Optimization of Collision Cell Potentials For Analysis of Opiates and their Glucuronyl Metabolites in a Triple Quadrupole Mass Spectrometer

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ABSTRACT

Purpose: To increase the sensitivity of MS/MS methods for quantitation of opiates and their metabolites by setting collision cell RF amplitudes and drag potentials to enhance transmission of or allow more time for the CID transitions of interest.

Methods: Tuning of the collision cell was performed by continuously infusing pure standards and running custom instrument control scripts to probe the effects of alternative RF amplitude and drag vane potentials. Potential changes of method selectivity were measured by introducing spiked samples via the Thermo Scientific™ VeriSpray™ PaperSpray ion source.

Results: Analytes of interest were found to fragment by fast and slow processes, sometimes dependent on collision energy, requiring qualitatively different tunings. Slow activation or fragmentation requires balancing the Q2 RF amplitude between optima for precursor and product ions and can benefit from using the drag vanes to retard transit of ions through the collision cell.

INTRODUCTION

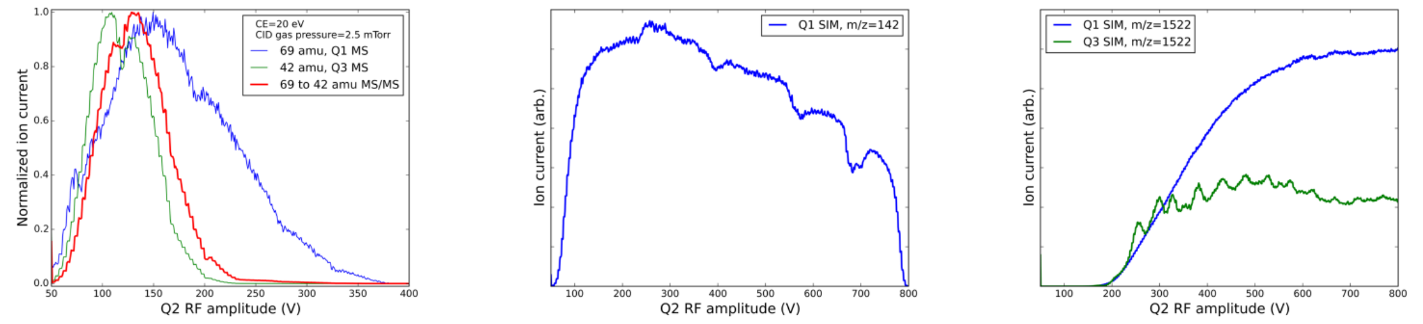
That collision energy must be tuned per precursor-product collision-induced dissociation (CID) transition for maximum sensitivity of targeted MS/MS (SRM) experiments is nowadays widely expected and understood. Collisional activations favoring formation of different products differ in both activation energy and in the number and nature of other activated states and dissociation channels present [1], making the shape of the tuning curve and the optimum collision energy highly variable and difficult to predict in practice.

Less commonly understood is the benefit of tuning ion-optical properties to favor particular CID pairs. At any given RF amplitude, radio-frequency quadrupole collision cells have a mass cutoff below which ions cannot be stably transmitted. The use of curved collision cells to reduce the flux of neutral ions at the detector and keep instruments compact introduces a need for higher (pseudo-)potentials to steer ions around the curve, which adds a high-mass cutoff. For low q ($q = \frac{2eV}{\omega^2 r_0^2 (\frac{m}{z})}$) Syka and Schoen [2] approximate the curve-induced motion away from the center of a collision cell as a function of time as

$$X_c = \frac{16E_z}{mRq^2\omega^2}\left(1 - \cos\frac{q\omega t}{2\sqrt{2}}\right).$$

The inverse square dependence on *q* implies an inverse square dependence on the RF amplitude *V* and (considering also the *m* in the denominator) a linear dependence on *m.* Representative Q2 (collision cell) tuning curves for low, middle, and high mass ions are as below in Figure 1.

Figure 1: Q2 RF tuning curves for various masses



Note that the transmission optima for the low mass ions are at RF amplitudes for which the high mass ion is not transmitted at all. We have shown previously that upwards of 2 × gain in signal intensity is possible if the Q2 RF amplitude is tuned specifically for each CID transitions from high to low mass [3].

Further investigation revealed that per-transition Q2 tuning provides benefit for some CID transitions but not others, even when the masses involved are similar, possibly due to different lifetime of the activated precursor ion. This suggested another variable to adjust. Modern instruments often use a weak axial drag field to move ions through the collision cell, allowing an increase in the number of SRMs that can be monitored without crosstalk. For slower or multi-step dissociation reactions turning off this axial field or using the same electrodes to slow the ion can increase the signal intensity.

Here we probe the benefits tuning the transmission properties of collision cell to enhance fragment production for two classes of molecule which have posed some challenges to analysts:

1. **Opiate glucuronides.** To yield useful fragment ions, opiate glucuronides must undergo a double fragmentation: loss of the glucuronic acid and fragmentation of the conjugated opiate into its product ions. Instead of quantitating the glucuronide metabolites and unconjugated drug molecules separately, protocols often call for hydrolysis of glucuronides with β–glucuronidase. Improving direct analysis of the glucuronides can enable "dilute-and-shoot" or ambient analysis and preserve the information about time and pattern of exposure lost when measurement of drug metabolites is pooled with measurement of drugs.
2. **Buprenorphine and norbuprenorphine.** Analysts' preferred CID products from these synthetic opiates are light (55 amu and 85 amu). Efficiency of production of these fragments differs between instrument manufacturers and from one MS product line to the next, especially between instruments with and without drag vanes. Increasing production of the light fragments would improve method portability and sensitivity.

MATERIALS AND METHODS

Sample Preparation

For exploratory analysis and instrument tuning, the (non-deuterated) reference standards listed in Table 1 were diluted serially to 1 µg/mL concentration in 1:1 water-methanol, 0.1% formic acid solution.

Table 1. Test compounds

	Source	Concentration	Catalog number
Buprenorphine HCl	Sigma-Aldrich	1 mg/mL	B7536
Norbuprenorphine	Sigma-Aldrich	1 mg/mL	N-059
Codeine-6-β-d-glucuronide (C6G)	Ceriliant	100 µg/mL	C-087
Morphine-3-β-d-glucuronide (M3G)	Ceriliant	100 µg/mL	M-018
Buprenorphine-D$_4$	Ceriliant	100 µg/mL	B-901
Norbuprenorphine-D$_3$	Ceriliant	100 µg/mL	N-920
Codeine-6-β-d-glucuronide-D$_3$	Ceriliant	100 µg/mL	C-138
Morphine-3-β-d-glucuronide-D$_3$	Ceriliant	100 µg/mL	M-017

To test improvement of method sensitivity, 1 mL of 1:1 water methanol samples and (for exploratory purposes) drug-free urine samples were spiked with 20 µL of mixed analyte solution and 5 µL of mixed deuterated internal standard solution diluted with 1:1 water-methanol to match the desired concentration (exclusive of the spiking volume) of analyte and 50 ng/mL of internal standard. Samples were mixed by vortexing, spotted, 10 µL per well, to Verispray sample plates, and set out overnight on the lab bench.

Instrument Tuning

All measurements were taken using a Thermo Scientific™ TSQ Quanits™ mass spectrometer., excepting those presented in Figure 1, which were taken using a TSQ Quantiva™. Optimal collision energies with 1.5 mTorr argon collision gas present in the collision cell and optimum product isolation masses were determined using the compound optimization feature of TSQ Quantis Tune instrument control software.

To tune the Q2 RF amplitude this voltage was continuously varied while monitoring a single SRM transition, averaging over 60 scans. Tuning of the drag potentials was investigated using a custom instrument control script (Figure 2). In brief: The front and rear drag potentials were iterated over combinatorially in 5 V steps, averaging 5 scans per measurement. Between measurements, the drag potentials were set to their standard value (50 V front, -50 V rear) and an unrecorded scan was run to clear any stalled ions.

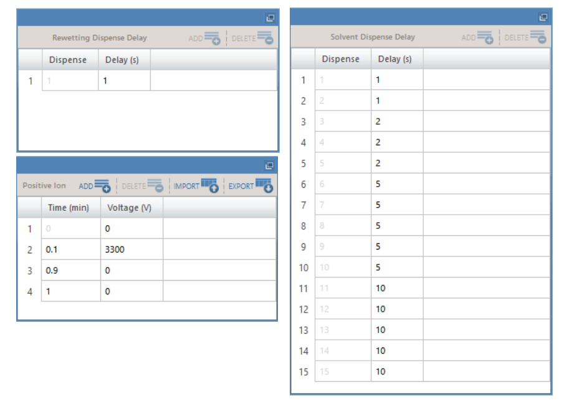
Paper spray sensitivity testing

Samples were delivered to the mass spectrometer using the VeriSpray ion source with 9:1 acetonitrile:water + 0.01% acetic acid solution used as both the rewetting and spray solvent. Rewetting and spray solvent was dispensed as in Table 3. The TSQ Quantis was run with the sweep cone removed and the following parameters set: 3800 V spray voltage, , capillary temperature 350 °C, Q1 peak width 0.7 Da, Q3 peak width 1.2 Da, 100 ms dwell time. The samples were run using the SRM scan definition in Table 4. Drag vanes were set to to nonstandard values custom values as by writing the desired settings to the instrument calibration file editing the instrument calibration file before the method run. Modified instrument control software was used to set the Q2 amplitude to user-defined, transition-specific values during the scan. XICs were integrated using Thermo Scientific™ TraceFinder™ software.

Table 2. Scan parameters

	Precursor	Product	CE (eV)	Q2 Amp. (V)
Buprenorphine	468.30	55.40	49	116
Norbuprenorphine	414.13	83.00	43	275
C6G	476.12	282.17	26	
M3G	462.14	165.00	62	
Buprenorphine-D$_4$	472.30	59.10	49	116
Norbuprenorphine-D$_3$	417.13	83.00	43	275
C6-β-d-glucuronide-D$_3$	479.12	285.17	26	
Morphine-3-β-d-glucuronide-D$_3$	465.14	165.00	62	

Table 3. Paper spray parameters



Rewetting Dispense Delay

	Dispense	Delay (s)
1	1	1

Positive Ion

	Time (min)	Voltage (V)
1	0	0
2	0.1	3300
3	0.9	0
4	1	0

Solvent Dispense Delay

	Dispense	Delay (s)
1	1	1
2	2	1
3	3	2
4	4	2
5	5	2
6	6	5
7	7	5
8	8	5
9	9	5
10	10	5
11	11	10
12	12	10
13	13	10
14	14	10
15	15	10

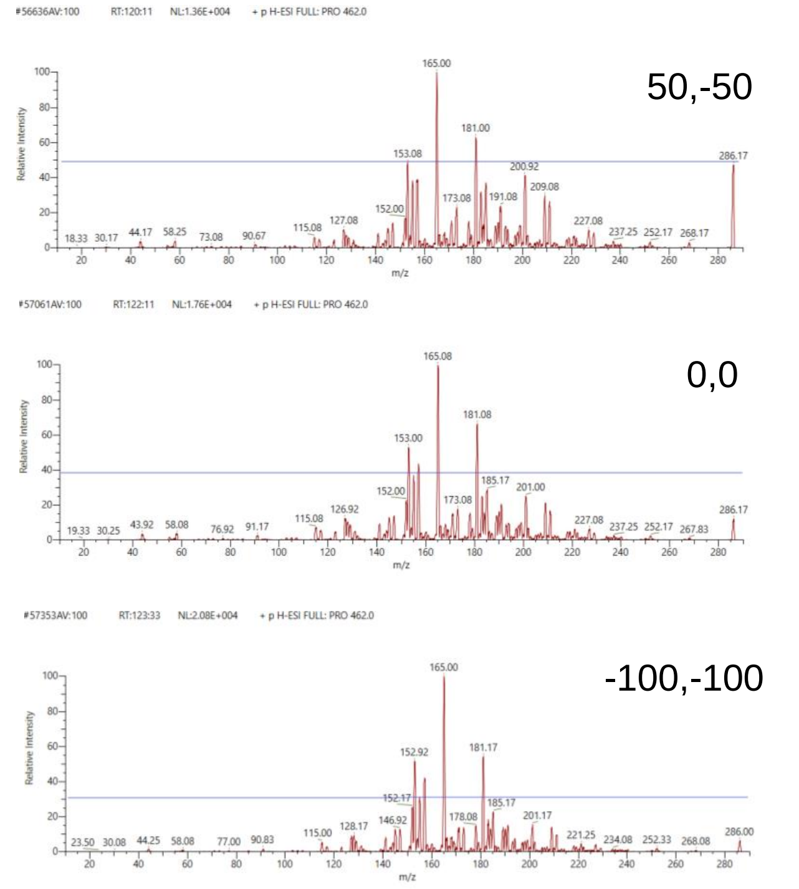
DRAG VANE TUNING

CID fragmentation of opiate glucuronides tends to produce the non-glucuronidated species in high abundance. Eliminating the axial drag field increases the production of more informative fragment ions. Figure 2 shows the resulting improvement in the product spectrum of M3G. The absolute intensity of most of the fragment ion peaks lighter than morphine + H$^+$ is enhanced with the drag vane potentials set to 0 V (relative to the DC offset applied to the Q2 quadrupole rods), while the morphine peak at 286 is diminished. Dropping the drag vane potentials 100 V while keeping the axial component flat results in a modest further improvement for some but not all peaks of the product spectrum. Similar improvement was observed for codeine-6-β-d-glucuronide.

The reason for improvement with front and rear drag vane potentials set to -100 V is not clear. With field penetration of less than 1% it is implausible that the 1 eV increase in collision energy would give the observed increase in absolute intensity of any fragment ion. To confirm this, the intensity of product ions set to their optimal collision energies (as determined by the TSQ Quantis Tune compound optimization feature) was measured with drag vanes at their standard tuning and at -100 V, -100 V; the same improvement on adjusting the drag vane potentials was observed.

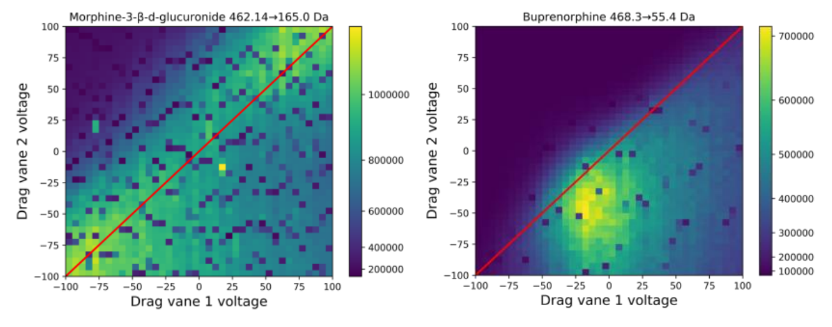
A more thorough investigation (Figure 3, left), stepping the drag vane potentials across their range combinatorially, shows that fragmentation of M3G is favored at nearly flat drag vane settings, slightly better with mild retarding potentials (above the red line) applied. Better performance at the high and low extremes may be due to fringe fields assisting entrance to or exit from the collision cell.

Figure 2: changes to M3G product ion spectrum on adjustment of the drag field.



Adjustment of the drag field enhances production of the lighter fragments of morphine-3-β-d-glucuronide at the expense of the glucuronic acid loss (morphine + H+) peak. Top: standard tuning. Center: drag vanes off. Bottom: Flat negative tuning. The thin blue line drawn on each plot corresponds to the absolute intensity of the morphine + H$^+$ fragment in the product spectrum at standard tuning.

Figure 3: SRM intensity, varying drag potentials.

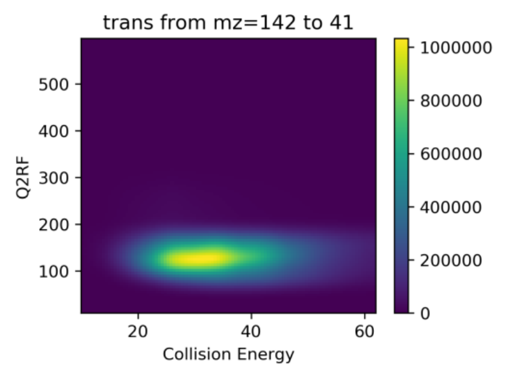


Dependence of the product ion intensity on front (vane 1) and rear (vane 2) drag vane potentials. The red line indicates flat settings, with no axial field.

This contrasts distinctly with the observed dependence of buprenorphine fragmentation on drag field tunings (Figure 3, right). Here the tuning of the 55 Da fragment a distinct optimum corresponding to an axial field assisting transit of ions through the collision cell. For M3G tuning is likely to be an optimization of slow fragment production, balancing increasing the time spent in the collision cell with the need to not stall the ions. For buprenorphine it is likely that production is saturated and tuning is an optimization of transmission.

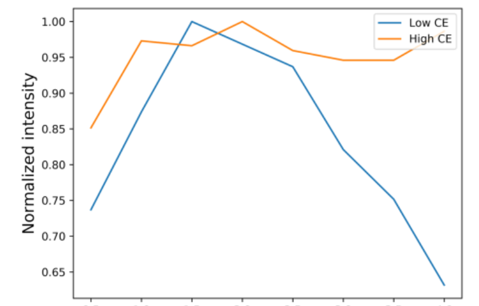
Q2 AMPLITUDE TUNING

Figure 4: Typical joint dependence on collision energy and Q2 RF amplitude



Dependence of 142→41 Da SRM signal intensity (tetramethylpiperidine) on collision energy and Q2 RF tuning . This is the most typical pattern observed for CID.

Figure 5: Sensitivity of buprenorphine 55 Da SRM intensity to collision gas pressure

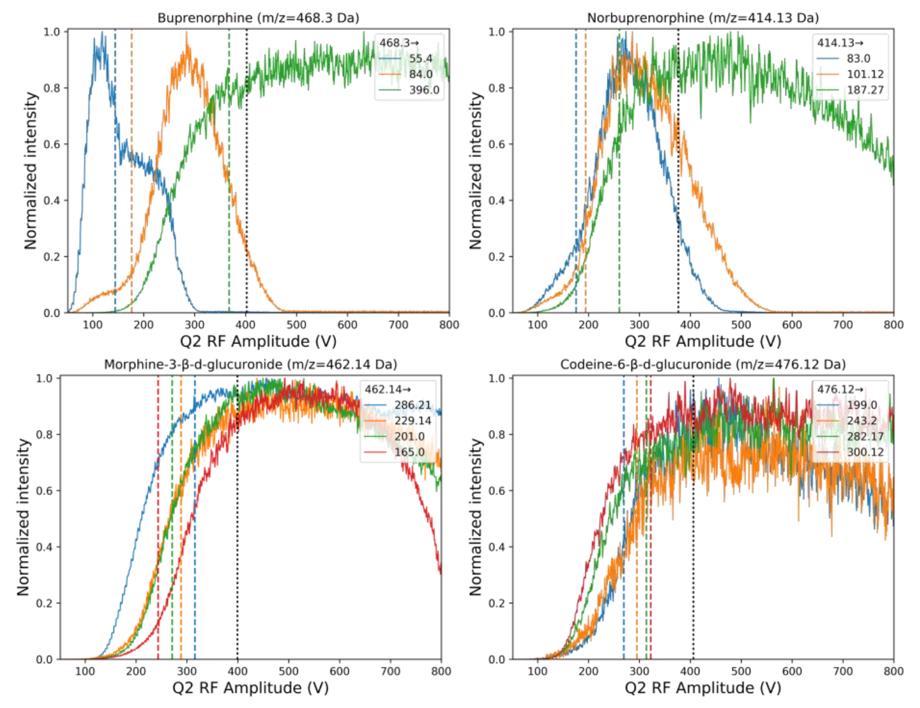


Setting the Q2 RF amplitude to favor either the high-CE or low-CE activation of the 468.2→55.4 Da transition of buprenorphine (see figure 7), we find that the low-CE process is highly dependent on collision gas pressure.

Figure 4 shows transition-specific variation of the optimal tuning of Q2, with some SRMs optimizing at the value that would best transmit an ion with the same mass and charge as the precursor, some—most distinctly, the 83 and 101 Da fragments of norbuprenorphine--optimizing at amplitudes intermediate between the precursor and product tunings. Two, the 55 and 84 Da fragments of buprenorphine, having a tuning that is a mixture between cases

That codeine and morphine glucuronide Q2 amplitude tunings mostly follow that of the precursor ion corroborates our interpretation of the behavior under flat or retarding drag vane potentials: either activation or fragmentation are slow and the precursor is transmitted deeply into the collision cell.

Figure 6: Q2 amplitude tunings for various opiate SRM transitions of interest.



Q2 (collision cell) RF amplitude tuning curves for various fragments of interest. The vertical dashed lines mark the expected optimal tuning for the product ion mass; the dotted black line marks the expected optimal tuning for the precursor mass.

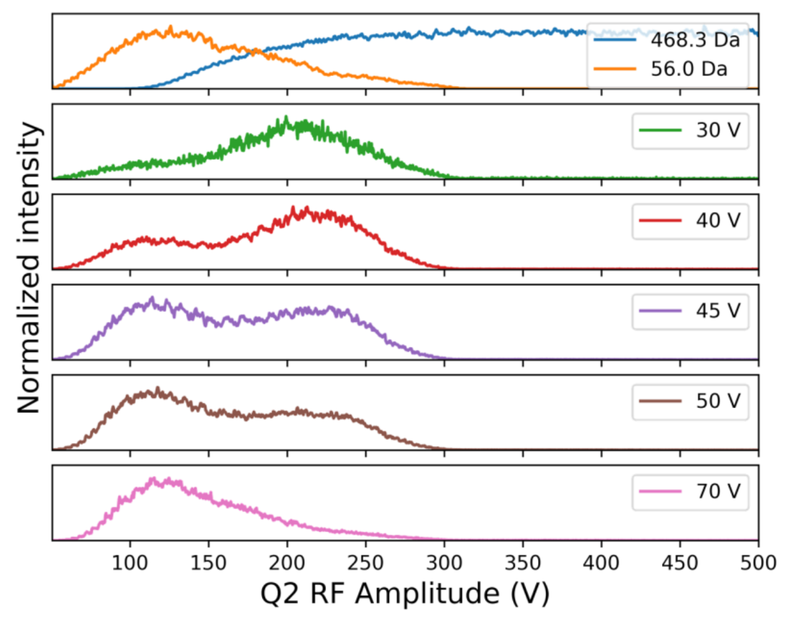
Moreover, the tuning curves for the codeine and morphine (glucuronic acid loss only) fragments are the leftmost of their respective series despite having the highest mass. It is likely, given the behavior shown in Figure 2, that these fragments are produced (on average) earlier and therefore closer to the entrance of the collision cell than the lighter fragments. A tuning that trades between precursor transmission deeper into the collision cell and product transmission out of the collision cell will in this case be biased more than the others toward transmission of the product.

Different modes of excitation tune differently

Measurement of the tuning curve for the 55 Da fragment of buprenorphine at multiple collision energies (Figure 7, second through bottom panels) shows clearly that the curve is the sum of two components corresponding to two different reaction pathways, one low RF amplitude component favored at high collision energy and a high RF amplitude component favored at low RF amplitude. Comparison of the CE=70 V Q2 RF tuning curve with the tuning of a background ion with m/z (56 Da) similar to that of the fragment shows that the low RF amplitude component corresponds to transmission of the product ion alone. The other component is located in a region of significant overlap between the precursor and product ion tuning curves suggesting that the precursor ion or an intermediate persists deeply into the collision cell.

The observed dependence of signal intensity on collision gas pressure, plotted in Figure 5, clarifies the different nature of the two pathways. If fragmentation occurs after a single collision near the entrance of the collision cell, saturation at low pressure, as observed, is to be expected. Saturation likely occurs as the probability of a that a collision exciting the correct vibrational mode occurs before the (unstable) precursor is lost. This could potentially be shown experimentally by careful measurement of the gas pressure dependence on slight detuning from the Q2 amplitude optimum.

Figure 7: Changes to product ion spectrum on adjustment of the drag field



Top panel: MS^1 (intact precursor) Q2 RF amplitude tuning curves for buprenorphine + H$^+$ and an unidentified 56 Da background ion. Second through bottom panels: Q2 RF amplitude tuning for the 468.2→55.4 Da transition of buprenorphine at various collision energies.

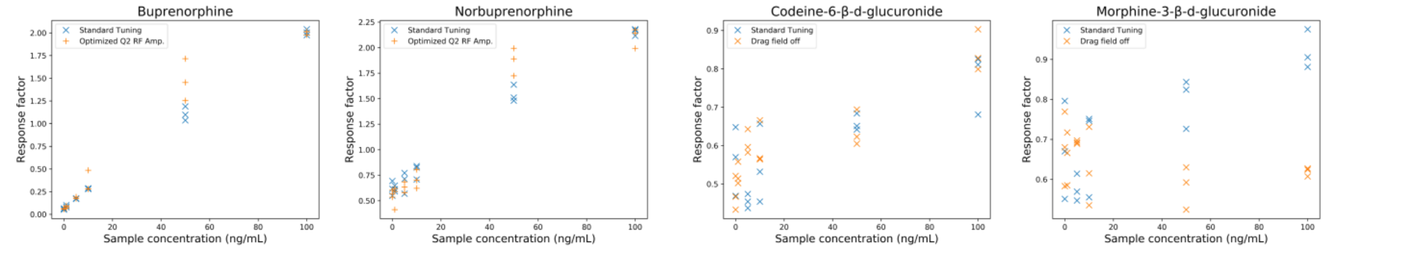
The stronger pressure dependence of the low-CE pathway suggests activation by multi-collision heating of the precursor. As pressure increases, fragmentation will happen earlier (on average); if the optimal pressure were to increase as the RF amplitude increases this would be demonstrated conclusively.

Strong coupling of Q2 tuning to collision energy is not generally observed; Figure 4 is representative of most CID transitions studied in preliminary investigations. Q2 amplitude typically optimizes at the optimum for the product mass, corresponding to activation near the collision cell entrance, or an intermediate value which can approach the optimum for the precursor mass. The "wing" on the tuning curve for the 84 Da fragment of buprenorphine and the more subtle shoulder in the tuning curve for the 83 Da fragment of norbuprenorphine suggests that in those cases there may also be two activation pathways at work.

Does Q2 tuning provide a general improvement of selectivity?

Since tuning of the collision cell in this fashion is an optimization for particular CID pathways, one might suspect that selectivity is increased. To probe this question, we prepared samples for analysis by paper spray MS/MS. Chemical background of urine matrix and paper was too great relative to the intensity of the protonated ions optimized above to give an informative result; for now we restrict our analysis to neat sample. Application of retarding or flat negative drag vane potentials without the extra collision-cell clearing step used to generate figure 3 was found to attenuate the signal due to ion stalling. A flat tuning of 0 V on the front and rear drag vanes was used for measurements of M3G and C6G. M3G and C6G, and separately buprenorphine and norbuprenorhine, were mixed and spotted together to VeriSpray cartridges for analysis as mixtures.

Figure 8: Comparison of paper spray MS/MS response with and without collision cell tuning.



Response factors for the compounds discussed previously, with and without custom collision cell tuning.

Absolute signal strength, quantified by integrating the XIC for the period of time when the spray voltage is on, was increased in most measurements. Internal standard and analyte ions were increased identically, as seen in the plots of response factors (Figure 8.) The exception is M3G, for which the response factors with the drag vanes turned off were flat. (Further investigation is necessary to determine the cause.)

At standard tuning (Figure 8, blue xes), norbuprenorphine, C6G, and M3G all suffer from considerable chemical background interference from the VeriSpray paper, as evinced by response factors approaching 0.5-0.6 if the trend in the data is extrapolated backwards. This is not reduced when the custom collision cell tunings are applied (orange +es); transition-specific Q2 RF amplitude and drag vane tuning does not provide enough increase in selectivity to appreciably reduce background interferences in general.

CONCLUSIONS

- Collision cell tuning for optimum signal intensity is sensitive to the nature and rates of the activation and dissociation processes; for optimum sensitivity tuning should be informed by chemistry. In particular, single-impact and multi-impact activation, and fast and slow dissociation require qualitatively different tunings.
- Reported differences in the abundance of the light CID fragments of buprenorphine and norbuprenorphine between different MS instrument product lines can be accounted for by considering the interplay of fragmentation process and the different collision cell configurations.
- Optimization of SRM intensity in this fashion does not come with a general increase of selectivity. Further method-development work and possibly the introduction of an orthogonal separation technique such as FAIMS is necessary.

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TRADEMARKS/LICENSING

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