Quadrupole Time-of-Flight Mass Spectrometry: A Paradigm Shift in Toxicology Screening Applications

*Darren R Allen, Brett C McWhinney

Department of Chemical Pathology, Pathology Queensland Central Laboratory, Brisbane, Qld 4006, Australia.

*For correspondence: Darren R Allen, darren.allen@health.qld.gov.au

Abstract

The screening of biological samples for the presence of illicit or legal substances is an important frontline tool in both clinical and forensic toxicology. In the clinical setting, drug screening is a useful tool for the clinician in improving patient care and guiding treatment. Analytical approaches for the screening of drugs in biological samples are extensive and well documented, though many rapid screening techniques often lack appropriate sensitivity and specificity, requiring careful clinical interpretation. The continuous emergence of new psychoactive substances presents a considerable analytical challenge in maintaining up-to-date methods for the detection of relevant drugs. Adapting and validating methods for the detection of new substances can be a complicated and costly undertaking. There is also a considerable lag time between the emergence of new drugs and the release of commercial assays for detection. Quadrupole time-of-flight mass spectrometry (Q-TOF-MS) has gained considerable attention over the last decade as an analytical technique that is capable of meeting the challenges of a rapidly changing drug landscape. Exhibiting both high sensitivity and specificity in drug detection, Q-TOF-MS also allows methods to be rapidly updated for newly emerging psychoactive agents. The coupling of Q-TOF-MS with techniques such as liquid or gas chromatography can provide both rapid and comprehensive screening solutions that are gaining popularity in the clinical laboratory setting.

Introduction

Q-TOF-MS is an analytical technique that advantageously combines the benefits of two different mass analysers. Utilising the high compound fragmentation efficiency of quadrupole technology in combination with the rapid analysis speed and high mass resolution capability of time-of-flight, a unique 'hybrid' analyser was introduced.¹ Originally developed in the early 1980s, the unique capabilities of the Q-TOF-MS were not fully utilised until the launch of the first commercial instrument in 1996.^{2,3} Since this time, Q-TOF-MS has become a widely accepted technique across numerous fields of biological and pharmaceutical research including metabolite identification, peptide analysis and drug discovery.⁴⁻⁶ In the clinical laboratory, the adoption of Q-TOF-MS as a routine analytical methodology has been prominent in the field of toxicology. Predominantly due to the comprehensive qualitative drug screening capability it offers, Q-TOF-MS is largely contributing to a paradigm shift in the detection methods of illicit and legal drugs in biological samples.⁷

In clinical practice, drug testing is used in a variety of clinical scenarios including the investigation of medication compliance, substance-induced disorders, drug abstinence monitoring and overdose management. Drug testing may also be performed in medico-legal scenarios including occupational drug testing and court-ordered monitoring. In the clinical setting, urine is the most commonly used specimen due to the relative ease of collection. Urine also offers extended detection times for drugs allowing the analysis of both parent drug and metabolites. Additional biological samples may also be used for drug detection including whole blood, serum, saliva, hair, nails, meconium and gastric contents. In the forensic setting, additional biological samples such as vitreous humour, teeth, and organs such as liver or brain may also be used for drug detection.^{8,9}

For decades, immunoassays have been the detection method of choice for the screening of illicit and legal drugs. Although immunoassays are rapid and generally low-cost techniques, their inherent limitations can reduce their overall clinical utility,

making clinical interpretation difficult.¹⁰ The use of mass spectrometry-based techniques for the screening of drugs in biological samples is not new. Compared with immunoassay, both liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) offer increased sensitivity and specificity in drug detection. Mass spectrometry-based techniques often require labour-intensive sample preparation such as derivatisation or hydrolysis of conjugated drugs, which can limit their utility where rapid drug screening is required. Capital cost of mass spectrometry instrumentation is also higher and experienced operators are generally required. Targeted mass spectrometry also requires the instrument to be specifically optimised for each analyte of interest, resulting in complex method development.¹¹ The application of Q-TOF-MS to comprehensive drug screening offers many advantages with regard to meeting the challenges of a rapidly changing drug landscape. This review examines the instrumentation and operating principles of Q-TOF-MS and its promising application in toxicology screening.

High Resolution Mass Spectrometry

High resolution mass spectrometry (HRMS) is defined by the Royal Society of Chemistry as ‘Any type of mass spectrometry where the “exact” mass of the molecular ions in the sample is determined as opposed to the “nominal” mass (the number of protons and neutrons).’¹² The performance of a high resolution mass analyser is usually expressed in terms of the instrument resolution. To determine the resolution of an instrument, the ‘full width at half maximum’ (FWHM) method can be used, where mass (m) is divided by the peak width at 50% of the peak height ($m/\Delta m_{50\%}$). A mass spectrometer is considered capable of high resolution analysis when $m/\Delta m_{50\%} > 10,000$ (**Figure 1**).^{13,14} Today a variety of high resolution mass spectrometers utilising alternative technologies are commercially available with variable resolution. These instruments include time-of-flight (TOF), fourier transform ion cyclotron resonance (FT-ICR) and orbitrap (OT) mass analysers. Over recent years, a reduction in the cost of instruments such as TOF and OT mass analysers has seen more high resolution mass analysers entering the clinical laboratory.¹⁵

Figure 1. Full width at half maximum (FWHM) method for the calculation of instrument resolution.

Q-TOF-MS: Instrumentation and Principles of Operation

The Q-TOF-MS is a 'hybrid' instrument combining quadrupole technologies with a time-of-flight mass analyser. Q-TOF-MS instrumentation closely resembles that of a triple-quadrupole mass spectrometer, though the third quadrupole has been replaced by a time-of-flight tube. The first quadrupole (Q1) is capable of operating as a mass filter for the selection of specific ions based on their mass-to-charge ratio (m/z), or in radio frequency (RF) only mode where all ions are transmitted through the quadrupole. The second quadrupole (Q2) acts as a collision cell where ions are bombarded by neutral gas molecules such as nitrogen or argon, resulting in fragmentation of the ions by a process known as collision induced dissociation (CID). The Q2 can also act in RF-only mode without subsequent fragmentation of ions. After leaving the quadrupole, ions are reaccelerated into the ion modulator region of the time-of-flight analyser where they are pulsed by an electric field and accelerated orthogonally to their original direction.¹⁶ All ions having acquired the same kinetic energy now enter the flight tube which is a field free drift region where mass separation occurs. Ions exhibiting a lighter mass will have a shorter time of flight, whereas heavier ions will take longer to traverse the flight path towards the detector.

Modern time-of-flight analysers also utilise a reflectron device which serves to correct for kinetic energy dispersion and spatial spread of ions that exhibit the same m/z , but have varying velocities. This reflectron correction allows ions of the same m/z to arrive at the detector at the same time. The reflectron device also increases the flight path length which improves mass resolution (**Figure 2**).¹⁶

Since Q-TOF-MS utilises quadrupole technology in conjunction with a time-of-flight analyser, two distinct scan types can be used for data acquisition. The first mode known as single MS mode uses the first and second quadrupole in RF-only mode to provide an accurate mass scan of the unfragmented precursor ion. The Q1 may also be used to select a specific mass or range of masses for transmission to the TOF analyser. The second mode (MS/MS) can utilise the Q1 in RF-only or mass filter mode to transmit ions into the collision cell (Q2) where CID occurs. The subsequent product ions and any unfragmented precursor ions are then transmitted to the TOF analyser where accurate mass measurement occurs. Alternating between these modes allows the Q-TOF-MS to simultaneously collect both precursor and product ion information.¹⁶ Detection of ions is achieved by a detector

Figure 2. Schematic diagram of a quadrupole time-of-flight mass spectrometer.

system known as a time-to-digital converter which converts the flight time of the ion into a mass signal.¹⁷

Q-TOF-MS: Data Acquisition

In 'targeted' data acquisition, the mass analyser is specifically optimised to detect only pre-determined compounds, subsequently excluding all other compound information in the sample. Today, triple quadrupole mass spectrometers are the instrument of choice for targeted analysis, and their application in toxicology has been prominent in targeted confirmatory analysis and quantification. A prominent feature of Q-TOF-MS that has contributed to its success in toxicology screening is the unique data acquisition methods that it utilises. Using the full scan capability of the time-of-flight analyser, data can be acquired in an unbiased or 'non-targeted' fashion without any prior knowledge of the compounds present in the sample.^{18,19} Non-targeted data acquisition modes can be classified into two groups: data-dependent acquisition (DDA) and data-independent acquisition (DIA). In DDA, the intact precursor ion is first measured, and then fragmented in the collision cell to obtain high resolution product ion spectra. DDA methods allow predefined criteria such as signal intensity to determine which precursor ions are selected for fragmentation. A disadvantage of the DDA approach is that potentially relevant analytes of low abundance may not be selected for further fragmentation. Also, in samples where precursor density is high, relevant analytes may fail to be detected in DDA acquisition due to the instrument acquiring product ions from previously selected targets. In DIA, there are no pre-determined criteria for triggering the acquisition of product ion spectra, allowing comprehensive MS/MS data collection.^{20,21} The use of non-targeted data acquisition also results in a significant increase in the amount of data collected by the instrument. An average triple quadrupole analysis may only contain several megabytes of data per injection compared to untargeted data collection via Q-TOF-MS which may exceed a gigabyte in size per sample injection. To manage this data appropriately, laboratories must allocate increased data storage capacity, improve hardware processing capability and implement appropriate archiving procedures.

Several unique DIA approaches have been developed for the comprehensive collection of both precursor and product ion spectra. One such acquisition mode is known as 'Sequential Window Acquisition of All Theoretical Mass Spectra' or 'SWATH'. In SWATH acquisition mode, data is collected by an initial precursor ion survey scan, followed by product ion generation from precursor ions selected in small Q1 isolation windows (≈ 20 Da wide). Using many consecutive isolation windows, the entire defined mass range is stepped through continuously during each scan cycle.^{20,21} SWATH acquisition also allows the use of variable user-defined Q1 isolation

windows. This has proven advantageous in mass regions where precursor density is high; resulting in improved analyte separation and selectivity.²¹ Another prominent DIA technique for comprehensive precursor and product ion monitoring is MS^E which utilises a rapid dual scanning mode. In MS^E, all compounds are transmitted through Q1 into the collision cell where they are subjected to alternating low and high collision energy. In low collision energy mode, precursor ions are focused through the collision cell, remaining generally intact for accurate mass measurement in the TOF analyser. During the high collision energy mode, precursor ions are subjected to a collision energy ramp from low to high where they undergo CID to form product ions which are subsequently measured.²²

Compound Identification

Compound identification via spectral library matching involves the comparison of mass spectra generated from unknown samples with a database containing spectra of known compounds. A major benefit of the use of spectral library matching in screening applications is the potentially unlimited number of compounds that can be included in a library, providing large comprehensive screening profiles. Comparison of spectra from unknown samples with those stored in the spectral library is performed by sophisticated computer algorithms that identify spectral matches, often with a scoring profile.²³

The use of spectral library matching to screen for drugs in biological samples is not a new concept, and has been successfully performed by GC-MS techniques for decades. The major advantage of using GC-MS with spectral library matching is the standardisation of mass spectrometric conditions, allowing a compound measured by a different system to produce the same results as the instrument that generated the reference spectra. This has resulted in the successful use of universal comprehensive spectral databases of compounds for use with GC-MS methods.²⁴ Low resolution mass spectrometers such as triple quadrupole or ion trap instruments coupled with liquid chromatography have also been applied to screening applications, though the generation of universal compound libraries has proven to be more challenging as the CID product ion spectra can vary considerably between different instruments.²⁵

In Q-TOF-MS, the non-targeted accurate mass acquisition of precursor ions and unique product ion spectra can provide an information-rich profile of an analyte. The use of CID spectra also gives the Q-TOF-MS additional identification confidence compared to accurate mass measurement alone where structural isomers exhibiting the same accurate mass exist e.g. morphine and hydromorphone. The coupling of Q-TOF-MS with chromatographic techniques such as liquid

chromatography and gas chromatography is also an important component of accurate compound identification, providing the additional analyte characteristic of retention time. In addition to the accurate mass of precursor and product ions, spectral libraries can incorporate additional analyte characteristics such as isotopic pattern and retention time that contribute to accurate compound identification.^{23,24}

In a recent study, the diagnostic performance of screening workflows utilising the incremental combination of alternative analyte characteristics was assessed. The analyte characteristics investigated were mass, isotope pattern, product ion spectra and retention time. It was noted that the inclusion of product ion spectra to the identification protocol provided the largest incremental improvement and was the most heavily weighted analyte characteristic at 70% of the total combined library score. The screening workflow utilising a combination of mass, isotope pattern and product ion spectra showed a positive predictive value of 82%. The addition of retention time to the workflow provided further analyte discrimination with the combined workflow exhibiting a positive predictive value of 96%.²⁶ The use of chromatography in non-targeted analysis was also shown to improve compound identification, particularly when complex biological matrices are analysed. Co-elution of compounds may cause ion suppression which can lead to a reduction in mass measurement accuracy, thus reducing confidence in compound identification. Relevant compounds with low signal-to-noise ratios due to ion suppression may also fail to be detected in the analysis. Improved chromatographic separation was also shown to improve compound identification by allowing the processing software to more effectively compartmentalise the data.²⁷

Studies on the optimisation of HRMS data analysis parameters have also been undertaken to investigate improved compound identification algorithms. A common software approach for compound identification includes a decision tree algorithm, where individual thresholds for each analyte characteristic must be met to provide a positive identification. In a study by Colby *et al.*, the evaluation of an alternative algorithm utilising a combined scoring approach, which considers all analyte parameters simultaneously, was assessed.²⁸ In this study, the combined library score method provided optimum drug identification with a positive predictive value of 94.7% and a negative predictor value of 99.4%. The combined scoring approach was seen to minimise false negative results by adding further flexibility to compound identification in cases where individual analyte characteristic scores were low. Drug identification workflows across different vendor platforms can also vary due to differences in hardware and software design, though their ability to perform comprehensive compound identification remains unchanged. In a study by Marin *et al.*,

the performance of compound identification across three LC-Q-TOF-MS platforms from different vendors was assessed with all platforms showing drug detection >90% for spiked plasma samples and >75% for spiked urine samples.²⁹

Application to Toxicology Screening

Toxicology screening has shown to be a useful adjunct to the clinician in improving patient care and treatment. In the emergency department, drug screening results rarely influence the emergency management of patients due to factors including turn-around time, limited specificity and sensitivity and complexity of interpretation.³⁰ In 2003, the National Academy of Clinical Biochemistry published recommendations for the use of laboratory tests to support poisoned patients in the emergency department. Highlighted here was the need for improvements in rapid drug detection techniques, and the development of new drug assays to meet changing drug abuse patterns.³¹ In recent years, the use of Q-TOF-MS in drug screening applications has proved a promising analytical technique in meeting many of the current and future challenges identified in drug detection.

Today, immunoassay methods remain the predominant technique for drug screening, with urine the predominant sample type utilised in the clinical setting. Immunoassays are rapid techniques that are easily automated on routine biochemistry platforms, which have made them a useful tool in the screening of a wide range of drug classes. Despite these advantages, there are a number of inherent limitations with immunoassays that must be considered when utilising their results in the clinical setting. Limited sensitivity and specificity in drug detection remains one of the greatest challenges which can lead to both false positive and false negative results. Other technical aspects which may complicate clinical interpretation include inconsistent cross-reactivity profiles between alternative immunoassay methods, variable cut-off levels for analyte detection and immunoassay interferences including adulteration.^{32,33} GC-MS has been considered the gold standard for systematic toxicological analysis for decades, offering the increased sensitivity and specificity of mass spectrometry with the use of comprehensive universal compound libraries. A disadvantage of GC-MS is that highly polar, non-volatile or thermally unstable compounds require chemical modification to allow analysis via GC-MS, which can result in more complex and labour-intensive sample preparation requirements.

Limitations of currently employed immunoassay screening techniques have been well documented in literature. In one such study by Marin *et al.*, 3571 urine samples that screened positive by immunoassay (EMIT® II) for the presence of amphetamine type substances produced 389 false positive

results (11.9%) when confirmed by LC-MS-MS.³⁴ A recent study also reviewed the utility of immunoassay-based drug screening in the compliance monitoring of patients treated for chronic pain. It was noted that immunoassay techniques for opiate detection showed 21% false negative results when compared to LC-MS-MS, indicating that immunoassays can be inadequately sensitive for medication compliance. The use of mass spectrometry-based techniques for compliance monitoring was also recommended in several clinical scenarios. For example, in order to simulate compliance, patients may add medication to their urine samples. Mass spectrometry techniques were able to detect the presence of parent drug in the sample and also indicate the absence of known urinary metabolites that would indicate appropriate compliance. The importance of identifying unknown medications or illicit drugs in patients was also recommended in cases of poly-pharmacy to detect potentially lethal drug interactions.³⁵

In recent years, applications utilising Q-TOF-MS techniques have addressed many of the limitations associated with immunoassay and GC-MS. With the inherent high sensitivity and specificity of mass spectrometry and the utilisation of vast compound libraries, rapid and comprehensive Q-TOF-MS screening solutions are becoming more commonly adopted in the clinical and forensic setting. The availability of large commercial libraries developed by vendors for use with their associated platforms has also largely eliminated the need for individual laboratories to invest in the in-house development of spectral libraries. The routine coupling of Q-TOF-MS with ultra-high performance liquid chromatography (UHPLC) has also simplified sample preparation and allowed the detection of a broader range of chemical substances. In the method described by Tsai *et al.*, using a simple 5-fold dilution of urine with deionised water, the screening of 62 drugs of abuse and their metabolites was achieved using UHPLC-QTOF-MS. In positive ionisation mode, 54 basic compounds could be detected in a 15 min runtime and 8 acidic compounds in a 12 min runtime in negative ionisation mode.³⁶

Using LC-Q-TOF-MS with a DDA, Broecker *et al.* developed a CID library containing more than 2500 compounds for systematic toxicological analysis in blood and urine.³⁷ This method included pharmaceuticals, illegal drugs, steroids, insecticides and herbicides. The CID accurate mass spectra were then added to an additional library containing the accurate mass and isotope patterns of 7500 compounds. The method could also utilise simple sample preparation techniques such as urine dilution, and acetonitrile protein precipitation for whole blood, serum and plasma. Samples were analysed in positive ionisation mode with a 24 min runtime using reverse phase chromatography. Venous blood samples (50 cases) that

had been previously analysed by HPLC coupled to a diode-array detector (HPLC-DAD) and GC-MS methods were re-analysed with the LC-Q-TOF-MS method allowing the detection of a further 31 compounds not identified on the previous toxicological reports. A recent study also compared LC-Q-TOF-MS with GC-MS for systematic toxicological analysis of 247 authentic serum samples and 12 post-mortem femoral blood samples.³⁸ The LC-Q-TOF-MS method was able to detect 335 compounds versus the 141 detected by the established GC-MS method. The increased detection rate with LC-Q-TOF-MS was due to increased sensitivity of the instrumentation and the ability to detect additional non-volatile, thermally unstable and highly polar compounds not amenable for GC-MS analysis.

In comparison to LC-Q-TOF-MS, the application of gas chromatography coupled with quadrupole time-of-flight mass spectrometry (GC-Q-TOF-MS) has appeared less prominent in toxicology screening in the clinical and forensic setting. GC-Q-TOF-MS has been more extensively utilised in environmental toxicology, which is likely a result of the historical precedence for the use of GC-based techniques in environmental science.³⁹ In the analysis of biological samples, GC-Q-TOF-MS has been successfully applied to the detection of designer steroids. In the method described by Abushareeda *et al.*, GC-Q-TOF-MS was applied to the analysis of anabolic androgenic steroids in human urine using enzymatic hydrolysis, liquid-liquid extraction and sample derivatisation.⁴⁰ The method also allowed quantitative analysis of six endogenous steroids and was successfully applied to specific steroid compounds that could not be ionised efficiently with LC-MS techniques. In a recent method by Polet *et al.*, GC-Q-TOF-MS was used for the detection of 294 doping substances in urine, 14 of which were quantitatively measured with a 14 min runtime following hydrolysis, liquid-liquid extraction and derivatisation.⁴¹ As an open screening technique, the method did not rely on a targeted analyte list, allowing faster identification of new compounds. The method also facilitated the rapid incorporation of new compounds without affecting method performance, as was experienced with the existing GC-MS screening method.

The coupling of ion-mobility spectrometry (IMS) technology with Q-TOF-MS platforms can also provide an additional degree of analyte separation complementary to that provided by chromatography and HRMS. IMS is advantageous in the separation of isomers and isobars that may exhibit the same chromatographic retention time as well as identical accurate mass. Ion-mobility techniques have been in use for many years and employ a range of alternative technologies. Commonly used IMS methods incorporated into Q-TOF-MS platforms include drift-time IMS and differential-mobility

spectrometry (DMS). In drift-time IMS, ions are separated in a drift tube based on differences in size and charge. The instrument measures the time taken for an ion to drift through a buffer gas under the influence of a low electric field. Ions with a higher charged state will exhibit a faster drift velocity. Also, ions with a larger collisional cross-section will undergo increased collisions with the buffer gas, resulting in a lower drift velocity.⁴² In DMS, ion mobility separation occurs between the electrospray ionisation (ESI) source and the sampling orifice of the mass spectrometer. Compounds are transported from the ESI source in a carrier gas (N₂) into a DMS cell that consists of two electrodes that apply a high-voltage radio frequency. Differences in the chemical structure of ions results in differential mobility as they move towards either electrode in the gas phase environment of the cell.⁴³

In addition to the use of urine and blood in toxicological analysis, Q-TOF-MS methods have been applied to a variety of alternative sample matrices such as oral fluid. In comparison to urine, oral fluid offers a much shorter window of detection for drugs indicating more recent use.^{44,45} In the method described by Griswold *et al.*, using oral fluid, LC-Q-TOF-MS was applied to the detection of fentanyl analogues and other clandestine opioids such as U-47700 in cases of heroin overdose. In this study, the detection of fentanyl occurred in 27 of the 30 patients tested, indicating that the addition of clandestine opioids to heroin presents an increased risk to health.⁴⁵ The use of hair samples in toxicological analysis provides a much longer window of detection than other commonly used biological samples, allowing the investigation of retrospective drug histories. Q-TOF-MS methods applied to hair analysis have provided large screening profiles for numerous drugs and psychoactive substances.^{46,47} General unknown screening by LC-QTOF-MS has also been applied to the analysis of nails which has demonstrated a strong correlation with hair, providing an additional matrix for use in retrospective drug abuse analysis.⁴⁸ In a method developed by Costa *et al.*, the coupling of a novel paper-spray ionisation source with Q-TOF-MS has allowed non-invasive qualitative detection of cocaine and cocaine metabolites from fingerprints.⁴⁹

The application of Q-TOF-MS to drug screening is providing the clinician with a far more detailed analysis of pharmaceuticals, illicit drugs and toxins that may be present in a patient sample. With this advantage, greater insight has been gained on drug use patterns, drug composition and associated adverse effects of illicit drugs. In a recent study by Pope *et al.*, analysis of urine results for cocaine-positive patients over a two-year period performed by LC-Q-TOF-MS showed the presence of levamisole in approximately 75% of cases.⁵⁰ Levamisole is an anti-helminthic drug commonly

used in veterinary medicine that has reportedly been used as a cocaine cutting agent. A variety of adverse medical conditions have been associated with levamisole use, with traditional screening applications failing to detect levamisole. Authors in this study highlighted the potential health risk associated with levamisole in increased cocaine use.

LC-Q-TOF-MS screening has also provided insight into the composition of drugs sold as the stimulant 3,4-methylenedioxymethamphetamine (MDMA) or 'Ecstasy', a commonly used drug in club and rave culture that has been linked to numerous intoxication cases. In a four-year study, oral fluid samples were collected from participants who indicated recent MDMA use. Samples were analysed by LC-Q-TOF-MS to extensively screen for the presence of additional therapeutic drugs, common drugs of abuse and novel psychoactive substances. Following analysis, additional stimulants were detected in oral fluid for 66 of the 223 participants (29.6%), indicating that various new synthetic drugs including the novel stimulants ethylone, methylone and dibutylone are likely being mixed with or substituted for MDMA which could lead to increased health risks for users.⁵¹

Non-targeted methods offer comprehensive and specific detection of all drugs present in a sample. Targeted methods are limited by the ability to only detect pre-determined compounds which can complicate interpretation, particularly where there is concurrent poly-pharmacy. In a recently published clinical case study, the interpretation of a positive result for methylamphetamine by targeted LC-MS-MS analysis in urine was complicated by the absence of detectable amphetamine, an expected metabolite via CYP2D6 oxidative demethylation. Previous LC-MS-MS confirmatory testing for methylamphetamine had shown appropriate metabolism of amphetamine, adding uncertainty to the current result. In this example, a patient case history of recently prescribed fluoxetine, a potent inhibitor of CYP2D6 activity, was considered the cause of the atypical metabolism pattern following co-ingestion with the methylamphetamine.⁵²

The ability of high resolution techniques such as Q-TOF-MS to see all detectable compounds in a sample may also complicate result interpretation. In a recently published case study involving HRMS in opioid compliance, the sensitive detection of additional metabolites from codeine use (hydrocodone, dihydrocodeine and hydromorphone) complicated the interpretation of whether the patient has taken additional non-prescribed opioids. In this case, laboratory consultation and subsequent quantification of the unexpected opioids indicated they were consistent with minor metabolism pathways subsequent to prolonged high dose codeine use.⁵³

New Psychoactive Substances: an Analytical Challenge

In the early 2000s, there began a rapid international emergence of new psychoactive drugs that continues today. These new substances, having almost no history in medicinal use, were distinguished from classical drugs of abuse by the classification of 'new psychoactive substances' or 'NPS'.⁵⁴ From the years 2009 to 2017, the World Drug Report 2018 indicates an international cumulative total of 803 individual NPS. By the end of 2017, the largest category of reported substances were the synthetic cannabinoids (251 substances) followed by the category termed 'other substances' (155 substances) and synthetic cathinones (148 substances).⁵⁵ This broad classification of new substances constitutes a diverse range of chemical classes and presents one of the greatest analytical challenges in the field of toxicology. Added to this challenge is the fact that many of these substances also show rapid transience on the drug scene which results in constantly shifting analytical targets. Many currently employed analytical techniques have attempted to detect these substances in a variety of biological matrices, though their utility has been critically questioned, particularly with respect to maintaining relevant analyte testing profiles.

The diagnostic efficiency of various immunoassay-based techniques for the detection of synthetic cannabinoids has also been investigated. A 2014 evaluation of an ELISA technique for the detection of 73 synthetic cannabinoids indicated that 55% of the synthetic cannabinoids were not detected by the assay due to low or absent cross-reactivity with the assay antibodies.⁵⁶ In an additional study by Franz *et al.*, the diagnostic efficiencies of two homogenous enzyme immunoassays (HEIA) were compared with a targeted LC-MS-MS technique. In a total of 549 screened samples, 7.7% of samples were confirmed positive for synthetic cannabinoids by LC-MS-MS. The HEIA technique failed to detect any positive samples, indicating insufficient cross-reactivity towards prevalent synthetic cannabinoids at the time of the study, leading the authors to strongly question the utility of the immunoassay technique.⁵⁷

The application of Q-TOF-MS techniques to the detection of new psychoactive substances has shown to be a promising approach in providing sensitive detection for a vast number of relevant substances. In a study by Kronstrand *et al.*, the comparison of an LC-Q-TOF-MS technique for synthetic cannabinoid detection with a commercial immunoassay technique (HEIA) showed that the immunoassay had acceptable cross-reactivity to only a very limited number of compounds. The LC-QTOF-MS method offered the distinct advantages of increased selectivity, as well as the ability to rapidly add new compounds to the library. Methods could also be updated with reduced development time and validation

requirements, proving a superior detection strategy.⁵⁸ In recent years, numerous methodologies have been published demonstrating the advantageous application of LC-Q-TOF-MS to the comprehensive, rapid and sensitive detection of new psychoactive substances with the advantage of allowing methods to be rapidly updated.^{59,60} The use of Q-TOF-MS in the detection of NPS in the environment is also providing insight into the current use of substances which can be beneficial in guiding the development of relevant analyte detection profiles. In a recent Australian study, LC-QTOF-MS utilising non-targeted SWATH acquisition analysis was performed on influent wastewater from all Australian states and territories, providing insight into national NPS use.⁶¹

Techniques for the rapid detection of NPS without the need for sample preparation are also gaining popularity. The ambient ionisation technique known as 'direct analysis in real time' (DART) allows direct analysis of sample by the mass spectrometer. In DART-MS, analyte is ionised via desorption from the sample surface through interaction with metastable species generated from the ion source.⁶² The use of DART ionisation coupled with high resolution instruments such as Q-TOF, TOF and Orbitrap has been largely adopted for the analysis of seized substances or herbal material often sold as 'legal highs'. In the absence of chromatographic separation, a limitation of this technique is the potential difficulty in differentiating isobaric compounds or accurately identifying compounds in complex mixtures.⁶² To potentially overcome these limitations, the method described by Gwak *et al.* incorporates the use of IMS coupled with Q-TOF-MS utilising a DART ionisation source. In this method, sample applied to a Teflon membrane substrate was used to introduce analyte into the system for the analysis of 35 NPS with an analysis time of less than 1 min from sample delivery.⁶³

Beyond Qualitative Screening: Quantification and Retrospective Analysis

Publications highlighting the advantages of Q-TOF-MS in drug screening applications have focused predominately on qualitative screening capability, though the quantitative ability of modern Q-TOF-MS instruments is gaining increased attention. Development in Q-TOF-MS technology over recent years has delivered improvements in the sensitivity, precision and linear dynamic range, which is further raising the profile of HRMS beyond qualitative applications. Although the linear dynamic range of Q-TOF-MS instruments can be several orders of magnitude lower than tandem quadrupole mass spectrometers, Q-TOF-MS is proving capable of performing robust quantitative workflows.⁶⁴ In a recent article by Rochat, a review of LC-high resolution and triple quadrupole mass spectrometer comparisons indicated that the instruments showed similar quantitative performance. It was also noted

by the author that the frequent claims of better sensitivity from triple quadrupole instruments is not related to the mass analyser itself, but rather the result of the latest hardware developments (e.g. improved ion optics) being introduced to triple quadrupole instruments first.⁶⁵

In a study performed by Morin *et al.*, the quantitative performance of triple quadrupole with Q-TOF-MS was investigated.⁶⁶ Highlighted in this article was the importance of conducting fair comparisons between Q-TOF and triple quadrupole instruments by the use of appropriately equivalent Q-TOF acquisition methods. The authors suggest that studies comparing generic Q-TOF-MS non-targeted acquisition modes such as TOF-MS with triple quadrupole are likely contributing to the reputation that Q-TOF-MS is a less sensitive and robust technique. Non-targeted acquisition modes are primarily designed for qualitative analysis, and quantitative comparison should be performed against targeted Q-TOF-MS acquisition modes such as SRM^{HR} (selective reaction monitoring – high resolution) to conduct a fair comparison. In the study, comparison of triple quadrupole SRM and Q-TOF SRM acquisition modes for quantification of large molecules showed that the sensitivity of Q-TOF using a targeted approach could match, and even exceed in certain circumstances, the sensitivity of triple quadrupole instruments.

Although Q-TOF-MS non-targeted acquisition modes are primarily designed for qualitative analysis, several published methods have also demonstrated robust quantitative workflows using these techniques. A 2015 study comparing different acquisition modes for quantitative analysis of the major cannabis metabolite 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) was investigated. Quantification using the acquisition mode MS^E showed excellent linearity and precision (CV <6%). Comparison of MS^E quantification with LC-MS-MS also showed excellent correlation ($r > 0.99$), with the lower sensitivity of the Q-TOF-MS method overcome by the use of a larger sample injection volume.⁶⁷ A 2018 study utilising Q-TOF-MS for the quantitative analysis of 30 drugs in hair with a non-targeted data acquisition (all ions mode) also showed acceptable method performance with reproducible stable ion ratios, and within-run precision between 1.4% and 6.7%.⁶⁸

A technique for expanding the linear dynamic range in Q-TOF-MS quantitative applications has involved the use of less abundant natural isotopologue signals. The application of this technique is easily facilitated in HRMS analysis as the data for all ions are automatically acquired. In a study by Liu *et al.* using UHPLC-Q-TOF-MS, the linear dynamic ranges of the four evaluated analytes were extended by 25–50 times

by the selective use of less abundant analyte isotopes, without compromising additional parameters such as mass accuracy, resolution and signal-to-noise ratio.⁶⁹

The unique non-targeted data acquisition of the Q-TOF also allows retrospective interrogation of data for the presence of newly recognised compounds following the addition of new compound information to a spectral library. This ability has proven advantageous in the detection of new compounds and metabolites from previously acquired data without the need for re-analysis. In a recently published LC-Q-TOF-MS method for the screening of fentanyl and fentanyl analogues in blood, method validation included retrospective analysis of 2339 previously acquired blood samples for the presence of newly added fentanyl analogues.⁷⁰

Conclusion

The application of Q-TOF-MS to the screening of drugs in biological samples is providing solutions to many of the analytical challenges facing drug detection today. With increased sensitivity, specificity, comprehensive analyte profiles, and the ability to rapidly update spectral databases for new compounds, Q-TOF-MS is becoming the analytical method of choice for comprehensive drug screening in the clinical laboratory.

Competing Interests: None declared.

References

1. Glish GL, Burinsky DJ. Hybrid mass spectrometers for tandem mass spectrometry. *J Am Soc Mass Spectrom* 2008;19:161-72.
2. Glish GL, Goeringer DE. Tandem quadrupole/time-of-flight instrument for mass spectrometry/mass spectrometry. *Anal Chem* 1984;56:2291-5.
3. Morris HR, Paxton T, Dell A, Langhorne J, Berg M, Bordoli RS, et al. High sensitivity collisionally-activated decomposition tandem mass spectrometry on a novel quadrupole/orthogonal-acceleration time-of-flight mass spectrometer. *Rapid Commun Mass Spectrom* 1996;10:889-96.
4. Xie C, Zhong D, Yu K, Chen X. Recent advances in metabolite identification and quantitative bioanalysis by LC-Q-TOF MS. *Bioanalysis* 2012;4:937-59.
5. Poon TC, Johnson PJ. Proteome analysis and its impact on the discovery of serological tumor markers. *Clin Chim Acta* 2001;313:231.
6. Ranasinghe A, Ramanathan R, Jemal M, D'Arienzo CJ, Humphreys WG, Olah TV. Integrated quantitative and qualitative workflow for in vivo bioanalytical support in drug discovery using hybrid Q-TOF-MS. *Bioanalysis* 2012;4:511-28.

7. Maurer HH, Meyer MR. High-resolution mass spectrometry in toxicology: current status and future perspectives. *Arch Toxicol* 2016;90:2161-72.
8. Moeller KE, Kissack JC, Atayee RS, Lee KC. Clinical Interpretation of Urine Drug Tests: What Clinicians Need to Know About Urine Drug Screens. *Mayo Clin Proc* 2017;92:774-96.
9. Gerostamoulos D. Urinary drug screening. *Aust Prescr* 2013;36:62-4.
10. Melanson SE. The utility of immunoassays for urine drug testing. *Clin Lab Med* 2012;32:429-47.
11. Gualé F, Shahreza S, Walterscheid JP, Chen HH, Arndt C, Kelly AT, et al. Validation of LC-TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens. *J Anal Toxicol* 2013;37:17-24.
12. Royal Society of Chemistry. High-resolution mass spectrometry. <http://www.rsc.org/publishing/journals/prospect/ontology.asp?id=CMO:0000498&MSID=c3tc00801k> (Accessed 8 January 2019).
13. Xian F, Hendrickson CL, Marshall AG. High resolution mass spectrometry. *Anal Chem* 2012;84:708-19.
14. Balogh MP. Debating Resolution and Mass Accuracy in Mass Spectrometry. http://alfresco.ubm-us.net/alfresco_images/pharma/2014/08/22/dfafa4af-cf02-43ff-bd24-3bc054805fb3/article-128057.pdf (Accessed 28 May 2019).
15. Jiwan JL, Wallemacq P, Hérent MF. HPLC-high resolution mass spectrometry in clinical laboratory? *Clin Biochem* 2011;44:136-47.
16. Chernushevich IV, Loboda AV, Thomson BA. An introduction to quadrupole-time-of-flight mass spectrometry. *J Mass Spectrom* 2001;36:849-65.
17. Gross JH. *Mass Spectrometry A Textbook*. 3rd ed. Heidelberg: Springer; 2017. p. 267-8.
18. Díaz R, Ibáñez M, Sancho JV, Hernández F. Target and non-target screening strategies for organic contaminants, residues and illicit substances in food, environmental and human biological samples by UHPLC-QTOF-MS. *Anal Methods* 2012;4:196-209.
19. Krauss M, Singer H, Hollender J. LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Anal Bioanal Chem* 2010;397:943-51.
20. Roemmelt AT, Steuer AE, Poetzsch M, Kraemer T. Liquid chromatography, in combination with a quadrupole time-of-flight instrument (LC QTOF), with sequential window acquisition of all theoretical fragment-ion spectra (SWATH) acquisition: systematic studies on its use for screenings in clinical and forensic toxicology and comparison with information-dependent acquisition (IDA). *Anal Chem* 2014;86:11742-9.
21. Zhang Y, Bilbao A, Bruderer T, Luban J, Strambio-De-Castillia C, Lisacek F, et al. The Use of Variable Q1 Isolation Windows Improves Selectivity in LC-SWATH-MS Acquisition. *J Proteome Res* 2015;14:4359-71.
22. Chindarkar NS, Wakefield MR, Stone JA, Fitzgerald RL. Liquid chromatography high-resolution TOF analysis: investigation of MSE for broad-spectrum drug screening. *Clin Chem* 2014;60:1115-25.
23. Colby JM, Rivera J, Burton L, Cox D, Lynch KL. Improvement of drug identification in urine by LC-QqTOF using a probability-based library search algorithm. *Clin Mass Spectrom* 2017;3:7-12.
24. Oberacher H, Arnhard K. Compound identification in forensic toxicological analysis with untargeted LC-MS-based techniques. *Bioanalysis* 2015;7:2825-40.
25. Maurer HH, Peters FT. Toward high-throughput drug screening using mass spectrometry. *Ther Drug Monit* 2005;27:686-8.
26. Colby JM, Thoren KL, Lynch KL. Suspect Screening Using LC-QqTOF Is a Useful Tool for Detecting Drugs in Biological Samples. *J Anal Toxicol* 2018;42:207-13.
27. Croley TR, White KD, Callahan JH, Musser SM. The chromatographic role in high resolution mass spectrometry for non-targeted analysis. *J Am Soc Mass Spectrom* 2012;23:1569-78.
28. Colby JM, Thoren KL, Lynch KL. Optimization and Validation of High-Resolution Mass Spectrometry Data Analysis Parameters. *J Anal Toxicol* 2017;41:1-5.
29. Marin SJ, Sawyer JC, He X, Johnson-Davis KL. Comparison of drug detection by three quadrupole time-of-flight mass spectrometry platforms. *J Anal Toxicol* 2015;39:89-95.
30. Hammett-Stabler CA, Pesce AJ, Cannon DJ. Urine drug screening in the medical setting. *Clin Chim Acta* 2002;315:125-35.
31. Wu AH, McKay C, Broussard LA, Hoffman RS, Kwong TC, Moyer TP, et al; National Academy of Clinical Biochemistry Laboratory Medicine. National academy of clinical biochemistry laboratory medicine practice guidelines: recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department. *Clin Chem* 2003;49:357-79.
32. Schütz H, Paine A, Erdmann F, Weiler G, Verhoff MA. Immunoassays for drug screening in urine : Chances, challenges, and pitfalls. *Forensic Sci Med Pathol* 2006;2:75-83.
33. Melanson SE, Baskin L, Magnani B, Kwong TC, Dizon A, Wu AH. Interpretation and utility of drug of abuse immunoassays: lessons from laboratory drug testing surveys. *Arch Pathol Lab Med* 2010;134:735-9.
34. Marin SJ, Doyle K, Chang A, Concheiro-Guisan M,

- Huestis MA, Johnson-Davis KL. One Hundred False-Positive Amphetamine Specimens Characterized by Liquid Chromatography Time-of-Flight Mass Spectrometry. *J Anal Toxicol* 2016;40:37-42.
35. Snyder ML, Fantz CR, Melanson S. Immunoassay-Based Drug Tests Are Inadequately Sensitive for Medication Compliance Monitoring in Patients Treated for Chronic Pain. *Pain Physician* 2017;20(2S):SE1-9.
 36. Tsai IL, Weng TI, Tseng YJ, Tan HK, Sun HJ, Kuo CH. Screening and confirmation of 62 drugs of abuse and metabolites in urine by ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. *J Anal Toxicol* 2013;37:642-51.
 37. Broecker S, Herre S, Wüst B, Zweigenbaum J, Pragst F. Development and practical application of a library of CID accurate mass spectra of more than 2,500 toxic compounds for systematic toxicological analysis by LC-QTOF-MS with data-dependent acquisition. *Anal Bioanal Chem* 2011;400:101-17.
 38. Grapp M, Kaufmann C, Streit F, Binder L. Systematic forensic toxicological analysis by liquid-chromatography-quadrupole-time-of-flight mass spectrometry in serum and comparison to gas chromatography-mass spectrometry. *Forensic Sci Int* 2018;287:63-73.
 39. Macherone A. Chapter 20 – The Future of GC/Q-TOF in Environmental Analysis. In: Ferrier I, Thurman EM, editors. *Comprehensive Analytical Chemistry* 2013;61:471-90.
 40. Abushareeda W, Lyris E, Kraiem S, Wahaibi AA, Alyazidi S, Dbes N, et al. Gas chromatographic quadrupole time-of-flight full scan high resolution mass spectrometric screening of human urine in antidoping analysis. *J Chromatogr B Analyt Technol Biomed Life Sci* 2017;1063:74-83.
 41. Polet M, Van Gansbeke W, Van Eenoo P. Development and validation of an open screening method for doping substances in urine by gas chromatography quadrupole time-of-flight mass spectrometry. *Anal Chim Acta* 2018;1042:52-9.
 42. Kanu AB, Dwivedi P, Tam M, Matz L, Hill HH Jr. Ion mobility-mass spectrometry. *J Mass Spectrom* 2008;43:1-22.
 43. Campbell JL, Le Blanc JC, Kibbey RG. Differential mobility spectrometry: a valuable technology for analyzing challenging biological samples. *Bioanalysis* 2015;7:853-6.
 44. Quintela O, Andrenyak DM, Hoggan AM, Crouch DJ. A validated method for the detection of Delta 9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta 9-tetrahydrocannabinol in oral fluid samples by liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. *J Anal Toxicol* 2007;31:157-64.
 45. Griswold MK, Chai PR, Krotulski AJ, Friscia M, Chapman BP, Varma N, et al. A Novel Oral Fluid Assay (LC-QTOF-MS) for the Detection of Fentanyl and Clandestine Opioids in Oral Fluid After Reported Heroin Overdose. *J Med Toxicol* 2017;13:287-92.
 46. Broecker S, Herre S, Pragst F. General unknown screening in hair by liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). *Forensic Sci Int* 2012;218:68-81.
 47. Aszyk J, Kot-Wasik A. The use of HPLC-Q-TOF-MS for comprehensive screening of drugs and psychoactive substances in hair samples and several “legal highs” products. *Monatsh Chem* 2016;147:1407-14.
 48. Krumbiegel F, Hastedt M, Tsokos M. Nails are a potential alternative matrix to hair for drug analysis in general unknown screenings by liquid-chromatography quadrupole time-of-flight mass spectrometry. *Forensic Sci Med Pathol* 2014;10:496-503.
 49. Costa C, Webb R, Palitsin V, Ismail M, de Puit M, Atkinson S, et al. Rapid, Secure Drug Testing Using Fingerprint Development and Paper Spray Mass Spectrometry. *Clin Chem* 2017;63:1745-52.
 50. Pope JD, Drummer OH, Schneider HG. The cocaine cutting agent levamisole is frequently detected in cocaine users. *Pathology* 2018;50:536-9.
 51. Krotulski AJ, Mohr AL, Fogarty MF, Logan BK. The Detection of Novel Stimulants in Oral Fluid from Users Reporting Ecstasy, Molly and MDMA Ingestion. *J Anal Toxicol* 2018;42:544-53.
 52. Hutcherson Wright A, Young JA, Elise Smith B, Saitman A. The Missing Metabolite: How Unexpected Urine Drug Metabolite Patterns May Lead to False Interpretations. *Clin Chem* 2018;64:1291-3.
 53. Cervinski MA, Jannetto PJ. A Question of Opioid Diversion or Compliance. *Clin Chem* 2019;65:236-40.
 54. King LA, Kicman AT. A brief history of ‘new psychoactive substances’. *Drug Test Anal* 2011;3:401-3.
 55. United Nations Office on Drugs and Crime. World Drug Report 2018. <https://www.unodc.org/wdr2018/> (Accessed 2 January 2019).
 56. Spinelli E, Barnes AJ, Young S, Castaneto MS, Martin TM, Klette KL, et al. Performance characteristics of an ELISA screening assay for urinary synthetic cannabinoids. *Drug Test Anal* 2015;7:467-74.
 57. Franz F, Angerer V, Jechle H, Pegoro M, Ertl H, Weinfurter G, et al. Immunoassay screening in urine for synthetic cannabinoids - an evaluation of the diagnostic efficiency. *Clin Chem Lab Med* 2017;55:1375-84.
 58. Kronstrand R, Brinkhagen L, Birath-Karlsson C, Roman M, Josefsson M. LC-QTOF-MS as a superior strategy to immunoassay for the comprehensive analysis of

- synthetic cannabinoids in urine. *Anal Bioanal Chem* 2014;406:3599-609.
59. Scheidweiler KB, Jarvis MJ, Huestis MA. Nontargeted SWATH acquisition for identifying 47 synthetic cannabinoid metabolites in human urine by liquid chromatography-high-resolution tandem mass spectrometry. *Anal Bioanal Chem* 2015;407:883-97.
60. Paul M, Ippisch J, Herrmann C, Guber S, Schultis W. Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach. *Anal Bioanal Chem* 2014;406:4425-41.
61. Bade R, Tschärke BJ, White JM, Grant S, Mueller JF, O'Brien J, et al. LC-HRMS suspect screening to show spatial patterns of New Psychoactive Substances use in Australia. *Sci Total Environ* 2019;650:2181-7.
62. Pasin D, Cawley A, Bidny S, Fu S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. *Anal Bioanal Chem* 2017;409:5821-36.
63. Gwak S, Almirall JR. Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). *Drug Test Anal* 2015;7:884-93.
64. Rochat B. From targeted quantification to untargeted metabolomics: Why LC-high-resolution-MS will become a key instrument in clinical labs. *Trends Analyt Chem* 2016;84(B):151-64.
65. Rochat B. Quantitative and Qualitative LC-High-Resolution MS: The Technological and Biological Reasons for a Shift of Paradigm. In: Ince M, Ince OK, editors. *Recent Advances in Analytical Chemistry*. Rijeka: IntechOpen; 2018. p. 1-18.
66. Morin LP, Mess JN, Garofolo F. Large-molecule quantification: sensitivity and selectivity head-to-head comparison of triple quadrupole with Q-TOF. *Bioanalysis* 2013;5:1181-93.
67. Chindarkar NS, Park HD, Stone JA, Fitzgerald RL. Comparison of Different Time of Flight-Mass Spectrometry Modes for Small Molecule Quantitative Analysis. *J Anal Toxicol* 2015;39:675-85.
68. Kronstrand R, Forsman M, Roman M. Quantitative analysis of drugs in hair by UHPLC high resolution mass spectrometry. *Forensic Sci Int* 2018;283:9-15.
69. Liu H, Lam L, Yan L, Chi B, Dasgupta PK. Expanding the linear dynamic range for quantitative liquid chromatography-high resolution mass spectrometry utilizing natural isotopologue signals. *Anal Chim Acta* 2014;850:65-70.
70. Noble C, Weihe Dalgaard P, Stybe Johansen S, Linnet K. Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MSE mode and retrospective analysis of authentic forensic blood samples. *Drug Test Anal* 2018;10:651-62.