Selectivity Challenges in Qualitative and Quantitative Analysis of Drugs and Metabolites

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ABSTRACT

In this presentation, we are demonstrating the key benefits of using differential mobility spectrometry for LC-MS analysis of model metabolites in biological matrices.

INTRODUCTION

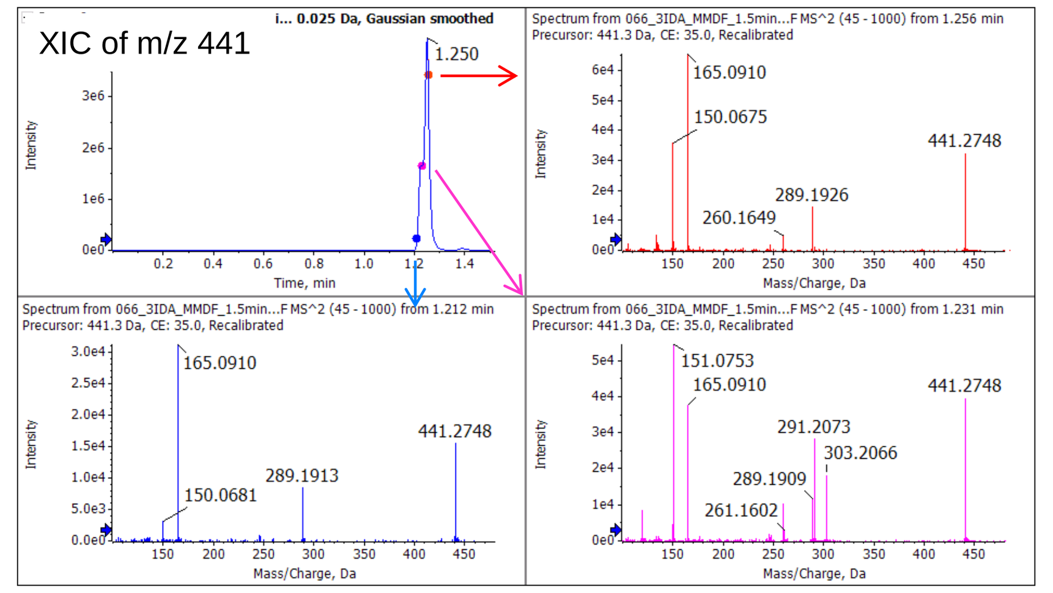


Figure 1. Common Example on Metabolite Analysis isobaric demethylated metabolites are co-eluted and require to separate for accurate metabolite identification and quantification. (XIC of *m/z* 441 and product ion spectrum)

Accurate metabolite identification and quantification are important for drug discovery and development studies. These studies can be challenging due to the limited amount of sample available from clinical or preclinical studies as well as the lack of authentic metabolite standard. There is a tradeoff between separation and acquisition time, typically, and method development for separation of isobaric metabolites and minimization of co-eluting contaminants with short run time can be time-consuming.

Ion mobility technology delivers a new dimension of selectivity and separation for metabolite identification in complex matrices, separation of isobaric metabolites and accurate quantification.

Ion Mobility - DMS

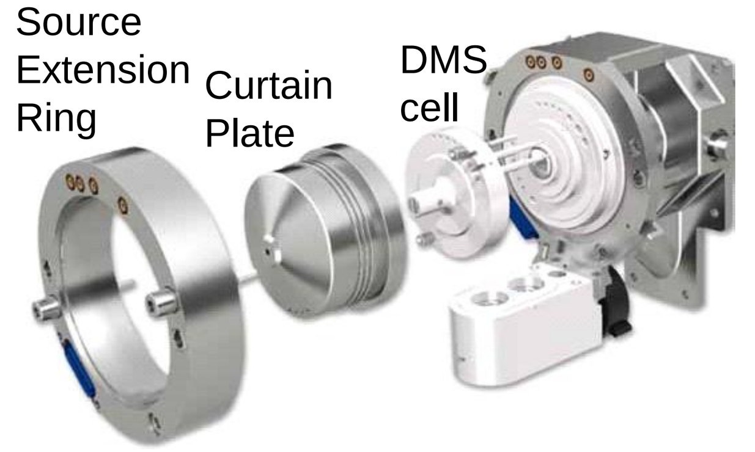


Figure 2. A prototype differential ion mobility spectrometry (DMS) device interfaced with an accurate mass instrument - DMS device is attached in front of the curtain plate and separates ions prior to entering the instrument orifice.

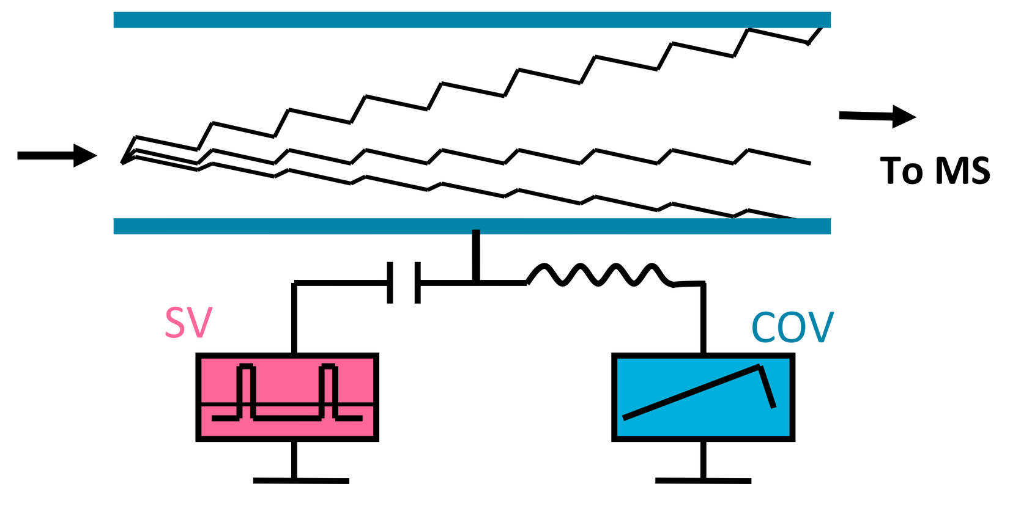


**Figure 3. Principle of DMS - ** Planar differential mobility device (DMS) separates ions based on differences in their chemical properties. A separation voltage (SV) is applied as the filtering voltage and the compensation voltage (COV) is applied as the restoring potential, which can be tuned for the compound of interest. Other co-eluting species that tune with different COV will be filtered away.

Key Benefits of DMS on QqTOF for Qual - Quant Workflow

- Separation of isobaric metabolites or co-administered drugs
- Reduction of background noise and co-eluting interference
- Reducing chromatographic run time and eliminating tedious LC method development and expensive LC columns - productivity increase and cost and time savings

For Qualification

- Enhanced and easier structure elucidation capabilities using clean product ion spectrum time - productivity increase and accurate results

For Quantification

- Selective and specific quantification, reduction in background noise and better peak integration, enhanced TOF-MS quant and eliminate MRM^{HR} method development - accurate results for PK profile and clearance rates, better S/N ratio and wider dynamic range, time savings and comprehensiveness

CHARANGES

- Tuning of DMS parameters
  - Time - UHPLC time frame
  - Samples - limited amount of samples, lack of authentic metabolite standard, complex matrices
  - Reproducibility of parameters
- Separation of co-eluting phase I isobaric metabolites
  - TOF-MS Quantitation
  - Comparison between Product Ion Spectrum for Precise Metabolite ID

Further Challenge

- Reduction of background noise and co-eluting interference
  Need rapid turnaround for optimization and simultaneous operation in UHPLC time frame

MATERIALS AND METHODS

Samples: ♦ Samples: mixture of isobaric metabolites, norverapamil and p-o-desmethyl verapamil (Fig.8), ♦ Matrix: water, rat plasma, and rat bile, ♦ Preparation: spiked into matrix at different concentration ranges (0.1 to 1000 ng/mL) **HPLC:** ♦ System: Shimadzu Prominence LC System (Shimadzu Corp), ♦ Column: Kinetex C18, 2X50mm, 2.6μm (Phenomenex Inc.), ♦ Mobile Phase: 0.1% formic acid in water / 0.1% formic acid in acetonitrile gradient (0.5-95%), ♦ Flow Rate: 0.4 mL/min, **Mass Spectrometry:** ♦ System: prototype DMS device interfaced with QqTOF system, ♦ Ion Mode: Positive (ESI / DuoSpray Ion Source), ♦ Scan Mode: TOF-MS, product ion scan, IDA (Information Dependent Acquisition), SWATH, ♦ Modifier: isopropanol, ♦ Run Time: 3.5min, **Software** (AB SCIEX): ♦ Acquiring: Analyst® TF 1.6 Software Components for SelexION Technology BETA, ♦ Processing: MultiQuant™ 2.1 Software, PeakView® 2.1 Software

RESULTS-1: Tuning DMS Parameters, Turnaround, Reproducibility of Parameters

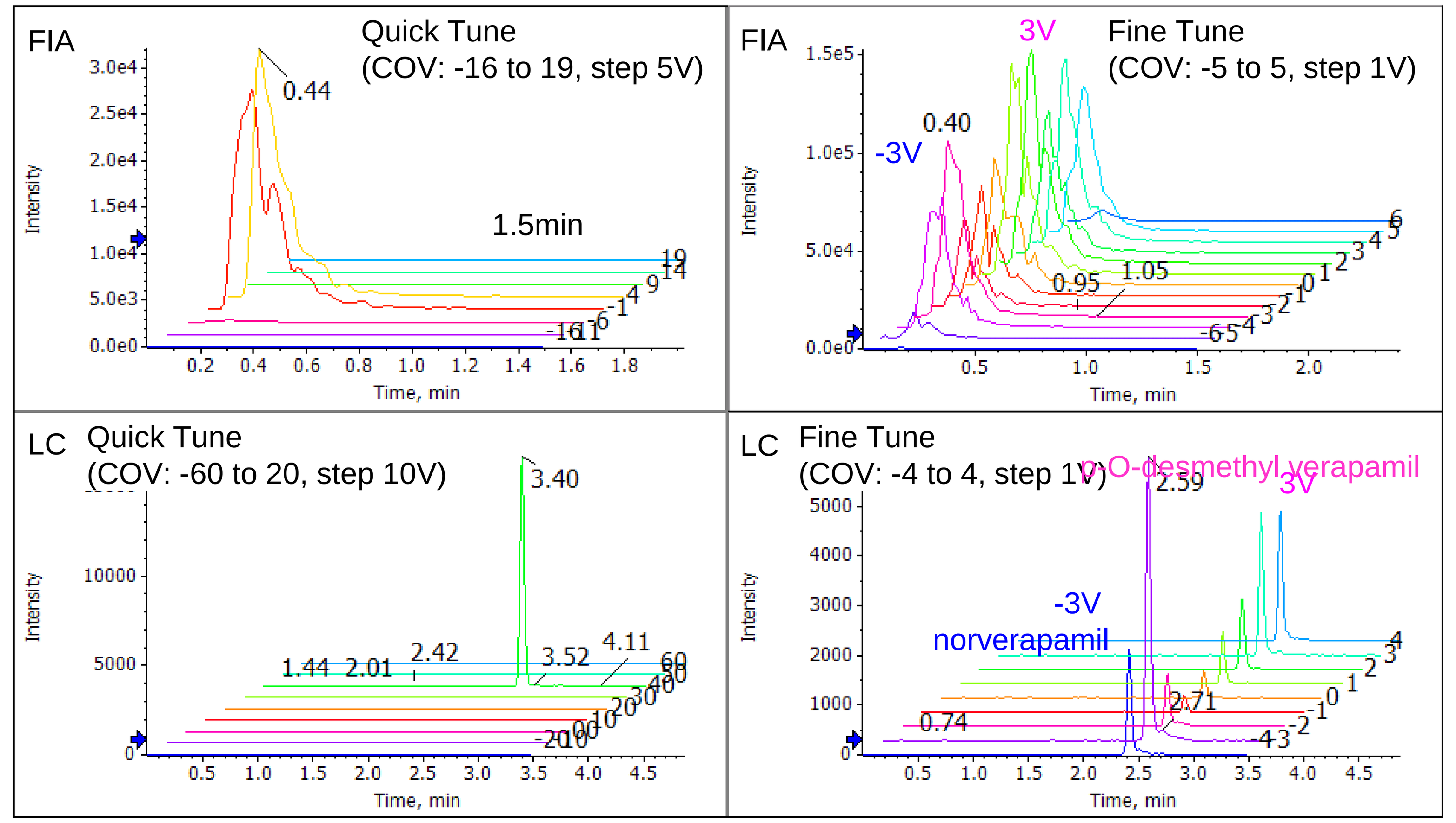


Figure 5. COV optimization for isobaric metabolites – COVs could be optimized for each metabolites in a few steps using quick and fine tunes. It was performed in FIA and UHPLC time flame and give the same optimal vales, -3V for norverapamil and 3V for p-O-desmethyl verapamil. It means limited amount of samples and samples in complex matrices can be optimized using this procedure.

RESULTS-2: Quantitation on TOF-MS

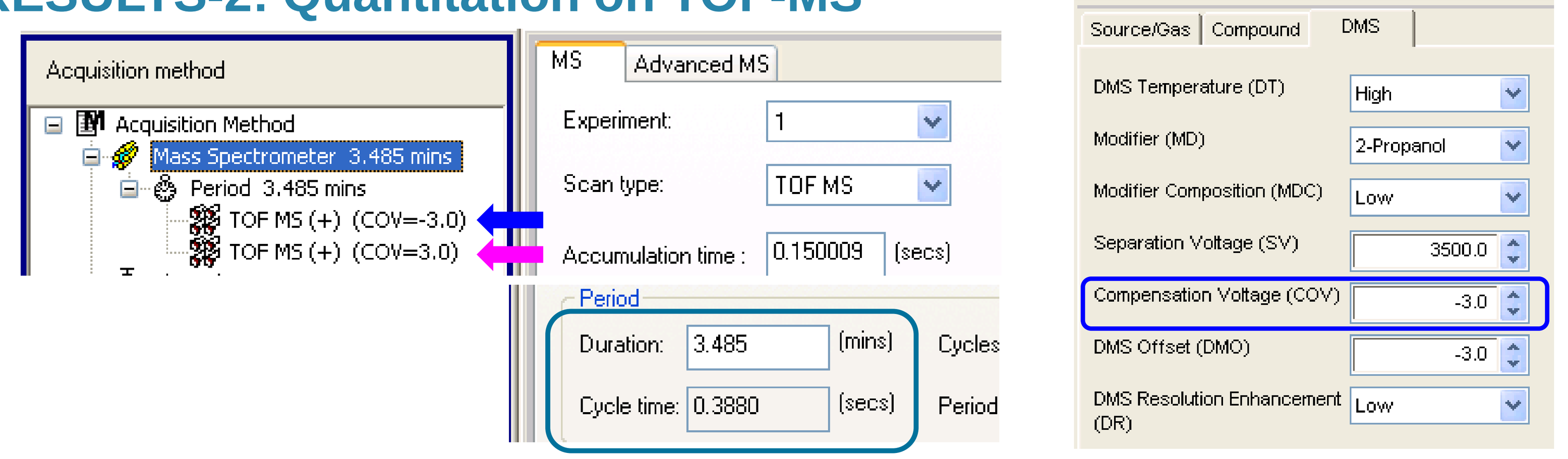


Figure 6. Acquisition Method: Norverapamil and p-O-desmethyl verapamil were acquired separately in different experiments with each optimal COV in the same run. The cycle time was 0.388sec and short enough for UHPLC 3.5min run.

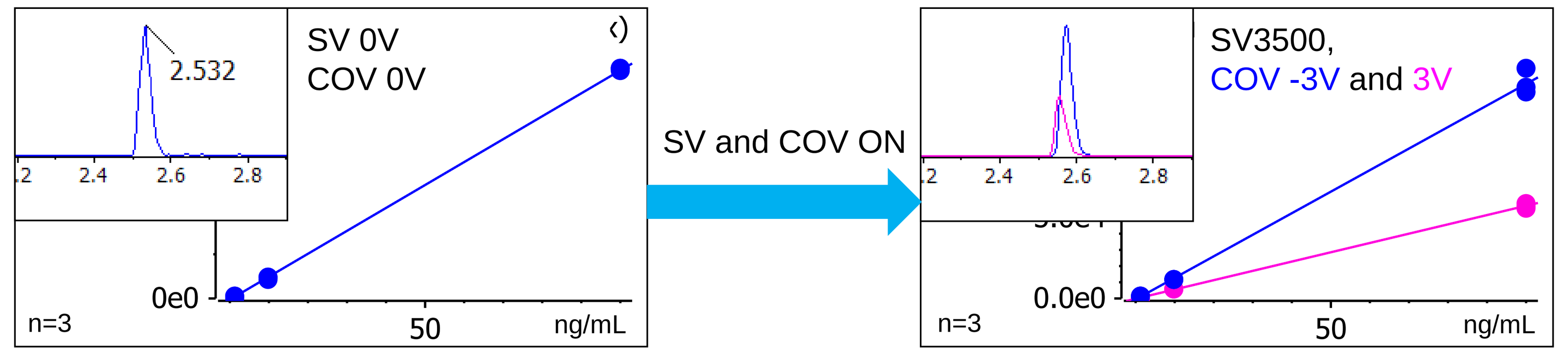
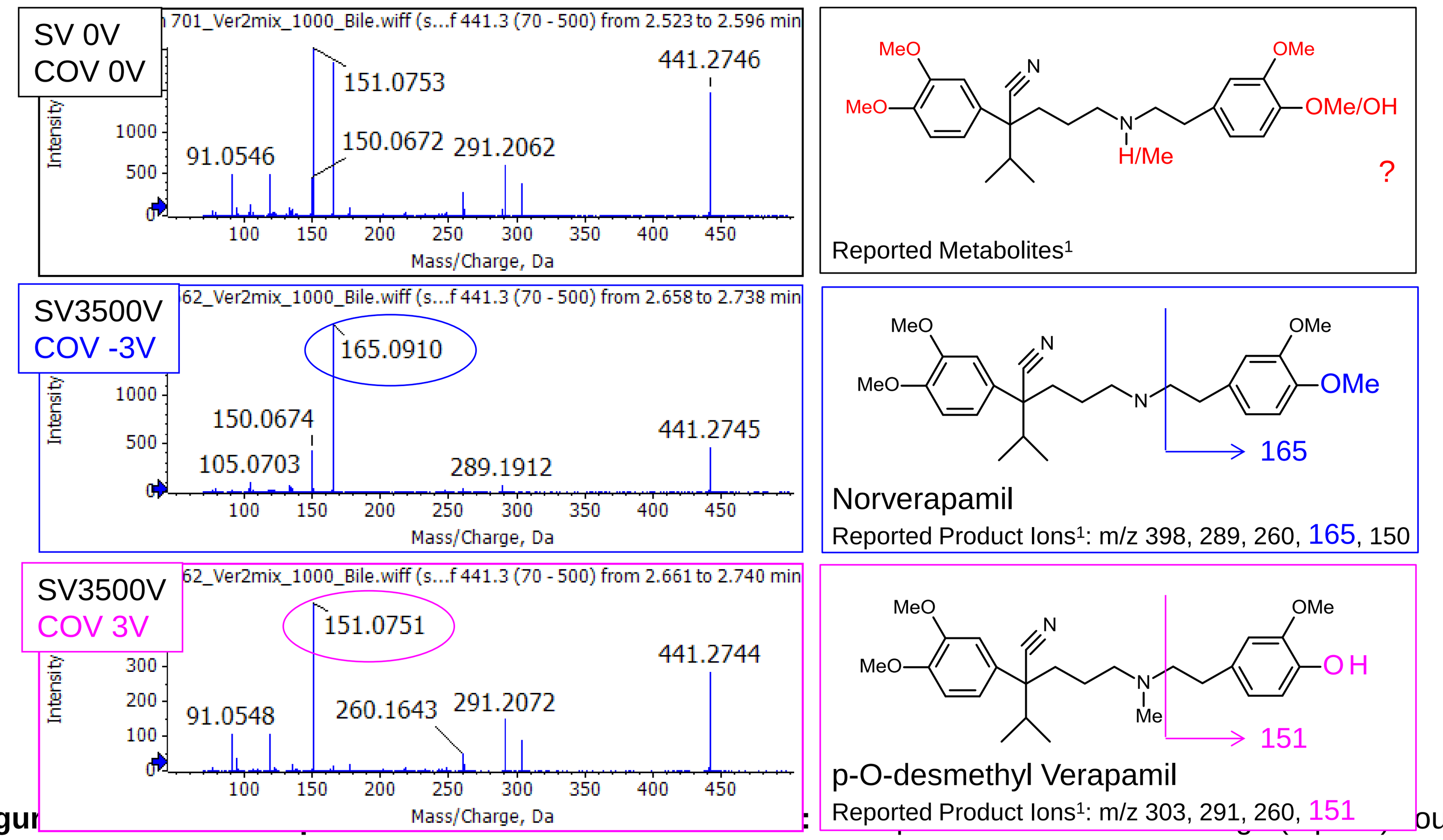


Figure 7. XIC and Calibration Curves of m/z 441 on TOF-MS with and without DMS: Both metabolites were detected and quantified as a single peak without DMS voltage (left). They were separated and quantified individually using the individual optimal DMS voltages.

RESULTS-3: Qualification for Metabolite ID



Figur… ould not point out the metabolic site. We could obtain individual product ion spectrum with DMS separation and found significant and structure related ions, *m/z* 150 and 165 in each spectra. In addition, these product ions were matched with ions in the literature[1].

RESULTS-4: Elimination of Background Noise and Co-eluting Interference and Optimization in UHPLC Time Frame

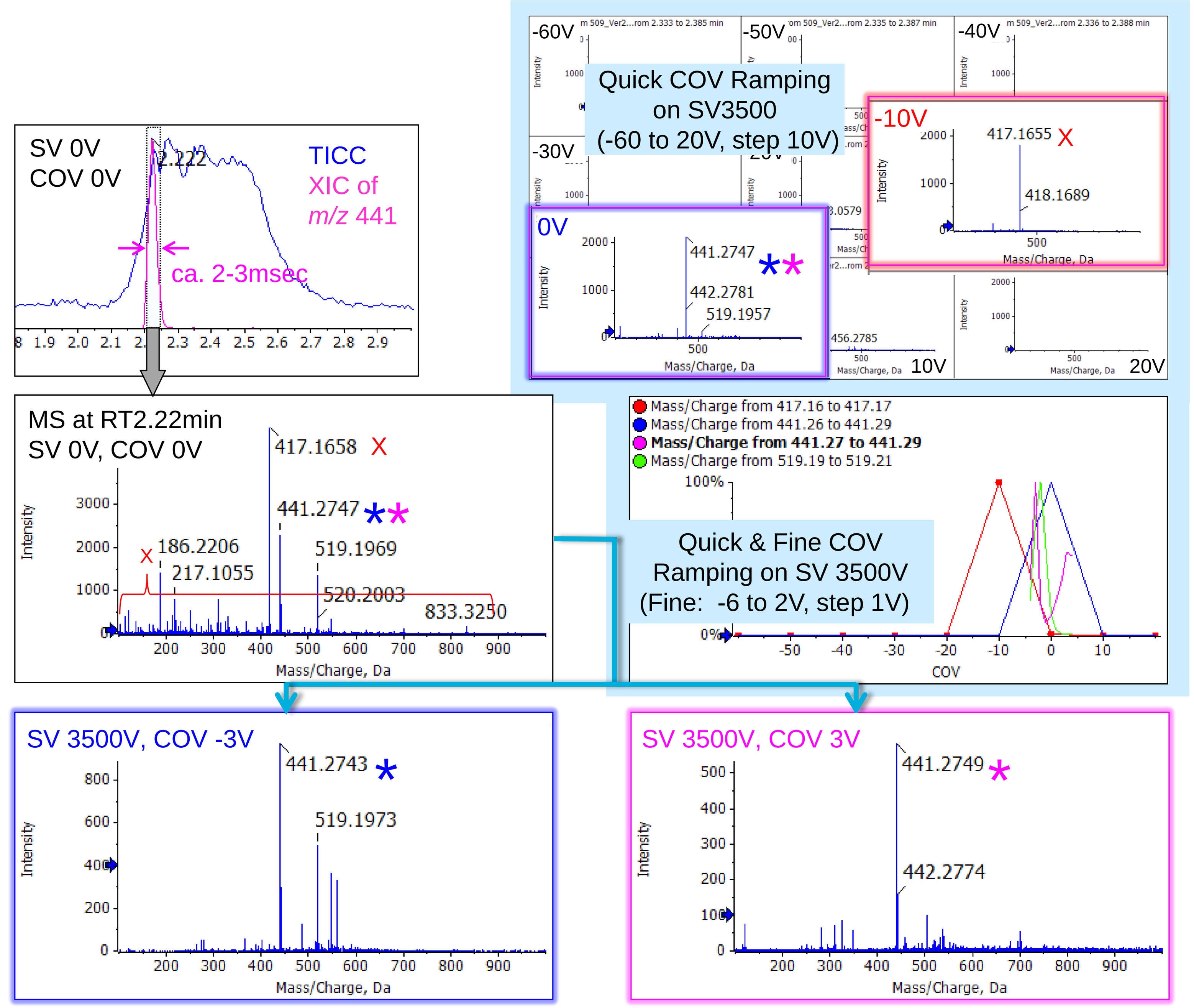


Figure 9. Workflow: Quick and fine tune were performed to eliminate background noise and co-eluted interference and optimization in narrow UHPLC peak. Most intense interference, m/z 417 was eliminated and two metabolites were separated with lower background noise.

CONCLUSIONS

- ✓ Optimization in UHPLC time scale
- ✓ Separation of co-eluted isobaric metabolites - TOF-MS Quantitation and Individual Product Ion Spectrum
- ✓ Reduction of background noise and co-eluting interference
- ✓ Confirmation of reproducible DMS parameters

Effect

- Eliminating LC, MRM^{HR} method development
- Selective, specific, comprehensive quantification and better S/N ratio and wider dynamic range
- Easy, precise and enhanced structure elucidation capabilities
- This technique is applicable to other applications, biopharma, lipids, metabolomics and others.

REFERENCES

1 Sun L et al / Acta Pharmacol Sin 2004 Jan 25 (1): 121-128

TRADEMARKS/LICENSING