Automated preparation of samples for fatty acid analysis using the Agilent workbench

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*Overview

- **Workbench?:**

What it does and it's function

- **Fat extraction methods we employ:**

Various methods in fat determination and samples that are analysed

- **How the workbench is incorporated into these methods:**

Its use in methods and its advantages

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* Agilent 7696A Sample prep Workbench
* Standalone automated sample preparation platform
* Capable of volumetric transfer of liquids between 2ml vials
* Performs accurate addition of reagents, dilutions, vortexing, heating of samples etc.
* Compliment to a GC/HPLC instrument

*Workbench

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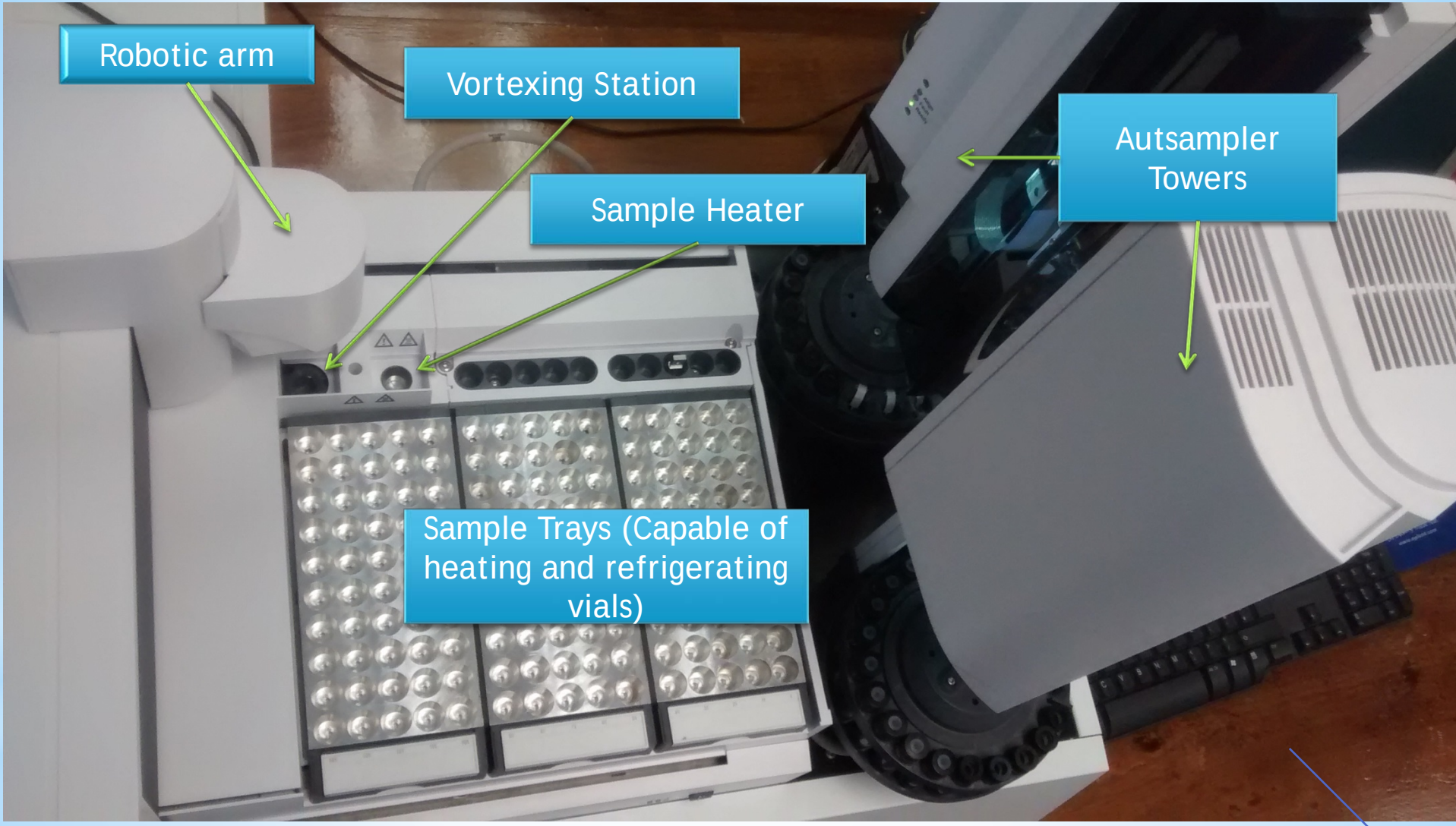

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*Automation

*Fat Extraction

- Triglyceride Determination→ Analysed as fatty acid methyl esters (FAMEs) using sodium methoxide in methanol (NaMeO)
- Free Fatty Acids→ Analysed as Free fatty acids, FAMEs using tetramethylammonium hydroxide (TMAH) or butyl esters using boron triflouride (BF3) in butanol
- Analysis of milk, cheese, milk powders, infant formula etc..

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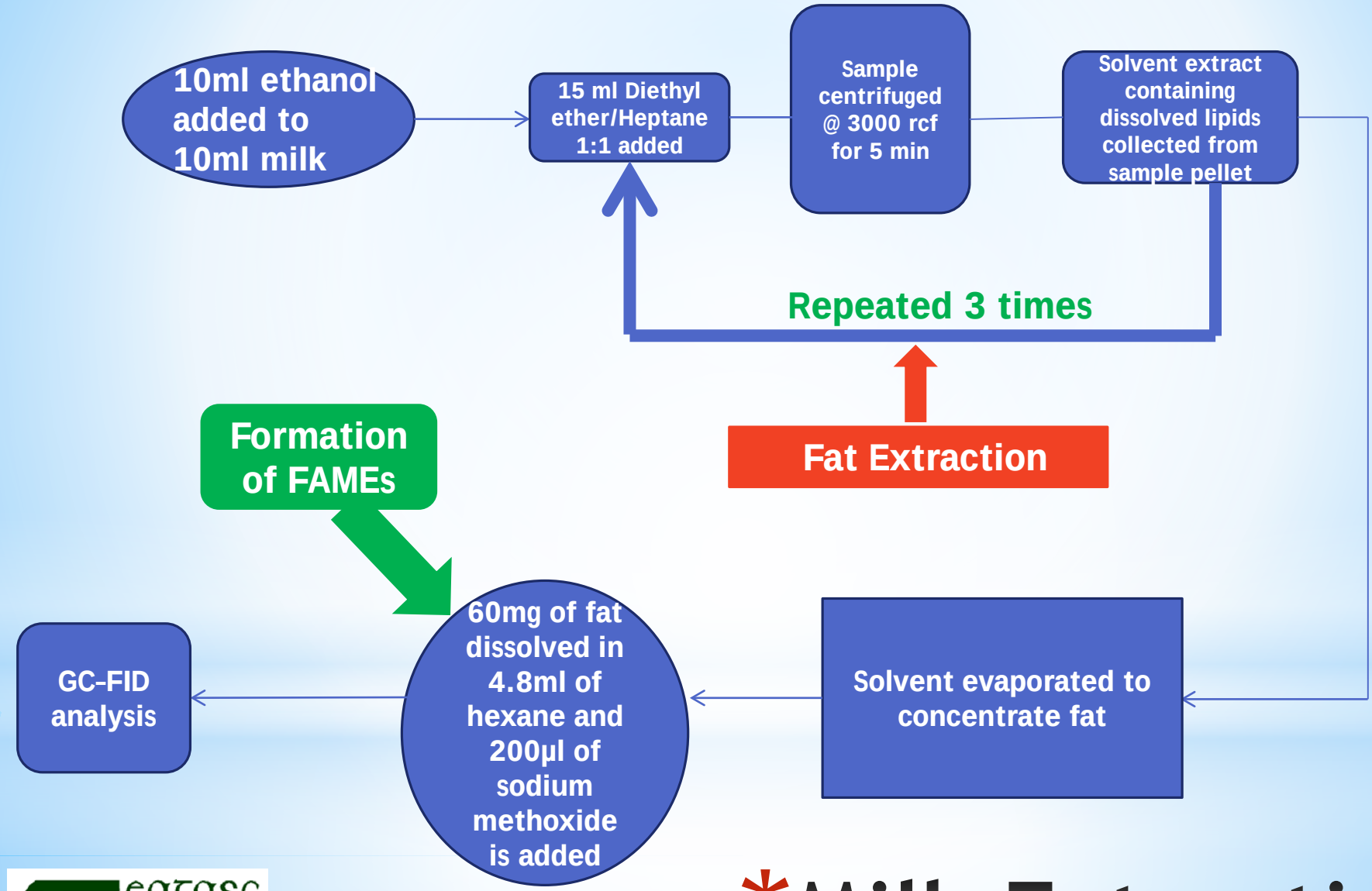

*Milk Extraction

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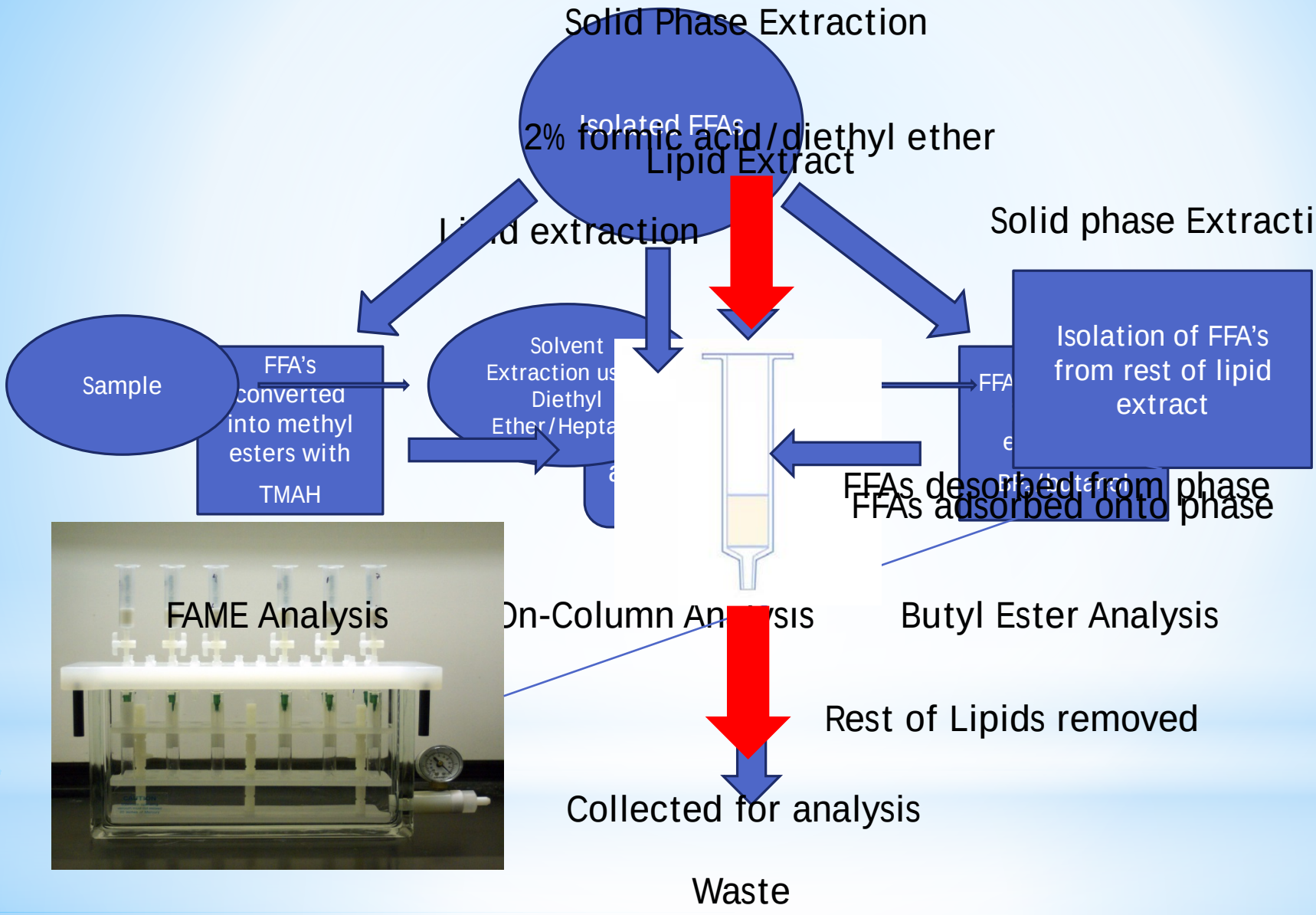

*FFA extraction

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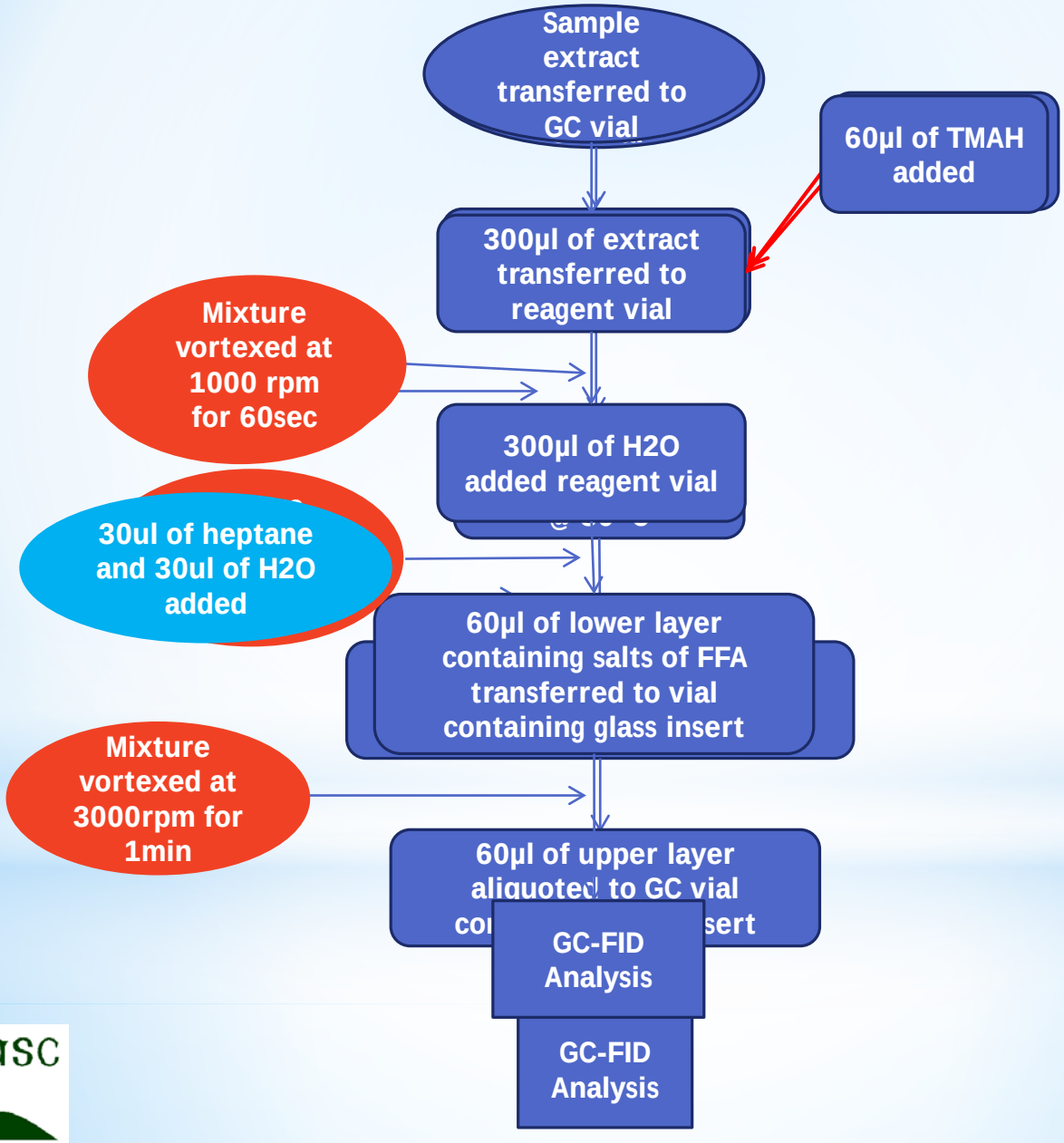
Methods:

* Derivitsation of triglycerides to FAMEs using NaMeO
* Derivitisation of FFAs to FAMEs using TMAH
* Derivisation of FFAs to butyl esters using BF3/Butanol

Incorporating the Workbench:

* Used to automate the derivitisation steps
* Used in sample and standard preparation eg. Dilution of sample extract, preparation of calibration levels etc.

Butyl Ester



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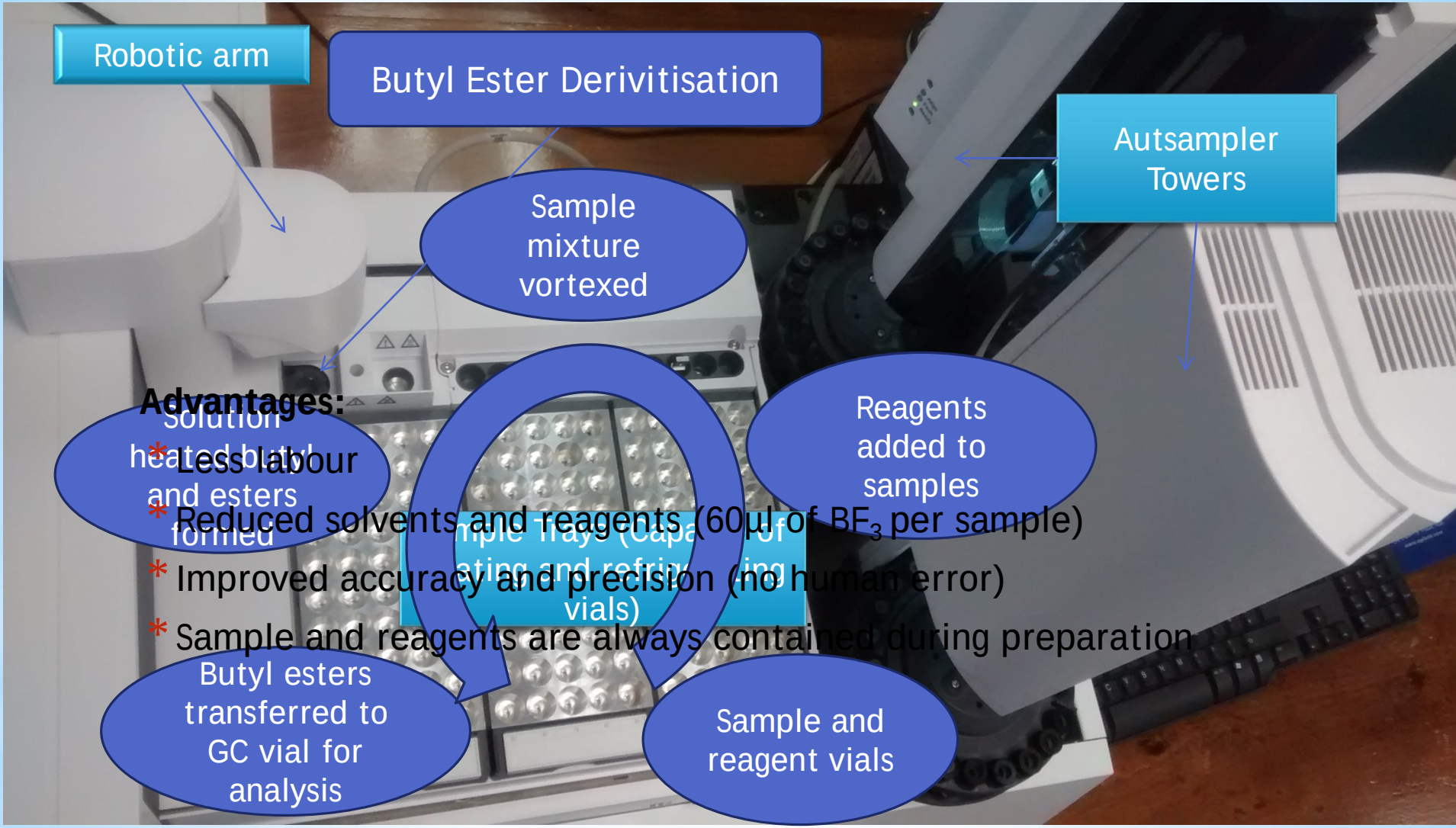


Advantages:

* Less labour
* Reduced solvents and reagents (60µl of BF_3 per sample)
* Improved accuracy and precision (no human error)
* Sample and reagents are always contained during preparation

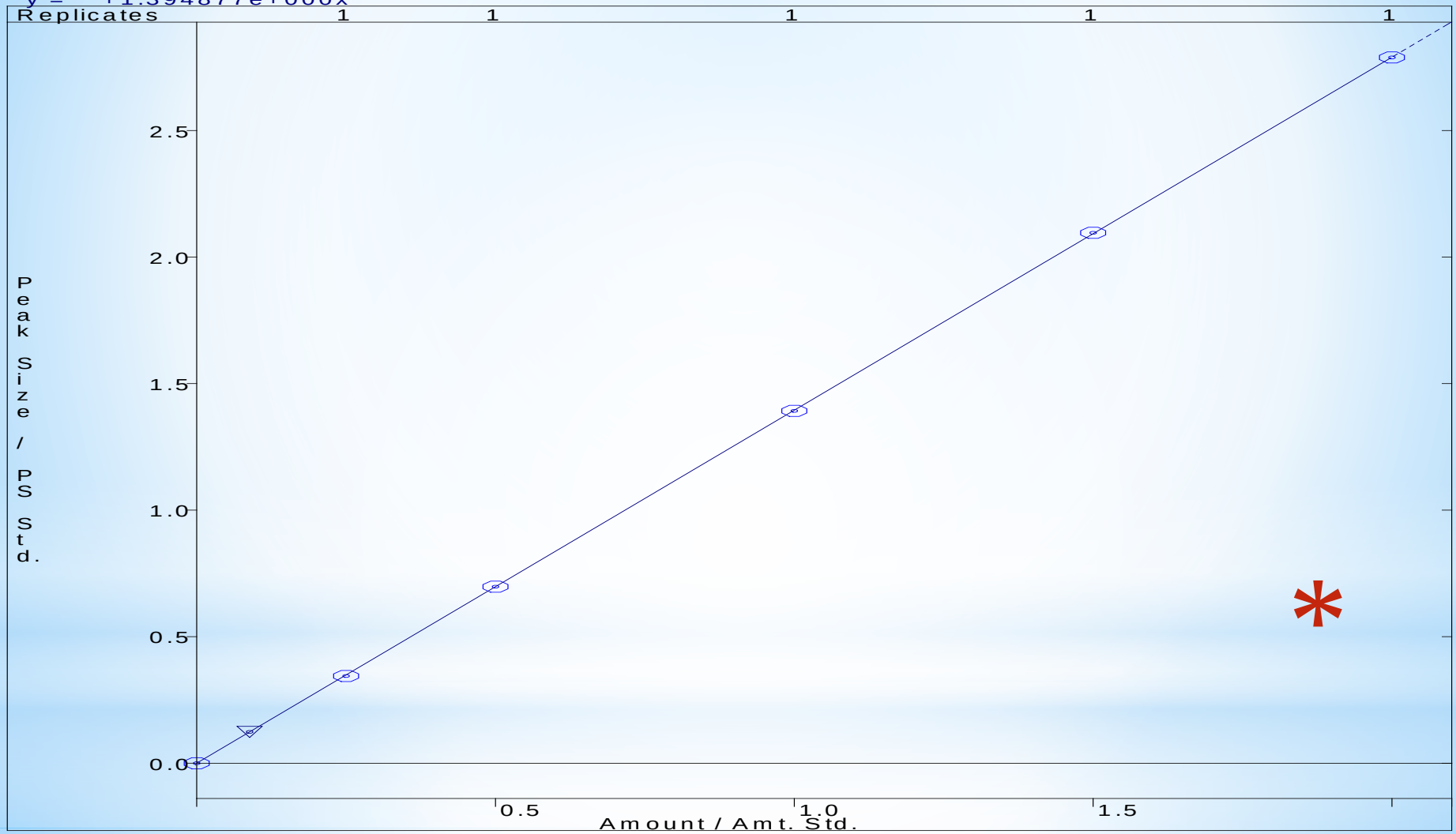
*Automation

Calibration Curve Report
File: c:\star\data\siobhan\18102012\ffa std c4-c18.mth
Detector: 3800 GC, Address: 44, Channel ID: Front
Analysis File: c:\docume~1\admini~1\locals~1\temp\run~1324.run

C10

Internal Standard Analysis
Curve Type: Linear
Origin: Force
y = +1.394877e+000x

Resp. Fact. RSD: 0.5560%
Coeff. Det.(r^2): 0.999995



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	On-Column	FAME	Butyl Ester	RSD (%)
Brie	1135	1067	1183	5.2
Processed Cheese	1104	952	1246	13.4
Light Cheddar	969	827	814	9.8
Mild Cheddar	1525	1627	1436	6.2
Special Reserve	2289	2316	1936	9.7
Blue Stilton	3109	2856	3355	8.0
Milk Powder	337	333	296	7.1
Infant Formula	173	227	217	13.8
Butter	1492	1295	1960	21.6
Yoghurt	182	246	223	14.9
Ice Cream	323	176	661	64.3
Milk	290	286	90	51.4
Nat. Butter Powder	73652	90949	67206	15.9
Nat.Cream Powder	48680	49274	44712	5.2
Nat. Cream paste	126615	114969	131622	6.9
Nat. Butter paste	106079	98129	106748	4.6
Nat. Blue Cheese paste	116085	104376	113377	5.5

Analysis of 17 dairy samples. Displayed are the average ppm (mg/kg) values obtained from six replicates (n=6)

	On-Column (R^2)	FAME (R^2)	Butyl Ester (R^2)
C4:0	0.9999	0.9978	0.9996
C6:0	0.9998	0.9995	0.9995
C8:0	0.9997	0.9997	0.9964
C10:0	0.9999	0.9999	0.9999
C12:0	0.9999	0.9998	0.9998
C14:0	0.9999	0.9998	0.9978
C16:0	0.9999	0.9999	0.9996
C18:0	0.9998	0.9999	0.9999
C18:1	0.9998	0.9998	0.9999
C18:2	0.9998	N/A	0.9999
C18:3	0.9994	N/A	0.9999
LOD (ppm)	0.7	5	8
LOQ (ppm)	3	20	20

Validation data obtained, included is correlation coefficient (R^2), limit of detection (LOD) and limit of quantification (LOQ)

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Comparison and validation of 2 analytical methods for the determination of free fatty acids in dairy products by gas chromatography with flame ionization detection

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*Any Questions?

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