Untargeted data independent acquisition analysis of drugs of abuse by LC-MS/MS, a generic approach for routine clinical pathology screening

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1: Introduction

Toxicological screening by high resolution accurate mass LC-MS/MS methods can benefit from higher confidence compared to nominal mass triple quadrupole methods however targeted MS/MS methods still require scheduled acquisition and can be limited to just the compounds selected for analysis. In this work a generic data independent acquisition (DIA) method was developed to detect and quantify a panel of drugs of abuse in human plasma using a high resolution accurate mass LC-MS/MS QTOF system. The method was designed as a generic data acquisition approach to extend the capability of LC-MS/MS analysis in routine clinical pathology for both targeted and untargeted data analysis.

2: Methods

Human plasma (BioIVT) was prepared using a QuEChERS protocol modified for clinical applications (Dulaurent *et al*. 2016) and spiked with a drugs of abuse panel creating calibration curves from 5 to 200ng/mL. Samples were separated using a biphenyl column (100 x 2.1mm 2.7um) binary gradient method over 10.5 minutes and analyzed by an LC-MS/MS Q-TOF (LCMS-9030, Shimadzu Corporation). All data was acquired using external mass calibration. MS acquisition scan range was m/z 100-500. Nineteen consecutive MS/MS scans were created isolating 20 Da ion width starting from m/z 120-140 through to 480-500; measurement mass range m/z 40-500, each MS/MS scan lasting 25 msec. Collision energy scanning (0-30V) enabled detection of precursor and product ions in MS/MS scans.

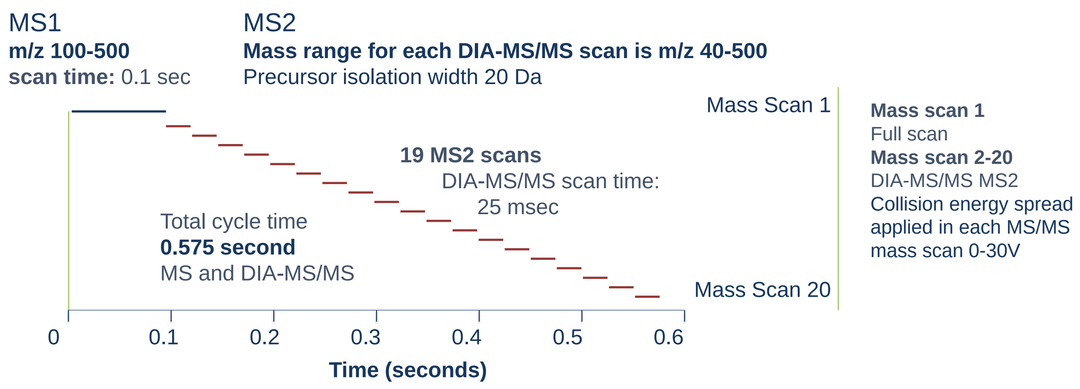


Figure 1. The untargeted DIA method was used to screen the drugs of abuse sample using the LCMS-9030 QTOF system. The LC-MS/MS method included full scan MS followed by sequential MS/MS scans.

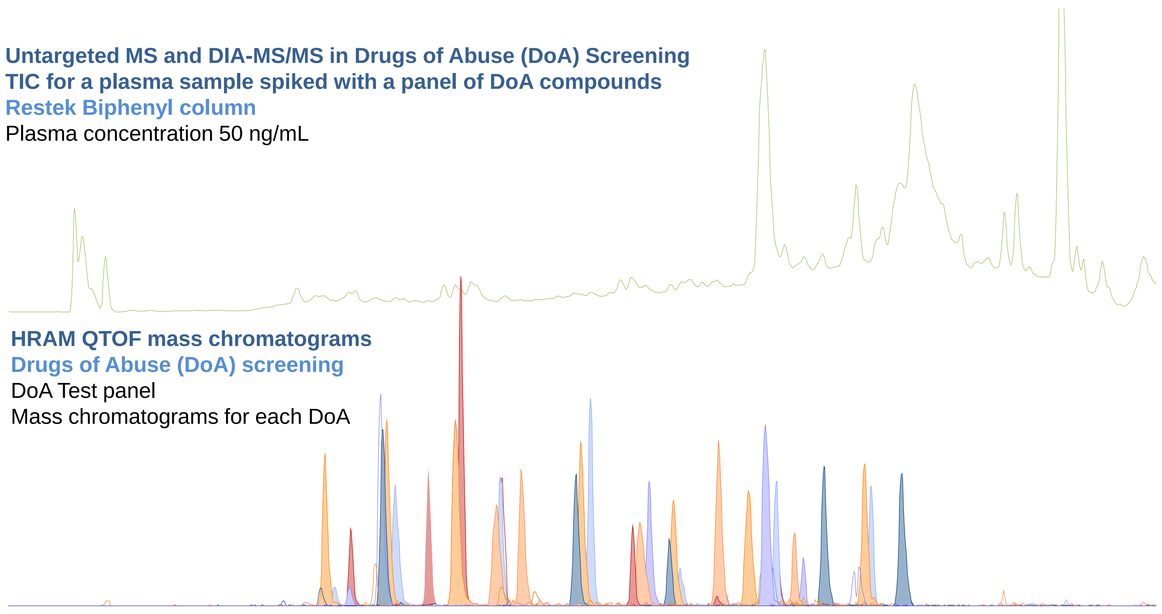
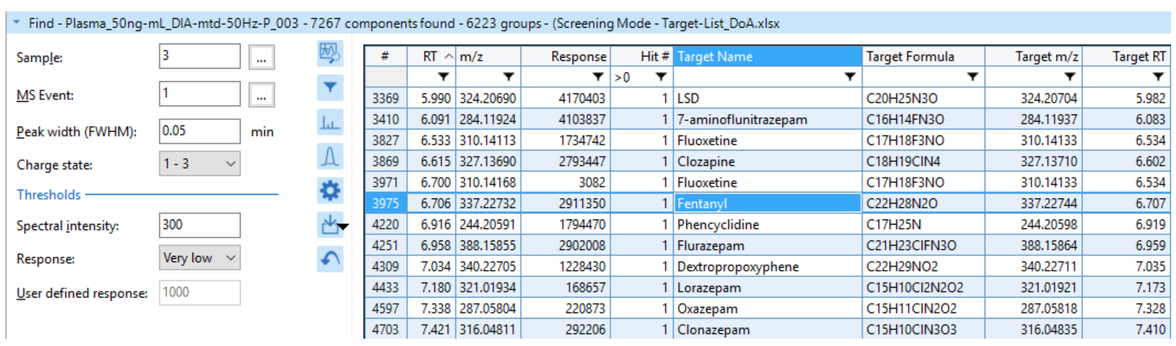


Figure 2. TIC and extracted mass chromatograms for a panel of drugs of abuse targets spiked at 50 ng/mL into a human plasma extract. To find a list of DoA targets, a component detection algorithm was used to deconvolute the data file using default search parameters.

3: Results

3-1. Component detection | FIND

Component detection was performed using an algorithm that was developed to locate covariant ions using a two-step process, 'search' followed by 'validation'. In the first step, a set of processed mass spectral data is created; masses from each scan are then correlated with equivalent masses from adjacent mass spectral scans to link data across the chromatographic space from which a peak identification method is applied. The second step in the algorithm further validates the results of the search process to accurately locate chromatographic components. Following component detection the screening workflow proceeds as outlined below.



#	RT	m/z	Response	Hit #	Target Name	Target Formula	Target m/z	Target RT
				>0				
3369	5.990	324.20690	4170403	1	LSD	C20H25N3O	324.20704	5.982
3410	6.091	284.11924	4103837	1	7-aminoflunitrazepam	C16H14FN3O	284.11937	6.083
3827	6.533	310.14113	1734742	1	Fluoxetine	C17H18F3NO	310.14133	6.534
3869	6.615	327.13690	2793447	1	Clozapine	C18H19ClN4	327.13710	6.602
3971	6.700	310.14168	3082	1	Fluoxetine	C17H18F3NO	310.14133	6.534
3975	6.706	337.22732	2911350	1	Fentanyl	C22H28N2O	337.22744	6.707
4220	6.916	244.20591	1794470	1	Phencyclidine	C17H25N	244.20598	6.919
4251	6.958	388.15855	2902008	1	Flurazepam	C21H23ClFN3O	388.15864	6.959
4309	7.034	340.22705	1228430	1	Dextropropoxyphene	C22H29NO2	340.22711	7.035
4433	7.180	321.01934	168657	1	Lorazepam	C15H10Cl2N2O2	321.01921	7.173
4597	7.338	287.05804	220873	1	Oxazepam	C15H11ClN2O2	287.05818	7.328
4703	7.421	316.04811	292206	1	Clonazepam	C15H10ClN3O3	316.04835	7.410

Data workflow

Acquire data using a MS and DIA-MS/MS method (as in Figure 1; changes applied to the mass range)
MS 100-500 Da (0.1 sec; mass range m/z 100-500) followed by 19 DIA-MS/MS sequential mass scans (each DIA-MS/MS mass scan 25 msec; MS/MS mass range m/z 40-500; precursor ion isolation 20Da);

Open the component detection research application
Select default intensity threshold and chromatographic peak width
Run the component detection algorithm for the 50 ng/mL spiked plasma sample
The plasma sample was prepared as a protein crash (using acetonitrile) with QuEChERS clean-up. The component detection algorithm found 6223 grouped compounds.

Apply a target list of DoA; the forensic toxicology screening library of 1278 compounds was filtered to search for classes of drugs of abuse (the library was filtered to 153 targets)
Open an Excel spreadsheet with a target list of drugs of abuse (the search criteria include name, formula, accurate mass and if available the retention time).
Using a filtered library of drugs of abuse compounds all targets were matched by accurate mass (within 5 ppm; below m/z 200 the mass tolerance was set to 1 mDa) and retention time (within 0.2 minute of the target list entries in the spreadsheet data base).

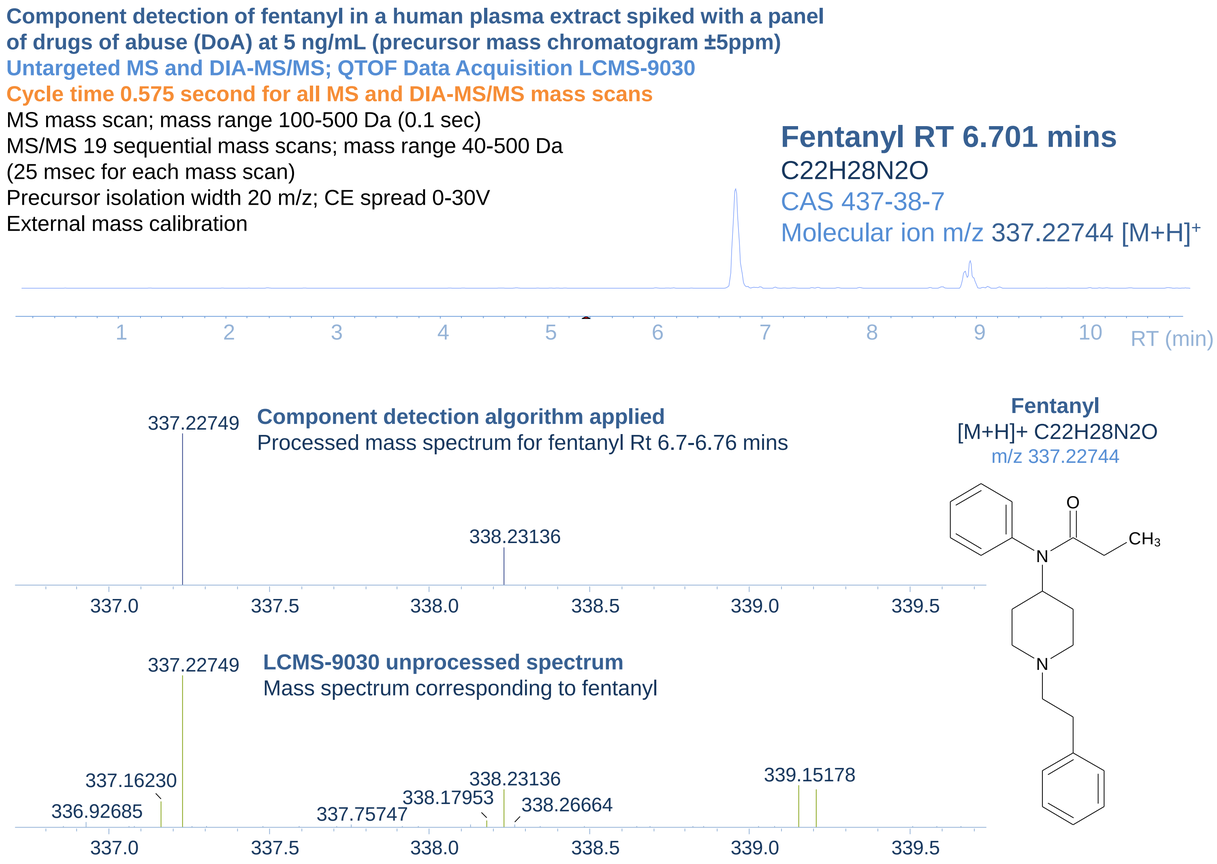


Figure 3. Mass spectrum for fentanyl processed by the component detection algorithm and the corresponding unprocessed mass spectrum. The component detection algorithm is designed to report a simplified "single component" output when multiple ionized species are present and the most accurate interpretation of isotopic distribution for each component.

3-2. Accurate mass assignment of MS/MS data | Assign

Using a research application software, a novel algorithm was applied to MS/MS data to predict and assign fragment ion formulae, structures and accurate masses. The algorithm functions by identifying groups of atoms that could be involved in a fragmentation mechanism, then uses the fragment ion data to confirm each putative mechanism. The process considers tautomeric shifts within the ionized precursor molecule; each charged tautomer is then analyzed for the presence of groups of atoms that could be involved in a putative fragmentation mechanism. Next the rearrangement of electrons is considered leading to homolytic or heterolytic cleavage or the creation of new bonds that did not originally exist. Product ion spectra were annotated (Fig. 4) and used to build quantitative accurate mass methods.

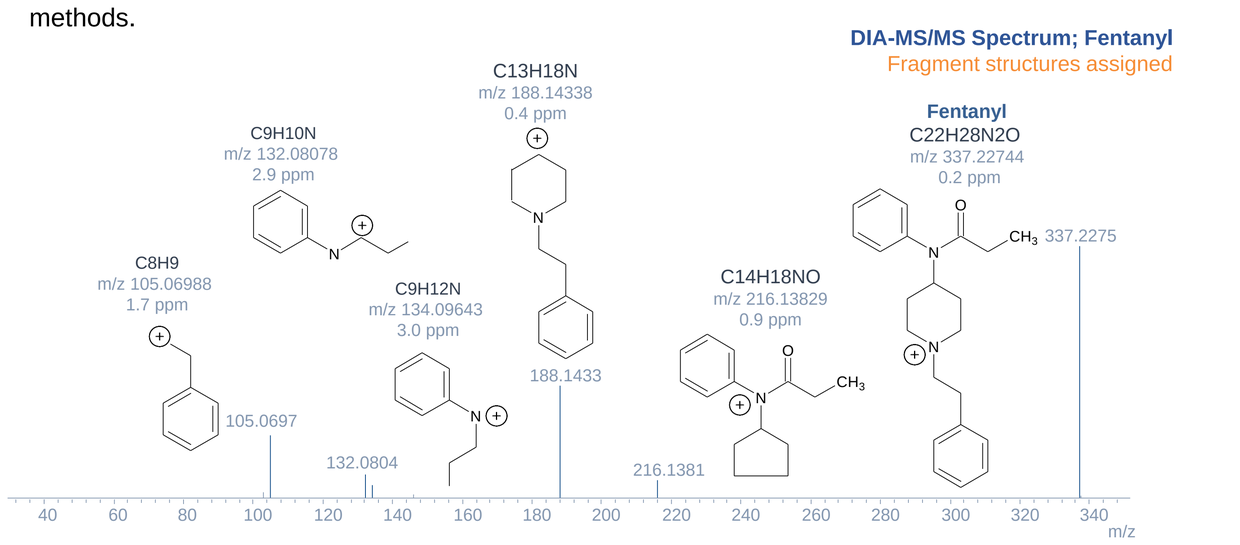


Figure 4. Assignment of DIA-MS/MS mass spectra for fentanyl in a human plasma extract using a collision energy spread of 0-30V.

3-3. Quantitation by accurate mass

The DIA-MS/MS method developed on a Q-TOF platform was designed to capture precursor and product ion spectra for all ionizing analytes providing options for targeted quantitation at either the MS or MS/MS level and retrospective untargeted analysis. This approach differs from a conventional targeted approach such as high resolution MRM which is designed to be highly specific for a panel of analytes. In this work, 41 drugs of abuse compounds were detected and quantified at the lowest calibration standard (5ng/mL) to an accuracy of 85-115% with linear and quadratic calibration curves up to 200 ng/mL for all targets using precursor DIA-MS/MS data. As a generic method this DIA-MS/MS acquisition method requires no other modification for both untargeted screening and quantitative analysis (Fig, 5).

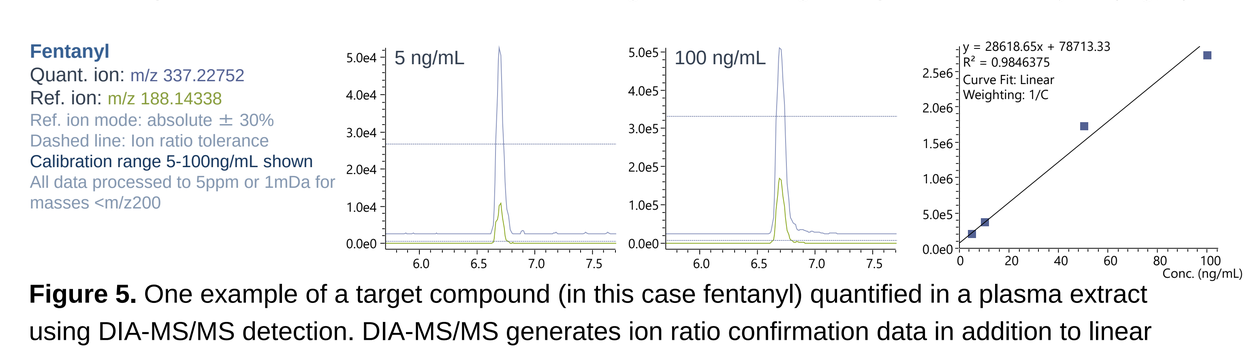


Figure 5. One example of a target compound (in this case fentanyl) quantified in a plasma extract using DIA-MS/MS detection. DIA-MS/MS generates ion ratio confirmation data in addition to linear or quadratic calibration data.

4: Conclusions

- Component detection successfully identifies compounds even at low concentrations.
- MS/MS fragment ion assignment can be used to calculate the accurate mass of fragment ions.
- Quantitative workflows are possible from untargeted generic methods such as DIA MS/MS.

5: References

Dulaurent, S., El Balkhi, S., Poncelet, L., *et al*. QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood **(2016)** *Anal Bioanal Chem*. 408(5):1467-74.

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