**Rapid Quantitation of Menstrual Blood Loss from Feminine Hygiene Products**

**Gene Ray, Pam Burnett, Ying Li and Dori Dadgar**

**KCAS, Shawnee, Kansas 66216 USA**

**INTRODUCTION**

Clinical trials involving uterine sparing procedures for treatment of fibroid symptoms or drug therapies to reduce heavy menstruation 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.

**RESULTS**

**Absorbance Optimization**

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

**Calibration Standards**

Calibration Standards were prepared directly into sodium hydroxide solution with limited portions of the venous blood sample (10 mL).

A 96-well plate design was used for sample analysis. Extracted samples were aliquoted in duplicate onto the plate, along with calibration standards QC samples and solvent blanks. A solution, containing trypan blue, was employed to monitor instrument performance throughout the study.

**Spectrophotometer**

Instrument: SpectraMax 190 (Molecular Devices)

Software: SoftMax Pro

Wavelength: 580 nm

Mode: Endpoint

**Optimal Absorbance & Kinetic Conversion to Alkaline Hematin**

- Selectivity
- Linearity & Best Fit
- Precision, Accuracy & Recovery
- Blood Recovery from Products
- Dilution
- Stability
- Presence of Anticoagulant

**VALIDATION DESIGN**

**Stability**

**Matrix stability**

Whole Blood, K$_2$EDTA Anticoagulant

4 °C: 56 days

Ambient: 43 days

30 °C: 55 days

45 °C: Unstable (7 days)

**Blood on Product**

Ambient: 2 ± 56 days

30 °C: 2 ± 28 days *

**Extract stability**

Alkaline Hematin

Ambient: 33 hours

* Blood stability for Kotex Maxipads only demonstrated up to 7 days at 30 °C.

**Anticoagulant**

Since calibration curves for the analysis of a given patient’s samples must be prepared from patient’s own venous blood, several days after collection, the presence of anticoagulant is essential. Freshly collected whole blood (with and without K$_2$EDTA) was diluted in extraction solvent and the absorbances read over 43 hour interval.

Absorbance readings were within ± 3% of each other at all time points.

**CONCLUSION**

This method has met or exceeded FDA guidance requirements and has been used for the determination of menstrual blood loss on over 85,000 feminine hygiene products from various clinical protocols.

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