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Trace Level Determination of Potential Genotoxic Impurity 2-Methoxy-5-Nitrophenol In Drug Substance

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ABSTRACT:

Trace level determination of 2-Methoxy-5-Nitrophenol (potential genotoxic impurity) in drug substances at pharmaceutical industry has been developed.

The accurate Quantization of 2-Methoxy-5-Nitrophenol was achieved on X-bridge C-18 column (250mm x 4.6mm, 5.0µm) with gradient elution at a flow rate of 1.0mL/min. Gradient elution containing mobile phase-A and mobile phase-B, 0.1% of Triethylamine in water was pH adjusted to 8.0 with Orthophosphoric acid used as mobile phase-A and Acetonitrile used as mobile phase-B. The elution of 2-Methoxy-5-Nitrophenol is monitored at 240nm, by using Ultra Visible / PDA detector at the level of 2.5ppm. 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. 2-Methoxy-5-Nitrophenol was uses the manufacturing process of Bosutinib. Hence 2-Methoxy-5-Nitrophenol was major possible genotoxic impurities of Bosutinib.

KEYWORDS: 2-Methoxy-5-Nitrophenol; Genotoxic impurity; HPLC; Bosutinib.

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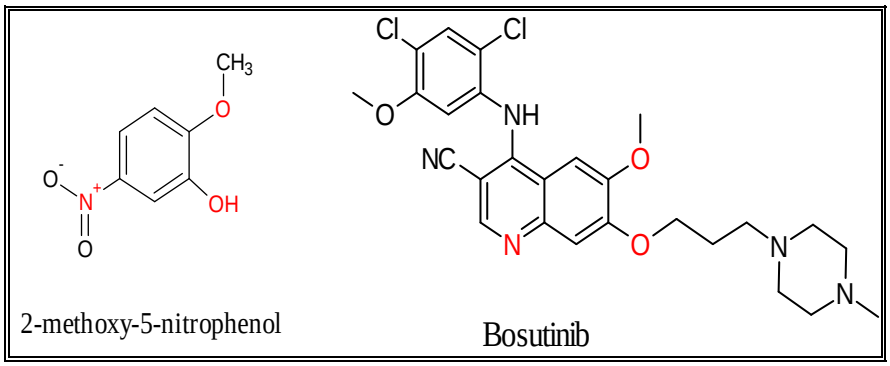
INTRODUCTION.

Back ground:

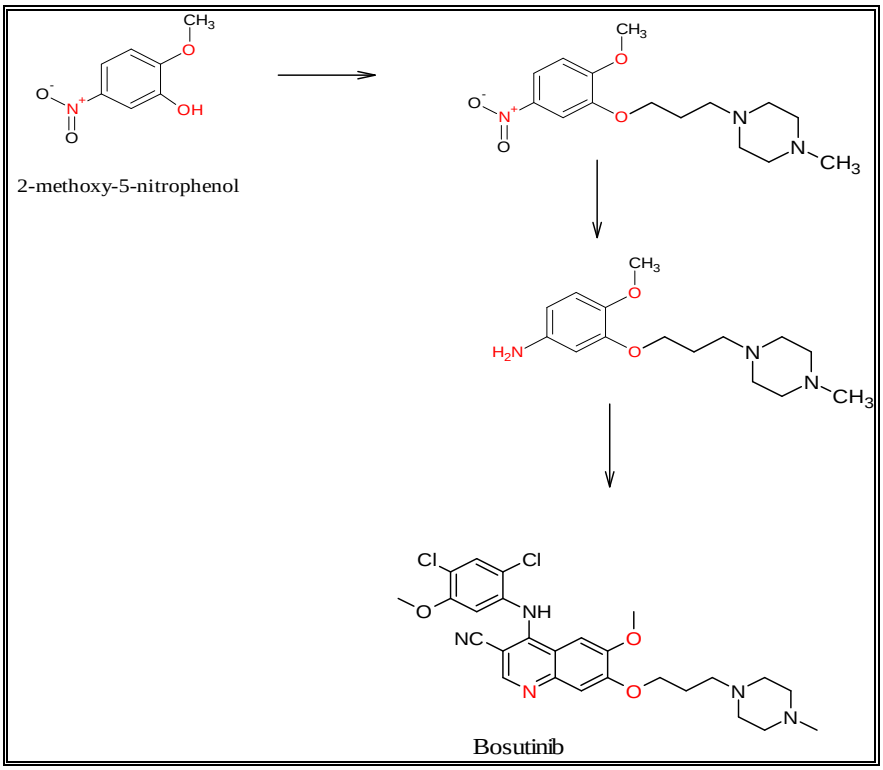
2-Methoxy-5-Nitrophenol is the genotoxic starting material[1] of Bosutinib. This compound was uses as one of the key starting material in the manufacturing process of Bosutinib. Which is active moiety in the molecule (Bosutinib).

As per the daily dosage limit of Bosutinib, limit of any genotoxic impurity[2] shall be control below 2.5ppm. Moreover Bosutinib is oncology drug/High potent (cancer drug). Hence Genotoxic impurities should be not available in the drug substances.

Structure of 2-Methoxy-5-Nitrophenol and Bosutinib was given below.



Bosutinib[3] was manufactures as per following process from 2-methoxy-5-nitrophenol. Which is the using as a starting materials in the manufacturing process of Bosutinib.



Due to the genotoxic nature of this impurity (2-Methoxy-5-Nitrophenol), a simple and accurate method for trace level determination of potential Genotoxic impurity was developed to quantify the impurities by HPLC in the Bosutinib drug substance. Advantage of this method is trace level[4] (2.5ppm) can be Quantify with regular HPLC analysis.

MATERIAL AND METHODS

Chemicals, standards and impurities

Acetonitrile (HPLC grade, Merck, India), Tri ethyl amine (AR grade, Merck, India), Orthophosphoric acid (AR grade, Merck, India), High pure water is from Milli-Q water purification system from Millipore and Hydrochloric acid (LR grade, Merck, India).

2-Methoxy-5-Nitrophenol (from Sigma Aldrich)

Bosutinib drug substance were obtained from Process Research department of Dr.Reddy's Laboratories, Hyderabad.

Equipments

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 2-Methoxy-5-Nitrophenol by HPLC in Bosutinib drug substances. The chromatographic method employs a mobile phase-A consisting 0.1% of triethylamine in water solution was pH adjusted to 8.0 with diluted Orthophosphoric acid and a mobile phase-B consisting acetonitrile. The method employs a gradient program (Time in min / %Mobile phase B) 0.01/10, 3/10, 20/48, 35/75, 55/75, 58/10, 65/10.

The method was developed using X-bridge C-18 column (250mm x 4.6mm, 5.0µm) column. The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 30°C, sample cooling rack temperature was maintained at 15°C and the wavelength was monitored at 240 nm. The injection volume was 50 micro liter (µL). Diluent is mixture of Acetonitrile and water in the ratio of 50:50 (v/v).

Preparations of Blank solution

Accurately transferred 2.0 mL of 1N Hydrochloric acid into a 10 mL of volumetric flask and made up to the mark with diluent (Acetonitrile: water)

Preparations of impurity stock solution

Accurately weighed and transferred 12.5 mg of impurity (2-Methoxy-5-Nitrophenol) 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 2.0 mL of 1N Hydrochloric acid 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.

Preparations of test sample solution

Accurately weighed and transferred 500mg of test sample in to 10 mL of volumetric flask, added 2.0mL of 1N Hydrochloric acid and sonicate to dissolve then made up to the mark with diluent.

Preparations of Impurity spiked with test sample solution

Accurately weighed and transferred 500mg of test sample in to 10 mL of volumetric flask, added 2.0mL of 1N Hydrochloric acid sonicate to dissolve and added 1.0 mL of impurity stock solution mixed well and made up to the mark with diluent.

RESULTS AND DISCUSSION (METHOD VALIDATION)

Linearity

Linearity test solutions for the content method are prepared from impurity stock solitons 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 impurities method were prepared by diluting impurity stock solutions to the required concentrations. The solutions are prepared at different concentration levels from LOQ to 3.75ppm. The correlation coefficients of 2-Methoxy-5-Nitrophenol was found 0.9999.

Linearity results were provided in table-1, Linearity curve for 2-Methoxy-5-Nitrophenol was given as Figure-1.

Table-1: Linearity result of 2-Methoxy-5-Nitrophenol:

Linearity		**Linearity of 2-Methoxy-5-Nitrophenol**
Level	**Concentration(ppm)**	**Area**
LOQ	0.60	4751
50%	1.25	9163
75%	1.88	13864
100%	2.50	18224
125%	3.13	22765
150%	3.75	27002
Slop		7102
Intercept		461.0

Correlation	0.99991

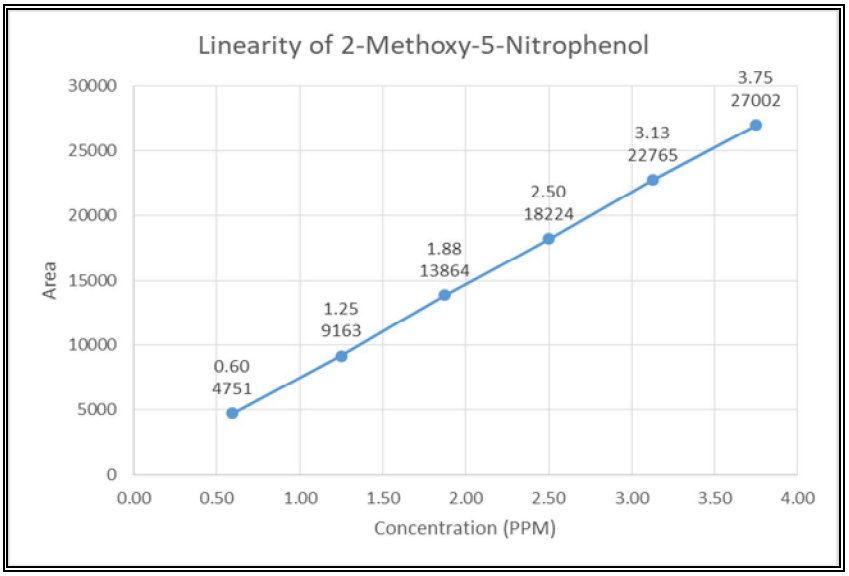


Figure-1: Linearity curve for 2-Methoxy-5-Nitrophenol

Limits of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ for 2-Methoxy-5-Nitrophenol 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 2-Methoxy-5-Nitrophenol and calculating the % R.S.D. of the area. Accuracy at LOQ level was evaluated in triplicate for the 2-Methoxy-5-Nitrophenol by spiking at the estimated LOQ level to test solution.

Limit of Quantitation[5] (LOQ) was found 0.60ppm and Limit of Detection[5] (LOD) was found 0.20ppm for 2-Methoxy-5-Nitrophenol with respect to test concentration.

Relative standard deviation for Limit of Quantitation (LOQ) found 2.20% for 2-Methoxy-5-Nitrophenol.

The limit of detection, limit of Quantitation and precision at LOQ values for 2-Methoxy-5-Nitrophenol are shown in Table-2 and Table-3.

Table-2: Concentration of LOD and LOQ for 2-Methoxy-5-Nitrophenol

Level	2-Methoxy-5-Nitrophenol
LOD	**0.20ppn**
LOQ	**0.60ppm**
Specification	2.5ppm

LOD and LOQ values with respect to test concentration.

Table-3: Precision Limit of Quantitation results for 2-Methoxy-5-Nitrophenol

LOQ	2-Methoxy-5-Nitrophenol
Preparation	**Area**
Preparation-1	4786
Preparation-2	4813
Preparation-3	4705
Preparation-4	4618
Preparation-5	4906
Preparation-6	4677
Mean	4751
SD	104.28
% RSD	**2.20**

Accuracy

The accuracy of the 2-Methoxy-5-Nitrophenol 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 50 mg/ml of test concentration. Recovery study of 2-Methoxy-5-Nitrophenol was performed at 1.25ppm, 2.5ppm and 5.75ppm levels and found that accuracy of the method falls in the range of 91.2% to 103.4%. Accuracy data is shown in the Table-4. The Accuracy study was performed with API samples of Bosutinib.

Accuracy result for 2-Methoxy-5-Nitrophenol was given in the table-4.

Table-4: Accuracy result for 2-Methoxy-5-Nitrophenol

2-Methoxy-5-Nitrophenol						
Levels	**Prep' n**	**Area**	**Added**	**Obtained**	**Recovery**	**Avg**
At LOQ	Prep'n-1	4044	0.60	0.54	91.2	**91.2**
	Prep'n-2	4115	0.60	0.55	92.8	
	Prep'n -3	3972	0.60	0.53	89.6	
At 50%	Prep'n-1	9467	1.24	1.27	102.5	**103.4**
	Prep'n-2	9647	1.24	1.30	104.4	
	Prep'n -3	9546	1.24	1.28	103.3	
At 100%	Prep'n-1	18765	2.48	2.52	101.6	**101.6**
	Prep'n-2	18567	2.48	2.49	100.5	
	Prep'n -3	18987	2.48	2.55	102.8	
At 150%	Prep'n-1	28935	3.72	3.88	104.4	**103.1**
	Prep'n-2	28562	3.72	3.83	103.1	
	Prep'n -3	28254	3.72	3.79	102.0	

Precision

The precision of the method is evaluated by analyzing six test samples of spiked with 2-Methoxy-5-Nitrophenol at 2.5ppm level. The Relative standard deviation is found to be 1.77% for 2-Methoxy-5-Nitrophenol. Precision data is shown in Table-5.

Table-5: Precision result for 2-Methoxy-5-Nitrophenol

Precision	**2-Methoxy-5-Nitrophenol**
Preparation	**Area**
Preparation-1	18318
Preparation-2	18941
Preparation-3	18813
Preparation-4	18136
Preparation-5	18403
Preparation-6	18232
Mean	18474
SD	327
% RSD	**1.77**

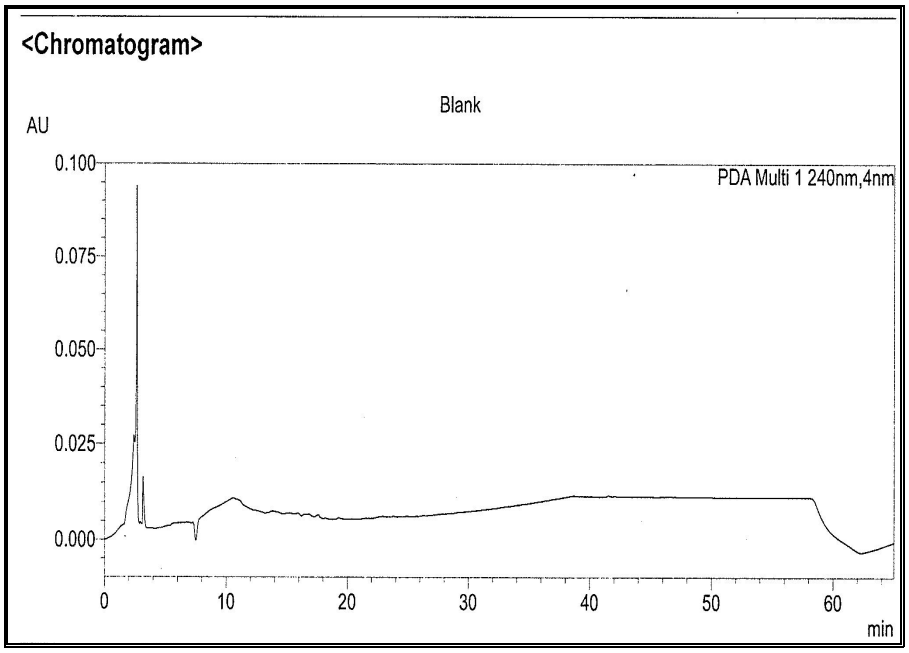


Figure-2: Blank run chromatogram at 240nm

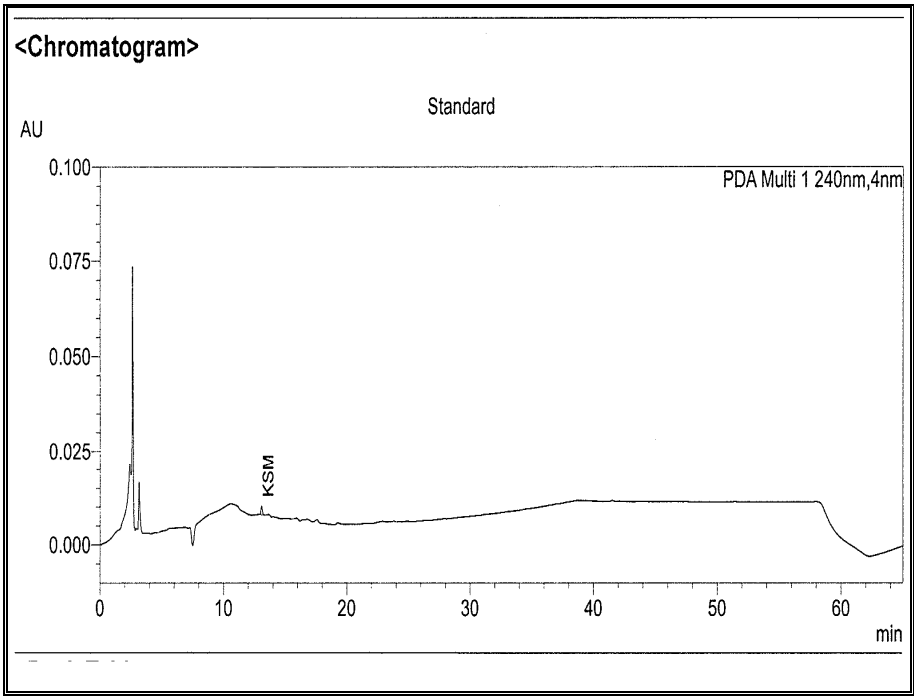


Figure-3: Impurity standard chromatogram at 240nm

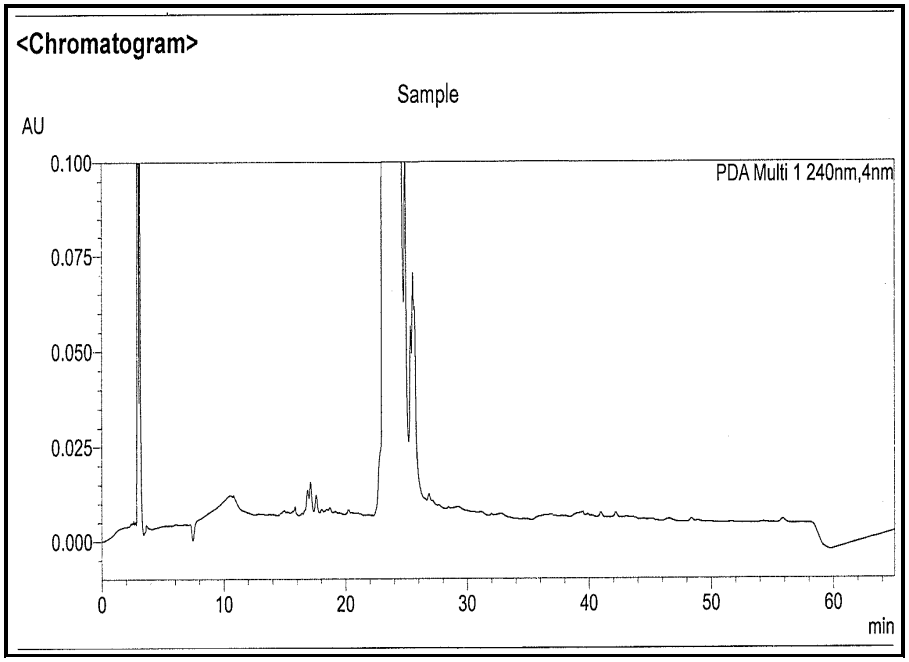


Figure-4: Bosutinib test sample chromatogram at 240nm

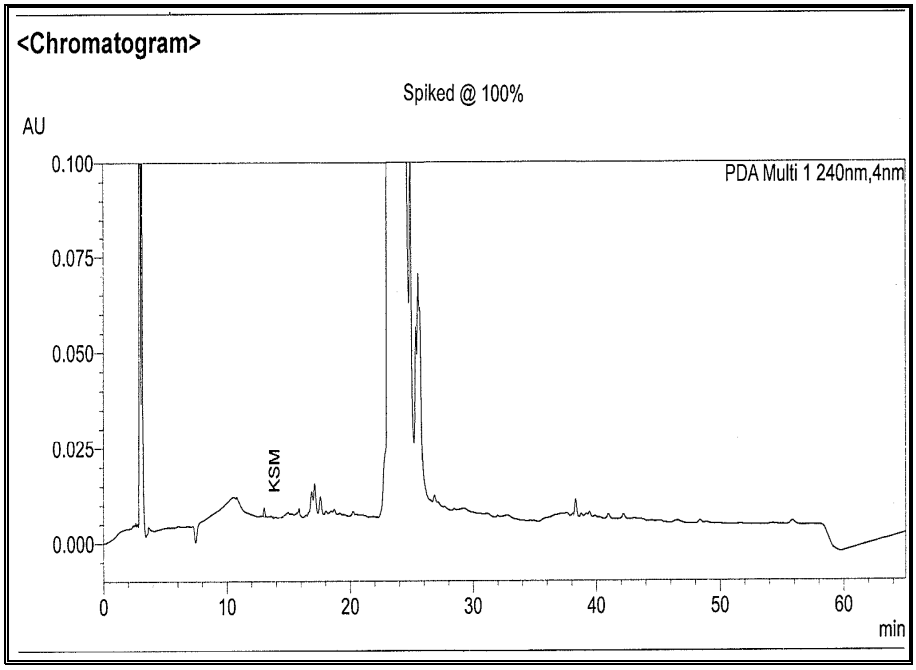


Figure-5: Impurity spiked to Bosutinib test sample chromatogram at 240nm

Summary:

All the validation results were summarized in the following table.

Table-6: Validation results for the parameters.

S. No	Validation parameter	Description	Result
1.	LOD	Impurity content (ppm)	0.2
2.	LOQ	Impurity content (ppm)	0.6
3.	Specification	Impurity content (ppm)	2.5
4.	Precision(n=6)	% RSD for area	1.77
5.	LOQ Precision(n=6)	% RSD for area	2.20
6.	Accuracy at LOQ	% Recovery	91.2
7.	Accuracy	% Recovery at 50%	103.4
		% Recovery at 100%	101.6
		% Recovery at 150%	103.1
8.	Linearity	Correlation coefficient	0.99991

CONCLUSION

A simple, sensitive and accurate method was developed for the trace level determination and quantitation of 2-Methoxy-5-Nitrophenol in pharmaceutical drug substance (Bosutinib) using regular high performed liquid chromatography. Selection of diluent (1N Hydrochloric acid) is the key step for the analytical approach which dissolves the Bosutinib compounds at such a high concentration (50mg/mL) and meets the specific requirement of analytical strategy. Since the trace level (2.5ppm) determination is the major task with regular HPLC. Method was developed with the wave length of 240nm is get high response for 2-Methoxy-5-Nitrophenol. X-bridge C-18 column (250mm x 4.6mm, 5.0µm) was used for the separation and retain purpose of the impurity (2-Methoxy-5-Nitrophenol) in

the column and good peak (Gaussian peak). This study was demonstrated the method was Linear accurate and precise at specification level and Quantitation level.

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