

PSB 121

Enhanced Fragmentation of Small Molecules in a Thermo Scientific LTQ Series Linear Ion Trap Mass Spectrometer Using Stepped Normalized Collision Energy

Page 1 of 2

Ion fragmentation is a crucial tool for structural elucidation of a variety of compounds in an ion trap mass spectrometer. The standard technique for ion fragmentation is Normalized Collision Energy™ (NCE) which improves fragmentation efficiency over a wide mass range, making it possible to obtain the maximum amount of structurally relevant information. Stepped Normalized Collision Energy (SNCE) is an enhancement to NCE for MS/MS analysis on mixtures of compounds where a variation in collision energies is required. By eliminating individual optimization of collision energies, SNCE simplifies the set-up for performing automated MS/MS experiments.

The SNCE process applies several different Normalized Collision Energy “steps” to a sample during ion dissociation. A diagram of the technique is shown in Figure 1. SNCE can sometimes improve ion dissociation by compensating for any secondary structural dependency, which may affect fragmentation efficiency. Stepped Normalized Collision Energy is useful for mixtures of compounds with different structures where each compound has a unique optimum collision energy level for fragmentation.

The parameters involved in setting up the SNCE experiment include: the central Normalized Collision Energy, the ion activation time, the range of Normalized Collision Energies and the number of steps. The range of SNCE energies will be centered on the value chosen for the Normalized Collision Energy. The activation time of each step is the total activation time divided by the number of steps. For a large number of steps, the total activation time should be increased to allow enough excitation at each Normalized Collision Energy level. This will increase the cycle time of the experiment, so the number of steps should be chosen carefully. Typical SNCE settings are shown in Table 1.

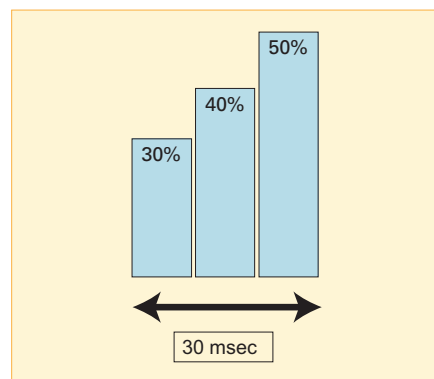


Figure 1. Diagram of Stepped Normalized Collision Energy Activation

Range	Steps	Collision Energy	Activation Time	Resulting Stepped Normalized Collision Energy
20	3	40	30 msec	First step at 30%, second step at 40%, third step at 50%, each step for 10 msec
20	2	40	30 msec	First step at 30%, second step at 50%, each step for 15 msec
20	3	40	60 msec	First step at 30%, second step at 40%, third step at 50%, each step for 20 msec
20	3	30	60 msec	First step at 20%, second step at 30%, third step at 40%, each step for 20 msec

Table 1: Typical SNCE Settings

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Page 2 of 2

The performance of the SNCE technique is demonstrated with a mixture of four compounds: neocuproine [$MH^+=209$], α -naphthylred [$MH^+=248$], ellipticene [$MH^+=247$] and triamterene [$MH^+=254$], each having two or more aromatic rings. Each of these compounds has a different energy threshold for fragmentation. Using the standard NCE technique with a setting of 40%, α -naphthylred was completely fragmented, neocuproine was not fragmented at all and ellipticene and triamterene were barely fragmented. At a Stepped Normalized Collision Energy of 40% with 3 steps and a 20% window, all of the compounds including the neocuproine were fragmented (Figure 2). Using SNCE on this mixture resulted in better fragmentation efficiency for all of the compounds.

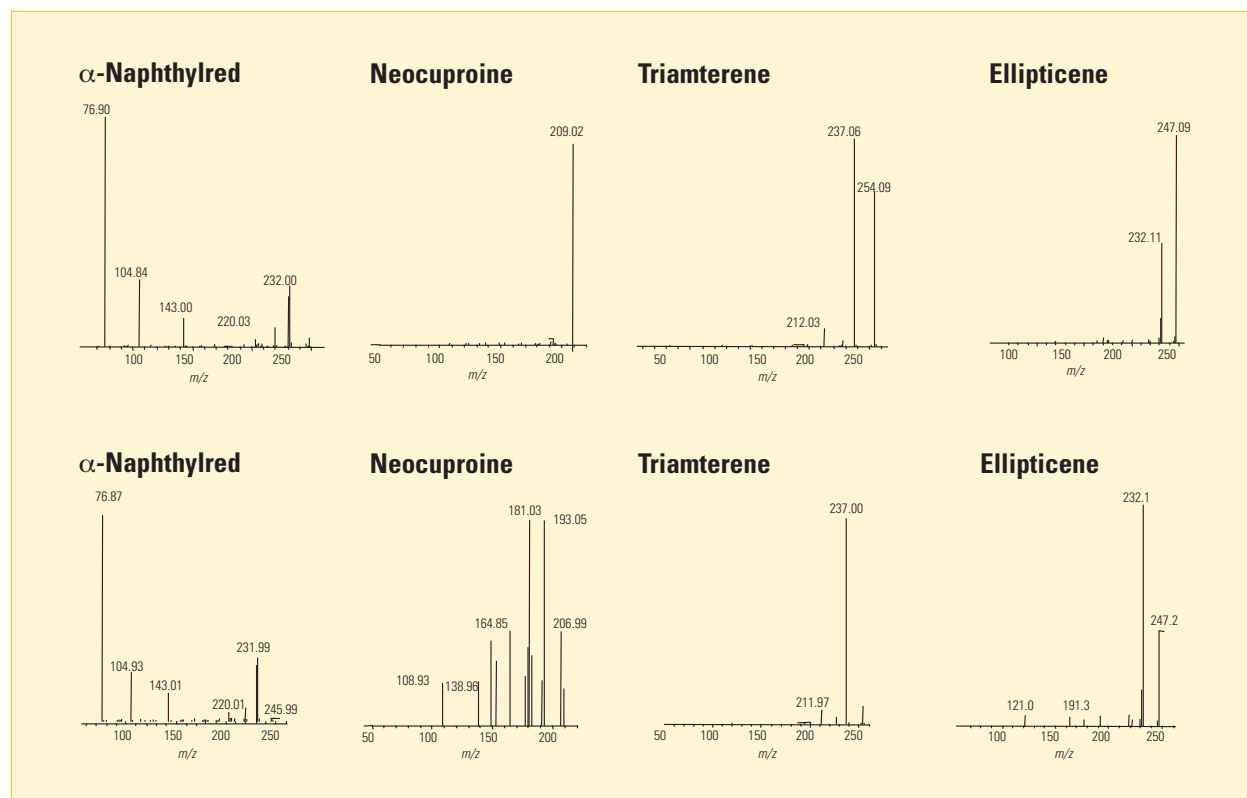


Figure 2: Fragmentation of α -naphthylred, neocuproine, ellipticene and triamterene with 40% NCE (top) and with SNCE, 40% midpoint, 20% range and three steps (bottom)