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Guide to automated sample preparation for GC and GC-MS

TriPlus RSH SMART Workflows

for Environmental, Food & Beverage, and Metabolomics samples

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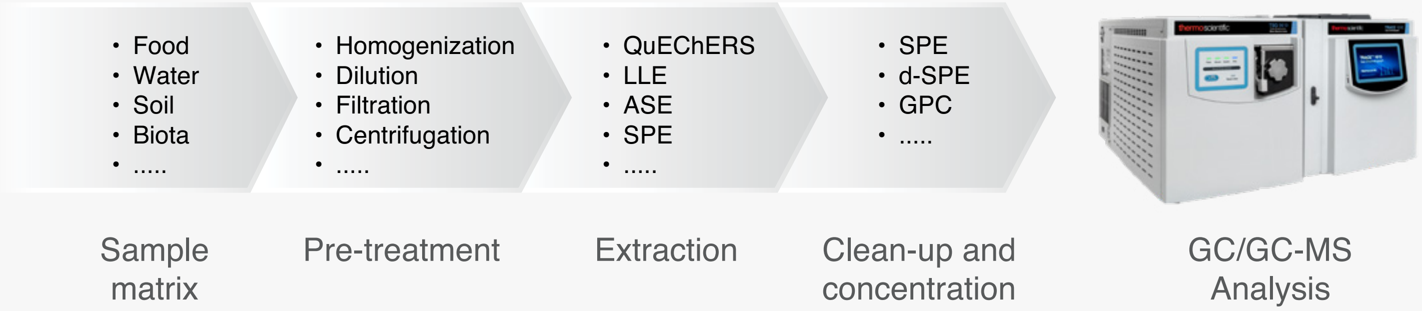
The role of sample preparation in gas chromatography

Gas chromatography is a separation technique widely used in many analytical testing and research laboratories to unravel samples composition or quantify impurities. It is a key analytical tool for many sample types like environmental matrices (water, soil, and air) for the detection of organic pollutants, drugs and pharmaceuticals for toxic impurities, biological samples for clinical studies, chemicals and petrochemicals, food samples for pesticides and other organic contaminants. However, most sample matrices are not suitable for direct injection into a GC system. Samples must be prepared to allow full vaporization before separation and detection at the required concentration limits.

Depending on the complexity of the sample matrix, the isolation of the volatile organic fractions may require extraction, cleanup, derivatization, and in many cases, a concentration step to reach the required sensitivity.

Most of the GC analysis time is taken by sample preparation, often involving several manual handling procedures, which are error-prone and a major source of analytical data variability.

This is why sample preparation represents the most important step prior to GC analysis.



Sample matrix
- Food
- Water
- Soil
- Biota
- .....

Pre-treatment
- Homogenization
- Dilution
- Filtration
- Centrifugation
- .....

Extraction
- QuEChERS
- LLE
- ASE
- SPE
- .....

Clean-up and concentration
- SPE
- d-SPE
- GPC
- .....

GC/GC-MS Analysis

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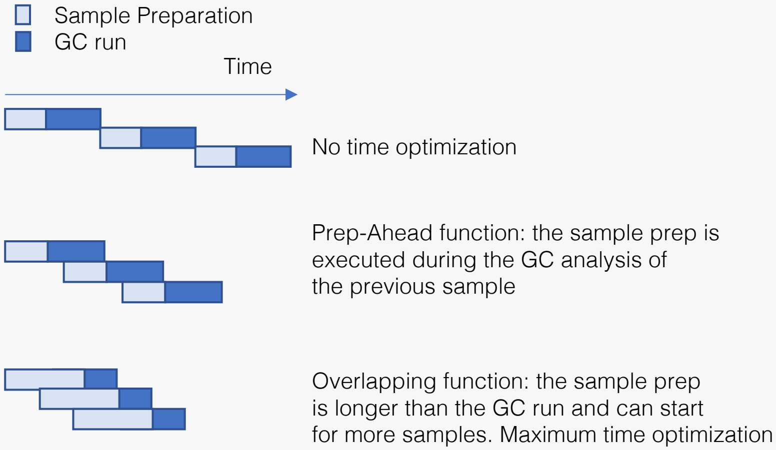
How automation helps modern laboratories

Sample preparation procedures often represent a tedious and time-consuming part of the analytical workflow for modern laboratories. Also, such procedures often require large quantities of expensive reagents and hazardous solvents that must be disposed.



No time optimization

Prep-Ahead function: the sample prep is executed during the GC analysis of the previous sample

Overlapping function: the sample prep is longer than the GC run and can start for more samples. Maximum time optimization

Increasing the level of automation for sample preparation is the way to relieve laboratories' workload while ensuring, through robotic operation, error-free and highly precise results.

Implementing automated sample preparation workflows for GC involves:

1. Saving labor time through unattended 24/7 operation
2. Saving money through scaling down sample and reagents volumes
3. Increasing confidence in analytical results
4. Reducing human exposure to hazardous chemicals
5. Approaching green chemistry techniques with less waste for more friendly environment procedures
6. Increasing sample throughput, thanks to the capability of overlapping operations while optimizing the overall cycle time.

Thermo Scientific automated sampling solutions combined with advanced and highly sensitive GC and GC-MS instruments are key to supporting modern laboratories to work more efficiently and match their productivity goal.

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TriPlus RSH robotic sample handling

The Thermo Scientific™ TriPlus™ RSH and RSH SMART robotic autosamplers offer a wide range of sample handling tools and accessories, to automate most of the sample preparation procedures.

The Automatic Tool Change (ATC) is the key to automatically swapping between up to six different syringe tools and handling a wide range of volumes and different reagents.

The TriPlus RSH SMART implements technological innovations that further expand automation, performance, and versatility for GC and GC/MS applications.

The SMART technology uses consumables equipped with a unique SMART chip for automatic ID, storage of operational parameters, operational ranges and usage history, enabling full traceability through Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) tools.

Heating

Vortexing

Dilution

Derivatization

Cooling

Centrifugation

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The tools you need to create a workflow

The TriPlus RSH and RSH SMART can be equipped with a wide choice of tools and accessories to automatically execute sample handling steps, required to build complex sample preparation workflows.

This eBook is a collection of PrepCycles, developed and validated to execute many sample handling and sample preparation workflows.

Vortex mixer for intensive sample mix

Agitator/incubator for HS, SPME or sample prep

Barcode reader for sample tracking

Controlled heating or cooling of samples

More sample trays for higher capacity

Dilutor with large reservoir

SPME/SPME Arrow fiber conditioning for SPME applications

Large solvent station

Large volume wash station

Standard wash station

Fast washing station

- Several tools are available to reliably automate common sample preparation procedures
- Ready-to-use pre-compiled set of operations (PrepCycles) are available as a default or on request
- Customized sequence of operations can be easily programmed by the user with the Sampling Workflow Editor
- Complex PrepCycles can be developed on-demand by the factory to satisfy specific requirements

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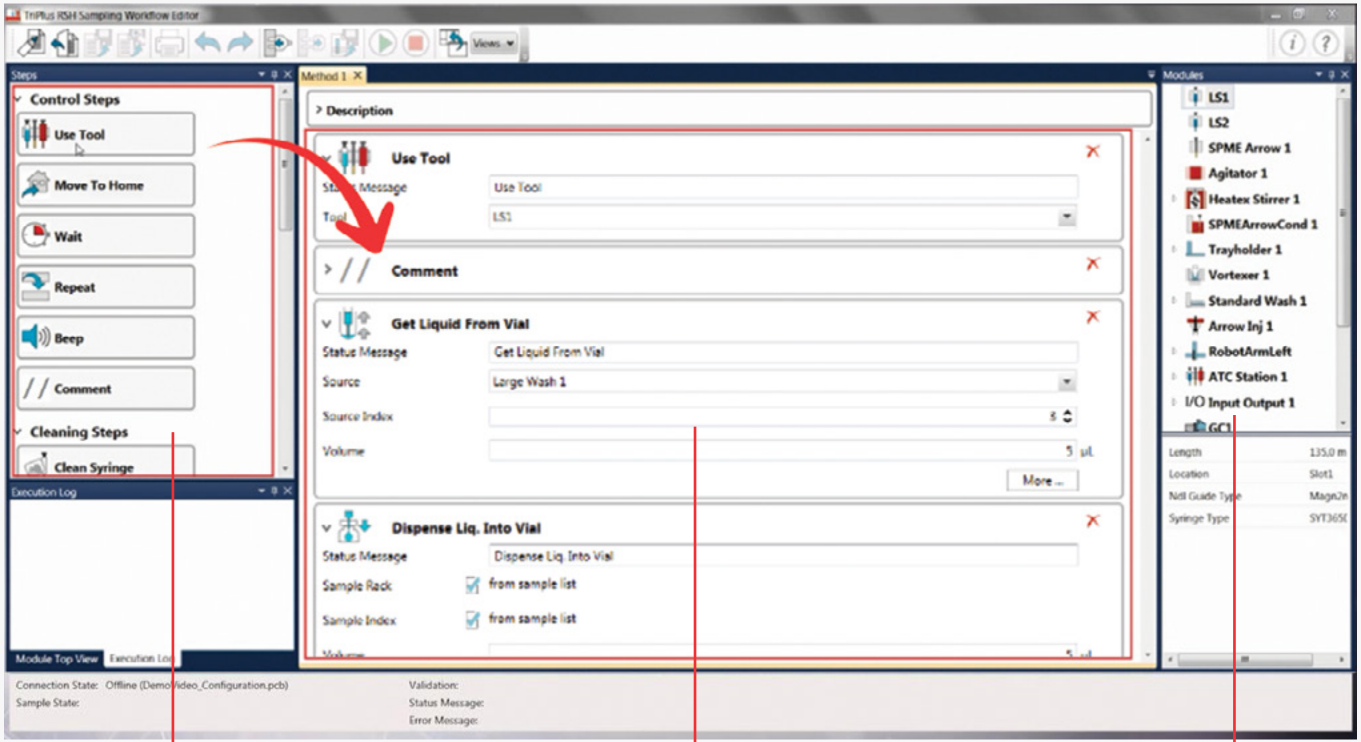
Sampling Workflow Editor software

The TriPlus RSH and RSH SMART can be easily programmed by the user to automatically perform the sequence of steps required to produce a customized sample preparation procedure.

The Thermo Scientific™ Sampling Workflow Editor is a very easy and intuitive software for programming your robotic autosampler and create a sequence of sample handling operations for the desired workflow.

This software gives higher flexibility to the operator who can create its own PrepCycle very easily, by listing every single step with a simple drag and drop approach.

To know more about how this software works, look at the Video Tutorial at www.thermofisher.com/Samplingworkfloweditor



Use Tool
Status Message: Use Tool
Tool: LS1

Comment

Get Liquid From Vial
Status Message: Get Liquid From Vial
Source: Large Wash 1
Source Index: 5
Volume: 5 µL

Dispense Liq. Into Vial
Status Message: Dispense Liq. Into Vial
Sample Rack: ☑ from sample list
Sample Index: ☑ from sample list
Volume: 5 µL

Length: 135.0 m
Location: Slot1
Ndl Guide Type: Magn2n
Syringe Type: SYT365(

Connection State: Offline (Demo ideo_Configuration.pcb)
Sample State:
Validation:
Status Message:
Error Message:

Steps Panel: lists the programmable actions according to the configuration

Method Panel: visualizes the steps of the programmed workflow

Modules Panel: displays modules and tools included in the configuration

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Calibration workflows

An accurate calibration of the instrument is essential in any chromatographic technique to perform quantitative analyses. A calibration curve is obtained by determining the correlation between the known concentration of target compounds and their analytical response (peak area or height).

This implies the preparation of standard solutions containing the analytes of interest at different concentration levels by diluting with solvent or with blank sample matrix, spanning the concentration range of interest.

Sometimes the procedures for setting up a calibration curve are more complex if additional sample preparation steps are included in the calibration.

The preparation of the calibration standards is a routine time-consuming operation, typically done manually, which however requires high accuracy and precision to avoid negative impact on quantitative results.

Automating the calibration method set up is simple and offers several advantages in terms of labor costs and data quality:

- Avoid tedious and time-consuming preparation steps
- Minimize the risk of errors due to incorrect measurement, mislabeling or cross-contamination, improving data confidence.
- Reduce the amounts of reagents, solvents and glassware

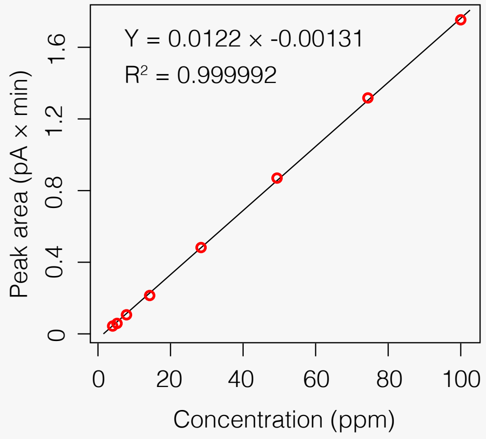
Most common calibration methods requiring sample handling

External calibration: standard solutions of target compounds at different concentration levels are prepared to create a calibration curve used to quantify those targets in an unknown sample.

Internal calibration: a fixed amount of an additional compound (i.e., Internal standard) is added to each sample. The concentration of targets is calculated based on the correlation between the peak area ratio and concentration ratio of the targets and the internal standard.

Standard addition: incremental amounts of standard at known concentration are added to each unkown sample. The difference of the detected peak areas between the unspiked and spiked unknown sample is used to quantify the target compounds.

The next sections illustrate the available PrepCycles to automate the preparation of calibration standards according to the most common calibration methods.



Example of an external calibration curve. Linear regression is typically used for most the detection systems, but quadratic, cubic or mixed models are also possible.

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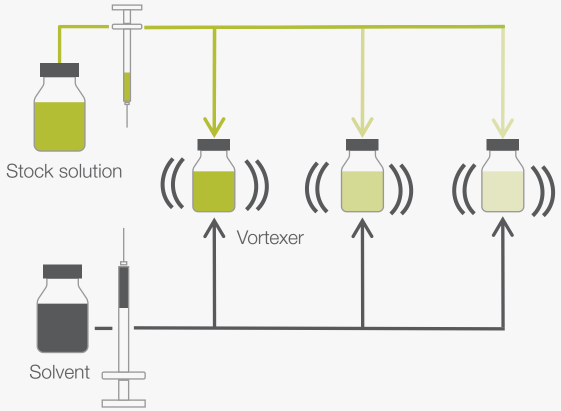
External standard calibration

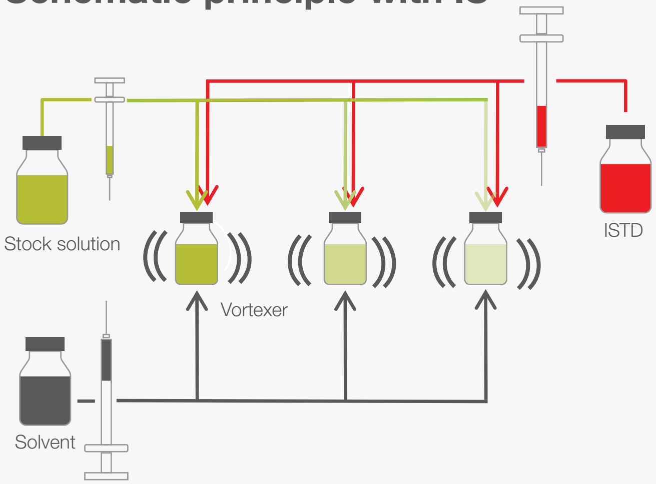
The Calibration Dilution Workflow PrepCycle is used to automate the off-line preparation of standard mixtures for external calibration by sequential dilution with a solvent, with or without Internal Standard (IS) addition.

Up to three series of nine vials each can be prepared, with dilution levels ranging from 1:2 to 1:1000.

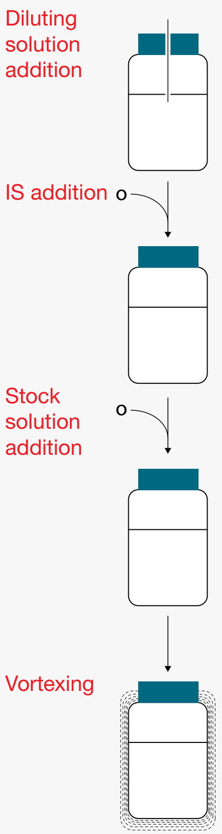
Schematic principle without IS



Schematic principle with IS



Workflow



Different volumes of the diluting solution are added to the vials, placed in fixed positions on the sample tray.

IS is added to all vials from a fixed position on the sample tray. This step is optional.

Different volumes of stock solution are added to the vials from a fixed position. Up to three series of nine vials each can be prepared, with range of dilution levels from 1:2 to 1:1000.

If a vortex mixer is available, a final vortexing step can be added (optional).

Highlights

- Available as default in Chromeleon and Thermo Scientific™ TraceFinder™ CDS
- This PrepCycle does not include on-line injection. In order to inject the calibration solutions, a separate sequence must be created
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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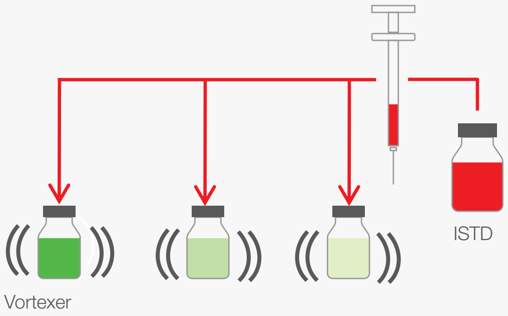
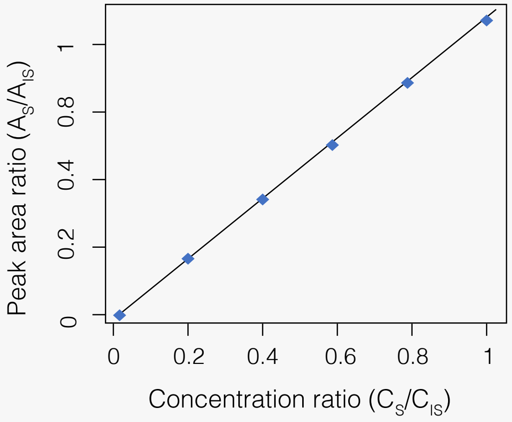
Internal standard calibration

The Internal Standard Addition PrepCycle is used to automate the Internal Standard (IS) addition in equal aliquot to existing calibration standard mixtures and samples.

Schematic principle

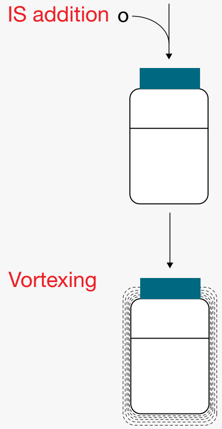


The IS is typically a compound which is not interfering with sample components in the chromatographic process. More than one IS can be used in case of a sample covering a wide range of volatility and polarity. The calibration plot for each target compound is built reporting the area counts ratio and the concentration ratio with the IS of reference. This is used to correct possible variation of the injected sample amount, since the ratio between the sample components and the IS is not affected.



Example of Internal Standard calibration with linear regression

Workflow



IS is added to all vials from a fixed position on the sample tray, solvent station or wash station

If a vortex mixer is available, a final vortexing step can be added (optional)

Highlights

- Available as default in Chromeleon and Trace Finder CDS
- Sampling Workflow Editor software allows to modify or create additional steps, for workflow customization
- This PrepCycle does not include on-line injection. In order to inject the samples, a separate sequence must be created

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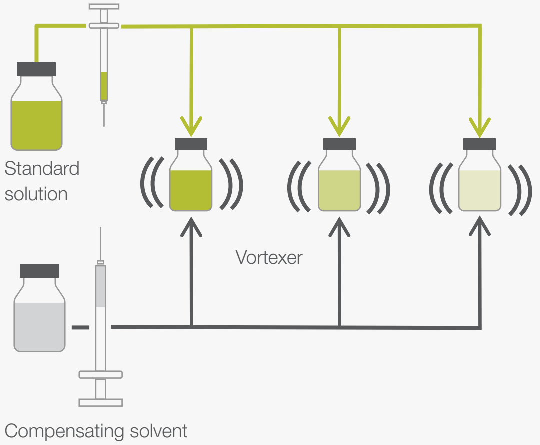
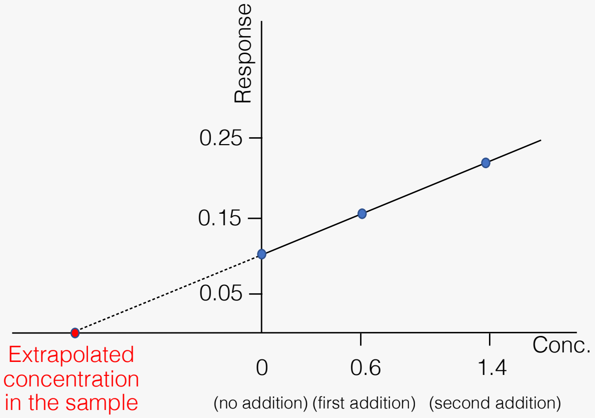
Standard addition calibration

The Standard Addition Calibration is used in case of sample matrices not easily reproducible for external calibration purposes. In this case, increasing aliquots of a standard mixture are added to the samples, and the increased response of the target compounds is plotted in the calibration curve.

Schematic principle

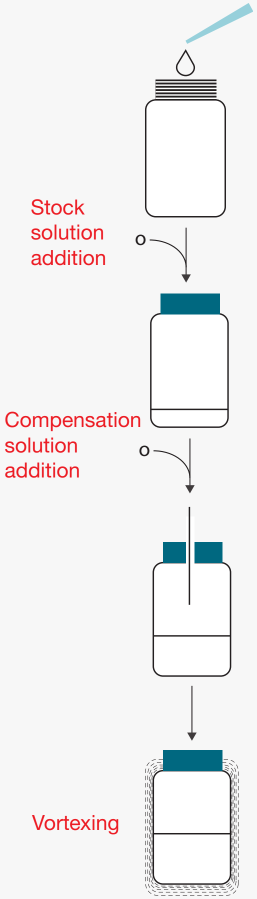


The increased standard aliquots added to the sample will sum up to the unknown amount in the sample, which can be then extrapolated.



Example of Standard Addition calibration with linear regression

Workflow



At the beginning of the procedure, the operator adds a sample aliquot to all vials dedicated to the calibration.

Increasing amounts of standard solution are added to the vials containing the sample. Up to six 1-100 uL amounts can be added from any position on a tray, wash station, or solvent station.

If required, different solvent amounts are added to compensate for volume differences in standard addition (optional).

If a vortex is included in the configuration, a final mixing step is possible.

Highlights

- This PrepCycle does not include online injection. In order to inject the samples, a separate sequence must be created
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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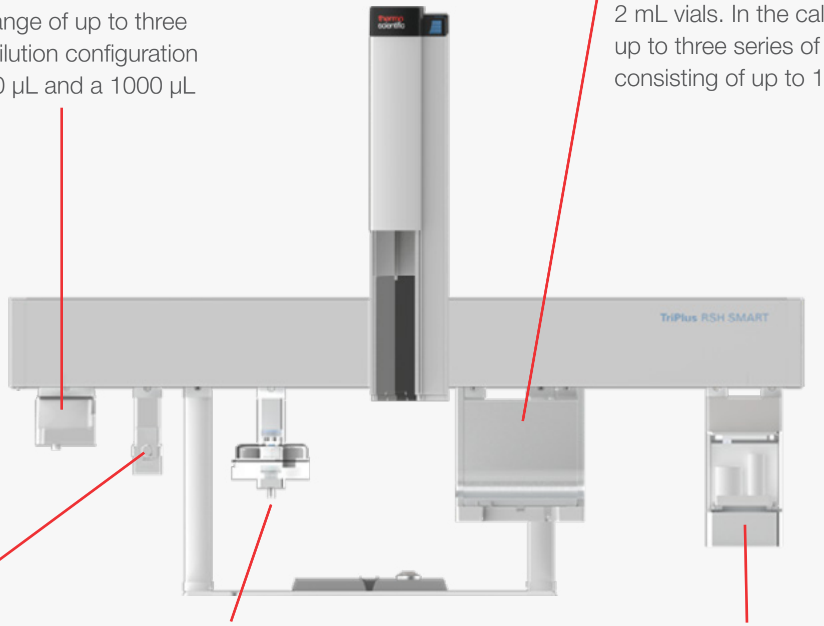
Automated calibration workflows

Minimum instrument set-up



Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools. The calibration dilution configuration requires liquid tools with a 10 µL and a 1000 µL syringe

Tray Holder

Holds up to 60 20 mL/10 mL vials or up to 162 2 mL vials. In the calibration dilution workflow, up to three series of vials can be prepared, each consisting of up to 10 concentrations

Standard Wash Station

Used for syringe washing or solvent and solution storage. In the calibration dilution configuration, this station holds the dilution solvent for 1–3 calibration solutions (positions 1–3) and the stock solution to be diluted (position 4)

Large Wash Station

Used for syringe washing or solvent and solution storage. In the calibration dilution configuration, this station is used exclusively for syringe washing. Positions 1 and 2 are used for washing after internal standard and stock solution addition, respectively

Vortex Mixer

Employed for sample mixing after reagent or standard addition

Resources

[Automated Workflows Brochure]

[TriPlus RSH SMART Web Page]

[Sampling Workflow Editor Tutorial]

Highlights

- Fits on a standard rail length
- Vortex Mixer optional but recommended
- Bench workstation compatible

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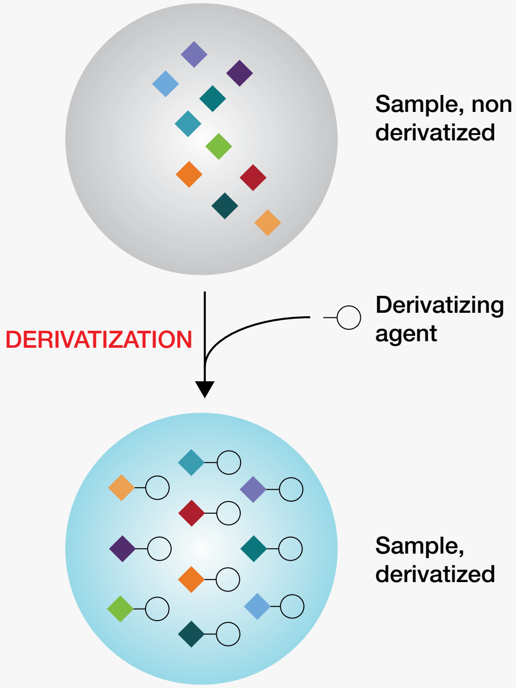
Derivatization workflows

Derivatization protocols are a common practice in GC and GCMS for changing the analytes' properties to improve their volatility, get a better separation and enhance the method sensitivity.

Derivatizing agents are reactive compounds capable of coupling to specific compound classes. Following derivatization, a stable new compound is generated bearing different physical-chemical properties.

Examples of common reactions are alkylation (e.g., methylation), formation of aryl derivatives, silylation (e.g., formation of trimethylsilyl derivatives), acylation (e.g., reactions with acyl chlorides or with chloroformates).



Why derivatize?

- To convert non-volatile/thermally unstable compounds into volatile/thermally stable adducts
- To introduce in the molecule a chemical group that significantly increases sensitivity with respect to a certain type of detector
- To increase analyte stabililty (even though, in some other instances derivatization results in products with limited stabillity over time)
- To obtain a similar response behavior from all analytes with respect to the detector (e.g., MS).

Automation greatly benefits derivatization workflows

Derivatization procedures are often complex and very time consuming and may involve the use of hazardous chemicals.

Automation provides several advantages:

- Significant labor time saving
- Increased sample throughput with optimized cycle time and 24/7 operations
- Enabled online GC injection after derivatization to prevent possible degradation of unstable adducts, or derivatization in batches for high productivity
- Minimized human exposure to hazardous chemicals
- Improved overall standardization and analytical performance repeatability, since every derivatization reaction may require specific conditions (e.g., agitation, temperature, pH).

Resources

[Guide to Derivatization for GC and GC-MS]

[Automated Workflow Solutions Web Page]

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Batch derivatization

The Batch Derivatization Workflow performs all the derivatization steps for a batch of up to six samples, with reagent addition, shaking, and incubation, allowing a wide range of customization options.

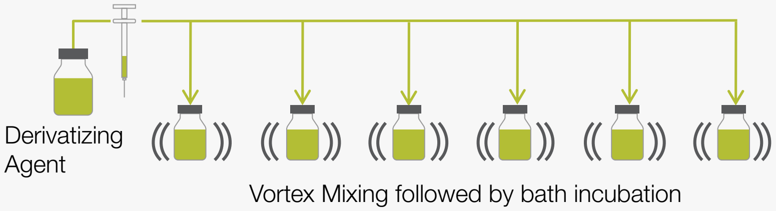
The sequence of operations can be performed twice for a two-step derivatization protocol. Alternatively, one of the two cycles can be used for internal standard addition.

Derivatization reactions can be achieved in the incubator at a controlled temperature or at ambient temperature.

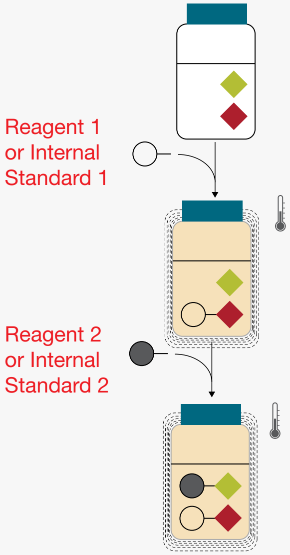
Samples can be stored in a temperature-controlled tray or drawer (optional).

Once derivatization is completed for the sample batch, a separate sequence must be created for injection into the GC system.

Schematic principle



Workflow



The steps "liquid addition/vortexing/ incubation" can be done once or repeated twice

In case of a two-step derivatization, two agitator/incubator modules can be installed to incubate the sample at different temperatures

Different solvents can be chosen for syringe wash before and after reagent addition reducing the risk of cross-contamination

Highlights

- Up to six samples prepared in batch before injection
- Optional second derivatization reagent or IS addition
- Optional second incubator for protocols including two different derivatization temperatures
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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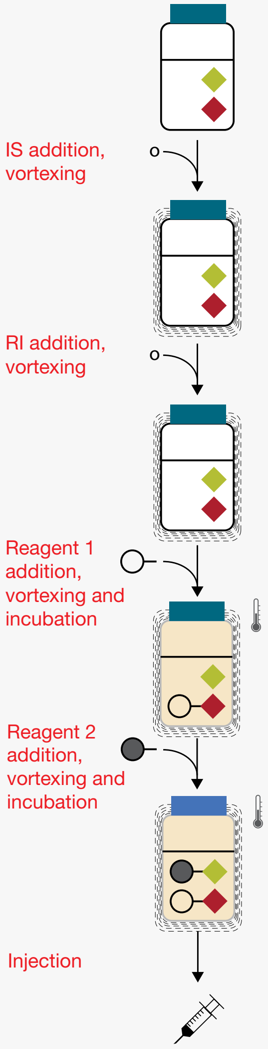
Sequential derivatization

Workflow



If required, the internal standard and the retention index standard can be added prior to derivatization

Up to three different syringes can be used to range the dispensing capability from 1 μL to 1000 μL

The sample can be vortexed applying different time and speed after each liquid addition

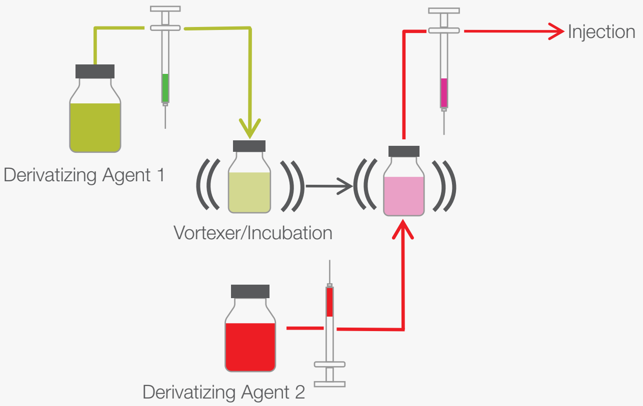
Two agitator/incubator modules can be installed to incubate the sample at different temperatures

Up to four different solvents can be chosen for syringe washing before and after reagent addition reducing the risk of cross-contamination

The Sequential Derivatization Workflow prepares each sample and injects it sequentially. The workflow includes two steps for the addition/vortexing of a standard mixture, typically an internal standard and a Retention Index standard, and up to two steps for possible addition of two different derivatization reagents.

This workflow permits the optimization of the overall cycle time thanks to overlapping with the GC run time. Minimizing the time between the derivatization process and the injection, it is preferred in the case of labile derivatives.

Schematic principle



Highlights

- Each sample is prepared and injected sequentially
- Steps overlapping for cycle time optimization
- Two different incubation temperatures can be handled
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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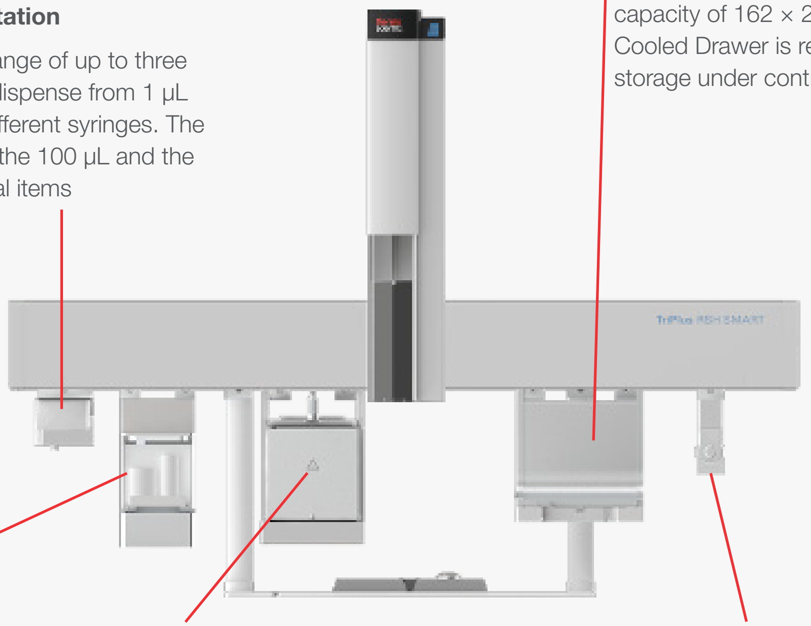
Derivatization workflow on a standard X-Rail

Minimum instrument set-up



Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools. It is possible to dispense from 1 μL up to 1000 μL using three different syringes. The 10 μL syringe is mandatory; the 100 μL and the 1000 μL syringes are optional items

Tray Holder

Used to store sample vials, it has a maximum capacity of 162 × 2mL vials. Alternatively, a Cooled Drawer is recommended for sample storage under controlled temperature (4–40 °C)

Vortex Mixer

Employed for sample mixing up to 2000 rpm after reagent or standard addition. Compatible with 0.5, 0.7, 2, 5, 10, or 20 mL vials

Agitator/Incubator

Used for sample incubation after derivatization reagent addition

Standard Wash Station

Used to wash the syringe before/after reagent addition and prior to injection with different solvents for one or more washing cycles. The Large Wash Station and Fast Washing Module are also compatible with this custom cycle

Resources

[Automated Workflows Brochure]

[TriPlus RSH SMART Web Page]

[Sampling Workflow Editor Tutorial]

Highlights

- Either batch or sequential workflow fits on a regular rail length
- Two incubators possible to handle different reaction temperatures
- Bench workstation compatible for off-line sample derivatization

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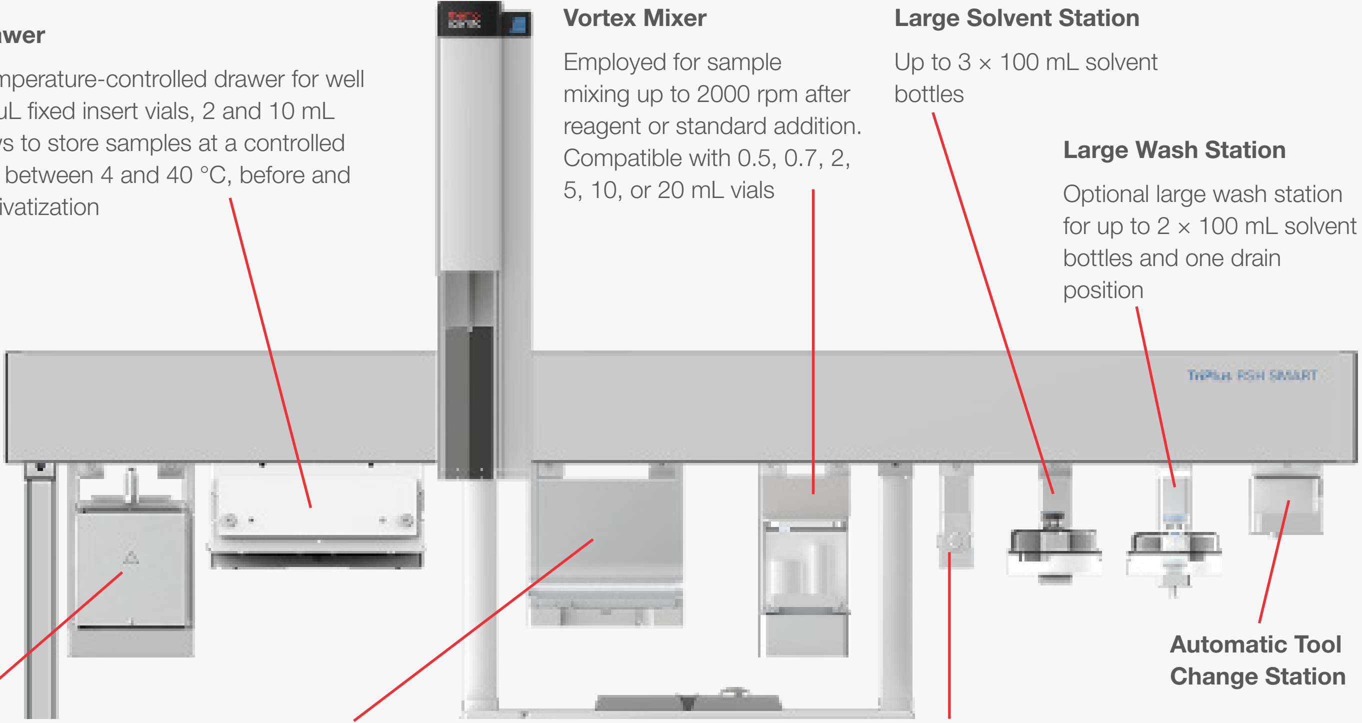
Derivatization workflow on the extended X-Rail

Extended instrument set-up



Cooled Drawer

Electrical temperature-controlled drawer for well plates, 300 µL fixed insert vials, 2 and 10 mL vials. It allows to store samples at a controlled temperature between 4 and 40 °C, before and after the derivatization

Vortex Mixer

Employed for sample mixing up to 2000 rpm after reagent or standard addition. Compatible with 0.5, 0.7, 2, 5, 10, or 20 mL vials

Large Solvent Station

Up to 3 × 100 mL solvent bottles

Large Wash Station

Optional large wash station for up to 2 × 100 mL solvent bottles and one drain position

Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools

Agitator/Incubator

Used for sample incubation after reagent addition. Two incubators can be configured to handle two different derivatization temperatures

Tray Holder

Used to store sample vials with a maximum capacity of 162 × 2 mL vials. Configured in addition to the Cooled Drawer to increase sample capacity

Standard Wash Station

Used for up to four different solvents for a total of 40 mL and 1 × 10 mL wast

Resources

Automated Workflows Brochure

TriPlus RSH SMART Web Page

Sampling Workflow Editor Tutorial

Highlights

- Flexible configuration with additional tools possible such as the Temperature Controlled Drawer
- Higher sample/solvent capacity for longer unattended sequences
- Bench workstation compatible for off-line sample derivatization

Dual-step sequential derivatization for metabolomics

The metabolome consists of low molecular weight (< 1500 Da) metabolites found in a biological system. Metabolomics aims to achieve a comprehensive characterization of the metabolome of cells, biofluids, tissues or organisms, known as metabolic profiling, useful for health assessment.

GC-MS is a powerful tool to characterize the metabolic profile and extensively used in metabolomic studies.

Discriminatory analysis (Volcano-plot)

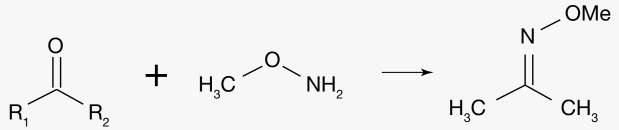
Metabolomics studies typically generate thousands of analytical features, collected over extended periods of time. Standardization is therefore one of the main requirements for the success of a metabolomics study as it improves confidence in data collected over time.

Statistical analysis is used to understand and interprete the data and reveal variables associated with different sample groups.

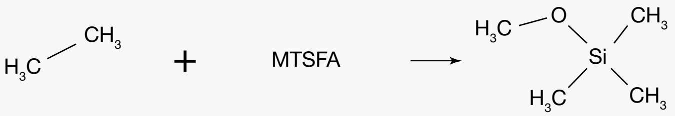
Low molecular weight compounds contained in biological samples are often non-volatile and unstable at the temperatures normally used in GC analysis.

Compound derivatization is necessary to increase the volatility of metabolites containing polar functional groups, such as carboxylic and amino groups.

A commonly used metabolomics workflow involves a dual-step derivatization consisting of methoximation, followed by silylation.



Methoximation



Silylation

This two-step derivation is preferable with a sequential workflow approach where single samples are prepared and injected sequentially for best integrity of labile TMS-metabolite.

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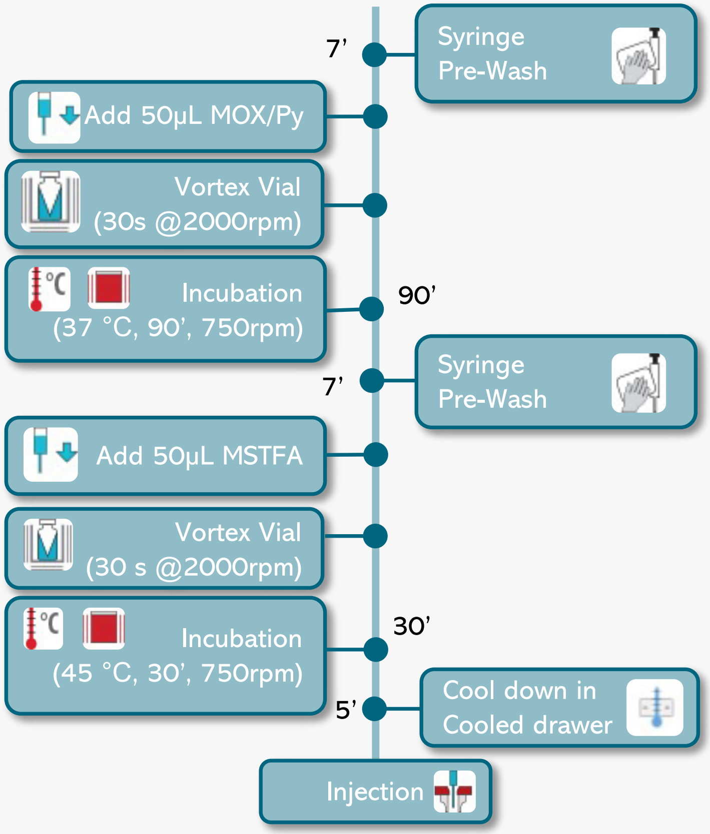
Sequential derivatization workflow in metabolomics

Dual-step automated workflow



7’

Syringe Pre-Wash

Add 50µL MOX/Py

Vortex Vial (30s @2000rpm)

Incubation (37 °C, 90’, 750rpm)

90’

7’

Syringe Pre-Wash

Add 50µL MSTFA

Vortex Vial (30 s @2000rpm)

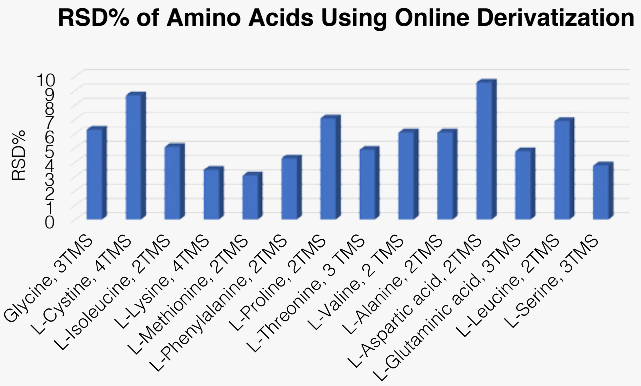
Incubation (45 °C, 30’, 750rpm)

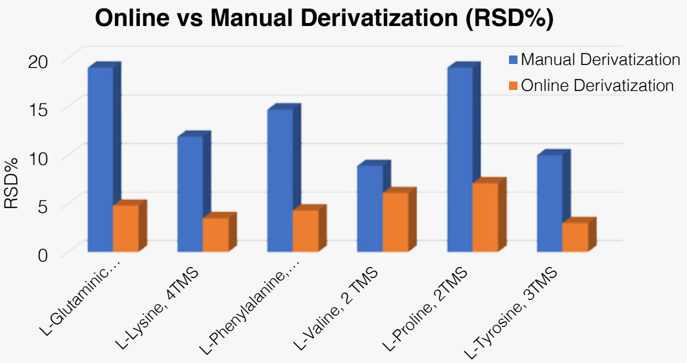
30’

5’

Cool down in Cooled drawer

Injection





Resources

Thermo Scientific Application Note AN10594

Presentation: Back To the Future

Sampling Workflow Editor Tutorial

Highlights

- The derivatized sample is immediately injected to prevent degradation
- Improved data precision
- 24 samples can be prepared and analyzed in 24 hours
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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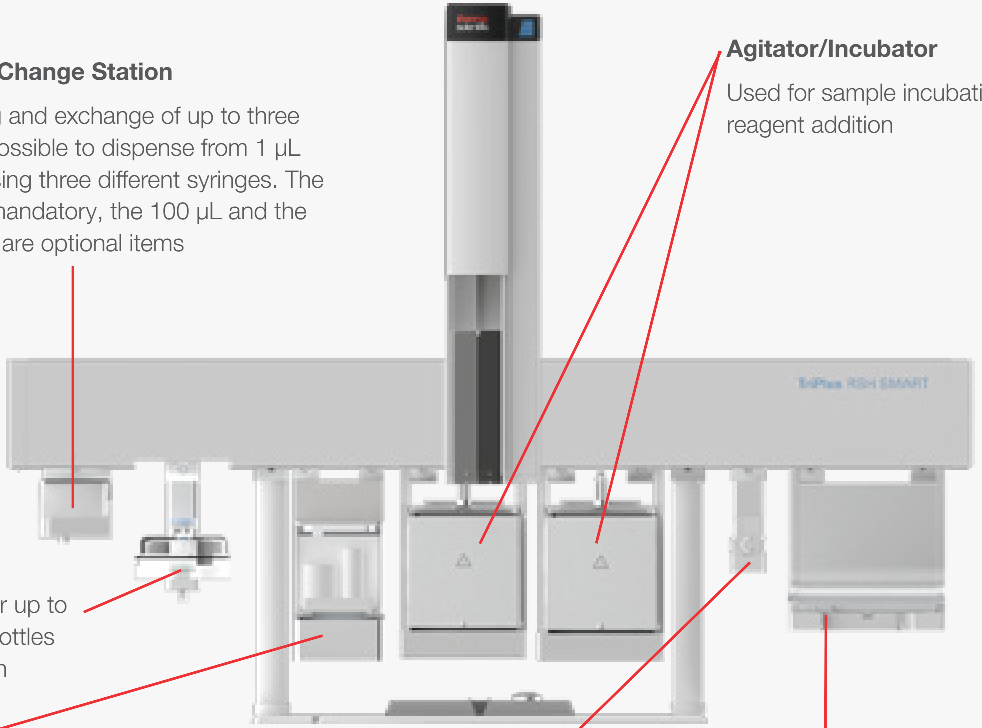
Sequential derivatization workflow for metabolomics

Instrument set-up



Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools. It is possible to dispense from 1 µL up to 1,000 µL using three different syringes. The 10 µL syringe is mandatory, the 100 µL and the 1000 µL syringes are optional items

Agitator/Incubator

Used for sample incubation after derivatization reagent addition

Large Wash Station

Large wash station for up to 2 × 100 mL solvent bottles and one drain position

Vortex Mixer

Employed for sample mixing up to 2000 rpm after reagent or standard addition. Compatible with 0.5, 0.7, 2, 5, 10, or 20 mL vials

Standard Wash Station

Used to wash the syringe before/after reagent addition and prior to injection with different solvents for one or more washing cycles. Large Wash Station and Fast Washing Module are also compatible with this custom cycle

Tray Holder

Used to store sample vials, it has a maximum capacity of 162 × 2 mL vials. Alternatively, a Cooled Drawer is recommended for sample storage under controlled temperature (4–40 °C)

Resources

Automated Workflows Brochure

TriPlus RSH SMART Web Page

Highlights

- Fits on standard rail length
- Two incubators are used simultaneously at different derivatization temperature
- Bench workstation compatible for off-line sample derivatization

Table of contents

Derivatization of melamine and its derivatives in dairy products

According to U.S. FDA protocol

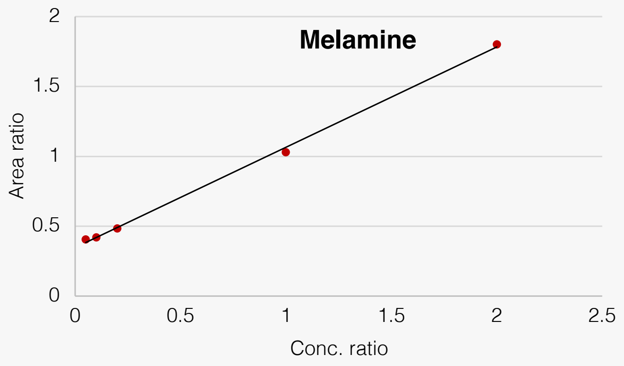
Melamine and its derivatives are inexpensive, nitrogen-rich compounds, which can be used as adulterants in food and/or animal feed to increase their market value by increasing their protein content.

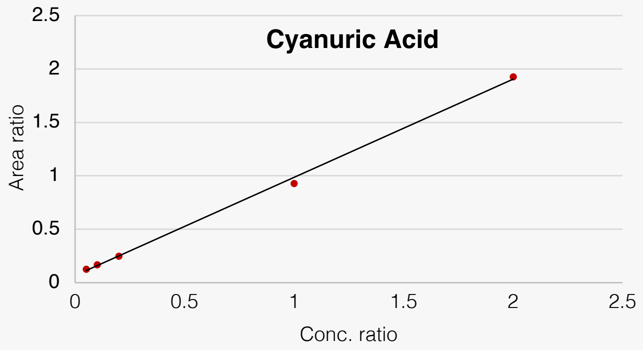
Milk and dairy products are particularly prone to such adulteration, which also impacts processed food containing dairy-based ingredients.

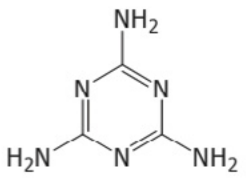
Cyanuric acid, ammeline, and ammelide are the by-products which are screened together with melamine in dairy products.

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

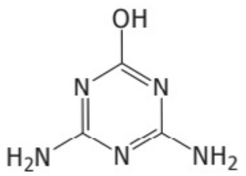
The derivatization procedure can be automated, with online injection into the GC-MS/MS system.



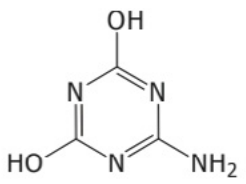




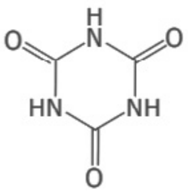
Melamine



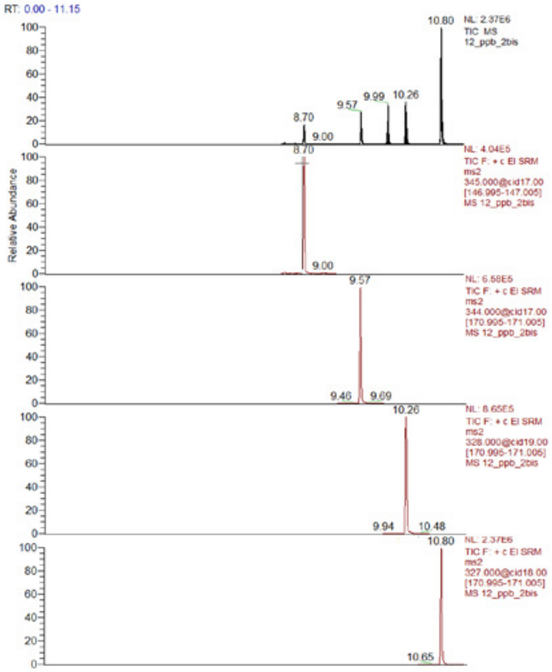
Ammeline



Ammelide



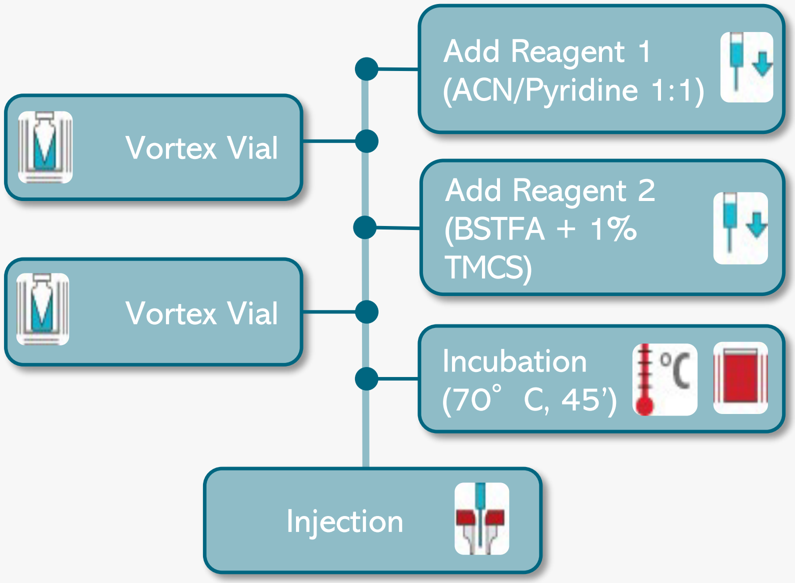
Cyanuric acid



SRM results from a milk sample spiked with 12 ppb of each compound

Derivatization of melamine and its derivatives in dairy products

Automated workflow



Add Reagent 1 (ACN/Pyridine 1:1)

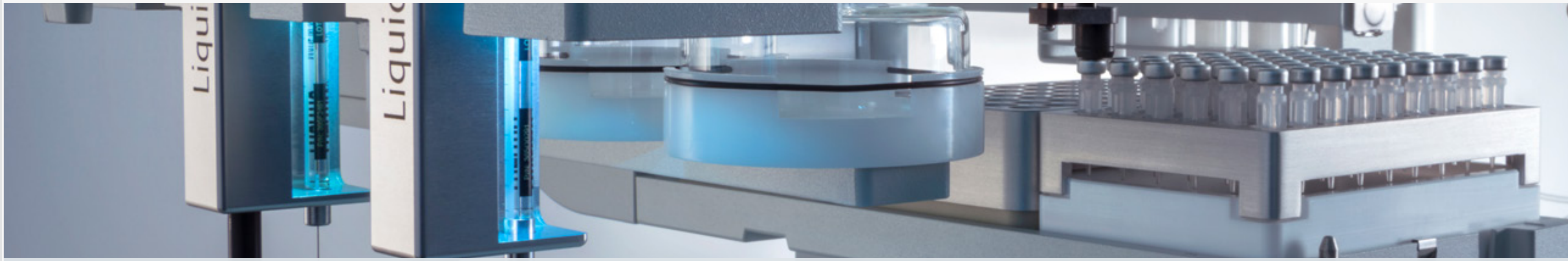
Vortex Vial

Add Reagent 2 (BSTFA + 1% TMCS)

Vortex Vial

Incubation (70° C, 45')

Injection



Resources

Poster Note PN10388

Sampling Workflow Editor Tutorial

Highlights

- Online injection in the GC-MS/MS system
- Improved data precision
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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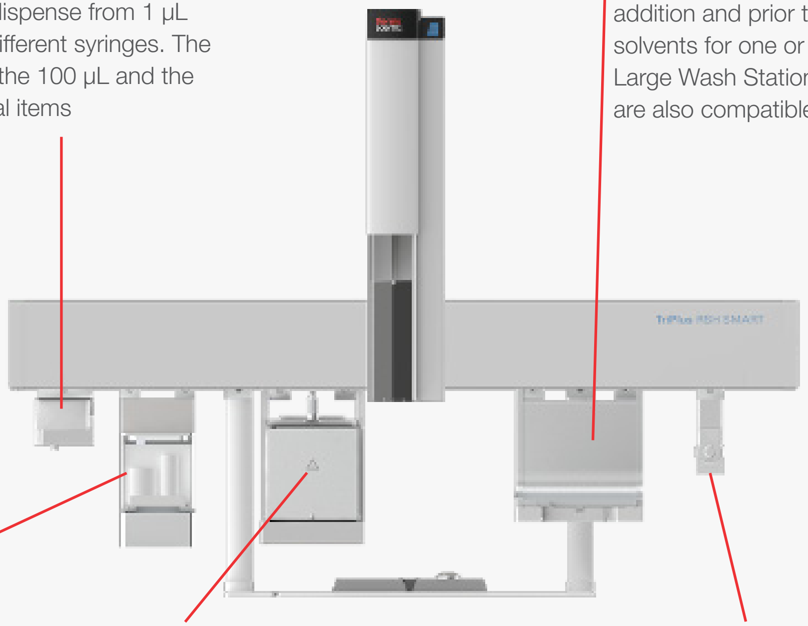
Derivatization of melamine and its derivatives in dairy products

Instrument set-up



Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools. It is possible to dispense from 1 µL up to 1,000 µL using three different syringes. The 10 µL syringe is mandatory, the 100 µL and the 1000 µL syringes are optional items

Standard Wash Station

Used to wash the syringe before/after reagent addition and prior to injection with different solvents for one or more of washing cycles. Large Wash Station and Fast Washing Module are also compatible with this custom cycle

Vortex Mixer

Employed for sample mixing after reagent or standard addition

Agitator/Incubator

Used for sample incubation after derivatization reagent addition

Tray Holder

Used to store sample vials, it has a maximum capacity of 162 × 2 mL vials. Alternatively, a Cooled Drawer is recommended for sample storage under controlled temperature (4–40 °C)

Resources

Automated Workflows Brochure

TriPlus RSH SMART Web Page

Highlights

- Fits on regular rail length
- Online injection to GCMS
- Bench workstation compatible for off-line sample derivatization

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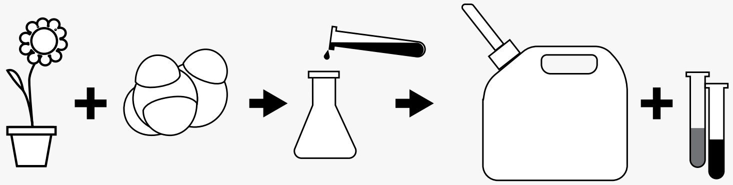
Derivatization workflow for biodiesel analysis

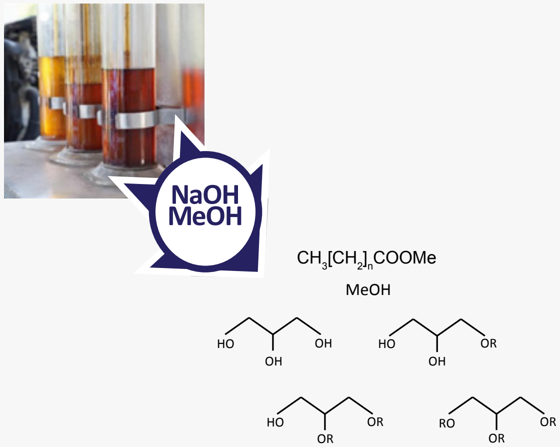
Free and total glycerol by Methods EN 14105 and ASTM D6584

Biodiesel is a clean burning alternative to fossil fuel, obtained from renewable sources such as vegetable or fat oil. The interest in biodiesel originates from its reduced environmental impact. Biodiesel is commercialized in pure form or in a blend with conventional diesel fuel and it can be used for heating or as engine fuel.



In biodiesel manufacturing, the raw oil is reacted with methanol in the presence of a strong base. The product is a mixture of fatty acid methyl-esters (FAMEs) and glycerol, along with unreacted mono-, di, and triglycerides and methanol.

Regulations set a maximum in the content of glycerin expressed as an aggregate of free and bonded forms.



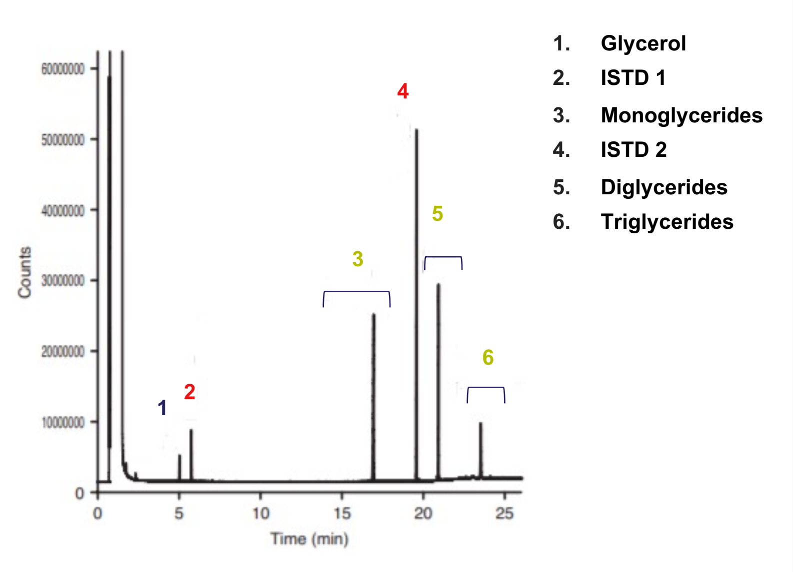
Mixture of compounds after transesterification of vegetable oil

In standard methods, the sample undergoes derivatization using MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide), which reacts with the free hydroxy groups.

The resulting adducts are analyzed by GC-FID. The reaction is finally quenched by adding heptane.

Dedicated PrepCycles are available to comply with methods EN 14105 (one IS) or ASTM D6584 (two IS), both with or without the calibration standards preparation.

Automation is greatly beneficial for this procedure which otherwise would require 1–1.5 hours of manual operation, including calibration standards preparation.



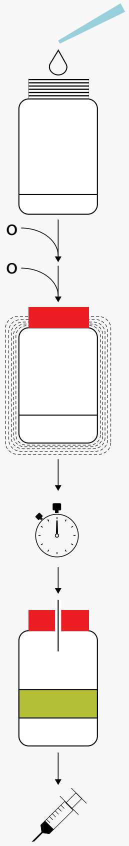
Example of chromatogram of biodiesel sample for free and total glycerol determination

Table of contents

Derivatization of free and total glycerol in biodiesel

Automated workflow

ASTM D6584 and EN 14105 PrepCycles are available also in the version including the calibration standards preparation, with 4 or 5-point calibration according to the method.



An aliquot of biodiesel is added manually at the beginning of the procedure

IS addition

Derivatizing agent addition

One or more internal standards are added to the samples or calibration standards, according to the method: butanetriol and tricaprin are used in ASTM D6584 whereas butanetriol in EN 14105

Vortexing

Derivatizing agent (MTSFA or MTSFA + pyridine) is added to the sample. This is followed by vortexing and a 15-minute wait

Waiting

8 mL of heptane are added to quench the derivatization reaction

Solvent addition

Injection can be carried out as an optional step

Injection

Resources

ASTM D6584-17

BS EN 14105:2020

Sampling Workflow Editor Tutorial

Highlights

- Dedicated PrepCycles for ASTM D6584 or EN 14105 methods, with or without calibration standards preparation
- Internal Standard addition included
- On-line injection is optional. The system can be used as a preparation bench station for off-line sample derivatization
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

Table of contents

Derivatization of free and total glycerol in biodiesel

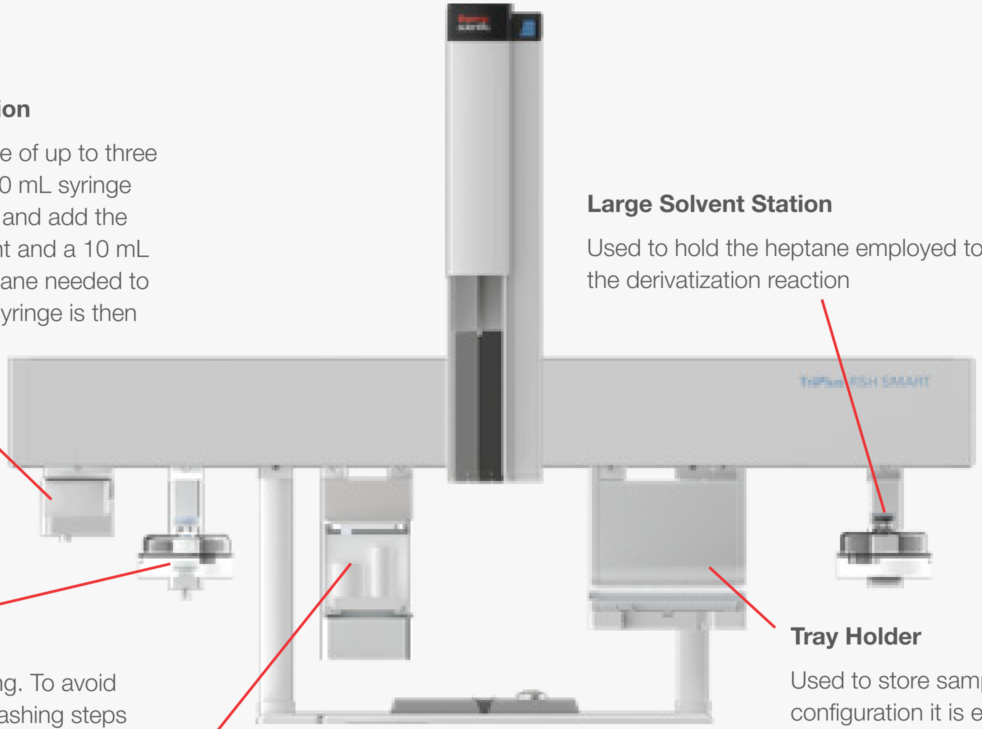
Instrument set-up

Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools: the cycle uses a 100 mL syringe to prepare the calibration curve and add the standards and derivatizing agent and a 10 mL syringe to add the 8 mL of heptane needed to quench the reaction. A 10 mL syringe is then used for GC injection

Large Solvent Station

Used to hold the heptane employed to quench the derivatization reaction



Large Wash Station

Used for generic syringe washing. To avoid cross-contamination, syringe washing steps performed before and after standard addition or MTSFA addition make use of dedicated reservoirs placed on the tray holder

Vortex Mixer

Employed for sample mixing after reagent or standard addition

Tray Holder

Used to store sample and solution vials, in this configuration it is equipped with three VT15 trays, each having a capacity of 15 × 10 mL vials. Tray 1 holds the derivatizing agent internal standard and stock solutions as well as dedicated wash solutions for each of the addition steps. Tray 2 and tray 3 hold samples and calibration solutions

Resources

Automated Workflows Brochure

TriPlus RSH SMART Web Page

Highlights

- Suitable for both ASTM and EN methods
- Fits on standard rail length
- On-line injection or bench configuration for off-line sample derivatization

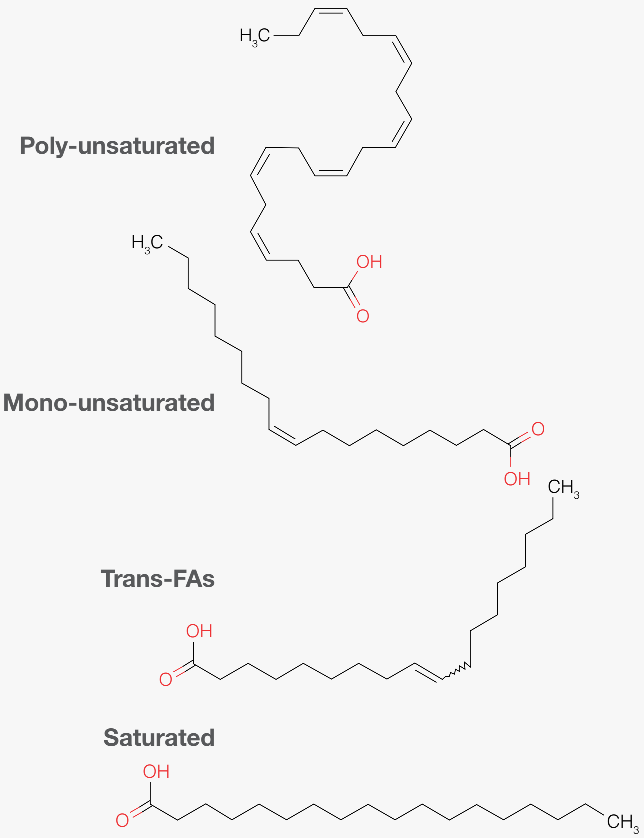
Table of contents

Derivatization workflow for fatty acids in food

Fatty acid composition provides critical information as to the quality and trading value of oil seeds and processed oil, as well as nutritional information on fat-containing food. Data on fatty acid composition also need to be reported in the label of conventional foods and dietary supplements.

Oils and fats in foods are composed of four different types of FAs: polyunsaturated, monounsaturated, saturated, and TFAs (Trans).

Since the amount and the composition of fat consumed every day has a major impact on health—especially towards the development of atherosclerosis—fatty acid composition is considered to be an important index of food quality.



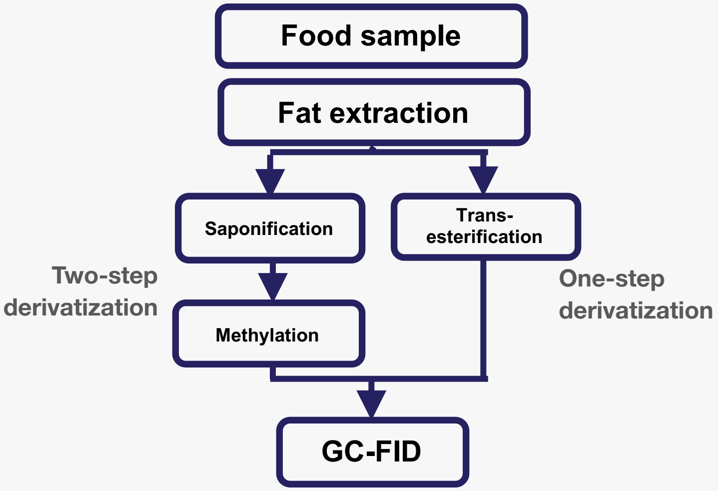
Examples of the different types of fatty acids

Fatty acid analysis in food proceeds through fat extraction, followed by derivatization and analysis by GC-FID. The derivatization step aims to convert triglycerides into fatty acid methyl-esters (FAMEs).

In case of common fats and oils, FAMEs can be directly prepared in a single transesterification step, but in general, such reaction involves two consecutive steps.

A first acid or basic hydrolysis produces free fatty acids (in an acidic solution) or their salts in case of a saponification reaction in a basic solution.

Then, the second step includes the methylation where the free FAs are esterified with methanol, in presence of an acid catalyst like BF3 (boron trifluoride) or a basic catalyst like NaOH or KOH (sodium or potassium hydroxide).



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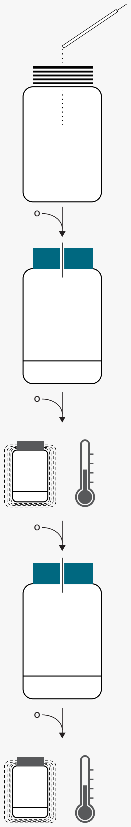
Fatty acid esterification

Automated workflow according to AOCS-CE 2-66



Add NaOH/MeOH

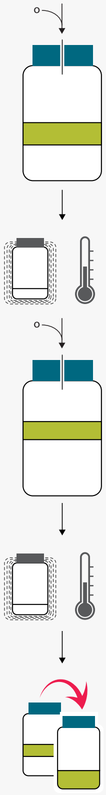
Shake and react in heated tray

Add BF_3/MeOH

Shake and react in heated tray

At the beginning of the procedure, an operator weighs an aliquot of extracted fat sample within a 2 mL vial

Saponification and methylation are carried out sequentially under temperature-controlled conditions



Solvent addition

Shake in heated agitator

Saturated NaCl addition

Shake in heated agitator

Transfer

Solvent (heptane or iso-octane) is added

FAME extraction is facilitated by shaking and salting out

The organic phase is transferred into a separate vial where it is ready for injection

Resources

Webinar: Benefits of Automated Sample Preparation Workflow to Reduce Costs and Increase Lab Efficiency: The Case of FAMEs

AOCS-CE 2-66 Method

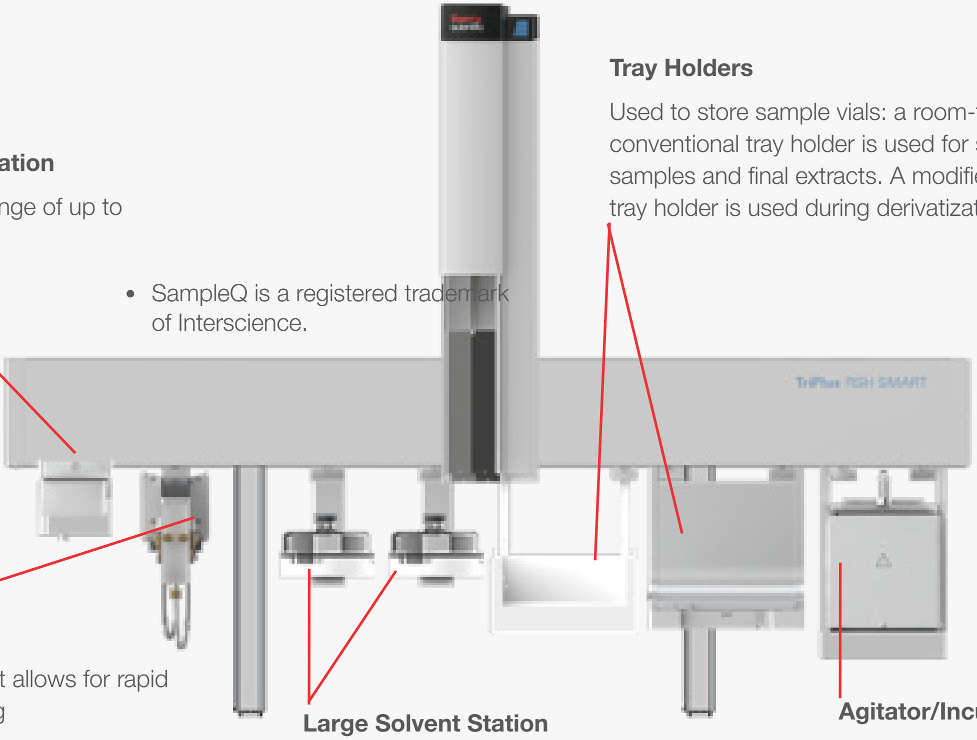
Highlights

- Low exposure to hazardous chemicals
- Increased data precision
- High sample throughput capabilities

Table of contents

Fatty acid esterification

Instrument set-up according to Method AOCS-CE 2-66



Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools

- SampleQ is a registered trademark of Interscience.

Tray Holders

Used to store sample vials: a room-temperature conventional tray holder is used for starting samples and final extracts. A modified heated tray holder is used during derivatization

Fast Wash Station

Used for all syringe washes. It allows for rapid and intensive syringe cleaning

Large Solvent Station

Holds three different 100 mL containers. Used for NaOH, BF3 and NaCl solutions and organic solvent

Agitator/Incubator

Used for vial shaking under temperature-controlled conditions

Resources

[Automated Workflows Brochure]

[SampleQ Application Note 20210208]

[Automated Workflow Solutions Web Page]

[SampleQ Web Page]

Highlights

- Method tested on customer's sample
- Bench station or on-line serving two GCs

SampleQ

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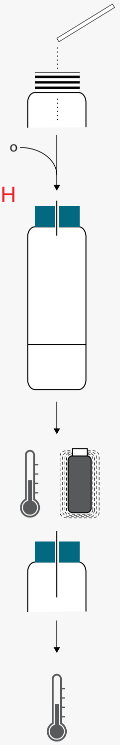
Fatty acid esterification

Automated workflow according to Method AOAC 996.01



At the beginning of the procedure, an operator weighs an aliquot of extracted fat sample within a 20 mL vial

IS addition
NaOH/MeOH addition

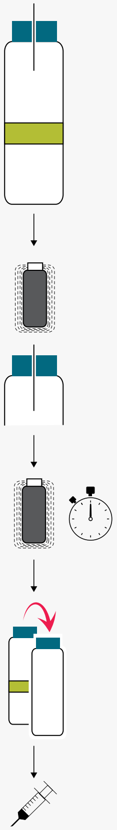
Incubate
Vortexing

Saponification proceeds for 10 minutes at 70 °C

BF_3/MeOH addition

Incubate

Fatty acid methylation proceeds for three minutes at 70 °C



Solvent addition

Vortexing

Saturated NaCl addition

FAME extraction is facilitated by vortexing and salting out

Vortexing
Wait

Transfer

The organic phase is transferred into a separate vial where it is ready for injection

Injection (optional)

Resources

[Webinar: Benefits of Automated Sample Preparation Workflow to Reduce Costs and Increase Lab Efficiency: The Case of FAMEs](#)

[AOAC 996.01 Method](#)

Highlights

- Increased data precision
- Low exposure to hazardous chemicals
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

Table of contents

Fatty acid esterification

Instrument set-up according to Method AOAC 996.01

Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools. This solution requires a tool holding a 10 mL syringe

Large Wash Station

Holds two 100 mL bottles and a waste port. For syringe cleaning this solution employs heptane and a saturated NaCl solution

Agitator/Incubator

Used for vial incubation and shaking during saponification and esterification

Tray Holder

Two VT15 trays hold up to 30 20 mL vials, used for derivatization and extraction. One VT54 tray holds 2 mL vials for the final extracts

Large Solvent Station

Holds three different 100 mL containers. Used for NaOH, BF3 and NaCl solutions and organic solvent (heptane)

Vortexer

Used for shaking after reagent and solvent addition

Resources

[Automated Workflows Brochure]

[TriPlus RSH SMART Web Page]

[Automated Workflow Solutions Web Page]

[Sampling Workflow Editor Tutorial]

Highlights

- Fits in a regular x-rail with 24 samples capacity
- Bench station or on-line injection

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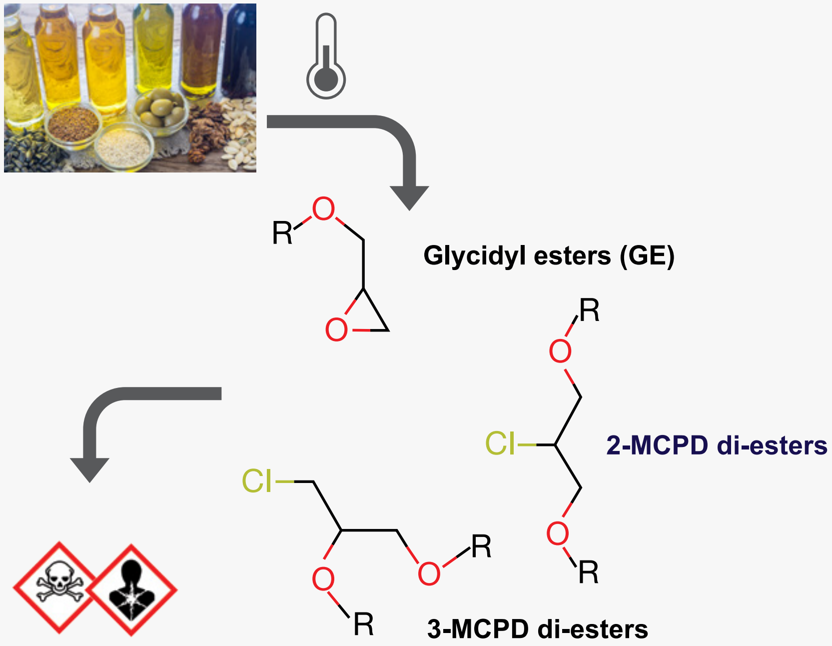
Derivatization workflow for MCPD and GE in edible oils and fats

3-MCPD (3-monochloropropane diol), 2-MCPD (2-monochlorropropane diol) and Glycidyl esters (GE) are sources of safety concern for food industry. These compounds are generated as by-products during high-temperature processing or refining of fats and edible oils.

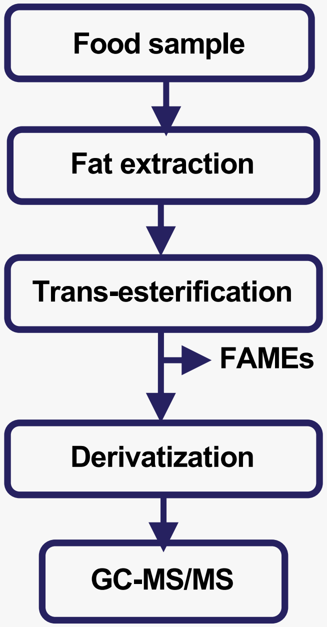
Depending on the type of food they may occur as a free substance, in the form of an ester with fatty acids or in both forms. Because of carcinogenic properties and overall toxicity, both the free form and their esters are highly regulated.

MCPD and GE are analyzed after transesterification and derivatization in order to report total MCPD and GE contents.

A common sample preparation involves the extracted fats to be first submitted to a transesterification step using sodium methoxide, which reacts as well with fatty acid glyceryl esters. The resulting FAMEs are separated by solvent extraction. Glycidol, 3-MCPD and 2-MCPD are then derivatized using poly-butyl-acrylate (PBA) and finally analyzed by GC-MS/MS).



By-products generated during high-temperature fatty food processing or refining


Food sample

Fat extraction

Trans-esterification

FAMEs

Derivatization

GC-MS/MS

Table of contents

Derivatization workflow for MCPD and GE in edible oils and fats

Official methods

The European Union defines fixed limits to the allowed maximum concentration of glycidol, 2- and 3-MCPD and the respective esters in different types of food.

Of the three AOCS offical methods currently used for the determination of these compounds in food and oils, AOCS Cd29c-13 shows the highest potential for quality control purposes as it allows for high turnaround through easier automation.

A recent validated modification of the AOCS Cd29c-13 method (Zwagerman method), permitting the direct detection of all the three analytes with one sample aliquot, offers the most suitable testing solution for QC laboratories.

	AOCS Cd29a-13 / ISO18363-3	**AOCS Cd29c-13 / ISO18363-1**	**Zwagerman method / ISO 18363-4**
Reaction Time	16 hours	5,5 min	12 min
Transesterification	Acidic, above Room Temp	Alkaline, Room Temp	Alkaline, below Room Temp
Components	3-MCPDe, 2-MCPDe and GE	3-MCPDe and GE (calculated)	3-MCPDe, 2-MCPDe and GE
Aliquots	1	2	1
Analysis	GC-MS	GC-MS	GC-MS/MS

	AOCS Cd 29a-13	**AOCS Cd 29b-13**	**AOCS Cd29c-13**	**Zwagerman Method**
Sample	100-110 mg	2x 100±0.5 mg	2x 100±0.5 mg	100-120 mg
Pre-treatment	THF /H^+/NaBr, 50°C, 15 min, extr, evap	Diethylether, -25°C	tBME, Room T	Toluene, 5°C
Trans-esterification	Acidic, H_2SO_4 / MeOH 40°C, 16h	Alkaline, NaOH / MeOH -25°C, 16h	Alkaline, $NaOCH_3$ / MeOH RT, 5.5 min	Alkaline, $NaOCH_3$ / MeOH 5°C , 5.5 min
Stop reaction	$NaCO_3$, Evap, RT	H^+/NaBr, -25°C, Evap	H^+/NaCl, RT H^+/NaBr, RT	H^+/NaBr, RT
FAME extraction	Na_2SO_4 + 2x extr n-heptane	2x extr iso-hexane	2x extr iso-hexane	2x extr n-hexane
Clean-up	-	3x Et_2O/EtAcO, Na_2SO_4	3x Et_2O/EtAcO, Na_2SO_4	-
Derivatization	PBA solution	PBA solution	PBA solution	PBA solution
Extraction and re-concentration	2x 1 ml n-heptane, Evap, 400µl n-heptane	Evap, 400µl iso-octane	Evap, 500µl iso-octane	Extr, 600µl n-hexane
Detection	MS-SIM	MS-SIM	MS-SIM	MS/MS
Difficulties for automation / manual prep	Evaporation steps, precipitation of salts	Evaporation steps + very low temp	Evaporation steps	Reliable automation

Resources

1. R. Zwagerman, P. Overman, Eur. J. Lipid Sci. Technol. 2019
2. 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by Acid Transesterification, Official AOCS Methods, AOCS 29a-13, approved 2013.
3. Determination of Bound MCPD- and Bound Glycidol- by Gas Chromatography/ Mass Spectrometry (GC/MS), Official AOCS Methods, AOCS 29b-13, approved 2013.
4. Fatty-acid bound 3-MCPD- and Glycidol- determination in oils and fats by GC/MS (Differential Measurement), Official AOCS methods, AOCS 29c-13, approved 2013

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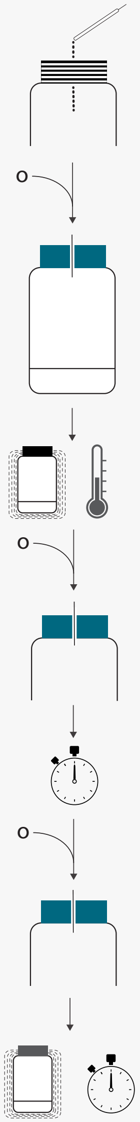
MCPD and GE transesterification and derivatization

Automated workflow according to modified AOCS Cd 29c-13 or ISO 18363-4 (Zwagerman method)



At the beginning of the procedure, the operator weighs 100–120 mg of fat in a 2 mL vial

Add tBME/toluene and IS

Fat is dissolved in *tert*-butyl-methyl-ether and homogenized. ^{13}C 3-MCPD ester is added as IS

Shake in heated tray, then vortex

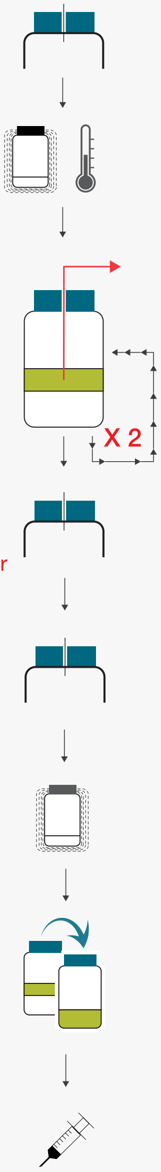
NaOCH$_3$/MeOH

Waiting

Transesterification step by adding NaOCH$_3$/methanol and stopped using acidified NaBr

Add acidified NaBr

Vortexing and waiting



Solvent addition

FAMEs are removed by extraction with iso-octane

Vortex and incubate

Remove organic phase

After extraction, the solvent is discarded, and this clean-up procedure is performed twice

Add PBA/ acetone/water

Final derivatization is achieved by addition of a poly-butyl-acrylate (PBA) solution

Solvent addition

The adducts are finally extracted and the organic layers are transferred in separate vials

Vortex

Transfer

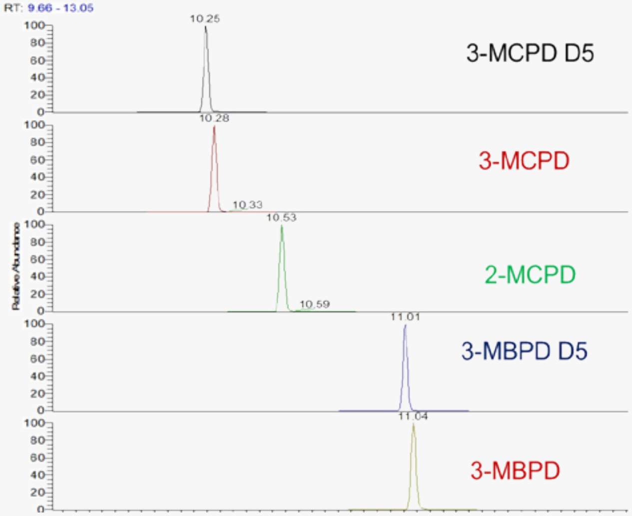
Inject

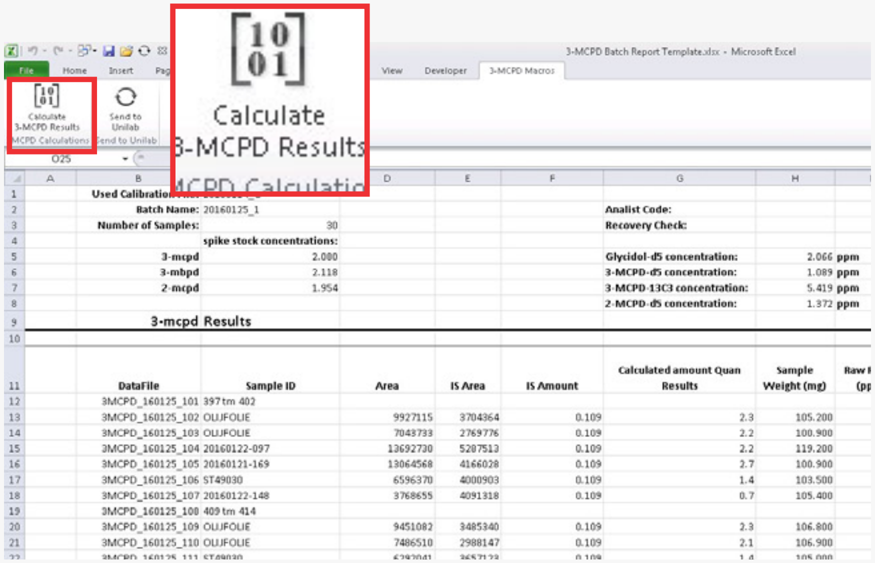
On-line or injection into the GC-MS (optional)

Table of contents

MCPD and GE transesterification and derivatization

ISO 18363-4 (Zwagerman method)





Batch Name: 20160125_1
Number of Samples: 30
spike stock concentrations:
3-mcpd 2.000
3-mbpd 2.118
2-mcpd 1.954

Analist Code:
Recovery Check:
Glycidol-d5 concentration: 2.066 ppm
3-MCPD-d5 concentration: 1.089 ppm
3-MCPD-13C3 concentration: 5.419 ppm
2-MCPD-d5 concentration: 1.372 ppm

3-mcpd Results

DataFile	Sample ID	Area	IS Area	IS Amount	Calculated amount Quan Results	Sample Weight (mg)
3MCPD_160125_101	397 tm 402					
3MCPD_160125_102	OLIJFOLIE	9927115	3704364	0.109	2.3	105.200
3MCPD_160125_103	OLIJFOLIE	7043733	2769776	0.109	2.2	100.900
3MCPD_160125_104	20160122-097	13692730	5207513	0.109	2.2	119.200
3MCPD_160125_105	20160121-169	13064568	4166028	0.109	2.7	100.900
3MCPD_160125_106	ST49030	6596370	4000903	0.109	1.4	103.500
3MCPD_160125_107	20160122-148	3768655	4091318	0.109	0.7	105.400
3MCPD_160125_108	409 tm 414					
3MCPD_160125_109	OLIJFOLIE	9451082	3485340	0.109	2.3	106.800
3MCPD_160125_110	OLIJFOLIE	7486510	2988147	0.109	2.1	106.900

	LOD (µg/Kg)	**LOQ (µg/Kg)**
3-MCPDe	10.0	15.0
GE	18.5	41.0
2-MCPDe	2.7	6.9

Resources

[SampleQ Technical Note SQ20170212](#)

[Webinar: An Automated Workflow Solution for the Determination of MCPDe and GE](#)

[Poster RAFA 2019](#)

[Article: Optimized Analysis of MCPD- and Glycidyl Esters in Edible Oils and Fats Using Fast Alkaline Transesterification](#)

[Analyte Guru Blog](#)

Highlights

- Determination of 2-MCPD is included in this method
- High sensitivity with Triple Quad and AEI source
- No evaporation step is necessary
- Only one injection per sample
- Full automated reporting

SampleQ™

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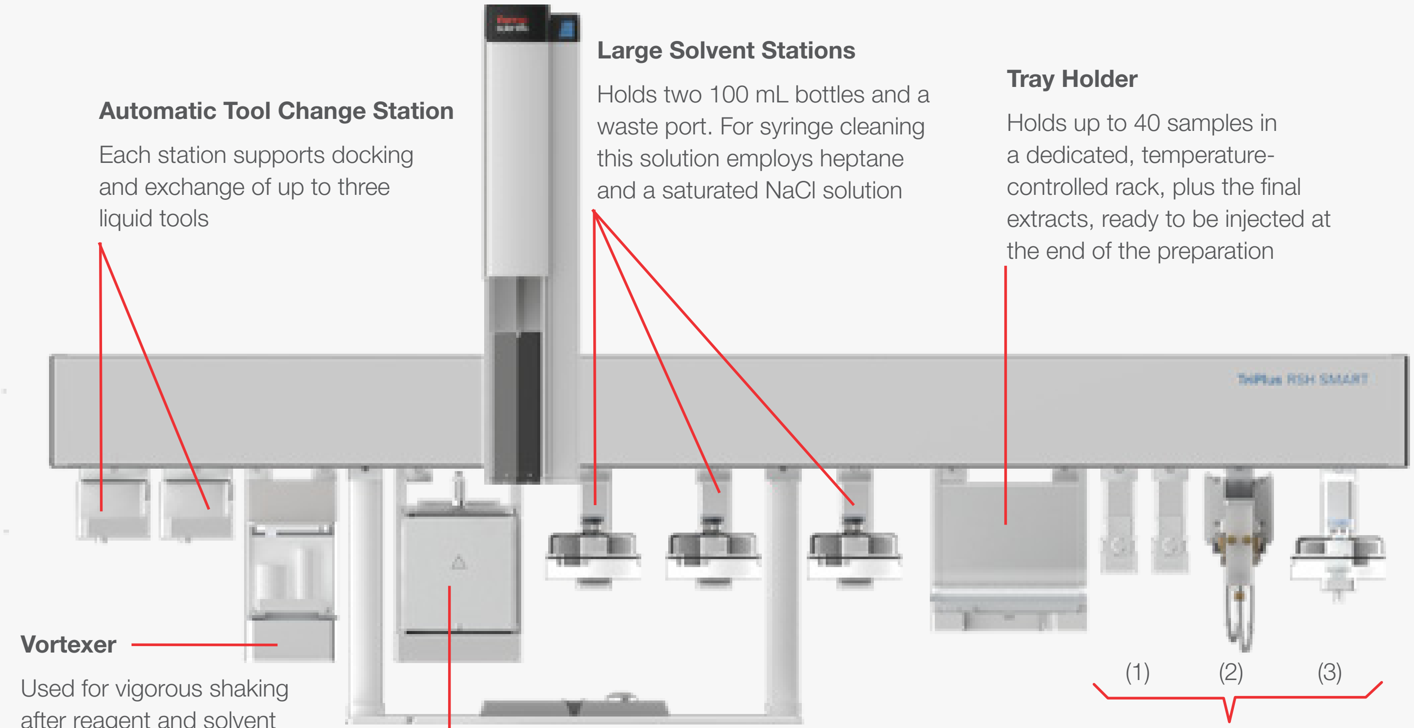
MCPD and GE transesterification and derivatization

Instrument set-up according to ISO 18363-4 (Zwagerman method)



Automatic Tool Change Station

Each station supports docking and exchange of up to three liquid tools

Large Solvent Stations

Holds two 100 mL bottles and a waste port. For syringe cleaning this solution employs heptane and a saturated NaCl solution

Tray Holder

Holds up to 40 samples in a dedicated, temperature-controlled rack, plus the final extracts, ready to be injected at the end of the preparation

Vortexer

Used for vigorous shaking after reagent and solvent addition

Agitator/Incubator

Used for vial incubation and shaking during sample dissolution, hydrolysis and esterification reactions and solvent extractions

Wash Stations

Different stations are required for the various washing steps, including two standard wash (1), one fast wash (2) and one large wash station (3)

Resources

[Automated Workflows Brochure]

[www.SampleQ.com]

[MCPD Automated Workflow Video]

Highlights

- > 100 sample positions
- Batch of 20 samples at once

SampleQ™

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Liquid-liquid extraction workflows

Liquid-liquid extraction (LLE) is the most widespread sample preparation technique before chromatography separations. LLE is a method based on the relative solubilities of compounds in two different immiscible liquids, usually water (polar) and an organic solvent (non-polar). During LLE, one or more compounds (or solutes) are transferred from one liquid phase to the other. At the end of the extraction (or partitioning), equilibrium is reached and one of the two phases will be solute-depleted while the other will be solute-enriched.

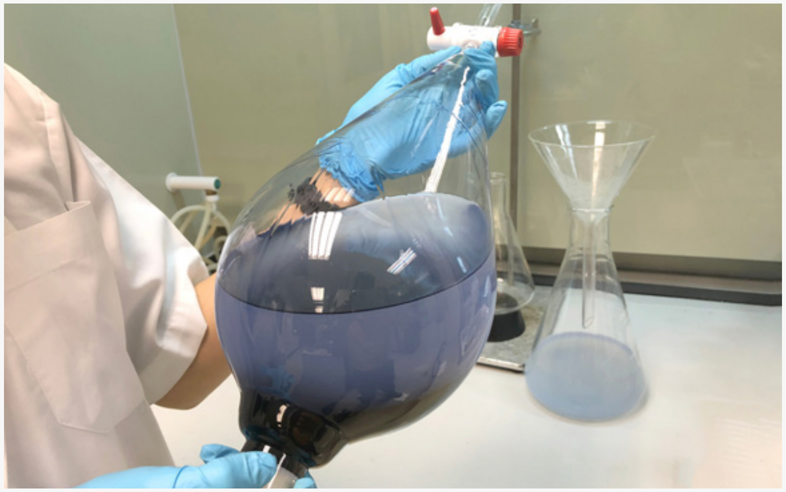
Liquid-liquid extraction seems relatively simple, but manual operations are time consuming, prone to human error, and inolve high solvent consumption.

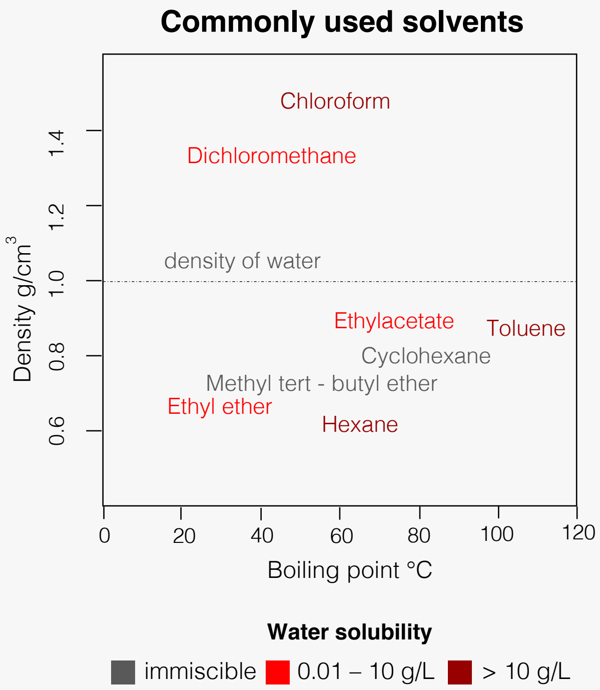
Automation is greatly beneficial in terms of time and cost savings:

- Highly standardized workflows, including agitation, centrifugation and solvent evaporation
- Scaled down of solvent and sample volumes
- Minimized direct contact of the operator with toxic solvents.

The choice of the right solvent for the extraction is a key parameter, ideally the solvent should display:

- Low reactivity and good dissolving power towards the compound -or the compounds- of interest
- Relatively high volatility, for easy removal after extraction
- Low or no miscibility and high enough difference in density compared to water, for easy phase separation
- Low melting/freezing point and low toxicity and flammability for easy handling and storage.




Commonly used solvents

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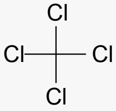
Automated LLE for organic contaminants (SVOC) in water

The presence of organic contaminants in waters and wastewaters represents a serious risk for the environment and human health. Organic contaminants in water belong to a wide variety of chemical structures, such as hydrocarbons, aldehydes, ethers, esters, phenols, organic acids, ketones, amines, amides, nitroaromatics, PCBs (also known as Aroclors), PAHs, phthalate esters, nitrosamines, haloethers, and trihalomethanes.

Sources of these compounds include pesticides and herbicides (containing phosphorus, sulfur, chlorine, or nitrogen), flame retardants, ingredients in cleaning agents and personal care products, solvents, and chemicals used in textile/electronic manufacturing and material manufacturing process additives.

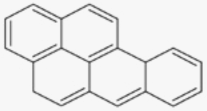
Regulatory bodies worldwide publish extensive lists of contaminants, which are regularly updated with new emerging pollutants. Strict performance limits are required by analytical determination in terms of sensitivity, accuracy, and linearity. This puts an increasing burden on contract testing labs in charge for water analysis.

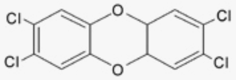
To date, LLE remains one of the go-to options for organic contaminant extraction from water samples. Laboratories look for options to save on solvent costs and to minimize sample preparation time. However, time-saving and cost-driven measurements should not compromise analytical results, hence the interest for reliable, automated sample preparation, coupled to state-of-the-art GC-MS.



Halogenated hydrocarbons

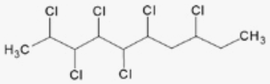
PAHs

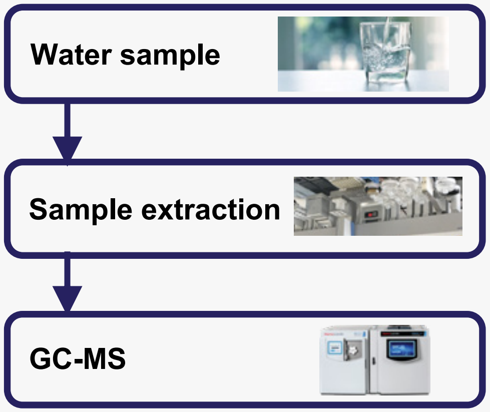




Dioxins

SCCPs





Water sample

Sample extraction

GC-MS

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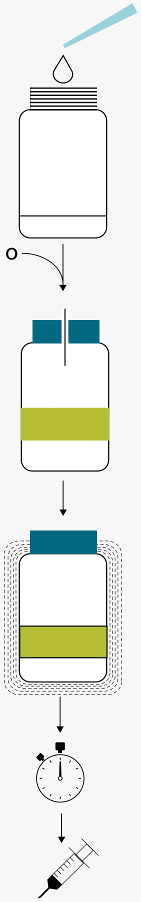
Automated LLE for organic contaminants (SVOC) in water

Automated workflow



At the beginning of the procedure, 10 mL of water sample are manually pipetted into a 20 mL headspace vial. Once the vial is placed onto the autosampler tray, the rest of the procedure is carried out automatically

IS addition
Solvent addition

10 µL of an Internal Standard mixture and 2 mL of solvent (pentane) are sequentially added to the sample by means of two separate liquid tools

Vortexing

A vortexing step (2,000 rpm for 1 minute) promotes analyte partitioning into the organic phase

Waiting

A five-minute waiting time allows for the organic layer to separate. The organic solvent is sampled and injected

Injection

A 200 µL syringe is used for large volume injection of 50 µL into a PTV injector

Suitable for...

Matrices

- Ground water
- Surface water
- Sea water
- Drinking water

Analytes

- Pesticides
- Phenoles
- Aromatic amines
- Chlorofenoxycarbonic acids
- PCBs
- PAHs
- Halo acetic acids

Resources

[Webinar: Improvement of Sensitivity, Accuracy and Precision of Quantitative Data Through Automation]

[Thermo Scientific Application Note AN10591]

[WaterWorld Article]

[Analyte Guru Blog]

Highlights

- In-vial extraction
- Scale down sample and solvent volumes
- LVI and Triple Quad with AEI ion source for high sensitivity in the low ppt range

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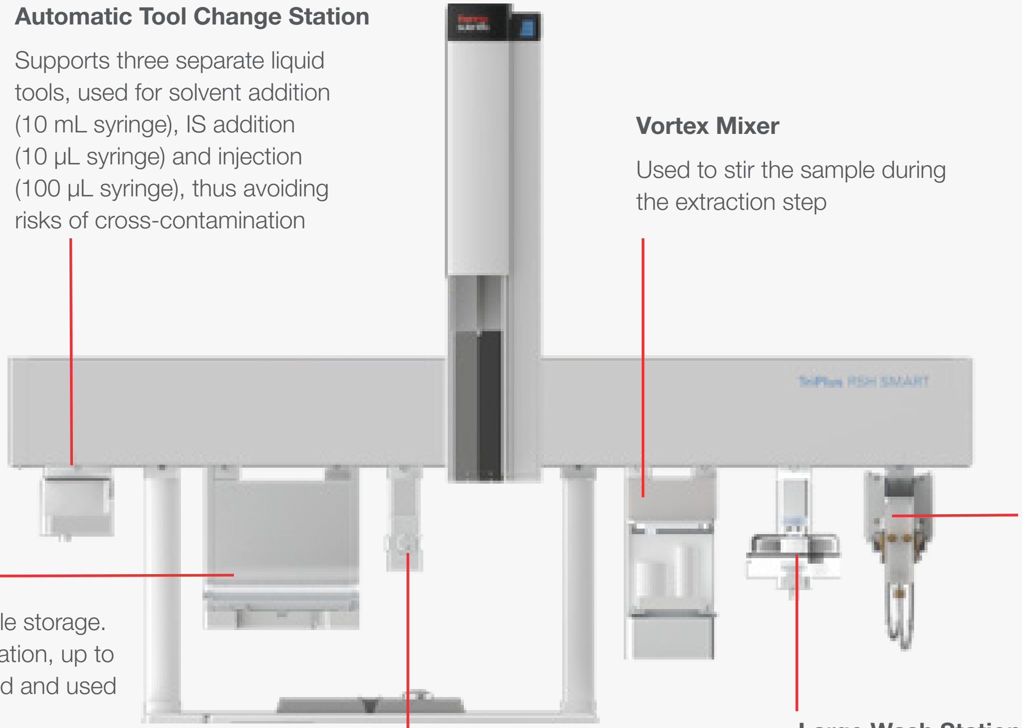
Automated LLE for organic contaminants (SVOC) in water

Instrument set-up



Automatic Tool Change Station

Supports three separate liquid tools, used for solvent addition (10 mL syringe), IS addition (10 µL syringe) and injection (100 µL syringe), thus avoiding risks of cross-contamination

Vortex Mixer

Used to stir the sample during the extraction step

Fast Wash Module

Provides the extensive washing needed for the 10 mL and 100 µL syringes

Tray Holder

Used for sample storage. In this configuration, up to 60 vials are held and used for extraction

Standard Wash Station

Used for syringe washing or solvent and solution storage. In this configuration, this station holds the internal standard solution

Large Wash Station

Used for syringe washing or solvent and solution storage. In this configuration, this station must be used to store solvent, and if necessary both positions can be used to increase capacity and throughput

Resources

Automated Workflows Brochure

SampleQ Web Page

Automated Workflow Solutions Web Page

Highlights

- Fits on regular x-rail
- On-line injection into the GC-MS/MS
- 60 vial positions for 24/7 operations

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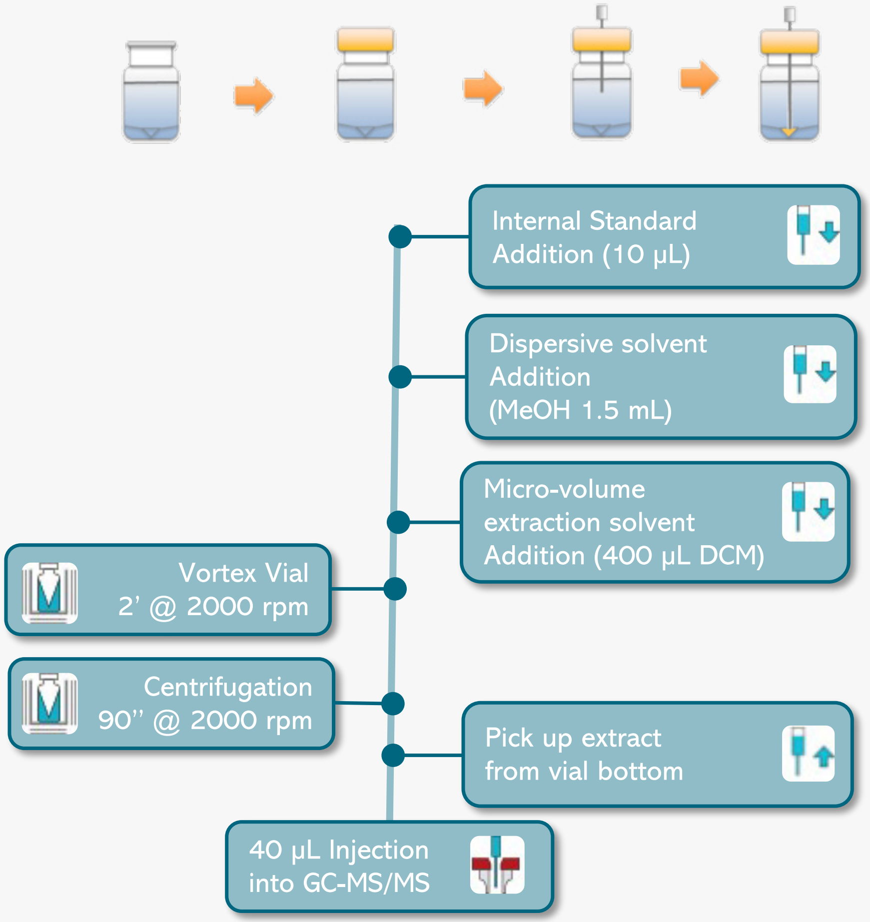
Dispersive micro-LLE for organic contaminants (SVOC) in water samples

Automated workflow



Internal Standard Addition (10 μL)

Dispersive solvent Addition (MeOH 1.5 mL)

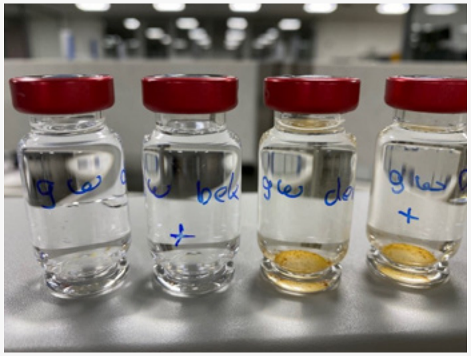
Micro-volume extraction solvent Addition (400 μL DCM)

Vortex Vial 2’ @ 2000 rpm

Centrifugation 90’’ @ 2000 rpm

Pick up extract from vial bottom

40 μL Injection into GC-MS/MS



- The workflow starts with 8 mL of water sample.
- A micro volume of extraction solvent is added in a dispersive way maintaining an elevated surface exchange, thus dramatically increasing the final concentration of the analytes in the organic phase.
- A centrifugation step is required to separate the organic solvent drop before injection in the GC-MS/MS.
- The enhanced enrichment along with Large Volume Injection (LVI) and Advanced EI MS source allow to reach sub-ppt sensitivity.

Resources

SampleQ Web Page

Highlights

- Sub-ppt sensitivity
- Compliant with the most stringent analytical requirements of current European regulations on water testing

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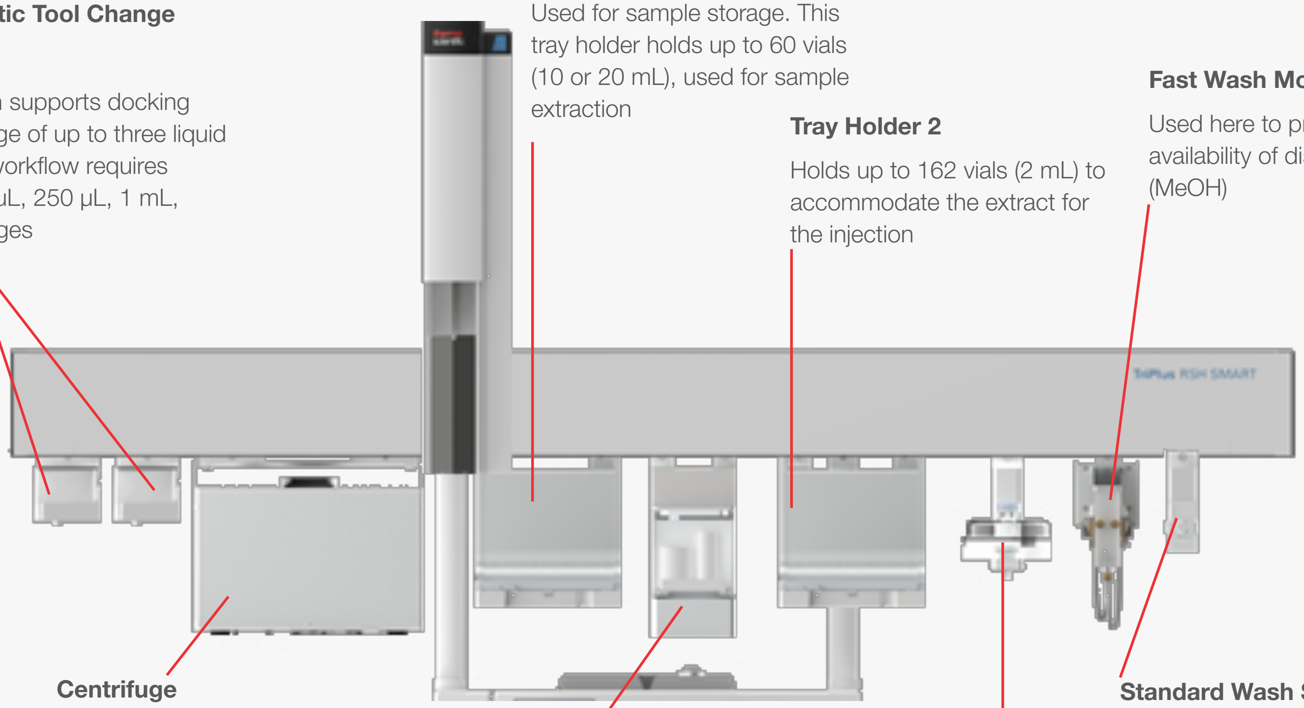
Dispersive micro-LLE for organic contaminants (SVOC) in water samples

Instrument set-up



2x Automatic Tool Change Stations

Each station supports docking and exchange of up to three liquid tools. This workflow requires 50 µL, 100 µL, 250 µL, 1 mL, 10 mL syringes

Tray Holder 1

Used for sample storage. This tray holder holds up to 60 vials (10 or 20 mL), used for sample extraction

Tray Holder 2

Holds up to 162 vials (2 mL) to accommodate the extract for the injection

Fast Wash Module

Used here to provide longer availability of dispersive solvent (MeOH)

Centrifuge

Supports centrifugation of 2, 10 or 20 mL vials. The centrifugation step facilitates phase separation after solvent extraction

Vortex Mixer

Used to add a stirring step during the extraction step

Large Wash Station

Used for syringe washing and solvent/calibration/IS solution storage

Standard Wash Station

Used to wash the syringes before/after IS addition and prior to injection with different solvents for one or more washing cycles

Resources

[Automated Workflows Brochure]()

[Automated Workflow Solutions Web Page]()

[TriPlus RSH SMART Web Page]()

Highlights

- Extended x-rail required
- Automatic six tools changer
- On-line injection into the GC-MS/MS, with 24/7 operations

SampleQ™

LLE for Hydrocarbon Index in water

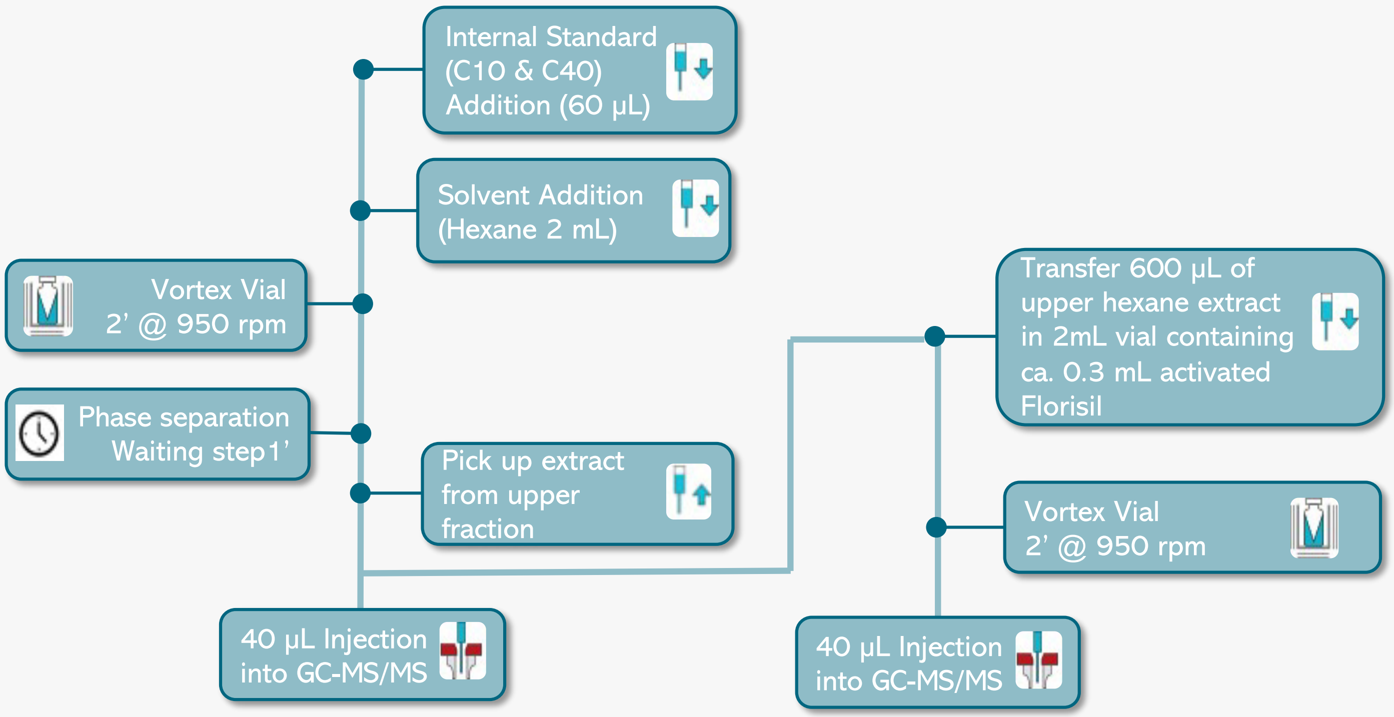
Automated workflow

A robust GC configuration with PTV injector and FID detector is used for automated in-vial extraction for the dermination of Hydrocarbon Index in water samples, followed by Large Volume Injection (LVI) for sensitivity in the low ppb levels.

Automation ensures high precision (RSD < 3%), limited water sample volume (10 mL), and very low solvent consumption (2 mL/sample). The clean up with Florisil is an optional step which can be done for positive samples only.

Extraction process

Clean-up proces



Internal Standard (C10 & C40) Addition (60 µL)

Solvent Addition (Hexane 2 mL)

Vortex Vial 2’ @ 950 rpm

Phase separation Waiting step 1’

Pick up extract from upper fraction

40 µL Injection into GC-MS/MS

Transfer 600 µL of upper hexane extract in 2mL vial containing ca. 0.3 mL activated Florisil

Vortex Vial 2’ @ 950 rpm

40 µL Injection into GC-MS/MS

Resources

[Presentation: Automated In-Vial Extraction of Surface Waters for the Analysis of Mineral Oil by GC-FID](#)

[SampleQ Web Page](#)

Highlights

- Fast method with Prep Ahead function
- Cycle time 21 min for 68 samples/24h
- Chromeleon CDS for instrument control, data acquisition, and reporting

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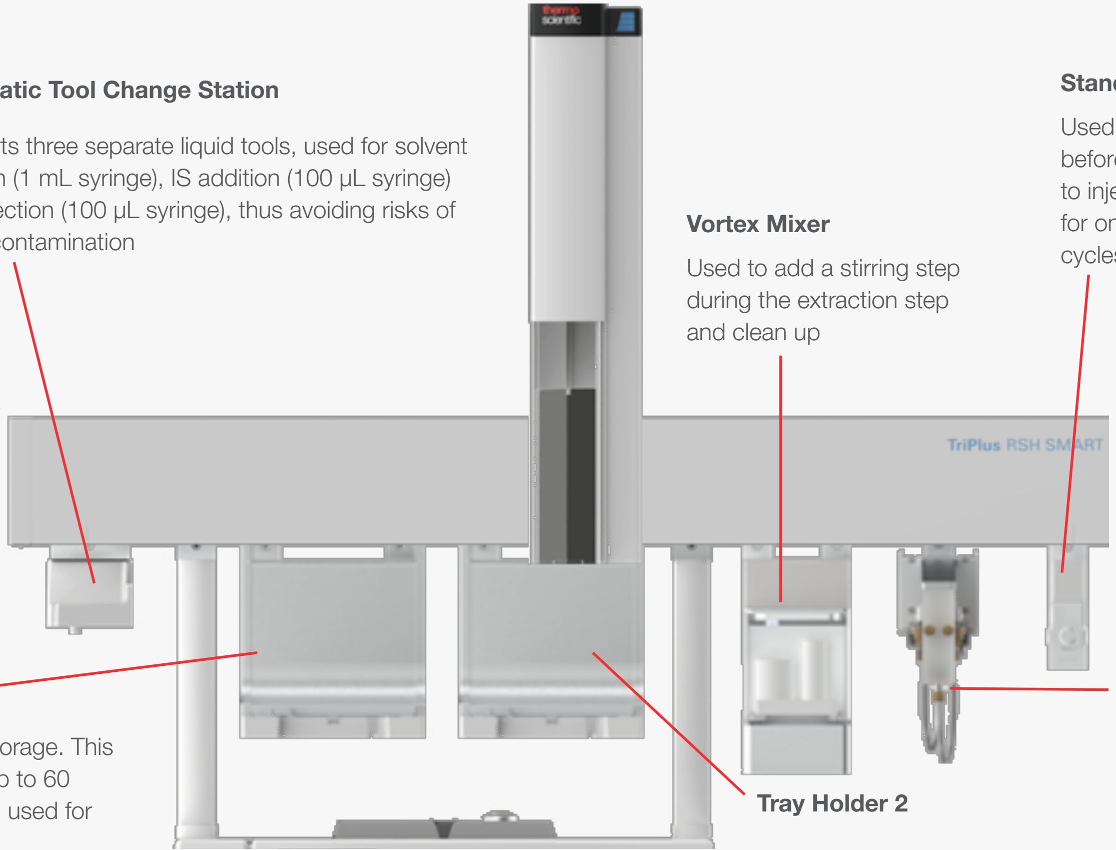
Automated LLE for Hydrocarbon Index

Instrument set-up



Automatic Tool Change Station

Supports three separate liquid tools, used for solvent addition (1 mL syringe), IS addition (100 µL syringe) and injection (100 µL syringe), thus avoiding risks of cross-contamination

Standard Wash Station

Used to wash the syringes before/after IS addition and prior to injection with different solvents for one or more of washing cycles

Vortex Mixer

Used to add a stirring step during the extraction step and clean up

Tray Holder 1

Used for sample storage. This tray holder holds up to 60 vials (10 or 20 mL), used for sample extraction

Fast Wash Module

Used to provide longer availability of extraction solvent (hexane)

Tray Holder 2

Used for sample storage, holding up to 15 vials (10 or 20 mL), during the clean up step, and up to 108 vials (2 mL) to accommodate the extract for the injection

Resources

Automated Workflows Brochure

Automated Workflow Solutions Web Page

TriPlus RSH SMART Web Page

Highlights

- Extended x-rail required
- On-line injection into the GC-MS/MS
- 24/7 operations

SampleQ

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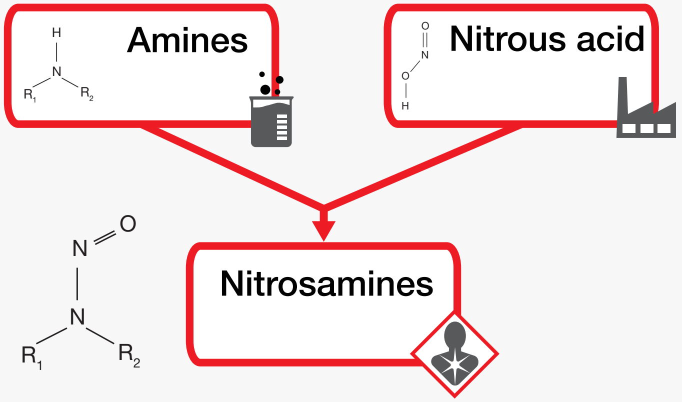
LLE of Nitrosamines in Metformin drug substance

Nitrosamines are considered a matter of concern as mutagenic carcinogens or probable carcinogens. Since 2018, the presence of nitrosamine impurities has been highlighted by the Food and Drug Administration (FDA) within several widely employed active principles such as angiotensin receptor blockers commonly known as 'sartans' used in the treatment of hypertension and metformin, which is employed to treat diabetes. This has sometimes led to these drugs being recalled.

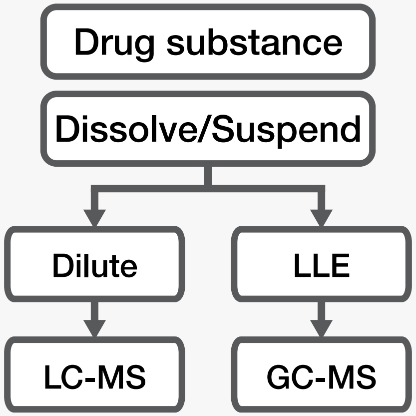


Nitrosamine impurities in pharmaceutical substances typically originate from the reaction between a secondary or tertiary amine present as an unintentional contaminant of raw materials, reagents, and solvents with nitrous acid, generated from nitrites through acid catalysis during the production process.

For LC-MS nitrosamine analysis, sample preparation involves a dissolution/suspension step with an aqueous/methanolic solution, followed by dilution and injection.

In the case of GC-MS, a liquid-liquid extraction step is also carried out to make the sample amenable to GC analysis.

Automation of the extraction procedures reduces the sample preparation time while improving data repeatability.



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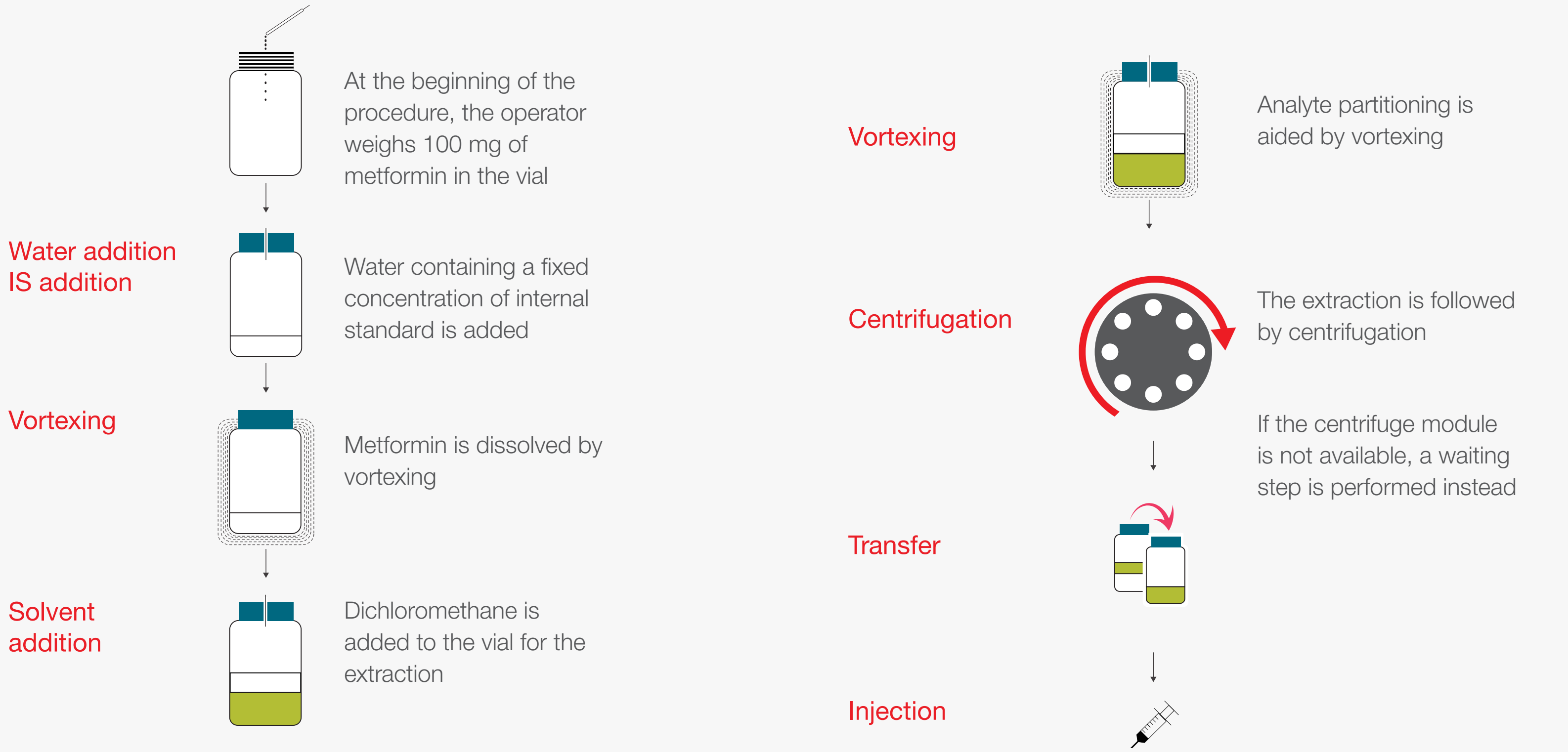
LLE of Nitrosamines in Metformin drug substance

Automated workflow



At the beginning of the procedure, the operator weighs 100 mg of metformin in the vial

Water addition
IS addition

Water containing a fixed concentration of internal standard is added

Vortexing

Metformin is dissolved by vortexing

Solvent addition

Dichloromethane is added to the vial for the extraction

Vortexing

Analyte partitioning is aided by vortexing

Centrifugation

The extraction is followed by centrifugation

If the centrifuge module is not available, a waiting step is performed instead

Transfer

Injection

Resources

[Webinar: Confidence in Process: A Fully Automated Solution for Nitrosamine Impurity Analysis Applicable to LC-MS and GC-MS]()

[Application Brief AB000390]()

[Analyte Guru Blog]()

Highlights

- Centrifugation is optional but recommended
- On-line injection is optional. The system can be used as a bench station for off-line sample preparation
- This PrepCycle can be reproduced with the SWE to modify or create additional steps

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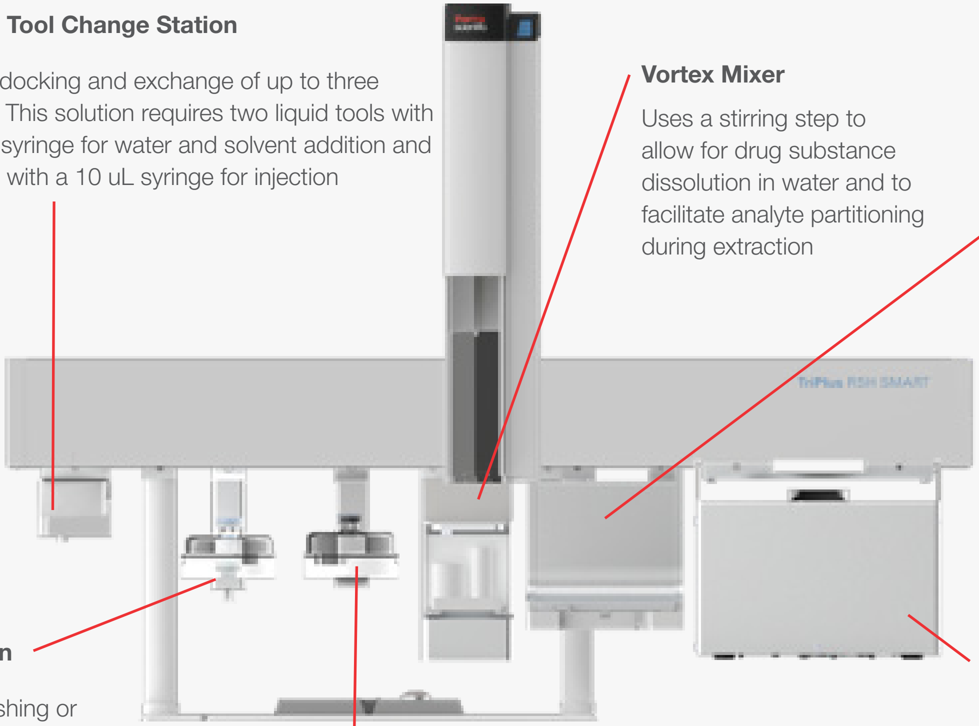
Automated LLE of Nitrosamines in Metformin drug substance

Instrument set-up



Automatic Tool Change Station

it supports docking and exchange of up to three liquid tools. This solution requires two liquid tools with a 1,000 uL syringe for water and solvent addition and a liquid tool with a 10 uL syringe for injection

Vortex Mixer

Uses a stirring step to allow for drug substance dissolution in water and to facilitate analyte partitioning during extraction

Tray Holder

Two holder slots are typically used for VT54 trays, holding up to 54 vials each and used for sample and extracts respectively. A cooled drawer can also be used

Large Wash Station

Used for syringe washing or solvent and solution storage. In this workflow, position 1 is filled with methanol and used for washing

Solvent Station

Used for 3 × 100 mL solvent bottles. Positions 1 and 2 are used for water and organic solvent

Centrifuge

Supports centrifugation of 2, 10 or 20 mL vials. The centrifugation step facilitates phase separation after solvent extraction

Resources

[Automated Workflows Brochure]

[TriPlus RSH SMART Web Page]

[Sampling Workflow Editor Tutorial]

Highlights

- Fits a regular x-rail
- Bench working station or on-line configuration

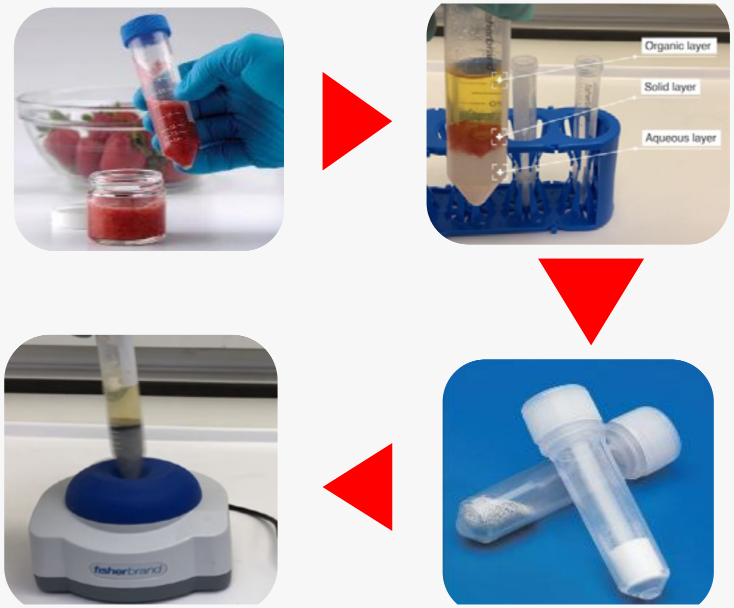
Automated µSPE clean-up of QuEChERS extracts

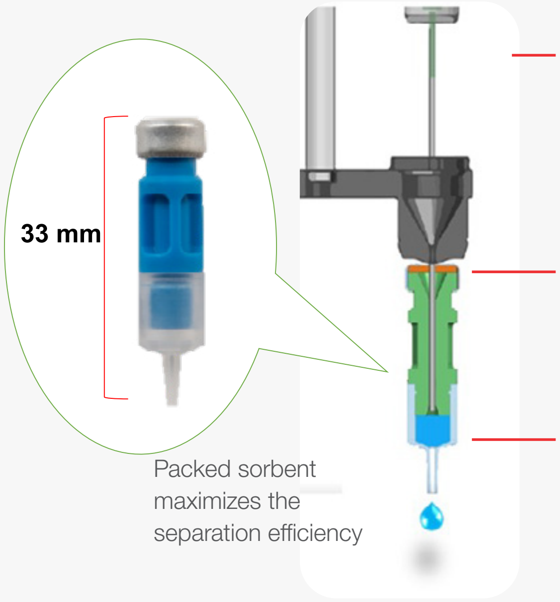
The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction and clean-up procedure was first developed in 2003 to simplify pesticide extraction from fruits and vegetables. Today, QuEChERS or QuEChERS-like workflows have been adopted by laboratories worldwide on a wide variety of compounds and sample matrices. Depending on target analytes, processed samples can be analyzed by means of GC-MS, HPLC-MS, or both.

Food extracts require a clean up step ususally performed with a solid-phase extraction (SPE) step. SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid sorbent phase. In the QuEChERS procedure, clean-up is carried out manually by dispersive solid-phase extraction (d-SPE): the sorbent is introduced directly into the sample, the suspension is then vigorously shaken and centrifuged, and an aliquot of the supernatant is finally analyzed.

In micro-SPE (µSPE), the conventional SPE cartridge is replaced by a miniaturized cartridge containing 45 mg of sorbent for GC analyses, and 30 mg for LC analyses. The µSPE clean-up procedure is fully automated through a dedicated tool of the TriPlus RSH or RSH SMART autosampler.

The autosampler syringe works as a LC pump. Low flow rates of approximatley 2 µL/s in the load and elution steps are used for sharp analyte/matrix separation.





Syringe accurately delivers sample and solvents at the desired volume and flow rate

Crimped-on septum ensures a closed flow path to the SPE sorbent and facilitate cartridge transport

Insertion of the syringe needle allows delivery of sample and solvents directly to the SPE sorbent

Packed sorbent maximizes the separation efficiency

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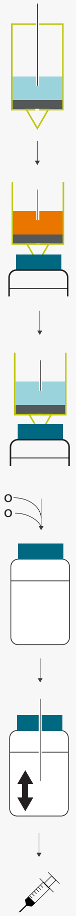
Automated µSPE clean-up of QuEChERS extracts

Automated workflow



Cartridge conditioning	The conditioning of µSPE cartridges is carried out to wet and settle the bed, activate the packing materials, and remove any residual process materials. This step is optional: It is generally carried out using acetonitrile, sometimes supplemented with acid and/or buffer solutions.
Cartridge loading and sample clean-up	The needle penetrates the septum and creates a seal so that the sample is forced through the sorbent bed by positive pressure when the plunger is depressed. The sample passes through the cartridge and is collected into a vial.
Cartridge elution	An additional cartridge elution step with solvent can be added to improve the recovery (optional)
Analyte protectant and IS addition	If required, analyte protectant and internal standard can be added to the cleaned-up sample. Analyte protectants are used in GC-MS analysis to stabilize response and maximize recovery. IS addition is useful to normalize analyte response.
Mixing	Sample mixing can be carried out by means of repeated aspiration and dispensing cycles.
Injection	Online configuration allows the processed sample to be injected immediately after clean up (optional).

Resources

Webinar: Automated Online µSPE Cartridge Clean-Up of QuEChERS Extracts Before LC-MS/MS and GC-MS/MS Analysis of Pesiticides in Foods

Thermo Scientific AN 65906

Thermo Scientific AN 65684

LCGC Article

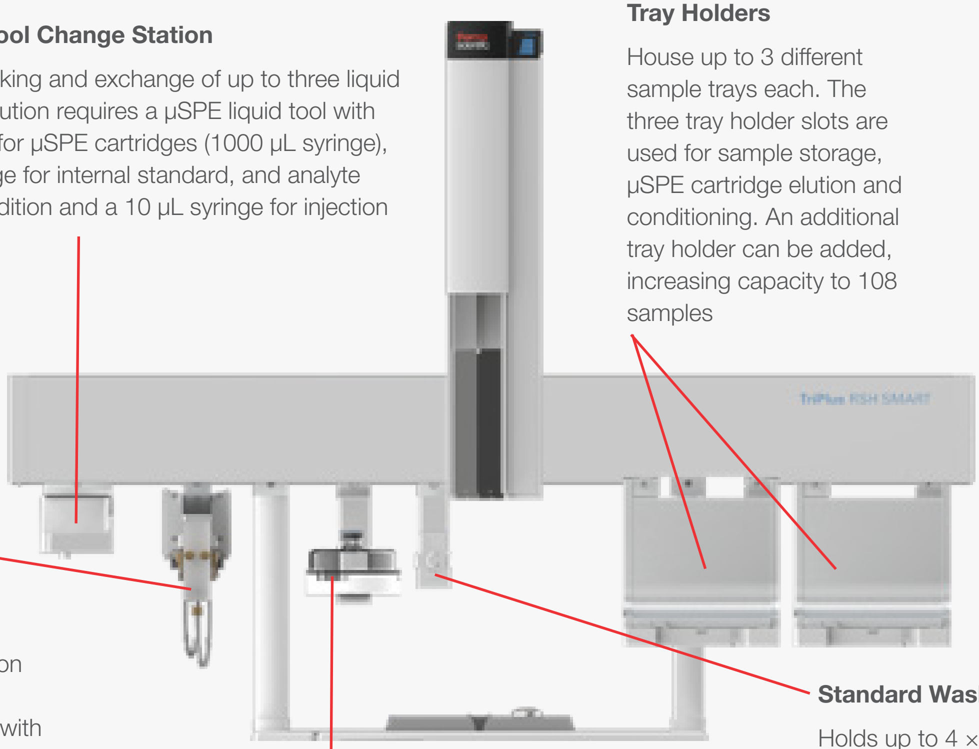
E. Hakme, M. Poulsen, J. of Chrom. A, Vol 1652 (2021)

Highlights

- Dedicated cartridges composition for GC-MS analyses
- Improved data repeatability
- No need for re-concentration step
- Avoid possible degradation or side reactions with on-line injection

Automated µSPE clean-up of QuEChERS extracts

Instrument set-up



Automatic Tool Change Station

Supports docking and exchange of up to three liquid tools. This solution requires a µSPE liquid tool with needle guide for µSPE cartridges (1000 µL syringe), a 25 µL syringe for internal standard, and analyte protectant addition and a 10 µL syringe for injection

Tray Holders

House up to 3 different sample trays each. The three tray holder slots are used for sample storage, µSPE cartridge elution and conditioning. An additional tray holder can be added, increasing capacity to 108 samples

Fast Wash Module

Provides sufficient wash solvent to clean the large volume sample preparation syringes. Wash 1 inlet is connected to a reservoir with MeOH/MeCN/H_2O (1:1:1) and Wash 2 is connected to a reservoir with Acetonitrile

Solvent Station

Holds 3 × 100 mL solvent bottles. Positions 1 and 2 are used for conditioning and elution solvents

Standard Wash Station

Holds up to 4 × 10 mL solvent vials and 1x 10 mL waste vial. Positions 1 and 2 are used for internal standard and analyte protectant, respectively. Positions 3 and 4 can be used for additional solvents

Resources

Automated Workflows Brochure

Automated µSPE Video

Automated Workflow Solution Web Page

Sampling Workflow Editor Tutorial

Highlights

- Fits a regular x-rail
- Overlapping of the clean-up with previous sample analysis (for on-line configuration)
- On-line injection or bench station for off-line sample preparation
- Suitable for cleaning-up samples for LC analysis

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Learn more at **thermofisher.com/triplusrsh**