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Medroxyprogesterone Acetate and Progesterone Measurement in Human Serum: Assessments of Contraceptive Efficacy

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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

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 [7] 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.

Currently, few methods exist that allow for accurate analysis of MPA concentrations in humans to facilitate the physician in optimizing or personalizing doses. The method described in this report offers a reliable, reproducible method for measurement of MPA as well as progesterone (P4) in a single serum sample and could be used to personalize the dosing regimen for individual patients.

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 MRM transition.

Stock solutions and standards

Individual stock solutions for MPA and progesterone (P4) were

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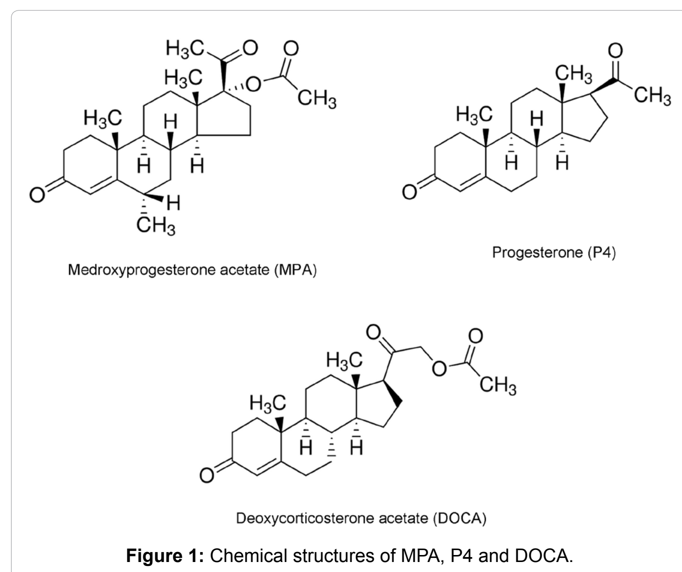

Figure 1: Chemical structures of MPA, P4 and DOCA.

made at a concentration of 1 mg/mL in methanol. Subsequent dilutions were made to yield mixed standards of 500 ng/mL, 50 ng/mL, and 5 ng/mL in methanol. The internal standard is deoxycorticosterone acetate (DOCA). The DOCA solution was made at 50 ng/mL in methanol. All standards were stored at -20°C. Stock solutions were stable for at least 3 months, dilutions were made monthly. Six calibration standards were prepared daily in extracted control serum matrix at concentrations of 0, 0.05, 0.1, 0.5, 1, 5, and 10 ng/mL with a fixed DOCA concentration at 5 ng/mL (Table 1).

HPLC mobile phases are as follows: Mobile phase A was 0.1% formic acid in HPLC grade water (Fisher). Mobile phase B was 100% Methanol (Optima Grade, Fisher).

Sample preparation

Blood samples were drawn from patients into serum tubes containing no anti-coagulant and allowed to clot at room temperature for 30 minutes. Serum was separated by centrifugation and samples were stored at -80°C until analysis. Serum samples were thawed on ice and 0.5 mL of serum was transferred into a 10 mL glass centrifuge tube. Samples were run in triplicate which required 1.5 mL of serum per analysis. Each serum sample was spiked with 10 µL of internal standard, 1 mL 100 mM potassium phosphate, pH 9, and 4 mL pentane was added. The mixtures were vortexed thoroughly to mix, shaken for 15 minutes at room temperature, and centrifuged at 1250×g for 5 minutes at 20°C. Occasionally a gel interface formed between the aqueous and organic layers. This was observed more often if the samples were placed on ice or refrigerated for an extended period of time. When this occurred, the samples were vortexed thoroughly and centrifuged at a higher rate to obtain a clean solvent phase separation (approximately1800-3000×g).

After centrifugation, the organic phase was collected into a separate 10 mL glass centrifuge tube and the extraction was repeated as above with 2 mL pentane. The organic phases from the first and second extraction were combined and subsequently dried under a stream of nitrogen gas. The dried samples were reconstituted in 100 µL HPLC water/methanol (50/50) being cautious to wash down the sides of the tube and mix thoroughly. A brief centrifugation at 1250×g, 2 minutes, 20°C was performed to concentrate the sample in the base of the centrifuge tube. The samples were then transferred into injection vials for analysis by LC/MS/MS.

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 × 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	Q3	Dwell Time	DP	EP	CE	CXP
	(amu)	(amu)	(msec)				
MPA	**387.234**	**327.100**	**100**	**46**	**10**	**19**	**8**
MPA	387.234	387.234	100	46	10	5	2
P4	315.246	315.246	100	91	10	5	2
P4	**315.246**	**297.200**	**100**	**91**	**10**	**23**	**6**
DOCA	373.184	373.184	100	76	10	5	2
DOCA	**373.184**	**331.200**	**100**	**76**	**10**	**25**	**8**

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.

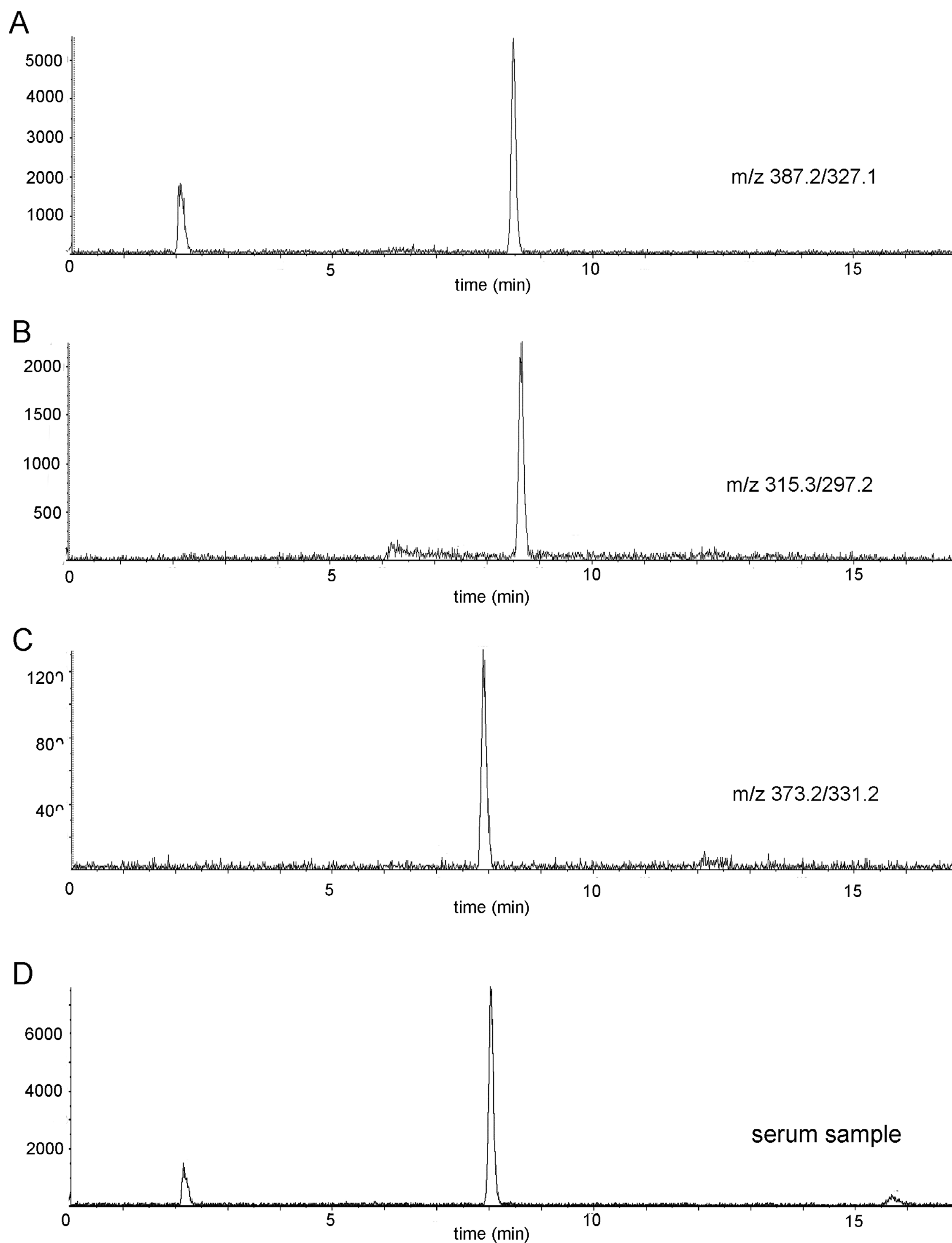

Figure 2: Chromatograms of MRM transitions. A) MPA standard 5 ng/mL; B) P4 standard at 5 ng/mL; C) DOCA standard at 5 ng/mL; D) serum samples at the MPA MRM.The peak at 2 min occurred intermittently and was non-linear with respect to the standards.

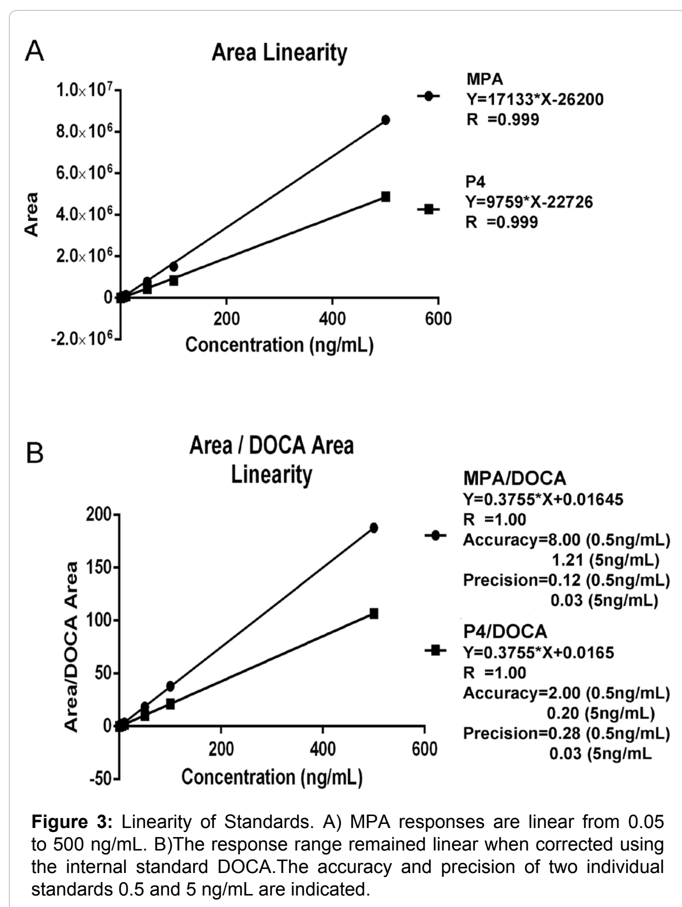

Figure 3: Linearity of Standards. A) MPA responses are linear from 0.05 to 500 ng/mL. B)The response range remained linear when corrected using the internal standard DOCA.The accuracy and precision of two individual standards 0.5 and 5 ng/mL are indicated.

	Mean*(SD)* ng/mL	**Intra-assay CV (%)**	**Inter-assay CV (%)**	**Recovery*(SD)* (%)**
MPA			v	
Serum, low	1.06*(0.31)*	32.83	29.41	65.86*(12.16)*
Serum, high	4.42*(1.20)*	10.98	27.28	61.11*(14.22)*
P4				
Serum, low	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

Table 2: Biological sample reproducibility.

serum. Recovery was calculated from the recovery of the internal standard from the samples after extraction. The mean recovery for all samples was 71.2% (± 12.5%). Individual recoveries of quality control samples are listed on Table 2.

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.

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 quadrapole) [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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