

Application News

GCMS-QP™2050 Gas Chromatography Mass Spectrometer

Determination of Nitrobenzene Compounds in Nifedipine by GCMS

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User Benefits

- ◆ The direct dissolution protocol without pre-treatment, significantly enhancing analytical efficiency.
- ◆ The specificity of the method is ensured by monitoring the characteristic ions of nitrobenzene compounds in selected ion monitoring (SIM) mode, which provides robust resistance to interferences.

■ Introduction

Nifedipine, a dihydropyridine calcium channel blocker with vasodilatory properties, is primarily indicated for the clinical management of hypertension and angina pectoris. Its synthetic route commences with 2-nitrotoluene as the starting material. Notably, the intermediate 2-nitrobenzyl alcohol, the byproduct 2-nitrobenzyl bromide, the reaction product 2-nitrobenzaldehyde, and potential impurities (3-nitrobenzaldehyde and 4-nitrobenzaldehyde) all contain nitrobenzene moieties, rendering them candidates for monitoring as potential genotoxic impurities. Previous studies have demonstrated that 2-nitrobenzaldehyde and 4-nitrobenzaldehyde exhibit mutagenicity in the Ames test, while 2-nitrotoluene has shown carcinogenicity in animal experiments. In accordance with the toxicological concern threshold (TTC) control approach specified in ICH M7, a threshold of 1.5 µg/day is recommended for genotoxic impurities in pharmaceuticals intended for lifelong administration.

In this study, a method for the determination of six genotoxic impurities in nifedipine was developed using a Shimadzu GCMS-QP2050 gas chromatography mass spectrometry. This method features simplified sample preparation, high sensitivity, and favorable repeatability, thereby enabling effective monitoring of the six nitrobenzene compounds as genotoxic impurities in nifedipine pharmaceutical.

■ Analytical Conditions

The analytical conditions are shown in Table1. GCMS-QP2050 system was used for this Application News.

■ Sample Preparation

Precisely weigh 50 mg of nifedipine sample into a 10 mL brown volumetric flask, dissolve in ethyl acetate and dilute to volume. Then analyze the solution.

■ Chromatogram of Mixed Standard

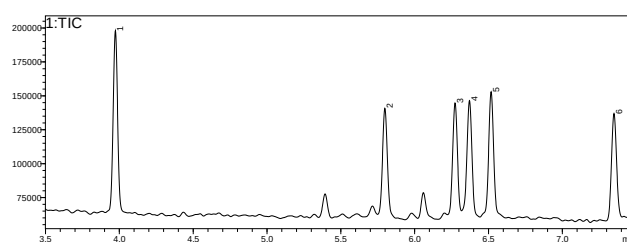


Fig. 1 TIC Chromatogram for Standard Sample (5 mg/L)

Table1 Analytical conditions

System	: GCMS-QP2050
Column	: SH-I-5Sil MS (30 m × 0.25 mm × 0.25 µm)
Column Temp. Program	: 100 °C (1 min)_8 °C/min_180 °C (2 min)_20 °C/min_250 °C (2 min)
Injection Temp.	: 250 °C
Injection Volume	: 1 µL
Carrier Gas Control Mode	: Constant linear velocity (36.5 cm/s)
Injection Mode	: Split
Split Ratio	: 10:1
Ionization Mode	: EI
Ion Source Temp.	: 230 °C
Interface Temp.	: 250 °C
Acquisition Mode	: SIM
SIM monitoring m/z	: Refer to Table 2

Table 2 Characteristic Ions for SIM Monitoring of Six Nitrobenzene Compounds

No.	Compound	CAS	RT (min)	Quantitative ion (m/z)	Qualitative ion(m/z)
1	2-Methyl-1-nitrobenzene	88-72-2	3.968	120	91, 65
2	2-Nitrobenzaldehyde	552-89-6	5.795	121	93, 104
3	1-Bromo-2-nitrobenzene	577-19-5	6.267	201	155, 173
4	4-Nitrobenzaldehyde	555-16-8	6.375	151	105, 77
5	3-Nitrobenzaldehyde	99-61-6	6.522	151	105, 77
6	2-Nitrobenzyl alcohol	612-25-9	7.348	77	79

■ Calibration Curves

Mixed standard solutions of six nitrobenzene compounds were prepared in ethyl acetate at five concentration levels (20、50、100、160、200 µg/L). Calibration curves for each compound exhibited excellent linearity ($R > 0.999$) as shown in Fig. 2. The SIM chromatograms of the six nitrobenzene compounds standard at 20 µg/L are displayed in Fig 3. Based on data for the 20 µg/L standard solution, Instrument Detection Limits (IDLs) for each target were calculated with a 3-fold signal-to-noise ratio (S/N). Results are shown in Table 3.

Table 3 Calibration Curve Information, Instrument Detection Limits

No.	Compound	Calibration Correlation (R)	IDLs (µg/L)
1	2-Methyl-1-nitrobenzene	0.9997	0.326
2	2-Nitrobenzaldehyde	0.9997	0.357
3	1-Bromo-2-nitrobenzene	0.9995	0.496
4	4-Nitrobenzaldehyde	0.9995	0.377
5	3-Nitrobenzaldehyde	0.9996	0.263
6	2-Nitrobenzyl alcohol	0.9995	0.270

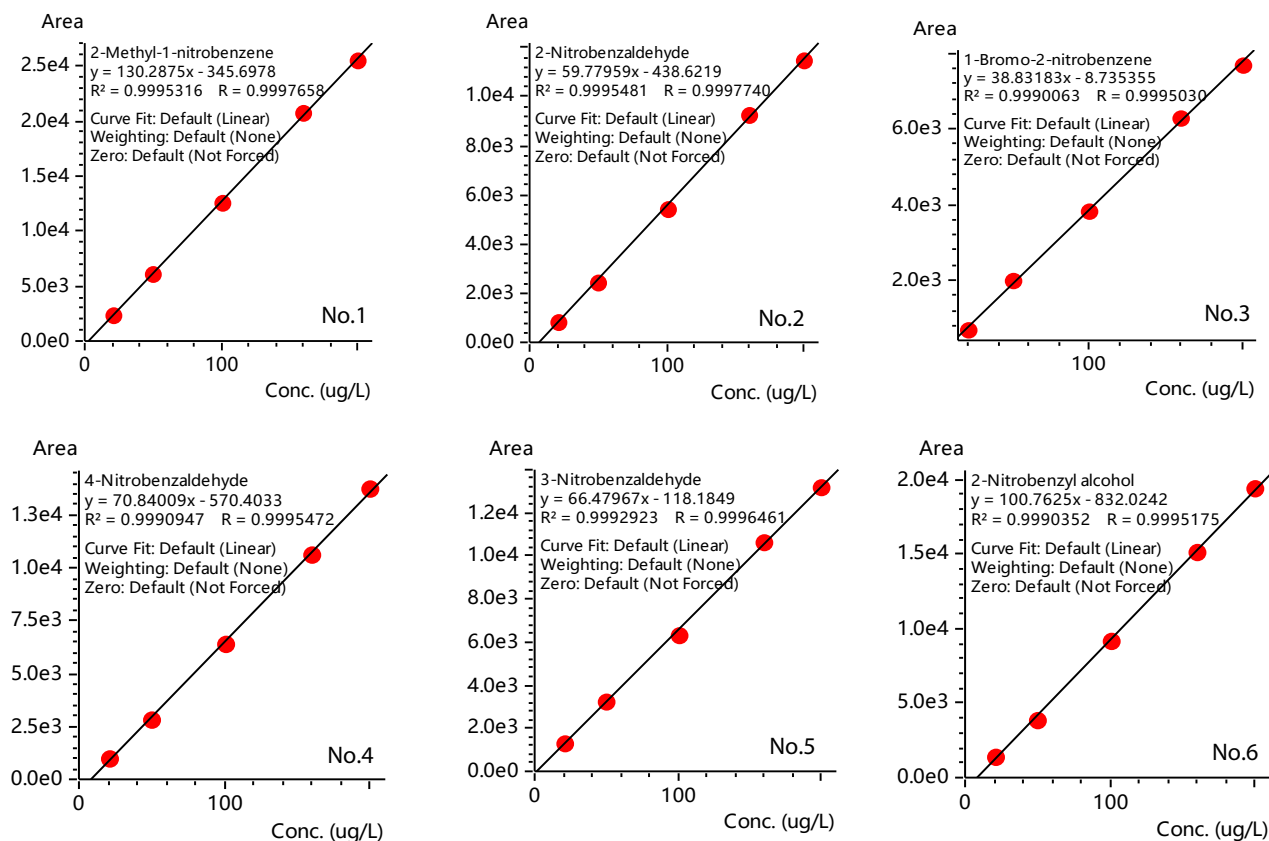


Fig. 2 Calibration Curves (20-200 µg/L)

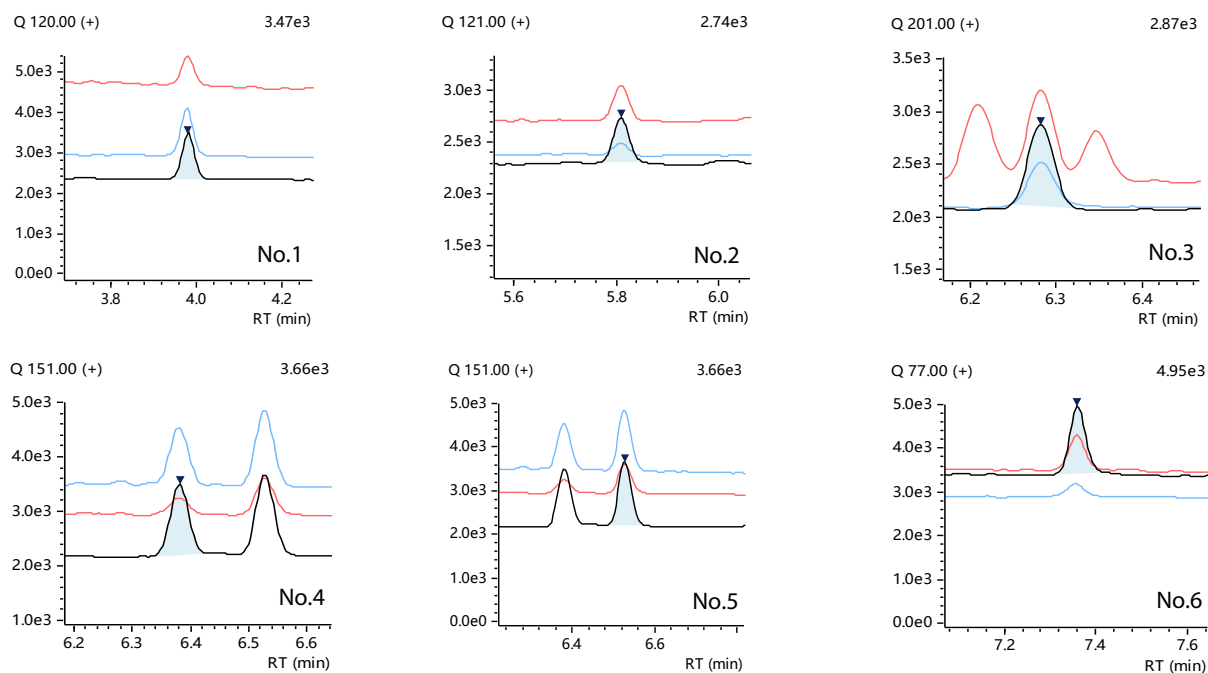


Fig. 3 Mass Chromatograms for the Compounds (20 µg/L)

■ Repeatability

A standard solution at a concentration of 50 µg/L was repeatedly analyzed six times to determine instrument repeatability. The peak area and RSD% values for each compound are shown in Table 4

■ Sample Testing and Recovery Test

The Nifedipine CR Tablets sample was analyzed according to the previously described methodology, demonstrating the absence of detectable genotoxic impurities as Fig 4. Subsequently, spike recovery experiments were conducted at three concentration levels (0.005, 0.01, and 0.032 µg/g) to validate the method's accuracy. The corresponding recovery data are systematically tabulated in Table 5.

■ Conclusion

A GC-MS method was established for the quantification of six nitrobenzene genotoxic impurities in nifedipine pharmaceuticals using a Shimadzu GCMS-QP2050 system. The calibration curves showed excellent linearity within the concentration range of 20-200 µg/L, with correlation coefficients ($R>0.999$). Precision was evaluated through six replicate injections of the 50 µg/L standard solution, and the relative standard deviations (RSDs) of peak areas for all analytes were less than 4%. Spike recovery experiments were performed at three concentration levels (0.005, 0.01, and 0.032 µg/g) in actual matrices, resulting in recovery rates ranging from 95.85% to 117.64%. This method is characterized by simplified sample preparation, high sensitivity (IDLs of 0.263-0.496 µg/L), and good repeatability. Overall, it provides a validated analytical strategy for quality control monitoring of the target genotoxic impurities.

Table 4 Repeatability Results

No.	Compound	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	RSD (%)
1	2-Methyl-1-nitrobenzene	3,958	3,917	4,098	4,218	3,953	4,034	2.80
2	2-Nitrobenzaldehyde	2,564	2,725	2,696	2,592	2,669	2,741	2.70
3	1-Bromo-2-nitrobenzene	1,985	2,022	2,045	1,991	1,898	2,078	3.10
4	4-Nitrobenzaldehyde	3,046	3,265	3,202	3,025	3,079	3,257	3.45
5	3-Nitrobenzaldehyde	3,426	3,470	3,453	3,417	3,476	3,623	2.16
6	2-Nitrobenzyl alcohol	4,474	4,244	4,427	4,321	4,429	4,433	1.98

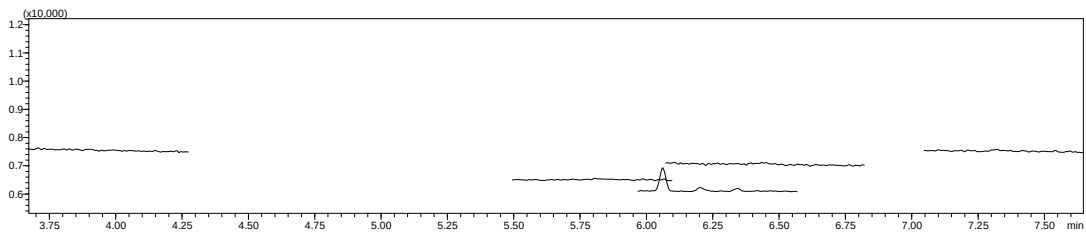


Fig. 4 Mass Chromatograms of Sample

Table 5 Results of Spiked Recoveries (n=3)

No.	Compound	0.005 µg/g		0.01 µg/g		0.032 µg/g	
		Results of Sample Testing (%)	RSD (%)	Results of Sample Testing (%)	RSD (%)	Results of Sample Testing (%)	RSD (%)
1	2-Methyl-1-nitrobenzene	95.85	3.93	104.59	1.95	106.01	4.08
2	2-Nitrobenzaldehyde	111.14	2.44	117.64	0.26	111.95	1.34
3	1-Bromo-2-nitrobenzene	100.61	3.82	116.74	4.27	108.69	3.34
4	4-Nitrobenzaldehyde	103.54	0.77	113.21	0.94	113.32	2.16
5	3-Nitrobenzaldehyde	106.00	5.24	114.92	3.07	113.96	2.78
6	2-Nitrobenzyl alcohol	107.61	4.92	113.06	4.13	105.13	0.83

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