RP-HPLC METHOD FOR THE SEPARATION OF PARACETAMOL, TIZANIDINE, ACECLOFENAC, CHLORZOXAZONE: APPLICATION TO TIZANIDINE DETERMINATION IN TABLET DOSAGE FORM

Ravisankar P^{*1,2}, Devala Rao G³

¹Department of Pharmaceutical Analysis and Quality Assurance, Vignan Pharmacy College, Vadlamudi, Guntur (A.P.), India

²Faculty of Science, Sri Chandrasekharendra Saraswathi Viswa MahaVidyalaya (SCSVMV University), Tamilnadu, India

³Department of Pharmaceutical Analysis, KVSRR Siddhartha College of Pharmaceutical Sciences, Vijayawada (A.P.), India

*Corresponding Author Email: banuman35@gmail.com

Article Received on: 21/03/13 Revised on: 09/04/13 Approved for publication: 20/05/13

DOI: 10.7897/2230-8407.04635

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

© All rights reserved.

ABSTRACT

New, rapid, precise, accurate and specific chromatographic method with UV detection was developed and validated for the separation of paracetamol, tizanidine, diclofenac and chlorzoxazone and applied for the determination of tizanidine in tablet dosage forms. RP-HPLC method was developed by using Welchrom C₁₈ Column (4.6 mm i.d. X 250 mm, 5 µm particle size), Shimadzu LC-20AT Prominence Liquid Chromatography. The mobile phase constituted of Phosphate buffer (pH-6.65): acetonitrile (60:40, v/v). The flow rate was set to 1.0 mL/min with the responses measured at 280 nm using Shimadzu SPD-20A Prominence UV-Visible detector. The retention times of paracetamol, tizanidine, aceclofenac and chlorzoxazone were found to be 3.200 min, 3.593 min, 7.717 min and 8.857 min respectively. This method successfully separated all the fore mentioned drugs in less than 10 min. tizanidine was found to give linear response in the concentration range of 2-10 µg/mL. Recovery studies were executed to ascertain the accuracy by standard addition method and average recovery was found to be 98.824 to 100.126 %. The limit of detection and limit of quantification were found to be 0.1761 µg/mL and 0.5336 µg/mL respectively. The proposed method is simple, economical, fast and hence can be applied for routine quality control analysis of tizanidine in tablet dosage form. It can also be extended for the determination of paracetamol, chlorzoxazone and aceclofenac.

Keywords: Isocratic RP-HPLC method, tizanidine, paracetamol, chlorzoxazone, aceclofenac.

INTRODUCTION

Paracetamol (PCM) is N-(4-hydroxyphenyl) acetamide (Figure 1(a)) is a potent analgesic and anti-pyretic. It is generally used in the treatment of headache, toothache, backache, dysmenorrhea, myalgia and neuralgia. Aceclofenac (ACF) is 2-[2-[2-[(2,6-dichlorophenyl) amino] phenyl]acetyl]oxyacetic acid (Figure 1(b)) is the most widely used NSAID presently. The primary mechanism of action of diclofenac is inhibition of Cyclooxygenase (COX) enzyme leading to the inhibition of prostaglandin biosynthesis. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis, ankylosing spondylosis, toothache and dysmenorrhea. Tizanidine (TIZ) is 5-chloro-N-(4, 5-dihydro-1H-imidazol-2-yl) benzo [c] [1,2,5] thia diazol-4-amine (Figure 1(c)) is a centrally acting skeletal muscle relaxant. It is a centrally acting α₂ adrenergic agonist¹. It is used to treat the painful muscle spasms, cramping and tightness of muscles caused by medical problems such as multiple sclerosis, spasticity, backache. Chlorzoxazone (CLZ) is 5-chloro-3H-benzoxazol-2-one (Figure 1(d)) is a centrally acting muscle relaxant. Chlorzoxazone is used to relieve pain and stiffness caused by muscle strains and sprains. It is used in combination with physical therapy, analgesics (such as aspirin or acetaminophen) and rest. Literature survey reveals that for the determination of above said drugs in biological fluids like plasma, blood, urine and pharmaceutical dosage

forms by spectrophotometry²⁻⁵, HPTLC⁶, High Performance Liquid Chromatography (RP-HPLC) with UV detection⁷⁻¹⁹, super critical fluid chromatography²⁰. The combination of PCM, TIZ, ACF and CLZ has not been adopted by any official pharmacopoeia. Literature survey reveals the report of a few analytical methods for the determination of PCM, TIZ, ACF and CLZ. So far there was no HPLC method available for the simultaneous separation of PCM, TIZ, ACF and CLZ and determination of TIZ alone. Therefore attempts have been made to develop and validate simple precise and sensitive isocratic RP-HPLC method for simultaneous separation of PCM, TIZ, ACF and CLZ and determination of TIZ in pharmaceutical formulations. When developing a new method one of the most salient goals is to achieve a steady and good separation. The selection of a highly reproducible HPLC method is vital if this goal is to be attained. Thus the present study illustrates development and validation of a novel, simple, rapid and efficient RP-HPLC analytical method with phenomenal separation of PCM, TIZ, ACF and CLZ with short retention time. This method also gives reduced run time with good resolution, excellent peak symmetry, use of reduced solvent consumption, providing reduced costs achieved without resorting to high back pressure. The proposed method was validated with respect to specificity, linearity, precision, accuracy, robustness, LOD and LOQ according to ICH guidelines²¹.

Figure 1(a): Paracetamol

Figure 1(c): Tizanidine

Figure 1(b): Aceclofenac

Figure 1(d): Chlorzoxazone

Figure 1: Structures of drugs investigated in the present study

MATERIALS AND METHODS

Chemicals and Reagents

The reference standards of PCM, TIZ, ACF and CLZ procured from Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India were used in the present study. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate, acetonitrile (HPLC grade) and triethylamine (HPLC grade) were purchased from Merck Pharmaceuticals Private Ltd., Mumbai, India. O-Phosphoric acid used was of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of TIZ (TIZAN) containing 4mg are manufactured by Sun Pharmaceutical Industries Pvt. Ltd., Mumbai, India. High purity HPLC grade water was used throughout the analysis. Mobile phase was filtered using 0.45µm nylon filter by Millipore (USA).

Instrumentation

HPLC was performed on an isocratic high performance liquid chromatography (Shimadzu LC-20AT Prominence Liquid Chromatography) equipped with a LC-20AT VP pump. Sample injection was performed via a Rheodyne (USA) sample injector with a 20µL loop and a Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C₁₈ Column (4.6mm i.d. X 250mm, 5µm particle size). The HPLC system

was equipped with "Spinchrom" software for data acquisition and quantification of peaks. The pH of the solution was measured using digital pH meter (Systronics model 802). A UV spectrum of all drugs was recorded for selection of working wavelength of detection by using a UV-Visible Spectrophotometer (Systronics model 2203). In addition an electronic balance (Shimadzu TX223L), a sonicator (spectra lab, model UCB 40) was used in this study.

Chromatographic conditions

Chromatographic separations were achieved using a C₁₈ (4.6mm i.d. X 250mm, 5µm particle size) analytical column. The mobile phase consisting of a mixture of Phosphate buffer (pH-6.65) and Acetonitrile in ratio of 60:40, v/v was selected as mobile phase and UV detection wavelength was 280nm with a flow rate of 1mL/min. the mobile phase was prepared freshly and passed through a 0.45µm membrane filter and degassed for 15min by ultrasonication. The mobile phase flow rate was maintained at 1mL/min and the injection volume was 20µL. The eluents were monitored at 280nm. The HPLC system and the column were kept in ambient temperature. Run time was 10min and retention time for TIZ was 3.600min. The optimized chromatographic conditions of TIZ are presented below in Table 1.

Table 1: Optimized Chromatographic Conditions and System Suitability Parameters for Tizanidine

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C ₁₈ Column (4.6 mm i.d. X 250mm, 5µm particle size)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	Phosphate Buffer(pH 6.65) : Acetonitrile (60:40, v/v)
Mobile phase	Phosphate Buffer (pH 6.65) : Acetonitrile (60:40, v/v)
Flow rate	1mL/min.
Detection wave length	UV at 280nm.
Run time	10 minutes
Column back pressure	125-130 kgf
Temperature	Ambient temperature(25°C)
Injection Volume	20µL
Retention time (t _R)	3.593 min
Theoretical plates[th.pl] (Efficiency)	9,524
Theoretical plates per meter[t.p/m]	190,472
Tailing factor (asymmetry)	1.074

Preparation of solutions and Reagents

Mobile phase

A freshly prepared 60:40, v/v, mixture of phosphate buffer and acetonitrile was used as mobile phase. The phosphate buffer was prepared freshly by dissolving 1.780g of KH_2PO_4 and 0.345g K_2HPO_4 in 600mL of HPLC grade water. The mixture was filtered through 0.45 μm nylon membrane filter and degassed by ultrasonication.

Stock and Working Standard Solutions

Accurately weigh 10mg of TIZ, dissolve in a 100mL volumetric flask with mobile phase and sonicate to mix well. This is stock standard solution of TIZ with concentration of 100 $\mu\text{g/mL}$. From the stock solution, working standard solutions were prepared in a concentration range of 2-10 $\mu\text{g/mL}$. Likewise, 10 $\mu\text{g/mL}$ of PCM, ACF and 5 $\mu\text{g/mL}$ of CLZ were prepared from 100 $\mu\text{g/mL}$ stock standard solutions of PCM, ACF and CLZ respectively into each 10ml volumetric flask.

Tablet Sample preparation

Accurately weighed and grind 20 tablets of TIZ in a mortar and triturated to a fine powder. From this, tablet powder which is equivalent to 4mg of TIZ was taken and the drug was extracted into 100mL of mobile phase in a beaker, stir and place in an ultrasonic bath until dissolution is complete.

Transfer this solution into a 100mL volumetric flask, rinse the beaker with mobile phase a few times, and transfer into the same volumetric flask. Add mobile phase to bring to volume. The resulting solution was filtered using 0.2 μm filter and degassed by sonication. This solution was further suitably diluted for chromatography to give a concentration of approximately 4 $\mu\text{g/mL}$.

Selection of detection wavelength

The overlain UV spectra of various diluted solutions of PCM, TIZ, ACF and CLZ in mobile phase were recorded using UV spectrophotometer. The isobestic point of maximum absorbance was observed at 280nm. This wavelength was used for detection of TIZ and PCM, ACF, CLZ.

Calibration curve for Tizanidine

Replicates of each calibration standard solutions (2, 4, 6, 8, 10 $\mu\text{g/mL}$) were injected using a 20 μL fixed loop system and the chromatograms were recorded. Calibration curves were constructed by plotting concentration of TIZ on X-axis and peak areas of standard TIZ on y-axis and regression equations were computed for TIZ. The linear regression data and calibration data of the proposed HPLC method for estimation of TIZ are presented in below in Table 2 and Table 3 respectively. The standard chromatogram of TIZ was shown in Figure 2. Calibration plot is shown in Figure 3.

Table 2: Linear Regression Data of the Proposed HPLC Method of TIZ

Parameter	Method
Detection wavelength(λ_{max})	UV at 280 nm
Linearity range ($\mu\text{g/mL}$)	2-10 $\mu\text{g/mL}$
Regression equation ($Y = a + bX$)	$y = 17.125x + 1.0169$
Slope(b)	17.125
Intercept(a)	1.0169
Standard error of slope (S_b)	0.150931
Standard error of intercept (S_a)	0.913937
Standard error of estimation (S_e)	1.262785
Regression coefficient (R^2)	0.9996
% Relative standard deviation* i.e., Coefficient of variation(CV)	1.1340
Percentage range of errors(Confidence limits)	
0.005significance level	1.358305
0.001 significance level	2.130599

Table 3: Calibration Data of HPLC Method for Estimation of TIZ

S. No.	Concentration, $\mu\text{g/mL}$	Retention time, (t_R)min	Peak area, mV.s.
1.	0	0	0
2.	2	3.597	34.982
3.	4	3.603	70.628
4.	6	3.597	104.942
5.	8	3.600	138.574
6.	10	3.593	170.738
Slope	17.1254	Correlation Coefficient [CC] (r)	0.9998
Intercept	1.0168	Squared CC (R^2)	0.9996

Assay of marketed formulations

From the above tablet sample preparation, 20 μL was injected 6times into the column under the predetermined optimized chromatographic conditions. From the peak area values of TIZ, the amount of drug present in the tablets was estimated

using the calibration curve method of the pure sample. The assay results are tabulated below in Table 4. Figure 4 illustrates the chromatogram of the sample formulation (TIZAN) of TIZ.

Figure 2: Standard chromatogram of TIZ (10µg/mL)

Figure 3: Calibration plot of TIZ

Table 4: Assay Results of TIZ Formulation

Formulations	Labeled amount	Amount found	% Assay± SD*
TIZAN4 mg tablets (Sun Pharma Industries Ltd., Mumbai, India)	4 mg	4.164mg	104.112±1.137 %

*Average of 6 determinations; SD is standard deviation

Figure 4: Chromatogram of formulation (TIZAN 4 mg tablets) of TIZ

Validation of the Proposed Method

Validation of the developed analytical procedure is the process by which it is established by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. Validation was performed in accordance with the International Conference on Harmonization (ICH). Validation parameters checked were specificity, precision,

accuracy, robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc. Specificity of the method was performed by

comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also that there is good correlation between the retention times of standard and sample. The specificity results are shown in Table 5. Therefore, it was concluded that the method is specific.

Table 5: Specificity Study for TIZ

Name of the solution	Retention time, (t _R)min.
Mobile phase	No peaks
Placebo	No peaks
TIZ, 10µg/mL	3.593 min.

Precision

Precision of an analytical procedure is the closeness of agreement (Degree of scatter) between a series of measurements obtained from multiple sampling of the sample homogeneous sample under the prescribed conditions. Precision of the method was performed as intra-day and inter-day precision. To study the intra-day precision, six replicate standard solutions of TIZ were injected. The %RSD was calculated and it was found to be 0.6876 which is within the acceptable criteria of not more than 2.0. Results of intra-day precision studies are shown in Table 6.

Table 6: Results of Precision Study (Intra-Day)

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	%RSD [#]
TIZ	10	1	169.884	0.6545
		2	170.126	
		3	171.062	
		4	170.186	
		5	168.762	
		6	172.029	

[#]Acceptance criteria < 2.0

For inter-day precision, six-replicate standard solution of TIZ was injected on the 2nd day of sample preparation. The %RSD was calculated and it was found to be 0.9629 which is

within the acceptable criteria of not more than 2.0. Results of inter-day precision studies are shown in Table 7.

Table 7: Results of Precision Study (Inter-Day)

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	%RSD [#]
TIZ	10	1	170.738	0.9963
		2	169.428	
		3	174.228	
		4	172.724	
		5	171.196	
		6	170.776	

[#]Acceptance criteria < 2.0

Accuracy (Recovery studies)

Accuracy of an analytical procedure is the closeness of agreement between the conventional true value or an accepted reference value and the value found. The accuracy was determined by adding a known amount of standard drug to the fixed amount of pre-analyzed tablet solution. %

Recovery was calculated by standard addition method which was performed at 80%, 100% and 120% level. The % recovery and %RSD was calculated and the results are presented in Table 8. Satisfactory recoveries ranging from 99.5264 to 100.5206% were obtained by the proposed method.

Table 8: Recovery Data of the Proposed RP-HPLC Method for TIZ

Recovery level	Amount added (mg)	Total amount (mg)	Amount found (mg)	Amount recovered (mg)	% recovery	Mean % Recovery ± SD	%RSD [#]
80%	3.176	7.3405	7.3304	3.1659	99.68	99.85 ± 0.3925	0.3931
	3.218	7.3825	7.3922	3.2277	100.30		
	3.192	7.3565	7.3429	3.1784	99.57		
100%	3.984	8.1485	8.1526	3.9881	100.10	100.03 ± 0.0951	0.0951
	4.026	8.1905	8.1932	4.0287	100.06		
	4.032	8.1965	8.1934	4.0289	99.92		
120%	4.822	8.9865	8.9273	4.7628	98.77	99.35 ± 0.5287	0.5322
	4.834	8.9985	8.9734	4.8089	99.48		
	4.806	8.9705	8.9612	4.7967	99.80		

[#]Acceptance criteria < 2.0

Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of the procedure's reliability during normal usage. For the determination of a method's robustness, a number of method parameters such as pH, flow rate, injection volume, detection wavelength, column temperature and mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. It was

observed that there were no marked changes in the chromatograms and the parameters are within the limits which demonstrated that the developed method was robust in nature. The complete results are shown in Table 9.

LOD and LOQ

The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit (LOQ) of an individual

analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated by formula $LOD = 3.3(SD)/S$ and $LOQ = 10 (SD)/S$, where

SD=standard deviation of response (peak area) and S= slope of the calibration curve. The LOD and LOQ of HCT by proposed method are abridged in Table 10.

Table 9: Robustness Results of TIZ

S. No.	Parameter ^a	Optimized	Used	Retention time (t_R), min	Plate count ^b	Peak asymmetry [#]	Remark
1.	Flow rate (± 0.2 mL/min)	1.0 mL/min	0.8 mL/min	3.926	9876	1.078	*Robust
			1.2 mL/min	3.286	9254	1.071	*Robust
2.	Detection wavelength (± 5 nm)	280 nm	275 nm	3.595	9538	1.068	Robust
			285 nm	3.597	9517	1.073	Robust
3.	Mobile phase composition (± 5 %)	60:40, v/v	65:35, v/v	3.242	9795	1.093	*Robust
			55:45, v/v	3.783	9286	1.085	*Robust

*considerable change in retention time

Table 10: LOD and LOQ

Limit of Detection (LOD)	0.1761 μ g/mL
Limit of Quantitation (LOQ)	0.5336 μ g/mL

RESULTS AND DISCUSSION

One of the salient aims for the present work is to achieve a consistent, reproducible separation. As a matter of fact selection of a phenomenal method is essential if this aim is to be attained. To optimize the HPLC parameters, several mobile phase compositions were tried. Decorous peak symmetry, plate count and resolution were obtained with mobile phase consisting of phosphate buffer (pH-6.65): acetonitrile in the ratio of 60:40 v/v. Optimized separation of PCM, TIZ, ACF and CLZ was achieved by using a C₁₈ column (250 mm X 4.6 mm i.d, 5 μ m particle size). Runtime was less than 10min at a flow rate of 1mL/min. The

quantification was achieved with detection at 280nm obtained from UV overlain spectra of PCM, TIZ, ACF and CLZ. The injection volume was 20 μ L. Under the conditions described above, separation of the mixture of PCM, TIZ, ACF and CLZ was achieved with a total run-time of 10min, with an elution window of 6min for all the four analytes. As shown in Figure 5, the resolution of all main peaks was found to be greater than 2.0. Exceptionally low asymmetry values were found for each analyte and peak shape was excellent as demonstrated by asymmetry values of less than 1.2 overall with the TIZ peak having an asymmetry value of 1.081. In all cases, the plate count was greater than 3000. The linear dynamic range of 2-10 μ g/mL met all specifications with $R^2 > 0.999$. Table 11 shows the data for separation chromatogram of mixture of PCM, TIZ, ACF and CLZ.

Table 11: Chromatogram Results of Proposed Combination Of PCM, TIZ, ACF and CLZ

Name of the Compound	Retention time (t_R), min	Peak No.	Assymetry [#]	Efficiency (th.pl) ^a	Theoretical plates/meter	Resolution ^s
PCM	3.200	1	1.078	10549	210978	-
TIZ	3.600	2	1.081	12559	251177	2.950
ACF	7.717	3	1.027	16831	336623	21.431
CLZ	8.857	4	1.026	22155	443095	4.748

^atheoretical plates > 3000, [#]acceptance criteria<1.5, ^sacceptance criteria>2.0

Table 12: Individual Chromatogram Results of Standards of PCM, TIZ, ACF and CLZ

Name of the Compound	Retention time (t_R), min.	Assymetry [#]	Efficiency (theoretical plates) ^a
PCM	3.197	1.072	10527
TIZ	3.593	1.074	12428
ACF	7.527	1.104	16280
CLZ	8.850	1.026	22088

^atheoretical plates > 3000, [#]acceptance criteria<1.5, ^sacceptance criteria>2.0

Figure 5: A typical chromatogram of Mixture of PCM, TIZ, ACF and CLZ

For the comparative evaluation of retention times and peak areas of PCM, TIZ, ACF and CLZ drugs, chromatograms of PCM, TIZ, ACF and CLZ drug standards were recorded

individually. The representative individual standard chromatograms of PCM, TIZ, ACF and CLZ are shown in Figure 6 - 9 and the results are presented in Table 12.

Figure 6: Standard chromatogram of PCM (10µg/mL)

Figure 7: Standard chromatogram of TIZ (10µg/mL)

Figure 8: Standard chromatogram of ACF (10µg/mL)

Figure 9: Standard chromatogram of CLZ (5µg/mL)

CONCLUSION

Rapid separation of PCM, TIZ, ACF and CLZ was successfully attained with a relatively short retention time, provides outstanding resolution, good peak shape, gives reliable and highly reproducible results on C₁₈ HPLC column. Separation of PCM, TIZ, ACF and CLZ mixture was achieved with a total run time of 10 minutes with an elution window of 6 minutes for all four analytes. Excellent values for precision, recovery and linearity were achieved together with low LOD and LOQ for TIZ. The ease in preparation of mobile phase and economy of the components of mobile phase show explicitly the applicability of this method the best choice in routine analysis of PCM, TIZ, ACF and CLZ in QA/QC departments in short period and even in low concentration levels.

ACKNOWLEDGEMENTS

The authors are indebted to Hetero Labs, Hyderabad, India for providing the samples of PCM, TIZ, ACF and CLZ. We are highly thankful to Dr. L. Rathaiah, Honorable Chairman, Vignan group of institutions, Vadlamudi, Guntur, for providing the necessary facilities to carry out this research work.

REFERENCES

1. Drug today medical journal, Lorina publication (India) Inc., Delhi-91, DT 78, 2012; 1: 343-51.
2. Ware Agasti L, Aher Sunil J, Musmade Deepak S, Gajare Suvarna P, Patil Poonam P. Simultaneous ratio derivative spectrophotometric estimation of aceclofenac and tizanidine with paracetamol in combined dosage forms. *Int Res J Pharm* 2012; 3(8): 205-8.
3. Garg G, Saraf S, Saraf S. Simultaneous estimation of aceclofenac, paracetamol and chlorzoxazone in tablets. *Indian J Pharm Sci* 2007; 69: 692-4. <http://dx.doi.org/10.4103/0250-474X.38481>
4. Wahbi AAM, Gazy AA, Abdel Razak O, Mahgoub H, Moneeb M. Simultaneous Determination of Paracetamol and Chlorzoxazone using Orthogonal Functions Ratio Spectrophotometry. *Saudi Pharm J* 2003; 11(4): 192-200.
5. Patel Satish A, Prajapati Kalpesh M. Spectrophotometric method for simultaneous determination of chlorzoxazone and diclofenac sodium in synthetic mixture. *Int Res J Pharm* 2012; 3(9): 293-6.
6. Kaul N, Dhaneshwar SR, Agrawal H, Kakad A, Patil B. Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in pharmaceutical dosage form. *J Pharm Biomed Anal* 2005; 37(1): 27-38. <http://dx.doi.org/10.1016/j.jpba.2004.09.036> PMID: 15664740
7. Ravisankar S, Vasudevan M, Gandhimathi M, Suresh B. Reversed-phase HPLC method for the estimation of acetaminophen, ibuprofen and chlorzoxazone in formulations. *Talanta* 1998; 46(6): 1577-81. [http://dx.doi.org/10.1016/S0039-9140\(98\)00043-5](http://dx.doi.org/10.1016/S0039-9140(98)00043-5)
8. Nimje H, Wate SP, Dharkar DP, Razdan R. Simultaneous RP-HPLC determination of nimesulide and tizanidine in tablets. *Indian J Pharm Sci* 2007; 69: 281-3. <http://dx.doi.org/10.4103/0250-474X.33158>
9. Siddiqui FA, Arayne MS, Sultana N, Qureshi F. Development and validation of stability-indicating HPLC method for the simultaneous determination of paracetamol, tizanidine, and diclofenac in pharmaceuticals and human serum. *J AOAC Int* 2011; 94(1): 150-8. PMID: 21391492
10. Mei-Ling Qia, Peng Wang, Lei Wang. Validated liquid chromatography method for assay of tizanidine in drug substance and formulated products. *Anal Chim Acta* 2003; 478(2): 171-7. [http://dx.doi.org/10.1016/S0003-2670\(02\)01525-8](http://dx.doi.org/10.1016/S0003-2670(02)01525-8)
11. Gandhimathi M, Ravi TK, Varghese SJ. Simultaneous LC determination of tizanidine and rofecoxib in tablets. *J Pharm Biomed Anal* 2005; 37(1): 183-5. <http://dx.doi.org/10.1016/j.jpba.2004.09.039> PMID: 15664760
12. Puranik M, Wadher SJ, Dhole S, Yeole PG. Simultaneous estimation of valdecoxib and tizanidine hydrochloride in tablets by RP-HPLC. *Indian J Pharm Sci* 2006; 68: 670-2. <http://dx.doi.org/10.4103/0250-474X.29647>
13. Shaikh KA, Devkhile AB. Simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone by RP-HPLC in pharmaceutical dosage form. *J Chromatogr Sci* 2008; 46(7): 649-52. <http://dx.doi.org/10.1093/chromsci/46.7.649> PMID: 18718143
14. Erdal Dinç, Abdil Ozdemir, Halil Aksoy, Dumitru Baleanu. Chemometric approach to simultaneous chromatographic determination of paracetamol and chlorzoxazone in tablets and spiked human plasma. *J Liq Chromatogr Relat Technol* 2006; 29(12): 1803-22.
15. Joshi R, Sharma R. Development and validation of RP-HPLC method for simultaneous estimation of three-component tablet formulation containing acetaminophen, chlorzoxazone and aceclofenac. *Anal Lett* 2008; 41(18): 3297-308. <http://dx.doi.org/10.1080/00032710802515086>
16. Pawar UD, Naik AV, Sulebhavikar AV, Datar TA, Mangaonkar K. Simultaneous determination of aceclofenac, paracetamol and chlorzoxazone by HPLC in tablet dose form. *J Chem* 2009; 6(1): 289-94.
17. Ali MS, Rafiuddin S, Ghori M, Kahtri AR. Simultaneous determination of Paracetamol, chlorzoxazone and related impurities 4-aminophenol, 4-chloroacetanilide and p-chlorophenol in pharmaceutical preparations by high-performance liquid chromatography. *J AOAC Int* 2007; 90(1): 82-93. PMID: 17373439
18. Shinde V, Desai B, Tendolkar N. Simultaneous Determination of Paracetamol, Diclofenac Sodium and Chlorzoxazone by HPLC from Tablet. *Indian J Pharma Sci* 1995; 57(1): 35-7.
19. Rathinavel G, Priyadarsin R, Thakur D, Premanand DC, Valarmathy J, Hemalatha S, et al. Validated RP-HPLC method for estimation of aceclofenac, paracetamol and chlorzoxazone in dosage form. *Der Pharma Chemica* 2010; 2(2): 286-96.
20. Bari VR, Dhorda UJ, Sundaresan M. A simultaneous packed column supercritical fluid chromatographic method for ibuprofen, chlorzoxazone and acetaminophen in bulk and dosage forms. *Talanta* 1997; 45(2): 297-302. [http://dx.doi.org/10.1016/S0039-9140\(97\)00153-7](http://dx.doi.org/10.1016/S0039-9140(97)00153-7)
21. ICH, Harmonized Tripartite Guideline. Validation of Analytical Procedure: Methodology (Q2B). International Conference on Harmonization; 1997.

Cite this article as:

Ravisankar P, Devala Rao G. RP-HPLC method for the separation of paracetamol, tizanidine, aceclofenac, chlorzoxazone: application to tizanidine determination in tablet dosage form. *Int. Res. J. Pharm.* 2013; 4(6): 156-163

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