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Journal of Chromatography A, 1124 (2006) 57–67
JOURNAL OF CHROMATOGRAPHY A
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Extraction and preconcentration of salbutamol and terbutaline from aqueous samples using hollow fiber supported liquid membrane containing anionic carrier

Yadollah Yamini [b], Curt T. Reimann [a], Alireza Vatanara [c], Jan Åke Jönsson [a,*]

[a] *Department of Analytical Chemistry, University of Lund, P.O. Box 124, 221 00 Lund, Sweden*
[b] *Department of Chemistry, Tarbiat Modarres University, P.O. Box 11415-175, Tehran, Iran*
[c] *Department of Pharmaceutics, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

Available online 22 May 2006

Abstract

This paper presents a new three-phase liquid-phase microextraction (LPME) strategy for extraction and preconcentration of salbutamol (SB) and terbutaline (TB) from aqueous samples, including urine. The drugs were extracted from 11 ml of aqueous sample (source phase; SP) into an organic phase with microliter volume located inside the pores of a polypropylene hollow fiber, and then back-extracted into 24 μl of a second aqueous solution as the receiving phase (RP), located in the lumen of the hollow fiber. In preliminary experiments, we tried to transport the drugs using a pH gradient between the two sides of the hollow fiber. Due to the existence of both amine and phenolic groups on the drugs, very little transport occurred and enrichment factors (EF) less than one were obtained. Further experiments were done in the presence of bis(2-ethylhexyl) monohydrogenphosphoric acid (D2EHPA) or methyltrioctylammonium chloride (Aliquat 336) in the organic phase, to extract drugs from acidic and basic matrices, respectively. Results showed that transport of drugs from alkaline solution into 1 M of sodium bromide occurred when the membrane was impregnated with dihexyl ether containing 20% Aliquat 336. To optimize the EF, the effects of different parameters such as the nature of organic solvent used to impregnate the membrane, compositions and volumes of SP and RP, type and concentration of carrier, extraction time and stirring rate were investigated. Optimal results were obtained in the presence of 0.005 M of NaOH (pH 11.70) in the SP, 1 M of NaBr in the RP, 20% of Aliquat 336 in dihexyl ether as membrane impregnation solvent, stirring rate of 500 rpm and extraction time of 60 min. Under these conditions, enrichment factors of 52.9 and 213.1, dynamic linear ranges of 20–5000 and 10–5000, and limits of detection of 2.5 and 0.5 ng/ml were obtained for salbutamol and terbutaline, respectively. Also determination of drugs in environmental water and urine samples in the range of nanograms per millilitre with RSDs < 10% was possible using HPLC–photodiode array detection or HPLC–MS.

Keywords: Three-phase LPME; Hollow fiber membranes; Salbutamol; Terbutaline; HPLC–MS; Water analysis; Urine

1. Introduction

The occurrence of drug residues in the aquatic environment emerged as a matter of public concern after elevated levels of clofibric acid were found in some Berlin groundwater samples in 1992 [1]. Recently, increased efforts to monitor drug residues in such environments have shown that this is a widespread problem: many pharmaceutical compounds can frequently be found in the effluent of sewage treatment plants and river water at concentrations of up to several micrograms per liter [2]. Conventional sewage treatment plants do not fully degrade residues of pharmaceuticals, so that they are introduced into the aquatic environment [3,4]. Thus, developing very sensitive methods for monitoring of known organic contaminants and rapidly tracking down new contaminants in surface water is a matter of high priority [5–9].

Salbutamol (SB), 2-(*tert*-butylamino)-1-(4-hydroxy-3-hydroxymethylphenyl) ethanol and terbutaline (TB), 1-(3,5-dihydroxyphenyl)-2-(*tert*-butylamino)ethanol belong to the class of β_2-agonist drugs [10,11]. β_2-Agonists possess a common β-phenyl-β-ethanol amine group but vary in that there are different substituents on the amine nitrogen and also on the phenyl ring. The presence of two hydroxyl groups on the phenyl ring of TB and one hydroxyl group and an additional $-CH_2OH$ substituent on the phenyl ring of SB increase their hydrophilicity (Fig. 1) [12] and thus render their direct extraction into hydrophobic media difficult ($K_{OW} < 1$).

* Corresponding author. Tel.: +46 46 222 8169; fax: +46 46 222 4544.
E-mail address: jan_ake.jonsson@analykem.lu.se (J.Å. Jönsson).

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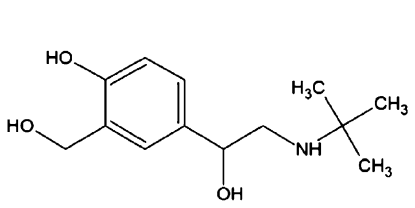
doi:10.1016/j.chroma.2006.05.001

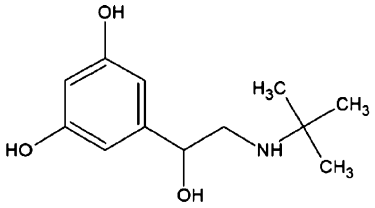


Salbutamol

pK_{a1} = 9.22 ±0.47, pK_{a2} = 9.83 ±0.47

log K_{OW} = 0.01 ±0.37

$(M + 1)^+$ = 240.2



Terbutaline

pK_{a1} = 9.12 ±0.1, pK_{a2} = 9.33 ±0.47

pK_{a3} = 10.77 ±0.1, log K_{OW} = 0.48 ±0.36

$(M + 1)^+$ = 226.1

Fig. 1. Structures of salbutamol and terbutaline and their pK_a and log K_{OW} values and monoisotopic masses (assuming ionization by single protonation). pK_a and log K_{OW} values were calculated using the ACD/pK_a (ver. 1.20) and ACD/log P DB (ver. 3.00) software programs (Advanced Chemistry Development, Toronto, Canada).

SB and TB are used for the treatment of pulmonary disorders, particularly in the treatment of bronchial asthma in humans [13]. As therapeutic agents, low doses of these drugs can be administered directly to the lung airways via inhalation in order to minimize negative side effects and maximize efficiency [14]. In addition to their anti-asthmatic effects, β_2-agonists have been pharmacologically proven to be able to increase muscle protein, reduce total body fat due to lipid removal from fat depots, and promote muscle growth. In animals, β_2-agonists, in addition to their regular role in veterinary medicine as bronchodilatory and tocolytic agents, increase the ratio of lean meat to fat (repartitioning) and improve feed conversion efficiency [15]. The World Anti-Doping Agency (WADA) has forbidden the oral or parenteral use of TB and SB due to their effects on the central nervous system and due to certain anabolic-like effects [16]. SB and TB appear in urine both as unaltered drugs and as sulfate-conjugated metabolites [12]. A concentration of free salbutamol in urine that exceeds 1 μg/ml constitutes a doping violation [17].

The most usual analytical method for determination of β_2-agonists in biological samples is gas chromatography–mass spectrometry (GC–MS) [12,18–22]. Derivatization has an important role in the determination of β_2-agonists by GC–MS and different derivatization procedures have been employed [12]. Unfortunately, derivatization is a complicated and time-consuming procedure. Hence, HPLC methods seem to be good alternatives to GC–MS analysis since the derivatization step is not required. HPLC methods using UV [23,24], fluorescence [25–27], electrochemical [28,29], and mass-selective [30–38] detections have been extensively employed in quantitative analysis of β_2-agonist in biological samples. Also some miscellaneous methods, such as capillary electrophoresis with UV detection [39], sequential injection analysis using spectrophotometric [40], chemiluminescence or fluorescence detection [41], and europium-enhanced fluorescence detection [42], have been employed for β_2-agonists determination in different samples.

Due to the complexity of the composition of the matrices and the trace amounts of analytes involved, an effective extraction/purification approach prior to final analysis is of vital importance for residue analysis of β_2-agonists, regardless of the chromatographic method used. Several different sample preparation procedures have been employed, either by themselves or in conjunction with one another, for extraction/purification of β_2-agonists before analysis: liquid–liquid extraction (LLE), solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD), immunoaffinity chromatography (IAC), dialysis, and supercritical fluid extraction (SFE) [13].

SPE has been commonly employed for extraction and preconcentration of β_2-agonists before final analysis. There is large flexibility with SPE due to different available analyte–extractant interactions, including adsorption, apolar interaction, polar interaction, ion exchange, and molecular imprinting [12,13,18–22,25–27,29–35,39,40]. Although SPE consumes much less time than LLE, it still involves large consumption of solvents and additionally suffers from clogging when handling real samples. The other methods mentioned above require specialized and/or expensive apparatus to perform.

For reduction of solvent usage in sample preparation, a miniaturized format of LLE called liquid-phase microextraction (LPME) was introduced [43]. In two-phase LPME the analytes are extracted from an aqueous sample matrix into an organic acceptor phase. Three-phase LPME involves extraction from an aqueous sample into an organic phase immiscible with water and located on top of the aqueous phase, followed by back-extraction into a separate microdrop of aqueous phase suspended from the tip of a microsyringe and penetrating into the organic phase [44]. In recent years the mechanical robustness of this scheme has been enhanced with the aid of a porous-walled polypropylene hollow fiber used to support the organic phase in the pores of the wall while holding the second aqueous phase in the central channel or lumen of the fiber [45–49].

In the present study, hollow fiber liquid membrane containing Aliquat 336, an anionic carrier, in three-phase LPME mode was used for extraction and preconcentration of SB and TB from aqueous samples. The enrichment factor (EF) was studied as a function of the nature of the immobilized organic phase, type and concentration of carrier in the membrane phase (MP), compositions of source (donor) phase (SP) and receiving (acceptor) phase (RP), volume of SP and RP, extraction time, and stirring