A Validated Ion Chromatography method for determination of Ammonium content in Omeprazole tablets

Swetha Parsha\textsuperscript{a,c}, Y Ravindra kumar\textsuperscript{a}, M Ravi chander\textsuperscript{b}

\textsuperscript{a} Dr. Reddy's Laboratories Ltd. Analytical Research & Development, IPDO, Bachupally, Kukatpally, Hyderabad – 500072, A.P, India
\textsuperscript{b} Department of Chemistry, Mahatma Gandhi Institute of Technology, A. P. India
\textsuperscript{c} Department of Chemistry, Jawaharlal Nehru Technological University, Hyderabad, India, India

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\textbf{ABSTRACT}
A simple, feasible, definite and strong Ion chromatography method was developed for the quantitative determination of ammonium content in Omeprazole tablets. The method was developed using Ion pac CS17 Column, 250 X 4.6mm X 5.0 µm column with mobile phase containing 1.5mM Methane sulfonic acid in water. The eluted compounds were monitored using conductivity detector. The unknown peak and ammonium peak were well separated with resolution more than 2.0. The developed method was validated as per International Conference on Harmonisation of technical Requirements for registration of pharmaceuticals for human use guidelines with respect to linearity (The high correlation coefficient >0.99), limit of detection, limit of quantification, exactness, precision and robustness. The Limit of detection, Limit of quantification values of Ammonium were 8ppm and 30ppm respectively.

Introduction
Omeprazole, chemically 6-Methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl) methylsulfinyl)-1H-benzo[d] imidazole (Figure-1). It is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal disease and Zollinger-Ellison syndrome. Omeprazole is a drug of proton pump inhibitor class. Omeprazole is a selective and irreversible proton pump inhibitor. It suppresses stomach acid secretion by specific inhibition of the H+/K + ATPase system found at the secretory surface of gastric parietal cells. Because this enzyme system is regarded as the acid (proton, or H+) pump within the gastric mucosa, omeprazole will inhibit the final step of acid production. Omeprazole will also inhibit both basal and stimulated acid secretion irrespective of the stimulus. It is thought essential to have an Ion chromatography method for the quantitative estimation of Ammonium in Omeprazole tablets. Ammonium content plays a major role in opening of the tablet. Hence keeping in mind the bioavailability and solubility of tablet in relation to opening of the tablet the reproducible method for estimation of Ammonium content has been developed. Main aim of the method is to develop a precise method for estimation of Ammonium content in Omeprazole tablets. The samples were provided by Dr Reddy's Laboratories. The analytical method for estimation of Ammonium content was subjected to validation according to the International Conference on Harmonization (ICH) guidelines[1-3].

Fig: 1 Structure of Omeprazole

\textsuperscript{*}Corresponding Author: Swetha Parsha, Dr. Reddy's Laboratories Ltd. Analytical Research & Development, IPDO, Bachupally, Kukatpally, Hyderabad – 500072, A.P, India E-Mail: swethaparsha50@gmail.com
Materials and reagents

Omeprazole tablets were supplied by Dr. Reddy’s Laboratories Limited, IPDO, Hyderabad, India. The analytical grade Ammonium Formate and Methane sulfonic acid were purchased from Merck, Darmstadt, Germany. Ion chromatography grade water was used for eluent and diluent preparation.

Chromatographic Conditions and Equipment

IC was carried out on a Dionex Ion chromatography system equipped with an electrochemical detector model ED50, Autosampler model AS50, Gradient pump GP50 with conductivity detection. The data processing was performed using chromeleon software. The chromatographic column used was Ion pac CG17 (guard) and CS17 (analytical), 250X 4.6mm, and 5µm particle size. Isocratic elution with the mobile phase composition contained 1.5mM Methane Sulfonic acid in IC grade water.

The eluent flow rate was 1.0 mL/min. The column oven is maintained at ambient temperature of 25ºC and the electrochemical detector was utilized to detect the output signal. IC grade water (diluent) is used for preparing the standards and samples. The microlitre volume of injection was 25.

Preparation of Stock Solutions

Ammonium standard solution (1000ppm) was prepared by dissolving proper amount of Ammonium Formate in diluent. Further 10ppm solution of Ammonium was prepared in diluent.

Method Validation

The described method has been expensively validated for estimation of Ammonium in Omeprazole tablets.

Precision

The precision study of IC method was performed by six times analysis of Omeprazole tablet samples spiked with specification limit of Ammonium. The percentage relative standard deviation of the % area of Ammonium was calculated. The precision study analysis was also performed with different analyst on different day otherwise referred as ruggedness or intermediate precision.

Linearity of Response

The linearity of the method indeed reveals the behavior of the method that is graphical representation of detector response versus different concentrations which was evaluated for Ammonium by injecting each separately prepared solution from the level of LOQ to 200% of the specification limit. The parameters like Correlation coefficient, Y-intercept at 100% level, slope of the calibration curve were calculated.

Robustness

The robustness of an analytical procedure is an assessment of the method efficiency to stay unaltered by tiny, but conscious changes in method parameters and provides an authenticity of regular usage. To determine the robustness of the method the experimental parameters were moderately modified. The system suitability exercise has been performed at flow rate of 1.10ml/min and 0.90ml/min in addition to the exact flow rate of 1.00ml/min as per method. Results were summarized and checked for the % RSD for Ammonium peak area at each varied flow rate.

Specificity

As per synthetic route Sodium ion may possibly present in the sample. Hence specificity has been carried out with respect to blank and Sodium. Specificity of this method was performed by injecting blank as diluent once, Ammonium 10ppm standard once, sample as such once, Sodium standard once, sample spiked with Sodium and Ammonium[4-12].

Results and Discussion

Method development and optimization

The core intention of this experimentation is to develop a stability indicating Ion chromatography method for determination of Ammonium content in Omeprazole tablets. Initially attempts were made by using Ionpac columns (CG12A, CS12A) using Methane sulfonic acid buffer. In this intended experimental conditions, peak shape of Ammonium is not satisfactory. The resolution between unknown impurity and Ammonium is poor, and peak shape for Ammonium was not good. Further attempts made with column and mobile phase optimization.

Trials performed with change in Column to CS17 (Analytical) and CG17 (Guard) and mobile phase as 3mM Methane sulfonic acid. Injected the standard in these conditions. Observed less resolution between unknown peak and Ammonium peak. Then trial performed with decrease in
mobile phase composition to 1.5mM Methane sulfonic acid. Used CG17 and CS17 column only. Observed increase in resolution between unknown peak and Ammonium peak in this method. As Ammonium is used in the process in outer coating of tablet, trials performed for analysis of sample at varied sonication times (10 minutes, 20 minutes, 30 minutes and 60 minutes) to know the effect of sonication on results. Observed no variation in results of Ammonium content in samples at different sonication times. Performed placebo interference study by injecting placebo into the system. Observed there is no interference in the sample from placebo, method was found to be specific with respect to placebo. Finally there was better separation with good peak shape using CG17 and CS17 column and Methane sulfonic acid as mobilephase. Observed good resolution between unknown peak and Ammonium peak. Analysed wide range of samples and calculated the amount of Ammonium.

Validation of the Method

System suitability

System suitability is established by injecting six standards of Ammonium standard solution (10 ppm) and calculated the % RSD for six injections of Ammonium peak area (Table 1).

Precision

In this study the RSD% of the amount of Ammonium in samples spiked with Ammonium was within 4.0%. The RSD% in the intermediate precision study was 8.2%. The RSD% values are presented in Table 2.

Limit of Detection and Quantification

The determined limit of detection, limit of quantification and precision at LOQ values for Ammonium were reported in Table 3.

Linearity

Linearity calibration plot for the method was obtained over the calibration ranges tested i.e., 4 ppm to 15 ppm and correlation coefficient obtained was greater than 0.99. Linearity calibration plot for the method was obtained over the calibration ranges tested i.e., LOQ to 150% (LOQ, 50%, 75%, 100%, and 150% of specification limit). The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined in Table 4.

Accuracy

The recovery of Ammonium was ranged from 95.4 to 103.3%. The percentage recovery of the Ammonium is listed in Table 5.

Robustness

In all the deliberately varied chromatographic conditions (Different flow rate), the system suitability acceptance criteria was within the allowable range. The values are presented in Table 6.

Specificity

Specificity is performed with blank and Sodium standard. The results were listed in Table 7.

Table 1: Method Validation results - System suitability

| Sr. No. | Ammonium area |
|---------|---------------|
| Injection – 1 | 1.3280 |
| Injection – 2 | 1.2834 |
| Injection – 3 | 1.2421 |
| Injection – 4 | 1.2873 |
| Injection – 5 | 1.2910 |
| Injection – 6 | 1.2818 |
| Average | 1.2856 |
| %RSD | 2.13% |

Table 2: Method Validation results - Method Precision and Intermediate precision

| Sr. No. | Ammonium ppm | Ammonium ppm |
|---------|--------------|--------------|
| Preparation – 1 | 192.9 | 157.0 |
| Preparation – 2 | 179.6 | 186.4 |
| Preparation – 3 | 187.4 | 157.4 |
| Preparation – 4 | 178.8 | 169.2 |
| Preparation – 5 | 172.1 | 148.0 |
| Preparation – 6 | 180.3 | 158.4 |
| Average | 181.9 | 162.7 |
| %RSD | 4.0 | 8.2 |

Table 3: Method Validation results - LOD, LOQ & LOQ Precision

| Sr. No. | Ammonium | Ammonium |
|---------|----------|----------|
| Area | 0.0076 | 0.0406 |
| Signal to noise | 2.9 | 10.3 |
| Conc. In ppm ((µg/g)) | 8.0ppm | 30ppm |
Table-3 (a)

| Sr No | Ammonium Area   |
|-------|-----------------|
| 1     | 0.0249          |
| 2     | 0.0244          |
| 3     | 0.0206          |
| 4     | 0.0203          |
| 5     | 0.0195          |
| 6     | 0.0200          |
| Avg   | 0.0216          |
| %RSD  | 11.07           |

Table-4: Method Validation results- Linearity

| Conc. in ppm | Area of Ammonium peak |
|--------------|------------------------|
| 0.300        | 0.0267                 |
| 0.671        | 0.0629                 |
| 1.006        | 0.1192                 |
| 1.341        | 0.1511                 |
| 2.012        | 0.2648                 |
| Correlation  | 0.995                  |

Table-5: Method Validation – Accuracy (Recovery) data

| Spike level % | % Recovery |
|---------------|------------|
| LOQ           | 103.3      |
| 50%           | 102.5      |
| 100%          | 99.3       |
| 150%          | 95.4       |

Table-6: Method Validation – Robustness data

| Sr No | Ammonium area at 0.9ml/min | Ammonium area at 1.0ml/min | Ammonium area at 1.10ml/min |
|-------|----------------------------|----------------------------|-----------------------------|
| 1     | 1.5276                     | 1.1659                     | 1.3055                      |
| 2     | 1.5324                     | 1.3019                     | 1.300                       |
| 3     | 1.516                      | 1.3198                     | 1.2992                      |
| 4     | 1.519                      | 1.3340                     | 1.3127                      |
| 5     | 1.5199                     | 1.4116                     | 1.1969                      |
| 6     | 1.5507                     | 1.4266                     | 1.3182                      |
| Avg   | 1.5276                     | 1.3266                     | 1.2887                      |
| %RSD  | 0.84%                      | 7.1                        | 3.54%                       |
Table-7: Method Validation –Specificity data

| Sample           | RT of Sodium | RT of Ammonium |
|------------------|--------------|----------------|
| Blank            | 10.437       | Not Detected   |
| Ammonium std     | NA           | 11.823         |
| Sample As Such   | 10.747       | 11.456         |
| Sample spiked    | 11.150       | 12.053         |
| Sodium std       | 10.763       | NA             |

Fig: 2 Linearity graph for Ammonium content in Omeprazole

Conclusion
The fast isocratic Ion chromatography method developed for the simultaneous trace level quantitative determination of Ammonium in Omeprazole tablets is reproducible, linear, rugged and robust. The validation results of the method also found to be satisfactory. This method can be used for regular analysis of trace level quantitative determination of Ammonium in Omeprazole tablets.

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Conflict of interest statement
We declare that we have no conflict of interest.

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