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A Novel Method for the Determination of Sildenafil (Viagra®) And Its Metabolite (UK-103,320) in Postmortem Specimens Using LC/MS/MS and LC/MS/MS/MS

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16. Abstract

During the investigation of aviation accidents, postmortem samples from victims are submitted to the FAA's Civil Aeromedical Institute for drug analysis. Because new drugs are continually being released to the market, it is our laboratory's responsibility to develop methods which can identify these new drugs. This paper presents a rapid and reliable method for the identification and quantitation of sildenafil (Viagra®) and its metabolite, UK-103,320. Sildenafil, when used properly, is relatively safe. There are, however, certain side effects that could create potential hazards. For example, Sildenafil has been shown to potentiate the hypotensive effects of nitrates commonly employed in the treatment of certain heart conditions. The procedure described herein incorporates solid-phase extraction and LC/MS/MS and MS/MS/MS utilizing an atmospheric pressure chemical ionization (APCI) ion trap mass spectrometer (MS) in the positive ionization (PCI) mode. Solid-phase extraction provided an efficient sample extraction yielding recoveries of approximately 80%. This method is highly selective and sensitive, having a limit of detection of 1 ng/mL for both compounds. Sildenafil and UK-103,320 were found to have a linear dynamic range of 2-800 ng/mL and 4-800 ng/mL, respectively. This procedure showed intra-day (within day) relative error of ≤ 6% and relative standard deviation (RSD) within 4% for both the 50 ng/mL and 200 ng/mL controls. The inter-day (between day) relative errors were ≤ 4%, while the RSD was within 12% for both control concentrations. Sildenafil and UK-103,320 were found to be stable in blood for at least one week at 4°C. This method was also used for the determination of sildenafil and UK-103,320 in postmortem fluid and tissue specimens collected from fatal aviation accident victims.

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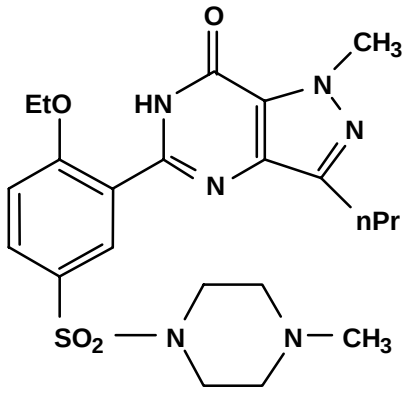
INTRODUCTION

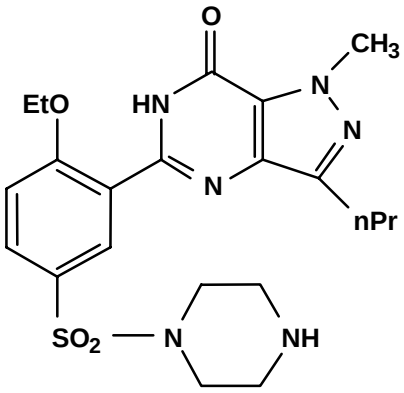
The Federal Aviation Administration's Civil Aeromedical Institute (CAMI) is responsible under the Department of Transportation (DOT) order 8020.11A, Chap 4, Par 170, to "conduct toxicologic analysis on specimens from … aircraft accident fatalities." Additionally, DOT order 1100.2C, Chap 53, Par 53-15 requires that CAMI "investigates selected general aviation and air carrier accidents and searches for biomedical and clinical causes of the accidents, including evidence of … chemical abuse." Therefore, as new drugs are released to the pharmaceutical arena, CAMI's Toxicology Research Laboratory must aggressively develop methods to identify and quantitate them.

Sildenafil (Viagra®), used for the treatment of erectile dysfunction (ED), is rapidly becoming one of the most popular and widely used drugs throughout the United States and Europe. The extensive use of sildenafil is exemplified by the fact that 6 million prescriptions for this agent were written during the first 6 months following its introduction (1). Contrary to popular belief, sildenafil is not an aphrodisiac, does not work in the absence of sexual arousal, and does not make a potent man more virile (2). Sildenafil (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo-[4,3-d]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine, see Figure 1) is a potent inhibitor of the cGMP-specific phosphodiesterase type 5 enzyme (PDE5) found predominantly in the penile corpus cavernosum (3). Cyclic guanosine monophosphate (cGMP), which is broken down by PDE5, is directly responsible for producing smooth muscle relaxation in the corpus cavernosum and allowing the inflow of blood. Thus, by inhibiting PDE5, sildenafil has the potential to improve male erectile function (4).

After oral administration, sildenafil is rapidly absorbed, reaching peak plasma concentrations in 30-120 minutes (5). It is metabolized in the liver predominantly to the active desmethyl metabolite,



(a) Sildenafil



(b) UK-103,320

Figure 1. Structures of sildenafil (a) and its metabolite, UK-103,320 (b).

UK-103,320. UK-103,320 (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenyl-sulphonylpiperazine, see Figure 1) exhibits approximately 50% of the potency of the parent drug and, hence, contributes to the observed pharmacological effects (5). Under steady-state conditions, the plasma concentrations of UK-103,320 are approximately 40% of those seen for sildenafil (6). Both sildenafil and its metabolite have a terminal half-life of about 4 hours.

Sildenafil, when used properly, is relatively safe. There are, however, certain side effects that could create potential hazards. For example, Sildenafil has been shown to potentiate the hypotensive effects of nitrates commonly employed in the treatment of certain heart conditions (7). Moreover, while sildenafil inhibits PDE5, it also has a high affinity for phosphodiesterase type 6 (PDE6), which is a retinal enzyme involved in phototransduction (5). The inhibition of PDE6 can result in the inability to discriminate between blue and green colors, resulting in a condition known as "blue tinge" (8). Although only about 3% of patients report visual disturbances, this blue-green impairment could cause problems in the execution of certain tasks. For example, this impairment could lead to a problematic situation for a pilot relying upon instruments during night flights or adverse meteorological conditions (9). Additionally, the increasing use of color video terminals in aviation is anticipated to increase the probability of a mishap for a color deficient pilot.

Because of its increasing popularity and potential side-effects, the need for a procedure to detect both sildenafil and its metabolite in biological samples is becoming increasingly important. This paper describes a novel method for the identification and quantitation of both sildenafil and UK-103,320 in postmortem fluids and tissues using solid phase extraction (SPE) and liquid chromatography (LC) with MS/MS and MS/MS/MS detection utilizing atmospheric pressure chemical ionization (APCI) ion trap mass spectrometry (MS) in the positive ionization (PCI) mode.

MATERIALS AND METHODS

Reagents, Standards and Supplies

All solvents were of HPLC-grade and were obtained from Fisher Scientific (Fischer Scientific Co., Fair Lawn, NJ). Double distilled water was prepared using a Millipore model Milli-QT bench-top purification device (Millipore, Continental Water Systems, El Paso, TX) and was used for all reagent preparations. Formic acid was obtained from Fisher Scientific. Sildenafil and UK-103,320 were obtained from Pfizer pharmaceutical company (Pfizer Ltd., UK). Medazepam was obtained from Sigma (Sigma Co., St. Louis, MO).

Standards of both sildenafil and UK-103,320 were prepared at 1 mg/mL in acetonitrile. A stock solution of the internal standard, medazepam, was prepared at 1 mg/mL in methanol. The HPLC buffer was 50 µM formic acid buffer adjusted to pH 4.50 with ammonium hydroxide.

Instrumentation

Analyte separation was achieved using a Hewlett Packard series 1100 HPLC (Hewlett Packard Co., Wilmington, DE) equipped with a LC-18 guard column (4.0 mm x 3.0 mm i.d., 3 µM particles) from Phenomenex (Torrance, CA) followed by a Supelcosil LC-18 column (150 mm x 4.6 mm i.d., 3 µM particles) from Supelco (Bellefonte, PA). Samples were injected using a Hewlett Packard G1313A autosampler. Identification and quantitation were accomplished using a Finnigan (Finnigan Corp., San Jose, CA) model LCQ atmospheric pressure ionization ion trap mass spectrometer. Control of the HPLC system, integration of the chromatographic peaks and communication with the APCI-MS system was accomplished using a Gateway 2000 personal computer using Finnigan LCQ software.

LC/MS/MS and MS/MS/MS Method

Initial ionization evaluation of the analytes using positive and negative chemical ionization indicated that positive chemical ionization (PCI) provided greater signal to noise ratios for this application. Prior to sample analysis, APCI PCI MS conditions were optimized by infusing, at 1 mL/min, UK-103,320 dissolved in the mobile phase. Tuning to the UK-103,320 $[M+H]^+$ ion (m/z 461.5) was accomplished using the auto-tune feature. Instrument tuning was also completed for the internal standard,

medazepam. Each sample analysis was split into 2 unique data collection segments. Segment 1 collected data for sildenafil and UK-103,320 and segment 2 collected data for medazepam. The operating conditions for segment 1, which analyzed sildenafil and the metabolite, were as follows: APCI capillary temperature, 250°C; APCI vaporizer temperature, 450°C; source voltage, 8.0 kV; source current, 5 µA; sheath gas flow (nitrogen), 80.0; auxiliary gas flow (nitrogen), 15.0; capillary voltage, 34.0 V; tube lens offset, 40.0 V; octapole 1 offset, -3.75 V; octapole 2 offset, -7.0 V; interoctapole lens voltage, -20 V; trap DC offset voltage, -10 V; multiplier voltage, 0.0 V; and 1 micro-scan having a maximum ion injection time of 200 ms. Additionally, segment 1 was split into 4 separate scan events, 2 scan events being dedicated to each compound. Scan event 1 performed MS/MS on the sildenafil parent ion (m/z 475.4) using a collision energy of 22%. Scan event 2 performed MS/MS/MS on the sildenafil daughter ion (311.1) using a collision energy of 16%. Scan event 3 performed MS/MS on the UK-103,320 parent ion (m/z 461.5) and scan event 4 performed MS/MS/MS on the UK-103,320 daughter ion (m/z 311.1) using collision energies of 24% and 20%, respectively. The second segment was dedicated to medazepam. The operating conditions for this segment were as follows: APCI capillary temperature, 250°C; APCI vaporizer temperature, 450°C; source voltage, 8.0 kV; source current, 5 µA; sheath gas flow (nitrogen), 80.0; auxiliary gas flow (nitrogen), 20.0; capillary voltage, 16.0 V; tube lens offset, 5.0 V; octapole 1 offset, -2.50 V; octapole 2 offset, -6.0 V; interoctapole lens voltage, -22 V; trap DC offset voltage, -10 V; multiplier voltage, 0.0 V; and 3 micro-scans having a maximum ion injection time of 200 ms. This segment consisted of 2 scan events. Scan event 1 performed MS/MS on the medazepam parent ion (m/z 271.1) and scan event 2 performed MS/MS/MS on the medazepam daughter ion (m/z 242.1) using collision energies of 20% and 16%, respectively.

The HPLC was run isocratic with a flow rate was 1 mL/min and a mobile phase composition of acetonitrile/formic acid buffer (87:13). The sample injection volume was 10 mL. Three unique ions having the highest abundance in the MS/MS mode were summed and used for quantitation of each analyte. Sildenafil's ions were 311.1, 313.2, and 377.1, which came from its parent ion 475.4. UK-103,320's ions were also 311.1, 313.2, and 377.1, which came from its parent ion 461.5. Only 2 ions, 242.1 and 254.1, were summed for medazepam. The full spectra of MS/MS and MS/MS/MS were used for analyte confirmation. Retention times were approximately as follows: sildenafil, 2.31 minutes; UK-103,320, 2.80 minutes; and medazepam, 3.72 minutes.

Calibrators and controls

Calibration curves were prepared in whole blood at concentrations ranging from 1-800 ng/mL. A minimum of 7 calibrator values were used to construct each calibration curve. Controls used for the determination of accuracy, precision, and stability were prepared in whole blood at 50 and 200 ng/mL using drug standards prepared separately from those used for the calibrators. Controls were prepared in pools large enough to provide samples for the entire study. The medazepam internal standard solution was prepared at a final concentration of 500 ng/mL. Medazepam, although structurally unrelated to sildenafil, has proven itself to be a robust generic internal standard through its extensive use in our laboratory.

Analyte concentrations were determined using an internal standard calibration procedure. Calibration curves were prepared by plotting a linear regression of the analyte/internal standard response factor versus the analyte concentration. Response factors were determined for each specimen, and the various analyte concentrations were then obtained from the respective calibration curves.

Sample Extraction

Calibrators, controls, and postmortem fluid and tissue specimens were prepared and extracted in the following manner. Tissues were homogenized with a Brinkmann Tissue Homogenizer (Brinkmann Instruments, Westbury, NY) following a dilution with water (1 g H_2O/1 g wet tissue). Three mL aliquots of fluids, controls and calibrators, and 2 g of the tissue homogenates were transferred to individual 15 mL screw-top vials. To each sample was added 500 ng internal standard as 1 mL of a 500 ng/mL stock solution. The samples were vortexed and allowed to stand for 10 minutes. To these were added 9 mL ice-cold acetonitrile, and the combinations were mixed on a rotary extractor for 15 minutes. Centrifugation at 820 x g for 5 minutes provided removal of cellular debris and proteins. The supernatant was transferred to 15 mL vials and evaporated in a water bath at 40°C

under a stream of dry nitrogen to a volume less than 1 mL. To this was added 4 mL 0.1 M phosphate buffer, pH 6.0. The extracts were transferred to solid-phase extraction (SPE) columns, which were preconditioned with 2 mL methanol, followed by 3 mL 0.1 M phosphate buffer, pH 6.0. The SPE columns were Bond Elute Certify® columns obtained from Varian (Varian Co., Harbor City, CA.). Care was taken not to dry the column prior to extract addition. Column flow rates of 1-2 mL/min were maintained in each step using a Varian 24 port pressure manifold with a nitrogen pressure of 3 psi. Once the samples had passed through the columns, the columns were washed with 1 mL of 1 M acetic acid, followed by 6 mL methanol, then dryed completely between each wash with 25 psi nitrogen for 5 minutes. The analytes were eluted off the columns with 4 mL of 2% ammonium hydroxide in ethyl acetate, which was prepared daily. Eluents were evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen, brought up in 50 µL acetonitrile and transferred to sample vials for analysis.

Recovery

The recovery of each analyte was determined using the following procedure (10). Briefly described, two groups of samples, X and Y, were extracted in the same manner as described above. Group X was spiked with drug prior to extraction, and group Y was spiked with drug following column elution. Following LC/MS/MS analysis the average response factor of the group X, divided by that for group Y, yielded the desired recovery values. The analyte concentration used for the recovery study was 100 ng/mL.

Standard Addition

Concentrations for tissue samples were determined using a standard addition method (11). Each tissue sample was prepared in duplicate, with 1 of the samples being spiked with 200 ng of sildenafil and UK-103,320. By a comparison of the spiked and unspiked sample values, a concentration was derived for each sample.

RESULTS AND DISCUSSION

The procedure described herein provides a fast, reproducible, and accurate method for the determination of sildenafil and its metabolite, UK-103,320, by incorporating solid-phase extraction and LC/MS/MS and MS/MS/MS utilizing an atmospheric pressure chemical ionization (APCI) ion trap mass spectrometer (MS) in the positive ionization (PCI) mode. The use of solid-phase extraction provided a cleaner sample and required less organic solvent than did an alternative liquid-liquid extraction procedure. The extraction efficiency for the SPE was also notably superior to that of the liquid-liquid extraction. The average recovery of sildenafil and its metabolite at a concentration of 100 ng/mL was 83 ± 9% and 78 ± 5%, respectively.

Sildenafil, UK-103,320 and medazepam peaks were completely resolved and experienced no interference from endogenous sample matrix components. All analytes were eluted from the column in less than 4 minutes and had theoretical plates ranging from 3000 to 9000. Figure 2 shows a representative LC/MS/MS chromatogram.

By incorporating APCI-MS in the PCI mode, simple MS spectra were produced consisting predominantly of the protonated $[M+H]^+$ ion. Using an LCQ ion trap we were then able to conduct MS/MS on these unique ions. The $[M+H]^+$ ions used for MS/MS were 475.4, 461.5, and 271.1 for sildenafil, UK-103,320 and medazepam, respectively. MS/MS/MS spectra were then obtained by conducting MS on the MS/MS daughter ions 311.1 of sildenafil and UK-103,320 and 242.1 of medazepam. In forensic analysis, it is desirable to provide full scan MS/MS and MS/MS/MS data for compound identification instead of selected ion monitoring (SIM) data. This is because a full spectrum provides an entire "fingerprint" of a compound, whereas SIM does not. Therefore, the MS/MS and MS/MS/MS full spectra were used for analyte identification. Full scan MS/MS and MS/MS/MS spectra for each analyte are shown in Figures 3 through 8.

LC retention times were additionally used as analyte acceptability criteria and were required to be within 2% of the average calibrator retention time. Typical retention times were 2.31, 2.80, and 3.72 minutes for sildenafil, UK-103,320, and medazepam, respectively.

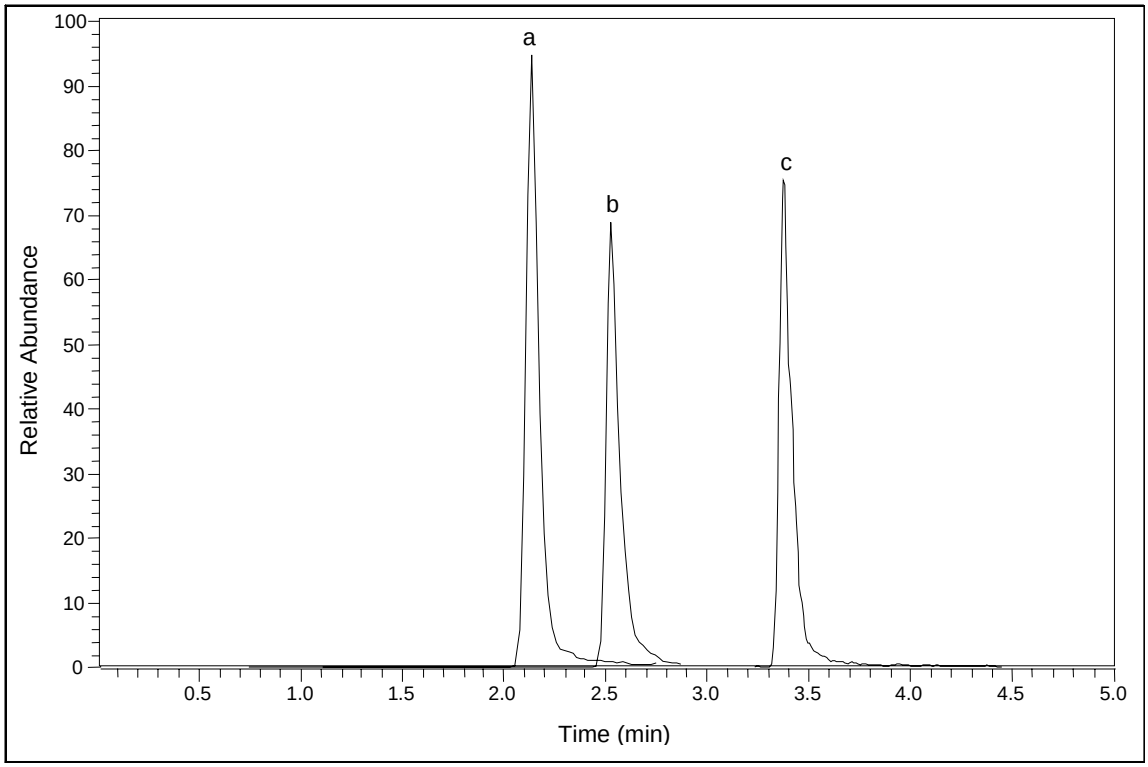


Figure 2. MS/MS chromatogram of sildenafil (a), UK-103,320 (b), and medazepam (c).

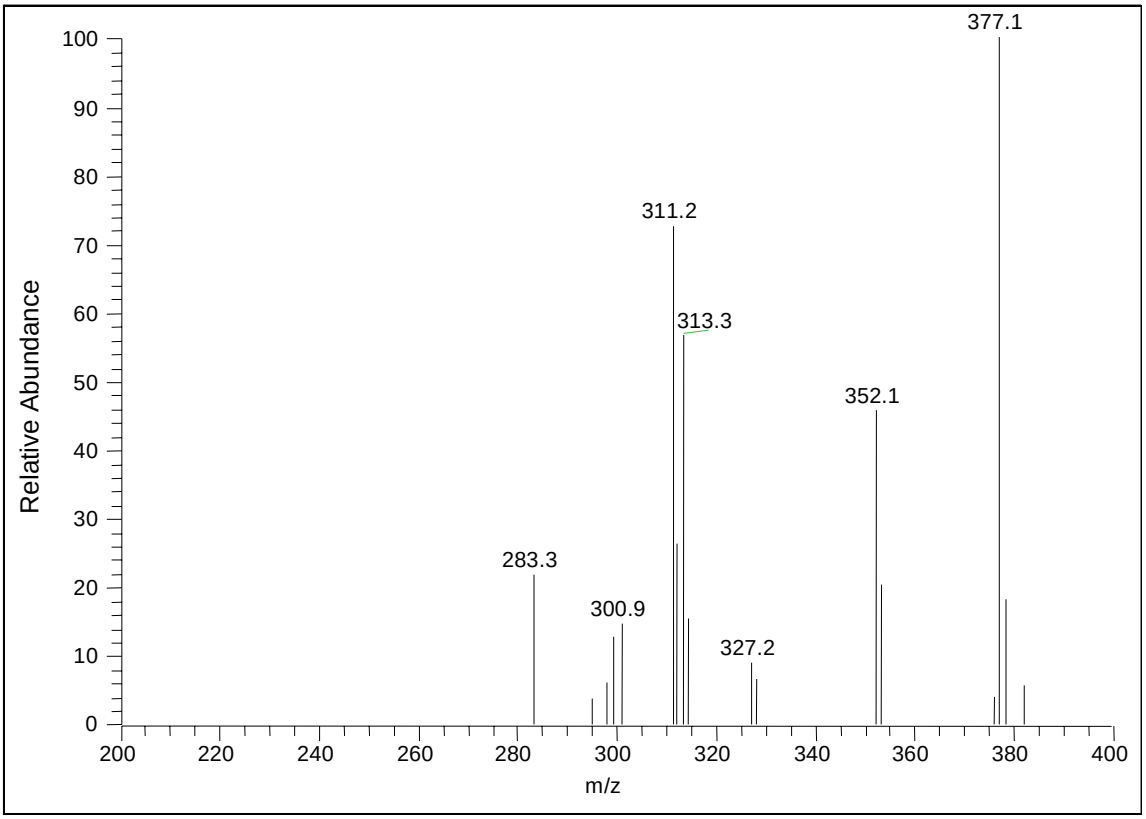


Figure 3. MS/MS spectrum of sildenafil (*m/z* 475.4 → spectrum).

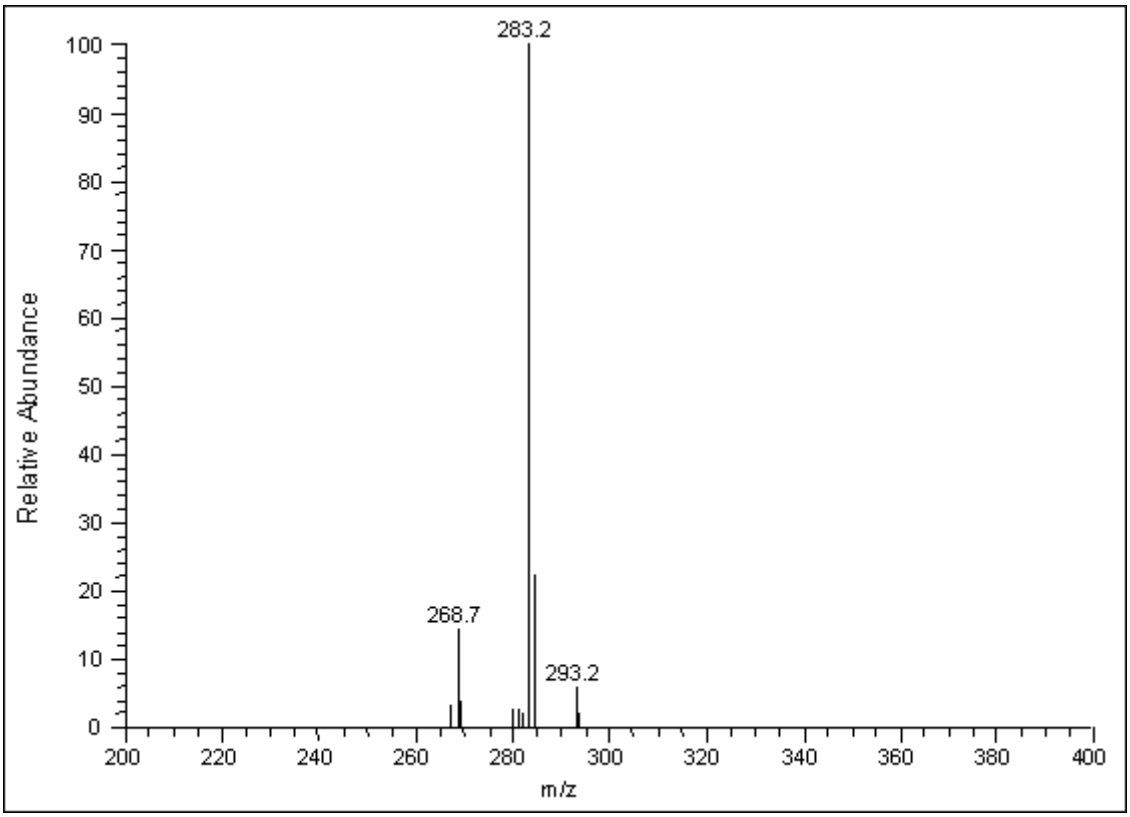


Figure 4. MS/MS/MS spectrum of sildenafil (*m/z* 475.4 → 311.1 → spectrum).

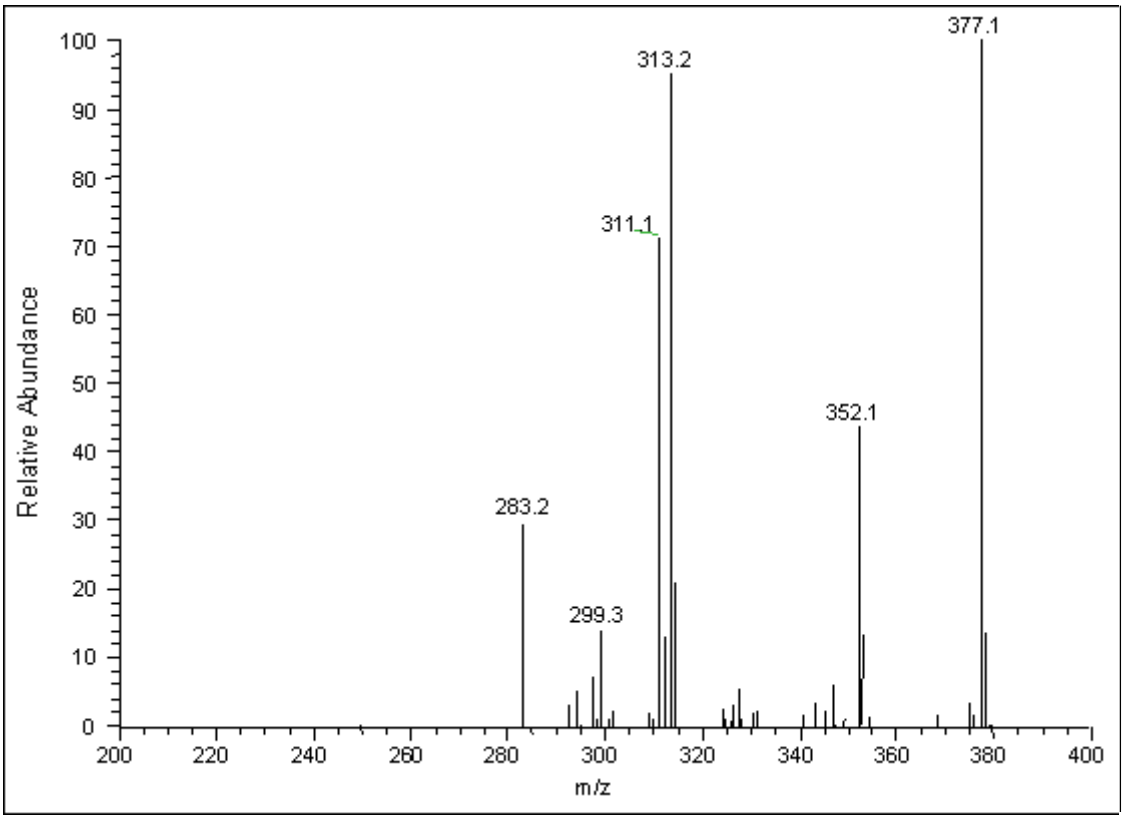


Figure 5. MS/MS spectrum of UK-103,320 (*m/z* 461.5 → spectrum).

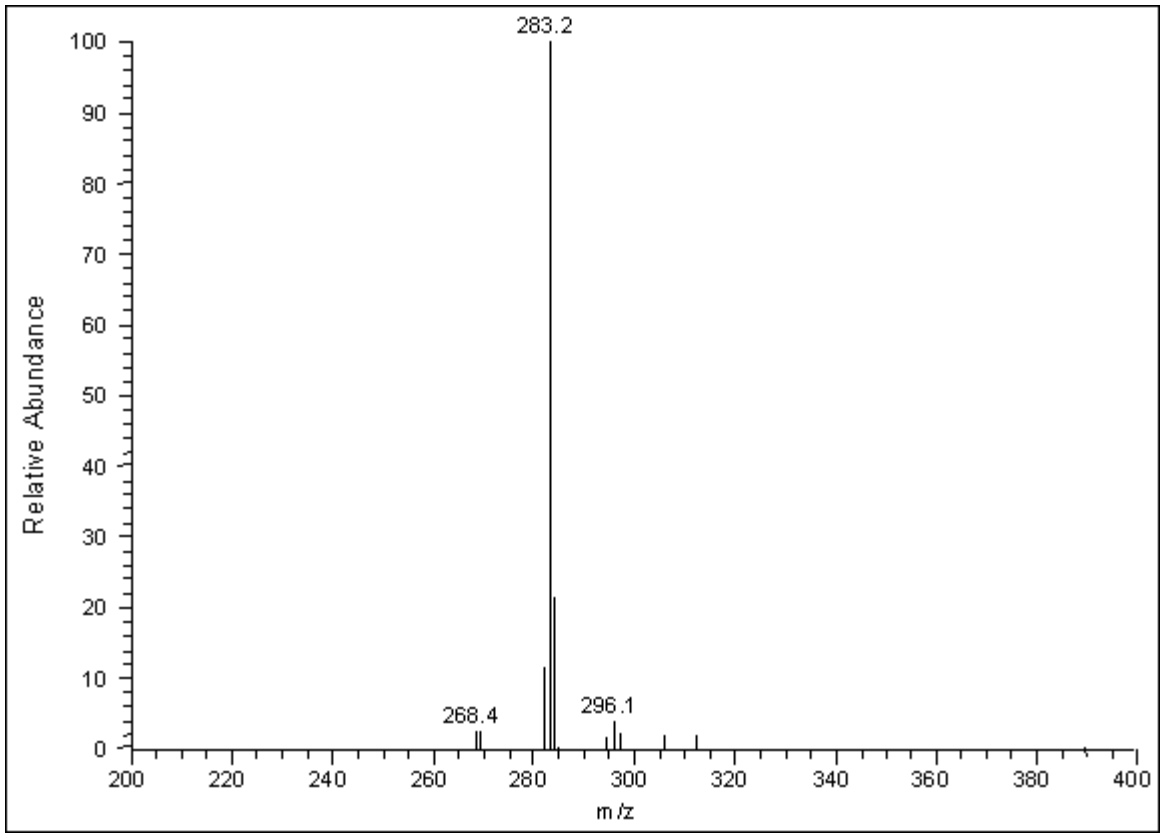


Figure 6. MS/MS/MS spectrum of UK-103,320 (*m/z* 461.5 → 311.1 → spectrum).

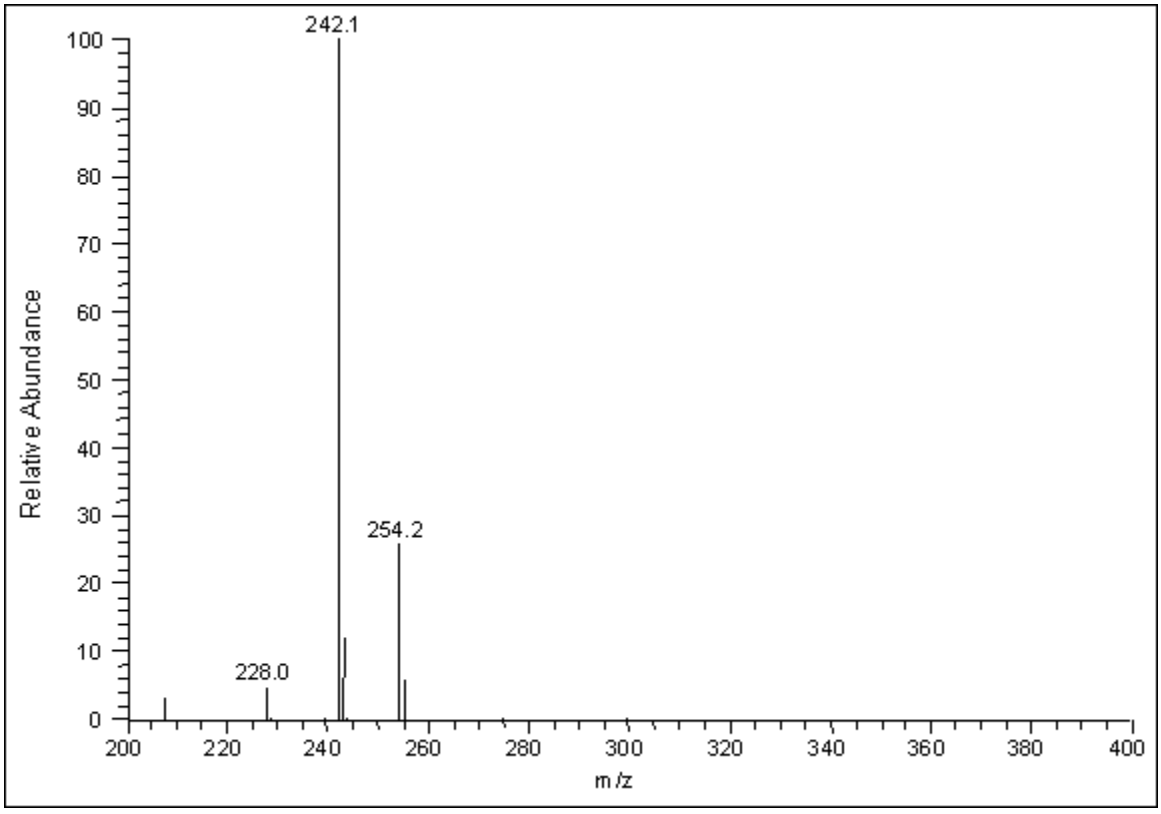


Figure 7. MS/MS spectrum of medazepam (*m/z* 271.1 → spectrum).

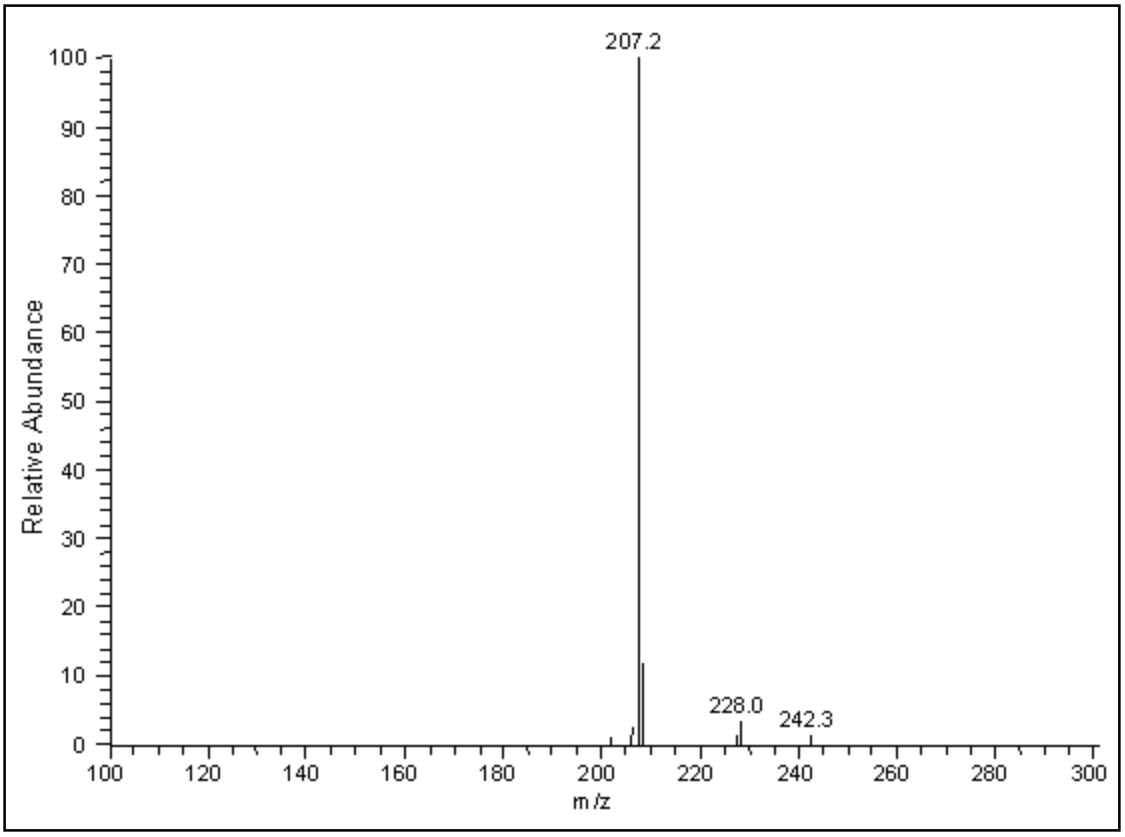


Figure 8. MS/MS/MS spectrum of medazepam (*m/z* 271.1 → 242.1 → spectrum).

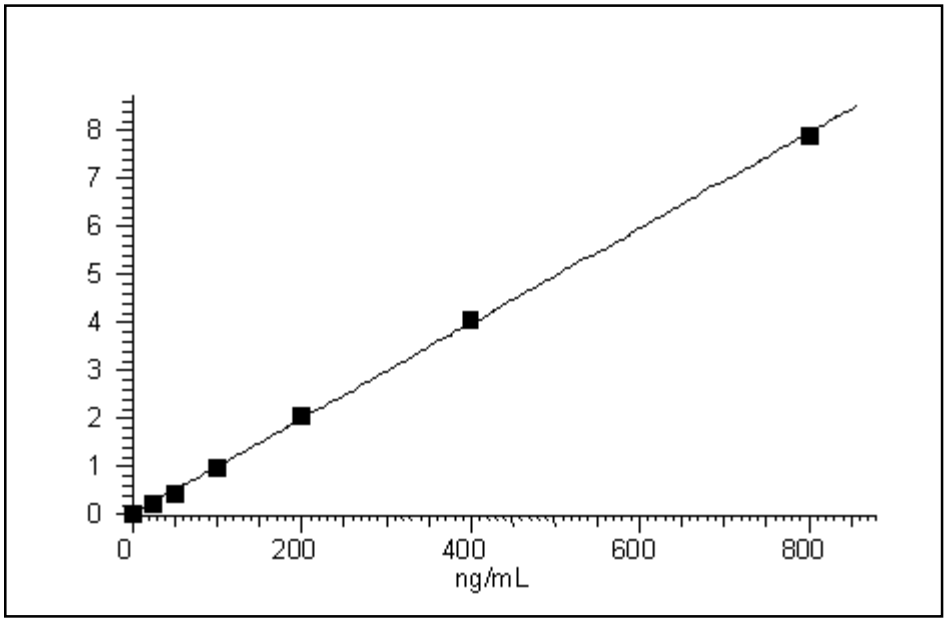


Figure 9. Representative calibration curve for sildenafil ($r^2 = 0.9996$)

Quantitation of the analytes was accomplished by initially summing the intensities of the 3 highest abundance ions in the MS/MS mode for each analyte. Since medazepam MS/MS spectrum has only 2 major ions, only 2 were summed for this compound. The area of the summed ions for sildenafil and UK-103,320, divided by the area of the summed ions of medazepam, gave a response factor that was then used for quantitation. The ions used for quantitation and identification are listed in Table 1.

The linear dynamic range (LDR), limit of detection (LOD), and lower limit of quantitation (LOQ) were determined by analysis of blood spiked with the analytes. The LDR of the calibration curves were 2-800 ng/mL for sildenafil and 4-800 ng/mL for UK-103,320. The correlation coefficients for both of these curves exceeded 0.999. A representative curve is shown in Figure 9. Non-linearity was observed at concentrations greater than 800 ng/mL. The LOD and LOQ determined for each analyte of interest are listed in Table 2. The LOD was defined as the lowest concentration of analyte having a minimum signal-to-noise ratio (S/N) of 5, in addition to meeting the MS/MS and MS/MS/MS full spectra identification and retention time criteria. The LOQ was defined as meeting all LOD criteria plus having an experimentally determined value within ± 20% of its prepared concentration. The LOD and LOQ for sildenafil were 1 ng/mL and 2 ng/mL, respectively. UK-103,320 had a LOD of 1 ng/mL and a corresponding LOQ of 4 ng/mL.

Sample carry-over was evaluated by analyzing a blank sample, which was run immediately following the 800 ng/mL calibrator. This blank showed no carry-over of sildenafil, its metabolite, or medazepam; therefore, carry-over was determined not to be a concern.

Method accuracy and precision were determined for sildenafil and UK-103,320. The accuracy was measured as the relative error between the experimentally determined and prepared concentrations of a sample, and precision was measured as the relative standard deviation (RSD) in the experimentally determined concentrations. Intra-day (within day) and inter-day (between day) accuracy and precision studies were performed using controls at 50 ng/mL and 200 ng/mL. These values were chosen as they represent typical lower and upper therapeutic concentrations (12). Intra-day relative errors in the 50 ng/mL and 200 ng/mL control groups were ≤ 6% for both analytes. Furthermore, the intra-day RSD was ≤ 4% for both the 50 ng/mL and 200 ng/mL sildenafil and

Table 1. Ions used for the quantitation and confirmation of sildenafil and UK-103,320.

Compound	Quantitation Ions, MS/MS (m/z)	Qualifier Ions, MS/MS/MS (m/z)
Sildenafil	311.1, 313.2, 377.1	283.2
UK 103,320	311.1, 313.2, 377.1	283.2
Medazepam	242.1, 254.1	207.2

Table 2. LDR, LOD, LOQ and recovery for sildenafil and UK-103,320.

Compound	LDR (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Recovery (%)
Sildenafil	2-800	1	2	83 ± 9
UK 103,320	4-800	1	4	78 ± 5

Table 3. Intra-day precision and accuracy validation of sildenafil and UK-103,320.

Intra-day precision and accuracy (n=3)				
	Sildenafil		UK-103,320	
Added conc. (ng/mL)	50	200	50	200
Mean ± S.D. (ng/mL)	51 ± 1	201 ± 3	47 ± 2	207 ± 1
R.S.D.	2%	1%	4%	1%
relative error	+2%	+1%	-6%	+4%

Table 4. Inter-day* precision and accuracy validation of sildenafil and UK-103,320.

Inter-day precision and accuracy (n=12)				
	Sildenafil		UK-103,320	
Added conc. (ng/mL)	50	200	50	200
Mean ± S.D. (ng/mL)	52 ± 4	199 ± 8	49 ± 6	196 ± 7
R.S.D.	8%	4%	12%	4%
relative error	+4%	-1%	-2%	-2%

*Three controls at each concentration were run on days 1, 2, 3 and 7.

Table 5. Stability of sildenafil and UK-103,320 maintained at 4°C for 7 days.

Stability at day 7 (n=3)				
	Sildenafil		UK-103,320	
added conc. (ng/mL)	50	200	50	200
mean ± S.D. (ng/mL)	52 ± 2	200 ± 4	43 ± 2	191 ± 2
R.S.D.	10%	2%	5%	1%
relative error	+4%	+2%	-14%	-5%

UK-103,320 controls. Intra-day results are summarized in Table 3. The inter-day accuracy and precision were determined by repeat analysis of the controls on days 1, 2, 3 and 7. The blood controls were prepared in pools on day 1 and stored at 4°C until extraction. The inter-day relative errors of the analytes at both concentrations did not exceed 4% of the target values. The RSD of the 50 ng/mL sildenafil and UK-103,320 control was 8% and 12%, respectively. The 200 ng/mL values for the same period had RSD values ≤ 4%. The inter-day results show that this method is both accurate and precise over a seven-day period (Table 4).

The stability of sildenafil and UK-103,320 in whole blood was evaluated by looking at the control values on day 7 (Table 5). The analytes showed no apparent decrease in concentration after 1 week at 4°C. In fact, all of the values were within 14% of their target concentrations. These results show that non-frozen samples are stable for a minimum of 1 week.

Table 6. Sildenafil and UK-103,320 levels in postmortem fluids and tissues.

Postmortem Fluid and Tissue* Concentrations				
	Victim #1		Victim #2	
Fluids (ng/mL)	Sildenafil	UK-103,320	Sildenafil	UK-103,320
Blood	8	18	0	2
Urine	13	583	0	11
Bile	158	6632	4	1181
Tissues (ng/g)				
Liver	47	77	1	6
Kidney	24	24	0	0
Heart	125	24	7	4
Muscle	35	35	0	0

*Tissue values determined using standard addition (11).

Postmortem Specimen Analysis

In fatal aviation accidents, specimens from the accident victims are routinely sent to the Federal Aviation Administration's Civil Aeromedical Institute for toxicological analysis. Postmortem fluid and tissue samples obtained from 2 separate aviation fatalities that had previously been screened positive for sildenafil and/or its metabolite, UK-103,320, were re-examined using this new method to determine levels of both analytes. The fluid and tissue samples that were tested from each victim were blood, urine, bile, liver, kidney, heart and muscle. Using the method presented in this paper, sildenafil and UK-103,320 were identified in both victims. The results are presented in Table 6. The highest levels of sildenafil and UK-130,320 found in both victims were in the bile. This is to be expected because the major excretion route for both analytes is in the feces (13).

It appears Victim 1 had taken sildenafil more recently than had Victim 2. According to published blood elimination curves (2), it appears that Victim 2 had taken sildenafil at least 15 to 20 hours prior to death. Victim 1 appears to have taken the drug 8 to 12 hours before death. Since sildenafil was taken at an unknown period of time prior to death in both cases, the concentrations and distributions determined yield very little information other than to demonstrate the widespread applicability of this new method.

CONCLUSION

The treatment of erectile dysfunction (ED) by sildenafil is both widespread and increasing. Thus, the possible occurrences of undesirable side effects are of increasing concern. With this in mind, a method for the identification and quantitation of sildenafil and its metabolite, UK-103,320, has been developed which is fast, reliable, and sensitive. By utilizing solid-phase extraction, a rapid and clean sample preparation was achieved with minimal solvent use. Additionally, the extraction provided very good analyte recoveries. The method described in this paper exemplifies the effectiveness of combining liquid chromatography with mass spectrometry for the determination of large nonvolatile and/or thermally labile compounds. APCI-MS in the PCI mode provided a "soft" ionization, which yielded a simple spectrum consisting mainly of protonated $[M+H]^+$ ions. The LCQ ion trap enhanced the specificity and sensitivity of the method by providing MS/MS and MS/MS/MS analyses of these unique $[M+H]^+$ ions. This methodology was demonstrated to be highly effective for the identification and quantitation of sildenafil and UK-103,320 in postmortem fluid and tissue samples.

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