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Trace Level Determination of Potential Genotoxic Impurity O-Toluidine (2-Methyl Aniline) in Drug Substance

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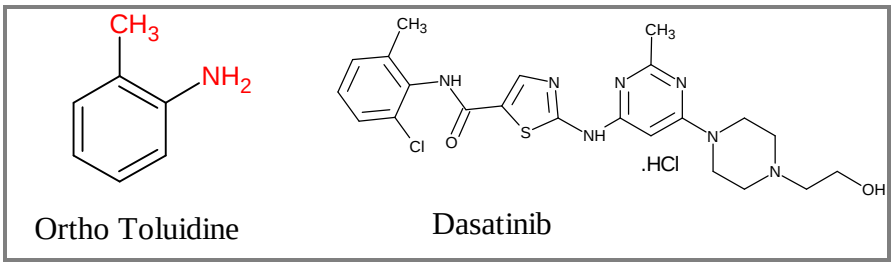
ABSTRACT

An analytical method has been developed for trace level determination of O-Toluidine (potential genotoxic impurity) in drug substances at pharmaceutical industry. The accurate Quantitation of O-Toluidine was achieved on Inertsil ODS-3V column (250 mm x 4.6 mm, 5.0 µm) with gradient elution at a flow rate of 1.0 mL min^{-1}. Gradient elution containing mobile phase-A and mobile phase-B, 0.015mM potassium dihydrogen phosphate in water used as mobile phase-A and Acetonitrile and water mixture was used as mobile phase-B. The elution of O-Toluidine is monitored at 210 nm, by using Ultra Visible / PDA detector at the level of 3 mg L^{-1}. The high correlation coefficient (R2>0.999) values indicated clear correlations between the investigated compound concentrations and their peak areas within the LOQ (limit of Quantitation) to 150% level. O-Toluidine was uses in manufacturing process of dasatinib. Hence O-Toluidine was major possible and potential genotoxic impurities of dasatinib.

Graphical Abstract



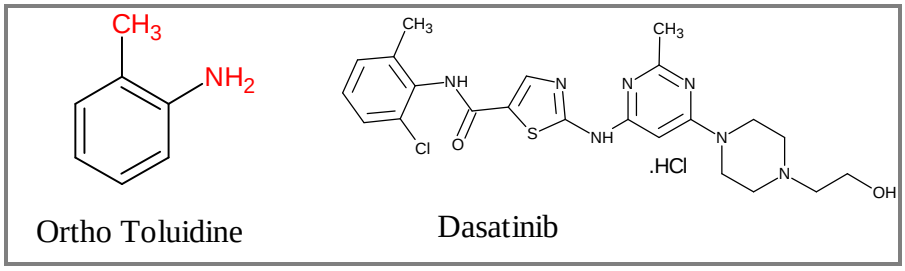
Structure of O-Toluidine and Dasatinib

Keywords: O-Toluidine, 2-methyl aniline, Genotoxic impurity, HPLC, Dasatinib.

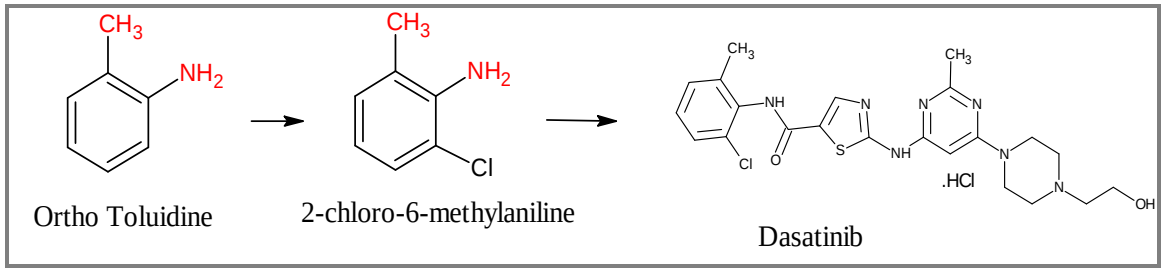
INTRODUCTION

O-Toluidine is the process possible genotoxic impurities of Dasatinib. This compound was uses as one of the key starting material (KSM) in the manufacturing process of Dasatinib, which is active moiety in the molecule (Dasatinib) [**1-5**].

As per the daily dosage limit of Dasatinib, any genotoxic impurity shall be control below 3 mg L^{-1}. Moreover Dasatinib is oncology drug/High potent (cancer drug). Hence Genotoxic impurities should be not available in the drug substances. Structure of O-Toluidine and Dasatinib was given below [**6-9**].



Structure of O-Toluidine and Dasatinib



Manufactures process of Dasatinib

Due to the genotoxic nature of this impurity (O-Toluidine), simple and accuate analytical methods was developed to determination/quantify the impurity by HPLC in the Dasatinib drug substance at trace level (3 mg L^{-1}). Advantage of this method is trace level (3mg $L^{-1)}$ can be Quantify with regular HPLC analysis.

MATERIALS AND METHODS

Chemicals, standards and impurities: Acetonitrile (HPLC grade, Merck, India), Potassium dihydrogen phosphate (AR grade, Merck, India), High pure water is from Milli-Q water purification system (Millipore), O-Toluidine (from Sigma Aldrich), Dasatinib drug substance were obtained from Process Research department of Dr.Reddy's Laboratories, Hyderabad.

Equipment: LC was carried out with Shimadzu HPLC with photodiode array detector. The output signal was monitored and processed by using LC solution software.

Chromatographic Conditions: A new gradient method is developed for determination of o-Toluidine content by HPLC in Dasatinib drug substances. The chromatographic method employs a mobile phase-A consisting 0.015 mM potassium dihydrogen phosphate in water used as mobile phase-A and Acetonitrile and water mixture in the ratio 90:10 (v/v) was used as mobile phase-B. The method employs a gradient program (Time in min / %Mobile phase B) 0.01/35, 15/35, 18/55, 23/85, 30/85, 30.1/35, 35/35. The method was developed using Inertsil ODS-3V (250 mm x 4.6 mm, 5.0 µm)

column. The flow rate of the mobile phase was 1.0 mL min^{-1}. The column temperature was maintained at 25°C, sample cooling rack temperature was maintained at 15°C and the wavelength was monitored at 210 nm. The injection volume was 20 µL. Diluent is Acetonitrile.

Preparations of Blank solution: Use Acetonitrile as blank (Figure 1).

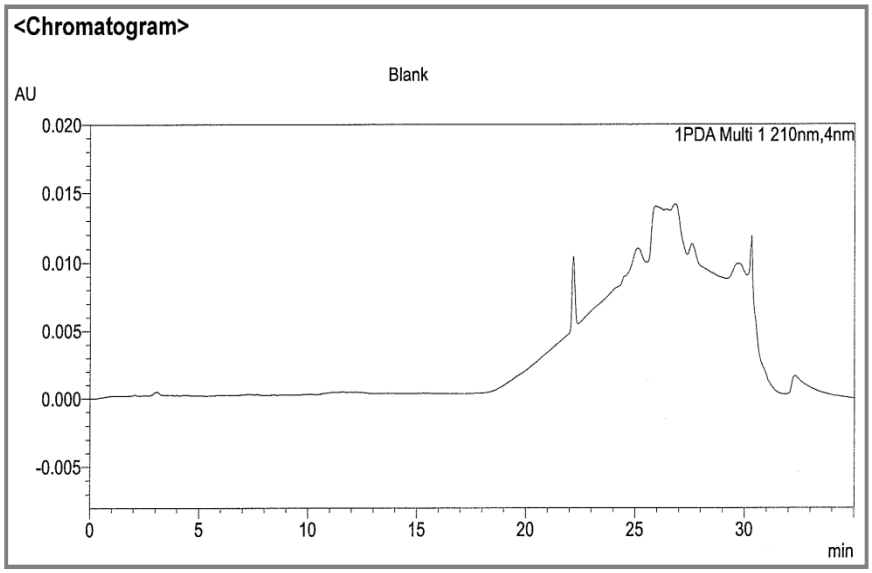


Figure 1. Blank run chromatogram at 210 nm.

Preparations of impurity stock solution: Accurately weighed and transferred 7.5 mg of impurity (O-Toluidine) into 100mL of volumetric flask, added 20mL of diluent and dissolved then made up to the mark with diluent and mixed well. Further transferred 1.0 mL of this solution in to 100 mL of volumetric flask and made up to the mark with diluent and mixed well.

Preparations of impurity standard solution: Accurately transferred 1.0 mL of impurity stock solution in to 10 mL of volumetric flask containing 5 mL of diluent, mixed well and made up to the mark with diluent (figure 2).

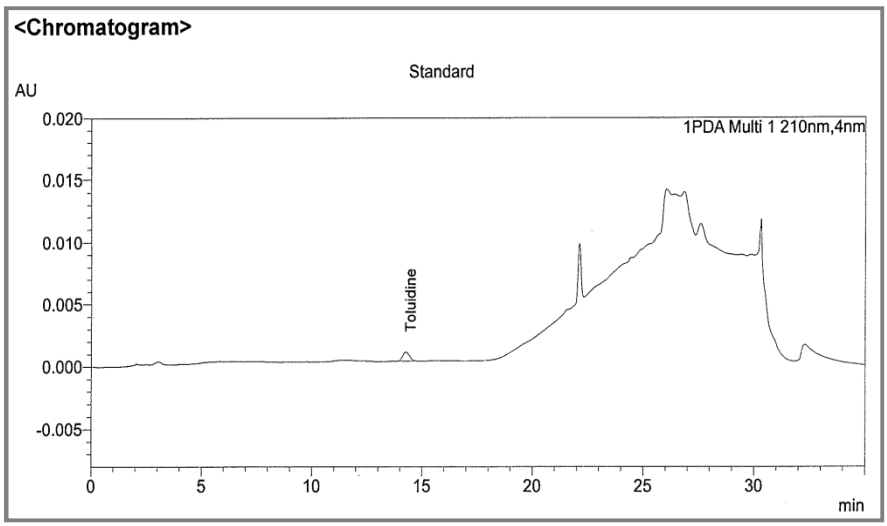


Figure 2. Impurity standard chromatogram at 210 nm.

Preparations of test sample solution: Accurately weighed and transferred 250mg of test sample in to 10 mL of volumetric flask, added 2.0mL of diluent and sonicate to dissolve then made up to the mark with diluent (Figure 3).

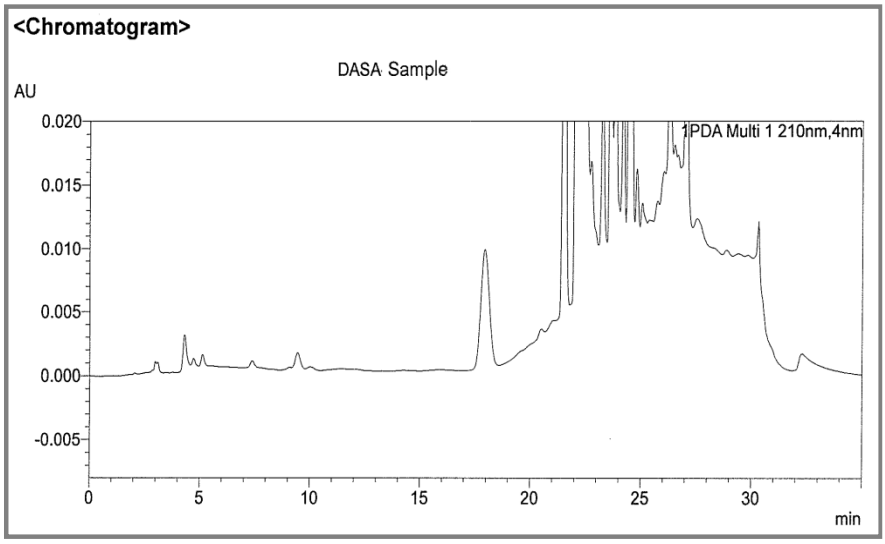


Figure 3. Dasatinib test sample chromatogram at 210 nm

Preparations of Impurity spiked with test sample solution: Accurately weighed and transferred 250 mg of test sample in to 10 mL of volumetric flask, added 2.0 mL of diluent and sonicated to dissolve then added 1.0 mL of impurity stock solution mixed well and made up to the mark with diluent (Figure 4).

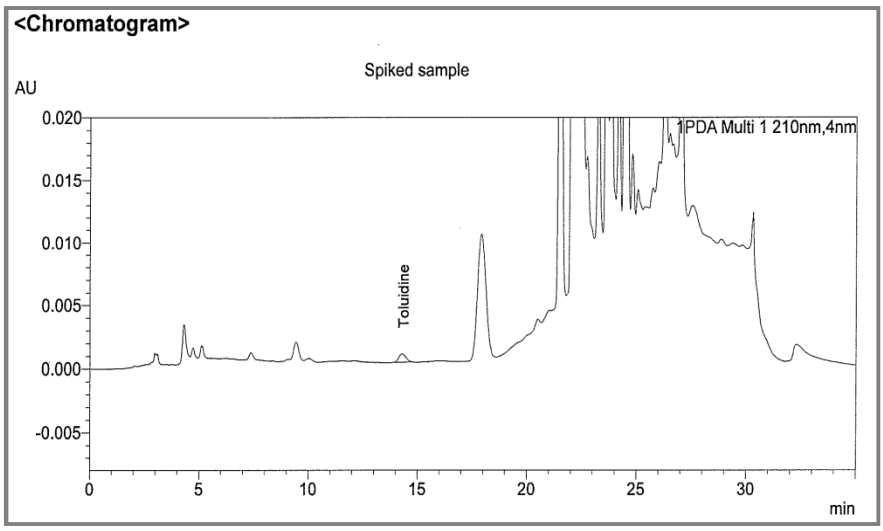


Figure 4. Impurity spiked to Dasatinib test sample chromatogram at 210 nm.

RESULTS AND DISCUSSION

Linearity: Linearity test solutions for the content method are prepared from impurity stock solutions at five concentration levels from 50 to 150% of analyte concentration (50, 75, 100, 125 and 150%). The peak area versus concentration data is treated by least-squares linear regression analysis. Linearity solutions for the method impurities were prepared by diluting impurity stock solutions to the

required concentrations. The solutions are prepared at different concentration levels from LOQ to 150% (4.5mg L^{-1}). The correlation coefficients of o-Toluidine were found 0.999.

Linearity results were provided in table1, Linearity curve for O-Toluidine was given as figure 5.

Table 1. Linearity result of O-Toluidine

Linearity		O-Toluidine
Level	**Concentration (mg L^{-1})**	**Area**
LOQ	0.60	2751
50%	1.50	6163
75%	2.25	8564
100%	3.00	11224
125%	3.75	13765
150%	4.50	16502
	Slop	3491
	Intercept	752.6
	Correlation	0.99981

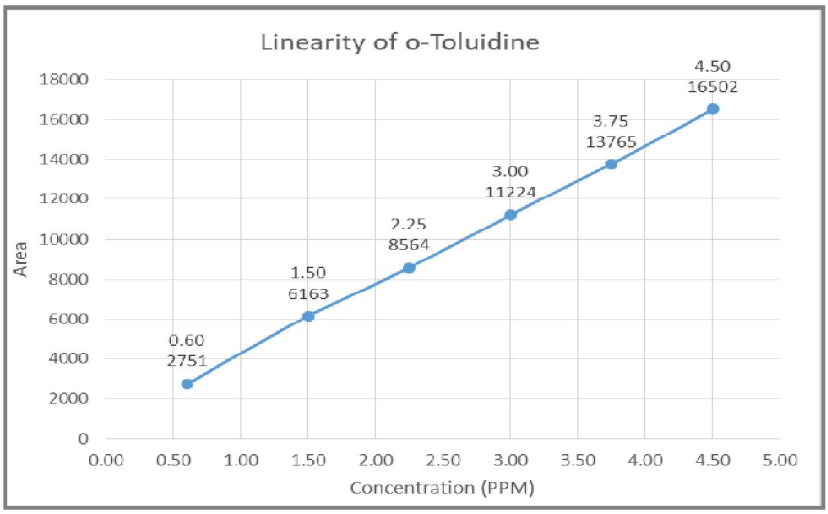


Figure 4. Linearity curve for O-Toluidine.

Limits of detection (LOD) and Limit of quantitation (LOQ): The LOD and LOQ for O-Toluidine was estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentration. Precision study was also carried at the LOQ level by injecting six individual preparations of O-Toluidine and calculating the % R.S.D. of the area. Accuracy at LOQ level was evaluated in triplicate for the O-Toluidine by spiking at the estimated LOQ level to test solution. Limit of Quantitation (LOQ) was found 0.60 mg L^{-1} and Limit of Detection (LOD) was found 0.20 mg L^{-1} for O-Toluidine with respect to test concentration. Relative standard deviation for Limit of Quantitation (LOQ) found 2.20% for O-Toluidine. The limit of detection, limit of Quantitation and precision at LOQ values for O-Toluidine are shown in table 2 and 3.

Table 2. Concentration of LOD and LOQ for O-Toluidine

Level	O-Toluidine
LOD	**0.20mg L^{-1}**
LOQ	**0.60mg L^{-1}**
Specification	3mg L^{-1}

LOD and LOQ values were with respect to test concentration.

Table 3. Precision Limit of Quantitation results for O-Toluidine

LOQ	O-Toluidine
Preparation	**Area**
Preparation-1	2786
Preparation-2	2813
Preparation-3	2705
Preparation-4	2618
Preparation-5	2906
Preparation-6	2677
Mean	4751
SD	104.28
% RSD	**2.20**

Accuracy: The accuracy of the O-Toluidine content method is evaluated in triplicate at three concentration levels, i.e. 50, 100 and 150% of the specification concentration along with Limit of Quantitation level. The recovery is calculated against 25 mg mL^{-1} of test concentration. Recovery study of O-Toluidine was performed at 1.5 mg L^{-1}, 3 mg L^{-1} and 4.5 mg L^{-1} levels and found that accuracy of the method falls in the range of 97.9% to 102.2%. Accuracy data is shown in the table 4. The Accuracy study was performed with API samples of Dasatinib. Accuracy result for O-Toluidine was given in the table 4.

Table 4. Accuracy result for O-Toluidine

O-Toluidine						
Levels	**Preparation**	**Area**	**Added**	**Obtained**	**Recovery**	**Avg**
At LOQ	Preparation-1	2144	0.60	0.61	101.4	**102.2**
	Preparation-2	2215	0.60	0.63	104.8	
	Preparation-3	2123	0.60	0.60	100.4	
At 50%	Preparation-1	5267	1.50	1.49	99.5	**98.4**
	Preparation-2	5147	1.50	1.46	97.5	
	Preparation-3	5196	1.50	1.47	98.3	
At 100%	Preparation-1	10765	3.00	3.05	101.8	**101.8**
	Preparation-2	10567	3.00	2.99	99.9	
	Preparation-3	10987	3.00	3.11	103.8	
At 150%	Preparation-1	15935	4.50	4.52	100.6	**97.9**
	Preparation-2	15362	4.50	4.36	97.0	
	Preparation-3	15254	4.50	4.32	96.2	

Precision: The precision of the method is evaluated by analyzing six test samples of spiked with O-Toluidine at 3 mg L^{-1} level. The Relative standard deviation is found to be 2.70% for O-Toluidine. Precision data is shown in table 5.

Table 5. Precision result for O-Toluidine

Precision	O-Toluidine
Preparation	**Area**
Preparation-1	10318
Preparation-2	10941
Preparation-3	10813
Preparation-4	10636
Preparation-5	10403
Preparation-6	10232
Mean	10557
SD	285
% RSD	**2.70**

Summary: All the results were summarized in the following table.

Table 6. Validation results for the parameters

S. No	Validation parameter	Description	Result
1	LOD	Impurity concentration (mg L^{-1})	0.2
2	LOQ	Impurity concentration (mg L^{-1})	0.6
3	Specification	Impurity concentration (mg L^{-1})	3.0
4	Precision(n=6)	% RSD for area	2.70
5	LOQ Precision(n=6)	% RSD for area	3.79
6	Accuracy at LOQ	% Recovery	102.2
		% Recovery at 50%	98.4
7	Accuracy	% Recovery at 100%	101.8
		% Recovery at 150%	97.9
8	Linearity	Correlation coefficient	0.99981

APPLICATION

Method was developed with the wave length of 210 nm is get high response for O-Toluidine. Inertsil ODS-3V column (250 mm x 4.6 mm, 5.0 µm) was used for the separation and retains purpose of the impurity (O-Toluidine) in the column and good peak (Gaussian peak). This study was demonstrated the analytical method was linear accurate and precise at specification level (3 mg L^{-1}) and Quantitation level (0.6 mg L^{-1}).

CONCLUSION

A simple, sensitive and accurate analytical method was developed for the trace level determination and quantitation of O-Toluidine in pharmaceutical drug substance (Dasatinib) using regular high performed liquid chromatography. Selection of diluent acetonitrile is the key step for the analytical approach which dissolves the Dasatinib compounds at such a high concentration (25 mg mL^{-1}) and meets the specific requirement of analytical strategy. Since the trace level (3 mg L^{-1}) determination is the major task with regular HPLC. Method was developed with the wave length of 210 nm is get high response for O-Toluidine. Inertsil ODS-3V column (250 mm x 4.6 mm, 5.0 µm) was used for the separation and retains purpose of the impurity (O-Toluidine) in the column and good peak (Gaussian peak). This study was demonstrated the analytical method was linear accurate and precise at specification level (3 mg L^{-1}) and Quantitation level (0.6 mg L^{-1}).

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