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Simultaneous Quantitation of Atenolol, Metoprolol, and Propranolol in Biological Matrices Via LC/MS

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16. Abstract

Hypertension is a growing medical concern in the United States. With the number of Americans suffering from hypertension increasing, the use of antihypertensives such as beta-blockers is increasing as well. In fact, three beta-blockers — atenolol, metoprolol and propranolol — were among the 200 most prescribed medications in the United States in 2003. Pilots that successfully manage their hypertension can remain certified to fly. The Federal Aviation Administration currently designates approximately 8% of active pilots as "hypertensive with medication." The Civil Aerospace Medical Institute (CAMI) performs toxicological evaluation on victims of fatal aviation accidents. At CAMI beta-blockers are analyzed using gas chromatography with mass spectrometric detection. We have, however, recently developed a liquid chromatography with mass spectrometric detection (LC/MS) method for the simultaneous quantitation of three commonly prescribed beta-blockers atenolol, metoprolol, and propranolol. One advantage of our LC/MS method is the specificity provided by an ion trap MS. Utilizing an ion trap MS, we were able to conduct MS/MS and MS/MS/MS on each analyte. This method also eliminates the time-consuming and costly derivitization step necessary during GC/MS analysis. Additionally, by utilizing this novel method, any concerns about beta-blocker metabolite and/or sample matrix interference are eliminated. The limits of detection for this method ranged from 0.39 - 0.78 ng/mL, and the linear dynamic range was generally 1.6 - 3200 ng/mL. The extraction efficiencies for each analyte ranged from 58 - 82%. This method was successfully applied to postmortem fluid and tissue specimens obtained from victims of three separate aviation accidents.

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INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) is responsible under Department of Transportation (DOT) order 8020.11B, 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 "investigate … general aviation and air carrier accidents and searches for biomedical and clinical causes of the accidents, including evidence of … chemical abuse." Therefore, CAMI's Toxicology Research Laboratory must aggressively develop analytical methods to identify and quantitate over-the-counter, prescription, and illicit drugs.

Hypertension is a growing medical concern in the United States. With an increasing number of Americans suffering from hypertension every year, the use of antihypertensive medications such as beta-blockers has increased as well. Three beta-blocker medications — atenolol, metoprolol, and propranolol — were among the 200 most prescribed drugs in the United States in 2003, ranked 4, 14, and 165, respectively.[1] Pilots that successfully manage their hypertension either with diet, exercise, and/or medication may remain medically certified to operate an aircraft. However, these pilots are closely monitored to ensure that their hypertension is properly controlled. The FAA classifies approximately 8% of all active civil aviation pilots as "hypertensive with medication."[2]

Toxicological evaluation of postmortem samples obtained from pilots is an important part of the investigation of fatal civil aviation accidents. During this evaluation it is not uncommon to detect beta-blocker compounds such as atenolol, metoprolol, or propranolol in the submitted biological samples. In forensic toxicology laboratories, these compounds are most commonly confirmed and/or quantitated by gas chromatography with mass spectrometric detection (GC/MS).[3-11] Liquid chromatography coupled with mass spectrometric detection (LC/MS), however, is becoming increasingly more prevalent in the field of forensic toxicology and is considered a superior alternative to GC/MS for the analysis of many compounds.[12]

The application of LC/MS provides several advantages over GC/MS. For many compounds, analysis by GC/MS first requires derivatization with costly derivatizing agents to increase compound volatility. This derivatization step is not only costly, it also increases the time required to analyze these drugs. Most compounds that require derivatization before GC/MS analysis can be analyzed by LC/MS, without this additional step. For example, beta-blockers require derivatization prior to GC/MS analysis, which can be accomplished with pentafluoropropionic anhydride (PFPA); however, this step is not necessary when these compounds are analyzed by LC/MS. Another specific LC/MS advantage for the analysis of these three beta-blockers is selectivity. Each of these compounds has metabolites that may be present when analyzing postmortem specimens. One metoprolol metabolite in particular, *o*-desmethylmetoprolol, when analyzed by GC/MS, has a similar retention time and nearly identical mass spectral fingerprint as atenolol, and may lead to false atenolol positives.[2]

There are very few analytical LC/MS methods published for the determination of beta-blockers from biological specimens.[13-15] Furthermore, we were unable to find any citation for the toxicological determination of beta-blockers in postmortem fluid and tissue specimens using LC/MS; in particular, atmospheric pressure chemical ionization (APCI) in conjunction with ion trap MS. This manuscript describes the validation and application of such a method.

MATERIALS AND METHODS

Chemicals and Solutions

All aqueous solutions were prepared using double deionized water (DDW), which was obtained from a Milli-QT_{plus} Ultra-Pure Reagent Water System (Millipore®, Continental Water Systems, El Paso, TX). All chemicals were purchased in the highest possible purity and used without any further purification. Atenolol, metoprolol,and propranolol were purchased from Sigma Chemical Company (St. Louis, MO). Atenolol-d7 was purchased from the Radian Corporation (Austin, TX). Methanol, acetonitrile, ammonium hydroxide, acetic acid, ethyl acetate, sodium fluoride, potassium phosphate monobasic, and nitric acid were purchased from Fisher Scientific (Pittsburgh, PA). Formic acid (97%) was purchased from ICN (ICN Biomedicals, Inc., Irvine, CA).

The pH of all solutions was measured using a Corning model 430 pH meter (Corning Life Sciences, Acton, MA) connected to a Corning 3-in-1 model pH electrode.

Two separate 10 mL stock solutions of atenolol, metoprolol, and propranolol were prepared independently at 1.00 mg/mL in methanol. Each of these stock solutions was derived from a unique lot of dry chemical obtained from the manufacturer. These two stock solutions were subsequently identified as calibrators and controls. Atenolol-d7 was employed as the internal standard for these experiments and was prepared at a concentration of 100 µg/mL in 10 mL of methanol. These methanolic solutions were stable for at least 12 months. However, for maximum assurance of the quality of data, we never employed any stock solution over 6 months old.

The aqueous portion of the HPLC buffer was 50.0 mM formic acid adjusted to pH 5.00 with conc. ammonium hydroxide. Aqueous buffer and acetonitrile were mixed in a 98:2 ratio, respectively, to help prevent the growth of microbes, and this mixture was filtered through a vacuum filtering apparatus that incorporated a 0.45 µm GH polypro 47 mm hydrophilic, polypropylene membrane filter obtained from Pall Gelman laboratory (Pall Corp., East Hills, NY). The primary organic component of the mobile phase was HPLC grade methanol, which was filtered prior to use through a vacuum filter apparatus that incorporated the same type of membrane filter. The elution gradient employed for these experiments utilized the previous aqueous mixture and methanol at an initial ratio of 90:10. This ratio was adjusted to 10:90 (aqueous mixture:methanol) at 5.00 min and returned to 90:10 (aqueous mixture:methanol) at 7.00 min. An equilibration time of 3.00 min was added to the end of this gradient elution profile for a total HPLC run time of 10.00 min.

Instrumentation

Analyte separation was achieved using a Hewlett Packard 1100 HPLC (Hewlett Packard Co., Wilmington, DE) equipped with a Security Guard™ C-8 guard column (4.0 mm x 3.0 mm i.d., 3 µm particles) from Phenomenex® (Torrance, CA), followed immediately by a Supelcosil™ LC-18 (150 mm x 4.6 mm i.d., 3 µm particles) analytical column from Supelco (Supelco/Sigma-Aldrich, Bellefonte, PA). Samples were injected using a Hewlett Packard G1313A autosampler. Identification and quantitation were accomplished using a Finnigan model LCQ atmospheric pressure chemical ionization (APCI) ion trap mass spectrometer (ThermoFinnigan Corp., San Jose, CA) that utilized nitrogen as the sheath gas and helium as the reagent gas. Control of the HPLC system, integration of the chromatographic peaks, and communication with the mass spectrometer were accomplished using a Gateway 2000 E-4600-SE personal computer using Xcalibur™ LC/MS software version 1.3 (ThermoFinnigan Corp., San Jose, CA).

LC/MS/MS and LC/MS/MS/MS Methods

For all determinations, the HPLC was operated in the gradient mode (discussed above) with a flow rate of 1.0 mL/min. The sample injection volume was 10 µL. The HPLC column was routinely equilibrated overnight prior to use. Following use, the column was washed and stored in a 50:50 mixture of methanol:H_2O. Initial ionization evaluation of these compounds by direct injection into the LCQ indicated that positive chemical ionization (PCI), creating the $[M+H]^+$ ions, was much more effective in signal production than negative chemical ionization (NCI), which formed the $[M-H]^-$ ions. APCI-PCI-MS conditions were optimized separately for each of the three compounds by infusing the desired compound at a concentration of approximately 10 µg/mL, prepared by dilution from the stock solutions using methanol, into the LCQ at a constant rate of 25 µL/min. Tuning the MS for the desired ions was then accomplished using the autotune feature of the Xcalibur™ software. As a result of preliminary APCI-PCI-MS investigations, each sample analysis was subsequently split into 3 unique data collection segments.

The operating conditions for segment 1, which was used for analysis of both atenolol-d7 and atenolol, were as follow: APCI capillary temperature, 150°C; APCI vaporizer temperature, 450°C; source voltage, 4.0 kV; source current, 5.0 µA; capillary voltage, 21.0 V; tube lens offset, 50.0 V; octapole 1 offset, -2.25 V; octapole 2 offset, -6.5 V; interoctapole lens voltage, -16.0 V; ion trap DC offset, -10 V; multiplier voltage, 0.0 V; micro-scan injection time, 200 msec. Segment 1 was further split into 3 separate scan events. Scan event 1 involved collection of the $[M+H]^+$ parent ions for both atenolol and atenolol-d7 at *m/z* 267.4 and *m/z* 274.3, respectively. Scan event 2 collected the atenolol daughter ion at *m/z* 190.1 following collision-induced dissociation (CID) of the parent ion using a collision energy of 38%. Scan event 3 collected the granddaughter ion at *m/z* 145.1 following CID of the daughter ion using a collision energy of 40%.

The operating conditions for segment 2, which analyzed for metoprolol, were as follows: APCI capillary temperature, 150°C; APCI vaporizer temperature, 450°C; source voltage, 5.0 kV; source current, 5.0 µA; capillary voltage, 17.0 V; tube lens offset, 45.0 V; octapole 1 offset, -3.25 V; octapole 2 offset, -7.5 V; interoctapole lens voltage, -20.0 V; ion trap DC offset, -10 V; multiplier voltage, 0.0 V; micro-scan injection time, 200 msec. Segment 2

was further split into 3 separate scan events. Scan event 1 involved collection of the $[M+H]^+$ parent ion at *m/z* 268.4. Scan event 2 collected the daughter ion at *m/z* 191.1 following CID of the parent ion using a collision energy of 40%. Scan event 3 collected the granddaughter ion at *m/z* 159.1 following CID of the daughter ion using a collision energy of 36%.

The operating conditions for segment 3, which analyzed for propranolol, were as follows: APCI capillary temperature, 150°C; APCI vaporizer temperature, 450°C; source voltage, 5.0 kV; source current, 5.0 µA; capillary voltage, 26.0 V; tube lens offset, 55.0 V; octapole 1 offset, -1.50 V; octapole 2 offset, -6.50 V; interoctapole lens voltage, -18.0 V; ion trap DC offset, -10 V; multiplier voltage, 0.0 V; micro-scan injection time, 200 msec. Segment 3 was further split into 3 separate scan events. Scan event 1 involved collection of the $[M+H]^+$ parent ion at *m/z* 260.3. Scan event 2 collected the daughter ion at *m/z* 183.1 following CID of the parent ion using a collision energy of 38%. Scan event 3 collected the granddaughter ion at *m/z* 155.1 following CID of the daughter ion using a collision energy of 38%.

Calibrator and Control Preparation

Calibration curves were prepared by serial dilution utilizing bovine whole blood as the diluent. Calibrators were prepared from one set of original stock standard solutions of atenolol, metoprolol, and propranolol. Controls were prepared in a similar manner to calibrators, using bovine whole blood as diluent but employing the second set of original stock solutions. Calibration curves were routinely prepared at concentrations ranging from 0.78-3200 ng/mL. A minimum of 7 calibrators were used to construct each calibration curve employed for quantitation. Controls used for the determination of accuracy, precision, and stability were prepared at 80 and 320 ng/mL. Controls were prepared in pools large enough to provide replicate samples for the entire study. The atenolol-d7 internal standard solution was prepared in DDW at a final concentration of 500 ng/mL by dilution from the 100 µg/mL stock solution.

Quantitation of atenolol, metoprolol, and propranolol in samples was achieved via an internal standard calibration procedure. Response factors for each compound were determined for every sample analyzed. The response factor was calculated by dividing the area of the analyte peak by the area of the internal standard peak. Calibration curves were derived by plotting a linear regression of the analyte/internal standard response factor versus the analyte concentration for each respective calibrator. These calibration curves were then used to determine the concentrations of atenolol, metoprolol, and propranolol in controls and specimens.

Sample Preparation and Extraction Procedure

Postmortem fluid and tissue specimens, calibrators, and controls were prepared and extracted in the following manner. Tissue specimens were homogenized using a PRO250 post-mounted homogenizer (Pro Scientific, Oxford, CT). The generator used with this homogenizer was 30 mm in diameter and set to rotate at 22,000 rpm. Tissues were homogenized following a 1:2 dilution with 1.00% NaF in DDW. Three mL aliquots of specimen fluids, calibrators, and controls, and 3.0 g aliquots of tissue homogenate were transferred to individual 16 x 150 mm screw top tubes. To each sample, 500 ng of internal standard was added as 1.00 mL of the 500 ng/mL stock internal standard solution. The samples were then vortexed briefly and allowed to stand for 10 min. Nine mL ice-cold acetonitrile was added to each sample. The mixture was then placed on a rotary mixing wheel and mixed for 15 min by simple rotation of the wheel at 15 rpm. Centrifugation at 820×g for 5 min provided removal of cellular debris and proteins. Following centrifugation, the supernatant was transferred to clean 16 x 125 mm culture tubes and evaporated in a water bath at 40°C under a stream of dry nitrogen to a volume less than 1 mL. Following acetonitrile evaporation, 3.00 mL 0.10 M phosphate buffer, pH 6.00 was added to each sample. The extracts were transferred to solid-phase extraction (SPE) columns, which had been pre-conditioned with 2.00 mL methanol, followed by 2.00 mL 0.10 M phosphate buffer, pH 6.00. Care was taken not to dry the column prior to extract addition. Bond Elute Certify® SPE columns, obtained from Varian (Varian Co., Harbor City, CA) were employed for this study. Column flow rates of 1-2 mL/min were maintained in each SPE step using a Varian 24 port Cerex™ SPE processor (Varian Co., Harbor City, CA) with a nitrogen pressure of 3 psi. Once each sample had passed through its respective column, the columns were washed with 1.00 mL of 1.00 M acetic acid then dried completely with 25 psi nitrogen for 5 min. Once dried the columns were again washed by adding 6.00 mL methanol to each. Following the methanol wash, the columns were again dried completely with 25 psi nitrogen for 5 min. The analytes were eluted off the columns with 4.00 mL of 2.00% ammonium hydroxide in ethyl acetate, which was prepared fresh daily. Eluents were evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen, reconstituted in 50.0 µL methanol, and transferred to LC sample vials for LC/MS analysis.

Recovery

The recovery of each analyte was determined using the following procedure.[16] Briefly, two groups, X and Y, of controls prepared using negative whole bovine blood as the diluent were extracted in the same manner as described immediately above. Group X was spiked with a precisely known amount of atenolol, metoprolol, and propranolol prior to extraction. Group Y was spiked with the same precisely known amount of atenolol, metoprolol, and propranolol following the solid phase extraction elution step. Upon analysis, the average response factor obtained from group X was divided by the average response factor obtained from group Y to yield the percent recovery value (100 * X/Y = % recovery) for each of these compounds.

RESULTS AND DISCUSSION

The procedure described herein provides a rapid, reproducible, and accurate method for the determination of the three commonly prescribed beta-blockers — atenolol, metoprolol, and propranolol. This procedure incorporates solid-phase extraction and LC/MS/MS and MS/MS/MS utilizing an APCI ion trap MS in the positive ionization (PCI) mode. SPE provided a cleaner sample and required less organic solvent than did an alternative liquid-liquid extraction procedure commonly employed in our laboratory.[17]

Atenolol/atenolol-d7, metoprolol, and propranolol peaks were completely resolved and experienced no interference from endogenous sample matrix components. All analytes were eluted from the column and detected in less than 8 min. Figure 1 shows a representative LC/MS/MS chromatogram.

An ion trap mass spectrometer is a collection device that allows for the "trapping," or isolation of ions from a target compound, followed by subsequent formation of unique spectra from these individual ions. APCI is a soft ionization technique and, when used in the PCI mode, becomes an excellent source of $[M+H]^+$ parent ions. This ionization technique, in combination with an ion trap mass analyzer, enabled us to perform MS/MS/MS on atenolol, metoprolol, and propranolol. The employment of an ion trap MS following LC separation eliminates any possible interference from either metabolites or endogenous matrix components. Atenolol had a $[M+H]^+$ parent ion at *m/z* 267.4. The parent ion was collected by the ion trap and subjected to collision-induced dissociation (CID), resulting in 2 predominant daughter ions at *m/z* 190.1 and 225.1. Collecting the *m/z* 190.1 ion and performing CID on it resulted predominantly in the granddaughter ion at *m/z* 145.1. The same process was employed for metoprolol, which had a parent $[M+H]^+$ ion at *m/z* 268.3, a predominant daughter ion at *m/z* 191.1, and a granddaughter ion at *m/z* 159.1 generated from *m/z* 191.1 fragmentation. Propranolol ionization resulted in a parent $[M+H]^+$ ion at *m/z* 260.3, a predominant daughter ion at *m/z* 183.1, and a granddaughter ion at *m/z* 155.1 generated from *m/z* 183.1 fragmentation.

The full-scan MS/MS and MS/MS/MS spectra for atenolol, metoprolol and propranolol provided the "fingerprints" used for analyte identification and confirmation. These full scan spectra are shown in Figures 2-7. The "fingerprint" criterion was fundamentally qualitative in nature. For example, in an MS spectrum with all the usual other peaks being below 15% of the base peak, we would never accept as valid a sample that produced any peak greater than 30% of the same base peak. Likewise, we would never accept as valid a sample in which all peaks were less than 5% of the same base peak. LC retention times were also used as analyte acceptability criteria. Retention times for each analyte were required to be within 2% of the average calibrator retention time for that analyte. Typical retention times were 4.26, 6.44, and 7.64 min for atenolol/atenolol-d7, metoprolol, and propranolol, respectively.

Quantitation of each analyte was accomplished by monitoring the highest abundance ion(s) in the MS/MS mode. The MS/MS spectrum for atenolol had 2 major ions; these ions were summed to derive the chromatographic peak for this compound. The chromatographic peak area of the monitored ions from the compounds of interest divided by the chromatographic peak area of internal standard, atenolol-d7, resulted in a response ratio that was utilized for quantitation. The ions selected from each compound for both quantitation and qualification are shown in Table 1.

The linear dynamic range (LDR), limit of detection (LOD), and limit of quantitation (LOQ) were initially determined by analysis of extracted bovine whole blood calibrators ranging in concentration from 0.39-6400 ng/mL. The LDR for each compound was determined following this analysis. The experimentally determined LDRs for atenolol, metoprolol, and propranolol were found to be 1.6-3200 ng/mL, 1.6-3200 ng/mL, and 0.78-1600 ng/mL, respectively. The correlation coefficients for each of these calibration curves exceeded 0.995 when a weighting factor of 1/X was employed. The LOD and LOQ values determined for each of these beta-blockers 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 "fingerprint" 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 atenolol were 0.78 ng/mL and 1.6 ng/mL, respectively. Metoprolol had a LOD of 0.39 ng/mL and a corresponding LOQ of 1.6 ng/mL, and propranolol had an LOD of 0.39 ng/mL and an LOQ of 0.78 ng/mL. As can be seen in Table 2, the average recovery of atenolol, metoprolol, and propranolol at a concentration of 300 ng/mL was 58 ± 9%, 80 ± 2%, and 82 ± 4%, respectively.

Carryover contamination was initially investigated and subsequently monitored by the use of solvent blank injections. A methanol blank injected following the highest extracted calibrator showed no carryover contamination. Subsequently, blanks were used throughout the sample sequence to verify that no sample-to-sample contamination occurred.

Intra-day (within day) and inter-day (between days) accuracy and precision were examined for this extraction. Accuracy was measured as the relative error between the experimentally determined and target concentrations of a sample. Precision was measured as the relative standard deviation (RSD) for the experimentally determined concentrations. Whole blood controls at 80 and 320 ng/mL were prepared in pools on day 1 and stored in the refrigerator at 4°C until extracted.

For intra-day analyses, a calibration curve was extracted along with 5 replicates of each control concentration on day 1 of the experiment. The intra-day relative error and RSD for atenolol were +1% and 4% at 80 ng/mL, and -9% and 4% at 320 ng/mL, respectively. The intra-day relative error and RSD for metoprolol were -1% and 5% at 80 ng/mL, and +1% and 3% at 320 ng/mL, respectively. The intra-day relative error and RSD for propranolol were -3% and 3% at 80 ng/mL, and +1% and 1% at 320 ng/mL, respectively. These data are summarized in the beginning of Table 3.

Inter-day accuracy and precision were evaluated by extracting 5 replicates of each of 2 control concentrations on days 2, 5, and 7 and basing quantitation on the calibration curve originally prepared on day 1. The results obtained after storage of each control lot at 4°C for 2, 5, and 7 days can be seen in Table 3. At 2 days of storage the relative error and RSD for atenolol were -10% and 5% at 80 ng/mL, and -15% and 2% at 320 ng/mL, respectively. The relative error and RSD for metoprolol were -13% and 15% at 80 ng/mL, and -5% and 5% at 320 ng/mL, respectively. The relative error and RSD for propranolol were -5% and 14% at 80 ng/mL, and -2% and 3% at 320 ng/mL, respectively. At 5 days of storage, the relative error and RSD for atenolol were -3% and 5% at 80 ng/mL, and -11% and 5% at 320 ng/mL, respectively. The relative error and RSD for metoprolol were -4% and 6% at 80 ng/mL, and -2% and 7% at 320 ng/mL, respectively. The relative error and RSD for propranolol were -8% and 9% at 80 ng/mL, and -2% and 6% at 320 ng/mL, respectively. At 7 days of storage, the relative error and RSD for atenolol were -4% and 5% at 80 ng/mL, and -13% and 1% at 320 ng/mL, respectively. The relative error and RSD for metoprolol were -1% and 3% at 80 ng/mL, and -0.3% and 2% at 320 ng/mL, respectively. The relative error and RSD for propranolol were -15% and 5% at 80 ng/mL, and -2% and 2% at 320 ng/mL, respectively.

After 7 days of storage at 4°C, the 80 ng/mL control was found to have an atenolol concentration of 77 ± 4 ng/mL, a metoprolol concentration of 79 ± 2 ng/mL, and a propranolol concentration of 68 ± 3 ng/mL. The 320 ng/mL control was found to have an atenolol concentration of 279 ± 4 ng/mL, a metoprolol concentration of 319 ± 7 ng/mL, and a propranolol concentration of 313 ± 5 ng/mL. These relatively minor decreases were found to be acceptable for general use; nonetheless, as a good laboratory practice and in an effort to maintain a high degree of accuracy, we would recommend (1) preparing new calibration curves at the beginning of each new analysis and (2) prompt toxicological analysis once a specimen has been thawed.

Metabolite Interference

The metoprolol metabolite *o*-desmethylmetoprolol, when present in postmortem specimens, has been shown to lead to false atenolol positives when specimens are analyzed by GC/MS.[2] We investigated *o*-desmethylmetoprolol, utilizing the newly validated LC/MS method to ensure that no interference occurred. The retention time for this compound was 5.20 min, which is between atenolol (4.26 min) and metoprolol (6.44 min). Additionally, as shown in Figures 8 and 9, the MS and MS/MS spectra for *o*-desmethylmetoprolol do not share any predominant ions with the MS/MS spectra obtained from atenolol; more specifically, the ions used for atenolol quantitation are not present in the MS/MS *o*-desmethylmetoprolol spectrum.

Method Application: Postmortem Specimen Analysis

Postmortem fluid and tissue specimens obtained from 3 separate aviation accidents over the past 2 years were analyzed. These 3 cases had previously been screened positive for atenolol, metoprolol, or propranolol by GC/MS and were re-examined using this novel method to obtain quantitative concentrations of these compounds in various fluid and tissue specimens. The fluid and tissue specimens selected for analysis were blood, urine, liver, kidney, and

skeletal muscle. The 3 cases chosen for this investigation had a majority, if not all, of the desired specimens available for analysis. The results of this analysis are shown in Table 4. The wide range of concentrations obtained (3 ng/g to 1268 ng/mL) demonstrates the utility of this novel method and its applicability in the field of forensic toxicology.

CONCLUSION

An LC/MS method that is rapid, reliable, and sensitive has been developed for the identification and subsequent quantitation of atenolol, metoprolol, and propranolol in postmortem fluid and tissue specimens. This method offers the selectivity afforded by LC/MS/MS/MS, which prevents interference from metabolites commonly encountered when analyzing postmortem specimens. This novel method eliminates the need for derivitization prior to analysis, saving both time and money. For the quantitation of 3 commonly prescribed beta-blockers, the relative simplicity of this procedure provides a viable alternative to GC/MS for the field of forensic toxicology.

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FIGURES AND TABLES

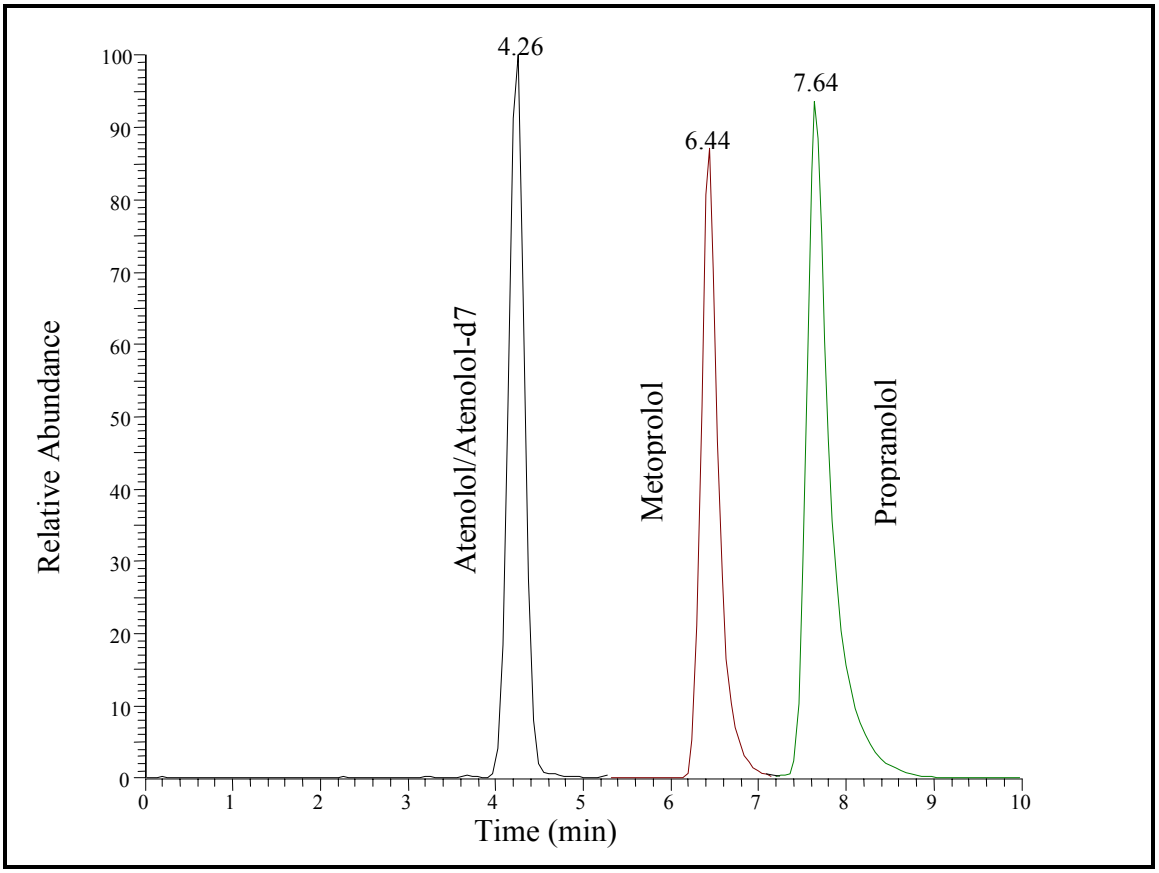

Figure 1. Representative concatenated chromatogram of atenolol/atenolol-d7, metoprolol, and propranolol in an extracted whole blood calibrator. Chromatographic peaks represent ions monitored in SIM mode for each compound as follows: atenolol-d7 MS ion at *m/z* 274.3; atenolol MS/MS ion at *m/z* 190.1; metoprolol MS/MS ion at *m/z* 191.1; and propranolol MS/MS ion at *m/z* 183.1. Peaks were obtained from a 10 µL injection of a 200 ng/mL calibrator. The chromatogram is constructed by monitoring for atenolol/atenolol-d7 from 0-5.2 min, for metoprolol from 5.2-7.1 min, and for propranolol from 7.1-10.0 min. The 100% relative abundance corresponds to the peak current observed for the atenolol/atenolol-d7 peak.

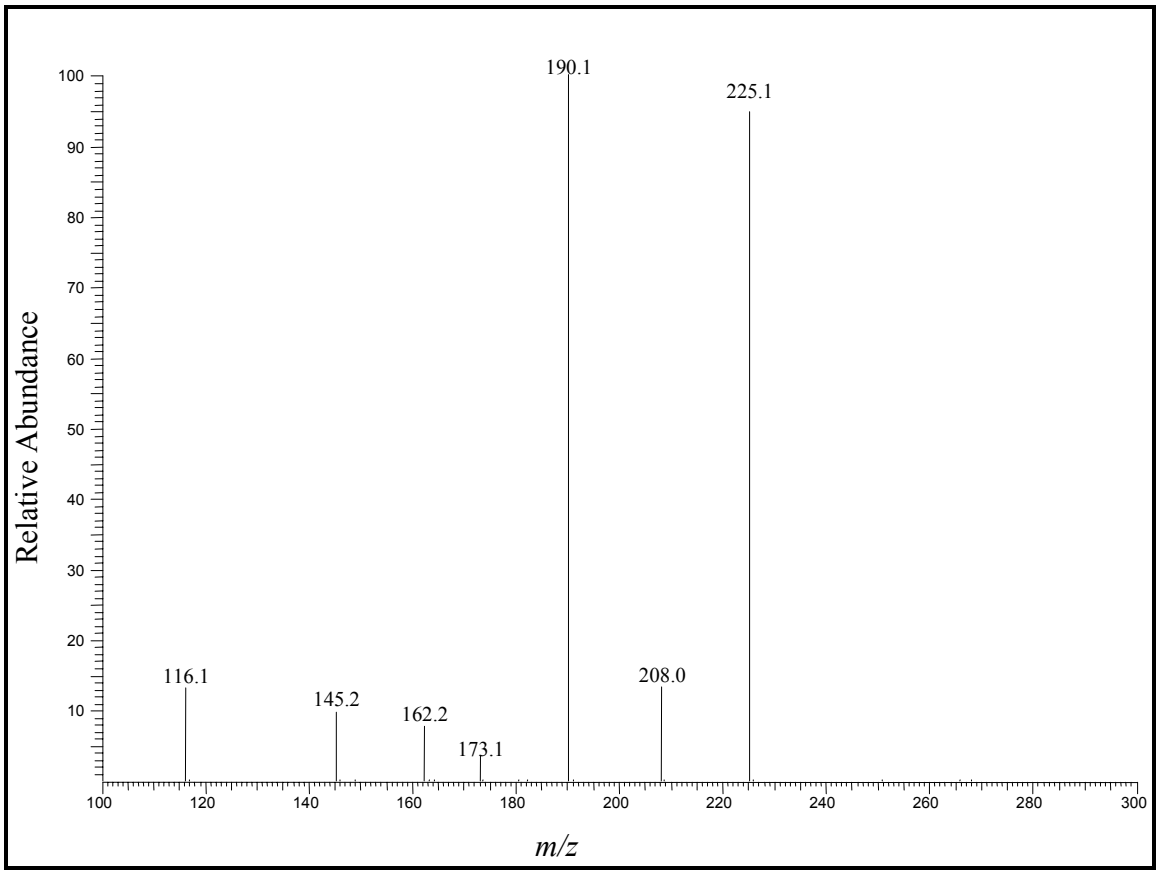

Figure 2. MS/MS spectrum of atenolol (*m/z* 267.4 → spectrum).

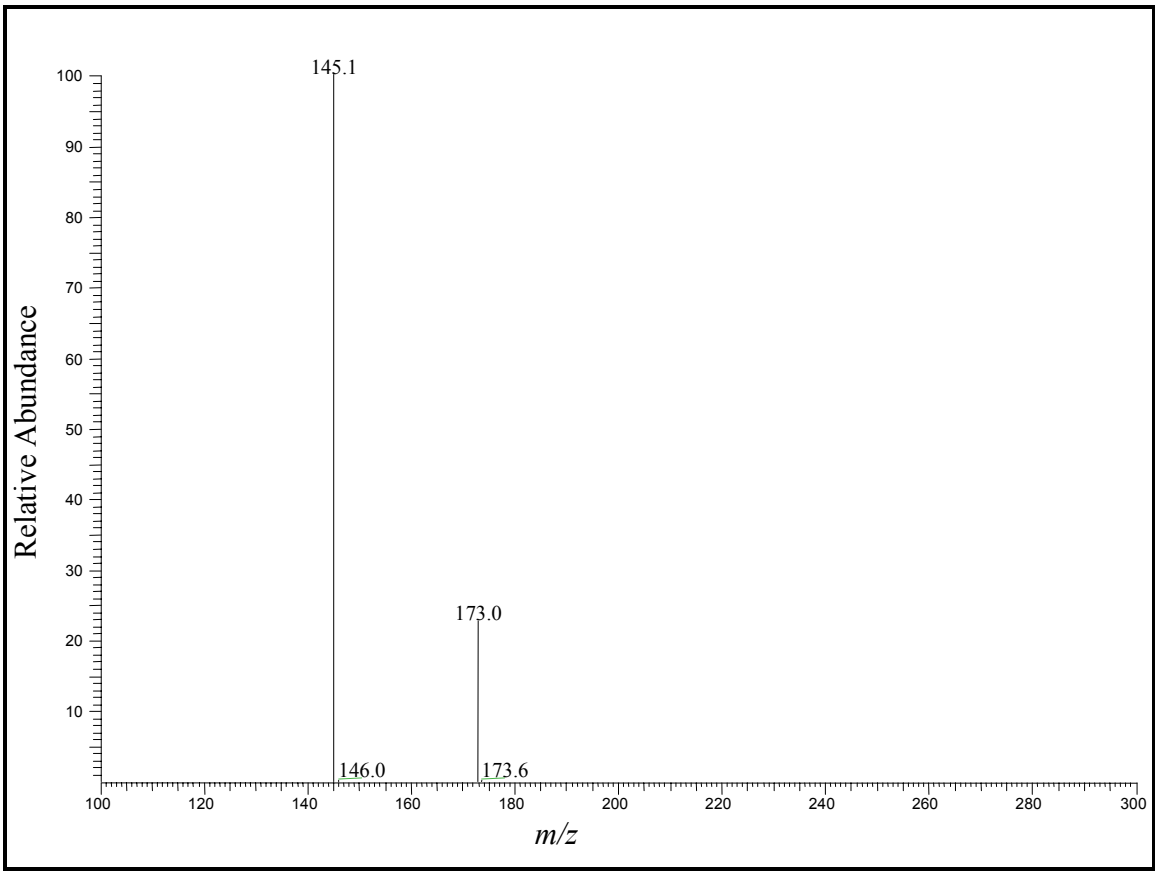

Figure 3. MS/MS/MS spectrum of atenolol (*m/z* 267.4 → 190.1 → spectrum).

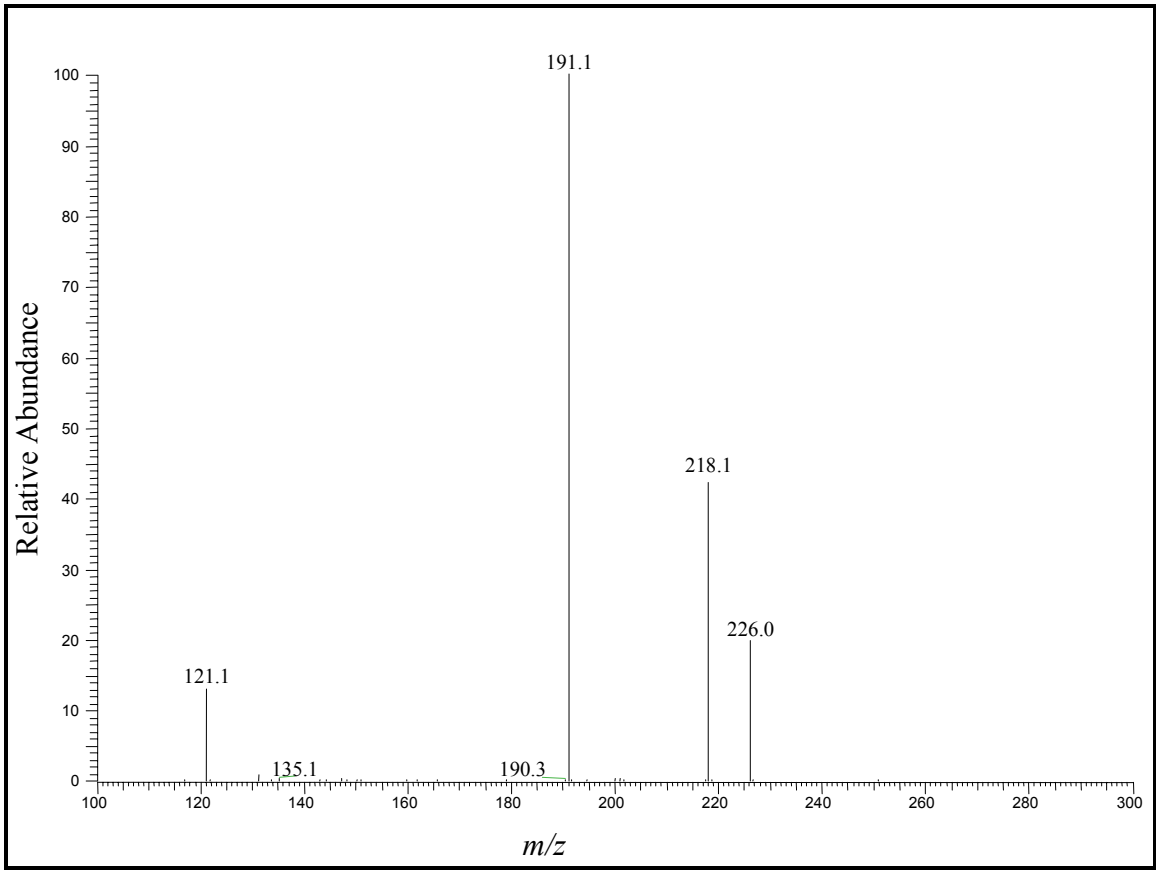

Figure 4. MS/MS spectrum of metoprolol (*m/z* 268.4 → spectrum).

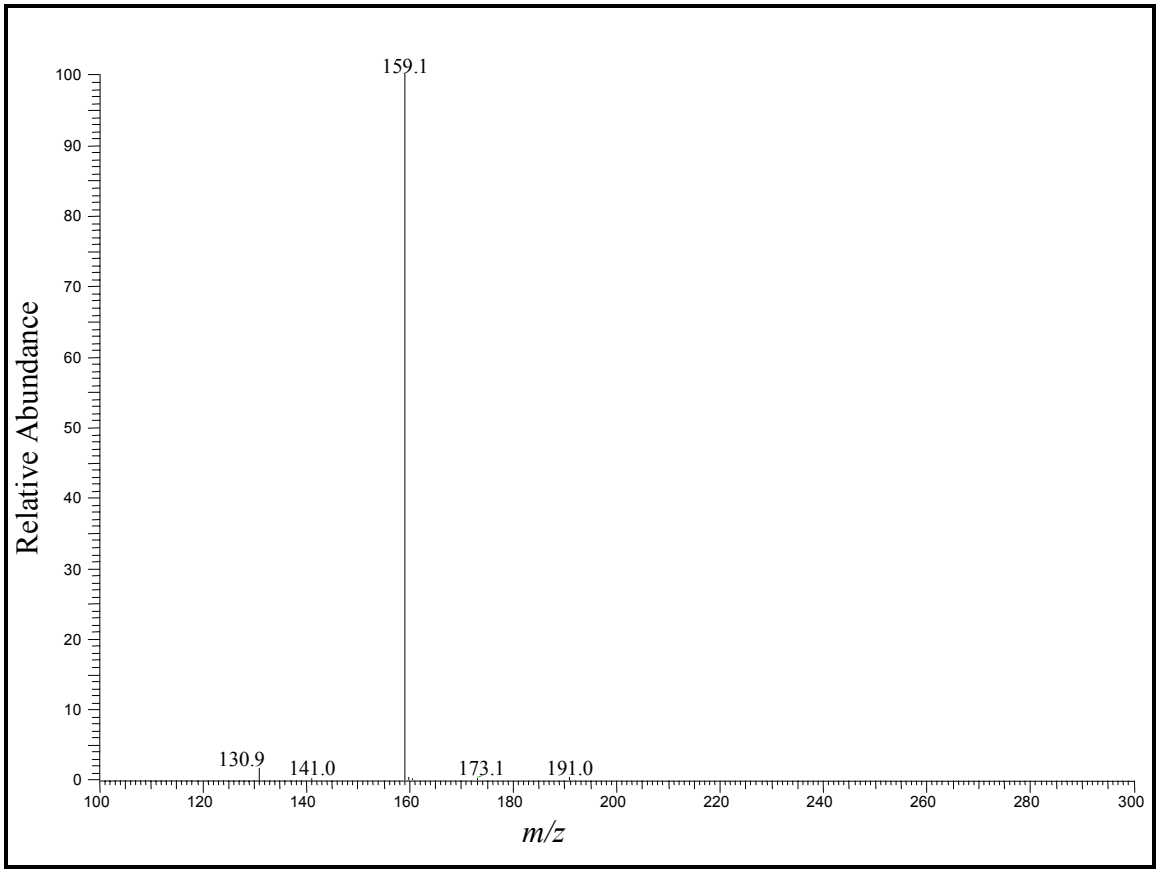

Figure 5. MS/MS/MS spectrum of metoprolol (*m/z* 268.4 → 191.1 → spectrum).

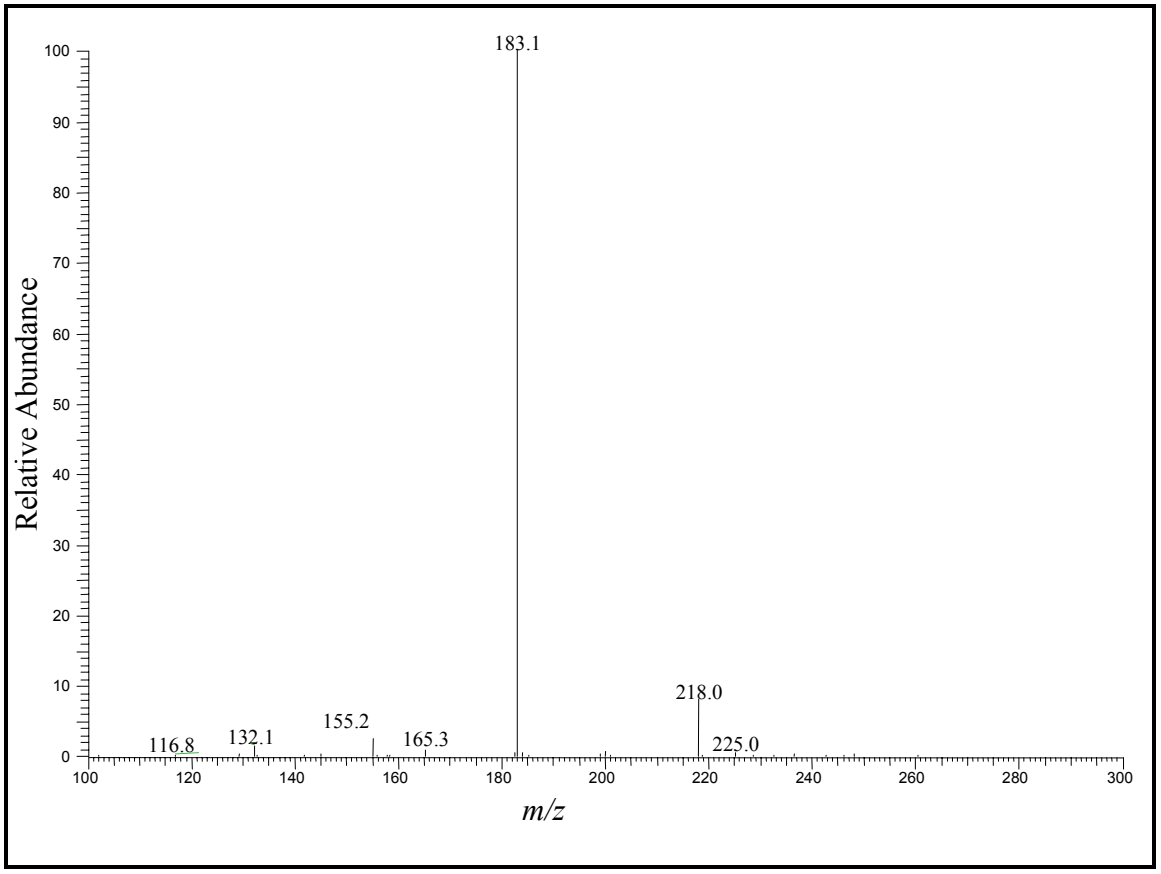

Figure 6. MS/MS spectrum of propranolol (*m/z* 260.3 → spectrum).

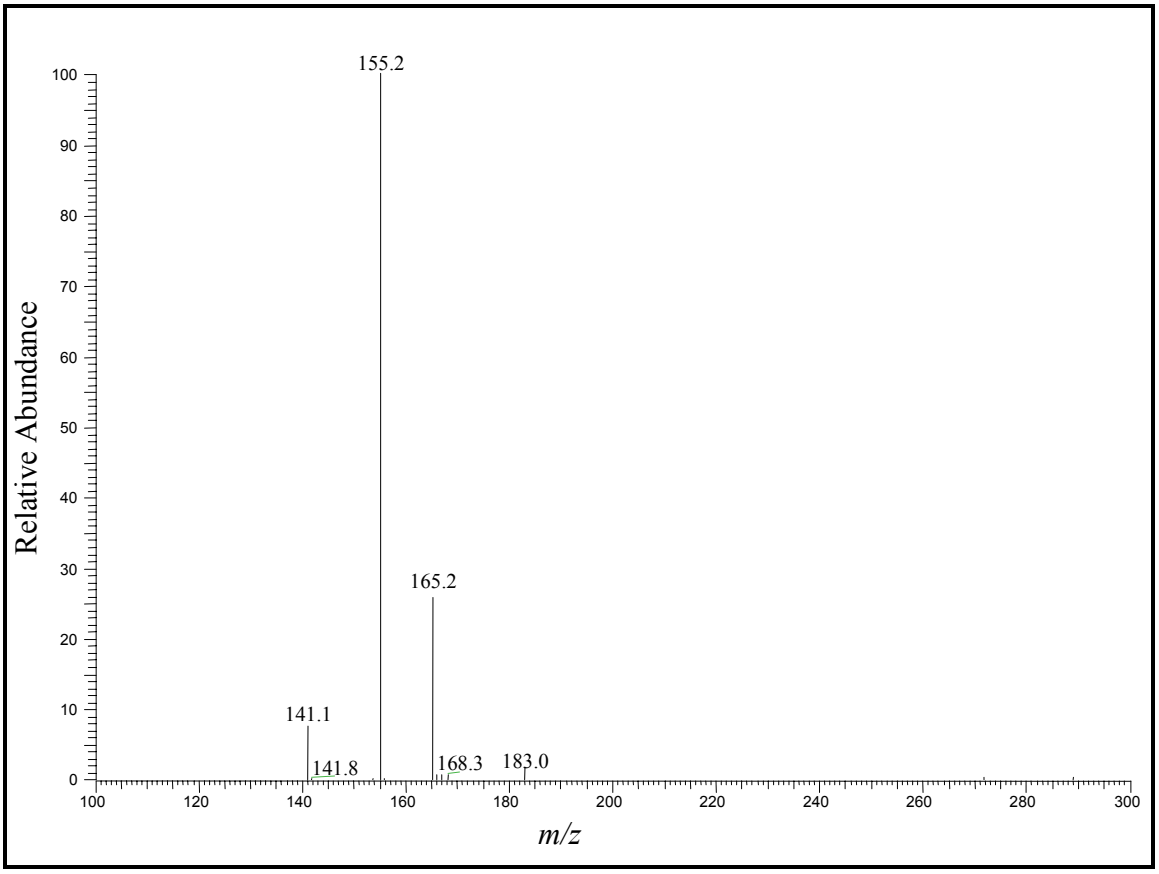

Figure 7. MS/MS/MS spectrum of propranolol (*m/z* 260.3→ 183.1 → spectrum).

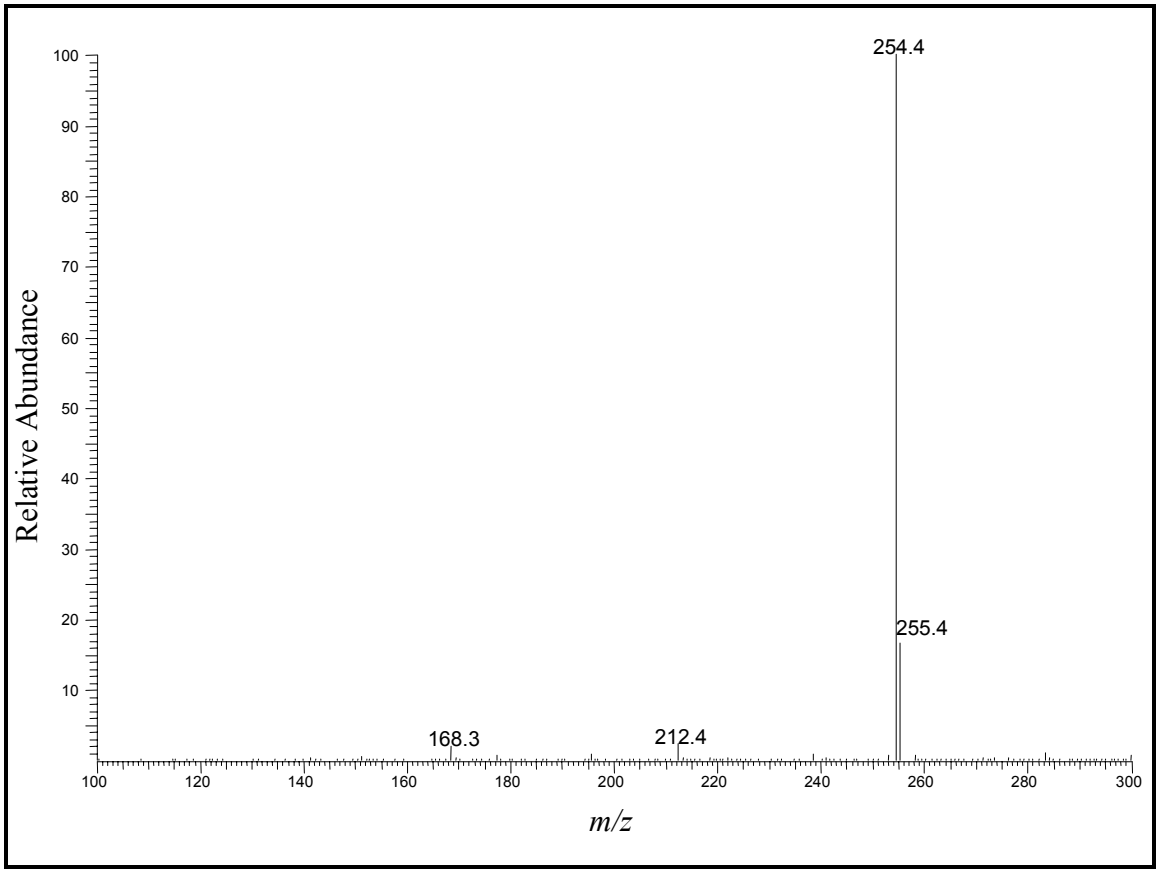

Figure 8. MS spectrum of *o*-desmethylmetoprolol.

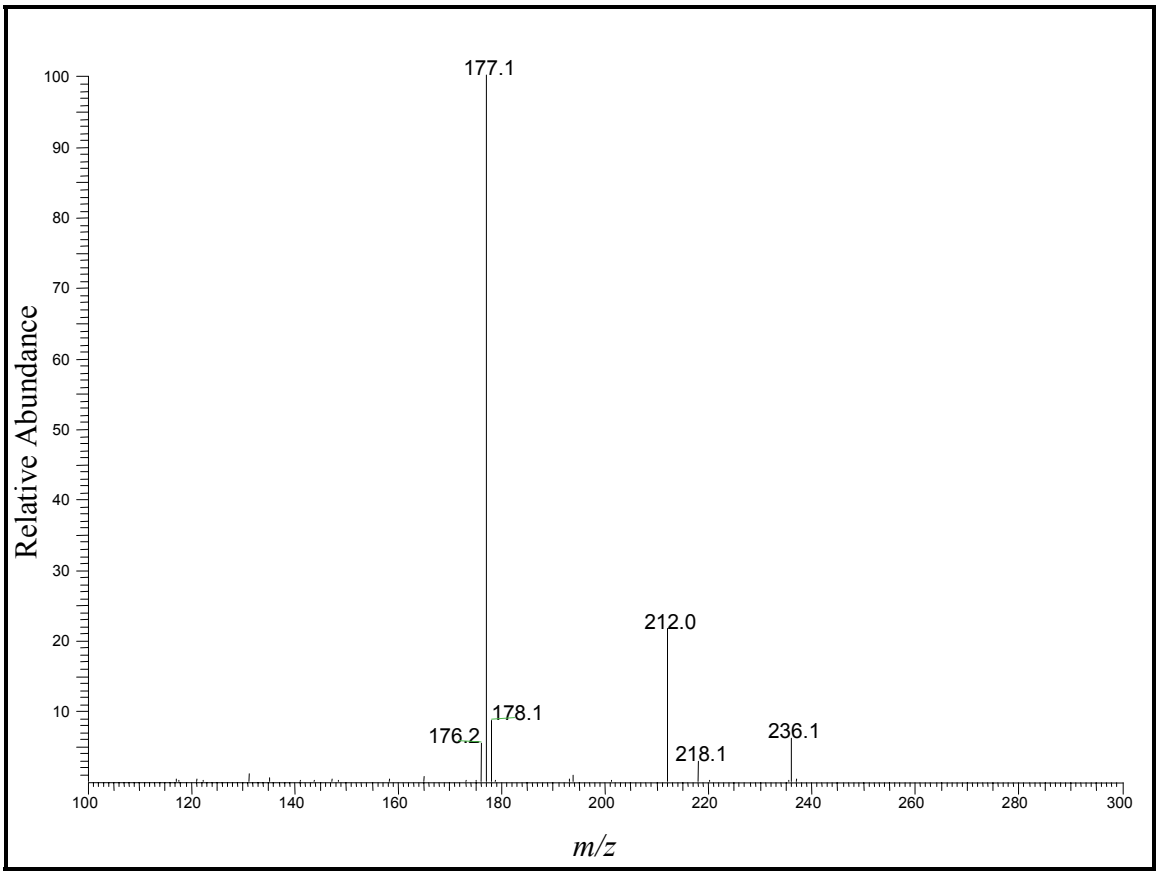

Figure 9. MS/MS spectrum of *o*-desmethylmetoprolol (*m/z* 254.4 → spectrum).

TABLES

Table 1. Ions used for the quantitation and qualification of atenolol, metoprolol, and propranolol.

Compound	Quantitation Ions, MS/MS (*m/z*)	Qualifier Ions, MS/MS/MS (*m/z*)
Atenolol	190.1, 225.1	145.1
Metoprolol	191.1	159.1
Propranolol	183.1	155.2

Table 2. LDR, LOD, LOQ, and recovery data for atenolol, metoprolol, and propranolol.

Compound	LDR (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Recovery (%)*
Atenolol	1.6-3200	0.78	1.6	58 ± 9
Metoprolol	1.6-3200	0.39	1.6	80 ± 2
Propranolol	0.78-1600	0.39	0.78	82 ± 4

*Recovery at 300 ng/mL, n=5

Table 3. Intra-day Accuracy and Precision for Repeated Determinations Over 7 Days*

	Atenolol		Metoprolol		Propranolol	
	Day 1		Day 1		Day 1	
Target Conc.	80	320	80	320	80	320
Mean ± SD	81 ± 3	291 ± 13	79 ± 4	325 ± 9	82 ± 2	323 ± 3
Relative Error	+1%	-9%	-1%	+1%	+3%	+1%
R.S.D.	4%	4%	5%	3%	3%	1%
	Day 2		Day 2		Day 2	
Target Conc.	80	320	80	320	80	320
Mean ± SD	72 ± 4	272 ± 5	70 ± 11	303 ± 15	76 ± 11	314 ± 11
Relative Error	-10%	-15%	-13%	-5%	-5%	-2%
R.S.D.	5%	2%	15%	5%	14%	3%
	Day 5		Day 5		Day 5	
Target Conc.	80	320	80	320	80	320
Mean ± SD	78 ± 4	284 ± 13	77 ± 5	314 ± 21	74 ± 7	314 ± 18
Relative Error	-3%	-11%	-4%	-2%	-8%	-2%
R.S.D.	5%	5%	6%	7%	9%	6%
	Day 7		Day 7		Day 7	
Target Conc.	80	320	80	320	80	320
Mean ± SD	77 ± 4	279 ± 4	79 ± 2	319 ± 7	68 ± 3	313 ± 5
Relative Error	-4%	-13%	-1%	-0.3%	-15%	-2%
R.S.D.	5%	1%	3%	2%	5%	2%

*n=5 at each concentration for each day, controls were run on days 1, 2, 5, and 7.

Table 4. Beta-blocker concentrations (ng/mL, ng/g) in postmortem fluids and tissues of 3 separate aviation fatalities.

	Blood	Urine	Liver	Kidney	Muscle
Case 1 Atenolol	81	1268	258	127	N/A*
Case 2 Metoprolol	57	73	303	249	51
Case 3 Propranolol	38	N/A*	172	25	3

* Specimen type not available for analysis.