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Full Length Research Paper

Development and validation of a reverse phase high performance liquid chromatography (HPLC) method for determination of tizanidine in human plasma

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A simple and cost effective high performance liquid chromatography (HPLC) method was developed for determination of tizanidine in human plasma using liquid extraction technique. The assay of tizanidine was performed after extraction of drug from plasma using diethyl ether as extraction solvent. The isocratic elution was performed in Agilent, Zorbax SB-C_{18}, 4.6 × 150 mm column maintained at 30°C with mobile phase containing acetonitrile and ammonium acetate in a ratio of 15:85 v/v, respectively. The linear relationship was found within the concentration range of 0.25 to 8 ng/ml, with a flow rate of 1 ml/min and detector wavelength of 230 nm. The evaluated validation parameters were found within the acceptable range. Use of simple HPLC technique with short retention time makes this method a convenient choice for assay of tizanidine in human plasma.

Key words: Tizanidine, bio-analytical method validation, linearity, accuracy, limit of detection (LOD), limit of detection (LOQ), liquid extraction

INTRODUCTION

Tizanidine hydrochloride is an imadazoline derivative that acts on centrally located alpha 2 receptors for producing monolytic response on skeletal muscles (Wagstaff and Bryson, 1997). It is used for the treatment of multiple sclerosis or spinal cord injury or spasticity associated with diseased condition (Sweetman, 2009). Tizanidine is also used for the relieving of pain with disorders like myofacial pain (Meythaler et al., 2001), refractory pain, neuropathic pain, chronic tension type headache and chronic daily headache (Saper et al., 2002). Tizanidine is widely absorbed in gastro intestinal (GI) tract. Peak plasma concentration is achieved in 1 to 2 h after oral administration. Tizanidine protein binding is 30% and it undergoes extensive first pass metabolism (Shanker et al., 2009). Tizanidine is chemically [5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole]and demonstrate basic and lipophilic properties. The drug is ionised in acidic environment and soluble in water (Qi et al., 2003).

Many researchers reported method for determination of tizanidine in human plasma. Lee et al. (2002) evaluated tizanidine by using gas chromatography-mass spectrometry.

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(Lee et al., 2002). Momo et al. (2010) determined tizanidine in human plasma and urine with use of liquid chroma-tography-tandem mass spectrometry in presence of maxiletine (Momo et al., 2010). Ulu et al. (2012) utilized a spectroflourimetric method based on dervitization for estimation of tizanidine in plasma, urine and dosage forms (Ulu et al., 2012).

In the current study, tizanidine hydrochloride was analyzed using simple high performance liquid chromato-graphy (HPLC) method. The low amount of drug in plasma was extracted by liquid-liquid extraction tech-nique. The method overcomes the issue of determination of low concentration of tizanidine in human plasma. The described method is specific and sensitive with short retention time (4.4 min) that makes it cost effective (Figure 1 and Table 1).

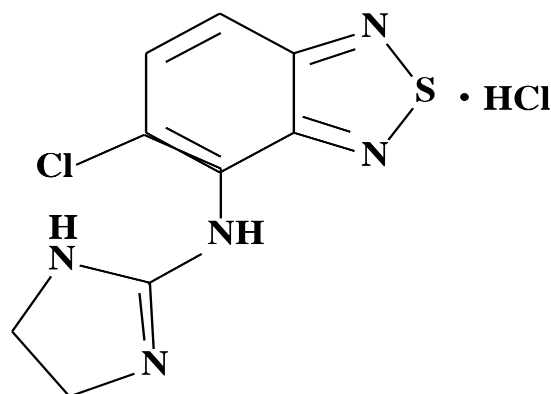

Figure 1. Tizanidine hydrochloride.

METHODOLOGY

Instruments and apparatus

HPLC system (Pump: LC-10 AT VP; detector: SPD-10A *VP*, Shimadzu Corp., Kyoto, Japan), Communication bus module (CBM-102), Guard column C18, 4.0 × 2 mm, HPLC Column (Agilent, Zorbax SB-C18, 4.6 × 150, 5 µm, USA), Column Oven (CTO-10A, Shimadzu Corp., Kyoto, Japan), HPLC Software Class GC 10 version 2.0.0.0, (Shimadzu Corp., Kyoto, Japan), Ultra Sonic bath (Clifton, Nickel Electro Ltd. Somerset, England), Centrifuge machine (Hereues, Osterode, Germany), Vortex mixer (Whirl mixer, England), Filtration assembly (Sartorius, Gorringen, Germany), Swinney Filter assembly (Millipore, England), Hamilton Microliter syringe (Hamilton, Switzerland), Micropippette (Mettler Toledo, Scwerzenbach, England), pH meter (Mettler Toledo, Scwerzenbach, England) were used in the study.

Preparation of chromatographic solutions

Mobile phase

The mobile phase consisted of acetonitrile and 0.1 M ammonium acetate in a ratio of 15:85 v/v. The mobile phase was filtered and sonicated before use.

Preparation of stock solutions and working standard

The standard stock solution was prepared in mobile phase by dissolving accurately weighed quantity of tizanidine to make 1 mg/ml. The standard samples for calibration curve in plasma were prepared by spiking standard stock solution in 1 ml of blank plasma for preparation of secondary stock solution of 100 ng/ml by serial dilution. Secondary stock solution of 100 ng/ml was diluted in plasma for 0.25, 0.5, 1, 2, 4 and 8 ng/ml concentrations.

Sample preparation for drug determination in plasma using HPLC

Sodium fluoride (20 mg) and 0.4 ml of sodium hydroxide (5 mol/L) were added in 1 ml of spiked plasma and vortex for 1 min. Diethyl ether (5 ml) was added and vortex for 10 min for extraction, later centrifuged for 5 min at 4500 rpm. Supernatant was transferred into another test tube and evaporated to dryness using gentle stream of nitrogen at 40°C. The sample was reconstituted in 70 µl of mobile phase, then vortex for 2 min and again centrifuged for 5 min at 4500 rpm. A quantity of 50 µl of the final sample was injected into the HPLC for analysis.

Chromatographic conditions

In the current study, the evaluation of tizanidine was performed at a flow rate of 1 ml/min with detection wavelength of 230 nm and column maintained at a temperature of 30°C.

Method validation

The reported method for determination **of nicergoline metabolite** in human plasma in which Zheng et al. (2012) used tizanidine hydrochloride as internal standard, was modified for estimation of tizanidine hydrochloride in plasma. The proposed method was validated as per International Conference on Harmonisation (ICH) guidelines (ICH, 2005) for selectivity, linearity, accuracy, precision, sensitivity and stability.

Selectivity

Selectivity is helpful for differentiation between drug and other components present in the sample. For selectivity of method, six different blank samples of plasma were run, and it was established at lower limit of quantification (LLOQ) of drug.

Linearity and calibration curves

The linearity was assessed between different concentrations of drug molecule and response of detector by calibration curve (Lister, 2005). The samples with concentrations of 0.25, 0.5, 1, 2, 4, and 8 ng/ml were analyzed in triplicate and calibration curve was constructed, and coefficient of correlation (r^2) was determined.

Intraday and interday accuracy and precision

Five samples of four different concentrations in plasma were analyzed at different time in the same day for intraday accuracy and

Table 1. System suitability parameters.

Parameter	Mean (n=5)	Relative standard deviation (%)	Limit
Retention time (min)	4.4	-	-
Area	22167	0.91	Less than 2
Tailing factor	1.36	0.56	Less than 2
Theoretical plates	6410	-	-

precision determination and analyzed for three consecutive days for interday accuracy and precision. The concentrations were calculated using standard calibration curves.

Lower limit of quantification (LLOQ) and limit of detection (LOD)

Lower limit of quantification (LLOQ) and limit of detection (LOD) were established by analysis of different low concentrations (0.05, 0.1, 0.15 and 0.25 ng/ml). For lower limit of quantification, the signal to noise ratios was observed for the lowest concentration and considered acceptable when response was 7 times of the noise. Lowest detectable and quantifiable concentration against standard was considered as LOQ. The concentration when signal to noise ratio was observed as 3 times has been considered as LOD.

Analytical recovery of method

The absolute recovery was established by comparative study of drug spiked in plasma and in mobile phase. Three different concentrations (2, 4 and 6 ng/ml) each with 5 replicates were examined for determination of recovery.

Plasma stability of the drug

Freeze-thaw and long term stabilities were carried out for the drug in plasma. The freeze and thaw stability was evaluated with selected low (1 ng/ml) and high (7 ng/ml) concentrations with 20 samples of each concentration. Samples were frozen at -20°C for 24 h. A set of all concentrations was thawed and assessed while remaining samples were frozen for the next 24 h. Other two sets of five samples of each concentration were analyzed with the same procedure while the last set was refrozen for next day analysis to complete the three freeze-thaw cycles. These samples were evaluated with reference to freshly prepared samples. The long term stability was performed by preparing fifteen samples of low and high concentrations in plasma. The samples were stored at -20°C. Five samples of each concentration were analyzed at the end of second week and the next five at the end of third week of storage with respect to its initial concentration.

RESULTS AND DISCUSSION

In the current method, tizanidine was extracted from plasma using liquid extraction which is extensively for determination of low drug concentrations in biological fluids (Ciccolini et al., 2001; Esrafili et al., 2007; Xiong et al., 2009). Gan et al. (2002) developed and validated the estimation of tramadol in human plasma using HPLC followed by liquid-liquid extraction. Nirogi et al. (2006) quantified tizanidine in human plasma after liquid-liquid extraction with liquid chromatography tandem mass spectrometry in the range of 50 to 5000 pg/ml. Siddiqui et al. (2011) validated simple HPLC method for simultaneous determination of paracetamol, tizanidine and diclofenac in biological fluids and found a linearity for tizanidine in the concentration range of 120 to 10,000 ng/ml.

Selectivity

Six blank plasma samples were run and no peak at the retention time of drug was detected in plasma. The drug sample was run in same condition and no interference was found. No interfering plasma peak was observed at the drug retention time proved a good selectivity of the method. Selectivity of the method was shown in Figure 2.

Linearity and calibration curves

Evaluations of samples with concentration 0.25, 0.5, 1, 2, 4 and 8 ng/ml were performed in triplicate. The standard calibration curve was linear with a mean r^2 of 0.9989 with percent accuracy between 90 to 104.6%. The linearity chromatogram and linearity curve of tizanidine are shown in Figure 3 and 4.

Accuracy and precision

The HPLC method was also validated for intraday and interday accuracy and precision. The intraday accuracy was found in a range of 90 to 96% while interday accuracy with value of 84 to 92% was observed as shown in Table 2. All the values complied with standard acceptable range of ±15 % for bioanalytical method accuracy and precision.

Lower limit of quantification and limit of detection

The five samples of each concentration (0.05, 0.1, 0.15 and 0.25) were analyzed for LLOQ and LOD determination.

Table 2. Accuracy and precision of tizanidine in plasma.

Parameter	Selected concentrations in method validation (ng/ml)			
	0.25	1	6	8
		Intraday		
Mean (n=5)	0.23	0.9	5.75	7.67
% Accuracy	92	90	95.833	95.875
Standard deviation	0.014	0.039	0.047	0.069
% Coefficient of variation	6.087	4.333	0.817	0.900
		Interday		
Mean (n=5)	0.21	0.85	5.5	7.24
% Accuracy	84	85	91.667	90.5
Standard deviation	0.017	0.043	0.052	0.069
% Coefficient of variation	8.095	5.059	0.945	0.953

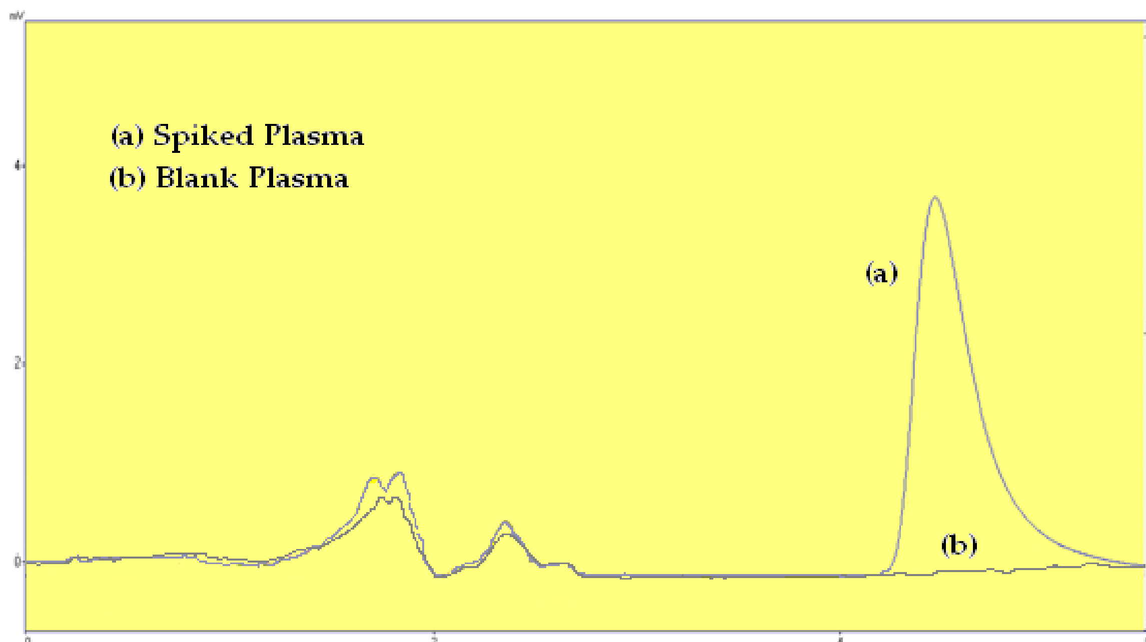

Figure 2. Chromatogram showing selectivity of the method.

The concentration 0.05 ng/ml was not detectable while the lower limit of detection was found as 0.1 ng/ml, and lower limit of quantification (LLOQ) value was 0.25 validated with accuracy of 94.4% that is within the specified limit of 20% and presented in Table 3.

Analytical recovery

Recovery of the method was performed for low, medium and high concentration within the calibration curve range. The method was found with good recovery with the mean analytical recovery of 97.135% for three selected concentrations of 2, 4 and 6 ng/ml as shown in Table 4.

Plasma stability of drug

Freeze and Thaw stability of the drug in plasma were evaluated for three freeze-thaw cycles and estimated accuracy were found to be 99.4, 97.8 and 98.2% for low concentration (1 ng/ml) and 98.66, 98.37 and 98.31% for high selected concentration (7 ng/ml) for freeze thaw cycle 1, 2 and 3, respectively. The average degradation of drug in three FT cycles was found to be 2.268 and 1.040% for concentration of 1 and 7 ng/ml, respectively.

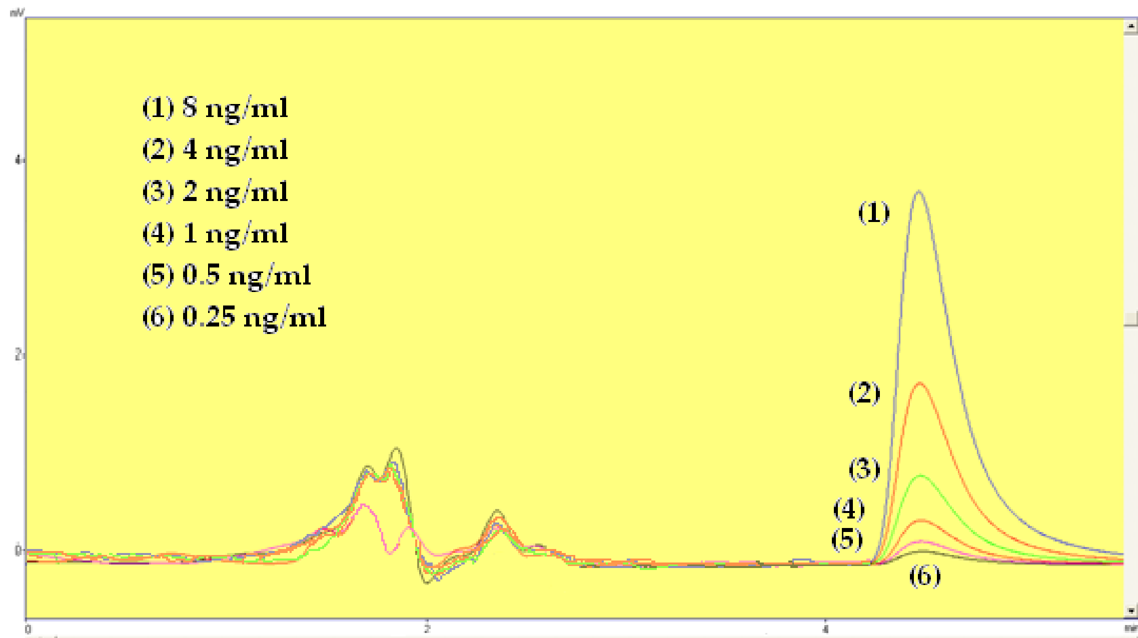

Figure 3. Linearity chromatogram of tizanidine in plasma.

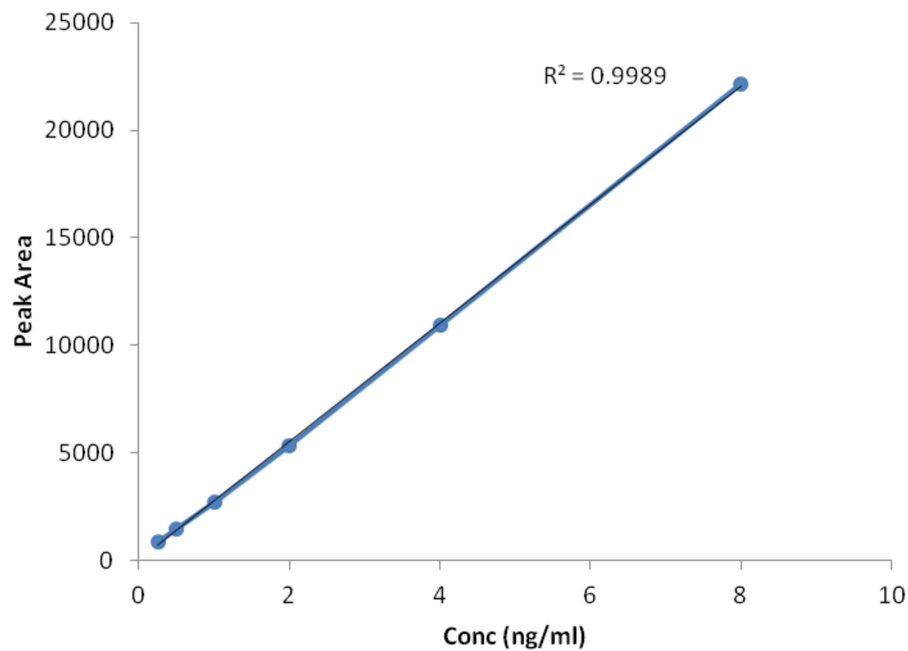

Figure 4. Linearity of different concentration of tizanidine.

Long term stability of the drug was performed for three weeks for low and high concentrations. More than 95% drug was found in the samples after three weeks that represent good long term stability of the drug in plasma.

The mean degradation of drug for both the concentrations of drug in plasma after three weeks was 3.695%. The results of freeze thaw stability and long term stability studies are presented in Tables 5 and 6.

Table 3. Limit of detection of tizanidine in plasma.

Conc. (ng/ml)	Calculated concentration (ng/ml)					Mean	Standard deviation	% Coefficient of variation	% accuracy
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5				
0.25	0.24	0.23	0.23	0.24	0.24	0.236	0.005	2.321	94.4
0.15	0.11	0.1	0.12	0.13	0.11	0.114	0.011	10.002	76
0.1	0.07	0.08	0.06	0.06	0.04	0.062	0.015	23.923	62
0.05	-	-	-	-	-	-	-	-	-

Table 4. Results of recovery studies.

S/No	Conc. (ng/ml)	Mean peak area in plasma (n=5)	Mean peak area in mobile phase (n=5)	% Recovery
1	2	5056.4	5204.6	97.153
2	4	10617.2	11051.6	96.069
3	6	15147.6	15428	98.183
Mean recovery				97.135

Table 5. Freeze and thaw stability of tizanidine.

Parameter	Low concentration (1 ng/ml)				High Concentration (7 ng/ml)			
	Fresh Sample	FT Cycle 1	FT Cycle 2	FT Cycle 3	Fresh sample	FT Cycle 1	FT Cycle 2	FT Cycle 3
Mean (n=5)	1.006	0.994	0.978	0.982	6.98	6.906	6.886	6.882
Standard deviation	0.030	0.021	0.015	0.018	0.027	0.062	0.057	0.066
% Coefficient of variation	2.949	2.086	1.517	1.822	0.392	0.896	0.825	0.955
% Accuracy	100.6	99.4	97.8	98.2	99.71	98.66	98.37	98.31

*FT – Freeze-thaw cycle.

Table 6. Long term stability of tizanidine in plasma.

Parameter	Low concentration (1 ng/ml)			High concentration (7 ng/ml)		
	Fresh sample	After 2 weeks	After 3 weeks	Fresh Sample	After 2 weeks	After 3 weeks
Mean (n=5)	1.012	0.982	1.0	7.032	6.888	6.748
Standard deviation	0.069	0.066	0.069	0.090	0.128	0.113
% Coefficient of variation	6.860	6.693	7.209	1.281	1.858	1.678
% Accuracy	101.200	98.200	95.400	100.457	98.400	96.400

Conclusion

The method has been validated successfully for the determination of tizanidine in human plasma sample. Validation parameters such as selectivity, linearity, accuracy, precision and stability showed good results and complied with standard acceptable range. Hence, this liquid extraction based HPLC method can be used effectively for the estimation of tizanidine in human plasma.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Ciccolini J, Catalin J, Blachon M, Durand A (2001). Rapid high-performance liquid chromatographic determination of docetaxel (Taxotere) in plasma using liquid–liquid extraction. J. Chromatogr. B:

Biomed. Sci. Appl. 759(2): 299-306.
Esrafili A, Yamini Y, Shariati S (2007). Hollow fiber-based liquid phase microextraction combined with high-performance liquid chromatography for extraction and determination of some antidepressant drugs in biological fluids. Anal. Chim. Acta. 604(2):127-133.
Gan S, Ismail R, Wan Adnan W, Wan Z (2002). Method development and validation of a high-performance liquid chromatographic method for tramadol in human plasma using liquid–liquid extraction. J. Chromatogr. B. 772(1):123-129.
ICH (2005). Validation of Analytical Procedures: Text and Methodology. International conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. International Conference on Harmonisation, Geneva, Switzerland, www. ich. org.
Lee J, Seo JH, Kim DH (2002). Determination of tizanidine in human plasma by gas chromatography–mass spectrometry. Analyst 127(7):917-920.
Lister AS (2005). Validation of HPLC methods in Pharmaceutical Analysis, 1 ed. Elseveir Inc., UK.
Meythaler JM, Guin-Renfroe S, Johnson A, Brunner RM (2001). Prospective assessment of tizanidine for spasticity due to acquired brain injury. Arch. Phys. Med. Rehab. 82(9):1155-1163.
Momo K, Homma M, Osaka Y, Inomata S, Tanaka M, Kohda Y (2010). Effects of mexiletine, a CYP1A2 inhibitor, on tizanidine pharmacokinetics and pharmacodynamics. J. Clin. Pharmacol. 50(3):331-337.
Nirogi RV, Kandikere VN, Shukla M, Mudigonda K, Maurya S (2006). Quantification of tizanidine in human plasma by liquid chromatography coupled to tandem mass spectrometry. Rapid Commun. Mass Spectr. 20(15):2286-2292.
Qi ML, Wang P, Wang L (2003). Validated liquid chromatography method for assay of tizanidine in drug substance and formulated products. Analyt. Chim. Acta. 478(2):171-177.
Saper JR, Lake AE, Cantrell DT, Winner PK, White JR (2002). Chronic Daily Headache Prophylaxis With Tizanidine: A Double-Blind, Placebo-Controlled, Multicenter Outcome Study. Headache 42(6):470-482.
Shanker G, Kumar CK, Gonugunta CSR, Kumar BV, Veerareddy PR (2009). Formulation and evaluation of bioadhesive buccal drug delivery of tizanidine hydrochloride tablets. AAPS Pharmscitech. 10(2): 530-539.
Siddiqui FA, Arayne MS, Sultana, N, Qureshi F (2011). 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. 94(1): 150-158.
Sweetman SC (2009). Martindale: The complete Drug Reference, 36th ed. Pharmaceutical Press, London.
Ulu ST (2012). Sensitive spectrofluorimetric determination of tizanidine in pharmaceutical preparations, human plasma and urine through derivatization with dansyl chloride. Luminescence 27(5):426-430.
Wagstaff A, Bryson H (1997). Tizanidine: A review of its pharmacology clinical efficacy and tolerability in the management of spasticity associated with cerebral and spinal disorders. Drugs 53(3): 435-452.
Xiong C, Ruan J, Cai Y, Tang Y (2009). Extraction and determination of some psychotropic drugs in urine samples using dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. J. Pharm. Biomed. Anal. 49(2): 572-578.
Zheng R, Wu YH, Jiang DX, Zhang D (2012). Determination of metabolite of nicergoline in human plasma by high-performance liquid chromatography and its application in pharmacokinetic studies. J. Pharm. Anal. 2(1):62-66.