Introduction

Medroxyprogesterone acetate (MPA) is widely used as an injectable contraceptive or as hormone replacement therapy. MPA has also been used to enhance growth in food animals. As such, it provides a significant risk for the population consuming the treated meats [1]. Over the years, multiple methods and techniques have been developed for measuring the levels of hormones such as MPA in animal fat, tissues, plasma, and urine [2-5]. The older techniques included enzyme immune assay [6] and gas chromatography-mass spectrometry (GC-MS) but more recently liquid chromatograph-mass spectrometry (LC-MS/MS) techniques have been developed.

In humans, MPA is administered as 150 mg intramuscularly or 104 mg subcutaneously at 3-month intervals as a contraceptive [8,9]. As with many drugs, the prescribed dosages are often higher than necessary for adequate contraceptive efficacy. Although ovulation is reported to resume when MPA serum concentrations decline to <0.1 ng/ml [10,11], several studies have demonstrated serum concentrations at the end of the 3-month dosing interval ranging from <0.04 ng/ml to 2.6 ng/ml with current MPA doses [9,10,12-14]. Moreover, single MPA doses of 25, 50, and 100 mg have been documented to inhibit ovulation for at least 3 months in several clinical studies [15-17]. Therefore, it appears that contraceptive doses of MPA could be lowered at least in certain populations without compromising efficacy.

Side effects of MPA administration can be significant and include decreases in bone mineral density (BMD) and increases in adiposity. In 2004, the FDA issued a black-box warning suggesting a 2-year limit on the use of MPA due to concerns about its effects on BMD [18]. In addition, studies have demonstrated increases in adiposity among adolescent and adult women while on MPA [19,20]. Consequently, personalizing the doses of MPA prescribed to achieve the necessary contraceptive action yet minimize the dose that the patient receives could be of vital importance to mitigate side effects.

Abstract

Medroxyprogesterone (MPA) is widely used as a contraceptive or for hormone replacement therapy after menopause. The use of MPA, specifically in adolescent girls, is linked to excess weight gain and decreases in bone mineral density. Metabolism and clearance of MPA is highly variable making this agent a candidate for individualized dosing strategies which could possibly decrease unwanted side effects. MPA has been measured in animal tissues and fluids for many years as a contaminant resulting from the use of growth hormones in food animals.

MPA has been measured by radioimmunoassay, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS/MS). LC-MS/MS techniques have proven to be both more specific than radioimmunoassay and required much simpler sample preparation techniques than GC-MS. Here we describe an LC-MS/MS method specifically designed for monitoring MPA and progesterone levels in human serum. The technique in this report involves minimal sample preparation and yields results with precision and accuracy suitable for clinical use.

Keywords: Medroxyprogesterone; LC-MS/MS; Measurement; Human serum

Materials and Methods

Materials and chemicals

Medroxyprogesterone 17-acetate (MPA), progesterone (P4) and deoxycorticosterone acetate (DOCA) was purchased from Sigma-Aldrich (St. Louis, MO) (Figure 1). The following chemicals were purchased from Fisher Scientific (Pittsburgh, PA): water (HPLC), potassium phosphate dibasic, pentane (HPLC), o-phosphoric acid, potassium hydroxide, methanol (optima LCMS), and formic acid. HPLC grade water was vital to our method as in-house water (deionized biological grade type 1, 18 mOhm resistivity) contained a contaminant that co-eluted with MPA at its MR transition.

Stock solutions and standards

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LC-MS/MS system

Analyses were performed on an ABI/Sciex 4000QTrap coupled with a Shimadzu 20 Series HPLC. MPA and P4 were separated using a Zorbax XDB-C8, 4.6 x 150 mm, 5 µm (Agilent) paired with a matching guard column. Mobile phase A was 0.1% formic acid in HPLC water and mobile phase B was methanol, with the gradient as follows: 60% B for 1 minute, linear to 90% B over 8 minutes, column wash at 90% B for 4 minutes, column re-equilibration at 60% B for 4 minutes resulting in a total run time of 17 minutes. The flow rate of 0.8 mL/min was held constant throughout the method. The column oven was maintained at 32°C throughout the method. The sample injection volume was 25 µL. Ions were generated in positive mode using atmospheric chemical ionization (APCI). The settings were as follows: CUR = 10; CAD = Medium; NC = 3; Temp = 360; GS1 = 30; Q1 and Q3 = Unit. The MRM pairs monitored are indicated in Table 1 (bold type indicates the transition used for quantification) and spectra of the specified transitions are presented in Figure 2.

Results

The standards and samples were analyzed using the MRM transitions noted in Table 1. The method was assessed for recoveries, range and limits of detection, precision, and accuracy.

Range and limits of detection

Standards were analyzed with concentrations from 0.005 to 5 ng/mL to determine the lower limits of detection (LOD). Using a signal to noise ratio of 3:1, concentrations below 0.05 ng/mL were not accurately detectable or in the linear response range. Consequently, the LOD was determined to be 0.05 ng/mL. Subsequently, standards were analyzed with concentrations from 0.05 to 500 ng/mL to determine the range of linear responses. Areas obtained from the MS analyses were plotted against the concentrations injected (Figure 3). Responses to all concentrations tested were linear. However, because ranges higher than 10 ng/mL would not be clinically relevant, higher concentrations were not explored further. The precisions and accuracies of two individual standards (10x) are also indicated on Figure 3.

Reproducibility and recovery

Quality controls were prepared by pooling serum from study participants receiving the currently approved MPA dose of 150mg. The serum from three participants 10 to 11 weeks post-treatment were pooled together for a lower concentration control, while alternatively the serum from three participants 1 to 2 weeks post-treatment were pooled together for a high concentration control. These were stored at -80°C and used for method validation. Data reported in Table 2 indicates the %CV for interday and intraday values for quality control

| Q1 (amu) | Q3 (amu) | Dwell Time (msec) | DP | EP | CE | CXP |
|---------|---------|------------------|----|----|----|-----|
| MPA     | 387.234 | 327.100          | 100| 46 | 10 | 19  |
| P4      | 315.246 | 315.246          | 100| 91 | 10 | 5   |
| DOCA    | 373.184 | 331.200          | 100| 76 | 10 | 25  |

Bold type indicates transition used for quantitation

Table 1: Precursor and product ions of each analyte and the settings used to generate ion pairs for multiple reaction monitoring.
Figure 2: Chromatograms of MRM transitions. A) MPA standard 5 ng/mL; B) P4 standard at 5 ng/mL; C) DOCA standard at 5 ng/mL; D) serum samples at the MPA MRM. The peak at 2 min occurred intermittently and was non-linear with respect to the standards.
There is variety among the LC-MS/MS methods including different ionization techniques (electrospray (ESI) [2,22], atmospheric chemical ionization (APCI) [4,5,23] and detectors (ion trap, triple quadrupole) [2,4,21]. Furthermore these techniques have been optimized for a variety of sample types which include animal fat, muscle, blood, and plasma. For these reasons it is difficult to compare actual analytical parameters. Only a few others have reported LC-MS/MS methods optimized for human serum and/or plasma [21,22].

The analytical method described here is straightforward, the sample preparation is minimal and the quantification provides sufficient sensitivity for clinical application.

This method could be easily translated into clinical practice for personalizing dosing and time interval to optimize contraceptive efficacy while minimizing unwanted side effects.

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**Conclusion**

Our data demonstrate a robust method for measuring medroxyprogesterone and progesterone in human serum samples. MPA is a potential contaminant in human food with undesirable side effects and the presence of MPA and other gestagens are extensively screened in meat products. For these reasons, MPA has been measured in animal tissues and fluids for many years using a variety of different techniques [2,6,7,21]. The LC-MS/MS techniques have proven to be far less labor intensive than the GC-MS methods used earlier and more specific than the EIA methods.

### Table 2: Biological sample reproducibility.

| MPA   | Mean (SD) ng/mL | Intra-assay CV (%) | Inter-assay CV (%) | Recovery (SD) (%) |
|-------|----------------|-------------------|--------------------|------------------|
| Serum, low | 1.06 (0.37) | 32.83 | 29.41 | 65.86 (12.16) |
| Serum, high | 4.42 (1.20) | 10.98 | 27.28 | 61.11 (14.22) |
| P4    | 0.18 (0.08) | 23.59 | 42.18 | * |
| Serum, high | 0.42 (0.12) | 14.47 | 27.50 | * |

*Both analytes were measured on a single sample so the recoveries were the same for both.*
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