UV-photodissociation on a triple quadrupole mass spectrometer to increase selectivity in LC-MRM analysis

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INTRODUCTION

Photodissociation has been used to study the structure and reactions of polyatomic ions. In contrast to activating the lowest-energy dissociation pathway, as in infrared multiphoton dissociation (IRMPD), ultraviolet photodissociation (UVPD) induced by single photon absorption leads to instantaneous promotion to an excited electronic state, followed by fragmentation. When applied to small molecule analysis, UVPD spectra can reveal fragment ions that are distinct from those generated by collision-induced dissociation (CID). Thus, UVPD, especially in tandem with CID, can offer unique insights into the structure of gas phase ions for structural elucidation, and be used to gain selectivity in quantitative analysis. Here, we present preliminary results on the use of UVPD implemented at the back end of a curved high-pressure collision cell to support qualitative MS/MS analysis, and MRM-like acquisition for quantitative analysis.

MATERIALS AND METHODS

Samples

All chemicals were either obtained from Sigma (St-Louis, MO) or from Toronto Research Chemical (North York, On) and used without any additional treatment. Blood collection tubes (BD Vacutainer®) were purchased from VWR (Mississauga, ON). The Vacutainer® back-ground matrix was prepared by shaking the tube filled with water, at 40 °C for 1 hour. The supernatant served as a surrogate interference matrix to mimic the presence of interferences for steroid analysis.

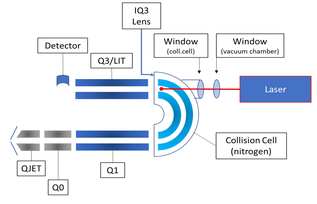
Mass spectrometry

UVPD and CID were performed on a research-grade hybrid quadrupole linear ion trap (QqLIT), based on QTRAP 5500 system geometry. The UV laser (355 nm) was operated at high pulse energy (80 µJ) and repetition rate of 10 kHz (MKS/Spectra-Physics, Irvine, CA). The laser beam was introduced between the rods of the collision cell via 2 windows, as depicted in Figure 1. For MRM-like analysis with UVPD, the voltage on the exit lens of the collision cell (IQ3) was adjusted to generate a small repulsive barrier during the excitation time, which was controlled by the pause time in the acquisition method.

LC-MS analysis

Separation was performed with a Shimadzu Nexera-UPLC system using an XBridge (2 x 50mm, 3 µm) column from Waters (Milford, MA). A linear gradient was used that transitioned from 98% water with 0.1% formic acid to 95% acetonitrile with 0.1% formic acid over 5 minutes.

Figure 1. UVPD Modified QqLIT.
Fragmentation region is located at the exit region of the high-pressure collision cell (IQ3).



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RESULTS AND DISCUSSION

Based on earlier work, operating UVPD at 355 nm offers advantages in terms of selective fragmentation [1]. Taking advantage of this selectivity, it is possible to fragment the compound of interest effectively while yielding little-to-no signal from concomitant background ions [1-3]. Here, the compound coverage of steroids and benzodiazepines was analyzed at 355 nm to evaluate this capability.

To perform UVPD in MRM-like mode, the exit lens of the collision cell was operated with a repulsive voltage to 'trap' the precursor and generate efficient UVPD fragmentation. Figure 2 shows the fragmentation efficiency for testosterone as a function of the potential difference between IQ3 and RO2 (cell rod offset). In all cases evaluated, IQ3 was optimal at 0.5 +/- 0.01 V, with respect to RO2, thus creating a 'pseudo-trap' region where the laser beam interacted with the precursor ion. Controlling the residence time of the precursor ion in that region at a given voltage, enabled the fragmentation efficiency of UVPD to be controlled. Figure 3 shows the fragmentation efficiency of testosterone as a function of the reaction time. Figure 4 is a representation of the potential barrier applied via IQ3 (exit lens of collision cell, Q2) to trap the precursor and fragment ions generated during UVPD. Figure 5 shows the impact of the IQ3 lens voltage with respect to RO2 on the potential field at the end of the collision cell and on the axial position of the collisional cooled ions. With a typical distance of 1 mm between IQ3 and Q2 rods, the observed optimal voltage of IQ3 (Figure 2) was in agreement with the predicted value. Therefore, on a given system configuration, IQ3 could be tuned to compensate for slight variability in distance and might not require additional tuning unless IQ3 was otherwise modified.

Figure 2. IQ3 optimization with UVPD-MRM mode.

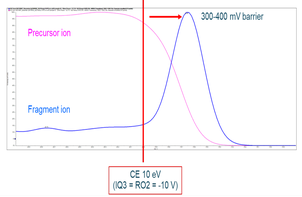


Figure 3. Fragmentation efficiency vs reaction time.

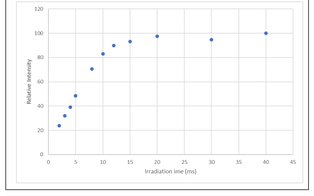


Figure 4. 'Pseudo-trap' used as reaction region generated by potential barrier.

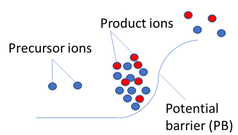
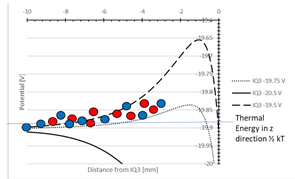


Figure 5. Impact of IQ3 lens location and voltage on the potential barrier at the exit of collision cell.



The selectivity of UVPD can be obtained by 2 approaches: 1) generating known fragment ions, common to CID, directly from UVPD of the intact precursor ion or 2) generating unique fragment ions not observed by CID. The first approach applies to large majority of steroid compounds when subjected to UVPD at 355 nm. Though no new fragments were generated with UVPD for the steroids evaluated (Figure 6A), this approach offered significant chemical noise reduction when compared to CID analysis. For quantitative studies, CID fragmentation can be tuned to maximize signal for a given fragment ion. Similarly, the collision energy can be adjusted to minimize interferences, though this approach is less frequently used, as it requires more development or tuning, and it can lead to compromised sensitivity. When using a 0.5 V potential barrier on IQ3, an 8 ms irradiation time, UVPD generated both major fragment ions of testosterone with efficiency >18% and depleted the residual precursor to 15% (Figure 7).

Using these conditions, the selectivity of detection of testosterone in surrogate-matrix was evaluated. The matrix used to generate background interference was from separation-gel of Vacutainer® tubes [4]. To generate a surrogate matrix, a Gold Cap Vacutainer®, was filled with water and shaken for 60 minutes at 40 °C. The water was then removed and used directly as matrix. Figure 8A shows that interferences were systematically observed in the MRM transition when CID fragmentation was used. Though interferences can be eliminated by different approaches, the detection of additional LC peaks was used to demonstrate some of the challenges faced in bioanalysis development. Conversely, when UVPD was used as the fragmentation approach, a single LC peak was detected for testosterone and the matrix blank yielded no detectable signal over the entire LC chromatogram. Similar observations were made for progesterone (8B).

Figure 6. UVPD and CID of testosterone and lorazepam.

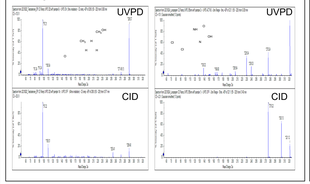


Figure 7. UVPD-MRM fragmentation efficiency for testosterone with irradiation time of 8 ms.

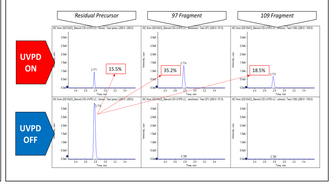


Figure 8. Interferences detected at different spike concentration in matrix for UVPD and CID, for testosterone (A) and progesterone (B).

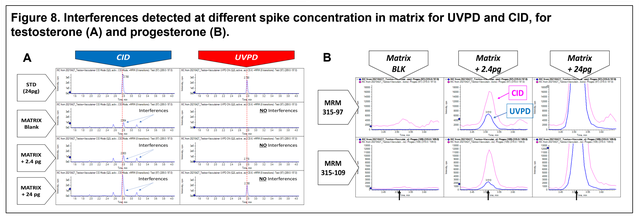


Figure 9. Impact of LC gradient on the detection of analyte and interference in UVPD and CID analysis for testosterone.

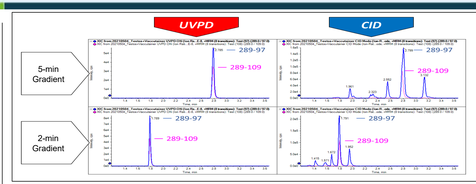
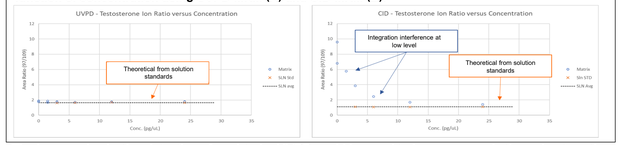


Figure 10. Peak area ion ratios for testosterone in solution standards and spiked in matrix as a function of concentration using UVPD-MRM (A) and CID-MRM (B).



The improved selective detection of analyte by UVPD could be used to improve sample throughput, as the analysis became less dependent on LC separation, as depicted in Figure 9. Using UPVD-MRM might lead to LC-free analysis for given compound classes using combination of UV wavelengths. Eliminating the risk of interferences detection via selective fragmentation can also improve the determination of ion ratio for confirmation analysis. The ion ratios observed in matrix at different concentrations matched those of the solution standards analyzed by UVPD (Figure 10A), whereas the ratios deviated from the expected values at low concentrations when analyzed by CID due to matrix interference (Figure 10B). Although this effect might have been corrected by adjusting the LC gradient, this difference in result by fragmentation technique demonstrates that the selective fragmentation achieved by UVPD-MRM might further alleviate the dependance of LC separation in quantitate analysis.

Conclusion

The selectivity of UVPD can lead to interference-free detection by MRM. When performed at high pressure, with high repetition rate laser and proper power, the reaction times required are compatible with MRM analysis. The fragmentation selectivity of UVPD opens possibilities in quantification by reducing the need for LC, since many interferences are not detected using this fragmentation method. The proposed implementation of this method enables one to easily alternate between conventional CID-MRM and UVPD-MRM.

References

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2. 2- Enjalbert, Q. *et al.* "*Photo-SRM:laser-induced dissociation improves detection selectivity of selected reaction monitoring mode*', Rapid Comm. Mass Spectrom., 2011 (25) 3375-3381
3. 3- Le Blanc, J.C.Y., *et al.* "*UV-Photodissociation on a hybrid QqLIT mass spectrometer to increase selectivity in LC Analysis*", 68th ASMS Reboot Conference, 2020 (WP-217)
4. 4- Wang, C. *et al.*, "*Validation of a Testosterone and Dihydrotestosterone Liquid Chromatography Tandem Mass Spectrometry Assay: Interference and Comparison with Established Methods*", Steroids, 2008 (73) 1345-1352

TRADEMARKS/LICENSING