Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN THEIR COMBINED TABLET DOSAGE FORM

AMIT SHAH*[1], PINAK PATEL[1], ANKIT PATEL[1]

Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Amrapali Township, Petlad - Khambhat Road, Dharmaj, Gujarat-388430, India. Email: amitsss15@gmail.com

Received: 27 Sep, 2012, Revised and Accepted: 02 Nov, 2012

ABSTRACT

A simple, accurate, precise and specific spectrophotometric method have been developed for simultaneous determination of Tolperisone Hydrochloride (TOL) and Diclofenac Sodium (DFS) in its combined tablet dosage form by using methanol as a solvent. The method involves solving of simultaneous equation based on measurement of absorbance at two wavelengths at 254 nm and 282 nm. Method follows Beer's linearity in the range of 5-35µg/ml for TOL and DFS both. The mean % recoveries were found to be in the range of 99.35 – 100.4% and 98.70 – 100.20 % for TOL and DFS respectively. Limit of Detection and quantitation was found to be 0.101µg/ml and 0.306µg/ml for TOL and 0.120µg/ml and 0.364µg/ml for DFS respectively. Assay results of market formulation were found to be 99.70 and 99.40 % for TOL and DFS respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of TOL and DFS in their combined Tablet dosage form.

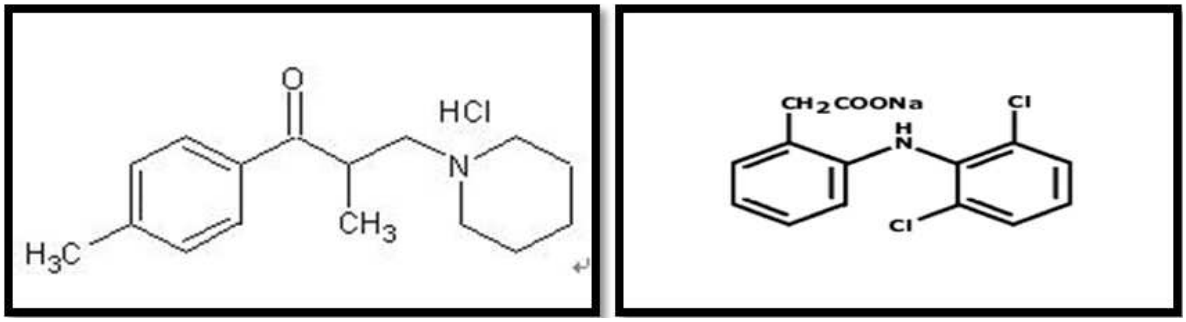
Keywords: Tolperisone Hydrochloride, Diclofenac Sodium, Simultaneous Equation Method, Analytical Method validation.

INTRODUCTION

TOL is chemically 2RS)-2-Methyl - 1 - (4-methylphenyl)- 3 - piperidin - 1 - yl propan -1-one monohydrochloride (Fig 1a), a piperidine derivative 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, myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, arthrosis of the large joints obliterating artherosclerosis of the extremity vessels, diabetical angthromboangitis obliterans, raynauds syndrome[7]. TOL is official in Japanese pharmacopoeia[1]. Chemically DFS is, sodium 2-[(2,6-dichlorophenyl)-amino]phenyl acetate (Fig 1b), used as analgesic and anti-inflammatory drug used in the treatment of rheumatoid arthritis, osteoarthritis and alkylosing spondylitis and also for a variety of non-rheumatic inflammatory conditions[21]. Diclofenac sodium is official in Japanese Pharmacopoeia[1,] British Pharmacopoeia[3], United States Pharmacopoeia[4] and Indian Pharmacopoeia[5].

The review of literature revealed that various analytical methods involving spectrophotometry[6-11], HPLC[12-19], HPTLC[20] have been reported for TOL in single form and in combination with other drugs. Several analytical methods have been reported for DFS in single form and in combination with other drugs including spectrophotometry[21-25], HPLC[26-32], LC-MS[33], HPTLC[34].

To the best of our knowledge, there is no published spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of TOL and DFS in combined tablet dosage form by Simultaneous equation method. The developed method was validated in accordance with ICH Guidelines[39] and successfully employed for the assay of TOL and DFS in their combined dosage form.



(a) **(b)**

Fig. 1: Chemical structure of (a) TOL and (b) DFS

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure TOL and DFS were kindly provided by Zydus Cadila Healthcare Ltd, Ahmedabad, Gujarat, India and Medico labs, Ahmedabad, Gujarat, India respectively as gratis samples. Analytical grade methanol was purchased from RFCL limited, New Delhi, India. Tablet of TOL and DFS in combined dosage form, TOLPIDOL-D, was procured from local market.

Instruments

Two spectrophotometers were used for study, A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study. Calibrated analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose.

Preparation standard stock solutions

Accurately weighed 100 mg of TOL and DFS standard were transferred to a separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml TOL and 1000 µg/ml DFS. From this solution 10 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml of TOL and 100µg/ml DFS.

Simultaneous equation method

5-35 µg/ml solutions of TOL and DFS were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm.This method of analysis was based on the absorption of drugs TOL and DFS at the wavelength maxima of each other. Two wavelengths were selected for the development of the simultaneous equations at 254nm and 282nm (figure 2). The absorptivity values E (1%, 1cm) were determined for two drugs at all selected wavelengths. The concentration of two drugs in mixture was calculated by using following equations.

$$C_X = (A_2\, ay_1 - A_1\, ay_2) / (ax_2\, ay_1 - ax_1\, ay_2)$$

$$C_Y = (A_1\, ax_2 - A_2\, ax_1) / (ax_2\, ay_1 - ax_1\, ay_2)$$

Where, C_x and C_y are the concentrations of TOL and DFS respectively in mixture and in sample solutions. A_1 and A_2 are the absorbences of sample at 254nm and 282nm, respectively, ax_1 and ax_2 are the absorptivity of TOL at 254nm and 282nm, ay_1 and ay_2 are the absorptivity of DFS at 254nm and 282nm. All standard and sample solutions absorbance was measured at 254nm and 282nm with their respective blanks.

Method validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (Coefficient of Variation - C.V.) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed method has been applied to pharmaceutical dosage form.

Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Linearity

Appropriate volume of aliquot from TOL and DFS standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give solutions containing 5-35 µg/ml TOL and DFS. All absorbance were measured at 254 nm and 284 nm for TOL and DFS respectively (n=6). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50, 100 and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Precision

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of TOL and DFS was carried out by estimating different concentrations of TOL (10, 20, 30 µg/ml) and DFS (10, 20, 30 µg/ml), 3 times on the same day and on 3 different days (first, second, fifth) and the results are reported in terms of C.V.

Detection limit and Quantitation limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\sigma/S$ criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness

The sample solution was prepared and then analyzed with change in the typical analytical conditions like stability of analytical solution.

Reproducibility

The absorbance readings were measured at different laboratory for sample solution using another spectrophotometer by analyst and the values obtained were evaluated using t- test to verify their reproducibility.

Determination of TOL and DFS in their Combined Dosage

Twenty tablets were weighed and powdered. A powder quantity equivalent to 150 mg TOL and 50 mg DFS was accurately weighed and transferred to volumetric flask of 100 ml capacity. 60 ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The above solution was filtered through whatman filter paper (0.45µ).The flask was shaken and volume was made up to the mark with methanol. From this solution 2 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 30µg/ml of TOL and 10µg/ml of DFS. The resulting solution was analysed by proposed methods. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

Simultaneous equation Spectrophotometric method for TOL and DFS combined dosage form- tablet

Owing to the solubility of TOL and DFS in the methanol it was selected as solvent. From overlain spectra of TOL and DFS it is clear that TOL exhibits λ_{max} at 254 nm and DFS exhibits λ_{max} at 282 nm. The overlain spectra of TOL and DFS reveals that the both the drug exhibits distinct λ_{max} and also both drugs shows absorbance at the λ_{max} of each other. For estimation of TOL and DFS using spectrophotometry simultaneous equation method was decided to be used. In this method two wavelengths are required. One wavelength is selected at which TOL shows maximum absorbance (254 nm), while second wavelength is selected at which DFS shows maximum absorbance (282 nm).

Simultaneous equation generated:

$C_x = (A_2 \times 0.0112 - A_1 \times 0.0374) / (0.0071 \times 0.0112 - 0.0502 \times 0.0374)$

Where,

1) A_1 and A_2 is absorbance of sample at 254 nm and 282 nm respectively

2) C_x is concentration of TOL in µg/ml

$C_y = (A_1 \times 0.0071 - A_2 \times 0.0502) / (0.0071 \times 0.0112 - 0.0502 \times 0.0374)$

Where,

1) A_1 and A_2 is absorbance of sample at 254 nm and 282 nm respectively

2) C_y is concentration of DFS in µg/ml.

The % recoveries were found to be in the range of 99.35 – 100.4% for TOL and 98.70 - 100.20% for DFS (Table 4). The precision of method was determined by repeatability, intraday and interday

precision and was expressed as the C.V. (Table 1) which indicates good method precision.

The Limit of detection for TOL and DFS was found to be 0.101µg/ml and 0.120µg/ml respectively. Limit of quantification for TOL and DFS was found to be 0.306µg/ml and 0.364µg/ml at 254 nm and at 282 nm respectively (Table 2-3).

The methods was found to be specific, as there was no interference observed when the drugs were estimated in presence of excipients and robust, as there was no significant change in absorbance up to 24 hours of preparation of solution in methanol. The proposed spectrophotometric method was successfully applied to TOL and DFS combined dosage form.

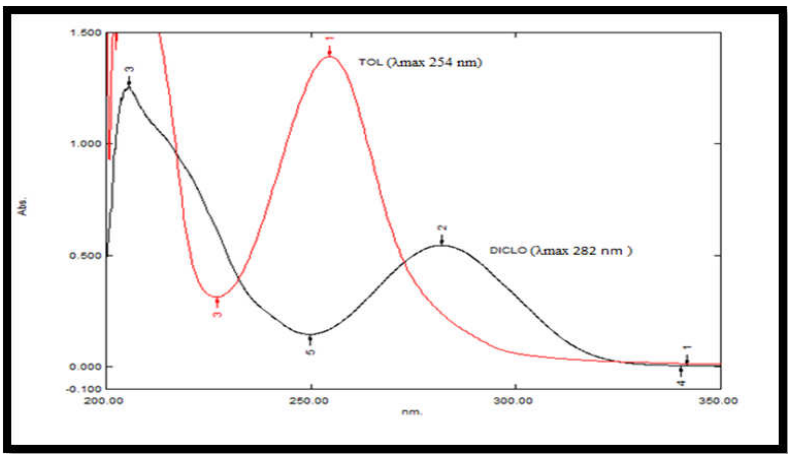


Fig. 2: Overlain spectrum of TOL (30µg/ml) and DFS (10µg/ml) in methanol

Calibration curves for TOL and DFS were plotted between absorbance and concentration (Fig. 3, 4). The following equations for straight line were obtained for TOL and DFS.

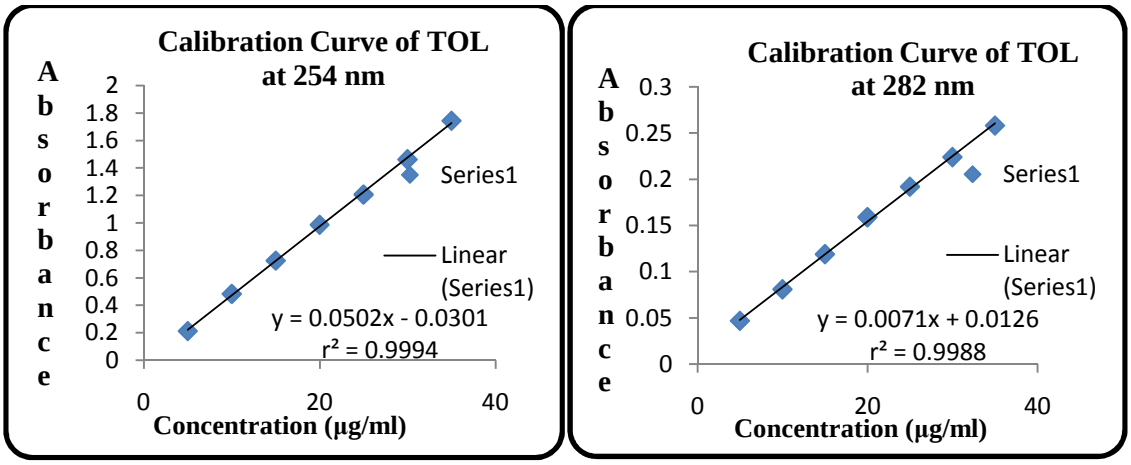


Fig. 3: Calibration Curve of TOL in Methanol at 254 nm and at 282nm

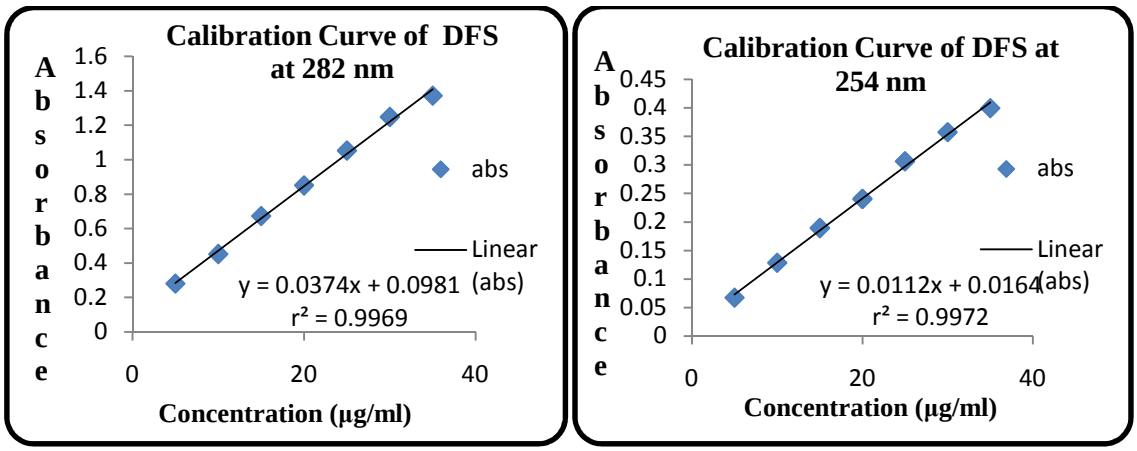


Fig 4: Calibration Curve of DFS in Methanol at 254 nm and at 282nm

Linear equation for TOL at 254 nm, **Y = 0.0502x - 0.0301;** Linear equation for TOL at 282 nm, **Y = 0.0071x + 0.0126**
Linear equation for DFS at 254 nm, **Y = 0.0112x + 0.0164;** Linear equation for DFS at 282 nm, **Y = 0.0374x + 0.0981**

Table 1: Summary of Validation Parameters of simultaneous equation method

Parameters	TOL	DFS
Recovery %	99.35 – 100.4	98.70 – 100.20
Repeatability(C.V.) (n=6)	0.37	0.33
Precision		
Intra-day (n=3)	0.19 - 0.97	0.18 – 0.50
Inter-day (n=3)	0.25 – 0.96	0.17 – 0.29
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 24 hrs.	Suitable for 24 hrs.

Table 2: Statistical data TOL by Simultaneous Equation method

Parameter	TOL (at 254 nm)	TOL (at 282 nm)
Range	5-35 µg/ml	5-35 µg/ml
Slope	0.0502x	0.0071x
Intercept	- 0.030217	0.01267
Regression Coefficient (r^2)	0.9994	0.9988
Standard deviation of Slope	0.000547	0
Standard deviation of Intercept	0.001538	0.000368
Limit of Detection (µg/ml)	0.101	0.171
Limit of Quantitation (µg/ml)	0.306	0.518

Table 3: Statistical data DFS by Simultaneous Equation method

Parameter	DFS (at 254 nm)	DFS (at 282 nm)
Range	5-35 µg/ml	5-35 µg/ml
Slope	0.0112x	0.0374x
Intercept	0.016733	0.01267
Regression Coefficient (r^2)	0.9972	0.9969
Standard deviation of Slope	0	0.0000816
Standard deviation of Intercept	0.000309	0.001364
Limit of Detection (µg/ml)	0.091	0.120
Limit of Quantitation (µg/ml)	0.276	0.364

Table 4: Accuracy data for TOL and DFS by Simultaneous equation method

% Level	Amount of drug added (µg/ml)		Amount recovered (µg/ml)		% Recovery	
	TOL (µg/ml)	DFS (µg/ml)	TOL (µg/ml)	DFS (µg/ml)	% TOL	% DFS
50 %	10	10	9.96	9.87	99.60	98.70
100 %	20	20	19.87	20.04	99.35	100.20
150 %	30	30	30.12	29.88	100.4	99.60

Table 5: Assay Results of Marketed Formulation

Formulation	Drug	Amount Taken (µg/ml)	Amount Found (µg/ml) (n = 3)	Labeled claim (mg)	Amount found per Tablet (mg)	% Label claim ±SD
TOLPIDOL-D (Tablet)	TOL	30	29.91	150	149.58	99.70 ± 0.2946
	DFS	10	9.94	50	49.70	99.40 ± 0.1743

CONCLUSION

The proposed Simultaneous equation method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of TOL and DFS in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of TOL and DFS in combined dosage form.

ACKNOWLEDGEMENT

Authors are thankful to Zydus Cadila Healthcare Ltd. (Ahmedabad, India) and Medico labs (Ahmedabad, India) for providing gratis sample with the great pleasure. The authors also thankful to Indubhai Patel College of Pharmacy and Research Centre (Dharmaj, India) for providing the necessary facilities for research work and to all the staff members and friends for their guidance and help throughout the research work.

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