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Quantification and Rapid Metabolite Identification in Drug Discovery Using API Time-of-Flight LC/MS

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Liquid chromatography/mass spectrometry (LC/MS), utilizing a time-of-flight (TOF) mass analyzer, has been evaluated and applied to problems in bioanalysis for pharmacokinetics and drug metabolism. The data obtained by TOF MS differ from those obtained using quadrupole mass spectrometer instruments in that full-scan spectra can be routinely collected with greater sensitivity and speed. Both quantitative and qualitative information, including compound concentration in rat plasma and full-scan atmospheric pressure ionization mass spectra, are concurrently obtained. This approach has been used to characterize the disposition of several drug compounds that have been simultaneously dosed to rats in a cassette format. Quantitation limits in the 5−25 ng/mL range (∼20 nM) were obtained from nominal mass chromatograms (0.5 Da resolution). A reference lock mass was used to provide accurate mass measurement to reach third decimal place accuracy in the monoisotopic molecular weight. An improvement in quantitation limits was demonstrated after using accurate mass determinations. Several possible preliminary drug metabolites were confirmed or refuted, based on accurate mass. The trend of metabolite formation and clearance was qualitatively evaluated.

The widespread use of liquid chromatography/tandem mass spectrometry (LC/MS/MS) in conjunction with atmospheric pressure ionization (API) has revolutionized bioanalytical chemistry and the drug discovery process. Because of the high degree of selectivity routinely provided by the multiple-reaction-monitoring experiment (MRM), bioanalytical method development time for quantitative determinations of one or several analytes has been reduced to a few days or less.[1−4] Quantitation limits for these types of methods in many common matrixes, such as plasma, serum, or cellular media, are generally less than a few nanograms per milliliter of sample. Although the sensitivity, selectivity, and efficiency of the MRM approach are excellent, one shortcoming is a scarcity of qualitative information needed to support the recognition and structural elucidation of metabolites that could be present.[5] Normally metabolite recognition and structure elucidation requires extensive method development in an effort that is separate and distinct from the quantitation of the known components present.[6] This additional effort can extend time requirements for drug discovery processes.

One alternative solution to this situation is the use of time-of-flight LC/MS (LC/TOF MS) to generate data that will simultaneously provide qualitative and quantitative information about drug candidate metabolism and disposition. During spectral scanning, the duty cycle of a quadrupole mass spectrometer is such that only a small fraction of the total time is spend monitoring any one ion. To obtain optimum signal-to-noise ratios (S/N), a quadrupole analyzer must allow a limited number of selected ions to pass. Most ions are filtered out, along with much of the qualitative information content.[7,8] Conversely, time-of-flight instruments inherently conserve, separate, and detect a significantly greater percentage (5−50%) of the ions that have been sampled into the high-vacuum region.[10] This enhanced ion throughput allows time-of-flight instruments to obtain full-scan spectra with better signal-to-noise characteristics than comparable spectra obtained with a scanned quadrupole. While time-of-flight data appear to be 1 order of magnitude more sensitive than data obtained from single-quadrupole instruments,[10] they cannot yet match the signal-to-noise ratios obtained from tandem mass spectrometry experiments such as MRM. The ability of time-of-flight instruments to collect and retain complete mass spectra can, however, greatly add to the information content of the LC/MS experiment.[11]

In this paper, we demonstrate the use of a liquid chromatography/time-of-flight mass spectrometry instrument to quantify and characterize the disposition of several example drug compounds that have been intravenously dosed into rats. The quantitative performance of this instrument is compared to that obtained using

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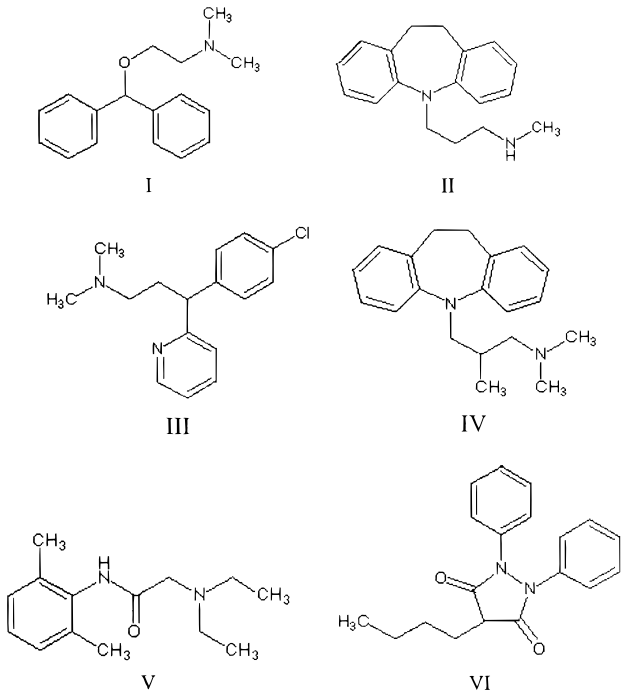

Figure 1. Chemical structures for test analytes used in this work: diphenhydramine (**I**), desipramine (**II**), chlorpheniramine (**III**), trimipramine (**IV**), lidocaine (**V**), and phenylbutazone (**VI**).

a triple-quadrupole instrument operating under a multiple-reaction-monitoring technique. Time-of-flight quantitation limits from the nominal mass and accurate mass experiments are compared. Using spectra in conjunction with predictions based on a general knowledge of drug metabolism pathways, the structures of proposed metabolites for each dosed compound are confirmed or denied. The lock-mass feature of the time-of-flight instrument allowed an exact molecular mass determination of each metabolite to be made, thereby providing molecular formula confirmation for unknown metabolites.

EXPERIMENTAL SECTION

Materials. Test compounds (Figure 1) were obtained from Sigma (St. Louis, MO). They are designated as diphenhydramine (**I**), desipramine (**II**), chlorpheniramine (**III**), and trimipramine (**IV**). Lidocaine (**V**) was chosen as an internal standard, and phenylbutazone (**VI**) was selected as a lock-mass reference compound. Liquid nitrogen, used in the mass spectrometer ion source as a drying and nebulizing gas, was purchased from AGA (Maumee, OH). Sodium hydroxide, ammonium acetate, potassium carbonate, and acetic acid were obtained from EM Science (Gibbstown, NJ). Reagent-grade water was prepared from in-house deionized water using a Milli-Q system (Millipore, Milford, MA). HPLC-grade ethyl acetate was purchased from Burdick & Jackson (Muskegon, MI). Rat plasma (heparinized) was from Pel-Freeze Biologicals (Rogers, AK). All reagents were used as received, without further purification.

Standard Preparation and Standard Curve Construction. A working solution (100 μg/mL) containing **I**−**IV** was made by dissolving the compounds in water/acetonitrile (50:50). The solution was spiked into blank rat plasma to form a high standard at 2000 ng/mL. A series of volumetric dilutions of the 2000 ng/mL standard with blank plasma was performed to obtain a standard curve. The final calibration standard concentrations were 2000, 1000, 500, 250, 100, 50, 25, 10, 5, 2.5, and 1 ng/mL. Plasma standards were stored at −20 °C until time of use. Aliquots (50 μL) of each standard and a blank were transferred into 1.1-mL tubes in a 96-well format. A 200 μg/mL working solution of lidocaine (IS) in water/acetonitrile (50:50) was prepared and stored at 4 °C. This solution was diluted with water/acetonitrile (70:30) to give a 100 ng/mL lidocaine (internal standard) solution.

Sample Collection and Preparation Procedure. Compounds **I**−**IV** were administrated as a single cassette dose (2.5 mL/kg or 10 mg/kg) to each rat by intravenous infusion for 5 min. Heparinized plasma samples were collected at predose (0 min) and 0.5, 1, 2, 4, and 6 h. Aliquots (50 μL) of the plasma samples were transferred to a 96-tube rack.

A semiautomated liquid/liquid extraction method was used for sample preparation.[12,13] An automatic liquid-handling workstation (Quadra-96 model 320, Tomtec, Hamden, CT) was used for all liquid transfers. Briefly, 25 μL of 100 ng/mL lidocaine (IS) solution, 100 μL of pH-adjustment solution (0.1 M KOH/K_2CO_3, pH 12), and 800 μL of ethyl acetate were added to each tube. The rack was placed in a shaker and shaken for 15 min. After centrifugation, the upper (organic) layer was transferred to a 96-well deep-well plate. The solvent in each well was gently dried by passing pressurized nitrogen gas across the surface. The samples were reconstituted with 100 μL of solvent (50:50 water/methanol) and vortexed briefly (~30 s).

Liquid Chromatography Experiments. Compounds **I**−**V** was separated isocratically, using a mixture of 50% methanol/50% 2 mM ammonium acetate (pH 4) as the mobile phase at room temperature at 200 μL/min. The separation column was packed with C18 material (YMC basic, 3 μm, and 2.0 × 50 mm, Wilmington, NC). Liquid chromatographic separations were performed using a quaternary solvent delivery system and autosampler (series 200, Perkin-Elmer, Norwalk, CT). Injection volume was 10 μL.

API-TOF MS Experiments. All API-TOF MS experiments were performed on a Micromass LCT instrument (Micromass, Beverly, MA) configured with a standard Z-Spray electrospray ionization source. Source conditions were as follows: Positive-ion electrospray, capillary voltage 3.5 kV, sample cone voltage 35 V, extraction cone voltage 10 V, source temperature 100 °C, and desolvation temperature 250 °C. Transfer optics settings were as follows: rf lens 200 V, rf dc offset 1 3.0 V, rf dc offset 2 1.0 V, aperture 10.0 V, acceleration 200.0 V, focus 1.0 V, and steering 0.0 V. Analyzer settings were as follows: MCP detector 2700 V, ion energy 38.0 V, tube lens 5.0 V, grid 2 55.0 V, TOF flight tube 4660 V, and reflectron 1782 V. The pusher cycle time was 55 μs. Data files were acquired in continuum (profile) mode, and spectra were stored from m/z 100 to 600 with a 500-ms accumulation time per averaged spectrum. Each averaged spectrum stored to the data system, therefore, contained ~9091 individual spectra (55 μs/spectra averaged over 500 ms).

Quadrupole MS/MS Experiments. A Quattro II LC/MS system (Micromass) operating under MassLynx 3.1 software, was

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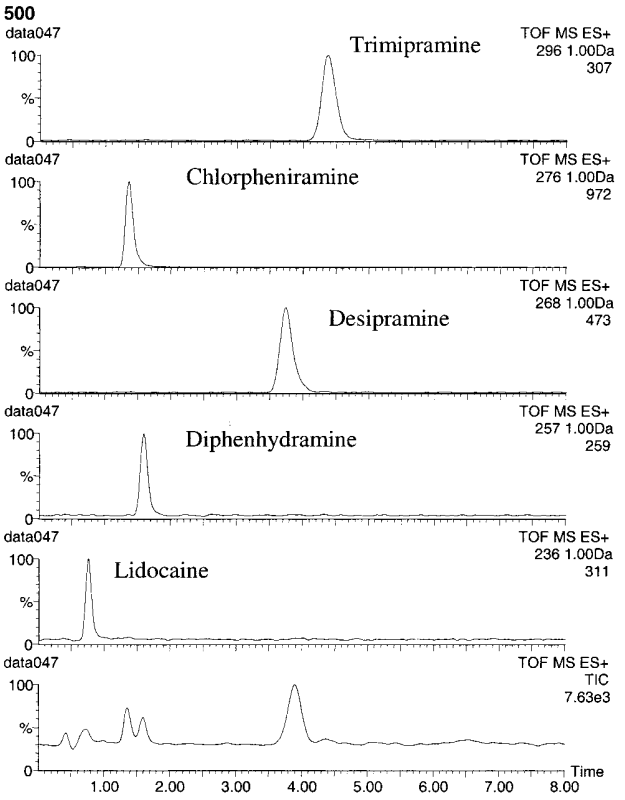

Figure 2. Total-ion chromatogram and extracted nominal mass chromatograms for **I**−**V** from LC/TOF instrument.

used for the quadrupole MS/MS experiments. The ion source was a standard Z-Spray electrospray configuration and was of the same design and generation as used for all time-of-flight experiments. The source conditions for positive ion experiments were typically as follows: capillary 3.5 kV, skimmer 1.5 V, rf lens 0.2 V, source temperature 100 °C, desolvation temperature 250 °C. Quadrupole 1 (Q1) parameters were as follows: LM resolution 14.0, HM resolution 14.0, ion energy 2.0, ramp 0.0, and lens 6 5 V. Quadrupole 3 (Q2) parameters were LM resolution 14.0, HM resolution 14.0, ion energy 1.0, ramp 0.0, lens 8 40 V, and lens 9 0 V. Multipliers were set at 650 V. The precursor and daughter ion combinations (m/z) for the five compounds were as follows: **I** 256 → 167, **II** 267 → 72, **III** 275 → 230, **IV** 295 → 100, and **V** 253 → 86. Dwell time for each channel was 100 ms. Interchannel delay was 30 ms.

Time-of-Flight MS Experiments. *TOF Calibration and Lock-Mass Procedures.* Three levels of mass calibration were utilized to obtain optimal mass accuracy measurements with the TOF instrument. Calibration methods used to improved mass accuracy were (1) appropriate setting of the L_{teff} value, (2) full-scan mass calibration with a reference standard, and (3) postcolumn addition of a reference lock-mass and subsequent mass-scale adjustment. Mass calibration to within nominal mass accuracy (±0.5 m/z) was obtained with appropriate adjustment of the L_{teff} value on the TOF time-to-digital converter (TDC). The L_{teff} value corrects for variations in the effective path length of the analyzer and is typically adjusted during instrument installation. A L_{teff} value of 1117.8 was used for these studies, based on installation specifications, and this value was confirmed prior to these experiments. A full-scan calibration was performed the day before the study to provide further correction of the mass scale. A poly(DL-alanine) stock solution (10 ng/mL) was prepared and introduced via flow injection into the LCT (a poly(DL-alanine) mass reference file was obtained from Micromass Inc., Beverly, MA). During acquisition, the ion signal intensity for poly(DL-alanine) was monitored to avoid detector saturation by observing ion signal peak tailing and total ion intensity. After a full-scan spectrum of poly(DL-alanine) was obtained, a time-of-flight calibration was performed relative to the mass reference file and this calibration was applied to all spectra. To obtain accurate mass measurements (defined as ±5 ppm mass accuracy) for the study samples, a calibration lock mass was added to the LC effluent postcolumn. Phenylbutazone ($(M + H)^+ = m/z$ 309.1603) at 50 μL/mL concentration was infused postcolumn at 1 μL/min using a Cole Parmer 74900 series infusion pump and a 500-μL Hamilton syringe. Narrow-bore PEEK tubing (0.005-in. i.d.) and a PEEK tee fitting provided the inlet plumbing. Solvent mixing between the chromatographic effluent and infusion solvent was sufficient to provide a stable phenylbutazone response. To avoid possible detector saturation effects, the infusion flow rate and phenylbutazone concentration were adjusted to maintain signal intensity for the lock mass between 150 and 250 ion counts for a 0.5−1.0-s spectrum average time. After data acquisition and chromatographic signal averaging, spectra were individually adjusted relative to the phenylbutazone lock-mass calibrator through software control.

Table 1. Comparison of Quantification Parameters for Compounds I−IV on LC/ES-TOF and LC/ES-Triple-Quadrupole (QQQ) MS

	corr coeff[a]	LOD[b] (ng/mL)	LOQ[c] (ng/mL)	concn (ng/mL)	precision[d] (% RSD)	accuracy (%)
			Diphenhydramine			
TOF	0.9988	10	25	50	6.4	111
				500	3.1	104
				1000	2.5	96
QQQ	0.9997	1	1	50	2.4	99
				500	1.8	101
				1000	2.3	99
			Desipramine			
TOF	0.9995	2.5	25	50	2.8	97
				500	4.2	102
				1000	2.3	97
QQQ	0.9978	1	1	50	2.7	106
				500	2.5	98
				1000	3.2	104
			Chlorpheniramine			
TOF	0.9944	5	5	50	2.3	101
				500	3.8	102
				1000	1.5	98
QQQ	0.9994	1	1	50	2.2	99
				500	2.5	98
				1000	12	102
			Trimipramine			
TOF	0.9995	5	25	50	3.7	100
				500	4.7	103
				1000	2.9	98
QQQ	0.9998	1	1	50	1.7	109
				500	1.2	102
				1000	2.7	103

[a] Curve type, second order; weighting, 1/x; origin, Exclude. [b] LOD, limit of detection. [c] LOQ, limit of quantitation (RE <20%) was based on quantitation of nominal mass chromatogram (±1 Da extracted from TIC). [d] Precision and accuracy were calculated based on four repeats for both TOF and triple quadrupole.

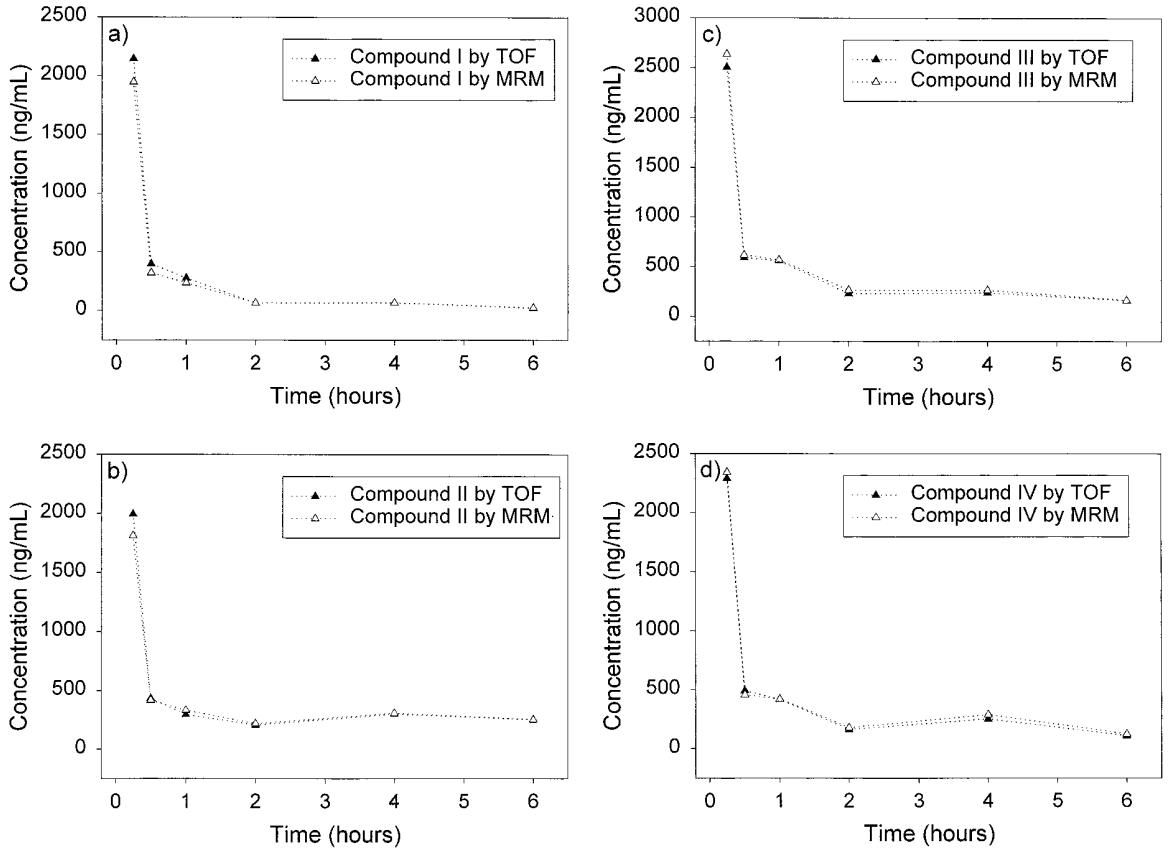

Figure 3. Disposition profiles for (a) **I**, (b) **II**, (c) **III**, and (d) **IV** as determined by MRM (triple-quadrupole) (△) and time-of-flight (○) instruments.

Quantitative Procedures. The Micromass LCT electrospray time-of-flight instrument was operated under MassLynx 3.3 software control. Nominal mass chromatograms (NMC, 1 ± 0.5 Da) were extracted from total-ion chromatograms (TIC), and the peaks were integrated and normalized to those of the internal standard area. The concentrations of **I**−**IV** in plasma samples were then calculated by comparison to the equation of a standard curve, constructed using a weighted ($1/x$ or $1/x^2$) quadratic model.

When accurate mass measurements were used to quantify unknowns, the spectrum of the drug was obtained first by scanning under the peak in NMC. The mass was corrected using the method described above, scanning at the top of the spectral peak to give an extracted accurate mass chromatogram (AMC) with a typical mass range of less than 0.1 Da. The same quantitative procedure as described for the nominal mass chromatograms was then applied to the extracted accurate mass chromatograms to obtain drug concentrations in unknowns.

Metabolite Structure Elucidation. Possible metabolites were predicted, based on a general understanding of drug metabolism pathways (e.g., N-dealkylation, aromatic ring hydroxylation, etc). The exact monoisotopic masses of possible metabolites were calculated based on their elemental compositions. The nominal mass chromatograms of these possible metabolites were then extracted from the total-ion chromatogram obtained from the TOF. A combined spectrum was obtained by scanning the maximum or tail part of the peak in the nominal mass chromatograms. The spectrum was calibrated by lock mass (phenylbutazone, 309.1603 Da) to give exact masses of unknown sample components.

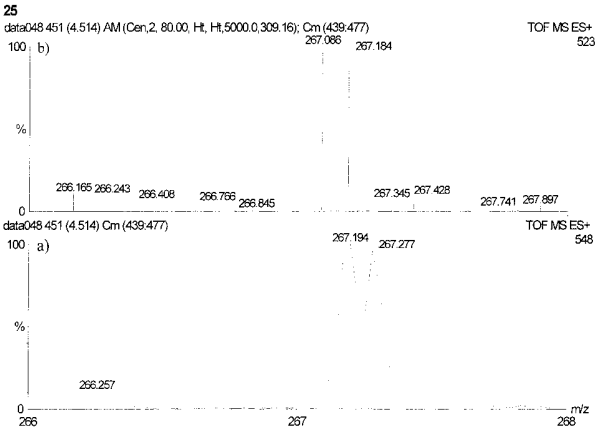

Figure 4. Continuum spectrum (a) and centered accurate-mass spectrum (b) of desipramine in plasma extract.

RESULTS AND DISCUSSION

General Assay Performance Characteristics. In this study, the quantitative performance of liquid chromatography/time-of-flight mass spectrometry and triple-quadrupole mass spectrometry (LC/MS/MS) were compared. The semiautomated liquid/liquid extraction and sample-handling procedures used here have been established and reported previously.[12,13] The procedure utilized a 96-well format that allowed a complete plate of samples to be assayed in approximately 2−3 h. Recovery for the test compounds ranged from 45 to 60% using ethyl acetate as the extraction solvent.

Figure 2 shows representative nominal mass selected-ion chromatograms for the test compounds included in this study. The analyte capacity factors ranged from 1.7 (chlorpheniramine)

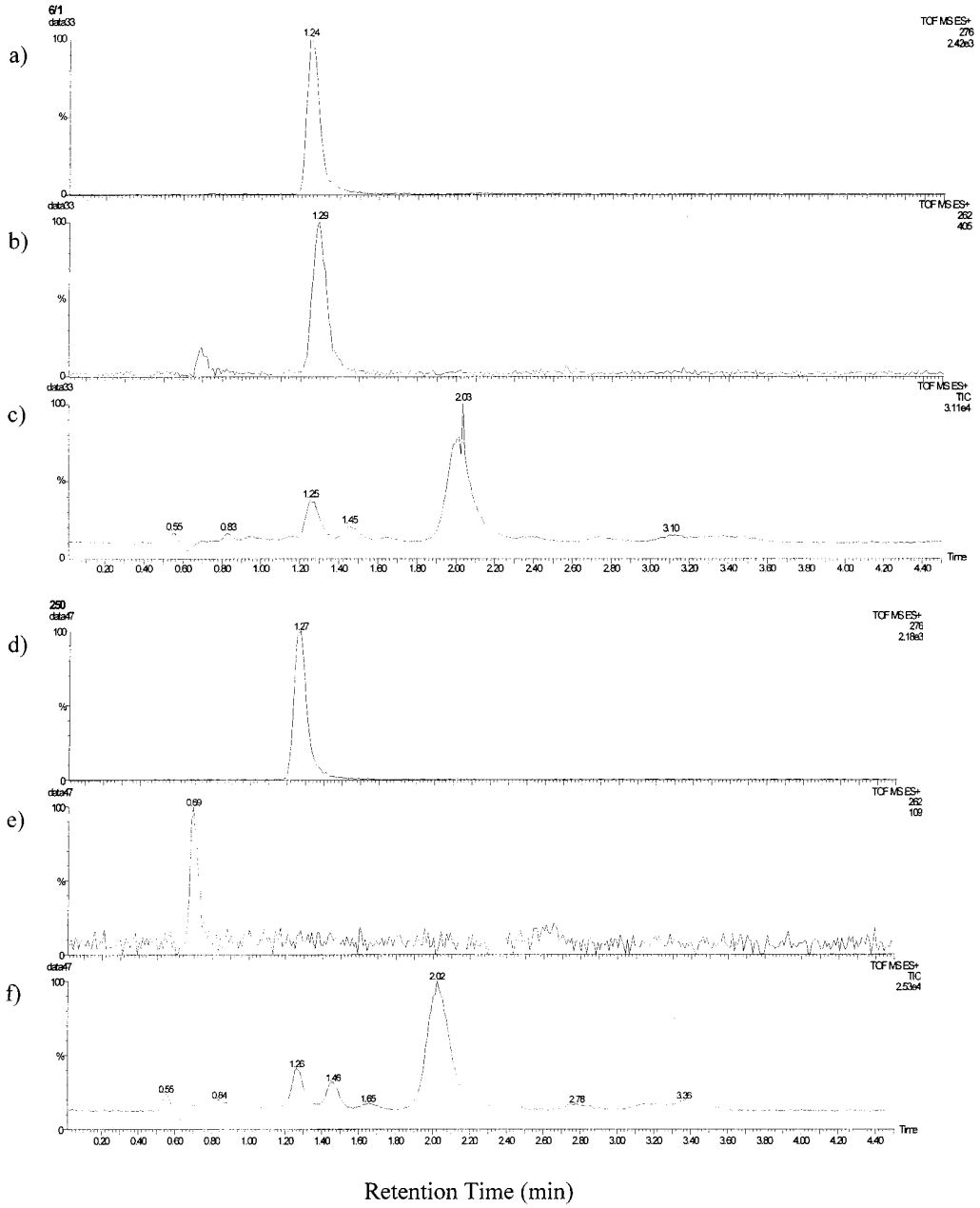

Figure 5. Selected-ion chromatograms for **III** and desmethyl metabolite from plasma sample extract. Chromatograms of samples from a dosed animal are indicated as (a) parent I channel (*m*/*z* 276), (b) desmethyl I channel, and (c) total-ion chromatogram. Chromatograms of spiked samples are indicated as (d) parent I channel, (e) desmethyl I channel, and (f) total-ion chromatogram.

to 6.3 (trimipramine). Adequate chromatographic capacity was desired for these separations so that unidentified biotransformation products that are potentially more polar than the parent drug compounds were retained adequately, without the deleterious effects of ion suppression.[14]

Quantification of Drugs in Plasma Using LC/TOF MS. *Linearity, Precision, and Accuracy.* For four example compounds, Table 1 lists several parameters related to quantitation: correlation coefficient, limit of detection, limit of quantitation, precision, and accuracy of replicate determinations ($n = 4$). Samples were from spiked rat plasma and prepared by liquid/liquid extraction over a concentration range of 1−2000 ng/mL. The parameters listed for TOF were calculated based on nominal mass chromatograms (±0.5 Da). A comparison of the results suggests that MRM gave lower detection limits than TOF for the four compounds studied. Approximately a 5−10-fold improvement in detection limit was obtained on the triple-quadrupole instrument using MRM. Multiple-reaction monitoring demonstrated ∼25-fold better limit of quantitation (<±20% relative error) than that of TOF based on nominal mass chromatograms. The quantitative capability of time-of-flight mass spectrometry appears competitive because it provided similar dynamic range (>2.5 orders of magnitude using a quadratic curve with $1/x$ weighting) with almost identical precision and accuracy over this range.

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Panels a−d of Figure 3 show concentration comparisons for **I**−**IV**, respectively, in samples from dosed rats. Each reported concentration was the average of samples from three individual animals. The concentration range observed for the four drugs was 20−2500 ng/mL. Dilution factors of 4 were applied to samples at 0.25 and 0.5 h. The average differences between TOF and MRM results were less than or equal to 10% over the entire concentration range for each of the compounds studied. These comparisons indicate that instrument performance between the time-of-flight instrument using nominal mass and the triple quadrupole using MRM quantitation was comparable and acceptable.

Improving Quantitation in TOF Mass Spectrometry with Accurate Mass Measurement. Using nominal mass chromatograms, quantitation results from the time-of-flight instrument were comparable to MRM when sample concentrations were above the lower limit of quantitation (25 ng/mL). Overall, the quantitation limit for nominal mass TOF was 5−25-fold worse than MRM, as described above. The potential utility of accurate mass chromatography was investigated as a means of improving the quantitation limit. One characteristic of modern orthogonal TOF MS is high-mass resolution, with the present instrumentation achieving mass resolution of 5000 (fwhm) or greater. Narrow mass range (∼0.1 Da) chromatograms can be extracted from total-ion chromatograms to improve the selectivity in situations where detection is chemical noise limited.

The mass spectrum in Figure 4a (lower trace) represents a continuum acquisition of desipramine from a rat plasma extract. Two masses at 267.277 and 267.194 Da, differing by 0.083 Da, represent desipramine and endogenous plasma interference, respectively. As shown in this figure, when nominal mass calibration is employed, these mass spectral peaks are ambiguous. After centering and calibration with a lock-mass compound, the mass-corrected spectrum in Figure 4b (upper figure) was generated. For the observed desipramine peak (267.184 Da) the mass difference from theoretical was only 7.5 ppm, as opposed to 374 ppm for the low-mass peak. The mass range of 267.227−267.327 Da could be extracted from the total ion chromatogram to give an exact mass chromatogram of desipramine for each sample, thereby improving selectivity.

The standard curve of desipramine at low concentration range (1−100 ng/mL) improved dramatically after application of exact mass quantitation. The scatter about the regression line (r^2) improved from 0.9865 to 0.9988, and the limit of detection was reduced to 1 ng/mL compared with a 25 ng/mL quantitation limit using nominal mass conditions. The limits of quantitation improved from 25 to 2.5 ng/mL (trimipramine), from 25 to 5 ng/mL (diphenhydramine), and from 5 to 2.5 ng/mL (chlorpheniramine) after using accurate mass quantitation. These quantitation limits are very similar to those obtained by MRM, indicating that selectivity improvements with accurate mass measurements can improve quantitation limits.

Screening for Structures of Unknown Metabolites. To obtain a chemical structure for unknown metabolites, knowledge of potential metabolic pathways is combined with molecular weight information obtained through time-of-flight mass spectrometry. For example, **III** has a molecular weight of 274 and forms an M + 1 ion with mass-to-charge ratio (m/z) of 275. This molecule is expected to biotransform to a desmethyl metabolite with a molecular weight of 260 and an M + 1 ion of 261. Panels a and b of Figure 5 show nominal mass chromatograms for parent drug and desmethylmethyl metabolite, with respective chromatographic retention of 1.24 and 1.29 min. A total-ion chromatogram (Figure 5c) represents the relative ion intensities of **III** and metabolite in this sample drawn at 1-h postdosing.

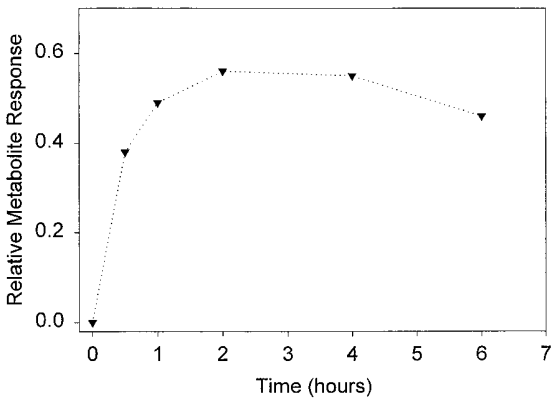

Figure 6. Ion intensity (relative to internal standard response) for the desmethyl metabolite of **III** as a function of time postdosing.

To verify the assignment of these two peaks as parent drug and metabolite, a visual comparison with standard and predose samples can be made. Parts d and e of Figure 5 represent an extracted plasma standard at 500 ng/mL of **III**. The peak for this compound has a similar retention time and is detected on the same mass chromatogram (m/z 275) as that for the sample. As is often the case for metabolites, no authentic standard was available for the desmethyl metabolite of **III**. Note that no peak is detected on the metabolite channel in the standard (m/z 261, Figure 5e) and predose samples. This rules out the possibility that the peak obtained for the desmethyl metabolite in the sample (Figure 5b) is an analytical artifact obtained through in-source fragmentation or some form of channel cross-talk.

As shown in Figure 6, a plot of ion intensity versus postdose sample draw time shows the formation and clearance of this compound and allows biological half-life to be calculated. By comparing half-life to the dosing interval, an estimate of metabolite accumulation could be made. This time course for formation and clearance of this component further supports the hypothesis that it represents a metabolite.

Elementary Structural Elucidation Using Accurate Mass Chromatograms. Using the accurate mass capability of the time-of-flight mass spectrometer, exact masses of unknown metabolites could be calculated with a high degree of precision. If these masses agreed with theoretical masses to within 10 ppm based on the predicted formula, the identity of unknown metabolites was confirmed. If the peak was absent from the exact mass chromatogram of the parent drug standard and predose samples, formation of the metabolite was further verified. If a component eluted with a similar retention time, but the measured accurate mass for the peak differed from the calculated mass of the predicted metabolite by more than a few ppm (∼10 ppm), then the peak was rejected as that metabolite. Assuming some knowledge of possible metabolites, if the determined masses for metabolites were close enough to the theoretical values, a unique molecular formula could be determined. If the predicted metabolites were not abundantly

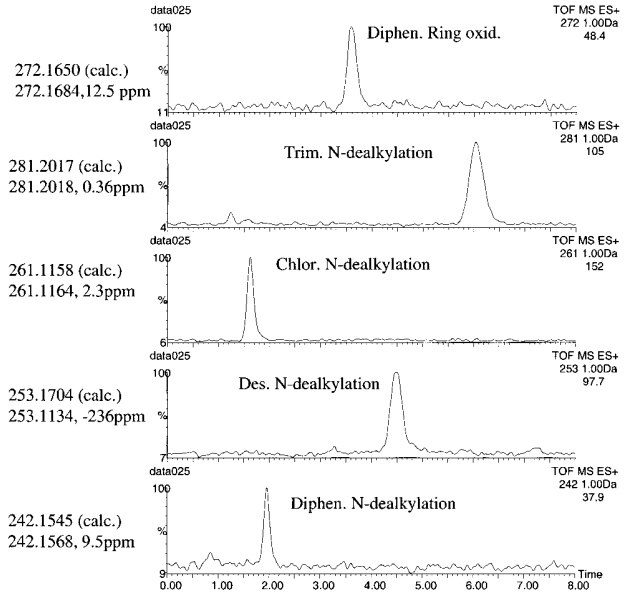

Figure 7. Accurate mass chromatograms (from top to bottom) for (a) **I** ring hydroxylate, (b) **IV** N-dealkylation, (c) **III** N-dealkylation, (d) **II** N-dealkylation, and (e) **I** N-dealkylation metabolites. Differences between theoretical and found masses added confidence to found metabolites.

formed and the signal-to-noise ratio was low (<80), mass errors of 10−15 ppm were observed. For abundant metabolites, mass errors of less than 3 ppm were achieved. Using these approaches, several metabolites were identified in the data set obtained from the cassette dosing of **I**−**IV**. Figure 7 shows the exact mass chromatogram for a number of metabolites of **I**−**IV** and differences in the masses found from the theoretical values.

CONCLUSIONS

These results demonstrate the utility of a time-of-flight LC/MS instrument to provide sensitive and highly selective quantitative assessments of simultaneous drug disposition profiles. The quantitation limits obtained for the TOF experiment were ∼25 times less sensitive than those obtained using a triple-quadrupole mass spectrometer operating under multiple-reaction monitoring. The linearity, accuracy, and precision of these determinations were in close agreement. The quantitation limits for the TOF improved when accurate mass capabilities were employed and were nearly equal to those obtained using MRM on a triple-quadrupole instrument when selectivity was chemical noise limited. The sample preparation technique used in these studies was liquid/liquid extraction, which is known to provide relatively clean sample extracts when optimized for a given chemical structure. Other commonly used high-throughput sample preparation techniques, such as sample dilution and protein precipitation, do not provide the same level of sample cleanup and would require evaluation to determine their compatibility with LC/TOF MS for applications involving analytical quantitation.

The availability of full-scan mass spectra throughout each LC/TOF chromatogram and accurate mass measurements provided qualitative information that could be used to ascertain whether certain predicted drug metabolites were present in samples. Although not a definitive approach, the knowledge of exact analyte masses allowed the determination of possible molecular formula and chemical structure for these metabolites. It seems feasible that this approach could be useful in providing metabolism information for early-phase drug discovery candidates, while simultaneously allowing for the quantitation of the parent drugs.

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