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Low Level Quantification of Potential Genotoxic Impurity in Omeprazole Drug Substance by UPLC

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ABSTRACT

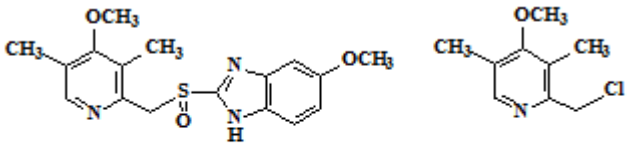
A new, sensitive and rapid Ultra-Performance Liquid Chromatography (UPLC) method developed and validated for the determination of potential genotoxic impurity namely 2-(Chloromethyl)-4-methoxy-3,5-dimethylpyridine (CMDP) at trace level in omeprazole by applying the concept of Threshold of Toxicological Concern (TTC), a limit of 12.5 ppm was calculated based on the maximum daily dose of omeprazole drug substance. The UPLC method was developed and validated by using Acquity UPLC HSS C18, 1.8 µ (100 × 2.1 mm) column with oven temperature maintaining at 40°C. 0.01 M Phosphate buffer pH 2.5 and acetonitrile were chosen as mobile phase A and B in gradient reverse phase mode. Chromatographic parameters i.e., Flow rate: 0.20 ml/min, wave length detection: 205 nm, injection volume: 5 µl and run time: 18 min were applied for this methodology. The proposed method is specific, sensitive, accurate and precise. The established limits of Limit of Detection (LOD) and Limit of Quantification (LOQ) for this impurity are found to be 1.1 ppm and 3.3 ppm respectively. The average recovery obtained was 100.6% at 4 levels in 12 determinations for CMDP in omeprazole drug substance. This method is a good quality control tool for quantitation of CMDP at very low level in omeprazole. The experimental results are discussed in detail in this research paper.

Keywords: Omeprazole, Genotoxicity, Development, Validation, UPLC

INTRODUCTION

Omeprazole is one of the most widely prescribed drugs internationally even if many advanced drugs in the same category are available thus large number of batches are manufactured perennially in a lot of pharmaceutical companies. Omeprazole, a substituted benzimidazole, is a prototype of H/K-ATPase inhibitor in gastric parietal cells [1,2]. Its therapeutic potential has been concern documented as a potent long-acting inhibitor of gastric acid secretion for the treatment of peptic ulcer, refractory gastroesophageal reflux disease, Zollinger-Ellison syndrome and other related hyper-secretory conditions [3,4]. Omeprazole is chemically known as 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, molecular formulae is $C_{17}H_{19}N_3O_3S$ and its molecular weight is 345.42. Omeprazole undergoes extensive hepatic metabolism [5]. Few methods for the determination of the impurities either in bulk drugs or pharmaceuticals have been reported. In the last few years, it can be observed an increased interest for identification and quantification of impurities in bulk drugs using new methodologies. For determination of omeprazole, its related substances and its enantiomer, so many methods are available in literature including pharmacopoeial monographs [6-10].

Guidelines from the International Conference on Harmonization (ICH) and European Medicines Agency (EMA) provide the limits for impurities in drug substances and drug products [11]. ICH limits do not apply to potential genotoxic impurities because of their adverse effects; hence it is necessary to determine limits based on the daily dose of the drug substance. This task drains process-development resources. To overcome this problem, scientists have to identify genotoxic impurities early in process development, develop analytical methods (i.e., for quantifying the genotoxic impurity), and demonstrate the necessary synthetic process controls. In general, TTC approach [12,13] is applied to control genotoxic impurities in drug substances. 2-(Chloromethyl)-4-methoxy-3,5-dimethylpyridine (CMDP) is used as a raw material in the preparation of omeprazole. CMDP structure having structural alert, hence it is required to control in omeprazole drug substance with not more than 12.5 ppm by TTC approach based on maximum daily dose of omeprazole. Both omeprazole and CMDP chemical structures have shown in Figure 1.



Omeprazole CMDP

Figure 1: Chemical structures of omeprazole and 2-(Chloromethyl)-4-methoxy-3,5-dimethylpyridine (CMDP)

For the lower sensitivity of CDMP impurity level, UPLC has been chosen an analytic technique, which can give good separations using columns, packed with smaller particles, with lower flow rates, greater speed with superior resolution and sensitivity. This work has not been reported in literature till date. This research work illustrates the development, optimization of UPLC method for the determination of CMDP and method validated accordance with ICH guidelines [14].

EXPERIMENTAL SECTION

Chemicals, reagents and samples

CMDP reference sample and omeprazole sample and omeprazole related substances were obtained from APL Research Centre-II (a division of Aurobindo Pharma Ltd., Hyderabad.). Analytical grade (AR grade) potassium dihydrogen orthophosphate, orthophosphoric acid, HPLC grade methanol, and acetonitrile were procured from E. Merck; India. Highly pure Milli-Q water was utilized by using millipore purification system.

Instrumentation and chromatographic conditions

The chromatographic separations were performed on UPLC system with acquity binary solvent manager, sample manager and Photo Diode Array (PDA) detector with Empower software data handling system. Mobile phase A: Dissolve 1.36 g of potassium dihydrogen orthophosphate in 1000 ml of water. Adjust pH 2.5 ± 0.05 with orthophosphoric acid. Mobile phase B: Acetonitrile. UPLC column: Acquity UPLC HSS C18, (100 × 2.1 mm) 100 mm long, 2.1 mm internal diameter, 1.8 µm particle diameter column (Make: Waters), Column oven temperature: 40°C. Flow rate: 0.20 ml/min, injection volume: 5 µl, detection wave length: 205 nm and pump is in gradient mode. The gradient program is as follows: Time (min)/A (v/v): B(v/v); $T_{0.01}$/85:15, T_7/85: 15, T_9/20:80, T_{15}/20:80, $T_{15.1}$/85:15, T_{18}/85:15. Standard solution run time: 8 min with initial gradient ratio, sample solution: 18 min. Diluent: A mixture of water and methanol in the ratio of 50:50 v/v. The retention time of CMDP peak is at about 4.3 min.

Preparation of solutions

Standard solution

Prepare a standard solution containing 0.175 µg/ml concentration in diluent using CMDP reference sample. Filter through 0.22 µ or finer porosity membrane filter.

Sample solution

Accurately weigh and transfer about 70 mg of sample into a 10 ml clean, dry volumetric flask, add 5 ml of methanol and sonicate to dissolve. Make up to volume with water (7000 µg/ml). Filter through 0.22 µ or finer porosity membrane filter. Note: Prepare fresh solution.

System suitability criteria

The column efficiency as determined from the CMDP peak is not less than 3000 USP plate count and USP tailing for the same peak is not more than 2.0 from standard solution chromatogram.

RESULTS AND DISCUSSION

Method validation

The developed and optimized method were established through the validation experiments per the ICH guidelines [14], individually in terms of specificity or selectivity, Limit of Detection (LOD) and Limit of Quantification (LOQ), accuracy, precision (system precision, method precision) and stability of standard and sample solutions.

Specificity

Specificity parameter is the capability of the method to establish the interest analyte in the presence of other related substances of drug substance. Solutions of CMDP, all known related substances were prepared individually and injected to confirm retention time. The solutions of omeprazole drug substance, omeprazole drug substance spiked with CMDP (control sample) and omeprazole drug substance spiked with all known related substances including CMDP (Spiked sample) were injected to confirm any co-elution with CMDP peak from any known related substances. Peak purity for CMDP were established by using waters Empower software and found to be passing (Purity angle should be less than purity threshold). Moreover, no peak is observed at the retention time of CMDP peak in the diluent chromatogram and all related substances are well separated from CMDP peak. Hence, this method is specific and selective. The typical UPLC chromatograms of diluent, standard solution, Omeprazole drug substance spiked with CMDP and Omeprazole drug substance spiked with all known related substances including CMDP are shown in the Figure 2. The specificity experiments data is given in Table 1.

Table 1: Specificity data

Control sample			
Name	**RT (min)**	**Purity angle**	**Purity threshold**
CMDP	4.043	2.426	3.662
Spiked sample			
Name	**RT (min)**	**Purity angle**	**Purity threshold**
CMDP	4.047	2.484	4.717

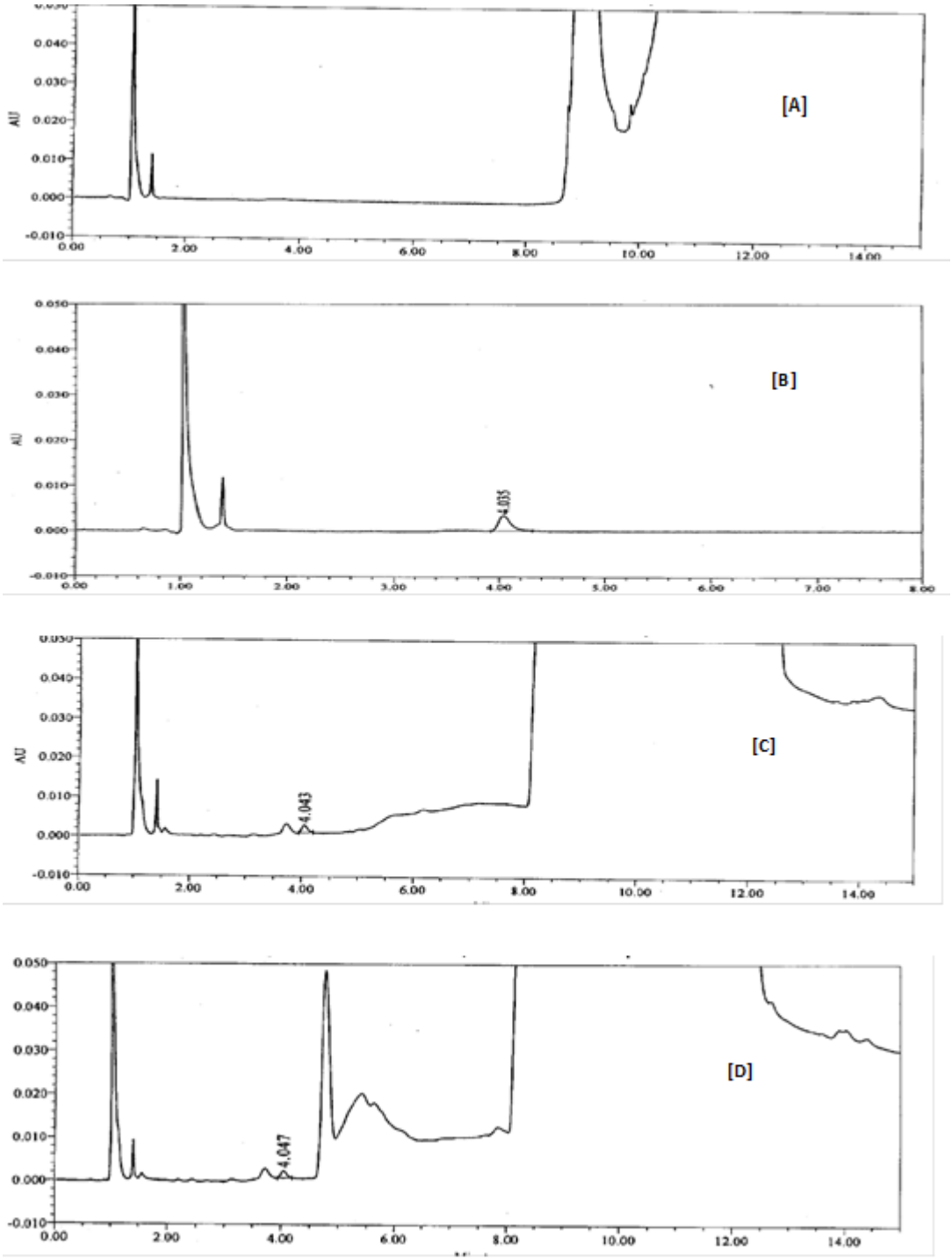


Figure 2 : A Typical UPLC chromatograms of diluent solution (A), Standard solution (B), Omeprazole spiked with CMDP (C), Omeprazole spiked with other related substances including CMDP (D)

LOD and LOQ

The LOD and LOQ values of CMDP were predicted from signal to noise ratio data. The predicted concentration of LOD/LOQ solutions were prepared and injected six times each in to UPLC by following the test method conditions and the results are found to be 1.1 ppm and 3.3 ppm.

Accuracy

The recovery experiments were performed by using standard addition technique. In this experiment, sample solutions were prepared in triplicate by spiking CMDP at levels of LOQ, 50%, 100% and 150% of specification limit (12.5 ppm) as per test method and injected each solution into UPLC as per methodology and the percentage recoveries were calculated. The fully validated recovery results are presented in Table 2.

Table 2: Accuracy data

CMDP (LOQ level)							
% Level/Sample ID		**Amount added (ppm)**		**Amount found (ppm)**		**% Recovery**	
LOQ Level Sample - 1		3.334		3.031		90.9	
LOQ Level Sample - 2		3.320		3.235		97.4	
LOQ Level Sample - 3		3.325		3.178		95.6	
Statistical analysis							
Mean	94.6	**SD**	3.36	**% RSD**	3.6	**95% Confidence interval (±)**	8.3

CMDP (50% to 150% level)					
Concentration/Sample ID	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical analysis**	
50% Level Sample 1	6.234	6.187	99.2	Mean	102.5
50% Level Sample 2	6.225	6.535	105.0	SD	2.97
50% Level Sample 3	6.216	6.415	103.2	% RSD	2.9
100% Level Sample 1	12.468	12.637	101.4	Mean	100.6
100% Level Sample 2	12.468	12.175	97.6	SD	2.69
100% Level Sample 3	12.504	12.860	102.8	% RSD	2.7
150% Level Sample 1	18.729	19.572	104.5	Mean	104.7
150% Level Sample 2	18.729	19.281	102.9	SD	1.91
150% Level Sample 3	18.756	20.013	106.7	% RSD	1.8

Overall statistical analysis							
Mean	102.6	**SD**	2.84	**% RSD**	2.8	**95% Confidence interval (±)**	2.2

Precision

System precision

The standard solution of CMDP was prepared and injected in six replicates in to UPLC and calculated the %RSD of peak areas of CMDP.

Method precision

It was demonstrated by preparing six sample solutions individually using a single batch of omeprazole drug substance spiked with CMDP at specification level and determined the CMDP content. Achieved results like %RSD and 95% confidence interval for six determinations are summarized in Tables 3a and 3b.

Table 3a: System precision data

Injection ID	**CMDP area**	**Statistical analysis**	
1	27166	Mean	26691
2	26653	SD	235
3	26574	% RSD	0.9
4	26565		
5	26614	95% Confidence interval (±)	247
6	26571		

Table 3b: Method precision data

Sample ID	**CMDP content (µg/g)**	**Statistical analysis**	
1	11.5	Mean	12.0
2	11.5	SD	0.59
3	11.5	% RSD	4.9
4	12.7	95% Confidence interval (±)	0.62
5	12.7		
6	12.1		

Solution stability

For the determination of stability of the standard and sample solutions, standard solution and sample solution spiked with CMDP at specification level were prepared as per methodology and analyzed initially and at different time intervals by keeping the solution at room temperature (~ 25°C) and refrigerator condition (~ 6°C). The % difference in the peak area obtained at initial and different time interval was found to be less than 8.0 for standard solution at room temperature (~ 25°C). The results concluded that standard solution is stable for at least 15 hours at room temperature. But the sample solution area was continuously increasing at room temperature and as well as at refrigerator condition. The results concluded that sample solution is stable for at least 2 hours at refrigerator condition.

CONCLUSION

The UPLC chromatography method was developed, optimized and validated for the determination of CMDP content in Omeprazole drug substance. Based on results of various validation parameters it was concluded that the method is specific, sensitive, precise and accurate and the method can be introduced into routine testing.

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