INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN SYNTHETIC MIXTURE

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Article Received on: 19/07/12 Revised on: 22/08/12 Approved for publication: 04/09/12

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of Diclofenac sodium and Tolperisone hydrochloride in bulk and synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Diclofenac sodium has absorbance maxima at 281 nm and Tolperisone hydrochloride has absorbance maxima at 255 nm in methanol. The linearity was obtained in the concentration range of 2-20 µg/ml and 2-20 µg/ml for Diclofenac sodium and Tolperisone hydrochloride, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 100.6 ± 0.41 and 99.64 ± 0.50 for Diclofenac sodium and Tolperisone hydrochloride, respectively. The method was successfully applied to laboratory prepared synthetic mixture because no interference from the mixture excipients was found. The suitability of this method for the quantitative determination of Diclofenac sodium and Tolperisone hydrochloride was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Diclofenac sodium and Tolperisone hydrochloride in combination. The results of analysis have been validated statistically and by recovery studies.

KEY WORDS: Diclofenac sodium, Tolperisone hydrochloride, Recovery, Simultaneous equations method, Validation.

INTRODUCTION

Tolperisone (TOL) is chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one (Figure 1) is a well known antispasmodic drug[1]. It is official in Japanese Pharmacopoeia (JP). JP[2] describe potentiometric method for its estimation. Literature survey reveals HPLC[3] and UV[4] methods for estimation of TOL in single dosage form. Literature survey also reveals HPLC[5] and UV spectrophotometry[6] methods for determination of TOL with other drugs in combination. Diclofenac sodium (DIC) is chemically 2-[2,6dichlorophenylamino] benzene acetic acid sodium salt[7] (Figure 2). Diclofenac sodium (DIC) is official in IP and BP. IP[8] and BP[9] describes liquid chromatography method for its estimation. Literature survey reveals HPLC[10-11] and UV[12] methods for determination of DIC in single dosage form. Literature survey also reveals HPLC[13-15], UV spectrophotometry[16] and HPTLC[17] method for the determination of DIC with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of TOL and DIC in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of TOL and DIC in synthetic mixture or dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their combined synthetic mixture.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

TOL and DIC bulk powder was kindly gifted by Torrent Research Centre, Gandhinagar, India and Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India respectively. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of standard TOL (10 mg) and DIC (10 mg) powder were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of TOL and DIC.

Methodology

The working standard solutions of TOL and DIC were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400 nm against methanol as blank. Maximum absorbance was obtained at 255 nm and 281 nm for TOL and DIC, respectively. These two wavelengths can be employed for the determination of TOL and DIC without any interference from the other components in their synthetic formulations.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [18]

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 2-20 µg/ml for TOL and 2-20 µg/ml for DIC. Accurately measured standard solutions of TOL (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0 ml) and DIC (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the solutions were measured at 255 and 281 nm against methanol as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions ($n = 6$) for TOL and DIC (6 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of TOL and DIC (4, 6, 8 µg/ml for TOL and 4, 6, 8 µg/ml for DIC). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of TOL and DIC by the standard addition method. Known amounts of standard solutions of TOL and DIC were added at 50, 100 and 150 % level to prequantified sample solutions of TOL and DIC (7.5 µg/ml TOL and 2.5 µg/ml DIC). The amounts of TOL and DIC were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines [18]

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Analysis of TOL and DIC from synthetic mixture

Tolperisone (75 mg) and diclofenac (25 mg) standard drug powder were accurately weighed and then mixed with commonly used formulation excipients like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution (0.2 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of TOL (15 µg/ml) and DIC (5 µg/ml). The responses of the sample solution were measured at 255 nm and 281nm for quantitation of TOL and DIC, respectively. The amounts of the TOL and DIC present in the sample solution were calculated by solving respective simultaneous equations for TOL and DIC as follows.

$Cx = (A2\ aY1 – A1\ aY2) / (aY1\ aX2 - aY2\ aX1)$

$Cy = (A1\ aX2 - A2\ aX1) / (aY1\ aX2 - aY2\ aX1)$

Where,

A_1 and A_2 are absorbances of mixture at 255 nm and 281 nm;

aX_1 and aY_1 are absorptivities of TOL and DIC respectively at 255 nm;

aX_2 and aY_2 are absorptivities of TOL and DIC respectively at 281 nm.

RESULTS

The standard solutions of TOL and DIC were scanned separately in the UV range and zero-order spectra for TOL and DIC were recorded. Maximum absorbance was obtained at 255 nm and 281 nm for TOL and DIC, respectively. These two wavelengths can be employed for the determination of TOL and DIC without any interference from the other drug in their combined synthetic mixture. Overlain zero-order absorption spectrum of TOL and DIC in methanol is shown in (Figure 3). Linear correlation was obtained between absorbances and concentrations of TOL and DIC in the concentration ranges of 2-20 µg/ml and 2-20 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of TOL were found to be 0.79 and 0.80 % at 255 and 281 nm, respectively. The RSD value of DIC was found to be 0.72 and 0.44 % at 255 and 281 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.31-0.89% and 0.33-0.58% for TOL at 255 and 281 nm, respectively and 0.30-0.53% and 0.50-0.65% for DIC at 255 and 281 nm, respectively) and intraday (0.29-0.82% and 0.44-0.77% for TOL at 255 and 281 nm, respectively and 0.44-0.98% and 0.43-0.78% for DIC at 255and 281 nm, respectively) variation for TOL and DIC, reveal that the proposed method is precise. LOD and LOQ values for TOL were found to be 0.26 and 0.23 µg/ml and 0.79 and 0.70 µg/ml at 255 and 281nm, respectively. LOD and LOQ values for DIC were found to be 0.22 and 0.14 µg/ml and 0.66 and 0.43 µg/ml at 255 and 281 nm, respectively. These data show that method is sensitive for the determination of TOL and DIC. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.64 ± 0.5 and 100.6 ± 0.41 for TOL and DIC, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine TOL and DIC in their combined synthetic mixture. The results obtained for TOL and DIC were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of TOL and DIC in synthetic mixture as well as in pharmaceutical dosage forms.

DISCUSSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of TOL and DIC in Synthetic mixture. The method utilizes easily available and cheap solvent for analysis of TOL and DIC hence the method was also economic for estimation of TOL and DIC from Synthetic mixture. The common excipients and other additives are usually present in the Synthetic mixture do not interfere in the analysis of TOL and DIC in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

ACKNOWLEDGEMENT

The authors are thankful to Torrent Research Centre, Gandhinagar, India and Acme Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of TOL and DIC, respectively for carry out the research work. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India for providing all the facilities to carry out the research work.

REFERENCES

1. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 13th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station 2006. p. 1698.

2. Japanese Pharmacopoeia. Society of Japanese Pharmacopeia. 15th ed. Shibuya Tokyo Japan 2006.p. 1190-1191.
3. Murali M, Satyanarayana PV. Simple Validated Iso-cratic RP-HPLC method for estimation of Tolperisone in Bulk and Pharmaceutical dosage Form. Der Pharma Chemica 2011; 3:13-19.
4. Koladia BB, Vaghela VM. UV Spectroscopic method for Quantitative Estimation of Tolperisone Hydrochloride in Bulk and Pharmaceutical dosage form. Int J PharmTech Res 2012; 4:1317-1322.
5. Liawruangrath S, Liawruangrath B, Pibool P. Simultaneous Determination of Tolperisone and Lidocaine by HPLC. J Pharm Biomed Anal 2001; 26:865-72.
6. Sharma KK, Patel PU. First derivative Spectroscopic method for Simultaneous Estimation of Paracetamol and Tolperisone in their combined dosage form. J Pharm Sci Bio Res 2012; 2:92-96.
7. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 13th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: 2006. p. 542.
8. Indian Pharmacopoeia. Vol. II. The Controller of Publication. 6th ed. Govt. of India. New Delhi 2010.p. 1199.
9. British Pharmacopoeia. Vol. I. Stationary office. London Medicines and Healthcare product regulatory agency 2010.p. 672.
10. El-sayed YM, Abdel-hameed ME, Suleiman MS, Najib NM. A Rapid and sensitive HPLC method for the Determination of Diclofenac Sodium in Serum. J Pharm Pra 2011; 40:757-729.
11. Mayee R, Rawat S, Thosar A, Atre K, Mane P. Development and validation of HPLC method for Determination of Diclofenac Sodium by Tape Stripping method. Asian J Pharm Bio Res 2011; 1:317-322.
12. Khaskheli AR, Abro K, Sherazi ST, Afridi HI, Mahesar SA, Saeed M. Simpler and Faster Spectrophotometric Determination of Diclofenac Sodium in Tablet, Serum and Urine samples. Pak J Anal Environ Chem 2009; 10:53-58
13. Gowramma B, Rajan S, Muralidharan S, Meyyanathan SN, Suresh B. Validated HPLC method for Simultaneous Estimation of Paracetamol and Diclofenac in Pharmaceutical formulation. Int J of ChemTech Research 2010; 2:676-680.
14. Mulgund SV, Phoujdar MS, Londhe SV, Mallade PS, Kulkarni TS, Deshpande AS et al. Stability indicating HPLC method for Simultaneous determination of Mephenesin and Diclofenac Diethyl amine. Ind J Pharm Sci 2009; 71:35-40.
15. Shinde VM, Desai BS. Simultaneous Estimation of Paracetamol and Diclofenac and Chlorzoxazone by HPLC from Tablet. Ind J Pharm Sci 1995; 57:35-37.
16. Revathi G, Rama Rao N, Venkata SP. Simultaneous UV-spectrophotometric determination and validation of Diclofenac Sodium and Rabeprazole Sodium using Hydrotropic agents in its Tablet dosage form. Int J Drug Dev Res 2012; 4: 316-324
17. Dhaneshwar SR, Bhusari VK. Validated HPTLC method for Simultaneous Quantitation of Diclofenac Sodium and Misoprostol in Bulk drug and formulation. Asian J Pharm Biol Res 2011; 1:15-21.
18. The International Conference on Harmonization. Q2 (R1). Validation of Analytical Procedure Text and Methodology. 2005.

Table 1: Regression analysis data and summary of validation parameters for TOL and DIC

Parameters	TOL		DIC	
Wavelength (nm)	255	281	281	255
Beer's law limit (µg /ml)	2-20	2-20	2-20	2-20
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.063x - 0.021 0.063 -0.021	y = 0.01x - 0.002 0.01 -0.002	y = 0.046x - 0.008 0.046 0.008	y = 0.015x - 0.001 0.015 0.001
Correlation coefficient (R^2)	0.9980	0.9960	0.9970	0.9970
LOD[a] (µg/ml)	0.26	0.23	0.14	0.22
LOQ[b] (µg /ml)	0.79	0.70	0.43	0.66
Repeatability (% RSD[c], n = 6)	0.49	0.99	0.98	1.0
Precision (% RSD, n = 3) Interday Intraday	 0.31-0.89% 0.29-0.82%	 0.33-0.58% 0.44-0.77%	 0.50-0.65% 0.43-0.78%	 0.30-0.53% 0.44-0.98%
Accuracy ± S. D[d]. (% Recovery, n = 5)	99.64 ± 0.5		100.6 ± 0.41	

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification [d]S. D. is standard deviation

Table 2: Recovery data of TOL and DIC

Drug	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 5)
TOL	7.5	50	99.92 ± 0.53
	7.5	100	99.80 ± 0.84
	7.5	150	99.19 ± 0.13
DIC	2.5	50	100.3 ± 0.63
	2.5	100	101.4 ± 0.28
	2.5	150	100.1 ± 0.35

S. D. = Standard deviation. n = Number of determinations.

Table 3: Analysis of TOL and DIC in synthetic mixture

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	TOL	DIC	TOL	DIC	TOL	DIC
I	150	50	150.04	50.15	100.0 ± 0.78	100.3 ± 0.98

S. D. = Standard deviation. n = Number of determinations.

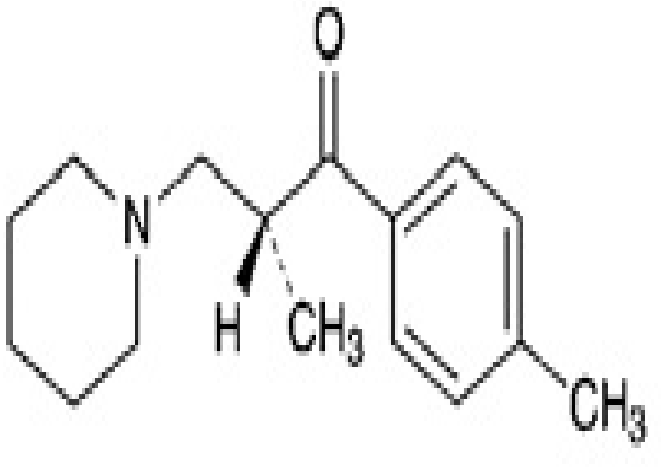

Figure 1: Chemical structure of Tolperisone (TOL)

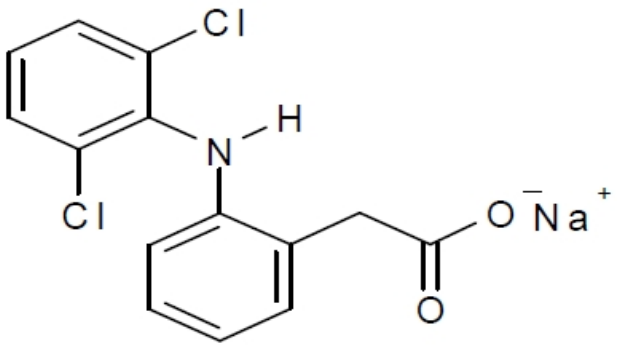

Figure 2: Chemical structure of Diclofenac Sodium (DIC)

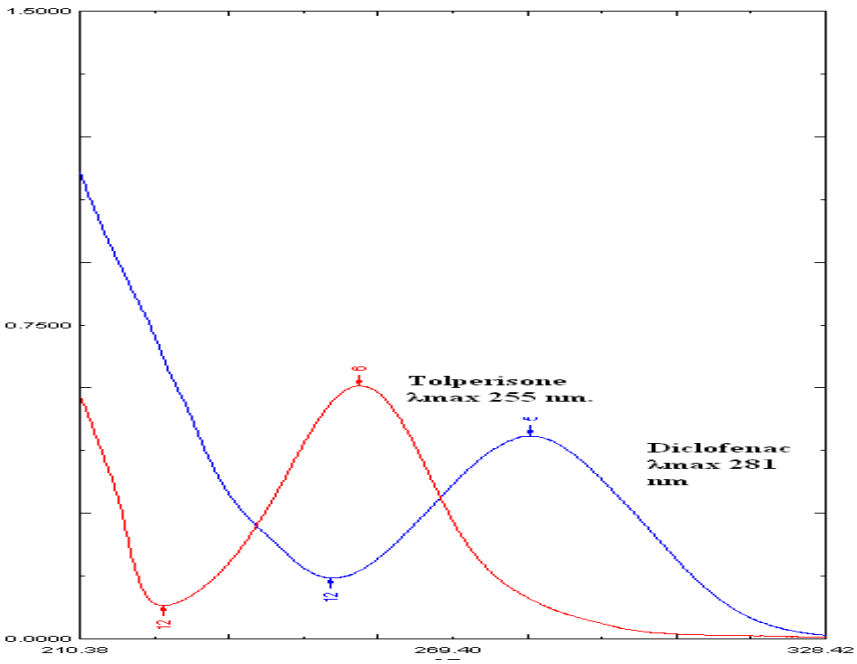

Figure 3: Overlain absorption spectra of TOL (255 nm) and DIC (281 nm) in methanol

Source of support: Nil, Conflict of interest: None Declared