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Application News

Analysis of Phenethylamines Using On-Column TFA Derivatization

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User Benefits

- Trifluoroacetyl (TFA) derivatization increases the ability to differentiate between phenethylamines which otherwise provide poor mass spectral information.
- Automatic derivatization shortens sample pretreatment time.
- Two-step injection is now a standard function of LabSolutions™ GCMS and is available through software updates.

■ Introduction

Phenethylamines, such as methamphetamine and MDMA, are illegal drugs that are abused worldwide. Phenethylamines are prone to adsorption due to their amine backbone, and analysis of the unchanged phenethylamines provides poor mass spectral information. Therefore, they are commonly derivatized to make them more easily differentiated. Trifluoroacetic (TFA) anhydride is typically used for the analysis of phenethylamines (Fig. 1). However, conventional derivatization by anhydrous TFA is time-consuming as it requires drying of the derivatization reagent after derivatization (Fig. 2).

This Application News presents the analysis of phenethylamines by automatic on-column derivatization using auto-injector AOC-30i and a LabSolutions GCMS system that is now equipped with 2-step injection as a standard function. On-column derivatization allows for the direct analysis of extracted urine samples without additional sample pretreatment, which can significantly shorten the time required for derivatization.

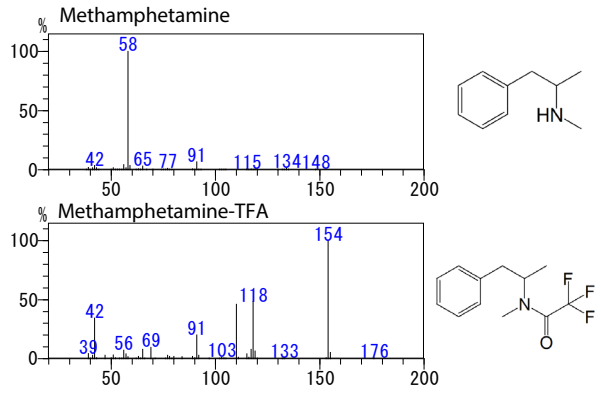

Fig. 1 Mass Spectra of Intact and TFA-Derivatized Methamphetamine

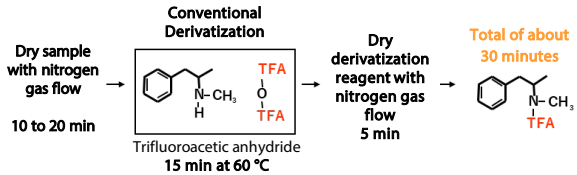

Fig. 2 Conventional TFA Derivatization Method

■ 2-Step Injection Functionality

GCMSsolution software required an optional software dedicated for performing 2-step injection; it is now available as a standard function in LabSolutions GCMS. For existing users, this function can be used by updating the software to the applicable version. (Refer to the notes.)

MBTFA (N-methyl-bis-trifluoroacetamide), which was developed specifically for on-column derivatization, was used as the derivatizing agent. MBTFA derivatizes amino groups quickly and selectively. In addition, hydroxyl groups are known to be less reactive. On-column derivatization using 2-step injection is performed by injecting samples into the GC-MS system according to the following steps (Fig. 3).

Step 1: MBTFA derivatizing agent => Air => Analytical sample, are successively drawn into the syringe.

Step 2: The sample and some of the air are injected into the injection port. The sample is vaporized in the injection port and introduced into the capillary column.

Step 3: The system waits a few seconds while the target analytes enter the capillary column. Then the MBTFA derivatizing reagent is injected.

Step 4: When the derivatizing agent passes over target analytes in the capillary column, phenethylamines are derivatized and then analyzed by GC-MS system.

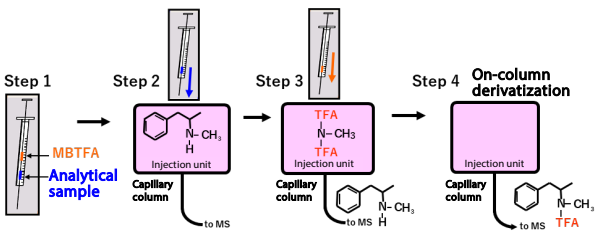

Fig. 3 On-Column Derivatization Flow Using 2-Step Injection

Notes:

- Two-step injection functionality is available in the following versions of LabSolutions.
  LabSolutions GCMS Ver. 5.131 or later
  LabSolutions LC/GC Ver. 5.131 or later
  LabSolutions DB GCMS Ver. 6.131 or later
  LabSolutions DB LC/GC Ver. 6.131 or later
- Two-step injection functionality can be used with AOC-20i + 20sU, AOC-30i, and AOC-30i + 20 sU autoinjector and sampler configurations.
- To use GCMSsolution, optional software is required.

■ Sample Pretreatment

Urine sample (2 mL) was adjusted to a pH of about 10 using a 5 % aqueous sodium carbonate solution. One milliliter of ethyl acetate was added, vortex-mixed, and centrifuged to recover the organic phase. Liquid-liquid extraction using ethyl acetate was performed twice. After mixing the organic phases, an appropriate amount of anhydrous sodium sulfate was added for dehydration. The final extract was used for analysis.

■ Analytical Conditions

The analytical conditions are shown in Table 1.

A screenshot of the 2-step injection parameter settings is shown in Fig. 4. A GCMS-TQ8040 NX system was used for this Application News, but a GCMS-QP2020 NX or GCMS-QP2050 system can also be used.

Table 1 Analytical Conditions

GC-MS:	GCMS™-TQ8040 NX
Autoinjector:	AOC-30i
Column:	SH-I-5Sil MS (30 m, 0.25 mm I.D., 0.25 µm)
Derivatizing Reagent:	MBTFA
[GC]	
Injection Unit Temp.:	250 °C
Column Oven Temp.:	100 °C (0 min) → (15 °C/min) → 170 °C → (20 °C/min) → 320 °C (2 min)
Carrier Gas Control:	Pressure (140 kPa)
Injection Mode:	Split
Split Ratio:	5
[MS]	
Ion Source Temp.:	200 °C
Interface Temp.:	250 °C
Data Acquisition Mode:	Scan (*m/z* 45 to 500)
Event Time:	0.3 sec
[2-Step Injection]	
Derivatizing Reagent Volume:	1 µL
Dwell Time:	4 sec
Injection Speed:	High
Air Aspiration Volume:	2 µL
Air Injection Volume at Sample Injection:	1 µL

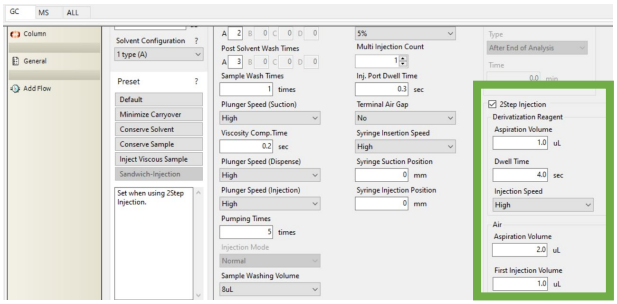

Fig. 4 2-Step Injection Parameter Settings

■ Results

Fig. 5 and Fig. 6 show the mass chromatograms and mass spectra obtained by analysis of a urine sample spiked at 0.1 µg/mL of the target phenethylamines and pretreated by liquid-liquid extraction, respectively. All target phenethylamines were detected with good sensitivity, and no peaks from respective unchanged drugs were detected, indicating a nearly 100 % derivatization efficiency. Fig. 6 shows the comparison of the methamphetamine-TFA mass spectrum obtained from a urine sample spiked at 0.1 µg/mL with the corresponding mass spectrum registered in the GC/MS forensic toxicology database. While not all contaminants from the urine sample could be subtracted, the results show that the fragment patterns from the methamphetamine-TFA closely matched the mass spectrum registered in the database.

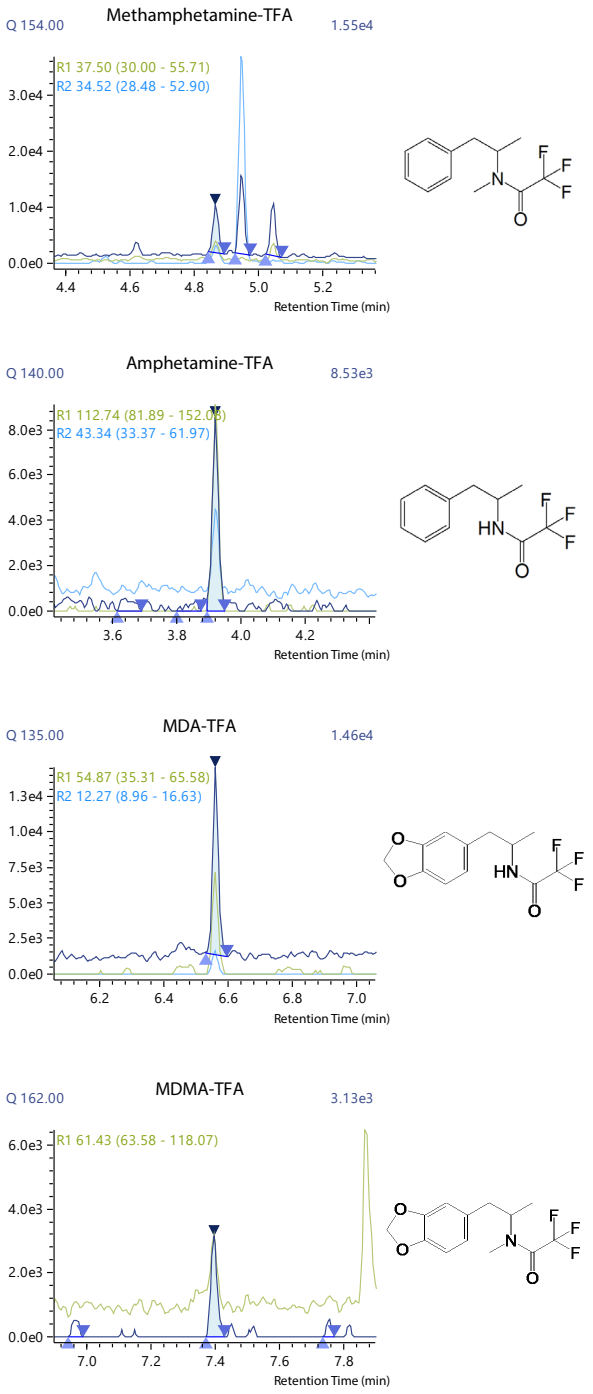

Fig. 5 Mass Chromatograms of 0.1 µg/mL Concentrations of Respective Drugs in Urine

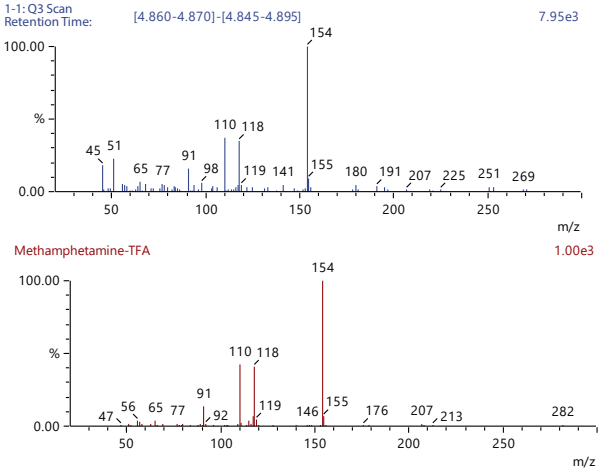

Fig. 6 Comparison of Methamphetamine-TFA Mass Spectra
Top: Mass Spectrum Detected from 0.1 µg/mL Concentration in Urine
Bottom: Mass Spectrum Registered in the Database

Calibration curves over the 0.1 to 10 µg/mL concentration range for the spiked phenethylamines in urine are shown in Fig. 7. Coefficient of determination (R^2) values were at least 0.9992, indicating good linearity.

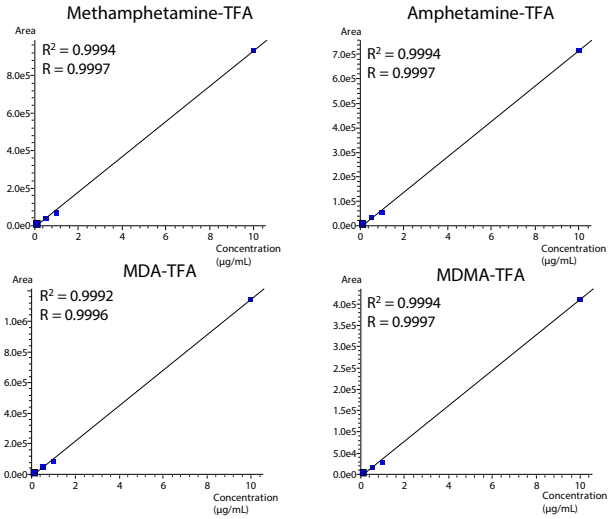

Fig. 7 Calibration Curves for the Target Phenethylamines
(0.1 to 10 µg/mL Concentration Range in Urine)

■ Application to Authentic Samples

Total ion current chromatograms (TICC) obtained from analyzing solvent-extracted authentic urine sample are shown in Fig. 8, and the corresponding chromatograms of the detected phenethylamines are shown in Fig. 9. Methamphetamine and amphetamine were detected in the urine samples. These results demonstrate the ability of qualitative and quantitative analysis using 2-step injection with high selectivity in authentic samples.

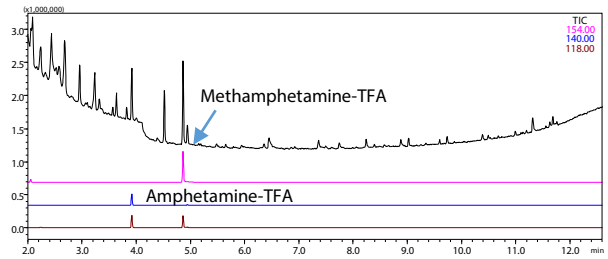

Fig. 8 Total Ion Current Chromatograms

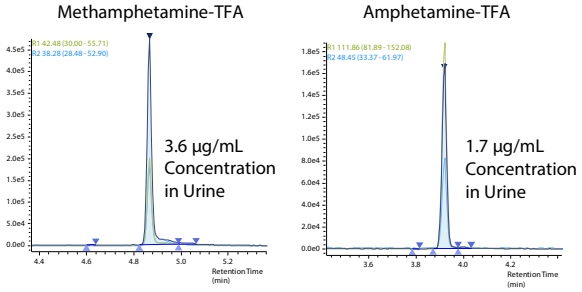

Fig. 9 Mass Chromatograms of Detected Drugs

■ Conclusion

The 2-step injection function automates the derivatization of phenethylamines, thereby reducing the time required for sample pretreatment.

The 2-step injection function now comes as standard feature in LabSolutions GCMS software, and can be used by existing LabSolutions GCMS users by updating the software.

However, this function is limited to use with phenethylamines as it requires optimization of the type of derivatizing reagent, target compound, and analysis conditions.

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