RESEARCH PAPER

QUANTIFICATION OF LOW LEVEL 2,3-DICHLORONITROBENZENE AS A GENOTOXIC IMPURITY IN ARIPIPRAZOLE BY HPLC.

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ABSTRACT:

The authors developed a simple liquid chromatographic method to determine 2,3-dichloro- nitrobenzene in the aripiprazole drug substance. The ICH recommendations were followed to validate the developed method for Specificity, LOD, LOQ, Method precision, and accuracy. The LOD and LOQ values were 66 ppm and 200 ppm, respectively.

KEYWORDS: Development, Validation, 2,3-Dichloronitrobenzene, Aripiprazole, Genotoxic impurity, and HPLC.

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INTRODUCTION

Dichloronitrobenzene is a light yellow to light brown crystalline powder. Molecular mass, density, boiling point, and empirical formula of the compound are 192 g/mol, 1.449 g/mL, 2700 °C, and C_6H_3ClNO2, respectively. It is combustible, and the structure of 2,3-Dichloronitrobenzene is shown in Figure 1.

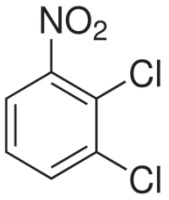

Figure 1

Various methods in the literature for determining Aripiprazole include HPLC [1-9], LC-MS/MS [10-11], GC-MS [12], spectrometry [13-14] and UHPLC-MS/MS [15-16]. However, no method is available for determining 2,3-Dichloronitrobenzene in Aripiprazole using high-performance liquid chromatography. In the present work, the authors have attempted to develop a method and its validation for determining the genotoxic impurity, 2,3-Dichloronitrobenzene, in the Aripiprazole bulk drug using HPLC. The synthesis of Aripiprazole involves 2,3-Dichloronitrobenzene as a raw material, which is a genotoxic compound [17-24]. Hence, an analytical method for 2,3-dichloronitrobenzene in Aripiprazole was developed and demonstrated stability-indicating behavior. The results are presented in this paper.

EXPERIMENTAL

Chemicals:

2,3-Dichloronitrobenzene was purchased from Tokyo Chemical Industry Limited; gradient-grade Acetonitrile (ACN) was purchased from Rankem. Aripiprazole samples are received from the local market. AR-grade potassium dihydrogen phosphate was purchased from Merck, Mumbai. High-purity water was prepared using a Millipore Milli-Q plus purification system.

Chromatographic conditions

Column: Inertsil ODS (250 mm x 4.6mm x 5 µ)
Flow: 1.5 mL/min
Pump mode: Isocratic
Injection volume: 20µL
Wavelength: 230 nm
Column oven: 30°C
Buffer: 0.05M KH_2PO_4
Mobile phase: ACN and buffer (60:40 v/v)
Diluent: Acetonitrile
Run time: 15 min.

Instrumentation:

Analysis was carried out on the LC system, a Waters Alliance e2695 separation module with a Waters 2489 UV detector (Waters Corporation, USA), using Empower-2 software.

Mobile phase preparation

Buffer:

6.80 g of KH_2PO_4 in 1 L of water; mix well until dissolved, then filter to degas.

Mobile phase:

ACN: Buffer (60:40 v/v)

Standard stock Preparation:

Weighed and transferred 10.0 mg of 2,3-Dichloronitrobenzene to a 100 mL volumetric flask and dissolved using Acetonitrile and sonicated for 5 minutes, diluted to 100mL with diluent. Taken 5mL from the above flask and diluted to 50mL using diluent

Standard Preparation:

Further, 5.0 mL of the above standard stock solution was transferred to a 50 mL volumetric flask and diluted with the diluent.

Test solution:

Weighed and transferred about 50.0 mg of Aripiprazole into a 50 mL volumetric flask, containing 25 mL of ACN, sonicated for 5 minutes to dissolve, made up with ACN, and filtered using a 0.45 µ filter. Filtered solution used for sample injection.

RESULTS AND DISCUSSION

Method development and optimization:

The authors aimed to develop an analytical method for determining 2,3-Dichloronitrobenzene in Aripiprazole at trace levels using HPLC. 2,3-Dichloronitrobenzene is a yellow solid at ambient temperature and is soluble in ethyl acetate, 1-propanol, and Acetonitrile (ACN). Initially, development starts with an isocratic program to elute 2,3-dichloronitrobenzene at 1.5 mL/min using an Inertsil ODS column (3 µ, 250 mm × 4.6 mm). ACN and buffer (60:40 % v/v) are used as the mobile phase. Buffer consisted of 0.05M potassium hydrogen phosphate (KH2PO4) in water (H2O) (6.8 g of KH2PO4 in 1L of H2O). The better peak shape and elution response for 2,3-Dichloronitrobenzene were obtained at an optimized injection volume of 20 µL, with a retention time of 7 minutes when the detector wavelength was set to 230 nm.

Method validation

The new method was validated for specificity, accuracy, sensitivity, precision, linearity, robustness, ruggedness, and solution stability using the ICH guidelines. Summary of the findings were provided in Table 1.

Table 1: Summarized results of method validation for 2,3-Dichloronitrobenzene

Validation parameter	Acceptance criteria	Our Result
Specificity	Method should be specific	Specific
Precision		
System precision	% RSD ≯ 15.0	0.24
Repeatability	% RSD ≯ 15.0	0.00
Intermediate precision	% RSD ≯ 15.0	0.00
LOD	Detectable Peak	66 ppm
LOQ	Quantifiable peak	200 ppm
	% RSD ≯ 15.0	0.72
Linearity	Correlation coefficient ≮ 0.99	0.999
Accuracy	Should be between 80-120%	97.2% to 99.7%
Robustness		
Flow (1.35 mL/min)	% RSD ≯15.0	0.65 %
Flow (1.65 mL/min)	% RSD ≯15.0	0.76 %
Column temperature (27°C)	% RSD ≯ 15.0	1.72%
Column temperature (33°C)	% RSD ≯ 15.0	1.48%

Table 2 Linearity Results

%Level	Concentration(ppm)	2,3-Dichloronitrobenzene Area
LOQ	210	4214.51
50	520	10273.60
80	830	15410.40
100	1040	20547.20
120	1240	24656.64
150	1530	31123.88
Correlation coefficient		20.718
Intercept		-251.410
Slope		0.999

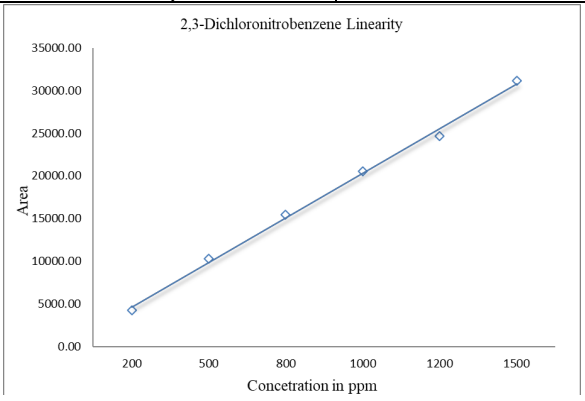

Figure 2, Linearity graph for 2,3-Dichloronitrobenzene,

CHROMATOGRAMS

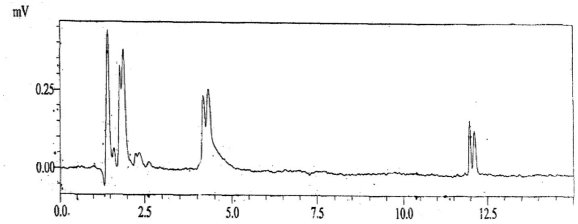

Figure 3: Blank chromatogram

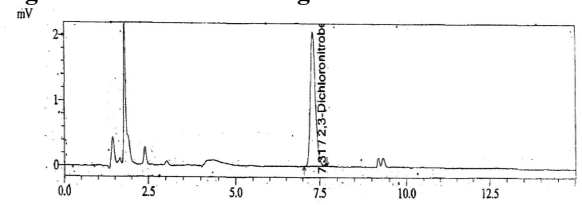

Figure 4: Standard chromatogram

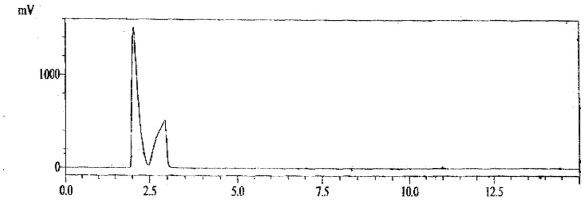

Figure 5: Sample chromatogram

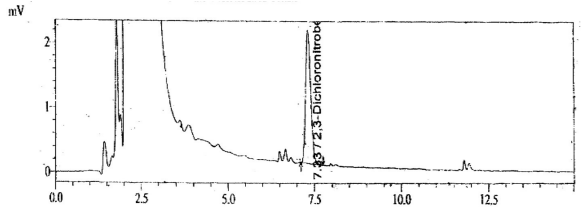

Figure 6: Spiked sample chromatogram

CONCLUSION

A new sensitive HPLC method was developed and validated for the trace analysis of 2,3-dichloronitrobenzene in the aripiprazole drug substance. The authors validated the developed method in accordance with the ICH guidelines. This method is sensitive enough to detect 66 ppm. Moreover, quantify the 2,3-dichloronitrobenzene at 200 ppm in pharmaceutical drug substances.

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