Rapid Quantitation of Menstrual Blood Loss from Feminine Hygiene Products

Gene Ray, Pam Burnett, Ying Li and Dari Dadgar
KCAS, Shawnee, Kansas 66216 USA

INTRODUCTION
Clinical trials involving uterine sparing procedures for treatment of fibroid symptoms or drug therapies to reduce heavy menstural require menstrual blood loss (MBL) assessment as an end point. The two reported approaches are visual assessment (pictogram scoring) and quantitative assessment via hemoglobin conversion to alkaline hematin. Pictogram assessments are typically unreliable with low correlation coefficients. To further complicate pictogram assessments, numerous product changes have recently been made by tampon and pad manufacturers, altering visual absorption patterns. The measurement of MBL by alkaline hematin has become the method of choice and FDA’s “Gold Standard” for MBL assessment.

METHOD
Alkaline hematin quantitation involves pummeling used products in a concentrated sodium hydroxide solution. Since hemoglobin values vary significantly, the method was designed measuring the resulting hematin absorbance against a calibration curve prepared from the subjects’ venous blood. A 10 mL K$_2$EDTA venous blood sample was required, following each subject cycle.

The method was evaluated using three types of feminine hygiene products (tampons, pads and pantiliners) from different manufacturers. Quality Controls (QCs) were prepared by placing known amounts of blood (2.5 to 30 mL) onto various products. The samples were placed into polypropylene bags, along with a specified volume of sodium hydroxide extraction solvent.

VALIDATION DESIGN
Optimal Absorbance & Kinetic Conversion to Alkaline Hematin
- Selectivity
- Linearity & Best Fit
- Precision, Accuracy & Recovery
- Blood Recovery from Products
- Stability
- Presence of Anticoagulant

RESULTS
Absorbance Optimization
A wavelength of 580 nm was selected based on defined plateau region with minimal absorbance from blank product extracts.

The QCs were placed into a Stomacher™ and pummeled until the extraction was complete. The resulting extracts were then incubated to permit the conversion of hemoglobin to alkaline hematin.

Spectrophotometer
- Instrument: SpectraMax 190 (Molecular Devices)
- Software: SoftMax Pro
- Wavelength: 580 nm
- Mode: Endpoint

Conversion stabilized after 2 hours at room temperature.

Selectivity
Various products were evaluated for background absorbances across manufacturers to the absorbance of the low calibration standard. A criteria of < 50% interference was employed, in which several products met this requirement.

Linearity & Best Fit
The method was evaluated using two analytical ranges, 2.5 to 50 mL blood (0.5 L extraction) and 5 to 100 mL blood (1 L extraction) using six calibration levels. The data was best fit using unweighted, 4-parameter regression analysis.

Precision & Accuracy

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean</th>
<th>CV [%]</th>
<th>Bias [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tampon</td>
<td>2.41</td>
<td>19.4</td>
<td>-3.4</td>
</tr>
<tr>
<td>Pad</td>
<td>2.68</td>
<td>15.9</td>
<td>-7.2</td>
</tr>
<tr>
<td>Pantiliner</td>
<td>2.37</td>
<td>15.9</td>
<td>-7.2</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENTS
We want to acknowledge the contributions of Jenny McNown, Rhonda Owsley and Lisa Turner in skillfully executing this study. We also want to thank Bonnie Dawson and Glen Dixon in the creation of the web portal and specialized databases.