



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR
DETERMINATION OF TIZANIDINE IN HUMAN PLASMA- A
REVIEW**

**G.LINO STEFFY ASTER, M.VIJEY AANANDHI, AFROZ PATAN AND
S.RAMACHANDRAN***

Department of Pharmaceutical Chemistry and Analysis, Vels Institute of Science Technology
and Advanced Studies (VISTAS), Chennai-600 117, Tamilnadu, India

*Corresponding Author: Dr. S.Ramachandran: E Mail: ramachandrans.sps@velsuniv.ac.in

Received 16th Sept. 2021; Revised 20th Oct. 2021; Accepted 19th Dec. 2021; Available online 1st Aug. 2022

<https://doi.org/10.31032/IJBPAS/2021/11.8.6319>

ABSTRACT

A straightforward and practical elite fluid chromatography (HPLC) strategy was produced for assurance of tizanidine in human plasma utilizing fluid extraction strategy. The measure of tizanidine was performed after extraction of medication from plasma utilizing diethyl ether as extraction dissolvable. The isocratic elution was acted in Agilent, Zorbax SB-C18, 4.6 × 150 mm section kept up with at 30°C with portable stage containing acetonitrile and ammonium acetic acid derivation in a proportion of 15:85 v/v, individually. The direct relationship was found inside the fixation scope of 0.25 to 8 ng/ml, with a stream pace of 1 ml/min and finder frequency of 230 nm. The assessed approval boundaries were found inside the OK reach. Utilization of straightforward HPLC strategy with short maintenance time makes this technique a advantageous decision for examine of tizanidine in human plasma.

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

INTRODUCTION

Tizanidine is an imidazole derivate with structural just like clonidine and has high valuable analgesic motion used as a muscle spasm and continual spasticity. It is presynaptic inhibition reduces the affinity for alpha 2 – agonist receptor. Its

apprehensive flex, as a result additionally performing as an analgesic, so its imidazole motion lets in tizanidine to lowers spasticity and motion resistance [1]. Data suggest that tizanidine has several pharmacologic residence which includes discount in the launch of excitatory amino acids and substance P in polysynaptic reduction the spinal pathways [2]. Modulation of the hyperexcitability of the

group 2 group 1 premotor neuronal lumbar pathway with the aid of tizanidine would provide in addition proof that this system perform position within the pathophysiology of spasticity [3]. The oral bioavailability of tizanidine is ready 21% specificity due to massive first – by pass metabolism and its suggest elimination half existence is about three hr [4].

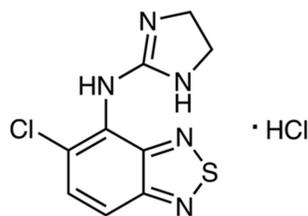


Figure 1: Structure of tizanidine hydrochloride

The post operative analgesic impact of tizanidine has been proven in a rat model, however to our information, the position of tizanidine in postoperative pain treatment has now not yet been investigated [5]. Its metabolism is particularly mediated by using cytochrome P450 1A2 – isoenzyme ciprofloxacin, a quinoline antibiotic has been shown to be clinically relevant inhibitor of CYP1A2. In an pharmacokinetic look at, ciprofloxacin extended the location under the plasma awareness curve of tizanidine by using a median of 10 fold (variety 6 fold to 24 fold) [6]. Tizanidine is chemically [5 chloro -4 (2 – imidazoline -2- yl amino) – 2,1,3 – benzothiadazole] and display basic and

lipophilic properties. The drug is ionised I acidic surroundings and soluble in water [7].

MATERIALS AND METHODS:

CHEMICALS AND REAGENTS:

Tizanidine hydrochloride is utilized to treat fits, squeezing and snugness of muscle brought about by clinical issue like numerous sclerosis, spastic diplegia, back torment or certain different wounds of the spine or focal sensory system. During the HPLC investigation for related substances of tizanidine hydrochloride 1 groups, integrated according to the course cycle impurity 2 was identified. The substance of this contamination was observed to be in the request for 0.05% to 0.015% w/w. The

disconnection and portrayal of this obscure cycle pollution 2 was accordingly, essential not exclusively to meet the tough administrative prerequisite, yet in addition to an understanding into the conceivable course of arrangement of the contamination. Test of tizanidine hydrochloride 1 was integrated and portrayed in Mylan India pvt. Ltd. earlier Merck advancement focus pvt. LTD, India. Sodium dihydrogen phosphate, methanol HPLC grade and phosphoric corrosive were acquired from Merck India Ltd., Mumbai, India. The chromatographic sanitization was performed on a NOVA prep 200 Merck Hitachi preparation HPLC framework comprising of L – 7400 UV identifier and HSM programming and a worked in autosample for part assortment . The immaculateness of division was kept on eye on Merck Hitachi framework comprising of L- 7100 siphon, L-7300 segment broiler, L- 7200 autosample, L- 7420 locator and HSM information securing programming [8].

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY RP-HPLC:

Switch stage chromatography utilizes hydrophobic reinforced pressing, generally with an octadecyl or octyl practical gathering and a polar fluid portable stage. Polar substances incline toward the portable stage and eluted first. Since the

hydrophobic person of the solutes will increment, and followed maintenance will likewise increment. For the most part, the lower the extremity of the portable segment, the upper is its eluent strength. The elution request of the classes of mixtures in table is turned around (consequently the name switch stage chromatography) [9].

HUMAN PLASMA:

Entire bloods was gathered into sodium heparin tube (9ml) for all investigations and furthermore in EDTA (6ml) tubes for the anticoagulant correlation study. Upon assortment, the examples were centrifuged 1500xg, 4c, 10 min after assortment supernants were frozen at -20 c or -80 c. Plasma medication, which is set to alter the treatment of skin illness like melanoma or even dental contamination. The guidelines of plasma medication depends on the immediate or circuitness utilization of an electrical release to an organic objective. The electrical release applying high voltage to a functioning with the assistance of working gases like helium, argon, nitrogen, oxygen, air or their admixture. At the point when a atmospheric pressure plasma source is worked in an encompassing atmosphere, it produces receptive oxygen species (ROS) and responsive nitrogen species (RNS) which are aggregately known as RONS. These RONS are the key species which have made plasma sources attainable for

use with different biomedical use, a particular objective might require an on site or off site therapy. With on location treatment, the dynamic release or plasma crest at encompassing temperature comes into direct contact with the natural objective. This might be useful for the treatment of seriously influenced wounds and for condition in which the plasma source is promptly accessible. During an off site treatment, natural arrangement is uncovered straightforwardly or by implication to the plasma hotspot for a forordained time frame [10].

METHOD VALIDATION:

After the advancement of the technique, approval of the created strategy for the assessment of TZN was proceeded according to the ICH rules [11, 12]. The advanced HPLC technique should be approved by various boundaries to ensure that the general exhibition quality on the methodology satisfies the requests only for the arranged reason [13].

The accompanying boundaries had been done with the technique approval [14, 15].

SYSTEM SUITABILITY:

Technique reasonableness is inspected to assess the advanced chromatographic conditions that are intended for examination. A piece of 10 $\mu\text{g mL}^{-1}$ of TZN standard arrangement was added to a spotless and dry volumetric flagon of 10 mL and it was broken up with the assist

portable with staging sufficient to secure a grouping of 10 $\mu\text{g mL}^{-1}$.

SPECIFICITY:

Explicitness will be the strategy capacity to remain unaffected inside the presence of some other part which may probably be existing, these involve contaminations, corrupted diluents, and items. Particularity was finished to ensure that the parts which are existing don't affect the example being dissected. 20 μl of standard arrangement and diluents had been acquainted straightforwardly with the HPLC framework and furthermore the not set in stone.

LINEARITY:

The strategy linearity is the capacity of it's to offer test outcomes that are explicitly corresponding to the analyte focus inside the example. Linearity examination was completed from 2-10 $\mu\text{g mL}^{-1}$ for TZN.

ACCURACY:

Exactness of the technique or still up in the air when the closeness of the test outcome got by that strategy coordinate against the genuine worth. Exactness was achieved by the arrival of known centralizations of standard medications. The strategy's precision was evaluated by estimating the information of recuperation of the recognized amount of analytes added.

PRECISION:

The accuracy of the insightful technique is the degree of arrangement between the

particular assessment results when the interaction is consistently applied to a few examples supported by the homogeneous test, accuracy of the examination strategies ordinarily communicates as relative standard deviation.

LIMIT OF DETECTION:

The restriction of identification of the technique is the base amount of medication parts that can without much of a stretch be assessed, in addition not by and large evaluated being an exact worth under the characterized trial factors.

LIMIT OF QUANTIFICATION:

The measurement furthest reaches of the strategy will be the most un-level of analyte which might be evaluated definitively and precisely.

ROBUSTNESS:

The strength of the scientific strategy is a level of the capacity of its to remain unaltered by some little variations for example stream rate adjustments, some modification in the proportion of the portable stage, and changes in the frequency.

RESULT AND DISCUSSION:

In the current technique, tizanidine was extricated from plasma utilizing fluid extraction which is widely for assurance of low medication fixations in natural liquids [16, 17] created and approved the assessment of tramadol in human plasma utilizing HPLC followed by fluid extraction

[18] measured tizanidine in human plasma after fluid extraction with fluid chromatography pair mass spectrometry in the scope of 50 to 5000 pg/ml [19] approved basic HPLC technique for concurrent assurance of paracetamol, tizanidine and diclofenac in natural liquids and discovered a linearity for tizanidine in the fixation scope of 120 to 10,000 ng/ml.

SELECTIVITY:

Six clear plasma tests were run and no top at the maintenance season of medication was identified in plasma. The medication test was run in same condition and no impedance was found. No meddling plasma top was seen at the medication maintenance time demonstrated a decent selectivity of the technique. Selectivity of the technique was displayed in

LINEARITY AND CALIBRATION CURVE:

Assessments of tests with fixation 0.25, 0.5, 1, 2, 4 and 8 ng/ml were acted in three-fold. The norm alignment bend was direct with a mean r^2 of 0.9989 with percent exactness between 90 to 104.6%. The linearity chromatogram and linearity bend of tizanidine are shown

ACCURACY AND PRECISION:

The HPLC technique was likewise approved for intraday and interday exactness and accuracy. The intraday exactness was found in a scope of 90 to 96% while interday exactness with worth of

84 to 92% was seen as displayed in **Table 1**. Every one of the qualities conformed to standard adequate scope of $\pm 15\%$ for bioanalytical technique.

LOWER LIMIT OF QUANTIFICATION AND LIMIT OF DETECTION:

The five examples of every fixation (0.05, 0.1, 0.15 furthermore, 0.25) were examined for LLOQ and LOD assurance. The focus 0.05 ng/ml was not discernible while the lower limit of detection was found as 0.1 ng/ml, and lower limit of quantification (LLOQ) esteem was 0.25 approved with precision of 94.4% that is inside the predefined furthest reaches of 20% and introduced in **Table 2**.

ANALYTICAL RECOVERY:

Recuperation of the strategy was performed for low, medium and high fixation inside the adjustment bend range. The strategy was found with acceptable recuperation with the mean scientific recuperation of 97.135% for three chose groupings of 2, 4 and 6 ng/ml as displayed in **Table 3**.

PLASMA STABILITY OF DRUG:

Freeze and Defrost dependability of the medication in plasma were assessed for three freeze-defrost cycles and assessed exactness were observed to be 99.4, 97.8 and 98.2% for low focus (1 ng/ml) and 98.66, 98.37 and 98.31% for high chose fixation (7 ng/ml) for freeze defrost cycle 1, 2 and 3, separately. The normal corruption of medication in three FT cycles was observed to be 2.268 and 1.040% for centralization of 1 and 7ng/ml, individually (**Figure 3**).

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 4 and 5**.

Table 1: Accuracy and precision of tizanidine in plasma

Parameter	Selected concentration in method validation ng/ml			
	0.25			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
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

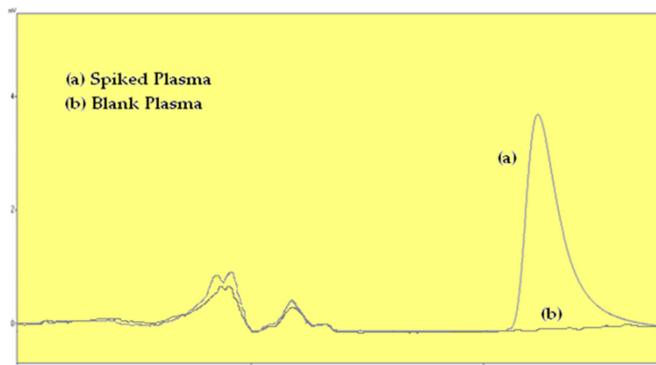


Figure 2: chromatogram showing selectivity of the method

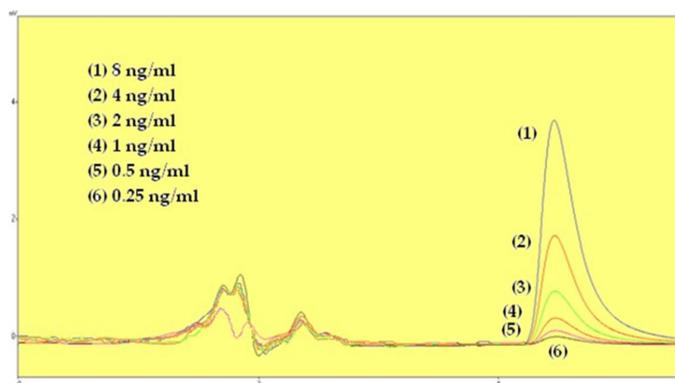


Figure 3: linearity of chromatogram of tizanidine in plasma

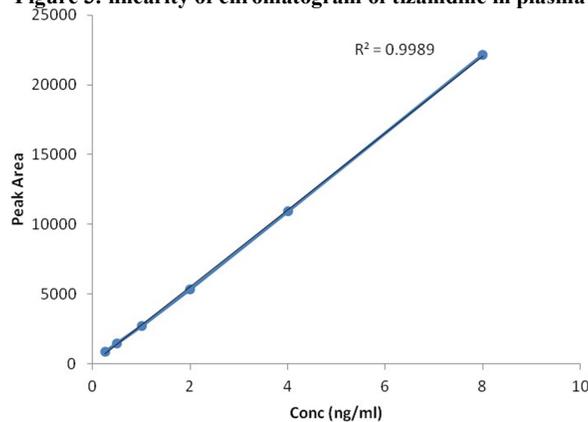


Figure 4: linearity of different co centration of tizanidine

Table 2: Limit of detection of tizanidine in plasma

Con(ng/ml)	Sample1	Sample2	Sample3	Sample4	Sample5	Mean	Standard deviation	%coffection variation	%accuracy
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 3: 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
				97.135

Table 4: Freeze and thaw stability of tizanidine

parameter	Low concentration (1ng/ml)				High concentration(7ng/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

Table 5: Long term stability of tizanidine in plasma

Parameter	Low concentration(ng/ml)			High concentration(ng/ml)		
	Fresh sample	After2weeks	After3weeks	Fresh sample	After 2weeks	After3weeks
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 strategy has been approved effectively for the assurance of tizanidine in human plasma test. Approval boundaries like selectivity, linearity, exactness, accuracy and soundness showed great outcomes and consented to standard satisfactory reach. Henceforth, this fluid extraction based HPLC technique can be utilized effectively for the assessment of tizanidine in human plasma.

ACKNOWLEDGEMENT

The authors are immensely thankful to the organization Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing helpful support for the study.

REFERENCE

[1] Padulles A and Lozanso T, Management of tizanidine

withdrawal syndrome: A case Report, SAGE journals, 2018.

[2] Semenchuk MR and Sherman S, Effectiveness of tizanidine in neuropathic pain: an open label study, J pain, 2000; 1(4): 285-92.

[3] Maupas E and Roques CF, Modulation of the transmission in the group 2 heteronymous pathways by tizanidine in spastic hemiplegic patients, J Neural Neurosurg Psychiatry, 2004; 75(1): 130-135.

[4] Granfors M.T and Backman J.T, Tizanidine is mainly metabolized by cytochrome P450 1A2 in vitro, British journal of clinical pharmacology, 2004; 57(3): 349-353.

[5] Taylan and Akkaya, Tizanidine for the management of acute postoperative pain after inguinal

- hernia repair A placebo controlled double – blind trial , European journal of Anaesthesiology, 2016; 33(3) : 215-222.
- [6] Tomka I.T and Kullak – ublick G.A, Analysis of drug-drug interaction in Swiss claims data using Tizanidine and ciprofloxacin as a prototypical contraindicated combination, The Annals of pharmacotherapy, 2018; 52(10); 983-991.
- [7] Yusuf R.I and Muhamad I.N, Development and validation of a reverse phase high performance liquid chromatography (HPLC) method for determination of tizanidine in human plasma , African journal of pharmacy and pharmacology, 2014; 8(7): 199-205.
- [8] Gore V.G and Kumar M, Isolation and characterization of a process in tizanidine hydrochloride, Indian journal of pharmaceutical sciences, 2010; 72(3): 360- 362.
- [9] Sanjay KD, Kumar HD. Importance of RP-HPLC in analytical method development: A review. Int. J. Pharm. Sci. Res., 3, 4626–33 (2012).
- [10] Kaushik N.K and Veerana M, Biological and medical application of plasma – activated media, water and solution, Biological chemistry, 2018; 400(1): 39-62.
- [11] Beckett, A.H., Stenlake, J.B., Practical Pharmaceutical Chemistry. 4th ed. CBS publishers and distributors. New Delhi 1997, pp. 284- 297.
- [12] Willard, H.M., Merritt, L.L., Dean, J.A., Settle, F.A., Instrumental method of Analysis. 7th ed. CBS publication and distributors. New Delhi, 1986, pp. 513-514.24. Gurudeep, C., Sham, K.A., Instrumental Methods of Analysis. 5th ed. Himalaya publishing home. Mumbai 2005, pp. 108-109.
- [13] Gurudeep, C., Sham, K.A., Instrumental Methods of Analysis. 5th ed. Himalaya publishing home. Mumbai 2005, pp. 108-109.
- [14] Beckett, A.H., Stenlake, J.B., Practical Pharmaceutical Chemistry. 4th ed. CBS publishers and distributors. New Delhi. 1997, pp. 163- 165.
- [15] International Conference of Harmonization ICH Q2 (R1). Validation of analytical procedures 2005: Text and methodology. Available from <https://www.ich.org/fileadmin/public%20website/ICH%20products/%20Guidelines%20Quality/%20Q>

- 2%20R1%20Guidline.pdf
[Accessed 21 July 2020].
- [16] 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.
- [17] 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.
- [18] 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.
- [19] 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.