Application Note

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Automated MRM Method Development for US EPA Method 8270 with the Agilent MassHunter Optimizer for GC/TQ

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Abstract

This application note demonstrates the use of Agilent MassHunter Optimizer for GC/TQ to enable highly automated, end-to-end development of multiple reaction monitoring (MRM) data acquisition methods. The Optimizer for GC/TQ uses spectral deconvolution to reliably identify precursor ions, even in the presence of chromatographic interferences. This tool enables significant time savings and reduces manual review when developing MRM data acquisition methods. A mix of 83 compounds related to US Environmental Protection Agency (EPA) method 8270 was used to challenge the process and evaluate the effects of coelutions on MRM method development.

Key advantages of the Optimizer tool include:

- Time-savings for developing an optimized MRM method
- Automation and reduced manual work
- Reproducibility
- A smooth transition of GC/MSD methods to GC/TQ
- Built-in review tools

Introduction

Development of GC/MS/MS MRM transitions is a challenging and time-consuming multistep process, which is frequently further complicated by analyte coelution and matrix interferences. This has traditionally required manual intervention by an experienced scientist. MassHunter Optimizer for GC/TQ enables automated optimization of the data acquisition parameters for MRM mode.

End-to-end MRM method development can be highly automated, with no user interaction. Alternatively, each of the optimization steps can be performed individually. These steps include:

- Identification of the analytes using library search of deconvoluted spectra
- Precursor ion identification
- Product ion identification at various collision energies
- Selection of product ions
- Optimization of collision energy

Several workflows available with the Optimizer for GC/TQ, such as *Start from scan data* and *Start from SIM ions*, allow new GC/TQ users to convert existing single quadrupole scan or SIM methods to triple quadrupole MRM methods. Existing TQ users can re-optimize collision energies for current MRMs and update their retention times under the new chromatographic conditions with the *Start with MRMs* workflow. For existing MRM methods, the retention time update function is available to ensure a seamless transition to modified chromatographic conditions, such as a new chromatographic stationary phase or GC oven temperature program.

In this application note, an MRM acquisition method was developed for 83 semivolatile compounds, including targets, surrogates, and internal standards for US EPA 8270. Four workflows were showcased, starting with different acquisition methods, such as scan, SIM, and MRM:

- When starting from scan data, 83 compounds were identified. Precursor and product ions were determined, and collision energies were optimized.
- The *Start from SIM ions* workflow enabled product ion identification for the imported SIM ions and collision energy optimization of the 351 MRM transitions for 83 compounds.
- Collision energies for 83 compounds were re-optimized with the *Start from MRMs* workflow.
- Retention times were updated for an MRM method using the *update retention time* function.

The 8270 "full mix" (AccuStandard), containing 83 semivolatile compounds, was used to challenge the process and evaluate the effects of coelutions. While the entire MRM development process was largely successful, coelutions can sometimes cause complications, requiring review and manual intervention.

Experimental

MassHunter Optimizer for GC/TQ is installed automatically with Agilent MassHunter GC/MS Data Acquisition Version 10.0 and above. It is supported for use with Agilent 7000 series and 7010 series GC/TQ. A desktop icon is created when a GC/TQ instrument is configured using the Agilent GC/MS configuration tool. To start MRM development, an existing data acquisition method is required. All GC parameters of the acquisition method will be retained when developing and optimizing MRM transitions.

This application note features four workflows, including *Start from scan data*, *Start from SIM ions*, *Start from MRMs*, and *Retention time update* when starting from MRMs. An Agilent 7890/7000D triple quadrupole GC/MS system, Agilent MassHunter GC/MS Data Acquisition Version 10.0, and Agilent MassHunter Unknowns Analysis Version 10.0 were used in this work. The starting acquisition method was previously optimized for successful GC/MSD analysis of semivolatiles meeting the performance requirements of the US EPA 8270 method.[1]

Start from scan data

The *Start from scan data* workflow included the following steps, performed sequentially:

- Acquisition or import of full scan data to identify target compounds
- Precursor ion identification
- Product ion identification
- Collision energy optimization

In this work, scan data were acquired with MS1 scan, a scan time of 450 ms, which results in a sampling rate of five samples per second. When starting from scan, the first step of MRM development is identification of the analytes using a library search of deconvoluted spectra. This allows correct identification of target analytes and enables reliable selection of precursor ions, even in the presence of chromatographic interferences such as column bleed or coeluting analytes or matrix interference. The spectral deconvolution and library search algorithms are similar to what is used with Agilent MassHunter Unknowns Analysis software. Library formats supported by the Optimizer for GC/TQ include *.L and *.mslibrary.xml. This provides the flexibility of using large spectral libraries such as NIST or small user-created libraries built with Agilent MassHunter library editor software. In this application note, the NIST17 spectral library was used for compound identification.

The latter three steps in MRM development can be automated, with no user intervention. Alternatively, the result of each step can be reviewed before proceeding to the next step. Before proceeding, the user may modify automated selections and choose additional ions if desired. In this application note, precursor and product ion selections were reviewed before proceeding to the following optimization steps.

Start from scan data workflow enabled to complete optimization with 12 injections in 6 hours.

Start from SIM ions

The *Start from SIM ions* workflow included the following steps performed sequentially:

- Import of SIM ions for target compounds as a .CSV file that included compound names and retention times. Alternatively, SIM ions may be imported from a previously created SIM acquisition method.
- Product ion scan and identification.
- Collision energy optimization.

Start from SIM ions workflow enabled to complete optimization with 11 injections in 5.5 hours.

Start from MRMs

The *Start from MRMs* workflow involved the following steps:

- Importing MRM transitions from a previously created MRM acquisition method or a .CSV file
- Collision energy optimization

All three of these workflows include the collision energy optimization step. When starting from scan or SIM, product ion identification is performed. With the *Start from scan data* workflow, the compound and precursor ion identification steps must be performed first.

After MRM development and collision energy optimization are complete, the developed acquisition method can be saved as a time-segment MRM method or a dynamic MRM (dMRM) method. The latter option allows the user to define minimum dwell time and number of cycles per second.

To update retention times, an existing MRM or SIM method is imported and a chromatographic run must be performed.

Start from MRMs workflow enabled to complete optimization with four injections in 2 hours.

Results and discussion

Start from scan data: library search and precursor ion identification

Eighty-three semivolatile compounds, including surrogates and internal standards, were identified using an acquired full scan chromatogram of an EPA 8270 standard "full" mixture by searching deconvoluted mass spectra against the NIST17 spectral library. These 83 semivolatile compounds were used to challenge the process and evaluate the effects of coelutions that may complicate the MRM development process and require manual review. Figure 1A demonstrates the Optimizer window after completing compound identification. The window includes:

- A compound table
- A GC/MS chromatogram with labeled peaks
- A deconvoluted mass spectrum for a highlighted compound
- Precursor ions available for a highlighted compound
- A summary of all precursor ions selected for all identified compounds

A Library match score is displayed in the table in Figure 1A under the Hit Score column. Information available in the library such as compound name, CAS number, molecular formula, and molecular weight is imported into the Compound Table in the Optimizer. A deconvoluted spectrum for each identified compound is displayed when selecting it in the compound table. A deconvoluted spectrum for a highlighted compound, 2-methyl-4,6-dinitrophenol (DNOC), is shown at the bottom right in Figure 1A. Spectral deconvolution enables correct compound identification and reliable choice of precursor ions, even in the presence of chromatographic interferences such as column bleed or coeluting peaks. The suggested precursor ions are highlighted in green.

Note the choice of precursors suggested by the software is based on abundance and *m/z* value. Also, no more than two ions from a cluster are selected. For example, *m/z* 168 was automatically selected as a precursor ion due to its higher *m/z* value and uniqueness despite being less abundant than ions *m/z* 51 and 53.

Precursors suggested by the software can be overwritten by the user by unchecking selected ions and checking other available ions.

The list of available precursor ions is displayed when selecting a corresponding compound in the compound table. Precursor ions available for DNOC are shown under the DNOC table, the second table in the Optimizer window in Figure 1A. The ions selected in the table were chosen as precursor ions automatically by the software, as the Optimizer method was set up to pick no more than four ions as precursors for each compound, as indicated in the precursor ion identification parameters in Figure 1B.

A

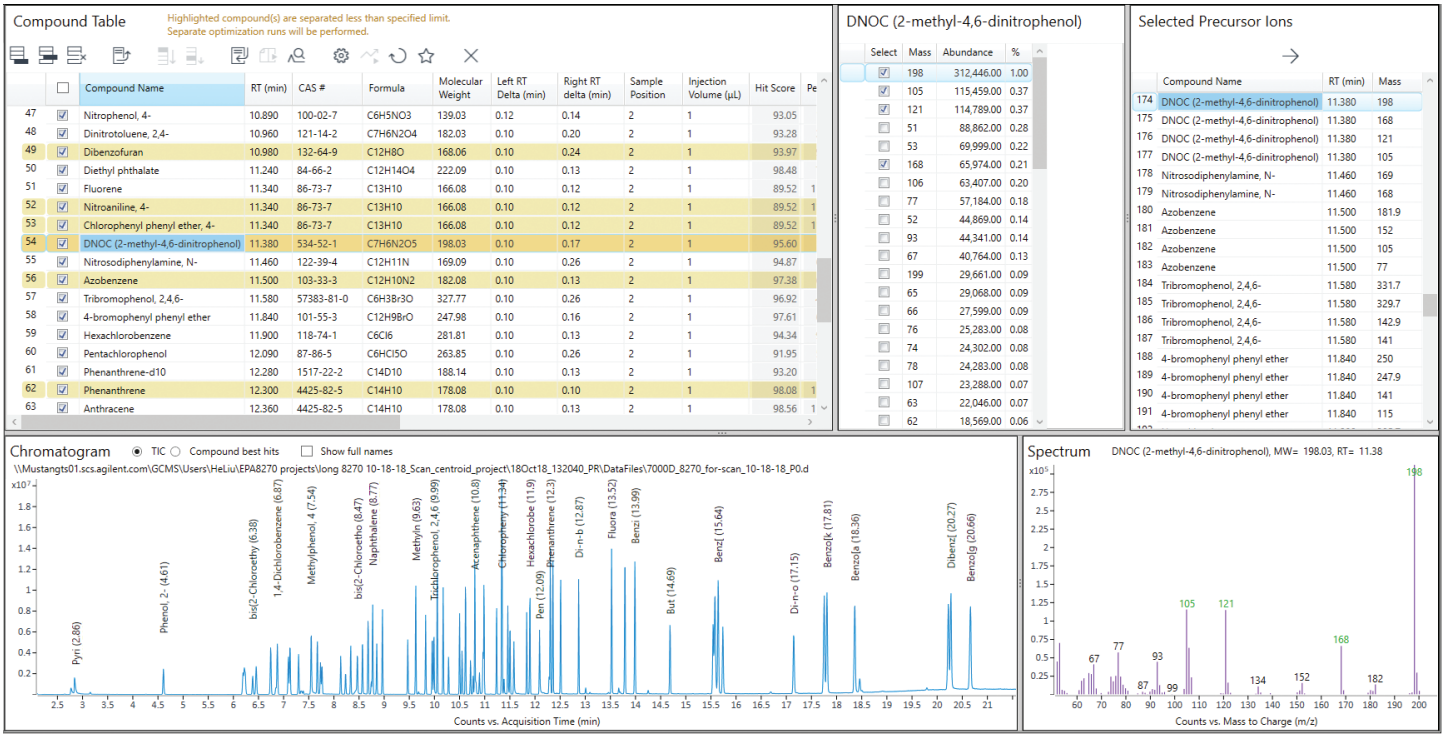


Figure 1A. Agilent MassHunter Optimizer for GC/TQ window displaying compound identification results.

The following MRM development steps (i.e., product ion identification and collision energy optimization) can be automated, requiring the user to review only the final optimized transitions. It can also be done manually, allowing the user to review the selection of product ions before performing the collision energy optimization step.

Start from scan data: product ion identification

Product ion optimization may require several injections depending on the number of precursor ions per analyte and how well the targets are chromatographically resolved. It is recommended to have the analytes chromatographically baseline-resolved to ensure the most effective MRM development. However, MRM development can be performed for coeluting compounds if their mass spectra differ and response abundance

B

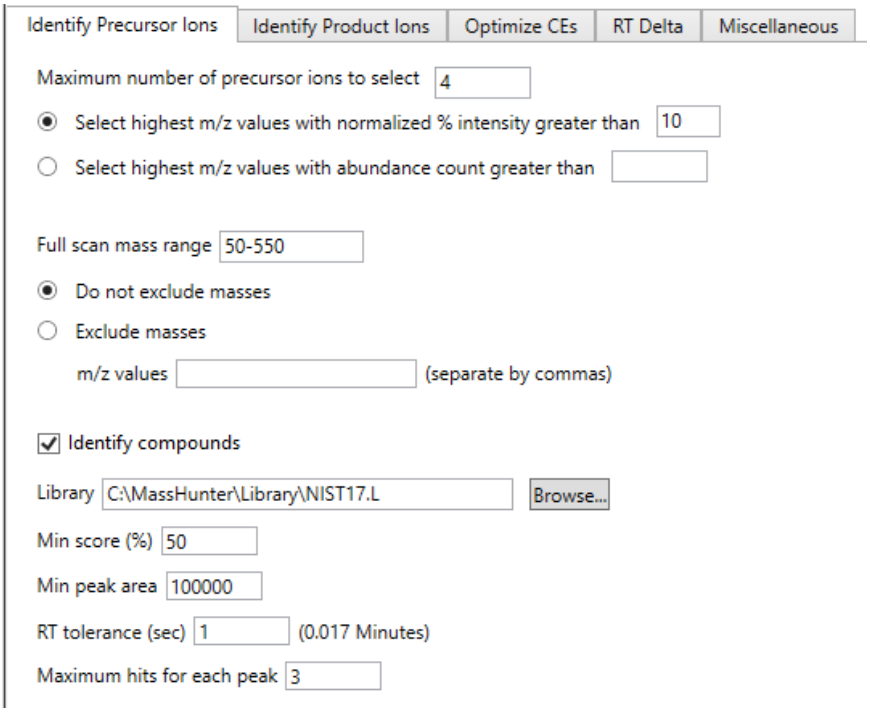


Figure 1B. Agilent MassHunter Optimizer for GC/TQ window displaying precursor identification parameters.

of the compounds is comparable. To perform MRM development for coeluting targets, additional injections may be needed.

In this work, a challenging mixture of 83 analytes was analyzed in under 22 minutes. Some of the 83 target compounds coeluted as indicated by yellow highlighting in the compound table in Figure 1A. To perform a product ion scan for all 83 targets, seven injections were required. If coeluting compounds were ignored, product ion identification for the remaining compounds could have been performed with three injections.

Product ion identification for each precursor ion is performed via product ion scans at multiple collision energies defined by the user under product ion identification parameters (Figure 2B). A maximum of four collision energies are permitted for product ion scan. In this work, product ion scan experiments were

B

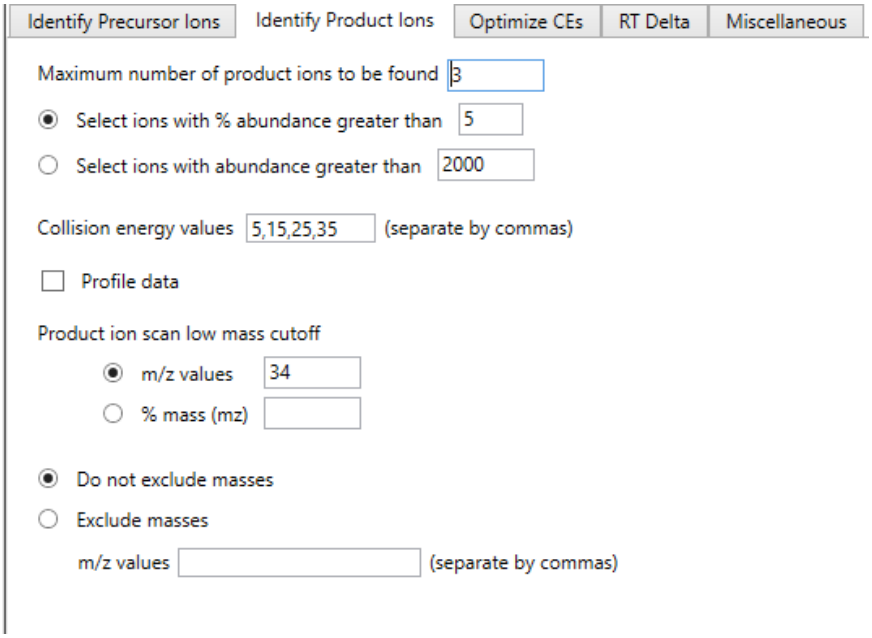


Figure 2B. Product ion identification parameters.

A

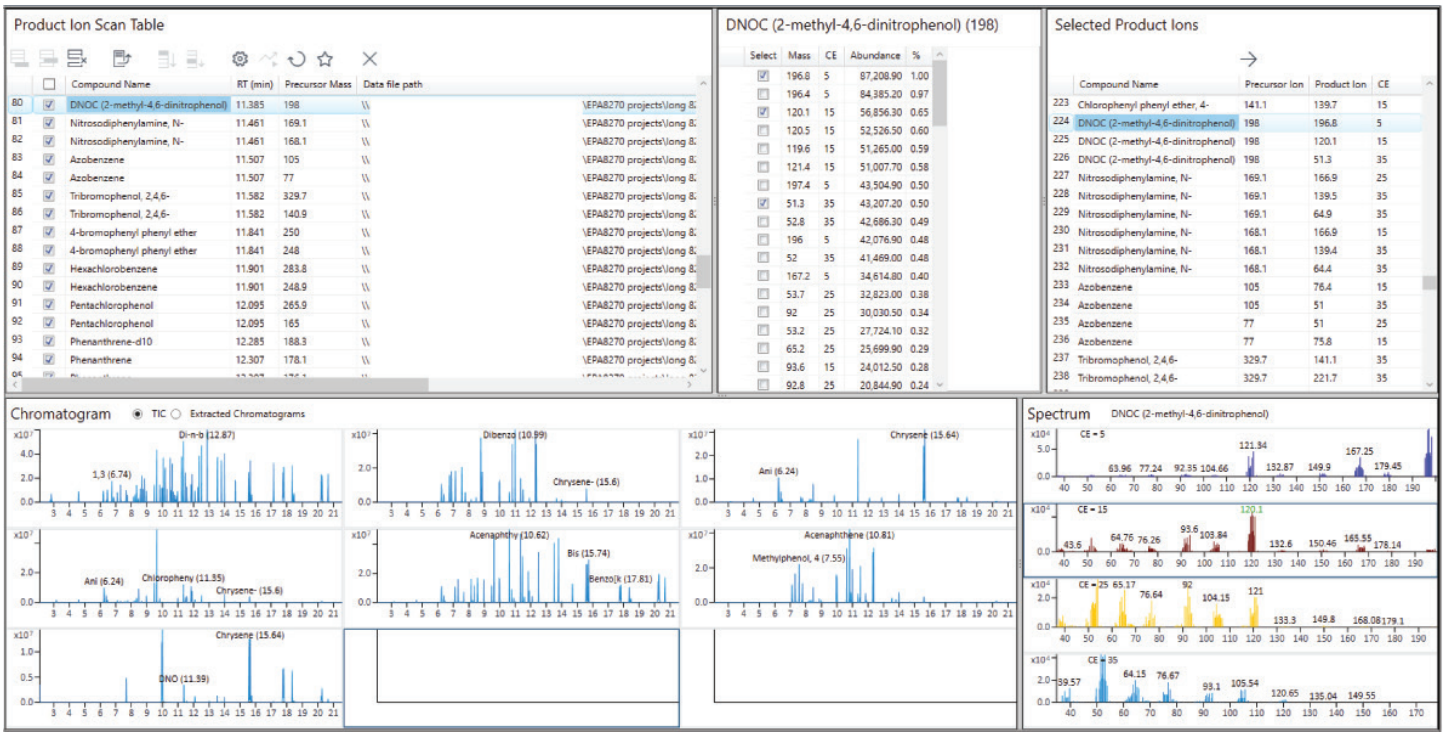


Figure 2A. Product ion identification results with precursor (*m/z* 198) for DNOC highlighted in the product ion scan table.

performed at default values of 5, 15, 25, and 35 eV. Product ion identification results are shown in Figure 2A, with DNOC highlighted in the product ion scan table. The window includes:

- A product ion scan table, in which each line corresponds to one precursor ion
- Total ion chromatograms (TIC) for product ion scan
- Product ion scan mass spectra for the highlighted precursor acquired at four different collision energies
- A table with product ions available for each precursor
- A summary of all product ions selected

Product ion identification parameters are shown in Figure 2B. Selection of product ions is based on their abundance and clustering consideration, as demonstrated for the *m/z* 198 precursor for DNOC in Figure 2A. If a manual revision of the product ion identification step is performed, product ions suggested by the software can be overwritten by the user by unchecking selected ions and checking available ones.

Start from scan data: collision energy optimization

Collision energy optimization can be performed around the value chosen in the previous step or over a user-defined range. In this work, collision energies were optimized around the collision energy found to be best out of the four values in the product ion identification step (Figure 3B). Collision energy optimization results are shown in Figure 3A, with the 198 → 120.1 transition for DNOC highlighted in the MRM transitions table. The window includes:

- An MRM transitions table, in which each line corresponds to one MRM transition
- A TIC acquired at various tested collision energy values
- An ion breakdown profile, which features a plot of the MRM transition abundance versus collision energy
- Collision energies with the corresponding abundances for the highlighted MRM transition

To perform collision energy optimization for MRMs developed for all 83 targets, it would take four injections. If coeluting compounds were ignored, collision energy optimization for the remaining MRM transitions could have been performed with just one injection.

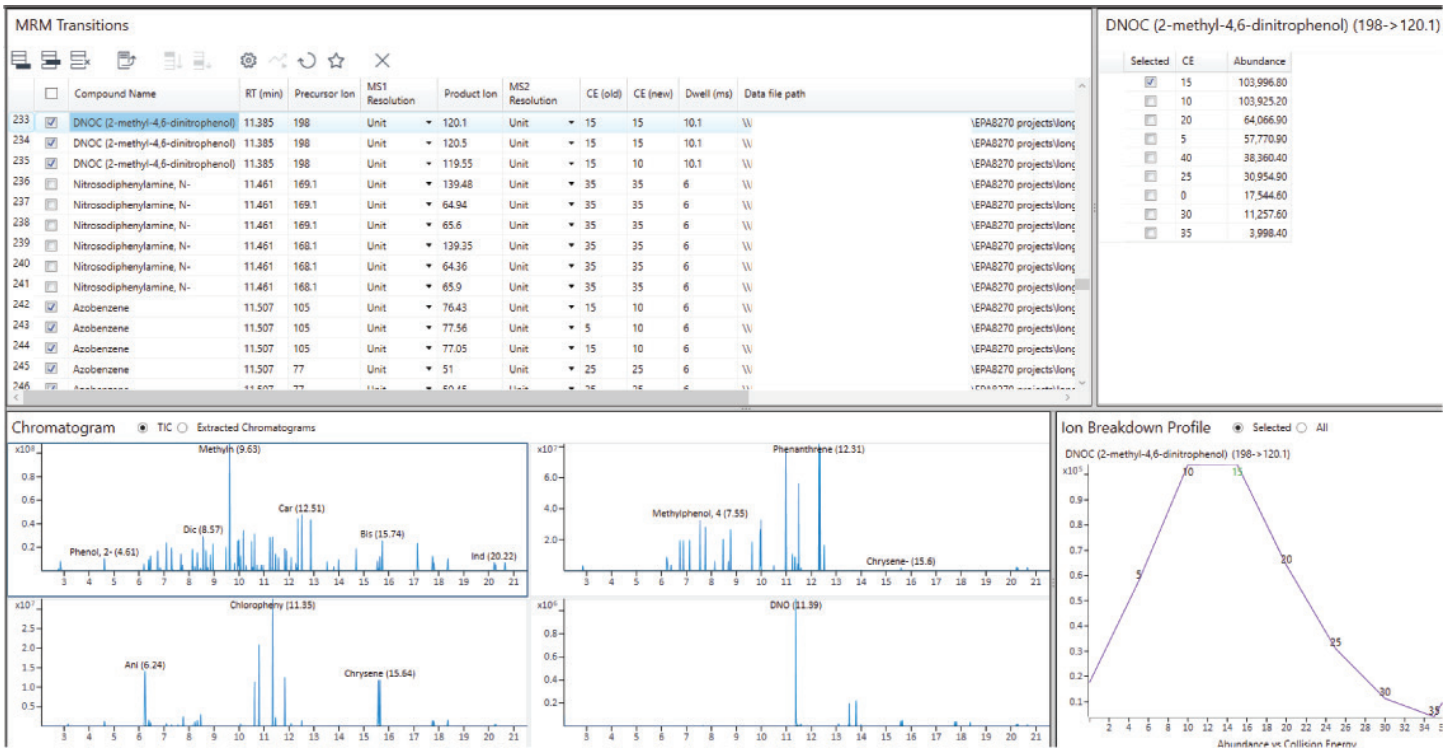


Figure 3A. Collision energy optimization results with the 198 → 120.1 transition for DNOC highlighted in the MRM transitions table.

Start from scan data: alternative workflows

The *Start from scan data* workflow was intentionally performed using a complex mixture of 83 semivolatile compounds to challenge the process and evaluate the effects of coelutions that may complicate the MRM development process and require manual review. An alternative approach to the optimization would be to split the 83 targets into several different mixes, thus minimizing coelution and the need for manual revision and user intervention. If splitting the sample appears laborious, an alternative workflow is to split the compound list into multiple projects. Then, compounds in each list are optimized separately and the results are saved as .CSV files. All .CSV files can be imported into a new project to create a complete MRM acquisition method. This approach allows minimizing coelution and decreasing the number of injections needed within one project for MRM development.

Start from SIM ions

To develop MRM transitions when a SIM acquisition method is available, such as from an existing GC/MSD method, a SIM acquisition method or a properly formated .CSV file can be loaded to initiate the *Start from SIM ions* workflow. In this work, 130 SIM ions for 83 compounds were imported as a .CSV file, containing information on compound names, ions, and retention times (Figure 4A). When starting from SIM ions, the imported ions are used as precursors, and MRM development starts with a product ion scan followed by collision energy optimization. If retention times under the current chromatographic conditions are different, the Optimizer allows the user to acquire and analyze SIM data to determine retention times. Updated retention times are displayed in the SIM ions table, as shown in Figure 4B.

B

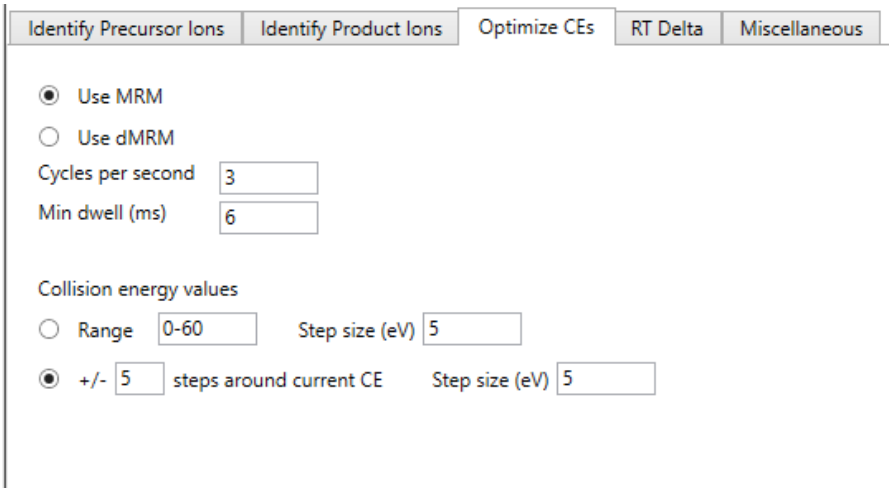


Figure 3B. Collision energy optimization parameters.

A

Compound Table

Highlighted compound(s) are separated less than specified limit.
Separate optimization runs will be performed.

		Compound Name	RT (min)	CAS #	Formula	Molecular Weight	Left RT Delta (min)	Right RT delta (min)	Sample Position	Injection Volume (µL)	Peak Area
28	☑	Naphthalene-d8	8.747				0.10	0.10	2	1	
29	☑	Naphthalene	8.773				0.10	0.11	2	1	
30	☑	Chloroaniline, 4-	8.850				0.10	0.15	2	1	
31	☑	Hexachlorobutadiene	8.965				0.10	0.10	2	1	
32	☑	Phenol, 4-chloro-3-methyl-	9.470				0.10	0.17	2	1	
33	☑	Methylnaphthalene, 2-	9.630				0.10	0.10	2	1	
34	☑	Hexachlorocyclopentadiene	9.830				0.10	0.10	2	1	
35	☑	Trichlorophenol, 2,4,5-	9.950				0.10	0.10	2	1	
36	☑	Trichlorophenol, 2,4,6-	9.990				0.10	0.10	2	1	
37	☑	1,1'-Biphenyl, 2-fluoro-	10.057				0.10	0.10	2	1	
38	☑	Chloronaphthalene, 2-	10.172				0.10	0.11	2	1	
39	☑	Nitroaniline, 2-	10.282				0.10	0.15	2	1	
40	☑	Dimethyl phthalate	10.500				0.10	0.10	2	1	
41	☑	Dinitrotoluene, 2,6-	10.550				0.10	0.10	2	1	
42	☑	Acenaphthylene	10.622				0.10	0.12	2	1	
43	☑	Nitroaniline, 3-	10.718				0.10	0.10	2	1	
44	☑	Acenaphthene-d10	10.772				0.10	0.10	2	1	
45	☑	Acenaphthene	10.807				0.10	0.10	2	1	
46	☑	Phenol, 2,4-dinitro-	10.830				0.10	0.10	2	1	
47	☑	Nitrophenol, 4-	10.896				0.10	0.10	2	1	
48	☑	Dinitrotoluene, 2,4-	10.966				0.10	0.13	2	1	
49	☑	Dibenzofuran	10.986				0.10	0.10	2	1	
50	☑	Diethyl phthalate	11.233				0.10	0.12	2	1	
51	☑	Fluorene	11.342				0.10	0.10	2	1	
52	☑	Nitroaniline, 4-	11.344				0.10	0.10	2	1	
53	☑	Chlorophenyl phenyl ether, 4-	11.349				0.10	0.10	2	1	
54	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385				0.10	0.14	2	1	
55	☑	Nitrosodiphenylamine, N-	11.461				0.10	0.10	2	1	
56	☑	Azobenzene	11.507				0.10	0.10	2	1	
57	☑	Tribromophenol, 2,4,6-	11.582				0.10	0.14	2	1	
58	☑	4-bromophenyl phenyl ether	11.841				0.10	0.10	2	1	
59	☑	Hexachlorobenzene	11.901				0.10	0.16	2	1	
60	☑	Pentachlorophenol	12.095				0.10	0.14	2	1	
61	☑	Phenanthrene-d10	12.285				0.10	0.10	2	1	

Figure 4A. Compound table listing target compounds imported as a .CSV file in the *Start from SIM ions* workflow.

In this work, 351 MRM transitions were developed for 83 target compounds, starting with 130 imported SIM ions. The product ion scan step required seven injections and was followed by four injections needed for collision energy optimization. Thus, all optimization for 83 compounds was completed in 5.5 hours. In this workflow, parameters used for product ion identification and collision energy optimization were the same as in the Start from scan data workflow, shown in Figures 2B and 3B. The result of MRM development when starting from SIM ions is displayed in the same way as in the *Start from scan data* workflow, as demonstrated in Figure 3A. Automated creation of the EPA 8270 MRM method enables successful migration from GC/MSD SIM using the Optimizer.

B

SIM Ions

		Compound Name	RT (old)	RT (new)	Mass	MS1 Resolution	Dwell (ms)	Data file path
70	☑	Dinitrotoluene, 2,4-	10.966	10.966	165	Unit	27.36	
71	☑	Dibenzofuran	10.986	10.986	168.1	Unit	28.26	
72	☑	Dibenzofuran	10.986	10.986	139.1	Unit	28.26	
73	☑	Diethyl phthalate	11.233	11.233	149	Unit	27.43	
74	☑	Fluorene	11.342	11.342	166.1	Unit	22.47	
75	☑	Fluorene	11.342	11.342	165.1	Unit	22.47	
76	☑	Nitroaniline, 4-	11.344	11.344	138	Unit	22.47	
77	☑	Nitroaniline, 4-	11.344	11.344	108	Unit	22.47	
78	☑	Chlorophenyl phenyl ether, 4-	11.349	11.349	204	Unit	23.12	
79	☑	Chlorophenyl phenyl ether, 4-	11.349	11.349	141.1	Unit	23.12	
80	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385	11.385	198	Unit	22.39	
81	☑	Nitrosodiphenylamine, N-	11.461	11.461	169.1	Unit	26.86	
82	☑	Nitrosodiphenylamine, N-	11.461	11.461	168.1	Unit	26.86	
83	☑	Azobenzene	11.507	11.507	105	Unit	30.81	
84	☑	Azobenzene	11.507	11.507	77	Unit	30.81	
85	☑	Tribromophenol, 2,4,6-	11.582	11.582	329.7	Unit	32.42	
86	☑	Tribromophenol, 2,4,6-	11.582	11.582	140.9	Unit	32.42	
87	☑	4-bromophenyl phenyl ether	11.841	11.841	250	Unit	46.68	
88	☑	4-bromophenyl phenyl ether	11.841	11.841	248	Unit	46.68	
89	☑	Hexachlorobenzene	11.901	11.901	283.8	Unit	53.84	
90	☑	Hexachlorobenzene	11.901	11.901	248.9	Unit	53.84	
91	☑	Pentachlorophenol	12.095	12.095	265.9	Unit	49.1	
92	☑	Pentachlorophenol	12.095	12.095	165	Unit	49.1	
93	☑	Phenanthrene-d10	12.285	12.285	188.3	Unit	42.02	
94	☑	Phenanthrene	12.307	12.307	178.1	Unit	42.03	
95	☑	Phenanthrene	12.307	12.307	176.1	Unit	42.03	
96	☑	Anthracene	12.355	12.355	178.1	Unit	46.81	
97	☑	Carbazole	12.512	12.512	167	Unit	73	
98	☑	Di-n-butyl phthalate	12.869	12.869	149	Unit	106.33	
99	☑	Fluoranthene	13.528	13.528	202.1	Unit	106.31	
100	☑	Fluoranthene	13.528	13.528	201.1	Unit	106.31	

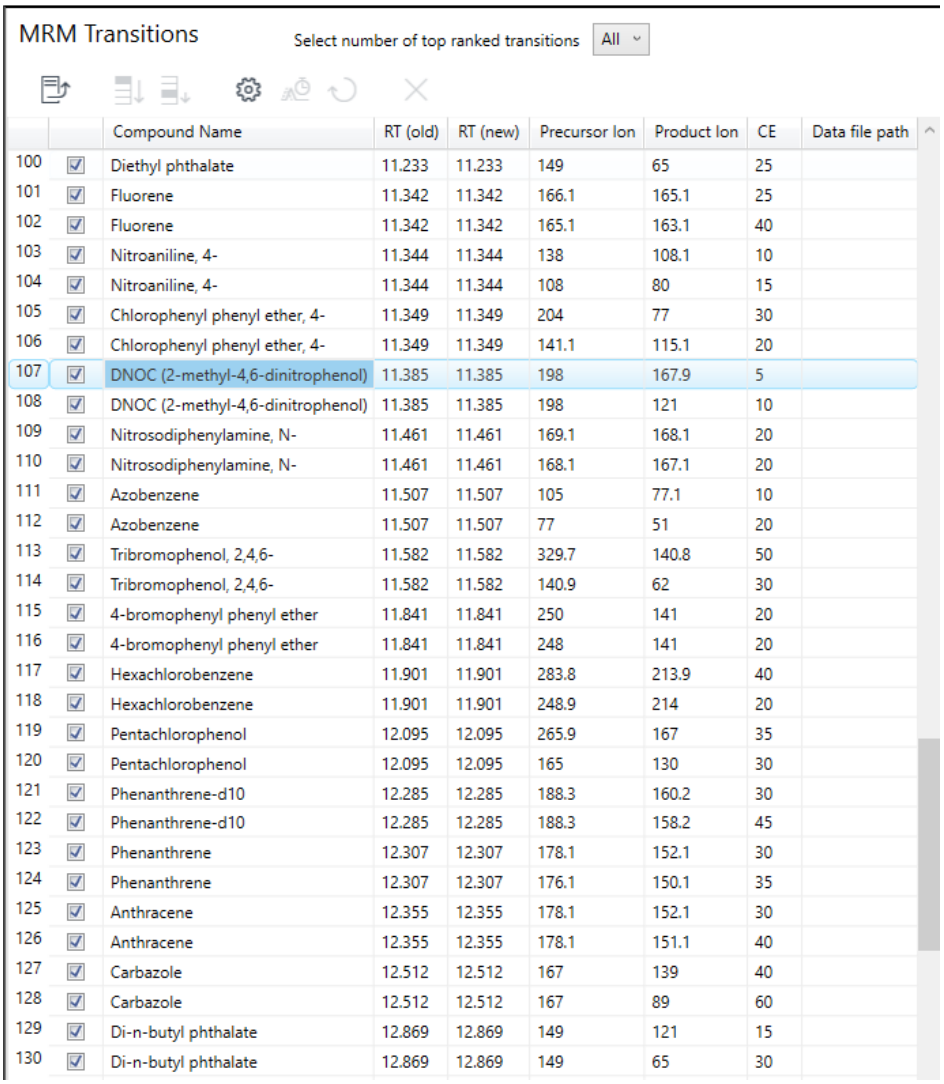
Figure 4B. Imported SIM ions with the original and updated retention times of target compounds in the *Start from SIM ions* workflow.

Start from MRMs

To re-optimize collision energies for existing MRM transitions, an MRM acquisition method is loaded to initiate the *Start from MRMs* workflow. In this work, 166 MRM transitions for 83 compounds were imported (Figure 5). If retention times under current chromatographic conditions are different, the Optimizer allows the user to acquire and analyze MRM or dMRM data to update retention times. Updated retention times are displayed in the MRM transitions table shown in Figure 5.

In this work, collision energies for 166 MRM transitions for 83 compounds were re-optimized in four injections. In this workflow, parameters used for collision energy optimization were the same as in the previously described *Start from scan data* and *Start from SIM ions* workflows, shown in Figure 3B. The result of collision energy optimization when starting from MRMs is displayed in a similar way as in *Start from scan data* or *Start from SIM ions* workflows, as demonstrated in Figure 3A with an additional column showing old collision energy values.

The *Start from MRMs* workflow may be particularly useful when enhancing method selectivity for analysis in matrix. Optimal collision energies determined in matrix may differ from the values optimized in solvent.



		Compound Name	RT (old)	RT (new)	Precursor Ion	Product Ion	CE	Data file path
100	☑	Diethyl phthalate	11.233	11.233	149	65	25	
101	☑	Fluorene	11.342	11.342	166.1	165.1	25	
102	☑	Fluorene	11.342	11.342	165.1	163.1	40	
103	☑	Nitroaniline, 4-	11.344	11.344	138	108.1	10	
104	☑	Nitroaniline, 4-	11.344	11.344	108	80	15	
105	☑	Chlorophenyl phenyl ether, 4-	11.349	11.349	204	77	30	
106	☑	Chlorophenyl phenyl ether, 4-	11.349	11.349	141.1	115.1	20	
107	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385	11.385	198	167.9	5	
108	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385	11.385	198	121	10	
109	☑	Nitrosodiphenylamine, N-	11.461	11.461	169.1	168.1	20	
110	☑	Nitrosodiphenylamine, N-	11.461	11.461	168.1	167.1	20	
111	☑	Azobenzene	11.507	11.507	105	77.1	10	
112	☑	Azobenzene	11.507	11.507	77	51	20	
113	☑	Tribromophenol, 2,4,6-	11.582	11.582	329.7	140.8	50	
114	☑	Tribromophenol, 2,4,6-	11.582	11.582	140.9	62	30	
115	☑	4-bromophenyl phenyl ether	11.841	11.841	250	141	20	
116	☑	4-bromophenyl phenyl ether	11.841	11.841	248	141	20	
117	☑	Hexachlorobenzene	11.901	11.901	283.8	213.9	40	
118	☑	Hexachlorobenzene	11.901	11.901	248.9	214	20	
119	☑	Pentachlorophenol	12.095	12.095	265.9	167	35	
120	☑	Pentachlorophenol	12.095	12.095	165	130	30	
121	☑	Phenanthrene-d10	12.285	12.285	188.3	160.2	30	
122	☑	Phenanthrene-d10	12.285	12.285	188.3	158.2	45	
123	☑	Phenanthrene	12.307	12.307	178.1	152.1	30	
124	☑	Phenanthrene	12.307	12.307	176.1	150.1	35	
125	☑	Anthracene	12.355	12.355	178.1	152.1	30	
126	☑	Anthracene	12.355	12.355	178.1	151.1	40	
127	☑	Carbazole	12.512	12.512	167	139	40	
128	☑	Carbazole	12.512	12.512	167	89	60	
129	☑	Di-n-butyl phthalate	12.869	12.869	149	121	15	
130	☑	Di-n-butyl phthalate	12.869	12.869	149	65	30	

Figure 5. MRM transitions table listing MRM transitions for target compounds imported from the MRM acquisition method, with the original and updated retention times from the *Start from MRMs* workflow.

Updating retention times

The *Update retention times* functionality available in the Optimizer for SIM and MRM acquisition methods is useful when altering chromatographic conditions and when retention time shift is expected. It allows retention time updating without user intervention. It is recommended to review the updated results if multiple compounds share the same SIM ions or MRM transitions. Figure 6A shows MRM chromatograms for five compounds before and after retention time update. Original and updated retention times for these compounds are shown in Figure 6B.

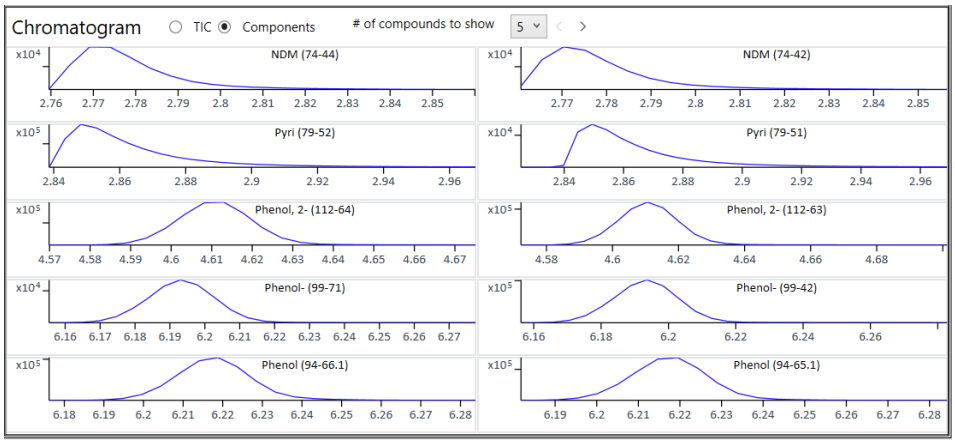
The Optimizer for GC/TQ enables significant time savings and reduces manual review when developing MRM data acquisition methods. Time and number of sample injections required for each of the discussed workflows are summarized in Table 1.

Reviewing results and saving method

When collision energy optimization is complete, the results are reviewed, and the acquisition method is saved. Information on all the developed transitions is shown in the expanded table view in Figure 7A. The number of top-ranked MRM transitions to be saved is defined by the number specified under *Select number of top ranked transitions*; only the checked MRM transitions will be included in the acquisition method. In this work, all the developed MRM transitions were selected and saved. To simplify method review, a nested view of the results table is available (Figure 7B).

The developed MRM acquisition method can be saved as either a time-segment MRM method or a dynamic MRM method (Figure 8). The user defines minimum dwell time and the number of cycles per second when saving a method. The developed transitions can also be exported as a .CSV file.

A



B

Compound Name	RT (old)	RT (new)
NDMA	2.770	2.769
NDMA	2.770	2.769
Pyridine	2.861	2.848
Pyridine	2.861	2.848
Phenol, 2-fluoro-	4.607	4.611
Phenol, 2-fluoro-	4.607	4.611
Phenol-d6	6.189	6.194
Phenol-d6	6.189	6.194
Phenol	6.215	6.219
Phenol	6.215	6.219
Aniline	6.235	6.240

Figure 6. MRM chromatograms for five compounds before and after retention time update (A) and the original and updated retention times for these compounds (B).

Table 1. Time and number of sample injections required for 8270 "full mix" optimization.

Workflow	Number of Injections Required In This Work*	Time**
Start from scan data	12	6 hours
Start from SIM ions	11	5.5 hours
Start from MRMs	4	2 hours
Updating retention times for small shift within time segment windows	1	0.5 hour
Updating retention times for big shift	6	3 hours

* Numbers in this table are specific for this 8270 study. Less interference between coeluting compounds will need fewer injections. More precursors per compound will need more injections for product ion scans.

** Instrument cycle time was 30 minutes.

A

Optimized MRM Transitions

Select number of top ranked transitions: All | Left RT Delta (min): 0.20 | Right RT delta (min): 0.20 | Overwrite RT Delta

Nested View

		Compound Name	RT (min)	Precursor Ion	MS1 Resolution	Product Ion	MS2 Resolution	CE	Abundance	%	CAS #	Formula	Molecular Weight	Left RT Delta (min)	Right RT delta (min)	Sample Position	Injection Volume (µL)
233	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385	198	Unit	120.1	Unit	15	56,856.34	1.00				0.10	0.14	2	1
234	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385	198	Unit	120.5	Unit	15	52,526.46	0.92				0.10	0.14	2	1
235	☑	DNOC (2-methyl-4,6-dinitrophenol)	11.385	198	Unit	119.55	Unit	10	51,265.01	0.90				0.10	0.14	2	1
236	☑	Nitrosodiphenylamine, N-	11.461	168.1	Unit	139.35	Unit	35	434,381.53	1.00				0.10	0.10	2	1
237	☑	Nitrosodiphenylamine, N-	11.461	169.1	Unit	139.48	Unit	35	260,821.55	0.60				0.10	0.10	2	1
238	☑	Nitrosodiphenylamine, N-	11.461	169.1	Unit	64.94	Unit	35	238,973.06	0.55				0.10	0.10	2	1
239	☑	Nitrosodiphenylamine, N-	11.461	168.1	Unit	64.36	Unit	35	230,751.62	0.53				0.10	0.10	2	1
240	☑	Nitrosodiphenylamine, N-	11.461	168.1	Unit	65.9	Unit	35	175,412.81	0.40				0.10	0.10	2	1
241	☑	Nitrosodiphenylamine, N-	11.461	169.1	Unit	65.6	Unit	35	149,990.39	0.35				0.10	0.10	2	1

B

Optimized MRM Transitions

Select number of top ranked transitions: All | Left RT Delta (min): 0.20 | Right RT delta (min): 0.20

☑ Nested View

	Compound Name	RT (min)	Left RT Delta (min)	Right RT delta (min)	CAS #	Formula	Molecular Weight	Sample Position	Injection Volume (µL)
⊟ 54	DNOC (2-methyl-4,6-dinitrophenol)	11.385	0.10	0.14				2	1

Precursor Ion	Abundance
⊟ 198.00	

Product Ion	Abundance	%	CE
120.10	56,856.34	1.00	15.00
120.50	52,526.46	0.92	15.00
119.55	51,265.01	0.90	10.00

⊞ 55	Nitrosodiphenylamine, N-	11.461	0.10	0.10				2	1
⊞ 56	Azobenzene	11.507	0.10	0.10				2	1

Figure 7. Results of MRM transition optimization in an expanded view (A) and a nested view with DNOC results expanded (B).

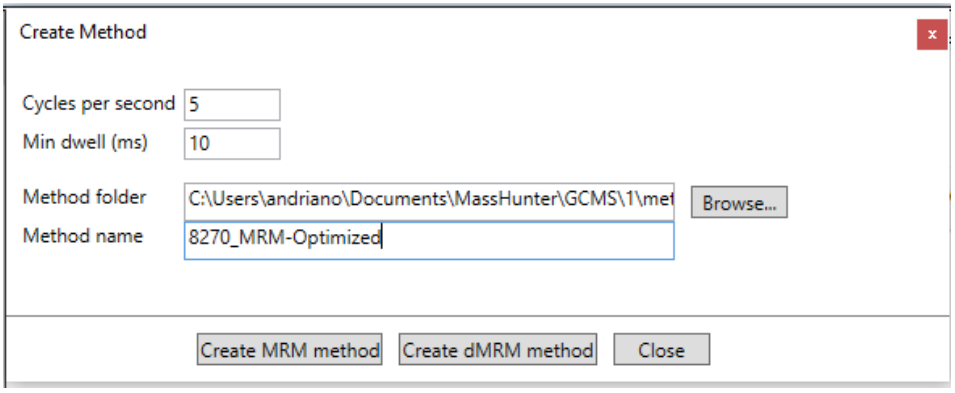


Figure 8. Creating a method with the Agilent MassHunter Optimizer for GC/TQ.

The developed and optimized MRM acquisition method was successfully used for semivolatiles analysis, meeting the performance requirements of the US EPA 8270E, with the results shown elsewhere.[2]

Conclusion

Four workflows were enabled with a highly automated optimization tool for MRM acquisition. MassHunter Optimizer for GC/TQ was showcased, featuring MRM method development for a complex "full mixture" US EPA 8270 containing 83 semivolatile targets. The 8270 "full mixture" was used to challenge the process and evaluate the effects of coelutions. Four workflows were started with different acquisition methods, such as scan, SIM, and MRM. When starting from scan data and SIM ions, MRM transitions were developed and collision energies were optimized. Automated creation of the EPA 8270 MRM method enabled successful migration from GC/MSD SIM using the Optimizer. When starting with an MRM acquisition method, retention times for the target compounds were updated and collision energies were re-optimized. The optimized results were saved as a dMRM acquisition method.

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