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Evaluation Of Analytical Method Using Pharmaceutical Drugs: Genotoxic Methyl P-Toulene Sulfonate

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Abstract:The genotoxic impurity methyl p-toulene sulfonate (MPT) poses a significant health risk in the pharmaceutical products. This has aimed on the development and evaluation of the analytical method based on the chromatographic conditions where gas chromatography-mass spectrometry (GC-MS) have quantified and detected the MPT in pharmaceutical drug formulation. The method was validated for selectivity, sensitivity, precision and accuracy and robustness following International Council for Harmonisation (ICH) guidelines. It has added on limits of detection (LOD) and Quantification (LOQ) as well as recovery rates were assessed. It has proposed on the methods which shows excellent specificity for MPT, with reliable detection and low concentration, making it promising tool with the pharmaceutical quality control and genotoxic impurity testing.

Index Terms - Genotoxic impurities, Methyl p-Toluene Sulfonate, Gas Chromatography-mass spectrometry (GC-MS), anti-biotic drugs, method validation.

I. INTRODUCTION

The presence of the genotoxic impurities (GTIs) has added on the pharmaceutical product with the anti-cancer medications and concerning on the mutations. The cancer and genetic disorders have made some changes in the agencies where the regulatory validation and analytical methods have ensured on the impurities within acceptable limits [1]. It has relatively managed the impurities and identified and quantified the GTIs in designated anti-cancer drugs or antibiotic drugs.

The study aims to use the cutting-edge technologies such as gas chromatography mass spectrometry (GCMS) to identify the potentially carcinogenic and genotoxic contaminants in newer medications in bulk (active pharmaceutical ingredients). It can present the implementation of the modern chromatographic method coupled with techniques where hyphenated mass detectors can calculate genotoxic impurities in the medications that are specific [2]. Necessary parameter such as precision, optimization, method creation, linearity extensive validation selectivity and robustness. Established analytical procedures are incorporated into analysis processes and regular quality control. The methods are compiled into a data and compared with the validation criteria as per the conclusion of US FDA, ICH, and EMEA guidelines [3].

1.1 Genotoxic Impurities

Methyl p-toulene sulfonate (MPT) has added on the presentation of the synthetic processes or contamination. It has posed on the significant risk to human health. This includes DNA damage through carcinogenicity. It has required stringent monitoring and control of such impurities in pharmaceutical drugs and regulatory compliance and patient safety.The role of metabolism, epoxides and in vivo with the alkenes and arenes have potentially manage the cause of the adverse effect than performed impurities with the

aromatic compounds. The aromatic amines generally covering primary and secondary aromatic amines which include electrophilic species and adding on the positive results in the Ames Test when S9 mixture exist [3]. 2, 4-Diaminotoluene, 2,4-diaminoethylbenzene with nitro group being a direct mutagen. This can present positive results for the p- aniside and p-chloroaniline and another compound.

In case of negative results, the p-Nitrophenol have possess the synthetic chemical adds on the fungicidal activity used for the materials in the synthesis of some drugs [5]. It has included the invitro test which range a little toxicity to maximum tolerated doses.

Another aromatic compound involving the Fentanyl impurities have produced seven aromatic degradants where N-phenyl-1-(2-phenylethly)-piperidin-4 amine (PPA) where the unscheduled DNA synthesis occurs [2]. There are probabilities of the compound to predict higher probabilities and Ames positive results.

Tremogenic impurities comprise have highly added APIs in the potentially pharmacopoeial APIs which includes pethidine and paroxetine (3-[(1, 3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl) piperidine) remains unclear with the toxic impurities. β lactum related impurities include the antibiotics cefotaxime and piperacillin [2]. The dimeric impurity of cefotaxime includes the storage processes which is structured as

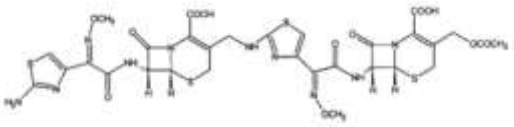

Fig. 1. Dimeric Impurity of Cefotaxime [2]

In vitro chromosomal have align with the concentration of 45mg per culture and no clastogenicity in mammalian cells in vitro test.

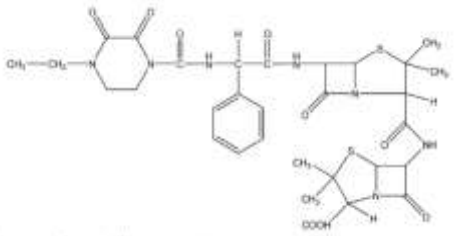

Fig. 2. Piperacillin Impurity-A [3]

The piperacillin impurity-A has prominent degradation of product which has included all strains such as TA97a, TA 98, TA 100, TA 102 and other. It has utilized the GIs where the pharmaceutical effects to monitor the outcome of the chemical synthesis with the conventional method GCMS [4].

2.0 EXPERIMENTAL METHODOLOGY

This study is conducted by GC-MS/MS system equipped with the Mass-detector: Head space injector and software for recording the chromatograms.

2.1Material and Method

2.1.1 Reagents, Materials and Standards

The reference of the reagent and solvents includes GCMS quality sourced from regional supplies and Analytical grade [7]. Anti-biotic Medications: sourced from laboratory covering methyl p-toulene sulphonate pure standards purity >99% obtained from Sigma- Aldrich. The solvents include Acetonitrile, Methanol (MeOH), dichloromethane (DCM), and hexane (Hex) were obtained from Merck and 4-bromotoulene (purity>98%) were used as internal standards.

2.2 Analytical Conditions and Equipment's

Qualitative and quantitative analysis to identify the analytical procedure with the impurities in the pharmaceutical ingredients with the steps covering sample preparation (adequate sample collection and preparation), chromatographic conditions (analytical techniques selection), GCMS analysis (Performing the analytics), Estimating the confidence interval to validation of data analysis [8].It includes the agilent 7890B GC coupled with MS detector 5977A model. The capillary column consists of the Agilent HP-5 (30m*0.25 mm*0.25µm film thickness. 10µL Hamilton syringe for sample injection and pipettes, volumetric flasks, and standard laboratory glassware.

3.1 Analytical condition of GCMS

3.2 Preparation of Standard Solution and Sample Solution

To accurately quantify the genotoxic impurities in anti-cancer medications, it has added on the concentration where the standard solutions serve as a reference for calibrating GCMS system and validating on the methods [15].

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3.3 Internal standard solution preparation

The use of the internal standards helps in compensating on the variation of the sample prepared for the injection volumes and improving the accuracy and precision of the analysis. The internal standard has chemically managed the analytes but it does not interfere with their detection [8]. It has added a similar retention time and response factor for the analytes where weigh of the GTIs quantify into a volumetric flask. This can dissolve in a suitable solvent with the desired concentration of 100 µg/mL to mix it thoroughly. Additionally, it has fixed volume of internal standard solution to each sample solution including the quality control of samples and calibrating standards of samples before analysis [7].

3.4 Standard solution preparation

There are series of standard solution used for calibration purposes with the way to cover the expected range of impurities in the sample. The pipette appropriate volumes of intermediate standard solution into volumetric flask and dilute to the mark of the solvent. It has included different solutions within mix of the solution thoroughly. It also prepares the quality control standards at low, medium, and high concentration within the calibration range [9]. This has set out the use of the QC standards and monitor the accuracy and precision of the analytical method during the routine analysis. The GC conditions includes injector temperature 250^0C and oven program with initial temperature of 70^0C which hold for 2 min and ramped to 250^0C and hold for 5 min. Carrier gas includes Helium at 1.0 mL/min (Constant flow mode) and split ratio of 1:10. While in MS condition, the Ionization mode including the electron impact ionization (EI) at 70 eV and Ion source temperature at 230 ^{0}C and Quadrupole temperature at 150^0C. It includes the mass range to m/z 50-350. The detection mode selected for ion monitoring (SIM) for MPT (m/z 174) and internal standard (m/z 109).

3.5 Sample solution separation

The separation of sample has involved a critical step to ensure the quantification of genotoxic impurities with the chromatographic conditions where the column, mobile phase and detector settings have utilized the flow rate, temperature, and gradient program optimization to achieve a good separation of the impurities from the API and each other. The injection of the prepared solutions typically ranging from 1-10 µL into the GCMS system is set out at equilibrated before injection [13]. The chromatographic data would use the calibration cure constructed from the standard solutions to quantify the impurities in the sample solution. The concentration is calculated with each impurity and compared within the regulatory limits. The formulation of APIs was in powdered sample of 100 mg with mixture of acetonitrile and water (1:1, v/v). after vortex mixing and centrifugation, the supernatant was filtered through a 0.45 µm nylon filter and diluted with solvent mixture for GC-MS analysis.

For calibration standards, the known quantities of MPT were spiked with blank matrix at various concentrations and internal standard were added with the calibration standards and sample at a concentration of 50 ng/mL to ensure accurate quantification.

3.6 Validation of Analytical Methods

The validation comes when the selectivity of the method is undertaken place. Henceforth, it can be done by analyzing the pharmaceutical formulation of interfering peaks from drug matrix. The GC-MS chromatogram obtained from a blank sample and spiked sample was carefully examined when compared with the internal standards of (4-bromotoulene) and other potential contaminants to form the specificity of the method. Validation is the way to ensure the criteria of the accuracy, precision, specificity, sensitivity, linearity and robustness. This can also lead with the linearity and range for setting out the concentration and the response [6]. The parameters have been evaluated as:

3.6.1 Determination of Specificity

Specificity is evaluated by analyzing the standard solution of API where potential impurities and methods are separately formed from the degradation of any products [8]. It has chromatographic conditions are optimized with the way to achieve distinct yet well-resolved peaks for each component.

3.6.2 Determination of Accuracy and Precision

It has been evaluated that accuracy is assessed on spiking the known quantities and impurities into the API and analyzing the samples with the recovery of the spiked impurities [10]. It has been directed that precision on the other hand is analyzing the same sample multiple times under the same conditions and calculating the

relative standard deviation (RSD). This has calculated both intra-day and inter-day. The accuracy has also set out to spike the known concentrations of MPT into blank samples and compared the measured concentration with true concentration. This recovery has been calculated by determining the percentage of MPT recovered from the spiked samples.

3.6.3 Determination of Linearity and Range

Linearity is evaluated by analyzing the standard solutions of impurities at different concentrations and plotting of peak versus concentration area. It has set out the response to the method where the linear response and correlation coefficient (R^2) is calculated with the linearity access [1]. For MPT the peak area ratio can be plotted as per the internal standards against the concentration. It has evaluated the construction range for curve to be ranged in between 0.1 to 100 ng/mL. The linearity of the method is assessed by calculating the correlation coefficient which nearly to be greater than 0.99.

3.6.4 Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is representing the lowest concentration of the impurity and are not quantified easily. The analyte can relate to the distinguished from background noise but it has not necessarily quantified as an exact value [4]. This can also typically include the low concentration of the analyte and calculated standard deviation of the response.

$$LOD = 3.3 * \sigma / S$$

σ Standard deviation of response for low concentration sample
S is the slope of the calibration curve.

$$LOQ = 10 * \sigma / S$$

LOQ is lowest concentration quantified with the acceptable precision and accuracy. It usually determines the signal-to-noise ratio method but for GTIs its highly selective method [6]. The LOD is likely to be defined as lowest concentration of MPT and reliably detecting the signal-to-noise ratio (S/N) of 3:1, while LOQ was the lowest concentration that could accurately quantify the S/N ratio of 10:1.

3.6.5 Determination of Robustness

The robustness is evaluated with the deliberated changes to chromatographic conditions such temperature and flow rate. It has represented the effects of the separation and quantification of the impurities. The method is considered that the robust yet consistent results under varied conditions are developed [8]. The robustness has certainly involve the effect of variations on the peak area of MPT under the evaluated conditions of GC-MS i.e., injection temperature, column temperature and carried flow rate.

IV. DISCUSSION

4.1 Interpretation of Discussion

The GCMS method have demonstrated with the high selectivity, reproducibility, sensitivity, and detection of the quantification of GTIs in anti-biotic medication. It has clearly set out the impurities from API where the optimization of the chromatographic conditions and retention times and peak shapes can be attained. It has validated on the results where the reliability of the satisfactory values can manage the robustness and LOD, LOQ along with the precision, accuracy, precision [12]. The mass matrix of the MPT also showcased the characteristic peaks with molecular ion at m/z 186 and fragment ions at 155 and confirming the identity of the compounds.

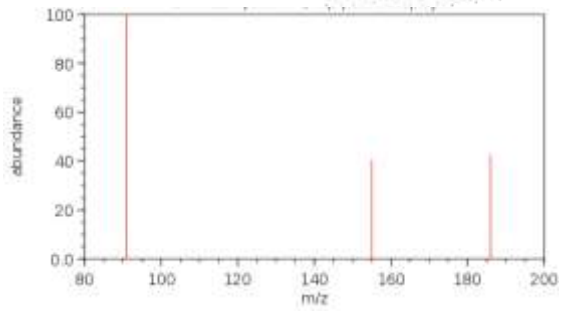

Fig 3. Methyl p-toulenesulfonate GCMS Mass Spectral

The method has added on the well-resolved peaks with no interference from blank peaks . This can confirm on the specificity of the method. The recovery might spike with the range of impurities from 95% to 105% and ranging on the high accuracy [11]. It has RSD values for precision below of 2% demonstrating excellent reproducibility. The method might showcase on the linear response of impurities over the concentration range of 0.1 to 10 µg/mL

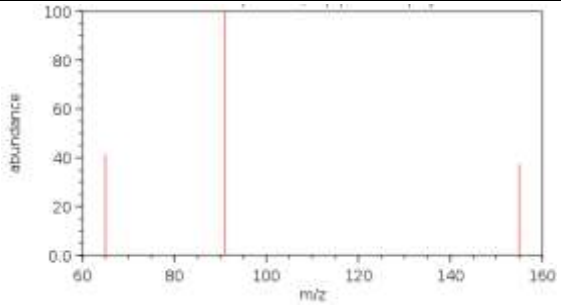

Fig 4. Methyl p-toulenesulfonate compound total peaks

It has correlated coefficient (R^2) greater than 0.999 [13]. The method is suitable with the impurities at trace levels and LOD values to range with 0.01 to 0.05 µg/mL, and the LOQ values to range with 0.05 to 0.1 µg/mL. It is the lowest detection limits demonstrated with high sensitivity in the method. The robustness remained at peak in the chromatographic conditions. It is consistent as the peak time did not change the pH, flowrate and affected the quantification of impurities and separation [15]. The method has turnout to be robust at minimal variation in peak area when small changes were made to the GC-MS conditions indicating the reliable yet slightly variant conditions.

5. Conclusion

The study has developed a validated GCMS method to determine the genotoxic impurities in the anti-cancer drugs which is robust, accurate and compliant with regulatory standards. It has ensured on the safety and efficacy of pharmaceutical products. This can integrate on the routine analysis to monitor with the control genotoxic impurities (API potential ingredients-based impurities) in pharmaceutical. The future direction of the research would integrate on the drugs PGIs and explore on the advanced detection techniques such as high-resolution mass spectrometry. It has also enhanced the detection of the characterization of GTIs. This can additionally link with the green chromatography methods and using hazardous solvents. It contributes to the sustainable analytical practices in the pharmaceutical industry. The method was successfully validated according to ICH guidelines and demonstrating the excellent linearity, precision, accuracy and robustness. It has also ensure the safety and quality of the drugs by monitoring the genotoxic impurities like MPT.

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