*Chromatography Technical Note No AS165*

Automation of Derivatisation Workflows for GC-MS Metabolomics Applications

Camilla Liscio, Anatune Ltd. Girton, Cambridgeshire (UK).

Introduction

Gas chromatography-mass spectrometry is often used for metabolomics applications because of its reproducibility and chromatographic resolution power. However many of the target metabolites (e.g. organic acids, amino acids, carbohydrates) would not be suitable for GC chromatographic analysis due to their physical and chemical properties. Hence, a chemical derivatisation prior to analysis is often required to increase their volatility and thermal stability and allow their successful detection.

Silylation reactions are often used to replace active hydrogens (e.g. -OH, -COOH, $-NH_2$, -SH) with an alkylsilyl group via bimolecular nucleophilic substitution (SN-2 mechanism) as shown in the equation below for the –OH functional group.

$$Compound\text{-}OH + R3Si – X \rightarrow Compound\text{-}O\text{-}Si\text{-}R3 + HX$$
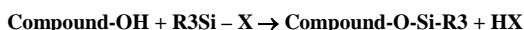
The choice of a silyl reagent is based on a variety of factors: its reactivity and selectivity toward the target compound, the stability of the final derivative, and the abundance and nature of reaction by-products.

Trimethylsilyl derivatives (TMS) are the most commonly used. Several reagents will give TMS derivatives. Amongst these, MSTFA (N-Methyl-N-trimethylsilylfluoroacetamide) offers high reactivity but also more volatile by-products. This allows the successful detection of early-eluting compounds which would be otherwise hidden in the chromatogram.

Recent studies [1] have shown the potential of trimethylsilylcyanide (TMSCN) as silylating reagent. The smaller molecular size offers higher reactivity towards sterically hindered functional groups and the only by-product HCN gives cleaner chromatograms.

On the other hand, sterically hindered reagents with bulkier R groups, even if generally less reactive, give more stable and less moisture sensitive derivatives.

N-tertbutyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) is widely used for the formation of tert-butyldimethylsilyl (tBDMS) derivatives for compounds such as amino acids and organic acids [2]. In fact, tBDMS derivatives are more stable and produce an intense ion peak $[M\text{-}57]^+$ specific for each molecule.

This application note describes the automation of three different derivatisation workflows for metabolomics applications: TMS using MSTFA, TMS using TMSCN and TBDMS using MTBSTFA.

Samples were fully prepared by the MPS and then injected directly on a GC/Q-TOF MS system for instrumental analysis.

Instrumentation

The fully automated derivatisation workflows for metabolomics applications was developed on a GERSTEL MultiPurpose Sampler (MPS) 2 XL Dual head (Figure 1) equipped with the following objects:

- Solvent reservoirs (5 positions)
- Standard Wash station (2 washes and 1 waste)
- Tray VT98
- Tray IS28
- Agitator
- GERSTEL Multiposition Vortexer (mVorx)
- GERSTEL MultiPosition Evaporation station (mVAP)
- Anatune CF-200 Robotic Centrifuges

GC-MS Analysis was performed using the Agilent 7890B Gas Chromatograph coupled to the Agilent 7200B Q-TOF High-Resolution Accurate-Mass Mass Spectrometer

Figure 1: GERSTEL Dual Head MPS for fully automated derivatisation workflows for metabolomics applications

Methods

Optimised TMS derivatisations:

The TMS derivatization was preceded by methoximation step (MOX) to allows reaction of carbonyl functional groups (>C=O) to form oxime derivatives. This step is crucial to prevent cyclization of reducing sugars, formation of keto-enol tautomers for aldehydes and ketones and decarboxylation.

Approximately 1 mg of dried marjoram was weighed in an ultra-high recovery vial. The sample was firstly added with 50µL of MOX solution (methoxyamine hydrochloride in pyridine containing adonitol as internal standard) and the mixture was incubated at 30 °C.

The silylating reagent (either MSTFA + 1% TMCS or TMSCN) was subsequently added to the sample and reacted at 37°C. After derivatisation, the sample was cooled at room temperature before injecting 1 µL for GC-MS analysis.

Optimised tBDMS derivatisation:

Approximately 1 mg of dried marjoram was weighed in an ultra-high recovery vial. The sample was firstly added with norvaline methanolic solution as internal standard (IS). The sample was then evaporated to dryness using the multiposition evaporation station (mVAP).

Acetonitrile and derivatising reagent MTBSTFA + 1% tBDMCSI were added to the sample and after mixing, the mixture was incubated at 90 °C (750 rpm agitation speed).

After derivatisation, the sample was cooled at room temperature before injecting 1 μL for GC-MS analysis.

GC/MS conditions:

GC:

- Column: HP-5MS Ultra inert 30 m x 0.25 mm x 0.25 μm
- Injection mode: Split 10:1
- Flow: 1 mL/min
- GC ramp: 50 °C held for 2 min, 10 °C/min to 300 °C, held for 8 min
- Auxiliary temperature: 280 °C

MS:

- Removable Ion Source (RIS) in Electron impact (EI) mode at 250 °C
- Collision cell: Nitrogen as collision gas 1.5 mL/min
- QTOF in 2GHz mode, scan range 35-500 m/z

Results and Discussion

Figure 2 shows the Total Ion Chromatograms (TICs) of a procedural blank and two analytical replicates for the three investigated derivatisation workflows: MOX-TMS using MSTFA, MOX-TMS using TMSCN and tBDMS using MTBTSFA.

Derivatisation was successfully achieved for all three methods as shown by the derivatised IS peaks present in all samples.

Retention times and peak areas for the internal standards are summarized in Table 1.
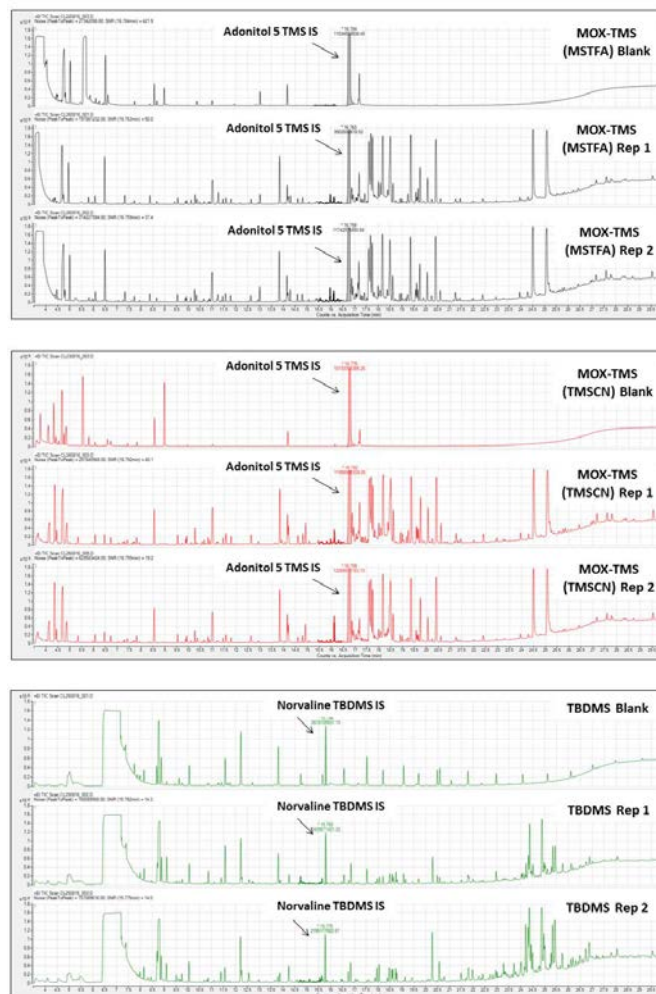

Figure 2: Total Ion Chromatograms (TICs) of procedural blank and two analytical replicates for the three automated derivatisation workflows: MOX-TMS using MSTFA (top, black traces), MOX-TMS using TMSCN (middle, red traces) and tBDMS using MTBTSFA (bottom, green traces).

MOX-TMS profiles using MSTFA and TMSCN were very similar but as stated by Khasimov et al [1], background contribution especially in the first part of the chromarogram was lower when using TMSCN.

The data files were processed using Agilent Technologies MassHunter Workstation Software Unknowns Analysis for deconvolution and library search. Figure 3 summarises the results for TMS and tBDMS derivatives hits with match factor above 70.
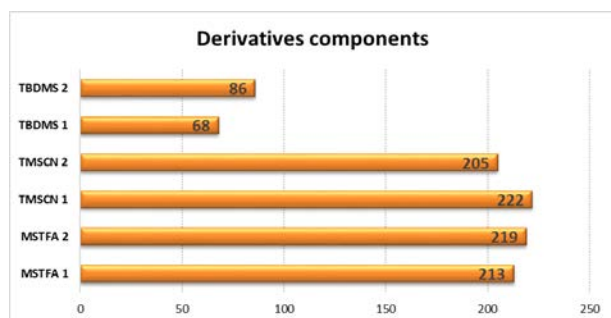
	Adonitol 5TMS IS		Norvalin TBDMS IS	
Sample	RT	Area	RT	Area
MOX-TMS (MSTFA) Blank	16.784	11706256702		
MOX-TMS (MSTFA) Rep 1	16.763	9872312548		
MOX-TMS (MSTFA) Rep 2	16.759	11716962160		
Average	*16.769*	*11098510470*		
Standard Deviation	*0.0134*	*1061932041*		
RSD%	*0.1*	*9.6*		
MOX-TMS (TMSCN) Blank	16.776	9958898954		
MOX-TMS (TMSCN) Rep 1	16.792	11956955338		
MOX-TMS (TMSCN) Rep 2	16.755	12089811103		
Average	*16.774*	*11335221798*		
Standard Deviation	*0.0186*	*1193780169*		
RSD%	*0.1*	*10.5*		
TBDMS Blank			15.794	2678705937
TBDMS Rep 1			15.792	2409571431
TBDMS Rep 2			15.775	2195177582
Average			*15.787*	*2427818317*
Standard Deviation			*0.0104*	*242280063*
RSD%			*0.1*	*10.0*

Table 1: Retention times and peak areas for the internal standard Adonitol 5TMS and Norvaline tBDMS in the investigated samples

Figure 3: Hits for TMS and tBDMS derivatives for the two sample replicates of the three automated derivatisation workflows.

MSTFA and TMSCN showed similar results for the TMS derivatisation.

On the other hand, tBDMS derivatisation gave a lower total number of derivatives but a higher percentage of hits scoring above 90 match factor (30% for TBDMS against 20% for TMS derivatives). Furthermore, tBDMS profiles showed the presence of several tBDMS derivatives for aminoacids and organic acids.

Conclusions

Three derivatisation workflows for metabolomics applications were fully automated: MOX-TMS using MSTFA, MOX-TMS using TMSCN and tBDMS.

All three derivatisation methods were successfully and reproducibly performed as shown by the internal standards Areas and RSDs%.

Several components hits were identified by the Agilent Unknown Analysis Software as target TMS and tBDMS derivatives with match factor above 70.

References

1.Khakimov et al. The use of trimethylsilyl cyanide derivatisation for robust and broad-spectrum high-throughput gas chromatography-mass spectrometry based metabolomics, Anal Bioanal Chem (2013) 405: 9193. doi:10.1007/s00216-013-7341-z

2. Ohie T. et al. Gas chromatography-mass spectrometry with tert-butyldimethylsilyl derivatisation: use of the simplified sample preparations and the automated data system to screen for organic acidemias, Journal of Chromatography B, 746 (2000) 63-73

Acknowledgements