0.5-3.5 µg/mL	0.5-3.5 µg/mL
Correlation coefficient	0.99953	0.999997	0.99999	0.99976
Slope	1009.80	565.97	966.91	578.67
Y - Intercept	1532.50	301.80	56.65	-158.29
R-square	0.99906	0.99993	0.99998	0.99953

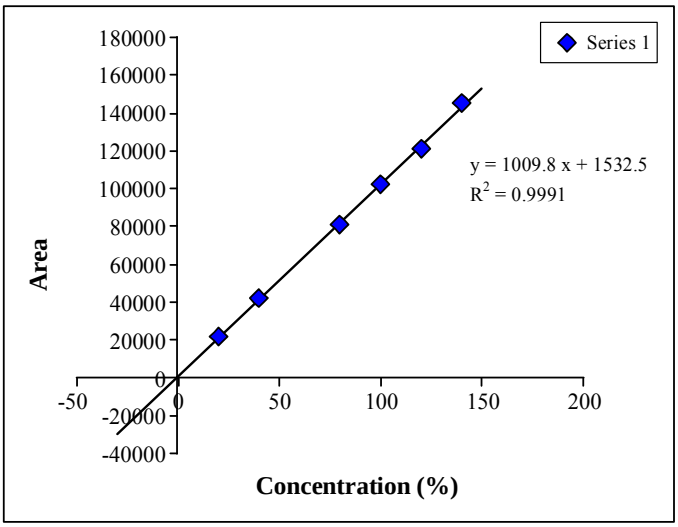

Fig. 5: Linearity graph of Tolperisone hydrochloride

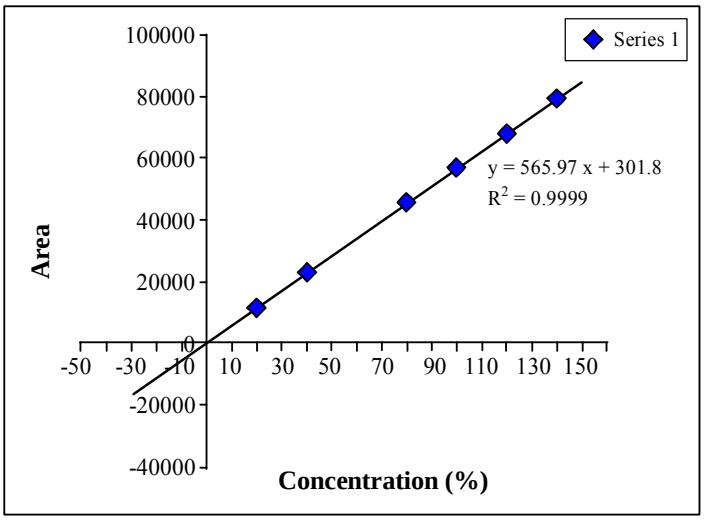

Fig. 6: Linearity graph of 4-MMP impurity

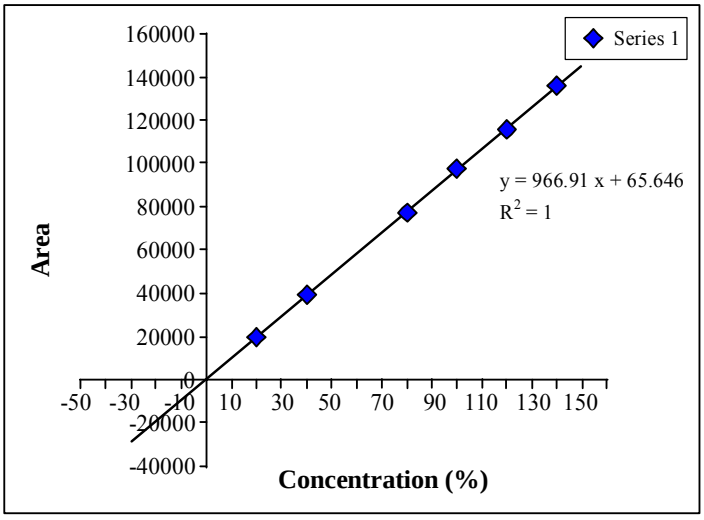

Fig. 7: Linearity graph of vinyl ketone impurity

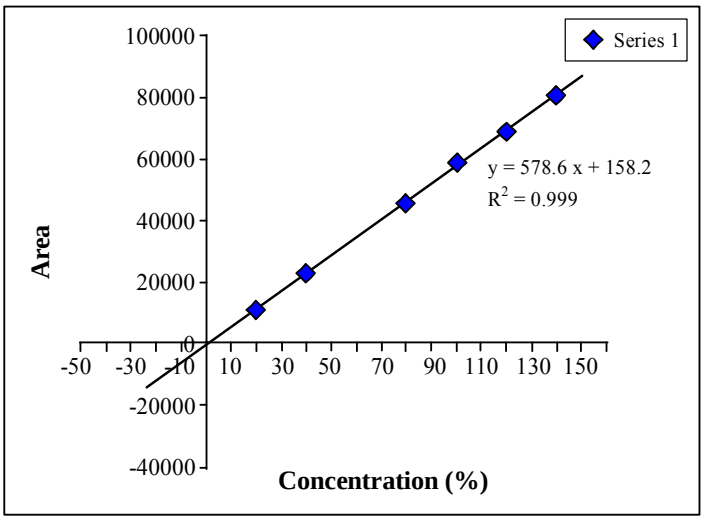

Fig. 8: Linearity graph of 2-methylhydroxy impurity

Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that maybe expected to be present. Typically, these might include impurities, degradants, matrix, etc. Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interference from the blank and placebo. Specificity of the peak purity of Tolperisone hydrochloride, 4-MMP, vinyl ketone and 2-methylhydroxy impurity were assessed by comparing the retention time of standard and the sample and good correlation was obtained. Injected the individual identification solutions of Tolperisone hydrochloride, 4-MMP, vinyl ketone and 2-methylhydroxy impurity each; for the identification purpose. All the peaks were found pure in presence of each other. Also there were no peaks when the placebo and blank were injected and no interferences, hence the method is specific. System suitability solution was injected to determine the resolution, tailing factor and theoretical plates for all the peaks.

Table 2 shows specificity study values observed for Tolperisone hydrochloride and it's impurities. Figs. 9, 10, 11 and 12 show Tolperisone hydrochloride, 4-MMP, vinyl ketone and 2-methylhydroxy impurity peak purity graph, respectively

Table 2: Specificity study values observed for Tolperisone hydrochloride and it's impurities

Specificity study	Tolperisone hydrochloride	4-MMP impurity	Vinyl ketone impurity	2-methyl hydroxy impurity
Retention time in minute	16.691	9.084	11.048	13.103
Relative retention time	1.0	0.54	0.66	0.79
Resolution	7.629	-	5.434	6.136
Tailing factor (NMT 2.0)	1.295	1.161	1.145	1.146
Theoretical plates (More than 2000)	15737.77	15480.46	16115.84	16472.84
Peak purity: Peak purity index	0.99999	0.99988	0.99996	0.99959
Blank/Placebo interference	Not detected	Not detected	Not detected	Not detected
% RSD peak area (NMT 2.0%)	0.75 %	0.19 %	0.28 %	0.61 %

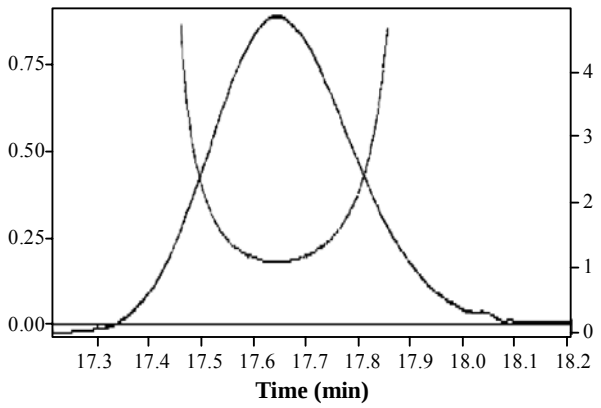

Fig. 9: Tolperisone hydrochloride standard peak purity graph

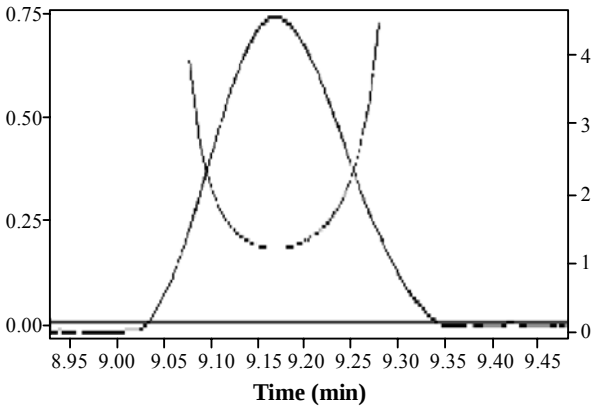

Fig. 10: 4-MMP impurity peak purity graph

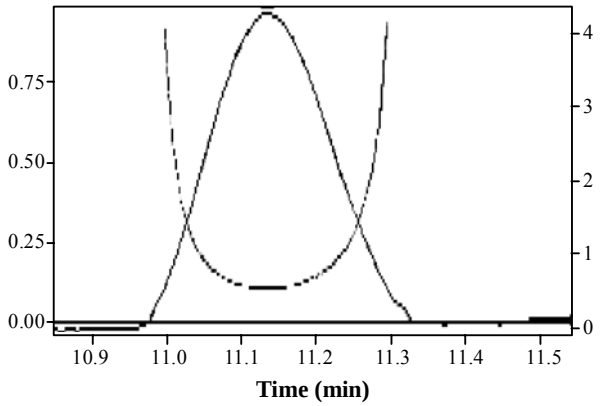

Fig. 11: Vinyl ketone impurity peak purity graph

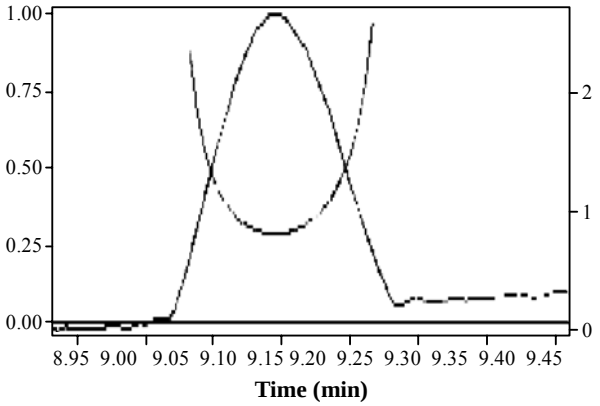

Fig. 12: 2-methyl hydroxy impurity peak purity graph

Precision and ruggedness

Precision was carried out for Inter and Intraday analysis for tablet dosage form. Precision was evaluated by carrying out six independent sample preparations of a single lot of formulation. The sample preparation for tablet dosage form was carried out in same manner as described in sample preparation for tablet and spiking of impurity solution to the concentration of 1.0 µg/mL of 4-MMP impurity and 2.5 µg/mL of each of vinyl ketone and 2-methylhydroxy impurity

Relative standard deviation (% RSD) was found to be less than 2%, which proved that the method is precise. Table 3 shows method precision and intermediate precision study.

Table 3: Method precision and intermediate precision study

Precision study	S. No.	4-MMP impurity (%)		Vinyl ketone impurity (%)		2-methyl hydroxy impurity (%)	
		Method precision	**Intermediate precision**	**Method precision**	**Intermediate precision**	**Method precision**	**Intermediate precision**
	1	0.199	0.198	0.499	0.496	0.495	0.497
	2	0.199	0.198	0.498	0.495	0.498	0.499
	3	0.199	0.198	0.497	0.495	0.497	0.497
	4	0.199	0.199	0.499	0.496	0.503	0.497
	5	0.200	0.199	0.499	0.497	0.501	0.497
	6	0.198	0.198	0.497	0.493	0.499	0.499
	Mean	0.199	0.198	0.498	0.496	0.499	0.497
	SD	0.0004	0.0005	0.0009	0.0008	0.0032	0.0009
	RSD	0.22	0.28	0.18	0.17	0.64	0.18
Precision - intermediate precision :	**Mean**	**0.199**		**0.497**		**0.498**	
	SD	**0.0006**		**0.0016**		**0.0023**	
	RSD	**0.32**		**0.32**		**0.47**	

Accuracy (recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by impurity standard addition method at 25, 100 and 125% concentration levels of impurity standard of concentration 1 µg/mL of 4-MMP, 2.5 µg/mL of vinyl ketone and 2-methyl hydroxy impurities each. Known amounts of standard solution of impurity were added to the pre-analyzed sample and were subjected to the proposed HPLC method. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.39% for 4-MMP impurity, 99.38% for vinyl ketone impurity and 100.22% for 2-methyl hydroxy impurity.

Table 4 shows results of recovery studies.

Table 4: Results of recovery studies

Recovery	4-MMP impurity	Vinyl ketone impurity	2-methyl hydroxy impurity
Level	% Recovery	% Recovery	% Recovery
25%	99.35	99.21	99.78
100 %	99.51	99.32	100.02
125 %	99.31	99.61	100.87
Mean	**99.39**	**99.38**	**100.22**
% RSD	**0.25**	**0.22**	**0.54**

Limit of quantification and limit of detection

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach, standard deviation of the response and slope (calibration curve method). LOQ and LOD were calculated as LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve. Limit of quantification of impurities 4-MMP, vinyl ketone and 2-methylhydroxy were found to be 0.02 µg/mL, 0.05 µg/mL and 0.08 µg/mL, respectively. Limit of detection of impurities 4-MMP, vinyl ketone and 2-methylhydroxy were found to be 0.01 µg/mL, 0.02 µg/mL and 0.03 µg/mL, respectively.

Robustness

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effect of change in mobile phase composition and change in pH of mobile phase was studied. Tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes like ± 2 mL in mobile phase composition and ± 0.2 change in pH.

Table 5 shows the results of different robustness parameter.

Stability of stock solution

During solution stability experiments, RSD for the impurities 4-MMP, vinyl ketone and 2-methyl hydroxy content were found 1.55%, 1.42% and 1.70%, respectively for tablet dosage form, which were within 5% RSD. Results of the solution stability experiments confirmed that standard solutions and sample solution in the diluent were stable for upto 12 hr during the analysis.

Table 5: Results of different robustness parameter

	Content	pH 2.3	pH 2.7	Mobile phase composition 57 : 43	Mobile phase composition 53 : 47
Tailing factor	Tolperisone HCl	1.305	1.311	1.315	1.197
Theoretical plates	Tolperisone HCl	16377.21	16348.56	16499.62	17329.21
Resolution	Tolperisone HCl	7.254	7.251	7.071	7.648
	4-MMP	-	-	-	-
	Vinyl ketone	5.908	5.904	5.833	6.022
	2-methyl hydroxy	2.608	2.611	2.286	2.889
% RSD	Tolperisone HCl	0.122	0.405	0.160	0.104
Retention time	Tolperisone HCl	17.494	17.493	16.758	18.197
Retention time for impurity	4-MMP	10.694	10.695	10.452	10.910
	Vinyl ketone	12.868	12.869	12.545	13.155
	2-methyl hydroxy	13.949	13.950	13.459	14.383
% RSD for Impurity content in Tablet	4-MMP	0.34	0.28	0.33	0.40
	Vinyl ketone	0.20	0.28	0.18	0.25
	2-methyl hydroxy	0.58	0.57	0.55	0.59

CONCLUSION

As described in ICH guidelines, the identification and isolation of impurities is a very important task during drug synthesis and storage. It can provide crucial toxicology and safety data of the final drug and dosage forms. We have identified three impurities in samples of Tolperisone hydrochloride drug formulation product, characterized by HPLC analytical data.

The HPLC method developed and validated allows a simple and fast quantitative determination of 4-MMP, vinyl ketone and 2-methyl hydroxyl impurities from tablet dosage formulation. A mobile phase composed of buffer and acetonitrile with a run time (40 min) and isocratic elution used were advantageous and made the routine analysis easy. Among the significant advantages of this method are simplicity, selectivity, accuracy and precision ensuring that it is suitable for determining the impurity content of Tolperisone hydrochloride in tablet dosage form.

ACKNOWLEDGEMENT

Author (S S S) would like to thank Dr. Mrs. Nandini R. Pai, D. G. Ruparel College Mumbai, for her needful suggestions during the research work. Also thankful to Mr. Prasad Gavde and Mr. Sameer, Assistant Scientist.

REFERENCES

1. The Nature and Origin of the Impurities in Drug Substances (1.2), Sdndor Ggrgg Chapter 1 (1998) pp 12-13.

2. T. V. R. Raju., Development and Validation of a Precise, Single HPLC Method for the Determination of Tolperisone Impurities in API and Pharmaceutical Dosage Forms, Sci. Pharm., **81**, 123-138 (2013).

3. Japan Pharmacopoeia xv, 1190-1191 (2006).

4. M. Murali and P. V. V. Satyanarayana, Simple Validated Isocratic RP–HPLC Method for Estimation of Tolperisone in Bulk and Pharmaceutical Dosage Form, Der. Pharma. Chemica., **3**, 13-19 (2011).

5. I. C. Nimila, P. Balan, N. Chiranjeevi, V. U. Maheswari and S. Rajasekhar, Method Development and Statistical Validation of UV Spectrophotometric Method for Tolperisone Hydrochloride in Bulk and Tablet Dosage Form, J. Pharm. Res., **4**, 1356-1357 (2011).

6. ICH, Harmonized Tripartite Guideline: Validation of Analytical Procedures: Methodology, Q2B (1996).

7. ICH, Harmonized Tripartite Guideline: Validation of Analytical Procedures, Q2A (1996).

8. Process for the Production of High-purity, 2,4'-dimethyl-3-piperidino-propiophenone (Tolperisone), Pharmaceutical Compositions that Contain the Latter, as well as Active Ingredient Formulations that Contain Tolperisone : Patent Application Number: 20100150995, Publication date:06/17 (2010).

9. Method for Producing Salts of Tolperisone, United States Patent 7385060, Publication Date: 06/10 (2008).

10. P. S. Praveen, B. Anupama, V. Jagathi and G. D. Rao, Spectrophotometric Determination of Tolperisone using 2,4-dinitro-phenyl-hydrazine Reagent, Int. J. Res. Pharm. Sci., **3**, 317-320 (2010).

11. L. Saisunee, L. Boonsom and P. Piyaporn, Simultaneous Determination of Tolperisone and Lidocaine by HPLC, J. Pharm. Biomed. Anal., **26**, 865-872 (2001).

12. J. W. Bae, Y. S. Park and U. D. Sohn, HPLC Determination of Tolperisone in Human Plasma, Arch. Pharm. Res., **29**, 339-342 (2006).

13. Jo´zsef Ba´lint, Gabriella Egri, Imre Markovits, Synthesis of some Tolperisone Metabolites in Racemic and Optically Active form, Tetrahedron: Asymmetry, **12**, 3417-3422 (2001).

14. Jo´zsef Ba´lint, a Imre Markovits, Synthesis, Resolution and Absolute Configuration of a Tolperisone Metabolite, J. Ba´lint et al., Tetrahedron: Asymmetry, **12**, 719-724 (2001).

15. S. Liawruangrath and B. Liawruangrath, HPTLC Determination of Tolperisone Hydrochloride, J. Pharm. Biomed. Anal., **20**, 401-404 (1999).

16. P. S. Praveen, B. Anupama, V. Jagathi and G. D. Rao, Spectrophotometric Determination of Tolperisone using 2,4-dinitro-phenyl-hydrazine Reagent, Int. J. Res. Pharm. Sci., **3**, 317-320 (2010).

17. L. Saisunee, L. Boonsom and P. Piyaporn, Simultaneous Determination of Tolperisone and Lidocaine by HPLC, J. Pharm. Biomed. Anal., **26**, 865-872 (2001).

18. V. Jagathi, M. Shaiba, K. Raghavi, M. Sindhura and R. Prashanthi, Assay of Tolperisone by Extractive Spectrophotometry, Res. J. Pharm. Bio. Chem. Sci., **3**, 654-657 (2010).

19. Gabor Orgovan, Karoly Tihanyi, Bela Noszal, NMR Analysis, Protonation Equilibria and Decomposition Kinetics of Tolperisone, J. Pharmaceut. Biomed. Anal., **50**, 718-723 (2009).