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Simultaneous Estimation of Tolperisone Hydrochloride and Diclofenac Sodium in Bulk and Tablet Dosage form by RP-HPLC Method

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ABSTRACT

A simple, fast, accurate and precise method has been developed for the simultaneous determination of tolperisone hydrochloride and diclofenac sodium from pharmaceutical formulation by Reversed-phase high performance liquid chromatography. The separation was carried out on C_{18} column using mobile phase consisting of a mixture of acetonitrile and water in the ratio 90 : 10 v/v (pH adjusted to 3.5 with orthophosphoric acid). The flow rate was maintained at 1.0 ml/min. The UV detection was carried out at wavelength 273 nm. The retention time for tolperisone hydrochloride and diclofenac sodium was found to be 2.769 min and 6.363 min respectively. Linear response obtained for tolperisone hydrochloride was in the concentration range 30-150 µg/ml (r^2 = 0.998) and diclofenac sodium in the range 10-50 µg/ml (r^2 = 0.999). The relative standard deviation in the tablets was found less than 2% for six replicates. The method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, limit of detection, limit of quantification and robustness.

Keywards: *Tolperisone hydrochloride, Diclofenac sodium, RP-HPLC, Validation.*

INTRODUCTION

Tolperisone hydrochloride (TPS) is chemically 2-methyl-1-(4-methyl phenyl)-3-(1-piperidyl) propane-1 one, a piperidine derivative as shown in **Fig. 1**. TPS is an aryl alkyl β-aminoketone having an asymmetric carbon atom α to the carbonyl group [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 artherosclerosis of the extremity vessels, Diabeticalangiopathy, thromboangitisobliterans, raynaudssyndrome [2]. TPS is official in Japan pharmacopoeia [3].

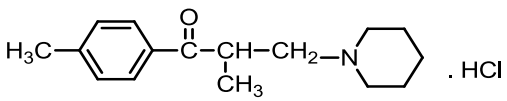

Fig. 1: Structure of Tolperisone Hydrochloride

Diclofenac sodium is a sodium [O-(2,6-dichlorophenyl)-amino-phenyl]acetate is a non-steroidal drug [4]. It has anti-inflammatory analgesic having potent cycloxygenase inhibition activityas shown in **Fig. 2**. DLF is used for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis and ankylosingspondilytis [5]. DLF is official in Indian pharmacopoeia [6].

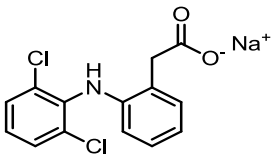

Fig. 2 : Structure Of Diclofenac Sodium

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Literature Survey revealed that numbers of method have been reported in literature for the individual analysis of TPS and DLF by UV spectrophotometric [7-9], RP-HPLC method and hydrotropic solubilization technique [10-12]. DLF is reported to be estimated by spectrophotometry, HPLC and HPTLC individually or in combination with other drugs like paracetamol, papaverine, thiocolchicoside and rabeprazole [13-15]. RP-HPLC method is available in literature for simultaneous determination of TPS with etodolac, paracetamol, lornoxicam [16]. However, there is no reported RP-HPLC method available for simultaneous estimation of TPS and DLF. The aim of the present work was to develop easy, economic, accurate, specific and precise chromatographic methods for simultaneous estimation of TPS and DLF on bulk drugs and combined marketed formulation and validation of newly developed analytical methods.

MATERIALS AND METHODS

Chemical and reagents:

Active pharmaceutical ingredient of TPS was received as gift sample from Richard themis Pvt LTD, Vapi Gujarat, India and DLF was received as gift sample from emcure pharmaceuticals Bhosri, Pune. Marketed formulation containing TPS and DLF (150:50 mg) were purchased from local pharmacy shop. Methanol (HPLC grade), water (HPLC grade), Acetonitrile (HPLC grade) and orthophosphoric acid (AR grade) were procured from Merck Chemicals, India.

Equipment and chromatographic conditions:

The HPLC system used was Waters 510 HPLC system equipped with a Rheodyne injector (20 µl) and UV detector. Chromatographic separation was carried isocratically at room temperature with a Purosphere STAR RP- C_{18} (250 mm × 4mm i.d., 5µm) column from Merck KGaA 64271 Darmstadt, Germany. Data acquisition was made with DataAce software.

The mobile phase consisted of acetonitrile and water in the ratio 90:10 v/v (pH adjusted to 3.5 with orthophosphoric acid). The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed. The injection volume was 20 µl and eluted at a flow rate of 1.0 ml/min. The detection wavelength was 273 nm.

Preparation of standard solutions:

Standard stock solutions (100µg/ml) of TPS and DLF were prepared by dissolving accurately weighed 10 mg of each drug separately in mobile phase in 100 ml volumetric flask and filtered through 0.45 µ nylon filter. The working standard solutions of these drugs were further diluted with mobile phase to get required concentration of TPS (15 µg/ml) and DLF (5 µg/ml).

Preparation of sample solutions:

Twenty tablets were weighed and crushed to a fine powder. The quantity of the powder equivalent to 150 mg of TPS and 50mg of DLF was weighed accurately and then transferred to 100 ml volumetric flask containing 70 ml of mobile phase. It was then sonicated for 15 min. The solution was filtered through a 0.45 µ nylon filter and volume was made up to the mark with mobile phase. The final dilution was made with mobile phase, contained about 15µg/ml and 5µg/ml of TPS and DLF respectively.

Method validation: [18-20]

Linearity and range:

Accurately measured standard working solutions of TPS 30, 60, 90, 120, 150ml and DLF 10, 20, 30, 40, 50 were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Calibration curves were plotted over a concentration range of 30-150 µg/mL for TPS and 10-50 µg/mL for DLF as shown in **Fig. 3 & 4**.

Accuracy (% recovery):

Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) by standard addition method. Twenty tablets were weighed and the average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 150 mg TPS was transferred to 50 ml volumetric flask to dissolve the drugs and then the volume was made up to the mark and sonicated for 15 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From the filtrate 0.1 ml was transferred to three 10.0 ml volumetric flasks and add 0.08 ml (Flask 1), 0.1 ml (Flask 2), and 0.12 ml (Flask 3) of stock solution of API and then made up to the mark with Mobile phase to make them 80%, 100% and 120% spiking. Each sample was prepared in triplicate at each level and injected.The results obtained for Accuracy are summarized in **Table 1**.

Precision:

The intra-day, inter-day, reproducibility was done to determine precision of the developed method. The intra-day precision of the developed HPLC method was determined by preparing the samples of the same batch in nine determinations with three concentrations and three replicate (n=3) each on same day. The Percentage R.S.D. of the results was used to evaluate the method precision. The inter-day precision was determined by assaying the samples in triplicate (n=3) per day for consecutive 3 days. The results obtained for precision are summarized in **Table 2**.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visually method or calculating the signal -to- noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline. In this method the limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visual method.

System suitability test:

Asymmetry of both the analytes' peak in standard should not be more than 2.0,Theoretical plates of both the analytes' peak in standard should not be less than 2000, Relative Standard Deviation for five replicates injections of both the standard preparation should not be more than 2.0%. The results obtained for system suitability are summarized in **Table 3**.

Robustness:

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate ±0.1, pH of mobile phase ±0.1, and ratio of Mobile phase±2. The results of robustness studies are shown in **Table 4**.

Assay:

Twenty tablets were weighed and finely powdered. The average weight of tablets is determined with the help of weight of 20 tablets. A portion of powder equivalent to 150 mg of TPS was accurately weighed into a 50 ml volumetric flask and 15ml mobile phase was added and sonicated for 15 min to effect complete dissolution of the TPS and DLF, then volume was made up to the mark with mobile phase. The solution was filtered through Whatman filter paper.The aliquot portion of the filtrate was further diluted to get final concentration 15µg/ml of TPS and 5 µg/ml of DLF. The % assay of the drugs was calculated. The proposed validated method was successfully applied to the simultaneous determination of TPS and DLF in tablet dosage form as shown in **Table 5**.

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of TPS and DLF in bulk as well as in pharmaceutical preparation was found to be simple, accurate, economical and rapid. The method was validated as per the ICH guidelines.

Linearity:

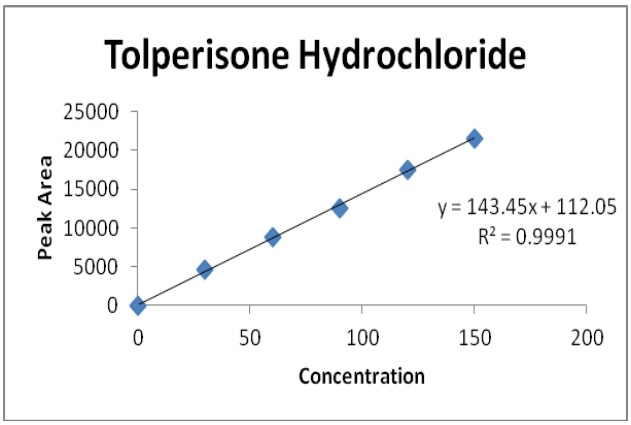

Fig. 3: Calibration Curve orTolperisone Hydrochloride

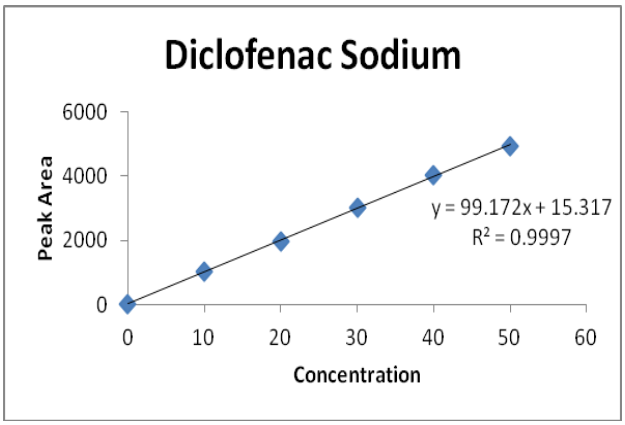

Fig. 4: Calibration Curve ForDiclofenac Sodium

Accuracy (%recovery):

Table No. 1: Result of recovery study

Level of recovery	% Recovery*		Standard Deviation		% R.S.D.	
	TOL	DLF	TOL	DLF	TOL	DLF
80 %	100.04	101.47	±0.098	±0.017	0.0097	0.0167
100 %	98.95	99.84	±0.640	±0.045	0.6513	0.0470
120 %	101.88	102.15	±0.012	± 0.023	0.0121	0.0218

*Average value ± SD of three determinations, SD is standard deviation and %RSD is relative standard deviation

Precision:

Table No. 2: Result of Precision study

Intra-day Precision Data

Drug	Mean*	S. D.	%R.S.D.
TOL	8901.127	± 0.180	0.00203
DLF	1981.437	± 0.203	0.01027

*Average of six determination

Inter-Day Precision Data

Drug	Mean*	S. D.	%R.S.D.
TOL	8709.374	± 0.34	0.00394
DLF	1702.500	± 0.27	0.01634

*Average of six determinations

System suitability testing:

Table No. 3: Result of system suitability parameters

Parameters	TPS	DLF
Retention time (min)	2.769	6.363
Tailing factor (limit <2)	1.479	1.3

Robustness:

Table No. 4: Result of Robustness study

Factor	Level	Retention time		Tailing factor		Peak Area		S.D.		% RSD	
pH (± 0.1)		TOL	DLF	TOL	DLF	TOL	DLF	TOL	DLF	TOL	DLF
3.4	-0.1	2.4	5.8	1.9	1.5	3990.11	978.43	± 0.180	± 0.32	0.0046	0.0331
3.5	0.0	2.6	6.3	1.9	1.5	4566.32	1014.19	± 0.017	± 0.004	0.0003	0.0004
3.6	+0.1	2.5	6.0	1.9	1.5	4788.79	1215.71	± 0.190	± 0.24	0.0041	0.0198

Factor	Level	Retention time		Tailing factor		Peak Area		S.D.		% RSD	
Flow Rate(±0.1ml/min)		TOL	DLF	TOL	DLF	TOL	DLF	TOL	DLF	TOL	DLF
0.9	– 0.1	2.4	5.8	1.9	1.5	3850.26	998.20	± 0.127	± 0.251	0.0032	0.0251
1.0	0.0	2.6	6.3	1.9	1.5	4567.44	1013.16	± 0.011	± 0.055	0.0002	0.0054
1.1	+ 0.1	2.5	6.0	1.9	1.5	4658.89	1110.22	± 0.011	± 0.011	0.0002	0.0010

Factor	Level	Retention time		Tailing factor		Peak Area		S.D.		% RSD	
Mobile Phase (±2 v/v)		TOL	DLF	TOL	DLF	TOL	DLF	TOL	DLF	TOL	DLF
88:12	-0.2	2.4	5.8	1.9	1.5	4067.78	999.52	± 0.0057	± 0.5000	0.0001	0.0500
90:10	0.0	2.6	6.3	1.9	1.5	4570.98	1019.56	± 0.0057	± 0.0377	0.0001	0.0377
92:08	+0.2	2.5	6.0	1.9	1.5	4950.76	1113.38	± 0.0057	± 0.1644	0.0001	0.0147

* Average of three determinations, S.D. is Standard deviation; RSD is the Relative Standard deviation

Application of developed method to pharmaceutical formulation:

Table No. 5: Result of assay of Tablet formulation :.

Sr. No	Drug	Amount of drug estimated* (mg/tablet)	% Label Claim*	S.D	% R.S.D.
1.	TOL	149.00	99.33	±0.3046	0.3067
2.	DLF	49.87	99.74	±0.6791	0.6809

* Average of six determinations, S.D. is Standard deviation

Table No. 6: Method validation and system suitability parameters

Parameters	TPS	DLF
Linearity range	30-150 µg/ml	10-50 µg/ml
Regression coefficients (r)	0.999	0.999
Limit of detection µg/ml	0.00532	0.0438

Limit of quantitation µg/ml		0.01621	0.1329
Precision	**Intra-day (%RSD)**	0.00203	0.01027
	Inter-day (%RSD)	0.00394	0.01634
Retention time (min)		2.769	6.363
Tailing factor (limit <2)		1.479	1.3

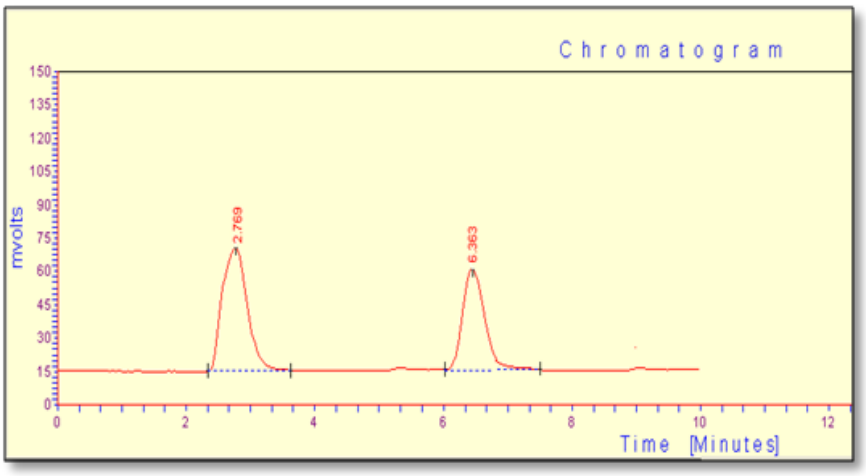

Fig. 5: Chromatogram of Tolperisone Hydrochloride & Diclofenac Sodium

CONCLUSION

The developed method was novel, simple, accurate, precise reproducible, economical, which would be used to estimate TPS & DLF in their combined dosage form in routine analysis.

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REFERENCES:

1. Gohil K, Shah B, Patel B, Patel P and Patel N. Method development and validation for estimation of tolperisone hcl in bulk and in tablet dosage form and its stress degradation study using spectrophotometric method, An International Journal of Pharmaceutical Sciences, **2012**; 27: 2750-2762.
2. Carolin N , Balan P, Chiranjeevi N , Uma V, Maheswari and KarthikeyanM. Method development validation and forced degradation studies of tolperisone hydrochloride by RP-HPLC method in bulk and tablet dosage form, International Journal of Pharmaceutical Biosciences, **2011**; 587-595.
3. Japanese Pharmacopoeia, Society of Japanese Pharmacopoeia, 15th Edition, Shibiya Tokyo, Japan, **2006**; 618.
4. Maryadele JO, Heckelman PE, Koch CB, Roman KJ. Editors. The Merck Index: an Encyclopedia of Chemicals, Drugs and Biologicals. 14th edition. Whitehouse Station, NJ, USA: Merck Research Lab, Division of Merck and co. Inc.; **2006**; 522, 3081.
5. Emami J, Ghassami N, Talari R. A. Rapid and sensitive modified HPLC method for determination of diclofenac in human plasma and its application in pharmacokinetic studies,sIsfahan University of Medical Sciences, **2007**; 15(3): 132-137.
6. Khaskheli A R, Sirajuddin, AfridiH.I, Mahesar S.A and Munawar Saeed. Simpler and Faster Spectrophotometric Determination of Diclofenac Sodium in Tablets, Serum and Urine Samples, Pak. J. Anal. Environ. Chem, **2009**; 10: 53-58.
7. Indian Pharmacopoeia, Government of India, Ministry of health and family welfare, **2007**; 2: 407-408.
8. Patel M.G., Parmar R.R. , Nayak P.P. and D. A. Shah. The simultaneous estimation of paracetamol and tolperisone hydrochloride in tablet by UV spectrophotometric methods, Journal of pharmaceutical science and bioscientific research, **2012**; 2: 63-67.
9. Patel M, Patani P, Shukla M. Method development and statistical validation of UV spectrophotometric method for estimation of tolperisone hydrochloride and paracetamol in synthetic mixture and combined dosage form, International Journal of Pharmaceutical Research and Bioscience **2012**.
10. BernadettStiedl, DorottyaKova Cs-Kiss, Krisztina Luda Nyi, Attila Bo Dis, ImreKlebovich, Istva N Antal. LC-UV assay Of Tolperisone Hydrochloride From Sustained Release Matrix Tablets Chromatography, **2012**; 71: 109-113
11. Mandava V. Basaveswara Rao, B.C.K. Reddy, T. SrinivasaRao and V. Prasanthi Sadineni Chowdaraiah. Estimation of diclofenac sodium pellets (extended release) in commercial dosage forms using a simple and convenient spectophotometric method, **2009**; 2: 488-490.
12. Sharma K. K. Patel P.U., First derivative spectrophotometric method for the simultaneous estimation of tolperisone and paracetamol in their combined dosage form journal of pharmaceutical science and bioscientific research, **2012**; 2: 92-96.
13. Shah U, Thula K, Raval M, Desai P. Development and validation of UV spectrophotometric methods for simultaneous estimation of tolperisone hydrochloride and paracetamol from combined tablet dosage form, International Journal of Biological &Pharmaceutical Research, **2012**; 3(4): 634-639.
14. Sengar M R, Gandhi S V, Patil U P, Rajmane V S. Simultaneous determination of diclofenac sodium and thiocolchicoside in fixed dose combination by spectrophotometrys, Asian Journal of Pharmaceutical and Clinical Research, **2010**; 3(2): 89-91.
15. Gunji R, Nadendla R, Ponnuru V. Simultaneous UV-Spectrophotometric determination and validation of Diclofenac Sodium and Rabeprazole Sodium using Hydrotropic agents in its tablet Dosage Form, International Journal of Drug Development & Research, **2012**; 4(1): 316-324.
16. Regina K, Determination of diclofenac sodium and papaverine hydrochloride in tablets by HPLC method, Acta Poloniae Pharmaceutical & Drug Research, **2008**; 65(4): 403-408.
17. Sireesha K, Ravi Kumar A. Simultaneous estimation of lornoxicam and tolperisone by RP-HPLC, International Journal of Advances in Pharmaceutical Research, **2012**; 3: 981-987.
18. Sethi PD. HPLC- High Performance Liquid Chromatography-Quantitative Analysis of Pharmaceutical Formulations. 1st edition. New Delhi: CBS Publication; **2001**; 3-72.

19. ICH Q2A, Text on validation of Analytical Procedures, Geneva: International Conference on Harmonization, **1994**; 1-5.

20. ICH, Q2B, Validation of Analytical Procedures: Methodology, Geneva: International Conference on Harmonization, **1996**; 1-8.

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