International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Novel Extraneous Peaks in GC Analysis from GC Diluents, Processing Solvents and API Containing Salts: Identification by Mass Spectrometry

Perla Ganesh[*1,4], *Apparao Sapala*[2], *N.Jagadeesh*[1], *Ch. B. V. Narasimha Raju*[1,4], *BM.Rao*[4], *Dharamasoth Rama Devi*[3] , *K. Basavaiah*[4]

[1]*Dr. Reddy's Laboratories Limited, API Plant-III, Plot No 116, IDA Bollaram, Medak District, Hyderabad 502325, Telangana, India.*

[2] *Department of chemistry, Vignan jyothi institute of information technology, Hyderabad, India.*

[3]*Dr. Samuel George Institute of Pharmaceutical Sciences, Andhra Pradesh, India.*

[4]*Department of Chemistry, Andhra University, Vishakhapatnam, India.*

Abstract

As per the set forth International Council for Harmonization (ICH) guidelines, analytical scientists intrigue to invest their time in identifying the potential and non-potential impurities which arises from residual solvents in Active Pharmaceutical Ingrediants (API) by Gas chromatographic (GC) analysis. These impurities can rise from numerous sources like synthesis, washing, purification, filtration, storage of the new drug, degradation of solvents, cross reactions and milling in analytical conditions. These peaks play a vital role in deciding the quality of the product and effect of the drug. Hence, identifying these impurities is a challenging task to improve the safety usage of the drug. Herein, we are reporting the impurities using GCMS analytical technique embodied with the elegant mechanistic strategy for their formations. We focus on the impurities arising from GC diluents, Processing solvents and API. We strategically investigated the formation of impurities due to the reaction of processing solvents with its minor impurities, degradation of residual solvents and Cross reactions among API containing excess salts with residual solvents. We extended our investigation on degradation of GC diluents and its cross reactions with excess salts of API.

Keywords: Extraneous peaks, GC, GC-MS, API containing salts, Processing solvents and GC diluents

Full-length article **Corresponding Author*, e-mail: pganesh168@gmail.com

1. Introduction

Pharmaceutical impurities are the unwanted new chemical entities which include byproducts (formed during synthesis), degradation products (emerged from manufacture process) and products from API processing solvents. According to ICH guidelines, impurities are classified in different types such as Organic impurities, Inorganic impurities and In-process product impurities etc. These impurities [1-2] in the drug play a significant role in impacting the quality and also affect the safety of the patient. Thus, Research on the formation of impurities based on different conditions is a challenging task to the scientists as it includes not only identifying the impurities but also requires critical analysis of its route cause and followed by specification of its quantity [3].

An analytical technique such as NMR, HPLC, and MASS spectroscopy (LCMS &GCMS) helps in structural elucidation [4] of pharmaceutical impurities. Among these analytical tools, GCMS is the important instrument to analyze specifically volatile compounds along with the non-volatile substances present in the API. In addition, GCMS is the most prominent tool for the separation and identification of components in compounds which is attributed to its sensitivity and effectiveness. Mostly the sample preparation method includes dilution of sample with solvents (GC diluents) that converts a real matrix into an appropriate sample for GC analysis. The GC diluent [5] helps to yield the exact result by reducing the concentration of the sample. In accumulation, the diluent should be able to dissolve an enormous diversity of samples even that possess high boiling point, Commonly used sample diluents [6] for GC analysis.

Processing solvents (or) Residual solvents (RS) are Volatile Organic Chemicals that are produced / used during the Active Parmaceutical Ingredients manufacturing process. These are difficult to remove completely. In order to assess pharma products for toxicity and adverse effects, RS analysis is essential. Their physicochemical properties also affect pharmaceutical products, posing potential health risks. It is therefore mandatory that health authorities in the world determine the RS levels in APIs and excipients [7]. It is therefore mandatory for health authorities around the world to accurately determine the levels of RS in APIs and excipients. Some of API/Drugs in parental form doesn't lend itself to being absorbed or utilized properly in Human bodies. The possible methods to prevail this are chemical and biopharmaceutical tweaks that involves the creation of

a 'salt form' of the Drug [8]. Hence, API/Drugs are often chemically made into their salt forms to the enhance solubility of the drug; absorption ability of drug into blood stream and thereby increase drug's effectiveness. Well known API/Drugs in salt form (Eg's: Clopidogrel bisulphate, Sitagliptin HCl, Dabigatron mesylate, and Sugammadex sodium) exhibiting therapeutic effects.

GC-MS technique was used to conclude extraneous peaks/extracellular peaks attributable to GC analysis using NIST library [9]. A GC-MS analysis uses 70ev energy to ionize the sample, so it is a hard ionization method. Therefore, GC-MS spectral data cannot be used to compile chemical structures like LC-MS spectral data. This may result in the formation of extraneous peaks/extraneous peaks due to the cross reaction between GC diluents, processing solvents and API containing salts.

2. Instrumentation

ALS-EI, HS/GC-EI methods were developed and optimized to identify the degradants, GC/EI- MS data of degradants were recorded by using Agilent 7890A Gas chromatograph equipped with 5975C Mass selective detector and G1888 network headspace sampler.

3. Results and Discussions

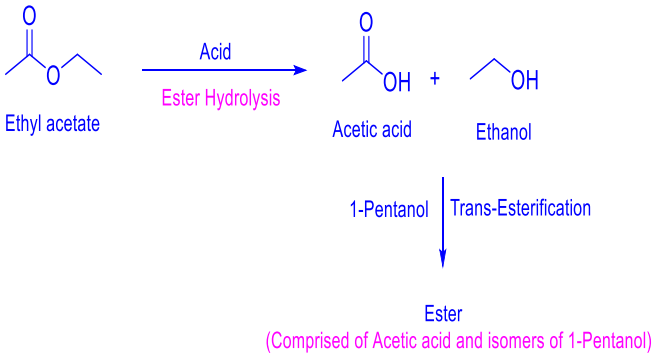
3.1. Processing solvents

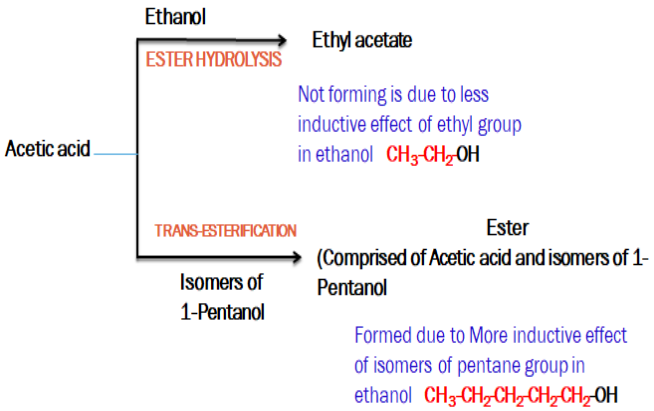
The API dissolved in organic solvents and also used in the continuous process such as work-up, separation of compounds and purification process. Many processes involve in removing these organic solvents, and the left-over solvent traces are called residual solvents (RS) or organic volatile impurities (OVI). Identifying these Residual solvents is essential as per ICH Guidelines Q3C (R6) and are presented here with individual data.

3.2. Ethyl acetate

Ethyl acetate (EtOAc) is an organic solvent which contains ester functional group. EtOAc is one of the common organic solvent used in the API which is attributed to its polarity, moderate boiling point and eco-friendly nature. During GC-analysis of API, peaks corresponding to EtOAc were observed. Intense analysis has been carried out to identify the extraneous peaks from GC-analysis. The most probable structures were shown below which corresponds to the observed peaks and shown in the Figure- 1.

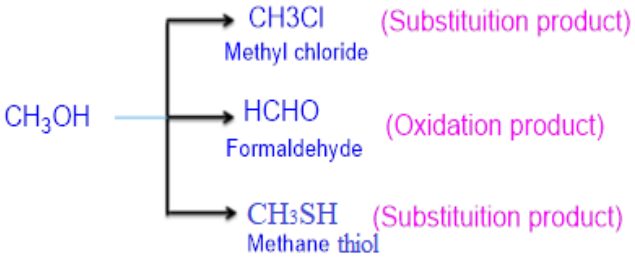
Interestingly, all the extraneous peaks comprised of ester groups with different side chain were observed. The observed extraneous peaks might be due to the Trans esterification reaction [10-12]. Based on the peaks from Tran's esterification reaction, we envisioned that the observed peaks might due to the exchange of 1-pentanol with an alcohol group of an ester group, GCMS Data of Unknown peaks from Ethyl acetate solvent from NIST library shown in Figure-2 &3. The chance of revisable formation of Ethyl acetate during esterification is less compared to the Trans-esterification, which is attributed to the presence of more electron releasing group in the isomers of pentanol than ethanol.





3.3. Methanol

Methanol contains –OH functional group. Methanol is a Low boiling, most polar solvent and ability to solubilize both lipophilic and hydrophilic. During GC-analysis of API, peaks corresponding to methanol were observed. The extraneous peaks were identified and co-related with the most probable structures with proper reasoning and are shown below:



The hydroxyl group in methanol would be a good leaving group in presence of acidic medium which facilitate the formation of Methyl chloride (in presence of Chlorinated API drugs) and formation of Methane thiol (in presence of thio sources) shown in Figure- 4-6.

3.4. Higher alcohol

We continued our investigation on next higher alcohol such as Ethanol, Isopropyl alcohol (IPA) and 2-butanol. (relation with methanol).

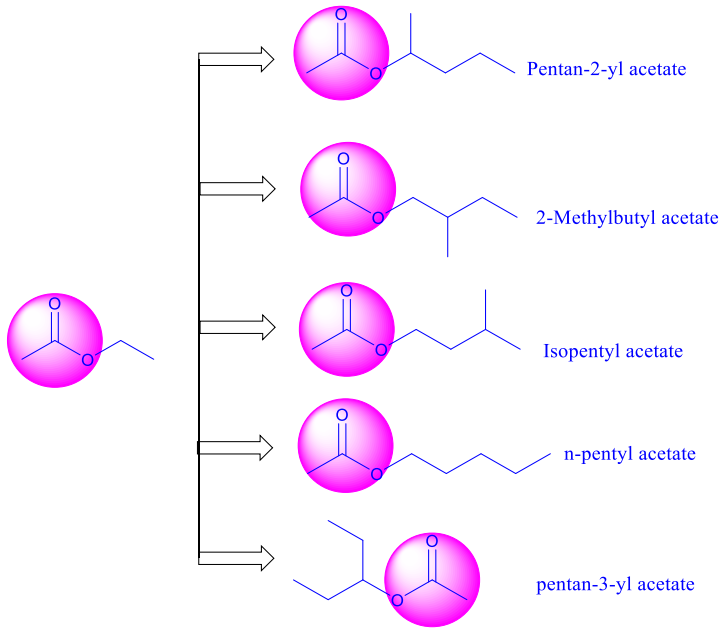


Figure 1: Schematic representation of the **EtOAc** extraneous peaks from GC-analysis.

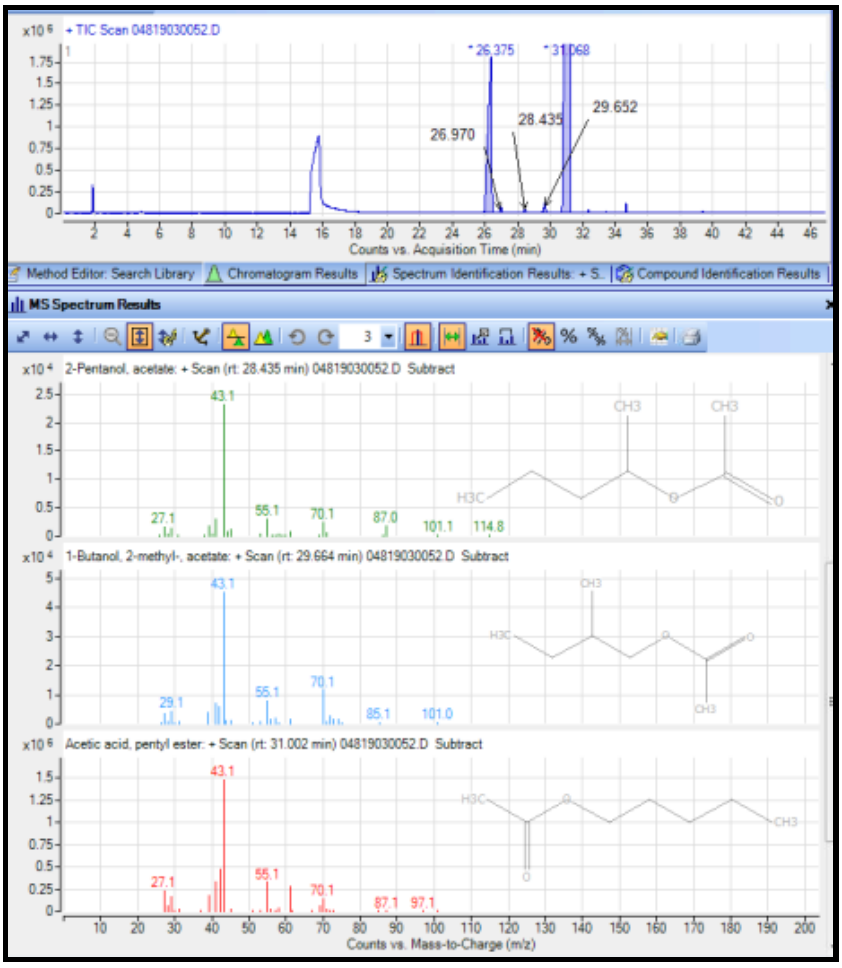


Figure 2: GCMS showing the peaks corresponding to ethyl acetate fragments

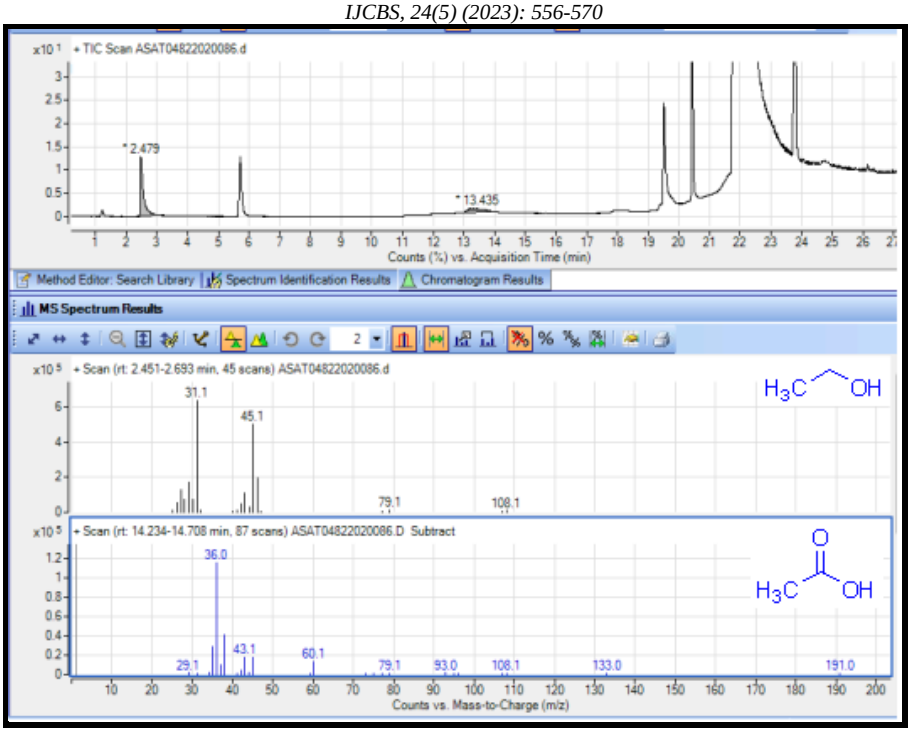


Figure 3: GCMS showing the peaks corresponding to ethyl acetate fragment

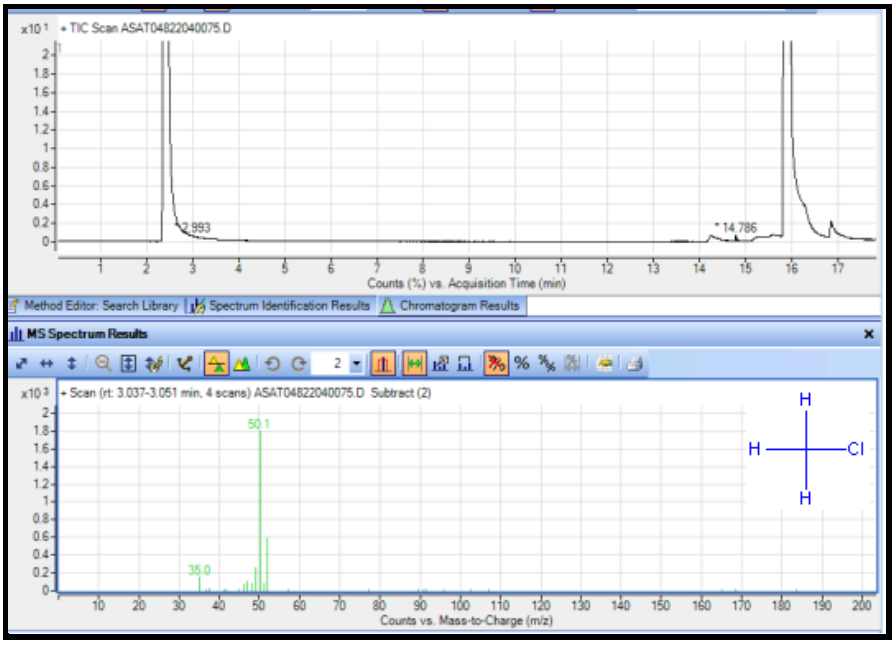


Figure 4: GCMS showing the peaks corresponding to methanol fragment

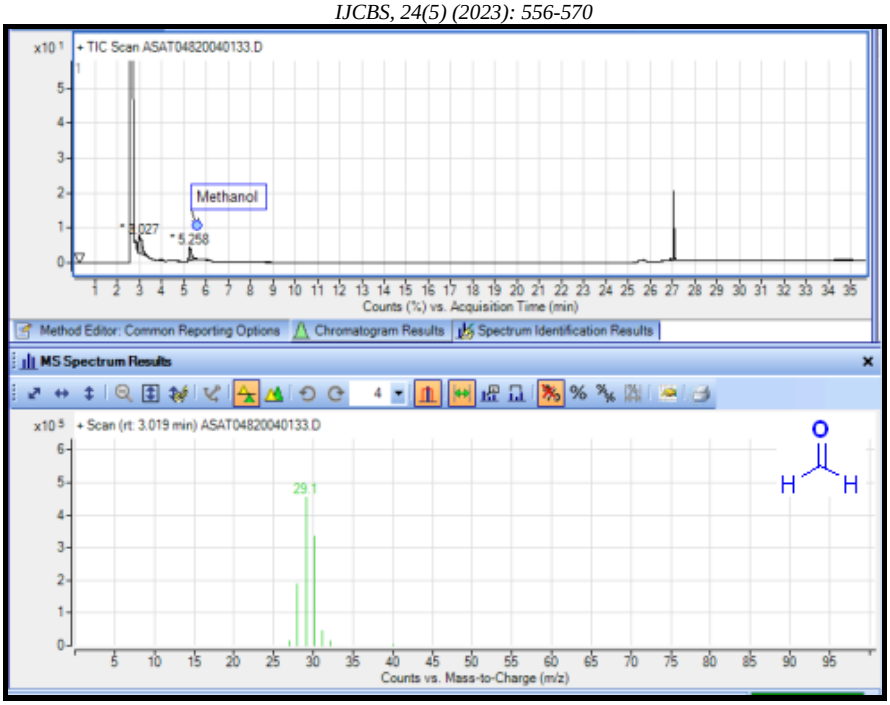


Figure 5: GCMS showing the peaks corresponding to methanol fragment

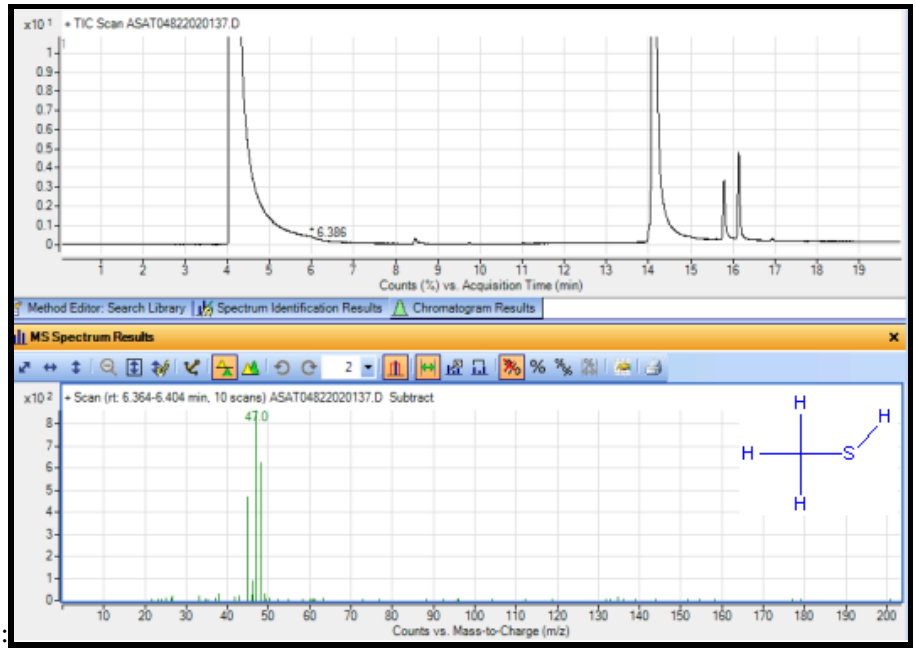


Figure 6: GCMS showing the peaks corresponding to methanol fragment

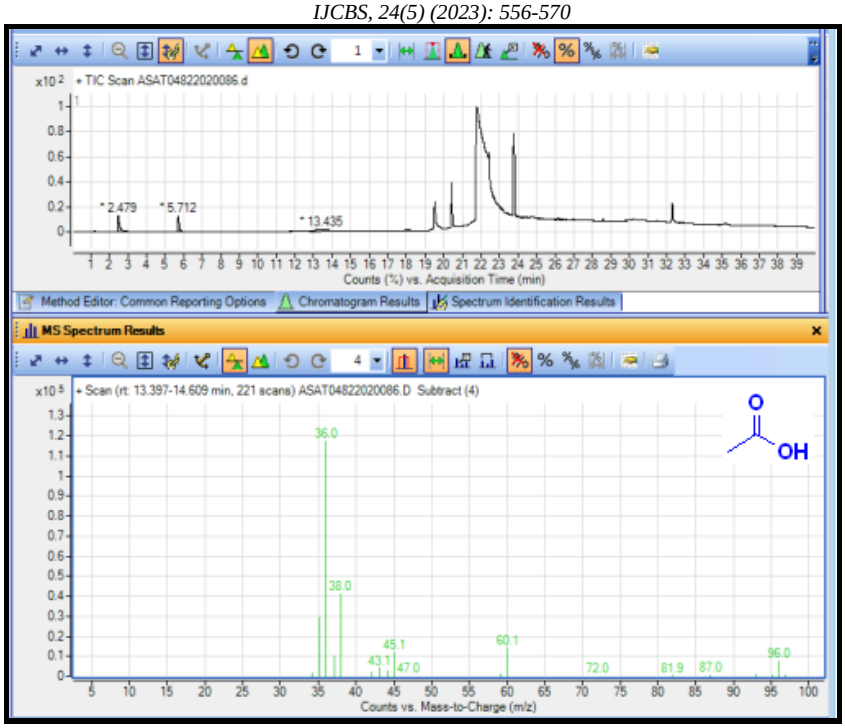


Figure 7: GCMS showing the peaks corresponding to ethanol fragment

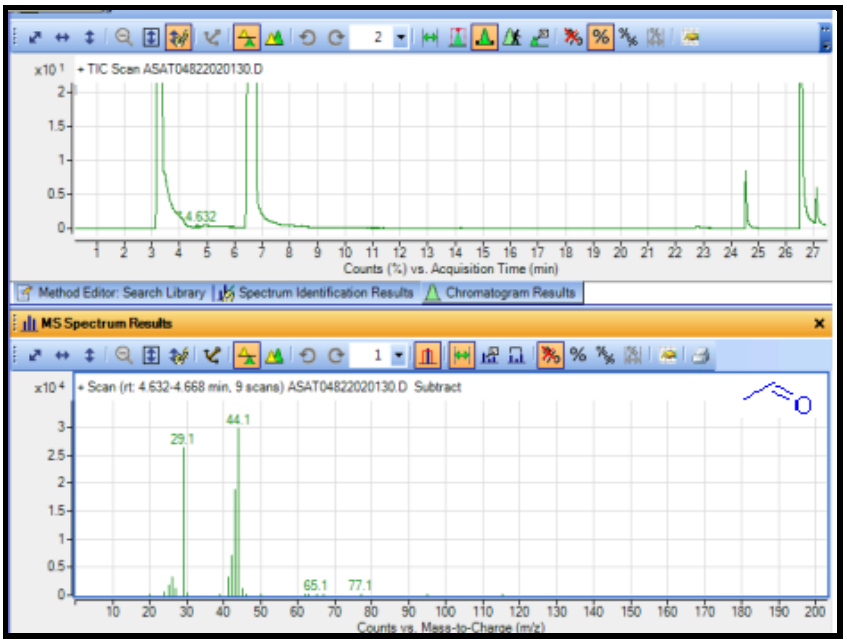


Figure 8: GCMS showing the peaks corresponding to ethanol fragment

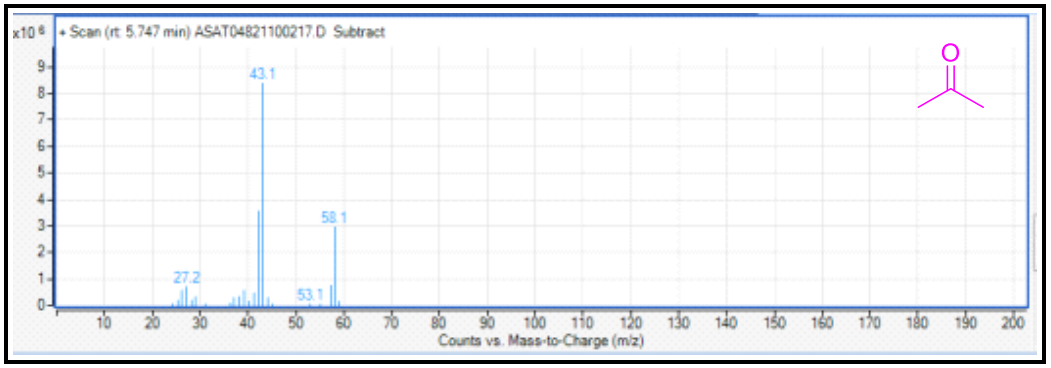


Figure 9: GCMS showing the peaks corresponding to isopropyl alcohol fragment

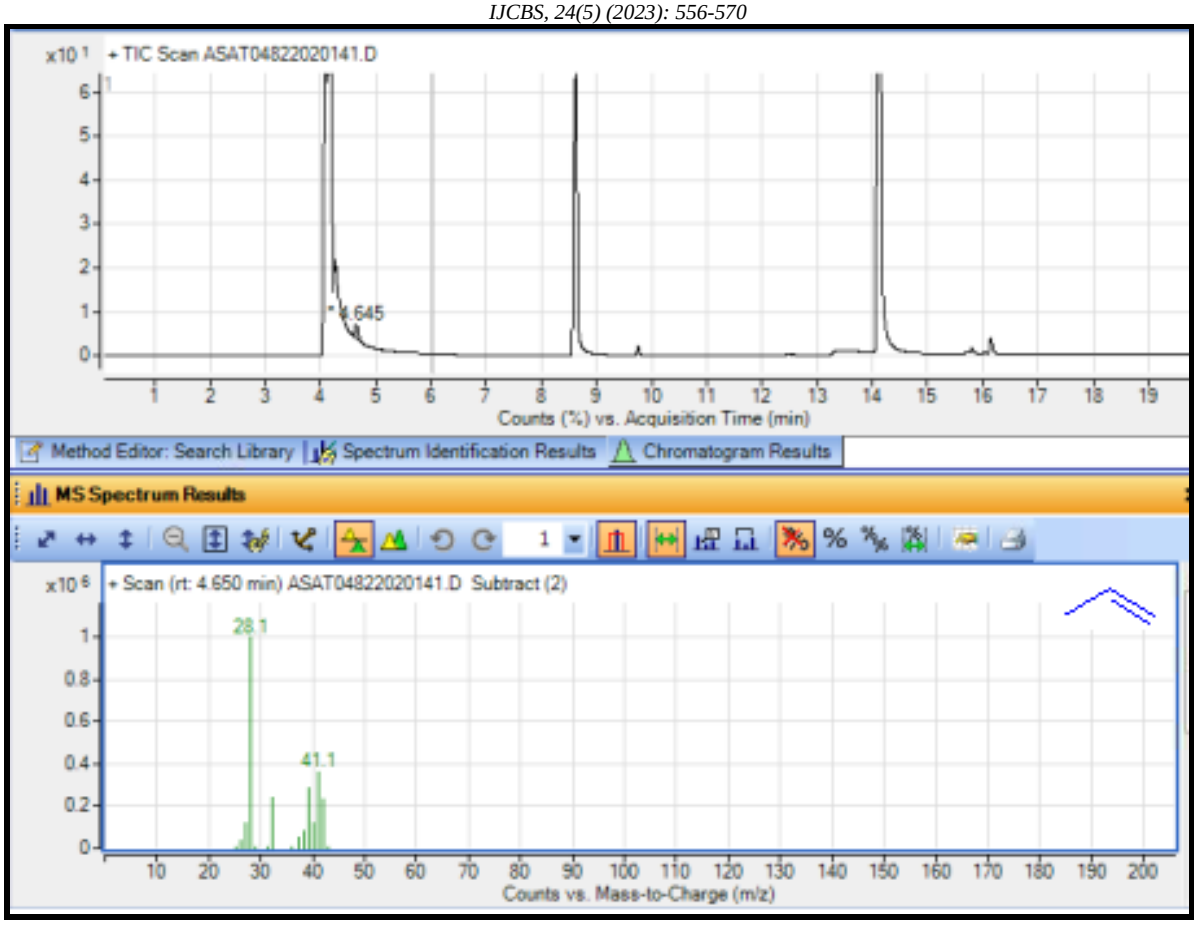


Figure 10: GCMS showing the peaks corresponding to isopropyl alcohol fragment

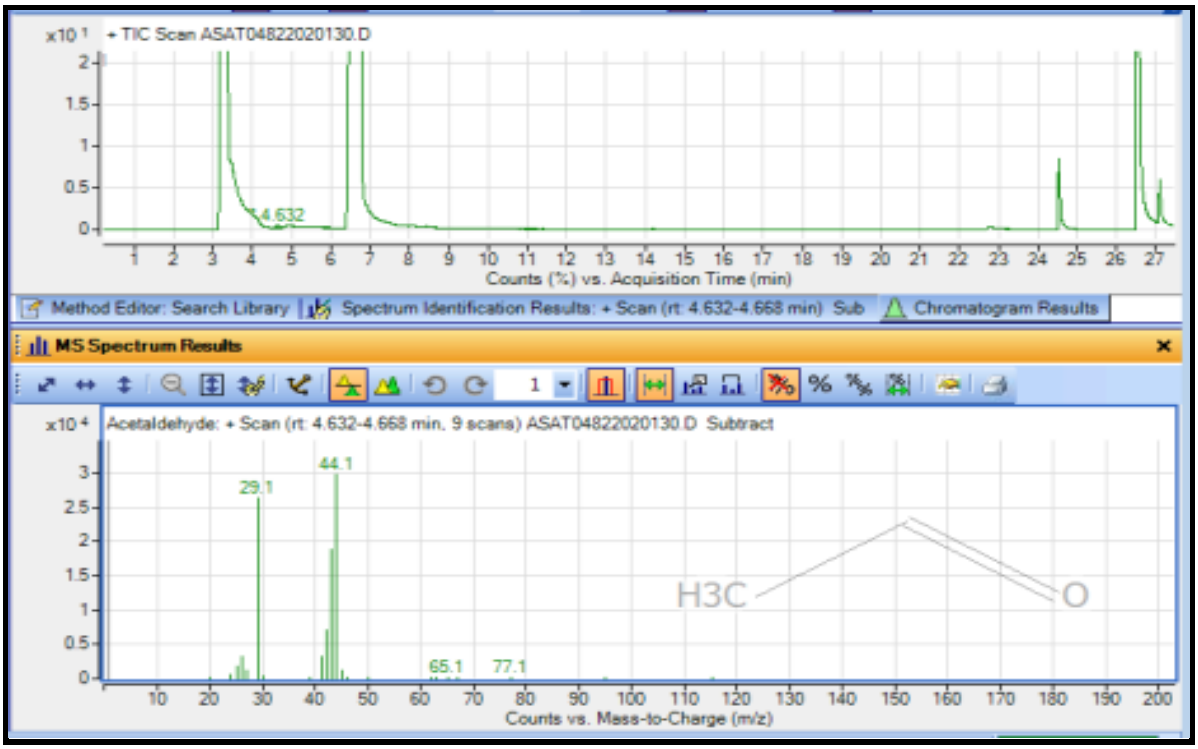


Figure 11: GCMS showing the peaks corresponding to isopropyl alcohol fragment

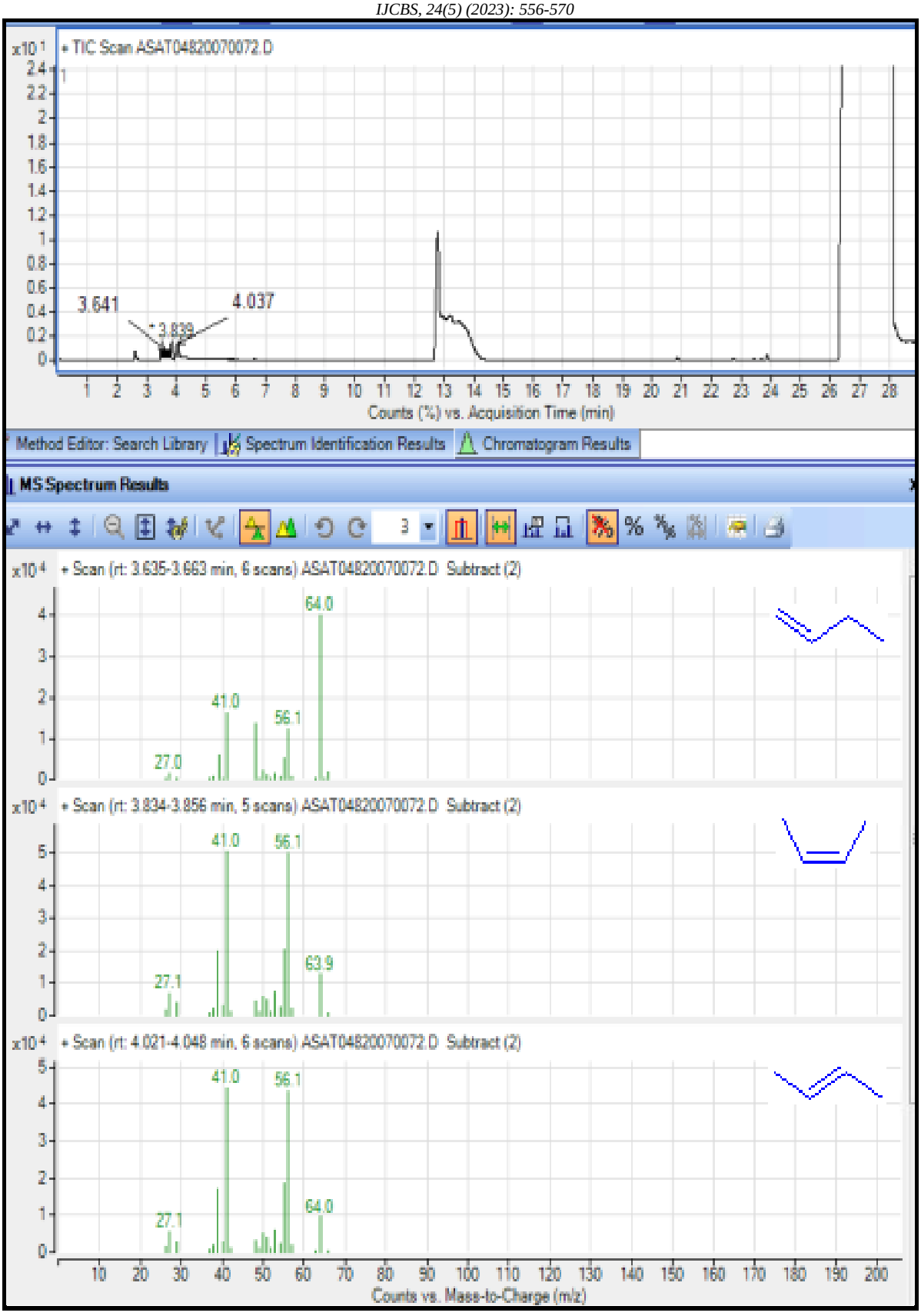


Figure 12: GCMS showing the peaks corresponding to 2-butanol fragments

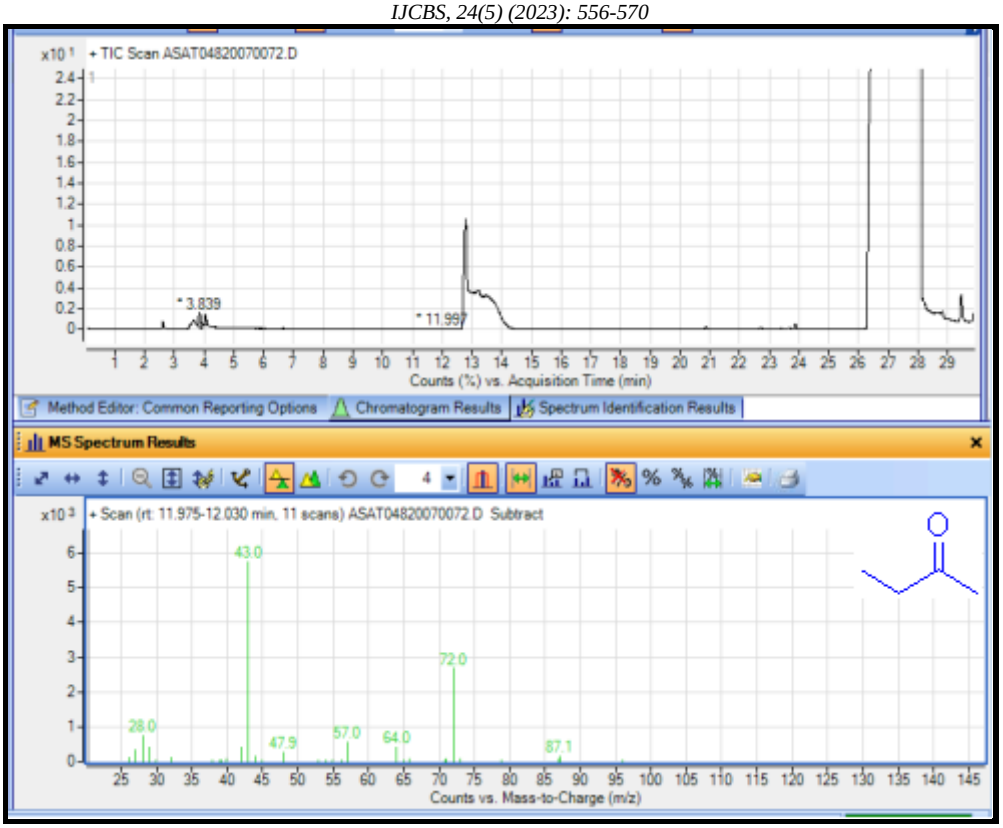


Figure 13: GCMS showing the peaks corresponding to 2-butanol fragment

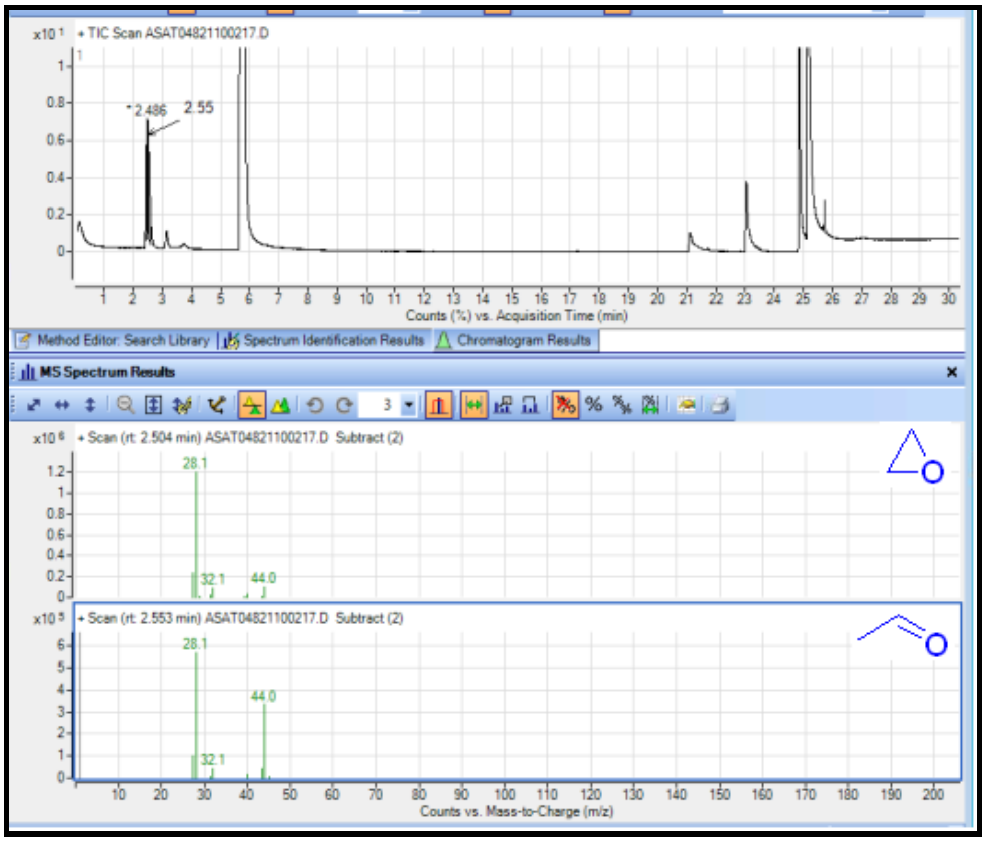


Figure 14: GCMS showing the peaks corresponding to Acetone fragment

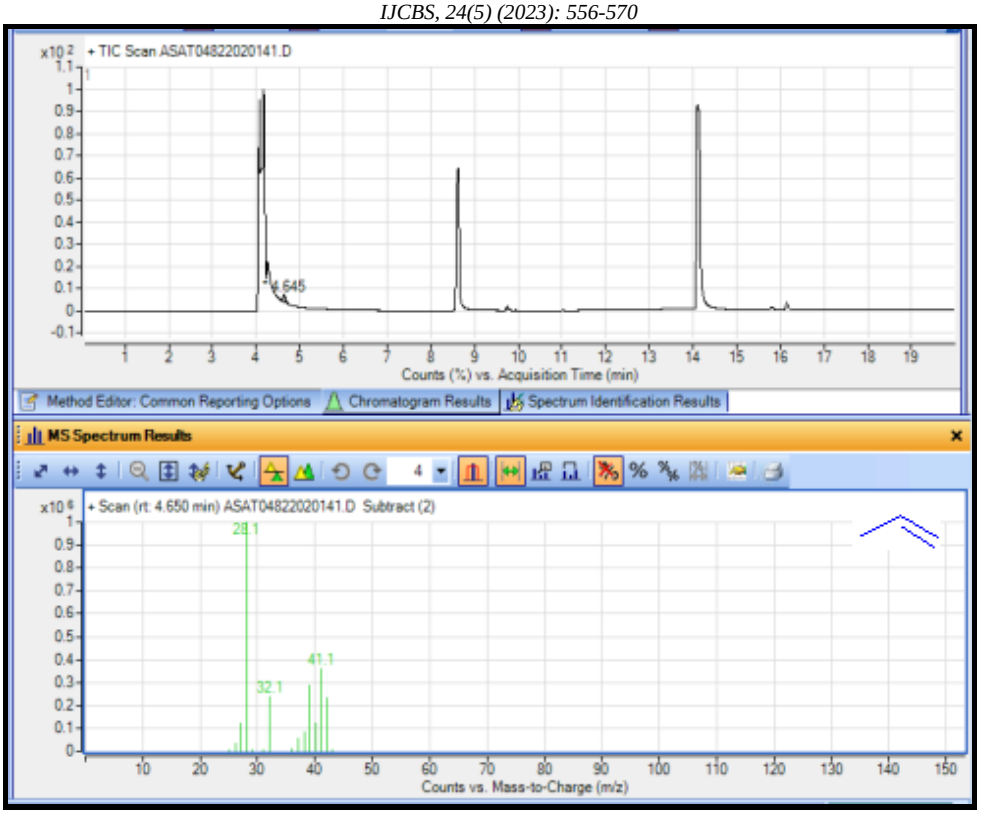


Figure 15: GCMS showing the peaks corresponding to Acetone fragment

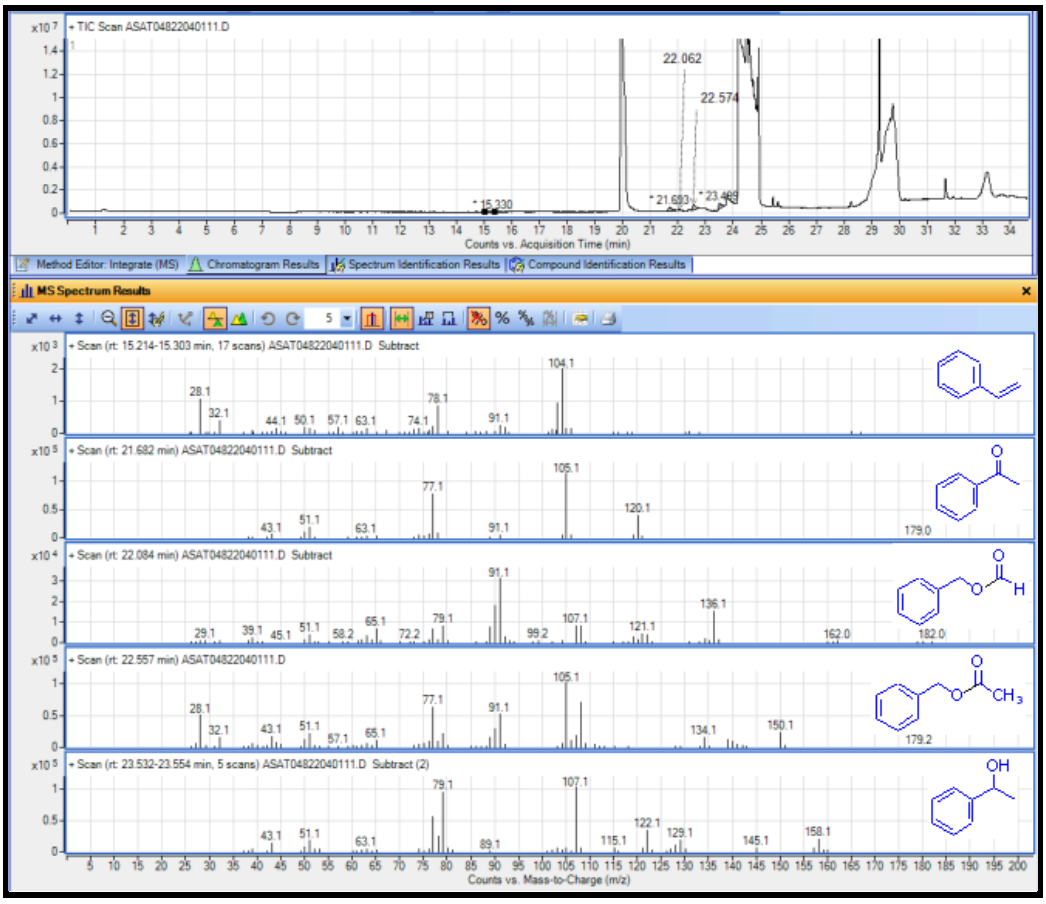


Figure 16: GCMS showing the peaks corresponding to Benzyl alcohol fragments

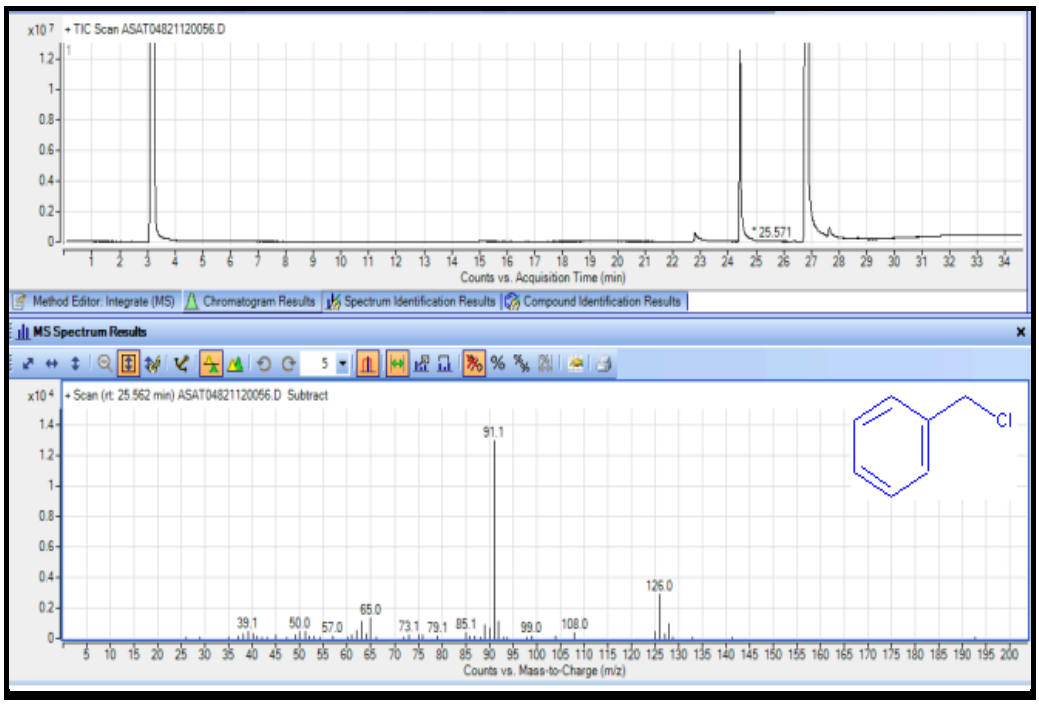


Figure 17: GCMS showing the peaks corresponding to Benzyl alcohol fragment

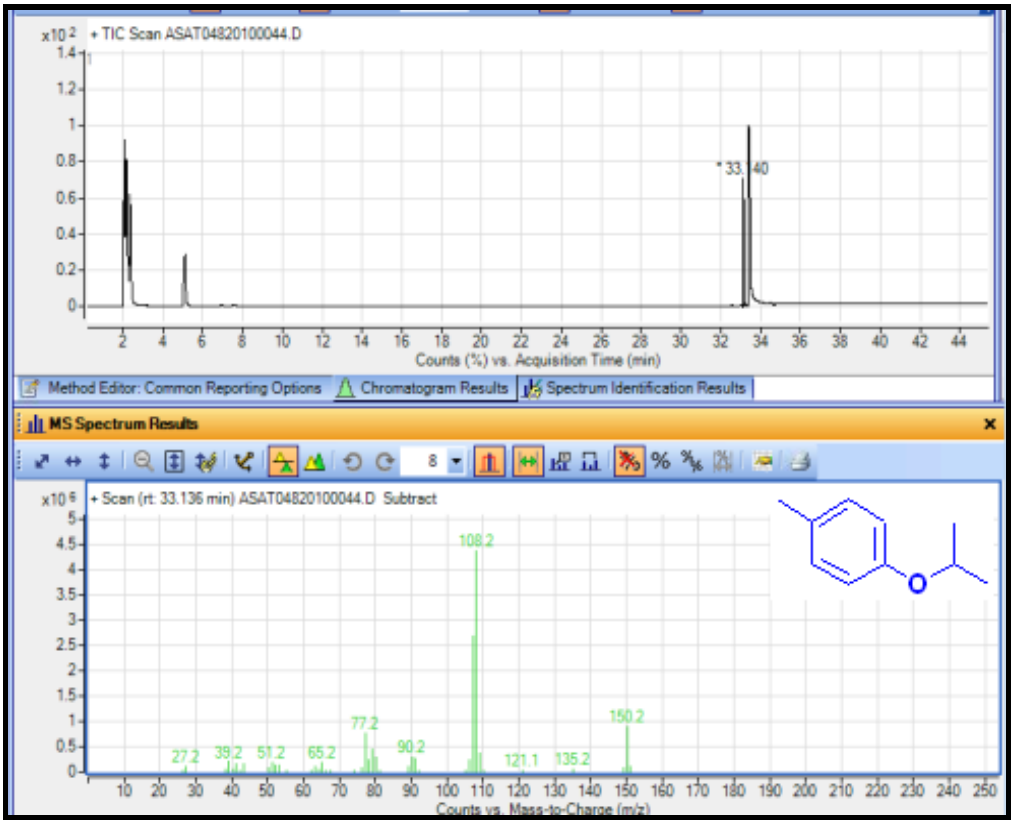


Figure 18: GCMS showing the peaks corresponding to P-Cresol fragment

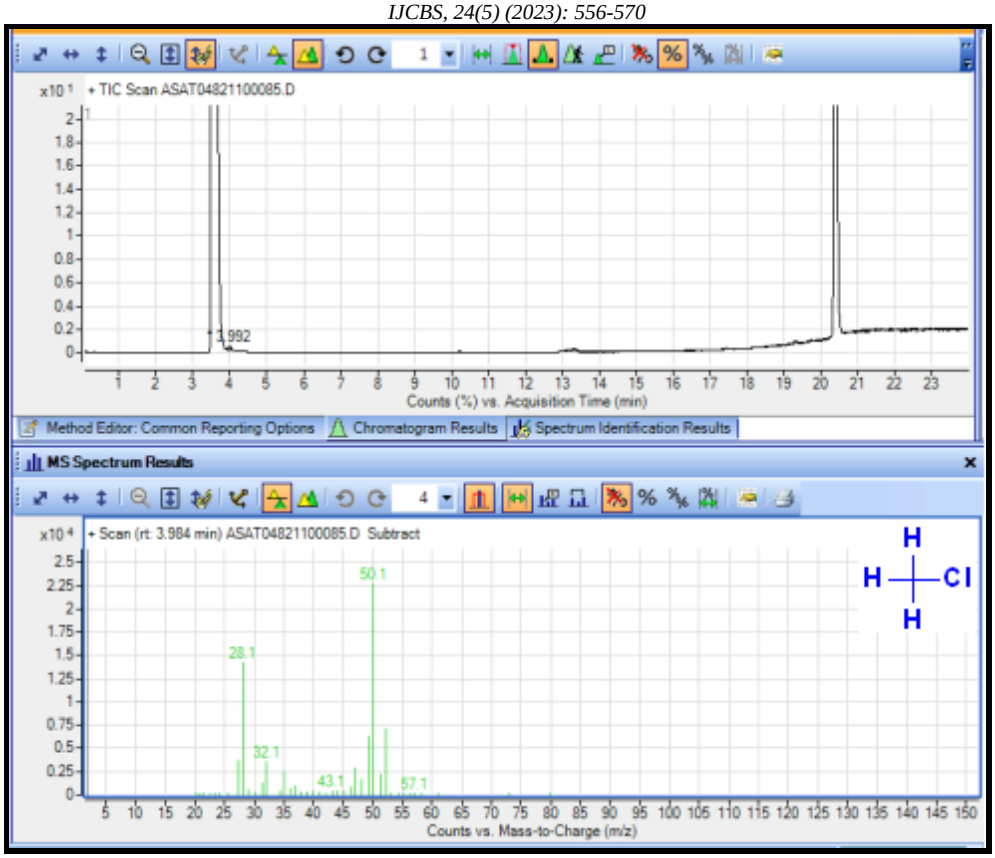


Figure 19: GCMS showing the peaks corresponding to NMP fragments

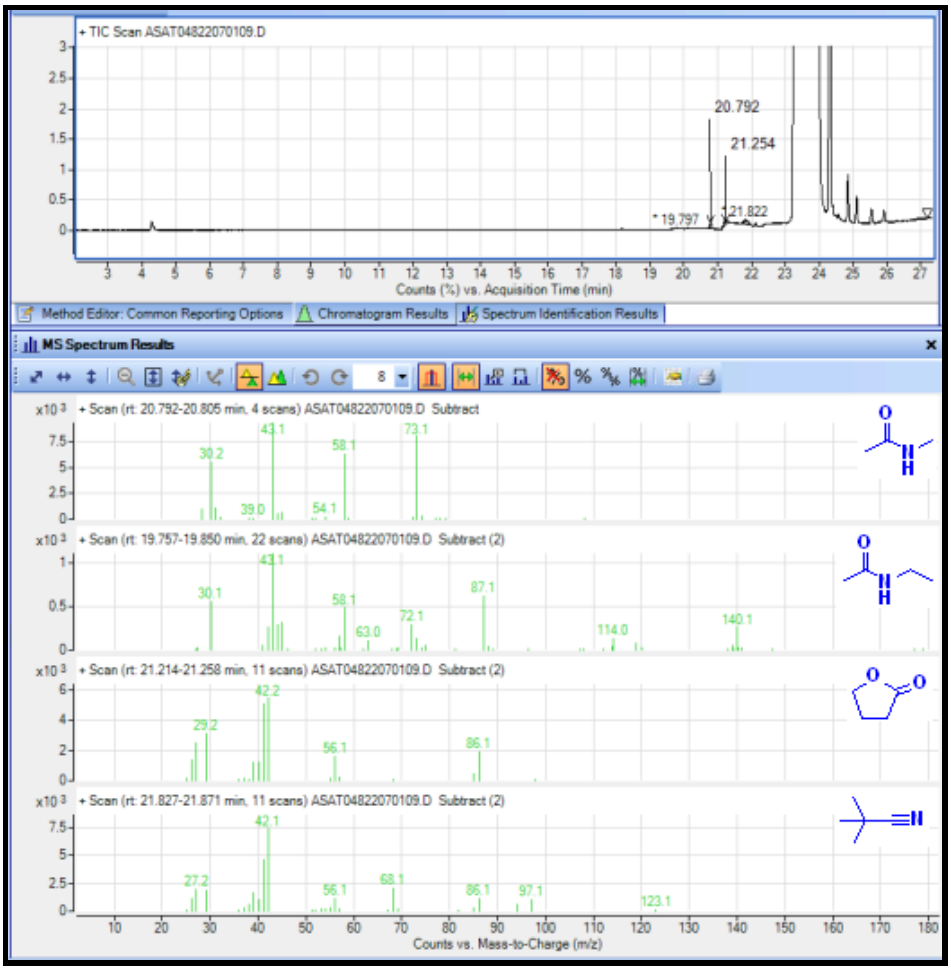
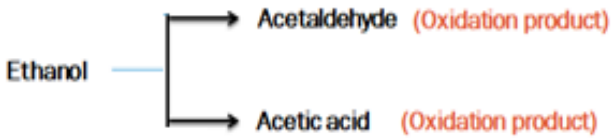


Figure 20: GCMS showing the peaks corresponding to NMP fragments

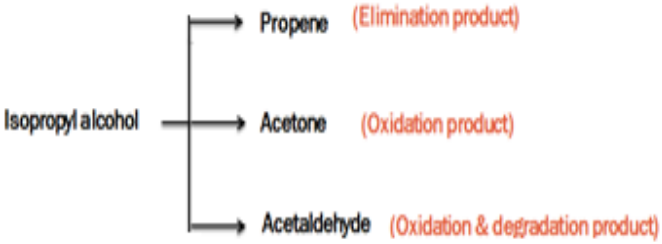
The extraneous peaks related to the higher alcohols were observed, during GC-analysis of API. Systematic approach and proper reasoning revealed the most relevant structures related to the higher alcohols. The formation of

possible products was named in the suitable type of reaction in the below mentioned scheme.

***3.4.1. Ethanol*:** possible formation of impurities given below and shown in Figure-7-8.

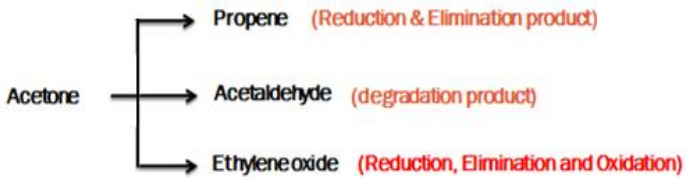


3.4.2. IPA: The formation of possible products was named with suitable type of reaction in the below mentioned scheme and corresponding data shown in Figure-9 - 11.



3.4.3. 2-Butanol: Presence of excess of H_2SO_4 along with API drugs would leads to form the mentioned impurities [13]. The Formation of possible products were named in the suitable type of reaction in the below mentioned scheme and shown in Figure-12-13.

3.4.4. Acetone: Acetone contains –ketone functional group, most polar solvent, Low boiling solvent, Ability to solubilize both lipophilic and hydrophilic group. Acetone mostly used for both organic and inorganic substances due to presence of polar and non-polar groups in the structure. Methane sulphonic acid is a strong acid, and which facilitate Carbonyl functional group of acetone for the formation different possible products. The formation of possible products was named in the suitable type of reaction in the below mentioned scheme and shown in Figures 14-15.



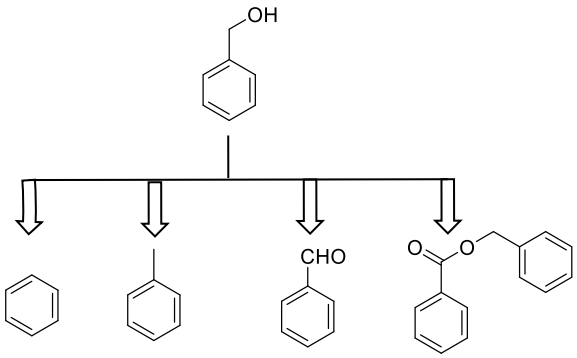
3.5. Diluents

3.5.1. Introduction

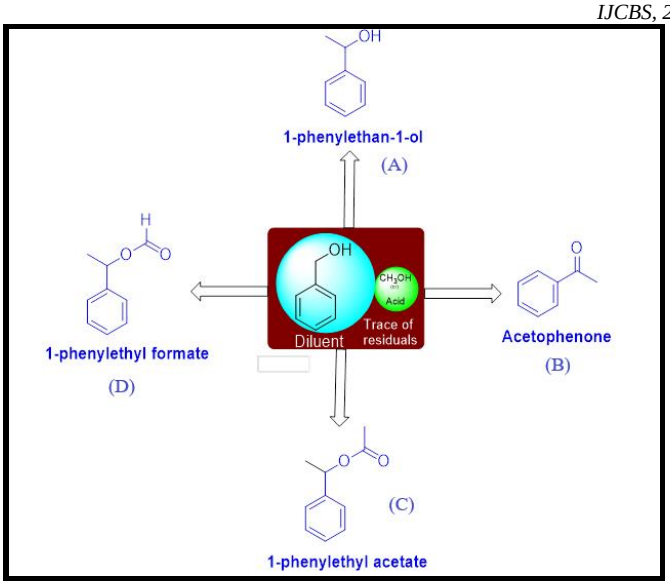
Mainly we focussed on the Diluents which are familiar usage in GC-Analysis. There are many samples that are not ready to be introduced directly into instruments, hence we use volatile organics as GC diluents, and dilution reduces the concentration of the sample and provides exact result to the analytes. Thee sample preparation procedures can be named as extraction, clean up, derivatization, transfer to vapour phase, and concentration. So, the basic concept of a sample preparation method is to convert a real matrix into a sample in a format that is suitable for GC analysis, It is worth noting that sample diluent has an important influence on GC by affecting sensitivity, equilibration temperature and time. Several commonly used sample diluents for GC analyses, such as benzyl alcohol, P-cresol, NMP, dimethyl sulfoxide, N,N-dimethylformamide, N,N-dimethylacetamide, 1,3-dimethyl-2-imidazolidinone, Methanol, and mixtures of water/DMF or water/DMSO.

3.5.2. Benzyl alcohol (BZA)

One of the well-known diluent for GC-Analysis is BZA due to its polarity, low toxicity, and low vapour pressure. The impurities reported by Dr Caitlin M. Crombie and et al [14] were shown in scheme 1. The new results observed when the diluent BZA treated with API (which is comprised of methanol or acid traces) and are shown in scheme.

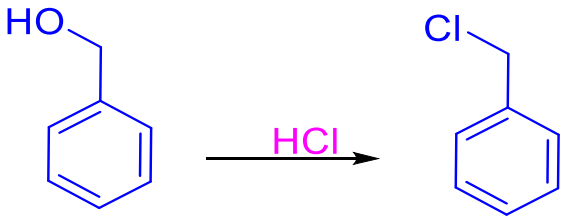


The formation of A can be achieved by methyl insertion reaction through radical mechanism. The obtained results from the GC-Analysis showed the peak corresponding to product A. The formation of B can be achieved by methyl insertion reaction through radical mechanism followed by oxidation reaction. The peak corresponding to product B is observed during the GC-Analysis of diluents with the traces of residuals. The formation of C might involves methyl insertion reaction through radical mechanism, oxidation of methanol and esterification reactions. The peak corresponding to product C is observed during the GC-Analysis of diluents with the traces of residuals. The formation of D might involves methyel insertion reaction through radical mechanism, oxidation of methanol and esterification reactions. The peak corresponding to product C is observed during the GC-Analysis of diluents with the traces of residuals, shown in Figure-16



Aceto phenone, Benzyl formate, Benzyl acetate & 1-Phenylethanol

When Benzyl alcohol will react with API containing excess of HCl then a substitutional reaction occurs to form benzoyl chloride as shown below and Figure-17

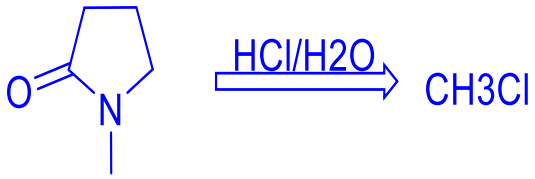


3.5.3. P-Cresol

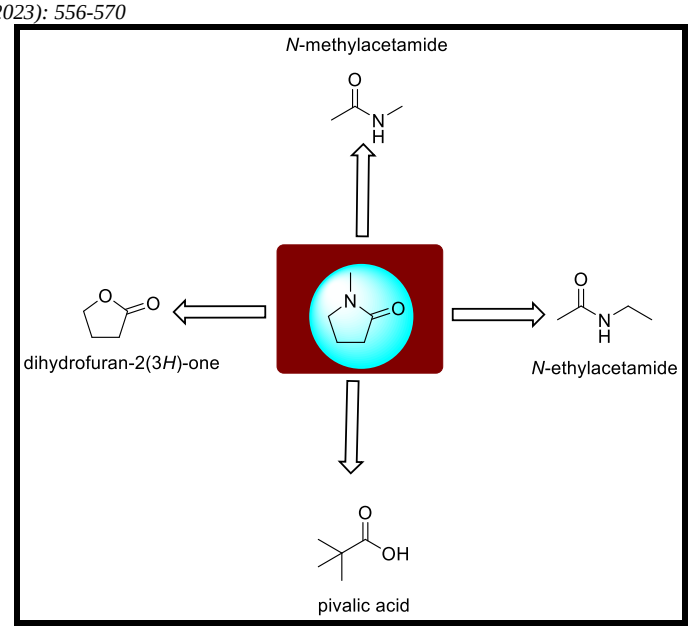
P-Cresol (Bp: 202°C) is a colourless solid that is widely used intermediate in the production of other chemicals. When P-Cresol diluent reacts with API containing IPA processing solvent the products formed would be 4-methylphenyl isopropyl ether shown in Figure-18.

3.5.4. N-Methylpyrrolidone (NMP)

The NMP (Boiling point: 202ºC) is miscible with water as well as with most of the common organic solvents. Furthermore, it is one of the dipolar aprotic solvents that include dimethylformamide and dimethyl sulfoxide. It is used in the pharmaceutical industries as a GC solvent, exploiting its non-volatility and ability to dissolve diverse materials. When NMP as GC diluent reacts with API containing excess of HCl/H_2O then the product would be Methyl chloride as shown below and in Figure-19.



NMP will expose to atmosphere conditions many degradant impurities may form like, 2,5-Pyrrolidinedione, N-Methyl acetamide, N-ethyl acetamide [15-17] and shown in Figure-20.



N-methyl acetamide, N-ethyl acetamide, butyrolactone & 2,2-dimethyl propanenitrile:

4. Conclusions

Apart from API degradation in GC analysis, extraneous/extraneous peaks could be caused by cross contamination of GC diluents or processing solvents or API containing excess of salts. These are some of possible degradants which would arise during processing of API, but not directly from the API. We also mechanistically demonstrated the formation of impurities with specific type of reactions. We strongly believe that this article helps in identifying the quality of the drug along with enough information about the route cause of the impurities.

Acknowledgments

Special thanks to the scientists from Analytical department Nuka Srikanth Kumar and Siva Manikyalarao rao Punjala and Marri. Veera Bhadra rao and wish to thank the management of Dr.Reddy's Laboratories Ltd. For permitting to carry out the present work. The authors also wish to thanks to, and the colleagues from Analytical Research & Development Department for supporting this work. Dr. Reddys communication number for this article: IPDOIPM-00697.

References

[1] H. Chandrawanshi, U. Pilaniya, P. Manchandani, P. Jain, N. Singh, K. Pilaniya. (2010). Recent Trends in the Impurity Profile of Pharmaceuticals. Journal of Advanced Pharmaceutical Technology and Research. 1(3): 302. https://doi.org/10.4103/0110-5558.72422.

[2] https://www.drugs.com/article/pharmaceutical-salts.html.

[3] C. Cheng, S. Liu, B.J. Mueller, Z. Yan. (2010). A Generic Static Headspace Gas Chromatography Method for Determination of Residual Solvents in Drug Substance. Journal of Chromatography. A. 1217(41): 6413–6421. https://doi.org/10.1016/j.chroma.2010.08.016.

[4] P. Ganesh, Ch. B.V.V.N. Raju, B.M. Rao, J. Nagadeep, V.K. Chakravarthy, K. Basavaiah, D.R. Devi. (2023). Characterization and Quantitation of N-Nitroso Duloxetine Impurity in Duloxetine Hydrochloride Drug Substance. Asian Journal of Chemistry. 35(11): 2789–2796. https://doi.org/10.14233/ajchem.2023.30576.

[5] https://www.intechopen.com/chapters/66518.

[6] K. Urakami, A. Higashi, K. Umemoto, et al., (2004). Matrix media selection for the determination of residual solvents in pharmaceuticals by static headspace gas chromatography. Journal of Chromatography A. 1057(2004): 203–210.

[7] https://www.google.com/search?q=ich+q3(6)&rlz=1C1GCEU_enIN985IN985&oq=ich+q3(6)&aqs=chrome..69i57j0i22i30j0i390l5.8642j0j15&sourceid=chrome&ie=UTF-8.

[8] D. Gupta, D. Bhatia, V. Dave, V. Sutariya, S. Varghese Gupta. (2018). Salts of Therapeutic Agents: Chemical, Physicochemical, and Biological Considerations. Molecules. 23(7): 1719. https://doi.org/10.3390/molecules23071719.

[9] https://www.sisweb.com/software/ms/nist.htm

[10] https://en.wikipedia.org/wiki/Transesterification.

[11] B.R. Buckley. (2005). Comprehensive Organic Functional Group Transformations II, https://doi.org/10.1016/B0-08-044655-8/00093-3.

[12] Ferreira, Arthur Batista, Lemos Cardoso, Abiney, Da Silva, Márcio José, Tin-Catalyzed Esterification and Transesterification Reactions: A Review. https://doi:10.5402/2012/142857

[13] K. Yarbagi, N. Rajana, J. Moses Babu, B. Venkateswara Rao and P. Douglas. International Journal of Pharmaceutical Science and Research. 7 (11).

[14] J.K. Crom rds, J. Qi, H. Jiang, C.J. Kiely, X. Liu, M.S. Skjøth-Rasmussen, G.J. Hutchings. (2021). Enhanced Selective Oxidation of Benzyl Alcohol via *In Situ* H_2O_2 Production over Supported Pd-Based Catalysts. ACS Catalysis. 11(5): 2701–2714. https://doi.org/10.1021/acscatal.0c04586.

[15] G. Lennon, S. Willox, R. Ramdas, S.J. Funston, M. Klun, R. Pieh, S. Fairlie, S. Dobbin, D.F. Cobice. (2020). Assessing the Oxidative Degradation of N-Methylpyrrolidone (NMP) in Microelectronic Fabrication Processes by Using a Multiplatform Analytical Approach. Journal of Analytical Methods in Chemistry. 1–12. https://doi.org/10.1155/2020/8265054.

[16] Praveen Kumar, Shilpi Verma, Ramanpreet Kaur, Josipa Papac, Hrvoje Kuˇsi´c, Urˇska Lavrenˇci˘c ˇStangar, Enhanced photo-degradation of N-methyl-2-pyrrolidone (NMP): Influence of matrix components, kinetic study and artificial neural network modelling, Journal of Hazardous Materials 434 (2022) 128807, https://doi.org/10.1016/j.jhazmat.2022.128807.

[17] Harry L, Campbell, Bradley A, Striebig, Evaluation of N-Methylpyrrolidone and Its Oxidative Products Toxicity Utilizing the Microtox Assay, Environmental Science & Technology / Vol. 33(11), 1999.