PRODUCT SUPPORT BULLETIN

Data Dependent Constant Neutral Loss Scan Function for Identification of Biotransformations Using Thermo Scientific Ion Trap Mass Spectrometers

PSB 118

Determination of the metabolic fate of drugs is an essential and important part of the drug development process. *In vitro* biotransformation studies are commonly performed in the early drug discovery to determine metabolic pathways and to generate Phase I and Phase II metabolites for further characterization. In Phase I reactions, the parent compounds undergo hydrolysis, oxidation and reduction. Phase II reactions involve glucuronidation, sulfation, methylation, acetylation, glutathione and amino acid conjugation. Each of these transformations shows a characteristic mass shift with respect to the molecular ion of the parent compound as listed in Table 1.

Biotransformation	*Change in mass*
Oxidation	M+16
N-hydroxylation	M+16
Reduction	M+2
Methylation	M+14
Acetylation	M+42
Sulfation	M+80, M+96
Glucuronidation	M+176, M+192
Taurine Conjugation	M+107
Cys Conjugation	M+121, M+119
GSH Conjugation	M+307, M+305

Table 1: Mass shifts for some common biotransformations

The wide variety of potential metabolites and resulting complex mixtures make their identification a challenging and labor-intensive task. The Data Dependent™ constant neutral loss (DDCNL) scan function, available on all Thermo Scientific ion trap mass spectrometers, significantly expedites identification of these metabolites.

A flow chart of the scan events for a DDCNL experiment is shown in Figure 1. In the DDCNL experiment, an MS scan is performed, followed by an MS/MS scan of the most intense peak to look for a user-defined neutral loss. If the MS/MS scan produces a fragment with the targeted neutral loss, an MS^3 scan of the product ion is initiated. However, if the fragments in the MS/MS scan do not correspond to the targeted neutral loss, the software moves on to the next most intense peak in the MS scan and performs another MS/MS experiment. This sequence is repeated for a preset number of ions in the MS scan. The triggering of the MS^3 scan event then points to the ions that exhibit a particular structural motif. The benefit of DDCNL is that by performing MS^3 scans only on those compounds that show the desired neutral loss, the mass spectrometer can rapidly scan for low intensity ions.

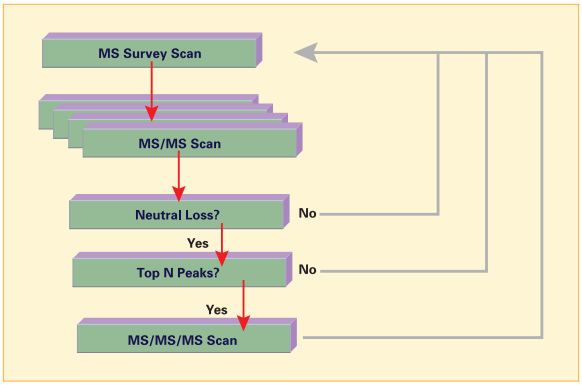

Figure 1: Flow chart for DDCNL scan events

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An application of DDCNL scanning to identify *in vitro* metabolites of glyburide is illustrated in Figure 2. This experiment was performed with a user-defined neutral loss of 125. By scanning the chromatogram in real time for a constant neutral loss of 125, the DDCNL scan function triggers an MS^3 scan for a metabolite at *m/z* 510. Thermo Scientific Metabolite ID™ and Mass Frontier™ software were subsequently used to identify the site of hydroxylation in this metabolite.

DDCNL is a technique that increases selectivity for identification of metabolic modifications. It also simplifies the analysis of complex mixtures in biological matrices, and enables characterization of low abundance species. This scan technique can be used for the analysis of biotransformations, and can also be applied to the analysis of compound transformations, such as degradation or

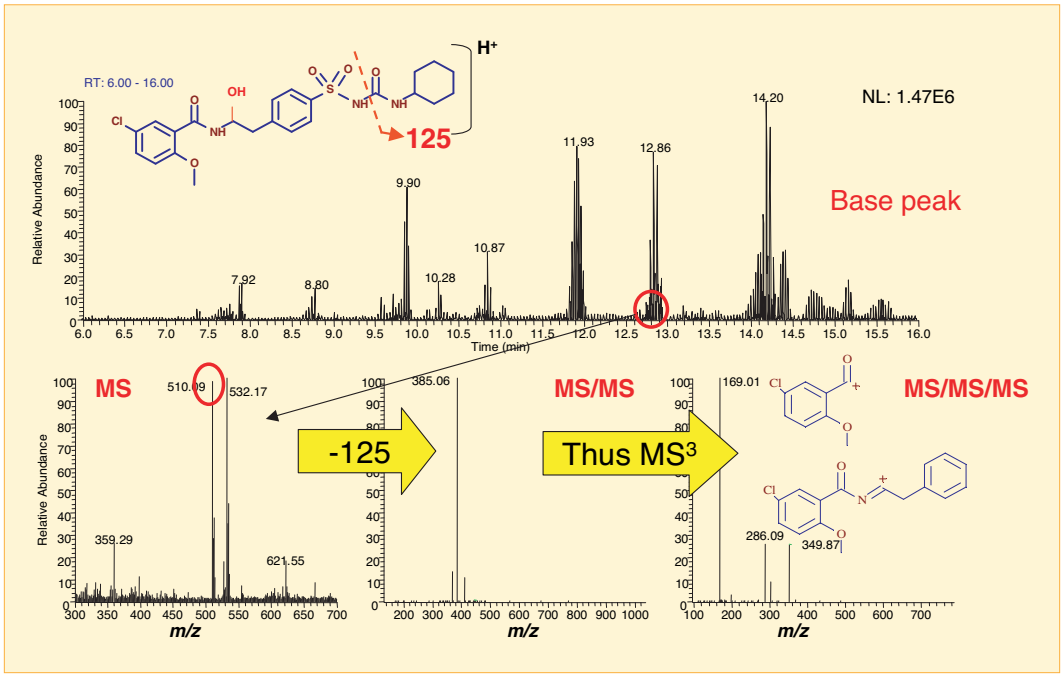

Figure 2: LC-MS/MS analysis with Data Dependent Constant Neutral Loss-triggered MS^3 for the analysis of *in vitro* metabolites of glyburide generated from incubation with rat liver microsomes.