Simultaneous Spectrophotometric Determination of Tizanidine and Diclofenac in Tablets

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Two simple, precise and accurate methods for simultaneous estimation of tizanidine hydrochloride and diclofenac sodium in combined dosage form have been described. Method 1 involves formation of Q-absorbance equation at 296 nm (isoabsorptive point) and at 281.3 nm while method 2 involves formation of simultaneous equation at 281.3 and 317.1 nm using methanol as solvent. Both the methods were validated and the results were compared statistically. They were found to be precise, accurate, and specific. The proposed methods were successfully applied to estimation of tizanidine and diclofenac in combined tablet formulation.

Diclofenac sodium (DCL) is a well-accepted non steroidal antiinflammatory drug (NSAID) and recommended in the treatment of local pain in order to reduce inflammation and pain. Frequently patients taking diclofenac and other NSAIDs suffer from muscle spasm causing the condition to be more painful and this also results in functional impairment[1]. Tizanidine hydrochloride (TZN), 5-chloro-N (4, 5-dihydro-1H-imidazol-2-yl) 2, 1, 3-benzothiadiazol-4-amine, is an α adrenergic receptor agonist, which is a centrally active skeletal muscle relaxant and is chemically different from other muscle relaxants[2]. In addition to its muscle-relaxant properties and central analgesic effect, TZN also has gastroprotective effect[3]. Hence it is used in the combination of NSAIDs for the treatment of local pain. DCL is official in various pharmacopoeias and reports are available for estimation of diclofenac in combined formulation containing other drugs using super critical fluid chromatography[4,5], HPLC[6-15] and UV Spectrophotometric[16-19] methods. Tizanidine hydrochloride is an imidazoline derivative and not official in any of the pharmacopoeia. Several analytical methods for the estimation of tizanidine using HPLC[20], isocratic SFC[21], polarography[22,23], colorimetry[24,25] and UV spectrophotometry[26] have been reported. Reddy *et al.*[27] reported simultaneous spectrophotometric estimation of tizanidine and nimesulide from combined dosage forms using methanol as a solvent. Moreover the literature survey revealed that so far no method has been reported for estimation of TZN and DCL in combined dosage forms, hence we attempted to develop simple, accurate and economic analytical method. This paper describes two simple UV spectrophotometric methods for simultaneous estimation of TZN and DCL in tablets using methanol as solvent. For simultaneous estimation, standard TZN had to be added to the samples in order to get measurable absorbance of TZN.

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An UV/Vis double beam spectrophotometer, model Spectrascan UV-2600 (Chemito Instruments ltd.) and Lambda 19 of Perkin Elmer, USA with 1 cm matched quartz cells were used. TZN standard stock solution (100 μg/ml) was prepared by weighing a 25 mg portion of TZN (Blue Cross Laboratories Ltd., Mumbai) standard and it was transferred to a 25 ml volumetric flask and volume made to 25 ml with methanol. From this solution, aliquot of 2.5 ml was withdrawn and it was diluted up to 25 ml using methanol. DCL (Mercury Laboratories Ltd., Vadodara) standard stock solution (100 μg/ml) was prepared by weighing a 25 mg portion of DCL standard in to a 25 ml volumetric flask and volume was made up to 25 ml with methanol. From this solution, aliquot of 2.5 ml was withdrawn and it was diluted up to 25 ml using methanol.

Selection of analytical wavelengths for the Q-absorbance method (Method 1) was done by taking pure drug samples of TZN and DCL, which were separately dissolved in methanol to give two solutions of 8 μg/ml. They were then scanned in the wavelength range of 200-400 nm. From the overlain spectra of both drugs (fig. 1) wavelengths 296 nm (isoabsorptive point) and 281.3 nm (λmax of DCL) were selected for the formation of Q-absorbance equation. For

calibration curves, stock solutions of TZN and DCL were appropriately diluted to obtain concentration range of 4–24 μg/ml for each drug. The absorbance of TZN measured at 296 nm and 281.3 nm and calibration curves were plotted. Similarly the absorbance of DCL measured at 296 nm and 281.3 nm and calibration curves were plotted. The absorptivities (A1%, 1 cm) of each drug at both the wavelengths were determined.

The absorbance and the absorptivity values at the particular wavelengths were calculated and substituted in the following equation to obtain the concentration. $C_{DCL}=(Q_M-Q_Y)xA_1/(Q_X-Q_Y)xa_{x1}$, $C_{TZN}=A1/a_{x1}-C_{DCL.}$ where, $C_{TZN.}$ C_{DCL} are concentration of DCL and TZN, respectively, A_1 is the absorbance of sample at 296 nm, a_{x1} is the absorptivity of DCL at 296nm, Q_X was obtained by using the equation, (absorptivity of DCL at 281.3 nm)/(absorptivity of DCL at 296 nm), Q_Y was obtained from (absorptivity of TZN at 281.3 nm)/(absorptivity of TZN at 296 nm), and Q_M from, (absorbance of sample at 281.3 nm)/(absorbance of sample at 296 nm).

For the selection of analytical wavelengths in simultaneous equation method (Method 2) the spectra of TZN and DCL of the method 1 were used and wavelength 317.1 nm and 281.3 nm (λmax of TZN and DCL, respectively) were selected for the formation of the simultaneous equations. For calibration curves, stock solutions of DCL and TZN were appropriately diluted to obtain TZN and DCL in the concentration range of 4–24 μg/ml. The absorbance of TZN and DCL were measured at 317.1 and 281.3 nm and calibration curves were plotted. The absorptivities of both the drugs at both the wavelengths were determined.

The absorbance and the absorptivity values at the particular wavelengths were calculated and substituted in the following equation to obtain the concentration. $C_{TZN}=(A_1a_{x2}-A_2a_{x1})/(a_{x2}a_{y1}-a_{x1}a_{y2})$, $C_{DCL}=(A_2a_{y1}-A_1a_{y2})/(a_{x2}a_{y1}-a_{x1}a_{y2})$, where, $C_{TZN.}$ C_{DCL} are concentration of DCL and TZN, respectively, A_1 is the absorbance of sample at 281.3 nm, A_2 is the absorbance of sample at 317.1 nm, a_{x1} is the absorptivity of DCL at 281.3 nm, a_{x2} is the absorptivity of DCL at 317.1 nm, a_{y1} is the absorptivity of TZN at 281.3 nm and a_{y2} is the absorptivity of TZN at 317.1 nm.

For the estimation of drugs from the commercial formulations twenty tablets of two brands Tizpa-D (Blue Cross Laboratoriess. Limited, Mumbai) and Tizaren (Sun Pharma limited, Mumbai), containing 2.288 mg tizanidine hydrochloride, equivalent to 2 mg tizanidine and 50 mg diclofenac sodium were weighed and finely powdered. For method 1 and 2, powder equivalent to TZN 1.144 mg and DCL 25 mg was accurately weighed and to this 12 mg of standard tizanidine hydrochloride was added (for standard addition). The mixture was then extracted with methanol and extract was filtered and filtrate was appropriately diluted to get final concentration 7.886 μg/ml of TZN and 15 μg/ml of DCL. Absorbance of this solution was measured at appropriate wavelengths and values were substituted in the respective formulae to obtain concentrations.

The overlain spectra of both the drugs showed that the peaks are well resolved thus satisfying the criteria for obtaining maximum precision based on absorbance ratios[29]. The criteria being the ratios, $(A_2/A_1)/(a_{x2}/a_{x1})$ and $(a_{y2}/a_{y1})/(A_2/A_1)$, should lie outside the range 0.1-2.0 for the precise determination of Y and X, respectively. Where, A_1, A2 represents the absorbance of the mixture at λ_1 and λ_2, a_{x1} and a_{x2} denote absorptivities of X at λ_1 and λ_2 and a_{y1} and a_{y2} denote absorptivities of Y at λ_1 and λ_2, respectively. In this context the above criteria was found to be satisfied for DCL (X) and TZN (Y) where λ_1 is 296 nm and λ_2 281.3 nm for Q-absorbance method and λ_1 is 281.3 nm and λ_2 is 317.1 nm for simultaneous equation method.

Fig. 1 shows three wavelengths that could serve as isoabsorptive point; they are 222 nm, 259 nm and 296 nm.

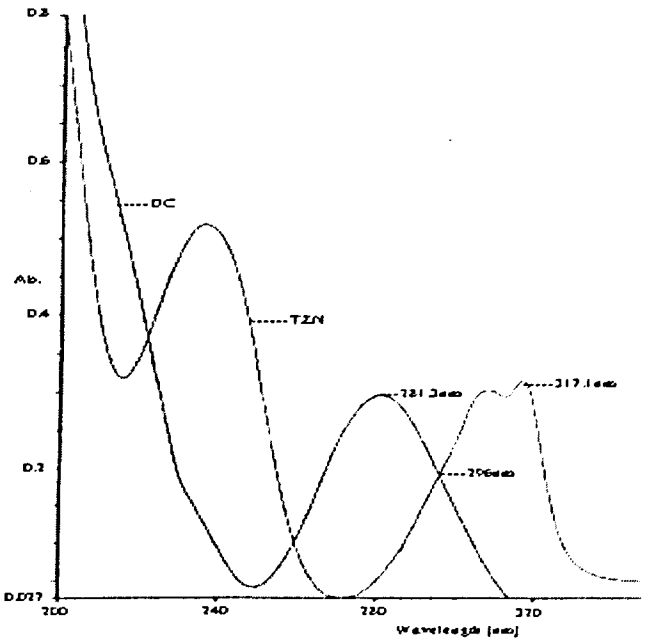

Fig.1: Overlain spectra of TZN and DCL in methanol. TZN is Tizanidine hydrochloride, DCL is Diclofenac sodium and Ab is absorbance.

By comparing the absorptivity of both the drugs at these wavelengths, 296 nm was found suitable for the analysis since both the drugs gave same absorptivity at this wavelength. Other wavelength i.e. the λmax of DCL selected at 281.3 nm. Hence 296 and 281.3 nm were selected for the formation of Q-absorbance equation.

In simultaneous equation method two wavelengths i.e. λmax of both the drugs were required. The spectra of TZN showed two distinct peaks, one at around 222 nm and other at 317.1 nm. The latter was selected for analysis of TZN since the former showed change in λmax with change in

TABLE 1: REGRESSION ANALYSIS OF CALIBRATION CURVES

Parameters	TZN			DCL		
Wavelength (nm.)	281.3	296	317.1	281.3	296	317.1
Concentration range (μg/ml)	4-24	4-24	4-24	4-24	4-24	4-24
Intercept	-0.0095	-0.0163	-0.0264	0.0067	-0.0157	-0.0040
(S.D.)	(0.0016)	(0.0029)	(0.0025)	(0.0025)	(0.0029)	(0.0020)
Slope	0.0085	0.0250	0.0450	0.0379	0.0250	0.0035
(S.D.)	(0.0002)	(0.0001)	(0.0003)	(0.0002)	(0.0002)	(0.0001)
Correlation coefficient (r^2)	0.9984	0.9994	0.9987	0.9997	0.9981	0.9976

Method 1 is Q- absorbance method while Method 2 is simultaneous equation; S.D. is standard deviation while r^2 is correlation coefficient.

TABLE 2: SUMMARY OF VALIDATION PARAMETERS

Parameters	Method 1		Method 2	
	TZN	DCL	TZN	DCL
Limit of quantification (μg/ml)	1	1	1	6
Linearity range (μg/ml)	1-24	1-24	1-24	6-24
Correlation coefficient	0.9984[a]	0.9997[a]	0.9984[a]	0.9997[a]
(r^2)	0.9992[b]	0.9981[b]	0.9987[c]	0.9976[c]
Accuracy %	99.1-100.1	98.9 – 100.8	97.1 – 99.1	98.0 – 101.4
Repeatability	0.019[a]	0.004[a]	0.019[a]	0.004[a]
(RSD, n=6)	0.007[b]	0.009[b]	0.008[c]	0.030[c]
Precision (%CV)				
Intraday (n=3)	1.50 -1.70[a]	0.63-0.96[a]	1.50 -1.70[a]	0.63-0.96[a]
	0.85-1.36[b]	1.04-1.60[b]	0.56-0.73[c]	1.90-3.22[c]
Interday (n=3)	1.26-2.09[a]	0.95-3.04[a]	1.26-2.09 [a]	0.95-3.04[a]
	1.41-2.21[b]	1.42-2.49[b]	0.57-1.53[c]	2.92-4.01[c]
Specificity	Specific	Specific	Specific	Specific

Method 1 is Q-absorbance method while Method 2 is the simultaneous equation method, RSD is the relative standard deviation while r^2 is the correlation coefficient, CV is the coefficient of variation. a; at 281.3 nm. b; at 296 nm, c; at 317.1 nm.

TABLE 3: VALIDATION OF EQUATIONS USING MIXED STANDARD DRUGS FOR METHOD 1 AND 2

Concentration μg/ml		% Recovery			
		Method 1		Method 2	
TZN	DCL	TZN	DCL	TZN	DCL
5	20	100.6±0.98	98.3±0.72	98.9±0.81	98.4±0.47
10	15	101.0±1.29	99.7±0.33	98.4±0.88	99.7±0.35
15	10	101.5±0.38	100.7±0.58	98.6±0.53	100.0±1.03
20	5	100.7±0.64	100.8±0.35	98.5±0.32	100.0±1.05

Method 1 is Q-absorbance method while Method 2 is the simultaneous equation method. Values for recovery are mean±s.d. for three determinations.

TABLE 4: ANALYSIS OF COMMERCIAL FORMULATIONS

Method	Tablet A		Tablet B	
	%TZN	%DCL	%TZN	%DCL
Method 1	100.5±1.19	99.8±0.60	100.8±1.20	100.1±0.67
Method 2	98.7±0.22	101.1±1.14	98.8±0.64	98.8±0.59

Method 1 is Q-absorbance method while Method 2 is the simultaneous equation method. Values for recovery are mean±s.d. for three determinations. Tablet A: Tizpa-D (Blue cross labs. Limited, Mumbai) and Tablet B Tizaren (Sun pharma limited, Mumbai), containing 2.288 mg tizanidine hydrochloride, equivalent. to 2 mg tizanidine and 50 mg diclofenac sodium.

concentration. The λmax of DCL was 281.3 nm, which was used for its estimation.

Regression analysis of the calibration curves of both the methods is presented in Table 1. The proposed methods were successfully used to estimate the amount of TZN and DCL present in two of the marketed tablet formulations containing TZN and DCL. The assay values for both the tablets were comparable with the corresponding labeled amounts as shown in Table 2. Validity of equations formed in method 1 and 2 was checked using mixed standards (Table 3). The validation parameters of proposed methods are summarized in Table 4. On observing the validation parameters, both the methods were found to be precise accurate and specific. When compared to method 2, method 1 gave assay results close to 100%. Though TZN had to be added in the proposed methods they are simpler and economical methods when compared to that of chromatographic estimation. Hence, the proposed methods can be employed for routine assay of tablets containing TZN and DCL.

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Spectrophotometric Determination of Hepatoprotective Principle of *Phyllanthus niruri* Linn

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A simple and sensitive spectrophotometric method in ultraviolet region has been developed for the determination of phyllanthin in different parts of *Phyllanthus niruri*. Phyllanthin shows maximum absorbance at 280 nm. Beer's law is obeyed in the concentration range of 5-50 μg/ml. The proposed method is precise, accurate and reproducible.

Phyllanthus niruri Linn. belongs to the natural order Euphorbiaceae. It is an annual herb and chiefly distributed in the tropical and sub-tropical regions of the world[1]. It is incorporated in large number of reputed hepatoprotective herbal formulations. A number of compounds have been isolated from the plant, which include lignans, alkaloids, flavonoids and sterols. The presence of phyllanthin, which belongs to the class of lignans is reported to the responsible for the hepatoprotective activity[2-5]. No standard method has been reported for the estimation of this active principle. An attempt is made here to determine the percentage of phyllanthin by spectrophotometer, using methanol as a blank.

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The percentage of phyllanthin was evaluated separately for leaf-midrib (including fruits) and stem-root by this method.

A Shimadzu model 250 UV/Vis spectrophotometer with 1 cm matched quartz cells was used. Silica gel G and methanol (analytical grade) were obtained from S. D. Fine Chemicals, Mumbai and all other organic solvents used were of laboratory grade. Fresh herbs of *P. niruri* were collected locally in the month of September, 2002 from two different locations viz., Osmania University Campus and Ghatkesar. Botanical identification was performed and a voucher specimen (PN/WP/02) is being maintained in the phytochemistry department of G. Pulla Reddy College of Pharmacy (GPRCP), Hyderabad, A.P. The stem-root was separated from the herb. Both the samples were air-dried at room temperature and ground into powder.