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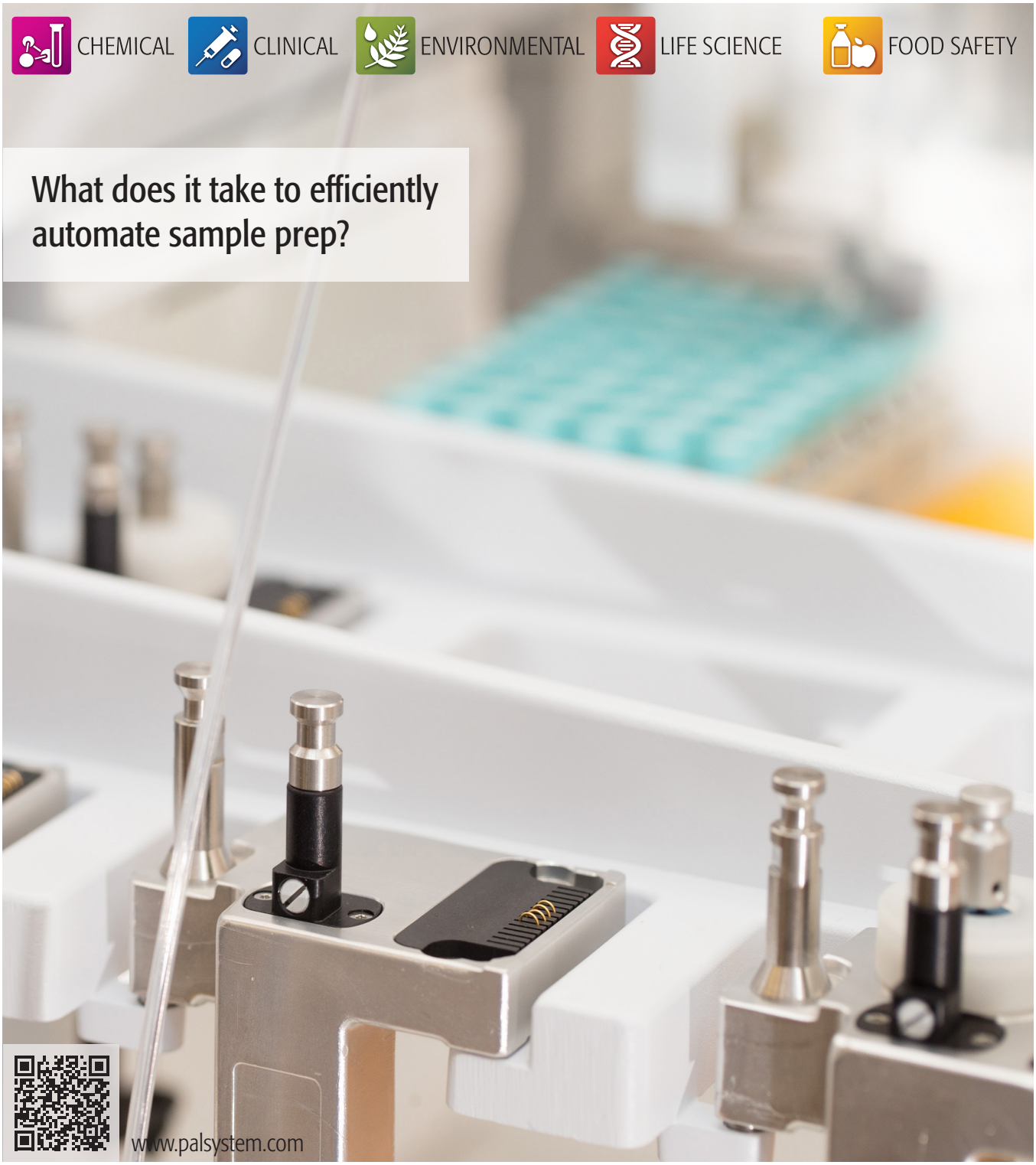
Ingenious sample handling

GC Application Note

CHEMICAL CLINICAL ENVIRONMENTAL LIFE SCIENCE FOOD SAFETY

What does it take to efficiently automate sample prep?



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Building sample prep workflows - what does it take?

It is remarkable that a large number of different sample prep workflows can be broken down to a small number of very similar operations. In this article we will look at the analysis of fatty acids as an example. The analysis of oils, fat and fat containing food by GC via fatty acid methyl esters (FAME) is frequently performed in governmental, quality control (QC) or contract research laboratories. Normally the samples are processed manually, which is labor intensive and exposes the lab personnel to potentially hazardous chemicals.

The procedure to generate and analyze FAME from the food samples is described in Fig. 1:

Manual step: Accurate weighing of sample (e.g. 1 drop = 15.3 mg), addtition of 1.53 mL dioxane, incl. three internal standards

- Transfer of 100 µL sample to a 2 mL vial
- Addition of 100 µL 5 % Na-methoxide in methanol (reagent)
- Mixing for 10 sec
- Reaction time 90 sec
- Addition of 1 mL n-heptane
- Mixing 10 sec
- Addition of 300 µL Na-citrate (15 % in water, to quench the transesterification)
- Vortexing 10 sec
- Wait for 60 sec (phase separation)
- Injection of 1 µL extract (upper phase) into the GC

Fig.1: Transesterification workflow for fatty acids, leading to FAME, with subsequent GC-analysis

A closer look reveals that the recurring steps of the procedure outlined in fig. 1 are of three types:

- Aspiration / dispensing of liquids
- Mixing
- Transport of objects (vials in this case)

Except for weighing of the sample the entire procedure can be accomplished with three basic operations. These operations can be readily transferred to a PAL RTC sample prep station. The figure on the next page describes the required steps.

Workflow

Sample transfer and addition of internal standard

- Pick up 100 µL syringe, sample
- Aspirate 100 µL sample and dispense into a 2 mL vial
- Wash syringe
- Change syringe (10 µL syringe, internal standard)
- Aspirate 10 µL of internal standard and dispense into a 2 mL vial

Derivatisation

- Change syringe (1000 µL syringe, reagent)
- Aspirate 100 µL 5 % Na-methoxide in methanol (reagent) and dispense into a 2 mL vial
- Transport vial to Vortex Mixer
- Vortex mixing 10 sec
- Transport vial to tray

>> Reaction time 90 sec

Liquid/liquid extraction

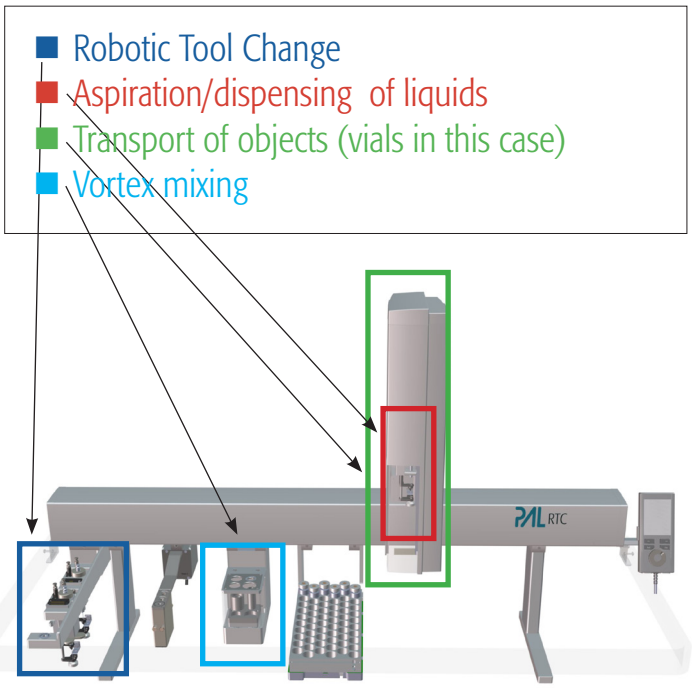
- Change syringe (1000 µL syringe, organic)
- Aspirate 1 mL n-heptane and dispense into a 2 mL vial
- Transport vial to Vortex Mixer
- Vortex mixing 10 sec
- Change syringe (1000 µL syringe, aqueous)
- Aspirate 300 µL Na-citrate (15 % in water, to quench the transesterification) and dispense into a 2 mL vial
- Transport vial to Vortex Mixer
- Vortex mixing 10 sec
- Transport vial to tray

>> Wait for 60 sec (phase separation)

Injection

- Change syringe (1 µL syringe, injection)
- Injection of 1 µL liquid/liquid extract (upper phase) to GC

Robotic Elements



Comment

The basic steps to perform the FAME workflow including subsequent GC analysis have been outlined here. Four basic operations are sufficient to accomplish the task, see above. The following pages describe in more detail how the generation of FAME is realized on a PAL RTC.

Always use the appropriate tool

- The key feature enabling the workflow described above is the Robotic Tool Change (RTC). This unique feature allows the PAL RTC to switch between different tools.

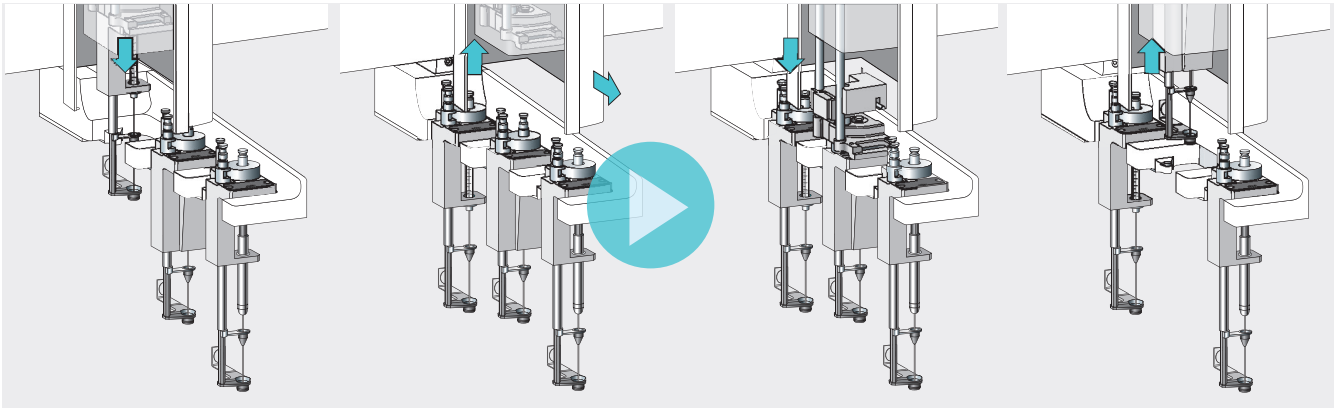


Fig. 1: Schematic of Robotic Tool Change, key feature enabling the use of different syringes or other devices during sample prep.

- The PAL RTC dispenses or injects µL to mL of liquids, always with the appropriate syringe for the required accuracy (Download pdf). It also performs headspace, SPME or ITEX (in-tube extraction) sample enrichment for GC. A listing and detailed description of all the available tools can be found here.

- When adding reagents or internal standards the resulting mixture needs to be completely homogenized. For efficient liquid/liquid extractions (LLE) vigorous mixing of the two phases is mandatory. The PAL RTC mixes solutions and suspensions thoroughly (see Link) in vials up to 40 mL.

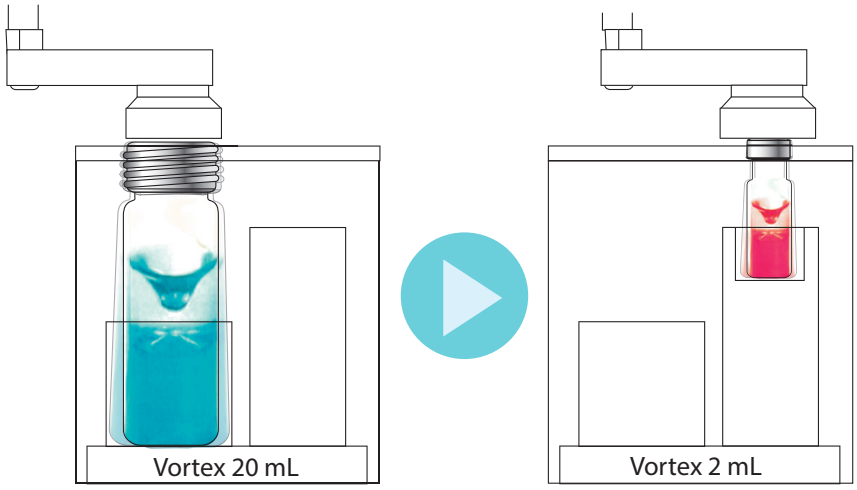


Fig. 2: The Vortex Mixer Module for vials up to 40 mL

- The PAL RTC transports objects (e.g. vials up to 40 mL), even larger ones like well plates (see video Link)

- Last (in the process), but not least is the fact that the PAL RTC performs the injection into the analyzer (LC, GC, spectrometer) with the appropriate tools.

- With PAL Sample Control setting up of methods is straightforward. It is interfaces directly to most chromatographic data systems.

Summary

With the simple building blocks described above applications like the ones listed below are easily realized. (The links lead to publications, posters or application notes on each topic):

- Standard addition prior to injection (different syringe types, mixer)
- Standard addition to GC headspace samples
- Serial dilution and automatic generation of calibration standards (Download pdf)
- Derivatisations like fatty acids to FAME (Download pdf) , aldehydes with PFBHA (Download pdf), steroids with MSTFA (doping analysis Link)
- Liquid/liquid extraction (Download pdf)
- Automated method development, e.g. applying tool change to select the optimal SPME fiber (Download pdf)

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Imprint

Date of print: 10.2015

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