Multi-Target Screening for 300 Drugs Using a Q TRAP® LC/MS/MS System and Library Searching

Purpose

A multi-target screening (MTS) toxicological analysis for drugs in blood and urine has been developed using a hybrid linear ion trap (LIT) mass spectrometer. An intelligent alternative to common GC/MS or LC/MS techniques used for target analysis, this analysis method rapidly detects, identifies, and confirms tranquilizers (benzodiazepines), hypnotics, antidepressants, neuroleptics, cardiac drugs, and drugs of abuse (opiates, cocaine, amphetamines, cannabinoids). In a single LC/MS/MS run, multiple reaction monitoring (MRM) and enhanced product ion (EPI) scans in an Information Dependent Acquisition (IDA) experiment with mass spectral library searching provides sensitive target analyte detection and quantitation, confirmatory MS/MS spectra, and subsequent identification with a mass spectrometric database.

Overview

With 5% of the world's total population over the age of 15 using illicit drugs[1], and several hundred thousands of drug intoxications per year in the western world alone, fast screening methods for drugs and pharmaceuticals are necessary for the detection of xenobiotics in clinical and forensic intoxication cases. Screening methods usually include immunoassay tests, available only for a small number of substance classes, and subsequent chromatographic methods such as GC/MS or HPLC with UV. For many of today's polar drugs and xenobiotics, GC/MS analysis is hampered by difficult derivatization protocols. HPLC is ideally suited for these polar compounds but even with diode array detection (DAD), UV detection lacks the necessary specificity. Further, UV analysis methods require long run times to minimize the potential for co-elution.

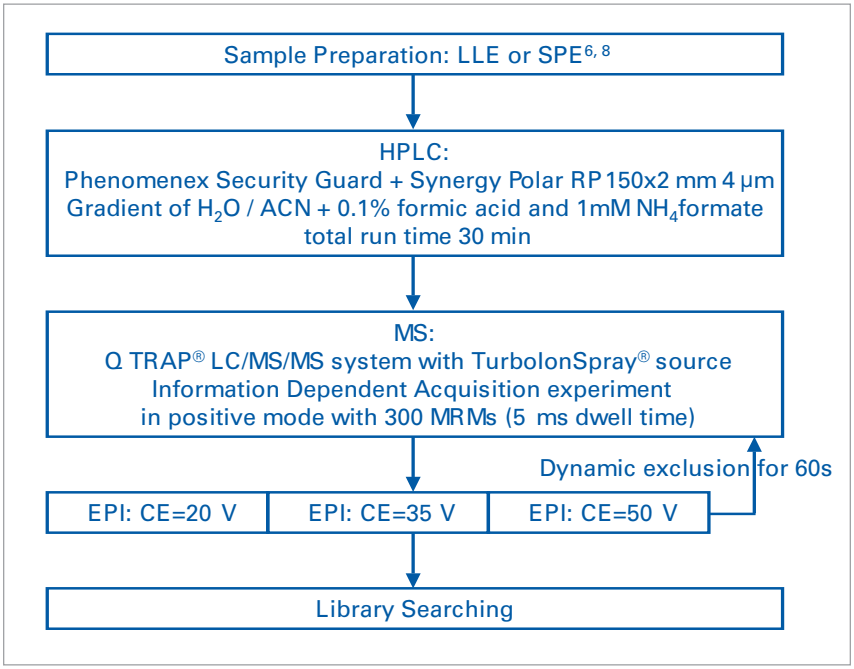

Figure 1. Workflow of multi-target screening method.

Since 1998, LC/MS screening for drugs has made progress with in-source collision-induced dissociation (CID) mass spectral libraries for general unknown screening.[2,3,4,5] LC/MS/MS target analysis has also been applied to forensic cases by Gergov et al[6], with subsequent identification of compounds by further analysis in product ion scan mode and MS/MS library searching.

The hybrid linear ion trap technology of the Q TRAP® system enables simultaneous detection of a drug and confirmation by IDA.[7] Compared to the common product ion scan mode of a triple quadrupole MS, confirmation can be achieved at significantly

higher sensitivity using the enhanced product ion (EPI) scan mode. In addition to superior sensitivity, the rapid scans of the Q TRAP® system provide excellent duty cycle.

Taking advantage of these analytical capabilities of hybrid LIT technology, a method was developed to detect, identify, characterize, and confirm 300 drugs typically targeted in forensic and clinical intoxication cases. While not demonstrated here, dividing the chromatogram into retention time windows (periods) can further increase the number of compounds that can be screened and confirmed in a single analysis.

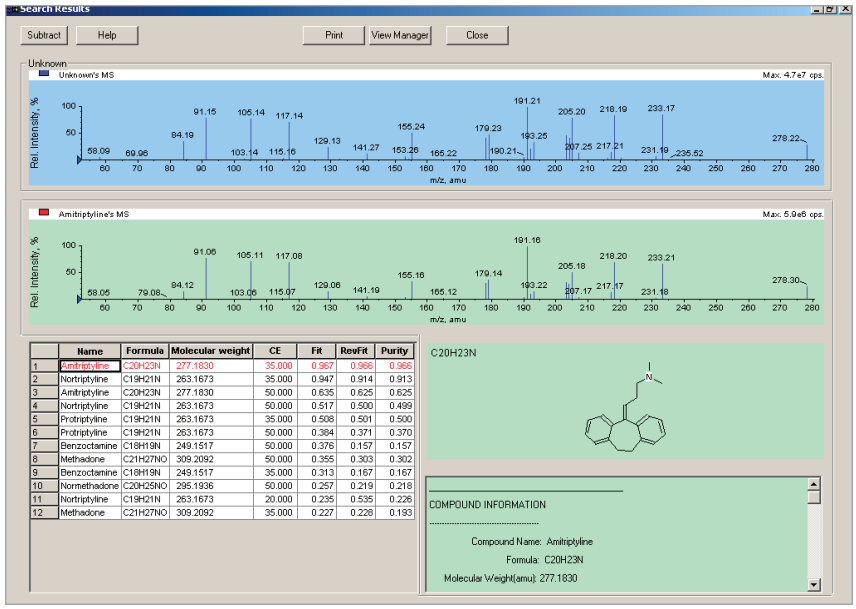

Figure 2. Amitriptyline identified by mass spectral library search (35 eV spectrum).

Key Features of Hybrid Linear Ion Trap Technology

- Exceptional triple quadrupole and ion trap sensitivity allows identification, characterization, confirmation, and quantitation of low abundance analytes with a high degree of confidence.
- Powerful workflows enable fast, efficient identification, characterization, confirmation, and quantitation—all in a single experiment.
- LINAC® collision cell permits greatly reduced dwell times without a loss in sensitivity allowing multi-target analyses.
- Broad linear dynamic range provides true triple quadrupole quantitation performance and enhances identification of ions in complex matrices.
- Powerful advanced scan modes, including neutral loss and precursor ion scans, can be used in flexible combinations to achieve unprecedented selectivity.

Experiment

Extraction was performed either by liquid liquid extraction (LLE) as described by Gergov *et al.*[8] or solid phase extraction (SPE) as described by Chen *et al.*[9] The subsequent analysis procedure is described in Figure 1. Liquid chromatography was carried out using a system equipped with a degasser, binary pump, column oven, and autosampler (Agilent 1100 Series). For the separation, an analytical column—Synergi Polar RP, 150 x 2 mm, 4 µm with Security Guard (Phenomenex)—was used. A gradient starting with 10% mobile phase B—acetonitrile 95% (v/v), 5% ammonium formate, and 1 mM formic acid 0.1% (v/v)—was increased linearly to 95% B over 20 minutes, kept constant at 95% B for 3 minutes, then decreased to 10% B in 0.1 minutes and re-equilibrated for 10 minutes. Mobile phase A consists of water, 0.1% formic acid, and 1 mM ammonium formate. The flow rate was 250 µL/min.

MS was carried out on a Q TRAP LC/MS/MS system with a TurboIonSpray® source. The chosen IDA survey scan contained 300 MRM transitions each with a 5 ms dwell time at one of the following given collision energies (CE): 20, 35, or 50 eV. In the case of a positive detection of a MRM transition, an EPI scan of the precursor ion is triggered as a dependent scan, yielding product ion mass spectra at three pre-selected collision energies with the high sensitivity of the linear ion trap. The EPI scan was carried out at the following three different CEs: 20, 35, and 50 eV. The IDA dependent scan intensity threshold was set to 500 cps. Dynamic exclusion was set to 60 seconds to allow for the detection of co-eluting and isomeric substances. Finally, the resulting EPI spectra were then searched against an existing mass spectral library for the identification of drugs present in the sample. Fit, reverse fit, and purity values are given as results in a mass spectral library search hit list.

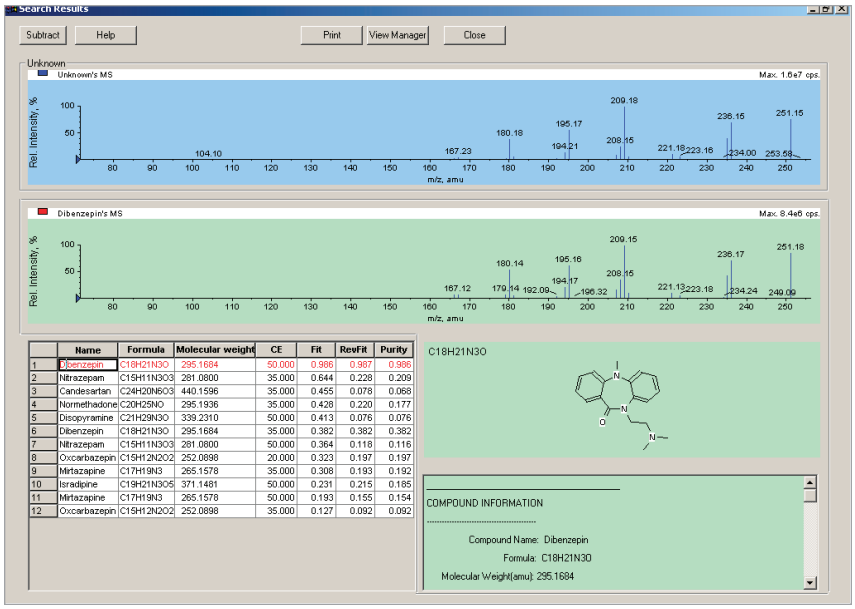

Figure 3. Dibenzepine identified by mass spectral library search (50 eV spectrum).

Results and Discussion

The multi-target screening method was successfully applied to samples of forensic cases. For comparison purposes, all compounds were first identified using established methods. The MTS method was then applied, yielding the identical results—in less than half the time required by conventional techniques.

Dynamic exclusion of detected MRM transitions was essential to detect co-eluting compounds; this is especially important for intoxication cases, where concentrations are usually 10 to more than 100 times higher than normal therapeutic concentrations. Examples of library searches are given in Figures 2 and 3. Figure 2 shows the library spectrum and the acquired sample spectrum of amitriptyline (MW 277) at a CE of 35 eV. Figure 2 also includes the library search fit values, all greater than 0.96 for this identification. The second compound in the list, nortriptyline (MW 263), can easily be excluded as a possibility, since it cannot produce the signal observed at m/z 278.

The fit value provides information about similarity of the signals in the library reference spectrum with those in the unknown spectrum. The reverse fit reflects the similarity of the signals in an unknown spectrum with those in a reference spectrum. Finally, the purity value is a combination of both fit values. Figure 3 illustrates another example for dibenzepine at a CE of 50 eV with the fit and purity values all > 0.98. Purity values above 0.9 were obtained for all cases. A library hit with fit, reverse fit and purity values all > 0.9 has a very high probability of being the correct identification. However, fit and purity values can be as low as 0.7, especially for compounds identified at very low concentrations.

Conclusions

The multi-target screening method described here rapidly and confidently detects and identifies 300 pre-selected target drugs and metabolites in blood samples of forensic and clinical cases. The method provides analytical results identical to established chromatographic screening methods, in considerably less time. The LINAC® collision cell in the Q TRAP® system enables extremely brief 5 ms dwell times, resulting in only a minor loss in sensitivity; in fact, compared to established methods, the MTS method shows higher sensitivity for most of the 300 screened compounds, as well as superior selectivity for greater confidence in identification and quantitation results.

Additionally, this method requires only a single sample injection—another important advantage over the published method of Gergov *et al*[6] that results in fewer analyses, shorter analysis time, and more information per experiment.

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For more information about the MTS method and library, please call 877-287-5700 (in North America) or 650-554-3011 (outside North America).

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