Journal of Pharmaceutical and Biomedical Analysis 127 (2016) 147–155

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development of SPME-LC–MS method for screening of eight beta-blockers and bronchodilators in plasma and urine samples

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ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 18 February 2016
Accepted 1 March 2016
Available online 4 March 2016

Keywords:
Solid phase microextraction
SPME
Liquid chromatography–mass spectrometry
Beta-blockers
Plasma
Urine
High throughput
Sample preparation

ABSTRACT

The current work describes the development and validation of a simple, efficient, and fast method using solid phase microextraction coupled to liquid chromatography–tandem mass spectrometry (SPME-LC–MS/MS) for the concomitant measurement of eight beta-blockers and bronchodilators in plasma and urine. The presented assay enables quantitative determination of acebutolol, atenolol, fenoterol, nadolol, pindolol, procaterol, sotalol, and timolol. In this work, samples were prepared on a high-throughput platform using the 96-well plate format of the thin film solid phase microextraction (TFME) system, and a biocompatible extraction phase made of hydrophilic-lipophilic balance particles. Analytes were separated on a pentafluorophenyl column (100 mm × 2.1 mm, 3 μm) by gradient elution using an UPLC Nexera coupled with an LCMS-8060 mass spectrometer. The mobile phase consisted of water-acetonitrile (0.1% formic acid) at a flow rate of 0.4 mL min^{-1}. The linearity of the method was checked within therapeutic blood-plasma concentrations, and shown to adequately reflect typically expected concentrations of future study samples. Post-extraction addition experiments showed that the matrix effect ranged in plasma from 98% for procaterol to 115% for nadolol, and in urine, from 85% for nadolol and pindolol to 119% for atenolol. The method was successfully validated using Food and Drug Administration (FDA) guidelines, and met all acceptance criteria for bioanalytical assays at five concentration levels for all selected drugs. The final protocol can be successfully applied for monitoring concentrations of the selected drugs in both plasma and urine matrices obtained from patients or athletes.

1. Introduction

Beta-blockers (β-blockers) are important clinical drugs used in the treatment of several cardiovascular disorders such as angina pectoris, cardio arrhythmia, and hypertension [1], whereas bronchodilators are used to control symptoms of asthma and chronic obstructive pulmonary diseases [2]. However, although these compounds have revolutionized the medical management of the above diseases, their use has been prohibited in-competition and out-of-competition for several sports, especially in activities that require vehicle control and bodily movements; as these compounds can induce a reduction in heart-rate and tremors, as well as improve general performance during competitions, they have been considered to be unsportsmanlike performance enhancers, as they give individual athletes an unfair advantage over others [3]. While typical oral doses of β-blockers and bronchodilators range from 5–100 mg, maximum blood concentrations of these compounds are below 1000 ng mL^{-1}; these drugs have a narrow therapeutic range (Table 1), and show toxicity at higher concentrations [4]. Considering these implications, high throughput screening and sensitive determinations of β-blockers and bronchodilators in biological matrices such as urine and plasma are essential in various aspects, such as doping control, toxicology, and therapeutic drug monitoring.

Different analytical detection and quantification techniques have been reported for analysis of β-blockers and bronchodilators in biological samples such as urine and plasma. These methods include thin layer chromatography [5], fluorescence [6], micellar electrokinetic chromatography (MEKC) [7], gas chromatography–mass spectrometry [8], high performance liquid chromatography (HPLC) with UV and fluorescence detection [9,10], LC–mass spectrometry [11–15], and matrix-assisted laser desorption/ionization-mass spectrometry [16]. Currently, chromatographic techniques coupled with mass spectrometry detectors are more widely used due to their comparative high sen-

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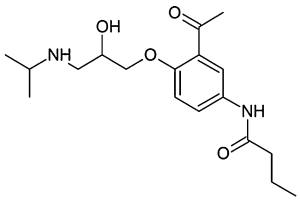
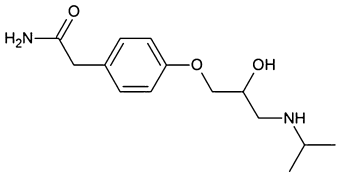
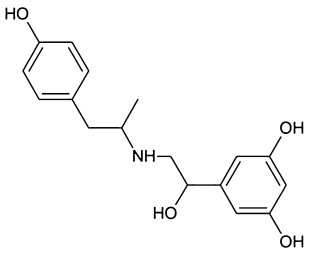
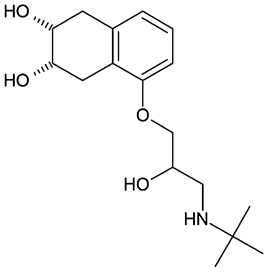
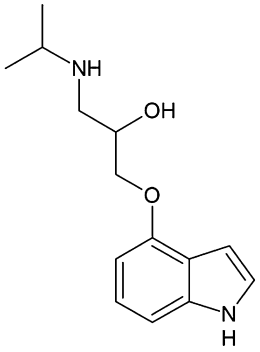
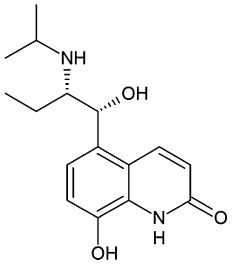
http://dx.doi.org/10.1016/j.jpba.2016.03.001
0731-7085/

sitivity and selectivity. However, most methods based on GC–MS require a time-consuming derivatization step of polar groups e.g., aminopropanol chain, resulting in poor chromatographic separation of several polar derivatives of β-blockers, as well as instability of some derivatives [17]. As an alternative method, LC–MS has been successfully applied for determination of β-blockers and bronchodilators. LC–MS is broadly used today, and offers good sensitivity, selectivity, and quantification of β-blockers in biolog-

Table 1
Structure and characteristics of the selected target compounds (β-blockers and bronchodilators).

Compound Name	Molecular Structure	Molecular mass ($g\,mol^{-1}$)[a]	logP [42]	Therapeutic blood-plasma concentration ($mg\,L^{-1}$) [4]
Acebutolol		336.43	1.71	0.2–2
Atenolol		266.34	0.16	0.1–1
Fenoterol		303.35	1.60	0.001–0.04
Nadolol		309.60	0.81	0.01–0.25
Pindolol		248.32	1.75	0.02–0.15
Procaterol		290.36	1.28	NF