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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF STRUCTURE RELATED GENOTOXIC IMPURITIES IN OMEPRAZOLE DRUG BY LC-MS/MS

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ABSTRACT

A simple, sensitive and accurate gradient reversed-phase LC-MS/MS method has been developed for the Determination of structure related impurities namely 2-chloro methyl -3,5-dimethyl-4-methoxy pyridine Hcl (CDMP), 4-methoxy-3,5-dimethyl-4-nitro pyridine-1-oxide (DNO) and 4-methoxy-2-amino aniline (MAA) in Omeprazole (API). These are Potential genotoxic impurities and hence need to be controlled in Omeprazole (API) before formulating. The analysis was Performed using SHIMADZU LC-MS/MS (tandem mass spectrometer) 8030 C_{18} column (YMC PACK PRO, 100×4.6, 3 µm) as a stationary phase with Column oven temperature ambient and PDA detection at 254nm. The separation was achieved using mobile phase Buffer (0.1%formic acid) pH 4: Methanol. The method was optimized based on the peak shapes and by comparing Retention time between 2-chloro methyl -3, 5-dimethyl-4-methoxy pyridine Hcl (CDMP), 4-methoxy-3, 5-dimethyl-4-nitro pyridine-1-oxide (DNO), 4-methoxy-2-amino aniline (MAA) and Omeprazole. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of linearity, precision, accuracy and specificity. The LOD and LOQ values were found to be 1.8µg/ml and 3.1µg/ml, respectively. The sample Concentration injected was 1mg/ml. The method is linear within the range of 3.1-18.6µg/ml for the Impurities.

KEYWORDS: Genotoxic impurities, Omeprazole, CDMP, DNO, MAA.

INTRODUCTION

An impurity in a drug substance as defined by the International Conference on Harmonization (ICH) Guidelines is any component of the drug substance that is not the chemical entity defined as the drug substance and affects the purity of active ingredient or drug substances. Similarly, an impurity in a drug product is any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product. Therefore any extraneous material present in the drug substance has to be considered an impurity even if it is totally inert or has superior pharmacological properties. The impurity profile of pharmaceuticals is of increasing importance as drug safety receives more and more attention from the public and from the media. Impurities could be forming from the impact of heat, light, and oxidants (including air) on the drug product and might be catalyzed or accelerated by trace metal impurities, changes in the pH of the formulation, interactions with packaging components, excipient and other active ingredients, in the case of combination products. Therefore, identification, quantification, and control of impurities in the drug substance and drug product, are an important part of drug development and regulatory assessment. (S. Lakshmana Prabu et al., 2010).

Genotoxic impurities

Genotoxic impurities induce genetic mutations, chromosomal rearrangements, chromosomal breaks and act as carcinogenic compounds .Genotoxicity deals with mutagenesis, carcinogenesis, and teratogensis. Impurities present in active pharmaceutical ingredients responsible for deleterious action on a cell's genetic material affecting its integrity. Therefore, exposure to even low levels of such impurities present in final active pharmaceutical ingredient (API) may be of significant toxicological concern. These compounds cause damage to DNA by different mechanism such as alkylation or other interactions that can lead to mutation of the genetic codes. Thus, the term "Genotoxic" is applied to those agents that interact with DNA and its associated cellular components (e.g. the spindle apparatus) or enzymes (e.g. Topoisomerases) (Usha Yadav et al., 2013)

- **Regulatory aspects**

The assessment of Genotoxic impurities and determination of acceptable limits for such impurities is difficult in active substances. The EMEA guideline recognizes the limitations and proposes the use of a "Threshold of toxicological concern" (TTC) for Genotoxic impurities. In EMEA guidelines, a threshold of toxicological concern (TTC) has been developed to define the

exposure level of any unstudied chemical that will not pose a risk of significant carcinogenicity or other toxic effects TTC originally developed as a Impurities can be analyzed by the following instruments "Threshold of regulation" at the FDA for food materials was established .Based on the analysis of 343 carcinogens from carcinogenic potency database. The TTC value was estimated to be 1.5µg/person/day. The concentration limit (in ppm) of Genotoxic impurity in drug substance derived from the TTC can be calculated based on the expected daily dose to the patient using equation:

Concentration limit (in ppm) =TTC (1.5µg day) /dose (g day)

Genotoxic Impurities can be analyzed by the following instruments

- High Performance liquid chromatography (HPLC).
- Gas chromatography (GC).
- UHPLC coupled with mass chromatography (LC/MS).
- UHPLC coupled with tandem mass chromatography (LC/MS/MS).
- Gas chromatography coupled with mass chromatography (GS/MS).
- Gas chromatography coupled with tandem mass chromatography (GC/MS/MS).
- Inductive couple plasma coupled with mass chromatography (ICP-MS).
- Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES).
- Nuclear Magnetic Resonance (NMR) Spectroscopy.
- Ion-Exchange coupled with mass chromatography (IE-MS)

Analysis of genotoxic impurities can be very challenging because they must be controlled at levels significantly lower than 0.01–0.03 %. Ideally, the analytical procedure should allow detection limits in the range of 1 to 5 ppm (0.0001–0.0005 % w/w). Such low levels require not only more sensitive analytical instruments, but also place higher demands on selectivity because a higher number of other organic impurities may be present at lower concentration ranges, e.g., from excipient.

The relatively large amount of API may also interfere with low-level impurities .so this low level genotoxic impurities can be analyzed by using LC-MS/MS which on comparing with other spectroscopy techniques very economic, accurate and sensitive.

MATERIAL AND METHODS

A. Drug and reagents

Formic acid and Methanol from Merck (Mumbai, India) , Water for UPLC studies was obtained Milli Q Milliporewater, pure form of Omeprazole(API) from hetero pharmaceuticals, Standards of (2-chloro methyl -3,5-dimethyl-4-methoxy pyridine Hcl (CDMP), 4-methoxy-3,5-dimethyl-4-nitro pyridine-1-oxide (DNO), 4-methoxy-2-amino aniline (MAA)) from hetero pharmaceuticals

B. Apparatus and equipment

Lc was carried out on SHIMADZU (LC/MS/MS 8030) Lab solutions software. Separations were achieved on YMC PACK PRO C18, 100×4.6, 3 µm. A ph/Ion analyzer (Labindia, made in) was used to check and adjust the ph of buffer solutions. Other. Small equipment were PCI sonicator (22L500/CC/DTC made in), precision analytical Balance (MX5, Mettler Toledo, Schwerzenbach, Switzerland).

C. Preparation of mobile phase

0.1 % of formic acid was dissolved in 1000ml HPLC grade water and adjusted to pH-4.

D. Chromatographic conditions

The analysis was Performed using SHIMADZU LC-MS/MS (tandem mass spectrometer) 8030 C_{18} column (YMC PACK PRO, 100×4.6, 3 µm) as a stationary phase with Column oven temperature ambient and PDA detection at 254nm. The separation was achieved using mobile phase Buffer (0.1%formic acid) pH 4: Methanol.

RESULTS AND DISCUSSION

ANALYTICAL METHOD DEVELOPMENT

Solubility of sample was done by different solvents (water, methanol, Acetonitrile) and the solubility of compound was determined as freely soluble in methanol.

Initially method development work was started by taking mass spectra from 10 -2000 of standard impurities. By observing the spectra of standard solutions channel no, 186,168, 138 (m/z) were taken and used for trials to develop a method.

From the above experiment it was found that three impurities CDMP, DNO and MAA can effectively be analyzed by using the LC-MS/MS method with buffer (pH 4), methanol with gradient program at a flow rate of 0.3ml/minute and detection wavelength of 254nm. The

retention times of the standard impurities were found to be 3.406, 14.66 and 11.786 minutes respectively.

ANALYTICAL METHOD VALIDATION

SYSTEM SUITABILITY /SYSTEM PRECISION

Accurately weighed and transfer about 10mg of MAA, CDMP, and DNO standards into a 20 ml of volumetric flask dissolved and diluted to volume with diluent and mix well. Transferred 0.2ml of above solution into a 10 ml volumetric flask dissolved and diluted to volume with diluent and mix well. Transferred 0.2 ml of above solution into a 20 ml volumetric flask dissolved and diluted to volume with diluent and mix well. Transferred 2.5 ml of MAA, CDMP, DNO standard stock solutions into 20ml volumetric flask and diluted to volume with diluent and mix well.

Preparation of test solution

Accurately weighed and transferred about 5mg of test sample into a 5ml volumetric flask, dissolved and diluted to the volume with diluent and mix well.

SPECIFICITY

Preparation of standard solution

Transferred 2.5 ml of MAA, CDMP, DNO standard stock solutions into 20ml volumetric flask and diluted to volume with diluent and mix well.

Preparation of blend solution

Accurately weighed and transferred about 5mg of test sample into a 5ml volumetric flask, dissolved and diluted to the volume with diluent and mix well.

ACCURACY

Accuracy was Performed by spiking the sample with known concentration of MAA, CDMP, and DNO specified in the method at four levels, i.e. LOQ, 50, 100, &150% with respect to sample concentration .calculate the contents of MAA, CDMP, and DNO With corrected contents of MAA, CDMP, and DNO in sample and determined the percentage recovery.

Linearity

Linearity is expressed in terms of variance around the slope of the regression line calculated in accordance to establish mathematical relationship between the test results obtained by the analysis of 3 standards solutions with varying concentration. Perform the analysis with different

concentrations (6 levels) i.e. LOQ, 50%, 75%, 100%,125% and 150% ,perform the linearity level- 1,2,3,4,5,6 solutions and calculate the correlation coefficient.

INTERMEDIATE PRECISION

Intermediate precision was carried out on different day, with different analyst, different instrument and with different column with fresh preparations.

LIMIT OF DETECTION (LOD)

Based on signal to noise ratio obtained from standard solution derive LOD concentration for 3 standards which will yield a signal to noise ratio about **3:1**, injected LOD standard solution.

LIMIT OF QUANTIFICATION (LOQ)

Based on signal to noise ratio obtained from standard solution derive LOD concentration for 3 standards which will yield a signal to noise ratio about **10:1**, injected LOD standard solution.

SAMPLE PREPARATION OF OMEPRAZOLE FOR BATCH ANALYSIS

Weighed 10mg of Omeprazole sample in 10ml volumetric flask, dissolved in methanol and diluted up the volume with methanol. Filter the solution using 0.45µ syringe filter. Injected solution to LC-MS/MS for the determination of impurities present in the sample. The batch of Omeprazole was analyzed under developed condition.

Blank

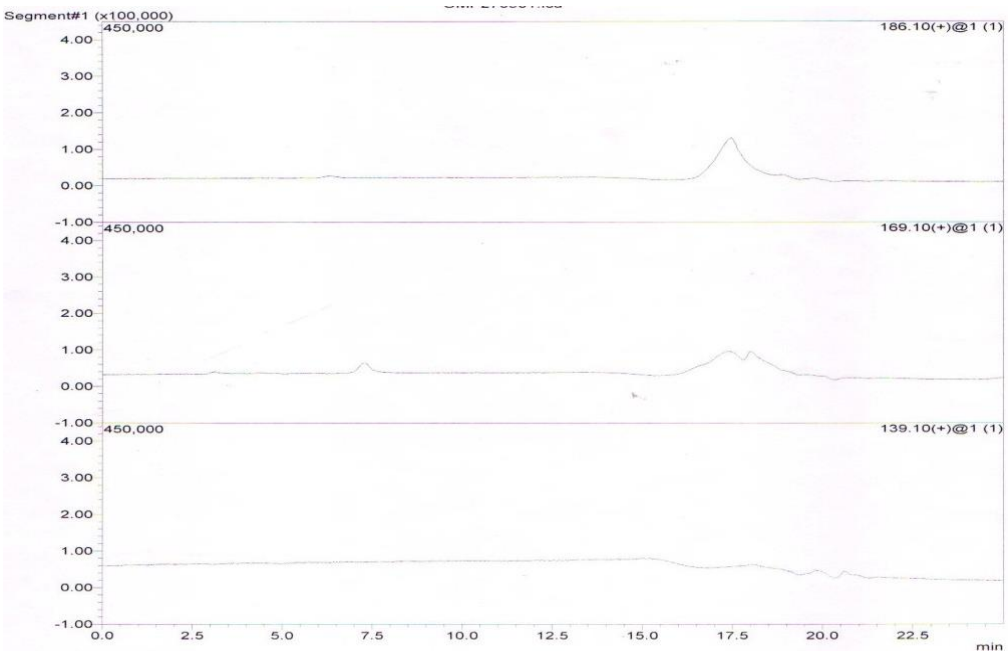
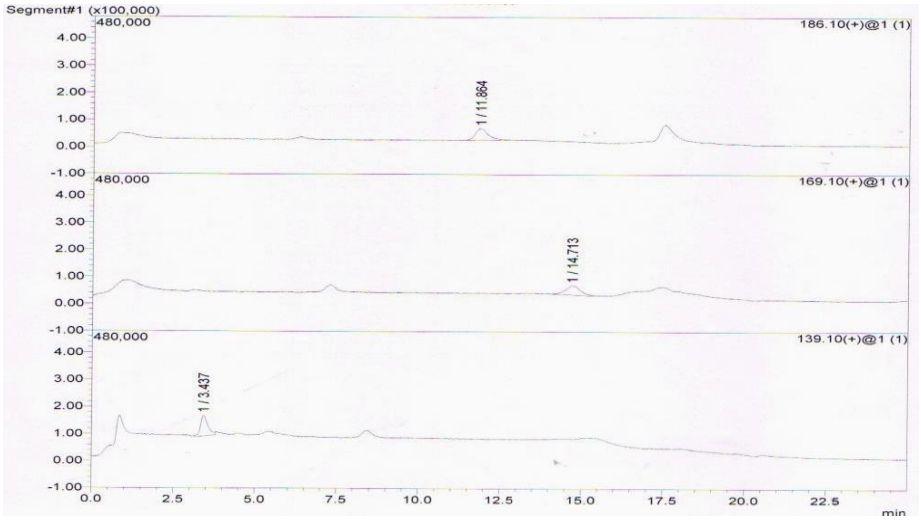


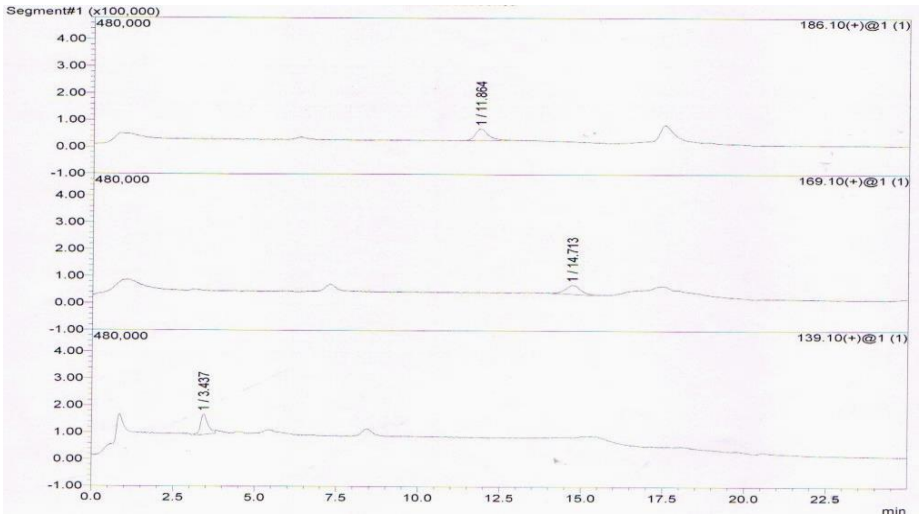
Fig no 11: Blank Chromatogra

METHOD VALIDATION

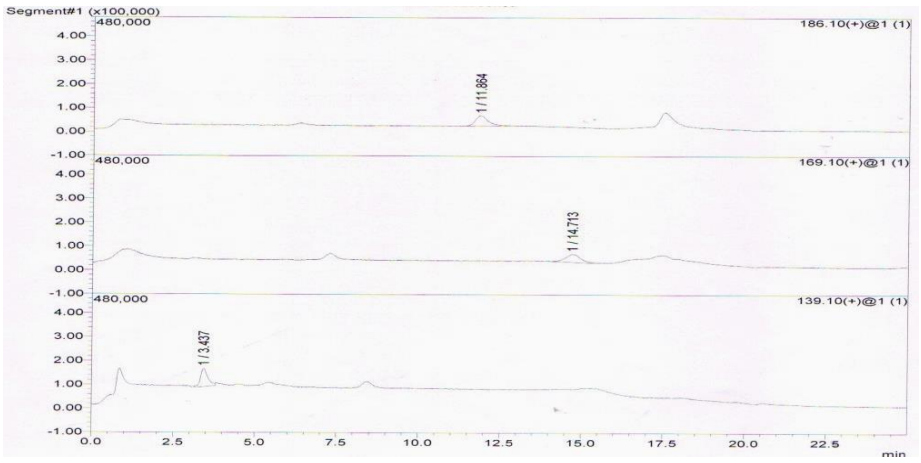
SYSTEM PRECISION



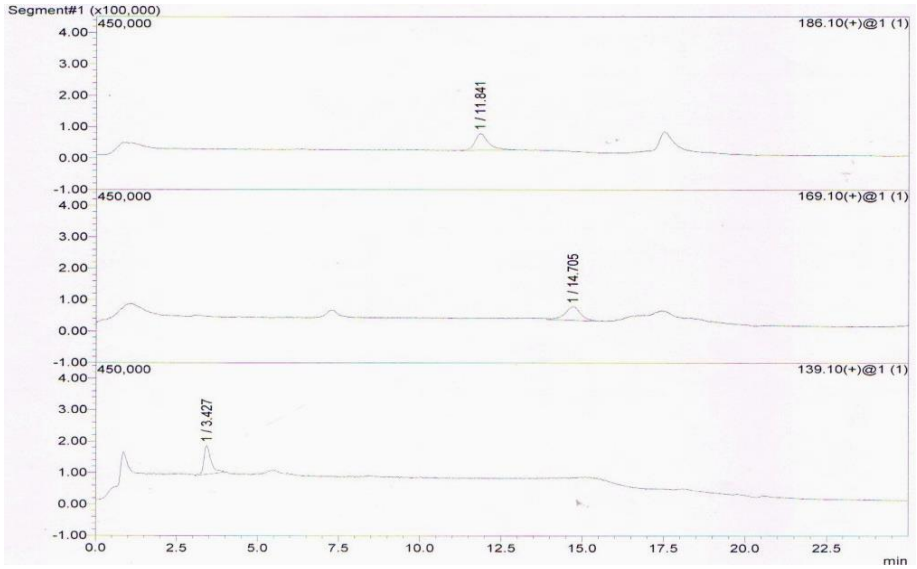
System Precision Chromatogram -1



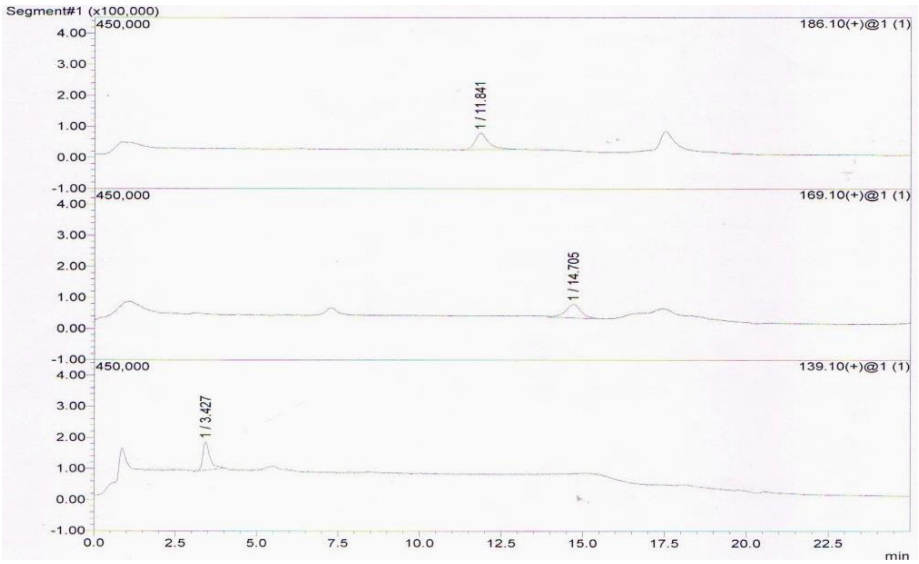
System Precision Chromatogram-2



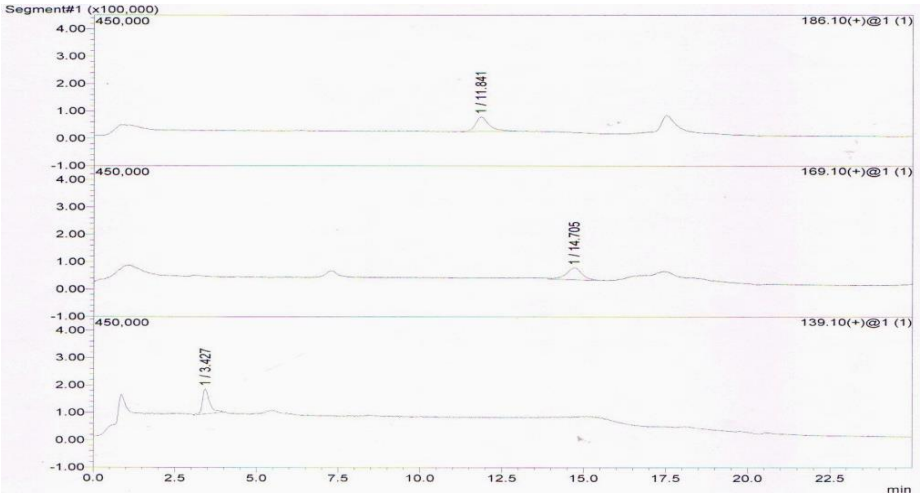
System Precision Chromatogram -3



System Precision Chromatogram -4

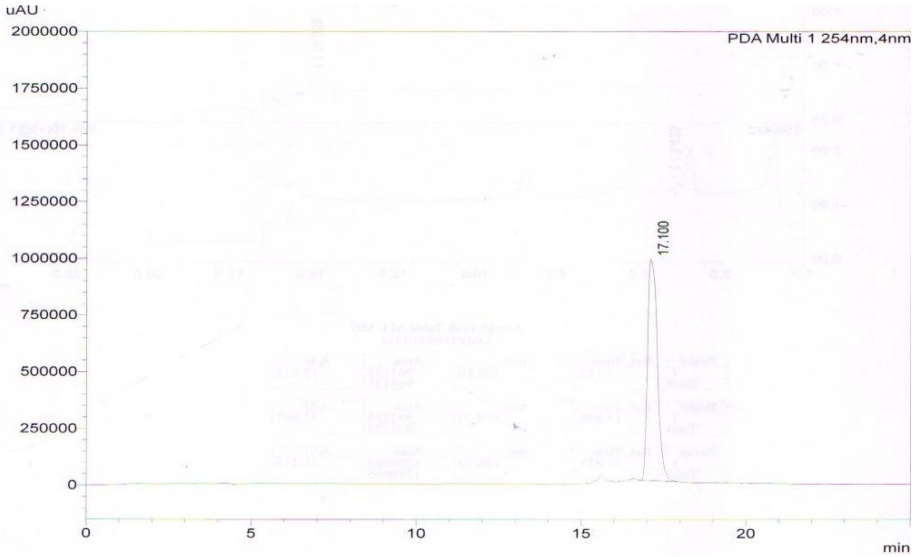


System Precision Chromatogram -5



Fig

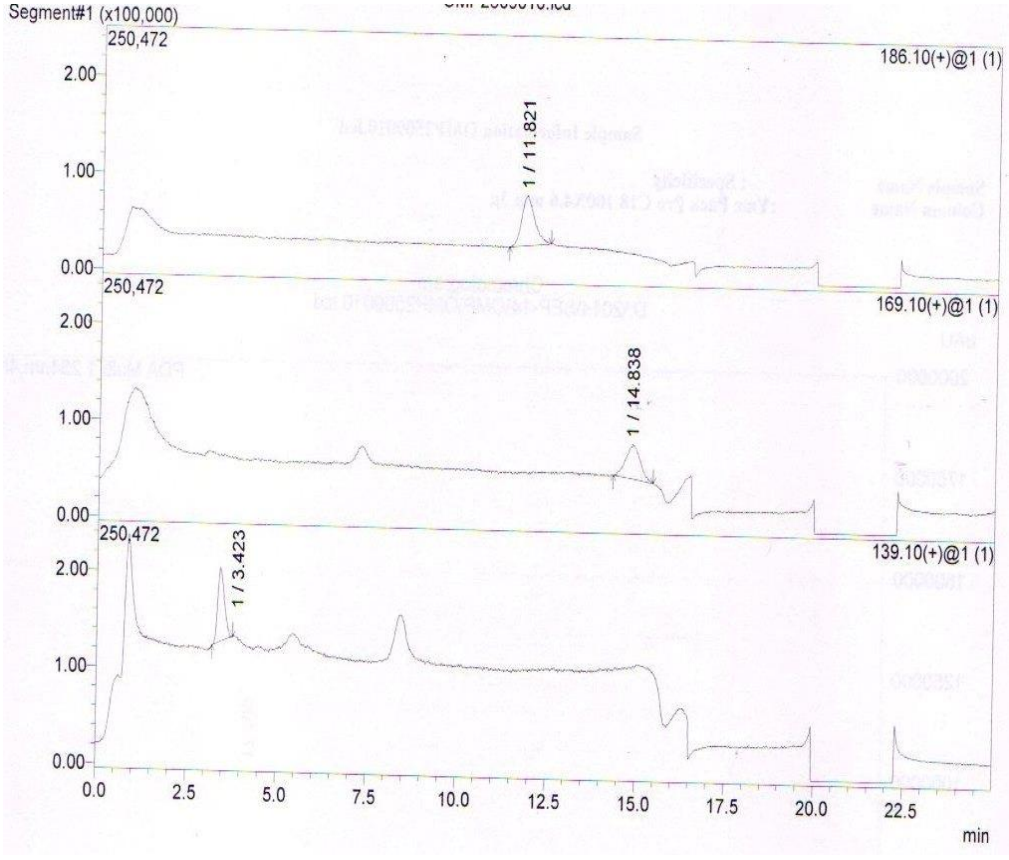
Specificity



Specificity Chromatogram of Omeprazole API

Observations of Omeprazole drug Chromatogram

Id	DRUG NAME	RETENTION TIME	AREA
OMP2509010	Omeprazole	17.100	20162673



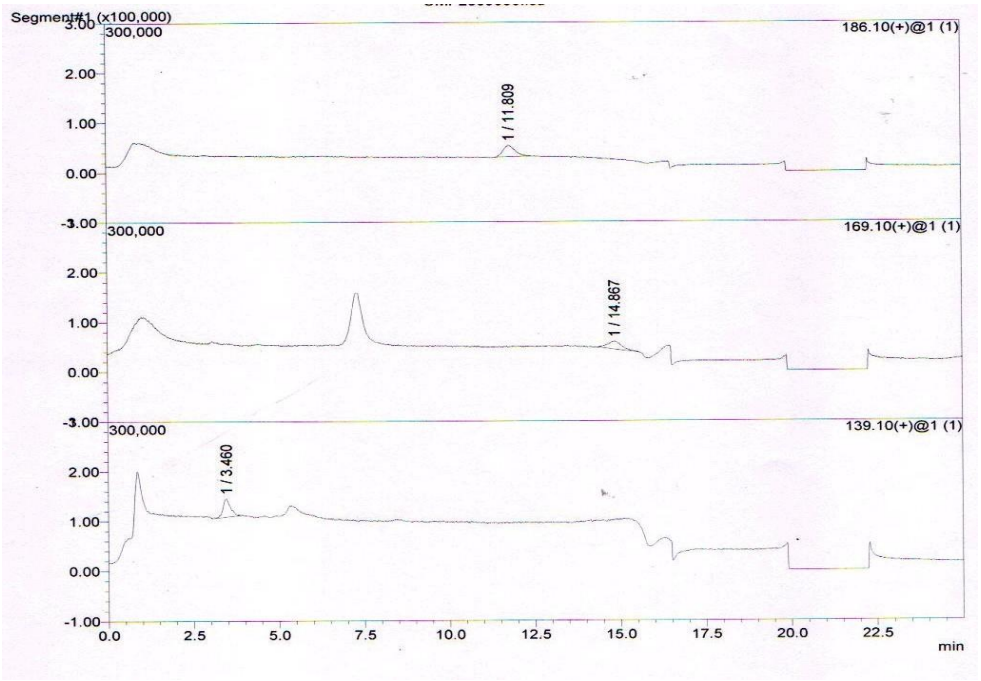
Specificity Chromatogram of CDMP, DNO, and MAA

Observations of impurities Chromatogram

ID	DRUG NAME	RETENTION TIME	AREA
Channel -186	CDMP	11.821	1290665
Channel -169	DNO	14.838	993726
Channel -139	MAA	3.423	961135

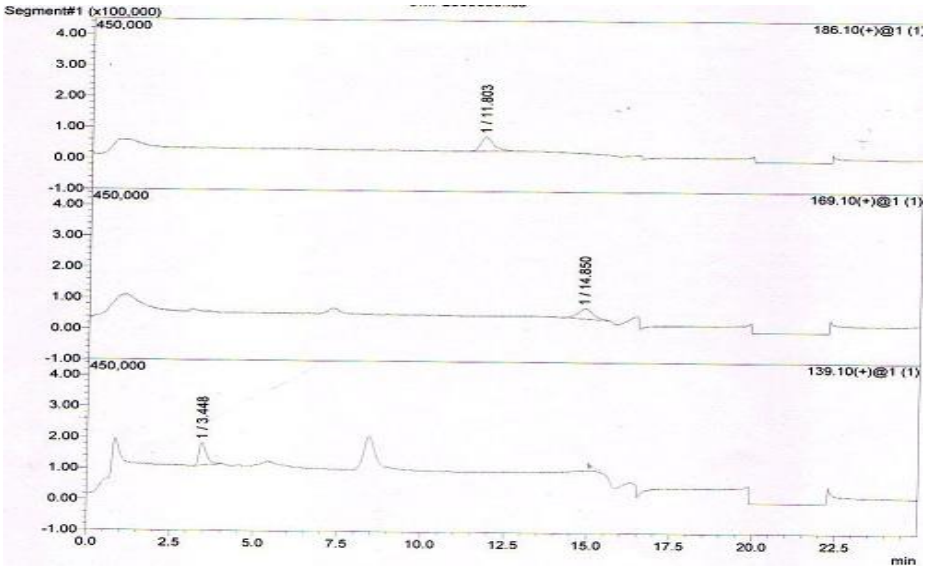
ACCURACY

Accuracy 50%



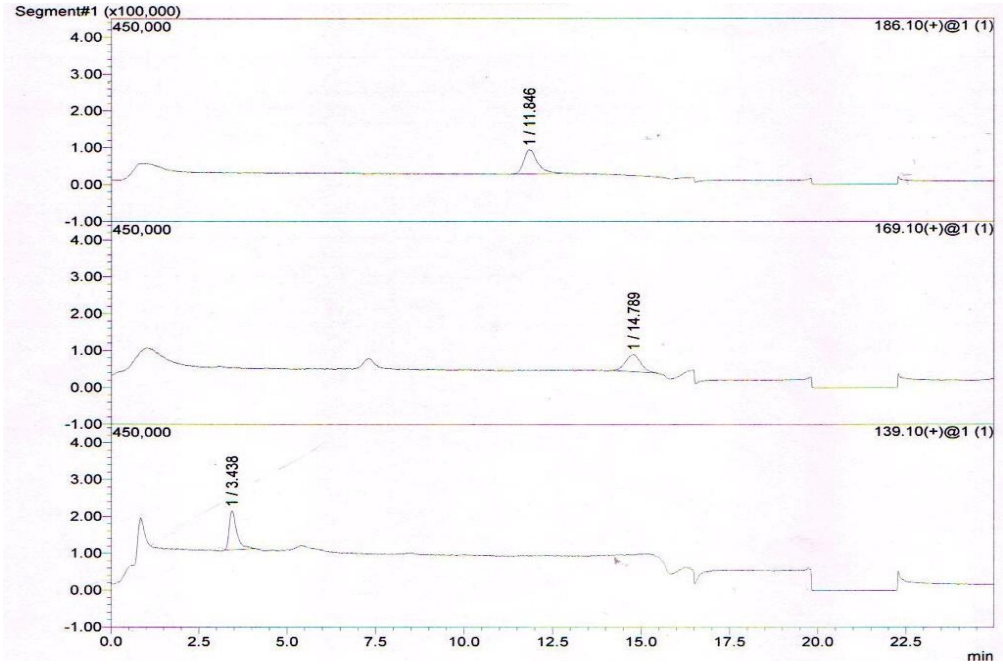
Accuracy 50 % Chromatogram

Accuracy 100%



Accuracy 100 % Chromatogram

Accuracy 150%



Accuracy 150 % Chromatogram

Accuracy Observation of MAA

% Accuracy	PEAK AREA	AMOUNT ADDED (µg)	AMOUNT FOUND (µg)	% RECOVERY	MEAN %RECOVERY
50	699941	6.15	4.93	80.16	80.48
	769898		5.0	81.3	
	699819		4.9	80	
100	889910	12.4	12.64	102	101.73
	898817		12.76	104	
	869890		12.3	99.19	
150	1050561	18.6	22.38	119	117
	1040165		22.1	118	
	1009691		21.5	115	

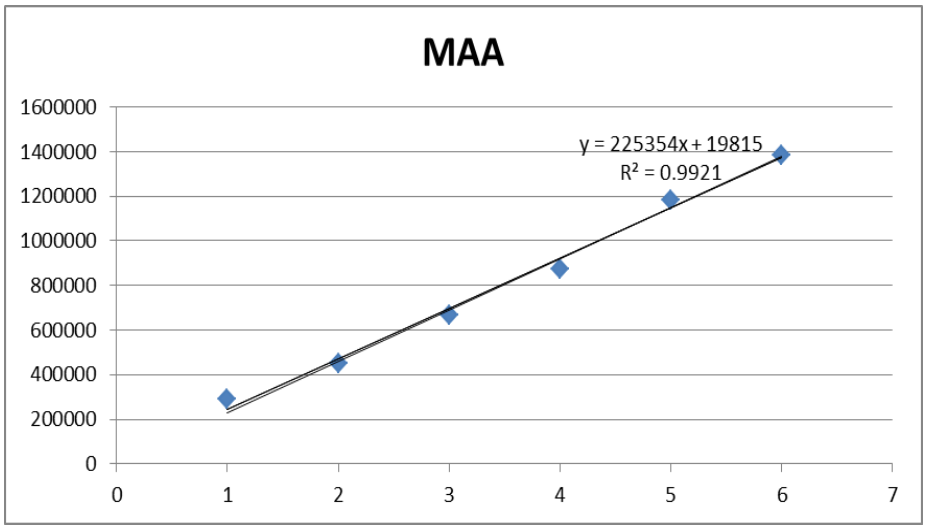
Accuracy Observation of DNO

%Accuracy	PEAK AREA	AMOUNT ADDED (µg)	AMOUNT FOUND (µg)	% RECOVERY	MEAN %RECOVERY
50	679123	6.15	4.9	80.16	80.38
	680212		4.89	80	
	685111		4.98	81	
100	890675	12.4	13	104.8	102.95
	869651		12.70	102.45	
	860675		12.68	101.6	
150	1000987	18.6	22.0	118	117.2
	990912		21.79	116.6	
	994123		21.8	117	

Accuracy Observation of CDMP

%Accuracy	PEAK AREA	AMOUNT ADDED (µg)	AMOUNT FOUND (µg)	% RECOVERY	MEAN %RECOVERY
50	772341	6.15	4.97	81.16	81.5
	779871		5.2	82.6	
	769074		4.95	81	
100	967698	12.4	12.46	100.8	99.4
	944769		12.1	97.8	
	956711		12.3	99.6	
150	1101203	18.6	21	116	116.23
	1096781		21.1	116.6	
	1094521		21.08	116	

Linearity Curve for MAA

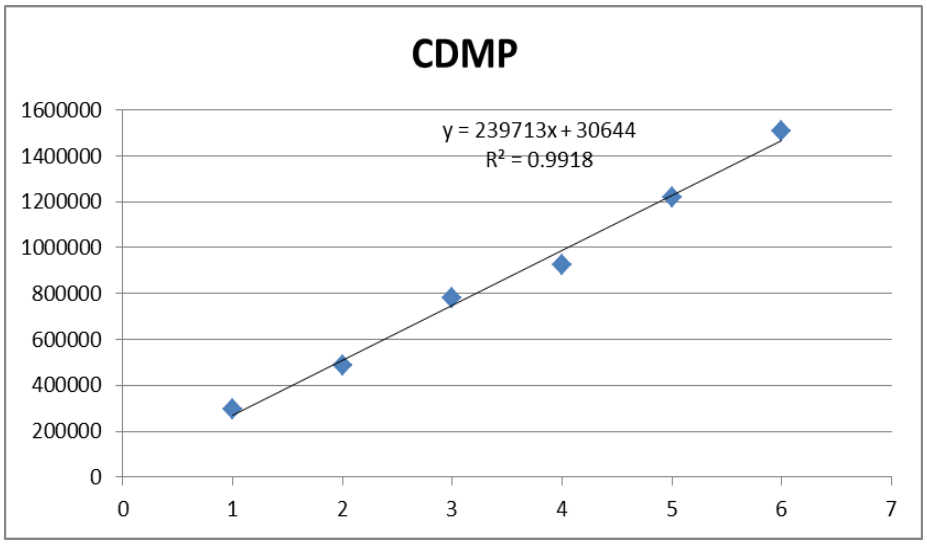


Linearity Curve for MAA

Linearity Observation of MAA

S.No	Linearity Level	Concentration	Area
1	25%	3.1	290119
2	50%	6.2	450334
3	75%	9.3	665289
4	100%	12.4	875900
5	125%	15.5	1185023
6	150%	18.6	1384664
	Correlation	Coefficient	0.992

Linearity Curve for CDMP

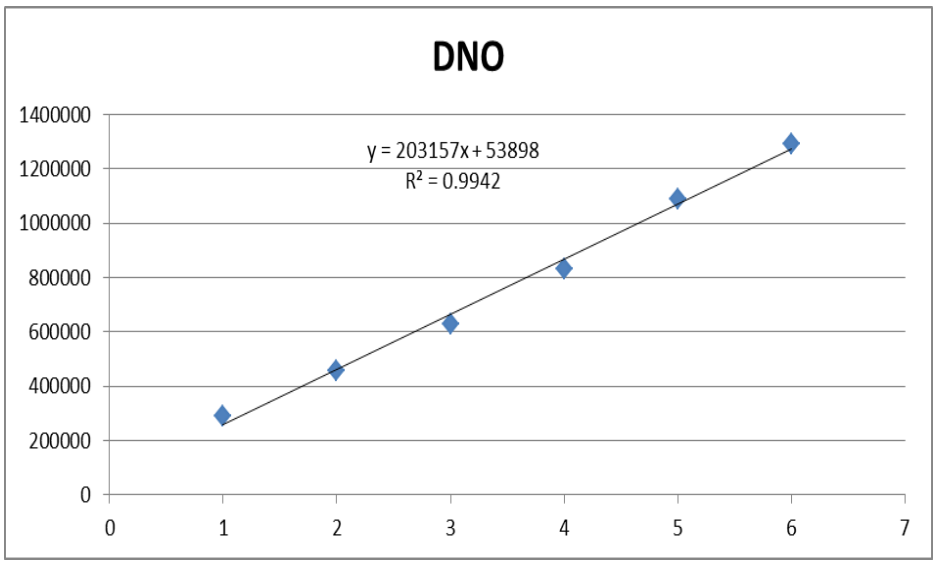


Linearity Curve for CDMP

Linearity Observation of CDMP

S. No	Linearity Level	Concentration	Area
1	25%	3.1	296809
2	50%	6.2	487617
3	75%	9.3	783039
4	100%	12.4	923163
5	125%	15.5	1219642
6	150%	18.6	1507557
	Correlation	Coefficient	0.991

Linearity Curve for DNO



Linearity Curve for DNO

Linearity Observation of DNO

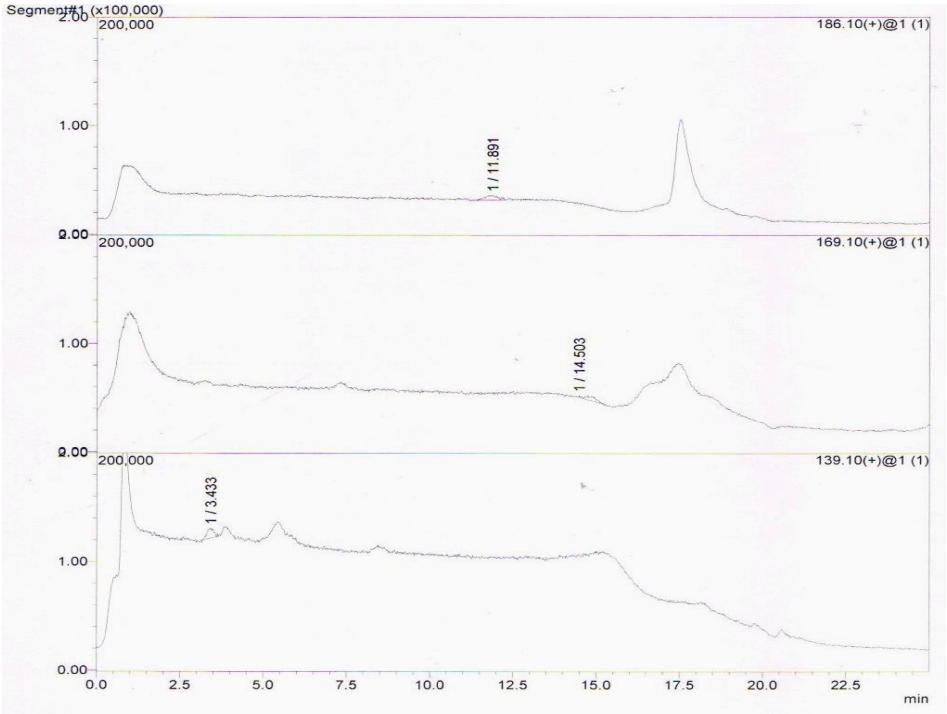
S.No	Linearity Level	Concentration	Area
1	25%	3.1	289382
2	50%	6.2	458458
3	75%	9.3	626705
4	100%	12.4	834094
5	125%	15.5	1089922
6	150%	18.6	1291124
	Correlation	Coefficient	0.994

INTERMEDIATE PRECISION

INJECTION	CDMP	DNO AREA	MAA AREA
Injection1	1023163	920949	908912
Injection2	990123	921990	954980
Injection3	997239	939806	948598
Injection4	949327	949803	939923
Injection5	1019876	935284	939857
Injection6	998903	925991	939899
Average	**996438.5**	**932304.3**	**938694.8**
Standard deviation Deviation	**26569.19**	**11372.33**	**15840.49**
% RSD	**2.6678**	**1.21980**	**1.687502**

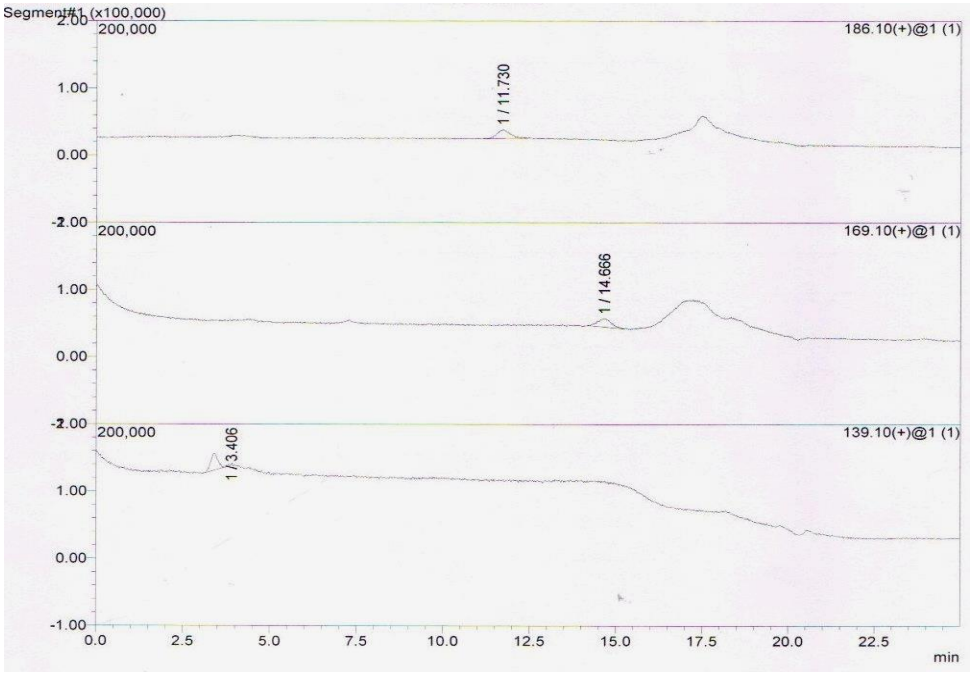
Observations of Intermediate Precision

LIMIT OF DETECTION



Chromatogram of Limit of Detection

LIMIT OF QUANTIFICATION

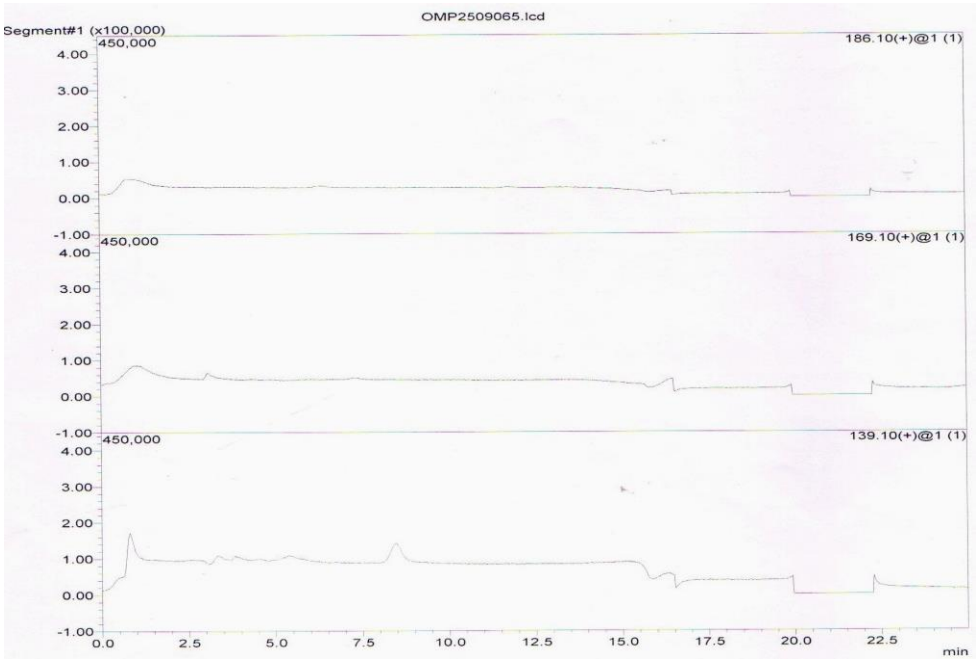


Chromatogram of Limit of Quantificatio

LOD and LOQ Results of Impurities

Compound name	LOD (µg/ml)	S/N Ratio	LOQ (µg/ml)	S/N Ratio
CDMP	1.08	4	3.1	12
DNO	1.08	5	3.1	11
MAA	1.08	4	3.1	11

Batch Analysis



Chromatogram of Limit of Detection

Results Obtained Form Batch of Omeprazole.

Compound name	CDMP	DNO	MAA
OMP2509065	Not detected	Not detected	Not detected

CONCLUSION

The proposed LC-MS/MS method is selective and sensitive for the quantification of Genotoxic impurities 2-chloro methyl -3, 5-dimethyl-4-methoxy pyridine hcl (CDMP), 4-methoxy-3, 5-dimethyl-4-nitro pyridine-1-oxide (DNO) and 4-methoxy-2-amino aniline (MAA) in Omeprazole (API). The method is capable of detecting three structure related impurities. Hence this method is useful for the detection of Potential Genotoxic impurities present in Omeprazole and the three impurities are within the limit, so this API drug can be used or promoted for next level of formulation.

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