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# Determination of Carbon Disulfide Content in Omeprazole Magnesium Samples by Using RP-HPLC

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**ABSTRACT**

Highly sensitive method for the determination of Carbon disulfide impurity content in Omeprazole Magnesium samples by using RP-HPLC has been presented in the present paper. Quantification of Carbon disulfide content in Omeprazole Magnesium sample by HPLC with UV Detector. Carbon disulfide was determined by RP-HPLC method using Inert Sustain swift C18 (250x4.6mm, 5µm) column as a stationary phase. Column temperature maintained 40°C and sample cooler temperature 5°C, Injection volume 20µL, Flow rarely was 0.8 ml/min, Carbon disulfide was detected using UV detector at the wavelength of 206 nm and run time was 40 minutes. The mobile phase used water and acetonitrile in isocratic mode. The method validation has been carried as per International Conference on Harmonization guidelines (ICH). The limit of quantitation (LOQ) was found at 6.07 ppm for Carbon disulfide.

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## INTRODUCTION

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and finalized formulations is one of the most challenging tasks of pharmaceutical analytical chemists in the industrial environment [1]. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products [2]. For these reasons, all major international pharmacopeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product [3-4].

The literature search revealed that papers on the degradation of Omeprazole [5] determination by UV spectrophotometry method [6] Omeprazole (OME) in human plasma and urine by LC-MS-MS [7] colorimetric method [8] determination of S-Omeprazole, R-omeprazole and racemic Omeprazole [9] are available, but as such there is no validated method available, which reports more known and unknown impurities precisely and significantly for Omeprazole (OME), as such and in the drug product.

Omeprazole is a highly effective inhibitor of gastric acid secretion used in the therapy of stomach ulcers and zollinger-ellison syndrome. The drug inhibits the H(+)-K(+)-ATPase (H(+)-K(+)-exchanging ATPase) in the proton pump of gastric parietal cells [10-11]. The chemical IUPAC name of Omeprazole is 6-methoxy-2-[(4-methoxy-3,5-dimethyl pyridine-2-yl)methylsulfinyl]-1H-benzimidazole. Its empirical formula is $C_{17}H_{19}N_3O_3S$, and its structural formula is shown in **Figure 1**[12]. Omeprazole Magnesium is a white to off-white free-flowing crystalline powder with a molecular weight of 713.1 g/mol. The salt is slightly soluble (0.25 mg/mL) in the water at 25°C, and it is soluble in methanol.

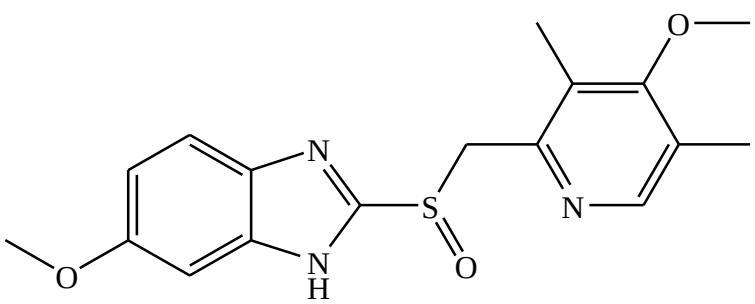


**Figure No. 1:** Chemical structure of Omeprazole Magnesium (OPM)

**Impurity structure:**

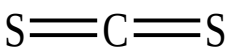

**Figure No. 2:** Chemical structure of Carbon disulfide (CDS)

In the literature, no analytical method was reported for the determination of Carbon disulfide (CDS) in Omeprazole Magnesium (OPM). Hence the author was aimed towards the development of rapid, specific, and robust methods for the determination of CDS in OPM at trace level concentration.

## MATERIALS AND METHODS

### Chemicals and reagents

Carbon disulfide (CDS) was purchased from Sigma-Aldrich, Mumbai, India. Acetonitrile and Milli-Q water HPLC grade procured from Merck, India.

**Mobile phase:** Water and Acetonitrile in the ratio of 70:30(%v/v) elution was the isocratic mode.

**Preparation of diluent:**

Acetonitrile was used as diluent.

**Preparation of CDS stock solution-1:** Transferred accurately 15µL of Carbon disulfide into a 10mL volumetric flask add 5ml of diluent sonicated to dissolve. Mixed well and made up to the mark with diluent.

**Preparation of CDS stock solution-2:** Transferred accurately 100µL of standard stock solution-1 into a 100mL volumetric flask add 50ml of diluent mixed well and made up to the mark with diluent.

**Standard solution:** Transferred 65µL of standard stock solution-2 into a 10mL volumetric flask containing about 5mL of diluent. Mixed well and made up to the mark with diluent. This solution is equivalent to 12.3 ppm of CDS concerning 1.0mg/mL of the sample solution.

**Preparation of sample spiked solution:**

Weighed 10mg of the Omeprazole Magnesium into a 10mL volumetric flask. Dissolved in 5mL of diluent and added 32µL of CDS standard stock solution-2 solution. Mixed well and then made up to the mark with diluent.

**Chromatographic conditions**

RP-LC analysis was carried out on Agilent-1200 (Agilent Corporation, USA) wavelength 206 nm. Inert Sustain C18 (250x4.6mm, 5µm) column was used as the stationary phase. The mixture of Water and Acetonitrile in the ratio of 70:30 (v/v) was used as a mobile phase in isocratic elution. The flow rate of the mobile phase was kept at 0.8mL/min. The injection volume was set as 20µL. Column oven temperature and autosampler temperature were set as 40°C and 5°C, respectively.

**RESULTS AND DISCUSSION**

**Method development**

A blend solution containing CDS and Omeprazole Magnesium was run in 1.0 mL/min flow rate. Omeprazole Magnesium and carbon disulfide impurity eluted at end of the Omeprazole magnesium peak moreover shortened and hence the flow rate of the mobile phase was decreased from 1.0 mL/min to 0.8 mL/ min. In this condition, Omeprazole Magnesium eluted at an optimum retention time, but the retention time of carbon disulfide was drastically increased. Hence, the elution order was observed from the chromatogram **(Figure 6)** Omeprazole Magnesium solution spiked with CDS (12.3 µg/mL).

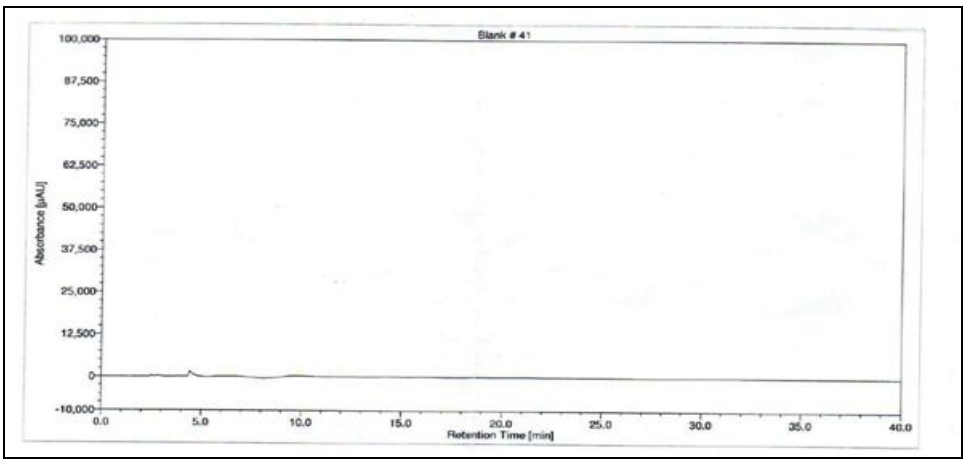


**Figure No. 3: Typical chromatogram of Blank**

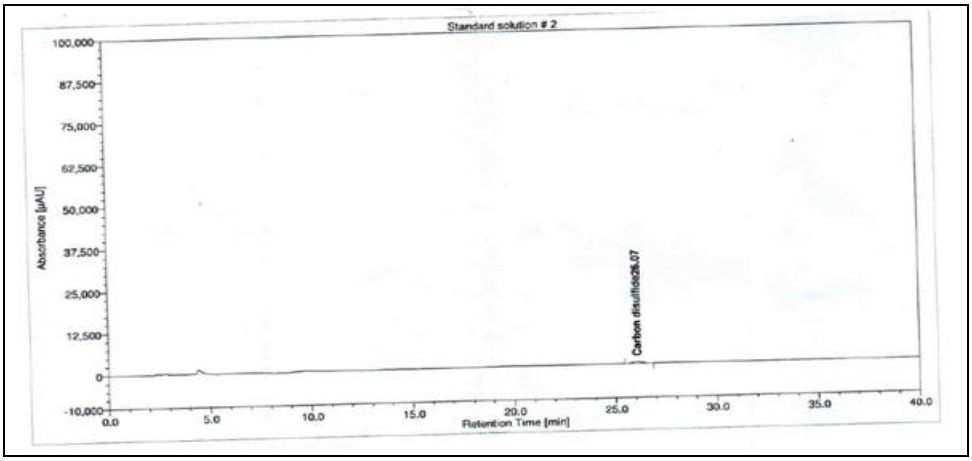


**Figure No. 4: Typical chromatogram of standard**

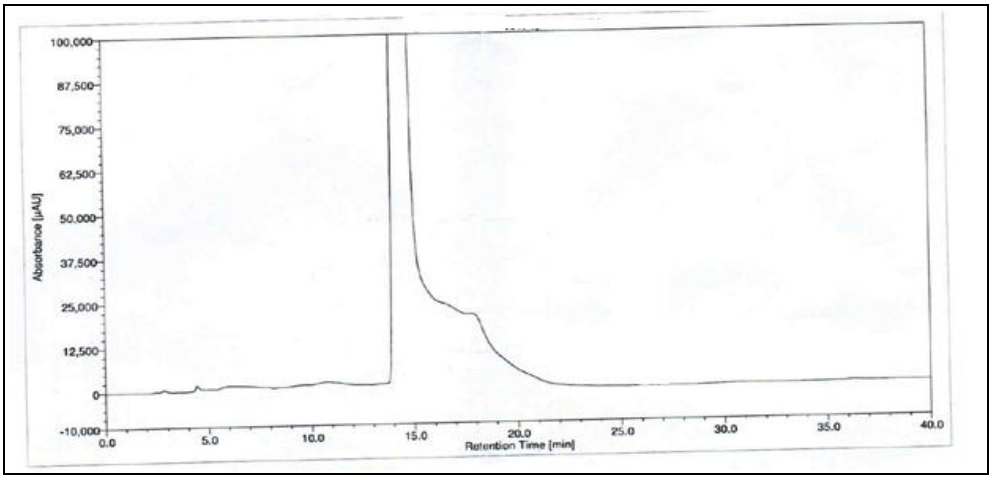


**Figure No. 5: Typical chromatogram of as such sample**

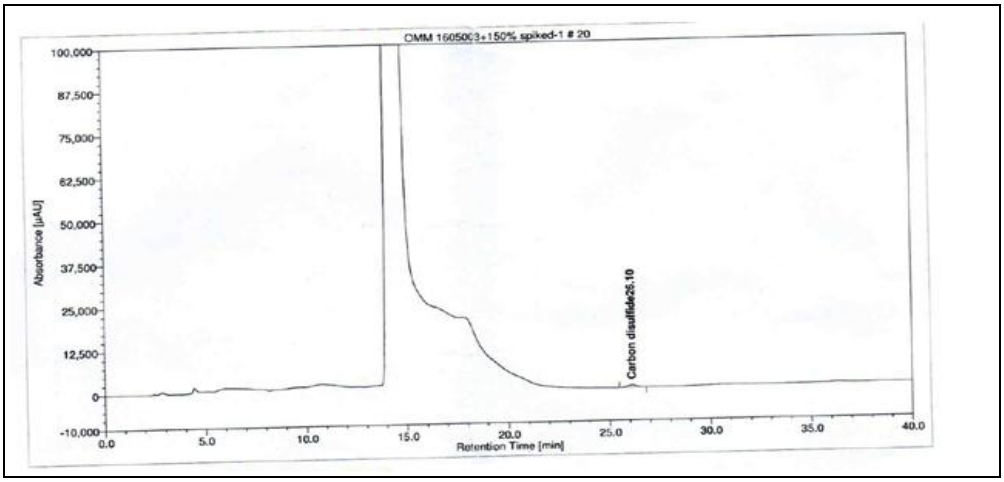


**Figure No. 6: Spiked CDS chromatogram of Omeprazole Magnesium**

**Method validation**

**Specificity**

**Blank interference**

A study to establish the interference of blank was conducted. Diluent was injected as per the test method.

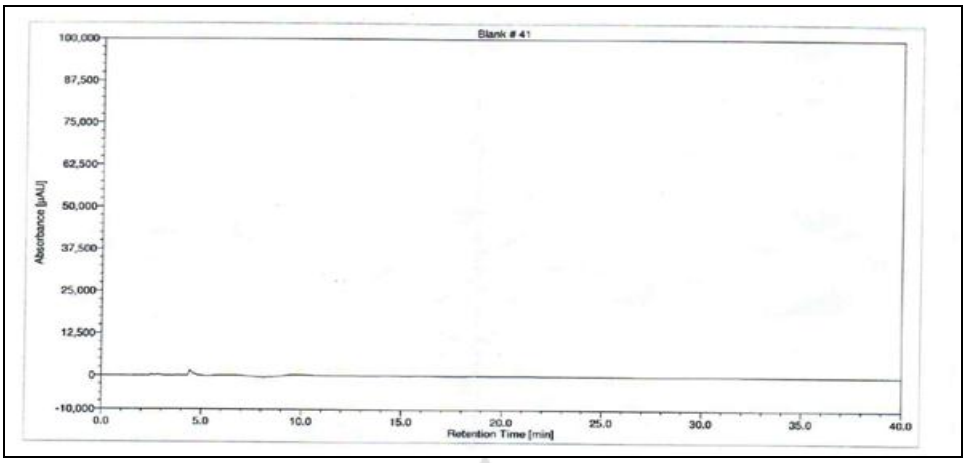


**Figure No. 7: Typical chromatogram of Blank**

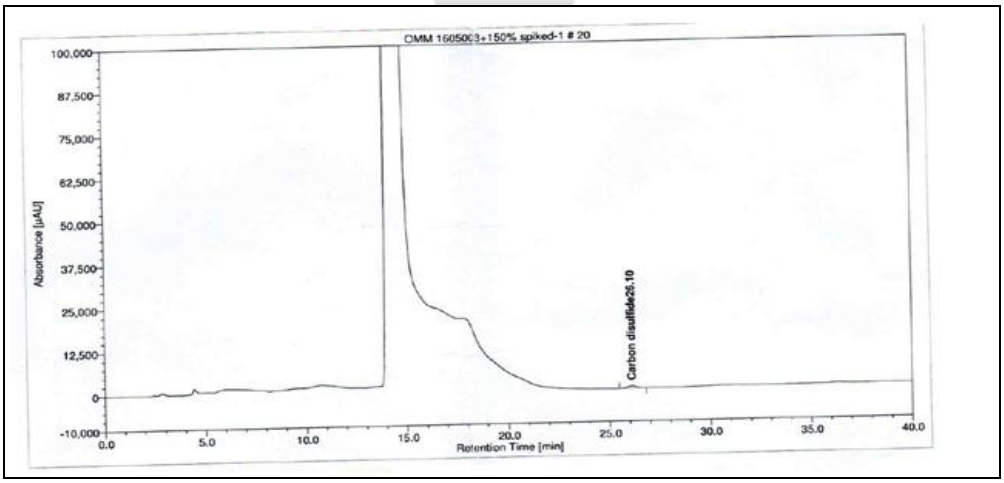


**Figure No. 8: Spiked CDS chromatogram of Omeprazole Magnesium**

It was observed that the Carbon disulfide peak was not co-eluting with the main analyte peak. Omeprazole Magnesium standard solution preparation and in spiked test preparation was calculated and found to be within the acceptable limit.

**Precision**

**System Precision:**

**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 Carbon disulfide.

**Table No. 1: System Precision data for Carbon disulfide**

| Injection No | Carbon disulfide |
|---|---|
| 1 | 15942 |
| 2 | 14892 |
| 3 | 15142 |
| 4 | 14959 |
| 5 | 15078 |
| 6 | 14753 |
| **Mean area** | **15128** |
| **SD** | **421.94** |
| **%RSD** | **2.79** |

The %RSD of peak area for Carbon disulfide was found to be 2.79% which is below 5.0% indicates that the system gives a precise result.

**Method Precision**

The precision of the impurity was determined by injecting six sample solutions spiked with impurities (Carbon disulfide) at the specification level. The samples were prepared as per the method and the result for the precision study is tabulated in **Table 2.**

**Table No. 2: Results of method precision**

| Inj. No | Carbon Disulfide |
|---|---|
| 1 | 14954 |
| 2 | 13852 |
| 3 | 15042 |
| 4 | 14163 |
| 5 | 13987 |
| 6 | 14254 |
| **Mean (%)** | **14375** |
| **SD** | **502.7499** |
| **% RSD** | **3.50** |

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

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

A solution containing 3.03 µg/ml of Carbon disulfide 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 three injections.

**Table No. 3: LOD for Carbon Disulfide**

| Name | Inj-1 | | Inj-2 | | Inj-3 | | Mean Area | Mean S/N |
|---|---|---|---|---|---|---|---|---|
| | Area | S/N | Area | S/N | Area | S/N | | |
| Carbon Disulfide | 2445 | 4.01 | 2542 | 3.03 | 2389 | 3.54 | 2459 | **3.53** |

A solution containing 6.07 µg/mL of Carbon disulfide 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 No. 4: LOQ for Carbon Disulfide**

| Component | Inj-1 | Inj-2 | Inj-3 | Inj-4 | Inj-5 | Inj-6 | Avg. | %RSD |
|---|---|---|---|---|---|---|---|---|
| Carbon Disulfide | 7935 | 7459 | 7321 | 7756 | 7556 | 7531 | 7593 | **2.89** |

The limit of limit of quantitation and detection of quantitation values obtained for Carbon disulfide was within the acceptance criteria.

**Linearity and Range**

The linearity is determined by injecting the solutions in duplicate containing carbon disulfide ranging from LOQ to 200% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Determine the response factor for each impurity concerning Omeprazole Magnesium. Report the linearity range as the range for determining the impurities. Results obtained are in the table & figure show the line of best fit for peak area versus concentration.

**Table No. 5: Linearity of detector response Carbon Disulfide**

| Level (%) | Concentration (ppm) | Mean Area |
|---|---|---|
| LOQ | 6.2 | 7354 |
| 75 | 9.1 | 10998 |
| 100 | 12.3 | 15178 |
| 125 | 15.5 | 19123 |
| 150 | 18.5 | 23005 |
| 200 | 24.6 | 28998 |
| $R^2$ Value | | 0.9960 |
| % Y-intercept | | 2.19 |
| Slope | | 1191.01 |
| Intercept | | 331.832 |

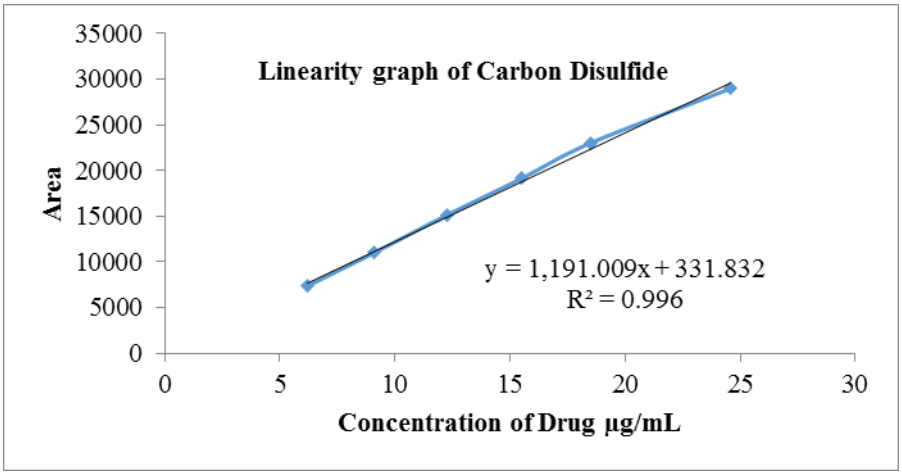


**Figure No. 9: Linearity of detector response for Carbon Disulfide**

The linearity results for Carbon Disulfide in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

**Accuracy**

Recovery of Carbon disulfide in Omeprazole Magnesium was performed. The sample was taken and varying amounts of Carbon disulfide 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 No. 6.**

**Table No. 6: Accuracy study of Carbon Disulfide**

| S.No. | Component Name | % Recovery level | | |
|---|---|---|---|---|
| | | LOQ | 100% | 150% |
| 1 | Carbon Disulfide | 101.90 | 90.10 | 93.60 |
| 2 | | 96.50 | 92.90 | 93.30 |
| 3 | | 103.20 | 91.60 | 95.60 |
| **Avg. Recovery** | | **100.5** | **91.6** | **94.2** |

A simple, economic, accurate, and precise HPLC method was successfully developed. In this method, it was carried out by using Inert Sustain swift C18 (250x4.6mm, 5µm)**.** Injection volume of 20µl is injected and eluted with the mobile phase used water and acetonitrile in isocratic mode, which is pumped at a flow rate of 0.8 ml/min. Column temperature 40°C and sample temperature 5°C. Detection was carried out at 206 nm. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, and precision.

For Selectivity, the chromatograms were recorded for standard and sample solutions of Omeprazole Magnesium and Carbon disulfide impurity. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of related substances in Omeprazole Magnesium. There is no interference of diluent at Omeprazole Magnesium and Carbon disulfide impurity. The limit of detection (LOD) and limit of quantitation (LOQ) for Carbon disulfide standard 3.03µg/mL and 6.07 µg/mL respectively.

The linearity results for Carbon disulfide in the specified concentration range LOQ to 200% are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for Carbon disulfide found to be 0.9960 respectively.

The accuracy studies were shown as % recovery for Carbon disulfide at the specification level. The limit of % recovered shown is in the range of 90 and 110% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of Carbon disulfide. The acceptance limit should be no more than 10, and the results were found to be 3.50% within the acceptance limits.

## CONCLUSION

The proposed RP-LC method that can quantify impurity Carbon disulfide in Omeprazole Magnesium at trace level concentration has been developed and validated as per ICH guidelines [13]. The effectiveness of the method was ensured by specificity, precision, Linearity, and accuracy. Hence, the method well suits for their intended purposes and can be successfully applied for the release testing of Omeprazole Magnesium into the market.

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