RESEARCH ARTICLE

RP-HPLC Method Development and Validation of Genotoxic Impurity 1-Acetyl-2-imidazolidinone content in Tizanidine Hydrochloride

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ABSTRACT

Tizanidine hydrochloride is widely used as a muscle relaxant as 2-6 mg tablet formulation which content 1-Acetyl-2-imidazolidinone as a genotoxic impurity. In this study, an RP-HPLC method was successfully developed and validated in accordance with regulatory requirements for determining 1-Acetyl-2-imidazolidinone in Tizanidine hydrochloride formulation. The sample preparation method is easy and free of toxic solvents. 215 nm observed as λ_{max}in scanning. The developed method is strictly linear in the range of 1.04-0.16µg/ml. The method was highly précised and robust. The proposed RP-HPLC method is suitable for both the qualitative and quantitative analysis of 1-Acetyl-2-imidazolidinone in Tizanidine hydrochloride formulations.

Keywords: RP-HPLC, Tizanidine Hydrochloride, Genotoxic impurity

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INTRODUCTION

Tizanidine hydrochloride is an α2 adrenalgic agonist having IUPAC name 5-Chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine hydrochloride and chemical formula $C_9H_8ClN_5S$. It is physically solid white-yellowish powder with drug content 99.0% - 101.0%.[1-12] The drug has low solubility in acetic acid and is freely soluble in water and ethanol due to salt form.[13-18] It widely used as muscle relaxant and control spasticity. It is mainly used in the treatment of pathological conditions like back pain, spastic diplegia, multiple sclerosis, and several other diseases which are associated with nervous system that responsible for muscular cramping, spasm and tightness. NSAIDs and many other formulation and synthetic molecules have been screened for muscle relaxant.[19-23] Tizandine in lower dose give synergetic effect with anti-nociceptive and anti-inflammatory effect of ketorolac and naproxane while reducing side effect.[24-25] 1-Acetyl-2-imidazolidinone is impurity found in Tizanidine hydrochloride which has genotoxic properties. At present, no chromatographical method is available for quantification as well as qualitative analysis for 1-Acetyl-2-imidazolidinone present in Tizanidine hydrochloride dosage form. In present study, RP-HPLC method developed and validated for 1-Acetyl-2-imidazolidinone content in Tizanidine Hydrochloride.

MATERIALS & METHOD

Chemicals and equipment's

All chemicals were procured from Sigma-Aldrich, India, and used without purification. HPLC analysis was performed on a Waters HPLC System 2695(Made in the USA), equipped with an autosampler 100-µl loop, Quaternary pump, thermostated column compartment, and photodiode array detector 2996. Separation was achieved on an Inertsil ODS-3V (250 mm × 4.6 mm i.d., 5 µm) make: GL Science and particle size in Armstrong unit was used for the analysis. A Shimadzu analytical balance (Model No. AP225WD, Switzerland) and an Aczet sonicator (India) were also utilized for sample preparation.

Preparation of mobile phase

For mobile phase A (MPA), 1.36 g of potassium dihydrogen phosphate was dissolved in 1000 mL of water, sonicated until fully dissolved, and filtered through a 0.45 µm PVDF membrane. Mobile phase B (MPB) consisted of acetonitrile and water mixed in a 90:10 %v/v ratio.

Standard preparation

1-Acetyl-2-imidazolidinone standard (4.16µg/ml) was freshly prepared by diluting 10.4 mg of compound in 20 ml diluent (Water: Acetonitrile (95:5 v/v). The mixture was sonicated for 10 minutes. Then, 5.0 mL of this solution was diluted to 50 mL with diluent, and 1.0 mL of this diluted solution was further diluted to 25 mL with diluent. The final standard solution was transferred to an HPLC vial and injected into the HPLC system.

Sample preparation

About 250 mg of sample was diluted with 25 ml of diluent (Water: Acetonitrile (95:5 v/v))(10000 µg/ml).Sonicated the solution for 10 min until it dissolved completely. The sample was placed in an HPLC vial and directly injected into the HPLC system. Chromatographic conditions are shown in Table 1 and chromatogram of blank, reference standard and sample are shown in Fig. 1.

Run Timing

For Standard 10 minutes and blank and sample 40 minutes. (As the peak of interest is eluted at about 7 minutes and the gradient is same up to 10 minutes).

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Table 1: Chromatographic conditions

Column:	Inertsil ODS-3V (250 x 4.6) mm, 5µm or equivalent	
Mobile Phase A:	1.36 g KH_2PO_4 in 1000 ml of water.	
Mobile Phase B:	Acetonitrile: Water (90: 10)	
Detector:	UVdetector (LC UV 100)	
Flow Rate:	1.0 ml/min	
Column oven temp.:	35°C	
Volume of each injection:	20 µl	
Detection wavelength:	215 nm	
Mode:	Gradient	
Time (In Min.)	% A	% B
0.01	95	5
10	95	5
13	25	75
22	25	75
25	95	5
35	95	5

Table 2: Calibrationcurve of 1-Acetyl-2-imidazolidinone.

Conc. (µg/ml)	Area Mean± SD	%RSD
1.04	21705	0.36
1.66	33915	0.40
2.08	45862	0.38
2.49	53017	0.21
3.12	67178	0.35
4.16	93116	0.75

Table 3: Precision data of 1-Acetyl-2-imidazolidinone.

Trial	Peak Area		Intraday Precision
1	44776	Time	Area (Mean±SD)(n=1)
2	44978	0 hr	45791.67
3	44931	14 hr	45856.6
4	45055	24 hr	45891.6
5	44842		
6	44912		InteradayPrecision
Mean	44915.67		
SD	98.46962	-	44802.6
%RSD	0.21%		

Method validation

Linearity and range

Linearity was measured by the correlation coefficient n a linear regression analysis. Six different concentration levels, ranging from 1.04 to 4.16 µg/mL, were analyzed to assess the linearity of the response. The calibration curve was constructed by plotting peak area against concentration.

Precision

Repeatability

System precision analysis was done with a standard of 1-Acetyl-2-imidazolidinone prepared at its test concentration of 4.16µg/ml and chromatograph in six replicates and calculated peak area of all with % RSD. Method precision was analysed with six different preparations of the standard.

Intraday and Interday Precision

It was determined by analysing standard reference standard of 1-Acetyl-2-imidazolidinone prepared solutions (4.16µg/ml) at three different times and the same time.

Accuracy

The accuracy of the method is directly related to the percentage recovery. A pre-quantified sample solution of the synthetic mixture was aliquoted into eighteen 10 mL volumetric flasks. Standard solutions of 1-Acetyl-2-imidazolidinone were spiked into these samples at four different concentration levels (50%, 100%, 120%, and 200%).

Robustness

Tiny meaningful changes in instrumental parameters like rate of flow, composition of mobile phase and detection wavelength were used to analyse the robustness of this method by injecting of standard solution of (concentration) 1-Acetyl-2-imidazolidinone and evaluating its effect by peak area measuring and calculated % RSD.

Specificity

The method's specificity was determined by examining the resolution factors of the 1-Acetyl-2-imidazolidinone peak in relation to the nearest adjacent peak, as well as among all other peaks.

RESULTS AND DISCUSSION

Selection of wavelength maxima

The solution of 1-Acetyl-2-imidazolidinone was scanned finely at 200-400 nm, and it was revealed that the solution showed the highest absorbance at 215 nm. without any interference.

Linearity and Range

The method demonstrates good linearity and range with a linear regression fit of R^2 = 0.9996 and with regression equation, y = 22854x - 2954.2. (Table 1) It shows strict linearity within the concentration range of 1-5 µg/mL. The residuals plot exhibits a random distribution around zero. Data for Calibration curve of 1-Acetyl-2-imidazolidinone is shown in Table 2 and the curve is shown in Fig.2. Figure 3 represents the overlay chromatogram of linearity.

Precision

In the system precision analysis, the %RSD of the peak area for 1-Acetyl-2-imidazolidinone, based on six replicate injections, was found to be 0.21%. The data is presented in Table 3.

Accuracy

The accuracy of 1-Acetyl-2-imidazolidinone measurement was assessed by preparing six sample solutions containing of 1-Acetyl-2-imidazolidinone at the LOQ (50%) along with three sample solutions at different concentration levels (50, 100,120 and 200% of the specified limit). Each solution was measured three times.

The percent recoveries for all preparations ranged from 96.48% to108.92% (Table 4.)

Limit of Detection and limit of quantification

The LOD and LOQ limits were validated with signal-to-noise ratio of ≥3 and 10. The LOD was determined to be 2.7 ppm, with a signal-to-noise ratio ranging from 3 to 4. While a range of 10–12 of ratio was observed for LOQ of 8.2 ppm, of which the % RSD of the peak area was 0.23% for six replicate injections.

CONCLUSION

The percentage assays of conc. of 1-Acetyl-2-imidazolidinone was found in the acceptable range. The %

Table 4: Accuracy data of 1-Acetyl-2-imidazolidinone.

% Level of spike	Amount. of std. in sample. (µg/ml)	Amount of .std. added (µg/ml)	Total amount. of. drug (µg/ml)	Totalamount of std. found (µg). Mean ± SD (n=3)	% Recovery
0	4.16	-	4.16	4.53 ± 0.91	108.923
50	4.16	2.08	6.24	6.01 ± 0.31	96.488
100	4.16	4.16	8.32	8.48 ± 1.02	101.938
120	4.16	4.99	9.15	9.08 ± 0.83	98.202
200	4.16	8.32	12.48	12.91± 0.68	103.489

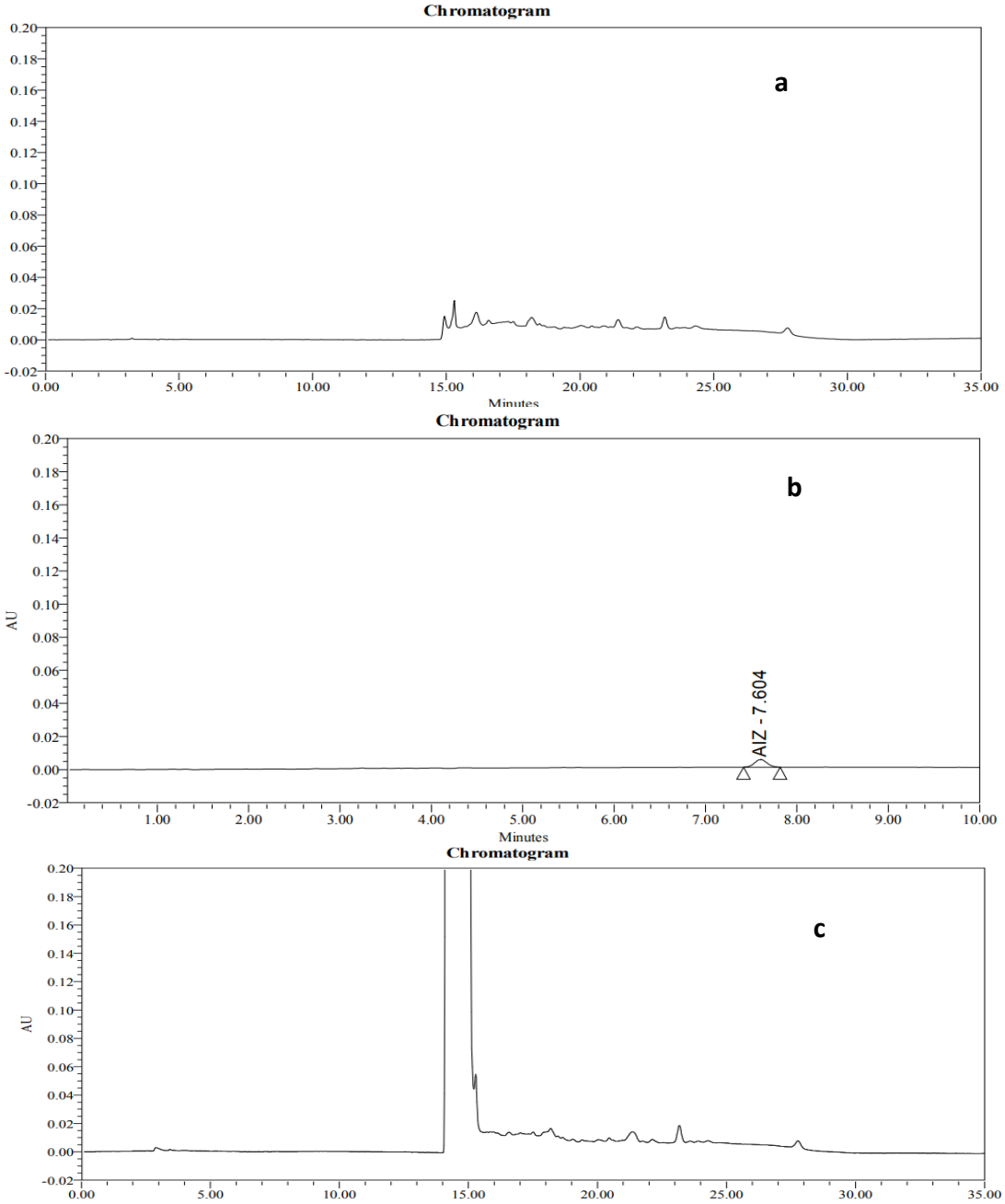

Figure1: Chromatograms of a;blank, b;reference standard and c;sample.

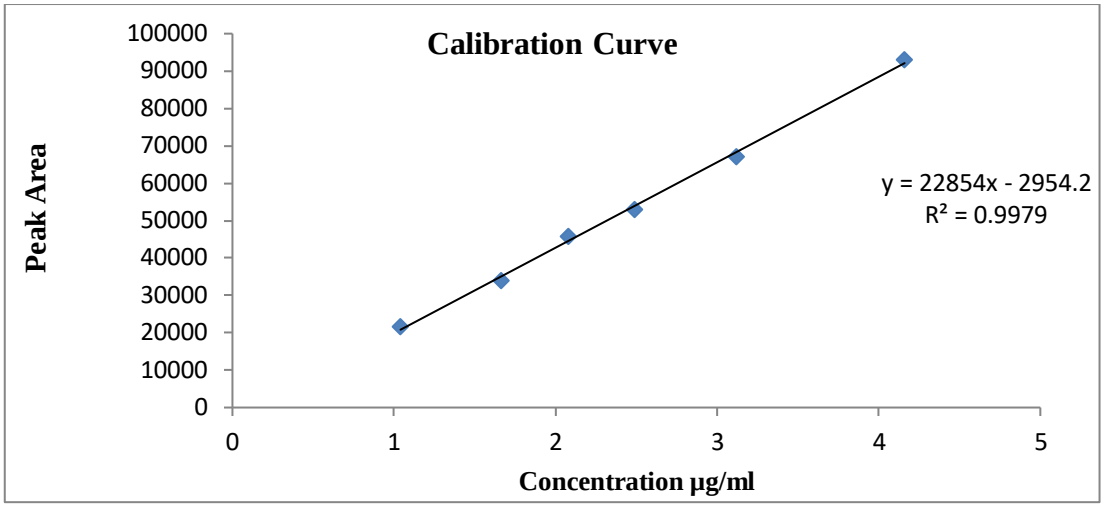

Figure 2: Calibration curve of 1-Acetyl-2-imidazolidinone.

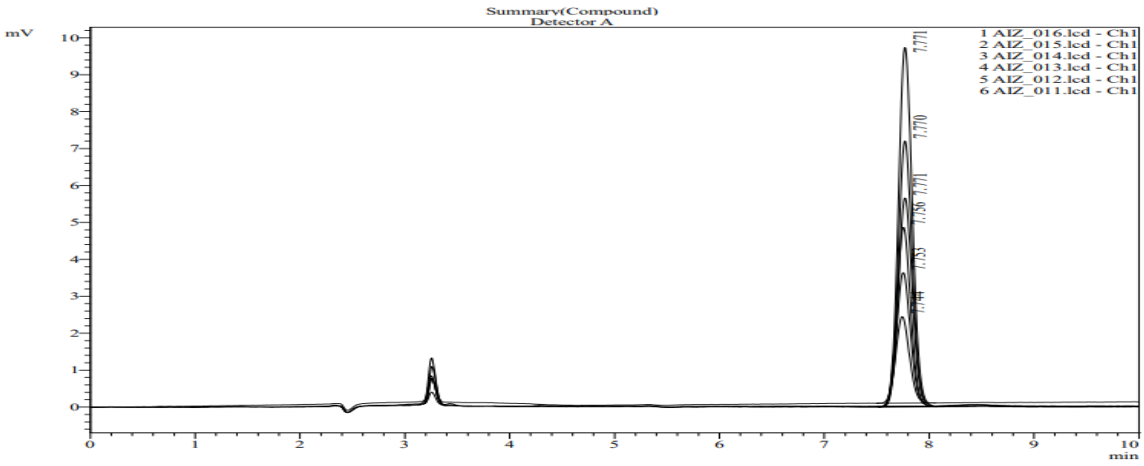

Figure 3: Overlay chromatogram of linearity

RSD is well within the prescribed limits as per ICH guidelines. The sample preparation is easy and used chemicals are non-toxic. A specific HPLC method using an ODS 3V column was adapted to achieve optimal chromatography for 1-Acetyl-2-imidazolidinone and Tizanidine Hydrochloride demonstrating for suitable specificity, linearity, accuracy and Robustness. The LOD of method was determined as 2.7 ppm based on the average signal-to-noise ratio. Hence this method can be used for 1-Acetyl-2-imidazolidinone analysis for drug substances. On basis of the above data it was can be that RP-HPLC method was successfully developed & validate for1-Acetyl-2-imidazolidinone content in Tizanidine Hydrochloride.

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