# The Agilent 7696A Sample Prep WorkBench

## Techniques and Strategies to Build Automated Sample Prep Methods

Jim McCurry
Senior Scientist
Little Falls Site
Wilmington, Delaware  USA



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# Outline

- What is the 7696A WorkBench
- Steps to translating and building WorkBench methods from manual methods
- Optimizing WorkBench method conditions
- Results using WorkBench Methods

# Agilent 7696A Features and Capabilities

**Simple Sample Prep:**

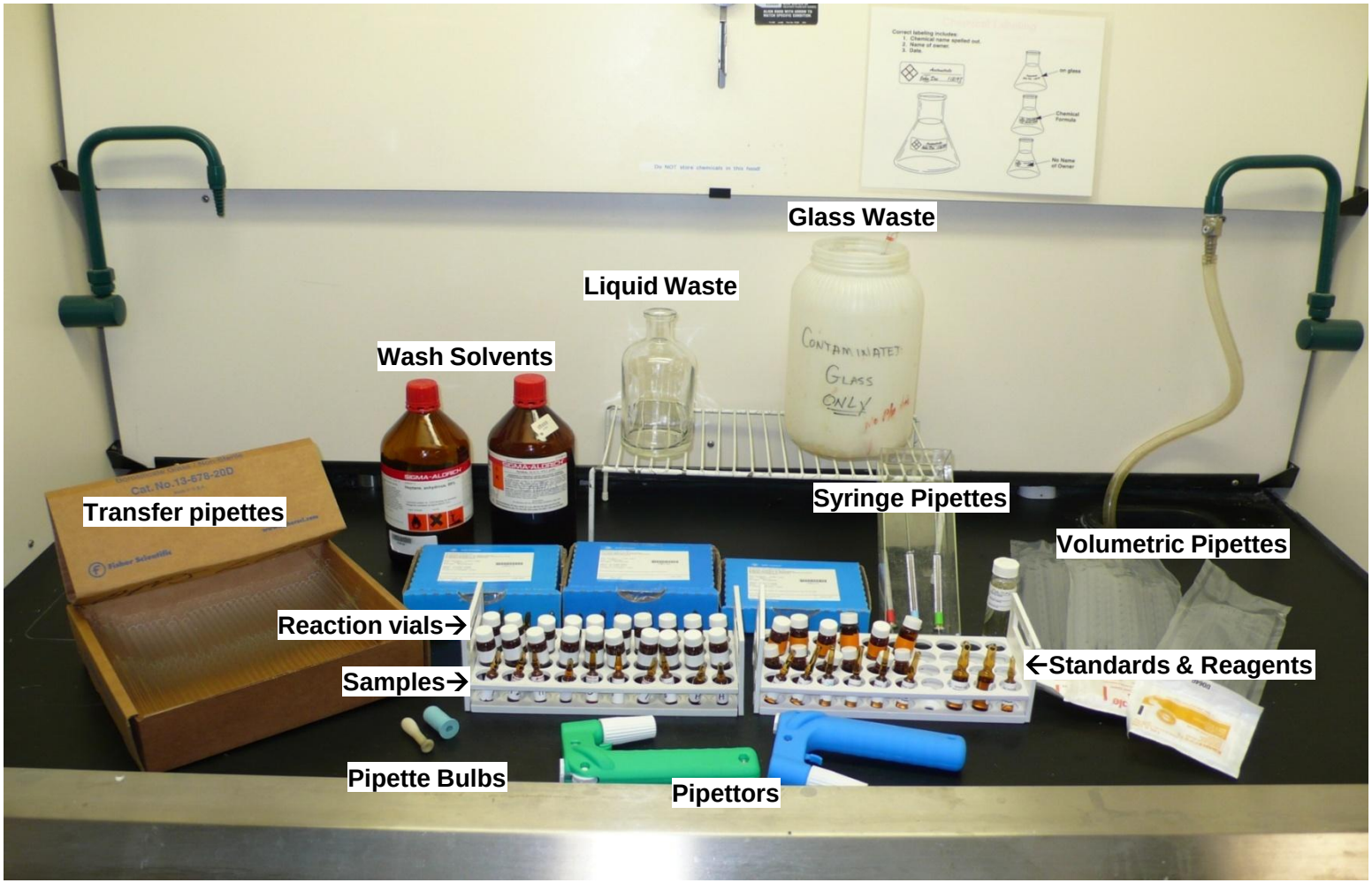
- **Dilution** / Aliquoting / Reconstitution
- **Additions** (standards, reagents, etc)
- **Heating** (Reactions, Derivatization, etc)
- **Mixing** – Vortex

**Additional Capabilities:**

- Sample Tray Heating – 50 vial positions*
- Sample Tray Peltier Cooling – 50 vial positions*
- Dedicated Sample Prep Software
- Liquid/Liquid Extraction

* Option / Accessory

# Typical Manual Sample Prep – ASTM D6584



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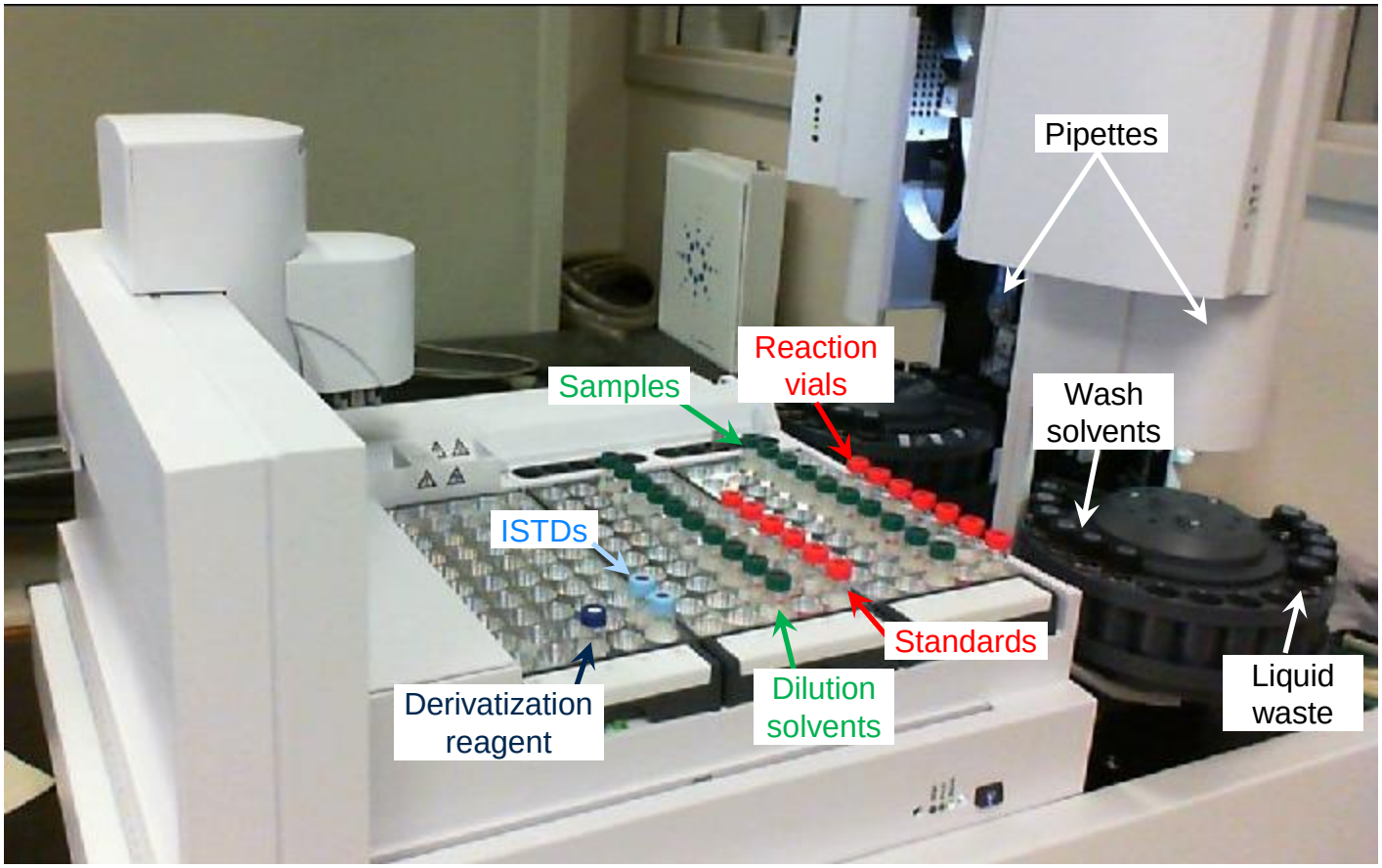
# WorkBench Sample Prep – ASTM D6584



# Translating Manual Methods to WorkBench

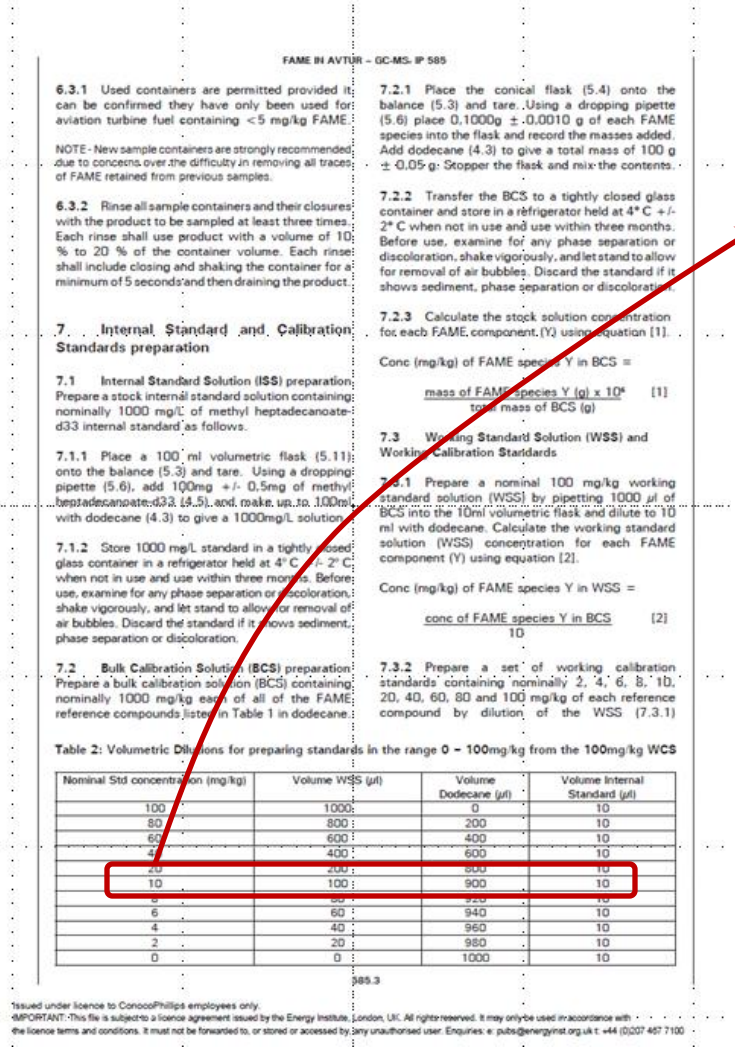
## IP 585 – GC/MS Analysis of Trace FAME in Jet Fuel

- Method measures 5 to 100 mg/kg total FAME contaminatation in Jet Fuel
- GC/MS analysis technique requires and expensive, deuterated internal standard
  - Methyl Heptadecanoate-d33 (C17:0d33)

- Step 1 – Convert Manual method to a step-by-step script
- Step 2 – Scale each step to fit into 2-mL ALS vials
- Step 3 – List resources need for each step
- Step 4 – Layout resources in WorkBench software
  - Chemicals, standards, solvents, reagents, syringes, wash solvents, waste vials
- Step 5 – Build method using WorkBench software
- Step 6 – Optimize WorkBench conditions
  - Syringe conditions, mixing, heating or cooling

# Translating Manual Methods to WorkBench

## Step 1 – Convert Manual method to a step-by-step script



FAME IN AVTUR – GC-MS- IP 585

**6.3.1** Used containers are permitted provided it can be confirmed they have only been used for aviation turbine fuel containing <5 mg/kg FAME.

NOTE - New sample containers are strongly recommended due to concerns over the difficulty in removing all traces of FAME retained from previous samples.

**6.3.2** Rinse all sample containers and their closures with the product to be sampled at least three times. Each rinse shall use product with a volume of 10 % to 20 % of the container volume. Each rinse shall include closing and shaking the container for a minimum of 5 seconds and then draining the product.

**7. Internal Standard and Calibration Standards preparation**

**7.1** Internal Standard Solution (ISS) preparation. Prepare a stock internal standard solution containing nominally 1000 mg/L of methyl heptadecanoate-d33 internal standard as follows.

**7.1.1** Place a 100 ml volumetric flask (5.11) onto the balance (5.3) and tare. Using a dropping pipette (5.6), add 100mg +/- 0.5mg of methyl heptadecanoate-d33 (4.5), and make up to 100ml with dodecane (4.3) to give a 1000mg/L solution.

**7.1.2** Store 1000 mg/L standard in a tightly closed glass container in a refrigerator held at 4° C +/- 2° C when not in use and use within three months. Before use, examine for any phase separation or discoloration, shake vigorously, and let stand to allow for removal of air bubbles. Discard the standard if it shows sediment, phase separation or discoloration.

**7.2** Bulk Calibration Solution (BCS) preparation. Prepare a bulk calibration solution (BCS) containing nominally 1000 mg/kg each of all of the FAME reference compounds listed in Table 1 in dodecane.

**7.2.1** Place the conical flask (5.4) onto the balance (5.3) and tare. Using a dropping pipette (5.6) place 0.1000g ± 0.0010 g of each FAME species into the flask and record the masses added. Add dodecane (4.3) to give a total mass of 100 g ± 0.05 g. Stopper the flask and mix the contents.

**7.2.2** Transfer the BCS to a tightly closed glass container and store in a refrigerator held at 4° C +/- 2° C when not in use and use within three months. Before use, examine for any phase separation or discoloration, shake vigorously, and let stand to allow for removal of air bubbles. Discard the standard if it shows sediment, phase separation or discoloration.

**7.2.3** Calculate the stock solution concentration for each FAME component (Y) using equation [1].

Conc (mg/kg) of FAME species Y in BCS =

$$\frac{\text{mass of FAME species Y (g)} \times 10^6}{\text{total mass of BCS (g)}} \quad [1]$$

**7.3** Working Standard Solution (WSS) and Working Calibration Standards

**7.3.1** Prepare a nominal 100 mg/kg working standard solution (WSS) by pipetting 1000 µl of BCS into the 10ml volumetric flask and dilute to 10 ml with dodecane. Calculate the working standard solution (WSS) concentration for each FAME component (Y) using equation [2].

Conc (mg/kg) of FAME species Y in WSS =

$$\frac{\text{conc of FAME species Y in BCS}}{10} \quad [2]$$

**7.3.2** Prepare a set of working calibration standards containing nominally 2, 4, 6, 8, 10, 20, 40, 60, 80 and 100 mg/kg of each reference compound by dilution of the WSS (7.3.1)

**Table 2:** Volumetric Dilutions for preparing standards in the range **0 – 100**mg/kg from the **100**mg/kg WCS

| Nominal Std concentration (mg/kg) | Volume WSS (µl) | Volume Dodecane (µl) | Volume Internal Standard (µl) |
|---|---|---|---|
| 100 | 1000 | 0 | 10 |
| 80 | 800 | 200 | 10 |
| 60 | 600 | 400 | 10 |
| 40 | 400 | 600 | 10 |
| 20 | 200 | 800 | 10 |
| 10 | 100 | 900 | 10 |
| 8 | 80 | 920 | 10 |
| 6 | 60 | 940 | 10 |
| 4 | 40 | 960 | 10 |
| 2 | 20 | 980 | 10 |
| 0 | 0 | 1000 | 10 |

385.3



Convert manual method to a “Script” of steps

| Steps | Manual Prep of 10 mg/kg IP585 Standard |
|---|---|
| 1 | Add 900 uL dodecane to vial |
| 2 | Add 100 uL working standard solution (100 mg/kg) |
| 3 | Add 10 uL ISTD solution (1000 mg/kg C17:0d33) |
| 4 | Mix |

| Steps | Manual Prep of Sample |
|---|---|
| 1 | Add 1000 uL of sample to vial |
| 2 | Add 10 uL ISTD solution (1000 mg/kg C17:0d33) |
| 3 | Mix |



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# Scaling Manual Prep for 2-mL WorkBench vials

## Step 2 – Scale each step to fit into 2-mL ALS vials

| Steps | Manual Prep | 10:1 Scaling --> | WorkBench Prep |
|---|---|---|---|
| 1 | Add 900 uL of dodecane to a vial | | Add 90 uL of dodecane to a vial |
| 2 | Add 100 uL of working calibration standard | | Add 10 uL of working calibration standard |
| 3 | Add 10 uL ISTD solution | | Add 1 ISTD solution |
| 4 | Mix | | Mix |

- Manual sample preps must be scaled to fit 2-mL vial
- Reduce amounts of solvents and expensive chemicals
- High recovery vials needed to contain 100 uL of final solution volume

# Translating Manual Methods to WorkBench

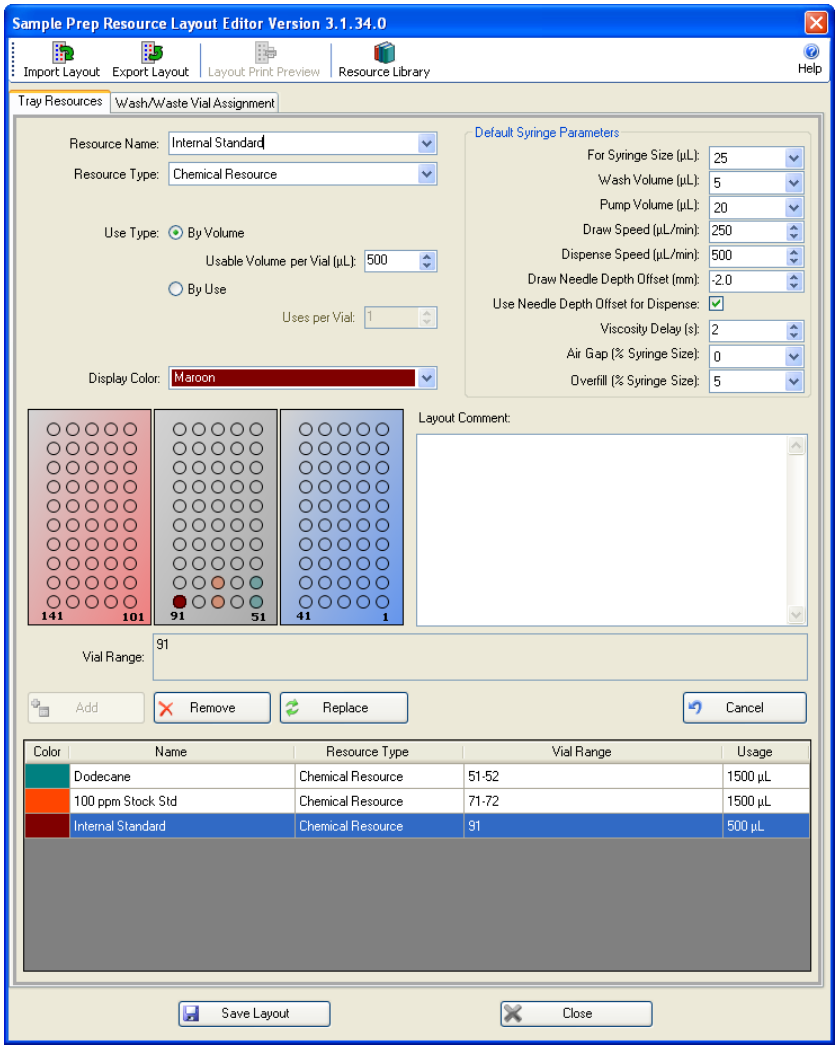
## Step 3 – List resources need for each step

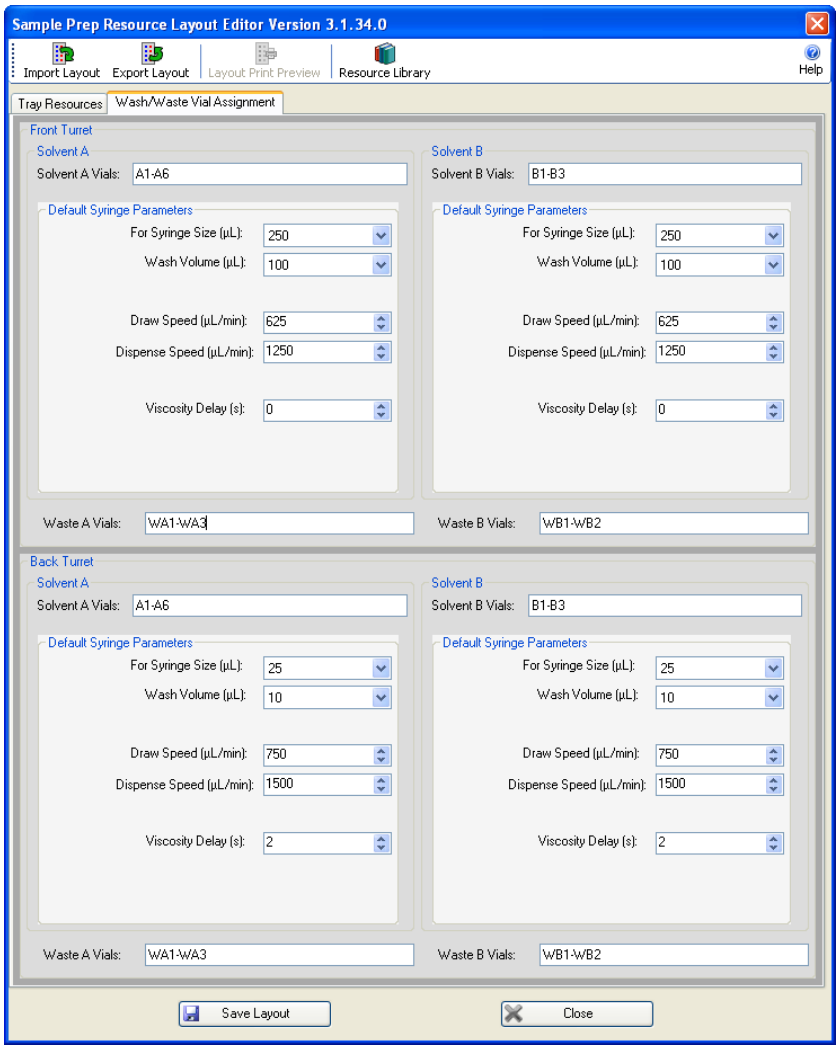
- Four chemical resources are needed for this sample prep
- Syringe size, draw speeds, and dispense speed are optimized for each type of chemical resource

| Resource | Tray Position | Syringe Size | Syringe Draw Speed | Syringe Dispense Speed |
|---|---|---|---|---|
| Dodecane | 51 - 52 | 250 uL | 2500 uL/min | 5000 uL/min |
| Stock Standard (100 mg/kg each FAME) | 71 -72 | 25 uL | 250 uL/min | 500 uL/min |
| ISTD (1000 mg/kg C17:0d33) | 91 | 25 uL | 250 uL/min | 500 uL/min |

# Translating Manual Methods to WorkBench

## Step 4 – Layout resources in WorkBench software

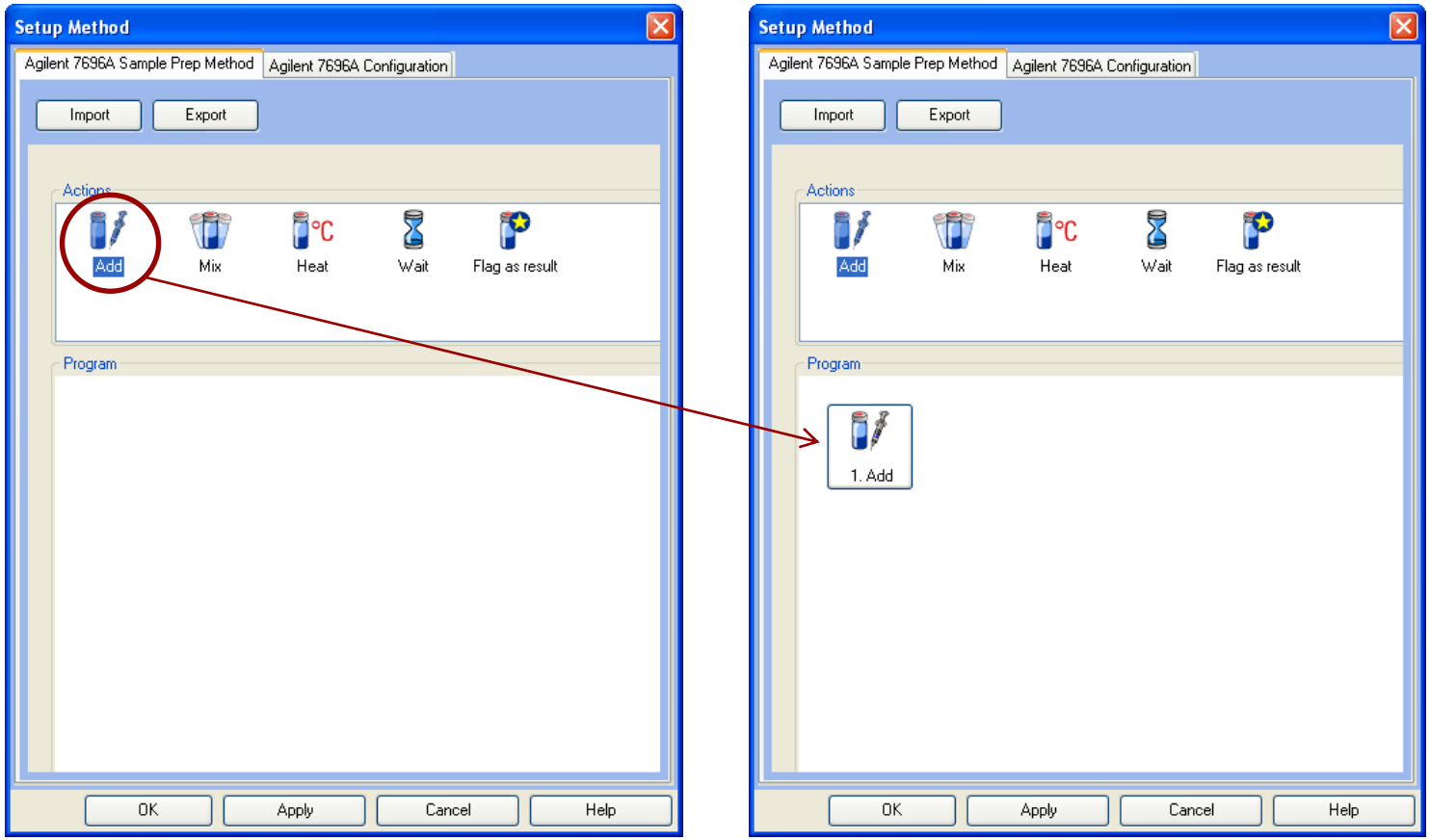


# Translating Manual Methods to WorkBench

## Step 5 – Build method using WorkBench software

- WorkBench software builds methods graphically
- “Drag and drop” WorkBench functions into the method program



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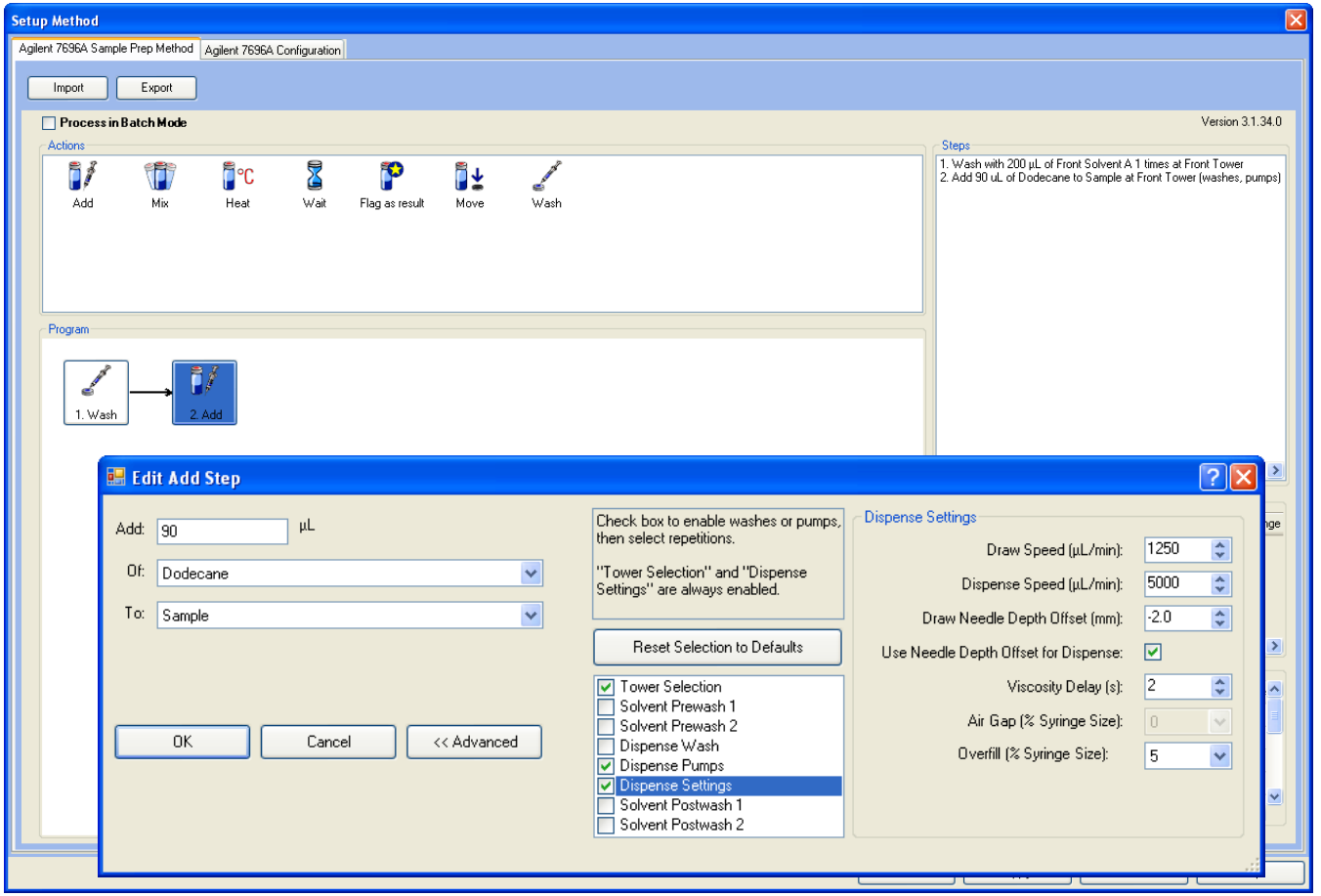
# Translating Manual Methods to WorkBench

## Step 5 – Build method using WorkBench software



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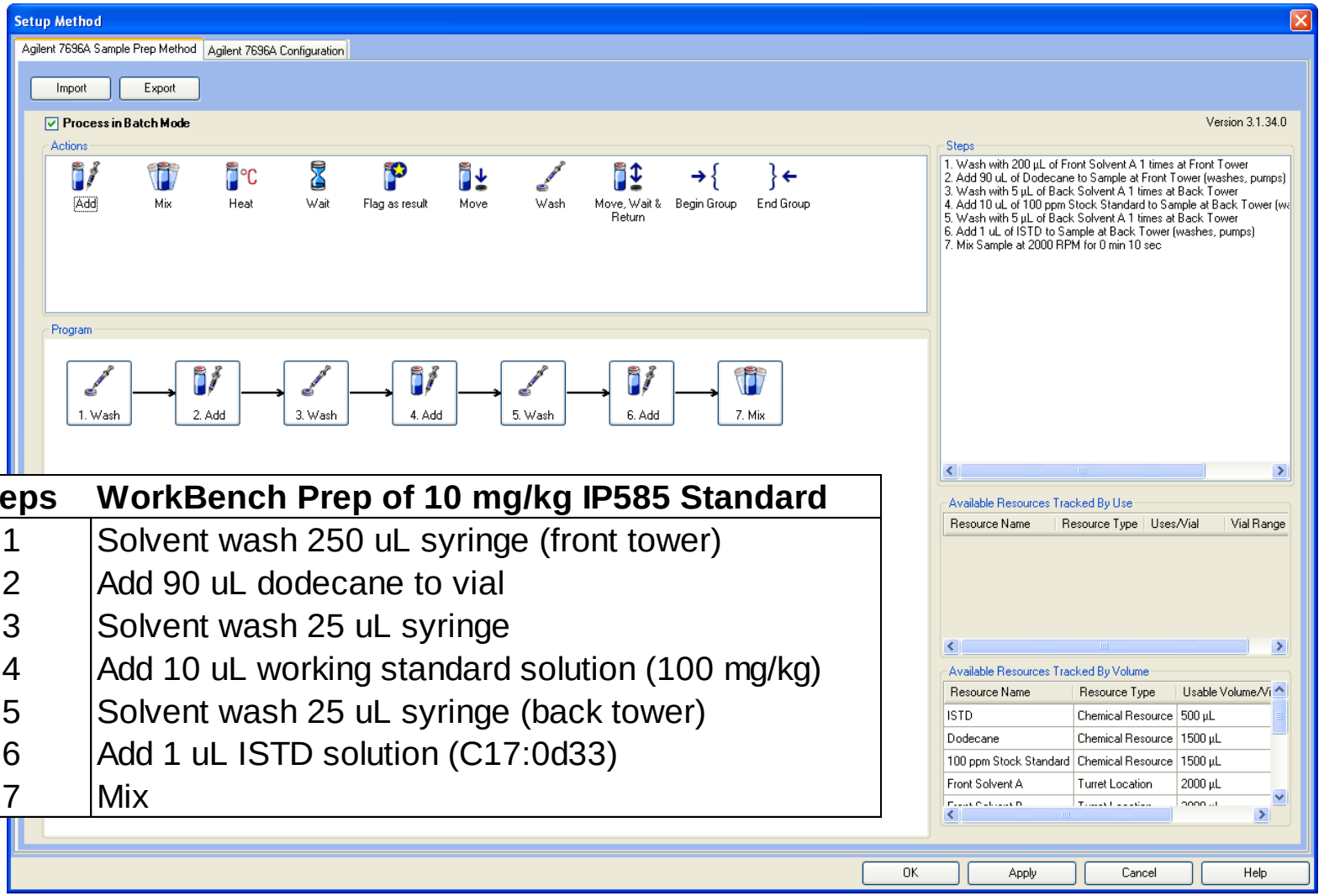
# Translating Manual Methods to WorkBench

## Step 5 – Build method using WorkBench software




| Steps | WorkBench Prep of 10 mg/kg IP585 Standard |
|---|---|
| 1 | Solvent wash 250 uL syringe (front tower) |
| 2 | Add 90 uL dodecane to vial |
| 3 | Solvent wash 25 uL syringe |
| 4 | Add 10 uL working standard solution (100 mg/kg) |
| 5 | Solvent wash 25 uL syringe (back tower) |
| 6 | Add 1 uL ISTD solution (C17:0d33) |
| 7 | Mix |



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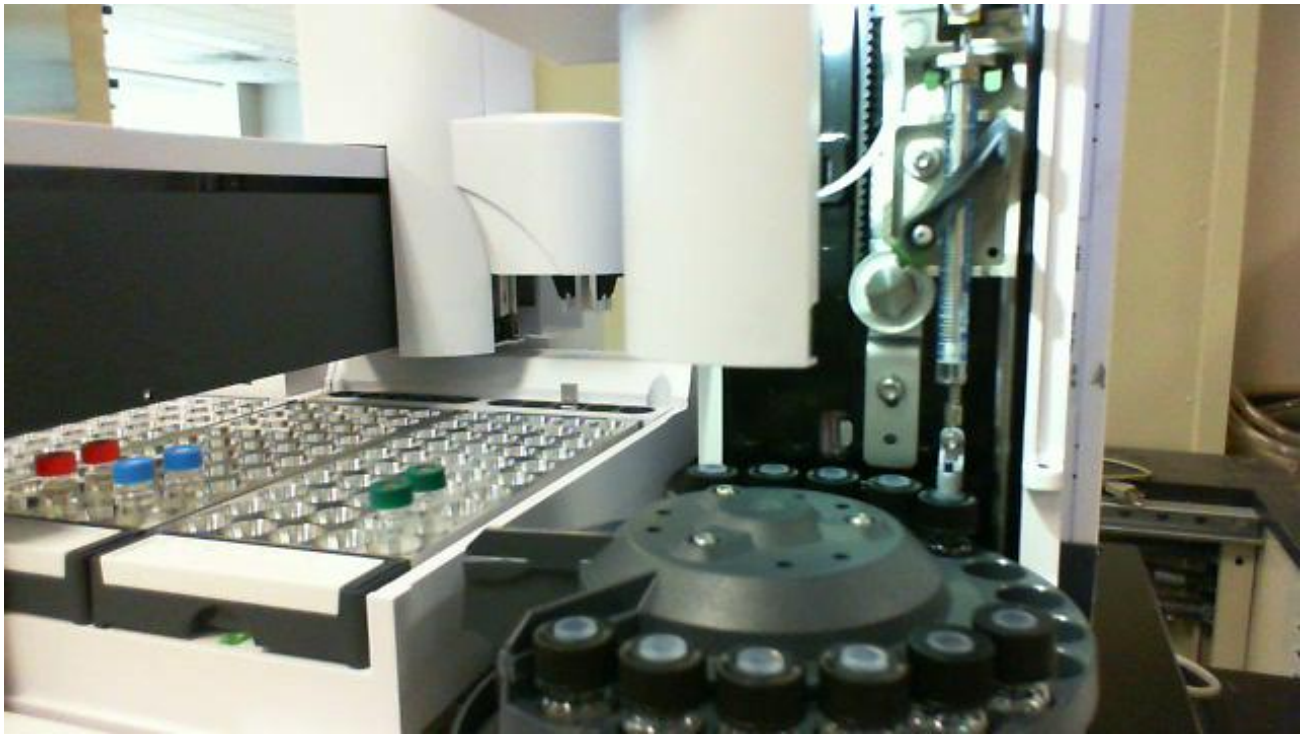
# Translating Manual Methods to WorkBench Syringe Bubble Formation

## Step 6 - Optimize WorkBench conditions

This Guy Likes Bubbles

WorkBench Does Not Like Bubbles

- Bubble formation in the syringe results in poor precision and accuracy when dispensing liquids



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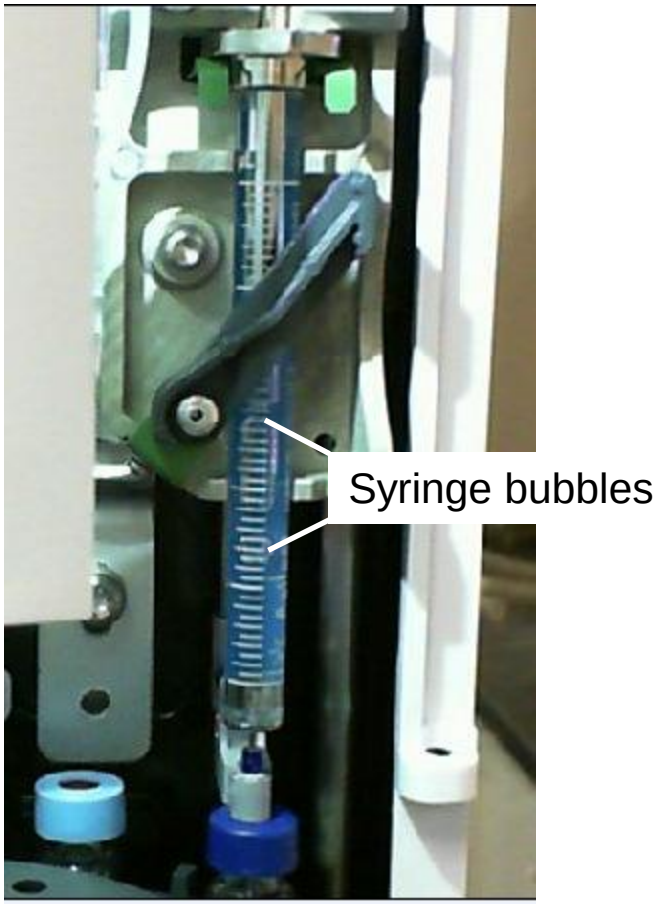
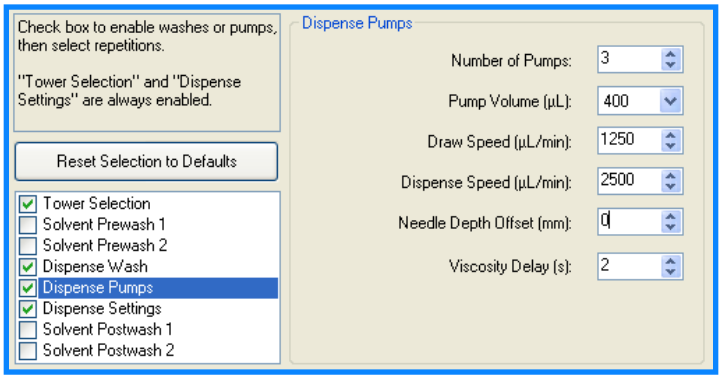
# What Causes Bubble Formation

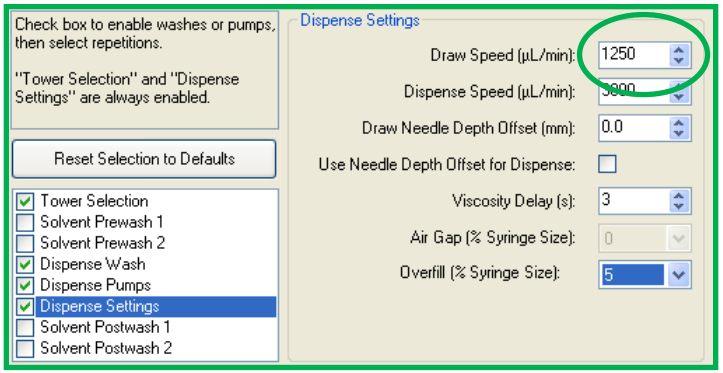
- Cavitation
  - rapid change in pressure at the face of the plunger
  - low-boiling liquids
- Solvent degassing
  - i.e. dissolved air in methanol
- In-Vial Vacuum Formation
  - usually found when withdrawing large volumes from vial
  - overfilled vials
  - viscous liquids



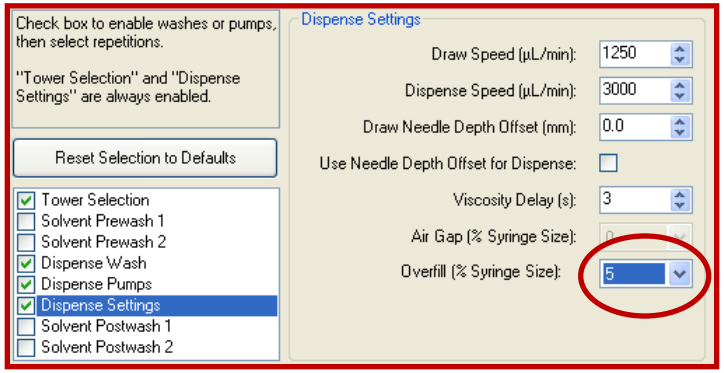
# Techniques to Prevent Syringe Bubbles




Use Dispense Pumps to blow out bubbles




Slower draw speeds reduce cavitation bubbles




Overfill keeps bubbles in the syringe and out of the vial



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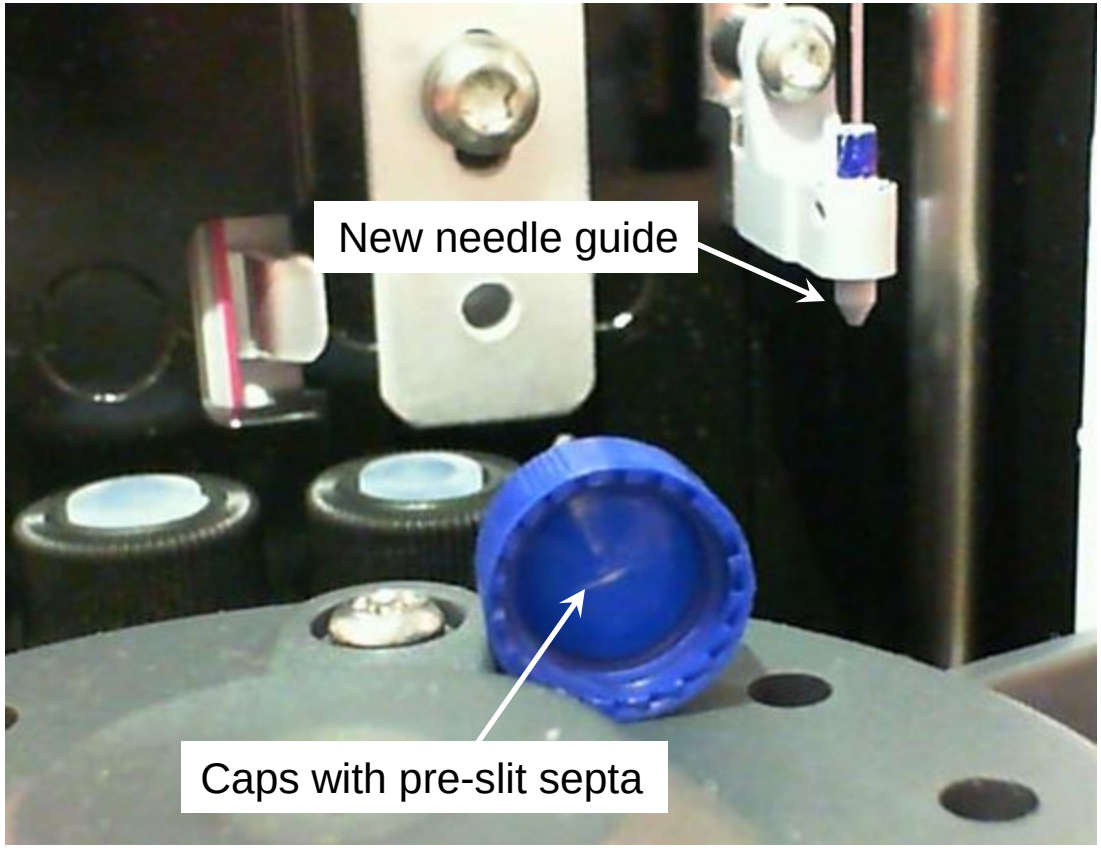
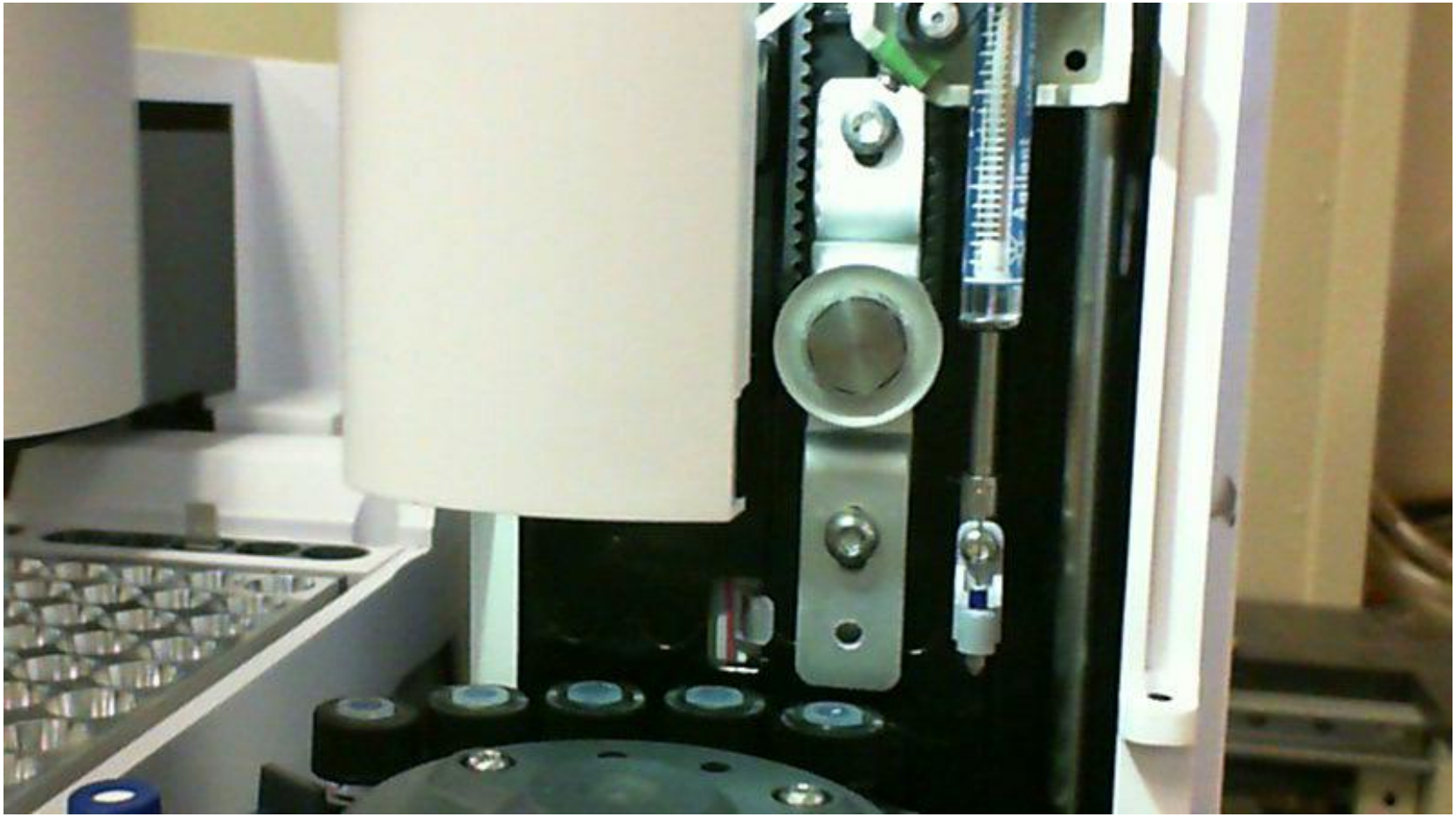
# Eliminating Syringe Bubbles Caused by In-Vial Vacuum

- Pre-slit septa in vial caps
- New needle guide design
- Extended nose on needle guide opens pre-slit septa
- Relieves vacuum inside vial
- Split reseals after needle withdraw
- Needle guide does not affect standard septa
- Not recommended for highly volatile samples

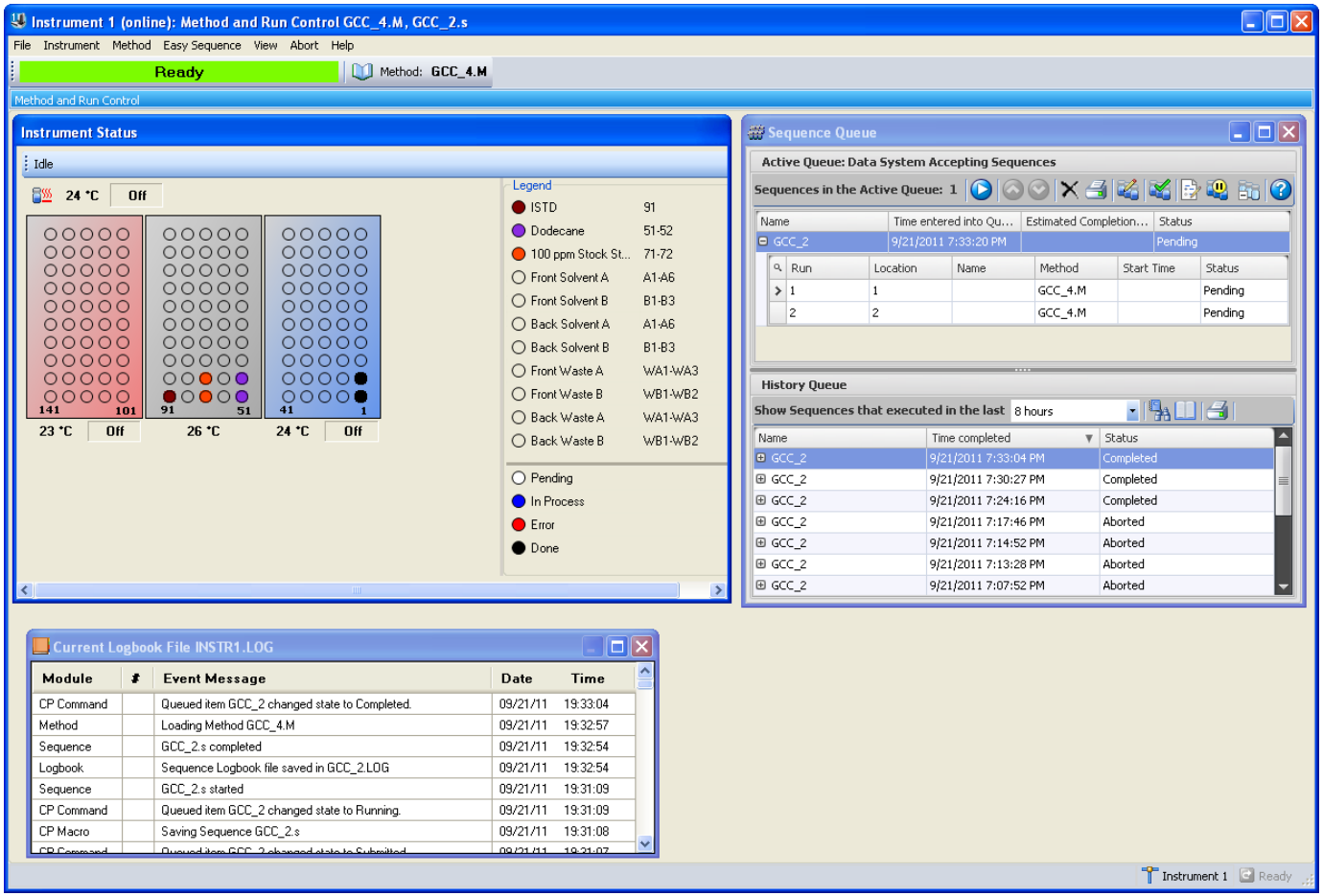


# Elimination of Syringe Bubble

- Pre-slit septa and new needle guide
- Slower draw speed (1250 uL/min)
- 5% Syringe overfill



# Running WorkBench Methods Using the Sequence Queue




- Methods are run using Sequence Queue
- Select the Method
- Choose the number of sample vials to run
- WorkBench will keep track of resource usage during sequence



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# Method IP585 – 10 mg/kg Standard Preparation

| Steps | WorkBench Prep of 10 mg/kg IP585 Standard |
|---|---|
| 1 | Solvent wash 250 uL syringe (front tower) |
| 2 | Add 90 uL dodecane to vial |
| 3 | Solvent wash 25 uL syringe |
| 4 | Add 10 uL working standard solution (100 mg/kg) |
| 5 | Solvent wash 25 uL syringe (back tower) |
| 6 | Add 1 uL ISTD solution (C17:0d33) |
| 7 | Mix |

# Method IP585 – 10 mg/kg Standard Preparation

| Steps | WorkBench Prep of 10 mg/kg IP585 Standard |
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| 3 | Solvent wash 25 uL syringe |
| 4 | Add 10 uL working standard solution (100 mg/kg) |
| 5 | Solvent wash 25 uL syringe (back tower) |
| 6 | Add 1 uL ISTD solution (C17:0d33) |
| 7 | Mix |

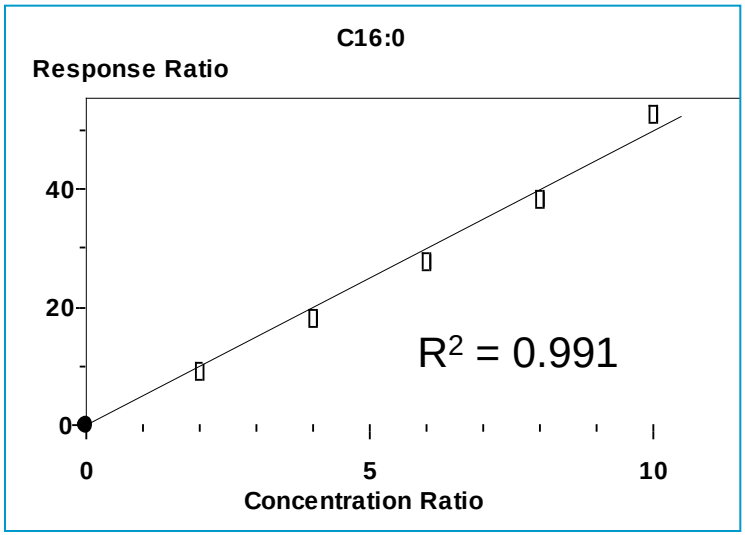
# Method IP585 – Complete Automation of Calibration Standards and Sample Preparation

- 2 Sets of Five Calibration Standards
  - Low Level Set: 0, 2, 46, 8, and 10 mg/kg FAMEs in C12
  - High Level Set: 20, 40, 60 , 80 and 100 mg/kg FAMEs in C12

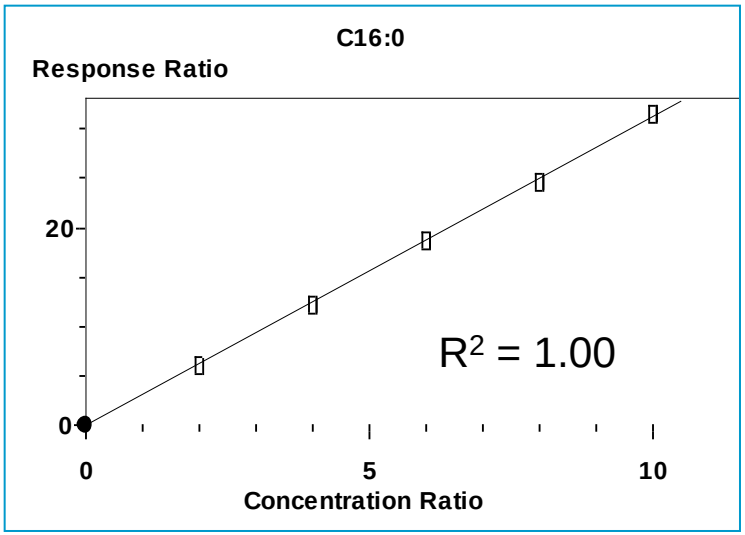
- 3 Jet Fuel Samples with Different Amounts of Total FAME
  - 3-Step WorkBench Sample Prep:
    - Add 100 uL sample to empty vial
    - Add 1 uL ISTD to sample
    - Mix
  - Each sample prepped in duplicate to measure repeatability (r)
  - One injection of each sample on 5975C GC/MS system
    - Configured for IP method 585

# Comparison of Manual and WorkBench Calibration

Methyl Stearate – Manual Prep
using 1 mL Cal Stds

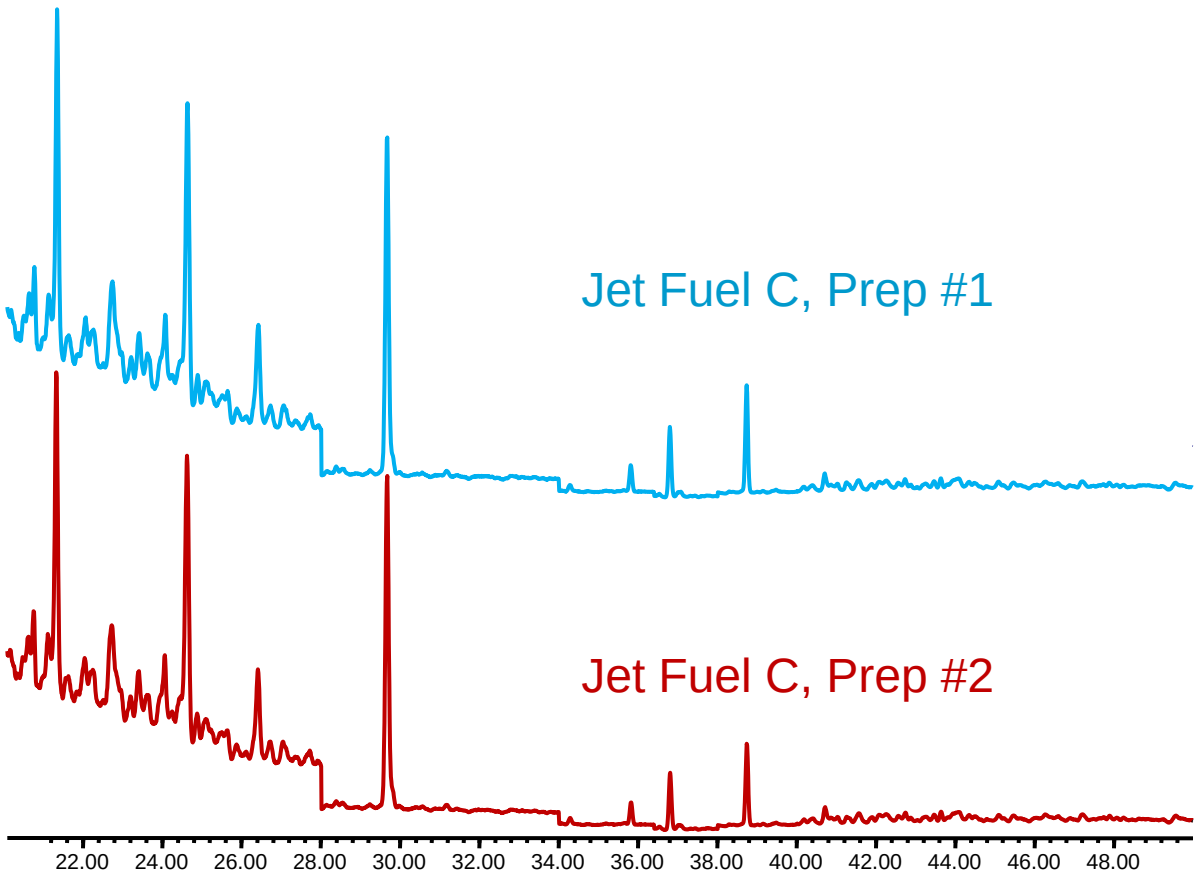



Methyl Stearate – 7696A Prep
Using 100 uL Cal Stds




**Better calibrations using fewer amounts of expensive compounds**

# WorkBench Prep Sample Precision



# WorkBench Prep Sample Precision

Consistent results obtained for each sample

Greatly exceeds IP585 repeatability (r) specification at all three levels of FAME content

**Jet Fuel Sample A**

| | Run 1 | Run 2 | Avg | Std Dev | RSD | r | r (IP585) |
|---|---|---|---|---|---|---|---|
| C16:0 | 17.53 | 17.25 | | | | | |
| C17:0 | 16.71 | 16.39 | | | | | |
| C18:0 | 16.56 | 16.32 | | | | | |
| C18:1 | 16.93 | 16.70 | | | | | |
| C18:2 | 17.22 | 16.99 | | | | | |
| C18:3 | 17.72 | 17.50 | | | | | |
| **Total** | **102.67** | **101.15** | **101.91** | **1.07** | **1.05%** | **1.52** | **19.63** |

**Jet Fuel Sample B**

| | Run 1 | Run 2 | Avg | Std Dev | RSD | r | r (IP585) |
|---|---|---|---|---|---|---|---|
| C16:0 | 6.05 | 6.17 | | | | | |
| C17:0 | 0.00 | 0.00 | | | | | |
| C18:0 | 2.10 | 2.13 | | | | | |
| C18:1 | 9.69 | 9.85 | | | | | |
| C18:2 | 28.52 | 28.90 | | | | | |
| C18:3 | 4.67 | 4.71 | | | | | |
| **Total** | **51.03** | **51.76** | **51.40** | **0.52** | **1.00%** | **0.73** | **11.39** |

**Jet Fuel Sample C**

| | Run 1 | Run 2 | Avg | Std Dev | RSD | r | r (IP585) |
|---|---|---|---|---|---|---|---|
| C16:0 | 1.20 | 1.30 | | | | | |
| C17:0 | 0.00 | 0.00 | | | | | |
| C18:0 | 0.20 | 0.20 | | | | | |
| C18:1 | 0.90 | 1.10 | | | | | |
| C18:2 | 1.90 | 2.00 | | | | | |
| C18:3 | 0.90 | 0.90 | | | | | |
| **Total** | **5.10** | **5.50** | **5.30** | **0.28** | **5.34%** | **0.40** | **3.86** |

# Summary

- Six simple steps used to translate a manual sample prep to an automated WorkBench Prep

- WorkBench hardware provide tools need for many liquid sample prep techniques
  - 2 syringe pipettes, wash solvents, waste containers, heating and cooling stations, mixing station

- WorkBench software provides easy, graphical user interface to build and run automated sample prep techniques
  - resource layout, drag-and-drop functions, Sequence Queue, logbooks
  - software automatically keeps track of resource usage
    - Warns user when resource are low
    - Warns user when resources are full (i.e. waste vials)