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Quantitation of atenolol, metoprolol, and propranolol in postmortem human fluid and tissue specimens via LC/APCI-MS☆

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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 to 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% to 82%. This method was successfully applied to postmortem fluid and tissue specimens obtained from victims of three separate aviation accidents.
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Keywords: Forensic science; Toxicology; Liquid chromatography/mass spectrometry; Atenolol; Metoprolol; Propranolol

1. Introduction

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

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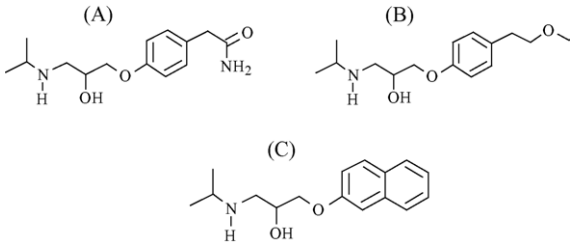

Fig. 1. Chemical structures of: (A) atenolol, (B) metoprolol, and (C) propranolol.

[1]. The chemical structures of these compounds can be seen in Fig. 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.

2. Materials and methods

2.1. 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 concentrate 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 min and returned to 90:10 (aqueous mixture:methanol) at 7 min. An equili-