analytical chemistry

pubs.acs.org/ac Article

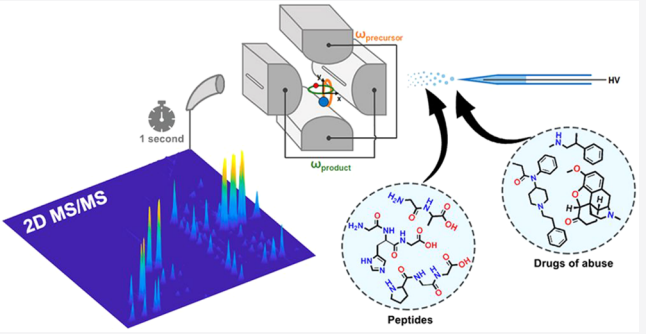
2D MS/MS Spectra Recorded in the Time Domain Using Repetitive Frequency Sweeps in Linear Quadrupole Ion Traps

Lucas J. Szalwinski, Dylan T. Holden, Nicolás M. Morato, and R. Graham Cooks*

Cite This: *Anal. Chem.* 2020, 92, 10016–10023 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Ion trap mass spectrometers have emerged as powerful on-site analytical platforms, in spite of limited mass resolution, due to their compatibility with ambient ionization methods and ready implementation of tandem mass spectrometry (MS/MS). When operated at constant trapping voltage, ions can be activated at their secular frequencies and all MS/MS experiments can be performed, including the two-dimensional tandem mass scan (2D MS/MS scan) in which all precursor ions and their subsequent product ions are both identified and correlated. In the new method of performing this 2D MS/MS experiment presented here, the precursor ions are excited by a nonlinear (inverse Mathieu q) frequency sweep while the resulting product ions are identified by their ejection time within a repeating orthogonally applied nonlinear (inverse Mathieu q) frequency sweep. This resulting compact representation contains the total fragmentation behavior of a collection of ionized compounds and captures detailed chemical information efficiently (typically in 1 s). The approach is implemented using a simple single mass analyzer instrument. This methodology was tested on three different multicomponent mixtures: drugs of abuse, peptides, and fentanyl analogs. The data are compared with those obtained by more common MS/MS scan methods.



INTRODUCTION

The use of tandem mass spectrometry (MS/MS) is often central to complex mixture analysis, especially for in situ measurements where chromatographic separations are not practicable.[1−3] The ideal portable instrument for characterization of complex mixtures would have a single mass analyzer and would use ambient ionization for little-to-no sample preparation.[4,5] Advances in ambient ionization methods are routinely transferred from benchtop instrument to portable instruments.[6,7] By contrast, most advances in mass analyzers moved in the direction of increased instrumental complexity toward devices that cannot be easily miniaturized.[8,9] Two-dimensional tandem mass spectrometry is an experiment that has been adapted for use in high performance instruments but it is also transferable to smaller systems. Figure 1 summarizes the three-dimensional data domain (two dimensions of mass/charge, one dimension of ion abundance) of 2D MS/MS. It includes recording the entire 2D domain (green) as well as several common one-dimensional m/z experiments (neutral loss, precursor and product ion scans) and the zero-dimensional multiple ion reaction monitoring (MRM) experiment. Acquisition of the full 2D MS/MS data domain as opposed to its subparts has the potential to provide comprehensive chemical characterization of a sample in a single scan.

Two-dimensional tandem mass spectrometry was first demonstrated by Pfändler working with an FT-ICR instrument and using a series of timed rf pulses to excite and de-excite ions to achieve 2D FT ICR data.[10] More recent FT-ICR-based improvements in 2D-MS/MS include coupling radius dependent fragmentation methods,[11] phase correction of the signal output,[12] and rf pulse sequence optimization.[13] Recently, our group demonstrated the experimental implementation of two-dimensional tandem mass spectrometry (2D MS/MS) in a single scan of a linear quadrupole ion trap.[14,15] The FT-ICR experiment provides high resolution on a wide range of precursor ions; however, this method requires ultrahigh vacuum instrumentation, a superconducting magnet, and lengthy acquisition times.[16,17] By contrast, the quadrupole ion trap method has modest vacuum requirements and short acquisition times, albeit with reduced mass range and resolution; as such it should be best suited to implementation

Received: April 21, 2020
Accepted: June 24, 2020
Published: June 24, 2020

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023

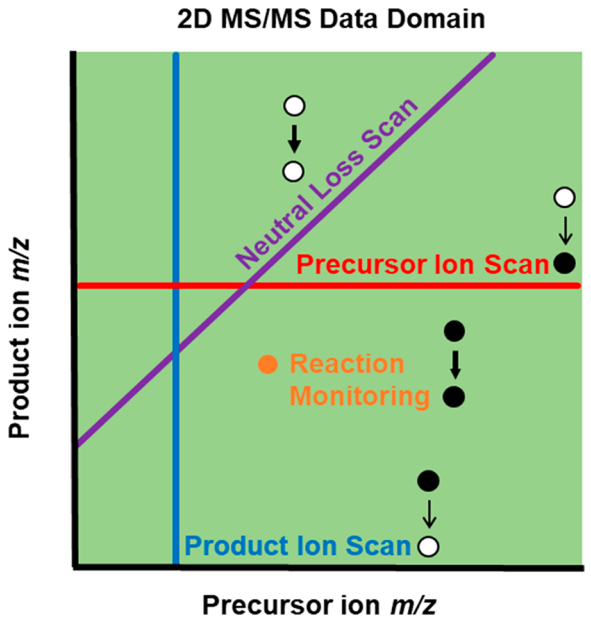


Figure 1. Summary of MS/MS scan modes represented in the 2D MS/MS data domain. Open circles indicate variable mass, while closed circle represents fixed mass. Narrow arrow indicates variable mass transition while block arrow indicates fixed or defined mass transition.

in the form of a miniature mass spectrometer for on-site analysis.[18,19]

The first experimental implementation of 2D MS/MS in a quadrupole ion trap used a nonlinear frequency (inverse Mathieu q) sweep[20] to dissociate precursor ions while a broadband signal waveform was used to eject all fragment ions.[14] The precursor ion m/z was determined from the time at which product ions were detected and the product ion m/z values were determined by analyzing the frequencies present in the detected signal. The resulting signal showed distinct product ion peaks with spacings corresponding to the secular period of motion of an individual product ion (Figure 2a). This motion,[21] termed "micropacket"[22] or "micromotion",[23] serves for mass analysis in the micropacket method of 2D MS/MS. Taking short fast Fourier transforms of the signal, the secular frequency of the ejected product ions can be determined and then translated to a mass/charge ratio (m/z) through calculation of the Mathieu β value, hence the Mathieu q-value, and so by solving the Mathieu equation, to the value of m/z.[24,25]

Most commercial ion trap detection circuits are too slow to detect ion micromotion, so a second method, frequency tagging, was implemented (Figure 2b).[14] In this method, each secular frequency was modulated by a unique low frequency beat. The detection circuitry was insensitive to the high frequency signal allowing only the detected beat frequency to be observed, converted to secular frequency, and hence to m/z in the same fashion as in the micropacket method.

The performance of the initial micropacket method was far superior to that of the frequency tagging method of 2D MS/MS because the frequencies being measured were much higher. The measurement of higher frequencies over the same time period provided higher frequency resolution, which in turn improved mass resolution. One major disadvantage of both these methods is that the transients used for the Fourier transforms last only for hundreds of microseconds, thus limiting frequency resolution. The trade-off between frequency

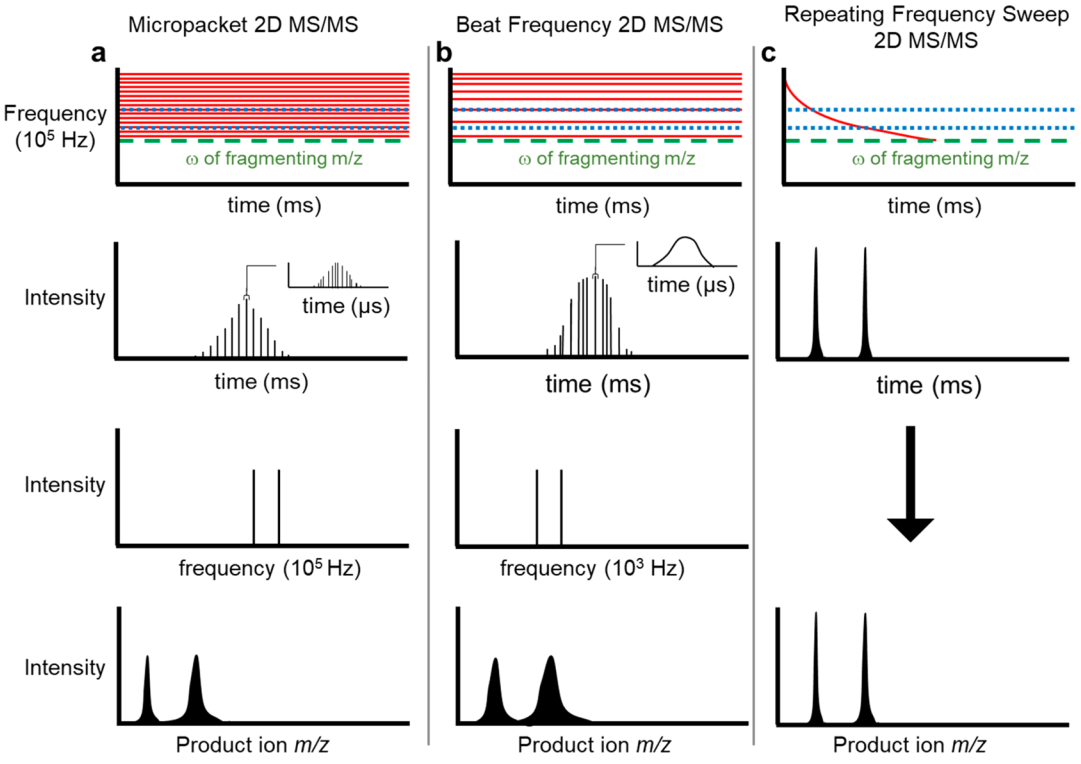


Figure 2. Comparison of the product ion ejection process for (a) micropacket, (b) beat frequency, and (c) repetitive frequency sweep methods of 2D MS/MS in a linear quadrupole ion trap. First row: waveform (red) and secular frequencies of product ions being analyzed (blue). Second row: signal obtained at the detector. Third row: fast Fourier transform of the detected signal. Fourth row: product ion spectra calibrated for m/z.

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023

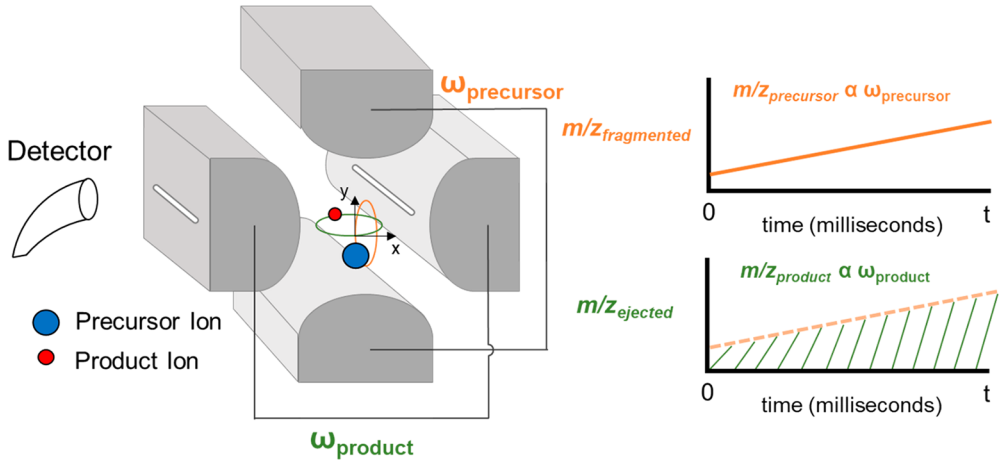


Figure 3. Methodology for performing time domain 2D MS/MS in a quadrupole ion trap. Precursor ions are fragmented by an auxiliary ac frequency applied to the rod pair used to create the field in the *y*-coordinate. The orthogonal rod pair provides a field in the *x*-coordinate that quickly sweeps ions into the detector as it passes through secular frequencies of the possible product ions. Product ion ejection is repeated multiple times over a single precursor m/z value in order to preserve precursor ion m/z information.

and time resolution is well-known. This limitation can be circumvented by avoiding frequency-based methods and the associated Fourier transforms when short analysis times are desired using micropacket techniques.[26] Furthermore, difficulties in discriminating frequency harmonics, as well as the nonlinear calibration, makes converting frequency to m/z challenging by this method. The new method presented in Figure 2c, avoids the need to use Fourier transforms by ejecting product ions using a nonlinear frequency sweep during which the time of product ion detection is linearly correlated to its m/z. This allows simple m/z calibration as well as improving product ion mass range and resolution.

EXPERIMENTAL SECTION

Chemicals. All drugs of abuse were bought from Sigma-Aldrich (St. Louis, MO) and diluted in 50:50 methanol/water containing 0.1% formic acid. The drug target mixture was diluted to 1000, 500, 400, 300, 200, 100, 50, and 25 ppb, while the internal standard, methamphetamine-d5, was held at 300 ppb when determining limits of detection (LOD). Two peptides, Gly-His-Gly and Ala-Gly, were purchased from Sigma-Aldrich while all others were from Bachem (Torrance, CA). The combined peptide mixture was diluted in acetonitrile to 5 ppm of each compound. The fentanyl analog mixture was diluted in methanol to a concentration of 5 ppm for each compound.

Ionization. Nanoelectrospray (nESI) was used for all experiments. Borosilicate glass capillaries (1.5 mm o.d., 0.86 mm i.d.) from Sutter Instruments (Novato, CA) were pulled to 5 μm tip diameters using a Flaming/Brown micropipette puller (model P-97, Sutter Instrument Co.). Solutions were pipetted into the capillary. An electrode holder (Warner Instruments, Hamden, CT) was used to place the tip of the capillary within 2 cm of the mass spectrometer inlet. A 1.5 kV potential was applied to generate electrospray.

Instrumentation. A modified Thermo Finnigan LTQ mass spectrometer (San Jose, CA) was used for all experiments. This instrument was previously modified so the rf trapping voltage could be controlled externally.[27] This allowed the rf voltage to be kept constant throughout the analytical scan to maintain the motion of trapped ions at a constant secular frequency. Additional modifications were made to apply externally controlled low voltage auxiliary waveforms individually to both the *x*- and *y*-rod pairs.[28] Nitrogen was used instead of helium as the bath gas in order to improve sensitivity.[29] An uncalibrated ion gauge reading of 1.5×10^{-5} Torr was used in the experiments.

Waveform Generation. Two Keysight 33612A arbitrary waveform generators with 64 megasample memory upgrades (Newark element14, Chicago, IL) were separately coupled to the *x*- and *y*-electrode pairs. The waveforms were calculated in Matlab (MathWorks, Natick, MA), exported as.csv files and imported into the waveform generator software.

Time-Domain 2D MS/MS Scan Methodology. The experiment is illustrated in Figure 3. Two simultaneous waveforms are applied orthogonally to fragment precursor ions and eject product ions. The waveform applied to fragment the precursor ions is swept nonlinearly through the ion secular frequencies in order to linearize the choice selection of m/z fragmented over time. The second waveform, applied to the *x*-rod pair to eject product ions, is composed of repeating nonlinear frequency sweeps. These repeated sweeps are identical in their frequency components, but the amplitude is dropped to zero during times when the applied frequency corresponds to m/z values higher than that of the ion being fragmented with the assumption that only singly charged ions are being interrogated. This modulation is used to avoid ejecting precursors before fragmentation occurs. The product ion scan rate is conserved throughout the scan while maintaining the linear time vs m/z correlation in the product ion spectrum. The two-dimensional spectra are produced by splitting the signal output into segments of the same length as the product ion sweep. The product ion m/z is determined by the signal location within each of these segments, while the precursor ion m/z is determined by the location of that segment within the entire scan.

Scan and Trapping Experimental Parameters. The m/z range analyzed was the same for both precursor and product ions. However, the LMCO was different for each experiment. The drugs of abuse, peptides, and fentanyl analogs had LMCO values of 80, 85, and 120 Th (m/z units), respectively. In each experiment, ion injection times were optimized to avoid space charging while providing adequate signal. The injection times for all of the above experiments

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023

were between 0.5 and 2 ms and they were held constant for the duration of each experiment. For the drug and peptide mixtures, the scan time was 900 ms with the product ion ejection waveform being repeated every 1.5 ms. The fentanyl mixture scan time was 1200 ms with the product ion ejection waveform also being repeated every 1.5 ms. The scan time for the tetraalkylammonium mixture was 600 ms with a product ion ejection sweep occurring every 1 ms. The precursor ion activation and product ion ejection time ranges were selected to provide a sufficient number of data points across each peak in both precursor and product ion dimensions. It would be assumed that slower scanning rates would improve both mass accuracy and resolution. However, both scan rates are limited to the fragmentation time of the precursor ion population. Better analytical performance would be expected if fragmentation methods that fragments precursor ions faster were used. Additional time, typically 10 s of milliseconds duration, was added between consecutive 2D MS/MS scans to avoid possible overlap of scans.

RESULTS

This new repetitive frequency sweep version of 2D MS/MS was explored in three cases involving complex mixture analysis and to which MS/MS has been applied previously. The first two examples concern detection and identification of known analyte(s) in a complex mixture. In one case there is an emphasis on detection of the analytes and in the other the emphasis is on identification of the analytes, operating on the assumption that the requirements for detection are less stringent than those for identification. In the context of MS/MS, detection of a compound might be accomplished using one or two transitions (precursor to product ions) whereas compound identification might require four or five such transitions. The first example consists in the determination of 12 drugs of abuse in a single scan in which a single precursor/product ion transition is used to go beyond detection and identification to quantification of the analyte at ppb levels. The second example compares the 2D MS/MS data with data previously collected using product ion scans to identify unknown peptides in a complex mixture. Lastly, fentanyl analogs are interrogated in a single scan to identify fragmentation patterns that could be used to identify unknown analogs for which no data are currently available.

Determination of 12 Drugs of Abuse. The ability to determine known drugs of abuse from a complex mixture is exemplified in Figure 4. The drugs of interest were determined by monitoring the abundance of a single precursor/product ion transition against a precursor/product ion transition for an internal standard. This represents the conventional method for quantitation of drugs of abuse, multiple reaction monitoring (MRM,•⇒•) in which two or more transitions are monitored.[30] A few precursor/product ion transitions can be used to confirm the detected analyte while a single transition is used as quantifier. This "scan" is rarely used in instruments other than triple quadrupoles; however, pseudo-MRM methods have been implemented in other instruments where the single individual transitions of interest can be extracted from a product ion scan.[31] The same method in which single transitions are extracted out of a higher dimensional spectrum is also useful for targeted drug quantification in 2D MS/MS. Acceptable quantitative performance was obtained for most of the drugs of abuse analyzed using methamphetamine-d5 as internal standard. With the single exception of 6-MAM, LOD's

# - Compound	Quantifier	LOD (ppb)
1 - Amphetamine	136 → 91	13.5 ± 6.9
2 - Methamphetamine	150 → 91	15.8 ± 4.5
3 - MDMA	194 → 163	5.7 ± 1.6
4 - MDEA	208 → 163	5.7 ± 1.6
5 - Acetyl Norfentanyl	219 → 84	20.7 ± 25.0
6 - Amitriptyline	278 → 233	15.1 ± 7.4
7 - Hydrocodone	300 → 199	24.3 ± 21.2
8 - Acetyl Fentanyl	323 → 188	4.1 ± 1.4
9 - 6-MAM	328 → 211	96.7 ± 54.3
10 - Fentanyl	337 → 188	27.2 ± 8.7
11 - Isobutryl Fentanyl	351 → 188	4.9 ± 1.5
12 - Furanyl Fentanyl	375 → 188	30.7 ± 12.0

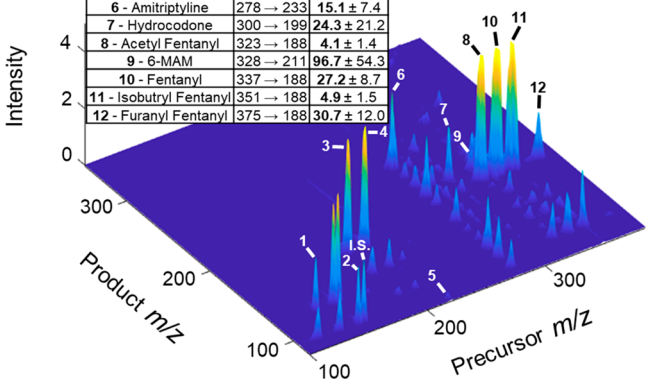


Figure 4. 2D MS/MS scan of 12 drugs of abuse quantified by a single internal standard, methamphetamine-d_5 (transition m/z 155 → 91). The signal intensity for each transition was determined as the integrated volume of signal divided by integrated volume of the internal standard signal. Limits of detection were estimated as the analyte concentrations that would provide signal/noise ratios of 3. All samples and blanks were run in triplicate.

were 50 ng/mL or less and correlation coefficients were 0.94 or better (see Figure S3 for additional detail).

Note that a single transition might be sufficient for detection, but several different transitions are needed to confirm the identification of a particular analyte. Figure 5a shows the extracted product ion scan for precursor m/z 278, amitriptyline, in the 2D MS/MS scan. The similarity to the product ion scan obtained on a commercial ion trap instrument (Thermo LTQ, Figure 5b) demonstrates how the same fragments can be used for confirmation in the 2D MS/MS scan. The differences in relative abundances of the product ions are attributed to the use of nitrogen as a bath gas which causes additional fragmentation of the primary product ion, m/z 233, and to the instrumental differences that affect energy transfer and ion transmission.

2D MS/MS for Identification of Peptides. Although Figure 5a shows the potential for compound identification, the canonical method for confirming the identity of an analyte is to compare the fragmentation data of the unknown to that for an authentic sample. A simple example of the application of this methodology using 2D MS/MS data is shown in Figure 6b where each peptide in a mixture was identified by matching its fragmentation data (extracted product ion scan) to data obtained in directly recorded product ion MS/MS scans after isolating the ionized peptide. In silico generation of fragment ion data could become an alternative to annotate newly acquired mass spectra quickly.[32] The comparison can be done quickly regardless of whether the data were acquired in silico or in situ, so the main limitation is how quickly experimental MS/MS data can be obtained. Figure 6 demonstrates the quality of the information generated in a 2D MS/MS scan in less than a second.

This figure shows data for a mixture of 10 peptides analyzed to determine how the extracted information obtained in a 2D MS/MS scan compares to conventional ion trap product ion spectra. Peptide identification typically is achieved by sequential fragmentation along the peptide backbone. The charge is typically retained along the backbone, so identi-

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023

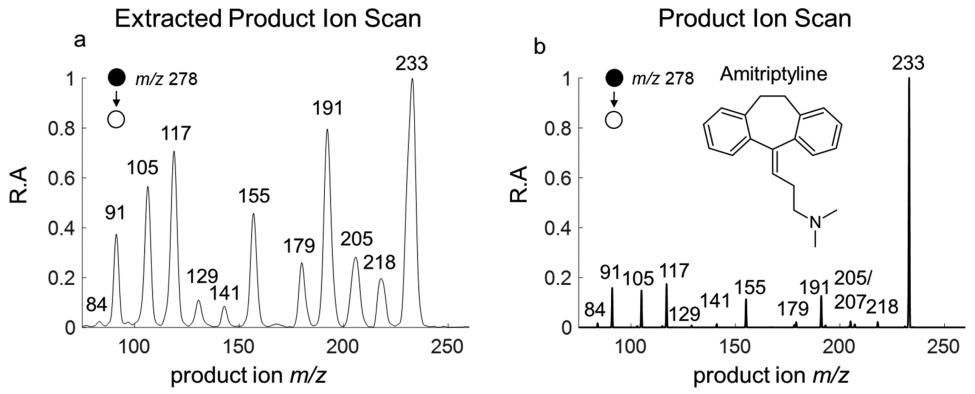


Figure 5. (a) Product ion scan of amitriptyline (m/z 278) extracted from the 2D MS/MS scan and (b) authentic product ion scan of amitriptyline (m/z 278). Note the similarity in products but the difference in resolution. The data in panel b were recorded in 0.1 s while the data in panel a were extracted from a larger data set collected over a period of 1 s with several hundred other product ion spectra being acquired at the same time.

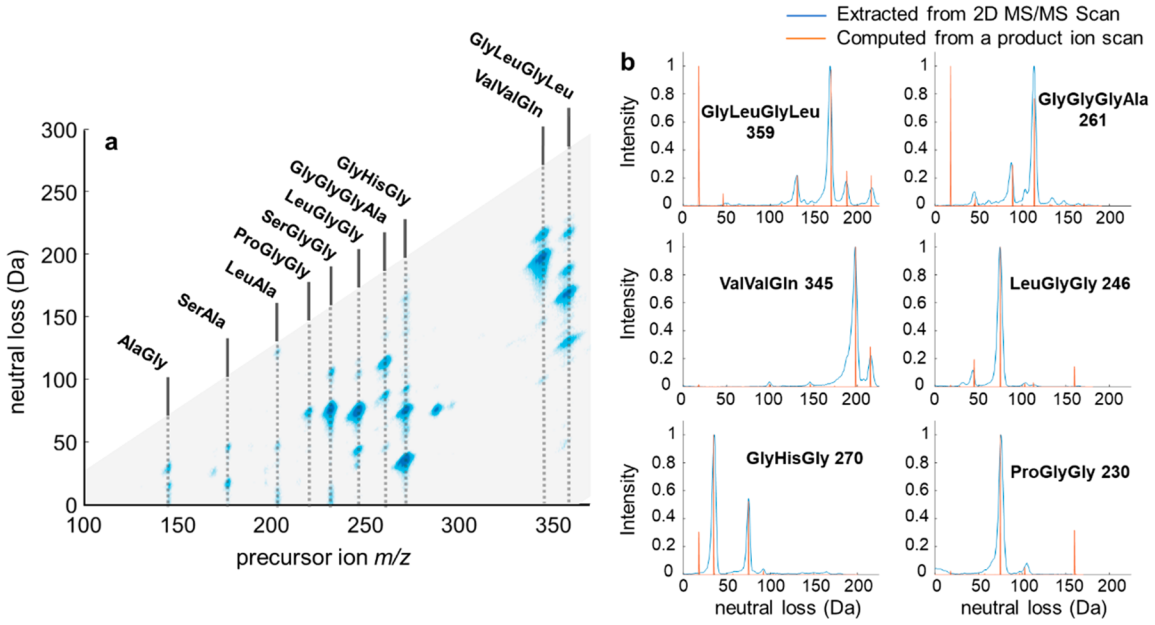


Figure 6. (a) 2D mass spectra of 10 small peptides showing precursor ion m/z plotted against neutral loss (instead of the more usual product ion axis) by subtracting a given product ion by its precursor ion m/z and (b) comparison of the neutral losses calculated from a 2D MS/MS scan (blue) with those derived from an authentic product ion scans (orange).

fication of the amino acid sequence can be based on neutral losses observed in the spectra. For this reason, it is sometimes more convenient to observe the neutral losses for each precursor ion rather than the more usual direct observation of product ions. This is readily accomplished by subtracting the product ion m/z from the precursor ion m/z at every point in the precursor/product ion plane and replacing the normal product ion axis with a neutral loss axis. It seen in Figure 6a, peptides with N-terminal glycine all undergo a neutral loss of 75 Da (neutral glycine) and peptides with N-terminal alanine undergo a neutral loss of 46 Da (CO + H_2O). Furthermore, the extracted data from the 2D MS/MS scan compare favorably with ion trap product ion scans, shown in Figure 6b. The notable differences being in the loss of water and the neutral loss of 160. The expected neutral loss of 160 gives a product ion, m/z 86 for precursor ion m/z 246, which lies near the low mass cut off, while a loss of water falls within the bandwidth of the frequency applied for fragmentation. This means the primary product of dehydration will be further fragmented. Figure 6b demonstrates that the 2D MS/MS scan captures the same depth of information obtained as the individual product ion scans. The decrease in resolution also is clear, however it does not severely limit successful matching of fragmentation patterns. This point is demonstrated in Figure S2 where all nine of the fragments observed in the extracted product ion scans were found also in the 2D MS/MS data with an average mass/charge error of 1.3 Th. An important point not yet considered is the efficiency with which the 2D data can be collected. A single product ion scan (180 ms) can be recorded faster than the whole 2D MS/MS scan (900 ms); however, the product ion scan acquisition time scales directly with the number of mixture components of interest, while the 2D MS/MS acquisition time is independent of that number. In the case shown in Figures S2 and 6, the 10-analyte mixture requires more than twice the time to analyze even if only the specified ten analytes are targeted. Furthermore, to generate the whole 2D MS/MS spectrum from product ion scans across the whole precursor mass range, 250 product ion scans from precursors separated by 1 Th would take 45 s compared to the constant 900 ms for the 2D MS/MS scan.

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023

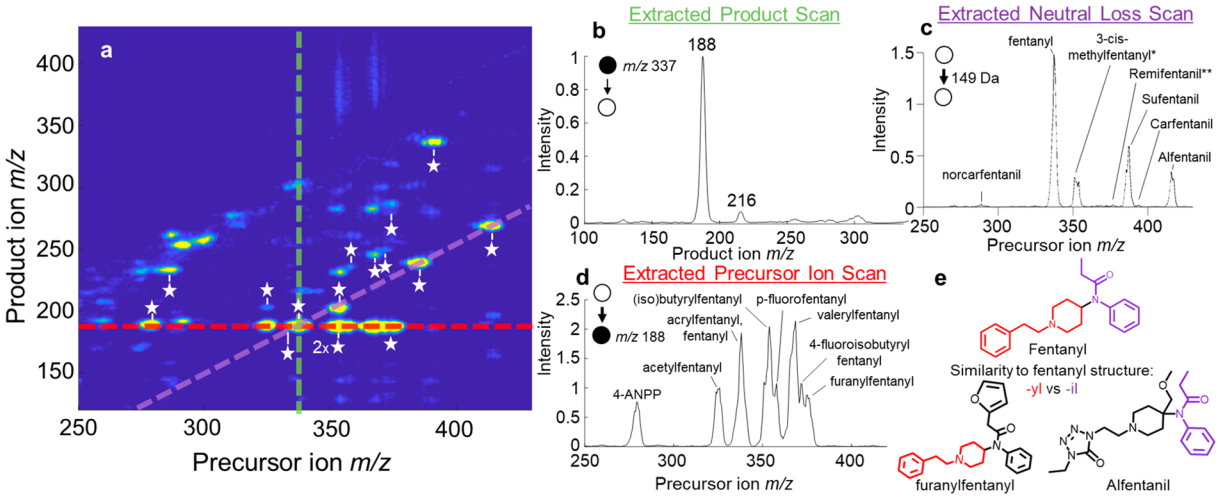


Figure 7. (a) 2D MS/MS scan of 16 fentanyl analogs in a mixture with extracted scans overlaid; (b) extracted product ion scan of fentanyl; (c) extracted neutral loss scan of 149 Da; (d) extracted precursor ion scan of m/z 188; and (e) structural comparison of the -yl vs -il fentanyl analogs. White stars indicate a known transition of each precursor ion analyzed. 3-*cis*-Methylfentanyl possesses the same conserved structural feature as the -il variants. The neutral loss of 149 Da for remifentanil is observed with a signal/noise ratio of 8 in the extracted product ion scan.

2D MS/MS for the Untargeted Detection of Fentanyl Analogs. The detection and subsequent identification of novel fentanyl analogs is representative of a larger challenge in analytical chemistry where the desired list of identifiable compounds is always larger than the known list of identifiable compounds. Data independent acquisition (DIA) methods are well suited to identifying truly unknown analytes. Among these methods is SWATH-MS on AB Sciex instruments where multiple precursor ion isolation windows allow for "shotgun CID" in which the precursor ion is replaced by retention time and the product ion/retention time relationship allows for improved detection coverage.[33] These methods have seen widespread use due to the ability to provide extensive amounts of fragmentation data for very complex samples. There are two offsetting features to these "shotgun CID" methods of DIA. First, these methods are used in practice to identify compounds already present in libraries, but they do not expand the library. Second, the requirement for LC is not amenable to portable instruments. A non-LC-based DIA method on a portable mass spectrometer would allow de novo on-site identification of compounds in complex samples.

The strength of DIA using 2D MS/MS is demonstrated for the case of a complex mixture of 16 fentanyl analogs (Figure 7). Those analogs with names ending in -yl have a conserved phenylpiperidine moiety while those ending in -il have a conserved *N*-phenylpropanamide moiety. These conserved moieties can be detected by monitoring the charged product, m/z 188, for -yls and the neutral product, loss of 149 Da, in the -ils. The parent compound, fentanyl, contains both functionalities and thus lies on the intersection of the neutral loss and precursor ion scan lines is in this rapid class differentiation experiment. All other compounds are detected on either the neutral loss or precursor ion scan line. It would be sufficient to detect either of these transitions to signal a potential fentanyl analog but, by obtaining the full 2D data, further identification can be obtained by simply extracting the product ion spectrum from the existing data. The coupling of a method of detecting known transitions to the identification of unknown analytes is an attractive feature of 2D MS/MS. Certainly this feature will facilitate library expansion and thus forensic analysis.

Identification of Nonlinear Precursor/Product Ion Relationships. The two one-dimensional MS^2 scans in which the product, whether charged or neutral, is conserved and these can be used to identify the corresponding precursors as belonging to a particular chemical class. The precursor ion scan implies that the transition will produce common charged product ions for precursors of the same or similar class. The neutral loss scan implies that the transition will produce a common neutral product molecule for precursors of the same or similar class. Both of these statements implicitly assume that addition or subtraction to the precursor molecule will produce the same addition or subtraction even in the products. Additions conserved on the charged product are expected to yield a common neutral loss while additions conserved on the neutral product yield constant product ions. This will indeed produce a linear relationship between increase of mass of the precursor and increase in mass of the product. However, this analysis does not cover all possibilities. For example, if a precursor is modified in more than one way (besides simple addition of a functional group), then the precursor would not be seen in the 1D MS/MS scans. An example of a chemical system not able to be related through a single 1D MS/MS scan is shown in Figure S4. The six tetraalkylammonium ions differ in the lengths of the four alkyl chains attached to the nitrogen center. No conserved neutral losses or product ions are observed; however, it can be seen that these analytes do share a common functional relationship. The identification of these complex relationships in mixtures could prove useful in situations where the chemical modifications are not limited to simple additions or subtractions.

Frequency vs Time Measurements for Product Ion Analysis in 2D MS/MS. Previous 2D MS/MS experiments using a linear quadrupole ion trap were performed using the same fragmentation waveform as done in this study but by ejecting the product ions using a sum of sines broadband waveform.[14] The product ion information was determined from iterative fast Fourier transforms across the mass spectrum. This produced a two-dimensional mass spectrum where the product ion m/z was determined by the frequency components found in each FFT and the precursor ion m/z was determined by the time at which the FFT was recorded. This

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023

previous micropacket method[14] demonstrated the power of acquiring MS/MS data across a range of masses. This was shown specifically by identifying fentanyls in a mixture.

However, there are two main shortcomings when performing the 2D MS/MS experiment by this method and both stem from the decreased frequency dispersion at lower q-values (higher m/z). The first shortcoming relates to the decreased product ion mass resolution in the higher m/z range covered, which is caused by decreased frequency dispersion in two ways. First, the product ion ejection broadband waveform will lose specificity in product ion selection with increasing m/z, as is the case for any ion trap method that uses ion secular frequencies for activation. This is mitigated by performing frequency scans in such a way that the frequency scan rate is varied across the mass range to make the mass scan rate constant.[20] However, applying this methodology to broadband ejection methods is not trivial. The second factor affecting resolution lies in the m/z calibration from frequency, as peaks of similar frequency widths will necessarily have decreased mass resolution as mass increases. These two effects result in wider peaks in frequency that are widened even further during the mass calibration.

The second shortcoming in the previous micropacket method using a broadband method for product ion analysis is that the mass range over which precursor ions can be detected in the 2D MS/MS scan is limited. Earlier, it was argued that there is a loss of resolution at higher product ion m/z; however, the loss in frequency dispersion also affects precursor ion measurements. The broadband waveform in the micropacket method was continuously updated during the scan to include product ions of higher mass. The highest mass product ion included in the broadband waveform in these experiments had a secular frequency not much greater (just 10 kHz) than frequencies present in the fragmentation waveform. This was done so that the product ion ejection waveform would not eject precursor ions. However, at higher precursor ion mass, the 10 kHz difference was insufficient, and precursors were ejected before fragmentation. The simple solution is to increase the separation between the two frequencies, but this results in loss of product ion mass range. A comparison between the micropacket method and this new repetitive frequency sweep is demonstrated in Figure S1. The improvement in product ion information comprehensiveness is presented in the product ion spectra extracted from this new repetitive frequency sweep (Figure S1b) and is comparable to the conventional product ion spectra (Figure S1c), whereas the extracted product ion spectra obtained by the micropacket method (Figure S1a) contains only one or two fragment ions.

CONCLUSIONS

A new method of performing a 2D MS/MS scan using a single mass analyzer is demonstrated. This method directly provides product ion m/z values from the ejection time during a product ion sweep. This avoids the frequency/time trade-off typical of the Fourier analysis inherent in the micropacket method of 2D MS/MS. The new method is contextualized using chemical analytes including mixtures of drugs of abuse and peptides. These examples outline the method's capability to obtain large amounts of MS/MS in very short analysis times (typically 1 s per scan). Comparisons to existing MS/MS scan modes are made by reducing the dimensionality of the 2D data after mass analysis to compare the data obtained by conventional methods. This data treatment is favorable to the conventional product ion scan as 100% of the data collected in that scan is shown, whereas less than 1% of the data acquired in the 2D MS/MS scans is included (since all other precursor ions are being analyzed simultaneously in this experiment). The difference between reducing dimensionality after analysis versus reducing the dimensionality before should not be underestimated, especially in the context of in situ analysis of complex mixtures of unknowns. These capabilities are expected to be most valuable in resource constrained environments. This new 2D MS/MS method provides combined detection/identification of the constituents of a complex mixture in a mass analyzer which has the potential to be miniaturized.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01719.

Additional experimental results as shown in Figures S1−S4 (PDF)

AUTHOR INFORMATION

Corresponding Author

R. Graham Cooks − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0002-9581-9603; Email: cooks@purdue.edu

Authors

Lucas J. Szalwinski − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0002-1145-0694

Dylan T. Holden − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States*

Nicolás M. Morato − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0003-0218-407X

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c01719

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge funding from NSF 1905087 and FLIR Systems, Inc. Dalton Snyder is acknowledged for his contribution to the concepts underlying this work and Greg Eakins and Mark Carlsen (Jonathan Amy Facility for Chemical Instrumentation at Purdue University) are acknowledged for important modifications to the LTQ instrument.

REFERENCES

(1) Dowling, S.; McBride, E. M.; McKenna, J.; Glaros, T.; Manicke, N. E. *Forensic Chemistry* **2020**, *17*, 100206.

(2) Morato, N. M.; Pirro, V.; Fedick, P. W.; Cooks, R. G. *Anal. Chem.* **2019**, *91* (11), 7450−7457.

(3) Kerpel dos Santos, M.; Gleco, E.; Davidson, J. T.; Jackson, G. P.; Pereira Limberger, R.; Arroyo, L. E. *Forensic Chemistry* **2018**, *8*, 134−145.

(4) Silva, L. C. da; Pereira, I.; Carvalho, T. C. de; Allochio Filho, J. F.; Romão, W.; Gontijo Vaz, B. *Anal. Methods* **2019**, *11* (8), 999−1013.

(5) Ma, X.; Ouyang, Z. *TrAC, Trends Anal. Chem.* **2016**, *85*, 10−19.

(6) Wickramasekara, S.; Kaushal, R.; Li, H.; Patwardhan, D. *Anal. Bioanal. Chem.* **2019**, *411* (12), 2707−2714.
(7) Fedick, P. W.; Pu, F.; Morato, N. M.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2020**, *31* (3), 735−741.
(8) Wilkinson, S. D.; Martin, S.; Orton, A. L.; Markandu, R.; Jones, B. C. *Rapid Commun. Mass Spectrom.* **2020**, *34* (10), No. e8735.
(9) Smith, D. F.; Podgorski, D. C.; Rodgers, R. P.; Blakney, G. T.; Hendrickson, C. L. *Anal. Chem.* **2018**, *90* (3), 2041−2047.
(10) Pfändler, P.; Bodenhausen, G.; Rapin, J.; Houriet, R.; Gäumann, T. *Chem. Phys. Lett.* **1987**, *138* (2), 195−200.
(11) van Agthoven, M. A.; Delsuc, M.-A.; Rolando, C. *Int. J. Mass Spectrom.* **2011**, *306* (2), 196−203.
(12) van Agthoven, M. A.; Kilgour, D. P. A.; Lynch, A. M.; Barrow, M. P.; Morgan, T. E.; Wootton, C. A.; Chiron, L.; Delsuc, M.-A.; O'Connor, P. B. *J. Am. Soc. Mass Spectrom.* **2019**, *30* (12), 2594−2607.
(13) van Agthoven, M. A.; Chiron, L.; Coutouly, M.-A.; Sehgal, A. A.; Pelupessy, P.; Delsuc, M.-A.; Rolando, C. *Int. J. Mass Spectrom.* **2014**, *370*, 114−124.
(14) Snyder, D. T.; Szalwinski, L. J.; St. John, Z.; Cooks, R. G. *Anal. Chem.* **2019**, *91* (21), 13752−13762.
(15) Snyder, D. T.; Demond, P. S.; Szalwinski, L. J.; Dhummakupt, E. S.; McBride, E. M.; Cooks, R. G.; Glaros, T.; Mach, P. M. *Int. J. Mass Spectrom.* **2019**, *444*, 116171.
(16) Chiu, C. K. C.; Lam, Y. P. Y.; Wootton, C. A.; Barrow, M. P.; Sadler, P. J.; O'Connor, P. B. *J. Am. Soc. Mass Spectrom.* **2020**, *31* (3), 594−601.
(17) Floris, F.; van Agthoven, M. A.; Chiron, L.; Wootton, C. A.; Lam, P. Y. Y.; Barrow, M. P.; Delsuc, M.-A.; O'Connor, P. B. *J. Am. Soc. Mass Spectrom.* **2018**, *29* (1), 207−210.
(18) Tang, Y.; Xu, Q.; Li, D.; Xu, W. *Analyst* **2020**, *145* (11), 3892−3898.
(19) Li, A.; Hansen, B. J.; Powell, A. T.; Hawkins, A. R.; Austin, D. E. *Rapid Commun. Mass Spectrom.* **2014**, *28* (12), 1338−1344.
(20) Snyder, D. T.; Pulliam, C. J.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2016**, *30* (22), 2369−2378.
(21) Julian, R. K.; Reiser, H.-P.; Graham Cooks, R. *Int. J. Mass Spectrom. Ion Processes* **1993**, *123* (2), 85−96.
(22) Remes, P. M.; Syka, J. E. P.; Kovtoun, V. V.; Schwartz, J. C. *Int. J. Mass Spectrom.* **2014**, *370*, 44−57.
(23) Hager, J. W. *J. Am. Soc. Mass Spectrom.* **2009**, *20* (3), 443−450.
(24) Snyder, D. T.; Pulliam, C. J.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2016**, *30* (10), 1190−1196.
(25) March, R. E.; Todd, J. F. J. Theory of Quadrupole Instruments. In *Quadrupole Ion Trap Mass Spectrometry*; John Wiley & Sons, Inc.: Hoboken, NJ, 2005; pp 34−72.
(26) Dziekonski, E. T.; Johnson, J. T.; Lee, K. W.; McLuckey, S. A. *Anal. Chem.* **2017**, *89* (20), 10965−10972.
(27) Snyder, D. T.; Cooks, R. G. *Anal. Chem.* **2017**, *89* (15), 8148−8155.
(28) Snyder, D. T.; Pulliam, C. J.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2016**, *30* (7), 800−804.
(29) Snyder, D. T.; Szalwinski, L. J.; Hilger, R.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2018**, *29*, 1355−1364.
(30) Kontostathi, G.; Makridakis, M.; Bitsika, V.; Tsolakos, N.; Vlahou, A.; Zoidakis, J. Development and Validation of Multiple Reaction Monitoring (MRM) Assays for Clinical Applications. In *Proteomics for Biomarker Discovery: Methods and Protocols*; Brun, V., Couté, Y., Eds.; Springer New York: New York, 2019; pp 205−223.
(31) Xu, Z.; Jiang, T.; Xu, Q.; Zhai, Y.; Li, D.; Xu, W. *Anal. Chem.* **2019**, *91* (21), 13838−13846.
(32) Allen, F.; Greiner, R.; Wishart, D. *Metabolomics* **2015**, *11* (1), 98−110.
(33) Gillet, L. C.; Navarro, P.; Tate, S.; Röst, H.; Selevsek, N.; Reiter, L.; Bonner, R.; Aebersold, R. *Mol. Cell. Proteomics* **2012**, *11* (6), 016717.

https://dx.doi.org/10.1021/acs.analchem.0c01719
Anal. Chem. 2020, 92, 10016−10023