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Development of RP-HPLC Method of Tizanidine HCL with Some Validation Parameter

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Abstract This study compiles the information for the development of analytical methods for estimation of the Tizanidine HCl that will be helpful for further research work on this drug and its impurity. The present Literature survey provides information about the Analytical methods like UV,TLC,RP-HPLC,HPTLC,UHPLC and other methods have been reported for Tizanidine HCl drug individually and along with other drugs. The analysis of published data revealed that, there was only UV spectroscopic method (calibration curve metod) is reported for estimation of Tizanidine HCl fixed dose combination. Estimation of Tizanidine HCl by superlative RP-HPLC method i.e. Mobile phase- Acetonitrile: phosphate buffer (pH: 7.5) (50:50%v/v), Column C18 (250mm*4mm*5µm), Flow rate- 1.0 ml/min, Wavelength: 318 nm. Optimized HPLC condition was validated by assessing validation parameters and it meet the acceptance criteria set by ICH. It was showed method was linear and precise. The validated RP-HPLC-PDA method can be used for routine analysis of Tizanidine HCl in tablet.

Keywords Tizanidine HCl, RP-HPLC, Method development and validation, ICH Q2 (R1) guideline

1. Introduction

A serious disease which is an immune-mediated inflammatory disease that attacks myelinated axons in the CNS. You work on your muscle groups in a certain order. When your body is physically relaxed, you cannot feel anxious. Ventilatory Failure: When administered in large doses, muscle relaxants paralyze the respiratory muscles and ventilation must be assisted. Residual Block: Residual block is common after the use of nondepolarizing relaxants.

Dantrolene: Dantrolene (Dantrium) is used to treat muscle spasms caused by spinal cord injury, stroke, cerebral palsy, or MS. It works by acting directly on the skeletal muscle to relax the muscle spasm. Side effects can include drowsiness, dizziness, lightheadedness, and fatigue. [1-8]

Zanaflex is a central alpha-2-adrenergic agonist indicated for the management of spasticity. Because of the short duration of therapeutic effect, treatment with

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Zanaflex should be reserved for those daily activities and times when relief of spasticity is most important Which is Mfg and marketed by Zydus Cadila.

Today, HPLC continues to evolve rapidly toward higher speed, efficiency, and sensitivity, driven by the emerging needs of life sciences and pharmaceutical applications. Figure 1.2. depicts the classical technique of Liquid Chromatography with a glass column that is packed with coarse adsorbents and gravity fed with solvents. Fractions of the eluent containing separated components are collected manually. This is contrasted with the latest computer-controlled HPLC, depicted in Figure 1.2., operated at high pressure and capable of very high efficiency. [9-10]

Fig. 1.1

(a) The traditional technique of low-pressure liquid chromatography using a glass column and gravity-fed solvent with manual fraction collection.

(b) A modern automated HPLC instrument capable of very high efficiency and pressure.

High-performance liquid chromatography (HPLC), sometimes called highpressure liquid chromatography, is a separation technique based on a solid stationary phase and a liquid mobile phase. Fig.1.3 It describes the work out flow about High performance liquid chromatography (HPLC).

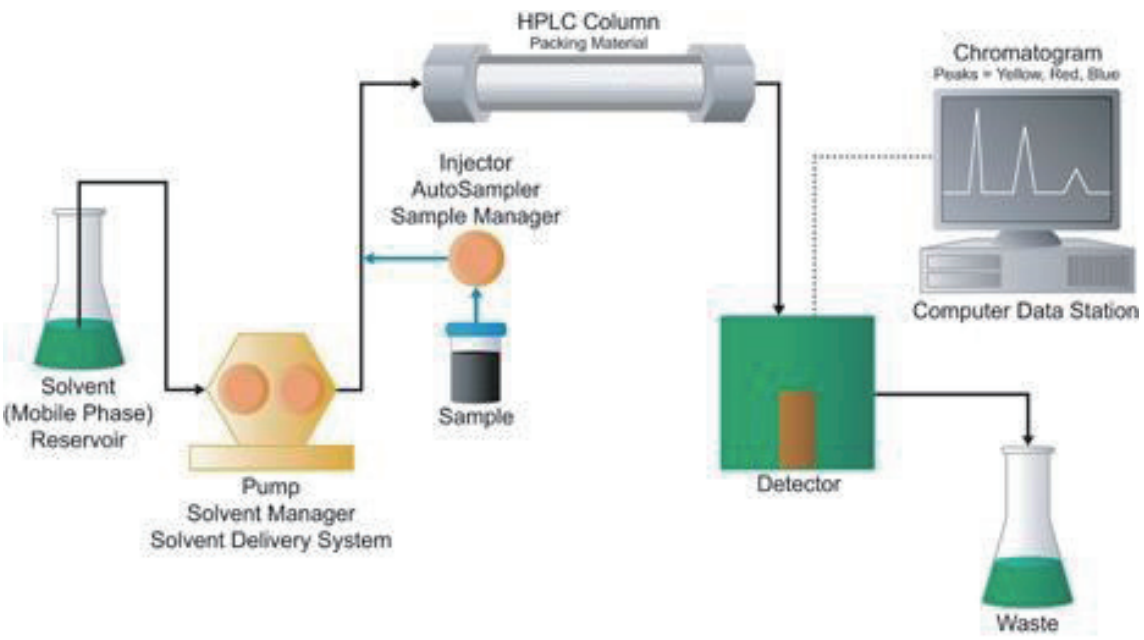


Fig.1.2 Block diagram of HPLC

Principle of separation

"The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated." An in-line detector monitors the concentration of each separated component band in the effluent and generates a trace called the "Chromatogram," shown in Figure1.4 [11-12]

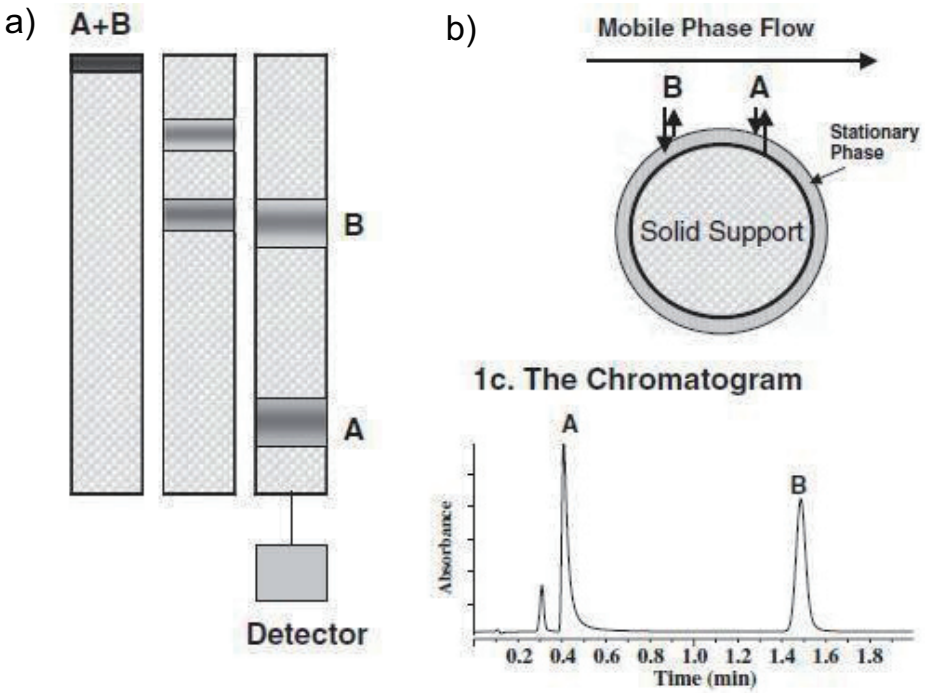


Fig.1.3.1 Schematic of the chromatographic process showing the migration of two bands of components down a column. **Fig.1.3.2** A chromatogram plotting the signal from a UV detector displays the elution of components A and B.

i.Normal phase:

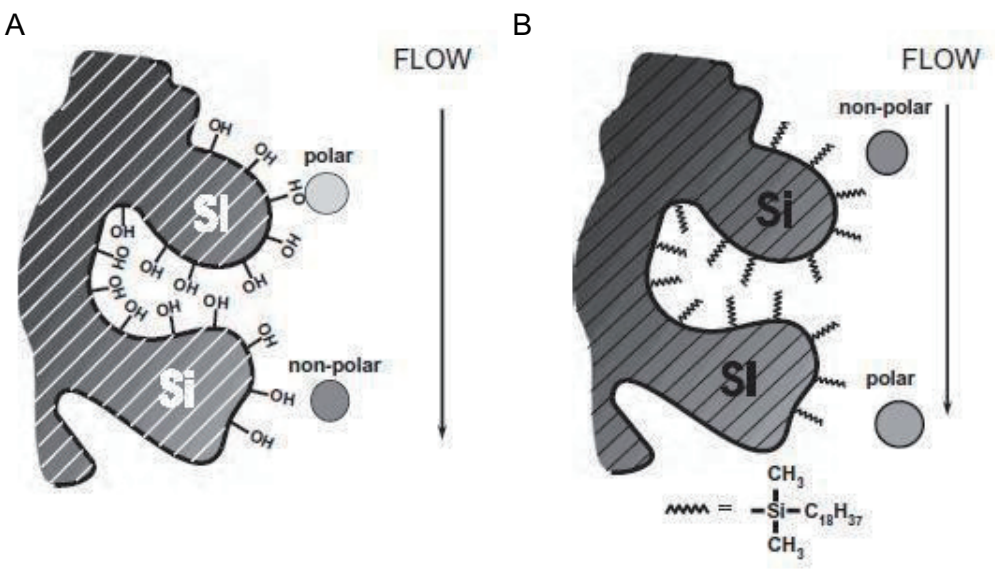
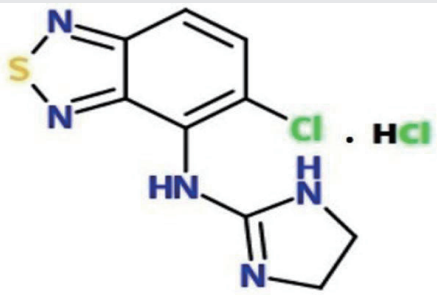


Fig.1.4.1 Schematic diagram depicting separation modes of Normal-phase chromatography (NPC) and **Fig.1.4.2** Reversed-phase chromatography (RPC).

2. Drug Profile:-tizanidine Hcl[6-8]

2.1 Table for Drug Profile of TIZANIDINE HCl

Name	Tizanidine HCl
Official in	Pharmacopoeia 2014
Description	Tizanidine is central α2 adrenergic receptor which is used for relieve the spasms and increased muscle tone caused by multiple sclerosis.
Structure	
Chemical formula	$C_9H_9Cl_2N_5S$
Mol.Weight	290.17 gm/mol
IUPAC name	5-chloro-n-(4,5 dihydro-1H-imidazole-2-Yl) 2,1,3,benzothiadiazol-4-amine HCL
Categories	Central α2 adrenergic receptor
Solubility	Tizanidine is lipid-soluble drug , only soluble in water that conc. Is greater than 20mg/ml and methanol
Pharmacology	Tizanide]ine HCL is a central α2 adrenergic receptor agonist and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. And indicate for the relief of the muscle spasticity it is a short duration of action.

2.2 Review of literature

Tizanidine HCl

Table 2.2 Official and Reported method for Tizanidine HCl [13-17]

Sr.no.	Official in	Method	Description	Ref. no.
1	Tizanidine HCl	"RP-HPLC"	**Column** Inertsil ODS 3V (250x4.6mm)um **Mobile phase:** 50 volume of triethylamine buffer Ph 3.0 and same volume of acetonitrile created. Mobile section sonicated 10 min to get rid of gases and filtered through 0.45um membrane for degassing from the mobile section **Flow rate**: 0.8ml/min **Detection**: 230 nm	4

2	I.P-2014	"RP-HPLC"	**Column** Inertsil C18 (25cmx4.6mm) **Mobile phase:** A mixture of 50volumes of a buffer solution prepared by dissolving 6.8 gm of monobasic potassium phosphate in 1000 ml of water adjusting the pH to7.5 with 5.3 M potassium hydroxide, and 50 volumes of acetonitrile, **Flow rate:** 1ml/min **Detection:** 230 nm	3
3	U.S.P-2014	RP-HPLC"	**Column** Inertsil C18 (25cmx4.6mm) **Mobile phase:** Prepare a filtered and degassed mixture of buffer solution and acetonitrile(80:20), make adjustment if necessary . **Flow rate**: 1ml/min **Detection**: 230nm	15
4	Tizanidine HCl & Diclofenac Sodium	"RP-HPLC"	**Column** 250mm hypersil C18 HPLC column **Mobile phase:** ACN: Phosphate buffer(50:50 v/v) **Flow rate**: 1ml/min **Detection**:220nm	16
5	Tizanidine HCl & Valdecoxib	"RP-HPLC"	**Column** HPLC Column 250x4.6mm C18 ,5u column Mobile phase: ACN:WATER (60:40) **Flow rate: 1ml/min** **Detection**:200-400nm	10
6	Tizanidine HCl & Meloxiczm	"RP-HPLC"	**Column** Hypersil ODS column (150x4.6 mm) 5u **Mobile phase:** Methanol: water (65:35) v/v **Flow rate**: 1ml/min **Detection:**230 nm	11
7	Tizanidine HCl & Aceclofenac	"RP-HPLC"	**Column** RP-intersile colunm250mmx4.6cm,50um Mobile phase: Phosphate buffer :ACN :Methanol (40:30:30v/v) **Flow rate:** 1.3ml/min **Detection:**260 nm	13

3. METHODS

3.1. SELECTION OF MOBILE PHASE

3.1.1 Preparation of Mobile phase

A mixture of 50 volumes of a buffer solution prepared by dissolving 6.8 gm of monobasic potassium phosphate in1000 of water adjusting the pH to 7.5 with 5.3 potassium hydroxide, and 50 volumes of acetonitrile.

3.1.2 Table for Selection of Mobile phase

Sr.no	Mobile phase	Flow rate(ml/min)	Ratio	Retention time	Remarks
1	Water: acetonitrile	1	30:70	6.090	Retention time reduced
2	Acetonitrile: phosphate buffer	1	50:50	2.067	Change in peak ,pick is sharp
3	Acetonitrile: phosphate buffer	1	50:50	2.090	Retention time almost same

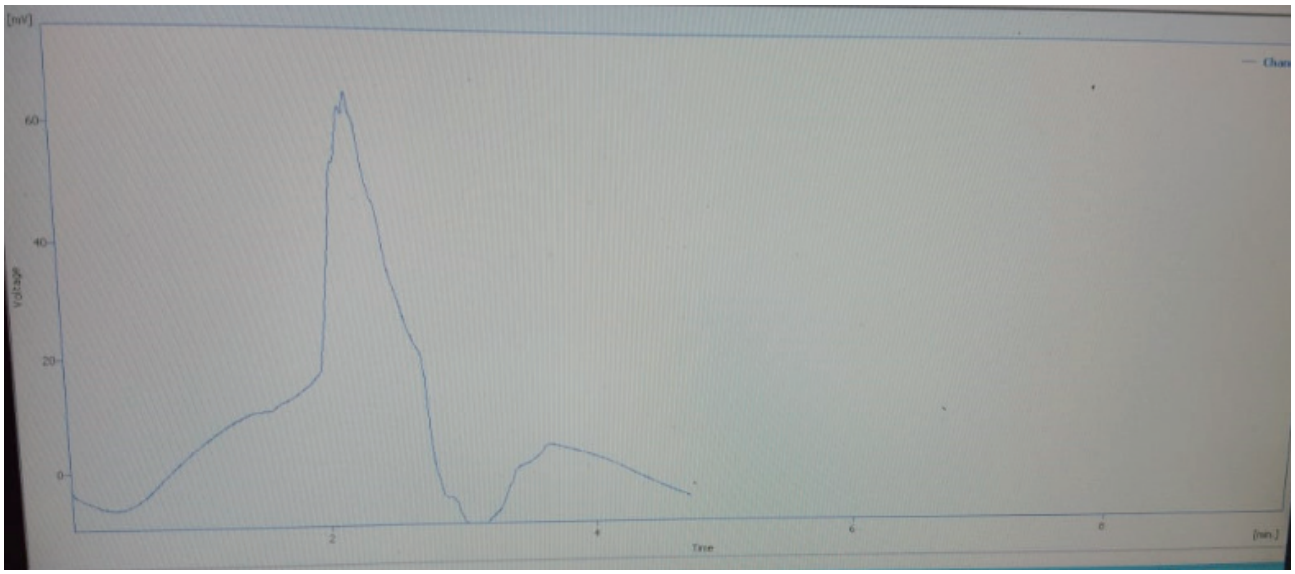


Fig.3.1

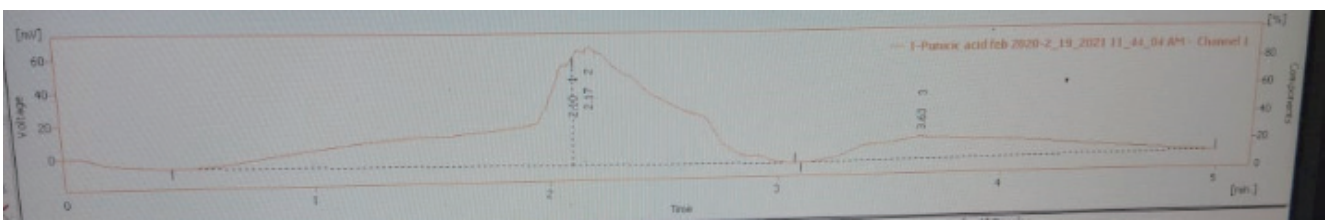


Fig.3.2

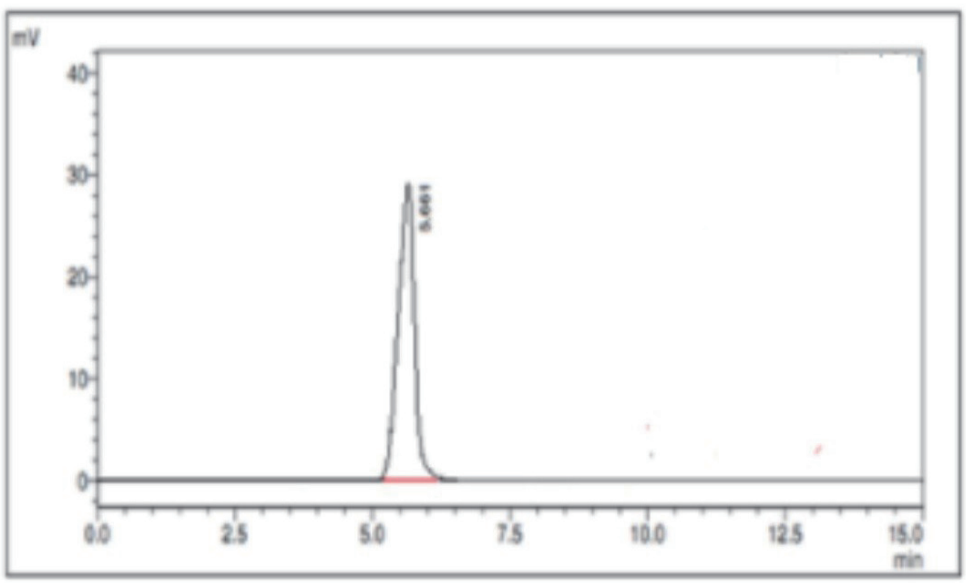


Fig.3.3

Mobile phase: Acetonitrile: phosphate buffer (50:50% v/v)

4. RESULT

Performed validated Parameter of this Drug

4.1. Linearity

Preparation of solution: A fine powder of Tizanidine HCl weight and transferred in the 100 ml volumetric flask. this flask was allow to dissolve intermittent sonication, mix. Was dilute with 100 ml of mobile phase thoroughly mixed and filtered with 0.45 µ nylon filter.

Tizanidine at 5 different concentrations ranging from 50% to 150% of nominal concentration such as 10 to 30ppm. Each of these dilution of different concentration was injected in duplicate into the column and the corresponding chromatograms were obtained.

Table 4.1.1 Data for linearity of Tizanidine HCl

Level	TZN.conc. in µg/ml	TZN avg. area
1	10	3906
2	15	5439
3	20	7607
4	25	8315
5	30	9860
6	35	11210

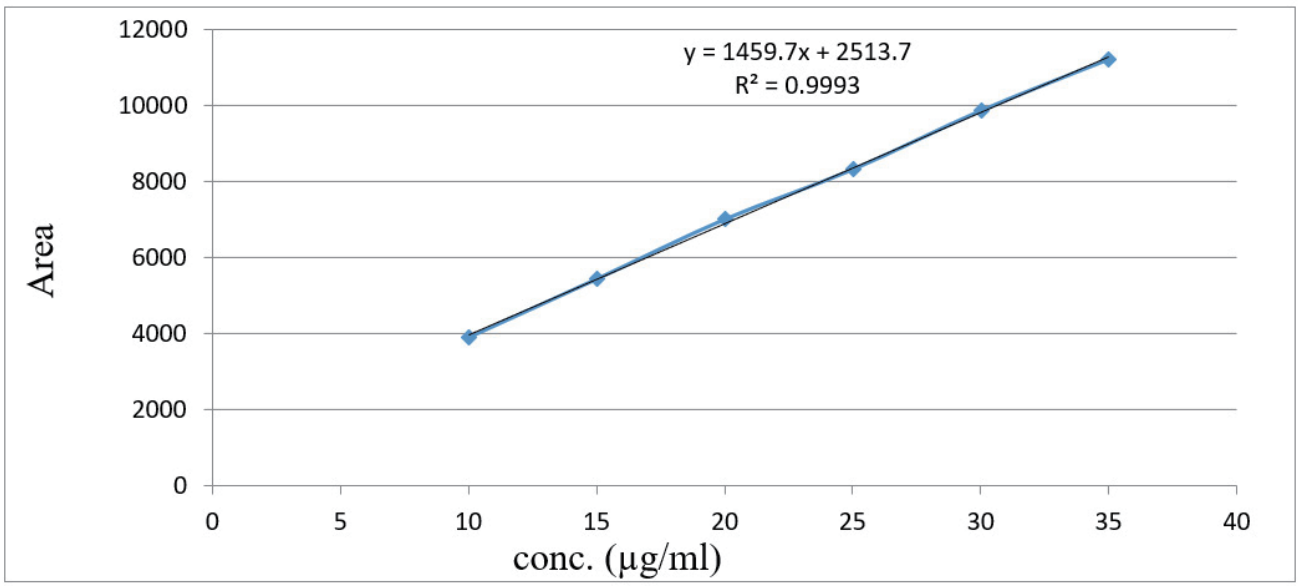


Fig. 4.1.1 Calibration curve of Tizanidine HCl

4.2. LOD and LOQ

"The LOD was estimated from the set of 3 calibration curves used to determination linearity. The LOD may be calculated as,

"Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves."

LOD = 3.3 × (SD/Slope)"

SD=7.420

3.3x (7.420/0.9993)

=24.50

"The LOQ was estimated from the set of 3 calibration curves used to determine linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)"

Where,

"SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves."

10x (7.42/0.993)

=74.72

Table 4.2.1 Data for LOD & LOQ of Tizanidine HCl

Drug	Lod	Loq
Tizanidine Hcl	24.50	74.72

4.3. Precision

Tizanidine at six different concentrations ranging from 50% to 150% of nominal concentration such as 10 to 30ppm.

4.3.1 Repeatability data for Tizanidine HCl

Conc. µg/ml	Area
10	7464.2565
10	7464.9865
10	7466.2597
10	7465.4591
10	7465.3267
10	7464.1294
Mean	7465.06965
SD	0.728716
%RSD	0.009

4.3.2 Intraday data for Tizanidine HCl

Conc. µg/ml	Area Mean ±SD (n=3)	%RSD
10	3902 ± 5	0.0188
20	7603 ± 6	0.0097
30	11204 ± 4	0.0065

4.3.3 Interday data for Tizanidine HCl

Conc. µg/ml	Area Mean ±SD (n=3)	%RSD
10	3901 ± 5	0.0187
20	7602 ± 3	0.0095
30	11204 ± 5	0.0065

4.4 Summary of validation parameter

Sr.no	Parameter	**Tizanidine HCl**
1	Linearity Range (µg/ml)	10-35 µg/ml
2	Regression Equation (y = mx + c)	y = 1459.7 + 2513.7
3	Slop(m)	1459.7
4	Intercept (c)	2513.7
5	Correlation coefficient (r)	0.9993
6	LOD(µg/ml)	24.50
7	LOQ(µg/ml)	74.72
8	Precision 1. Repeatability 2. Intraday 3. Interday	0.72 0.0188 - 0.0065 0.0187 - 0.0065

5. CONCLUSION

RP-HPLC method was developed and validate with some Parameter This method is found to be simple and precise. All the results were found satisfactory and the method can be apply for estimation of TIZANIDINE HCl in API.

Conflict of interest

There are no conflicting interests, as the authors have stated.

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