Rapid Quantification of Medroxyprogesterone Acetate (MPA) in Human Plasma by LC-MS/MS

Pamela Hummert,1 Madhuri Manohar,1 Wutyi S. Aung,1 and Mark A. Marzinke1,2*

Background: Medroxyprogesterone acetate (MPA) is a common contraceptive agent taken both orally and as a subcutaneous or intramuscular injection. Current LC-MS/MS methods for MPA quantification require large sample volumes and low-throughput analytical run times. Therefore, there are opportunities to improve upon existing methods for MPA quantification.

Methods: MPA was extracted from 600 μL plasma, evaporated to dryness, and the reconstituted solution was injected onto a Waters Acquity liquid chromatography (LC) system via an Agilent Zorbax Eclipse-Plus C18 2.1 × 50 mm (5.0 μm) column. MPA and its internal standard were monitored on a QTRAP® 5500 mass analyzer operated in positive ionization mode. The method was validated according to the Food and Drug Administration Bioanalytical Method Validation guidelines.

Results: The analytical measuring range of the assay was 200 – 10,000 pg/mL. QC samples prepared at the lower limit of quantification (LLOQ; 200 pg/mL) and low (600 pg/mL), mid (1750 pg/mL), and high (8500 pg/mL) levels showed interassay precision and accuracy ≤15.2% and ≤±9.6%, respectively. Stability-challenged samples yielded ≤15% from freshly prepared samples. Dilutional and matrix effects studies were also acceptable. The assay was also assessed in participants prescribed depot medroxyprogesterone acetate; observed concentrations were within the dynamic range of the assay.

Conclusions: An LC-MS/MS method for the quantification of MPA in plasma has been developed and validated. The described method is sufficiently sensitive and robust to quantify MPA in plasma and meets the criteria to support clinical trials.

IMPACT STATEMENT
The LC-MS/MS method for MPA quantification described in this work will facilitate an increased ability to perform drug–drug interaction studies in populations using oral or topical formulations of MPA as contraceptive agents.

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Nonstandard abbreviations: MPA, medroxyprogesterone acetate; DMPA, depot medroxyprogesterone acetate; IM, intramuscular; UHPLC, ultra high-performance liquid chromatography; SPE, solid-phase extraction; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.
Medroxyprogesterone acetate (MPA)³ is a synthetic analog of the steroidal hormone progesterone and differs from the endogenous hormone through the presence of a methyl group at carbon 6 and an acetate group at carbon 17 (1). While MPA is an agonist for the progesterone receptor, the progestin also shows an affinity for both the androgen and glucocorticoid receptors (2). MPA has long demonstrated high efficacy as a contraceptive agent and has been used as a hormonal therapy in premenopausal populations for the treatment of secondary amenorrhea, dysmenorrhea, and endometriosis (3–5). As a contraceptive agent, MPA inhibits the release of gonadotropin-releasing hormone, thereby decreasing the production and release of follicle-stimulating hormone and luteinizing hormone, resulting in inhibition of follicular development and the prevention of ovulation (6). Consequently, women using MPA have decreased progesterone concentrations due to ovulation suppression, as well as decreased estradiol concentrations and endometrial atrophy (7–9).

MPA can be administered orally or as a long-acting injectable; the use of depot medroxyprogesterone acetate (DMPA) removes the need for daily drug administration and reduces the risk of treatment failure. DMPA can be injected intramuscularly or subcutaneously, and while both modes are effective in preventing ovulation, they differ in their formulations and pharmacokinetics; traditional DMPA treatment is achieved using an intramuscular (IM) injection (7–10). For IM DMPA injections, there was a study that assessed the pharmacokinetics after an IM injection of DMPA containing 150 g/L of drug, in which the concentration of MPA peaked at 2.5 ng/mL after approximately 14 days. The MPA drug concentrations fell below 1 ng/mL 56 days post-DMPA injection (11). A recent analysis evaluated the MPA concentrations in women who received a subcutaneous (104 mg/0.65 mL drug) injection in the upper arm. Peak MPA concentrations of 0.95 ng/mL were achieved 2–14 days post-dosing; mean MPA concentrations at days 91, 104, and 120 ranged from 0.33 to 0.43 ng/mL (12). Therefore, robust assays are required for the quantification of MPA after IM or subcutaneous application for drug interaction and pharmacokinetic studies.

Various methods have described the quantification of MPA in serum or plasma, and the majority of approaches exploit LC-MS/MS techniques (12–20). The majority of methods for MPA quantification via LC-MS/MS require large sample volumes, typically in excess of 1 mL; extensive sample preparation; and analytical run times in excess of 10 min per sample (15–19). Efforts have been made recently to reduce sample volumes and improve sample preparation; however, issues such as long analytical run times and assay sensitivity were not overcome (19). Here, we present a rugged, sensitive, LC-MS/MS method for the quantification of MPA in plasma with an analytical run time of 4.0 min.

**EXPERIMENTAL METHODS**

Chemicals

MPA (C₂₄H₃₄O₄) and its isotopically labeled internal standard, medroxyprogesterone-d₆, 17-acetate (C₂₄H₂₈D₆O₄), were obtained in powder form from Toronto Research Chemicals. The structure for MPA is depicted in Supplemental Fig. 1 (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol1/issue2). Human plasma K₂EDTA was obtained from Bioreclamation (IVT) and Biological Specialty Corporation. For correlation studies, plasma was separated from whole blood collected in K₂EDTA Vacutainer Tubes from individuals actively using DMPA via an institutional review board–approved protocol through the Johns Hopkins University School of Medicine. HPLC and optima LC/MS–ultra high-performance liquid
chromatography (UHPLC) grade methanol, optima LC/MS–UHPLC grade water, and glacial acetic acid were obtained from Fisher Scientific.

Preparation of reagents and standards

Stock solutions of MPA were prepared in methanol at final concentrations of 1.0 g/L, and working solutions were prepared at 1000, 100, and 10 ng/mL via dilution with methanol. Before the generation of matrix-based calibrators, plasma was screened, and only MPA-free plasma was used for the generation of calibrator and QC materials. Calibration standards at final concentrations of 200, 350, 500, 800, 1000, 2500, 5000, 7500, and 10 000 pg/mL were prepared by spiking human K2EDTA plasma with appropriate volumes of the aforementioned working solutions. QC materials were prepared from an independently weighed master stock of MPA at the lower limit of quantification (LLOQ, 200 pg/mL), as well as low (600 pg/mL), mid (1750 pg/mL), and high (8500 pg/mL) QC levels. A stock solution of the isotopically labeled internal standard, MP-d6, 17-acetate, was prepared in methanol to a final concentration of 1 g/L, and an intermediate stock was prepared at 1 μg/mL. From the intermediate stock solution, an internal standard working solution of 24 ng/mL in methanol was prepared via dilution.

Sample preparation

Once thawed, 600 μL spiked plasma was combined with 650 μL water containing 1% acetic acid followed by the addition of 50 μL of the 24 ng/mL MP-d6, 17-acetate, in methanol in a 12 × 75 mm borosilicate glass tube. Mixtures were vortexed for 10 s and then subjected to centrifugation in a Jouan CT4i Centrifuge (Thermo Scientific) for 5 min at 1000g at 4 °C. Postcentrifugation, the entire supernatant was transferred to methanol preconditioned Oasis HLB 3cc Vac (60 mg) solid-phase extraction (SPE) cartridges (Waters Corporation) attached to a vacuum manifold (Waters Corporation). After samples were applied to the cartridges, vacuum pressure was applied (~10 in.Hg), and cartridges were subsequently washed with 1 mL of 5% methanol in water and 1 mL water. After washing, vacuum pressure was applied at ~25 in.Hg for 10 min at room temperature. Samples were eluted with 1 mL methanol/1% acetic acid. Vacuum pressure was applied for 10 min at ~25 in.Hg to elute samples. Eluents were transferred to 1-mL glass inserts, evaporated to dryness under a dry nitrogen stream, and reconstituted in 60 μL of a 1:1 water:methanol solution. Ten microliters of reconstituted material was injected into the LC-MS/MS system for analytical separation and mass spectrometric analysis.

Separation and instrument acquisition parameters

Chromatographic separation was performed using a Waters Acquity LC system consisting of a binary solvent manager, a sample manager (maintained at 4 °C), and a 10-μL injection loop. An Agilent Zorbax Eclipse-Plus C18 5.0 μm, 2.1 × 50 mm (Agilent), column was used for analytical separation; the column was maintained at ambient temperature. A mobile phase system comprised of water containing 0.1% acetic acid (mobile phase A) and 100% methanol (mobile phase B) was used for separation. The chromatographic separation and parameters are detailed in Supplemental Table 1 in the online Data Supplement. MPA was eluted at 1.27 min, and the total analytical run time was 4.0 min.

MPA quantification was achieved using a QTRAP® 5500 mass analyzer (SCIEX) equipped with an electrospray ionization source. Optimization of mass spectrometric parameters was achieved via the direct infusion of MPA and its internal standard into the mass analyzer. The instrument was operated in selective reaction monitoring and positive ionization mode. The following settings were optimal for MPA and MP-d6, 17-acetate, ionization: ion spray voltage: 5500; cur-
tain gas: 15; source temperature: 650 °C; source gas 1 and 2: 50.

Ion transitions monitored were as follows: m/z 387.2→285.1 (qualifier ion) and m/z 387.2→327.2 (qualifier ion) for MPA and m/z 393.3→330.3 and 393.3→288.4 for the isotopically labeled internal standard. Analyte-specific ionization parameters included declustering potentials of 95 and 75, collision energies of 25 V and 21.92 V, and collision cell exit potentials of 13 V and 15.9 V for MPA and its internal standard, respectively.

**Data evaluation**

Analyst® 1.6.2 Software (Version 1.6.2) (SCIEX) was used for the acquisition and analysis of chromatographic data. Microsoft Office Excel 2010 was used to determine intra- and interassay means, SDs, and coefficients of variation (%CVs; the SD divided by the mean, followed by multiplication by 100), as well as the percent deviation (a measure of accuracy) from theoretical concentrations (%DEV; the theoretical value minus the experimental mean value, divided by the experimental mean value, multiplied by 100) and the percent difference from nonchallenged QC materials (%DIF; the mean of the unchallenged samples minus the mean of the challenged samples, i.e., stability challenges, divided by the mean of the challenged samples, multiplied by 100). Outliers were determined using Grubb’s Outlier Test.

**METHOD VALIDATION**

The MPA assay was validated in accordance with the recommendations endorsed by the Food and Drug Administration Guidance for Industry, Bioanalytical Method Validation guidelines (20). The assay was tested and validated for intra- and interassay precision and accuracy, calibration curve analysis, dilutional analysis, stability challenges, carryover, selectivity, and matrix effects.

**Precision and accuracy studies**

Intraassay precision was determined through the analysis of 6 preparations and injections of QC materials extracted from plasma. The observed means, SDs, and %CVs were calculated at the previously described LLOQ, as well as low, mid, and high QC levels (n = 6). Interassay precision was assessed at all QC levels over 3 independent experiments; results were determined from run-specific calibration curves. Intra- and interassay accuracies were determined from 6 individual samples per QC value within an experiment and between 3 independent experiments. Acceptability criteria for the intra- and interassay precision and accuracy were ≤±20% for the LLOQ and ≤±15% for low, mid, and high QC levels.

**Calibration curve analysis**

Calibration standards were analyzed at the beginning and end of each run; the first set of calibrators (n = 9) was run in ascending order and the second in descending order to demonstrate the absence of carryover between samples. Calibration curve analysis was performed using the ratio of the peak area of MPA, and its internal standard with a weighted linear 1/x regression.

**Dilutional integrity**

Extended linearity beyond the established analytical measuring range (10 000 pg/mL) was also evaluated. Dilutional integrity was performed by preparing a solution at 3 times the highest calibrator for a final concentration of 30 000 pg/mL. This solution was diluted 4-, 8-, and 16-fold with drug-free plasma. Extended linearity was determined by setting theoretical values at the calculated dilutions (7500, 3750, and 1875 pg/mL, respectively) and determining the %DEV of samples analyzed in quadruplicate. To further characterize the dilutional integrity of samples containing MPA, mid and high QC levels were diluted 2- and 4-fold with drug-free plasma, and accuracy was determined.
by setting theoretical values at the calculated diluted concentrations (875 and 437.5 pg/mL for mid QC; 4250 and 2125 pg/mL for high QC, respectively). Samples were analyzed in quadruplicate.

Stability

Stability was determined via a number of challenges to ensure the fidelity of the method. For injection matrix studies, QC materials were processed as previously described and reanalyzed 24 h after being maintained at 4 °C. Sample matrix stability was performed by maintaining QCs at room temperature for 24 h before processing, extraction, and analysis. Freeze-thaw studies were performed on QC materials that went through three freeze-thaw cycles, transitioning from −80 °C to room temperature. Long-term stability for MPA in sample matrix maintained at −80 °C was tested at 3 months. For all stability challenges, materials were prepared and analyzed in replicates of 4, with the exception of injection matrix stability, which was conducted with 6 replicates. Stability was determined through the calculation of the %DIF between stability-challenged samples and freshly extracted and analyzed materials. Acceptable stability studies yielded a %DIF ≤±15% from freshly prepared QC materials.

Carryover, selectivity, and signal-to-noise ratio

To ascertain carryover for the MPA assay, 3 injections of upper limit of quantification (ULOQ) samples were injected followed by 3 injections of blank plasma samples. For selectivity, 6 lots of K2EDTA plasma from male donors (to serve as MPA-negative samples) were processed and subjected to LC-MS/MS analysis. Further, the signal-to-noise ratio of blank K2EDTA samples was evaluated to determine if signal of the LLOQ was 5 times the observed background noise level of blank plasma.

Matrix effects

Matrix effects, as well as extraction efficiency and processing efficiency, were determined as per the approach described by Matuszewski et al. (21). Three sets of QC samples were prepared in 6 independent lots of drug-free plasma. An unextracted set was prepared at low, mid, and high QC concentrations in the absence of matrix. Post-extracted samples were prepared by spiking MPA at the aforementioned concentrations into extracted plasma. Finally, a pre-extracted set was prepared in plasma and processed as previously described. Raw peak areas for both MPA and the internal standard were analyzed to determine matrix effects (comparison of post-extracted to unextracted samples), extraction efficiency (comparison of pre-extracted to post-extracted samples), and processing efficiency (comparison of pre-extracted to unextracted samples).

MPA quantification in study participants

To evaluate the robustness of the described method, plasma samples were collected from 5 study participants actively using DMPA as a contraceptive agent. All participants were managed with the 150 g/L DMPA formulation, and drug was administered as an IM injection. One blood sample was collected from each of the 5 participants. Samples analyzed ranged from 8 to 98 days post–IM injection.

RESULTS

Method development

MPA was quantified using the transition of m/z 387.2→285.1, a previously published transition that was identified after direct infusion of the compound into the mass analyzer (Fig. 1) (16). Multiple approaches for sample preparation were assessed, including protein precipitation. However, protein precipitation results in a higher background signal-to-noise ratio and poorer peak shape when compared to solid-phase extraction (data not shown).
Precision and accuracy studies

Intra- and interassay precision and accuracy were observed at the LLOQ, as well as low, mid, and high QC levels. The intra- and interassay precision for MPA in plasma ranged from 1.1% to 11.3% and 4.0% to 15.2%, respectively. Intra- and interassay accuracy for MPA in plasma ranged from −12.6% to 15.0% and −10.3% to 9.6%, respectively. All observed results were in accordance with the aforementioned acceptability criteria. A full summary of precision and accuracy studies is illustrated in Table 1.

Calibration curve analysis

The MPA standard curve was generated using a linear 1/x regression of calibrator; the average regression from 3 independent runs was ≥0.999. Further, the %CV at the lowest calibrator

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**Table 1. Intraassay and interassay precision and accuracy results.**

<table>
<thead>
<tr>
<th>QC level, pg/mL</th>
<th>Intraassay precision and accuracy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Interassay precision and accuracy&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, pg/mL</td>
<td>SD, pg/mL</td>
</tr>
<tr>
<td>LLOQ, 200</td>
<td>191.5</td>
<td>7.3</td>
</tr>
<tr>
<td>Low, 600</td>
<td>541.2</td>
<td>33.2</td>
</tr>
<tr>
<td>Mid, 1750</td>
<td>1876.7</td>
<td>41.8</td>
</tr>
<tr>
<td>High, 8500</td>
<td>9131.7</td>
<td>279.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 6 for each level of QC; representative data from a single analytical run.

<sup>b</sup> n = 15 for LLOQ; 18 for low, mid, and high QC levels; complex precision and accuracy from 3 analytical runs.
(200 pg/mL) was 19.8% across 3 runs; remaining calibrators ranged from 1.8% to 11.3%. The accuracy of calibration standards, which was determined as the mean back-calculated drug concentrations, ranged from −3.7% to 9.0%. A representative calibration curve is illustrated in Supplemental Fig. 2 in the online Data Supplement.

**Dilutional integrity**

When establishing the analytical measurement range of the assay, studies were also conducted to assess the dilutional integrity of samples that may be volume limited or above the upper limit of quantification. A plasma sample spiked with 3 times the ULOQ (30 000 pg/mL) and diluted 4-, 8-, and 16-fold yielded %CVs ranging from 4.9% to 8.5% and percent accuracies ranging from −6.9% to 3.3%. To characterize the applicability of the method to volume-limited samples, 2- and 4-fold dilutions were performed on mid and high QC samples. Precision and accuracy ranged from 2.8% to 7.4% and −12.5% to 8.7%. These data demonstrate that biological samples may be diluted for quantitative analysis from the established calibration curve.

**Stability**

The stability of MPA was assessed following challenges in reconstitution solution (injection matrix), in plasma matrix (sample matrix), as well as challenged to 3 freeze-thaw cycles. Overall acceptability was determined through the calculation of the %DIF, which compares initially or freshly analyzed samples with challenged QC samples. Comparison of reconstituted samples maintained at 4 °C for 24 h with previously analyzed samples yielded %DIFs ranging from −4.8% to −0.9%. For sample matrix analysis, comparison of room temperature–incubated QC samples to freshly prepared and tested QC plasma samples yielded differences ranging from −3.1% to 0.7%. Freeze–thaw studies were also conducted in replicates of 4 and compared with freshly prepared calibrators and QC levels. Differences ranged from −7.3% to 2.3%. Results of all stability challenges met previously described acceptance criteria. Please see Table 2 for more details.

Long-term stability studies were conducted in replicates of 6 and compared to results of the calibrators and QC samples processed and analyzed 3 months earlier. Differences between samples post-storage for 3 months at −80 °C and those previously analyzed ranged from initially −3.4% to 5.4% across the low, mid, and high QC levels.

**Matrix effects and selectivity studies**

The observed background in drug-free plasma was determined to have an insignificant effect on the response of MPA at the LLOQ concentration, which elutes at 1.27 min. The LLOQ concentration exhibited a signal-to-noise ratio of at least 5 times in drug-free plasma (see Fig. 3 in the online Data Supplement). Carryover studies, which assessed a blank injection after 3 injections of an ULOQ sample, indicated a signal intensity <18.3% of the peak

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Table 2. MPA stability studies.

<table>
<thead>
<tr>
<th>QC level, pg/mL</th>
<th>Injection matrix stability, n = 6</th>
<th>Sample matrix stability, n = 4</th>
<th>Freeze-thaw stability, n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mean</td>
<td>Treated mean</td>
<td>% Difference</td>
<td>Control mean</td>
</tr>
<tr>
<td>Low QC, 600</td>
<td>635.3</td>
<td>623.2</td>
<td>−1.9</td>
</tr>
<tr>
<td>Mid QC, 1750</td>
<td>1856.7</td>
<td>1868.3</td>
<td>0.6</td>
</tr>
<tr>
<td>High QC, 8500</td>
<td>8717.0</td>
<td>8410.0</td>
<td>−3.5</td>
</tr>
</tbody>
</table>
signal intensity observed at the LLOQ following the first post-ULOQ injection (Fig. 2).

A summary of matrix effects for MPA is detailed in Table 3. Matrix effects, which were quantitatively determined through the comparison of the post-extracted sample set to the unextracted sample set, illustrated substantial ion suppression observed for MPA. These data illustrate that the solid-phase extraction approach also isolated other substances that affect the ionization of MPA. However, relative matrix effects were negligible, since the isotopically labeled internal standard

Fig. 2. Carryover analysis of MPA and MP-d6, 17-acetate.
Chromatograms of the highest MPA calibrator (10 000 pg/mL) (A) and its internal standard (B) are shown. Post-injection blanks for MPA (C) and MP-d6, 17-acetate (D), demonstrate carryover <20% of the LLOQ for MPA.
also exhibited substantial suppression. Notably, there was ≤5.5% difference in matrix effects for MPA and MP-d6, 17-acetate, across tested concentrations (Table 3). Further, overall recovery efficiency, which was measured via the comparison of peak area responses of the pre-extracted sample set to the peak area responses of the post-extracted sample set were <5% different between MPA and its internal standard at any QC level. Similar observations were also made for overall processing efficiency.

**MPA quantification in study participants**

Commercial laboratories do not offer methodologies for MPA quantification. Thus, to assess the fidelity of the assay, plasma collected from 5 female study participants all prescribed DMPA were processed and quantified to assess the dynamic range of the assay. Plasma was collected one time per individual, and the participants had received the DMPA dose at different times, so the days post-injection range from 8 to 98 days across the 5 female participants. More information concerning the study set is included in Table 4. MPA concentrations ranged from 491 to 3365 pg/mL, respectively, for the participants who were on DMPA for 8–98 days post-injection.

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>DMPA dose information</th>
<th>Days post-DMPA injection</th>
<th>MPA, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPA-PL-001</td>
<td>150 g/L IM injection</td>
<td>77</td>
<td>1255</td>
</tr>
<tr>
<td>MPA-PL-002</td>
<td>150 g/L IM injection</td>
<td>91</td>
<td>491</td>
</tr>
<tr>
<td>MPA-PL-003</td>
<td>150 g/L IM injection</td>
<td>98</td>
<td>518</td>
</tr>
<tr>
<td>MPA-PL-004</td>
<td>150 g/L IM injection</td>
<td>81</td>
<td>953</td>
</tr>
<tr>
<td>MPA-PL-005</td>
<td>150 g/L IM injection</td>
<td>8</td>
<td>3365</td>
</tr>
</tbody>
</table>

**Table 3. Matrix effects, recovery efficiency, and processing efficiency of MPA and its internal standard MP-d6, 17-acetate.**

<table>
<thead>
<tr>
<th>QC level (µg/mL)</th>
<th>QC level, %a</th>
<th>QC level, %b</th>
<th>QC level, %c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low QC, 600</td>
<td>51293</td>
<td>11077</td>
<td>153167</td>
</tr>
<tr>
<td>Mid QC, 1750</td>
<td>447833</td>
<td>23400</td>
<td>033333</td>
</tr>
<tr>
<td>High QC, 47900</td>
<td>489667</td>
<td>114767</td>
<td>479500</td>
</tr>
</tbody>
</table>

- Matrix effects, % = peak area of (post-extracted samples/un-extracted samples) * 100.
- Recovery efficiency, % = peak area of (pre-extracted samples/post-extracted samples) * 100.
- Processing efficiency, % = peak area of (pre-extracted samples/un-extracted samples) * 100.
DISCUSSION

The method described above facilitates the rapid quantification of MPA in human plasma with an analytical measuring range of 200–10 000 pg/mL. Of note, significant ion suppression was observed for MPA, indicating that the solid-phase extraction did not fully remove interfering substances that could affect MPA ionization. Thus, the use of a structural analog or a nonisotopically labeled internal standard could have a significant effect on MPA quantification due to ionization variability. However, because comparable suppression was observed for the MP-d6, 17-acetate, internal standard, MPA can be quantified using the described analytical approach even in light of substantial ion suppression. The relative matrix effects, as well as the recovery and processing efficiency, were comparable between MPA and its internal standard across all tested concentrations. Furthermore, the recovery efficiencies for MPA and its internal standard ranged between 86% and 95.1% for the QC levels tested, reflecting nearly complete recovery of drug and internal standard postprotein precipitation.

The validated LC-MS/MS method for MPA quantification is also simplified, with streamlined sample preparation and improved throughput in comparison to previous reports, while maintaining a robust, functional assay analytical measurement range for study samples. Previously published methods for MPA are laborious and involve analyte extraction through iterative rounds of liquid-liquid extractions, pooling of eluents, and high volume requirements, typically in the 1.0–3.0 mL range (15–17). Further, analytical run times typically range from 10 to 14 min, which is unrealistic for high-throughput testing required for supporting larger clinical trials (15–19). Through the optimization of sample extraction and chromatographic separation parameters, a method was developed that requires 600 μL plasma and has an analytical run time of 4 min with a C18 column with a 5.0-μm bore size. A recent report highlights the development and validation of a multiplexed method for quantification of chemical castration agents, including MPA, via LC-MS/MS (19). Their approach compared various sample extraction approaches, and they were able to successfully isolate analytes of interest from serum via a double protein precipitation approach. While this method results in decreased sample volume (0.1 mL), analytical run times were still long (12.0 min), and the LLOQ for MPA was 0.5 ng/mL, which is insufficient for detecting MPA concentrations >3 months post-IM injection.

Although the assay does not improve upon previously published LLOQs (15), our data demonstrate that the assay can successfully quantify drug in plasma longer than 3 months after IM injection. The described method is appropriate for quantification of MPA in plasma and can be used in investigations for understanding potential drug–drug interactions between MPA and other concomitant medications (11, 23). This is an area of interest, since drug–drug interactions may significantly affect drug pharmacokinetics. Studies have demonstrated drug–drug interactions between antiretrovirals such as the protease inhibitors lopinavir and ritonavir with the hormonal contraceptive ethinyl estradiol, resulting in decreased drug concentrations of the hormonal contraceptive (24). The decreased hormonal contraceptive concentrations may thus have an impact on potency of the contraceptive agent. Further, ongoing clinical studies by our group are aimed at evaluating the potential interaction of MPA with antiretroviral agents that are used for pre-exposure prophylaxis. Characterization of potential drug–drug interactions in this example is important in ensuring not only proper contraceptive protection, but also sufficient antiretroviral therapeutic efficacy (11, 24). Additionally, the use of DMPA vs oral formulations may influence compound pharmacokinetics and pharmacodynamics in the back-
ground of treatment for chronic illnesses and disease states such as epilepsy (22, 23, 25).

CONCLUSIONS

An LC-MS/MS method has been developed and validated for the quantification of MPA in plasma in accordance with Food and Drug Administration recommendations. The method’s analytical measuring range meets the sensitivity needs in supporting clinical studies focused on drug pharmacokinetics. The method involves streamlined sample preparation, lower volume requirements, and shorter analytical run times.

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REFERENCES


