A HIGHLY SELECTIVE METHOD FOR THE ANALYSIS OF DROSPIRENONE IN HUMAN PLASMA
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Several common birth control formulations contain both drospirenone and ethinyl estradiol. A highly selective and sensitive analytical method for the analysis of drospirenone in human plasma has been developed for use in bioequivalence studies. The solid-phase extraction (SPE) and UPLC®/MS/MS methodologies are described as well as performance against validation parameters.

EXPERIMENTAL CONDITIONS

System: Waters ACQUITY UPLC® System with a Waters Quattro Premier® Triple Quadrupole Mass Spectrometer operated in positive ion MRM mode
Column: ACQUITY UPLC BEH C18 Column, 2.1 x 50 mm, 1.7 μm Part Number: 186002350
Mobile Phase: A: 0.1% HCOOH in water B: MeOH (drospirenone) or ACN (ethinyl estradiol)
Gradient: 70% A to 98% B over 2 min, hold 0.5 min, reset to initial (3 min total cycle time)
Flow Rate: 0.6 mL/min
Injection: 40 μL
Temperature: 55 °C
Desolvation Gas Flow: 750 L/hr
Source Temperature: 120 °C
Desolvation Temperature: 350 °C
Collision Cell Pressure: 2.6 x 10⁻³ mbar
MRM Transitions: 
drospirenone 367 → 96.6 ethinyl estradiol 530 → 171

Figure 1: Structures for ethinyl estradiol (17-ethynylestradiol) and drospirenone.
SOLID-PHASE EXTRACTION (SPE)

SPE Device: Oasis® MAX, 30 mg 96-well plate
Part Number: 186000373
Condition: 500 µL MeOH
Equilibrate: 500 µL water
Load: 250 µL human plasma diluted and acidified 1:1 with 4% H3PO4 in water
Wash 1: 500 µL 5% NH4OH in water
Elute 1: 2 x 125 µL 100% ACN
Elute 2: 2 x 125 µL 2% formic acid in 100% ACN

Directly inject elute 1. Dry down elute 2 and reconstitute in 50 µL of 10 mM sodium bicarbonate buffer, pH 10.5, add 50 µL of 1 mg/mL dansyl chloride solution in acetone and derivatize at 60 °C for 20 minutes.

RESULTS AND DISCUSSION

Mixed-mode SPE is highly selective and has been shown to be a very powerful tool in reducing matrix effects for bioanalytical assays. As this method is to be used in the determination of bioequivalence, it must meet all of the FDA requirements for validation, including the recent guideline relating to variability of matrix effects. The FDA now recommends that matrix effects be quantitated in 6 sources of matrix and that the variability in matrix effects not exceed ± 15%. Variability of matrix effects was determined to be less than ± 15% across all sources (Table 1).

![Human Plasma Source % Matrix Effects for Drospirenone](image)

<table>
<thead>
<tr>
<th>Human Plasma Source</th>
<th>% Matrix Effects for Drospirenone</th>
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</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td>7.4</td>
</tr>
<tr>
<td>Donor 2</td>
<td>5.9</td>
</tr>
<tr>
<td>Donor 3</td>
<td>3.8</td>
</tr>
<tr>
<td>Donor 4</td>
<td>-6</td>
</tr>
<tr>
<td>Pooled Sample 1</td>
<td>-5.1</td>
</tr>
<tr>
<td>Pooled Sample 2</td>
<td>-6</td>
</tr>
</tbody>
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Table 1: Calculated matrix effects for drospirenone in 6 sources of human plasma prepared by mixed-mode anion exchange SPE.

We included 4 individual donors and 2 lots of pooled plasma in the analysis to prove that the assay is selective for drospirenone. Minimization of variability across different sources of matrix (i.e., individual subjects in clinical studies), has been a major point of discussion in relation to Incurred Sample Reanalysis (ISR.) Many researchers believe matrix effects arising from sample variability are one of the primary causes for failure in repeat analysis.

SPE recovery was calculated for both drospirenone and ethinyl estradiol. Drospirenone was found in eluate 1 (bound by reversed phase) as expected and ethinyl estradiol was found in eluate 2 (bound by anion exchange to the resin). Analyte SPE recovery and RSDs are shown in Table 2.

![Table 2: SPE recoveries (n=8) and RSDs for drospirenone and ethinyl estradiol.](image)

<table>
<thead>
<tr>
<th></th>
<th>% SPE Recovery</th>
<th>% RSD</th>
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</thead>
<tbody>
<tr>
<td>Drospirenone</td>
<td>109</td>
<td>7.2</td>
</tr>
<tr>
<td>Ethinyl Estradiol</td>
<td>89</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Table 2: SPE recoveries (n=8) and RSDs for drospirenone and ethinyl estradiol.

The method is linear (1/x weighting) for the determination of drospirenone in human plasma from 0.5 to 250 ng/mL. Correlation coefficients were greater than 0.997, and deviations from the actual concentrations were less than 15%, including at the lower limit of quantitation (LLOQ). Sensitivity at the LLOQ of 0.5 ng/mL is shown in Figure 2. Calculated concentrations for QC’s at 0.75, 25 and 200 ng/mL were all within 15% of expected values.

![Figure 2: UPLC/MS/MS analysis of a blank plasma sample (A) and 0.5 ng/mL drospirenone extracted from human plasma (B).](image)
CONCLUSION

A sensitive, robust, and reliable UPLC/MS/MS method was developed for quantification of drospirenone in human plasma to support a bioequivalence study. Matrix effects were virtually eliminated through the combination of mixed-mode SPE and UPLC/MS/MS. Combining SPE in a 96-well format with 3-minute cycle times yielded sample throughput of over 400 samples a day without compromising data quality.

REFERENCES