Journal of Chromatography B, 1022 (2016) 93–101

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Salting-out assisted extraction method coupled with hydrophilic interaction liquid chromatography for determination of selected β-blockers and their metabolites in human urine

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form 1 April 2016
Accepted 5 April 2016
Available online 7 April 2016

Keywords:
HILIC
SALLE
β-Blockers
Metabolites

ABSTRACT

In this study, a new analytical method was developed and validated for the simultaneous analysis of β-blockers (metoprolol, propranolol, carvedilol) and their metabolites (5′-hydroxycarvedilol, *O*-desmethylcarvedilol, α-hydroxymetoprolol, *O*-desmethylmetoprolol, 5-hydroxypropranolol) in human urine. A salting-out assisted liquid-liquid extraction (SALLE) procedure was used for sample preparation. Several parameters affecting the extraction efficiency and method sensitivity including the type and volume of the extraction solvent, the type and quantity of the inorganic salt, extraction time and sample pH were investigated. Hydrophilic interaction liquid chromatography-ultraviolet detection (HILIC-UV) was used for the determination of all analytes. During method development, the effects of mobile phase components (type, pH, concentration of salt, organic modifier type and content, flow rate, column temperature) on the retention and separation of β-blockers and metabolites on the five different HILIC columns were examined. The method was linear for concentrations ranging from 0.1 to 8.0 μg/mL, with determination coefficients higher than 0.993 for all analytes. The limits of quantification were in the range from 0.1 to 0.2 μg/mL. Intra- and inter-day precision ranged from 0.1 to 8.9%, and accuracy was within ± 13% interval for all analytes. Under the optimized conditions, extraction efficiency was greater than 83.4% for determined compounds. The validated method was then applied to the measurement of β-blockers and their metabolites in human urine samples.

1. Introduction

It is a matter of concern that the World Health Statistics 2014 released by the World Health Organization (WHO) confirms that approximately 33.3% of the population suffers from cardiac problems (data from 194 countries) [1]. The most common cardiovascular diseases are high blood pressure (hypertension), coronary heart disease, heart failure, stroke and congenital cardiovascular defects. β-Adrenoceptor antagonists, also called β-blockers, are the most commonly used drugs for treating these ailments. They can also be used for the treatment of glaucoma, thyreotoxicosis (as an adjunct), anxiety and benign essential tremor [2,3]. Serious side-effects of β-blockers include bradycardia, aggravation of cardiac failure, bronchoconstriction, hypoglycemia and fatigue. An overdose of β-blockers may lead to life-threatening situations [4].

Pharmacokinetic and pharmacodynamic studies are important for the determination of the metabolism of drugs and for the design and development of safe medication. For these studies, effective, efficient, selective and reproducible analytical methods are required. The analysis of β-adrenergic blockers in human plasma, urine and other biological samples provides useful information for clinical studies. For instance, cases of intoxication, assessing the therapy compliance of patients, doping control and pharmacokinetic interactions with other drugs (lipid soluble β-blockers with calcium antagonists) need selective, efficient and reproducible methods [5].

In recent years, numerous analytical approaches have been proposed for the determination of β-blockers and their metabolites in biological fluids. An overview of all of these analytical methods can be found in a recent and comprehensive review by Saleem et al. [5–21]. To date, several chromatographic methods have been reported for the trace determination of β-blockers and their metabolites in biological samples, including high performance liquid chromatography (HPLC) in reversed phase (RP) with ultravi-

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http://dx.doi.org/10.1016/j.jchromb.2016.04.010
1570-0232/

Table 1
Accuracy precision and recovery data of β-blockers and their metabolites (n = 6).

Analyte	$C_{nominal}$[a] (μg/mL)	Intra-day		Inter-day		ER[d] (%)
		RSD[b] (%)	RE[c] (%)	RSD[b] (%)	RE[c] (%)	
O-DMCAR	3.2	0.22	−0.70	2.2	−1.4	99.3
	1.6	1.3	0.78	3.4	2.5	98.2
	0.16	3.1	12	4.9	13	97.1
CAR	3.2	0.63	0.56	1.9	2.5	97.8
	1.6	0.65	1.2	2.8	3.6	99.6
	0.16	7.5	1.3	8.9	9.7	90.7
5′-HCAR	6.4	0.38	−0.53	3.5	−4.5	92.5
	3.2	0.46	−2.0	2.7	−5.4	99.7
	0.32	6.9	−0.57	8.1	−9.1	104
PRO	3.2	1.1	−0.29	3.0	−4.1	98.4
	1.6	0.26	−0.35	4.8	−5.8	98.8
	0.16	4.0	−9.8	6.3	−9.9	98.2
5-HPRO	3.2	1.2	−2.1	2.5	−4.3	95.1
	1.6	1.8	−3.0	3.4	−4.6	96.2
	0.16	2.4	−4.2	4.6	−6.8	95.8
MET	6.4	0.65	−0.57	2.7	−3.6	99.5
	3.2	0.57	1.1	3.4	4.7	99.1
	0.32	1.9	−0.51	4.7	−5.7	93.8
O-DMMET	6.4	0.15	−0.55	2.1	−2.8	88.0
	3.2	0.61	0.22	1.8	3.7	93.1
	0.32	4.1	1.8	6.3	7.8	85.1
α-HMET	6.4	0.73	0.33	3.9	5.2	86.7
	3.2	0.92	1.0	2.8	5.4	102
	0.32	2.2	−3.2	4.2	−7.6	83.4

[a] Nominal concentration.
[b] Relative standard deviation.
[c] Relative error.
[d] Extraction recovery.

olet (UV), fluorescence, electrochemical, chemiluminescence (CL) or mass spectrometric detection (MS or MS/MS) [5–21]. Most chromatographic methods are based on gas chromatography-mass spectrometry (GC–MS), requiring the derivatization of the polar groups (aminopropanol chain) of compounds [22–27].

β-Blockers have been investigated in several biomatrices, including plasma, urine, seminal fluid, breast milk, and brain tissue. To date, to the best of our knowledge, there are only two reports dealing with the quantification of β-blockers with other drugs in influent wastewater (omeprazole, pantoprazole, ranitidine, citalopram, fluoxetine, paroxetine, venlafaxine, tramadol, nebivolol, metoprolol, atenolol, bisoprolol and metformin) and in human plasma (atenolol), using hydrophilic interaction liquid chromatography (HILIC) [28,29].

Sample preparation procedures of β-blockers and their metabolites, in most cases, include either liquid-liquid extraction (LLE), or solid-phase extraction (SPE). However, these procedures are time consuming, generally labor intensive and require large quantities of expensive, toxic and environmentally hazardous organic solvents [30]. Salting-out is a process involving the addition of electrolytes to the aqueous phase in order to increase the distribution ratio of a particular solute. Salting-out assisted liquid/liquid extraction (SALLE) with a water miscible organic solvent has shown distinctive advantages in bioanalytical research. SALLE is superior to LLE in terms of extract cleanness; moreover, SALLE uses environmentally safe reagents compared with common sample preparation techniques [31–35].

In this work, a simple, fast and environmentally friendly SALLE methodology in combination with HILIC-UV was developed and validated for the quantitative determination of β-blockers and their metabolites in human urine samples. Important parameters influencing the sample extraction technique and analytes separation were also optimized in order to obtain maximum extraction efficiency, sensitivity and selectivity. Additionally, the study aimed to improve our understanding of the HILIC mechanisms involved in the separation of β-blockers and their metabolites. The combination of a simple sample treatment such as SALLE with the HILIC technique was found to provide a useful method for monitoring β-blockers and their metabolites in human urine samples.

2. Experimental

2.1. Chemicals and reagents

Metoprolol (MET) and propranolol (PRO) was purchased from Sigma-Aldrich (St. Louis, MO). Carvedilol (CAR), 5′-hydroxycarvedilol (5′-HCAR), *O*-desmethylcarvedilol (*O*-DMCAR), α-hydroxymetoprolol (α-HMET), *O*-desmethylmetoprolol (*O*-DMMET), 5-hydroxypropranolol (5-HPRO) were purchased from Toronto Research Chemicals Inc. (North York, Canada). HPLC-grade water, methanol, ethanol, isopropanol, acetonitrile, ammonium acetate, ammonium formate, 25% ammonia solution and acetic acid were obtained from Merck (Darmstadt, Germany). Analytical-grade acetonitrile, methanol, ethanol, isopropanol, sodium chloride (NaCl), potassium chloride (KCl), magnesium sulfate ($MgSO_4$), ammonium chloride (NH_4Cl) and ammonium sulfate ($(NH_4)_2SO_4$) were purchased from POCH S.A. (Gliwice, Poland).

2.2. Preparation of standard solutions, calibration standards and quality control (QC) solutions

Stock standard solutions of β-blockers and their metabolites were prepared in methanol at concentrations of 1 mg/mL. Working solutions of β-blockers and their metabolites with concentrations