

# DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF PROCESS RELATED IMPURITIES IN DAPOXETINE HYDROCHLORIDE

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## ABSTRACT

A sensitive and stable High Performance liquid chromatographic method has been developed for the identification of three process related impurities viz impurity-A, impurity-B and impurity-C in Dapoxetine hydrochloride. The method was optimized based on the peak shape and resolution of impurity-A, impurity-B and impurity-C. Phenomenex Luna C18 column (250mm×4.6 mm, 5µm) was used for optimization of the method. The mobile phase for A pump consisted of 0.1% trifluoro acetic acid in water and for that of B pump it consisted of 0.1% trifluoro acetic acid in acetonitrile. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of quantitation limit (QL), detection limit (DL), linearity, precision, accuracy, specificity and robustness. The QL and DL values for impurity-A were found to be 0.02% and 0.005%, for that of impurity-B were found to be 0.028% and 0.009% and for that of impurity-C were found to be 0.048% and 0.016% respectively, with respect to sample concentration. The method was linear within the range of QL to 200% for all the three impurities. Thus, the newly developed method was found to be accurate, efficient and stable. The characterizations of these impurities were carried out for the confirmation of respective structures using nuclear magnetic resonance spectroscopy (NMR). A degradation study was also performed for Dapoxetine hydrochloride.

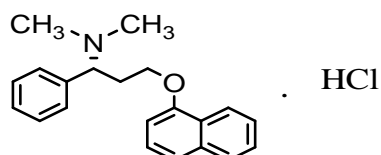
**Keywords:** Development; Validation; Dapoxetine hydrochloride; HPLC; Impurities.

## 1. INTRODUCTION

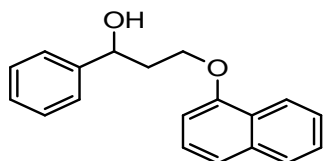
Dapoxetine hydrochloride is a selective short acting potent serotonin reuptake inhibitor (SSRI) Antidepressant proposed to be used for premature ejaculation. Utility for this indication was envisaged based on delayed ejaculation being a recognized side effect of the SSRI class in treatment of depression<sup>1</sup>. Dapoxetine hydrochloride is designated chemically as (S)-N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine with empirical formula of C<sub>21</sub>H<sub>23</sub>NO and molecular weight of 305.41<sup>2</sup>. Dapoxetine hydrochloride is a drug specifically developed

for the on-demand treatment of premature ejaculation. Premature ejaculation has been estimated to occur in 4-39% of men<sup>3,4-7</sup>. Dapoxetine hydrochloride is a SSRI with a short half-life developed specifically for the treatment of men with premature ejaculation,<sup>8-11</sup> however, slightly different from SSRIs Zoloft, Paxil and Prozac widely prescribed for depression and other psychiatric disorders such as bulimia or anxiety. Although use of antidepressant SSRIs such as fluoxetine, sertraline and paroxetine may increase the ejaculation latency time<sup>12-14</sup>. These SSRIs do not reach peak plasma concentration for

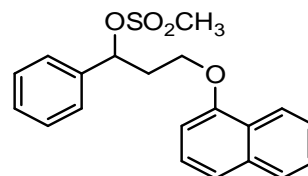
several hours after administration and may require a long lead in doing period for efficacy<sup>15</sup>. Very few methods are reviewed for Dapoxetine hydrochloride which reveals that High performance Liquid Chromatography method is described for the determination of Dapoxetine and its mono- and di-desmethyl metabolites in human plasma<sup>16</sup>. Development and validation of RP-HPLC method for the determination of Dapoxetine hydrochloride in pharmaceutical formulation using an experimental design<sup>17</sup>. Development and validation of dual wavelength UV spectrometric method for simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in their combined tablet dosage form<sup>18</sup>. Validated HPLC method for simultaneous quantitation of Tadalafil and Dapoxetine hydrochloride in bulk drug and formulation. Development and validation of RP-HPLC method for the estimation of Dapoxetine hydrochloride in tablet forms. Determination of Dapoxetine hydrochloride in bulk and in tablet dosage forms by RP-HPLC method. Highly selective, sensitive, rapid HPLC method for quantification of Dapoxetine hydrochloride in human plasma<sup>19-22</sup>. During the analysis of different Dapoxetine batches by HPLC-UV method, three unknown impurities were detected. Till date no report is available in literature regarding these impurities. Therefore, the present work was initiated to investigate the nature and origin of the impurities and characterized by NMR and Mass. The developed method was selective and specific for the determination of Dapoxetine hydrochloride related substances in Active Pharma Ingredient.



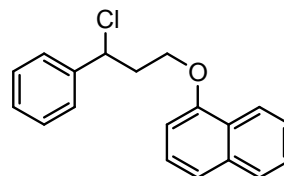
**Fig. 1: Dapoxetine Hydrochloride**



**Fig.2:3-(naphthalene-1-yloxy)-1-phenylpropan-1-ol (Impurity-A)**



**Fig. 3: 3-(naphthalene-1-yloxy)-1-phenylpropyl methanesulfonate. (Impurity-B)**



**Fig. 4: 1-(3-chloro-3-phenylpropoxy)naphthalene (Impurity-C)**

**Chemical structures of Dapoxetine hydrochloride and its process related impurities are shown in figures 1-4.**

## 2. EXPERIMENTAL

### 2.1 MATERIALS AND REAGENTS

HPLC grade Acetonitrile was purchased from Merck, water used was from a milli-Q purified system, Millipore, and trifluoroacetic acid was purchased from spectrochem. Hydrochloric acid, sodium hydroxide and hydrogen peroxide were also purchased from Merck. Dapoxetine hydrochloride of sample purity 99.94% were used in this study. Process related impurities, that is-Impurity-A, Impurity-B and Impurity-C were obtained from Chemical Research and Development Department, Troy Life Sciences, Bangalore.

### 2.2 Equipments and Chromatographic conditions

The HPLC system consisted of a Shimadzu prominence separate module LC-10AD equipped with PDA detector water Empower-2 software was used for the data acquisition and processing. Phenomenex Luna C18 (2) HPLC column of 250 mm X 4.6 mm id, 5 $\mu$ m particle size was used. The column was maintained at 25°C $\pm$ 2°C. The mobile phase for pump-A was 0.1% Trifluoroacetic acid in water and mobile phase for pump-B was 0.1% Trifluoroacetic acid in Acetonitrile. The flow rate was set at 1.0ml/min and UV detector 225 nm. The injection volume was 10 $\mu$ l for a sample concentration of 0.1%. The gradient elution was (T<sub>min</sub> A: B) T<sub>0</sub>65:35, T<sub>2</sub>65:35, T<sub>17</sub>35:65, T<sub>30</sub>15:85, T<sub>35</sub>15:85, T<sub>45</sub>35:65, T<sub>50</sub>65:35, T<sub>55</sub>65:35. The diluents used was Acetonitrile throughout the analysis.

### 2.3 Sample preparation for forced degradation studies

Stress degradation studies were performed as per ICH guidelines Q1 (R2) to demonstrate the stability indicating nature and specificity of the proposed method. About 50mg each of Dapoxetine hydrochloride sample was transferred into different 50ml volumetric flasks and subjected to forced degradation studies under acidic (1N HCl for 3h) and basic conditions (1N NaOH for 3h). The stressed samples of acid and base degradation were neutralized with NaOH and HCl respectively and made up to volume with diluents. Oxidative degradation was carried out using 30% hydrogen peroxide (80°C for 3h). Solid state stability of the drug substance was carried out by (a) thermal degradation at 105°C for 24h and (b) photolytic degradation was performed by keeping 1g of each solid sample in two separate loss on drying bottles (LOD bottles). In photo stability chamber, samples were exposed to get a minimum exposure of 1.2million lux hours for light and 200W h/m<sup>2</sup> for ultra violet region. Samples were withdrawn at appropriate time and subjected to LC analysis, using 1mg/ml concentration.

### 2.4 Preparation of stock solutions for method validation

A test preparation of 1mg/ml of Dapoxetine hydrochloride API sample was prepared by dissolving in diluent (Acetonitrile). A stock solution of impurities was prepared by dissolving 10mg of each impurity-A, impurity-B and impurity-C in diluent and made up to 10ml with diluent. 1ml of each individual stock solution was transferred into a 100ml volumetric flask and made up to volume with the diluent. From the above solution, 5ml of each individual impurity solution was transferred to 50ml volumetric flask and made up to volume with diluent. The standard solution of each impurity was prepared at 0.1% with respect to sample concentration (1mg/ml).

### 2.5 NMR spectroscopy

<sup>1</sup>H NMR spectra were recorded at 400 MHz, using Bruker 400MHz spectrometer (Bruker, Falladen, Switzerland) equipped with a 5 mm BBO probe and Z-gradient shim system. The <sup>1</sup>H spectra were recorded with 1s pulse repetition time using 30° flip angle. Samples were dissolved in deteriorated chloroform. The <sup>1</sup>H chemical shift values were reported on the  $\delta$  scale in ppm relative to TMS. All spectra were recorded with sample spinning.

### 2.6 Mass Spectrometry

The Mass spectrometry studies were performed on Agilent LC/MS Quadruple 6180 using electrospray ionization source, atmospheric pressure ionization. The typical electrospray source conditions were spray voltage, 5Kv, capillary voltage, 15-20V, heated capillary temperature 250°C, and tube lens offset voltage 20V, sheath gas (N<sub>2</sub>) pressure, 20 psi.

## 3. RESULTS AND DISCUSSION

### 3.1 Method development

The main criterion for the method development was to separate the three process related impurities, using HPLC. The difficulty was faced while separating the impurities and in obtaining good shape, better resolution and thus, to optimize an effective and sophisticated method. Several trials were carried out and different columns were used for developing a suitable method. For instance, when column Inertsil ODS with buffer consisting of sodium di-hydrogen orthophosphate, triethyl amine and pH-7.0 were used, the peak shape was unsatisfactory. The same was true when X-Terra RP 18 column and buffer containing ammonium acetate with pH-8.5 were used. When Phenomenex C 18column was used with buffer chosen to be tri-ethyl amine in water, with pH-4, failed to obtain desired resolution. However, when the buffer mobile phase-A was slightly altered by taking 1.0ml trifluoro acetic acid in 1000ml water and mobile phase-B with 1.0ml of Trifluoroacetic acid in 1000ml Acetonitrile with Phenomenex C18 column gave good peak shape, but significant resolution between impurity-B and impurity-C was not obtained. Optimum resolution between impurity-B and impurity-C were achieved after several trials for gradient profile, chromatographic conditions were finalized as described under section high performance liquid chromatographic conditions. In this method Impurity-A RRT was about 1.95, Impurity-B RRT was about 2.58 and impurity-C RRT was about 2.65. The response factor was found to be 0.93, 1.11 and 2.1 for impurity-A, impurity-B and impurity-C respectively.

Figure 5-7 depicts the experimental chromatograms of crude sample, QL solution and DL solution respectively.

### 3.2 RESULTS OF FORCED DEGRADATION STUDY

Dapoxetine hydrochloride was found to be stable in acidic, alkaline and oxidative stress conditions. Solid stress study confirmed that molecule is stable in thermal, photolytic

conditions. From the degradation studies and peak purity test results derived from PDA detector it was confirmed that the spectral purity of Dapoxetine hydrochloride peak was homogenous thus confirmed the stability indicating power of the newly developed method.

Figure 8-10 showing the experimental chromatograms of degradation studies and Table 1 shows the characterization data of impurities.

#### 4. METHOD VALIDATION

A newly developed and optimized method was validated for quantitation limit (QL), detection limit (DL), linearity, precision, accuracy, specificity and robustness as per ICH guidelines. Validation was carried out for all three process related impurities, viz. impurity-A, impurity-B and impurity-C. The level of impurities observed in crude sample was around 0.3%. Validation study was carried out for impurity-A, impurity-B and impurity-C. The selectivity was checked by injecting 1mg/ml of Dapoxetine hydrochloride solution containing 0.1% of all impurities monitored throughout the validation. Method validation results are summarized in table 2.

##### 4.1 Specificity

To demonstrate the specificity of HPLC method, in all the impurity spiked samples, the purity angle obtained for Dapoxetine hydrochloride and impurity peaks was less than purity threshold demonstrating spectral homogeneity. During this study impurity-A, impurity-B and impurity-C got well separated from each other and as well as from Dapoxetine hydrochloride which proved that the adopted method was specific.

##### 4.2 Accuracy and precision

The precision of the related substance method was checked by injecting six individual preparation of (1 mg/ml) Dapoxetine hydrochloride spiked with 0.1% impurities percentage RSD for peak areas of each impurity was calculated and study was also performed the same procedure on a different day. The intermediate precision of the method was also evaluated by a different analyst and different instrument in the same laboratory. Percentage RSD of areas of each impurity was less than 5.0, confirming good precision. Accuracy was validated through recovery experiments by spiking known amount of impurity (0.05%, 0.075%, 0.10%, 0.125% and 0.15%) with Dapoxetine hydrochloride with respect to sample concentration (1mg/ml).

Each parameter was analyzed in triplicates and percent recoveries were calculated.

##### 4.3 Linearity, RRF, Quantitation limit and detection limit

The linearity was established by measuring area responses for impurity-A, impurity-B and impurity-C, linearity ranging from QL to 200% (0.05%, 0.075%, 0.10%, 0.125%, 0.15% and 0.2%) with respect to sample concentration (1mg/ml). Seven concentrations were prepared across the range and injected in triplicates. The average area calculated was plotted against the concentration. The correlation co-efficient obtained was greater than 0.99 for all impurities. The results are presented in figures 11-13. The response factor for impurity-A was 0.93, impurity-B 1.11 and impurity-C was found to be 2.1. The quantitation limit (QL) and detection limit (DL) for Dapoxetine hydrochloride impurities were determined by signal to noise ratio method.

##### 4.4 Robustness and ruggedness

Experimental conditions were deliberately altered to determine the robustness of the method. Chromatographic conditions such as flow rate and column temperature were changed. Keen observations revealed that no significant changes were discovered when flow rate was changed (0.9 and 1.1 ml/min). Surprisingly, there was no drastic change even when column temperature was kept at 30°C. The resolution between impurity-B and impurity-C remained unaffected. The results are shown in table-3.

##### 4.5 Solution stability and mobile phase stability

Required analysis was carried out regarding solution stability and mobile phase stability. No significant changes were observed in both the solutions, also it was found to be stable up to 72hrs.

#### 5. CONCLUSION

Hence, method suggested was found to be simple, accurate, selective and equally sensitive. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is a stability indicating and can be conveniently used by quality control department to determine the related substances in regular Dapoxetine hydrochloride production samples.

#### 6. ACKNOWLEDGEMENT

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**Table 1: Comparative study of <sup>1</sup>H NMR**

Compound	Solvent	<sup>1</sup> H NMR
Impurity-A	CDCl <sub>3</sub>	d 6.7-8.2 (m, 12H Ar CH), 5.2 (t, 1H CH), 4.3 (q, 2H, CH <sub>2</sub> ), 2.4 (t, 2H, CH <sub>2</sub> )
Impurity-B	CDCl <sub>3</sub>	d 6.8-8.2 (m, 12H Ar CH), 3.7 (t, 2H CH <sub>2</sub> ), 4.3 (q, 1H, CH), 4.2 (q, 2H, CH <sub>2</sub> ), 2.3 (s, 3H, CH <sub>3</sub> )
Impurity-C	CDCl <sub>3</sub>	d 6.8-8.1 (m, 12H Ar CH), 4.8 (t, 1H CH), 3.9 (q, 2H, CH <sub>2</sub> ), 2.3 (t, 2H, CH <sub>2</sub> )

**Table 2: Method validation summary report**

System Suitability			
Parameter	Impurity-A	Impurity-B	Impurity-C
RT	26.347	34.715	35.652
RRT	1.95	2.56	2.65
Linearity (r)	0.9996	0.9992	0.9994
Response Factor	0.93	1.11	2.1
Quantitation limit (%)	0.02	0.028	0.048
Detection limit (%)	0.005	0.009	0.016
Precision at QL (RSD)	2.09	1.02	2.19
% Recovery at QL (n=3)	100.12	99.81	100.14
Accuracy (% Recovery)			
50%	98.51	100.92	94.55
75%	98.68	99.64	102.28
100%	99.82	100.83	99.23
125%	100.53	98.91	97.92
150%	100.31	99.57	99.16

number of determinations; RT, retention time; RRT, relative retention time; R<sup>2</sup>, correlation coefficient

**Table 3: Robustness of the LC method**

Validation Parameter	Resolution between Impurity-B and Impurity-C
Flow rate (ml/min)	
0.9	3.18
1.0	3.22
1.1	3.06
Column temperature (°C)	
25	3.36
30	2.88

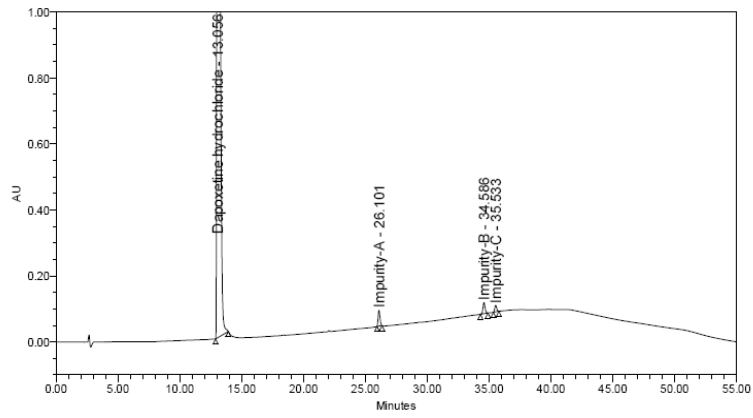


Fig. 5: Typical Chromatogram of Crude sample

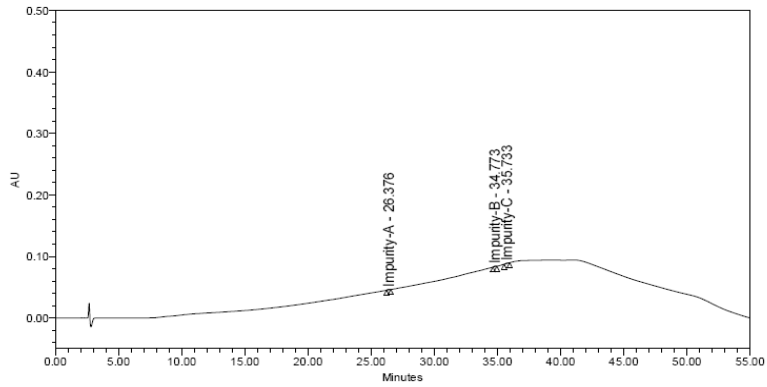


Fig. 6. Typical Chromatogram of QL Solution

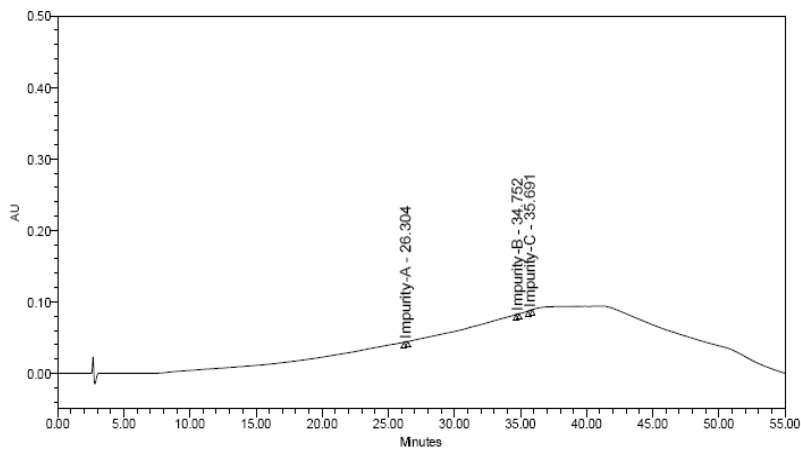


Fig. 7: Typical Chromatogram of DL Solution

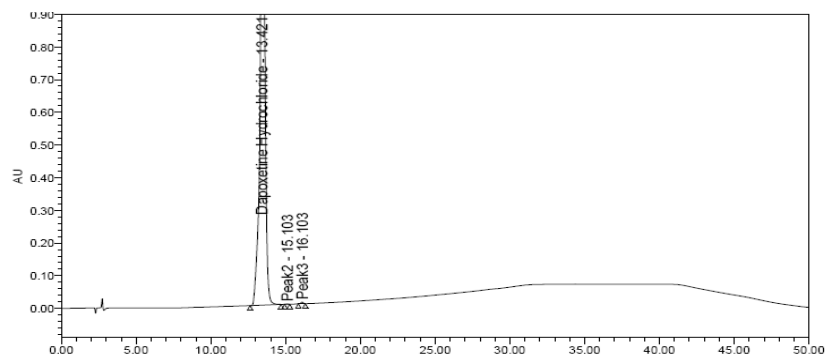


Fig. 8: Sample Chromatogram of acid hydrolysis (1.0N HCl)

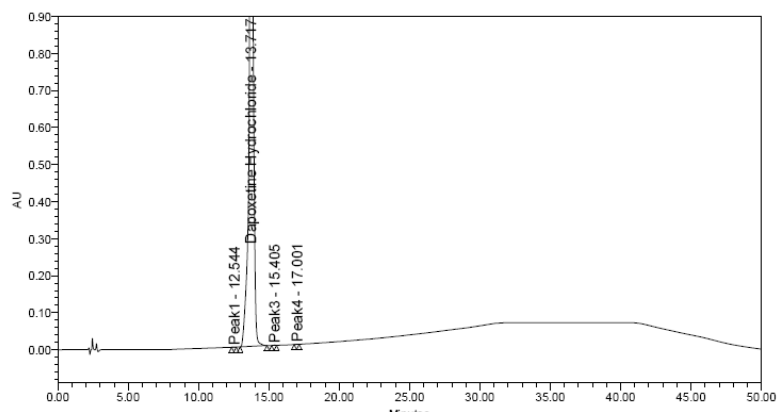


Fig. 9: Sample Chromatogram of base hydrolysis (1.0N NaOH)

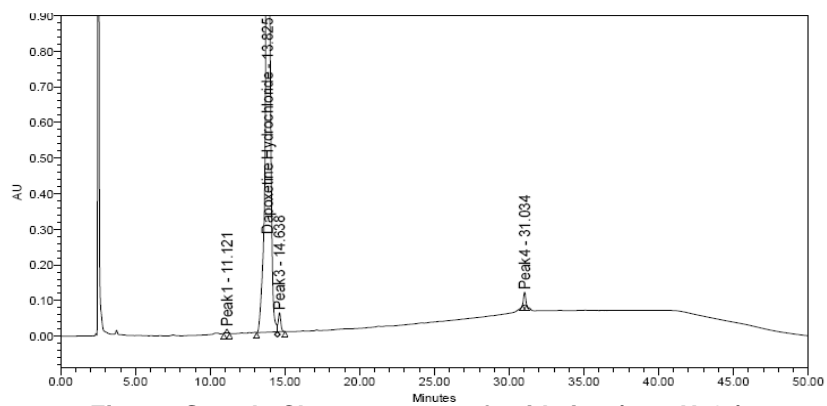


Fig. 10. Sample Chromatogram of oxidation (10% H<sub>2</sub>O<sub>2</sub>)

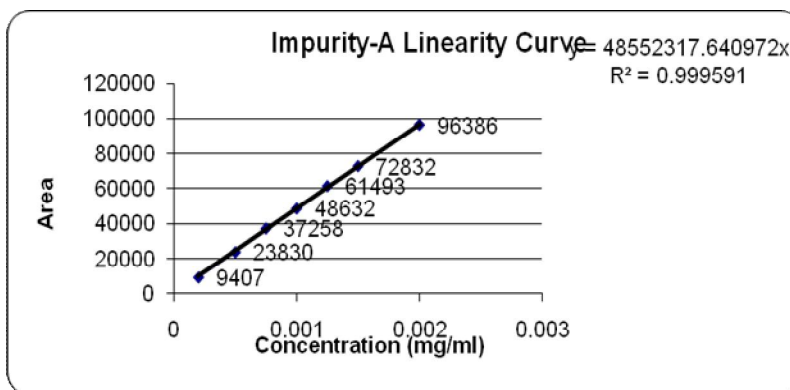


Fig. 11: Impurity-A Linearity curve

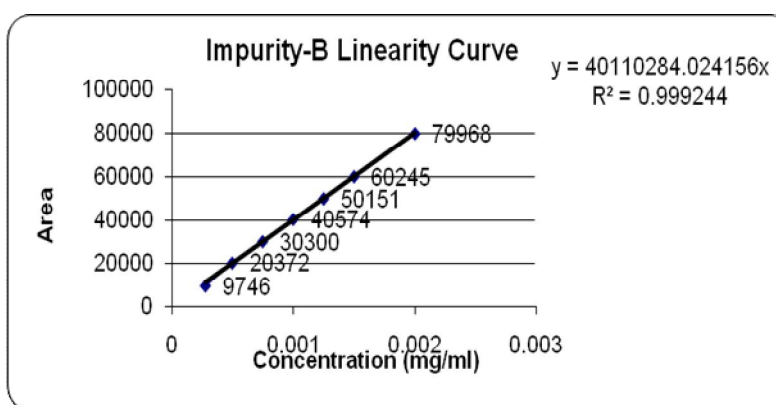


Fig. 12: Impurity-B Linearity curve

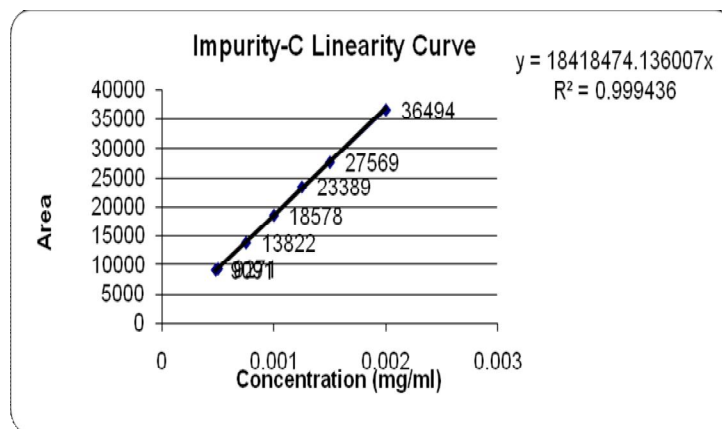


Fig. 13: Impurity-C Linearity curve



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