Determination Of Genotoxic Impurity Methyl P-Toluene Sulfonate in Pharmaceutical Drug by Chromatographic Technique

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ABSTRACT

The determination of genotoxic impurities (GIs) in pharmaceutical drug is critical to ensure drug safety and compliance with regulatory standards. It has identified and quantified GIs in Methyl p-Toluene Sulfonate and Doxofylline with use of Gas Chromatography-Mass Spectrometry (GC-MS). The method was developed and validated in accordance with ICH guidelines focusing on specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). It has analysed GC-MS analysis was performed using a DB-5 capillary column, with an electron ionization source and selective ion monitoring (SIM) mode to ensure high sensitivity for trace-level detection. The developed method demonstrated excellent specificity with well-resolved chromatographic peaks and minimal interference from the drug matrix. It has added on calibration curves for both drugs showed good linearity (R2>0.999) over a concentration range of 0.1 ppm to 10 ppm. The accuracy was confirmed by recovery studies with yield results within the acceptable range of 95-105%. The precision studies revealed that intra-day and inter-day relative standard deviations (RSD) below 2% and 3% respectively. The LOD and LOQ for methyl p-toluene sulfonate were 0.05 ppm and 0.1 ppm respectively while for Doxofylline have 0.07 ppm and 0.15 ppm. The method showcases the robustness under slight variation with analytical conditions. It can be concluded that the routine monitoring of genotoxic impurities in pharmaceutical formulations ensuring compliance with regulatory safety limits and contribution to the safety and efficacy of pharmaceutical products.

Keywords: Genotoxic Impurities, Methyl p-Toluene Sulfonate, Doxofylline, GC-MS, Method Validation, Limit of Detection (LOD), Limit of Quantification (LOQ), Recovery Precision, Pharmaceutical Safety.

I INTRODUCTION

The presence of genotoxic impurities in pharmaceutical poses a significant health risk due to their ability to cause DNA damage and potentially lead to cancer. It has involved regulatory authorities such as FDA and EMA which requires rigorous testing and control measure for genotoxic impurities in drug substances and product. Methyl p-Toluene Sulfonate (MPTS) is commonly used as reagent in pharmaceutical synthesis while Doxofylline is widely used as bronchodilator. However, these both compounds can be associated with genotoxic impurities, which must be detected and quantified to ensure patient safety and regulatory compliance.

Gas Chromatography-Mass Spectrometry (GC-MS) is one of the effective analytical techniques for detecting the trace level of organic impurities in pharmaceutical products as they have high sensitivity, accuracy, and precision to identify the complex structures. The paper discusses a validated GC-MS method development for detection of genotoxic impurities in MPTS and Doxofylline by utilizing the essential tool of pharmaceutical manufacturers and controlling quality in laboratories.

The study describes the GC-MS methodology for impurity detection followed by

experimental results and concluding remarks and future recommendations.

II PROPOSED METHOD

2.1 GC–MS Methodology for Impurity Detection

The GC-MS technique combines with the separation of the power of Gas Chromatography (GC) with analytical sensitivity and molecular identification capabilities of Mass Spectrometry (MS). In this study, the GC-MS method was optimized for analysis of MPTS and Doxofylline and their potential genotoxic impurities. It has involved the stages for detecting the impurities. They are as follows:

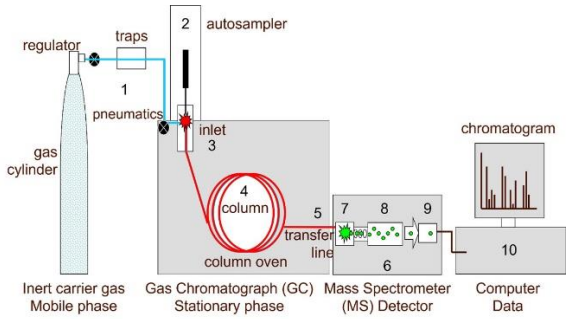

Figure 1: GC-MS Instrument

First stage of GC-MS involves the preparation of the sample. The pharmaceutical drug is first extracted using a solvent typically methanol or acetonitrile, to dissolve the active pharmaceutical ingredient (API) and focus on detection of impurities. Second stage is chromatographic Separation; it is the prepared sample which is injected into a gas chromatograph equipped with the capillary column. The sample is vaporized and separated into the individual component based on the volatility and interaction with stationary phase of the column. Third stage is Mass Spectrometric detection; the separated components are then detected using mass spectrometer. This can ionize the component and measure the mass-to-charge ratio (m/z) of resulting ions. It provides detailed information about the molecular structure of each impurity. Lastly, the data analysis to identify the potential genotoxic impurities based on the characteristic ion fragments and retention times and quantification of impurities is performed by comparing the peak areas of those of known standards.

The sample preparation is crucial to ensure accurate detection of impurities. The steps generated dilution, extraction, and filtration for the spiking sample with known standards are essentially managed for calibration and quantification. The sample is diluted in a suitable organic solvent like methanol to achieve trace-level impurity analysis. The impurities area extracted using techniques like liquid-liquid extraction or solid-phase extraction which depending on their physicochemical properties. The sample are also filtered with the particulate matter to remove the GC-MS performance for spiking of the sample.

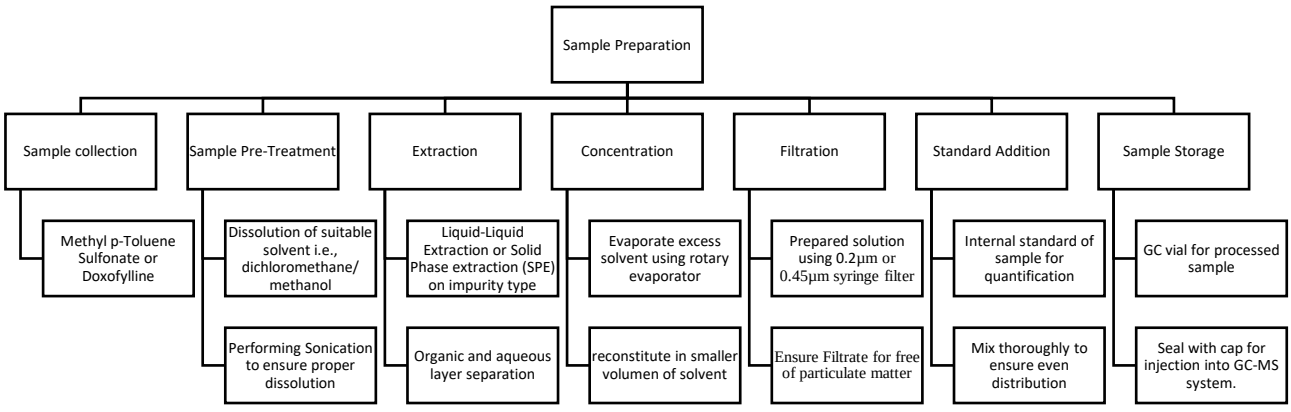

Figure 2: Sample Preparation Block Diagram

The prepared sample focuses on the drug named Doxofylline, a xanthine derivative to be well-established bronchodilator which is used for the management of the asthma and chronic obstructive pulmonary disease (COPD) which belong to the methylxanthine drug of the class. It includes theophylline with the improved pharmacological and safety profiles. The drug would significantly therapeutic benefit which add on the efficacy with alleviating the respiratory distress with the minimal adverse effect associated with traditional xanthine's. The chemical name of doxofylline is 7-(1,3- Dioxolan-2ylmethyl) theophylline. The molecular formula includes $C_{11}H_{14}N_4O_4$ with molecular weight at 266.25 g/mol. The mechanism of action to inhibits phosphodiesterase enzymes leading to increased intracellular cyclic AMP (cAMP) levels. The relaxation of bronchial smooth muscle with elevated cAMP results with the reduced airway resistance and improved airflow. The theophylline has demonstrated with the selective inhibition of phosphodiesterase IV (PDE4) with the associated way to anti-inflammatory effects. This can also reduce the activity on adenosine receptors with the way to result in fewer cardiovascular and central nervous system side effects to be attained.

2.2. Identification and Quantification of Genotoxic Impurities

Genotoxic impurities (GIs) in pharmaceuticals are significant concern due to their potential to cause damage to DNA, mutation, or cancer in humans. Regulatory guidelines such as those from international council for Harmonisation (ICH M7) emphasize to need the control of GIs in drug substances and products to ensure safety and compliance. Gas Chromatography- Mass Spectrometry (GC-MS) is a widely used analytical techniques for identification of GIs owing to high sensitivity, specificity and detection to volatility and semi-volatile impurities at trace levels.

The identification process begins with thorough understanding of the synthetic route and degradation pathways of drug substance. It has added on the alkylating agents, sulfonates, and halides to arise with by-products, intermediates, and degradation products to concern with impurities. The study also relates to chromatographic separation with sample injected into the GC system where the volatility and interaction with stationary phase. The impurities eluting from GC column are ionized and detected in the mass spectrometer. The determination of molecular weights and fragmentation patterns to be undertaken. The library matching spectra compared to known database (NIST library) to identify impurities. The structural elucidation to achieve through fragmentation analysis.

Quantification ensures the GIs to present the level below regulatory threshold with parts-per-million (ppm) range. It has presented the internal standards and calibration curves. The quantification process involves standard preparation at various concentrations involving the GI with sample to account for the variation in injection and analysis. Henceforth, the continuous advancement in instrumentation enhances efficacy in safeguarding public health.

2.3. Method Validation

The validation of an analytical method is crucial which ensure reliable yet accurate and reproducible detection and quantification of genotoxic impurities (GIs). It has critical parameters following the regulatory bodies which include validated parameters like specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). Each parameter is detailed in context of GCMS as follows:

2.3.1 Specificity

The GCMS offering the high specificity through chromatographic separation and mass spectral analysis have ensure that the physical separation of GIs have identified each compound based on its unique fragmentation pattern. It has analyzed a blank matrix (drug substance without GIs) to ensure no interference. The spike of the sample with known GIs and then verification of the detection without co-elution or overlapping peaks are fragmented for confirming the analysis.

2.3.2 Linearity

Linearity response has accurate quantification across the range of the interest typically in parts-per-million (ppm) levels for GIs. It has plotted the peak area against concentration to create a calibration curve with the calibration standards with GI at concentration to assess the correlation coefficient (R^2) with ≥0.99 for acceptable linearity.

Y= 58770.6648x-1540.1915…. (1)

2.3.3 Accuracy

Accuracy with GCMS have remark on the precise quantification using calibration standards and internal standards. The spike known concentration of GI into the sample matrix have validated approach. The spiked samples and compared measure concentration with known concentration can be calculated with the recovery percentage to typically fall within 95-105%.

2.3.4 Precision

Precision assesses with the reproducibility when validated properly. It analyzes the multiple replicates of the same sample within a single day. It performs analyses on different days or intermediate precision and calculating the relative standard deviation which should be usually below 2%.

2.3.5 Limit of Detection (LOD)

LOD is smallest concentration of GI to reliably detected but not necessarily quantified. The GCMS achieve low LODs to high sensitivity and selective detection capabilities. It determines the signal-to-noise (S/N) ratio for low concentration which is typically relied with 3:1 ratio.

$$LOD = 3.3 \frac{\text{x mean standard deviation of response}}{\text{Slope of the curve}}$$

$$LOQ = 10 \frac{\text{x mean standard deviation of response}}{\text{Slope of the curve}}$$

2.3.6 Limit of Quantification (LOQ)

LOQ is the smallest concentration of the GI that can be quantified with acceptable accuracy and precision. The GCMS provide precise quantification even at trace levels to ensure compliance with regulatory threshold. It has established the S/N ratio at 10:1 to define the LOQ with the predefined accuracy with 80%- 120% and precision criteria.

Y=60382.2x-1095…… (2)

III EXPERIMENT AND RESULT

The method was applied to both MPTS and Doxofylline Samples. After Sample Extraction and Preparation, the GC-MS system was set up with the parameters like column: DB-5ms (30m*0.25 mm * 0.25 µm). The experimental analysis utilizing GCMS and analysing the protocols and validated guidelines for MPTS and Doxofylline. The Materials and Reagents include Methyl p-Toluene Sulfonate and Doxofylline drug samples. It has analytical-grade reagents and solvents i.e., methanol, acetone, and water with calibration standards for GIs. The GC-MS instrumentation equipped with electron ionization (EI) source with mass range of 50-500 m/z. The sample prepared is dissolved in 10mg of the drug sample in 10 mL methanol with spiking standards where the GI were spiked into blank matrix samples for calibration. The filtration process utilizes filtered samples through 0.45 µm PTFE filters to remove particulates. The serial dilutions were prepared for linearity studies with 0.1 ppm to 10 ppm. The chromatographic conditions injection volume 1 µL, Carrier gas helium at 1 mL/min and Oven Temperature initially at 500C held for 1 min. The Ramp 1covers increase to 2000C at 150C/min while Ramp 2 covers increase to 2800C at 100C/ min held for 5 min with run time 20 minute. The mass spectrometric conditions include ionization mode for analysis for Selective Ion Monitoring (SIM) for higher sensitivity. GCMS-TQ8040with Mass- Detector and LC Solution software of Shimadzu. The Impurity peaks should be resolved from each other within specificity. No interference should be observed due to diluent at the retention time of Impurity (Methyl p-Toluene Sulphonate). Signal to noise ratio: 19 is the S/N ratio for the peak due to Methyl p-Toluene Sulphonate (MTS) in the chromatogram obtained with reference solution. It is concluded that the analytical method for Methyl p-Toluene Sulphonate of Doxofylline is specific.

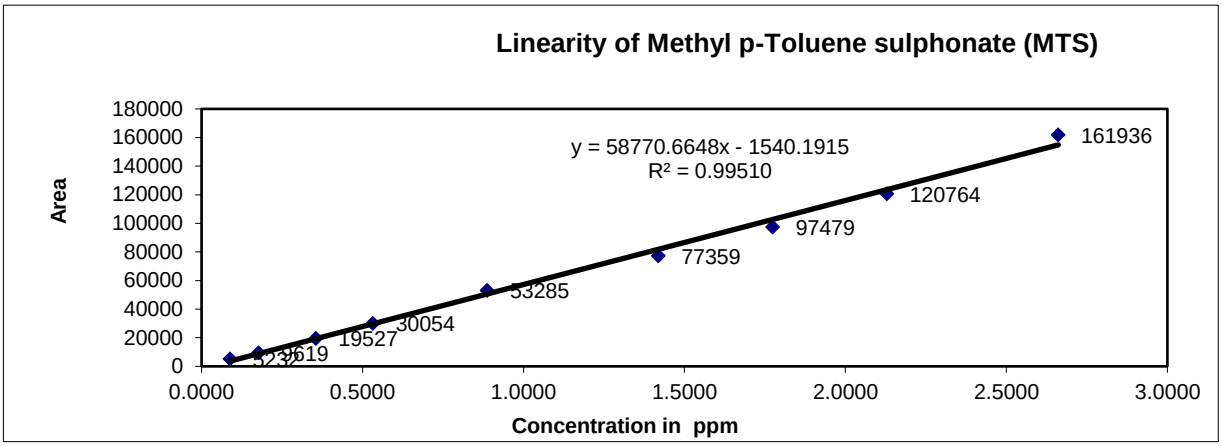

Figure 3: (a) Linearity of Methyl p-Toluene Sulphonate (MTS)

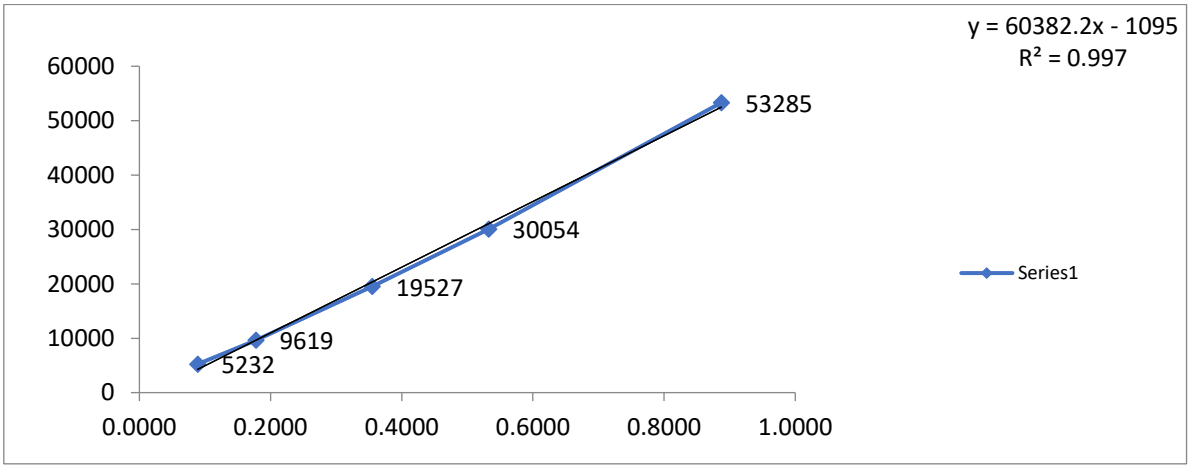

Figure 3: (b) LOD/LOQ for Methyl p-Toluene Sulphonate

$$LOD = 3.3 \frac{\text{x mean standard deviation of response}}{\text{Slope of the curve}} = 3.3 \frac{\text{x1036.4604}}{60382.2} = 0.057\ \mu g/mL$$

$$LOQ = 10 \frac{\text{x mean standard deviation of response}}{\text{Slope of the curve}} = 10 \frac{\text{x 1036.4604}}{60382.2} = 0.172\ \mu g/mL$$

The calculated value of Limit of Detection for Methyl p-Toluene Sulphonateis0.057 ppm. Hence the concentration 0.06 ppm is considered as limit of detection w.r.t. test. The calculated value of Limit of Quantitation for Methyl p-Toluene Sulphonateis0.172ppm Hence the concentration 0.17ppmis considered as limit of quantitation w.r.t. test.

The LOD for Methyl p-Toluene Sulphonate in Doxofylline found 0.006 ppm and S/N Ratio is found 33.8 found %RSD is 6.45% for LOD level. The LOQ for Methyl p-Toluene Sulphonate in Doxofylline were found0.017 ppm and S/N Ratio is 94.2 found %RSD is 2.77% for LOQ level. From the above results, it is concluded that the GCMS method for determination of Impurity (Methyl p-Toluene Sulphonate) in Doxofylline has LOQ limit for Methyl p-Toluene Sulphonate is 0.17 ppm and LOD limit Methyl p-Toluene Sulphonate0.06 ppm w.r.t Test. The linearity study was carried out for individual known Impurity of Doxofylline (i.e. Methyl p-Toluene Sulphonate (MTS)at 5.0% to 150 % of the standard

concentration. In this study the solution of individual Impurity at Nine different levels was prepared by dilution of stock solution fall known Impurity solution and analysed in triplicate. The value of Correlation Coefficient (r2) is found 0.99510or Methyl p-Toluene Sulphonate which are within the acceptance criteria. %RSD of all individual injection for all concentration are found within acceptance criteria i.e. NMT 15.0%. Based on the data it is concluded that the method is capable to determine the Impurity of Doxofylline in the linear range from 5.0% to 150 % of the standard concentration for Impurity (Methyl p-Toluene Sulphonate) which are within the acceptance criteria. From the above results, it is concluded that the GCMS method for determination of Impurity (Methyl p-Toluene Sulphonate) in Doxofylline has LOQ limit for Methyl p-Toluene Sulphonate is 0.17 ppm and LOD limit Methyl p-Toluene Sulphonate0.06 ppm w.r.t Test.

The Precision area have shown that the %RSD for Area and RT of Methyl p-Toluene Sulphonate. Impurity from six replicate is found 6.26% and 0.03%which are within the acceptance criteria i.e. NMT 15.0%. Based on the above data it is concluded that the Instrument have a good degree of precision for Impurity (Methyl p-Toluene Sulphonate) of Doxofylline.

The percentage recovery of Impurity should be 80.0 % to 120.0 % and % RSD should not be more than 15.0 % (If spiked Test sample analysed). In this study six replicate (in duplicate) sample preparations from Doxofylline at 100% test concentration (spiked) and duplicate for as such were prepared and analysed as per methodology to determine the Reproducibility of the analytical method. Mean from six replicate sample preparations, relative standard deviation is reported for results. The % RSD for sum of Methyl p-Toluene Sulphonate Impurity (Spiked) from six replicate sample preparations of Doxofylline for Impurity (Methyl p-Toluene Sulphonate) is found 3.06% which are within the acceptance criteria i.e. should not be more than 15.0%.and % Mean Recovery for Methyl p-Toluene Sulphonate is found 90.52% which are within the acceptance criteria i.e. 80% to 120%.

The percentage recovery for Methyl p-Toluene Sulphonate in Doxofylline were found within the range of 84.09 % to 118.87% and %RSD is 10.33 % for total recovery for LOQ level to 150% of Specification Limit. it is concluded that the GCMS with HS method for determination of (Methyl p-Toluene Sulphonate) Impurity in Doxofylline is accurate.

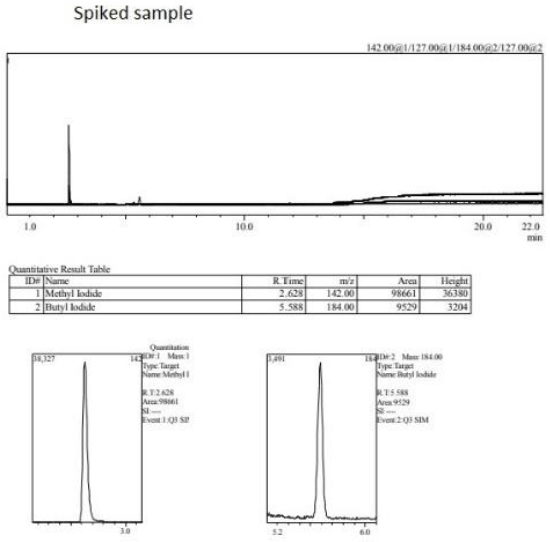

Figure 4: Spiked Sample for Quantitative Result

The chromatogram analysis has presented that the standard and blank solution have confirmed that their identity of the compounds is detected in the spiked sample. The peak is sharp and well-resolved which indicates the chromatographic conditions and successful spiking. The area and height of the methyl iodide is larger than the blank and standard which indicates that spiked concentration of Methyl Iodide in the sample is more. This can also present the height to be higher concentration compared to Butyl Iodide. The area has persistent with the Butyl Iodide where the blank has slightly higher concentration of Butyl Iodide and peak with the alignment of the expectation for its concentration. The SIM mode also ensure that there is high sensitivity

and selectivity for the analytes and help in the detection of added impurities in the spiked sample.

The observation has also shown that there is no interference from the matrix or diluent at the retention times of the analytes and indicating on the method specificity. The increase in the peak area for both analytes have also successfully demonstrated the spiking and quantification. The peaks are likely to meet with the strong results. This can also ensure on the reliability of the detected results. It can be concluded that the spiked sample contains detectable levels of both Methyl Iodide and Butyl Iodide with increased peak areas and heights compared to the blank solution and standard solutions. The chromatographic method demonstrates with the results that are excellent for sensitivity, specificity, and accuracy for the impurity detection in the spiked samples. The data confirmed the analytical method is effective and identify and quantify the impurities in the sample matrix.

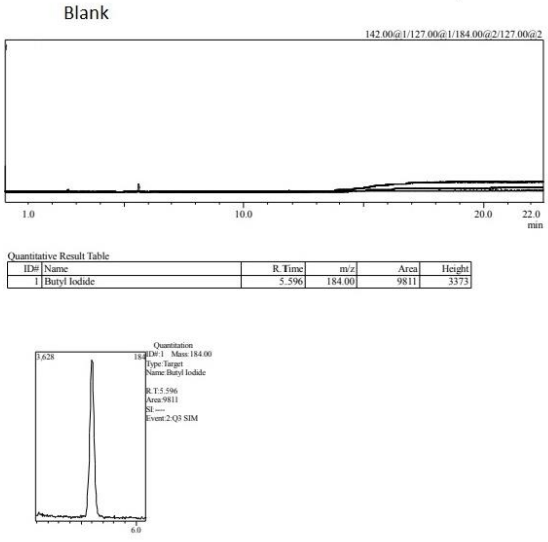

Figure 5: Blank Sample of MTS and BTS

The chromatogram analysis has added on the peak observation at 5.596 minutes corresponding to Butyl Iodide for the blank sample. There no peak is detected and indicating that there is absence in the blank sample. The baseline presents a stable yet minimal noise and no significant interference with Butyl Iodide peak area at 9811 and peak height at 3373 where the significantly smaller yet compared to reference solution suggest the trace amounts of Butyl Iodide in the blank. This suggests that the careful handling and cleaning of the equipment's to prevent the contamination or carry over the analysis during the process. The analytical method has demonstrated the specificity and no additional peak or interference are observed with the blank chromatogram.

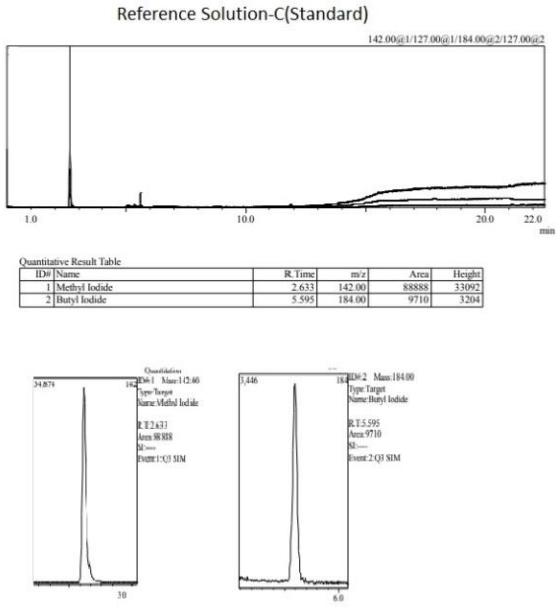

Figure 6: Reference Solution Standard-C Sample for MTS and BTS

The chromatogram interprets on the retention time (RT) of methyl iodide detection at 2.633 minutes and Butyl Iodide detection at 5.595 minutes. The retention times indicates distinct peaks for the two compounds and demonstrating a good separation and resolution between them. The Peak shape of the both appear to be sharp and symmetric which indicate on the optimal chromatographic conditions to be more effective for the separation. With the quantitative results to present that the methyl Iodide have significantly larger (88888)

compared to Butyl Iodide (9710) with indication of higher concentration of Methyl Iodide in the reference solution. The height has also added on the Methyl Iodide to be higher peak height than Butyl Iodide to have consistent way its higher concentration. The spectral data have included the mass-to-charge ratios (m/z) with the confirmed values at 142 and 184 m/z for the compounds at unique mass spectra. The event mode has both compounds which detected using SIM (Selected Ion Monitoring mode and enhancing sensitivity and specificity to the target ions. The chromatograms and spectral data have confirmed to no overlapping peaks and indicating that the specific detection of the Iodide without interference. The signal intensity has also been adequate with both compounds and ensuing reliable detection. It has distinctive peak area and retention time suggesting to suitable way for quantifying these analytes in complex matrices. The result has demonstrated the effective separation and detection of methyl Iodide and Butyl Iodide in the reference solution. The method has shown high sensitivity and specificity and mass spectra confirming the identity of the analytes. The method suitability has included for impurity analysis in pharmaceutical formulations.

Sr. No.	**Title**	**Acceptance criteria**	**Result**
1.0	Specificity	The Impurity peaks should be resolved from each other. No interference should be observed due to diluent at the retention time of Impurity	No interference observed due to diluent at the retention time of MTS& BMS
2.0	Linearity and Range for Impurity	The correlation coefficient should not be less than 0.99.	Methyl p-Toluene Sulphonate Correlation Coefficient (r^2) = 0.9951 Which are within the acceptance criteria. Range is method for 0.17 ppm to 2.66 ppm w.r.t. Test.
3.0	LOD (Methyl p-Toluene Sulphonate)	Signal to noise ratio should not be less than 3:1	S/N Ratio is 33.8, found for Methyl p-Toluene Sulphonate and LOD is found 0.06 ppm w.r.t. Test.
3.1	LOQ (Methyl p-Toluene Sulphonate)	Signal to noise ratio should not be less than 10:1 % RSD should be NMT15.0%	S/N Ratio is 94.2, found for Methyl p-Toluene Sulphonate and LOQ is found 0.17 ppm w.r.t. Test. %RSD is 7.75 %
4.0	Precision		
4.1	Instrument Precision	% Relative standard deviation for RT and Area Impurity (Methyl p-Toluene Sulphonate) in six results of Doxofylline should not be more than 15.0 %	(Methyl p-Toluene Sulphonate) %RSD is found 0.03 and 6.26for RT and Area which are within the acceptance criteria i.e. MT 15.0%
4.2	Method	% Relative standard deviation for Impurity (Methyl p-Toluene Sulphonate) in six results	%RSD for individual Impurity (Methyl p-Toluene Sulphonate) in six results of

	Precision	of Doxofylline should not be more than 15.0 %. In Spiked samples. % Recovery should be 80.0% to 120.0%	Doxofylline. Methyl p-Toluene Sulphonate (spiked) – 3.06% % Recovery is 85.83 % to 95.70 % for Methyl p-Toluene Sulphonate
4.3	Intermediate Precision	% Relative standard deviation for Impurity (Methyl p-Toluene Sulphonate) in six results of Doxofylline should not be more than 15.0 %. in Spiked samples. % Recovery should be 80.0% to 120.0% % Variation between MP and IP should be NMT 15.0%	RSD for individual Impurity (Methyl p-Toluene Sulphonate) in six results of Doxofylline are Methyl p-Toluene Sulphonate. (spiked) – 4.22% % Recovery is 83.33 % to 93.52% for Methyl p-Toluene Sulphonate 3.0888% Variation between MP and IP which are within the acceptance criteria i.e. NLT 15.0%
5	Accuracy	The percentage recovery for each level should be between 80.0 to 120.0%. Overall % Relative standard deviation for recovery at all the levels should not be more than 15.0 %.	% Recovery at LOQ Level % RSD =3.43% Minimum =84.86% Maximum =91.54% Recovery at 50% % RSD = 5.44% Minimum =98.97% Maximum =115.66% % Recovery at 100% % RSD = 3.98% Minimum =106.43% Maximum =118.87% % Recovery at 150% % RSD = 3.37% Minimum =105.70% Maximum = 115.59% Overall % RSD = 10.3%

6	Robustness	The percentage recovery for each level should be between 80.0 to 120.0%. % Variation between Method Precision and Robustness (all changes) should be NMT 15.0%	Percentage recovery: **(-Flow 5%)** = % Recovery is 92.10% **(+Flow 5%)** =% Recovery is 90.50% **HS Temp. -5°C** =% Recovery is 96.18% **HS Temp. -5°C** =% Recovery is 89.28% % Variation between Method Precision and Robustness **(-Flow 5%)** = 0.1030 **(+Flow 5%)** = 2.9418 **HS Temp. -5°C** =-4.9066 **HS Temp. -5°C** =4.7915

Table 1: Validation Test Results for Impurity (Methyl p-Toluene Sulphonate) in Doxofylline

IV CONCLUSION

Aziz

The present study overlooks on the various impurities which are reported to be a part of the pharmaceutical industry. This has included various drug molecule which are human carcinogenic and genotoxic in nature. This estimation has hyphenated techniques where the GCMS have used the in-vitro estimation of the drug and other impurities within it. The study has also validated the method to be used in the interactions between Doxofylline and excipients like Methyl p-Toluene Sulfonate in marketed formulation through drug safety and efficacy assurances with the results serving as a baseline. It also submits the details to regulatory agencies for impurity profiling in pharmaceuticals. This can add on the quantification of Methyl p-Toluene Sulfonate and Butyl Methane Sulfonate impurities in Doxofylline. This can also ensure its relevance in the pharmaceutical and manufacturing research in future. The doxofylline represents the significant advancement in xanthine-based bronchodilator therapy. However, the combination therapy with corticosteroids or beta-agonists are better for controlling the respiratory disorders. This has also sustained the release formulations to enhance patient compliance and prolonged therapeutic effects. The validated GC-MS method turns out to be effective for routine monitoring of genotoxic impurities in pharmaceutical formulations. It also ensures the regulatory safety standards, safeguarding drug quality and patient health.

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