



Fig. 1. AB Sciex QTRAP 5500 mass spectrometer

Introduction:

Indole- and indazole-3-carboxylates (I3Cs) are types of synthetic cannabinoids (SCs). SCs are a class of novel psychoactive substances (NPS) or 'legal highs'. Their incidence of use is increasing [1] as they are often difficult to detect. Currently, their structures and properties are not fully known, as well as their pharmacokinetics [2]. This means that their effects cannot be characterised, making them potentially dangerous. This is a cause for concern, especially while they remain largely undetectable.

This research is motivated towards confirmation and continuation of previous work [3], and aimed to identify any potential diagnostic analytes, as well as to develop a method for detection, which could prove useful in the fields of forensics and toxicology.

Aims & Objectives:

- Confirm the structures of six synthetic cannabinoids (Figure 3) by mass spectrometry, and determine their fragmentation patterns.
- Develop a repeatable method for simultaneous identification of a range of synthetic cannabinoids, after their extraction from biological matrices.
- Use this method to identify the presence of NPSs and/or their metabolites in clinical samples.

Methods:

- Extracted I3C samples from plasma and urine, and used the mass spectra to confirm the structures of each of the 6 compounds.
- Reverse phase C18 HPLC (high performance liquid chromatography) was used to separate compounds.
- Detected which NPS may be present in blood samples from hospital patients by use of HPLC-MS/MS.
- The process of mass spectrometry (MS) is illustrated in Figure 2.

Sample extraction by Isolute SLE+ solid phase extraction



Liquid chromatography with H₂O/MeOH + 0.1% FA gradient



Mass spectrometry including MS/MS, MRM, EMS, EPI, product ion scan, precursor scan, and MRM-triggered MS/MS



Data collection by Analyst and review with PeakView software

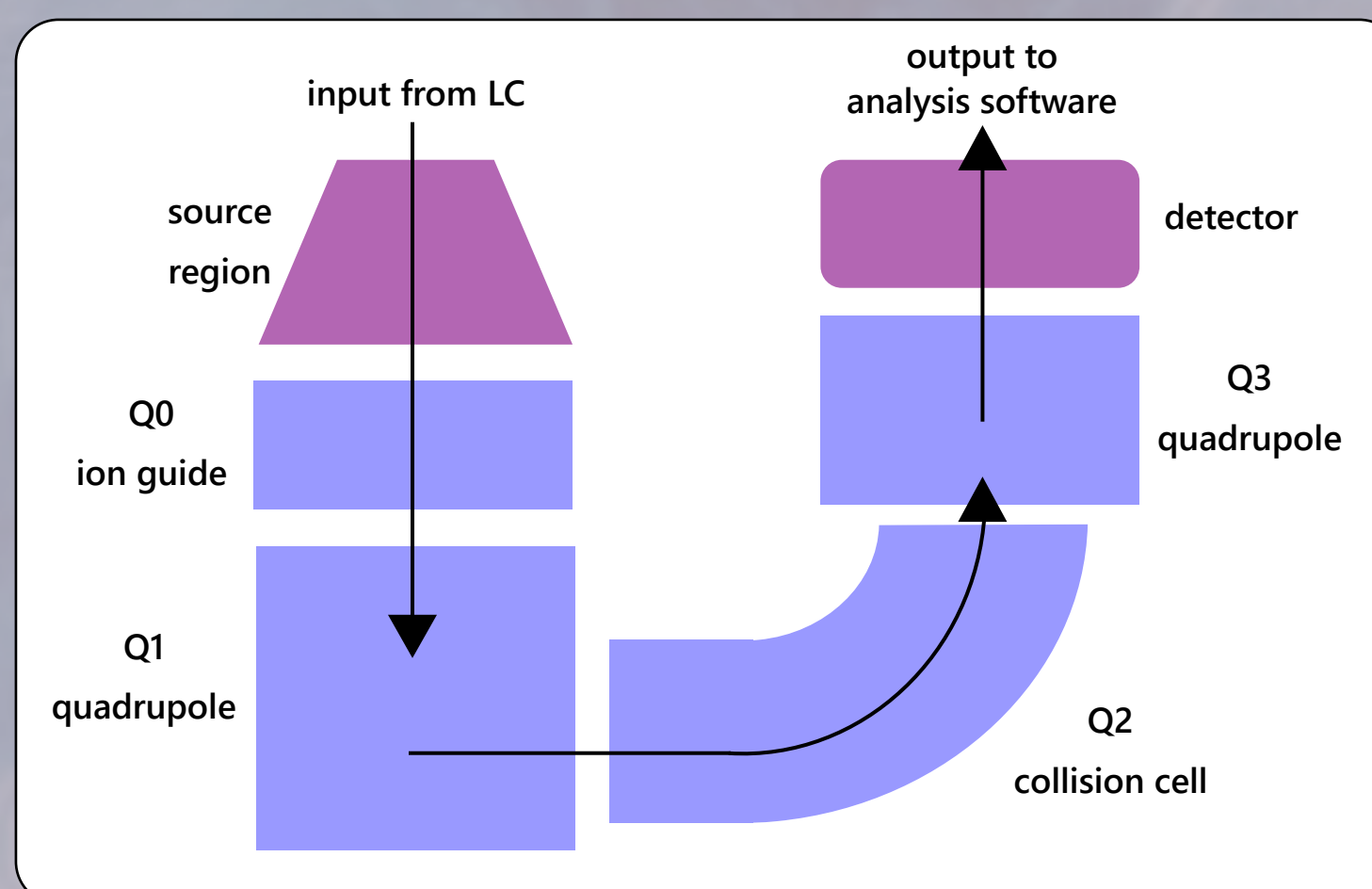


Figure 2. Mass spectrometer: Samples separated by liquid chromatography (LC) enter the mass spectrometer at the source region. Q0 is an ion guide which focuses a beam of ions to increase sensitivity and resolution. Q1 and Q3 are similar quadrupoles which can either be used as a filter to select for specified ions, or can be non-resolving and act as an ion guide only (similar to Q0). Q2 is a U-shaped collision cell containing collision gas, which causes ions to fragment. The detector detects a current produced by the fragmented ions and sends this information to a computer. Information is displayed as a mass spectrum, using Analyst software.

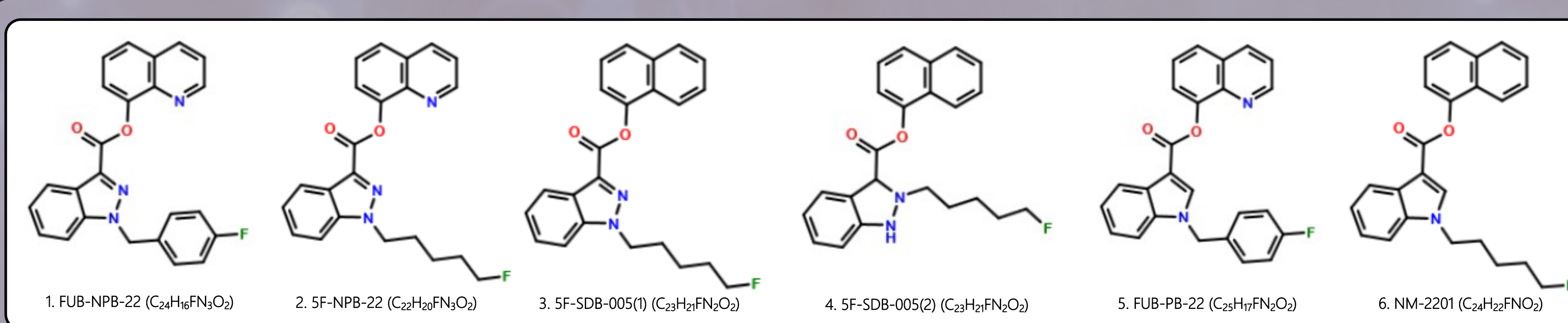


Figure 3. Synthetic cannabinoids: FUB-NPB-22 contains key structures of quinoline, carbonyl, indazole and fluorobenzene. 5F-NPB-22 contains naphthalene, carbonyl, indazole and fluoropentyl groups. 5F-SDB-005(1) and 5F-SDB-005(2) are stereoisomers, both containing naphthalene, carbonyl, indazole and fluoropentyl groups. They differ in the attachment of the fluoropentyl group - (1) has this group associated with N1 and (2) has its fluoropentyl group associated with N2. FUB-PB-22 contains quinoline, carbonyl, indole and fluorobenzene groups. NM-2201 contains naphthalene, carbonyl, indole, and fluoropentyl groups. Structures drawn using Elemental, via Chempicker.



Figure 4. Example result from LC-MS/MS: 10ng mix of all compounds after dilution with water, extraction from plasma, and reconstituted with mobile phase, run on an MRM-MS/MS scan. The first peak is at 16 minutes, showing that this compound was first to leave the LC column. The blue trace shows the MRM result, and the pink trace shows data-dependent transition from MS/MS scan. An MRM scan is 'multiple reaction monitoring', and is used to detect only ions which produce a specific fragment. MRM peaks can be highlighted to identify specific ion transitions. The bottom trace shows the mass spectrum (fragmentation pattern) of this compound.

Results:

- Results were recorded and displayed using Analyst and PeakView respectively. An example PeakView trace is shown in Figure 4, showing that 5F-SDB-005 has a parent ion of 377.2Da and major fragments of 233.0Da, 213.06Da, and 144.9Da.
- MRM result from a blood sample (Figure 5) showed a transition from 310.2Da to 265.1Da, indicating the presence of methadone.
- MRM of the clinical sample did not detect heroin or any I3Cs.
- A scan of another clinical sample suggested the presence of 5F-AKB-48, which is a novel synthetic cannabinoid [4].

Conclusions & Discussion:

- Successfully developed a repeatable LC-MS method for analysis of a mixture of drugs, when extracted from biological matrices.
- Confirmed structures of synthetic cannabinoids identified in [3] and their fragmentation patterns, as well as an isomer of 5F-SDB-005.
- The blood sample which was screened for the presence of NPSs tested positive for methadone, but negative for heroin and I3Cs.
- Another sample suggested that a non-I3C cannabinoid (5F-AKB-48) may have been present, but the ratio between peaks was too great to be confident about this result.
- Results were confirmed to prove that the LC-MS method was successful.
- Future research could be directed towards furthering knowledge by producing full descriptions of the pharmacokinetics of NPSs.

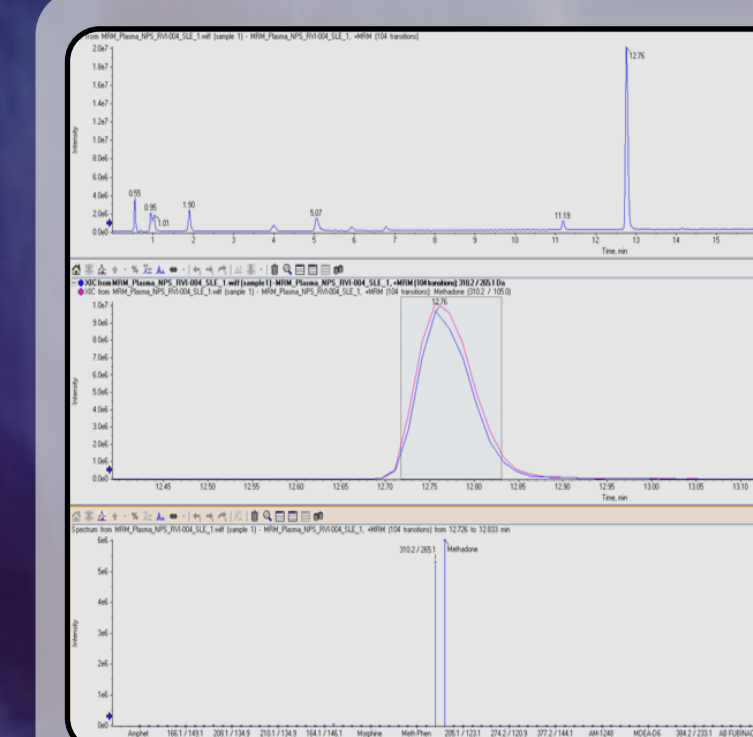


Figure 5. Drug screening: Top trace shows an MRM result from scan of blood sample from hospital, with a major peak at RT 12.62, which is expected of methadone. The mass spectrum at the bottom of the image shows that the peaks match up with the products of methadone fragmentation. They are present at similar intensities, so confirm the presence of methadone in the plasma sample.

References:

- Banister SD, Stuart J, Kevin RC, Edington A, Longworth M, Wilkinson SM, Beinat C, Buchanan AS, Hibbs DE, Glass M, Connor M, McGregor IS, Kassou M. (2015) 'Effects of Bioisoteric Fluorine in Synthetic Cannabinoid Designer Drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, AND STS-135.' *ACS Chem Neurosci*. [Epub ahead of print]
 - Gandhi AS, Zhu M, Pang S, Wohlfarth A, Scheidweiler KB, Liu H, Huestis MA. (2013) 'First Characterisation of AKB-48 Metabolism, a Novel Synthetic Cannabinoid, Using Human Hepatocytes and High-Resolution Mass Spectrometry.' *The AAPS Journal*. 15:1091-1098.
 - I3C research project based on work by: Shevyrin V, Melkozerov V, Eltsov O, Baranovsky A, Shafran Y. (2014) 'Synthetic cannabinoids as designer drugs: New representatives of indol-3-carboxylates series and indazole-3-carboxylates as novel group of cannabinoids. Identification and analytical data.' *Forensic Science International*. 244:263-275.
 - Scheidweiler KB, Jarvis MJ, Huestis MA. (2014) 'Nontargeted SWATH acquisition for identifying 47 synthetic cannabinoid metabolites in human urine by liquid chromatography-high-resolution tandem mass spectrometry' *Anal Bioanal Chem* 407:883-897
- Background image: <http://iwallpapers.com/18864-science-molecule-chemistry-wallpaper/>
 Mass spectrometer image: <http://notjenck.com.ar/aplicaciones/notjenck25/deteccion-de-peptidos-en-matrices-complejas-con-el-esquema-de-trabajo-midas/>