Information Dependent Acquisition—The Next Generation of Data Dependent Experiments for LC/MS/MS Analysis

Purpose

Information Dependent Acquisition (IDA) is a powerful extension of Analyst™ Software that maximizes the information content generated in a single LC/MS/MS run. The IDA program includes specific mass and retention time (SMART) filters that allow mass, isotope, and time-based windowed exclusion lists. IDA with SMART filters can be used on all LC/MS/MS systems from Applied Biosystems/MDS SCIEX. When you use IDA with triple quadrupole systems, you can achieve unprecedented selectivity of candidate ion species with Precursor Ion and Neutral Loss Scan functions. Simple user interfaces allow rapid specification of SMART filters and Mass Inclusion lists to further maximize the information content of an LC/MS/MS run.

Overview

Data dependent experiments are widely used to maximize the structural information obtained in a single LC/MS/MS analysis. DDE involves initial acquisition of a set of survey data, processing the survey data to determine the ion(s) of interest (candidates) and the acquisition of dependent scans on the candidates. The dependent scan is typically a product ion scan and may be performed under different experimental conditions, such as varying collision energy to maximize fragmentation information.

IDA with SMART filters improves the utility of DDE by maximizing the useful information extracted from a single LC/MS/MS run. Choosing appropriate candidate ions is critical, since incorrect selection either generates irrelevant MS/MS spectra or misses important data. Single MS scans are commonly used for the survey. Masses are selected on criteria such as ion intensity, charge state, and isotope pattern. Dynamic exclusion has been introduced to ensure that chosen precursor ions will not be reconsidered as candidates until a given period has elapsed, typically the time required to elute a single chromatographic peak. The analysis of minor components is, however, still challenging because the ions of interest may never exceed the threshold. The power of IDA in Analyst software comes from the comprehensive candidate ion...
selection criteria combined with the addition of precursor and neutral loss scans as survey scans. This unique feature of MS/MS survey scans significantly increases the specificity of candidate ion selection.

IDA has been implemented within Analyst™ software using looped experiments, proven technology that can rapidly determine the mass of candidate ions, and bases dependent experiments on these candidate masses. This approach achieves unparalleled flexibility. Users can specify unique and multiple survey scans and set multiple dependent scans, such as product ion scans at different collision energies, for the candidate masses. IDA uses the familiar Analyst software interface for experiments, which minimizes the learning curve. Figure 1 shows the ion selection, exclusion, and inclusion criteria available. Ions may be excluded for the entire LC/MS run or at specific retention times. The latter allows mass spectral interferences to be ignored at specified periods in the HPLC run even though they may be isobaric with species of interest. Excluding ions from the entire run lets users ignore consistent background ions of no interest.

When an IDA file is opened in Analyst processing software, a combined total ion chromatogram (TIC) corresponding to the sum of all experiments is displayed. This combined TIC can readily be split into traces for the separate IDA experiments, or individual MS and MS/MS spectra can be directly generated by double-clicking in the combined TIC.

**Key Features**
- Advanced Data Dependent Experiments (DDE) on all Applied Biosystems/MDS SCIEX LC/MS/MS systems for intelligent information collection
- Use of Precursor Ion and Neutral Loss Scan functions to increase specificity of candidate ion selection
- Specific mass and retention time (SMART) filters to maximize information content of data
- Looped experiments in Analyst software for multiple dependent scans to accommodate variable collision energies

**Experimental Conditions**
Urine samples from subjects exposed to monoacetylmorphine (MAM) were filtered using 0.45 µm filters, diluted 1:1 (v:v) with water and injected directly. Chromatography was performed using an Agilent Technologies 1100 Series HPLC System and a Hypersil® Aquasil C-18 column (2 x 100 mm, 5 µm). Solvent A consisted of water with 0.2% formic acid and solvent B consisted of acetonitrile with 0.2% formic acid. A gradient from 5% B to 95% B over 5 minutes at 200 µL/min was performed. MS analysis results were acquired on an API 2000™ LC/MS/MS System using a TurboIonSpray® source set to 450°C.

**Results**
Figure 2 illustrates data obtained from a typical IDA analysis using full scan MS as the survey scan. This clearly demonstrates the importance of candidate ion selection. While useful dependent product ion spectra were obtained for several metabolites (“+”), some were missed because they did not exceed the threshold specified (“0”). Spectra were also obtained from large peaks that were not of interest even though they are isobaric with actual metabolites (“I”). These can be excluded using an appropriate exclusion window.

Figure 3 demonstrates the ability of neutral loss scans to reduce the overall number of candidate ion selections for IDA analysis. In this particular case, a neutral loss scan of
176 was performed to identify glucuronide metabolites. The neutral loss survey scan clearly improves the selectivity, focusing the candidate selection on ions of interest when compared to the MS survey scan of Figure 2. The added specificity of this survey scan also provides information from relatively minor peaks that other components in the single MS survey scan might have obscured. In Figure 3, the peaks labeled 1 and 2 are morphine glucuronides and the peak labeled 3 is codeine glucuronide as determined by the subsequent dependent product ion scan.

The product ion scan data of m/z 476.3 in Figure 3 shows the loss of 176 expected for a glucuronide metabolite but limited other structural information for the molecule. However, the application software allows automatic execution of collision induced dissociation (CID) in conjunction with MS/MS scans providing greater structural information. The bottom pane of Figure 3 illustrates this capability. A higher orifice voltage provides data that contains more information about the core structure of the molecule.

**Conclusions**

IDA can generate a large amount of information from single-injection LC/MS/MS analysis. The usefulness of the information depends on the success of the selection criteria in distinguishing compounds of interest from background material. The use of highly specific MS/MS survey scans, such as neutral loss and precursor ion scans, greatly reduces the irrelevant data obtained, also allowing the detection of the components of interest at much lower concentrations. Precursor ion and neutral loss scans may be used either to detect specific metabolites such as glucuronides (as illustrated here), sulfates, or other specific compound classes. The successful use of MS/MS techniques for IDA survey scans requires the analytical system to generate very high quality spectra at rapid scan speeds. The LINAC™ collision cell in the API 2000™ and API 3000™ LC/MS/MS systems makes these systems ideal for the IDA software application. The patented LINAC collision cell technology allows very fast neutral loss, precursor ion, and product ion scans without degradation of mass spectral data. This also enables increased sample throughput with fast chromatography.

Although not shown here, the IDA application can use more than one survey scan, a unique feature that
further increases information obtained from metabolite samples. SMART filters allow the user to obtain maximum coverage of the species eluting from the column. In applications where the retention times of interferences are known, windowed exclusion is extremely valuable. For example, in the case of peptide digest analysis, autolysis peaks can interfere with the detection of important digest products. The masses of known autolysis products can be ignored at predefined retention times with windowed exclusion, allowing isobaric peptides to be examined elsewhere in the chromatogram.

When the goal is to generate the maximum information with the highest degree of confidence from compounds that are in low abundance, IDA with SMART filters is the answer. IDA with SMART filters is a powerful application of looped experiments for bioanalytical samples that maximize experimental information from a single LC/MS/MS acquisition. IDA, coupled with the proven reliability of Applied Biosystems/MDS SCIEX LC/MS/MS systems with the innovative LINAC collision cell, guarantees the highest quality MS/MS spectra even at the most demanding scan rates required by fast chromatography. In brief, IDA with SMART filters delivers more information with the highest confidence.

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