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Research Article

DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD OF SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise and accurate reverse phase liquid chromatographic method has been developed for the simultaneous estimation of Tolperisone Hydrochloride and Paracetamol in tablet formulations. The chromatographic separation was achieved on Symmetry C_{18} (250mmx4.6mm) analytical column. A mixture of acetonitrile: water (40:60v/v) (pH 3.0) was used as the mobile phase at the flow rate of 0.7ml/min and detector wavelength at 258nm (Isobestic Point) respectively. The retention time of Tolperisone HCl and Paracetamol were found to be 2.25 and 3.29 respectively. The validation of the proposed method was carried out for linearity, accuracy, recovery, precision, limit of detection and limit of quantification and robustness. The linear dynamic ranges were 2-10µg/ml for Tolperisone HCl and Paracetamol. The percentage recovery of Tolperisone Hydrochloride and Paracetamol were obtained from the range of 99.85-100.26%w/w and 99.88-100.69% w/w respectively. Limit of detection and quantification for Tolperisone HCl were 0.003µg/ml and 0.03µg/ml and Paracetamol 0.002µg/ml and 0.02µg/ml respectively. The developed method can be used for routine quality control analysis of titled drugs in combination of tablet formulation.

Keywords: Tolperisone HCl, Paracetamol, Simultaneous estimation, RP-HPLC method.

INTRODUCTION

Tolperisone Hydrochloride (TOLP) chemically 2-methyl–1-(4 –methyl phenyl)–3-(1-piperidyl) propane-1 one is a piperidine derivative[1] and the structure was shown in fig 1. It is a centrally acting muscle relaxant which is used in the treatment of different pathological conditions like multiocular sclerosis, myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, Arthrosis of the large joints obliterating atherosclerosis of the extremity vessels, diabetical angiopathy, thromboangitis obliterans , reynaud's syndrome[2]. Tolperisone Hydrochloride is official in Japan pharmacopoeia[3].The literature survey revealed that there are some analytical methods reported for Tolperisone Hydrochloride either individually like visible spectrophotometric method [4-6], HPTLC[7] or in combination with other drugs by RP-HPLC[8] and also reported on biological fluids[9].

Paracetamol (PARA) is N-(4 –hydroxyphenyl) acetamide, a Para-aminophenol derivative and the structure was shown in fig 1. It has analgesic, antipyretic properties and weak anti-inflammatory activity. Paracetamol is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia1(B.P). The I.P. & B.P. both suggest titrimetric and UV spectrophotometric assay method for Paracetamol in bulk and tablet formulations. Detailed survey of literature for Paracetamol revealed several methods based on techniques viz. HPLC[10-15], spectrophotometric[16-20] for its determination in pharmaceutical dosage form and in human plasma.The objective of the present work is to develop and validate new analytical method for simultaneous determination of Tolperisone HCl and Paracetamol in tablet dosage form.

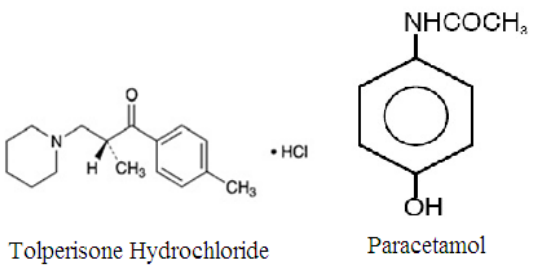

Fig. 1: Structure of Tolperisone Hydrochloride and Paracetamol

MATERIALS AND METHODS

Materials and Chemicals

Pure sample of Tolperisone Hydrochloride and Paracetamol were obtained from Amanath pharmaceuticals, Pondicherry. The purity range of TOLP was 99.79% w/w. Tablet formulation containing Tolperisone HCl 150mg and Paracetamol 500mg (Grandix Pharmaceuticals, Chennai), was used for the estimation. HPLC water prepared by using Millipore Q_3 purification system. HPLC grade Acetonitrile was procured from Merck (Mumbai, India). Analytical grade Orthophosphoric acid was purchased from nice chemicals (Mumbai, India). Digital balance (Sartorius BT 224S), Digital pH meter MK-VI were employed for the estimation.

Preparation of Mobile Phase

The mobile phase was prepared by mixing of Acetonitrile with water (pH 3.0) ((40:60v/v) and the water pH adjusted to 3.0 by using O-phosphoric acid. The mobile phase was sonicated for 15min and then it was filtered through a 0.45µ whatmans filter paper.

Preparation of Standard Solution

A stock solution (1000µg/ ml) of the standard drug was prepared by mixing of 25mg of TOLP and 25mg of PARA in 25 ml volumetric flask containing a mixture of acetonitrile:water (pH 3.0) (40:60v/v), sonicated for about 10min and then made up to the volume. The stock solution were suitably diluted to produce a concentration of 10 µg/ml of TOLP and 10µg/ml of PARA respectively.

Instrumentation and Chromatographic Conditions

A high performance liquid chromatographic system (WATERS Corporation, LC-2489 and USA) with an auto sampler was used for analysis. The data was recorded using WATERS EMPOWER software. The purity determination performed on a stainless column (Symmetry) 250mm long 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5µm diameter (250mmx4.6mm). The overlain spectrum of TOLP & PARA is shown in fig 2. The optimized chromatographic conditions were listed in table 1 and the chromatogram was showed in fig 3.

Sample Preparation

Twenty tablets were weighed and finely powdered and the average weight was determined. A portion of powder equivalent to

the weight of 25mg PARA was weighed accurately into 25ml volumetric flask and 20 ml of mobile phase was added and sonicated for 15 minutes to effect complete dissolution of TOLP and PARA, the solution was then made up to volume with mobile phase. The solution was filtered through whatmans filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 6µg/ml of TOLP and 20µg/ml of PARA. Twenty micro liters of the test solution was injected and chromatogram was recorded for the same and the amounts of the drugs were calculated.

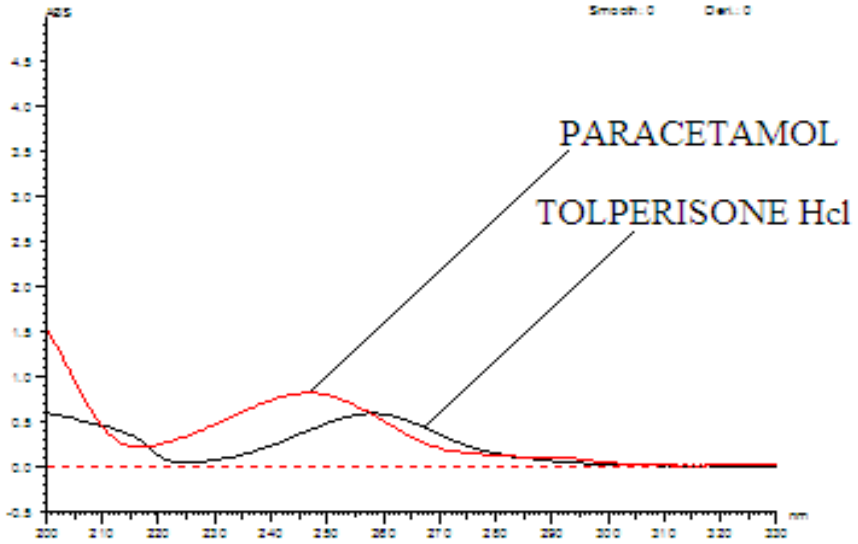

Fig. 2: Overlain spectrum of a mixture of standard Tolperisone HCl **and Paracetamol**

Table 1: Optimized Chromatographic Conditions

Parameter	Optimized conditions
Instrument	WATERS -HPLC, 2695seperation module
Column	C_{18}, 5µ, 250mmx4.6mm
Mobile phase *	Acetonitrile: water (40:60v/v) (pH 3.0 was adjusted with dilute Orthophosphoric acid)
Flow rate	0.7ml/min
Detection	258nm (Isobestic point)
Injection volume	20µl
Temperature	Ambient

*Filtered through a Whatmans filter paper

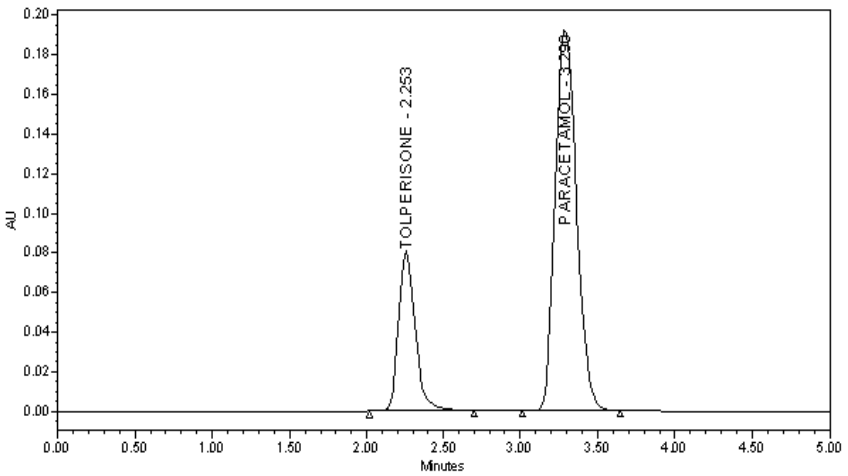

Fig. 3: Typical chromatogram of a mixture of standard Tolperisone HCl **and Paracetamol**

Validation

Linearity and range

Transfer 0.2ml to 1.0ml of aliquots of standard stock solution (1000 µg/ml) of TOLP and PARA into a five individual 100ml volumetric flask and diluted up to the mark with mobile phase to get the final concentrations ranging from about 2 to 10µg/ml of both the drugs. Triplicate injections of 20µl were made two times for each concentration and chromatographed under the conditions as described above. Peak areas of two drugs were recorded with the UV detector set at 258nm.On plotting the peak area Vs respective concentration of TOLP and PARA, they were found to be linear in the range of 2 to 10µg/ml with coefficient correlation (r^2) 0.9997and 0.9999. Typically the regression equation for the calibration curve was found to be y=16981x+374 for

TOLP and y=19342x-5028 for PARA. The linearity curve and results were shown in fig 3, 4 and table 2.

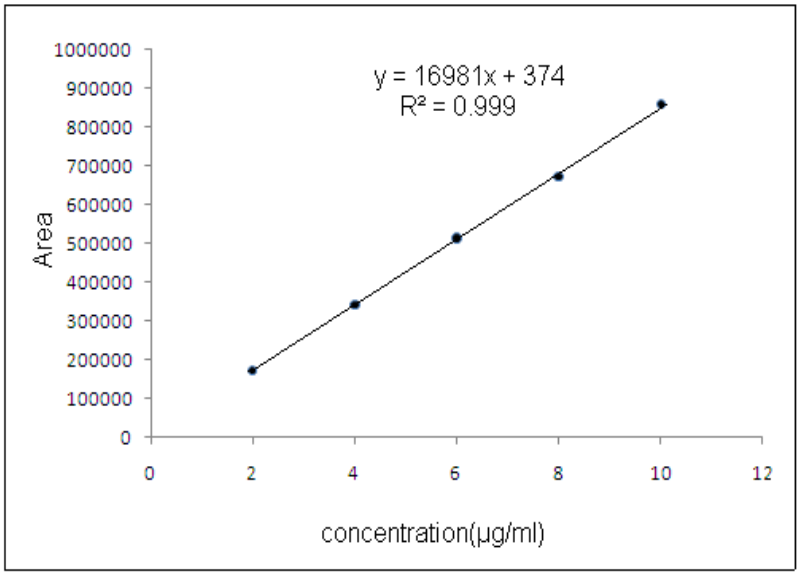

Fig. 3: **Calibration curve of Tolperisone** HCl

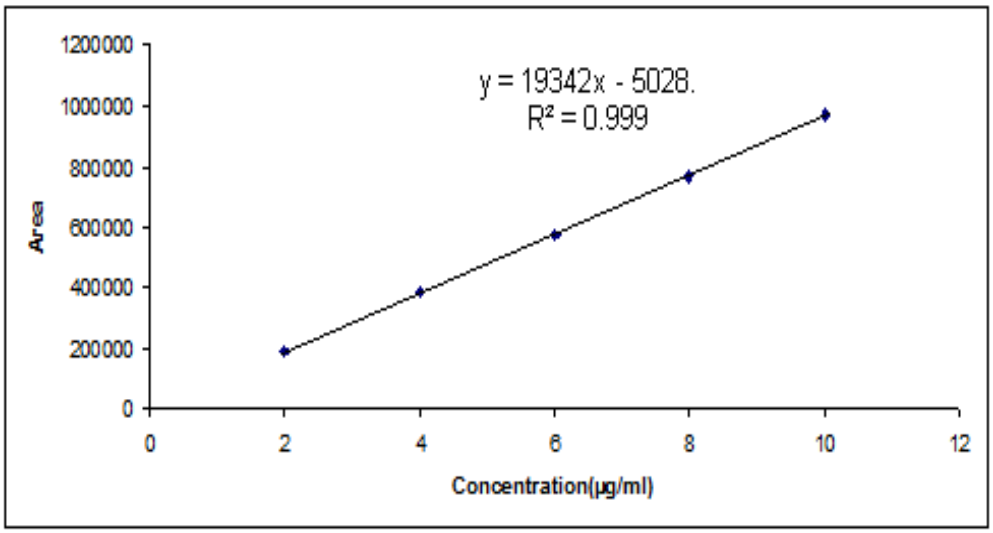

Fig. 4: Calibration curve of Paracetamol

Table 2: Result of Linearity and range

Tolperisone HCl		**Paracetamol**	
Concentration in µg/ml	**Peak Area**	**Concentration in µg/ml**	**Peak Area**
2	174414	2	190781
4	344061	4	381489
6	515127	6	572423
8	673627	8	765789
10	858705	10	965759
Correlation Coefficient (r2)	**0.9997**	**Correlation Coefficient (r2)**	**0.9999**
Intercept (y)	**3742.4**	**Intercept (y)**	**-5028.6**

Table 3: Validation and System Suitability Parameters

Parameters	**Tolperisone HCl**	**Paracetamol**
Theoretical plates*	1938	2815
Retention time*	2.25	3.29
USP tailing factor*	1.32	1.205
%R.S.D*	1.0744	1.0164
LOD (µg/ml)	0.003	0.03
LOQ (µg/ml)	0.002	0.02

*Mean of six determinations

Specificity

The specificity of the RP-HPLC method was determined by complete separation of TOLP and PARA as shown in fig 3 with parameters like retention time (Rt), resolution (Rs) and tailing factor (T).Here tailing factor for peaks of TOLP and PARA was less than 2% and resolution was satisfactory. The retention time for TOLP and PARA were found to be 2.25 and 3.29 respectively, for six replicates. The peaks obtained for TOLP and PARA were sharp and have clear baseline separation. The system suitability parameter was reported in table 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing six replicates analysis of the working solution.

The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch. Statistical evaluation of tablet analysis was carried out (table 4).The intraday and interday precision were determined and results of which are given in table 5.

Accuracy

Accuracy of the method was calculated by recovery studies. It is carried out by preparing the samples at a level of 50%, 100% and 150% of target concentration. The samples were prepared in triplicate in each level. The results of studies along with its evaluation are given in table 6.

Table 4: Statistical Evaluation of Tablet Analysis

Drug	%Label Claim	S.D	%RSD
Tolperisone HCl	100.23	1.090	1.087
Paracetamol	100.51	0.3994	0.3974

*Mean of six determinations

Table 5: Intraday and Interday Precision

	Tolperisone HCl		Paracetamol	
	%Label Claim*	%RSD	%Label Claim*	%RSD
Intra day	100.24	1.0985	100.56	0.3142
Inter day	100.22	1.077	100.46	0.4807

*Mean of six determinations

Table 6: Recovery Studies of TOLP and PARA in Combined Dosage Form

Drug	Recovery	% Recovered*	SD	%RSD
Tolperisone HCl	50%	99.85	1.738	1.741
	100%	100.24	1.101	1.098
	150%	100.26	0.8680	0.865
Paracetamol	50%	99.88	1.021	1.022
	100%	100.56	0.315	0.314
	150%	100.69	1.041	1.033

*Mean of three determinations

Limit of Detection and Limit of Quantification

LOD and LOQ for TOLP and PARA were determined (table 3) by calibration curve method. Solution of both TOLP and PARA were prepared in the rage of 2-10µg/ml and injected. Peak area of analysis was plotted against concentration. LOD and LOQ were calculated by using following equations.

$$LOD = \frac{3.3 \times Syx}{b} \qquad LOQ = \frac{10 \times Syx}{b}$$

Where Syx is residual variance due to regression; b is slope

LOD and LOQ for TOLP were 0.003µg/ml and 0.03µg/ml respectively and for PARA 0.002 µg/ml and 0.02 µg/ml respectively.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was investigated under a variety of conditions including changes of pH of mobile phase, flow rate, percentage of acetonitrile in the mobile phase. The results are given in table 7.

Table 7: Result of Robustness Study of TOLP and PARA

Factor	Level	Tolperisone HCl		Paracetamol	
		R_t	Peak area	R_t	Peak area
pH of mobile phase	2.8	1.30	571762	3.259	1571481
	3.2	2.247	615469	3.156	1691885
Flow rate (ml/min)	0.6	3.028	627180	4.433	1726890
	0.8	1.87	598960	2.687	1311539
% Acetonitrile	38	2.348	602469	3.289	168653
	42	2.198	639069	3.016	1692666

RESULT AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both TOLP and PARA. The optimized mobile phase containing acetonitrile: water (pH 3) (40:60 v/v) and the water pH adjusted to 3 with O-phosphoric acid was found to be satisfactory and gives two symmetric and resolved peaks for TOLP and PARA. The order of the elution was TOLP followed by PARA at 2.25 and 3.29 min respectively (fig 5). The flow rate was 0.7ml/min with UV detection

at 258nm (isobestic point).The calibration curve were found to be linear in the range of 2 to 10µg/ml for TOLP and PARA with a correlation co-efficient of 0.9997 and 0.9999 respectively. Typically the regression equation for the calibration curve was found to be y=16981x+3742 for TOLP and y=19342x-5028 for PARA. The quantification limit for TOLP and PARA were 0.03µg/ml and 0.02µg/ml respectively. The low %RSD value for intraday and interday precision revealed that the proposed method is robust and rugged. The results obtained by the proposed method were close to the label claim of both drugs. The lower values of % RSD in Table 5, 6 indicate that the method is precise and accurate. The percentage recovery of TOLP and PARA were obtained from the range of 99.85-100.26%w/w and 99.88-100.69% w/w respectively. No interfering peaks were found in the chromatogram indicating that the excipients used in tablet formulations did not interfere with the estimation of drug by the proposed HPLC method.

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Tolperisone HCl and Paracetamol in tablet formulation. The method is very simple and specific as both peaks are well separated from its impurities and excipients peaks with total runtime of 5minutes, which makes it especially suitable for routine quality control analysis work.

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