Mass Defect Filtering: a New Tool to Expedite Screening, Dereplication and Identification of Natural Products

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Introduction

Conventional screening for bioactive natural products in drug discovery involves bioassay-directed isolation and purification, which is time-consuming and expensive, and requires large amounts of crude plant or microorganism extracts. The use of LC/MS has played a valuable role in the screening of natural products as part of drug development programs over the past decade, facilitating rapid confirmation of known compounds, dereplication of known active compounds, rapid discovery of new compounds, etc. Our group successfully applied LC/MS in natural product screening for acetogenins using atmospheric pressure in-source collision-induced dissociated (CID) techniques over a decade ago. Recently, the technique of Mass Defect Filtering (MDF) has revolutionized the drug impurity and metabolite identification by LC-MS, and thus is being widely used in drug development. This new technology has been demonstrated applicable to natural product screening since analogue natural products also have similar mass defects. The objective of this study was to apply the MDF technology in combination with high resolution LC/MS and MS/MS in natural product chemistry to expedite the screening, dereplication and identification of natural products.

Experimental

Liquid chromatography:

Pump:	Shimadzu LC-20AT Pumps
Mobile Phases:	A: 10 mM ammonium acetate pH 5.6
	B: MeOH

Mass Spectrometry:

MS Systems:	Thermo LTQ Orbitrap XL
Ion spray (IS):	4.5 kV
Capillary temp:	350 °C
Sheath gas:	60
Auxiliary gas:	40

Group	Compound Name	Formula	MW (Da)	Mass defect (mDa)	ΔMW from average	ΔMD from average
Flavone	Luteolin	$C_{15}H_{10}O_6$	286	47.7	-9	-14.5
	Apigenin	$C_{15}H_{10}O_5$	270	52.8	-25	-9.4
Flavonol	Kaempferol	$C_{15}H_{10}O_6$	286	47.7	-9	-14.5
	Quercetin	$C_{15}H_{10}O_7$	302	42.7	7	-19.5
	Myricetin	$C_{15}H_{10}O_8$	318	37.6	23	-24.6
	Isorhamnetin	$C_{16}H_{12}O_7$	316	58.3	21	-3.9
	Pachypodol	$C_{17}H_{14}O_7$	344	89.6	49	27.4
	Rhamnazin	$C_{17}H_{14}O_7$	330	74.0	35	11.8
Flavanone	Hesperetin	$C_{16}H_{14}O_6$	302	79.0	7	16.8
	Naringenin	$C_{15}H_{12}O_5$	272	68.5	-23	6.3
	Eriodictyol	$C_{15}H_{12}O_6$	288	63.4	-7	1.2
	Homoeriodictyol	$C_{16}H_{14}O_6$	302	79.0	7	16.8
Flavanonol	Taxifolin	$C_{15}H_{12}O_7$	304	58.3	9	-3.9
Isoflavone	Genistein	$C_{15}H_{10}O_5$	270	52.8	-25	-9.4
	Daidzein	$C_{15}H_{10}O_4$	254	57.9	-41	-4.3
	Glycitein	$C_{16}H_{12}O_5$	284	68.5	-11	6.3
Flavan	Catechin	$C_{15}H_{14}O_6$	290	79.0	-5	16.8
		Average	**295**	**62.2**		

Table 1. Mass and mass defect values for common flavanoids.

Results and Discussion

1. Principles of MDF in Screening Natural Products

Many natural products exist as isomers or analogues, and the mass defects of the same series of natural products are similar, i.e. the different flavonoids as shown in Table 1.

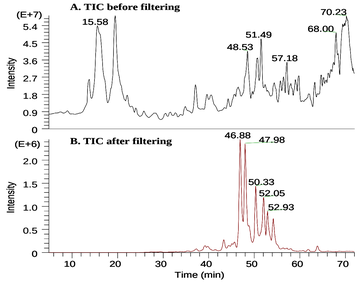

Figure 1. Total ion chromatograms of the full scan data comparing the unfiltered chromatogram (A) to the mass defect filtered chromatogram (B).

With a mass defect window set approximately ±?0 mDa around the mass defect of an applied filter template over a mass range of ±?0 Da around the mass of the filter template, the majority of interference ions falling outside the specified range will be automatically removed, and the resultant simplified data could facilitate the screening of natural products.

Figure 1 illustrates the effectiveness of the MDF (MetWorks 1.2) using Peimine as a filter template for detecting alkaloids in the crude extract of *Fritillaria thunbergii*. As shown in Figure 1A, a very large number of ions were widely spread from 10 to 70 min. The ion signals of the alkaloid analogues were buried in the TIC before MDF processing. However, when an MDF was applied to the whole LC-MS data set, the resultant TIC was a remarkable contrast (Figure 1B), in which the alkaloid analogues were easily detected in the much cleaner and simplified ion chromatogram.

The protonated ions at *m/z* 444, *m/z* 446, *m/z* 460, and *m/z* 462 were obscured by many matrix or surrounding interference ions in the unprocessed full-scan MS data (Figure 2A). Figure 2B depicts the same mass spectrum processed with mass defect filtering. After MDF processing, the majority of the interference ions were removed and the protonated ions at *m/z* 444, *m/z* 446, *m/z* 460, and *m/z* 462 became predominant in the mass spectrum.

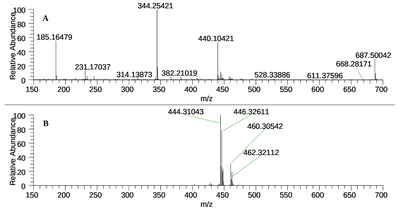

Figure 2. Mass spectra of minor alkaloids with *m/z* 444, *m/z* 446, *m/z* 460, and *m/z* 462 in the extract of *Fritillaria thunbergii* obtained by LC/MS without (**A**) and with (**B**) MDF processing.

2. Strategy of Selecting MDF Template

Natural source organisms typically contain many novel and structurally diverse chemical types.

To screen different types of compounds, various MDF templates should be designed.

For a series of natural analogues, the compound with mass defect and nominal mass values close to the respective mean mass defect and nominal mass values should be selected.

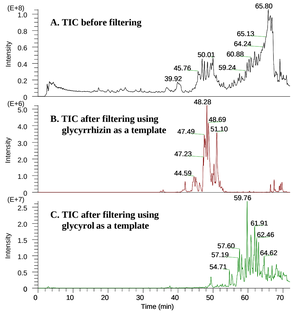

Figure 3. Total ion chromatograms of the full scan data comparing the unfiltered chromatogram (**A**) to the mass defect filtered chromatograms using glycyrrhizin as a template (**B**) and glycyrol as a template (**C**).

3. Application in Natural Product Screening and Dereplication

Figure 3 shows the effectiveness of MDF for detecting disaccharide conjugates in the crude extract of *Radix Glycyrrhizae*. The unprocessed base peak chromatogram showed no distinct peaks (Figure 3A). After MDF processing, the disaccharide conjugates were easily detected in the ion chromatogram from ca. 40 to 55 min region (Figure 3B); and the coumarins or flavonoids were easily detected in the ion chromatogram after ca. 55 min region (Figure 3C).

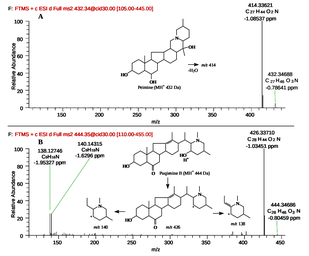

Figure 4. Product ion spectra of (**A**) *m/z* 432 ($C_{27}H_{46}O_3N$) and (**B**) *m/z* 444 ($C_{28}H_{46}O_3N$) from *Fritillaria thunbergii*.

4. Rapid Partial Identification of Natural Products

As shown in Figure 4, an alkaloid with a protonated formula of $C_{28}H_{46}O_3N$ (*m/z* 444) can be easily identified as a veratraman-type alkaloid based on the comparison of its product ion spectrum with that of Peimine (*m/z* 432), acquired by data dependent FT-MS^2 scan.

For the compound with *m/z* 444, two fragment ions at *m/z* 138 and *m/z* 140 were observed, consistent with typical dissociation from veratraman-type alkaloids. The fragmentation pathway of Puqienine B is shown in Figure 4, but this compound is not necessarily Puqienine B solely based on the LC/MS/MS data.

Conclusion

MDF has been demonstrated to be a powerful tool in the natural product screening, dereplication and rapid identification. Different type of natural products can be screened with the post-acquisition MDF processing using various templates. The known compounds can be easily dereplicated and the new compounds can be easily identified or partially identified. The MDF processing may be also useful in Traditional Chinese Medicine fingerprint and processing.