

# DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHODS BY LC-MS/MS FOR THE DETERMINATION OF PROCESS RELATED GENOTOXIC IMPURITIES IN LANSOPRAZOLE

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## ABSTRACT

A rapid and sensitive liquid chromatography-mass spectrometric (LC-MS/MS) quantification method has been developed and validated for the determination of genotoxic impurity in lansoprazole (LNZ) dosage form. Initially, the method was developed for the separation of genotoxic impurity of LNZ by HPLC. After successful separation of the impurities on HPLC, the method was transferred to LC-MS/MS to achieve the required sensitivity. The chromatographic separation was achieved on a Hypersil BDS C-18 (150 X 4.6 mm, 5 $\mu$ m) column using mobile phase containing buffer (0.01M Ammonium acetate), Acetonitrile and methanol in 50:45:5 v/v ratio. Methanol was used as diluent. The flow rate was 0.8mL min<sup>-1</sup> and elution was monitored at 205 nm. The mass of 2-Chloromethyl-3-methyl-4- (2,2,2-trifluoroethoxy) pyridine Hydrochloride was m/z 240(M+H<sup>+</sup>) with electrospray ionization with positive mode with ion spray voltage is 4500 volts. Lansoprazole and 2-Chloromethyl-3-methyl-4- (2,2,2-trifluoroethoxy) pyridine Hydrochloride elution order was observed from total ion chromatogram in scan mode. Validation was done in selective ion monitoring (SIM) mode. The calibration curves obtained were linear (R<sup>2</sup>-0.9984) over the concentration range of 0.90- 2.40  $\mu$ g/mL. The overall average recovery was found to be 97.6-98.2%. The developed method was validated with respect to specificity, LOD, LOQ, linearity, accuracy, precision as per ICH guidelines.

**Keywords:** Lansoprazole, Liquid Chromatography, HPLC, LC-MS/MS, SIM, Potential Genotoxic Impurity, Identification.

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## INTRODUCTION

Lansoprazole, chemically describes 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-enzimidazole, is an important proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the gastric (H<sup>+</sup>, K<sup>+</sup>) ATPase enzyme system. Lansoprazole is effective in the treatment of various peptic diseases, including gastric and duodenal ulcer and reflux esophagitis, Zollinger- Ellison syndrome and functional dyspepsia.<sup>1-5</sup>

In the synthesis of Lansoprazole (**1**), 2-Chloromethyl-3-methyl-4- (2,2,2trifluoroethoxy) pyridine Hydrochloride (**2**) is key and important intermediate. The definition of genotoxicity is broad and includes both direct and indirect effects on DNA.<sup>6</sup>

Genotoxic impurities could cause somatic mutations, carcinogenic or cause cancer on humans.<sup>7-8</sup> As per the regulatory guidelines, a threshold of toxicological concern (TTC) value of 1.5 $\mu$ g day<sup>-1</sup> in the taking of genotoxic impurities are permitted in the drug product.

Based on the literature of the survey, there were several analytical methods for the determination of genotoxic impurities in lansoprazole limit of detection was 0.9 ppm. These include; High-performance liquid chromatography (HPLC), Electrochemical and spectrophotometric methods.<sup>9-13</sup>

The literature reports a few spectrophotometric methods for the quantitation of genotoxic impurities. Some of the reported HPLC methods need sophisticated instruments or do not describe analytical parameters that are very important for the validation of analytical procedure such as accuracy, specificity, linearity, the limit of detection (LOD) limit of quantitation (LOQ). It is important to develop and validate analytical methods for its determination of genotoxic impurities.

The objective of the present study to develop highly sensitive, selective and accurate method development on LC-MS/MS technique to get good resolution between Lansoprazole and genotoxic impurity. The developed method was validated in terms of specificity, the limit of detection (LOD), the limit of quantitation (LOQ), precision, linearity, and accuracy as per ICH guidelines.<sup>14</sup>

Lansoprazole empirical formula is  $C_{16}H_{14}F_3N_3O_2S$  with a molecular weight of 369.37 and the chemical name is (*RS*)-2-([3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl)-1*H*-benzo[*d*]imidazole.<sup>15</sup>

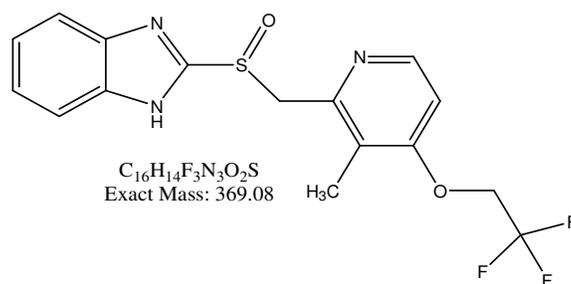


Fig.-1: Chemical Structure of Lansoprazole

The chemical formula of Genotoxic impurity of Lansoprazole is  $C_9H_{10}Cl_2F_3NO$ , Molecular weight: 276.08 and the chemical name is 2-Chloromethyl-3-methyl-4-(2,2,2-trifluoroethoxy) pyridine Hydrochloride.

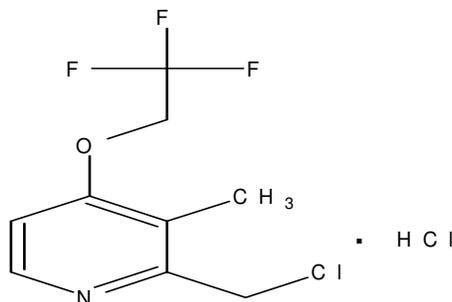


Fig.-2: Genotoxic impurity of Lansoprazole

## EXPERIMENTAL

### Chemicals and Materials

Lansoprazole and 2-Chloromethyl-3-methyl-4-(2,2,2-trifluoroethoxy) pyridine Hydrochloride impurity were received from the process research department of Matrix laboratories ltd., Hyderabad, India. Ammonium acetate, Triethylamine, Acetonitrile, and methanol were purchased from Merck Chemicals (Mumbai, India).

### Equipment

An Agilent 1200 LC system coupled with an API 3000 system (Applied Biosystems, Canada) with analyst software, version 1.6.2. LC.

### Preparation of Standard Solution

Weighed 10.0 mg of 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride into a 100 mL volumetric flask and diluted to volume with diluent. Dilute 1.0 mL of this solution into a 100 mL

volumetric flask and diluted to volume with diluent. Further diluted 0.9 mL of this solution to 100 mL with diluent.

### Preparation of Sample Solution

Weighed about 100 mg of sample into a 10 mL volumetric flask, and diluted volume with diluents and made up to volume with diluent and mixed well.

### LC-MS/MS Conditions

Method Validation is carried out on an API 3000 LC-MS/MS system (Applied Biosystems, Canada) with analyst software version 1.6.2. Lansoprazole and 2-Chloromethyl-3-methyl-4- (2,2,2-trifluoroethoxy) pyridine Hydrochloride impurity were separated on a Hypersil BDS C-18 (150 X 4.6 mm, 5 $\mu$ m) column using mobile phase containing buffer (0.01M Ammonium acetate), Acetonitrile and methanol in 50:45:5 v/v ratio. Methanol was used as diluent. The flow rate was 0.8 mL min<sup>-1</sup> and elution was monitored at 205 nm. The injection volume for the sample was kept 10 $\mu$ L and the run time was 10 min. The electrospray ionization with positive mode was used for LC-MS/MS with ion spray voltage is 4500 volts. Lansoprazole and 2-Chloromethyl-3-methyl-4- (2,2,2-trifluoroethoxy) pyridine Hydrochloride impurity elution order was observed from total ion chromatogram in scan mode. The mass spectrum of 2-Chloromethyl-3-methyl-4- (2,2,2-trifluoroethoxy) pyridine Hydrochloride impurity a parent peak at m/z 240(M+H<sup>+</sup>). Validation was done in selective ion monitoring (SIM) mode. Representative selective ion chromatogram was presented in Fig.-3.

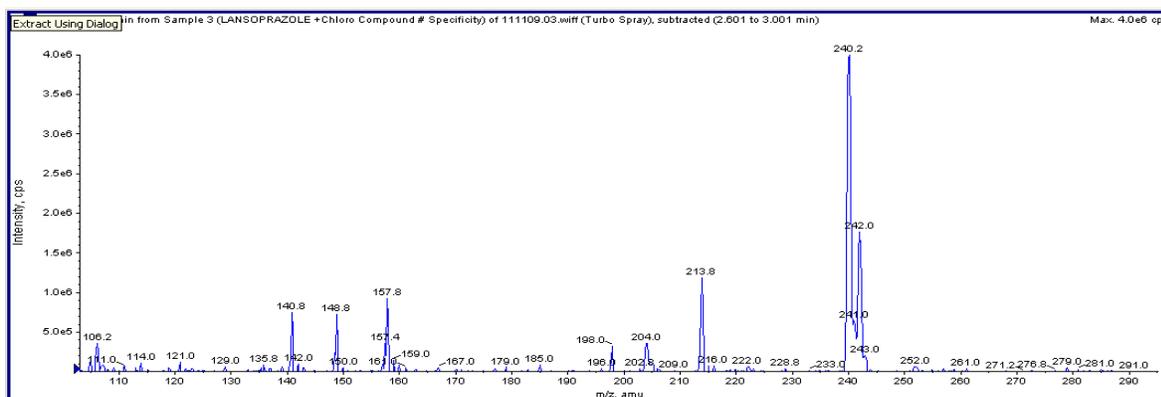


Fig.-3: Selective Ion Chromatogram of 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride

## RESULTS AND DISCUSSION

### LC-MS/MS Method Development

Method development trials were carried out on the HPLC technique consist of UV detector. Method development trials started with various buffer solutions such as monobasic, dibasic phosphate with acetonitrile and methanol as mobile phase in isocratic and gradient modes. Screened various stationary phase columns such as Octadecylsilane (C18), Phenyl (-NH<sub>2</sub>) and Cyano (CN). After several trials failed to get the sensitivity, selectivity and detection level of the genotoxic impurity, The technique was changed from UV detector to mass detector to obtain desired detection level and sensitivity. Further method development trials carried out on LC-MS/MS technique. Applied various buffer solutions as mobile phases such as Trifluoroacetic acid, Formic acid, Ammonia and Ammonium acetate with organic solvents such as methanol and acetonitrile. The analysis was carried out with various stationary phases such as Octadecylsilane (C18), Phenyl (-NH<sub>2</sub>) and Cyano (CN) and Dodecyl silane (C8) to get sensitivity and lower detection level. Based on method development trials method conditions and sample concentrations were optimized. The separation was achieved finally on Hypersil BDS C-18 (150 X 4.6 mm, 5 $\mu$ m) column using mobile phase containing buffer (0.01M Ammonium acetate), Acetonitrile and methanol in 50:45:5 v/v ratio. Methanol was used as diluent. The flow rate was 0.8 mL min<sup>-1</sup> and elution was monitored at 205 nm. The injection volume for the sample was kept 10 $\mu$ L and the run time was 10 min.

### Specificity

2-chloro methyl-3-methyl-4-(2,2,2-trifluoro.ethoxy)pyridine hydrochloride impurity and Lansoprazole was freshly prepared at a concentration of 0.1mg/ml in diluent. In this method 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride impurity was monitored by its molecular ion  $m/z$  240 (Protonated), which is specific fragment ion for 2- chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride. Lansoprazole was monitored by its molecular ion 370 (Protonated). Table 1 summarizes the retention time (RT) values and mass number ( $m/z$ ) values obtained 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride and a representative selective ion chromatogram was presented in Fig.-4.

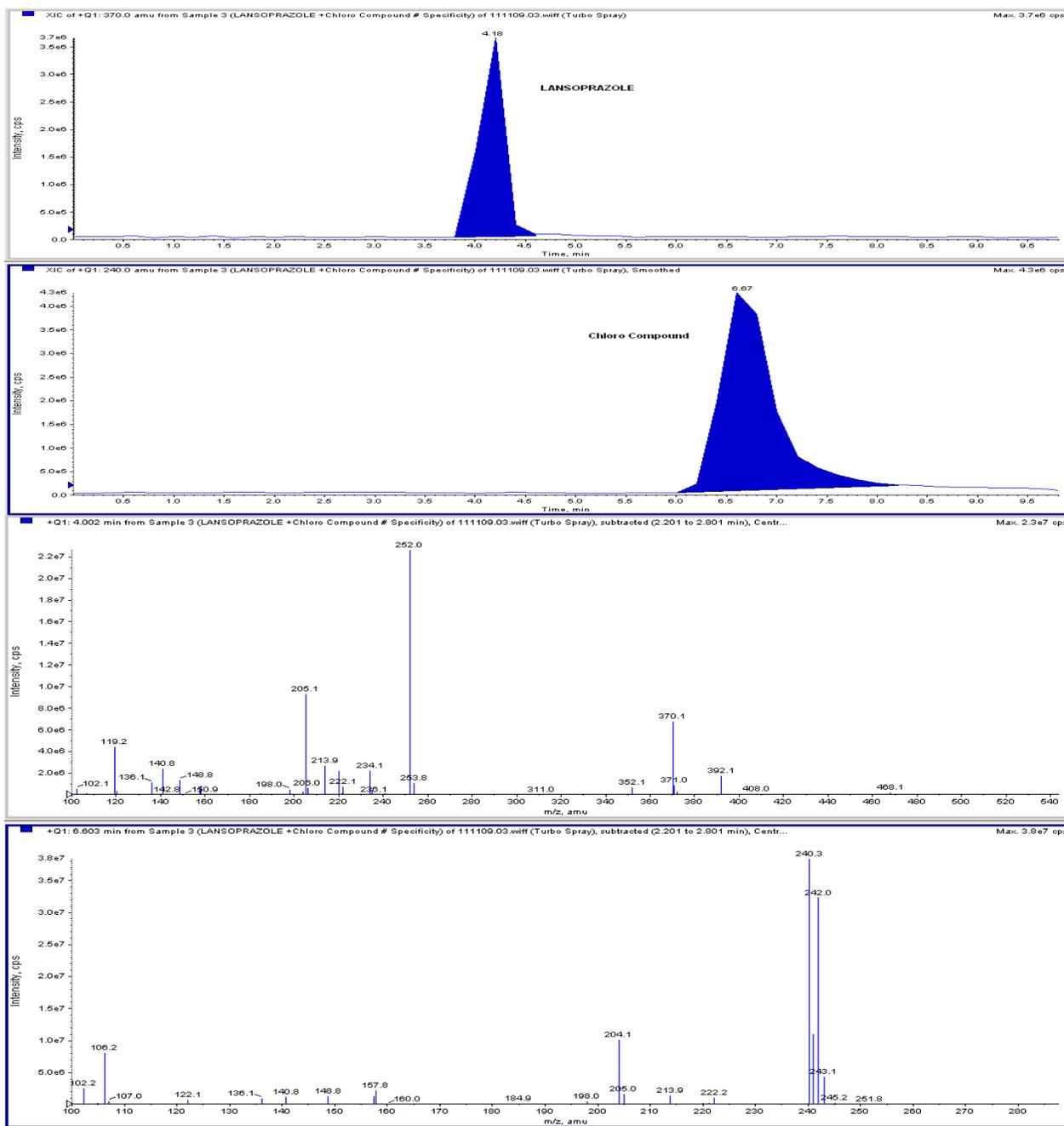


Fig.-4: Selective Ion Chromatogram of Lansoprazole and 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride

Table-1: Summary of Retention Time Values for 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride and Lansoprazole.

Name of the Component	Retention time (minutes)	m/z (Protonated)
2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride	6.73	240
Lansoprazole	4.13	370

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

To establish the limit of detection (LOD) and limit of quantitation (LOQ) of 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride impurity was prepared 5 ppm solution with respect to sample concentration (Lansoprazole) was injected. Based on the signal to noise ratio of impurity a series of solutions prepared to achieve the signal to noise ratios (S/N) of 3:1 and 10:1 respectively. The results are summarized in Table-2 and the representative extracted ion spectrum was presented in Fig.-5 and Fig.-6.

Table-2; Summary of Limit of Detection and Limit of Quantitation Results for 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride.

Name of the impurity	Test parameters	Results
2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride	LOD	0.1 ppm
	LOQ	0.3 ppm

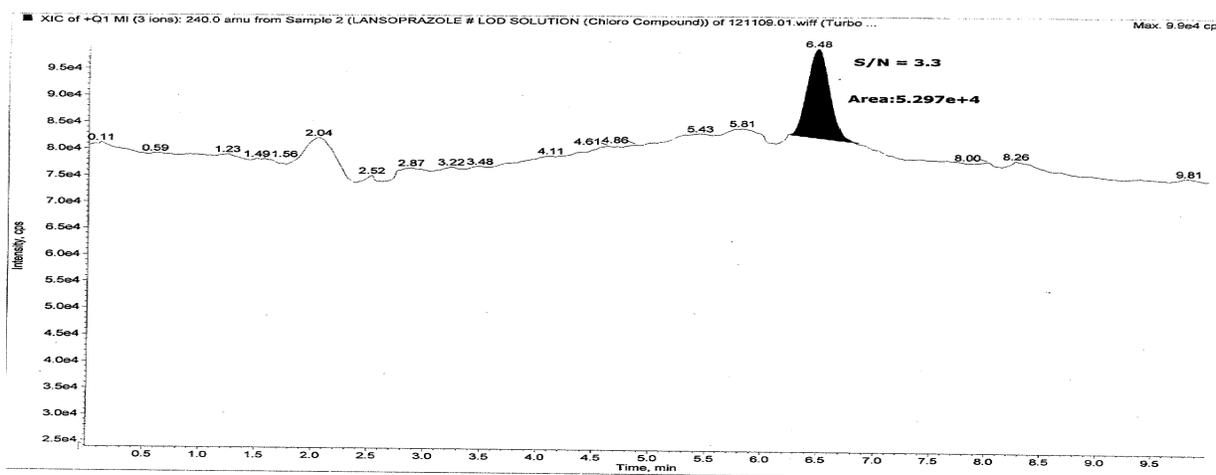


Fig.- 5: LOD of 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride

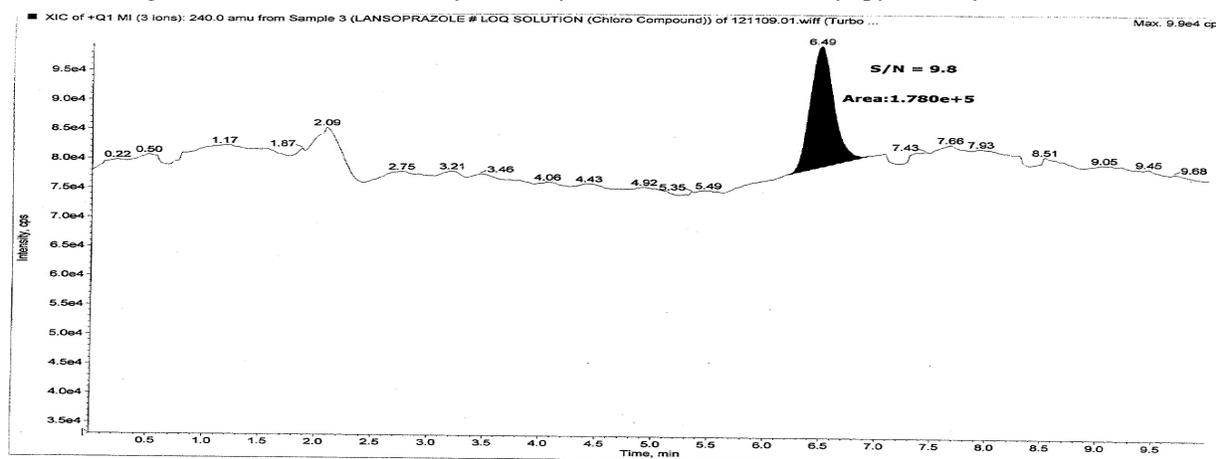


Fig.-6: LOQ of 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride.

### Precision at LOQ

The precision at LOQ of 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride impurity was evaluated by injecting LOQ solution six times into system. The %RSD of impurity peak areas were found to be 1.9.

### Linearity

To demonstrate the linearity of detector response for 2-chloro methyl-3-methyl-4-(2, 2, 2-trifluoro ethoxy) pyridine hydrochloride impurity, injected the solutions with concentrations ranging from LOQ level to 2.40ppm. Plot a graph of concentrations on X-axis versus peak areas on Y-axis and determined the slope, Intercept, Multiple R, R square from linearity graph. The corresponding results and linearity graph were summarized in Table-3 and Fig.-7.

Table-3: Linearity Data for 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride.

Parameter	Result
Slope	195066.6
Intercept	-4160.0
Multiple R	0.9992
R Square	0.9984

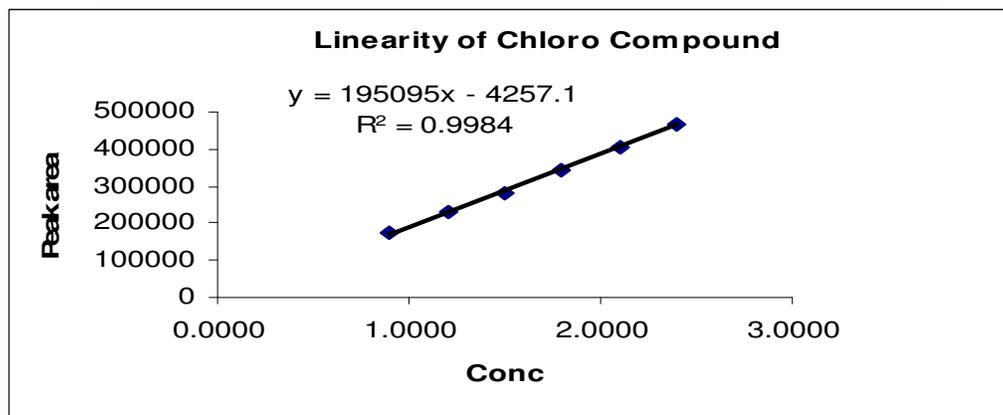


Fig.-7: Linearity Graph for 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoroethoxy) pyridine hydrochloride.

### Accuracy

The accuracy at the limit of Quantitation was determined by analyzing a solution containing Lansoprazole spiked with 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride impurity at LOQ level of the working strength of API. The percentage recovery obtained for the 2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride impurity. The results are summarized in Table-4.

Table-4: Percentage Recovery 2-Chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride at LOQ Level

Name of the impurity	Theoretical Conc. in ppm	Measured Conc. in ppm	% Recovery
2-chloro methyl-3-methyl-4-(2,2,2-trifluoro ethoxy) pyridine hydrochloride	0.3000	0.2880	97.6
	0.3000	0.2897	98.0
	0.3000	0.2939	98.2

### Sample Analysis

In order to verify the sensitivity and specificity of this method in a real-time situation, the present method was used to quantitate the Genotoxic impurity of lansoprazole using the lansoprazole 30mg tablet and the impurity was quantitated at 0.3 ppm.

## CONCLUSION

The results presented in this paper demonstrate that the successful development and validation of a simple, sensitive and selective LC-MS/MS method for the determination of 2-Chloromethyl-3-methyl-4-(2,2,2-trifluoroethoxy) pyridine. This method is highly sensitive (LOQ: 0.3ppm). Moreover, the total analysis time 10.0 min is the shortest. Thus, the advantage of this method is that a relative number of samples can be analyzed in a short time, thus increasing the output (i.e 6 samples in 1h). From the results of all the validation parameters, it can conclude that The method can be applied for routine samples analysis to quantitate the Genotoxic impurity of lansoprazole.

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