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High performance liquid chromatography/ion-trap mass spectrometry for separation and simultaneous determination of ethynylestradiol, gestodene, levonorgestrel, cyproterone acetate and desogestrel

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Abstract

A fast and highly sensitive high performance liquid chromatographic/ion-trap mass spectrometric method (LC/MS) has been developed for simultaneous determination of ethynylestradiol (EE2), gestodene (GES), levonorgestrel (LNG), cyproterone acetate (CPA) and desogestrel (DES). Among three types of sorbents tested (C8, C18 and phenyl) from two suppliers, the best separation was achieved on reverse phase Zorbax SB-Phenyl column using aqueous methanol as a mobile phase. A linear gradient profile from 70 up to 100% (v/v) in 7th min, kept constant at 100% up to 10th min and followed by a negative gradient to 70% of methanol up to 12th min was used for elution. Applicability of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) and influence of the mobile phase composition, its flow rate, capillary/vaporizer temperature of API source and in-source fragmentor voltage ionization are discussed. The on-column limits of quantification (10 S/N) were 300 pg of EE2, 14 pg of GES and LNG, 4 pg of CPA and 960 pg of DES per injection (1 μL) using APCI with data collection in selected ion monitoring (SIM) mode. The analytical performance of the method was evaluated using the determination of EE2, GES, LNG, CPA and DES in contraceptives and river water samples.

Keywords: Liquid chromatography; Ion-trap mass spectrometry; Electrospray ionization; Atmospheric pressure chemical ionization; Ethynylestradiol; Gestodene; Levonorgestrel; Cyproterone acetate; Desogestrel

1. Introduction

Chemicals that disrupt endocrine functions have been found in the environment. They are often linked to adverse effects on the reproductive system in wildlife and humans. Many reasonable suspicions have been raised concerning the environmental impact of synthetic contraceptive chemicals as well as the endogenous estrogens excreted by humans and animals. More stable estrogens, such as ethynylestradiol (EE2) and progestogens (or progestins), such as levonorgestrel (LNG), gestodene (GES), cyproterone acetate (CPA) and desogestrel (DES) are used more frequently for medical purposes (e.g. contraception, treatment of prostate and breast cancer, treatment of infertility).

Desogestrel and gestodene represent the latest generation of progestins and begin to replace levonorgestrel in oral contraceptives. The increasing use of incoming substances together with their high physiological activity and higher stability may lead to their ubiquitous occurrence in the environment.

The estrogens content in medical preparations, used in the management of menstrual and menopausal disorders as well as for contraception, is usually in a daily range 20–50 μg. As for the progestin content, it varies depending on the type of contraceptive. Thus, in combined oral formulations the progestin content is in a daily range 0.05–2 mg whereas it is lower in progestin-only contraceptives [1,2].

For many years, immunoassay methods have been the most sensitive analytical procedures available for the determination of estrogens in biological samples. These methods are sensitive, but are time consuming and prone to cross reactivity by endogenous steroids, co-administrated steroids and their metabolites. Gas

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chromatographic/mass spectrometric (GC/MS) methods typically employ some type of extraction (liquid–liquid or solid phase), and one or multiple steps of derivatization [2,4,5].

Recently, liquid chromatography coupled with electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) or atmospheric pressure photospray ionization-tandem mass spectrometry has been applied for the quantitative analysis of estrogens in environmental [3–9] and biological samples [10–13]. Liquid chromatography with tandem mass spectrometric detection was demonstrated to be superior to immunoassay methods or GC/MS in terms of selectivity, sensitivity, simplicity and analytical throughput [1,2,4,5].

Ethynylestradiol together with levonorgestrel belong to the commonly investigated estrogens in water, effluents of wastewater treatment plants, sewage sludge, soils, sediments and biological samples such as plasma and urine. Many methods based on a combination of a single extraction step, eventual cleanup and analysis (e.g. for water sample, solid-phase extraction, followed by chromatographic separation with mass spectrometric detection) have been published [1–6,10,11,14–17].

The use of electrospray-tandem mass spectrometry (ESI-MS–MS) in negative ionization mode for ethynylestradiol and in positive ionization mode for levonorgestrel has become dominant technique of their determination. Reported limits of detection (LODs) varied from 0.08 to 10 ng L^{-1} (resp. ng kg^{-1}) of ethynylestradiol and 1 to 20 ng L^{-1}(resp. ng kg^{-1}) of levonorgestrel in dependence on sample matrix, method of sample preparation and type of mass spectrometer used. The lowest detection limits were achieved using a triple–quadrupole mass spectrometer. Only limited number of information concerning cyproterone acetate, desogestrel and gestodene mass spectrometric determination have been published [12,18–20].

The aim of this study was to evaluate a liquid chromatographic/ion-trap mass spectrometric method (LC/MS) for the simultaneous determination of cyproterone acetate, gestodene and desogestrel together with ethynylestradiol and levonorgestrel and to test the applicability of the proposed method for their determination (see Fig. 1 for their molecular structures) in pharmaceutical dosage forms and in real and spiked water samples.

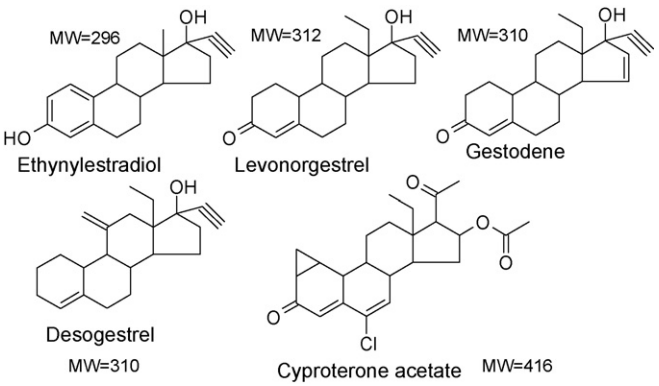

Fig. 1. Molecular structures of the investigated estrogen and progestins.

2. Experimental

2.1. Chemicals

Ethynylestradiol (EE2) was obtained from Riedel-de Haen (Seelze, Germany). *D*(−)-norgestrel (levonorgestrel, LNG) and cyproterone acetate (CPA) were purchased from Sigma–Aldrich (St. Louis, MA, USA). Gestodene (GES), desogestrel (DES) were obtained from Council of Europe, European Pharmacopoeia (Strasbourg, France). Stock standard solutions of each of the compounds ($c = 50\ \mu g\ mL^{-1}$) were prepared in methanol and stored in a refrigerator in darkness at 5 °C. The stability of the stock solutions of standards was controlled for 2 month and no change in concentration was observed. Working solutions were prepared daily by mixing and diluting the stock solutions with methanol.

The LC/MS Chromasolv® acetonitrile and methanol from Riedel-de Haen were used. Ammonium formiate (for MS), ammonium hydroxide solution (Trace Select Ultra) and formic acid (for MS) were purchased from Fluka Chemie (Buchs, Switzerland). De-mineralized water obtained by reverse osmosis using an AquaDem 02 (Aqua Osmotic, Tišnov, Czech Republic) was further purified using a MILLI-Q-RG (Millipore, Bedford, MA, USA).

2.2. High-performance liquid chromatography

An Agilent 1100 chromatographic system (Agilent, Waldbronn, Germany) equipped with a vacuum degasser, a quaternary pump, an autosampler, a column thermostat and a diode array detector was used. UV–vis spectra were automatically acquired for all peaks (range 190–400 nm, 2 nm steps). The system was coupled on-line to an ion-trap mass spectrometer Finnigan LCQ Advantage Max (San Jose, CA, USA). The XCalibur software (Version 1.4) controlled the whole liquid chromatographic/mass spectrometric system.

Following chromatographic columns were investigated: Zorbax SB-Phenyl (100 mm × 2.1 mm i.d., 3.5 μm particle size), Zorbax SB-Phenyl (75 mm × 4.6 mm i.d., 3.5 μm particle size), Zorbax Eclipse XDB C18 (50 mm × 2.1 mm i.d., 1.8 μm particle size), Zorbax Eclipse XDB C8 (50 mm × 2.1 mm i.d., 1.8 μm particle size), Zorbax Eclipse XDB C18 (50 mm × 4.6 mm i.d., 1.8 μm particle size), all products of Agilent (Palo Alto, CA, USA); Luna C8(2) (50 mm × 2.0 mm i.d., 3 μm particle size), Luna C18(2) (50 mm × 2.0 mm i.d., 3 μm particle size) and Luna Phenyl-hexyl (50 mm × 2.0 mm i.d., 3 μm particle size), all product of Phenomenex (Torrance, CA, USA).

The best results were obtained with Zorbax SB-Phenyl columns using an aqueous methanol as a mobile phase. A linear gradient profile from 70 up to 100% (v/v) of methanol in 7th min, kept constant at 100% of methanol up to 10th min and followed by a negative gradient to 70% of methanol up to 12th min was used for elution. The column was equilibrated prior to injection of each sample with the mobile phase containing 70% (v/v) of methanol for 5 min. The flow rate was 0.25 mL min^{-1} (2.1 mm columns) and 1.0 mL min^{-1} (4.6 mm columns), respectively. The column temperature was maintained at 35 °C. The