New Software Tool to Easily and Quickly Generate Automated Sample Preparation Workflow

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ABSTRACT

Purpose: this work illustrates the benefits of automated sample preparation for GC/GC-MS and introduces a new software tool for an easier and more versatile control of the Thermo Scientific™ TriPlus™ RSH™ Autosampler.

Methods: modern instrumentation allows to fully automate basic operations involved in sample preparation procedures, such as sample dilution, standard addition, heating, mixing. Examples of fully automated sample derivatization workflows are presented, covering different application fields as food analysis and metabolomics.

Results: the versatility of a new intuitive and easy to use software tool enhances the automation capabilities of the robotic autosampler to perform sample preparation procedures in a fully unattended way with significant gains in time and reliability of the operations.

INTRODUCTION

When gas chromatography (GC) or gas chromatography mass spectrometry (GC-MS) analysis is performed, several procedures are required in the laboratory to prepare the sample prior to the injection, with the purpose of making it amenable to GC analysis, or to increase sensitivity through analytes enrichment, or simply to prepare standard solutions for instrument calibration.
These procedures normally involves multiple steps such as extraction, clean up, dilution, standard addition, derivatization, mixing and heating.

Sample preparation still represents the bottleneck for many analytical workflows: a large percentage of the time spent in the laboratories is dedicated to sample preparation, especially when achieved manually. Moreover, about 30 percent of the possible sources of error is due to sample processing (sample loss, contamination, modification etc...) with important consequences on the analytical data accuracy and precision.

For these reasons, automating sample preparation is a real step forward as it significantly reduces the time required to complete the analytical method and increase the consistency of the results, providing higher sample throughput, unattended routine operations, better data quality and reduced cost per sample, thus increasing the overall laboratory productivity.

SAMPLE PREP AUTOMATION

TriPlus RSH autosampler and PrepCycles

The TriPlus RSH autosampler offers advanced capabilities in sample handling, beyond automated sample injections: liquid addition, mixing, heating, cooling are just some of the operations available. Several tools are available to reliably automate the most common operations, achieve the highest level of sample handling flexibility and satisfy the most stringent productivity requirements.

The Automatic Tool Change (ATC) is fundamental as it can hold multiple syringes and automatically change them, for example to handle different liquid volumes during specific operations such as standard addition or dilution or to minimize possible cross contamination.

Basic and advanced sample preparation procedures can be achieved either batching samples before the GC analysis or sequentially, preparing and injecting sample after sample, optimizing the overall cycle time through overlapping capabilities.

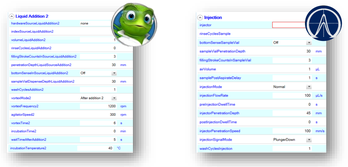
The TriPlus RSH sample handling operations are completely controlled and managed by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) and Thermo Scientific™ TraceFinder™ software. A few ready-to-use pre-compiled set of basic operations, known as TriPlus RSH PrepCycles, are currently available to cover the most common sample handling activities as a default. Additional dedicated Prep Cycles can be developed on-demand by the factory to satisfy specific requirements.

- **Internal Standard Addition:** automatic addition of a settable amount of ISTD to the sample before the injection
- **Standard Addition:** add increasing amounts of standard to any sample. Calibrating by standard additions is commonly used in headspace and SPME analyses.

- **Batch or Sequential Derivatization:** derivatization steps for repeated batches of samples or sequentially for immediate injection to prevent sample degradation.
- **Sequential Dilution:** automatic dilution of a stock solution
- **Ambient Temperature Headspace:** perform static headspace injection skipping the incubation step, for temperature sensitive samples
- **Ambient Temperature Solid Phase Microextraction (SPME):** perform SPME injection skipping the incubation step, for temperature sensitive samples

Ready-to-use PreCycles are imported and integrated in TraceFinder and Chromeleon CDSs: parameters are listed and assigned to specific workflow steps for an easy and intuitive setting (Figure 1)

Figure 1. TriPlus RSH PrepCycle parameters setting

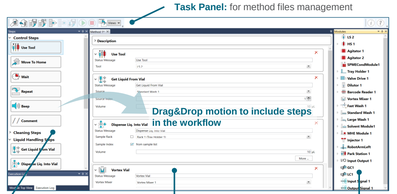


Sampling Workflow Editor software tool

The possibility to customize the sequence of operations on a specific workflow or quickly test different sample preparation conditions is a desirable feature for many laboratories, especially when dedicated to method development .

The Sampling Workflow Editor software tool adds this flexibility providing a simple, user friendly and intuitive way to program a sample preparation workflow. All the configured tools are automatically recognized by simply connecting the Sampling Workflow Editor software to the TriPlus RSH autosampler. Then, the sequence of steps required to compose the desired workflow is programmed by drag-and-drop motion.
The user-friendly interface also gives access to all the commands from one single screen, making them readily available (Figure 2).

Figure 2. TriPlus RSH Sampling Workflow Editor software interface



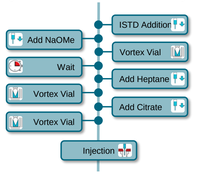
SAMPLE PREPARATION WORKFLOWS

Derivatization is one of the most common process required in GC laboratories to make samples that have poor volatility or poor thermal stability suitable to the GC analysis. The derivatization workflows for three typical applications were programmed with the Sampling Workflow Editor software tool: the Fatty Acids Methyl Esters (FAMEs) profiling, the analysis of Melamine in diary products, and the volatile metabolites profiling. A description of the preparation steps and results obtained are described. The TriPlus RSH equipped with the necessary tools was used with a Thermo Scientific™ TSQ™ 8000 GC-MS/MS for the analysis of melamine in diary product and with a Thermo Scientific™ ISQ™ 7000 GC-MS/FID for FAMEs and metabolites profiling.

Automated Sample Prep for FAMEs Analysis

Fatty acid profiling is a key analytical determination for the food industry, impacting several segments such as quality control, nutritional labeling, food design as well as vegetable oils blending and counterfeit assessment. The method applied enables the determination of total fat content, quantitative analysis of saturated and unsaturated cis- and trans-fatty acids in different matrices, such as vegetable oils, butter and cheese.
For the transesterification of fatty acid esters Na-methoxide was used as reactant as it transesterifies triglycerides in a very short time (90 sec) at ambient temperature, providing a fast, efficient and very robust method for fat analysis in food samples.

Figure 2: Derivatization workflow for fatty acids profiling



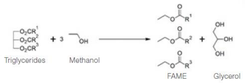


Figure 3 shows a good chromatographic separation achieved for all FAMEs obtained by the sample preparation procedure described. The fatty acids composition detected for different vegetable oils is shown in Table 1.
The TriPlus RSH autosampler was equipped a Vortex mixer for promoting the reaction and Large Solvent and washing stations to accomodate reagents and solvents in addition to standard vial trays. Two syringes, one 100 uL for the reagents and one 10 uL dedicated to the injection, were used to manage different volumes and avoid any cross-contamination. The Automatic Tool Change enabled the unattended change of the syringes according to the workflow.

Figure 3: Typical result of butter FAMEs. Complete GC separation within 11 minutes

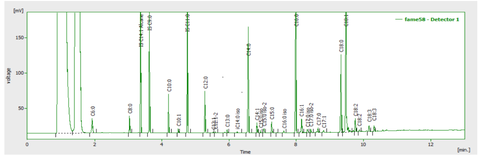


Table 1: Fatty acids % composition of different vegetable oils.

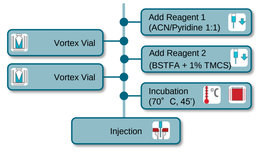
Coconut Oil	%	Peanut Oil	%	Safflower Oil	%	Olive Oil	%	Sunflower Oil	%
C8:0	7.5	C16:0	8.9	C16:0	6	C16:0	12.3	C16:0	4.7
C10:0	5.8	C18:0	3.2	C16:1	0.1	C16:1	0.7	C16:1	0.1
C12:0	45.8	C18:1	68.8	C18:0	2.5	C17:0	0.1	C18:0	1.9
C14:0	18.5	C18:2	16.3	C18:1	17.1	C17:1	0.2	C18:1	13.3
C16:0	9.3	C18:3	0.1	C18:2	73.2	C18:0	2.4	C18:2	57.1
C18:0	2.9	C20:0	1.3	C18:3	0.3	C18:1	74.5	C18:3	0.2
C18:1	8.2	C20:1	1.4	C20:0	0.4	C18:2	8.2	C20:0	0.3
C18:2	21			C20:1	0.2	C18:3	0.8	C20:1	0.2
						C20:0	0.5		
						C20:1	0.4		

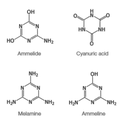
This example shows the capabilities of the TriPlus RSH autosampler to fully automate the sample preparation and analysis for FAMEs profiling. The proposed protocol allows a quick and reliable process increasing the system productivity, limiting solvents and reagents consumption compared to manual processes and preventing humans' exposure to hazardous chemicals.

Melamine and its derivatives in diary products by GCMS according to U.S. FDA protocol

Melamine and Cyanuric acid are inexpensive, nitrogen rich compounds which can be used as adulterants in food and/or feed to increase their market value by increasing their protein content. Milk and dairy products are particularly prone to such adulteration, this way impacting also the processed food containing dairy-based ingredients. Cyanuric acid, ammeline, and ammelide are the by-products which are screened together with melamine in dairy products.
Following the sample extraction, the TriPlus RSH autosampler can automate the derivatization procedure, with subsequent injection into the GC-MS/MS system.

Figure 4: Derivatization workflow for derivatization of milk extract, melamine and by-product



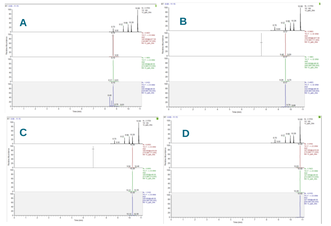


A milk sample was extracted according to the US FDA method. The TriPlus RSH autosampler was then used to add 300 µL of a solution of acetonitrile and pyridine (1:1) to the sample extract and then the vial is vortexed for 30 seconds. Next, 200 µL BSTFA with 1 % TMS were added to the vial which was vortexed for 1 min and then incubated at 70 ° C for 45 min.
The samples were run onto a Thermo Scientific™ TRACE™ 1300 series GC coupled with a TSQ 8000 triple quadrupole MS. The column used is a TR5-MS 30m 0.25mm 0.25 µm. The Mass Spectrometer was operated in SRM with retention times, collision energies, and precursor ions optimized for each compound (Table 2).

Table 2. SRM methods retention times, collision energy and precursor ions for each compound

Compound Name	RT	Window (min)	Mass	Product Mass	Collision Energy (Ev)
Cyanuric acid	8,70	0,7	345	147	17
Cyanuric acid	8,70	0,7	345	215	8
Cyanuric acid	8,70	0,7	345	330	8
Ammelide	9,58	0,7	344	171	17
Ammelide	9,58	0,7	344	286	8
Ammelide	9,58	0,7	344	329	8
DACP	9,99	0,7	273	171	17
DACP	9,99	0,7	273	237	8
Ammeline	10,26	0,7	328	171	19
Ammeline	10,26	0,7	328	214	8
Ammeline	10,26	0,7	328	285	8
Melamine	10,80	0,7	327	171	18
Melamine	10,80	0,7	327	213	8
Melamine	10,80	0,7	327	285	8

Figure 5. SRM results for the compounds screened. (A) Cyanuric Acid, (B) Ammelide, (C) Ammeline and (D) Melamine from a milk spiked with 12 ppb each.



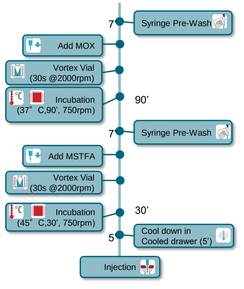
The system comprised of the TriPlus RSH autosampler equipped with incubation oven, Vortex and two different syringes with the ATC used to change them according to the workflow. Coupled to a TRACE 1300 Series GC with TripleQuad MS detector, the system provided a sensitive and reliable analytical platform capable of derivatizing and analyzing a high number of samples without any supervision, improving laboratory throughput with excellent analytical performances.

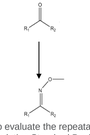
Two steps (Metoxymation/Silylation) online derivatization

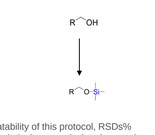
Gas chromatography–mass spectrometry (GC-MS) is an established analytical tool used successfully to determine the metabolic profile of biological sample in particular because it provides a wide metabolite coverage and access to comprehensive spectral libraries.
However, this advantage is only exploitable after a derivatization step to convert the polar/semipolar metabolites into volatile analogues, thus making them more amenable to GC separation.
Silylation is one of the most common derivatization procedures: it introduces a substituted silyl group (R3Si) to target compounds by replacing the active hydrogen to generate a less polar and more volatile derivative. Despite its diffusion, the relative instability of TMS derivatives is a source of significant variability throughout an analytical batch. The proposed procedure aims to automate the sample preparation through a two-step derivatization fully performed in automatic by the TriPlus RSH.

The TMS derivatization workflow is shown in Figure 6. All the required tools for online derivatization are mounted on the standard TriPlus RSH autosampler rail. The cooler drawer keeps all the samples at a constant 4 ° C environment with dried nitrogen gas continuously purged into it, which helps to prevent breakdown after derivatization. Two incubators can be used simultaneously on the standard TriPlus RSH rail for two-step derivatization at different temperatures. The automatic tool change station allows three syringes to be used in this preparation cycle. The Derivatized samples were analyzed immediately after derivatization on the GC-MS to avoid sample deterioration and improve the repeatability.

Figure 6. Two-step trimethylsilyl derivatization workflow

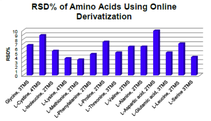


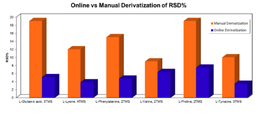




To evaluate the repeatability of this protocol, RSDs% (Relative Standard Deviation) were calculated on a mixture of 14 amino acids derivatized and analyzed on a Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS high resolution accurate mass (HRAM) mass spectrometer. The average RSD% was 5.85% which demonstrated a very good reproducibility considering that 10-15% is generally accepted as a good repeatability for GC-MS metabolomics. In contrast, manual derivatization performed by preparing 26 samples manually using the same standard with the same derivatizing reagents produced an average 14% RSD% (Figure 7).

Figure 7. SRM methods retention times, collision energy and precursor ions for each compound





To avoid false identification, erroneous quantitation, and interferences from matrix ions in the extracted ion chromatogram, acquiring reliable accurate mass measurements is essential for metabolomics studies using HRAM GC-MS. An outstanding mass accuracy (<1 ppm) was maintained across all quantitation ions during a 24-hour non-stop test for this automated online derivatization protocol.

CONCLUSIONS

The TriPlus RSH represent a valuable solution to automate the sampling preparation, providing high quality results and robust performance, reaching the ultimate 24/7 productivity and contributing to maximize the cost/sample ratio.
The TriPlus RSH Sampling Workflow Editor is a effective tool to program custom sample preparation workflows on the TriPlus RSH as demonstrated by the FAMEs, Melamine and the two-step TMS derivatization examples. Its extreme ease of use make it a real breakthrough in workflow compilation.
The robotic capabilities of the TriPlus RSH autosampler make it able to perform daily sample preparation workflow automatically and reliably, increasing repeatability from batch to batch, reducing manual and time-consuming lab work thus increasing the laboratory productivity.

REFERENCE

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