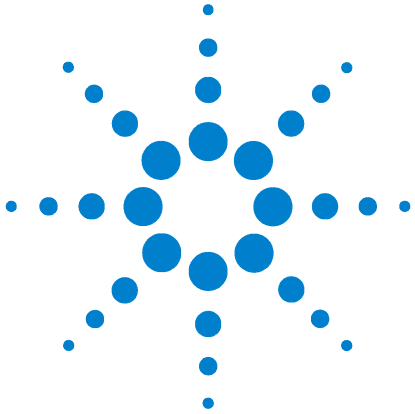

Agilent G1674AA Deconvolution Reporting Software (DRS) Solution for Forensic Toxicology

Getting Started

Agilent Technologies

Notices

Manual Part Number

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Contents

1 Decision Process to Create Your Method Name for the G1674AA Forensic Toxicology Data Base Library (DBL)

For convenience, you may want to print this manual to refer to as you proceed through the following chapters and steps. For this chapter, you also may want to have paper and pen/pencil at hand to write down each piece of the method file name you will create.

This process facilitates choosing best method(s) and files to load and set up for your system. It begins by building a method file name based upon the following series of questions about your intended application situation:

Step 1 *All* method file names begin with **FT**, for **F**orensic **T**oxicology. At this point then, your file name is simply **FT**.

Step 2 Column Stationary Phase (**5** *or* **35**)

Method sets are provided for both DB-5MS and DB-35MS stationary phases:

- DB-5MS method sets are typically the better choice because runs end at a lower temperature (325 °C) relative to DB-35MS methods (345 °C).
- DB-35MS method sets are disadvantaged in that they require the final temperature to be adjusted at setup to obtain desired retention time matches. DB-35MS methods are provided here for:
  - Use in confirmation.
  - And/Or for those labs which run other methods requiring DB-35MS on the same instrument performing forensic toxicology work.

Now choose *either* DB-5MS (preferred) *or* DB-35MS as stationary phase:

- For DB-5MS, this is represented by **5** as the next part of the method file name.

- For DB-35MS, this is represented by **35** as the next part of the method file name.

At this point then, your method file name will be *either* **FT5** *or* **FT35**.

Step 3 Analysis Speed (**_1X_** *or* **_2X_** *or* **_3X_** *or* **_4X_** *or* **_6X_**)

- Methods are provided for five different analysis speeds: 1X , 2X , 3X , 4X , and 6X:
  - *Base* method sets (1X) have a 10 °C per minute oven ramp. They have highest chromatographic resolutions but require longest analysis times. Thus, for typical screening applications, 1X method sets are unnecessarily long.
  - Numbers 2, 3, 4, and 6 represent method sets designated by the multiple by which the oven ramp is increased, and by the factor by which total analysis time is reduced, relative to the associated base method sets. Thus, 4X method sets run at 40 °C per minute and their total run times are one-fourth that of their associated base method sets.

For most toxicology screening applications, 2X , 3X , or 4X are most likely chosen. Best choice is governed by:

- Oven ramping capability of the gas chromatograph (GC)
- Pumping capacity of the mass spectrometer (MSD)
- Data gathering and processing speed of the MSD

If, for example, the system is a GC with 120-V oven, an MSD with diffusion pump, and with the column connected directly into the MSD, then only 1X or 2X methods can be used.

Method sets 3X , 4X , and 6X require the fast oven (240 V) and performance turbopump because column flow rates exceed 2 mL per minute.

- 6X method sets also require the oven *pillow* accessory to be used to attain the necessary oven rate of 60 °C per minute (use of the pillow requires that the MSD, inlet, and, if used, nitrogen phosphorus detector (NPD) are all located in back GC positions).

Choose the best speed to run based on your column choice and hardware configuration using appropriate information in the following Conditions Tables 1, 2, 3, *or* 4.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions for DB-5MS with Vacuum Outlet					
	Original 1X Method	**2X Method**	**3X Method**	**4X Method**	**6X Method**
Biggest 4 Ions	**FT5_1X_VAC.m**	**FT5_2X_VAC.m**	**FT5_3X_VAC.m**	**FT5_4X_VAC.m**	**FT5_6X_VAC.m**
Column Bleed Optimized Ions	**FT5_1X_VAC_BL.m**	**FT5_2X_VAC_BL.m**	**FT5_3X_VAC_BL.m**	**FT5_4X_VAC_BL.m**	**FT5_6X_VAC_BL.m**
Fatty Acid Matrix Optimized Ions	**FT5_1X_VAC_FA.m**	**FT5_2X_VAC_FA.m**	**FT5_3X_VAC_FA.m**	**FT5_4X_VAC_FA.m**	**FT5_6X_VAC_FA.m**
GC					
Agilent Technologies 6890 or					
7890 with Autiinjector and Tray					
Inlet	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless
Mode	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure
Injection Type	Splitless	Splitless	Splitless	Splitless	Splitless
Injection Volume (µL)	1.0	1.0	1.0	1.0	1.0
Inlet temp (°C)	280	280	280	280	280
Pressure, nominal (psig)	19	4.75	14.4	28.7	11.2
RT Locking Compound	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)
RT Locking Time (min)	17.122	8.561	5.707	4.281	2.854
Purge Flow (mL/min)	50	50	50	50	50
Purge Time (min)	1	0.75	0.5	0.4	0.25
Gas type	Helium	Helium	Helium	Helium	Helium
Oven					
Voltage (VAC)	120 or 240	120 or 240	240	240	240 and Pillow**[1]**
Initial Oven Temp (°C)	100	100	100	100	100
Initial Oven Hold (min)	1	0.5	0.33	0.25	0.167
Ramp Rate (°C/min)	10	20	30	40	60
Final Temp (°C)	325	325	325	325	325
Final Hold (min)	5	2.5	1.67	1.25	0.833
Total Run Time (min)	28.5	14.25	9.5	7.13	4.75
Equilibration time (min)	0.5	0.5	0.5	0.5	0.5
Column					
Type	DB-5MS	DB-5MS	DB-5MS	DB-5MS	DB-5MS
Agilent Part Number	122-5532	122-5512	122-5512	122-5512	Custom
Length (m)	30	15	15	15	10
Diameter (mm)	0.25	0.25	0.25	0.25	0.25
Film thickness (µm)	0.25	0.25	0.25	0.25	0.25
Nominal Initial Flow (mL/min)	**1.8**	**1.2**	**2.7**	**5.9**	**3.1**
Outlet pressure	Vacuum	Vacuum	Vacuum	Vacuum	Vacuum
MSD					
Agilent Technologies 5975 or					
5973 Inert with Performance Electronics					
Suggested Minimum Vacuum					
Pump	Diffusion	Diffusion	Performance Turbo	Performance Turbo	Performance Turbo
Tune File	Atune.U	Atune.U	Atune.U	Atune.U	Atune.U
Mode	Scan	Scan	Scan	Scan	Scan
Solvent delay (min)	2.8	1.4	0.8	0.7	0.45
EM voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage
Low mass (amu)	40	40	40	40	40
High mass (amu)	570	570	570	570	570
Threshold	0	0	0	0	0
TID	on	on	on	on	off
Sampling	2	2	1	1	0
Quad temp (°C)	150	150	150	150	150
Source temp (°C)	300	300	300	300	300
Transfer line temp (°C)	300	300	300	300	300
[1] requires Injection port and MSD interface in back positions and G2646-60500 oven pillow					

Conditions Table 1 GC and MS Conditions for DB-5MS with Vacuum Outlet

Table 2. Gas Chromatograph and Mass Spectrometer Conditions for DB-5MS with Atmospheric Outlet					
	Original 1X Method	**2X Method**	**3X Method**	**4X Method**	**6X Method**
Biggest 4 Ions	**FT5_1X_ATM.m**	**FT5_2X_ATM.m**	**FT5_3X_ATM.m**	**FT5_4X_ATM.m**	**FT5_6X_ATM.m**
Column Bleed Optimized Ions	**FT5_1X_ATM_BL.m**	**FT5_2X_ATM_BL.m**	**FT5_3X_ATM_BL.m**	**FT5_4X_ATM_BL.m**	**FT5_6X_ATM_BL.m**
Fatty Acid Matrix Optimized Ions	**FT5_1X_ATM_FA.m**	**FT5_2X_ATM_FA.m**	**FT5_3X_ATM_FA.m**	**FT5_4X_ATM_FA.m**	**FT5_6X_ATM_FA.m**
GC					
Agilent Technologies 6890 or 7890 with Autiinjector and Tray					
Inlet	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless
Mode	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure
Injection Type	Splitless	Splitless	Splitless	Splitless	Splitless
Injection Volume (µL)	1.0	1.0	1.0	1.0	1.0
Inlet temp (°C)	280	280	280	280	280
Pressure, nominal (psig)	33	17.8	25.6	33.5	23.1
RT Locking Compound	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)
RT Locking Time (min)	17.138	8.569	5.713	4.285	2.856
Purge Flow (mL/min)	50	50	50	50	50
Purge Time (min)	1	0.75	0.5	0.4	0.25
Gas type	Helium	Helium	Helium	Helium	Helium
Oven					
Voltage (VAC)	120 or 240	120 or 240	240	240	240 and Pillow**[1]**
Initial Oven Temp (°C)	100	100	100	100	100
Initial Oven Hold (min)	1	0.5	0.33	0.25	0.167
Ramp Rate (°C/min)	10	20	30	40	60
Final Temp (°C)	325	325	325	325	325
Final Hold (min)	5	2.5	1.67	1.25	0.833
Total Run Time (min)	28.5	14.25	9.5	7.13	4.75
Equilibration time (min)	0.5	0.5	0.5	0.5	0.5
Column					
Type	DB-5MS	DB-5MS	DB-5MS	DB-5MS	DB-5MS
Agilent Part Number	122-5532	122-5512	122-5512	122-5512	Custom
Length (m)	30	15	15	15	10
Diameter (mm)	0.25	0.25	0.25	0.25	0.25
Film thickness (µm)	0.25	0.25	0.25	0.25	0.25
Nominal Initial Flow (mL/min)	**3**	**2.2**	**4**	**6.2**	**5**
Outlet pressure (psig)	3.8	3.8	3.8	3.8	3.8
MSD					
Agilent Technologies 5975 or 5973 Inert with Performance Electronics					
Suggested Minimum Vacuum Pump	Performance Turbo	Performance Turbo	Performance Turbo	Performance Turbo	Performance Turbo
Tune File	Atune.U	Atune.U	Atune.U	Atune.U	Atune.U
Mode	Scan	Scan	Scan	Scan	Scan
Solvent delay (min)	2.8	1.4	0.8	0.7	0.45
EM voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage
Low mass (amu)	40	40	40	40	40
High mass (amu)	570	570	570	570	570
Threshold	0	0	0	0	0
TID	on	on	on	on	off
Sampling	2	2	1	1	0
Quad temp (°C)	150	150	150	150	150
Source temp (°C)	300	300	300	300	300
Transfer line temp (°C)	300	300	300	300	300

[1] requires Injection port and MSD interface in back positions and G2646-60500 oven pillow

Conditions Table 2 GC and MS Conditions for DB-5MS with Atmospheric Outlet

Table 3. Gas Chromatograph and Mass Spectrometer Conditions for DB-35MS with Vacuum Outlet					
	Original 1X Method	**2X Method**	**3X Method**	**4X Method**	**6X Method**
Biggest 4 Ions	**FT35_1X_VAC.m**	**FT35_2X_VAC.m**	**FT35_3X_VAC.m**	**FT35_4X_VAC.m**	**FT35_6X_VAC.m**
Column Bleed Optimized Ions	**FT35_1X_VAC_BL.m**	**FT35_2X_VAC_BL.m**	**FT35_3X_VAC_BL.m**	**FT35_4X_VAC_BL.m**	**FT35_6X_VAC_BL.m**
Fatty Acid Matrix Optimized Ions	**FT35_1X_VAC_FA.m**	**FT35_2X_VAC_FA.m**	**FT35_3X_VAC_FA.m**	**FT35_4X_VAC_FA.m**	**FT35_6X_VAC_FA.m**
GC					
Agilent Technologies 6890 or					
7890 with Autiinjector and Tray					
Inlet	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless
Mode	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure
Injection Type	Splitless	Splitless	Splitless	Splitless	Splitless
Injection Volume (µL)	1.0	1.0	1.0	1.0	1.0
Inlet temp (°C)	280	280	280	280	280
Pressure, nominal (psig)	20.3	3.75	13.1	22.2	10
RT Locking Compound	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)
RT Locking Time (min)	18.272	9.136	6.091	4.568	3.045
Purge Flow (mL/min)	50	50	50	50	50
Purge Time (min)	1	0.75	0.5	0.4	0.25
Gas type	Helium	Helium	Helium	Helium	Helium
Oven					
Voltage (VAC)	120 or 240	120 or 240	240	240	240 and Pillow**[1]**
Initial Oven Temp (°C)	100	100	100	100	100
Initial Oven Hold (min)	1	0.5	0.33	0.25	0.167
Ramp Rate (°C/min)	10	20	30	40	60
Final Temp (°C)	345 **[2]**	345 **[2]**	345 **[2]**	345 **[2]**	345 **[2]**
Final Hold (min)	9	4.5	3	2.25	1.50
Total Run Time (min)	34.5	17.25	11.5	8.625	5.75
Equilibration time (min)	0.5	0.5	0.5	0.5	0.5
Column					
Type	DB-35MS	DB-35MS	DB-35MS	DB-35MS	DB-35MS
Agilent Part Number	122-3832	122-3832	122-3832	122-3832	Custom
Length (m)	30	15	15	15	10
Diameter (mm)	0.25	0.25	0.25	0.25	0.25
Film thickness (µm)	0.25	0.25	0.25	0.25	0.25
Nominal Initial Flow (mL/min)	**1.9**	**1.1**	**2.4**	**4.3**	**2.82**
Outlet pressure	Vacuum	Vacuum	Vacuum	Vacuum	Vacuum
MSD					
Agilent Technologies 5975 or					
5973 Inert with Performance Electronics					
Suggested Minimum Vacuum					
Pump	Diffusion	Diffusion	Performance Turbo	Performance Turbo	Performance Turbo
Tune File	Atune.U	Atune.U	Atune.U	Atune.U	Atune.U
Mode	Scan	Scan	Scan	Scan	Scan
Solvent delay (min)	3	1.4	0.8	0.65	0.41
EM voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage
Low mass (amu)	40	40	40	40	40
High mass (amu)	570	570	570	570	570
Threshold	0	0	0	0	0
TID	on	on	on	on	off
Sampling	2	2	1	1	0
Quad temp (°C)	150	150	150	150	150
Source temp (°C)	300	300	300	300	300
Transfer line temp (°C)	300	300	300	300	300
[1] requires Iniection port and MSD interface in back positions and G2646-60500 oven pillow					

Conditions Table 3 GC and MS Conditions for DB-35MS with Vacuum Outlet

Table 4. Gas Chromatograph and Mass Spectrometer Conditions for DB-35MS with Atmospheric Outlet					
	Original 1X Method	**2X Method**	**3X Method**	**4X Method**	**6X Method**
Biggest 4 Ions	**FT35_1X_ATM.m**	**FT35_2X_ATM.m**	**FT35_3X_ATM.m**	**FT35_4X_ATM.m**	**FT35_6X_ATM.m**
Column Bleed Optimized Ions	**FT35_1X_ATM_BL.m**	**FT35_2X_ATM_BL.m**	**FT35_3X_ATM_BL.m**	**FT35_4X_ATM_BL.m**	**FT35_6X_ATM_BL.m**
Fatty Acid Matrix Optimized Ions	**FT35_1X_ATM_FA.m**	**FT35_2X_ATM_FA.m**	**FT35_3X_ATM_FA.m**	**FT35_4X_ATM_FA.m**	**FT35_6X_ATM_FA.m**
GC					
Agilent Technologies 6890 or					
7890 with Autiinjector and Tray					
Inlet	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless	EPC Split/splitless
Mode	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure	Constant Pressure
Injection Type	Splitless	Splitless	Splitless	Splitless	Splitless
Injection Volume (µL)	1.0	1.0	1.0	1.0	1.0
Inlet temp (°C)	280	280	280	280	280
Pressure, nominal (psig)	32.5	18	25.5	33.4	22.5
RT Locking Compound	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)	Prodifen (SKF-525a)
RT Locking Time (min)	18.272	9.136	6.091	4.568	3.045
Purge Flow (mL/min)	50	50	50	50	50
Purge Time (min)	1	0.75	0.5	0.4	0.25
Gas type	Helium	Helium	Helium	Helium	Helium
Oven					
Voltage (VAC)	120 or 240	120 or 240	240	240	240 and Pillow **[1]**
Initial Oven Temp (°C)	100	100	100	100	100
Initial Oven Hold (min)	1	0.5	0.33	0.25	0.167
Ramp Rate (°C/min)	10	20	30	40	60
Final Temp (°C)	345 **[2]**	345 **[2]**	345 **[2]**	345 **[2]**	345 **[2]**
Final Hold (min)	9	4.5	3	2.25	1.50
Total Run Time (min)	34.5	17.25	11.5	8.625	5.75
Equilibration time (min)	0.5	0.5	0.5	0.5	0.5
Column					
Type	DB-35MS	DB-35MS	DB-35MS	DB-35MS	DB-35MS
Agilent Part Number	122-3832	122-3832	122-3832	122-3832	Custom
Length (m)	30	15	15	15	10
Diameter (mm)	0.25	0.25	0.25	0.25	0.25
Film thickness (µm)	0.25	0.25	0.25	0.25	0.25
Nominal Initial Flow (mL/min)	**2.9**	**2.2**	**3.9**	**6.1**	**4.8**
Outlet pressure (psig)	3.8	3.8	3.8	3.8	3.8
MSD					
Agilent Technologies 5975 or					
5973 Inert with Performance Electronics					
Suggested Minimum Vacuum					
Pump	Performance Turbo	Performance Turbo	Performance Turbo	Performance Turbo	Performance Turbo
Tune File	Atune.U	Atune.U	Atune.U	Atune.U	Atune.U
Mode	Scan	Scan	Scan	Scan	Scan
Solvent delay (min)	3	1.4	0.8	0.65	0.41
EM voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage	Atune voltage
Low mass (amu)	40	40	40	40	40
High mass (amu)	570	570	570	570	570
Threshold	0	0	0	0	0
TID	on	on	on	on	off
Sampling	2	2	1	1	0
Quad temp (°C)	150	150	150	150	150
Source temp (°C)	300	300	300	300	300
Transfer line temp (°C)	300	300	300	300	300
[1] requires Iniection port and MSD interface in back positions and G2646-60500 oven pillow					

Conditions Table 4 GC and MS Conditions for DB-35MS with Atmospheric Outlet

These Tables provide comparative information useful in deciding best speed(s) to run. If you are uncertain, 2X is a good place to start. If a chosen speed turns out to be too fast, try the next slower speed.

If your MSD is an older model without Performance Electronics (models earlier than 5973 Inert with Performance Electronics), methods faster than 3x may be too fast. With older electronics, high scan speeds may result in significant signal losses.

In the method file naming process, the stationary phase portion from Step 2 is now followed by the speed designation choice. For example, a DB-5MS method which runs at 30 °C per minute at this point now would have the name **FT5_3X_** .

Step 4 Column Outlet Pressure (**ATM_** *or* **VAC_**)

Method sets are provided for operating the column at either vacuum outlet pressure or at somewhat above atmospheric pressure.

- Vacuum outlet methods (**VAC_**) are used when the column end is inserted directly into the MSD interface.
- **ATM_** methods are for use with Agilent Capillary Flow Technology (CFT) devices where the column end is typically operated at 3.8 psi above atmospheric pressure.

Having retention times collected at the column outlet pressure to be used provides better retention time matching, especially for those compounds eluting near the end of the run.

Note that CFT devices can provide significant advantages to toxicology screening analyses. For example, using a two-way splitter, column effluent may be split between the MSD and an NPD. Added information provided by the NPD is often useful in screening samples.

CFT devices also allow changing or servicing the column without venting the MSD. Another major advantage afforded by the devices is the ability to backflush the analytical column at the end of each run:

- By removing heavy matrix material from the head of the column at the end of each run, column and detector maintenance are substantially reduced.
- Carryover and ghost peaks from previous runs are reduced or eliminated.

At this time, choose either VAC or ATM methods based upon the hardware setup you are using.

In the naming convention, the stationary phase prefix and speed designation are now followed by the outlet pressure term:

- Vacuum outlet methods have **VAC_** in the name
- CFT methods have **ATM_**

For example, following from the example in Step 3, a DB-5MS method running at 30 °C per minute, and which is connected directly to the MSD, would have the name, thus far, of **FT5_3X_VAC_** .

Step 5 Ions Used for Quant Database (**nothing** *or* **_BL** *or* **_FA**)

Three different versions of each method set are provided based upon choice of ions used in the quant database:

1 A method using the largest four ions in a compound's spectrum is supplied.
   - The target ion is the ion with the largest abundance.
   - The three qualifiers are the next three largest ions assigned in order of decreasing abundance.
   - The naming convention to designate the largest four ions method sets is a *null* suffix (*nothing* at all).

These method sets are provided for legacy reasons and are used in some more advanced approaches.

The drawback of the largest four ions approach is that, in some cases, the signal-to-noise performance suffers. For example, if the biggest ion for a compound is 207, and the stationary phase has its largest bleed ion at 207, the signal-to-noise at that mass can be significantly reduced. The same problem is seen with low masses such as 44, where CO_2 and other background gases can result in interferences and increased noise.

2 To reduce this signal-to-noise problem, a second method set is provided where ions chosen for the quant database are selected to give optimal signal-to-noise ratios relative to both column bleed and background gases:
   - These methods would normally be chosen as they typically give best overall performance.
   - The naming convention to designate these method sets optimized for column bleed is the suffix **_BL** .

3 A third method type is provided where choice of ions has been optimized for samples having large amounts of fatty acids as typically seen in blood samples:
   - These methods give best signal-to-noise ratios in high fatty acid matrices.
   - They are *not* the best choice for samples having low levels of interfering fatty acids.
   - The naming convention to designate these method sets optimized for fatty acids is the suffix **_FA** .

Now choose the method set type best fitting your situation. In most cases, method sets optimized for column bleed (**_BL**) are the best place to start.

Continuing with the example from Step 4, the method name is one of the following three:

- **FT5_3X_VAC** – representing a DB-5MS vacuum outlet method running at 30 °C (3X) and using the largest four ions
- **FT5_3X_VAC_BL** – the same method but optimized for column bleed ions
- **FT5_3X_VAC_FA** – the same method but optimized for fatty acid ions

This completes the process for creating the name of the specific method best suited to your application situation.

1 Decision Process to Create Your Method Name for the G1674AA Forensic Toxicology Data Base Library (DBL)

2 Forensic DBL Files Setup

Step 1 Eight methods are preloaded and preconfigured for DRS:

FT5_1X_VAC_BL.m

FT5_2X_VAC_BL.m

FT5_3X_VAC_BL.m

FT5_4X_VAC_BL.m

FT5_1X_ATM_BL.m

FT5_2X_ATM_BL.m

FT5_3X_ATM_BL.m

FT5_4X_ATM_BL.m

If your derived method name(s) is/are in this list, proceed to Step 2, part a. Otherwise, proceed to Step 2, part b.

Step 2 Copy files to appropriate target folder locations:

a The eight preconfigured methods are located in the folder:

<drive>:\msdchem\MSDemo\FT Example Methods

Copy your selected method(s) into your method folder, typically:

<drive>:\msdchem\1\METHODS

where **<drive>** is typically your **C:** drive. Recognize that an error made here will likely lead to failures in actual operation.

Continue now to Chapter 3, “DRS System Verification”.

b For all non-preloaded / non-preconfigured methods, you must perform the following file **Copy** operations. Recognize that error(s) made here will likely lead to failures in actual operation. Also recognize that, where **<name>** is indicated, this is your derived method name (Chapter 1) and **<drive>** is typically your **C:** drive:

✔ Locate your method(s), **<name>.m**, to be used in the folder:

<drive>:\Program Files\Agilent\Forensics DBL\Method

Copy your selected method(s) to your method folder, typically:

<drive>:\msdchem\1\METHODS

✔ Copy files **FT35.L** and **<name>.scd** *from* the folder:

<drive>:\Program Files\Agilent\Forensics DBL\GCMS Libraries

to the folder:

<drive>:\DATABASE

✔ Copy the files **<name>.msl** and **<name>.cid** *from* the folder:

<drive>:\Program Files\Agilent\Forensics DBL\Libraries

to the folder:

<drive>:\NIST05\AMDIS32\LIB

NOTE **.msl** and **.cid** file names do *not* include the portion representing your ions choice situation. Thus, for example, file **FT35_3X_ATM.msl** supports all three choice situations: **FT35_3X_ATM.m**, **FT35_3X_ATM_BL.m**, or **FT35_3X_ATM_FA.m** .

✔ Copy the file **<name>_Test.D** *from* folder:

<drive>:\Program Files\Agilent\Forensics DBL\Data

to the folder:

<drive>:\ msdchem\1\DATA

NOTE **<name>_Test.D** names do *not* include the portion representing your ions choice situation. Thus, for example, test data file **FT35_3X_ATM_Test.D** supports all three choice situations: **FT35_3X_ATM.m** , **FT35_3X_ATM_BL.m** , or **FT35_3X_ATM_FA.m** .

This completes copying of your files to their working folder locations.

3 DRS System Verification

Generating a Test Forensics Toxicology DRS Report

As an example, and to test the DRS process, you can perform an offline manual data analysis exercise to produce a forensic toxicology DRS report using the **FT5_2X_VAC_BL** method case:

Step 1 Start the Data Analysis ChemStation.

Step 2 From the MSD, follow the menu path:
Spectrum > AMDIS > Analyze > Settings....

Step 3 In the resulting dialog boxes, verify and, if necessary, set parameters on **Identif.** and **Deconv.** tabbed views as in Figure 5 on page 18 and in Figure 6 on page 19, respectively.

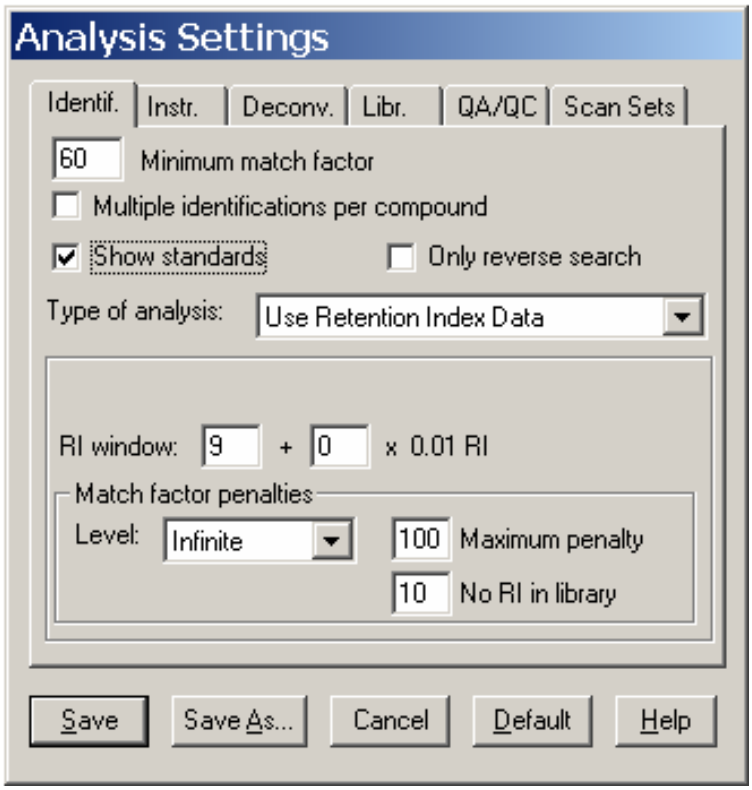

Figure 5 AMDIS Analysis Settings– **Identif. settings**

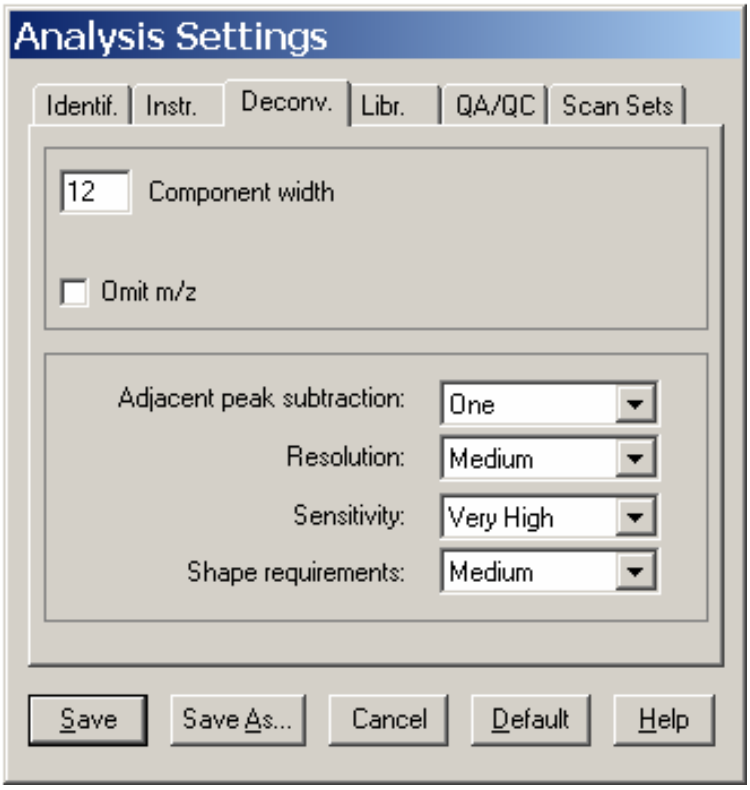

Figure 6 AMDIS Analysis Settings– **Deconv. settings**

Step 4 Select **Save** (settings are permanently saved in the AMDIS initialization file, **onsite.ini**). If prompted to **Reanalyze**, select **No**.

Step 5 **Exit** AMDIS.

Step 6 Load the appropriate ChemStation method: select **Method > Load Method...** and, in this case, browse to and to select **FT5_2X_VAC_BL.M**, then select **OK**.

Step 7 Load the associated ChemStation data file: select **File > Load Data File...** to browse to and to select **FT5_2X_VAC_Test.D**, then select **OK**. The selected data then appears.

Step 8 Again from the ChemStation, open the DRS Method Configurator: **DRS > Method Configurator**. The following default view appears:

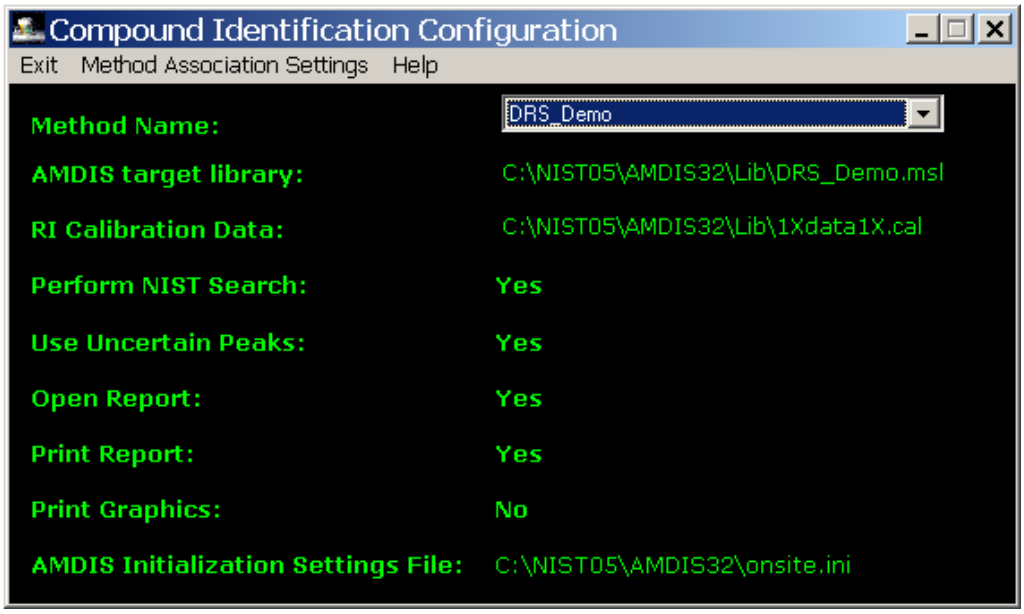

Figure 7 Configurator: default start view

Step 9 From the **Method Name:** dropdown list, select **FT5_2X_VAC_BL**.

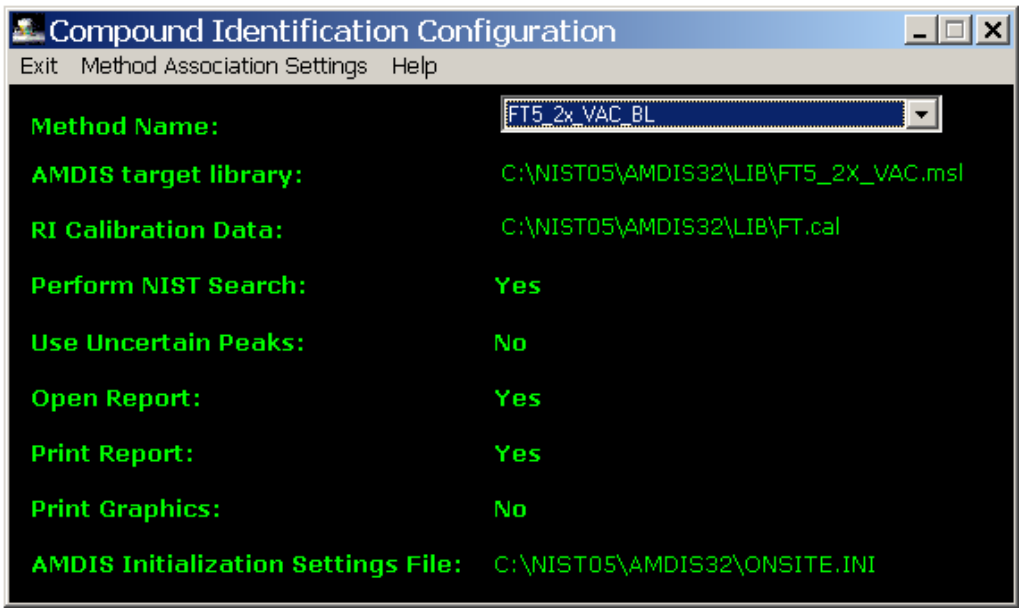

Figure 8 Forensic toxicology configuration

NOTE The **Method Name:**, as it appears in the DRS Configurator, does *not* include **.m** as an extension. This is intentional and is *not* an error: if any extension is added to the Configurator **Method Name:** entry, failure may occur.

NOTE Folder paths shown in Figure 7 and Figure 8 on page 20 are based upon default NIST and AMDIS installation locations. If you installed these software applications elsewhere, you should see paths reflecting your local situation. If needed, you can use **Edit Settings ...** via provided Browse buttons, ..., to update specific file locations:

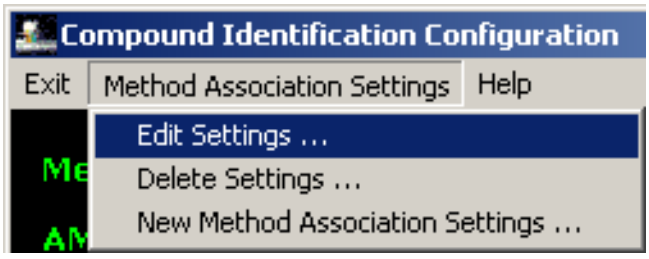

Figure 9 Configurator menu: **Edit Settings ...**

Step 10 **Exit and Save** to accept and to preserve these initialization settings:

Figure 10 Configurator menu: **Exit and Save**

Step 11 To generate the desired DRS report *automatically* at the end of the ChemStation's data analysis process, you must enter a post-run call to macro **trifecta.mac.** Do the following:

a Select **Method > Edit Entire Method**, then check *only* **Method information**, and select **OK**.

b In the resulting view, make the following changes:

- *Unselect* (disable) the **Data Acquisition** check box since data already exists and is loaded (Step 7).
- *Unselect* (disable) the **Data Analysis** check box since DRS, rather than the ChemStation, is to perform the analysis and to produce the report.
- Select (check) the **Post-Run Cmd/Macro** check box and enter **<drive>:\MSDChem\msexe\trifecta.mac** in the **Data Analysis** field to run **trifecta.mac** at the end of the analysis. **<drive>** is typically your **C:** drive. Enter useful explanatory text, if desired, into the **Method Comments:** field:

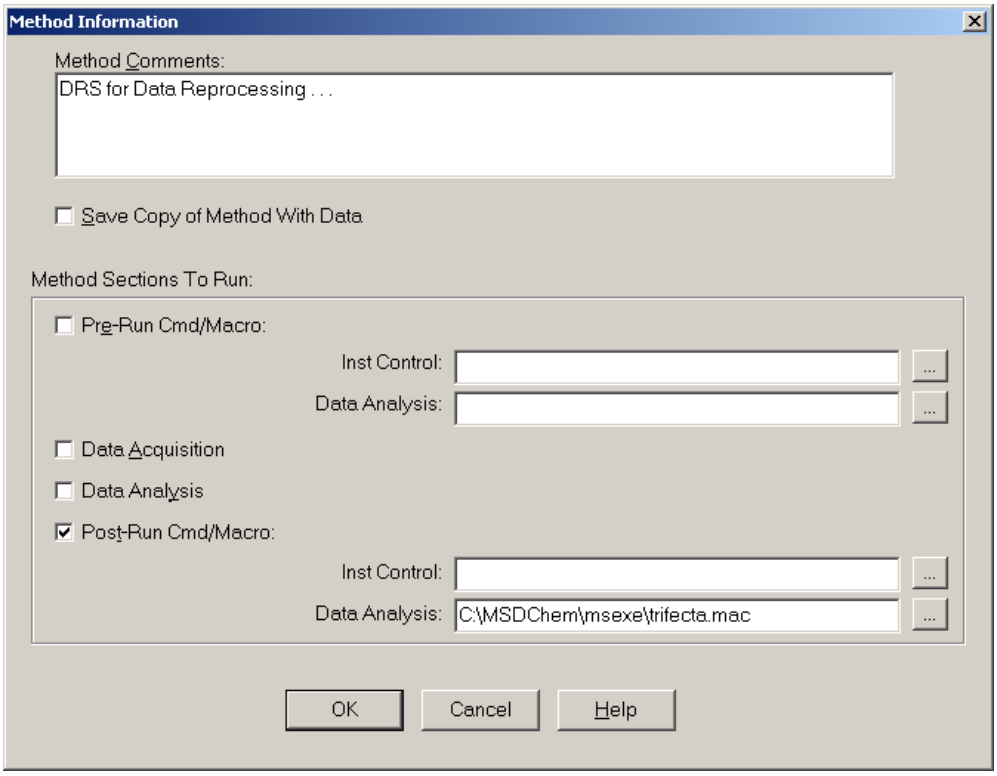

Figure 11 MS Method Information: **Post-Run Cmd/Macro** entry

Recognize that, should you *not* want to run DRS with this Method in the future, you need only *uncheck* (disable) the **Post-Run Cmd/Macro** check box. The macro call itself need not be removed.

Step 12 Run DRS on the loaded data file: from the ChemStation, select **DRS > Quant + DRS single file**.

When complete, the DRS software generates a deconvolution report which should appear similar (but not necessarily identical) to Figure 12 on page 24 or Figure 13 on page 25, depending upon the DRS version in use.

MSD Deconvolution Report
Sample Name: 25 drug mix
Data File: C:\msdchem\MSDemo\FT5_2X_VAC_Test.D
Date/Time: 04:46 PM Tuesday, Apr 8 2008

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (~ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
1.579	64040	Phenylethylamine, Beta-	7.09				
1.7657	60151	Amphetamine		89	-1.8	93	1
1.823	60151	Amphetamine	250.73				
2.013	122098	Phentermine	82.3	90	1.9	77	3
2.014	537462	Methamphetamine	261.53				
2.0805	537462	Methamphetamine		97	-0.2	91	3
3.0875	54115	Nicotine	361.46	93	-0.4	92	2
3.424	43021267	Anhydroecgonine Methyl Ester	5.02	87	0.2	71	2
3.452	771982	Phencyclidine artifact	329.05	89	-0.4	86	1
3.9479	4764174	Methylenedioxyamphetamine (MDA)	172.76	65	-0.5	75	4
4.287	42542109	Methylenedioxymethamphetamine (MDMA)	2265.24	93	-0.2	94	1
4.5739	14089522	Methylenedioxyethylamphetamine	664.13	96	-0.3	95	1
5.6467	57421	Meperidine	149.92	99	-0.3	95	1
6.146	54910893	Fluoxetine	8.45				
6.4900	77101	Phencyclidine	150.71	99	-0.6	94	1
7.7230	76993	Methadone		85	-0.3	77	2
8.075	50362	Cocaine	168.85	93	-0.2	95	1
8.175	72695	Nortriptyline	0.22				
8.3349	529384	Cocaethylene		59	-0.5	45	8
8.5587	302330	SKF-525a		90	-0.1	93	1
8.6934	604751	Oxazepam	64.13	94	-0.1	84	1
8.9781	76573	Codeine	109.55	82	-0.2	93	1
9.048	846491	Lorazepam	27.91	73	-0.3	69	1
9.1821	439145	Diazepam	116.29	95	-0.1	95	1
9.258	125291	Hydrocodone	127.56	80	0.3	94	1
9.3212	1972083	Tetrahydrocannabinol	129.86	99	-0.0	95	1
9.5900	76426	Oxycodone	243.51	81	0.1	92	1
9.8333	846504	Temazepam	121.97	82	0.1	79	1
9.9145	1622624	Flunitrazepam	165.01	99	0.2	91	1
9.916	999517021	Desmethyldoxepin (cis) AC	741.71				
9.9754	561273	Diacetylmorphine	135.29	99	0.3	86	1
10.020	34084509	7-Aminoflunitrazepam	3.08				
10.565	146225	Nitrazepam	651.08	85	-0.0	89	1
10.8721	1622613	Clonazepam		93	0.4	83	1
10.918	4959175	Clonazepam-M (amino-)	2.03				
11.254	999501029	Sulfamethazine AC	21.38				
11.254	28981977	Alprazolam	248.52	71	1.0	91	1
11.9903	50373	Lysergide (LSD)		92	1.0	88	1
12.0866	57249	Strychnine		80	1.5	82	1
12.850	19794935	Trazodone	282.1	94	2.5	86	1

Figure 12 Example Forensic Toxicology DRS report (using G1716AA DRS, version A.03)

MSD Deconvolution Report
Sample Name: 25 drug mix
Data File: C:\msdchem\1\DATA\FT5_2X_VAC_Test.D
Date/Time: 3:09:39 PM Tuesday, May 20, 2008

Adjacent Peak Subtraction = 1
Resolution = Medium
Sensitivity = Very High
Shape Requirements = Medium

The NIST library was searched for the components that were found in the AMDIS target library.

			Amount (~ng)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num.
1.579	64040	Phenylethylamine, Beta-	7.09					
1.7679	60151	Amphetamine			88	-1.7	92	1
1.823	60151	Amphetamine	250.73	770.3				
2.013	122098	Phentermine	82.3	82.58	90	1.9	77	3
2.014	537462	Methamphetamine	261.53	3345.77				
2.0805	537462	Methamphetamine			97	-0.2	91	3
3.0875	54115	Nicotine	361.46	297.61	93	-0.4	92	2
3.4199	43021267	Anhydroecgonine Methyl Ester	5.02	3.89	83	-0.2	66	2
3.452	771982	Phencyclidine artifact	329.05	297.69	88	-0.3	87	1
3.9479	4764174	Methylenedioxyamphetamine (MDA)	172.76	154.5	65	-0.5	75	4
4.2865	42542109	Methylenedioxymethamphetamine (MDMA)	2265.24	1986.18	92	-0.4	94	1
4.5739	14089522	Methylenedioxyethylamphetamine	664.13	599.34	96	-0.3	95	1

Figure 13 Example Forensic Toxicology DRS report (using G1716AA DRS, version A.04), part 1 of 2

5.6463	57421	Meperidine	149.92	135.44	99	-0.3	95	1
6.146	54910893	Fluoxetine	8.45					
6.4900	77101	Phencyclidine	150.71	137.11	99	-0.6	94	1
7.7208	76993	Methadone		276.04	85	-0.4	77	2
8.075	50362	Cocaine	168.85	155.49	93	-0.2	95	1
8.175	72695	Nortriptyline	0.22					
8.5587	302330	SKF-525a		218.53	90	-0.1	93	1
8.695	604751	Oxazepam	64.13					
8.9741	76573	Codeine	109.55	88.3	83	-0.5	84	1
9.0457	846491	Lorazepam	27.91	18.07	70	-0.6	48	1
9.1821	439145	Diazepam	116.29	96.56	95	-0.1	95	1
9.2549	125291	Hydrocodone	127.56	118.3	80	0.0	94	1
9.3216	1972083	Tetrahydrocannabinol	129.86	123.46	99	-0.0	95	1
9.594	76426	Oxycodone	243.51	206.49	79	0.4	90	1
9.8333	846504	Temazepam	121.97	104.99	82	0.1	78	1
9.9119	1622624	Flunitrazepam	165.01	125.9	99	0.1	90	1
9.916	999517021	Desmethyldoxepin (cis) AC	741.71					
9.9723	561273	Diacetylmorphine	135.29	106.71	99	0.1	85	1
10.020	34084509	7-Aminoflunitrazepam	3.08					
10.565	146225	Nitrazepam	651.08	569.55	85	-0.0	89	1
10.8747	1622613	Clonazepam		264.35	93	0.5	84	1
10.918	4959175	Clonazepam-M (amino-)	2.03					
11.2495	28981977	Alprazolam	248.52	154.84	71	0.7	89	1
11.254	999501029	Sulfamethazine AC	21.38					
11.990	50373	Lysergide (LSD)		234.45	92	1.0	88	1
12.0866	57249	Strychnine		210.55	78	1.5	84	1
12.850	19794935	Trazodone	282.1	221.41	94	2.2	87	1

Figure 14 Example Forensic Toxicology DRS report (using G1716AA DRS, version A.04), part 2 of 2

This completes the process of producing an example post-run manual DRS analysis and report.

4 Chromatographic Considerations

Step 1 If not already done, install the column for your specific method as listed in Conditions Tables 1, 2, 3, or 4.

Step 2 If you are using a CFT device (for example, a splitter to an NPD) at the end of the column. Make sure CFT restrictors are chosen to accept column flow for your chosen method as listed in Conditions Tables 1, 2, 3, or 4.

Total flow out the CFT restrictors should be at least 30% greater than the column flow listed in the Conditions Table for your method. This ensures a flow rate sufficient to prevent the GC from becoming *Not Ready* as required in the Retention Time Locking calibration step described in Chapter 5.

This completes chromatographic considerations associated with your method(s).

5 Retention Time Calibration and Locking

Supplied forensic toxicology DBL methods were each originally constructed by adding data analysis components to the original default ChemStation method.

Step 1 Load your chosen method into the ChemStation Instrument session. You may be asked some configuration questions upon loading the method.

Step 2 Enter all acquisition parameters for the method as listed in the specific Conditions Table used in determining your method.

Step 3 Inject 1 µL of a 5 ng/µL solution to run a sample of the retention time locking compound, proadifen (SKF-525a, CAS number: 302-33-0) to confirm that the method is working appropriately.

Step 4 Verify that retention time of proadifen is within about ± *1%* of the locking time listed in the specific Conditions Table (1, 2, 3, *or* 4) you used for vacuum outlet methods (**VAC_**), or ± *2%* for CFT outlet methods (**ATM_**).

If it is *not*, adjust inlet pressure in 1-psig steps and rerun the standard for each step until the retention time falls within the range. If retention time is too short, decrease pressure; if it is too long, increase pressure.

Step 5 Change the MSD solvent delay time to about one half of the locking time for proadifen and **Save** the method. This prevents the electron multiplier from being exposed to the solvent peak at the higher flow rate runs for the retention time locking calibration procedure.

Step 6 Run the retention time locking calibration procedure and lock the retention time of proadifen to that listed in the specific Conditions Table (1, 2, 3, *or* 4) you used in determining your Method (this procedure is described in your ChemStation's Help topic, "*To Lock an MS method*").

Step 7 After retention time locking is successfully completed, set the MSD solvent delay time back to that listed in the given Conditions Table and **Save** the method.

Step 8 Run a calibration sample containing drugs from the FT DBL to confirm the method is working properly. It is best to have a mixture of drugs spanning the retention time range of the method. It is especially important to have some early- and late-eluting compounds:

- Amphetamine, phentermine, and methamphetamine are good early test compounds.
- LSD, strychnine, and trazodone are good late test compounds.

Step 9 Inspect the chromatogram to confirm the solvent delay is set appropriately to prevent the back end of the solvent peak from producing full-scale response in the MSD. If this is *not* the case, as necessary, either shorten the splitless purge time or lengthen the solvent delay.

Step 10 Inspect retention times of drugs in the test mixture to confirm they fall within **± *0.12*** minutes of retention times listed in the specific method calibration table you are creating.

Compound retention times are found by loading the method in **Data Analysis** and then selecting the **Calibrate/Edit Compounds** menu item. If using DB-35 (**35**) methods, final temperature of your oven program may need to be adjusted in 1 °C increments from the nominal 345 °C to make retention times of late-eluting compounds such as LSD, strychnine, and/or trazodone, fall into range.

This completes retention time calibration and locking considerations associated with your method(s).

6 Final Response Factor Calibration

Method calibration tables (quant database) supplied with the FT DBL methods contain only approximate response factor calibrations for each compound. These response factor calibrations have units labeled **~ng**, where ~ indicates each given value to be an approximation:

- The purpose of these ~ calibrations is to provide a *very* approximate indicator of the amount of each compound found in the screening process.
- They are intended to provide an estimated value which can be used as a guide in preparation of a compound-specific, true calibration standard with which to calibrate for each specific compound desired.
- These responses must never be used to report *true* quantitative results.

The provided approximate calibration values are adjusted *after* the method is set up, and with the MSD already having been autotuned, but *before* any actual quantitative calibration is performed. The adjustment is done by injecting a sample containing 5 ng/µL of proadifen (this sample solution also could be the retention time locking calibration sample).

Step 1 In Data Acquisition, **Load** the FT DBL method you are using.

Step 2 Run the 5 ng/µL proadifen sample and generate a quant report.

Step 3 In Data Analysis, **Load** the just-completed data file from the previous step and generate a report.

Step 4 Check the reported amount: if the reported amount is 233.57 **~ng**, for example, then approximate response factors in the quant database *all* need to be multiplied by 233.57/5 to *normalize* them to be equivalent to that of the *5* ng/µL proadifen sample.

This normalization is done by selecting menu item:
Calibrate > Update... > Global Update > Set Other (via command)...

For this example, enter the command **Cresp[1]=Cresp[1]*233.57/5** (note the required use of square brackets, "**[ ]**") and select **OK**:

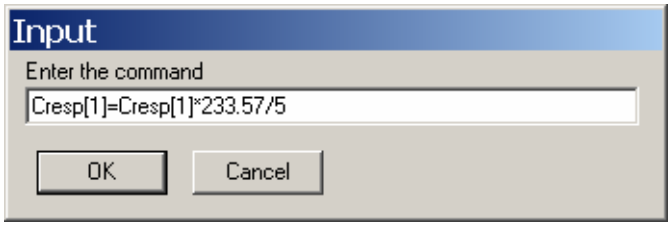

Figure 15 Changing all RFs by command entry

This step adjusts *all* calibration response factors by the same scaling value, in this example case, of 233.57/5.

NOTE

This step must be done *before* you do any actual individual component response factor calibrations. If done later, this adjustment step will incorrectly change your actual calibrated response factor values.

In entering your actual response factor calibration values, change amount units from **~ng** to your working units so, when reports are generated, they correctly indicate the source of your response factor calibrations.

This completes response factor calibration considerations associated with your method(s).

7 DRS Setup for Data Acquisition

Upon completing Chapter 3, Chapter 4, Chapter 5, and Chapter 6, you are now ready to perform final steps necessary to run your sample analyses. Three cases are to be considered:

Case 1 Your chosen method is *specifically* **FT5_2X_VAC_BL.m** as was used in Chapter 3, "DRS System Verification". In this case, you need only reopen the ChemStation view for the post-run call to macro **trifecta.mac** and make *one* change: to do this, proceed *directly* to Chapter 8, "MS ChemStation: DRS Post-Run Call".

Case 2 Your chosen method is one of the *other* seven preloaded / preconfigured ChemStation methods:

FT5_1X_VAC_BL.m

FT5_3X_VAC_BL.m

FT5_4X_VAC_BL.m

FT5_1X_ATM_BL.m

FT5_2X_ATM_BL.m

FT5_3X_ATM_BL.m

FT5_4X_ATM_BL.m

For this second case, do the following:

1 From the ChemStation, open the DRS Configurator: **DRS > Method Configurator** . The following default view appears:

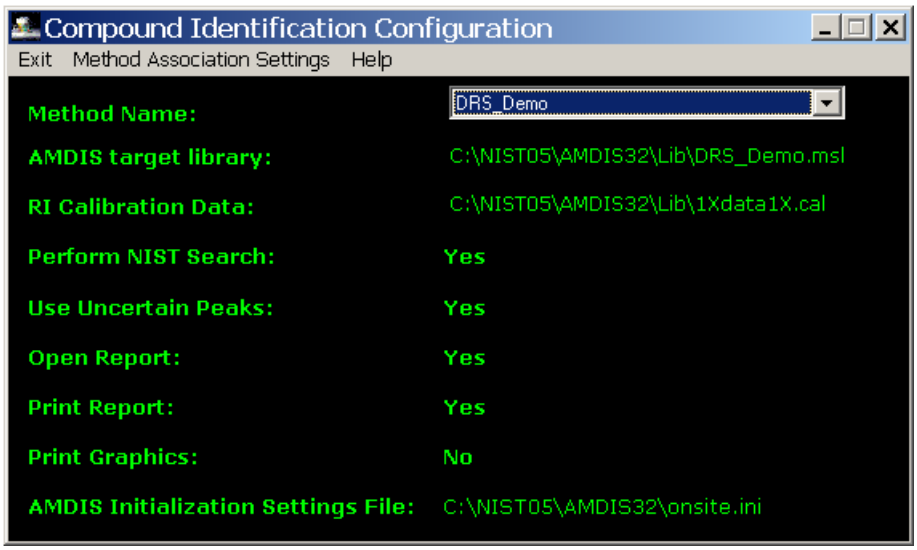

Figure 16 Configurator: default start-up view

2 Open the **Method Name:** list and select your chosen method name. Remember that, intentionally, there is no **.m** extension as part of the name.

3 **Exit and Save** to accept and to preserve these DRS Configurator method settings:

Figure 17 Configurator menu: **Exit and Save**

4 Reopen the ChemStation view for the post-run call to macro **trifecta.mac** and make *one* change: to do this, proceed *directly* to Chapter 8, “MS ChemStation: DRS Post-Run Call”.

Case 3 Your chosen method is one of the *other* 52 ChemStation methods *not* originally preloaded and preconfigured.

For this situation, do the following:

1 From the ChemStation, open the DRS Configurator: **DRS > Method Configurator** . The following start-up default view appears:

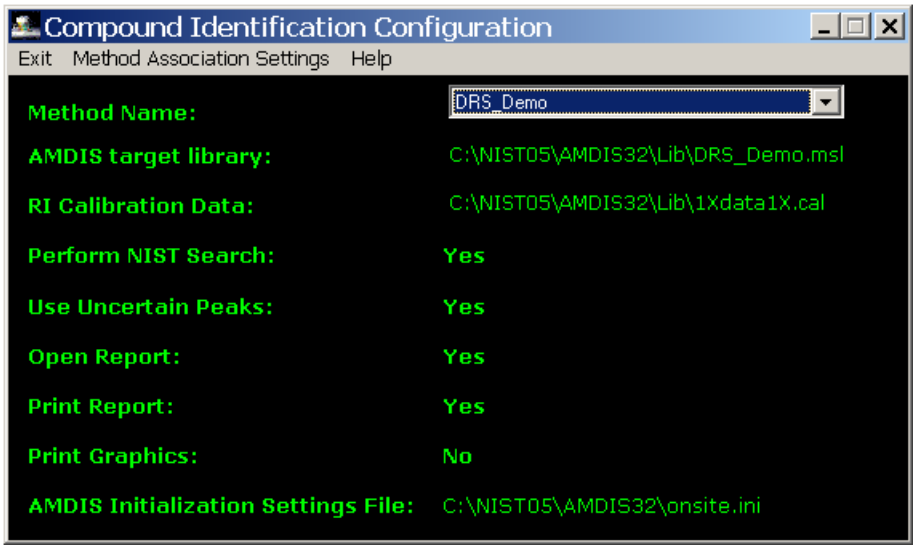

Figure 18 Configurator: default start-up view

2 Manually add your DRS Configurator method name to the **Method Name:** list by selecting: **Method Association Settings > New Method Association Settings ...** .

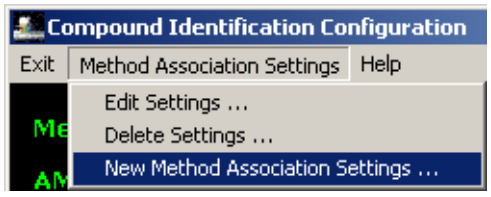

Figure 19 Configurator menu: **New Method Association Settings ...**

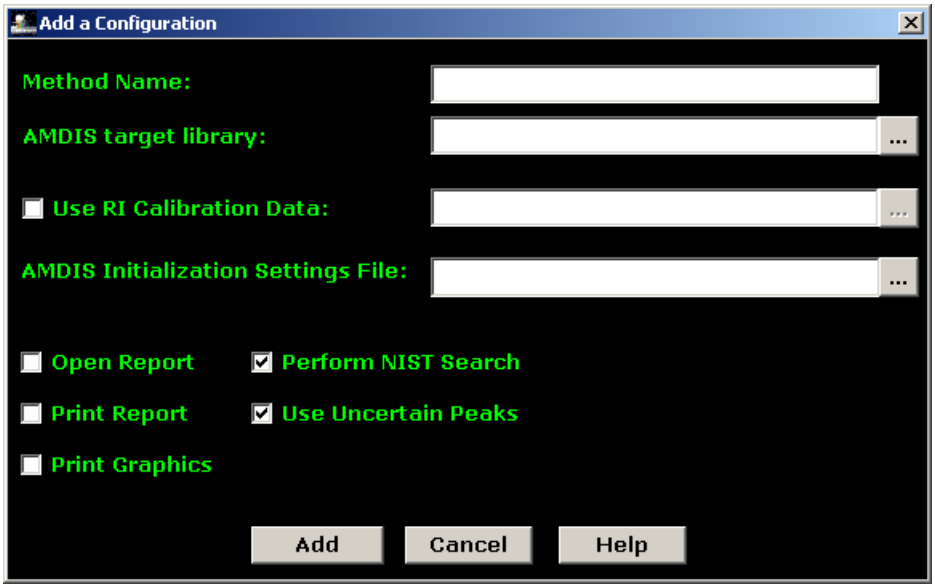

Figure 20 Configurator: **Add a Configuration**

3 Enter necessary information for your chosen method. As an example, suppose you determined the name of your chosen method (Chapter 1) to be **FT35_3X_VAC_FA**. The following entries and settings would be made:

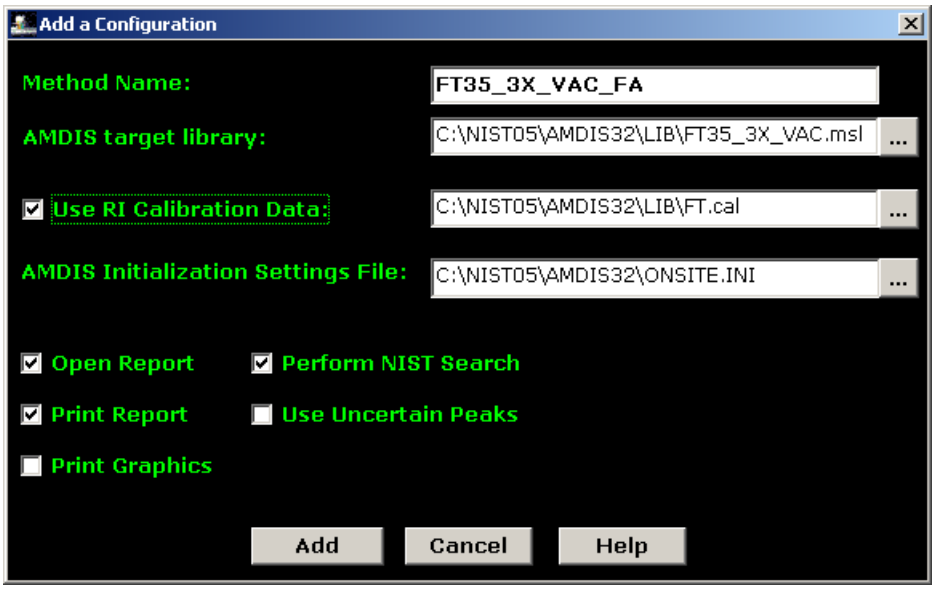

Figure 21 Configurator: adding a given forensic toxicology configuration

NOTE

1 There must be *no* **.m** extension included as part of the **Method Name:** entry.

2 For *any* **FT** DRS Configurator method, entries and settings here are the same *except* **Method Name:** and **AMDIS target library:** which are both method dependent.

3 Be careful typing entries: an incorrect or missing character will likely cause failure.

4 With entries and settings made for your chosen method, select **Add** to accept the new information. The following confirmation view appears:

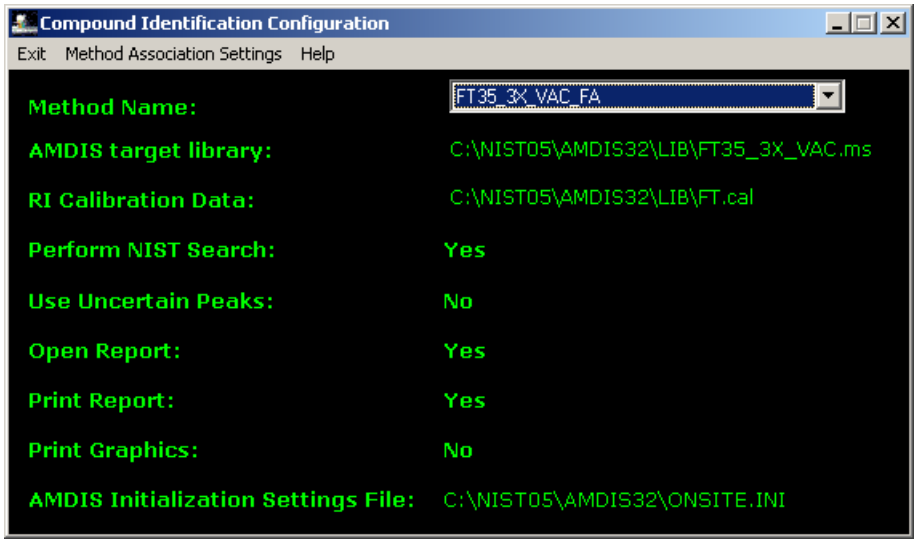

Figure 22 Configurator: verifying the added forensic toxicology configuration

NOTE

For **AMDIS target library:** , final entry character(s) may be truncated depending upon total line length. This is not a problem functionally.

5 **Exit and Save** to accept and to preserve these new DRS Configurator method settings:

Figure 23 Configurator menu: **Exit and Save**

NOTE You can return to this information for editing purposes at any time by opening the DRS Configurator, selecting your method, and then following the menu, **Method Association Settings > Edit Settings ...** .

6 Reopen the ChemStation view for the post-run call to macro **trifecta.mac** and make *one* change: to do this, proceed *directly* to Chapter 8, “MS ChemStation: DRS Post-Run Call”.

8 MS ChemStation: DRS Post-Run Call

To complete the process to generate an automatic DRS analysis and report at completion of each of your analyses, do the following:

Step 1 If not *already* loaded, load the appropriate ChemStation method: select **Method > Load Method...** and browse to and select your chosen method, **<name>.m** , then select **OK**.

Step 2 Select **Method > Edit Entire Method** , then check *only* **Method information**, and select **OK**.

Step 3 Select (enable) the **Data Acquisition** check box since new data does not presently exist (see Figure 24 on page 40).

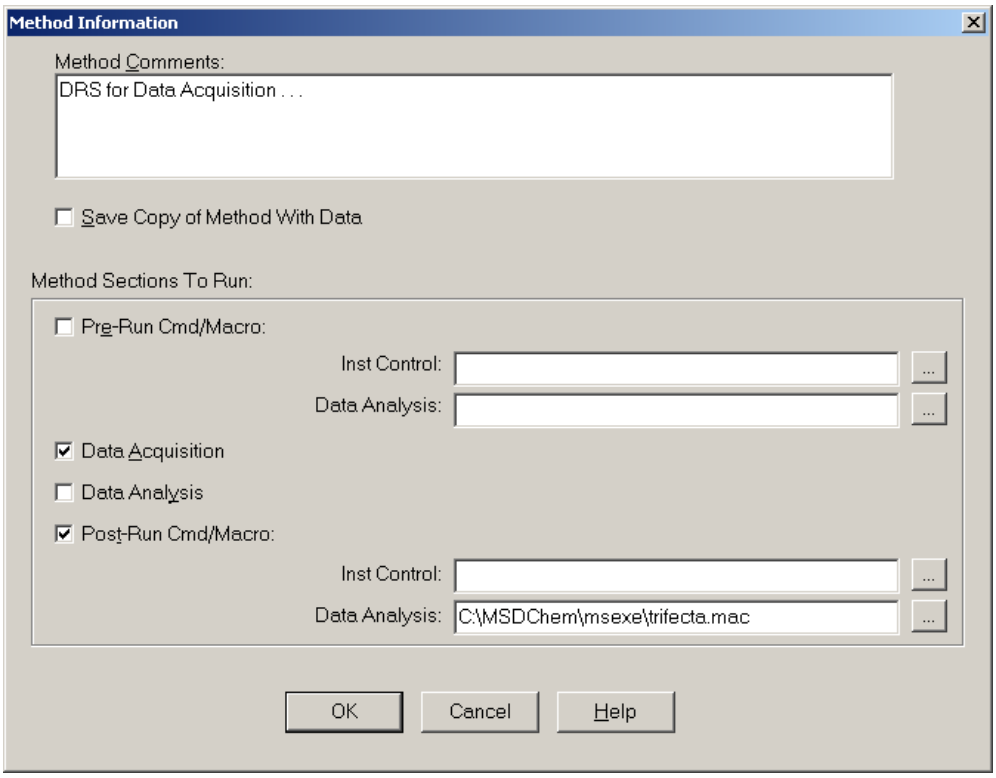

Figure 24 MS Method Information: Data Acquisition & **Post Run Cmd/Macro** entry

Step 4 **Save** the method to preserve this change.

You now should be ready to perform an analysis to generate a DRS report for your chosen method.

As references for additional information, see:

- "*Acquiring and Processing New Data Using a Sequence*" in DRS Help for additional information regarding performing multiple analyses via a Sequence.
- "*Sequence Reprocessing Mode*" in DRS Help for additional information regarding using a sequence to reprocess multiple existing data files.
- "*DRS Troubleshooting and Additional Information*" in DRS Help for problems encountered.

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