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Graphite-based microextraction by packed sorbent for online extraction of β-blockers from human plasma samples

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ABSTRACT

In the present work a new graphitic material (Carbon-XCOS) was used as a sorbent for microextraction by packed sorbent (MEPS). The β-blockers metoprolol and acebutolol in plasma samples were extracted and detected online using Carbon-MEPS syringe and liquid chromatography and tandem mass spectrometry (LC–MS/MS). Factors affecting the MEPS performance such as conditioning, washing and elution solutions were investigated. The validation of the bioanalytical method was performed using human plasma. The standard curve ranged from 10 to 2000 nM and the lower limit of quantification (LLOQ) was set to 10 nM. The method validation showed good accuracy and precision for the quality control (QC) samples at three concentration levels (30, 800 and 1600 nM). The accuracy values of the QC samples were in the range of 86–108% ($n = 18$). The precision values of intra- and inter-day for QC samples ranged from 4.4% to 14.4% (RSD) for the both studied analytes. The coefficient of determination (R^2) values were ≥0.999 ($n = 3$).

1. Introduction

Due to the complexity of the biological matrices, sample preparation has an important role in the analytical process. Samples such as blood, plasma or urine contain various quantities of salts, proteins, acids, bases, organic and inorganic compounds that can have similar chemical properties to the analytes of interest. Therefore, sample preparation is an essential part in the analysis of biological samples to transform the complex sample into a more suitable and cleaner form for analysis. The major objectives of the sample preparation are eliminating the interfering substances and enrichment of the analytes. An ideal and reliable sample preparation method should be fast, selective, accurate and fully automated. In addition, it should be more environmentally friendly.

During the last two decades, the interest for miniaturization of sample preparation techniques was growing. Microextraction by packed sorbent (MEPS) is a miniaturized form of solid phase extraction (SPE). MEPS was invented a decade ago [1–3]; it is a miniaturized sample preparation technique that is fast and efficient, and it uses small amounts of solvent and sample. It has been successfully applied for the extraction of a wide range of analytes from complex matrices in different research fields, such as in environmental analysis, bioanalysis and food analysis [4–14]. Most studies using MEPS involve silica based sorbents, and few have reported using other sorbents [2]. In order to enlarge the number of potential applications sorbent materials with peculiar characteristics should be investigated. Carbonaceous sorbents were introduced as sorbents for SPE in the 1980s for the extraction of nonpolar and moderately polar analytes. The interest in carbonaceous sorbent increased due to its ability of retaining very polar and highly hydrophilic compounds [15,16]. Graphitic carbon has unique retention mechanism and unique selectivity because of its large specific surface area and polarizability which provides sufficient retention both, for polar and nonpolar analytes and keeps a linear sample capacity over a large concentration range [17]. In addition pure graphitic materials are stable across the pH range from 1.0 to 14.0, so eluent of any pH can be used. Furthermore some graphitic sorbents, such as porous graphitic carbon (PGC) have sufficient hardness across high pressures or temperatures and also has unchanging surface energy that provides a linear adsorption isotherm [18]. In liquid chromatography the graphitic stationary phases have demonstrated a unique capability in separating of closely related compounds diastereo- and positional-isomers and polar compounds that have high solubility in water, which are difficult to retained by other sorbents due to their low affinity for reversed-phase sorbents. Furthermore when used for SPE graphitic sorbents express selectivity when fractioned elution conditions are optimized [19,20]. In few previous studies β-blockers have been successfully separated by PGC [21,22]. Recently a new composite

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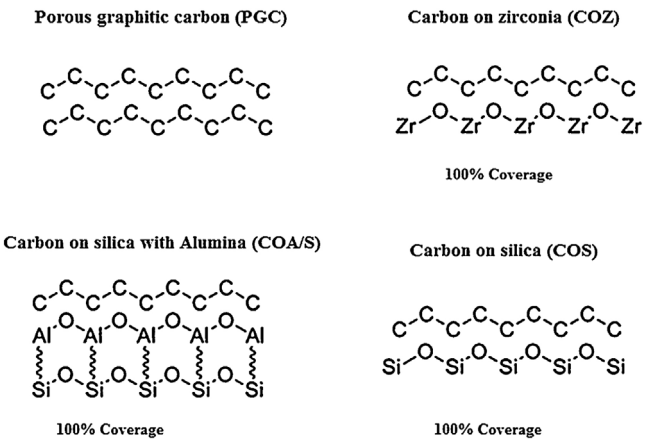

Fig. 1. Structure of the graphitic sorbent.

graphitic materials (CarbonX®) were produced by coating stable substrate with graphene are commercially available [23].

The aim of this study was to investigate the use of a new graphitic material as sorbent for microextraction by packed sorbent. In the present study a bioanalytical method for β-blockers in plasma samples using graphite-based sorbent and MEPS–LC–MS/MS was developed and validated.

2. Experimental

2.1. Chemicals

Acebutolol, metoprolol and pentycaine (IS) were obtained from AstraZeneca (Gothenburg, Sweden). Analytical grade acetonitrile, methanol, formic acid and were obtained from Merck (Darmstadt, Germany). Milli-Q Plus water purification system from Millipore Corporation (Bedford, USA) was used for water purification.

2.2. Graphitic carbon material

Two new graphitic composite materials, namely CarbonX® COA and COS evaluated as sorbent was provided by United Science Corporation (Center City MN, USA). A schematic view of the structure of this material is shown in Fig. 1. In the present study the method validation for acebutolol and metoprolol in human plasma was carried out utilizing CarbonX® COA in MEPS online with LC–MS–MS.

2.3. Instrumentation

Liquid chromatography (LC) system involves two pumps (Shimadzu, Kyoto, Japan) and CTC-Pal autosampler (Analytics AG, Zwingen, Switzerland). The analytical column was a Zorbax (50 mm × 2.1 mm, SB-C18, 3.5 μm) (Agilent, CA, USA). The mobile phase A was 0.1% formic acid in water and phase B was 0.1% formic acid in acetonitrile. The mobile phase flow rate was 0.2 mL/min and injected sample volume was 30 μL. A gradient LC method was used and described in Table 1. A Hettich centrifuge (Tuttlingen, Germany), was used for plasma centrifugation (the samples were centrifuged for 2.0 min at speed of 3500 rpm).

Table 1
LC gradient.

Time (min)	*B* (%)	Flow rate (mL)
01:00	10	0.2
03:00	90	0.2
05:00	90	0.2
05:10	10	0.2
06:00	10	0.2

Table 2
MS parameters for the studied analytes.

Compound	Precursor ion	Daughter ion	Cone (V)	Collision (eV)
Metoprolol	268.16	116.34	30	20
Acebutolol	337.15	116.1	25	22
Pentycaine (I.S.)	303.3	154.25	35	20

Temperatures – desolvation: 350 °C, cone: 150 °C.

A Quattro-micro tandem mass spectrometry (Waters, Manchester, UK) was used. Nitrogen was used as drying gas (400 L h^{-1}), and nebulizing gas (20 L h^{-1}). The cone and desolvation temperatures were 150 °C and 350 °C, respectively. The argon was used as the collision gas (AGA, Lidingö, Sweden).

The tuning parameters of mass spectrometry were provided by direct infusion of standard solution containing metoprolol, acebutolol or pentycaine (500 nM each). The scan mode was multiple reaction monitoring (MRM) using precursor ions at $[M+H]^+$ (*m*/*z*: 337, 268 and 303) and the product ions *m*/*z*: 116.1, 116.3 and 154.3 were used for quantification of acebutolol, metoprolol and penticaine, respectively. After optimization, the capillary voltage, extractor and the RF lens were set at 3.0 kV, 5 V, and 0.2 V, respectively. The cone voltage and collision energy values were reported in Table 2. MassLynx version 4.1 was used for data collecting and processing.

2.4. Sample preparation process

Two stock solutions (1.0 mM each) of metoprolol and acebutolol were prepared in methanol and kept in 4 °C (one was used for preparation of standards and the other was used for preparation of quality control (QC) samples). Two spiked pooled plasma samples (spiking solution: 100 μM) were prepared by adding of 0.5 ml of stock solution to 4.5 ml plasma and these were used as working solution one for preparation of standard solutions and one QC samples. Daily, examination of standard solutions was showed that the analytes solutions were stable for 3 weeks. The standards samples (10, 25, 50, 100, 500, 1000, 1500 and 2000 nM) and QC samples at three levels (QCL (low): 30, QCM (medium): 800 and QCH (high): 1600 nM) were prepared from the working solutions in plasma. All samples were vortexed and centrifuged at 3500 rpm for 3 min. Then, 500 μL (supernatant) of every sample was taken and diluted by Milli-Q water 4 times followed by online MEPS extraction and LC–MS/MS. The blank human plasma was pooled from five objects.

2.5. Method validation

Method validation was run according to FDA guidelines [24]. Each calibration curve had eight calibration points in the range of 10–2000 nM and the quality control samples prepared in three different levels low, medium and high concentration. The lower limit of quantification (LLOQ) was 10 nM. The calibration curves in plasma samples were tested with linear and quadratic regressions. The quadratic regression gave accurate results and therefore the quadratic equation was used in this study. The calibration solutions were prepared daily before each assay.

The peak area ratios for the analytes and IS were measured. Quality control samples (QC) at three different concentrations (high: H, medium: M and low: L) for three assays (run at different days) were used to calculate accuracy and precision. Each assay contained standard samples (S1–S8) and 6 of QC samples at each level (6 QCH, 6 QCM and 6 QCL).

The accuracy is calculated as the ratio of measured experimental value and true value [Accuracy = (measured value/true value)*100].