

Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX, HS-20 NX Trap

Analysis of Methyl Ethane Sulfonate in Nintedanib Esylate API Sample Using Headspace-Trap Technique

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User Benefits

- ◆ Shimadzu GCMS-TQ8050 NX with HS-20 NX autosampler works with LabSolutions GCMS 21CFR compliant software which extends its utilization in compliance environment like pharmaceuticals
- ◆ Trap headspace enables concentrating the analytes in cold trap with multi-injection count and helps to achieve higher sensitivity over static headspace, especially for trace level quantitation

■ Introduction

Overview: Alkyl and aryl sulfonic acids have been used in the synthesis of active pharmaceutical ingredients (APIs) in various measures. They are known for their acid catalytic properties, sulfonamide intermediates and as agents to enhance solubility through salt formation. However, as the reactions progress, they form their respective esters, which are well-known potent genotoxic impurities (GTIs), that act as DNA-alkylating agents in biological systems posing mutagenic and carcinogenic risks even at trace levels. Hence, it is necessary to categorize, qualify, and control these impurities to limit the potential carcinogenic risks, as mentioned in ICH M7(R2).

Nintedanib Esylate API belongs to a family of kinase inhibitors, used primarily to treat idiopathic pulmonary fibrosis (IPF). It acts as a triple angiokinase inhibitor which targets receptors involved in pulmonary fibrosis. The synthesis of the API often involves the reaction of Nintedanib free base with Ethane Sulfonic Acid in a process called as salification. The salt formed in the form of yellow crystals is used further in formulations to enhance stability as well as solubility. The by-product of this reaction, often due to presence of residual short-chain alcohols, is Methyl Ethane Sulfonate (MES).

Figure 1 depicts the structure of Nintedanib Esylate.

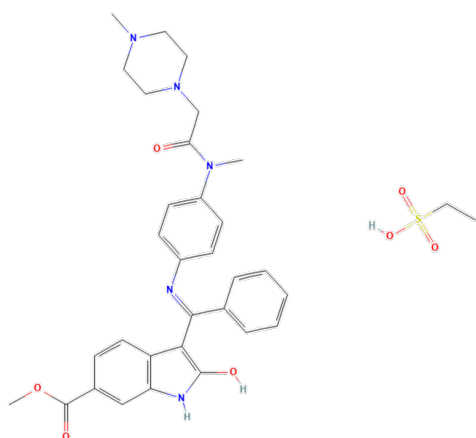


Figure 1 : Nintedanib Esylate API ^[1]

As per the ICH M7(R2) guidelines, a TTC (Threshold of Toxicological Concern) based acceptable intake of a mutagenic impurity of 1.5 µg/day which can be used for most pharmaceuticals as a default value, to derive an acceptable limit for control ^[2,3]. Nintedanib Esylate is supposed to be administered orally. Strength of the Nintedanib Esylate API in most of the medicines is 150 mg per capsule and prescribed dose is twice in a day. ^[4]

Irrespective of the limit calculated from maximum daily dose, Shimadzu has worked upon developing a method for quantification of MES at lowest possible detection so that it will be beneficial for the concern pharmaceuticals even if daily dose exceeds 0.3 g/day.

Need for a robust analytical method

Nintedanib Esylate API shows high solubility in acidic pH of about 3. To inject acidic aqueous media into GC via direct injection technique creates the risk of very high vapor pressure at the injector port. Further, its entry into the column might affect the stationary phase, thereby drastically reducing the shelf-life of the column. As a result, sensitivity of the analytical method becomes questionable. In order to overcome the said issues, it is imperative to develop a robust analytical method pertaining to high sensitivity, repeatability and reproducibility of the results.

In this regard, Shimadzu GCMS-TQ8050 NX triple quadrupole system with HS-20 NX (Trap) as shown in Figure 2, was used to develop a selective and sensitive method for the determination of Methyl Ethane Sulfonate in Nintedanib Esylate API at trace levels.



Figure 2 : Shimadzu GCMS-TQ™8050 NX with HS-20 NX (Trap)

Further, development and partial validation were performed at significantly lower concentrations which clearly evidences the capability of instrument and suitability of the developed method.

To evaluate the method suitability, few validation parameters were performed such as system suitability, LOQ precision, Linearity and accuracy in terms of spike recovery. Refer Table 1 for the concentration levels of method validation parameters.

Table 1: Summary of MES concentrations in validation parameters

Parameters	Conc. as such (ppb)	Conc. w.r.to sample (ppb)
System precision	5	50
Precision at LOQ level	0.3	3
Linearity	0.1 to 10	1 to 100
Accuracy	0.3 to 10	3 to 100

■ Experimental

Analysis was performed on triple quadrupole mass spectrometer. MRM transitions were optimized using MRM optimization tool available with Shimadzu by injecting 10 ppm MES standard. The best three MRM transitions were used for analysis and those were as reported in Table 2.

Table 2. MRM transitions and their CEs

MRM-1		MRM-2		MRM-3	
Transition (amu)	CE (V)	Transition (amu)	CE (V)	Transition (amu)	CE (V)
96.00 > 32.10	5	96.00 > 31.00	11	66.00 > 48.00	9

Where amu is atomic mass unit and V is volt.

Transition-1 was used for quantification and rest two transitions were used for qualification purpose.

Instrument parameters were as reported in Table 3.

Table 3. Instrument parameters

GCMS parameters	
Instrument used	GCMS-TQ8050 NX with HS-20 NX (Trap)
Column	SH-1701 (0.32 mm I.D x 30 m, d.f. = 1.0 µm) (P/N: 221-75782-30)
Injection Mode	Split
Split ratio	5
Flow control mode	Linear velocity
Linear velocity	45 cm/s
Carrier gas	Helium
Temp. Program	70 °C (2 min), 25 °C/min to 150 °C (2 min), 30 °C / min to 260 °C (4.13 min)
Ionization Mode	Electron Impact
Ion source Temp.	230 °C
Interface Temp.	250 °C
Acq. Mode	MRM
Detector Voltage	+0.4 kV (Relative to the tuning result)

Parameters for Headspace-Trap auto sampler

Mode	: Trap
Oven Temp.	: 120 °C
Sample Line Temp.	: 150 °C
Transfer Line Temp.	: 170 °C
Trap cooling Temp.	: 80 °C
Trap Desorb. Temp.	: 280 °C
Trap Equilibrium Temp.	: 80 °C
Shaking Level	: 5
Multi Injection Count	: 5
Pressurization Gas Pressure	: 150 kPa
Dry Purge Gas Pressure	: 0 kPa
Equilibrating Time	: 5 min
Pressure equilibration time	: 0.5 min
Load time	: 0.5 min
Load equilibration time	: 0.1 min
Injection time	: 10 min
Needle flush time	: 10 min
GC cycle time	: 20 min

Solution Preparations: Prepared the solutions as per procedure mentioned below.

Preparation of diluent: Carefully transferred about 0.83 mL conc. HCl (~35%) into a suitable container prefilled with 60-70 mL water and diluted to 100 mL with water and mixed thoroughly. Used this solution as diluent.

Extraction solvent: Used ethyl acetate as extraction solvent.

Preparation of blank: Transferred 2 mL diluent and 2 mL ethyl acetate in a 15 mL centrifuge tube and vortexed the mixture for 5 minutes. Centrifuged the same tube for 5 minutes below 5 °C temperature. Allowed the solution to settle down and transferred 100 µL of ethyl acetate layer into 20 mL HS vial, designated as blank solution and injected.

Preparation of standard solutions:

Diluted stock solution: Weighed about 50 mg of MES reference standard in 50 mL volumetric flask containing 5 mL methanol. Diluted to the mark with methanol. The concentration of this stock solution was 1000 ppm. Using this standard stock solution, 100 ppb standard solution was prepared in methanol and used further for linearity standard preparation.

Linearity level preparation: Prepared the linearity levels as mentioned in the below Table 4..

Table 4. Linearity standard solutions

Linearity Level	Volume of 100 ppb standard solution (mL)	Final dilution with Diluent (mL)	Conc. of MES (ppb)
Level-1	0.01	10	0.1
Level-2*	0.03	10	0.3
Level-3	0.05	10	0.5
Level-4	0.1	10	1
Level-5	0.3	10	3
Level-6*	0.5	10	5
Level-7	1.0	10	10

* Level-2 and Level-6 represents the concentration at LOQ level and system suitability level, respectively.

Transferred 2 mL of each linearity level solution along with 2 mL ethyl acetate into individual 15 mL Centrifuge tubes and vortexed for 5 minutes. Centrifuged each tube for 5 minutes below 5 °C temperature. Allowed the solutions to settle down and carefully transferred 100 µL of ethyl acetate layer from each linearity level solution mixture into respective 20 mL HS vials, sealed them and submitted for analysis.

Preparation of sample: Transferred 2 mL of diluent in 15 mL centrifuge tube. To this, added 200 mg of Nintedanib Esylate API sample and vortexed to dissolve the content. Added accurately 2 mL ethyl acetate to the same tube and vortexed for 5 min. Centrifuged the solution for 5 min below 5 °C temperature and allowed the mixture to settle. Carefully transferred 100 µL of upper organic layer into 20 mL HS vial, sealed immediately and injected.

Preparation for spiked sample for accuracy study: For accuracy study, samples were prepared following the above-mentioned method. Instead of diluent, 2 mL each of respective linearity level solutions were added. Accuracy study was performed for recovery of MES at four different levels including LOQ level.

Method precision: For method precision study, six replicates of spiked sample (level-6) were injected. Content in these injections were calculated using average area in system suitability injections and %RSD of content is evaluated

■ Results and discussions

Specificity: A comparison between the chromatograms of blank (ethyl acetate) and working standard solution (linearity level-6) shows that there is negligible interference from blank for MES i.e., below the LOD level, indicating that the method is specific. Figure 3 depicts overlay of the blank solution and 5 ppb standard injection.

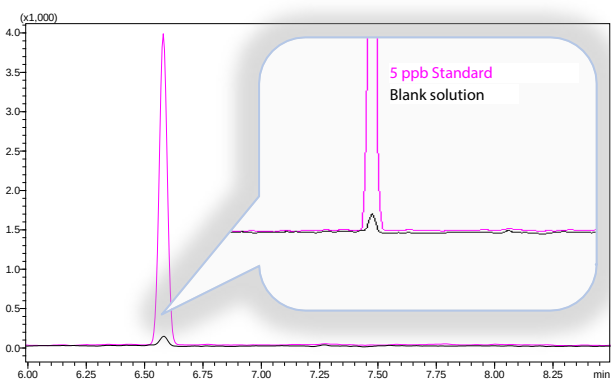


Figure 3 : Overlayed chromatogram of 5 ppb STD and blank solution

LOD and LOQ evaluation: For evaluation of LOD, duplicate injections of linearity level-1 were injected. Similarly, for Precision at LOQ level, six replicates of linearity level-2 were injected, and the summary of signal-to-noise ratio and %RSD were as shown in table 5 and reference chromatograms are shown in Figure 4.

System precision parameters: Six replicates of linearity level-6 solution were injected, and the results were shown in table 5.

Table 5. System suitability parameters of MES

Validation parameter	As such conc. (ppb)	Conc. w.r.t sample (ppb)	% RSD	S/N ratio
LOD	0.1	1	NA	15
LOQ precision	0.3	3	4.11	20
System suitability	5	50	3.63	NA

NA: Not applicable

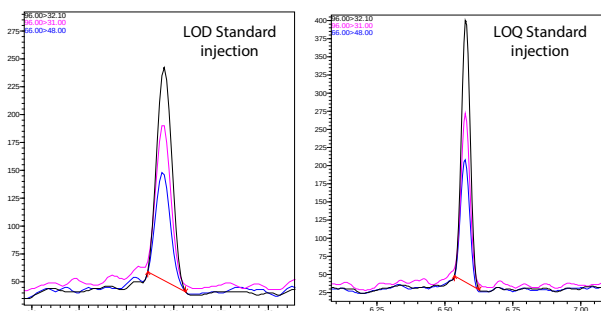


Figure 4 : Chromatograms of LOD and LOQ standard solution

Linearity: To evaluate the linearity, seven level calibration standards ranging from 0.1 ppb to 10 ppb were injected in duplicate. The correlation coefficient (r^2) was found to be 0.9999. Figure 5 depicts the calibration curve (a) and overlayed chromatograms of linearity levels (b).

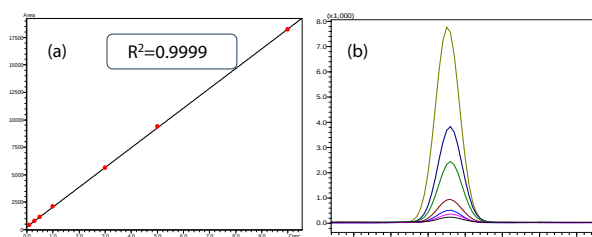


Figure 5 : Calibration curve (a) and Overlay of linearity levels (b)

Accuracy: Method accuracy was evaluated by performing %recoveries at four spike levels. Summary of the accuracy parameter is reported in the Table 6. %Recoveries for all the spike level were found to be well within the acceptance criteria of 70% to 130%.

Table 6. Summary of recovery at different accuracy levels

Accuracy level	Amount spiked as such (ppb)	Amount in sample (ppb)	Average rec. amt. (ppb)	Average recovery (%)
Level-1	0.3	ND	0.369	123.00
Level-2	1	ND	0.998	99.85
Level-3	5	ND	4.646	92.91
Level-4	10	ND	9.260	92.60

Figure 6 depicts the overlayed chromatograms of all spiked level with test sample injections.

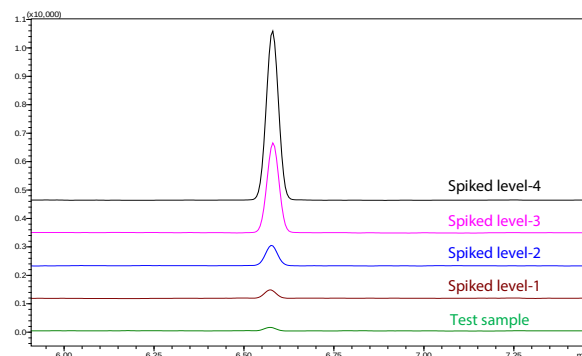


Figure 6 : Overlayed chromatograms of Accuracy samples

Method precision: To evaluate the method precision, six replicates of samples spiked at limit level were injected. %RSD of the content detected in these injections was found to be well within the acceptance criteria of not more than 15 %. Refer table 7 for the results.

Table 7. Summary of method precision

Precision	Amount spiked as such (ppb)	Average rec. amt. (ppb)	% RSD
Replicate-1	5	4.66	2.15
Replicate-2	5	4.64	
Replicate-3	5	4.76	
Replicate-4	5	4.50	
Replicate-5	5	4.57	
Replicate-6	5	4.76	

Figure 7 depicts the overlayed chromatograms of all spiked level with test sample injections.

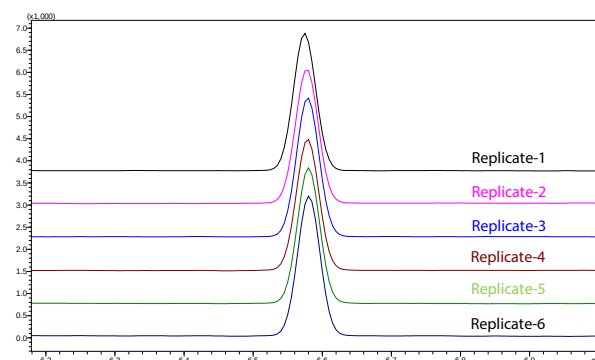


Figure 7 : Overlayed chromatograms of method precision samples

Batch analysis: For batch evaluation, four different batches of the Nintedanib Esylate were analyzed. The results were as mentioned in table 8 and the reference chromatograms are shown in figure 8.

Table 8. Summary of batch evaluation

Test samples	Avg. conc. (ppb)	Avg. conc. w.r.t sample (ppb)
Batch -1	1.95	19.5
Batch -2	3.67	36.7
Batch -3	56.18	561.8
Batch -4	2.96	29.6

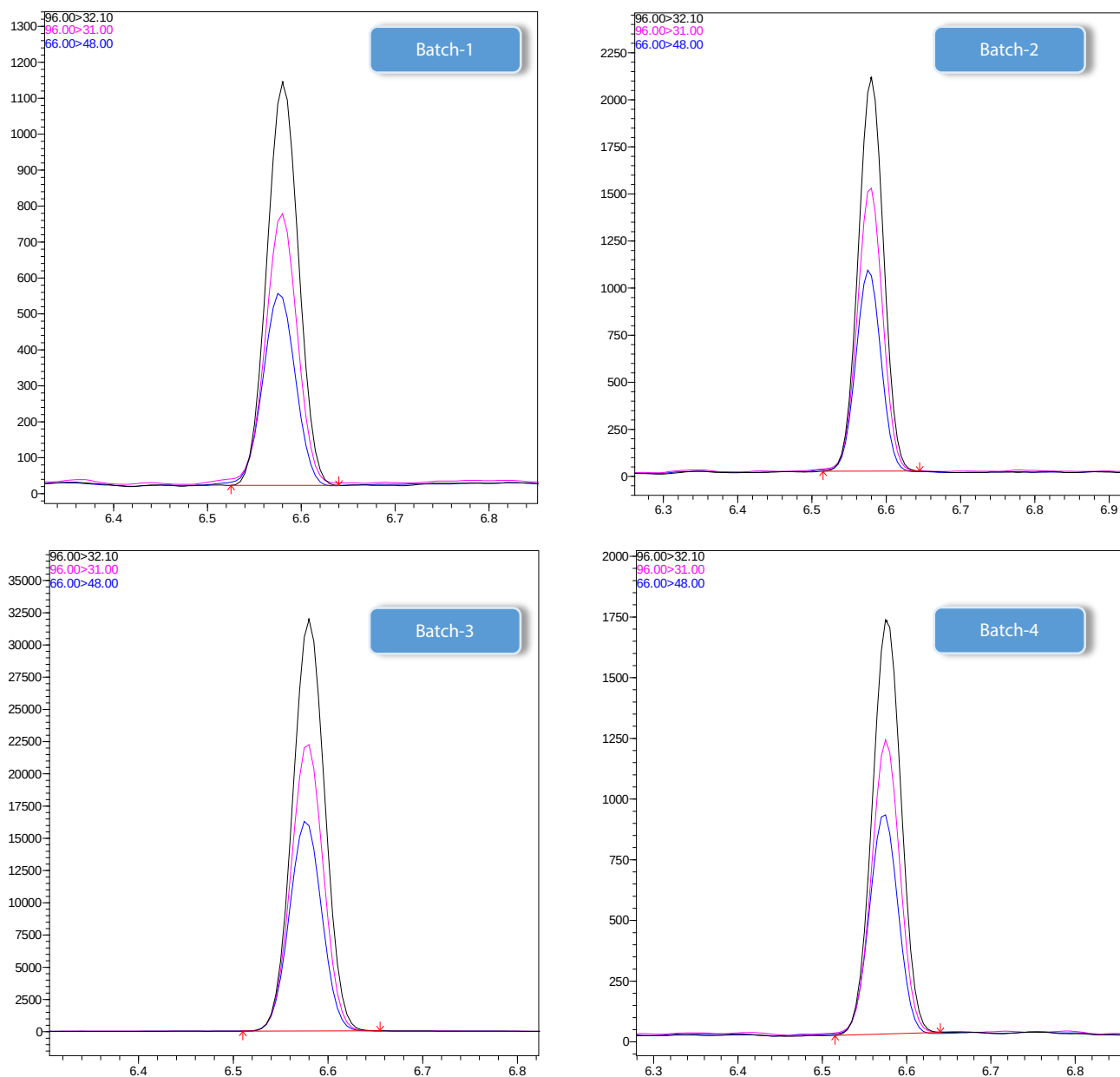


Figure 8 : Chromatograms of batch samples

■ Conclusion

1. Shimadzu GCMS-TQ8050 NX with HS-20 NX (Trap) autosampler enables accurate quantification of Methyl Ethane Sulfonate in Nintedanib Esylate API sample at ppb level.
2. Trap mode in the HS-20 NX auto sampler benefits in extracting the analyte multiple times which enhances sensitivity.

■ References

1. National Center for Biotechnology Information (2026). PubChem Compound Summary for CID 135476717, Nintedanib Esylate. Retrieved April 17, 2026 from <https://pubchem.ncbi.nlm.nih.gov/compound/Nintedanib-Esylate>.
2. Guideline on the Limits of Genotoxic Impurities issue by European Medicines Agency : EMEA/CHMP/QWP/251344/ 2006
3. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7 (R2)
4. Maximum daily dose of Nintedanib Esylate, <https://www.drugs.com/dosage/nintedanib.html>

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