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A Selective and Sensitive Method Development and Validation by LC-MS/MS Approach for Trace Level Quantification of Two Potential Genotoxic Impurities in Albendazole Drug Substance

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ABSTRACT

A simple new reverse-phase liquid chromatography coupled with mass spectrometric (LC-MS/MS) method was developed and validated for the quantification analysis of two potential genotoxic impurities, 2-nitro-4-thio cyanato aniline (GTI-I) and 2-nitro-4-propyl thio aniline (GTI-II) in albendazole drug substance. GTI-I and GTI-II were identified as potential genotoxic impurities from DEREK nexus software. The method utilizes Waters X-Bridge shield RP 18 (250 mm x 4.6 mm ID, 3.5µm) column with negative ion electrospray ionization in multiple reaction monitoring (MRM) detection mode. The isocratic program was developed. The solution-A was 0.01M ammonium formate in water, adjusted pH 6.0 with formic acid and solution-B was acetonitrile: methanol (50:50). The mobile phase was prepared 40:60 (v/v) ratio (solution-A: solution-B). The column oven temperature was maintained 40ºC and flow rate was used 1.0 mL/min. The method was validated as per International Conference on Harmonization (ICH) guidelines and quantitate up to 0.5 ppm of GTI-I and GTI-II. The correlation coefficient found for the linearity study was >0.9996 in each case. The %recovery of the added impurities found the range 96.0 to 104.0.

KEY WORDS: Albendazole, LC-MS/MS, Genotoxic impurities, multiple reaction monitoring (MRM), Derek nexus.

1. INTRODUCTION

Albendazole (Figure.1a) is a medicine used for the treatment for different type of parasitic worm infestations. Albendazole is useful for filariasis, giardiasis, trichuriasis, neurocysticercosis and ascariasis. Albendazole chemically is carbamic acid, [5-(propylthio)-1H-benzimidazole-2-yl]-, methyl ester. It has an empirical formula $C_{12}H_{15}N_3O_2S$ and molecular weight 265.33. 2-nitro-4-thio cyanato aniline (GTI-I) (Figure.1b) and 2-nitro-4-propyl thio aniline (GTI-II) (Figure.1c) chemicals are used in Albendazole process before stage.

Starting materials, intermediates, reagents and by-products as impurities in final product during the synthesis of active pharmaceutical ingredients (APIs). These impurities may be potential genotoxic impurities and potential to induce chromosomal breaks and genetic mutations and may cause cancer for humans. European Medicines Agency and ICH [ICH M7] have enclosed guidelines for genotoxic impurities. These guidelines anticipated for genotoxic impurities, toxicological concern value (1.5 µg/day) in drug substance.

It was identified that GTI-I and GTI-II were potential genotoxic impurities using DEREK nexus software. LHASA predictions derived out of DEREK nexus report clearly indicates that GTI-I and GTI-II is a very plausible entity that shows potential carcinogenicity and skin sensitisation in mammal & mutagenicity in vitro in bacterium is plausible. Though GTI-I and GTI-II are known potential carcinogen. The regulatory authorities proposed limit to be 1.8 ppm for GTI-I and GTI-II in the drug substance. It is essential to control and prove that these impurities are not carry forward to till final stage. A simple, selective, accurate and rapid method developed and method validated as per ICH guidelines in reputes of precision, linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), accuracy and robustness.

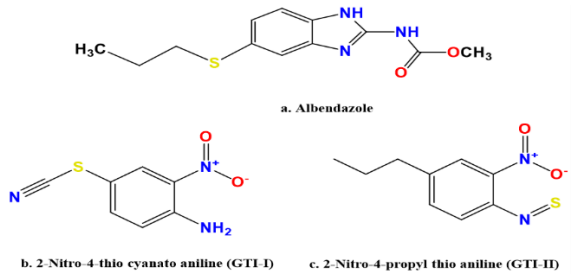

Figure.1. The Chemical structure of Albendazole, GTI-I and GTI-II

2. MATERIALS AND METHODS

Chemicals and reagents: Formic acid, ammonium formate, acetonitrile and methanol were procured Hiper grade from Merck (Mumbai, India). Albendazole, GTI-I and GTI-II were attained from Cipla Ltd (R&D), Bangalore, India.

Instrumentation: The mass spectrometer system used was an Applied Bio system Sciex (QTRAP-5500 series and Switzerland). LC was used Agilent HPLC (1200 series, Germany). Additional equipment used such as PCI sonicator (22L500/CC/DTC) and pH meter (Lab India, PICO$^+$, Mumbai, India).

Chromatographic conditions: Waters X-Bridge shield RP 18 (250 mm x 4.6 mm ID, 3.5µm) procured from Waters corporation (Massachusetts, US) analytical column was used. The solution-A was 0.01M ammonium formate in water, adjusted pH 6.0 with formic acid solution and solution-B was acetonitrile: methanol (50:50). The mobile phase was prepared 40:60 (v/v) ratio (solution-A: solution-B). The column oven temperature was set 40ºC, flow rate was used 1.0 ml/min and the injection volume 10 µl. Negative ion electrospray ionization probe & multiple reaction monitoring (MRM) detection mode were used for LC-MS/MS method for quantification of GTI-I and GTI-II content in albendazole drug substance. The MS parameters were presented in Table.1.

Table.1. Mass spectrometer conditions

Parameter	Albendazole	GTI-I	GTI-II
MRM monitoring for m/z transition	264.0 > 232.0	194.0 > 163.0	211.0 > 168.0
Ionization mode	Negative	Negative	Negative
Declustering potential (V)	-42	-40	-23
Entrance potential (V)	-15	-10	-12
Collision energy (V)	-28	-20	-19
Collision exit potential (V)	-18	-16	-11
Ion spray voltage (V)	-4500	-4500	-4500
Source temperature (ºC)	450	450	450
Curtain gas flow (psi)	40	40	40
Ion source gas1	50	50	50
Ion source gas2	50	50	50

Sample and standard preparation: The test concentration of Albendazole was fixed 10 mg/mL based on the mass detector response. Solution-A was 2% formic acid in water and. Solution-B was methanol. The diluent was used 20:80 (v/v) ration (solution-A: solution-B). The GTI-I and GTI-II standard solutions were prepared with a concentration of 0.5 ppm (LOQ level), 0.9 ppm (50%), 1.8 ppm (Limit Level), 2.7 ppm (150%) and 3.6 ppm with respect to the test concentration. All standard and sample solutions were prepared in amber colored glassware.

3. RESULTS AND DISCUSSION

Method development: There are some literature reports available for the determination of Albendazole in oral suspension and pharmaceutical dosage forms. No literature available for the determination of GTI-I and GTI-II content in Albendazole. For better sensitivity LC-MS/MS technique used for method development trails. The concluding chromatographic condition was achieved on a Waters X-Bridge shield RP 18 (250 mm x 4.6 mm ID, 3.5µm) column, column oven temperature was set 40ºC, flow rate was 1.0 ml/min and MS parameters were set to get maximum sensitivity for the impurities of GTI-I and GTI-II. Before obtaining the final method, the method was analyzed with different stationary phase columns which includes Phenyl, C18, C8, cyano and amino. In addition to that the trails were passed out through different mobile phase additives such as acetic acid, formic acid, ammonium acetate and mixture of acetonitrile and methanol.

Method Validation: The method was checked by injecting 1.8 ppm solutions of Albendazole, GTI-I and GTI-II with respect to test concentration for specificity. The retention time of Albendazole, GTI-I and GTI-II observed at 6.81, 5.29 and 12.22 minutes respectively. The specificity chromatograms show in figure.2.

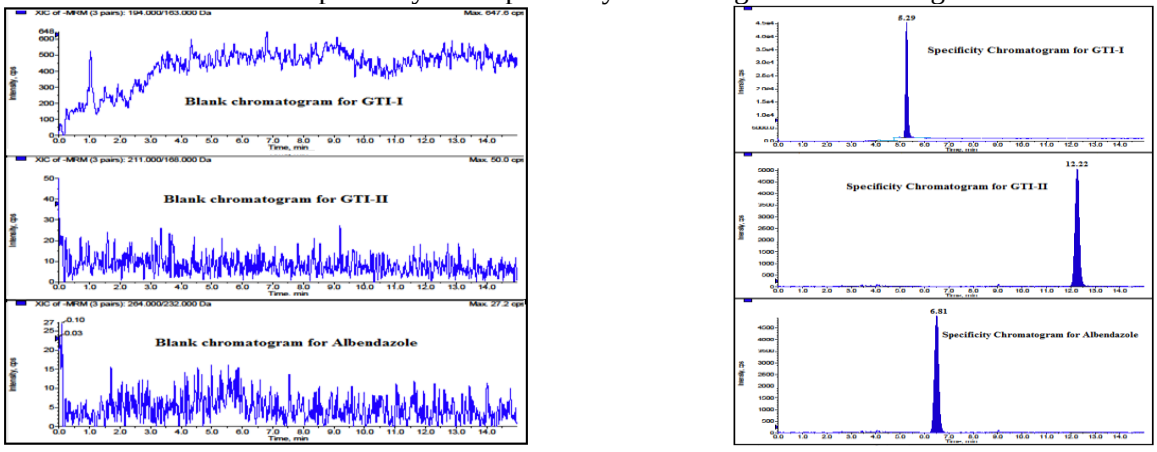

Figure.2. Specificity chromatograms of Albendazole, GTI-I and GTI-II

Limit of detection (LOD) and limit of quantification (LOQ) were calculated from S/N (signal to noise) ratio. The LOD and LOQ for GTI-I and GTI-II were found with the concentration of 0.15 ppm and 0.5 ppm for both impurities to obtain the S/N ratio 3 and 10 respectively. The developed method was confirmed over a concentration of five levels 0.5-3.6 ppm (LOQ, 50%, 100%, 150% and 200%) for linearity. The concentration of ppm in X-axis and peak areas in Y-axis used for calibration curve. The correlation coefficient, slope and intercept values were found through regression analysis and data are represented in Table.2. For method precision, six individual solutions were prepared by spiking Albendazole with the impurities of 1.8 ppm with respect to Albendazole 10 mg/mL concentration. Intermediate precision was performed by different column, different instrument and different day. For all the above determinations were calculated %RSD and originate below 10. The recovery studies by the standard addition method were performed. Accordingly, the accuracy was performed at LOQ, 50%, 100% and 200% in triplicate. The recovery found for both the genotoxic impurities, GTI-I and GTI-II were within the 94% to 104%. LOQ level chromatograms of accuracy was shown in figure.3. Robustness of the method was determined by making slight and deliberate changes in experimental conditions. Robustness were performed by mobile phase changed 0.1 units i.e. 1.0 to 1.1 mL/min, 1 to 0.9 mL/min for flow rate and temperature was altered by 2ºC i.e. 38ºC and 42ºC. The % RSD values from robustness study and method precision were calculated and found to be below 10 for GTI-I and GTI-II, demonstrated that the method was robust. GTI-I and GTI-II were quantitatively spiked at limit level concentration 1.8 ppm and stored at 5ºC for solution stability. The spiked solution was injected, initially and different intervals. The % variation were calculated for GTI-I and GTI-II content in the initial and different interval and found below 10. This indicates that the sample solution was stable up to 14 hours at 5 ºC. Validation results were summarized and presented in table.2.

Table.2. Summary of Analytical method validation results

Parameter	Results	
	GTI-I	GTI-II
Limit of detection (ppm)	0.15	0.15
Limit of quantification (ppm)	0.5	0.5
Linearity range (ppm)	0.5-3.6	0.5-3.6
Correlation coefficient	0.9999	0.9997
Slope	78158	59974
Intercept	-1893	3178
Method precision (%RSD)	0.97	0.88
Intermediate precision (%RSD)	1.97	2.38
% Recovery at LOQ (0.5 ppm)	96.4-102.3	97.1-103.9
% Recovery at 50% (0.9 ppm)	98.7-103.5	97.2-103.7
% Recovery at 100% (1.8 ppm)	97.1-103.9	96.7-102.9
% Recovery at 200% (3.8 ppm)	99.7-103.8	97.5-103.1

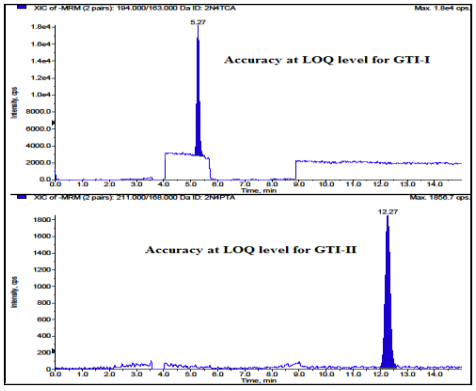

Figure.3. Accuracy at LOQ level for GTI-I and GTI-II

4. CONCLUSION

A simple isocratic RP-LC-MS/MS method was developed for the concurrent determination of GTI-I and GTI-II in albendazole. The anticipated LC-MS/MS method was validated as per ICH guidelines. It is simple, sensitive, selective, precise, accurate and robust. The developed method could be used for GTI-I and GTI-II content in albendazole for routine quality control release and stability studies.

5. ACKNOWLEDGEMENTS

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REFERENCES

Albendazole monograph USP 40-NF 45, The United States Pharmacopeial Convention, 12601 Twinbrook parkway, Rockville, MD, 20852, 2604.

Bolt HM, Foth H, Hengstler JG, Degan GH, Carcinogenicity categorization of chemicals-new aspects to be considered in a European perspective, Toxicology Letters, 151, 2004, 29-41.

Deductive Estimation of Risk from Existing Knowledge (DEREK) nexus, marketed by LHASA Ltd, Leeds, Yorkshire, U.K, DEREK nexus program Version Derek Nexus: 3.0.1, Nexus: 1.5.1, 2017.

Guideline for Assessment and Control of DNA Reactive (Mutagenic) Impurities in pharmaceuticals to Limit Potential Carcinogenic risk, M7 ICH, 2014.

European medical agency: Guideline on the limits of genotoxic impurities, EMA/CHMP/SWP/431994/2007 3, 2010, 1-6.

ICH validation of analytical procedures: text and methodology Q2 (R1), International Conference on Harmonization, 4, 2005, 1-13.

Jacobson-Kram D, McGovern T, Toxicological overview of impurities in pharmaceutical products, Advance Drug Delivery Reviews, 59, 2007, 38-42.

Krishnaiah YSR, Latha K, Karthikeyan RS, Satyanarayana V, HPLC method for the estimation of albendazole in pharmaceutical dosage forms, Asian journal of chemistry, 14, 2002, 67-71.

Mariene M, Fregonezi-Nery, Marcela M, Baracat Erika RM, Kedor-Hackman, Rafael Mota Pinheiro, Determination of albendazole in oral suspension, Analytical letters, 34, 2001, 1255-1263.