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Analysis of Genotoxic Impurities like Ethane Sulfonic acid esters in pharmaceutical substance by GC-MS/MS

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Introduction

Residual genotoxic impurities, and particularly alkyl esters of alkyl or aryl sulfonic acids, have been, and probably remain, a significant safety concern to drug regulators. Since the sulfonate moiety is readily displaced by a variety of nucleophiles, such esters can act as DNA alkylating agents in biological systems and have been shown to exert genotoxic effects in bacterial and mammalian cells [1]. Chemicals like Ethane sulfonic acid used in the process of pharmaceutical synthesis are likely to generate Ethane Sulfonic acid ester with alcohols as a reaction by-products. These Ethane sulfonic acid esters (Esylates) that include Methyl Ethane Sulfonate (Figure 1) and Ethyl Ethane Sulfonate (Figure 2) are Potential Genotoxic Impurities[1] (PGI's) and are of great concern to pharmaceutical manufacturers.

A TTC (Threshold of Toxicological Concern) based acceptable intake of a mutagenic impurity of 1.5 µg per person per day is considered to be associated with a negligible risk and can, in general, be used for most pharmaceuticals as a default value, to derive an acceptable limit for control[2][3].

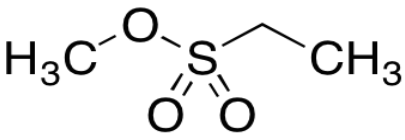

Figure 1. Methyl Ethane Sulfonate (MES)

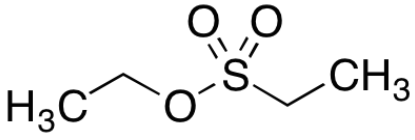

Figure 2. Ethyl Ethane Sulfonate (EES)

Shimadzu GCMS-TQ8050 triple quadrupole system (Figure 3) was used to develop selective and sensitive method for the determination of Ethane Sulfonic acid ester PGI's (Methyl Ethane Sulfonate and Ethyl Ethane Sulfonate) at trace levels.

Methods and Materials

Sample Preparation

- **Standard Stock Solution :**

Individual standards of MES and EES were procured from Toronto Research Chemicals (TRC), Canada. From these individual standards, stock solution containing mixture of MES and EES (5000 ppb each) was prepared in ethyl acetate.

- **Calibration Levels:**

Standard stock solution was further diluted with Iso-octane to prepare the concentration levels of 1.0 ppb, 2.5 ppb, 5 ppb, 10 ppb, 25 ppb, 50 ppb and 100 ppb.

- **Sample Preparation (50 mg/mL):**

About 75 mg Nintedanib Esylate API was taken in 2.0 mL Eppendorf tube and 1.5 mL of Iso-octane was added. The solution was vortexed for about 1.0 minute and filtered through 0.22 µ Nylon syringe filter. The resultant filtrate was taken for GC-MS/MS analysis.

- **Spike Sample Preparation:**

For recovery studies, Nintedanib Esylate API sample was spiked at the concentration level of 2.5 ppb, 5 ppb, 10 ppb, 25 ppb and 50 ppb. The spiked sample solution was vortexed for about 1.0 minute and filtered through 0.22 µ Nylon syringe filter.

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Figure 3. GCMS-TQ8050 Triple quadrupole system by Shimadzu

Key Features of GCMS-TQ8050

1. **Enhanced Sensitivity :** OFF-AXIS Ion Optics and newly designed high-sensitivity shielded detector offers outstanding noise elimination, enabling the system to reliably detect at femtogram level.

2. **Durable Hardware :** The contamination-resistant ion source and the new detector with over five times longer service life ensure reliable, long-term analysis.

3. **Superior Performance :** A new turbo-molecular pump with higher evacuation performance results in higher sensitivity and improves analysis accuracy for ultra-trace concentration levels. UFSweeper technology achieves high-speed MRM analyisis (800 transitions/ sec).

4. **Reliable Operations :** The Smart MRM technology that optimizes sensitivity helps accurately create methods for ultra-trace analysis. The enhanced accuracy control function provided by LabSolutions Insight software improves the reliability of analyzing data acquired from simultaneous multicomponent analysis.

GC-MS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8050 as per the conditions shown in below Table 1.

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Table 1. Analytical conditions

Chromatographic parameters	
Column	: Rtx-1701 (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection Mode	: Split
Split Ratio	: 5.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 40.2 cm/sec
Column Flow	: 1.20 mL/min
Injection Volume	: 1.0 µL
Column Temp. Program	:

Rate (˚C/min)	Temperature (˚C)	Hold time (min)
	70.0	2.00
25.00	150.0	2.00
30.00	260.0	14.13

Total Program Time	: 25.00 min
Mass Spectrometry parameters	
Ion Source Temp.	: 230.0 ˚C
Interface Temp.	: 270.0 ˚C
Ionization Mode	: EI
Acquisition Mode	: MRM
CID Gas	: Argon

MRM Method development

For MRM optimization, about 5 ppm standard mixture was analyzed using scan mode. For individual components, precursor ions were selected. Using selected precursor ions, product ion scan was performed with different Collision Energies (CE) ranging from 1 to 45 V with CE intervals of 2 V. For each component, six MRM transitions with appropriate CEs were determined (Figure 4). All the above steps were simplified with the help of Smart MRM optimization tool. These MRM transitions were registered to Smart Database and the final MRM method with overlapping segments and optimum dwell time was generated. MRM chromatogram of 100 ppb standard mixture acquired using this method is shown in Figure 5.

Table 2. Method creation in GCMS-TQ8050 using Smart MRM

Step.1	Step.2	Step.3	Step.4
Measure in SCAN mode and determine Pre-cursor ion	**MRM Optimization Tool:** Create batch sequence and method files with different collision energies automatically.	**MRM Optimization Tool:** Analyzes acquired data files and selects the best transitions and collision energy automatically. The result is registered to Smart MRM database.	**Smart MRM Database:** Creates method with overlapping segments and optimum dwell time.

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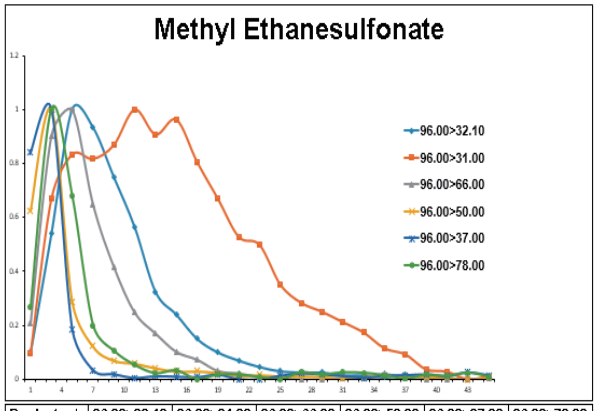
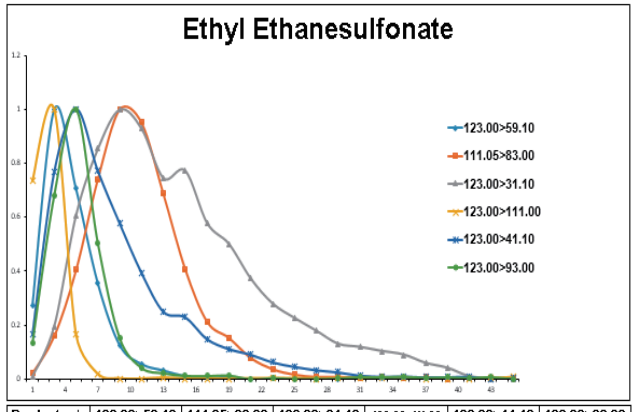


Product m/z	96.00>32.10	96.00>31.00	96.00>66.00	96.00>50.00	96.00>37.00	96.00>78.00
Max Int.	17335	11307	10085	6855	2673	2154
CE	5	11	5	3	3	3



Product m/z	123.00>59.10	111.05>83.00	123.00>31.10	123.00>111.00	123.00>41.10	123.00>93.00
Max Int.	20982	15664	14597	10468	8874	7399
CE	3	9	9	3	5	5

Figure 4. CE Optimization using Smart MRM optimization tool

Results

The mixture of MES and EES was analyzed using the method created as mentioned in Table 2. The sulfonic acid esters were quantitatively extracted from sample and evaluated statistically with respect to precision, linearity and recovery (Table 3).

Linearity plot for standards ranging from 2.5 ppb to 100 ppb concentration levels showed linear response with r^2 > 0.999 and %RSD (n=6) < 7.5% at LOQ (Figure 6 & 7). Recovery was calculated by external standard method. For all the spiked levels, the recovery obtained was found to be in the range of 70% to 130%. On the basis of statistical data obtained, the method was proved to be highly sensitive, accurate and reproducible

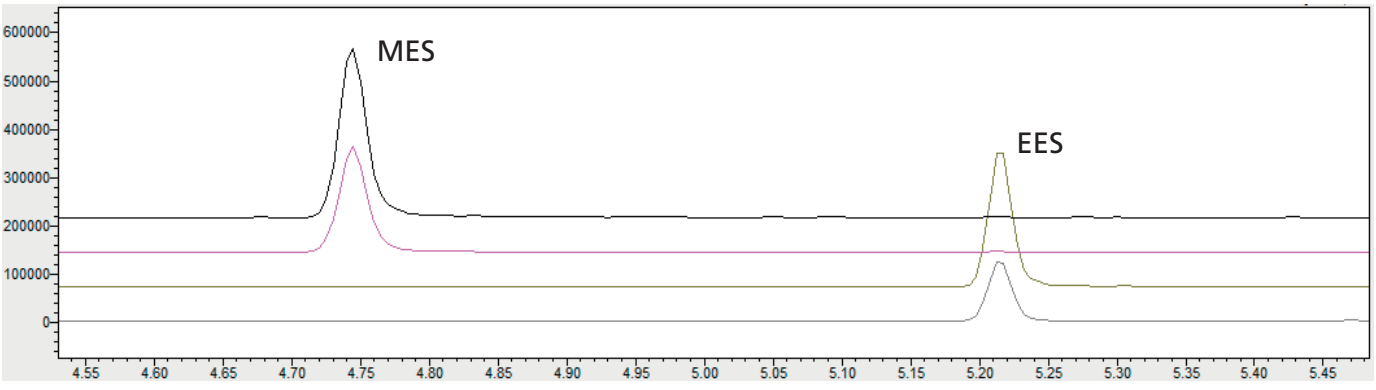


Figure 5. MRM chromatogram for 100 ppb standard mixture

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Table 3. Statistical summary table

Name	Target MRM (m/z)	Linearity Range (ppb)	r^2	LOQ (ppb)	LOQ % RSD (n=6)	LOD (ppb)	% Recovery Range at 2.5 ppb (n=3)	% Recovery Range at 5 ppb (n=3)	% Recovery Range at 10 ppb (n=3)
MES	96.00>32.10	2.5 - 100	0.9998	2.5	3.1	1.0	100±25	100±7	100±3
EES	123.00>59.10	2.5 - 100	0.9996	2.5	7.3	1.0	100±20	100±16	100±8

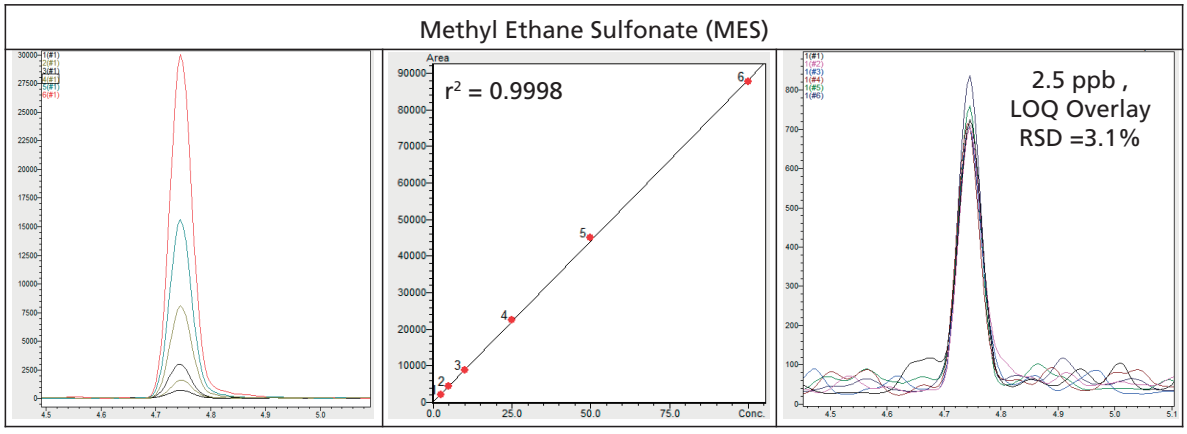

Figure 6. Linearity overlay, graph and LOQ precision overlay for MES

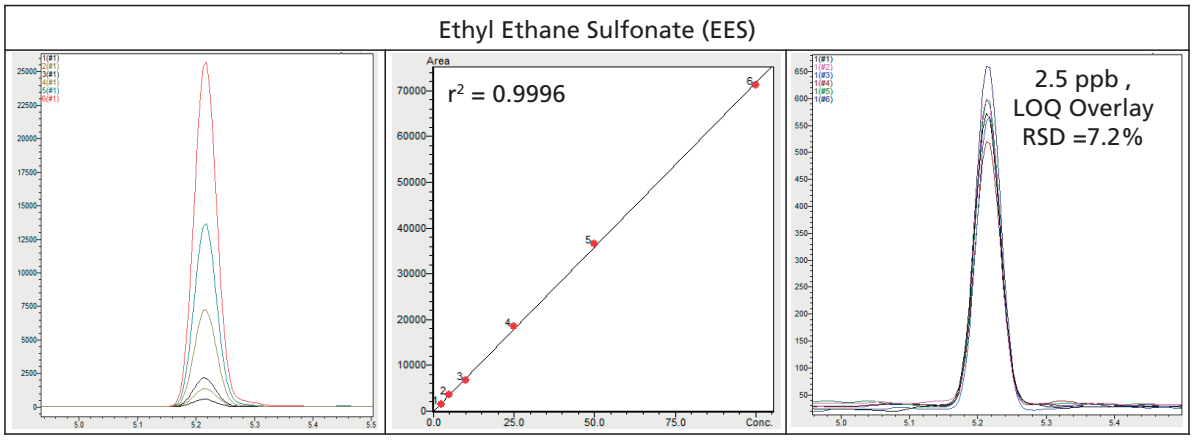

Figure 7. Linearity overlay, graph and LOQ precision overlay for EES

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Conclusion

- A highly sensitive and selective method was developed for PGI's like Ethane sulfonic acid esters (MES and EES) by using Shimadzu GCMS-TQ8050.
- Developed MRM method can be used for screening and quantification of Ethane sulfonic acid esters in various pharmaceutical substances.

References

[1] David P. Elder[a] and David J. Snodin[b](2009). Drug substances presented as sulfonic acid salts: Overview of utility, safety and regulation. Journal of Pharmacy and Pharmacology, Vol. 61, pp. 269-278.
[2] EMEA/CHMP/QWP/251344/2006.
[3] M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit potential carcinogenic risk guidance for industry (2015), The International Council for Harmonization(ICH).

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