A Method to Quantify Dapivirine in Small Hair Samples as a Metric of Adherence and Exposure to the Dapivirine Vaginal Ring: Implications for Real-World Roll-Out

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on behalf of the UCSF Hair Analytical Laboratory (HAL) and the Microbicides Trials Network (MTN)-025/HOPE study team

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Background

- A vaginal ring (VR) containing dapivirine (DPV) was assessed as an HIV prevention tool in open-label trials, including the MTN-025/HOPE trial.
- MTN-025/HOPE was an open-label extension trial to the MTN-020/ASPIRE trial, which assessed the continued safety of and adherence to the DPV VR for the prevention of HIV-1 acquisition in former MTN-020 participants (ClinicalTrials.gov number NCT01617096 and NCT02858037).
- Two clinical pharmacokinetics studies reported that the median DPV plasma concentration of DPV from the VR were 231 ± 46 pg/mL (at 421 hours, MTN-013)[1] and 273.5 ± 98.2 pg/mL (at 4 weeks, MTN-024/IPM 031).[2]
- Hair is easier to collect and store (at room temperature without biohazardous precautions) than plasma and used VRs, and hair levels[3] of antiretrovirals have proven useful as biomarkers of adherence and long-term exposure to oral PrEP and ART[4-6] in multiple settings.

Objective

- To develop an assay to measure DPV concentrations in small amounts of hair (~2 mg) from participants using the DPV VR in MTN-025 via a high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique.

Methods

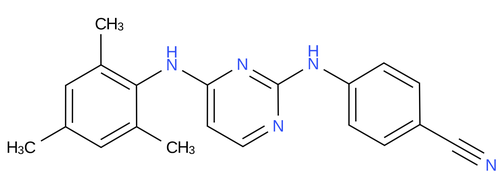
Participant samples

- Hair samples (20-30 strands) for the DPV method validation were collected from participants in MTN-025/HOPE.

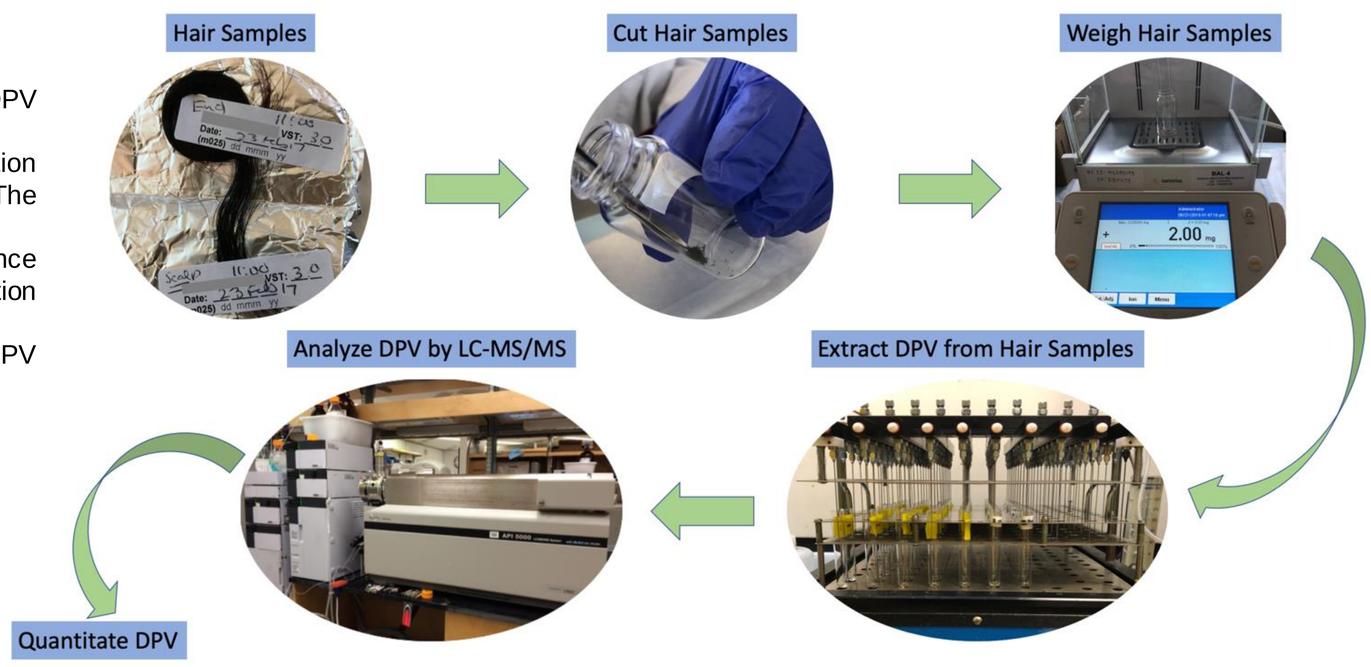
DPV quantitation in hair sample

- Sample preparation consisted of hair incubation (2 mg) in acidified methanol containing $^{2}H_{11}$-DPV (internal standard, IS) for ≥16 hours.
- Sample evaporated to dryness and reconstituted with 0.2 M ammonium bicarbonate solution (pH=8.0). DPV was then extracted via liquid-liquid extraction using methyl *t*-butyl ether. The organic layer was evaporated to dryness and reconstituted in LC-MS/MS mobile phase.
- DPV extracted from the hair was then analyzed by the LC-MS/MS system (Shimadzu Prominence UFLC coupled to Sciex API 5000 triple quadrupole mass spectrometer) via multiple reaction monitoring electrospray in positive ionization mode.
- Quantitation of DPV in hair was determined by plotting peak area ratios of DPV to $^{2}H_{11}$-DPV versus the nominal concentration of DPV.

Dapivirine



Procedure of Sample Preparation, Extraction and Quantification of DPV from Hair Sample



Results

- Our analytical method exhibited high sensitivity (lower limit of quantitation: 0.0100 ng/mg hair) and a wide linear dynamic range (standard curve range: 0.0100-10.0 ng/mg hair) using 2 mg of hair.
- Precision (defined by the % coefficient of variation) and accuracy (defined by % relative error) were both <15%.
- We quantified DPV concentrations in hair from eleven participants on DPV-based regimens. The average DPV concentration was relatively low, 0.0248±0.0198 ng/mg hair, and the range between 0.00980 and 0.0691 ng/mg hair.

DPV Intra-day Validation

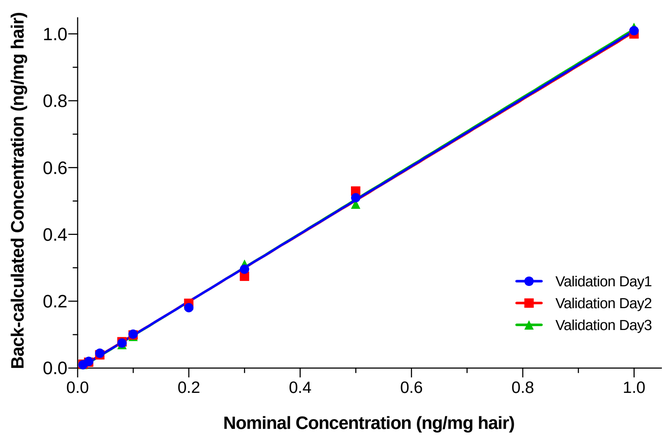
Quality Control*	LLOQ 0.0100 ng/mg hair	Low 0.0300 ng/mg hair	Medium 0.100 ng/mg hair	High 0.750 ng/mg hair
Mean ± SD	0.0101 ± 0.0010	0.0300 ± 0.0031	0.106 ± 0.012	0.730 ± 0.033
%CV**	9.90	10.3	11.3	4.52
%RE***	+1.00	0	+6.00	-2.67
n	6	6	6	6

DPV Inter-day Validation

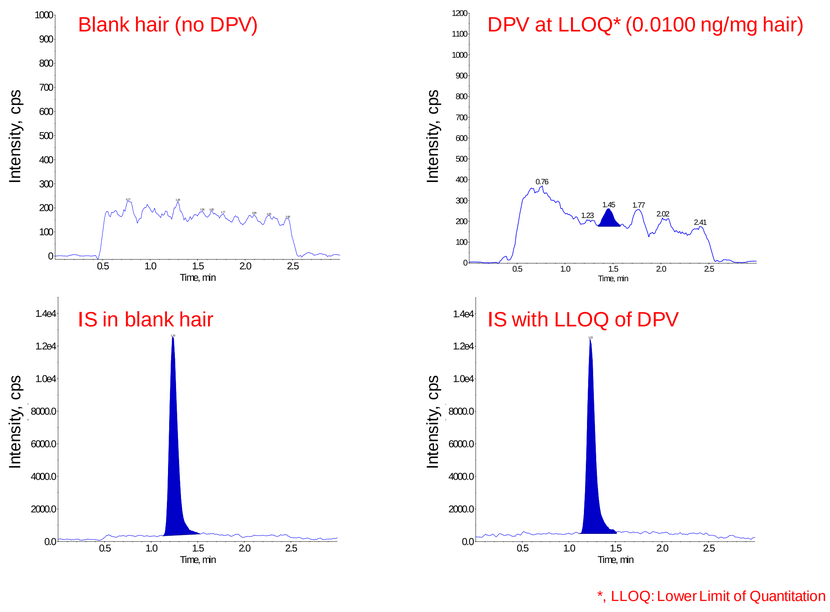
Quality Control	LLOQ 0.0100 ng/mg hair	Low 0.0300 ng/mg hair	Medium 0.100 ng/mg hair	High 0.750 ng/mg hair
Mean ± SD	0.0107 ± 0.0013	0.0301 ± 0.0040	0.102 ± 0.010	0.746 ± 0.043
%CV**	12.1	13.3	9.80	5.76
%RE***	+7.00	+0.333	+2.00	-0.533
n	18	18	18	18

*Blank hair with internal standard (IS) and DPV spiked at various concentrations, ** Percent of coefficient variation, ***Percent of relative error

DPV Standard Curve

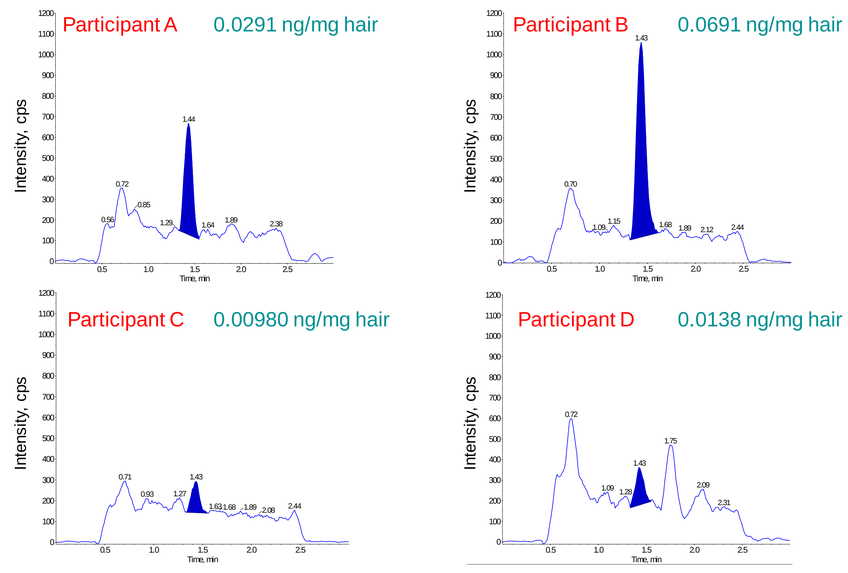


Represented Chromatogram of DPV

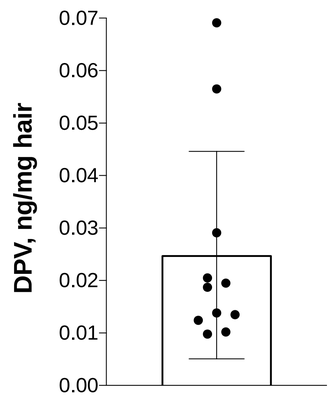


*, LLOQ: Lower Limit of Quantitation

Represented Chromatogram of DPV from Participant Hair



DPV Concentrations in Participant Hairs



Conclusion

- The DPV VR releases DPV in a sustained manner, maintaining high vaginal topical concentrations; it is well-tolerated and has the potential to be an important new tool for PrEP in women.
- The UCSF Hair Analytical Laboratory (HAL) which has extensive experience in developing hair-based ARV assays, has developed a sensitive, specific, accurate and precise method to measure DPV in small hair samples (20-30 strands).
- DPV concentrations of hair of 11 participants in MTN-025 demonstrated a wide linear range; further work to measure DPV in all MTN-025 participants is ongoing.
- DPV concentrations in hair may serve as a practical measure of adherence to the DPV VR once approved

References

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