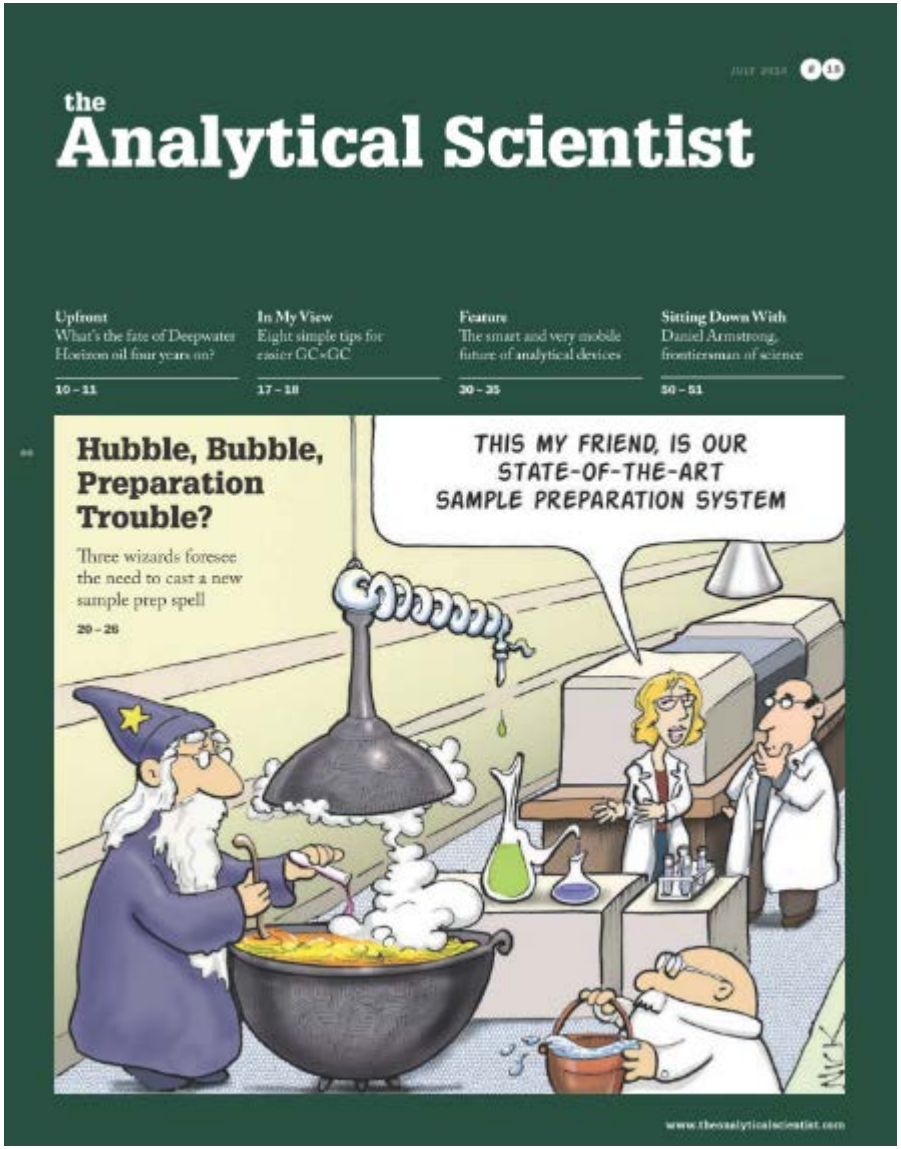
PAL System Applikationen

Mevio Heierli

PAL SYSTEM
Ingenious sample handling

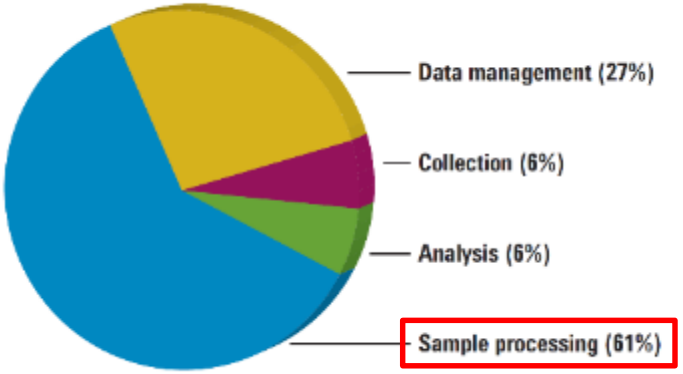
Sample Preparation?



PAL SYSTEM
Ingenious sample handling

Einige Zahlen

Time Spent on Typical Chromatographic Analysis

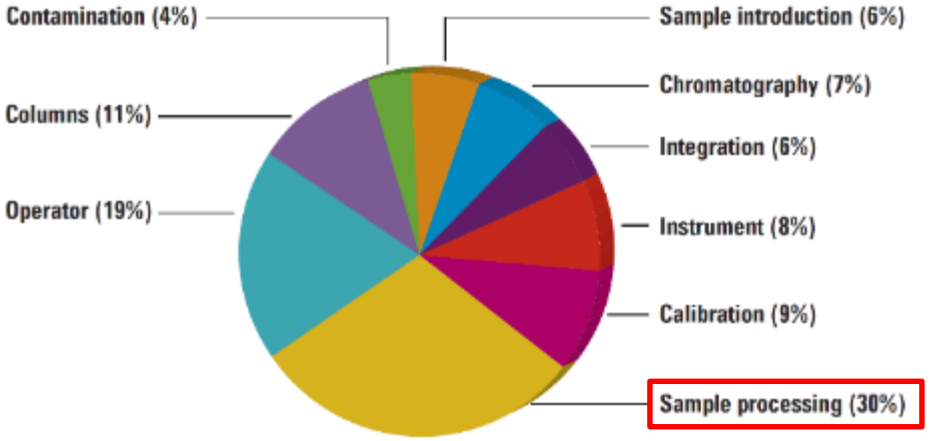


Data taken from Agilent Technologies survey

61% of time is spend for sample processing

30% of errors are coming form sample processing

Sources of Error Generated During Chromatographic Analysis



Data taken from Agilent Technologies survey

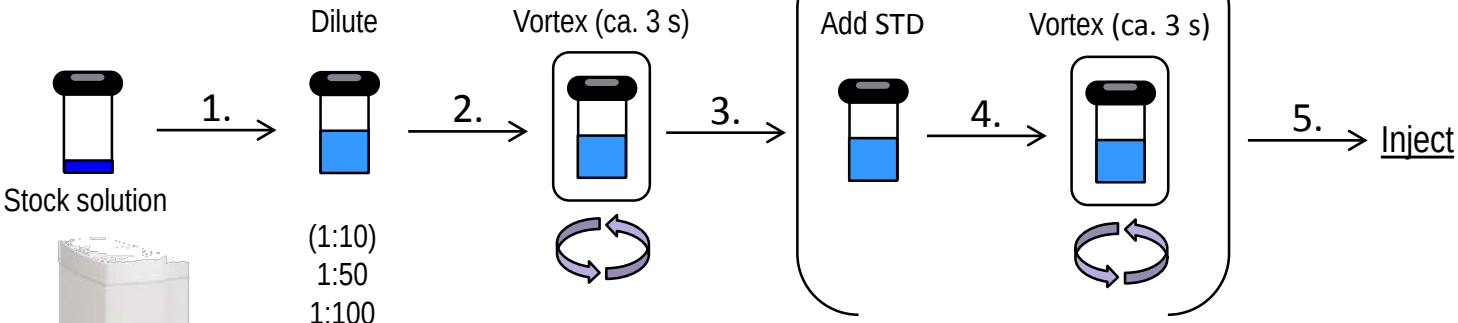
PAL SYSTEM
Ingenious sample handling

Standard Addition
Serial Dilution

Standard Addition – Serial Dilution

General Steps:

1. Dilute
2. Vortex
3. Add STD
4. Vortex
5. Inject



Kowal S, Balsaa P, Werres F, Schmidt TC;
Anal Bioanal Chem. 2013 Jul;405(19):6337-51
Fully automated standard addition method for the quantification of 29 polar pesticide metabolites in different water bodies using LC-MS/MS.

PAL SYSTEM
Ingenious sample handling


Dilution
Workstation

Dilution Workstation

- Show the reproducibility and accuracy of automated dilutions with a PAL RTC
- Test is done on a 14 compound mixture
- Range of dilution
  - Stock solution at 4 mg/mL
  - Dilutions from 400 to 1 µg/mL (9 vials) in hexane

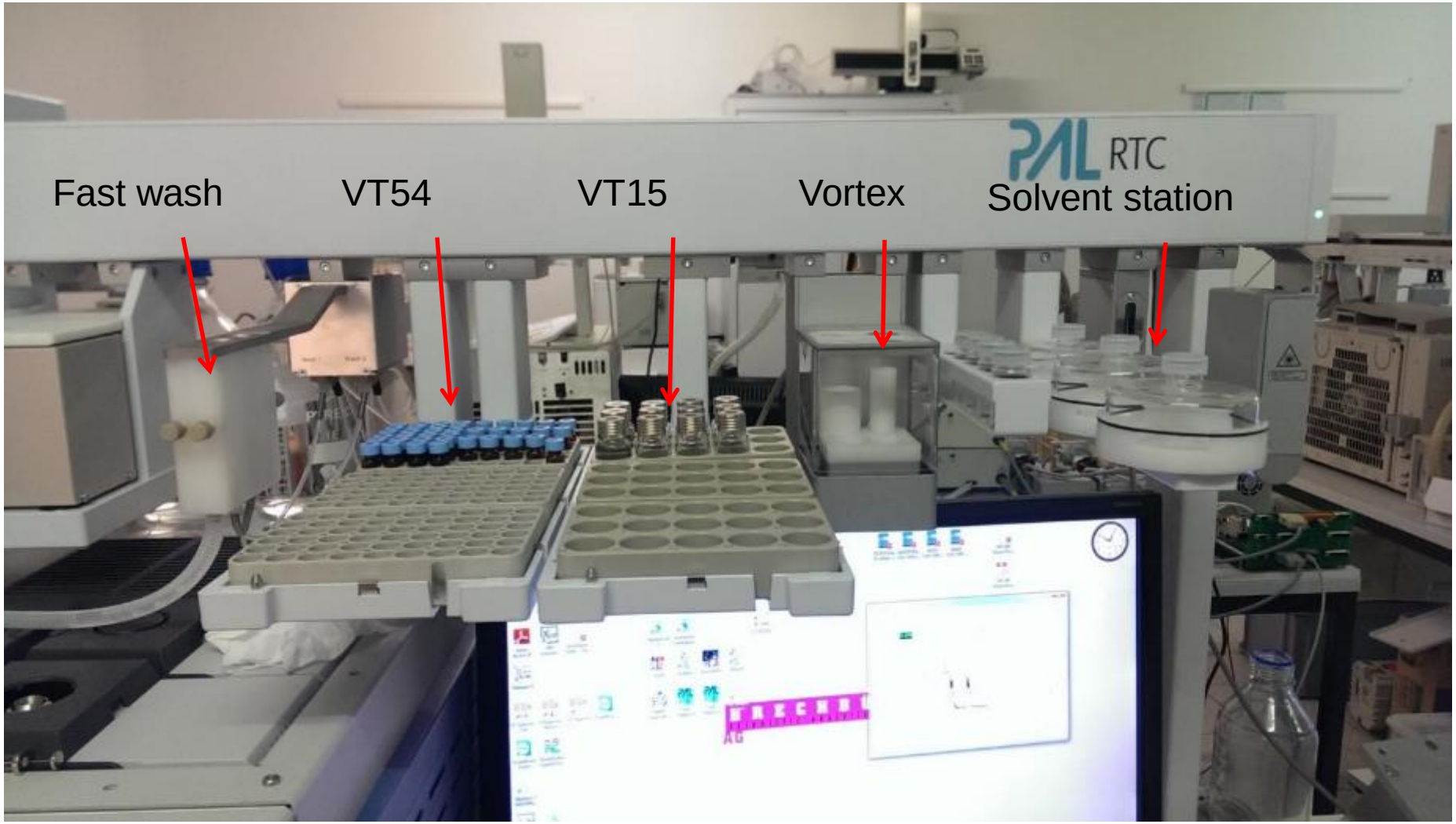
Many thanks to Philippe Mottay, Brechbühler AG, Schlieren, Switzerland

PAL Setup

- PAL RTC equipped with
  - 2 park stations
  - 2x 1000 µL syringe
  - 2x 100µL syringe
  - 2x 10µL syringe
  - Vortex mixer
  - Solvent module
  - Fast wash station
  - VT54 tray
  - VT15 tray
- Software PAL Sample Control

PAL SYSTEM
Ingenious sample handling

PAL Setup



PAL SYSTEM
Ingenious sample handling

Verification of method with GC compatible compounds

- The method was tested with GC compatible compounds
- Mixture of 14 compounds at 4 mg/ml
- Range of dilutions
  - 400; 200;100; 40; 20; 10; 4; 2; 1 µg/ml in Hexane
- Measured by GC/FID (Thermo Trace 1310)
- Method:
  - 40°C, 4 min to 260°C @15°C/min hold 1.5 min.
  - Split injection (20/1) at 260°C, column flow 2 ml/min
  - Detector at 270°C

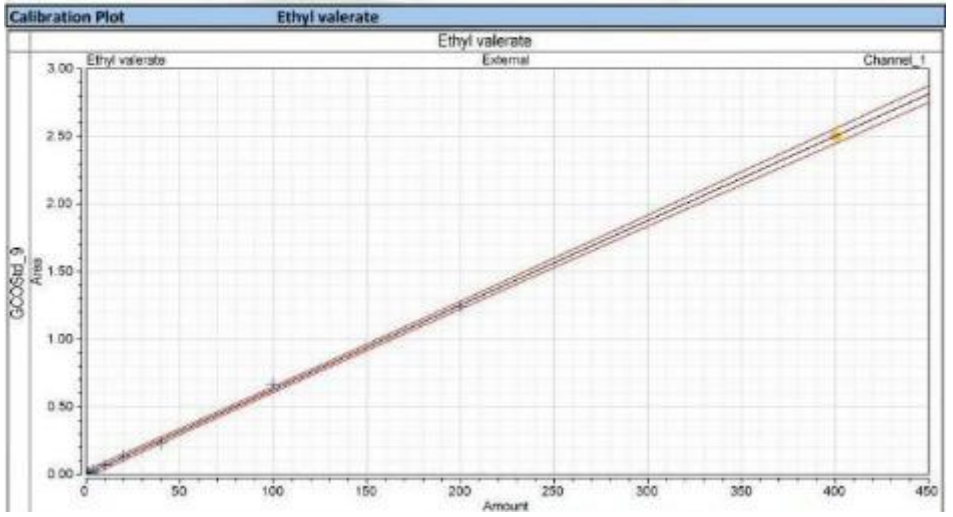
Calibration results

Calibration Details	Butyl acetate		
Calibration Type	Lin, WithOffset	Offset (C0)	0.0020
Evaluation Type	Area	Slope (C1)	0.0078
Number of Calibration Points	9	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9999

Calibration Plot — Butyl acetate

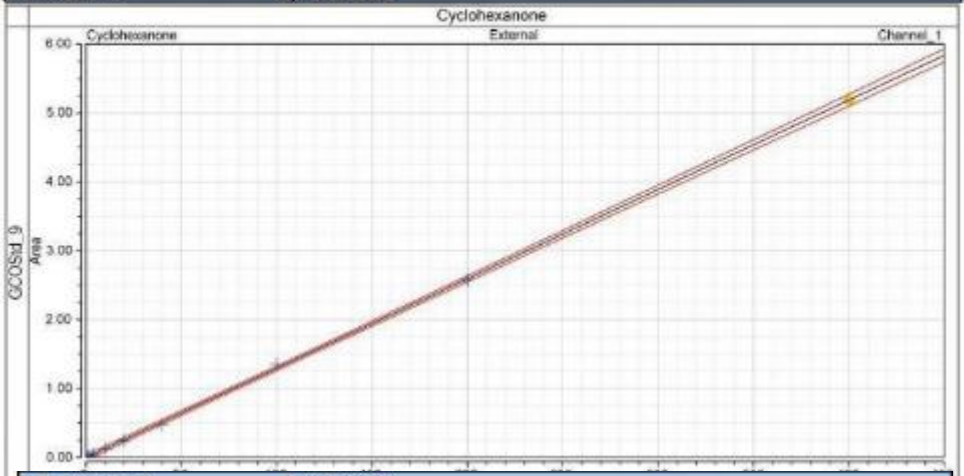
Calibration Details	Ethyl valerate		
Calibration Type	Lin, WithOffset	Offset (C0)	0.0023
Evaluation Type	Area	Slope (C1)	0.0062
Number of Calibration Points	9	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9996

Calibration Plot — Ethyl valerate



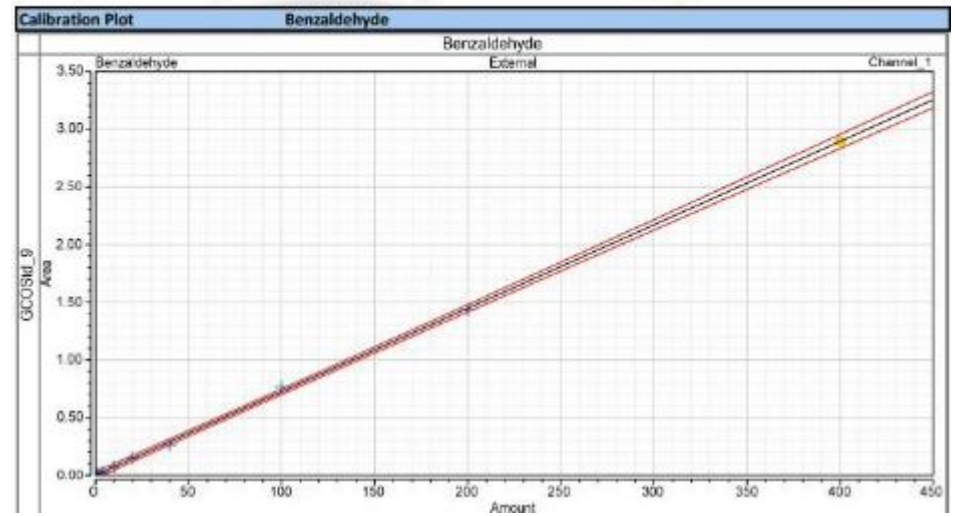
Calibration Details	Cyclohexanone		
Calibration Type	Lin, WithOffset	Offset (C0)	-0.0047
Evaluation Type	Area	Slope (C1)	0.0130
Number of Calibration Points	9	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9998

Calibration Plot — Cyclohexanone



Calibration Details	Benzaldehyde		
Calibration Type	Lin, WithOffset	Offset (C0)	0.0017
Evaluation Type	Area	Slope (C1)	0.0072
Number of Calibration Points	9	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9996

Calibration Plot — Benzaldehyde



Calibration results

Compound Name	R square
Butyl acetate	0.9999
Cyclohexanone	0.9998
Ethyl valerate	0.9996
Benzaldehyde	0.9996
Beta-pinene	0.9995
C10	0.9995
Limonene	0.9995
Linalool	0.9995
Benzyl acetate	0.9995
Menthol	0.9995
Citronellol	0.9995
Geraniol	0.9995
Coumarin	0.9997
Alpha Ionone	0.9995

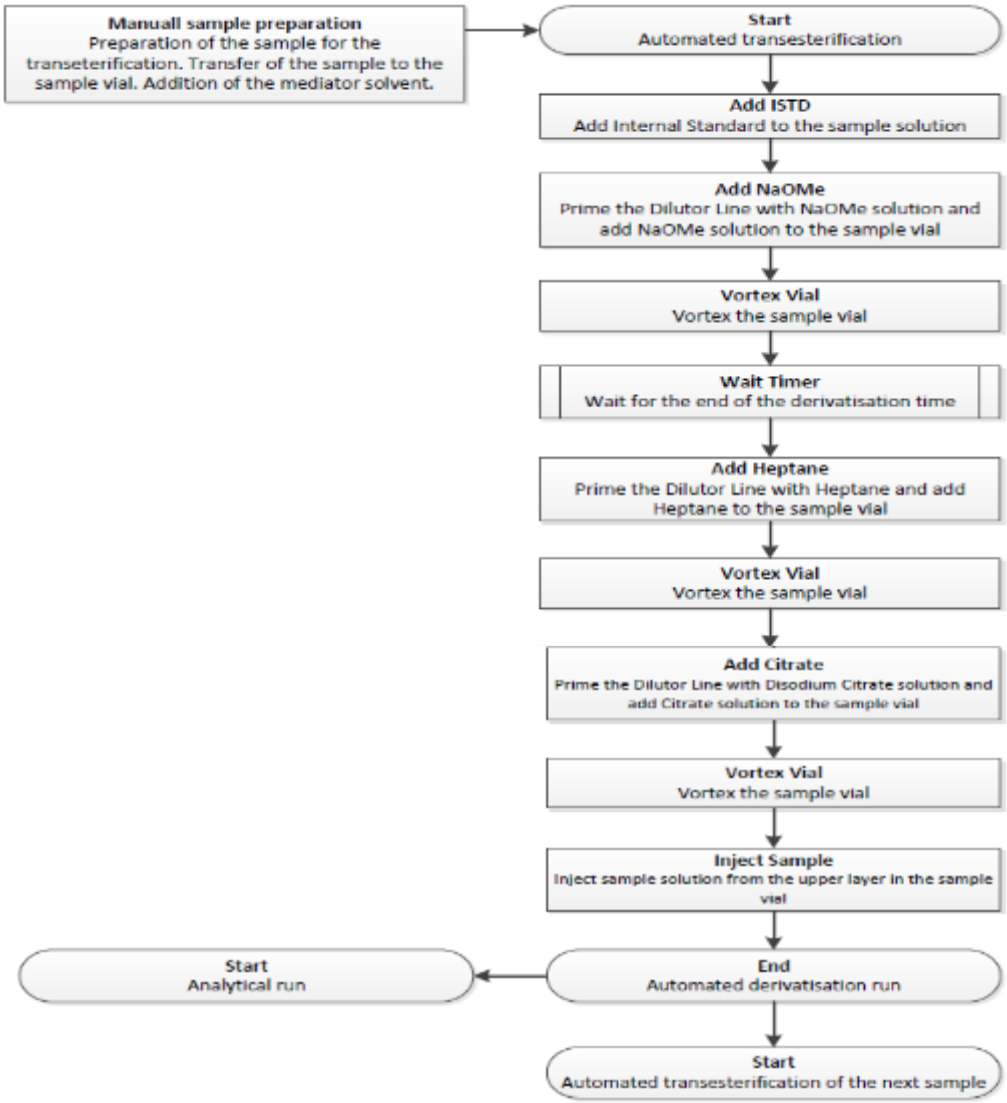
PAL SYSTEM
Ingenious sample handling

Derivatisation Workflow

Determination Fatty Acids as FAME by GC/MS

- Determination of fatty acid composition and content of foods
- Determination of Biodiesel composition
- Trans-esterification of fatty acids to FAME is a very common and at the same time tedious procedure.
- Automation increases productivity and prevents exposure of humans to hazardous chemicals.

Derivatisation Workflow FAME



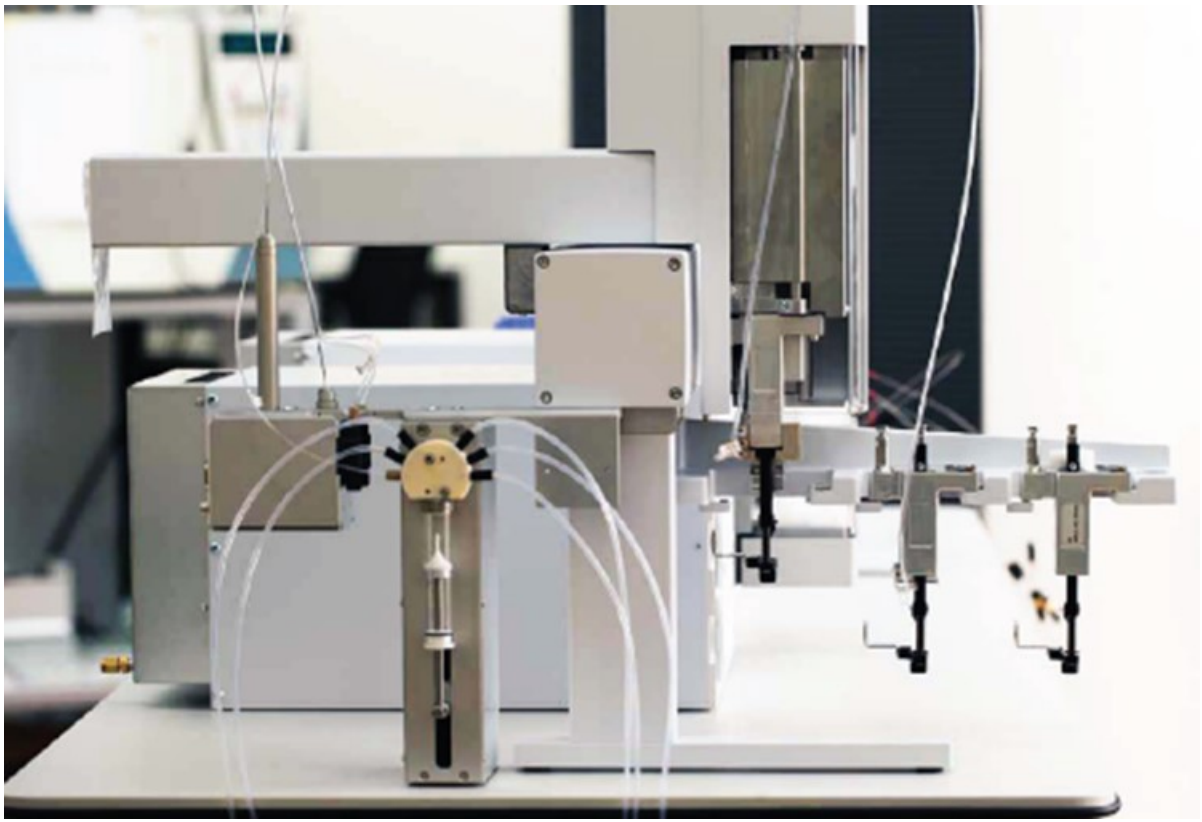
Generation of Fatty Acid Methylesters (FAME) with 1 min. Transesterification for GC/MS analysis

According to Eidg. Untersuchungs-methode 269.1

PAL SYSTEM
Ingenious sample handling

5 Port Dilutor Module

- Addition of Methyl ester / Heptane / Citrate
- Wash steps trough the dilutor



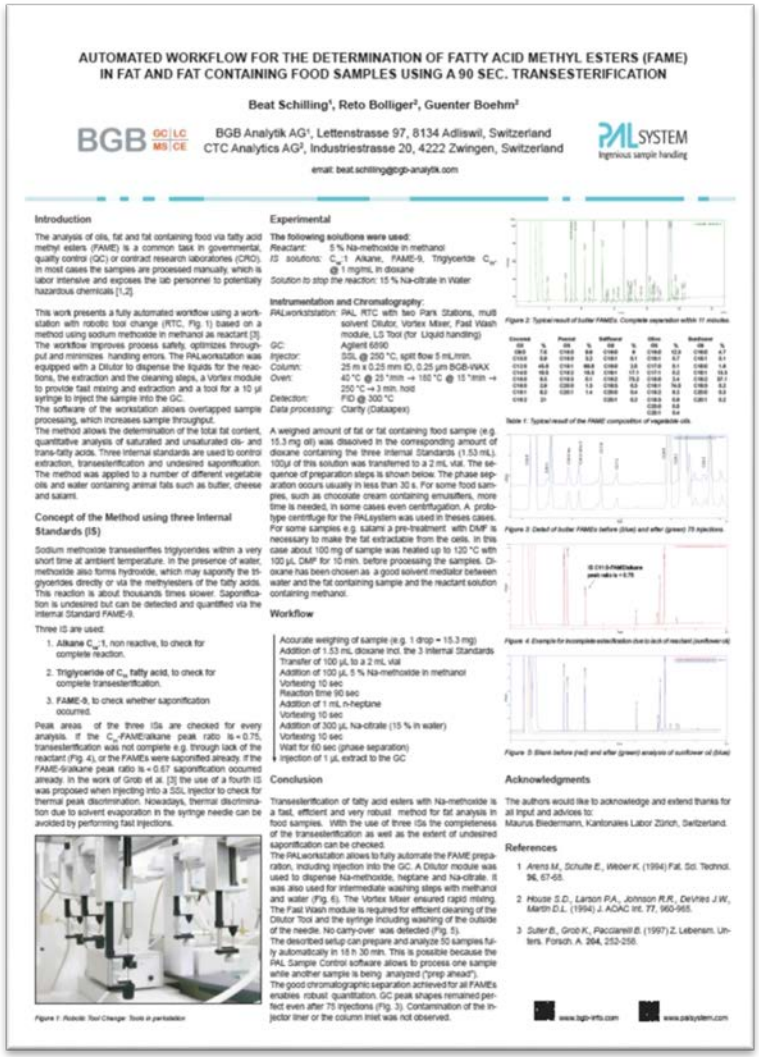
PAL SYSTEM
Ingenious sample handling

Poster presented at ISCC, Riva 2014

Automated Workflow for the Determination of Fatty Acid Methyl Esters (FAME) out of Fat and Fat Containing Food Samples using a 90 sec. Transesterification.

Beat Schilling[1], Reto Bolliger[2], Guenter Boehm[2]

[1] BGB Analytik AG, 8134 Adliswil, Switzerland, [2] CTC Analytics AG, 4222 Zwingen, Switzerland



AUTOMATED WORKFLOW FOR THE DETERMINATION OF FATTY ACID METHYL ESTERS (FAME) IN FAT AND FAT CONTAINING FOOD SAMPLES USING A 90 SEC. TRANSESTERIFICATION

Beat Schilling[1], Reto Bolliger[2], Guenter Boehm[2]

BGB Analytik AG[1], Lettenstrasse 97, 8134 Adliswil, Switzerland
CTC Analytics AG[2], Industriestrasse 20, 4222 Zwingen, Switzerland

email: beat.schilling@bgb-analytik.com

Introduction

The analysis of oils, fat and fat containing food via fatty acid methyl esters (FAME) is a common task in governmental, quality control (QC) or contract research laboratories (CRO). In most cases the samples are processed manually, which is labor intensive and exposes the lab personnel to potentially hazardous chemicals [1,2].

This work presents a fully automated workflow using a workstation with robotic tool change (RTC, Fig. 1) based on a method using sodium methoxide in methanol as reactant [3]. The workflow improves process safety, optimizes throughput and minimizes handling errors. The PALworkstation was equipped with a Dilutor to dispense the liquids for the reactions, the extraction and the cleaning steps, a Vortex module to provide fast mixing and extraction and a tool for a 10 µl syringe to inject the sample into the GC.
The software of the workstation allows overlapped sample processing, which increases sample throughput.
The method allows the determination of the total fat content, quantitative analysis of saturated and unsaturated cis- and trans-fatty acids. Three internal standards are used to control extraction, transesterification and undesired saponification. The method was applied to a number of different vegetable oils and water containing animal fats such as butter, cheese and salami.

Concept of the Method using three Internal Standards (IS)

Sodium methoxide transesterifies triglycerides within a very short time at ambient temperature. In the presence of water, methoxide also forms hydroxide, which may saponify the triglycerides directly or via the methylesters of the fatty acids. This reaction is about thousands times slower. Saponification is undesired but can be detected and quantified via the internal Standard FAME-9.

Three IS are used:

1. Alkane C_{xx}-1, non reactive, to check for complete reaction.
2. Triglyceride of C_{xx} fatty acid, to check for complete transesterification.
3. FAME-9, to check whether saponification occurred.

Peak areas of the three ISs are checked for every analysis. If the C_{xx}-FAME/alkane peak ratio is < 0.75, transesterification was not complete e.g. through lack of the reactant (Fig. 4), or the FAMEs were saponified already. If the FAME-9/alkane peak ratio is < 0.67 saponification occurred already. In the work of Grob et al. [3] the use of a fourth IS was proposed when injecting into a SSL injector to check for thermal peak discrimination. Nowadays, thermal discrimination due to solvent evaporation in the syringe needle can be avoided by performing fast injections.

Experimental

The following solutions were used:
Reactant: 5 % Na-methoxide in methanol
IS solutions: C_{xx}-1 Alkane, FAME-9, Triglyceride C_{xx} @ 1 mg/mL in dioxane
Solution to stop the reaction: 15 % Na-citrate in Water

Instrumentation and Chromatography:

PALworkstation:	PAL RTC with two Park Stations, multi solvent Dilutor, Vortex Mixer, Fast Wash module, LS Tool (for Liquid handling)
GC:	Agilent 6890
Injector:	SSL @ 250 °C, split flow 5 mL/min.
Column:	25 m x 0.25 mm ID, 0.25 µm BGB-WAX
Oven:	40 °C @ 25 °/min → 160 °C @ 15 °/min → 250 °C → 3 min. hold
Detection:	FID @ 300 °C
Data processing:	Clarity (Datapex)

A weighed amount of fat or fat containing food sample (e.g. 15.3 mg oil) was dissolved in the corresponding amount of dioxane containing the three internal Standards (1.53 mL). 100µl of this solution was transferred to a 2 mL vial. The sequence of preparation steps is shown below. The phase separation occurs usually in less than 30 s. For some food samples, such as chocolate cream containing emulsifiers, more time is needed, in some cases even centrifugation. A prototype centrifuge for the PALsystem was used in theses cases. For some samples e.g. salami a pre-treatment with DMF is necessary to make the fat extractable from the cells. In this case about 100 mg of sample was heated up to 120 °C with 100 µL DMF for 10 min. before processing the samples. Dioxane has been chosen as a good solvent mediator between water and the fat containing sample and the reactant solution containing methanol.

Workflow

- Accurate weighing of sample (e.g. 1 drop = 15.3 mg)
- Addition of 1.53 mL dioxane incl. the 3 Internal Standards
- Transfer of 100 µL to a 2 mL vial
- Addition of 100 µL 5 % Na-methoxide in methanol
- Vortexing 10 sec
- Reaction time 90 sec
- Addition of 1 mL n-heptane
- Vortexing 10 sec
- Addition of 300 µL Na-citrate (15 % in water)
- Vortexing 10 sec
- Wait for 60 sec (phase separation)
- Injection of 1 µL extract to the GC

Conclusion

Transesterification of fatty acid esters with Na-methoxide is a fast, efficient and very robust method for fat analysis in food samples. With the use of three ISs the completeness of the transesterification as well as the extent of undesired saponification can be checked.
The PALworkstation allows to fully automate the FAME preparation, including injection into the GC. A Dilutor module was used to dispense Na-methoxide, heptane and Na-citrate. It was also used for intermediate washing steps with methanol and water (Fig. 6). The Vortex Mixer ensured rapid mixing. The Fast Wash module is required for efficient cleaning of the Dilutor Tool and the syringe including washing of the outside of the needle. No carry-over was detected (Fig. 5).
The described setup can prepare and analyze 50 samples fully automatically in 18 h 30 min. This is possible because the PAL Sample Control software allows to process one sample while another sample is being analyzed ("prep ahead").
The good chromatographic separation achieved for all FAMEs enables robust quantitation. GC peak shapes remained perfect even after 75 injections (Fig. 3). Contamination of the injector liner or the column inlet was not observed.

Acknowledgments

The authors would like to acknowledge and extend thanks for all input and advices to:
Maurus Biedermann, Kantonales Labor Zürich, Switzerland.

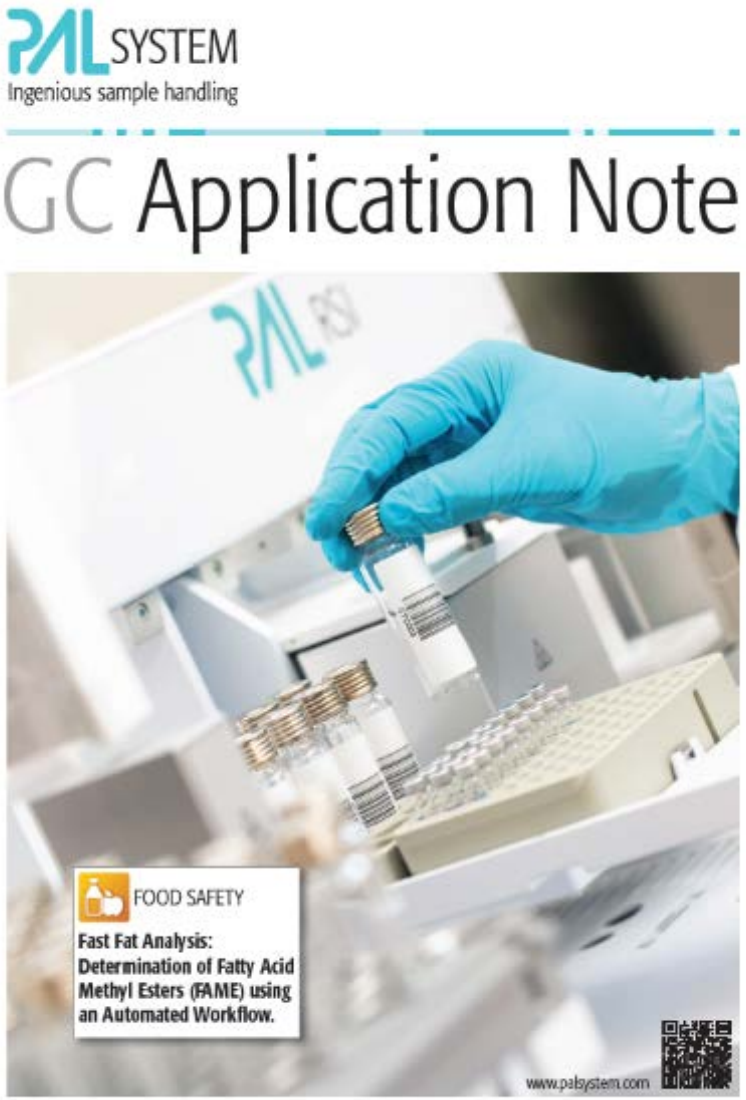
References

1 *Arens M., Schulte E., Weber K.* (1994) Fat. Sci. Technol. 96, 67-68.
2 *House S.D., Larson P.A., Johnson R.R., DeVries J.W., Martin D.L.* (1994) J. AOAC Int. 77, 960-965.
3 *Suter B., Grob K., Pacciarelli B.* (1997) Z. Lebensm. Unters. Forsch. A. 204, 252-258.

- Fast and reliable derivatization
- Very good accuracy and precision
- Excellent separation of different FAMEs
- High productivity
- Traceability

PAL SYSTEM
Ingenious sample handling

Application Note(s) auf www.palsystem.com



PAL SYSTEM
Ingenious sample handling

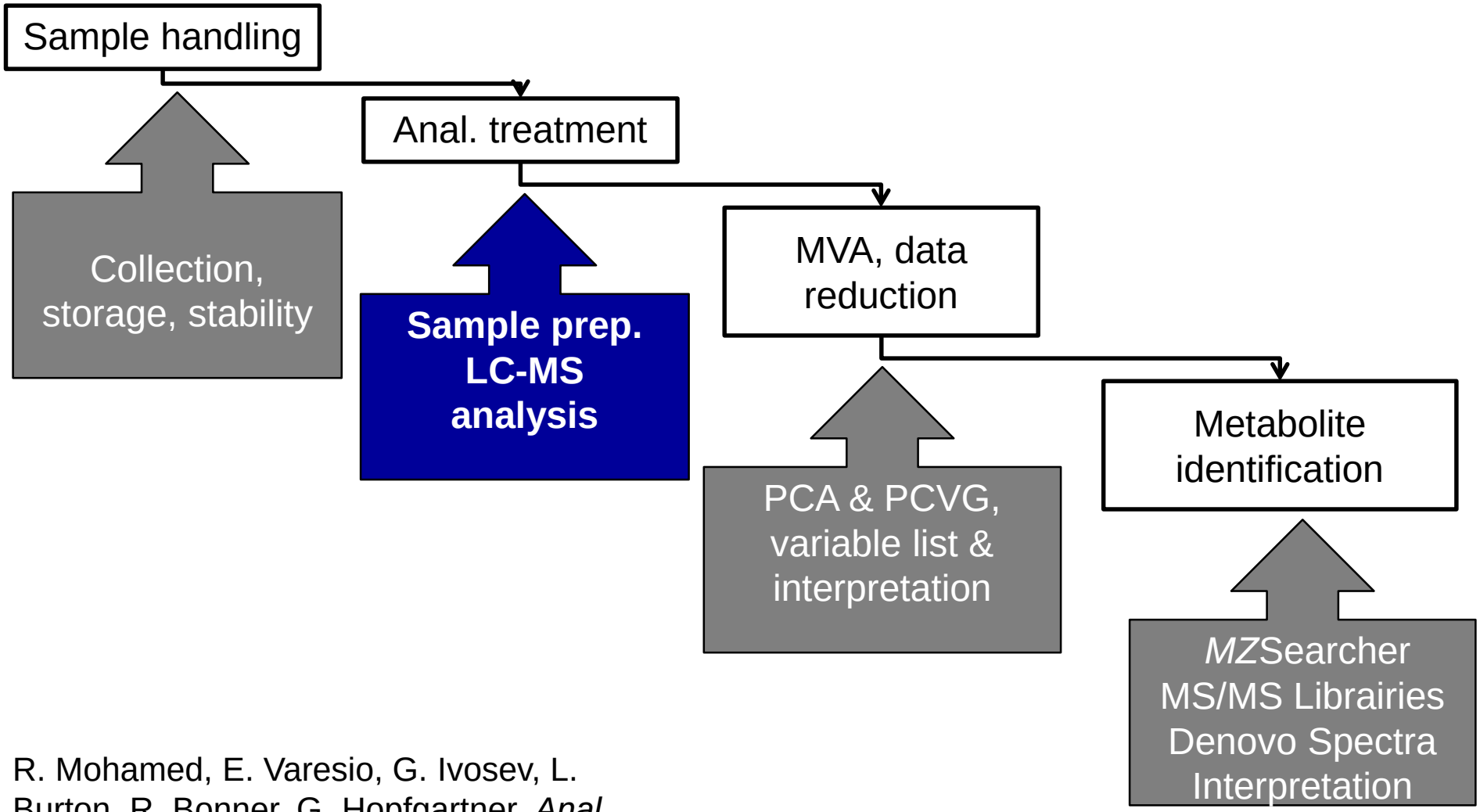
Integrated Platform Including Bligh and Dyer Extraction and Dual-Column UHPLC-MS/MS Separations for Metabolomics Studies

Gérard Hopfgartner, Sandra Jahn and Emmanuel Varesio

Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences
EPGL, University of Lausanne, University of Geneva
30 Quai Ernest Ansermet, CH-1211 Geneva 4, Switzerland

CTC Sunday Workshop @ IMSC 2014
Sunday, August 24th 2014, Geneva, Switzerland

Identification of Endogenous Metabolites from *Chlamydomonas reinhardtii* Algae



R. Mohamed, E. Varesio, G. Ivosev, L. Burton, R. Bonner, G. Hopfgartner. *Anal. Chem*, 81(18), 7677-7694, (2009).

Integrated Bligh and Dyer Extraction Workflow

b) Automated on-line sample preparation with RTC platform

Aspirate upper fraction

Aspirate lower fraction

Add 225 µL H_2O + 225 µL $CHCl_3$
Vortex for 10 s
Centrifuge for 5 min. (4000 rpm, ≈ 900 x g)

1) Aspirate 500 µL of **upper fraction**
(H_2O-MeOH fraction)

2) Aspirate 250 µL of **lower fraction**
($CHCl_3$ fraction)

Dilution 5-fold by adding 60 µL
to 240 µL of dilution solvent
(≈ 10% MeOH in samples)

pH 3 **pH 8**

Add chromatographic standards

Evaporate to dryness
with $N_{2\ gas}$
(0.35 bar, 35°C,10 min.)

Evaporation device

Reconstitute in 150 µL MeOH
Add chromatographic standards

Alternately inject 25 µL on C_{18} column
(UHPLC system 1)

Inject 5 µL on C_8 column
(UHPLC system 2)

UHPLC Conditions and Timings

UHPLC system 1

Aqueous fraction (AQ)

Flow = 400 µl/min

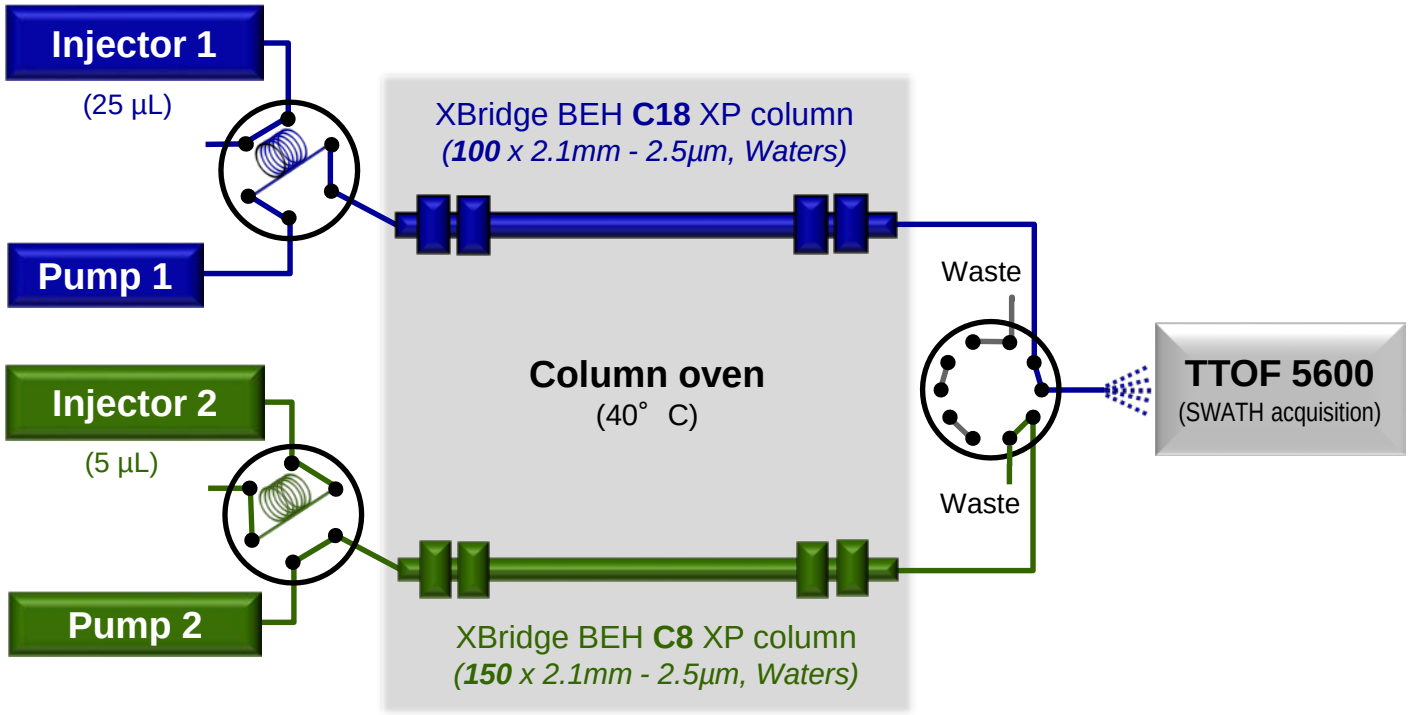
A) 5 mM NH_4Formate (**pH 3.0**)
B) ACN + 0.1% FA
C) 0.025% NH_4OH (**pH 8.3**)
D) ACN + 0.0125% NH_4OH

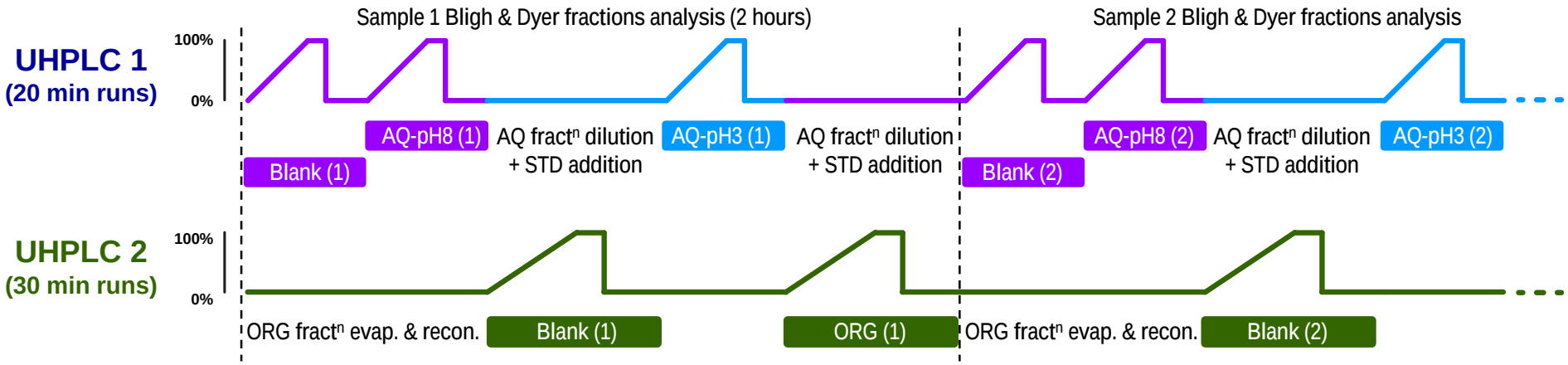
UHPLC system 2

Organic fraction (ORG)

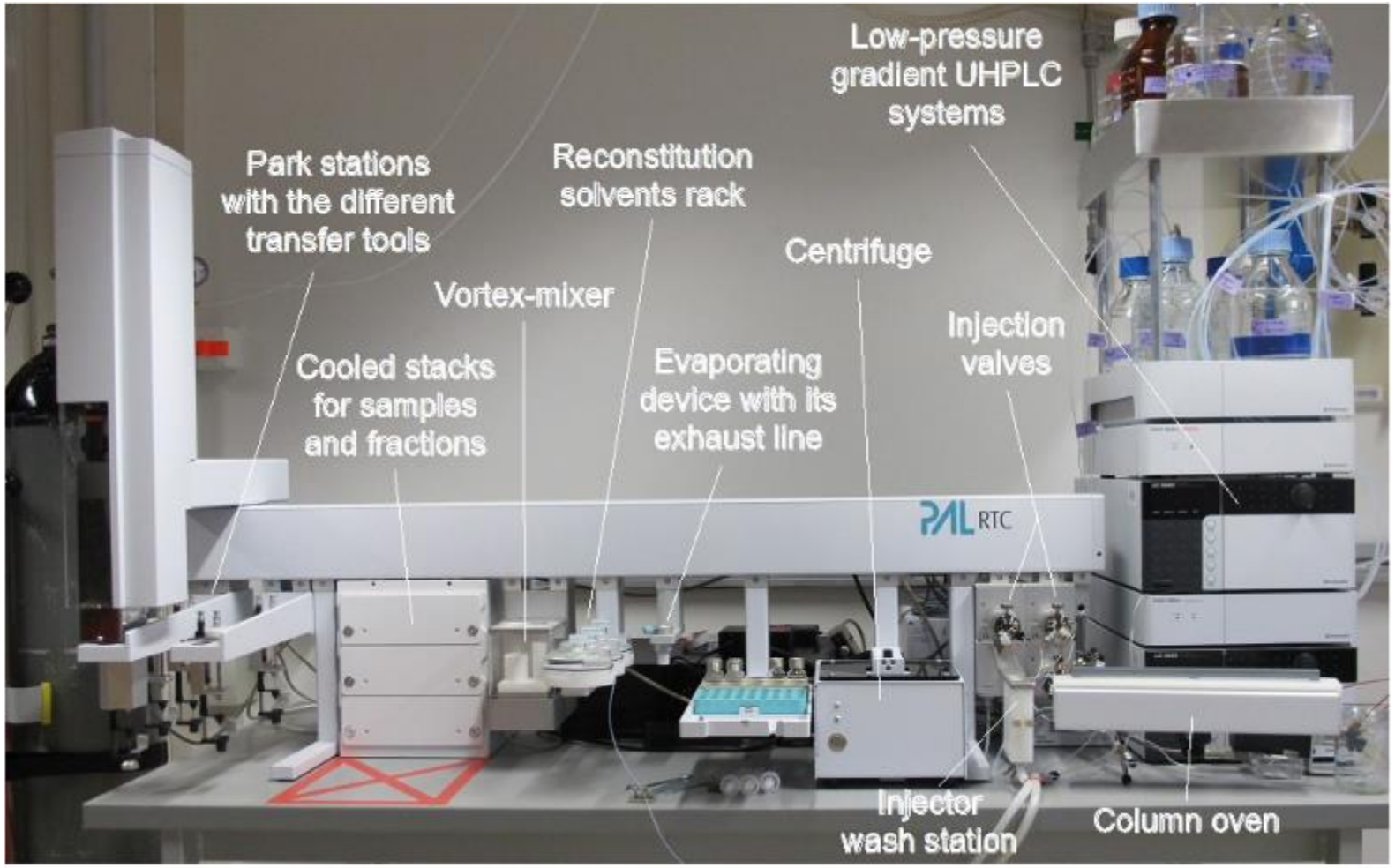
Flow = 300 µl/min

A) 5 mM NH_4Acetate (pH 4.2)
B) ACN + 0.1% AA





Instrumental Platform



- Robotic Tool Change (RTC) PAL system with several modules (CTC Analytics)
- Two quaternary LPG Nexera LC30AD UHPLC pumps (Shimadzu)
- TripleTOF 5600 mass spectrometer with CDS device (AB SCIEX)


Thak you very much!