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Article

Comparison of Different Time of Flight-Mass Spectrometry Modes for Small Molecule Quantitative Analysis

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Currently, the use of time of flight (TOF)-mass spectrometry (MS) in quantitative analysis of small molecules is rare. Recently, the quantitative performance of TOF mass analyzers has improved due to the advancements in TOF technology. We evaluated a Q-TOF-MS in different modes, i.e., Q-TOF-full scan (Q-TOF-FS), Q-TOF-enhanced-full scan (Q-TOF-En-FS), MS^E, Q-TOF-targeted (Q-TOF-TGT), Q-TOF-enhanced-targeted (Q-TOF-En-TGT), and compared their quantitative performance against a unit resolution LC–MS-MS (tandem quadrupole) platform. The five modes were investigated for sensitivity, linearity, signal-to-noise ratio, recovery and precision using 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) as a model compound in electrospray ionization (ESI) with negative polarity. Preliminary studies indicated that Q-TOF-FS mode was the least linear and precise; hence, it was eliminated from further investigation. Total imprecision in remaining four modes was <10%. The Q-TOF-En-FS and Q-TOF-En-TGT showed better signal intensity than their respective modes without enhancement. Overall, peak signal intensity was the highest in MS^E mode, whereas the signal-to-noise ratio was the best in the Q-TOF-En-TGT mode. Relatively, MS^E and Q-TOF-En-TGT modes were the best overall performers compared with the other modes. Both MS^E and Q-TOF-En-TGT modes showed excellent precision (coefficient of variation <6%), patient correlation ($r > 0.99$) and linearity (range, 5–455 ng/mL) for THC-COOH analysis when compared with LC–MS-MS. We also investigated the performance of the same four modes using methamphetamine in positive ESI. Quantitative data obtained by Q-TOF-En-TGT and MS^E, using both positive and negative ESI, suggest that these modes performed better than the other modes. While unit resolution LC–MS-MS remains the optimal technique for quantification, our data showed that Q-TOF-MS can also be used to quantify small molecules in complex biological specimens.

Introduction

Currently, quantitative analysis of small molecules is predominately performed by unit resolution liquid chromatography–tandem quadrupole mass spectrometry (LC–MS-MS). These LC–MS-MS assays typically use multiple reaction monitoring (MRM) to measure two or more product ions and an isotopically labeled internal standard. Product ion ratios are used for compound identification and peak area ratios relative to internal standards are used for quantification. Under certain conditions, some compounds fail to produce two product ions with a consistent ratio, for example when (i) the analyte is present at a relatively low concentration, (ii) there is a co-eluting compound with similar ions and (iii) the fragmentation reaction does not produce diagnostic product ions. Another limitation of MRM-based methods is that they can only measure/monitor a set list of analytes and hence lack the ability to perform untargeted screening.

Non-targeted (broad-spectrum) drug screening can be achieved using high-resolution MS (HRMS) with different mass analyzers including time of flight-MS (TOF-MS) (1–3). HRMS can provide specificity based on accurate mass analysis (3) and has the capability of retrospective analysis. Most toxicology laboratories have been reluctant to use HRMS for quantitation due to concerns related to sensitivity, dynamic range and precision when compared with LC–MS-MS instruments (4). Developments in HRMS platforms have improved its quantitative capability, enabling both highly specific screening and acceptable quantitation (5–9). The number of reports on quantitative HRMS applications in the toxicology laboratory is fairly limited (10–12). In addition, there is no literature comparing various scanning modes available with current Q-TOF-MS instrumentation. Our goal was to evaluate various Q-TOF-MS scanning modes for quantitative analysis of small molecules. The analysis of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine specimens was selected as the model system for negative ions because it is present at relatively low concentrations in a complex sample matrix. There are several published LC–MS-MS methods to quantify cannabinoid metabolites in urine (13–18), but to the best of our knowledge, there are no reports on quantitative analysis of tetrahydrocannabinol metabolites in human urine by Q-TOF-MS. Methamphetamine was used as a model compound to investigate the performance of Q-TOF-MS in the positive ionization mode.

For THC-COOH, initial experiments involved comparing precision, response (area), signal-to-noise (S/N) ratio and linearity of Q-TOF-full scan (Q-TOF-FS), Q-TOF-enhanced-full scan (Q-TOF-En-FS), MS^E, Q-TOF-targeted (Q-TOF-TGT), Q-TOF-enhanced-targeted (Q-TOF-En-TGT) modes and LC–MS-MS. A detailed description of these various modes of operation is presented in the results and discussion section of this study. After initial experiments, the Q-TOF-FS mode was eliminated from further investigation due to limited precision, linearity and sensitivity when compared with the other modes. MS^E, Q-TOF-En-FS, Q-TOF-TGT and Q-TOF-En-TGT modes were further investigated for within- and between-run precision, linearity, sensitivity, accuracy and patient correlation. Similarly, methamphetamine was investigated as a proof of concept representing the analytes that are analyzed in electrospray ionization (ESI), positive mode. The novelty of this study is not in the development of a new assay for measuring THC-COOH or methamphetamine, but in the evaluation of the quantitative performance of different scanning modes that are now available on a variety of TOF and Q-TOF instruments.

Experimental

Reagents

THC-COOH, methamphetamine and their deuterium-labeled analogs (THC-COOH-d_3 and methamphetamine-d_5) used as an internal standard were purchased from Cerilliant (Round Rock, TX, USA). All solvents were high-performance liquid chromatography (HPLC) grade or better. LC-MS grade solvents (methanol and acetonitrile) and hydrochloric acid (trace metal grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). LC–MS grade ammonium acetate, formic acid, aqueous ammonia solution (20–22%), potassium hydroxide, sodium tetraborate decahydrate and glacial acetic acid were obtained from Sigma-Aldrich (St Louis, MO, USA). Water was purified in-house by an ELGA Purelab Ultra Analytic purifier (Siemens Water Technologies, Lowell, MA, USA). Verex Cert+ 9 mm maximum recovery vials were purchased from Phenomenex (Torrance, CA, USA). Twenty milligram, 1-mL of CEREX solid-phase extraction (SPE) strong anion exchange cartridges were utilized for preparing samples for THC-COOH analysis (SPEware Corporation, Baldwin Park, CA, USA). Lyophilized quality control (QC) samples (3,000 ng/mL) and drug-free (human) urine (DFU) were purchased from UTAK Laboratories (Valencia, CA, USA) or the College of American Pathologists (CAP, Northfield, IL, USA).

Calibrators and sample preparation: THC-COOH

A solution of 100 μg/mL THC-COOH (Cerilliant Corporation) was diluted with methanol to prepare working stock solutions at 0.1, 0.4, 2.0, 4.8, 6.5 and 9.1 μg/mL concentrations. Calibrators at 5.0, 19, 98, 238, 327 and 455 ng/mL were prepared as follows. Twenty microliters of aqueous ammonia solution (20–22%) was added to six labeled wide mouthed 2-mL max recovery glass vials. 100 μL of each methanolic working stock calibration solution was added to the matching vials followed by addition of 1.88 mL of DFU. The pH of the resulting calibrator solutions was found to be ~10. The vials were capped and vortexed for at least 20 s and mixed on the rocker for at least 30 min. QC samples were prepared by adding distilled water to lyophilized QC specimens (UTAK Laboratories). Low (22.5 ng/mL) and high (225 ng/mL) QC samples were prepared by mixing a QC sample with DFU. Internal standard (THC-COOH-d_3) stock solution used was at 100 μg/mL (methanol) and stored at −20°C. A working stock of internal standard (2 μg/mL) was prepared by 1 : 50 dilution of the stock solution with methanol.

Fifty microliters of the internal standard solution was pipetted into a 10-mL screw cap glass tube. One milliliter of DFU, calibrator, QC specimen or patient urine specimen was added. One hundred microliters of 10 M potassium hydroxide solution was added in each tube. Tubes were capped and incubated at 60°C for 15 min to hydrolyze the glucuronide. After cooling to room temperature, tubes were centrifuged (900 *g*) for 2 min.

CEREX SPE strong anion exchange cartridges were loaded to the CEREX 48 Pressure Processor (SPEware). SPE cartridge conditioning was not required. Samples were decanted onto the strong anion exchange columns and loaded by applying positive pressure (9–12 psi) using nitrogen gas. Columns were washed with 1 mL of de-ionized water : acetonitrile : ammonium hydroxide solution (85 : 15 : 1; v/v/v) and then dried with a flow of nitrogen gas (≥80 psi) for 3 min. Columns were washed a second time with 1 mL of ethyl acetate with minimal positive pressure and then dried with nitrogen (≥80 psi) for 3 min. The analyte was eluted with 1 mL of hexane : ethyl acetate : glacial acetic acid (80 : 18 : 2; v/v/v) under minimal pressure into a maximum recovery vial (Phenomenex). Eluents were dried under nitrogen at 40°C in a CEREX 48 Sample Concentrator (SPEware Corp.). Eluted samples were reconstituted in 100 μL of 70 : 30, acetonitrile : water, vortexed for 10 s, and loaded onto the autosampler for injection (15 and 2 μL for Q-TOF-MS and LC–MS-MS analysis, respectively).

Calibrators and sample preparation: methamphetamine

The target concentrations of the calibrators were 100, 500, 1,000, and 5,000 (7,500 and 10,000 for linearity) ng/mL, prepared by diluting freshly opened methamphetamine solution (1 mg/mL, Cerilliant Corporation) with drug-free urine.

Methamphetamine-d_5 internal standard (50 μL at 0.15 ng/μL concentration in methanol) was added to each 1.5 mL screw cap autosampler vial labeled as patient specimen, QC or calibrator. A calibrator solution, patient specimen or QC material (50 μL) was added to the respective vials. Saturated borate buffer (50 μL) was added to each vial followed by 1–2 s (vortex) mixing and addition of 600 μL of extraction solvent (75% ethylacetate/25% heptane). Each vial was screw capped and vortexed vigorously on the multi-tube vortexer for 2 min. The vials were centrifuged for 10 min at 900 *g*. The upper organic layer (200 μL) was transferred to a second set of appropriately labeled crimp seal autosampler vials each containing 50 μL of 1% HCl in methanol. Content of the vials was evaporated to dryness under nitrogen at 50° C for 3 min. 750 μL of reconstitution solvent (40% methanol and 60% water) was added to each vial followed by vortex mixing for 1–2 s. Four and 30 μL of reconstituted sample was injected on LC–MS-MS and each Q-TOF-MS mode, respectively.

Liquid chromatography: THC-COOH

Chromatographic separation was performed by Acquity UPLC (Waters Corporation, Milford, MA, USA). Phenomenex Kinetex C8 column (50 × 2.1 mm, 2.6 μm; Phenomenex) was used for chromatographic separation. The autosampler temperature was set at 4°C and column oven at 40°C throughout analyses. Gradient elution was performed with 5 mM ammonium formate in water adjusted to pH 3 (A) and 0.1% formic acid in acetonitrile (v/v) (B) at a flow rate of 0.35 mL/min. The initial gradient conditions were 30% B, hold for 0.1 min, then increase to 95% B at 1.8 min. Ninety-five percent B was maintained until 2.3 min, at which time, the column was re-equilibrated to 30% B over 0.1 min and held for 0.2 min. Total running time was 2.6 min in each analysis.

Liquid chromatography: methamphetamine

Chromatographic separation was performed by Acquity UPLC (Waters Corporation). The UPLC HSS PFP (2.1 × 50 mm, 1.8 μm) column with a guard column (C18, 2.1 mm; Phenomenex) was used. The autosampler temperature was set at 4°C and column oven at 35°C throughout analyses. Gradient elution was performed with 2 mM ammonium acetate in water (0.1% formic acid, v/v) (A) and 2 mM ammonium acetate in methanol (0.1% formic acid, v/v) (B) at a flow rate of 0.40 mL/min. The initial

gradient of 40% B was held for 1.0 min, then increased to 70% B at 1.01 min. Seventy percent B was maintained until 2.0 min, followed by re-equilibration to 40% B at 2.01 min and held for 1.0 min. Total running time was 3.0 min in each analysis.

Mass spectrometry: THC-COOH and methamphetamine

Xevo G2 Q-TOF and Xevo TQ-S tandem MS (Waters Corporation) were used. MS parameters were optimized via a direct infusion of methamphetamine and THC-COOH (500 ng/mL in the initial mobile phase) separately at 10 μL/min (Table I). Optimized source parameters were as follows: capillary, 2.50 kV (THC-COOH) and 0.8 kV (methamphetamine); source temperature, 150°C; desolvation temperature, 500°C (for THC-COOH) and 600°C (for methamphetamine); and desolvation gas flow 1,000 L/h. Q-TOF was operated at ~20,000 (FWHM) resolution. Data acquisition and analysis were performed using the MassLynx™ V4.1 software, SCN862 and SCN901 (Waters Corporation), respectively. The effect of lock mass on the intensity of analyte was evaluated by comparing the data obtained with and without lock mass. Leucine enkephalin was used as the lock mass standard and the data were acquired every 15 s during the sample analysis using a baffle to isolate lock mass spray from sample analysis spray. A separate MRM-based LC–MS-MS (UPLC-Xevo TQ-S) was developed specifically for comparing the results with the various Q-TOF-MS modes of analysis (Table I). This LC-MS-MS was operated at unit mass resolution (±0.7 amu FWHM).

Validation

Sensitivity, linearity, imprecision and analytical recovery were investigated to evaluate method integrity.

The sensitivity of the different Q-TOF-MS modes was tested by comparing the signals obtained for analyte (peak area) in blank samples (10 samples) with those obtained from samples fortified at a concentration corresponding to the lower limits of quantitation (LLOQ). LLOQ was calculated by interpolation of the value corresponding to 10 times the S/N ratio for drug-free matrix samples in the calibration curve. The values obtained were validated by spiking drug-free urine and demonstrating acceptable bias and imprecision along with acceptable chromatographic peak shape and a relative retention time (to the internal standard) of 1.02 ± 0.02. The criteria for accuracy were within 20% of the target concentration and for precision was coefficient of variation (CV) <20% ($n = 5$). Imprecision and bias were evaluated at two QC concentrations spanning the dynamic linear range. Within-run % CV was evaluated by five determinations per concentration in 1 day. Between-day precision was done for each QC level ($N = 5$) for 5 days. Total CV was evaluated for five replicates per concentration on 5 days (N total = 25). Imprecision data using QC material was obtained using four modes of the Q-TOF-MS as well as LC–MS-MS. Statistical data were generated using EP evaluator version 10 (Data Innovations LLC, South Burlington, VT, USA).

Linearity was performed using a Clinical and Laboratory Standards Institute document EP6-A (19). Five sets of eight levels of THC-COOH calibrators ranging from 5 to 2,000 ng/mL were prepared by mixing stock solution of THC-COOH (100 μg/mL, in methanol) with DFU. The linearity of all five sets of calibrators was assessed by linear regression analysis. Five sets of six levels of methamphetamine calibrators ranging from 100 to 10,000 ng/mL were prepared by mixing stock solution of methamphetamine (100 μg/mL, in methanol) with DFU. The linearity of all five sets of calibrators was assessed by linear regression analysis.

Comparison of four Q-TOF-MS modes

Each of the Q-TOF-MS modes was first optimized for ionization of THC-COOH and methamphetamine (Table I). We also compared the THC-COOH and methamphetamine concentrations measured using the four different Q-TOF-MS modes and LC–MS-MS for 48 and 61 patient specimens, respectively. These patient specimens were selected because they were found to be positive on by immunoassay screening. THC immunoassay was performed by Roche Cobas analyzer (Roche Diagnostics, Mannheim, Germany) with a cutoff for a positive result of 100 ng/mL. Methamphetamine was screened using the amphetamines II immunoassay on the Roche Cobas analyzer (Roche Diagnostics) with a cutoff for a positive result of 1,000 ng/mL. Q-TOF-MS data on samples were obtained by all four modes and compared with those obtained by LC–MS-MS.

The effect of lock mass on Q-TOF-MS data

The effect of lock mass was evaluated in MS^E and Q-TOF-En-TGT modes. We compared the peak signal intensity of six THC-COOH calibrators, three patient urine samples and four QCs (two each from UTAK Laboratories and CAP) obtained with and without

Table I
Parameters Used for Analysis of THC-COOH and Methamphetamine in Four Q-TOF-MS Modes and LC–MS-MS

	Q-TOF-En-FS	MS^E	Q-TOF-TGT	Q-TOF-En-TGT	LC–MS-MS
THC-COOH					
Quantitation transition/ion	245.1547	343.1915	343.1915 → 245.1547	343.1915 → 245.1547	343.5 → 245.3[a]
Qualifier transition/ion	299.2017	299.2017	343.1915 → 299.2017	343.1915 → 299.2017	343.5 → 191.3[b]
Target enhancement (m/z)	350	NA	NA	350	NA
Collision energy	30 V	6*/30**	30 V	30 V	30[a] and 30[a]
Methamphetamine					
Quantitation transition/ion	119.0855	150.1283	150.1283 → 119.0855	150.1283 → 119.0855	150.0 → 119.1[c]
Qualifier transition/ion	91.0542	119.0855	150.1283 → 91.0542	150.1283 → 91.0542	150.1 → 91.1[d]
Target enhancement (m/z)	150	NA	NA	150	NA
Collision energy	10 V	6*/10**	10 V	10 V	10[c] and 10[d]

NA, not available; *, collision energy used for low energy scan; **, collision energy used for high energy scan; V, volts.
[a,b,c,d]The collision energies used for respective transitions.

lock mass. Leucine enkephalin was used as the lockspray reference compound [$(M-H)^-$, 554.2771]. Lockspray data were collected and the lock spray mass correction was applied to evaluate the effect of lock mass in MS^E and Q-TOF-En-TGT modes. All other data were obtained without lock mass data.

Results and discussion

Xevo G2 Q-TOF is a hybrid HRMS type instrument. It consists of a unit resolution quadrupole and a high-resolution TOF mass analyzers working in tandem separated by a collision cell. This instrument can be operated in five different modes, i.e., Q-TOF-FS, Q-TOF-enhanced, MS^E, Q-TOF-TGT and Q-TOF-En-TGT modes.

Target enhancement in enhanced modes

In the older generation of orthogonal acceleration (oa)-TOF instruments, a continuous stream of ions is conveyed into the acceleration region in front of the pusher plate (20–22). A pulse is applied to the pusher plate at a regular interval to push a batch of ions of various m/z from the continuous stream of ions. The duty cycle of these oa-TOF instruments is dependent on the flight time of the heaviest ion in the batch of ions pushed in the flight tube and it is relatively small for smaller ions compared with heavier ones. There is a time gap between consecutive pulses since the next pusher pulse is applied when the previous batch of ions reaches the detector. Under these settings, the ions continuously entering the acceleration region are lost due to the lack of pusher pulse when the ions from previous batch are still traveling in the flight tube.

The Q-TOF-MS instrument used in our investigation uses a traveling wave technology in the collision cell (23, 24) that transfers ions to the TOF in packets instead of as a continuous stream. When the target enhancement mode is used, the timing of the packets released from the traveling wave collision cell (the trap) into the TOF is m/z dependent. By synchronizing the pusher pulse to the desired m/z range arriving from the collision cell (23), the duty cycle for the target m/z range of ions is enhanced (23). This technique is advantageous in situations where recording of a wide mass range is not essential and allows for faster scanning over a limited m/z range to enhance sensitivity.

Q-TOF modes

(i) *Q-TOF-FS mode*: in this mode, the quadrupole mass analyzer acts as a radio frequency (RF)-only device that allows all ions to pass through and enter in the collision cell. Ions entering the collision cell are kept intact by applying minimal collision energy followed by full-scan analysis in the TOF analyzer.

(ii) *MS^E mode*: non-targeted drug screening and confirmation can be achieved in one analysis by Q-TOF-MS using various techniques such as 'All Ions MS-MS (Agilent Technologies)', MS^E (Waters Corporation), and MS^{ALL} and Sequential Window Acquisition of all Theoretical Fragment Ion Spectra (SWATH) (SCIEX) available from different MS vendors. These techniques obtain precursor and fragment ion information for the analyte in a single run using similar techniques. MS^E is a patented technology available only on Waters Corporation's newer Q-TOF-MS instruments. In this mode, the quadrupole acts as a RF-only device which transfers all ions to collision cell. MS^E is a dual scan mode where the instrument acquires low and high energy scan alternatively. For the low energy scan, minimal collision energy is applied in the collision cell; hence, minimal or no fragmentation takes place. All ions enter the TOF analyzer to produce a low energy scan that provides intact precursor ion information. To acquire the high energy scan, all ions entering in the collision cell are subjected to a collision energy ramp. Hence, all ions experience the collision energy and undergo fragmentation. Fragments are then analyzed in the TOF to obtain a high energy scan that provides fragment ion information. Each raw data file in MS^E mode has two channels; Channel 1 contains low energy scans (precursor ions), whereas channel 2 consists of high energy (fragment ions) scans. Figure 1 shows an example of data acquired using MS^E. Channel 1 shows the THC-COOH peak at 1.86 min consisting of low energy scans obtained by applying minimal collision energy, 6 V. Corresponding averaged mass spectrum (Part A) shows m/z 343.1917, which is an intact ion of THC-COOH with minimal fragmentation. For THC-COOH analysis, m/z 343.1917 obtained from low energy scan was used as the quantitative ion. The peak at 1.86 min in Channel 2 consists of high energy scans obtained by applying a collision energy of 30 V and its averaged mass spectrum (Part B) shows major fragments of THC-COOH at m/z 299.2020 and 245.1549 as well as the intact THC-COOH ion at m/z 343.1917. Similarly for methamphetamine, channel 1 (low energy scan) produced predominantly m/z 150.1283 and was used as the quantitation ion. Channel 2 (high energy scan) produced m/z 91.0542 and 119.0855 which were used as qualifier ions.

(iii) *Q-TOF-En-FS mode*: This mode functions as a Q-TOF-FS mode described above except that the analysis in the TOF analyzer is carried out differently. A desired mass range of ions entering the TOF analyzer can be enhanced as described above under 'target enhancement in the enhanced mode'.

(iv) *Q-TOF-TGT and Q-TOF-En-TGT mode*: These modes function in a similar fashion as tandem quadrupole instruments except that the second quadrupole is replaced with TOF mass analyzer. In this mode the Q-TOF functions by selecting a precursor ion in the quadrupole, fragmenting the selected ion in the collision cell followed by product ion analysis in the TOF. Enhancement of ions in the Q-TOF-TGT mode is achieved as described under 'target enhancement in the enhanced mode'.

When a m/z is selected for enhancement, this leads to improved intensity of masses below the set mass. When operated in the enhanced mode, the intensity of the ions in the desired range is higher than when operated in the non-enhanced mode. In the negative mode, THC-COOH forms an ion at m/z 343.1915. At collision energy of 30 V, this ion produces product ions at m/z 245.1547 and 299.2017 with optimal intensity. Hence, in all modes, we kept the collision energy at 30 V except during low energy scan in the MS^E mode. In the positive mode,

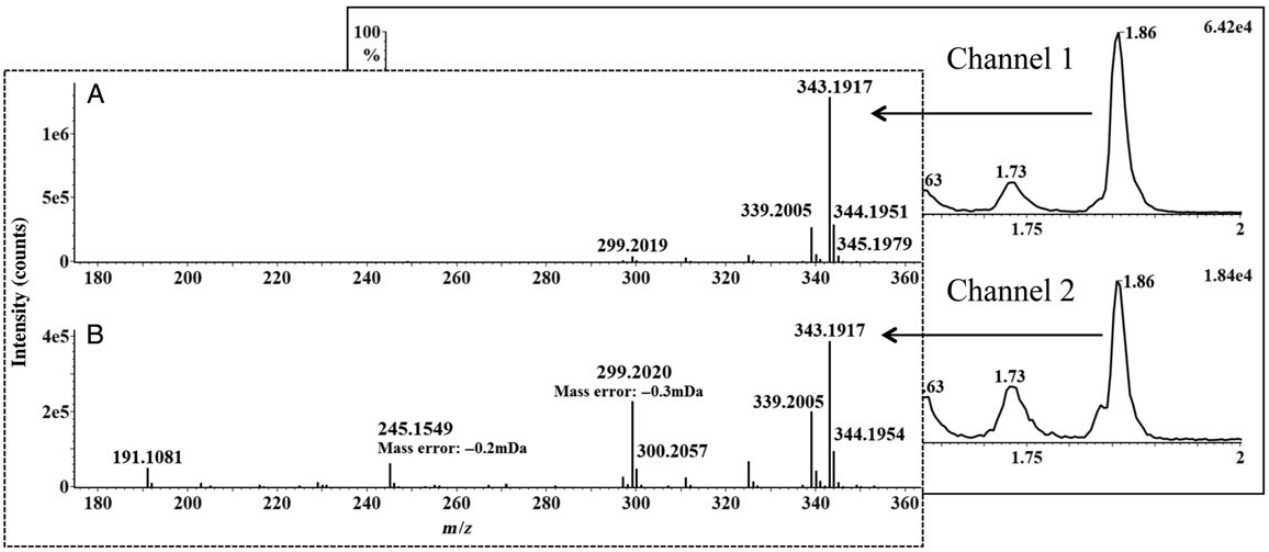

Figure 1. MS^E data of THC-COOH. Channels 1 and 2 are total ion chromatograms of low and high energy scans, respectively. A and B are averaged mass spectra of peaks at 1.86 min in the Channel 1 and 2, respectively.

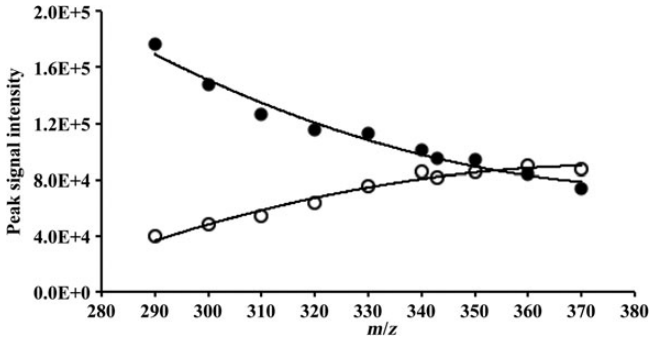

Figure 2. Target enhancement: the optimization of THC-COOH product ions. Peak signal intensity of product ions varies with different enhanced targets in the TOF analyzer. Black circles indicate intensity of m/z 245.1547 and white circles indicate intensity of m/z 299.2017.

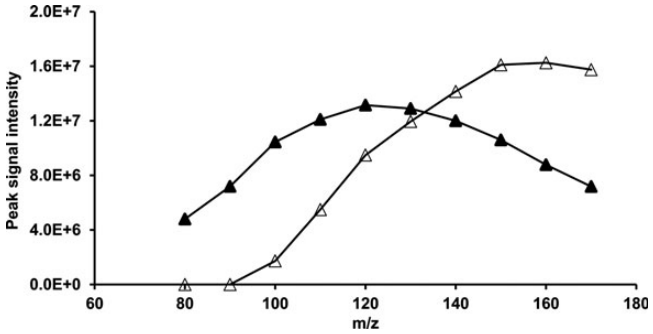

Figure 3. Target enhancement: the optimization of methamphetamine product ions. Peak signal intensity of product ions varies with different enhanced targets in the TOF analyzer. Black triangles indicate intensity of m/z 91.0542 and white triangles indicate intensity of m/z 119.0855.

methamphetamine forms an ion at m/z 150.1283. At collision energy of 10 V, this ion produces product ions at m/z 91.0542 and 119.0855 with optimal intensity. Hence, for methamphetamine, we kept the collision energy at 10 V for all modes except during low energy scan in the MS^E mode.

To enhance the intensity of THC-COOH product ions in the TOF analyzer, we evaluated various target m/z for enhancements by selecting m/z 290–370 with an increment of 10 Da. Figure 2 shows the plot of enhanced target and corresponding intensities of m/z 245.1547 and 299.2017 at that target. The peak signal intensity of m/z 245.1547 fragment ion decreased when the target for enhancement varied from m/z 290 to 370. The peak signal intensity of m/z 299.2017 fragment ion increased as the target for enhancement increased until it plateaued at m/z 340. There was no significant change in intensity between m/z 340 and 370. The optimal peak signal intensity for the combination of the two product ions monitored was m/z 350 (Figure 2). This target enhancement was used for both Q-TOF-enhanced and Q-TOF-En-TGT modes. Similarly, methamphetamine product ions were also investigated for enhancements by selecting m/z 80–170 with an increment of 10 Da (Figure 3). We observed that the optimal peak signal intensity for m/z 91.0542 and 119.0855 product ions was obtained at the target enhancement of m/z 150.

Data Processing

To determine the optimum parameters for data processing, a set of data were processed with different mass extraction windows. We used absolute mass windows of ± 0.005, 0.0125, 0.025, 0.050, 0.250, 0.500 Da (data not shown). As anticipated, a wider mass window of ± 0.500 Da yielded the most signal intensity where as smaller mass windows provided better S/N ratios. We processed the data using ± 0.500 Da for all of the data presented in this manuscript in order to achieve the desired LOD of 5 ng/mL for THC-COOH.

Linearity

All four modes showed excellent linearity for THC-COOH and methamphetamine. THC-COOH was linear from 5 to

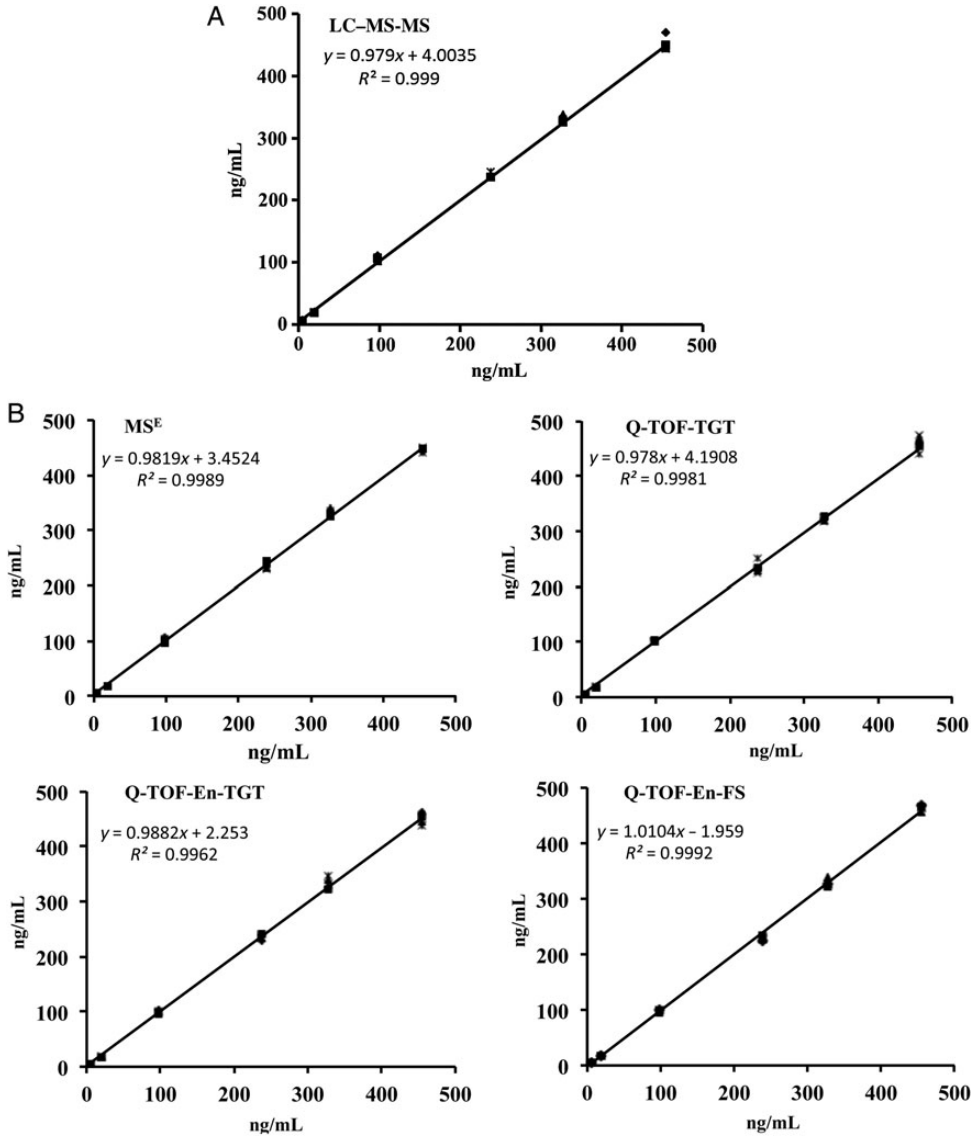

Figure 4. Cumulative calibration curves for THC-COOH in urine obtained by four modes of Q-TOF-MS. Six point calibrators were tested five times at each concentration. *X*-axis indicates expected concentration and *y*-axis indicates measured concentration.

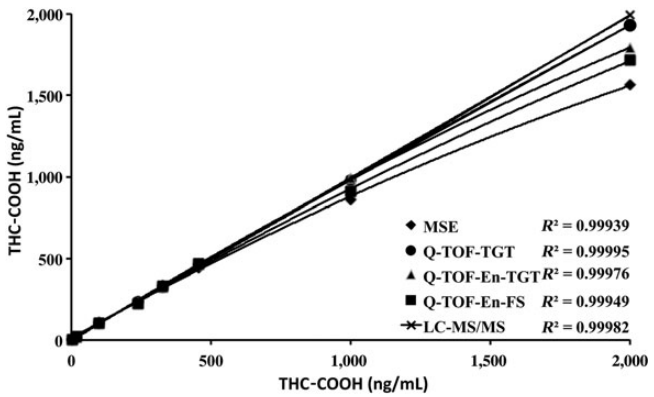

Figure 5. Extended calibration curves (polynomial fit) for THC-COOH in urine obtained by four Q-TOF-MS and LC–MS-MS over a range of 5–2,000 ng/mL. *X*-axis indicates expected concentration and *y*-axis indicates measured concentration.

455 ng/mL as demonstrated by calibrators (THC-COOH fortified into DFU). Slope, *y*-intercept and coefficient of determination (R^2) of TOF modes and LC–MS-MS are described in Figure 4, where the *x*-axis represents the expected THC-COOH concentrations and *y*-axis represents the measured THC-COOH levels. All R^2 values in TOF modes and LC–MS-MS exceeded 0.99. Linear regression analysis showed slopes ranged from 0.980 (Q-TOF-TGT) to 1.0104 (Q-TOF-En-FS) and *y*-intercept distributed between −1.959 (Q-TOF-En-FS) and 4.191 (Q-TOF-TGT). For LC–MS-MS, the slope was 0.979 and the *y*-intercept was 4.004. Also, we investigated the linearity of the four Q-TOF-MS modes over 5–2,000 ng/mL concentration range. Above 455 ng/mL some of the Q-TOF-MS modes demonstrated non linearity requiring a polynomial fit as shown in Figure 5. Although R^2 values for modes were >0.999, Q-TOF-TGT mode showed the best linearity when compared with LC–MS-MS linearity. Using positive ESI, for

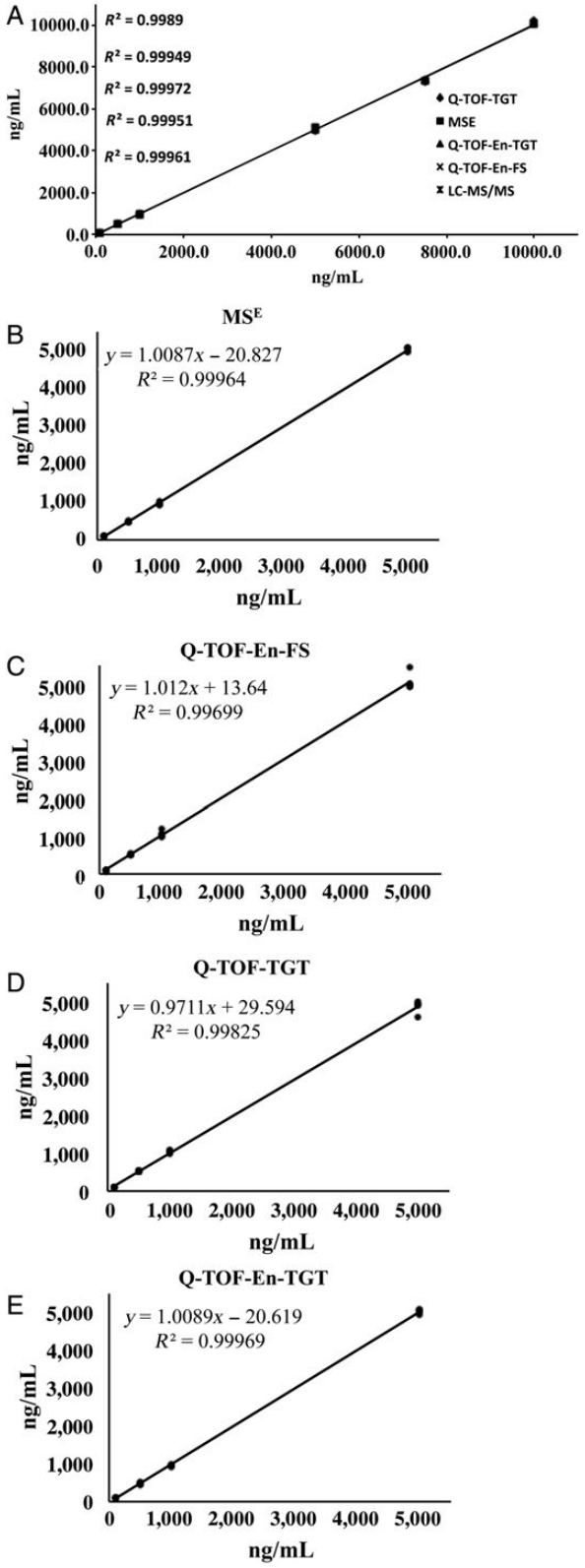

Figure 6. Plot A: extended calibration curves for methamphetamine in urine obtained by four Q-TOF-MS modes and LC–MS-MS over a range of 100–10,000 ng/mL. *X*-axis indicates expected concentration and *y*-axis indicates measured concentration. Plots B–E: cumulative calibration curves for methamphetamine in urine obtained by four modes of TOF-MS. Four point calibrators were tested five times at each concentration. *X*-axis indicates expected concentration and *y*-axis indicates measured concentration.

Table II
Imprecision Data for THC-COOH and Methamphetamine in Urine by Four Q-TOF-MS modes

Mode	Within-run CV (%, $n = 5$)		Between-day CV (%, $n = 5$)		Total CV (%, $n = 25$)	
	Low	High	Low	High	Low	High
THC-COOH						
MS^E	2.8	2.6	4.5	2.6	5.3	3.7
Q-TOF-TGT	4.8	3.3	3.1	0.8	5.7	3.4
Q-TOF-En-TGT	2.6	2.8	4.9	4.6	5.6	5.3
Q-TOF-En-FS	6.3	3.1	6.2	3.0	8.8	4.3
LC–MS-MS	2.3	2.7	6.5	3.4	6.9	4.4
Methamphetamine						
MS^E	2.4	2.3	4.4	6.5	5.0	6.9
Q-TOF-TGT	4.4	2.1	2.7	4.1	5.2	4.7
Q-TOF-En-TGT	3.9	2.0	5.1	4.9	6.4	5.3
Q-TOF-En-FS	4.2	3.4	3.6	4.3	5.6	5.5
LC–MS-MS	1.2	1.3	1.0	2.1	1.6	2.5

THC-COOH low- and high-quality control target concentrations were 22.5 and 225 ng/mL, respectively. Methamphetamine low- and high-quality control target concentrations were 300 and 3,000 ng/mL, respectively.
CV, coefficient of variation.

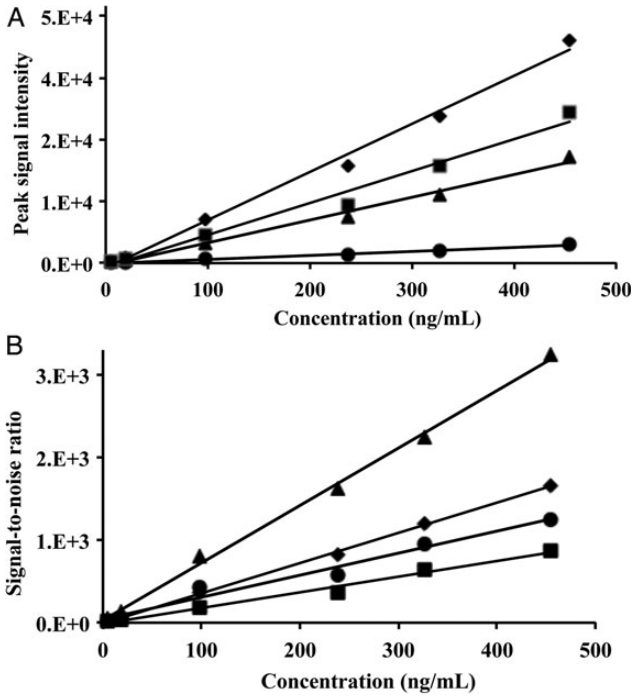

Figure 7. Peak signal intensity (A) and S/N ratio (B) for THC-COOH calibrators in four Q-TOF-MS modes. Each point indicates the mean value of five replicates. Diamond, MSE mode; circle, Q-TOF-TGT mode; triangle, Q-TOF-En-TGT mode; square, Q-TOF-En-FS.

methamphetamine, all modes showed excellent linearity in both 100–5,000 and 100–10,000 ng/mL concentration ranges (Figure 6).

Imprecision and bias

Imprecision and analytical recovery was evaluated at two concentrations (22.5 and 225 ng/mL) of THC-COOH across the linear dynamic range (Table II). Overall, within-run, between-run and total CV for the four modes were 2.6–6.3, 0.8–6.2 and 3.4–8.8%, respectively. For LC–MS-MS, within-run, between-run

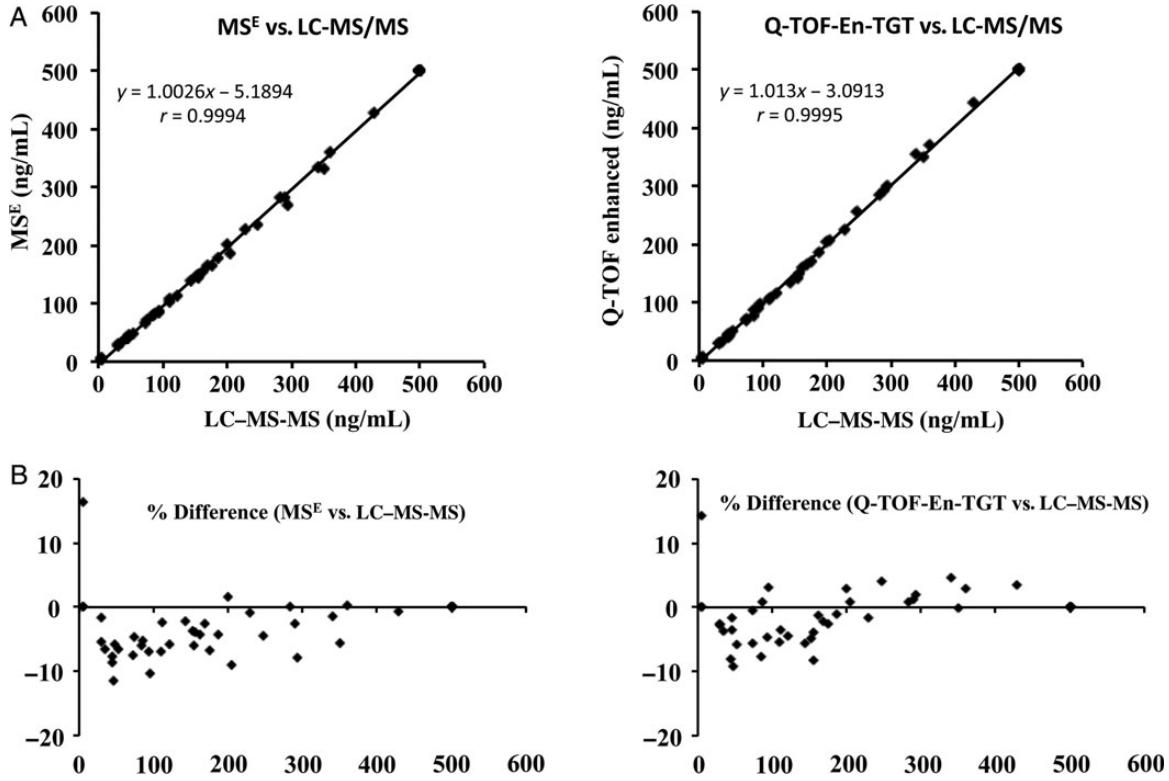

Figure 8. Patient correlation between two Q-TOF-MS modes and LC–MS-MS. Comparison of THC-COOH values measured by TOF (MS^E mode and Q-TOF-En-TGT mode) and LC–MS-MS (A), and percent difference (B).

and total CV were 2.3–2.7, 3.4–6.5 and 4.4–6.9%, respectively. Specific imprecision data for TOF and LC–MS-MS modes of analysis are described in Table II. Overall, total imprecision for TOF modes was <9% which was similar to the precision of LC–MS-MS (<7%). Compared with LC–MS-MS, the analytical recovery in four modes of Q-TOF-TGT ranged from 95.4 to 99.0%. For methamphetamine, overall imprecision was better than THC-COOH (Table II). The imprecision was <7% for all modes while for LC–MS-MS, it was <2.6%.

Peak signal intensity and S/N ratio of calibrators in four Q-TOF-MS modes when compared with LC–MS-MS

The main sensitivity difference between the Q-TOF-MS and LC–MS-MS instruments used in this investigation arises from the stepwave ions optics used in the LC–MS-MS, which is absent in the Q-TOF-MS. In addition, the aperture on the LC–MS-MS instrument that allows ions into the mass analyzer region is larger than the one on the Q-TOF-MS instrument. These differences in ions optics result in an increase in the ion flux on a tandem quadrupole instrument. Peak signal intensity of the quantitation ion of THC-COOH calibrators was the strongest in the MS^E mode and the weakest in the Q-TOF-TGT mode (Figure 7A). Methamphetamine showed strongest signal intensity in the Q-TOF-En-FS mode, followed by MS^E, Q-TOF-En-TGT and Q-TOF-TGT. However, as expected the S/N ratio for THC-COOH was highest in the Q-TOF-En-TGT mode, and the lowest in the Q-TOF-En-FS mode (Figure 7B). The MS^E mode collects data in a full-scan mode and hence showed the strongest peak signal intensity. Based on peak signal intensity, linearity and S/N ratio, MS^E and Q-TOF-En-TGT modes were selected as the best Q-TOF-MS modes for further study (patient comparisons). Compared with LC–MS-MS, the main drawback of Q-TOF-MS, regardless of scan mode, was that this instrument was less sensitive. We overcame this issue by injecting a larger sample volume (15 µL in Q-TOF-MS modes vs. 2 µL in LC–MS-MS for THC-COOH and 30 µL in Q-TOF-MS modes vs. 4 µL in LC–MS-MS for methamphetamine).

Patient specimen data by TOF and LC–MS-MS

Since MS^E and Q-TOF-En-TGT modes provided the optimal S/N, linearity and precision, we selected these two modes to compare with LC–MS-MS for quantifying THC-COOH concentrations in 48 patient urine samples (Figure 8A). Correlation coefficients (r) for MS^E and Q-TOF-En-TGT mode were 0.9994 and 0.9995, respectively. The mean concentration difference was −3 and −1% for MS^E and Q-TOF-En-TGT modes, respectively (Figure 8B). Overall difference between Q-TOF-En-TGT mode and LC–MS-MS was <10%, with exception of one specimen. About 94% of patient samples (45/48 patients) showed <10% concentration difference between MS^E and LC–MS-MS.

For methamphetamine, 61 patient (urine) specimens were analyzed using MS^E and Q-TOF-En-TGT modes; the resulting concentrations were compared with concentration obtained by LC–MS-MS. MS^E and Q-TOF-En-TGT showed excellent correlation with LC–MS-MS values (Figure 9). The correlation coefficients (r) for MS^E and Q-TOF-En-TGT modes were 0.9991 and 0.997, respectively. Overall difference between MS^E and LC–MS-MS was <10% with the exception of one specimen (% difference—

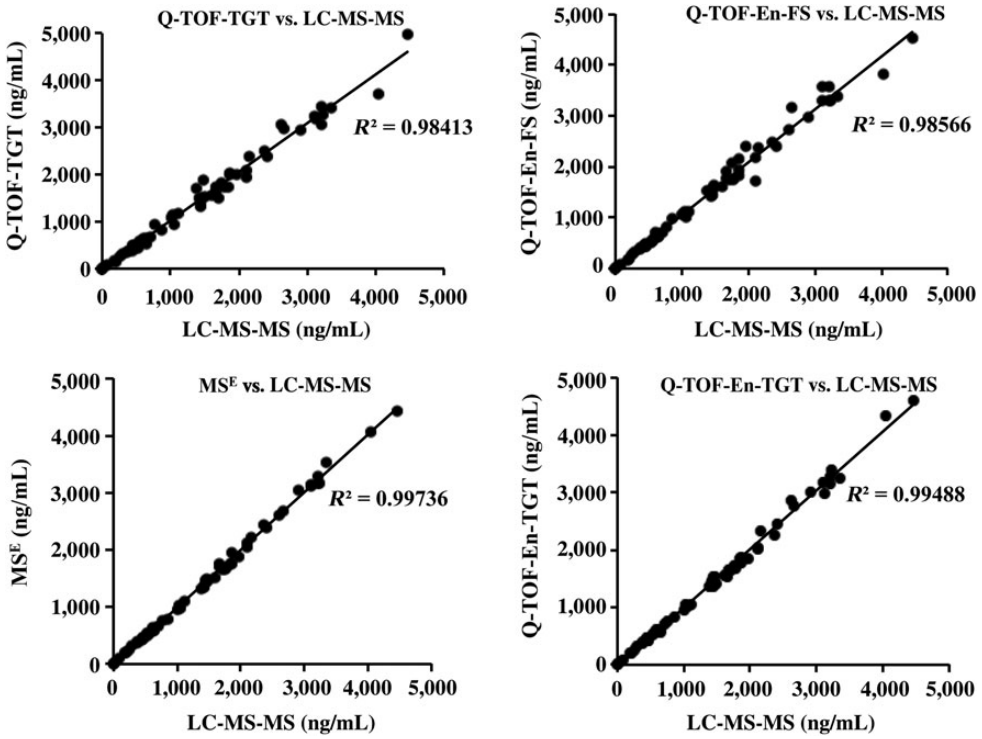

Figure 9. Patient ($n = 61$) correlation between four Q-TOF-MS modes and LC–MS-MS. Comparison of methamphetamine values measured by Q-TOF-MS (Q-TOF-En-FS, MS^E, Q-TOF-En-TGT and Q-TOF-TGT modes) and LC–MS-MS.

23%), while between Q-TOF-En-TGT mode and LC–MS-MS was also <10% except two specimens (14.3 and 11.5%).

The effect of lock mass in TOF data

Xevo G2 Q-TOF is equipped with a dual electrospray (probe) ion source that can spray a sample (LC inlet) and a calibration standard solution (lock spray) in a rapidly alternating manner. The two probes are located at a 90° angle to each other. A baffle is used to allow spray from one electrospray to enter the MS at a given time and thus minimizes the effect of the lock spray on ionization of the analyte. The lock mass is the m/z of an ion of the calibration standard (leucine encephalin), which is introduced separately into the ion source during analysis. The lock mass permits real-time recalibration of mass accuracy by correcting any sources of measurement error that the analyte may encounter throughout a sample run. During the sample run, lock mass data are acquired every 15 s for a total of 0.45 s (0.15 s/scan × 3 scans) at which only lock mass data are acquired and not sample data. Thus, the time is lost to acquire lock mass data, which would have been otherwise used to acquire sample data.

We investigated the effect of lock mass on the signal intensity of THC-COOH. The peak signal intensity of THC-COOH calibrators without lock mass was higher than those with lock mass in both MS^E and Q-TOF-En-TGT modes. On an average the signal decreased by about 50% when the lock mass data were acquired. This indicates that the acquisition of lock mass data adversely affects the duty cycle of the instrument, lowering the peak signal intensity of the analyte of interest. However, the quantitative accuracy of THC-COOH was not affected by the use of lock mass. In QC materials and patient urine samples, mean THC-COOH concentration without lock mass was 3.6% higher than that with lock mass in MS^E mode and 1.3% lower in Q-TOF-En-TGT mode, respectively. As expected, without the lock mass data, the mass accuracy was adversely affected.

Ion ratios

The ion ratio (ratio of peak areas of quantitation and qualifier ion) of an analyte should remain constant under a set of LC–MS-MS conditions. Ion ratios have been an essential component for LC–MS-MS identification of small molecules. In both Q-TOF-TGT and Q-TOF-En-TGT, the precursor ion is selected by the quadrupole and is subjected to controlled fragmentation in the collision cell followed by analysis of fragment ions in the TOF. Ion ratios in the Q-TOF-TGT and Q-TOF-En-TGT modes can be calculated in a similar manner as the LC–MS-MS mode. In the MS^E mode, the low energy scan produces analyte ions without significant fragmentation. For THC-COOH, m/z 343.1915 produced in low energy scan was used as the quantitation ion. The high energy scan captures fragments of precursor ions entering the collision cell without any pre-selection. To calculate ion ratios in the MS^E mode, we used two fragment ions (m/z 245.1547 and 299.2017) from the high energy scan. For Q-TOF-FS and Q-TOF-En-FS, we applied a collision energy (30 V) to produce m/z 245.1547 and 299.2017 ions. These two ions were used to obtain the ion ratios for Q-TOF-FS and Q-TOF-En-FS modes as well. In full-scan modes such as MS^E, Q-TOF-FS and Q-TOF-En-FS, precursor ion selection is not programmed but the fragmentation of all ions is achieved by applying high

collision energy to all ions entering the collision cell. Under such scenarios, even though we obtain fragment ion, the lineage of such fragments is not definitive. Hence, it is necessary to have a TOF analyzer with ~20,000 resolution (FWHM), which results in mass accuracy of $\leq$5 ppm in order to discern the desired fragment ions from possible interferences. The ion ratio of all true positives were within a set limit of $\pm$ 30% except two specimens were the concentration were $<$100 ng/mL. Both specimens failed the ion ratio in MS^E and Q-TOF-En-FS modes.

Conclusions

We investigated four modes (MS^E, Q-TOF-En-FS, Q-TOF-TGT and Q-TOF-En-TGT) of a Q-TOF-MS for quantitation of THC-COOH in urine. Sensitivity, linearity and precision of these different TOF scanning modes were compared. We also examined the effect of lock mass corrections on quantitative accuracy. Overall, Q-TOF-En-TGT and MS^E modes performed better than the other modes. Both MS^E and Q-TOF-En-TGT modes showed excellent precision (CV, $<$6%), and linearity for THC-COOH and methamphetamine analyses. When compared with LC–MS-MS on patient specimens, both MS^E and Q-TOF-En-TGT modes showed excellent correlation ($r > 0.99$). The sensitivity of Q-TOF-MS was lower than the LC–MS-MS. We overcame this challenge by injecting larger volume of specimens on Q-TOF-MS. While unit resolution LC–MS-MS remains the most sensitive technique for quantitation, our data demonstrate that Q-TOF-MS can also be used to quantify small molecules of toxicological interest.

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