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Rapid determination of 18 glucocorticoids in serum using reusable on-line SPE polymeric monolithic column coupled with LC-quadrupole/orbitrap high-resolution mass spectrometer

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ABSTRACT

A simple, rapid and sensitive method for the simultaneous determination of 18 glucocorticoids in serum was developed by coupling on-line solid-phase extraction (SPE) polymeric monolithic column to a liquid chromatography-quadrupole/orbitrap high-resolution mass spectrometer. A simple poly(ethylene glycol dimethacrylate) monolith column (10 mm × 2.1 mm i.d.) was fabricated, and the morphology, surface area and extraction performance of the monolithic column were characterized. Serum samples were extracted by acetonitrile (ACN). Then, online SPE was achieved on the synthesized monolithic column using a 10 mmol/L ammonium acetate solution as the loading solvent. After the transfer from the monolith into analytical column (Capcell Pak ADME column) using ACN, the adsorbed analytes were separated on the analytical column and detected with a high-resolution hybrid quadrupole/orbitrap mass spectrometer with full scan/ddMS^2 scan mode Under optimized conditions, the method was linear with target linear correlation coefficient (R^2) higher than 0.995. Detection limits were in range of 0.1–0.6 ng/mL, and the quantification limits were 0.3–1.5 ng/mL. The recovery was between 71.9% and 89.2% in three spike levels with precision (n = 5) of 5.40–12.1%. The serum sample was directly analyzed after a simple extraction procedure, and the on-line SPE and determination were achieved within only 16 min. The method was used to analyze the dynamic contents variation of cortisone and hydrocortisone in serum before and after the surgery.

1. Introduction

Glucocorticoids (GLUs) are a class of steroid hormone secreted by adrenal cortex–mainly cortisol and hydrocortisone. They play a key role in the regulation of biosynthesis and metabolism for sugar, fat and protein. They have function in immune response inhibition, anti-inflammatory, anti-viral, and anti-shock activity [1]. Currently, the GLUs have been used to treat at least 200–300 kinds of diseases [2]. They are not only used in a variety of alternative treatments of adrenal cortical dysfunction disease, but also widely used in the treatment of autoimmune disease, allergic disease, hematological system disease, asthma, organ transplantation, skin diseases, eye diseases, etc. [3]. GLUs are also widely used clinically, which can lead to side effects including aggravation of the central nervous system and endocrine system disorders [4]. The inappropriate or long-term use of GLUs can lead to a variety of drug-induced diseases–some even life-threatening. GLUs are prohibited in sports when used by systemic administrations [5]. Consequently, monitoring of GLUs in urine [6–12], serum [12–16] and other biological samples [17–19], is important to study regulation mechanism, clinical therapeutic effect, side effects, etc. of GLUs.

Currently, several methods can measure GLU including gas chromatography (GC) [20,21], high performance liquid chromatography (HPLC) [22–24], gas chromatography-mass spectrometry (GC–MS) [25] and liquid chromatography-mass spectrometry (LC–MS) [6–18,26–29]. The sensitivity and selectivity of GC and HPLC is lower than MS methods. GC–MS requires cumbersome derivative process, and HPLC–MS is preferred for trace analysis of pharmaceuticals because of its high sensitivity and specificity [30]. It requires better purification steps to reduce the matrix effect and ensure instrument stability when HPLC–MS is used. The traditional pretreatment methods such as LLE or off-line SPE are time-consuming and laborious, while on-line purification methods can overcome the disadvantages of off-line methods [31–35]. When a common SPE method [16] was used in the determination of GLUs in human plasma, HLB column was usually chosen, and

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the procedure including extraction, centrifugation, SPE cleanup, evaporation by nitrogen, and reconstitution. The whole process usually needs half day to deal with several samples. While the online SPE method [34] only including extraction step, and it is effective for direct and fast analyzing complicated samples. However, commercialized online purification columns are expensive and rare.

Monolithic columns with a unique structure possess some exceptional characteristics, which make them an excellent tool in the hands of analytical chemists, not only for separation but also for sample preparation [36]. Monolithic columns are a new type of sample pretreatment material and play key roles in many fields including life science, environmental science, medicine and health, food industry, etc. [37,38]. Monolithic columns are widely used in pre-treatment steps with excellent performance [39,40]. Monolithic columns are composed of functional monomer, crosslinking agent and pore forming agent through in situ polymerization. This makes a continuous rod with a large specific surface area, good permeability, simple preparation, and controllable pore structure. It is especially suitable for purification as an on-line solid phase extraction column. In our previous works, two on-line cleanup LC–MS/MS methods were developed for determination of contaminants in food using monolithic SPE columns [41,42].

Here we describe a simple and practical ethylene glycol dimethacrylate (EDMA) monolithic column. The monolithic column was combined to LC-quadrupole/orbirap high-resolution mass spectrometer to determine 18 GLUs including triamcinolone, cortisone, prednisolone, prednisone, hydrocortisone, fludrocortisone, 6-alpha-methylprednisolone, betamethasone, flumethasone, dexamethasone, beclomethasone, flurandrenolide, fluoromethalone, triamcinolone acetonide, budesonide, prednicarbate, halcinonide and amcinonide in serum.

2. Materials and methods

2.1. Chemicals and reagents

Ethylene dimethacrylate (EDMA) was purchased from Aladdin (Shanghai, China). Polyethylene glycol 400 (PEG-400) and *n*-propanol were acquired from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Azobisisobutyronitrile (AIBN) was supplied by Tianjin Damao Chemical Reagent Factory (Tianjin, China) and recrystallized with ethanol prior to use. Ammonium acetate (NH_4OAc) and formic acid (FA) were obtained from Dikma (Beijing, China). HPLC-grade methanol (MeOH), acetonitrile (ACN), isopropyl alcohol and acetone were purchased from Fisher Scientific (Geel, Belgium). Glucocorticoid standard substances were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

2.2. Preparation and characterization of poly (EDMA) monolithic columns

The EDMA monolithic column was prepared by in-situ polymerization. The polymerization mixture was prepared from EDMA, *n*-propanol, PEG-400 and the AIBN initiator (1% w/w with respect to monomers) according to the proportions specified in Table 1. After sonication for 30 min, the transparent and monophasic solution was carefully introduced into a stainless steel chromatographic column (10 mm × 2.1 mm i.d.). The column was placed in a 65 °C water bath for 24 h with both ends of the stainless steel chromatographic column sealed with plastic stoppers. The resulting column was connected to the HPLC system and washed with MeOH to remove the porogen and other impurities in the polymer rod.

Toluene was chosen as the marker analyte because EDMA monolithic extractor is a typical hydrophobic column. The retention factor (k) of toluene of each monolithic extractor was calculated to evaluate the corresponding hydrophobicity. Here, k was obtained from the formula $k = (t_R - t_0)/t_0$, wherein t_R is the retention time of toluene on different monolithic column at 0.3 mL/min with mobile phase of MeOH-water(1 + 9); t_0 is the retention time of solvent MeOH under the same condition (dead time). The scanning electron microscopy (SEM) images of the monoliths were obtained using an S-4800 scanning electron microscope (Hitachi, Tokyo, Japan).

Table 1
Composition of pre-polymerization mixture, pressure, and k of the monolithic column.

Monolith	EDMA (mmol)	1-Propanol (mL)	PEG400 (mL)	Pressure[a] (bar)	k[b]
M1	3.0	0.6	0.875	shrinked	–
M2	3.0	0.8	0.875	1.9	7.6
M3	3.0	1.0	0.875	3.1	9.6
M4	3.0	1.2	0.875	2.7	8.8
M5	2.8	1.0	0.875	2.5	5.3
M6	3.3	1.0	0.875	20	10.1
M7	3.8	1.0	0.875	over pressure	–

[a] Pressure measured at 0.3 mL/min using MeOH as mobile phase.
[b] k was calculated from the equation $k = (t_R - t_0)/t_0$, where t_R is the retention time of toluene on different monolithic column under flowrate of 0.3 mL/min with mobile phase of MeOH-water(1 + 9), and t_0 is the retention time of solvent MeOH under the same condition, being dead time.

2.3. Equipment

2010 liquid chromatograph (SHIMADZU, Japan), and on-line cleaning liquid chromatography-*Q* Exactive spectrometer (ThermoFisher, USA) were used in this experiment. This is composed of CTC multifunctional autosampler (with 100 μL quantitative ring), two quaternary gradient liquid pumps with pressure tolerance of 1250 bar, six-way valve switching device, multiple column switching device and quadrupole/orbitrap (Q/Orbitrap) hybrid mass spectrometer. A Sigma 3K-15 centrifuge (Sigma, USA), PT2100 homogenizer (KINEMATICA, Switzerland), DKZ-2 electrothermal constant temperature oscillation basin (Shanghai Jinghong Experimental Equipment Company, China), and a Milli-Q purification system (Millipore, USA) were also used.

2.4. Sample preparation

The test samples were got from patients undergoing anorectal operation. The blood was collected at baseline with postoperative days 1, 2, and 3. The blooding time was between 7:00am and 8:00am. The serum samples (about 3 mL) were frozen at −80 °C after serum separation. Meanwhile, some serum samples were collected for method validation.

Frozen serum samples were thawed at room temperature, 1.0 mL was weighed and 5 mL acetonitrile was added with vortexing for 1 min; extracts were centrifuged at 4500 r/min for 10 min, and about 1 mL supernatant was collected and filtered with 0.22 μm organic membrane filter.

2.5. Analytical conditions

2.5.1. Online SPE HPLC

In the online purification flow path, the mobile phase A was 0.1% formic acid aqueous solution, B was acetonitrile, C was acetone-isopropanol-acetonitrile (1 + 1 + 1, V/V/V), and sample injection volume was 50 μL. A Capcell Pak ADME column (150 × 2.1 mm, 4.6 μm) was selected as analytical column, 0.1% formic acid solution (D) and acetonitrile (E) were used as the mobile phases. The online purification process included loading, eluting, washing and re-initializing; specific chromatography conditions are listed in Table 2. The flow chart and valve configuration of on-line clean-up instrument refer to the supporting information Fig. S2 in reference [41].

2.5.2. Q/Orbitrap MS

Electrospray ionization source (ESI) was used with a capillary