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Dual quantification of dapivirine and maraviroc in cervicovaginal secretions from ophthalmic tear strips and polyester-based swabs via liquid chromatographic–tandem mass spectrometric (LC–MS/MS) analysis

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ABSTRACT

Background: Topical microbicidal agents are being actively pursued as a modality to prevent HIV viral transmission during sexual intercourse. Quantification of antiretroviral agents in specimen sources where antiviral activity is elicited is critical, and drug measurements in cervicovaginal fluid can provide key information on local drug concentrations. Two antiretroviral drugs, dapivirine and maraviroc, have gained interest as vaginal microbicidal agents, and rugged methods are required for their quantification in cervicovaginal secretions.

Methods: Cervicovaginal fluid spiked with dapivirine and maraviroc were applied to ophthalmic tear strips or polyester-based swabs to mimic collection procedures used in clinical studies. Following sample extraction and the addition of isotopically labeled internal standards, samples were subjected to liquid chromatographic–tandem mass spectrometric (LC–MS/MS) analysis using a Waters BEH C8, 50 mm × 2.1 mm, 1.7 μm particle size column, on an API 4000 mass analyzer operated in selective reaction monitoring mode. The method was validated according to FDA Bioanalytical Method Validation guidelines.

Results: Due to the disparate saturation capacity of the tested collection devices, the analytical measuring ranges for dapivirine and maravirocin cervicovaginal fluid on the ophthalmic tear strip were 0.05–25 ng/tear strip, and 0.025–25 ng/tear strip, respectively. As for the polyester-based swab, the analytical measuring ranges were 0.25–125 ng/swab for dapivirine and 0.125–125 ng/swab for maraviroc. Dilutional studies were performed for both analytes to extended ranges of 25,000 ng/tear strip and 11,250 ng/swab. Standard curves were generated via weighted ($1/x^2$) linear or quadratic regression of calibrators. Precision, accuracy, stability and matrix effects studies were all performed and deemed acceptable according to the recommendations of the FDA Bioanalytical Method Validation guidelines.

Conclusions: A rugged LC–MS/MS method for the dual quantification of dapivirine and maraviroc in cervicovaginal fluid using two unique collection devices has been developed and validated. The described method meets the criteria to support large research trials.

1. Introduction

Despite the multiple modalities available for the treatment and management of HIV/AIDS, more than 34 million individuals are currently infected with the virus, with more than 2.5 million new infections occurring annually [1]. There are numerous antiretroviral (ARV) drugs approved by the Food and Drug Administration (FDA) for disease treatment, and maximal viral suppression is typically observed in response to combinatorial ARV therapies [2]. In addition to disease management, ARV drugs have also been effective in HIV prevention in a number of settings, including pre-exposure prophylaxis (PrEP) in HIV un-infected individuals, as well as treatment as prevention in HIV-infected individuals [3,4]. However, there are numerous impediments to treatment success,

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including high-pill burden or oral therapeutic regimens, lack of regimen adherence, variable drug efficacy and potential ARV drug resistance, and decreased drug concentrations at the site of transmission [5–7]. Based on these challenges, topical administration of microbicidal agents has been pursued as a viral prevention strategy.

Since heterosexual transmission accounts for the highest proportion of HIV-1 infection globally, topical application of vaginal microbicides has been pursued to facilitate increased localized drug concentrations and subsequently, decreased transmission events [8–15]. One of the first studies that assessed the effectiveness of a topical microbicide was the Center for the AIDS Program of Research in South Africa (CAPRISA) 004 trial, a two-arm double-blinded placebo-controlled study to assess the safety and efficacy of the nucleotide reverse transcriptase inhibitor (NRTI) tenofovir [12]. The study concluded that topical administration of tenofovir as a gel formulation reduced HIV acquisition by 39% overall, and 54% in women with high adherence to microbicidal application.

With the success of the CAPRISA 004 trial, as well as additional tenofovir-containing PrEP studies, other drugs have also been evaluated as vaginal microbicidal agents, including the non-nucleoside reverse transcriptase inhibitor (NNRTI) dapivirine and the CCR-5 antagonist maraviroc. These agents elicit their antiviral activity through the noncompetitive binding and subsequent inhibition of the viral reverse transcriptase enzyme and the abrogation of the protein interaction with the host cell CD4 receptor via allosteric binding to the host cell CCR-5 co-receptor molecule, respectively [16–18]. While maraviroc has been well tolerated and elicits viral suppression following oral administration, dapivirine has primarily been pursued as a microbicidal agent [11,19–21]. Topical administration of dapivirine was well tolerated in both reservoir and matrix-based silicone elastomeric ring formulations, and localized concentrations were several orders of magnitude higher than required for in vitro viral inhibition [10,22]. Following the insertion of a matrix-based ring containing 25 mg of dapivirine, average concentrations at the C_{max} ranged from 850.7 to 1913 μg/g within cervicovaginal fluid, depending on where the fluid was collected in relation to the ring; the reservoir ring released less drug and the C_{max} ranged from 7.6 to 14.4 μg/g following the application of the reservoir-formulated ring [10]. There have been other formulations used in phase I studies, including gel and film-based delivery devices, to assess tolerability and the compartmentalized pharmacokinetics of the NNRTI [9,11,23]. Conversely, several studies in nonhuman primates have pursued the topical application of maraviroc, where it has shown protection from infection at the site of transmission [15,24]. Currently, ongoing studies are looking at the safety, efficacy and compartmentalized pharmacokinetics of both dapivirine and maraviroc as vaginal microbicides independently, or in combination [25,26].

Methods with a wide analytical measuring range are required for the quantification of drugs in luminal fluids for compartmentalized pharmacokinetic analysis. Cervicovaginal secretions are heterogeneous fluids collected within the vagina, and are comprised of cervical mucus containing mucins and glycoproteins, as well as sloughed vaginal epithelial cells, lymphocytes, eosinophils and a number of other cell types [27]. Further, cervicovaginal fluid is typically acidic due to the presence of *Lactobacillus*-containing microflora. One important pre-analytical consideration is the collection device used for the acquisition of cervicovaginal fluid. Previously implemented collection devices include Tear Flo$_{TM}$ Test Strips and Alpha Med Tear Test Ophthalmic Strips, as well as a variety of swabs and sponges [11,23,28].

Due to the unique composition of the matrix, as well as the analytical measuring range that may be encountered following topical administration of these drugs, optimized methods are required for the expedient and accurate quantification of these analytes in cervicovaginal secretions. While literature has referenced the quantification of dapivirine in cervicovaginalmatrices with lower limits of quantification (LLOQ) ranging from 20 ng/g cervicovaginalfluid to 40 pg/collection device, and maraviroc quantification with a LLOQ of 50 ng/ml, the full validation metrics have never been described [10,11,14,22]. Further, a method for the dual quantification of both the NNRTI dapivirine and the CCR-5 antagonist maraviroc has not been previously reported. Our group has previously developed and validated liquid chromatographic–tandem mass spectrometric (LC–MS/MS) assays for the quantification of dapivirine and maraviroc in plasma, with lower limits of quantification of 20 pg/ml and 500 pg/ml, respectively [29,30]. However, a method for quantification in cervicovaginal fluid must be amended for a wide analytical measuring range, with extensive linearity to accommodate previously reported localized drug concentrations. The described analytical method is currently being implemented for dapivirine and maraviroc quantification in cervicovaginal secretions in a number of completed and ongoing clinical trials, and meets the needed criteria for accurate and precise drug measurements.

2. Experimental

2.1. Chemicals

Dapivirine, also known as TMC120 (4-[[4-[(2,4,6-Trimethylphenyl)amino]-2-pyrimidinyl]amino]benzonitrile), as well as the deuterated internal standard $^{2}H_4$-dapivirine, were obtained from the International Partnership for Microbicides (IPM) in powdered form. Maraviroc (4,4-difluoro-N-[(1S)-3-[(3-exo)-3-[3-methyl-5-(1-methylethyl-d6)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]cyclohexanecarboxamide), and its isotopically labeled internal standard, $^{2}H_6$-maraviroc, were purchased from Toronto Research Chemicals (TRC, North York, ON). The chemical structures for both dapivirine ($C_{20}H_{19}N_5$) and maraviroc ($C_{29}H_{41}F_2N_5O$) are depicted in Fig. 1. Blank cervicovaginal fluid was obtained from healthy human subjects consented to an institutional review board (IRB)-approved protocol for biological sample collection. HPLC-grade and LC–MS/MS grade acetonitrile and LC–MS grade water were purchased from Fisher Scientific (Pittsburgh, PA). Proteomics-grade formic acid was purchased from Proteochem (Denver, CO).

2.2. Preparation of reagents and standards

Stock solutions of dapivirine and maraviroc were prepared in acetonitrile at final concentrations of 1 mg/ml each. Stock solutions of labeled internal standards were prepared in acetonitrile at final concentrations of 0.5 mg/ml ($^{2}H_4$-dapivirine) and 1 mg/ml ($^{2}H_6$-maraviroc). The dapivirine and maraviroc stock solutions were diluted with acetonitrile to generate working solutions of 100, 10, 1 and 0.1 μg/ml for both compounds. Cervicovaginal fluid was collected using an Instead Softcup (Evofem Inc., San Diego, CA) from healthy volunteers as per the aforementioned IRB-approved protocol. Specifically, secretions were self-collected by placing the Softcupin the vaginal cavity for 30 s; post-removal, the apparatus was transferred to a conical tube and centrifuged at 300 × *g* for 10 min at room temperature (25 °C). Following centrifugation, collected fluid was diluted 20-fold with water. Calibrators and quality control samples were prepared by spiking working solutions into diluted cervicovaginal fluid. The calibrator solutions were prepared at concentrations of 2, 5, 10, 25, 100, 250, 500 (for ophthalmic tear strips) or 750 (for polyester-based swabs) and 1000 ng/ml for dapivirine, and 1, 2, 5, 10, 50, 125, 300 and 1000 ng/ml for maraviroc. Calibrator solutions were maintained in 2.0 ml glass vials and stored at −80 °C. Calibrator solutions were then placed onto