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GC-MS GCMS-QP™ Series

Application News

Analysis of Nitroglycerin Metabolites in Blood Plasma Using NCI-GC/MS

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User Benefits

- NCI-GC/MS analysis can detect nitroglycerin metabolites and nitric acid metabolites with high levels of sensitivity.
- The same GC-MS system configuration can measure both nitroglycerin metabolites and nitric acid metabolites.
- It may be possible to predict optimal nitroglycerin dosage from the quantitation results of metabolites.

■ Introduction

Nitroglycerin (glyceryl trinitrate [GTN]) is an explosive compound and raw material of dynamite. GTN also has medical uses as an antianginal agent that induces vasodilation.

Upon administration, nitroglycerin is metabolized into glyceryl dinitrate (GDN) and glyceryl mononitrate (GMN) in the liver and blood, and nitric oxide (NO) derived from nitrogen dioxide (NO_2) is released into cells. Nitrogen oxide increases cyclic GMP production by activating guanylate cyclase, which subsequently decreases intracellular Ca^{2+} levels and relaxes the myocardium.

Nitroglycerin is effective in treating patients with acute heart failure or chronic conditions such as angina, but requires proper dosing due to anemia-related side effects such as hypotension-induced lightheadedness and dizziness.

Negative chemical ionization (NCI) is a type of chemical ionization that specifically detects compounds with electron affinity. Compounds that are detected with low sensitivity by electron ionization (EI) can be detected with high sensitivity by NCI by adding fluorine derivatives with electron affinity such as pentafluorobenzyl (PFB).

This Application News describes an investigation into using an NCI-GC/MS analytical system to measure nitroglycerin metabolites and nitric acid metabolites in order to predict the optimal dosage of nitroglycerin, a nitrate medication.

1. Analysis of Nitroglycerin and its Metabolites

■ Method

The compounds chosen for analysis were nitroglycerin (GTN) and the metabolites 1,2-glyceryl dinitrate (1,2-GDN), 1,3-glyceryl dinitrate (1,3-GDN), and 1-glyceryl mononitrate (1-GMN). *o*-iodobenzyl alcohol was used as an internal standard, and an internal standard solution of 2.5 µM *o*-iodobenzyl alcohol was prepared with ultrapure water for addition to samples.

200 µL of a mixed standard aqueous solution prepared to a predetermined concentration was placed in a 10 mL polypropylene tube and 10 µL of the internal standard solution was added (concentration in aqueous solution: 250 nM). Next, 4 mL of toluene was added and the mixture was stirred for 20 minutes. The mixture was then centrifuged at 10 °C and 3000 rpm for 5 min and the organic phase was isolated. This organic phase was dried under nitrogen gas flow, 200 µL of 2 % triethylamine in hexane was added, and after stirring, the solution was transferred to a 2 mL GC vial with a vial insert.

Nitroglycerin readily adsorbs to glassware. This property has posed a significant challenge in the measurement of nitroglycerin levels without derivatization, but this adsorption was prevented by adding triethylamine to act as a masking agent.

200 µL of blood plasma was also placed in a 10 mL polypropylene tube and prepared for analysis by the same procedure.

The analytical conditions used are shown in Table 1. Ionization was performed by EI and NCI (reagent gas: methane).

Although nitroglycerin is prone to thermal decomposition and tends to require a programmable temperature vaporizing (PTV) inlet, setting a low injection unit temperature of 150 °C allowed for detection.

Table 1 GC-MS Configuration and Analytical Conditions

GC-MS:	GCMS-QP2020 NX	
Auto-injector:	AOC-30i/20s U	
Column:	InertCap 17MS (30 m, 0.25 mm, 0.25 µm)	
[GC]		
Injection Temp.:	150 °C	
Column Oven Temp.:	50 °C (1 min) → (10 °C /min) → 200 °C (2 min)	
Injection Mode:	Splitless	
Carrier Gas:	He	
Carrier Gas Control:	Linear velocity (40.7 cm/sec)	
Injection Volume:	2 µL	
[MS]		
Ionization Mode:	NCI	
Reagent Gas and Pressure:	Methane (300 kPa)	
Ion Source Temp.:	200 °C	
Interface Temp.:	200 °C	
Data Acquisition Mode:	Scan/SIM	
Scan Range:	*m/z* 40 to 300	
Monitoring *m/z*:	1-GMN	91.0
	1,2-GDN	62.0, 46.0
	1,3-GDN	62.0, 46.0
	GTN	62.0
	o-Iodobenzyl alcohol	127.0

■ Results

Fig. 1 shows the total ion current chromatogram (TICC) measured in NCI mode of a sample containing each compound of interest added to ultrapure water and prepared for analysis. Fig. 2 shows the SIM chromatogram of a 5 nM standard sample and Fig. 3 shows calibration curves measured in the range 5 to 2,000 or 5 to 5,000 nM. Nitroglycerin and glyceryl nitrate metabolites were all detected at a concentration of 5 nM, but were not detected at any concentration when triethylamine was not added.

This inability to detect nitroglycerin and glyceryl nitrate metabolites when triethylamine was not added was shown to be caused by adsorption to the GC vial and vial insert.

The correlation coefficients (R^2) of the calibration curves were 0.997 or higher, indicating good linearity over the wide concentration range. Fig. 4 shows measurements taken from blood plasma collected from a subject at constant time period after nitroglycerin was administered. The metabolites glyceryl mononitrate (GMN) and glyceryl dinitrate (GDN) were detected at high concentrations, thus providing insight into drug metabolism behavior over time.

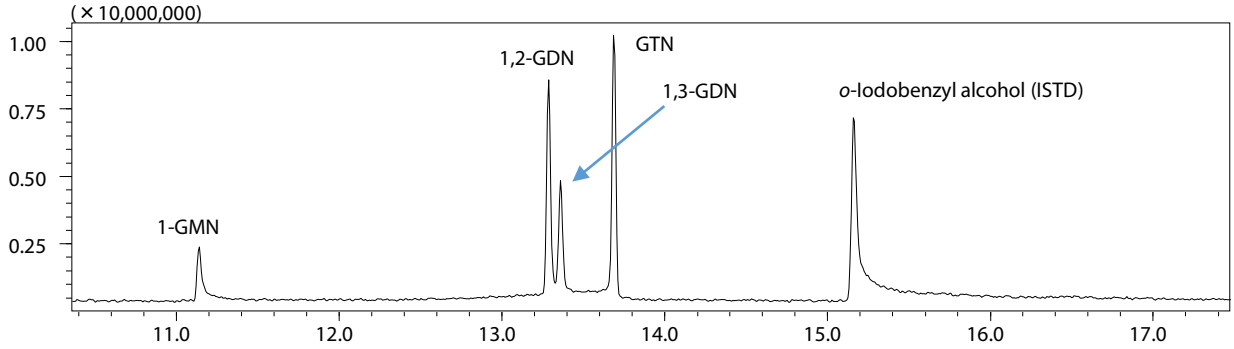


Fig. 1 Total Ion Current Chromatogram (TICC) of Nitroglycerin and Glyceryl Nitrates

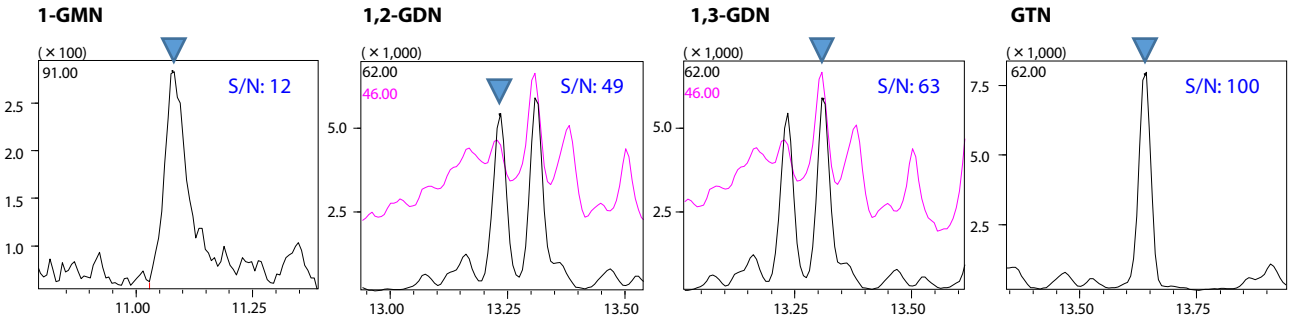


Fig. 2 SIM Chromatogram of Nitroglycerin and Glyceryl Nitrates in Standard Sample (Concentration: 5 nM)

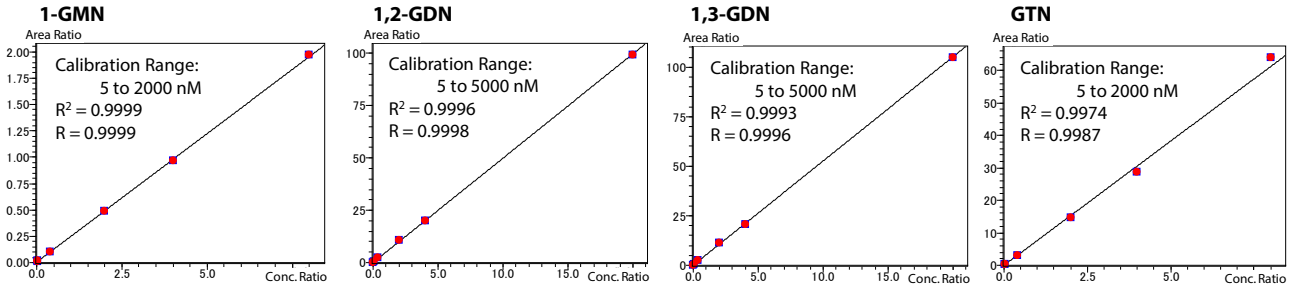
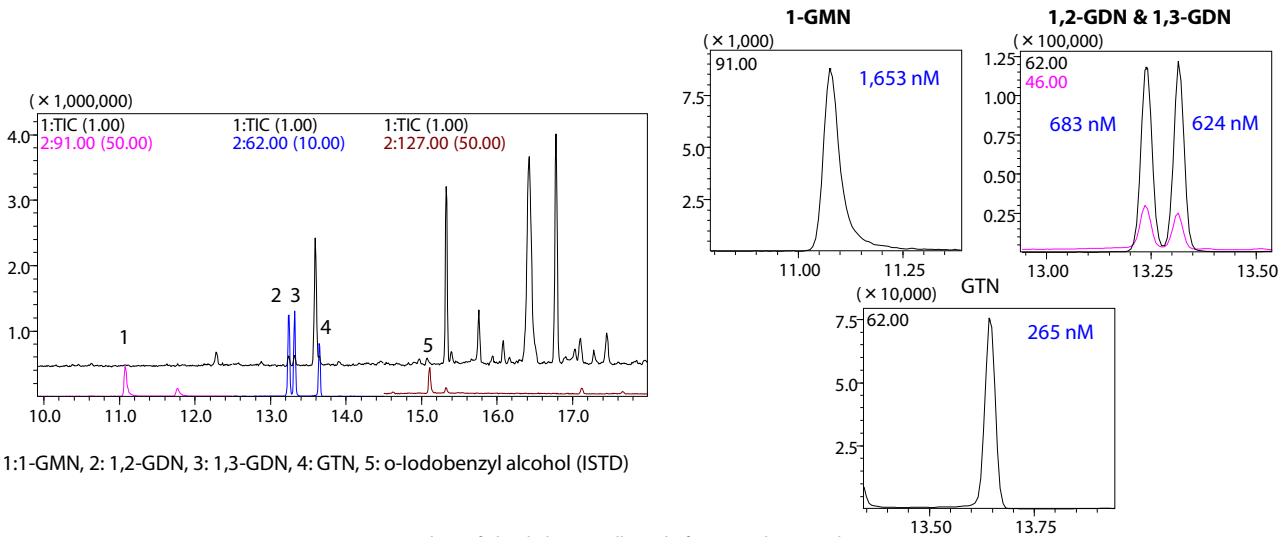


Fig. 3 Calibration Curves of Nitroglycerin and Glyceryl Nitrates



1:1-GMN, 2: 1,2-GDN, 3: 1,3-GDN, 4: GTN, 5: o-Iodobenzyl alcohol (ISTD)

Fig. 4 Analysis of Blood Plasma Collected After Nitroglycerin Administration

2. Analysis of Nitric Acid Metabolites

■ Method

Calibration curves of two nitric acid metabolites (a nitrate and a nitrite) were prepared with ultrapure water. The same nitrate and nitrite labeled with ^{15}N were also used as internal standards. Internal standard solutions of ^{15}N-NO_2 at 100 µM and ^{15}N-NO_3 at 1000 µM were prepared with ultrapure water.

100 µL of an aqueous solution of the nitrate and nitrite prepared to a predetermined concentration was placed in a microtube and 10 µL of the internal standard solution was added (10 µM ^{15}N-NO_2 and 100 µM ^{15}N-NO_3 in aqueous solution). 400 µL of acetone and 10 µL of pentafluorobenzyl bromide (PFBBr) were also added and the mixture was heated at 50 °C for 60 min to induce PFB derivatization. After the reaction, the mixture was left to cool to room temperature, and acetone was removed by vaporization under nitrogen gas flow. Next, 1000 µL of toluene was added, the mixture was stirred for 1 min with a vortex mixer, centrifuged at 3,500 rpm for 5 min, and the organic phase was collected in a GC vial. 100 µL of blood plasma was also placed in a micro-vial and prepared for analysis by the same procedure.

Table 1 shows the final equipment configuration and analytical conditions. The equipment configuration, ionization method, and analysis column were optimized so that the same system configuration could also be used to measure the nitroglycerin metabolite glyceryl nitrate.

Analysis revealed that NCI was at least 100 times more sensitive than EI in measuring the nitrate and nitrite derivatives. Fig. 5 shows the mass spectra of the ^{15}N-labeled nitrate and nitrite derivatives used as internal standards recorded using NCI. No molecular ions were detected in a derivatized form and ions of *m/z* 100 or higher were fragment ions derived from PFB. Accordingly, ions with PFB removed by ionization were chosen for selective ion monitoring.

Table 2 GC-MS Configuration and Analytical Conditions

GC-MS:	GCMS-QP2020 NX	
Auto-injector:	AOC-30i/20s U	
Column:	InertCap 17MS (30 m, 0.25 mm, 0.25 µm)	
[GC]		
Injection Temp.:	250 °C	
Column Oven Temp.:	70 °C (1 min) → (20 °C/min) → 300 °C (3 min)	
Injection Mode:	Splitless	
Carrier Gas:	He	
Carrier Gas Control:	Linear velocity (40.2 cm/sec)	
Injection Volume:	1 µL	
[MS]		
Ionization Mode:	NCI	
Reagent Gas and Pressure:	Methane (300 kPa)	
Ion Source Temp.:	200 °C	
Interface Temp.:	250 °C	
Data Acquisition Mode:	Scan/SIM	
Scan Range:	*m/z* 40 to 300	
SIM Monitored *m/z*:	^{15}N-NO_3-PFB (ISTD)	63.0
	^{14}N-NO_3-PFB	62.0
	^{15}N-NO_2-PFB (ISTD)	47.0
	^{14}N-NO_2-PFB	46.0

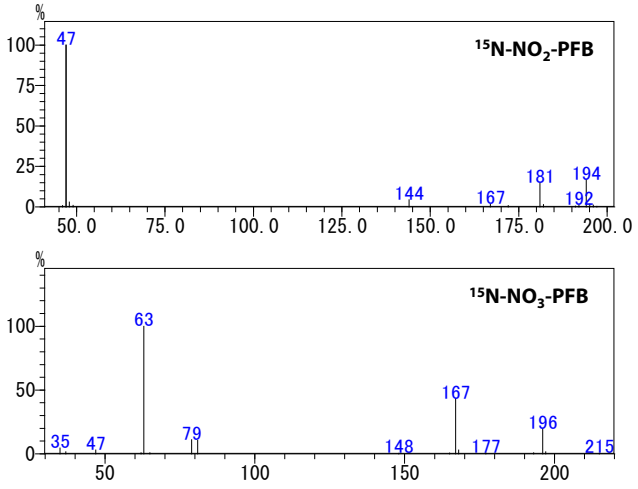


Fig. 5 NCI Mass Spectra of ^{15}N-Labeled Nitrate and Nitrite Derivatives

■ Results

Fig. 6 shows the SIM mass chromatogram recorded at the minimum concentration point on the calibration curves (NO_2: 0.75 µM, NO_3: 7.5 µM) and Fig. 7 shows the calibration curves themselves. The sensitivity of the analytical system was sufficient to detect nitrate and nitrate derivative concentrations of several µM without enrichment and both calibration curves show good linearity with $R^2 = 0.9998$ for NO_2 in the range 0.75 to 100 µM and $R^2 = 0.9979$ for NO_3 in the range 7.5 to 1000 µM.

Fig. 8 shows measurements taken from blood plasma collected from a subject after being administered nitroglycerin.

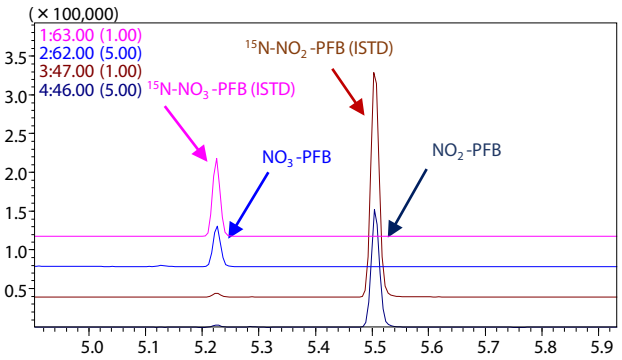


Fig. 6 SIM Mass Chromatogram of Nitrate and Nitrite Derivatives
(NO_2: 0.75 µM, NO_3: 7.5 µM)

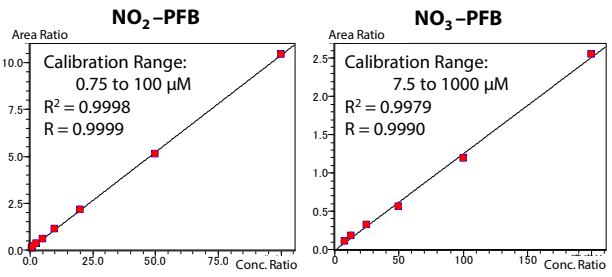


Fig. 7 Calibration Curves for Nitrate and Nitrite Derivatives

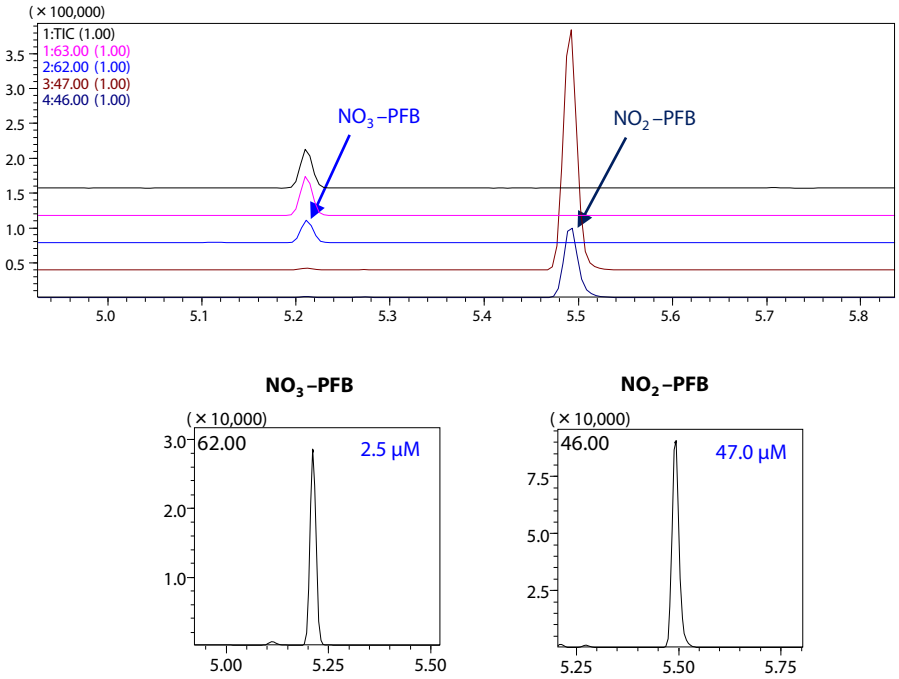


Fig. 8 Measurements from Blood Plasma Collected from Subject Administered Nitroglycerin

■ Conclusion

An analytical system for measuring nitroglycerin, nitroglycerin metabolites, and nitric acid metabolites was established using NCI-GC/MS. Derivatization methods and masking agents that prevent adsorption allowed high-sensitivity detection with NCI and allowed quantitation of nitroglycerin and its metabolites at concentrations of several nM in blood plasma and nitric acid metabolites at several µM.

It may be possible to predict the optimal nitroglycerin dosage as an antianginal agent for reducing related side effects by measuring nitroglycerin metabolite levels.

Although this study does not investigate the behavior of metabolite levels over time, such behavior could be used to manage administration at an optimal dose.

< References >

- Dimitrios Tsikas, Anal. Chem, 2000, 72, 4064-4072
- Peter Akrill, John Cocker, Journal of Chromatography B, 778 (2002) 193–198
- Martin Jorgensen, Michael P. Andersen, Journal of Chromatography, 517 (1992) 167-l 70

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