Development of GC method for analyzing Potential Genotoxic Impurities at low-level determination in Atorvastatin Calcium

Abstract

Atorvastatin Calcium (ATC) is a specific HMG-CoA reductase inhibitor utilized in the avoidance and treatment of cardiovascular infection in those at high danger and treat strange lipid levels. For the quantitative analysis of four PGIs in the medicine ingredient ATC, an ICH-approved Gas chromatography (GC) approach has been developed. Within the examined fixation range, the novel approach was explicit, exact, precise, and direct. Pollutant D, with 0.6 ng/mL, had the lowest recognition level among the four PGIs, which were all below 10 ng/mL. Every contaminant from ATC has been effectively chromatographically separated into its component parts. At a low level, ATC's technique was found to be highly effective in terms of pollution reduction. Proposed techniques are relied upon to measure the strength of ATC creation and to examine PGIs as a reference for ATC assessments. This method can be used to check for pollution levels in the manufacturing process of pharmaceuticals. The API's PGI levels will be kept low thanks to the GC's oversight. As a result, this study's findings will help ensure the safe use of APIs during clinical therapy.

Keywords: *Gas chromatography, Potential genotoxic impurities (PGIs), Atorvastatin Calcium (ATC) and Analytical method validation.*

Introduction

Atorvastatin Calcium (ATC) is a particular HMG-CoA reductase inhibitor utilized in the anticipation and treatment of cardiovascular illness in those at high danger and treat unusual lipid levels. Four potential genotoxic debasements (PGIs) are involved in the assembling system of Key beginning material/(tert-butyl 2-((4R,6R)- 6-(2-aminoethyl)- 2,2-dimethyl-1,3-dioxane-4-yl) acetic acid derivation) by means of side-effect arrangement, coming about because of the crude material Methane sulfonic corrosive (MSA) and the remaining reagents of ATC.

Atorvastatin Calcium has a place with a gathering of medications called HMG CoA reductase [1–2] inhibitors, or "statins." It is utilized along with diet to bring down blood levels of "awful" cholesterol (low-thickness lipoprotein, or LDL), to expand levels of "good" cholesterol (high-thickness lipoprotein, or HDL), and to bring down fatty substances (a sort of fat in the blood). Atorvastatin is utilized to treat elevated cholesterol and to bring down the danger of stroke, cardiovascular failure, or other heart confusions in individuals with type 2 diabetes, coronary illness, or other danger factors. Drug industrial facilities in most of the nations are supported for ATC creation, and arrangements of the medication have accomplished huge monetary and social advantages.

Four conceivably genotoxic contaminations (PGIs); specifically, Impurity-A/Methyl Methanesulfonate, Impurity-B/Ethyl Methanesulfonate, Impurity-C/Isopropyl Methanesulfonate, and Impurity-D/Butyl bromide, with primarily alarming utilitarian groups [3] have been distinguished during ATC blend. Four PGIs (pollutants A-D) associated with the union of the beginning material of ATC were identified. Among these pollutants, contaminations A-C are the side-effects, coming about because of the association of crude material Methane sulfonic corrosive (MSA) and the protic interaction solvents, viz., Methanol, Ethanol, and Isopropanol; debasement D is the remaining reagent at the critical beginning material. The location and measurement of such debasements during drug creation is astoundingly difficult. PGIs can prompt chromosomal breaks, hereditary transformations, or revisions in mammalian cell systems [4-6]. Contaminations amazingly influence the virtue of the last medication substance. Totally dispensing of PGIs from the medication substance has been accomplished through the execution of the cycle in the plan space. However, working the item in nonstop modescould prompt certain progressions simultaneously,

like limit upgrades which might bring about the advancement of contaminations. Consequently, the decrease of pollutions (PGIs) to the least level (beneath toxicological concern (TTC) limit) in dynamic drug fixings (APIs) is pivotal. Consequently, a new and legitimate strategy for the discovery and measurement of contaminations has been created.

The presence of potential PGIs has likewise drawn in the consideration of administrative specialists, and important rules have been delivered to the drug industry [7-9]. These rules propose an edge of TTC of 1.5 µg/day for drug plans and as far as possible and PGIs is set based on the most extreme day-by-day portion of the medication. For instance, for ATC with a most extreme everyday dose of 20 mg, the assessed allowed level of these pollutions is 2.5 ppm.

The discovery and measurement of contaminations, particularly genotoxic pollutants, in APIs is an obligatory prerequisite carried out by administrative authorities [10]. As a result fostering a delicate and approved technique is needed for the dependable assessment of PGIs in ATC and the commercialization of APIs.

Mesylate esters of aliphatic alcohols are potentially cancer-causing agents. Consequently, administrative specialists made it compulsory to present the contamination profile for the medication substances produced.

In this exploration, a new and touchy GC strategy with a satisfactory restriction of measurement (LOQ) values were set up and approved for the quantitative assurance of PGIs in ATC. The detailed techniques are approved by International Council for Harmonization (ICH) rules as far as LOQ, explicitness, exactness, accuracy, and linearity [11] are concerned. In this current review, the GC technique was utilized for the measurement of four PGIs in ATC because of its high selectivity and affectability. Our outcomes are anticipated to be huge for the protected utilization of this API for the drawn-out clinical avoidance and treatment of planned illnesses.

Material and Methods

Materials

The ATC drug substance is produced by Dr Reddy's Laboratories Ltd. The four PGIs (debasements A-C and D) were obtained from SRL and Spectrochem separately. The purities of the mixtures (>99.5%) utilized in the study were evaluatedby utilizing the suitable HPLC techniques.

All of the reagents used were HPLC-grade, with a purity of 99 percent. AR-grade methanol was bought from Spectrochem, India and used to set up the versatile stages. Ultrapure water was ready from a Milli-Q water filtration framework (Bedford, MA, USA). Acetonitrile was provided by SD Fine Ltd.,

Instrumentation

Gas chromatography investigation was done on Agilent framework (7890A) with Auto fluid sampler with Empower programming. MMS, EMS, IPMS and BB were isolated on AT-5 narrow segment (Agilent Technologies, USA, 30 m×053 mm i.d.×5.0 µm film). A 1µl infusion volume of 18.75 ppm combination arrangement with 1:1 split bay was chosen for infusion. The GC broiler temperature program used an underlying temperature of 85°C and an underlying holding season of 5 min, and afterward expanded at 2°C/min to 120°C holding season of 2 min. and afterward last temperature expanded at 50°C/min to 250°C was held for 18 min. Segment stream rate 5.0 psi. The injector and finder temperature for GC were 160°C and 260°C respectively.

Planning of Standard and Sample Solutions

The ATC arrangementsat a high grouping of 20 mg/mL in Acetonitrile were evaluated to assess the levels of contaminations (A-D) and survey p-GTI levels. Stock norms of the analysts (contaminations A–D) were ready at a centralization of 10 mg/mL in Methanol. Concequentl, standard blend arrangements containing the four contaminations at a centralization of 200 ng/mL (likeness 10 ppm) in Methanol were gotten by weakening the stock norms for investigation in ATC clumps.

Strategy Validation

The proposed technique as approved by the measures of ICH rules [13], including explicitness, linearity, LOD, LOQ, exactness, accuracy, and arrangement solidness was adopted.

Linearity

The linearity of the strategy was checked by plotting adjustment bends between the pinnacle regions versus the grouping of Impurities A-D over the reach 2.0-28 ppm. The slant, catch and relationship coefficient esteems were gotten from liner least-square relapse treatment. The straight reach was sufficiently represented by utilizing a six-point alignment diagram. The incline, block, and relationship coefficient were gotten from least-squares direct relapse examination. The relationship coefficient esteems detailed in (Table 1) demonstrate the best linearity of the technique. The relationship coefficient esteems revealed in Table-1 shows the best linearity of the strategy.

Cutoff of Quantification and Limit of Detection

Definitively measure the proper measure of 20 ng/mL arrangement under the linearity and weaken with Methanol quantitatively and stepwise if vital. The weakened arrangements were independently infused into the chromatograph. LOQs and LODs were characterized as the fixations that could be distinguished. The restrictions of quantitation of Impurity A-D were 6.1, 3.8, 2.5 and 2.0 ppm respectively. The restriction of recognition of Impurity A-D were 1.9, 1.5, 1.0 and 0.8, not set in stone at the most minimal fixations at which signal-to-clamor proportion is 3 and 10.

Precision

The precision of the technique was assessed through spiked recuperation tests. Legitimate pollutants A-D were spiked into 20.0 mg/mL ATC in three-fold utilizing fixation levels of 80% (160 ng/mL), 100% (200 ng/mL), and 120% (240 ng/mL). Great recuperations in the scope of 94.32%–107.43% with RSD esteems beneath 6.5% were accomplished.

Accuracy and Solution Stability

Accuracy was inspected by infusing six individual arrangements of the standard combination arrangement containing contaminations at the cutoff level (200 ng/mL). The RSD% of region for every pollutant was determined. The arrangement security of the debasements in the example arrangement was set up by investigating the standard blend arrangements at various time spans (2, 4, 8, 12, and 24 h) at room temperature. The technique approval results summed up in Table 1 show that our set up strategy can dependably measure these PGIs in ATC.

The accuracy of the technique was assessed interms of repeatability and transitional accuracy. The repeatability is controlled by ascertaining the overall standard deviation (% RSD) of six duplicate conclusions by infusing newly pre-arranged 18.75 ppm blend arrangement independently around the same time. For transitional accuracy, 18.75 ppm blend arrangement was infused on six unique days.

The low % RSD esteems by means of pinnacle regions affirm the great accuracy of the created strategy (Table 1). Debasements A-D was not identified in the lab (R&D) tests at 100mg/mL were investigated in the current technique, attributable to high purirty. Henceforth, the Accuracy of the technique was controlled by spiking Impurities A-D combination at four fixation levels (LOQ, half, 100% and 150% of 18.75ppm detail level) to 1 g of Atorvastatin Calcium and making the volume to 10 ml with diluent. Every assurance was done for multiple times. The recuperation information introduced in (Table 1) shows the exactness of the strategy. Clear and standard chromatograms are displayed in Figure 1-5. In the changed gas chromatographic states of ±5°C on the underlying broiler temperature and transporter gas stream ±0.5 psi, the maintenance times and pinnacle spaces of Impurities A-D were observed to be same demonstrating the heartiness of the strategy.

Arrangement dependability has been set up for a time of 24 h and the Similarity factor of the standard arrangements of Impurities A-D are 0.97, 0.96, 0.96 and 1.03 individually.

Test Analysis

The approved GC strategy was applied to gauge the previously mentioned PGIs in three clusters of ATC tests. The test convergence of ATC was 20.0 mg/mL, and that of the standard blend containing contaminations A-D was 200 ng/mL. The outcomes are recorded in Table 1. The levels of all PGI contaminations were underneath the characterized satisfactory TTC limits, in this manner demonstrating that all pollutants are very much controlled.

Results and Discussion

Insightful Method Development

This work intended to foster a touchy and dependable GC strategy to evaluate PGIs in Atorvastatin Calcium/ATC. Division of ATC and its four PGIs done on an AT-5 (30 m×0.53 mm×5.0 µm) hairlike section under programming temperature.acetonitrile was utilized as diluent. This strategy was approved according to International Conference on Harmonization rules (Q2R1). The constraint of quantitation of Impurity A-D are 6.1, 3.8, 2.5 and 2.0 ppm separately. The constraint of discovery of Impurity A-D are 1.9, 1.5, 1.0 and 0.8, still up in the air at the most reduced focuses at which signal-to-clamor proportion is 3 and 10. The created GC strategy was upgraded dependent on the goals of Impurities A-D and approved according to ICH rules. The strategy well suits for the expected reason.

Toxnet logical information uncovers that Impurities A-D are distinguished as class-1 according to ICH M7. As far as possible were set up as 18.75 ppm for the four PGIs (Impurities A-D) by thought to be the greatest day by day measurement of Atorvastatin Calcium 80 mg.

Explicitness

The relating chromatograms of contaminations A-D and ATC are displayed in Figure 1-5. The chromatograms show that the created techniques can effectively isolate the PGIs from each other and from the fundamental medication.

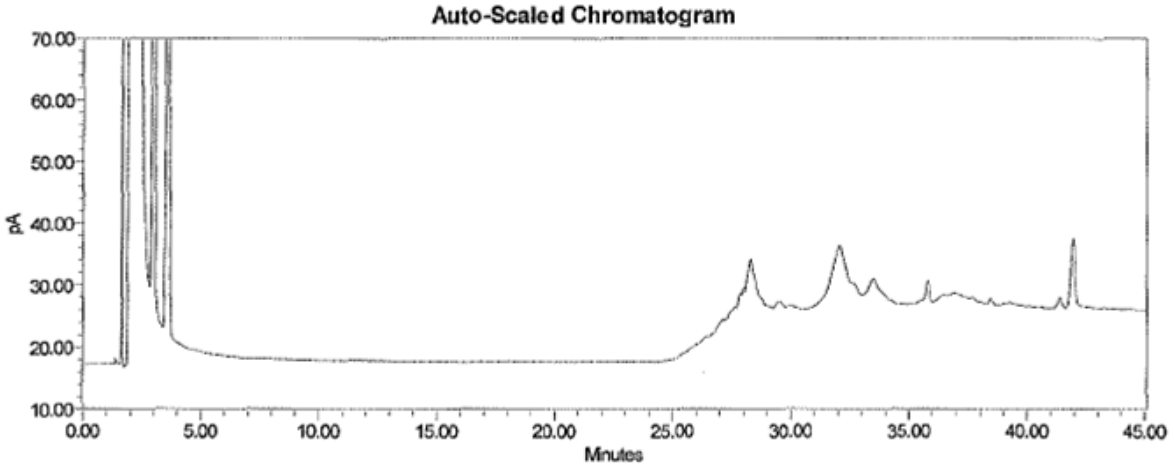

Figure-1: Typical GC Chromatogram of Diluent blank

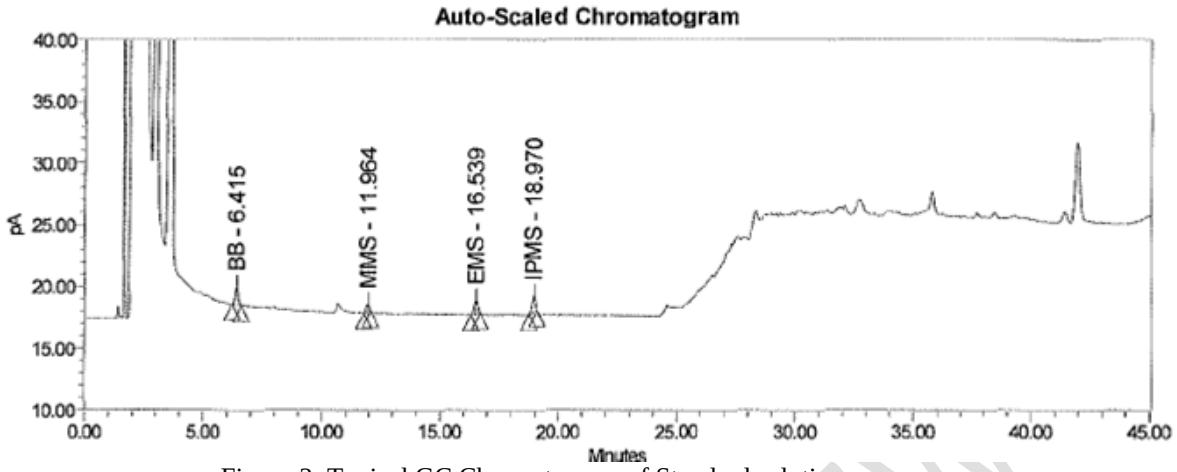

Figure-2: Typical GC Chromatogram of Standard solution

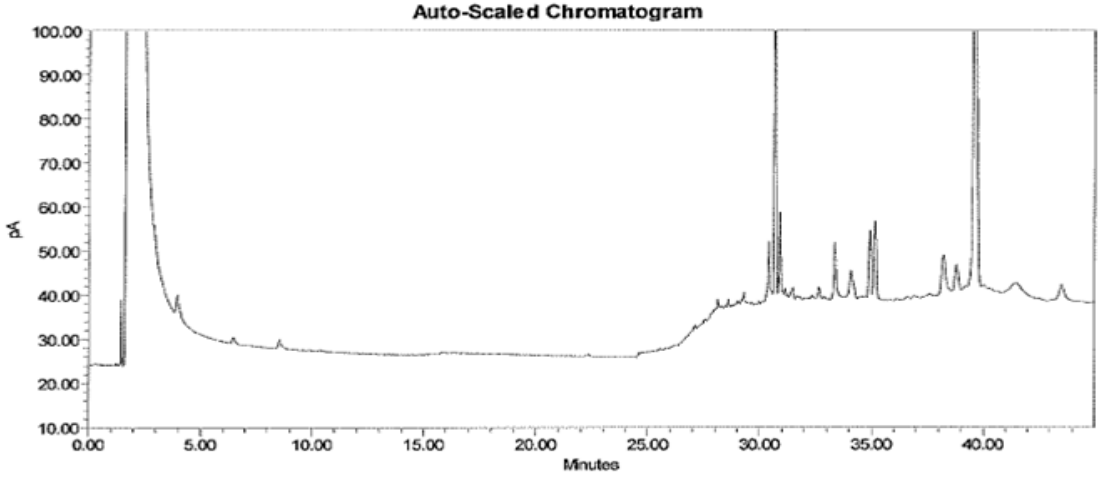

Figure-3: Typical GC Chromatogram of Test sample-1.

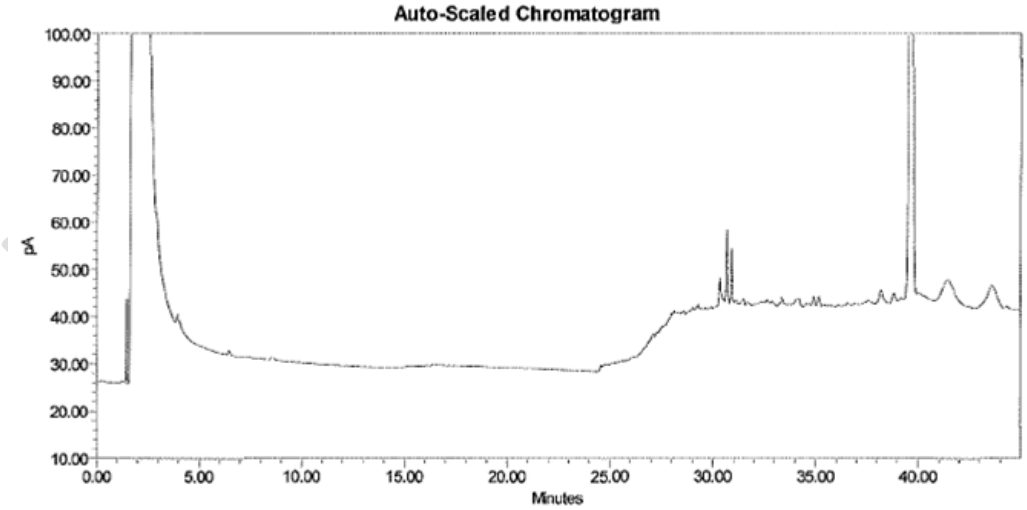

Figure-4: Typical GC Chromatogram of Test sample-2.

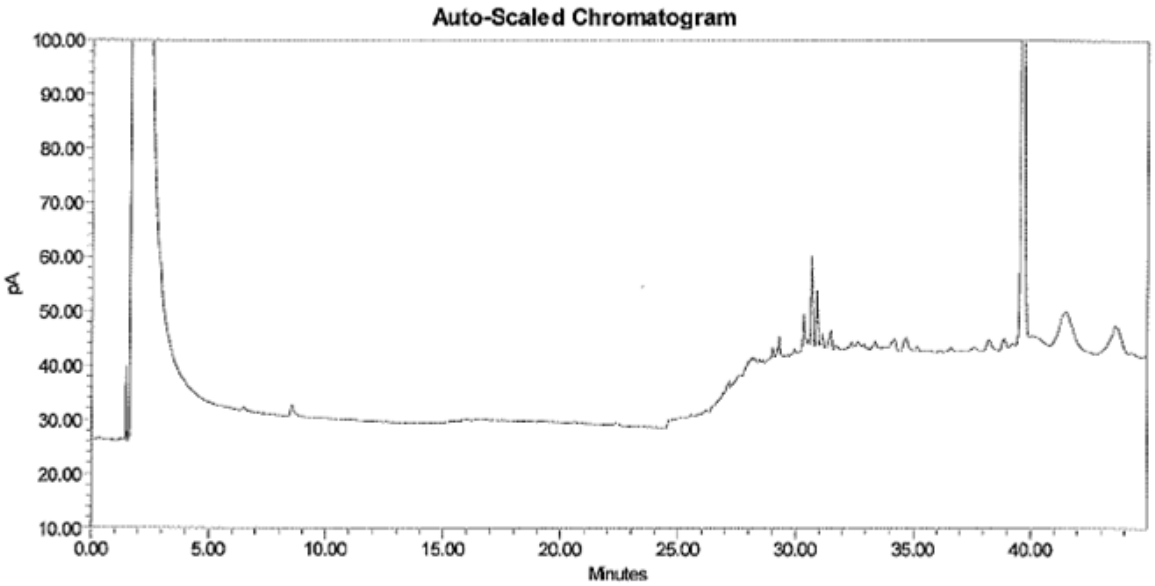

Figure-5: Typical GC Chromatogram of Test sample-3.

Figure 1-5: Typical ATC chromatograms of a mixed solution of impurities A-D and ATC (100 ng/mL).

#Imp-A/MMS, Imp-B/EMS, Imp-C/IPMS and Imp-D/BB.

Affectability

The LOD and LOQ of all PGIs were broke down by infusing weakened arrangements with known focuses. LODs and LOQs identified with pollutions A-D at 20.0 mg/mL. Among these pollutions, D uncovered the most fragile reaction and, accordingly, had the least affectability; paradoxically, contamination A-C were shown the most noteworthy affectability. These low LOQ esteems were considered agreeable and satisfactory for the particular investigation.

Test Analysis

The **validation of GC method**is summarized in Table 1. The test grouping of ATC was 20.0 mg/mL, and that of the standard combination containing debasements A-D was 200 ng/mL.The levels of all PGI contaminations were beneath the characterized accepted TTC limits, and it showed that all debasements are very much controlled.

Parameter	MMS	EMS	IPMS	BB
LOD (ppm)	1.9	1.5	1.0	0.8
LOQ (ppm)	6.1	3.8	2.5	2.00
Linearity Range (ppm)	2.0-28.0	2.0-28.0	2.0-28.0	2.0-28.0
Correlation Coefficient	0.998	0.994	0.996	0.996
% Y Intercept	2.2	4.5	2.4	-2.2
Precision at LOQ (%RSD)	5.40	9.18	5.80	2.24

Method Precision (%RSD)	7.14	4.95	7.55	3.98
Intermediate Precision(%RSD)-Ruggedness	7.11	4.17	4.17	3.61
% Recovery				
at LOQ	105.7-111.9%	102.3-110.2%	99.6-103.4%	86.2-93.7%
at 50%	93.9-110.7%	88.7-94.2%	87.5-89.2%	92.1-97.9%
at 100%	105.1-110.2%	99.0-100.8%	108.9-110.7%	96.5-98.2%
at 150%	99.5-104.0%	88.9-93.3%	91.1-99.9%	90.0-92.0%
Robustness				
Oven temperature condition-1 (80°C) %RSD	4.07	4.25	5.39	5.40
Oven temperature condition-2 (90°C) %RSD	5.22	2.76	4.62	3.46
Column flow rate condition-1 (4.5psi) %RSD	4.17	3.46	2.52	2.76
Column flow rate condition-2 (5.5psi) %RSD	3.07	0.99	1.12	2.13

Table-1: Summary of the validation of GC method

Conclusion

A delicate GC technique has been created and approved by ICH rules for the quantitative examination of four PGIs in the medication substance ATC. The new technique was explicit, exact, precise, and direct inside the surveyed fixation range. The recognition levels of the four PGIs were underneath 10 ng/mL, particularly for pollutant D at 0.6 ng/mL. Effective chromatographic division of every pollutant from ATC was completed.

Quantitative examination of the pollutions in substance clumps of ATC showed the high effectiveness of this strategy at a low level. As a flexible and advantageous strategy, the proposed technique is relied upon to be utilized in assessments of the strength of ATC creation and examination of PGIs as reference. This technique is appropriate in checking the level of pollutions during drug fabricating. The GC can guarantee low measures of PGIs in the API. Therefore, the aftereffects of this study will assist in guaranteeing protected utilization of APIs during clinical treatment.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

References

1. K. L. Dobo, N. Greene, M. O. Cyr, S. Caron, and W. W. Ku. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. Regulatory Toxicology and Pharmacology 2006; 44(3):282-293.
2. J. Ashby and R. W. Tennant. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutation Research/Reviews in Genetic Toxicology1991; 257(3): 229.
3. L. Ma, Y. N. Ma, Z. Chen, and Y. Jiang. Structure alerts of genotoxic impurities. Chinese Journal of New Drugs 2014; 23(18): 2106.
4. D. Q. Liu, M. Sun, and A. S. Kord. Recent advances in trace analysis of pharmaceutical genotoxic impurities. Journal of Pharmaceutical and Biomedical Analysis 2010; 51(5): 999.
5. M. H. A. Rahman, D. R. Gullick, J. Hoerner, and M. G. Bartlett. Determination of genotoxic impurities monomethylsulfate and dimethyl sulfate in active pharmaceutical ingredients. Analytical Methods 2017; 9 (7): 1112.
6. H. Lee. Pharmaceutical Industry Practices on Genotoxic Impurities, CRC Press, Boca Raton, FL, USA 2014.
7. European Directorate for the Quality of Medicines & HealthCare (EDQM), The European Pharmacopeia, EDQM, Strasbourg, France. 2017: 9.
8. The United States Pharmacopeial Convention, 40, Rockville, MD, USA 2017.
9. Chinese Pharmacopeia Commission, Pharmacopoeia of the People's Republic of China, China Medical Science Press, Beijing, China 2015.
10. K. McDonald. ICH Q11: development and manufacture of drug substances chemical entities and biotechnological/biological entities. Generics and Biosimilars Initiative Journal. 2012; 1(3): 142.
11. P. Borman and D. Elder. Q2(R1): validation of analytical procedures: text and methodology, in Proceedings of the International Conference on Harmonisation (ICH), Geneva, Switzerland 2005.