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DETERMINATION OF GENOTOXIC IMPURITY 2,3-DIMETHYLANILINE IN PARACETAMOL AND MEFENAMIC ACID GFOS USING RP-LC

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ABSTRACT

Highly sensitive method for the determination of genotoxic impurity such as 2,3-Dimethylaniline in Paracetamol and Mefenamic acid GFOS using RP-LC has been presented in the present paper. Quantification of 2,3-Dimethylaniline content in Paracetamol and Mefenamic acid GFOS samples by HPLC with UV Detector. 2,3-Dimethylaniline was determined by RP-LC method using Inertsil ODS-3V (250X4.6mm, 5µm) column as stationary phase. Mobile phase consisted of pH 6.50 phosphate buffer is used as Mobile phase-A and Methanol and water in the ratio of 90:10 v/v used as Mobile Phase-B, with the help of the gradient elution. Column temperature maintained 40°C, Injection volume 25µL, Flow rare was 1.0 ml/min and the separated 2,3-Dimethylaniline was detected using UV detector at the wavelength of 230 nm, sample cooler temperature 25°C and run time was 40mintues. The method validation has been carried as per International Conference on Harmonization guidelines (ICH). Limit of quantitation (LOQ) was found 0.08ppm for 2,3-Dimethylaniline.

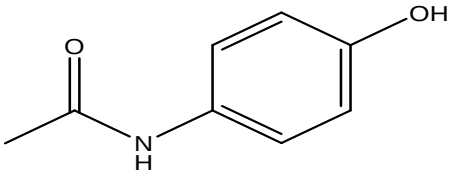
KEYWORDS: Genotoxic impurity, Paracetamol and Mefenamic acid GFOS, RP-LC method, validation and limit of quantitation.

Abbreviations
PCMA= Paracetamol and Mefenamic acid, 2,3-DMA= 2,3-Dimethylaniline.

1.0 INTRODUCTION

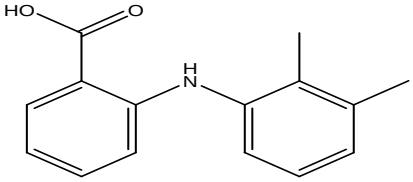
Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose.[1] These limits generally fall at low µg/mL levels. HPLC, GC methods (or final drug substance methods) are suitable for their determination. Their applications are oriented towards the potential identification and quantitation of trace level of impurities in drug substances.[2]

Paracetamol (PC) is chemically N - (4-hydroxyphenyl) acetamide and is used as analgesic and anti-pyretic agent Paracetamol has a narrow therapeutic index – the therapeutic dose is close to the toxic dose. Mefenamic acid (MA) is 2-[(2, 3-dimethylphenyl) amino]benzoic acid Mefenamic acid, an anthranilic acid derivative, is a member of the fenamate group of nonsteroidal anti-inflammatory drugs (NSAIDs). It exhibits anti-inflammatory, analgesic, and antipyretic activities. Similar to other NSAIDs, Mefenamic acid inhibits prostaglandin synthetase. The chemical structure of Paracetamol and Mefenamic acid is shown in **Figure: 1.0 and 2.0.**



Paracetamol

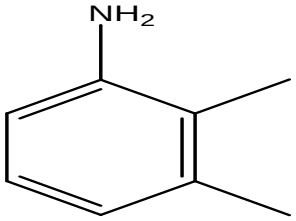
Figure: 1.0 Chemical structure of Paracetamol.



2-(2,3-dimethylphenyl)aminobenzoic acid

Figure: 2.0 Chemical structure of Mefenamic acid.

1.1 Impurity structure



2,3-Xylidine

Figure: 3.0 Chemical structure of 2,3-Dimethylaniline.

Literature survey reveals that various analytical techniques like, UV spectrophotometry,[3-7] spectrofluorimetric,[8] high performance liquid chromatography HPLC,[9-15] and high performance thin layer chromatography HPTLC,[16,17] were reported for the analysis of Paracetamol and Mefenamic acid in pharmaceuticals. Few HPLC methods have been reported for the simultaneous determination of Paracetamol and Mefenamic acid.

In literature, no analytical method was reported for the determination of 2,3-Dimethylaniline in Paracetamol and Mefenamic acid GFOS samples. Hence the author was aimed towards the development of rapid, specific and robust methods for the determination of 2,3-Dimethylaniline in Paracetamol and Mefenamic acid GFOS at trace level concentration.

2.0 Experimental

Chemicals and reagents

2,3-Dimethylaniline purchased from Sigma-Aldrich., Mumbai, India. Potassium dihydrogen ortho phosphate, Disodium hydrogen orthophosphate, Methanol and HPLC grade water were procured from Merck, India.

Mobile Phase-A

Weighed and transferred 1.7 grams potassium dihydrogen ortho phosphate and 1.8 grams Disodium hydrogen orthophosphate into a 1000 mL of Milli-Q-water sonicated to dissolve , and then adjusted to pH 6.5±0.05 with diluted ortho-phosphoric acid. Filter through 0.45µ membrane filter paper.

Mobile phase-B: Methanol and Water in the ratio of 90:10(%v/v).

Preparation of diluent

Methanol and Water in the ratio of 95:5 (%v/v).

Preparation of 2,3-Dimethylaniline stock solution: Accurately weighed and transferred 10 mg of 2,3-Dimethylaniline standard into a 20mL volumetric flask. Add 5ml of diluent and sonicated for 5 minutes. Make up the volume with diluent.

Standard solution: Transferred 5ml of 2,3-Dimethylaniline stock solution into a 100mL volumetric flask made up to the mark with diluent. This solution is equivalent to 0.25ppm of 2,3-DMA with respect to 2.5mg/mL of sample solution.

Preparation of sample solution

Take 20 sachets of Paracetamol and Mefenamic acid and weigh them (W_1).Now remove the powder from sachets & weigh empty sachets (W_2).Calculate the weight of the sachets contents by subtracting the empty sachets weight from $W_3=(W_1-W_2)$. Calculate a weight equivalent to 250mg of Mefenamic acid take into a 100ml volumetric flask, add 50ml of diluent sonicated for 30min. Filtered through 0.45µ PVDF filter.

Preparation of sample spiked solution

Weighed 250mg of the sample into a 100mL volumetric flask. add 50ml of diluent sonicated for 30min, then added 5 ml of 2,3-Dimethylaniline stock solution and mixed well and then made up to the mark with diluent. Filtered through 0.45µ PVDF filter.

Chromatographic conditions

RP-LC analysis was carried out on Agilent-1200 (Agilent Corporation, USA) wavelength 230 nm. Inertsil ODS-3V (250X4.6mm, 5µm) column was used as stationary phase. pH 6.50 phosphate buffer used as Mobile phase-A and Mobile Phase-B consisted of Methanol and water in the ratio of 90:10 v/v. in the gradient elution. The flow rate of the mobile phase was kept at 1.0mL/min. The injection volume was set as 25 µL. Column oven temperature and auto sampler temperature were set as 40°C and 25°C, respectively.

3.0 RESULTS AND DISCUSSION

3.1. Method development

A blend solution containing 2,3-DMA and PMCA was run in 1.5 mL/min flow rate. 2,3-Dimethylaniline closely eluted to placebo peak and hence the flow rate of the mobile phase was decreased from 1.5 mL/min to 1.0 mL/ min. In this condition impurity and placebo peaks are well separated. Hence, the elution order was observed from the chromatogram **(Figure.7.0)** Paracetamol and Mefenamic acid sample solution spiked with 2,3-DMA (0.25µg/mL).

3.2. Method validation

3.2.1 Specificity

Blank interference

Blank was prepared and injected as per test method. It was observed that no blank peaks were interfering with analytical peaks.

Placebo interference

Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering with analytical peaks.

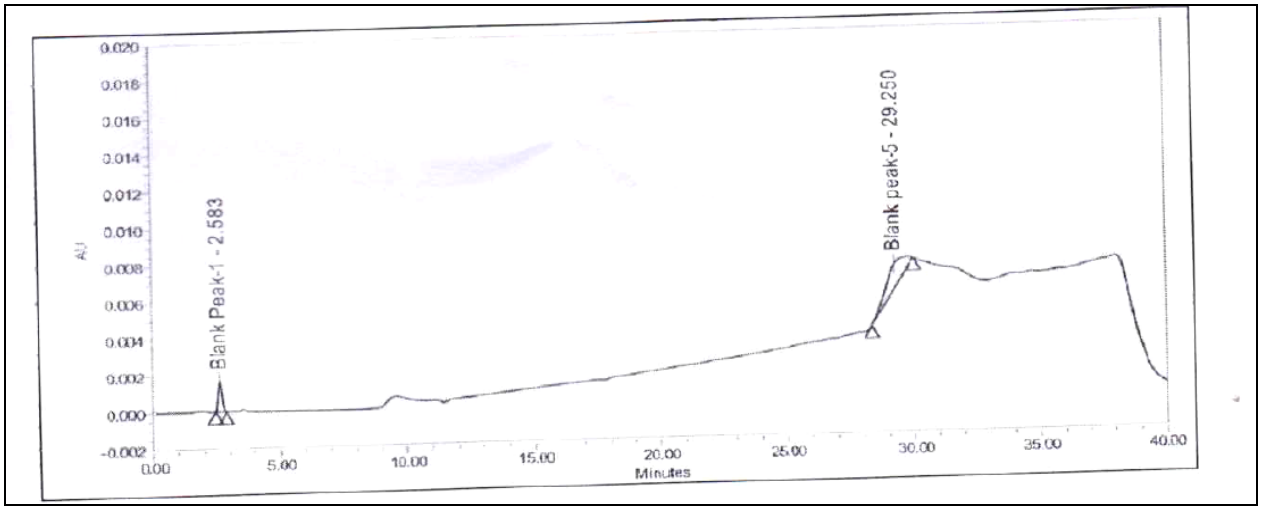


Figure 4.0: Typical chromatogram of Blank.

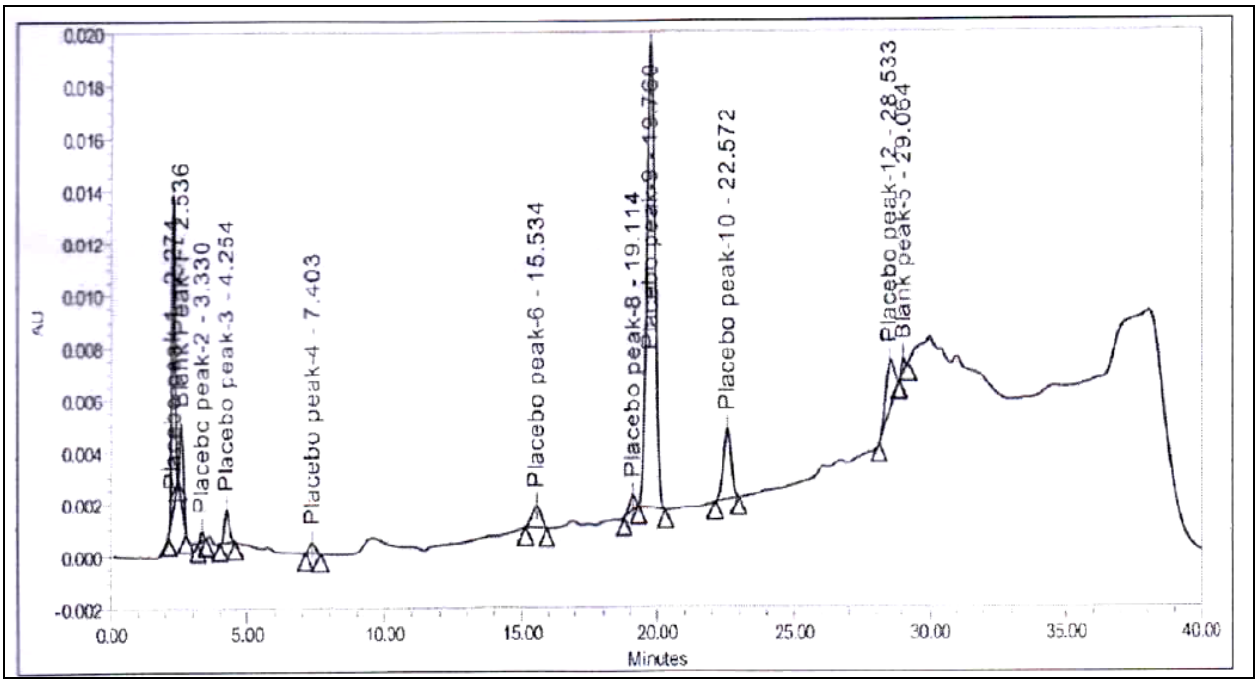


Figure 5.0: Typical chromatogram of Placebo.

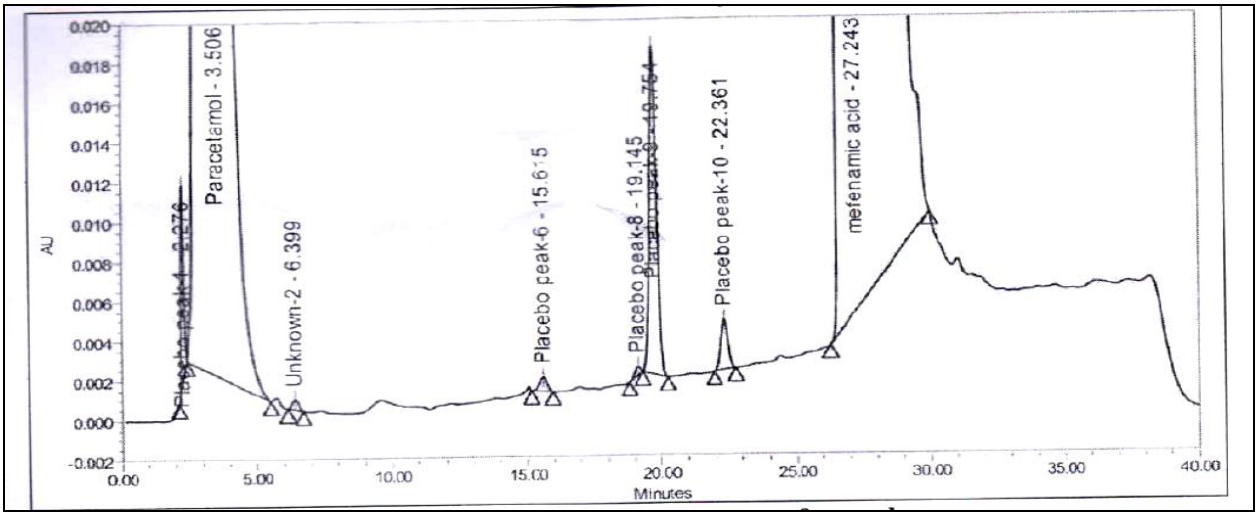


Figure 6.0: Typical chromatogram of sample.

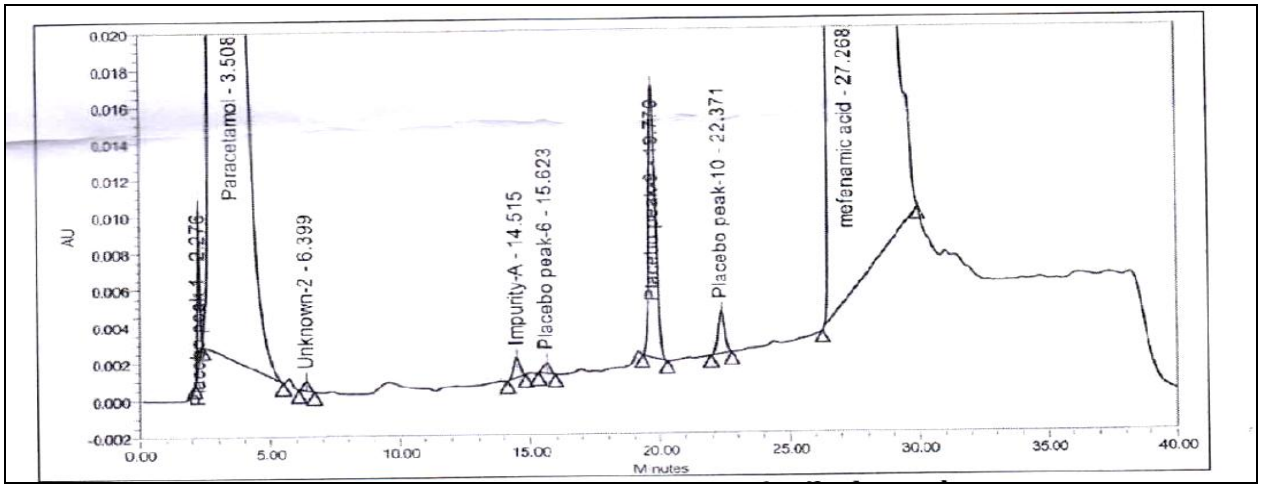


Figure 7.0: Typical chromatogram of spiked sample.

It was observed that no interference of blank and placebo at 2,3-dimethylaniline impurity retention time.

3.3 Precision

3.3.1 System Precision

Perform the analysis of reference solution (Diluted standard) six times and determine the percentage relative standard deviation of peak area of replicate injections of 2,3-DMA.

Table 1.0: System Precision data for 2,3-Dimethylaniline.

Inj. No	2,3-Dimethylaniline
1	0.244
2	0.255
3	0.249
4	0.238
5	0.261
6	0.254
Mean (%)	**0.2502**
% RSD	**3.31**

The %RSD of peak area for 2,3-Dimethylaniline was found to be 3.31% which is below 5.0% indicates that the system gives precise result.

3.3.2 Method Precision

Precision was determined by injecting six sample solutions spiked 2,3-DMA at specification level. The samples were prepared as per the method and the result for precision study is tabulated in **Table: 2.0.**

Table 2.0: Results of method precision.

Inj. No	2,3-Dimethylaniline
1	0.256
2	0.247
3	0.241
4	0.255
5	0.253
6	0.261
Mean (%)	0.25
% RSD	**2.82**

The method precession was performed with six replicate solutions of standard solutions prepared and the system suitability parameters found were within the acceptance criteria.

3.4 Limit of detection (LOQ) & Limit of Quantitation (LOD)

A solution containing 0.02 µg/ml of 2,3-DMA standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections.

Table 3.0: LOD for 2,3-Dimethylaniline.

Name	Inj-1		Inj-2		Inj-3		Mean Area	Mean S/N
	Area	S/N	Area	S/N	Area	S/N		
2,3-DMA	1954	4.01	1978	3.03	1818	3.54	**1916**	**3.53**

A solution containing 0.08 µg/mL of 2,3-Dimethylaniline standard was injected six times. The RSD of areas, deviations of each six replicates from the linear regression curve and average deviation for each standard were calculated. The results are presented in the following tables:

Table 4.0: LOQ for 2,3-Dimethylaniline.

Component	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg.	%RSD
2,3-DMA	6447	6528	5998	6321	6478	6011	**6297**	**3.76**

The limit of limit of quantitation and detection of quantitation values obtained for 2,3-Dimethylaniline impurity was within the acceptance criteria.

3.5 Linearity and Range

The linearity is determined by injecting the solutions in duplicate 2,3-Dimethylaniline ranging from LOQ to 150% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Report the linearity range as the range for determining the 2,3-Dimethylaniline impurity.

Table 5.0: Linearity of detector response 2,3-Dimethylaniline.

Level	Concentration (PPM)	Mean Area
LOQ	0.080	6447
50%	0.125	10226
100%	0.249	19880
120%	0.299	23335
150%	0.374	29476
Correlation coefficient		0.9998
% Y-intercept		1.87
Slope		77646.61
Intercept		371.26

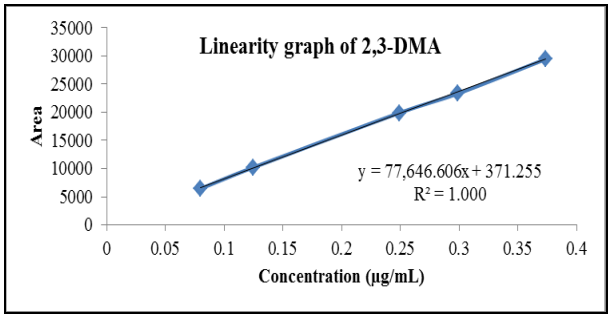


Figure: 8.0 linearity of detector response for 2,3-Dimethylaniline.

3.6 Accuracy

Recovery of 2,3-DMA impurity was performed. The sample was taken and varying amounts of 2,3-DMA impurity representing LOQ to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table 6.0.**

Table 6.0: Accuracy study of 2,3-Dimethylaniline.

S. No.	Theoretical (%)	% Mean Recovery 2,3-Dimethylaniline
1	LOQ	99.7
2	50	99.5
3	100	99.8
4	150	99.1

4.0 RESULTS AND DISCUSSION

The specificity of the developed LC method was indicated by 2,3-DMA solution (0.25µg/mL each) with respect to 2.5mg/mL of PCMA was injected separately and S/N ratios were recorded. These solutions were further diluted to achieve the signal-to-noise (S/N) ratios at about 3 and 10 for determining LOD and LOQ, respectively for both the methods. The precision of the methods was checked by injecting LOQ solutions for six times. The value of RSDs for area of 2,3-DMA was calculated.

The intermediate precision of the method was also verified on six different days in the same laboratory using the LOQ level solutions. The low RSD values ensured the precision of the developed method. Linearity test solution for 2,3-DMA was prepared individually at six concentration levels in the range of LOQ to 150% of the specification level 0.25µg/mL. LOQ and sixth levels were injected six times and other four levels were injected thrice. The average peak areas versus concentrations were subjected to least-squares linear regression analysis. The derived correlation coefficients were above 0.9998 indicating the best fitness of the linearity curves of the developed method. Standard addition experiments were conducted in triplicate preparations to determine accuracy of the methods at LOQ level and recoveries of all the genotoxins were determined. The recoveries were found to be in the accepted range. The system suitability of the method was ensure by getting the %RSD less than 10.0 for six injections of the 2,3-DMA in RP-LC method at specification level. Paracetamol and Mefenamic acid GFOS at trace level concentration have been developed and validated as per ICH guidelines.[17]

5.0 CONCLUSIONS

The proposed RP-LC method that can quantify genotoxic 2,3-Dimethylaniline in Paracetamol and Mefenamic acid GFOS at trace level concentration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensure by the specificity, precision, accuracy and robustness. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of Paracetamol and Mefenamic acid GFOS into the market.

6.0 ACKNOWLEDGMENT

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