CT-p-9

A systematic and comprehensive method for general toxicological screening using QTrap LC-UV-MS-MS

SIMON ELLIOTT[1], FRANS SCHOUTSEN[2] and BÉNÉDICTE DURETZ[3]

1 Regional Laboratory for Toxicology, Birmingham, United Kingdom;

2 Applied Biosystems, Warrington, United Kingdom;

3 Applied Biosystems, Les Ulis, France.

AIMS: LC-MS has become a standard technique of analysis in clinical and forensic toxicology. Various published methods focus on particular compounds or drug classes. The method presented outlines a systematic protocol for "unknown drug screening" utilising the novel features of the QTrap hybrid linear ion-trap coupled with the existing advantages of diode-array UV detection.

METHODS: To provide optimal conditions for elution separation of drugs, metabolites and matrix artefacts, a multi-step HPLC gradient is used based on a Gemini 5? C18 analytical column with acetonitrile, 1mM ammonium formate and 0.1% formic acid mobile phase. The analytical run time is 20 minutes with 3 minutes equilibration with a flow rate of 0.8 mL/min with UV-MS line splitting. Retention indices (RIs) are the preferred retention parameter for compound identification based on a series of retention markers. UV spectra are obtained between 200-595 nm.
For the MS, following positive mode ionisation, an enhanced MS (EMS) survey scan is acquired with information-dependent criteria for acquisition of an enhanced product ion (EPI) scan at different collision energies. This results in fragmentation of the most abundant precursor ion to characteristic product ions that can be compared with a spectral library. The EMS will detect a large array of ionised compounds and is used for general unknown screening but has lower sensitivity than traditional targeted screening using multiple reaction monitoring (MRMs). MRM analysis is used for detection of specific compounds that may be present at much lower concentrations. This is performed in both positive and negative ion mode with multiple injections of the extract if required. Liquid-liquid extraction is a flexible and amenable method of extraction for both EMS and MRM analysis and can produce much cleaner extracts and greater sensitivity than simple dilution or protein precipitation methods; although these methods can be used if chemical extraction is difficult. Solid-phase extraction is used for specific analysis of glucuronides (if required) followed by MRM or constant Neutral Loss (NL) mass spectral detection.

RESULTS: By utilising the advantages of HPLC, UV-DAD and the various MS scan methods available, this system was found to have the following advantages compared to existing systematic methods for LC-MS: 1. The use of RIs provide a more robust elution identifier, 2. The incorporation of UV-DAD provides an additional identification parameter, 3. The ability to obtain full scan MS data even following MRM acquisition, 4. The use of EMS as opposed to targeted screening (e.g. MRM data) provides general screening for a wider range of compounds that would not be detected if the MRM was not known, 5. Spectral information at different collision energies provides greater possibilities for identification and confirmation, 6. Analysis for glucuronide metabolites allows detection of latent drug use.

The methods have been to applied to a wide range of casework and in particular have resulted in the important detection of drug metabolites and taxine alkaloids (toxic component of the Yew tree). Taxine alkaloids are rarely suspected and like certain drug metabolites are unlikely to be part of a typical targeted screening system and would not be found. Identification of drug metabolites is particularly important in determining drug history and may also provide some pharmacological information. The requirement for a "catch all" system using DAD and EMS also removes the possibility that unknown or new compounds would be missed (e.g. new synthetic drugs) - this proved important in a benzylpiperazine (BZP) case.

CONCLUSIONS: The methods provide a comprehensive detection tool for clinical and forensic toxicological analysis of varying biological fluids that can be complimentary to existing techniques and assays as well as increasing the laboratory's analytical repertoire. The "catch all" approach also has advantages over existing systematic MS detection methods.

KEYWORDS: *Screening, QTrap*

Corresponding author: Benedicte.duretz@applera.com