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Molecularly imprinted polymer cartridges coupled on-line with high performance liquid chromatography for simple and rapid analysis of human insulin in plasma and pharmaceutical formulations

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ABSTRACT

In this paper, a novel method is described for automated determination of human insulin in biological fluids using principle of sequential injection on a molecularly imprinted solid-phase extraction (MISPE) cartridge as a sample clean-up technique combined with high performance liquid chromatography (HPLC). The water-compatible molecularly imprinted polymers (MIPs) were prepared using methacrylic acid as a functional monomer, ethylene glycol dimethacrylate as a cross-linker, chloroform as a porogen and insulin as a template molecule. The imprinted polymers were then employed as the solid-phase extraction sorbent for on-line extraction of insulin from human plasma samples. To achieve the best condition, influential parameters on the extraction efficiency were thoroughly investigated. Rapid and simple analysis of the hormone was successfully accomplished through the good selectivity of the prepared sorbent coupled with HPLC. Limits of detection (LOD) and quantification (LOQ) of 0.2 ng mL^{-1}, 0.7 ng mL^{-1}, and 0.03 ng mL^{-1}, 0.1 ng mL^{-1} were obtained in plasma and urine respectively. The obtained data exhibited the great recoveries for extraction of insulin from human plasma and pharmaceutical samples, higher than 87%.

1. Introduction

Insulin is the most important regulatory hormone that is central to regulating carbohydrate and fat metabolism in the body. It plays a vital role in the control of glucose homeostasis, consisting of 51 amino acids shared between two intramolecular chains and with a molecular weight of about 5800. Insulin is not a stable entity. However, enables molecules in its vicinity to be modified by chemical reactions. So, during storage, use of pharmaceuticals preparation and during inappropriate sample preparations, insulin is degraded by hydrolytic reactions or is transformed by formation of intermolecular covalent bonds with other insulin molecules, leading to higher molecular weight transformation products.

The insulin has been separated and quantified using different methods such as capillary electrophoresis [1], HPLC method [2,3], LC/MS analysis [4], immunoassays [5], electrochemical method [6], amperometry [7], surface plasmon resonance [8] and mass-sensitive biosensors [9,10]. Solid-phase extraction (SPE), nowadays, is a well-founded technique and has been used for the preconcentration and clean up of numerous different kinds of compounds in a variety of matrices before the chromatographic separation [11,12]. However, it is considered as an appropriate sample preparation technique, but the selectivity of the SPE sorbents has still remained as a challenging demand sought after by many research to enhance the selectivity of the method particularly in complex matrices. Hence, molecularly imprinted polymers (MIP) are currently being investigated by many researchers [13,14]. They are composed of immune sorbents (ISs) in which its affinity and selectivity stem from antigen–antibody interactions. Therefore, a selective extraction of the target analyte and of similarly structured compounds could be easily achieved [15,16]. One of the interesting configurations of the SPE sorbents could be considered as on-line combination with HPLC if the applied solid support is pressure resistant [17–21]. Despite their important interest as a selective sample pretreatment sorbents, the development of ISs is time consuming and relatively expensive. These drawbacks have contributed to the development of molecularly imprinted polymers (MIPs). MIPs are synthetic materials possessing specific cavities specially designed for the recognition of an analyte of interest. Their synthesis procedures for SPE application are mainly based on strong noncovalent interactions (such as hydrogen bonds or ionic interactions) between a target molecule (template) with functional monomers, followed by polymerization in the

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presence of a cross-linker, usually in a non-protic and weakly polar solvent. Once the template is removed, selective molecular recognition sites, often described as three-dimensional shapes in the polymer, are available for the selective rebinding of the target molecule and some structurally related compounds. The first application was carried out by the group of Sellergren in 1994 for the extraction of pentamidine present at low concentration level in urine [22]. Nowadays, MIPs have been largely applied to the extraction of target analytes from various complex matrices [23,24]. Concerning the pharmaceutical field, many examples deal with the extraction of a biomolecule by MIP from tablets [25–27], plasma [28–33] or from urine [34,35] samples. Most of these applications were based on off-line procedures. A very few applications were carried out in on-line mode [33,34,36–38] while trends in analytical chemistry are for high throughput approaches that require to minimize the time spent performing analysis. Therefore, MIP cartridges coupled on-line with high performance liquid chromatography (HPLC) can allow a straightforward and fast pretreatment due to their specific recognition properties. We applied MIPs as artificial receptors for off-line solid-phase extraction of bromhexine [31], metoclopramide [32], verapamil [39], and tramadol [40] in biological fluids. Recently, the applicability of an on-line solid phase extraction method using molecularly imprinted monolithic column was developed for the assay of tramadol in urine and plasma samples [41].

In this work a novel automated method has been developed for the selective extraction and further chromatographic determination of insulin based MIPs. It was intended to be used as the selective SPE cartridge sorbents for efficient sample clean-up of the analyte from complex biological matrices. The new SPE cartridge sorbents allows the sensitive, simple and inexpensive extraction and determination of the target drug in human plasma and urine samples while various pharmaceutical compounds found in the biological fluid were not well retained on the MIP support. To the best of our knowledge, it is rarely reported that the on-line MIP–SPE coupled to HPLC system is employed to monitor insulin.

2. Experiment section

2.1. Reagents

Prior to use methacrylic acid (MAA) from Merck (Darmstadt, Germany), it was distilled in vacuum in order to remove the stabilizers. Ethylene glycol dimethacrylate (EGDMA) and 2, 2′-azobis isobutyronitrile (AIBN) as the initiator of polymerization from Sigma-Aldrich (Steinheim, Germany) were used without any purification. All solvents used in chromatography analyses were HPLC grade and purchased from Merck. Pure insulin was supplied by Sigma (Munich, Germany). All solutions were made with deionized water purified using a Milli-Q Plus System (ELGA Corp., Purelab UHQ) and dissolved in water to obtain concentrations of 10, 50, 100, 150 and 200 μg mL^{-1}. It was also some insulin formulations which were purchased from post-market and analyzed as real samples: Insulin regular human (Lansulin® R, 100 IU 1 ml, Vial, Exir pharmaceuticals), Insulin Isophane (NPH)-(Lansulin® N, 1000 IU 1 mL^{-1}, Vial, Darou Pakhsh Pharmaceuticals) and Insulin Aspart (NovoRapid®, 100 IU 1 mL^{-1}, Pen, Nove Nordisk, Australia) were obtained as pharmaceutical preparations. All experiments and validation steps were performed utilizing fresh freeze plasma obtaining from healthy volunteers in Tehran University Hospital (Tehran, Iran) and kept frozen until use after gentle thawing. All standard solutions were freshly prepared daily and stored at 2–8 °C. Aliquots of standard stock solution of insulin were dispensed into 10 mL volumetric flasks and the flasks made up to volume with the mobile phase to give final concentrations range of 0.2–250 ng mL^{-1}.

2.2. Apparatus

An Alliance HPLC instrument from Waters Company used to separate and analyze insulin in biological samples. The chromatographic system composed of a multisolvent gradient Water pumps, a Water 2996 photodiode array detector and an online degasser. Chromatographic separation was achieved on an ACE C18, 5 μm, 4.6 mm × 250 mm column. Regarding the mobile phase, a degassed mixture of methanol:phosphate buffer (60:40) was prepared and delivered via P_A pump in the first stage of a gradient elution process. This is for eluting of our analyte from SPE cartridge. In the second stage, a prefiltered mixture of 60 volume of 1 mM sodium sulfate and 0.2% triethylamine in water (pH = 3.2 by *o*-phosphoric acid) and 40 volume of acetonitrile were prepared and delivered via P_B and P_C pumps at flow rate of 1.0 mL min^{-1}. This time programmed moving up the insulin through the SPE cartridge and analytical column is indicated in the Table 1. All of the analyses were carried out at an operation wavelength of 214 nm and HPLC data were acquired and processed using a PC and Millennium 2010 chromatogram manager software (Version 2.1 Waters). The mean retention time of insulin was 4.45 min. Re-equilibration of the column was accomplished within 30 min.

Scanning electron microscopy (SEM, PhilipsXL30 scanning microscope, Philips, the Netherlands) was employed to determine the shape and surface morphology of the produced polymer particles.

Table 1
The optimized sequence for the performance of the automated SPE–HPLC system.

Pump type duration (min)	P_1	P_2	P_3	P_4	P_5	P_6	P_A	P_B	P_C	Pump action
SPE phase										
0–1	Del.	Asp.	Asp.	Asp.	Asp.	Asp.	Off	Off	Off	Conditioning with methanol
1–2	Asp.	Del.	Asp.	Asp.	Asp.	Asp.	Off	Off	Off	Conditioning with ultra-pure water
2–3	Asp.	Asp.	Del.	Asp.	Asp.	Asp.	Off	Off	Off	Conditioning with ammonium phosphate
3–8	Asp.	Asp.	Asp.	Del.	Asp.	Asp.	Off	Off	Off	Loading insulin into the MIP cartridge
8–9	Asp.	Asp.	Asp.	Asp.	Del.	Asp.	Off	Off	Off	Washing with 0.1 M HCl
9–10	Asp.	Del.	Asp.	Asp.	Asp.	Asp.	Off	Off	Off	Washing with ultra-pure water
HPLC phase										
10–13	Asp.	Asp.	Asp.	Asp.	Asp.	Asp.	On	Off	Off	Eluting insulin from SPE cartridge with methanol:phosphate buffer 100%→0%
12–25	Asp.	Asp.	Asp.	Asp.	Asp.	Asp.	Off	On	On	Eluting insulin through HPLC column with 1 mmol sodium sulfate and 0.2% triethylamine in water:acetonitrile (60:40) 0%→100%
25–30	Asp.	Asp.	Asp.	Asp.	Asp.	Del.	Off	Off	Off	Regeneration of SPE cartridge with dichloromethane

Del. = Deliver, and Asp. = Aspirate.