Exploring the Benefits of Automated Unattended Sample Derivatization Prior to Gas Chromatography Analysis

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Overview

Purpose: Exploring the possibility to perform the sample preparation steps necessary for the derivatization of analytes in a fully automated and reliable way with the use of the same robotic sampler used for sample injection into the gas chromatograph.

Methods: As an example of application, we screened dairy products for melamine and its derivates presence via GC-MS/MS.

Results: The outcomes are positive, and precise results show it is possible to reliably automate sample preparation operations with full automatization of GC-MS analysis.

Introduction

Sample derivatization is often used in GC and GC/MS analyses to enhance the volatility of certain compounds. Strong intermolecular attractions between polar groups such as NH, SH or OH, groups can create hydrogen bonds that lead to molecular attraction and reduce volatility. Replacing hydrogen in these groups by alkylation, acylation, or silylation significantly increases volatility, especially in compounds with multiple polar groups. Typically, the replacement of active hydrogens with trimethylsilyl groups leads to volatile derivatives that can readily undergo GC analysis.

A typical silylation reaction requires the addition of a reagent such as MSTFA, TMCS, or BSTFA in presence of a base in an anhydrous and heated environment and may last several minutes. The length of the reaction, and the fact that often these additions of liquid reagents must be carried out manually are downsides of the process and negatively affect overall laboratory throughput.

The introduction of a system capable of handling much of the sample preparation process automatically can increase laboratory throughput and free the operator from the most tedious, time-consuming and error-prone steps.

The Thermo Scientific™ TriPlus™ RSH autosampler is an example of such a system.

The TriPlus RSH autosampler is a versatile automatic sampling platform that is also capable of advanced sample preparation cycles, enabling excellent precision and reproducibility and ensuring unattended and reliable operations.

FIGURE 1. TriPlus RSH autosampler, Thermo Scientific™ TRACE™ 1310 GC and TSQ™ 800 mass spectrometer

Preparation Cycles

The TriPlus RSH has the capability to perform sample preparation by using prep cycles. Prep cycles are pre-compiled, software-driven sets of operations for the TriPlus RSH autosampler that allow users to go beyond standard injection functions. Using prep cycles, the instrument can perform sample preparation procedures, such as calibration curve building, dilutions or derivatizations in an unattended, reliable and fully automated fashion.

The TriPlus RSH autosampler can automate these cycles with the innovative Automatic Tool Changing Station (ATC), which can hold multiple syringes and automatically change them, as an example for switching from liquid to headspace to SPME analyses in the same sequence or simply to handle different liquid volumes with the utmost precision (See Figure 2.)

FIGURE 2. TriPlus RSH ATC station.

The derivatization prep cycle can handle the entire process unattendedly and reliably. It offers the possibility of adding derivatizating agents and internal standards, vortexing and incubating samples into an oven or into cooled trays, according to the protocol followed, for a user-selectable time. At the end of a derivatization batch, samples can be automatically injected by the same sampler into a GC or a GC/MS.

The derivatization prep cycle is able to handle liquid volumes for samples, standards, and reagents between 1 mL and 10 mL , choosing the proper syringes accordingly, and can work with vial volumes from 2–100 mL, offering the possibility to scale down the volumes used, thus saving money and reducing the chemical waste generated.

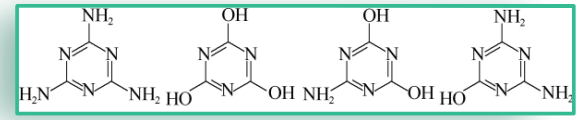
Automation of Sample Derivatization Procedures in Food Safety Studies

The cycle has been used to perform the derivatization step and the injections for the analysis of melamine, cyanuric acid, ammeline, and ammelide by GC-MS in milk products. The sample preparation procedure is carried out manually, in parallel, to validate the results obtained.

Melamine is an industrial chemical, commonly used in the manufacture of plastics and colorants; cyanuric acid, ammeline, and ammelide are the metabolites found after ingestion of melamine (Figure 3).

With GC-MS/MS, it is possible to determine the content of the four compounds with excellent sensitivity and specificity. The extracted samples must be derivatizated before the analysis.

FIGURE 3. Structure of melamine, cyanuric acid, ammelide, and ammeline.



Methods

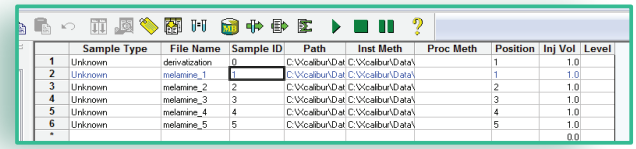
The sample preparation and analysis has been performed with the TriPlus RSH autosampler coupled with a TRACE 1310 Gas Chromatograph and a TSQ 8000 triple quadrupole mass spectrometer. The data were collected and processed with Thermo Scientific™ Xcalibur data system.

Sample Preparation

Common retail milk products were analyzed in parallel with standard solutions of the four analytes, plus the internal standard, prepared at various concentrations. Sample derivatization has been executed manually and *via* the TriPlus RSH autosampler to compare the results obtained. The sample extraction protocol used follows the US FDA methods for the screening of melamine, ammelide, ammeline, and cyanuric acid (version 2.1) [1]

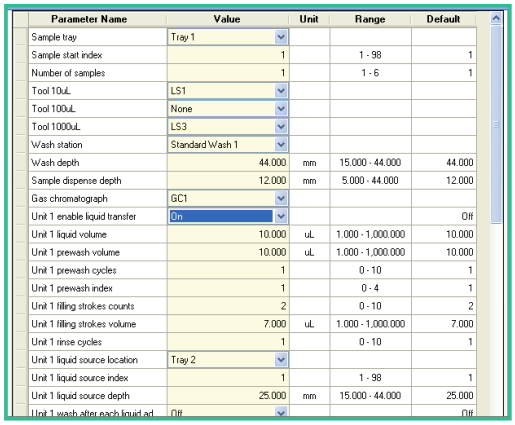
The TriPlus RSH autosampler is used to add 300 µL of a solution of acetonitrile and pyridine (1:1) to the sample extract and then the vial is vortexed for 30 seconds. Next, 200 µL BSTFA with 1 % TMS is added to the vial and is vortexed for 1 min and then incubated at 70 °C for 45 min. The previous operation, like the subsequent GC/MS injection, is performed in an unattended fashion by the autosampler. The user must have the requested hardware configuration and syringes, load the samples and reagents onto the tray, and load the prep cycle into a CDS sequence and run it for the number of samples to be analyzed. The entire sample preparation procedure uses the first line of the sample sequence; after that, the usual injection sequence occurs.

FIGURE 4. Xcalibur CDS samples calibration sequence.



	Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol	Level
1	Unknown	derivatization	0	C:\Xcalibur\Dat	C:\Xcalibur\Data\		1	1.0	
2	Unknown	melamine_1	1	C:\Xcalibur\Dat	C:\Xcalibur\Data\		1	1.0	
3	Unknown	melamine_2	2	C:\Xcalibur\Dat	C:\Xcalibur\Data\		2	1.0	
4	Unknown	melamine_3	3	C:\Xcalibur\Dat	C:\Xcalibur\Data\		3	1.0	
5	Unknown	melamine_4	4	C:\Xcalibur\Dat	C:\Xcalibur\Data\		4	1.0	
6	Unknown	melamine_5	5	C:\Xcalibur\Dat	C:\Xcalibur\Data\		5	1.0	
*								0.0	

FIGURE 5. Automated Derivatization Prep cycle user interface.



Parameter Name	Value	Unit	Range	Default
Sample tray	Tray 1			
Sample start index	1		1 - 98	1
Number of samples	1		1 - 6	1
Tool 10uL	LS1			
Tool 100uL	None			
Tool 1000uL	LS3			
Wash station	Standard Wash 1			
Wash depth	44.000	mm	15.000 - 44.000	44.000
Sample dispense depth	12.000	mm	5.000 - 44.000	12.000
Gas chromatograph	GC1			
Unit 1 enable liquid transfer	On			Off
Unit 1 liquid volume	10.000	uL	1.000 - 1,000.000	10.000
Unit 1 prewash volume	10.000	uL	1.000 - 1,000.000	10.000
Unit 1 prewash cycles	1		0 - 10	1
Unit 1 prewash index	1		0 - 4	1
Unit 1 filling strokes counts	2		0 - 10	2
Unit 1 filling strokes volume	7.000	uL	1.000 - 1,000.000	7.000
Unit 1 rinse cycles	1		0 - 10	1
Unit 1 liquid source location	Tray 2			
Unit 1 liquid source index	1		1 - 98	1
Unit 1 liquid source depth	25.000	mm	15.000 - 44.000	25.000
Unit 1 wash after each liquid ad	Off			Off

Gas Chromatography

- Oven: starting temperature 75 °C for 1 minute, raise to 300 at 15 °C/min, hold for 10 minutes
- Injector: 250 °C, carrier gas Helium 1 mL/min, splitless mode for 120 seconds

Column: Thermo Scientific™ TRACE TR5-MS 30 m x 0,25 cm x 0,25 µm

Mass Spectrometry

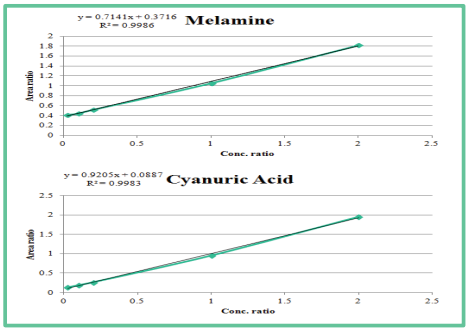
The TSQ 8000 mass spectrometer was operated in SRM mode with retention times, collision energies, and precursor ions optimized for each analyte. The temperature of transfer line and ion source were, respectively 250 °C and 270 °C.

Results

None of the analyzed commercially available milk samples were positive for the presence of melamine or the other target compounds.

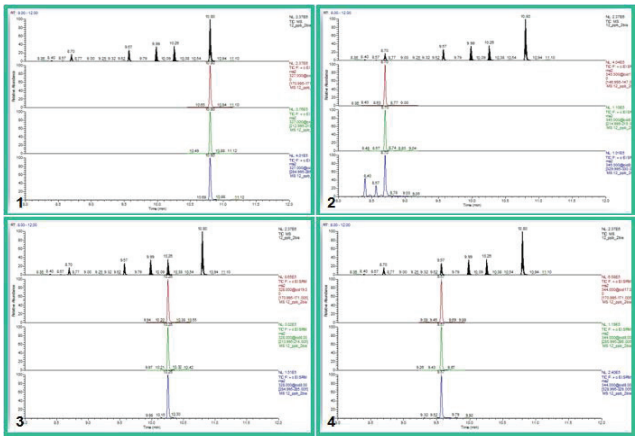
A milk sample was spiked with the target analytes at a concentration of 12 ppb each and tested via GC-MS/MS. Standard solutions of the four analytes were analyzed at concentrations of 2.5, 5, 10, 50, and 100 ppb each, and showed excellent results in terms of linearity.

FIGURE 6. Linearities for melamine and cyanuric acid shown as examples.



The results for the milk samples, the standard spiked samples, and the standard solutions obtained with the derivatization procedures done manually were in line with those obtained using the TriPlus RSH autosampler prep cycle.

FIGURE 7. SRM results for the compounds screened. 1.) Melamine, 2 .) Cyanuric Acid, 3.) Ammeline, 4.) Ammelide.



Conclusion

The TriPlus RSH autosampler is a versatile automatic sampling system that is also capable of advanced sample preparation cycles, enabling excellent precision and reproducibility and ensuring unattended and reliable operations.

The powerful platform comprised of the TriPlus RSH autosampler, a TRACE 1300 Series GC, and TSQ 8000 MS, is a sensitive and reliable analytical system capable of derivatizing and analyzing a high number of samples unattendedly, improving laboratory throughput with excellent analytical performances.

An example has been shown of the automated derivatization procedure for the determination of contaminants like melamine and related by-products in milk, which provided outstanding linearity and recoveries.

Similar automated cycles could be used to accelerate the derivatization procedures for biological and food samples prior to GC and GC/MS analysis, reducing operator time and the chance for errors, minimizing carryover, and reducing the amounts of chemicals used and waste generated, thus decreasing the cost of chemical waste handling.

References

1. FDA GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide and Cyanuric Acid.

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