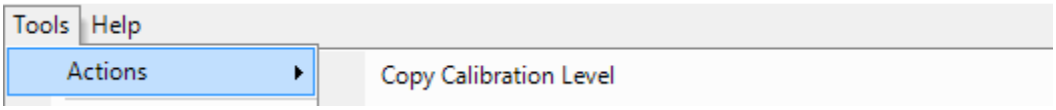
Agilent Technologies

Semi Quant→Copy Calibration Level -->The Script

In MassHunter Quantitative Analysis software, there is a script that allows specific compounds to be used for semi quantitative analysis. Proper implementation of this script requires that specific compounds groups be established referencing the individual compounds whose response factors will be used to "semi-quantitate" unknown compounds. This utility differs from the **Relative ISTD** global parameter and the **Copy Calibration Levels To** command in the sense that it is based on the compound group (**Cmpd.Group**) concept referencing the target or internal standards (ISTD).

1. The script is activated in the **Method Editor** from **Tools→Actions→Copy Calibration Level** command.



2. In this example, the target compounds Amp, Cocaine and Meth are being quantitated with the response factor from MDMA. The internal standards Amp-d5, Cocaine-d3 and Meth-d5 are using the response factor established for MDMA-d5. In the **Method Editor**, open the **Cmpd. Group** column by right clicking and selecting **Add/Remove Columns** and under the Quantifier header **Add** the **Cmpd. Group** column.
3. The next step involves establishing the link from the compound that is to be "semi-quantitated" to the measured compound and the measured compound's calibration curve. This is accomplished by placing the name of the measured compound in the **Cmpd. Group** field. In the table below Amp will be "semi-quantitated" based on the measured response of MDMA. This step should only have to be done once and the links can be saved as part of the quantitation method.

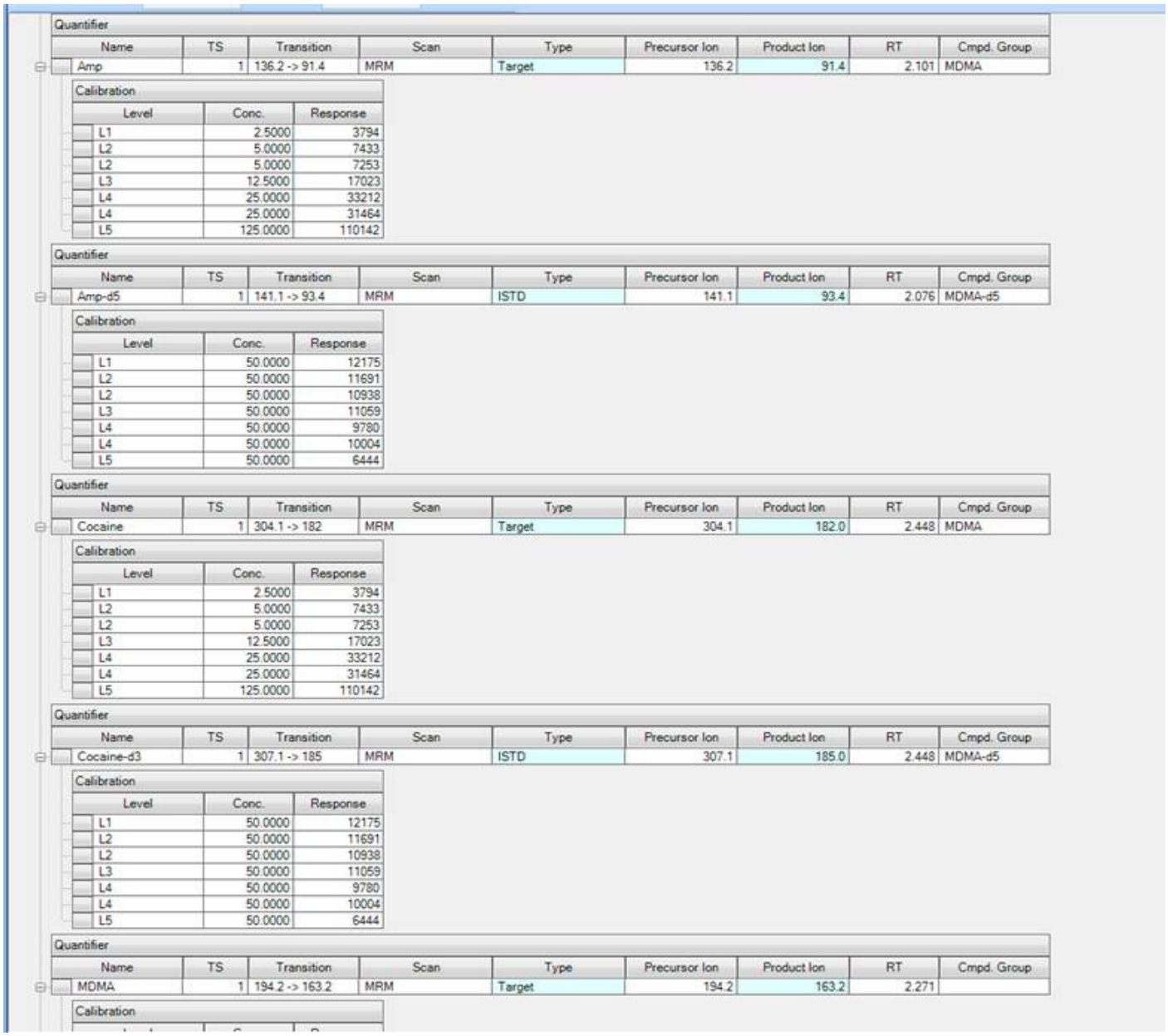
Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Cmpd. Group
Amp	1	136.2 -> 91.4	MRM	Target	136.2	91.4	2.101	MDMA
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	141.1	93.4	2.076	MDMA-d5
Cocaine	1	304.1 -> 182	MRM	Target	304.1	182.0	2.448	MDMA
Cocaine-d3	1	307.1 -> 185	MRM	ISTD	307.1	185.0	2.448	MDMA-d5
MDMA	1	194.2 -> 163.2	MRM	Target	194.2	163.2	2.271	
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	199.2	164.3	2.268	
Meth	1	150.1 -> 119.3	MRM	Target	150.1	119.3	2.237	MDMA
Meth-d5	1	155.1 -> 92.3	MRM	ISTD	155.1	92.3	2.231	MDMA-d5

4. In the **Batch Table→ Analyze Batch** and examine the calibration curve for MDMA and MDMA-d5 and make any necessary corrections such as manual integrations on calibration

standards or excluding data points from the calibration curve. This goal is to have valid calibration curves on any measured compound whose calibration curve will be used by other "semi-quantitated" compounds in a next step.

5. In the **Method Editor**, click on **Tools→Actions→Copy Calibration Level** to run the script. The script will only run in the Method Editor. Calibration information including Level name, Conc. and response of measured compounds will be copied automatically to target or "semi-quantitated" compounds.



Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Cmpd. Group
Amp	1	136.2 -> 91.4	MRM	Target	136.2	91.4	2.101	MDMA

Calibration

Level	Conc.	Response
L1	2.5000	3794
L2	5.0000	7433
L2	5.0000	7253
L3	12.5000	17023
L4	25.0000	33212
L4	25.0000	31464
L5	125.0000	110142

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Cmpd. Group
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	141.1	93.4	2.076	MDMA-d5

Calibration

Level	Conc.	Response
L1	50.0000	12175
L2	50.0000	11691
L2	50.0000	10938
L3	50.0000	11059
L4	50.0000	9780
L4	50.0000	10004
L5	50.0000	6444

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Cmpd. Group
Cocaine	1	304.1 -> 182	MRM	Target	304.1	182.0	2.448	MDMA

Calibration

Level	Conc.	Response
L1	2.5000	3794
L2	5.0000	7433
L2	5.0000	7253
L3	12.5000	17023
L4	25.0000	33212
L4	25.0000	31464
L5	125.0000	110142

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Cmpd. Group
Cocaine-d3	1	307.1 -> 185	MRM	ISTD	307.1	185.0	2.448	MDMA-d5

Calibration

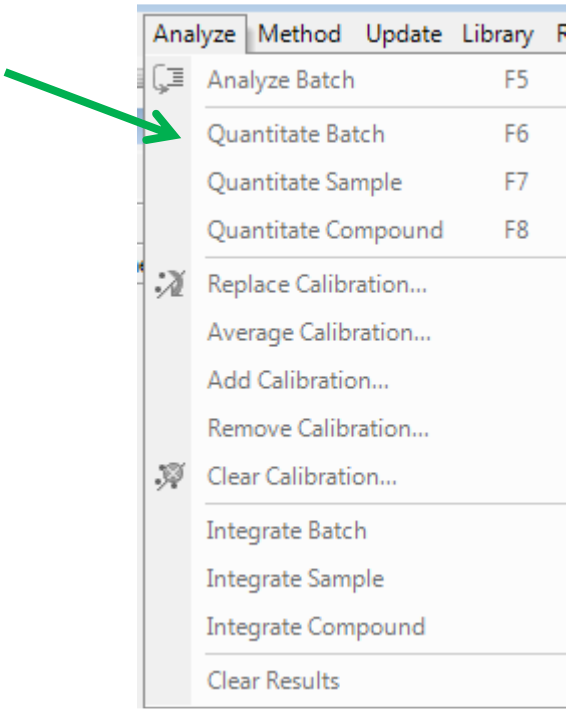
Level	Conc.	Response
L1	50.0000	12175
L2	50.0000	11691
L2	50.0000	10938
L3	50.0000	11059
L4	50.0000	9780
L4	50.0000	10004
L5	50.0000	6444

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Cmpd. Group
MDMA	1	194.2 -> 163.2	MRM	Target	194.2	163.2	2.271	

Calibration

6. Exit the **Method Editor** and **Quantitate** the batch. Do NOT use **Analyze Batch** or the **F5** key. At this point or any point thereafter **Analyze Batch** will create a new calibration curve based any **Cals** or **CCs** in the **Batch Table**. If the **Analyze Batch** command is used go back to step 4 to recover.



7. The batch has now been quantitated using the response factors from MDMA and MDMA-d5 and applied to the remaining targets and ISTDs.