A comparison of strategies for optimizing source parameters in multi-component LC-MS/MS methods: a journey from uninspired to super nerdy

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Takeaway: For **best results** with **source optimization**, choose a methodical approach for test conditions and **focus on the critical few analytes.** Don't waste time and effort on sensitive analytes!

Background and Aims

Question: What is the best (fastest and easiest) way to **optimize the conditions of the mass spectrometer** to meet the sensitivity and performance needs of a multi-analyte method with **hundreds of compounds?** We wanted to investigate different approaches to optiizing the source conditions of a SCIEX Triple Quad 4500 system running a panel of drugs and metabolites.

The approaches

1. Simple: This is a popular method of "guessing" or "borrowing from other methods."
2. Thorough: This approach involves testing a range of parameters. Tests were conducted either one factor at a time (OFAT), where one parameter was changed for each test run while others remained unchanged, or using a limited combinatorial design, where an array of values for parameters such as temperature (TEM) and ion spray voltage (ISV) were tested together in different combinations.
3. Sophisticated: The technique used here was a **fractional factorial design (FFD)** approach, which involves using a carefully chosen **fraction** of a complete set of **factorial** experiments. Initial experiments used a "high" and "low" value for each parameter and are randomized and mixed, with the results informing the second round of experiments.[1]

Option 1. Simple: go with **experience**

For option 1, method source parameters were selected to be generic and were based entirely on guidelines for method development by the authors. Little consideration was given to analyte class or type.
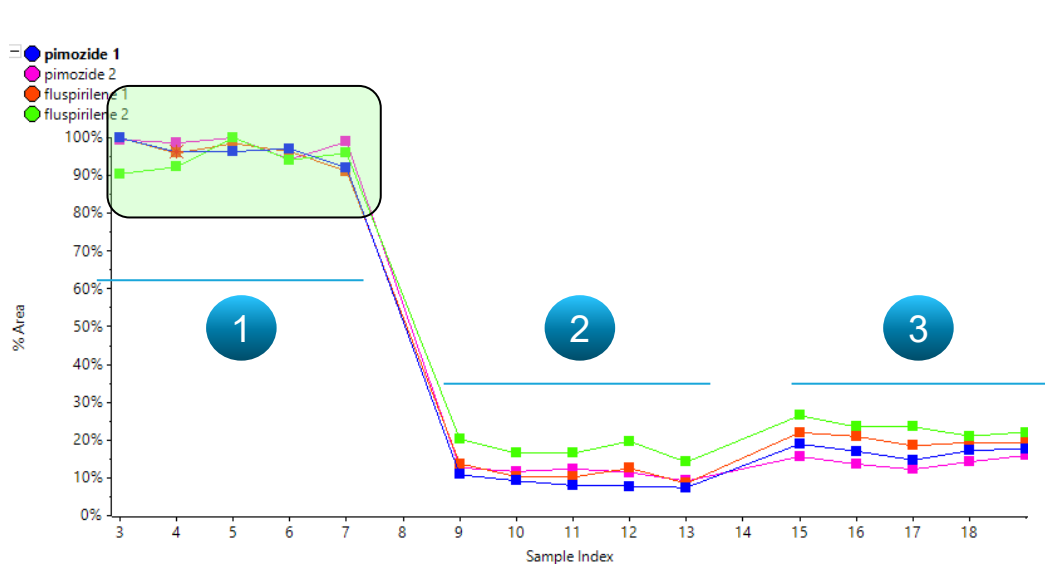
Figure 2. Area vs. n=5 injections for option 1 (left, highlighted), option 2 (centre) or option 3 (right) for selected analytes, where a clear preference for option 1 can be observed.

Final parameters

ISV	4500
TEM	600
Gas 1	50
Gas 2	70
CAD	9
CUR	30

Scorecard (option 1)

✔ Ease of use
✔ Ease of understanding
✔ Result
✔ Time (almost zero)
⚠ Future reliability
✔ Overall

*Graphic indicates value vs. available range of parameter; color indicates confidence after evaluation (red=low to green=high with gray indicating it was not evaluated)

Option 2. Thorough: **t**ry all the conditions methodically

Using a custom method builder[4], methods were built to test a range of parameters either one at a time (OFAT) or in a combinatorial manner (illustrated below). Samples were queued overnight and manually evaluated based on area without using statistical tools.
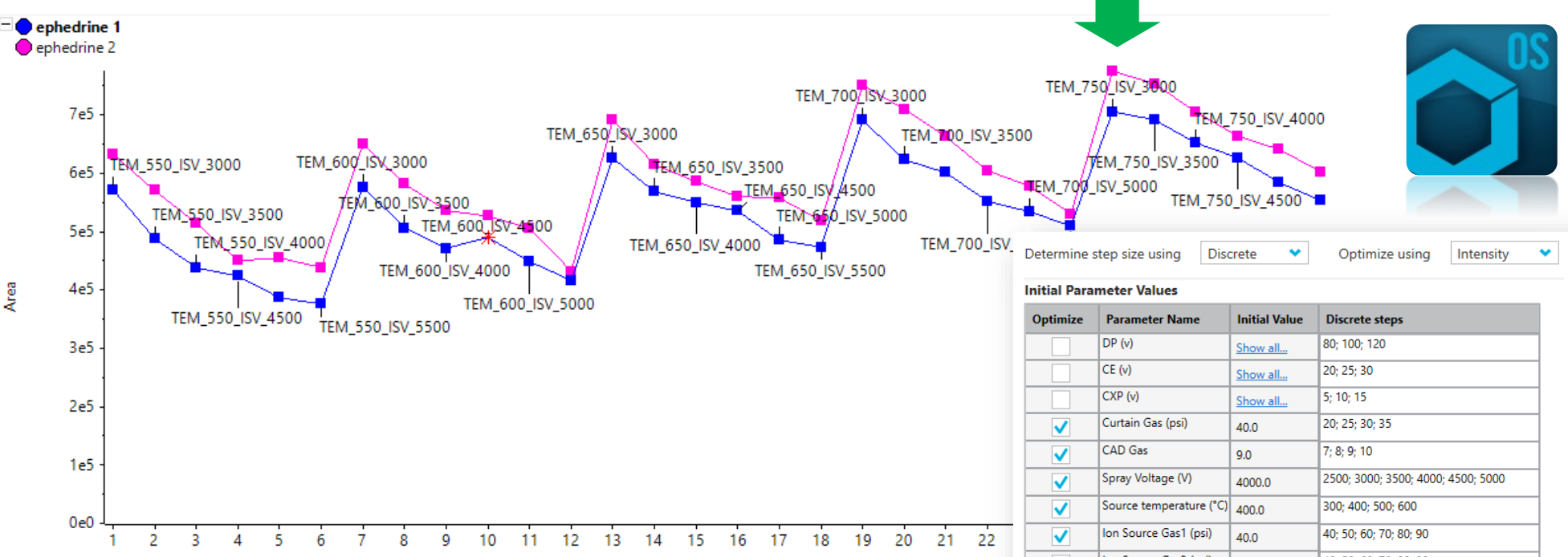
Figure 3. Metric plot of ephedrine response vs a combination of ion spray voltage (3000 to 5500 V) and temperature (550 to 750 ºC). The green arrow indicates the best conditions for area response in this case.
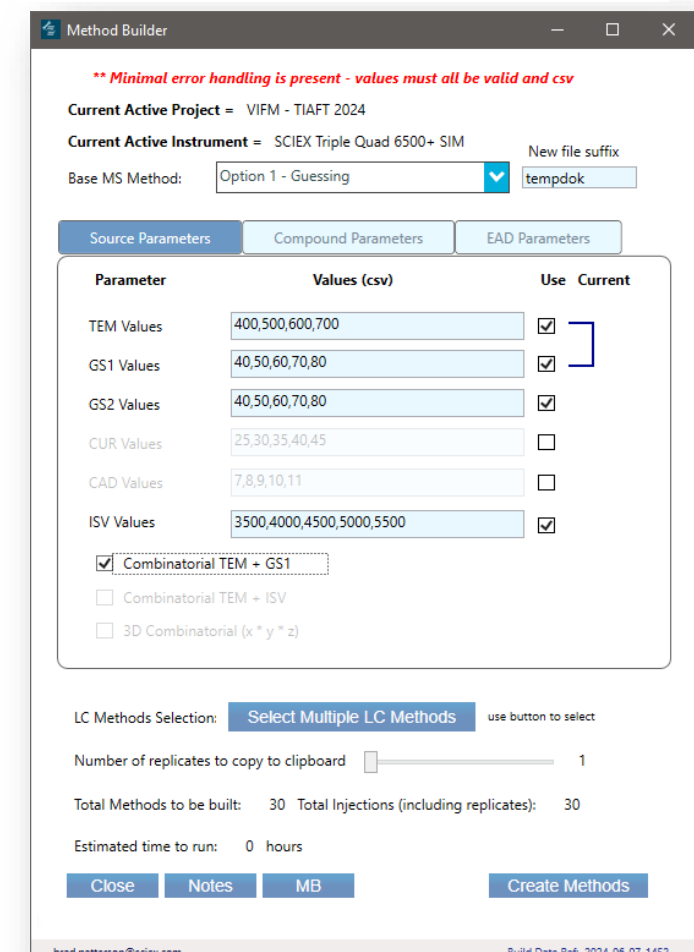
Figure 4. Built-in flow injection functionality can also be implemented.

Figure 5. Custom method builder constructed using the SCIEX Control API available from SCIEX[4].

Figure 6. Area vs. n=5 injections for option 1 (left), option 2 (centre; highlighted) or option 3 (right). For many analytes, suboptimal CAD led to a lower performance than option 3 even though conditions were similar.

Final parameters

ISV	1600
TEM	700
Gas 1	50
Gas 2	80
CAD	7
CUR	30

Scorecard (option 2)

⚠ Ease of use
⚠ Ease of understanding
⚠ Result
⚠ Time (quick setup)
✔ Future reliability
⚠ Overall

*see graphic in option 1 panel for details

The LC-MS/MS Method

- 327 compounds with **previously optimised** compound-specific parameters[2] – such as declustering potential (DP) and collision energy (CE) were used and kept constant throughout the optimization experiments.

- **A new** HPLC gradient was applied, and data were collected using the Scheduled MRM algorithm. No modifications were made to the LC method while working work with source parameters.

- The best conditions for each compound were examined for options 2 (manually driven) and option 3 (statistically-driven) to guide the selection of a final "best" method based on the peak area response of the analytes, with a focus on the poorest responding analytes (area).

- Replicate (n=5) injections of the final conditions were used for a final evaluation, and SCIEX OS software was used for automatic processing (peak integration).
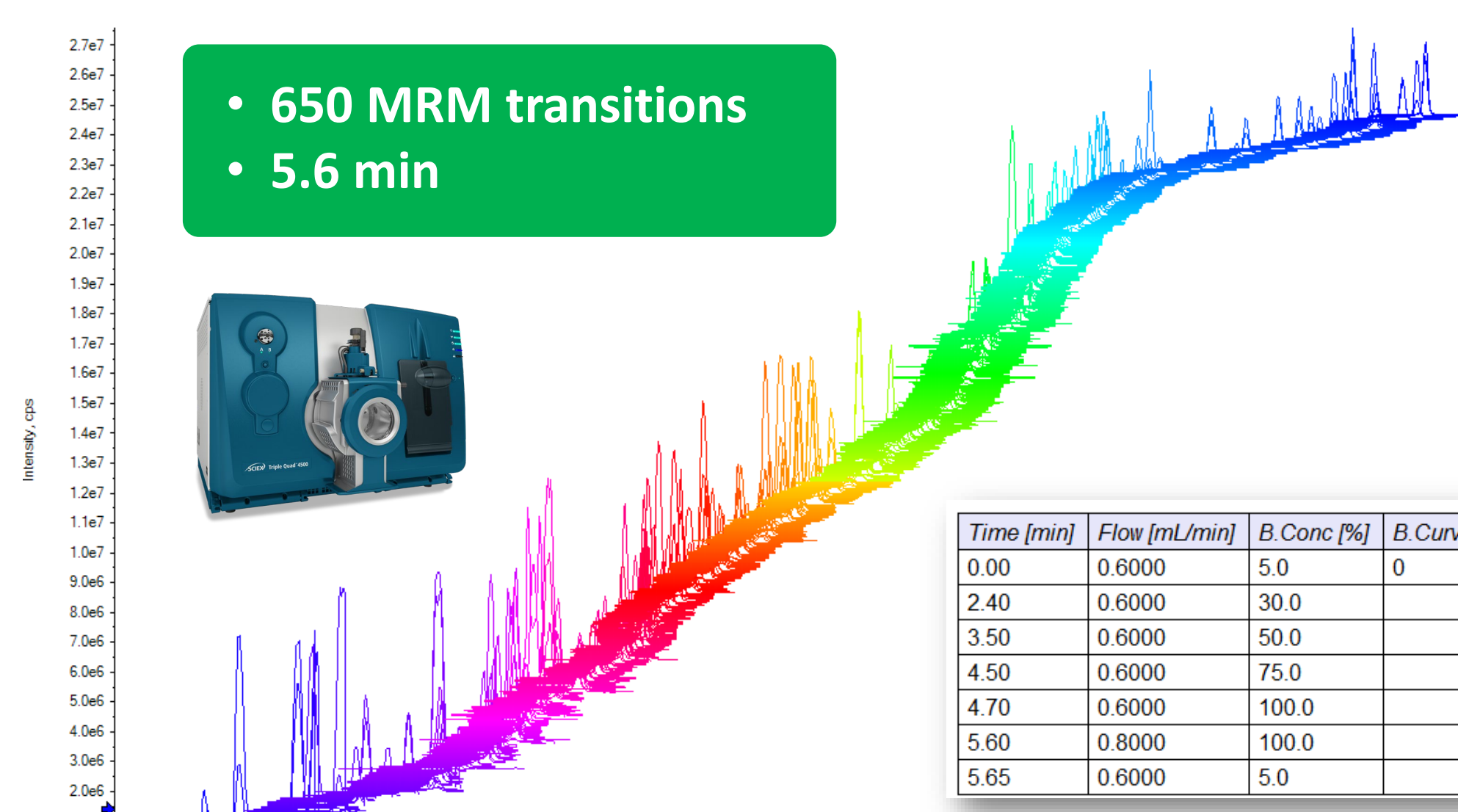
- 650 MRM transitions
- 5.6 min

Time [min]	Flow [mL/min]	B.Conc [%]	B.Curve
0.00	0.6000	5.0	0
2.40	0.6000	30.0	
3.50	0.6000	50.0	
4.50	0.6000	75.0	
4.70	0.6000	100.0	
5.60	0.8000	100.0	
5.65	0.6000	5.0	

Column: Phenomenex Kinetex™ Biphenyl Column (50 x 3)
Mobile phase A: water, 0.1% formic acid, 50 mM NH_4Ac
Mobile phase B: acetonitrile, 0.1% Formic acid
Gradient: see accompanying table
Injection volume: 1 µL (solvent standard)

Source parameters names:
*Ion spray voltage (**ISV**)
*Ion source temperature (**TEM**)
Gas 1 (nebulizer, **GS1**)
Gas 2 (heater gas, **GS2**)
Curtain gas (**CUR**)
*Collision gas (**CAD**)

*High-impact parameters

Figure 1: Example extracted ion chromatogram (XIC) offset in the y-axis direction.

Need more detail? Just ask!

Option 3. Sophisticated: **f**ractional factorial design (FFD)

The table below shows the initial set of 16 methods which are evaluating 6 parameters in the first round of testing. Conditions were generated by JMP Software[3] and were run in triplicate, interspersed with control conditions. This design screens conditions without needing to test every possible parameter value in the available range.

Pattern	#	Ion source voltage	Source temperature	GS-1 nebuliser gas	GS2 heating gas	Curtain gas	Collision gas
	1	2000	450	70	60	20	7
	2	5000	450	70	30	20	11
	3	2000	650	70	30	35	7
	4	5000	650	70	60	35	11
	5	5000	450	70	60	35	7
	6	2000	450	70	30	35	11
	7	5000	650	70	30	20	7
	8	2000	650	70	60	20	11
	9	5000	650	30	60	35	7
	10	2000	650	30	30	35	11
	11	5000	450	30	30	20	7
	12	2000	450	30	60	20	11
	13	2000	650	30	60	20	7
	14	5000	650	30	30	20	11
	15	2000	450	30	30	35	7
	16	5000	450	30	60	35	11

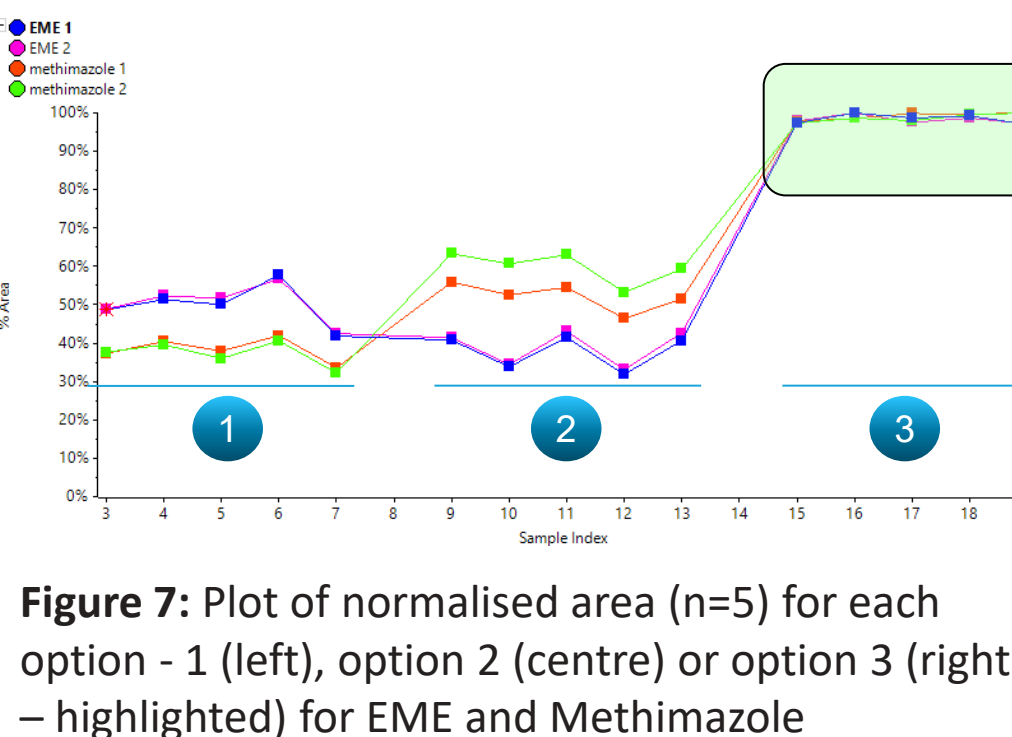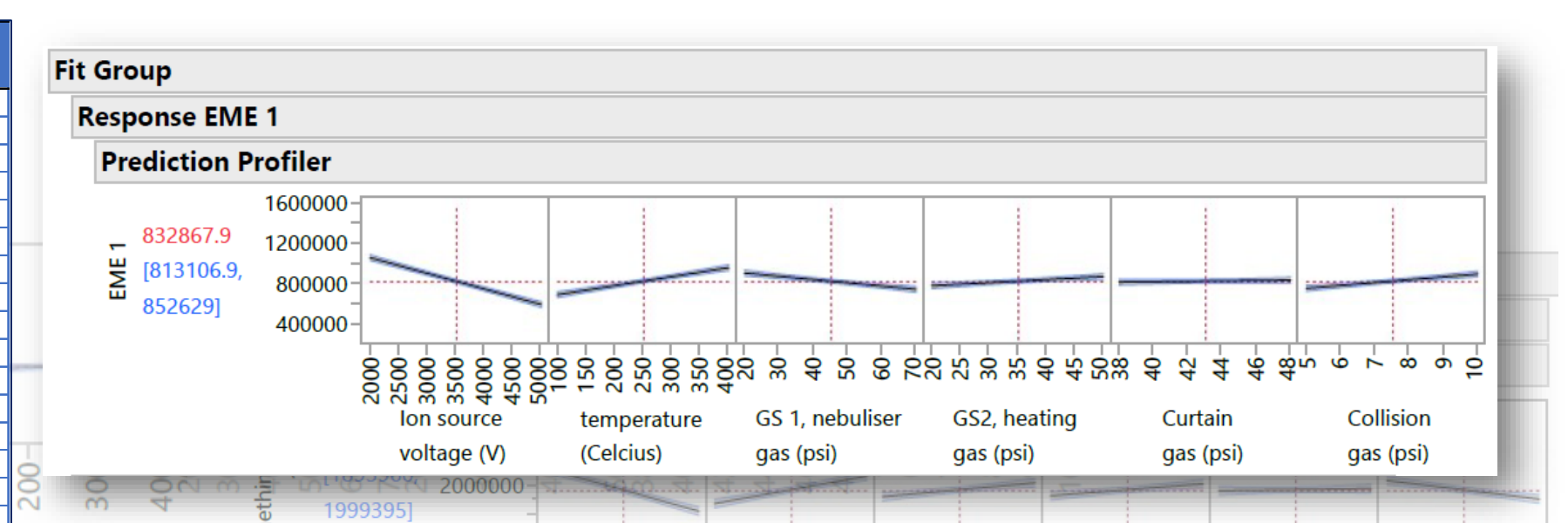Fit Group — Response EME 1 — Prediction Profiler

Figure 7: Plot of normalised area (n=5) for each option - 1 (left), option 2 (centre) or option 3 (right – highlighted) for EME and Methimazole

Final parameters

ISV	1750
TEM	710
Gas 1	68
Gas 2	74
CAD	8
CUR	30

Scorecard (option 1)

🔴 Ease of use
🔴 Ease of understanding
⚠ Result
⚠ Time (multiple days)
✔ Future reliability
⚠ Overall

*see graphic in option 1 panel for details

During evaluation, a decision was made to only use the quantifier ion from a compound, which reduced the number of results to process to approximately 300. Unfortunately, this decision failed to consider that the collision gas (CAD) parameter can affect fragmentation and lead to a suboptimal result for several of the MRMs.

Conclusions

1. Simple: Surprisingly, this generic method produced good results. Even though no compound-by-compound evaluation was performed, the areas and reproducibility were very good for this set of compounds. This option is a great starting point and could potentially get you 90% of the way there for most compound mixtures!
2. Thorough: This method effectively demonstrated how various changes affected certain compounds, but the volume of compounds to review meant led to some errors introduced by human bias. The best results would be obtained by focusing on the **lowest responding** analytes. This could be done overnight using instrument idle time.
3. Sophisticated: While the setup requires some knowledge and specialized software, the results were good and could be semi-automated to save more time. In this set of 650 MRMs, many early eluting compounds showed **improved** performance, but the method was not ideal for later eluting compounds. Better results could have been achieved by focusing only on the lowest responding analytes. Importantly, all fragments should be included to account for any effects of the collision gas (CAD) on the final method.
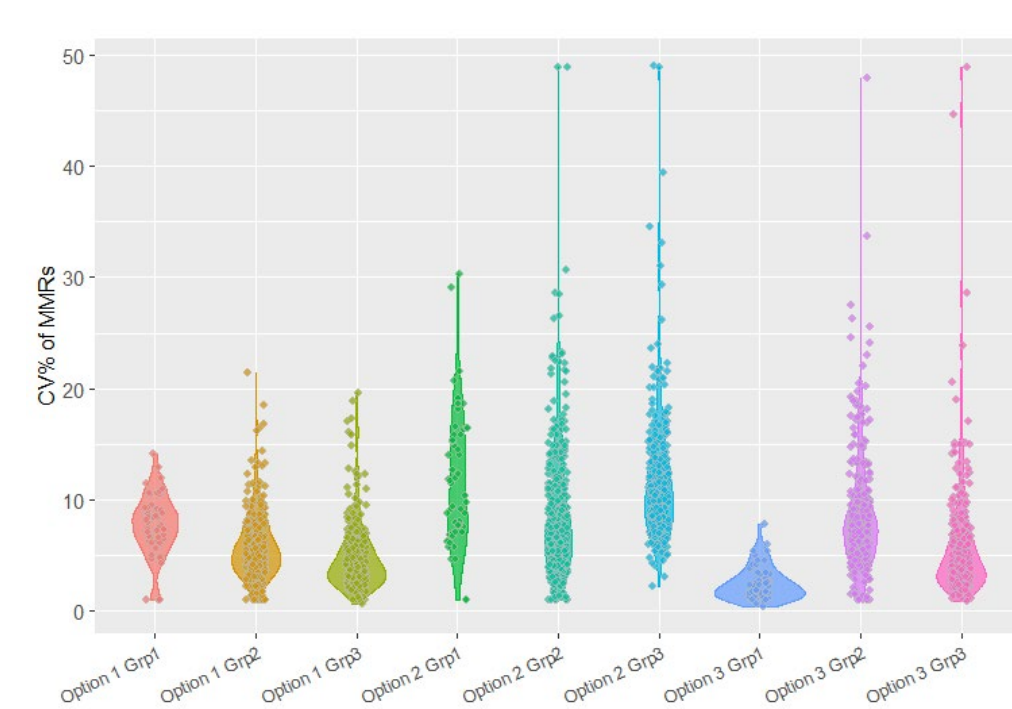
Figure 8. Violin plot of area CV% (n=5) for early (Grp1), middle (Grp2) and late (Grp3) eluting compounds for options 1, 2 and 3. Note the improvement in option 3, Grp 1 vs. other options.
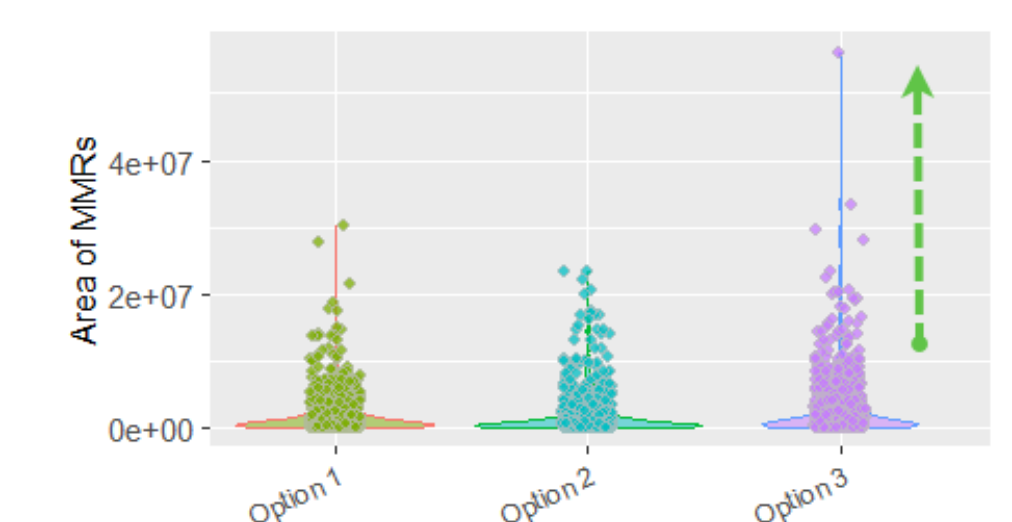
Figure 9: Peak areas for option 1, 2 and 3.

References

1. Dhayaalini Nadarajan, et al., Application of design of experiment for quantification of 71 new psychoactive substances in influent wastewater. Analytica Chimica Acta, 2024 Sep 8;1321: 343036. doi: 10.1016/j.aca.2024.343036..
2. Di Rago, et al. (2020). High Throughput Detection of 327 Drugs in Blood by LC-MS-MS with Automated Data Processing. J Anal Toxicol. 2021 Feb 13; 45(2): 154–183. doi: 10.1093/jat/bkaa057.
3. JMP Statistical Discovery home page. https://www.jmp.com/en_us/home.html (accessed October 2024).
4. Patterson, B. Custom method builder (created using the Control API SDK 1.5 available from SCIEX). Method builder is designed and written by Brad Patterson; not available through SCIEX. Email: brad.patterson@sciex.com.

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