MAT JOURNALS

Journal of Pharma and Drug Regulatory Affairs
Volume 1 Issue 2

Development of a Highly Sensitive Method for Quantitative Estimation of Dimethyl Sulfate Impurity in Neostigmine Methylsulfate Drug substances by Using GC-MS

Amit Gosar, Amit Joglekar, Tabrez Shaikh*, Sandeep Mhatre, Swapnil Patil
Research scientist
Indoco Remedies Limited, Analytical Research & Development Department,
Navi Mumbai, Maharashtra, India
Email: *tabrezs@indoco.com
DOI: http://doi.org/10.5281/zenodo.3248738

Abstract

A selective and highly sensitive gas chromatography-mass spectrometry (GC-MS) method was developed for the determination of Dimethyl Sulfate (DMS) impurity in Neostigmine Methylsulfate drug substance. The method was validated as per International Council for Harmonisation (ICH) guidelines, for which limit of detection and limit of quantitation obtained were 25.48 ppm and 77.20 ppm, respectively. The regression coefficient found for the linearity study was 0.9986. The recovery of the spiked Dimethyl Sulfate in drug substance obtained was in the range of 84.40% to 88.24% ensured the accuracy of the method The method precision with relative standard deviation (RSD) for repeatability and intermediate precision was less than 5%. The method can be adapted to determine Dimethyl Sulfate in Neostigmine Methylsulfate drug substance (API).

Keyword: *Dimethyl Sulfate, development, GC-MS, neostigmine methylsulfate, validation*

INTRODUCTION

Neostigmine is approved for the treatment of myasthenia gravis and reversal of nondepolarizing muscle relaxants [1−4]. The chemical name for Neostigmine Methylsulfate is [3-(dimethylcarbamoyloxy)phenyl]-trimethylazanium;methyl sulfate [1]. Its molecular formula is $C_{13}H_{22}N_2O_6S$, which corresponds to a molecular weight of 334.3 (Fig. 1).

Dimethyl Sulfate is a chemical reagent used in the synthesis of Neostigmine Methylsulfate as a reagent. Dimethyl Sulfate is an alkylating agent used in organic synthesis. Its molecular formula is $C_2H_6O_4S$ (Fig. 1). Dimethyl Sulfate is considered to be genotoxic as an experimental result shows it to be weak mutagenic [5−6]. Dimethyl Sulfate structure also shows alert with QSAR software. Hence, Dimethyl Sulfate should be controlled in drug substances to the threshold of toxicological concern (TTC) level 1.5 µg/day in drug substances [4]. A limit for the Dimethyl Sulfate was set based on the basis of maximum daily dose (MDD) of Neostigmine Methylsulfate 5 mg/day for which its limit comes out to be 300 ppm Neostigmine is an approved drug for the treatment.

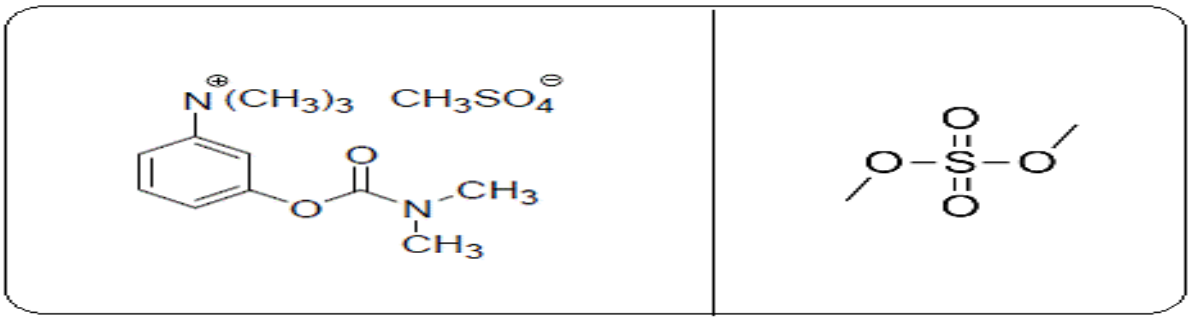

Figure 1: *Structure of neostigmine methylsulfate and dimethyl sulfate.*

EXPERIMENTAL

Material

Neostigmine Methylsulfate sample was obtained from chemical research and development (CRD) department of Indoco, while Dimethyl Sulfate used as a standard was purchased from the Merck chemicals.

Chemical and Reagents

Methyl tertiary butyl ether A.R grade was purchased from J.T. Baker while Sodium chloride and sodium sulfate (A.R grade) were purchased from Merck chemicals. Water used for the preparation of solution was from Milli-Q water purification system (Merck).

Instrumentation

GC separation was performed using gas chromatograph from Shimadzu Model QP2020 equipped with temperature programming capability, injector, capillary column, and Mass analyser with single quadrupole along with GC MS solution software. The analytical balance used was of Sartorius make with model ME 235P.

Chromatographic Condition

The separation was achieved using a DB-624 capillary column (Agilent) having 30 m length, 0.32 mm internal diameter and 1.8 µm thickness in a split mode (1:2). The initial GC oven temperature was kept at 40°C for 4 min. It was increased to 220°C at a heat rate of 20°C /min. It was then held at 220°C for 20 min. Helium (99.999%, purity) was used as the carrier gas and flow rate was kept constant at 1.5 mL/min.

Mass Condition

The mass spectrometer was operated in electron impact mode (EI) (70 eV of ion energy) with 4.9 min solvent delay, SIM acquisition mode with fragment 95 m/z. Mass interference and mass source kept at 200°C and 220°C.

Optimisation of Extraction Procedure

In order to achieve the highest recoveries for the compounds under investigation, different organic solvents for extraction were tried such as Methyl tertiary butyl ether, hexane dichloromethane. Best recovery for analyte was obtained with Methyl tertiary butyl ether solvent and extraction time 2 min.

PREPARATION OF SOLUTIONS

Preparation of Sodium Chloride Solution

Weigh and transfer 2.9 g of sodium chloride into 50 mL volumetric flask, dissolve and dilute upto the mark with water.

Preparation of Blank Solution

Transfer 5 mL of sodium chloride solution into 50 mL centrifuge tube, add 5 mL of diluent, mix and collect MTBE layer (upper layer). To this MTBE layer, add approx. 0.5 g of sodium sulfate and take MTBE extract for analysis.

Preparation of Standard Solution

Weigh and transfer about 0.15 g of Dimethyl sulfate standard into 50 mL volumetric flask. Dissolve and dilute upto the mark with diluent. Transfer 0.5 mL from this solution into 50 mL volumetric flask and dilute upto the mark with diluent. Further, transfer 5 mL from this solution into 50 mL volumetric flask and dilute upto the mark with diluent.

Preparation of Test Solution

Weigh and transfer about 0.05 g of sample into 50 mL centrifuge tube, add 5 mL of Sodium chloride solution, mix well. Then add 5 mL of diluent, mix and collect MTBE layer (upper layer). To this MTBE layer, add approx. 0.5 g of sodium sulfate and take MTBE extract for analysis.

RESULT AND DISCUSSION

Method Optimization

Several methods are reported for the determination of Dimethyl sulfate [7−9] but not available for specific product

Neostgmine Methylsulfate. Dimethyl Sulfate is a highly polar molecule and used as a solvent and methylating agent, hence mass spectrophotometer was selected using gas chromatography technique for its determination. The method was optimized for GC and mass condition with which retention time for Dimethyl Sulfate obtained was about 9.6 minute. Chromatographic run time kept was optimized to 25 minutes.

Method Validation

The analytical method validation work was conducted according to the ICH (International Conference on Harmonization) guidelines [10−13]. The parameters with which analytical method is validated are specificity, limit of detection, limit of quantitation, linearity, accuracy, precision and robustness.

Specificity

The specificity of the method was established by observing the interference at the Dimethyl Sulfate peak from the sample and blank peaks. The retention time of Dimethyl Sulfate is 7.0 min. The retention time of DMS was compared for Standard and spiked test sample which was matching. Hence, the method has been demonstrated for specificity.

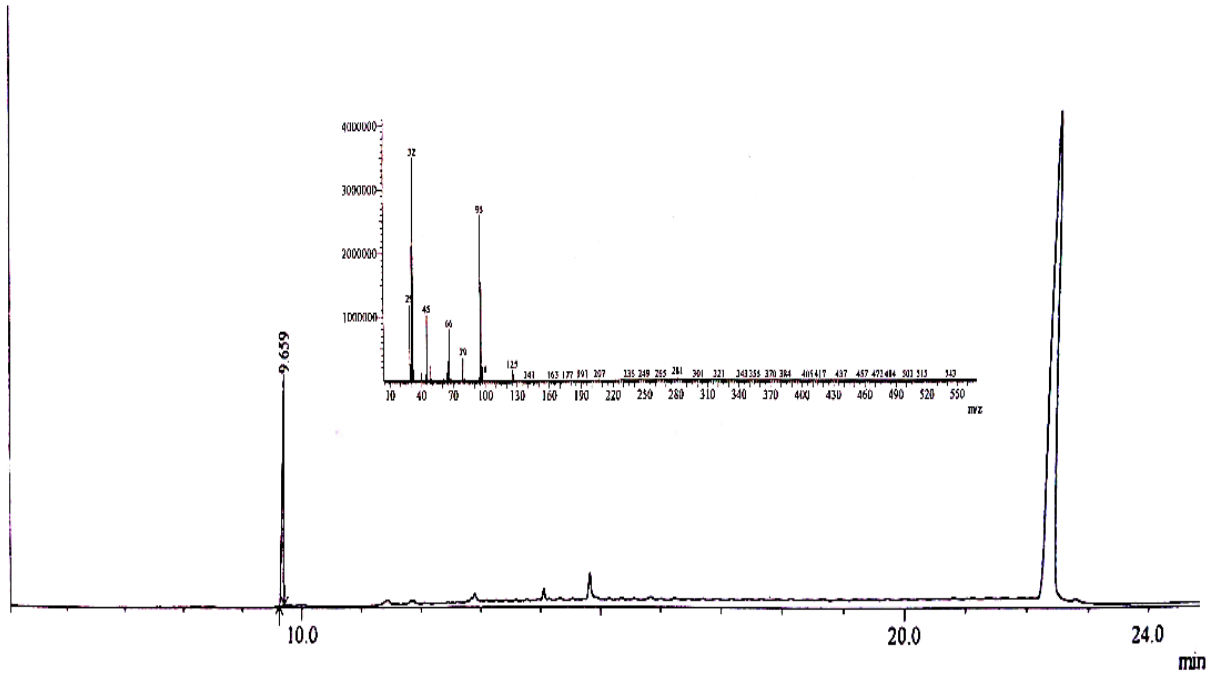

Figure 2: *Chromatogram and mass spectra of DMS.*

Limit of detection and Limit of quantitation

Series of standard solutions of Dimethyl Sulfate was prepared in a concentration ranging from 50% to 150% of target concentration (300 ppm w.r.t. sample). Limit of detection (LOD) and Limit of quantitation (LOQ) was calculated based on the residual standard deviation of the regression line and slope method. Limit of detection obtained for Dimethyl Sulfate was 25.48 ppm and Limit of quantitation 77.20 ppm.

Linearity

Series of linearity solution of Dimethyl Sulfate impurity were prepared from LOQ to 150% of target concentration (200 ppm w.r.t sample). Linearity curves were drawn by plotting the peak area of Dimethyl Sulfate against its corresponding concentration of linearity solution (Fig. 3). The observed regression coefficient for linearity curve was 0.9986 and % y-intercept was -0.86 %.

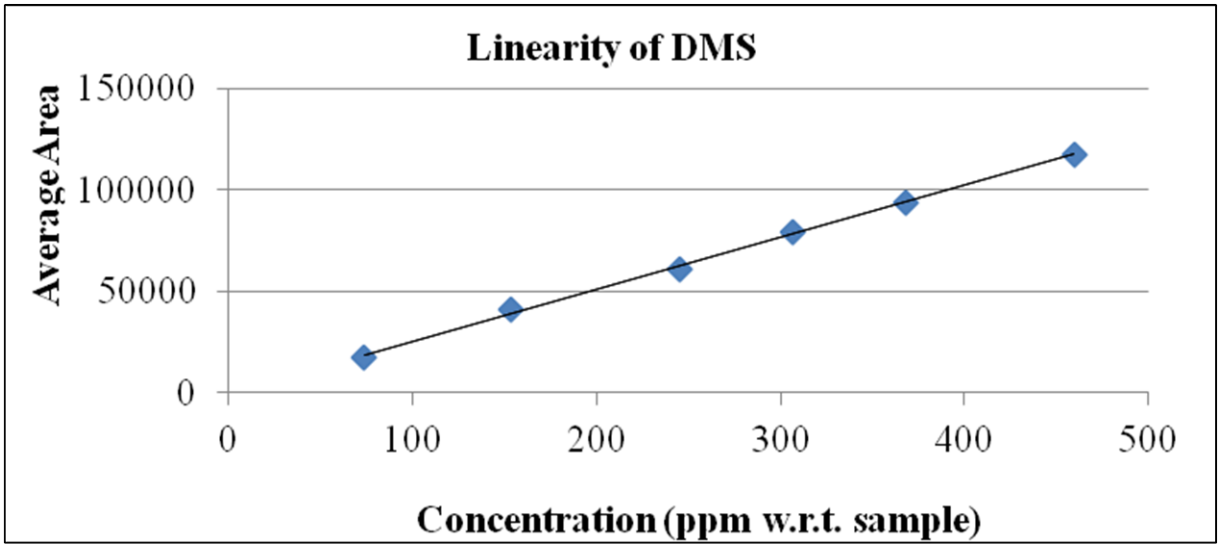

Figure 3: *Linearity graph of dimethyl sulfate.*

Precision

System precision was established by injecting six replicate injections of standard solution where % Relative standard deviation (%RSD) for Dimethyl Sulfate peak area was 1.57. To establish repeatability of the method, six spiked test solutions were prepared by spiking the Dimethyl Sulfate at its limit level concentration. % RSD for Dimethyl Sulfate content for six spiked solutions was 1.29. Similarly, intermediate precision was established by analysing the spiked test sample by a different analyst on a different day for which % RSD obtained was 1.12 including cumulative to repeatability was 1.60. This proves that the method is highly precise for the detection of Dimethyl Sulfate.

Accuracy

The accuracy of the method was determined for the related substances by spiking of known amounts of Dimethyl Sulfate in drug substances at levels LOQ, 80%, 100% and 120% of the specified limit. The method was highly accurate for recovery of Dimethyl Sulfate in the drug substances in the range of 84.40 and 88.24 %.

Robustness

The robustness of the method was established by making a deliberate change in carrier gas flow rate (1.4 ml/min and 1.6 ml/min) and ion source temperature (210°C and 230°C). % RSD for three determination for each change was less than 10.

CONCLUSION

An optimized GC-MS method was developed to determine the Dimethyl Sulfate content in Neostigmine Methylsulfate drug substances. Since molecular mass is more specific for each compound, no interferences were observed in Dimethyl Sulfate determination due to other impurities. An advantage of this method is its low detection limit. The developed method is simple and direct and does not require any other derivatization. This GC-MS method was validated which proves the method to be simple, highly precise, linear, accurate and rugged. Hence, this method can be used in the quality control department for routine low-level Dimethyl Sulfate analysis for pharmaceutical drug substances.

ACKNOWLEDGEMENT

We truly acknowledge and appreciate the support of our chemical research and development (CRD) team of Indoco in providing the sample for the study.

REFERENCES

1. M. B. Walker (1935), "Case showing the effect of prostigmin on myasthenia gravis", *Proceedings of the Royal Society of Medicine,* Volume 28, Issue 6, pp. 759–761.
2. R. N. Banerjee, A. L. Sahni, K. A. Chacko, K. Vijay (1972), "Neostigmine in the treatment of Elapidae bites", *The Journal of the Association of Physicians of India,* Volume 20, Issue 7, pp. 503–509.
3. G. S. Murphy, J. W. Szokol, J. S. Vender, J. H. Marymont, M. J. Avram (2002), "The use of neuromuscular blocking drugs in adult cardiac surgery: results of a national postal survey," *Anesthesia and Analgesia,* Volume 95, Issue 6, pp. 1534–1539.
4. M. M. Mehndiratta, S. Pandey, T. Kuntzer (2011), "Acetyl cholinesterase inhibitor treatment for myasthenia gravis", *Cochrane Database of Systematic Reviews,* Volume 2, Article ID CD006986
5. Selva M, Perosa A. (2008), "Green chemistry metrics: a comparative evaluation of dimethyl carbonate, methyl iodide, dimethyl sulfate and methanol as methylating agents", *Green Chemistry,* Volume 10, Issue 4, pp. 457−464.
6. Jarrahpour A, Zarei M. (Apr 8, 2009), "DMF-dimethyl sulfate as a new reagent for the synthesis of β-lactams", *Tetrahedron Letters,* Volume 50, Issue 14, pp. 1568−1570.
7. Zheng J, Pritts WA, Zhang S, Wittenberger S. (Dec 5, 2009), "Determination of low ppm levels of dimethyl sulfate in an aqueous soluble API intermediate using liquid–liquid extraction and GC–MS", *Journal of pharmaceutical and biomedical analysis,* Volume 50, Issue 5, pp. 1054−1059.
8. Gilland JC, Bright AP. (Jun 1, 1980), "Determination of dimethyl and diethyl sulfate in air by gas chromatography", *American Industrial Hygiene Association Journal,* Volume 41, Issue 6, pp. 459−462.
9. Khan M, Jayasree K, Reddy KK, Dubey PK (Jan 25, 2012), "A validated CE method for determining dimethylsulfate a carcinogen and chloroacetyl chloride a potential genotoxin at trace levels in drug substances", *Journal of pharmaceutical and biomedical analysis,* Volume 58, pp. 27−33.
10. Guideline (2014), "ICH Harmonized Tripartite. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic RISK M7", *International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva.*
11. Snodin DJ, McCrossen SD. (Jul 1, 2012), "Guidelines and pharmacopoeial standards for pharmaceutical impurities: overview and critical assessment", *Regulatory Toxicology and Pharmacology,* Volume 63, Issue 2, pp. 298−312.
12. Chan CC, Lee YC, Lam H, Zhang XM (Apr 23, 2004), "Analytical method validation and instrument performance verification", *John Wiley & Sons,*
13. Guideline IH (Nov 2005), "Validation of analytical procedures: text and methodology Q2 (R1)", *International conference on harmonization,* Geneva, Switzerland, pp. 11−12.