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Nota de aplicación

Accurate Compound Identification from Complex Natural Product Samples Using SONAR

Giorgis Isaac, Jimmy Yuk, Lee A. Gethings, Robert S. Plumb

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the power of SONAR for confident and increased accurate compound identification of multiple co-eluting compounds from complex natural product samples such as traditional medicine.

Benefits

SONAR is a data independent acquisition mode that uses the quadrupole as a mass filter, which is continuously scanning to generate MS/MS data.

Introduction

Yu Ping Feng San (YPFS) is a classical TCM formulation which has been traditionally used for treatment of immune system related diseases.[1] It is a complex mixture of three herbs; Radix Saposhnikoviae (RS; Fang feng), Radix Astragali (RA; Huangqi), and Rhizoma Atractylodis Macrocephalae (RAM ; Baizhu).[1,2] In any non-targeted discovery experiment, the goal is to identify as many compounds as possible. However, compound identification has always been a challenging step due to the combination of multiple herbs and the presence of multiple co-eluting closely related chemical constituents in such complex mixtures. The LC-MS/MS data generated contains fragment ions from multiple precursors due to compound coelution. This makes the fragment data more complex and hence difficult to make correct compound identification. SONAR is a data independent acquisition mode that uses the quadrupole as a mass filter, which is continuously scanning to generate MS/MS data.[3,4] Here we describe the application of SONAR for improved spectral clarity and confident compound identification from complex natural product samples.

Results and Discussion

Traditional DIA approaches work well for many sample types but can struggle when samples are extremely complex. The data independent SONAR acquisition mode has been used for confident and increased accurate compound identification of co-eluting compounds from a complex three-herb mixture traditional Chinese medicine YPFS. Each of the three herbs were ground individually into powder and mixed in a ratio of 1:2:2 (RS:RA:RAM) to obtain the YPFS formula. A 45 g powder sample of YPFS formula was immersed in 360 mL cold water for 30 min and decocted by boiling for 45 min. This operation was repeated once again.

The total extracts were combined and evaporated to dryness. The decoction was concentrated and dried. The dried extract was reconstituted in methanol (10 mg/mL), centrifuged and filtered through a 0.2 µm filter. Chromatographic separation was performed on a RP ACQUITY UPLC HSS T3 Column (2.1 x 100 mm, 1.8 µm) coupled to a Xevo G2-XS QTof Mass Spectrometer operating in SONAR mode with a quadrupole window of 10 Da, scanning over a mass range of 50–2000 *m/z* with a scan rate of 0.1 sec. Data was also collected using a traditional DIA method such as MS^E for comparison purposes.

Figure 1 shows representative complex base peak ion chromatogram of YPFS extract in positive ion mode. Figures 2A and 2B show extracted ion spectra of prim-O-glucosylcimifugin acquired using a traditional DIA without resolving quadrupole and SONAR with a resolving quadrupole respectively. In complex natural product

samples there are multiple co-eluting compounds which often confound structural analysis. The presence of these co-eluting precursor ions with prim-O-glucosylcimifugin in Figures 2A and 3A provides high complexity with 97 high energy fragment ions, making compound identification very complex and challenging. Moreover the presence of such high number of fragment ions can increase false positive compound identification because there is a higher probability that these fragments could match arbitrarily to other co-eluting fragment ions. On the other hand when the data is acquired using SONAR (Figures 2B and 3B) cleaner precursor and fragment ion spectra are generated; this is because SONAR performs high energy fragmentation in a selected narrow precursor mass window to provide specific fragment ions. The precursor spectra contains only the parent ion prim-O-glucosylcimifugin $[M+H]^+$ and $[M+Na]^+$. This provides eight clean relevant fragment ions generated only from the parent ion prim-O-glucosylcimifugin which leads to correct and confident compound identification.

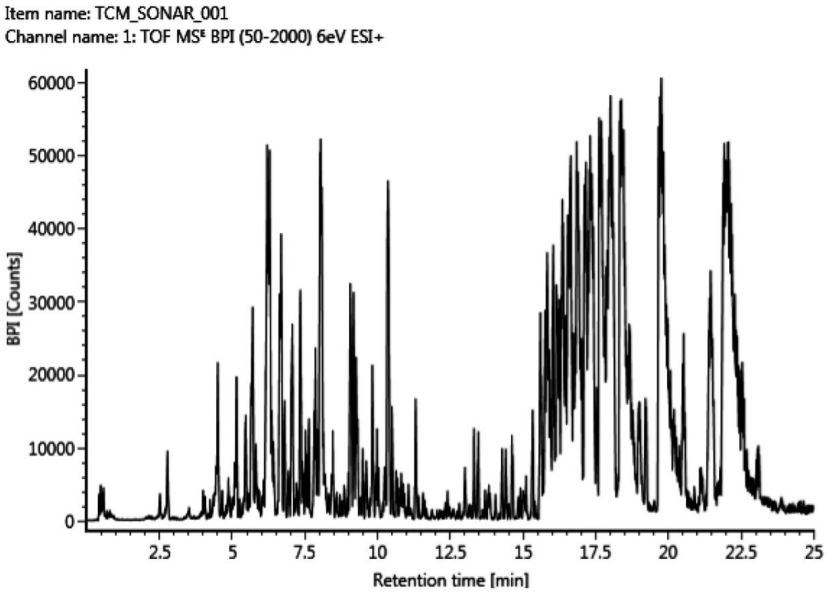

Figure 1. Representative base peak ion chromatogram in positive ion mode from a complex traditional Chinese medicine mixture, Yu Ping Feng San (YPFS) which contains three herbs.

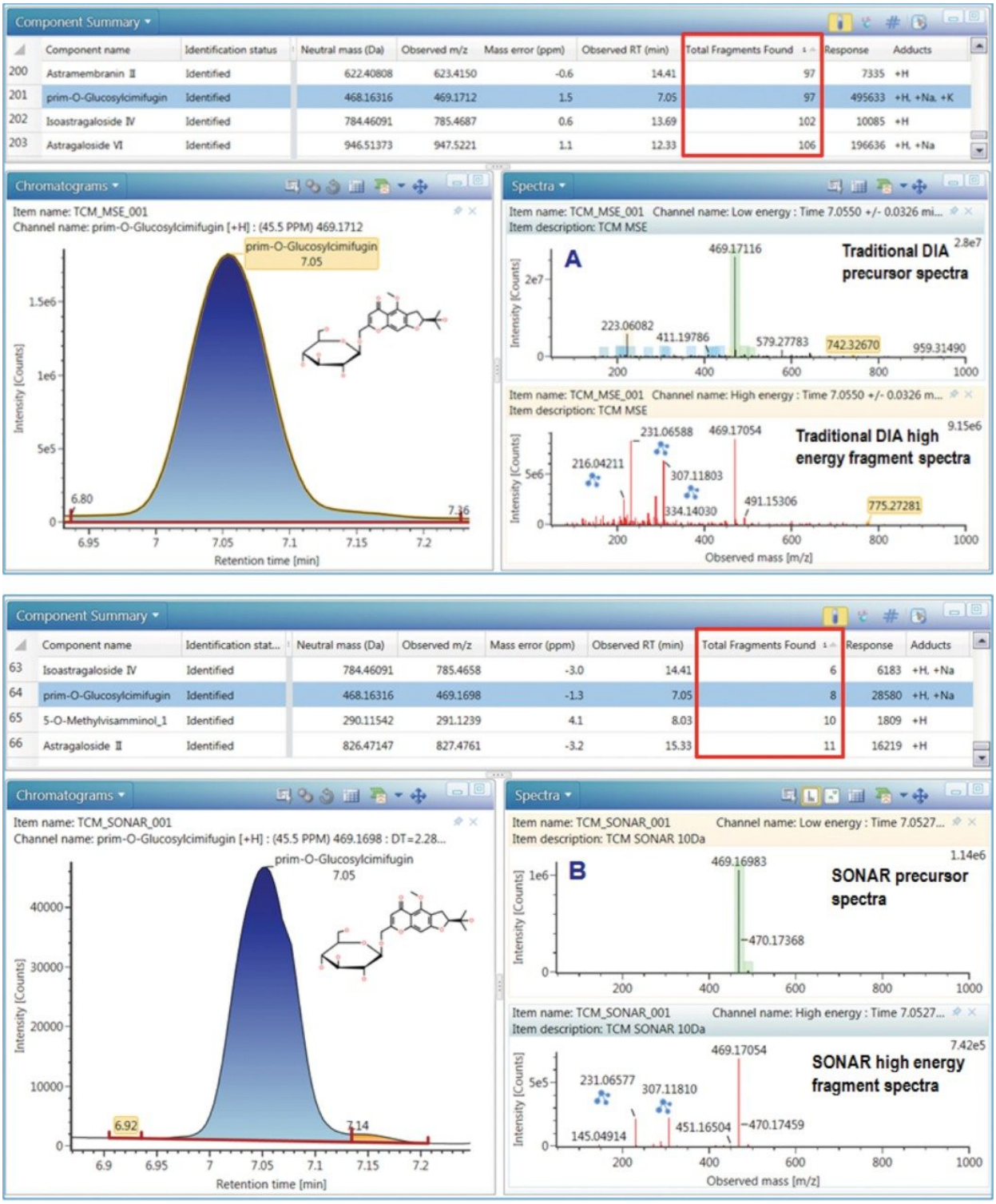

Figure 2. Extracted ion spectra of prim-O-glucosylcimifugin acquired using (A) traditional DIA without resolving

quadrupole. As shown in the red box, 97 high energy fragment ions were found. (B) SONAR with resolving quadrupole. As shown in the red box, 8 relevant high energy fragment ions were found.

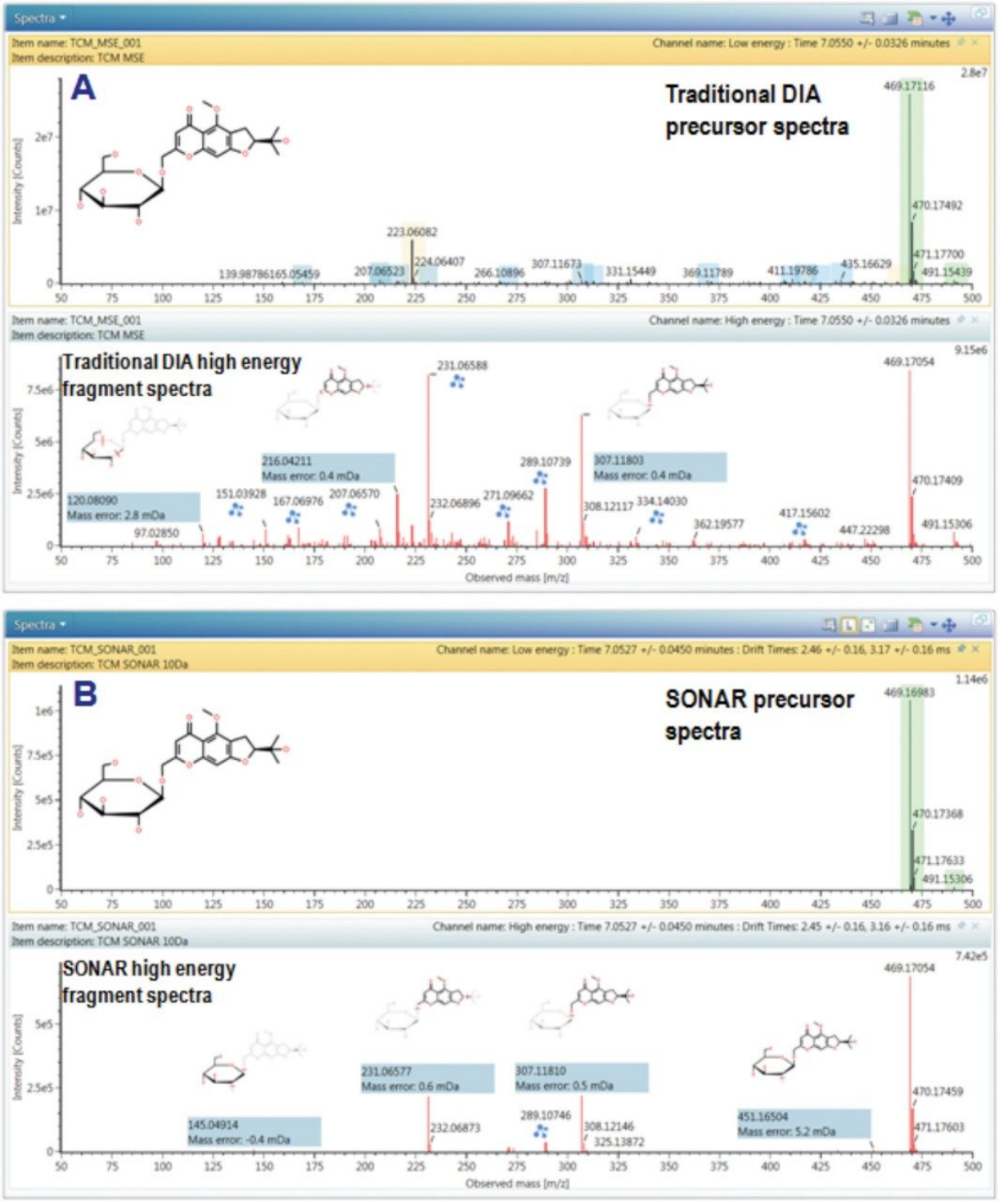

Figure 3. Zoom in extracted ion spectra of prim-O-glucosylcimifugin acquired using (A) traditional DIA without

resolving quadrupole provides complex high energy fragment ions generated from multiple co-eluting precursor ions. (B) SONAR with resolving quadrupole provides cleaner and relevant high energy fragment ions generated only from the precursor ion prim-O-glucosylcimifugin.

Conclusion

The data independent SONAR acquisition mode has been used for the confident and increased accurate compound identification of multiple co-eluting closely related chemical constituents from complex natural product samples. Compared to the traditional DIA method, the specificity of SONAR provides cleaner precursor and high energy fragment ion spectra, which results in confident compound identification.

References

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