Published in final edited form as:

Bioanalysis. 2013 February ; 5(3): . doi:10.4155/bio.12.319.

Antiretroviral bioanalysis methods of tissues and body biofluids

Robin DiFrancesco^{*}, Getrude Maduke, Rutva Patel, Charlene R Taylor, and Gene D Morse
HIV Clinical Pharmacology Quality Assurance & HIV Clinical Pharmacology Research Programs,
University at Buffalo School of Pharmacy & Pharmaceutical Sciences, Translational
Pharmacology Research Core, New York State Center of Excellence in Bioinformatics & Life
Sciences, 701 Ellicott Street, Buffalo, NY 14203, USA

Abstract

Research in the many areas of HIV treatment, eradication and prevention has necessitated measurement of antiretroviral (ARV) concentrations in nontraditional specimen types. To determine the knowledgebase of critical details for accurate bioanalysis, a review of the literature was performed and summarized. Bioanalytical assays for 31 ARVs, including metabolites, were identified in 205 publications measuring various tissues and biofluids. 18 and 30% of tissue or biofluid methods, respectively, analyzed more than one specimen type; 35–37% of the tissue or biofluid methods quantitated more than one ARV. 20 and 76% of tissue or biofluid methods, respectively, were used for the analysis of human specimens. HPLC methods with UV detection predominated, but chronologically MS detection began to surpass. 40% of the assays provided complete intra- and inter-assay validation data, but only 9% of publications provided any stability data with even less for the prevalent ARV in treatments.

Background

Current HIV clinical research objectives

The current focus of HIV clinical pharmacology and therapeutics research is in prevention of transmission, eradication of latent HIV reservoirs, treatment of co-infection with hepatitis C virus, tuberculosis and malaria, as well as the development of nanomedicine and new formulations for dose optimization. To determine compartmental distribution of antiretrovirals (ARVs) to latent viral reservoirs, prevent viral transmission, assess adherence or long-term ARV exposure, as well as investigate the etiology of adverse ARV effects, such as mitochondrial toxicity, cognitive impairment and muscle wasting, the collection and analysis of samples other than blood and its products is required. Consequently, many research protocols that include HIV clinical pharmacology objectives are developed to include the collection, handling and bioanalysis of uncommon specimen types such as biofluids and tissues. New analyses of tissues and biofluids have been reported with

© 2013 Future Science Ltd

^{*}Author for correspondence: Tel.: +1 716 881 7465, Fax: +1 716 849 6890, rda@buffalo.edu.

Financial & competing interests disclosure

This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, NIH, Department of Health and Human Services, under contract number HHSN272200800019C. The Clinical Pharmacology Quality Assurance (and Quality Control) Program gratefully acknowledges the contributions of the Clinical Pharmacology Quality Assurance (and Quality Control) Program Laboratory and Program staff. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: WWW.FUTURE-SCIENCE.COM/DOI/SUPPL/10.4155/BIO.12.319

correlation to clinical outcomes and minimum inhibitory/effective antiviral concentrations. ARV concentrations in hair have recently been used to correlate with plasma concentrations and provide a noninvasive tool to quantitate long-term, systemic ARV exposure for HIV treatment and adherence [1–5]. Saliva drug concentrations have also been correlated with free-drug and plasma concentrations [6,7]. Cerebrospinal fluid (CSF) concentrations have been of interest for studies focused on HIV-associated neurologic disease and to calculate the ARV central penetration effectiveness score [8]. Vaginal and cervicovaginal fluids (CVF) or semen drug concentrations are currently being determined in pre-exposure prophylaxis and to prevent HIV transmission [9,10]. The reported concentrations in each of these specimen types may be normalized for protein content [11], cell number [12] or cell type [13], or other physiological or chemical characteristics that assist in data interpretation and for PK and PD modeling.

Importance of the specimen integrity & identity

The proper collection, storage, processing and bioanalysis of the contents of these matrices, as well as the appropriate use of concentration results in interpreting outcomes, have not been standardized. Data published from clinical studies may or may not reference or include methods for sample handling and bioanalytical methods (herein referred to as ‘methods’). While the proper collection, handling and storage of these specimens are essential to the validity of the drug concentration results, standardized methods are currently lacking. Published ARV methods that test tissues and biofluids offer an opportunity to identify specimen handling data that may expedite current research initiatives. Pre-analytical details that support the integrity of future specimens may provide researchers with valuable information for these special specimen types.

Dependability of bioanalytical methods

Methods to quantitate drugs and metabolites in nontraditional, or ‘rare’, matrices such as tissues and biofluids are not commonly required for pharmaceutical bioanalysis and are not currently regulated by US FDA guidelines [14]. However, some publications may contain important bioanalytical details that have been employed, such as precautions and conditions for stable sample preparations, prior to chromatographic analysis and the corresponding parameters of the method operation (i.e., MS detection modes). Those that have been validated and include the supporting validation data in the published method should be considered as the most dependable and valuable to the researcher, whereas those that omit these key data should be closely examined before adopting information.

Interpreting results

Finally, the appropriate use of drug concentration results from specific specimen types should consider the biological and physicochemical qualities of specimens between and within individuals and methods to provide a meaningful normalization of drug concentrations. Validation data, such as LLOQ, variability, accuracy, specificity and selectivity, can also provide a baseline for the further development or adaptation of specific methods.

Rationale for this review

This review provides a summary of pre-analytical, analytical and postanalytical material from the literature of published methods of bioanalysis for ARV concentrations in nontraditional specimens such as tissues and biofluids.

Literature searches & data compilation

Multiple Pubmed literature searches were performed to identify published ARV methods for the following specimen types:

Biofluids: amniotic, breast milk, cerebral spinal, cervical/vaginal, intestinal, saliva, semen, sputum;

Tissues: brain, colomic, fetal, glandular, gastrointestinal, hair, heart, kidney, liver, lung, lymphatic, muscle, nails, placental tissue, spleen, testes and vagina.

The searches did not include blood, blood cells, plasma or urine. Keywords for the searches included the drug name plus the various tissues and fluids. All approved ARVs were cross-referenced with the specimen types. The publications included analytical methods used to characterize ARVs in humans and animals.

Extraction of data

Important variables relevant to method validation and application were extracted from the publications for support of specimen handling and analysis in future clinical studies. For each publication, the ARVs and metabolites analyzed were noted. Specimen collection, handling (including addition of stabilizers), processing steps (e.g., flash freezing or homogenization prior to storage), and storage techniques, as well as the stability data associated with these steps, were identified. Stability data included duration of stability, temperature and condition of specimen. In addition to the type of analytical method used, supporting validation data, variation, accuracy and LLOQ were collected.

Classifying the specimens

After compiling the reported data from all references, final categories of specimen types and analytical method types were utilized to consolidate and coordinate all data. The tissue categories were internal tissues, hair (the only external tissue found), placenta and fetal tissue. The biofluid categories included amniotic fluid, breast milk, CSF/extracellular cerebral fluid, CVF, saliva, and semen/seminal fluid.

Enumerating results

To simplify the enumeration process for bioanalytical details, references that were cited and used for more than one specimen type were counted as a separate method (e.g., if a reference measured an ARV in both seminal plasma and saliva, it was counted as one method for seminal plasma and one method for saliva). This approach accounts for enumerated variables that differ depending on the specimen type. In addition, for references that indicated the use of multiple methods for bioanalysis, each method noted was counted as a separate method. When ‘% of methods’ is noted, the denominator is based on the above enumerations. When ‘% published’ is noted, the percentage is derived specifically from the number of total references.

Bioanalysis methods for ARVs in tissues & biofluids

The literature search resulted in the identification of 205 publications identifying methods used for measuring ARV in the nontraditional tissues and biofluids of interest [3–7,11,15–213]. 12% of the publications found measured ARV in both tissues and biofluids of interest; 5% of publications indicated multiple methods were used. As defined above for enumeration, 35–37% of the tissue or biofluid methods quantitated more than one ARV. 18 and 30% of tissue or biofluid methods, respectively, analyzed more than one specimen type. 20 and 76% of tissue or biofluid methods, respectively, were used for the analysis of human

specimens. Notably, hair, saliva and CVF methods were only used for human specimens. Methods for all currently marketed ARVs were identified for at least one specimen type with the exception of tipranavir. Other than for zidovudine phosphorylated metabolites, only one other method for nucleoside reverse transcriptase inhibitors (NRTI) phosphorylated metabolites (stavudine triphosphate) was identified [203].

Sample collection

For tissues (including internal, fetal and placental), 76 of the 84 references provided a collection method. Of these, most tissues, except hair, were surgically extracted, especially when taken from animals. Human hair was collected by manually cutting a length from the head. Exsanguination of blood from tissues was not indicated in the methods used except for fetal tissue; exsanguination is important due to the contamination of blood from the vessels in the tissue. Fluids were collected using a variety of techniques appropriate to the fluid location. With the exception of breast milk, across all categories, nearly all of the methods provided a collection description. Amniotic fluid was collected during surgical procedures such as laparotomy, during delivery or directly withdrawn through a catheter, the uterine vein or a syringe aspiration. Breast milk was collected manually for five of the 15 methods, by an electric pump for one of the 15, but nine of 15 of the breast milk methods provided no collection information. Most of the CSF specimens were collected by cisternal or lumbar puncture, the latter being preferred. The two references collecting extracellular cerebral fluid used microdialysis probes in animals. A total of 23 of 43 references for seminal fluid/semen reported using masturbation; one reported using electroejaculation. Saliva and CVF collection methods varied the most and nearly all references reported the method used for collection. Saliva was collected under stimulated (Salivette®) or unstimulated conditions (expectoration) into vessels or with swabs (Salivette swab). CVF was collected by aspiration, lavage, or Sno-Strip™ or blotting paper. Neither sample rejection criteria nor notable precautions were provided for tissues or biofluids.

Sample handling & storage

Only one of the references used a buffer addition to CVF after collection for stabilization [212]. Both tissues (23%) and biofluids (5%) used cooling by placement on ice (or refrigeration) or flash freezing in liquid nitrogen in an effort to minimize or slow any decomposition or metabolism of the analytes of interest within the specimen. 63% of the methods indicated whether the tissue was homogenized prior to or after storage. Of the 63%, most tissues (60%) were stored frozen and then were thawed and homogenized prior to analysis rather than before storage (40%). 79% of the tissues and 60% of the biofluids indicated storage conditions. When indicated, freezer storage was most often indicated as an ultracold freezer (30% tissues, 65% biofluids) or by a typical freezer (52% tissues, 33% biofluids). When storage for hair samples was indicated (67%), the samples were wrapped in foil to protect from light and held at ambient temperature with or without enclosure in a desiccated bag.

Bioanalytical methods

Table 1 summarizes the method types for tissues and biofluids. The early references were more likely to have used HPLC with UV detection and, conversely, the newer references were more likely to have used a MS for detection. Some ligand-binding methods (e.g., enzyme immunoassay and radioimmunoassay) were used to measure didanosine, stavudine and zidovudine. The use of UPLC was cited in more recent methods.

ARVs measured

The number of methods by drug and by specimen category is depicted in Figure 1 and displayed by areas of HIV research. These research areas include HIV prevention (genital tract specimens), **HIV treatment and adherence** (hair, saliva specimens), **HIV eradication** (sanctuary site specimens such as CSF and internal tissues) and **HIV maternal-to-child transmission** (amniotic fluid, breast milk, fetal and placental tissue). Overall, methods for CSF analysis predominated for biofluids, while internal organs were the most commonly investigated tissues. Excluding metabolites, zidovudine (n = 81 methods) was measured most often in all categories, followed by indinavir and lamivudine (n = 37), lopinavir and ritonavir (n = 32, 34), stavudine and didanosine (n = 27), nelfinavir and nevirapine (n = 24), abacavir (n = 17), and efavirenz and saquinavir (n = 15). Very few methods measured the currently favored ARVs atazanavir or tenofovir (n = 11), maraviroc or raltegravir (n = 10), darunavir (n = 4), emtricitabine (n = 3), etravirine (n = 1) and none were identified that measured tipranavir or the phosphorylated NRTI for abacavir, lamivudine, didanosine, dideoxycytidine or emtricitabine. There was a negative correlation with the chronology of drug development and the number of ARV methods published that assayed alternative specimens (data not shown).

Assay validation

Validation data was provided for methods for 54% of the tissue methods and complete data, including variation and accuracy at three levels of concentrations, which were provided for most of the 54% (74%), although in some cases it was difficult to determine if the validation data were specific to the matrix. Internal tissue methods provided the least (26%) validation data. For biofluids, 61% of the biofluid methods included validation data; variation and accuracy at three levels of concentrations for 68% of those reporting validation data.

Stability data

Minimal stability data for the analytes in tissues or biofluids for postcollection processing, time of storage and/or handling were reported. Table 2 summarizes stability data that were published that indicated specimen types, analytes, time and conditions of stability tested. The table also cites the reference numbers. Only 9% of the references provided stability data. For the current agents of most interest, very limited or no stability data were found: atazanavir (none), darunavir (none), efavirenz (hair – 4 months ambient) [5], emtricitabine (none), lopinavir (hair – 4 months ambient) [5], maraviroc (none), raltegravir (none) and tenofovir (vaginal tissue – 2 months ultracold) [55]. For di- or tri-phosphorylated forms of NRTI, stability data for only zidovudine forms were identified [100].

Reporting of results

Concentration data were reported in mass or molar units per volume of tissue homogenate or biofluid. Some tissues methods normalized homogenate results by the weight of the tissue specimen and reported per gram unit of tissue rather than volume of homogenate. One CSF method used normalized CSF concentration by the individual total CSF protein concentration, however, there was no attempt to normalize results for other biofluids, which can also vary in protein content [11].

Key discussion points

It is clear that many physiological and practical considerations should be deliberated and with the exception of the actual method, standardized methods and approaches ought to be utilized in clinical research practices. For the method of ARV measurement, even though the FDA bioanalytical method guidance requires validation only when matrices can be obtained

in adequate quantities, the pivotal nature of drug concentration data within current research initiatives may outweigh the justification of FDA guidance exemptions. These points and others follow.

A good specimen is vital

The characterization of specimen collection and handling is critical to interpreting concentration results and their relationship to therapeutic outcomes. The data collected show little variability in collection techniques, except for saliva and CVF. Methods for CVF include lavage, direct aspiration and use of Sno-Strip devices (or similar products) for collection. For CVF collection methods, the use of TearFlo® or Sno-Strip collection devices have been recommended by one set of investigators to minimize contamination of the sample with tissue cells and blood, as well as standardize the location and total volume of the collection [150]. For saliva samples, stimulated collection devices or stimulated or nonstimulated expectoration were the most common collection methods, however, saliva itself can vary in viscosity. A higher level of stimulation decreases the viscosity of the sample. Therefore, stimulation may allow for a more homogenous sample representing free-drug concentration whereas an unstimulated sample may contain more cells and other debris such as mucus and glycoproteins.

A 2011 review article reported on both the female and male genital tract samples that have been collected and analyzed for ARV concentration. Although the review is focused on the postdose ARV genital tract to blood plasma ratio, the authors included various types of collection (for female only; male collections were identical) and types of analysis including the LLOQ for either plasma or the matrix [214]. The article also discusses the specimen collection method, the variability of the specimens collected and the effects on the integrity and interpretability of the sample results.

Lastly, it is important to consider conditions that render the biological samples unusable. Contamination with blood is one example, but there are additional variables (other than contamination) to be considered.

Lability & stability

Maintaining the stability of drugs within specific matrices is essential to assuring accuracy in the values reported. The contents of most tissues and biofluids contain enzymes and the pH range of some may be considerable. Since the data supporting ARV stability during specimen collection was rarely reported, laboratories will need to determine the validity of adopting the specimen collection process prior to application to clinical specimens. Moreover, data to support the stability of drugs and metabolites after freeze-thaw treatment or for long-term storage of specimens were insufficient for the application to most multicenter clinical studies. This last point of discussion should also serve as a reminder that tissue and biofluid samples may be limited by volume and/or require special handling and storage conditions that are routinely different than that of blood, plasma or urine. However, this should not excuse the testing of ARV stability under the special handling and storage conditions required, even if the measure is solely qualitative.

Methods from the past to the present

Chronologically, the primary method of analysis was HPLC with UV detection followed by single or triple quadrupole MS detection in more recent years. These methods represent a foundation upon which newer, more complex technology can be built. The bioanalytical field can continue to expect less HPLC and more UPLC, which produces shorter analysis times and utilizes less solvent. Detectors will also continue to become more sensitive, specific and selective to allow for measurements below the femtomole range, in targeted

tissues or biofluids, with small differences in mass or chemical properties, respectively. These detection improvements will ultimately be needed as the development of nanoformulations matures and products are approved based on tissue-targeted concentrations.

Although not reported in this literature review, quantitation of ARVs in internal tissues using radioactively labeled drugs in animals was common. Radioactively labeled compounds can be more sensitive, but are often less specific and are subject to more matrix effects. The same is true of ligand-binding methods for small molecules such as ARVs. Few references cited the use of ligand-binding methods for ARV measurement and, in all of these cases, references were dated. Therefore, traditional methods of ligand-binding are unlikely to be used in future studies.

Method validation versus publication

Overall, the reporting of key validation data varied indicating that publications are not consistent to support their methods. It appeared as though the reporting of precision and accuracy of the methods was >50%. While this is encouraging and should facilitate the transfer of these validated methods to new applications, it was sometimes unclear as to whether data were derived from validation in plasma matrices or in the matrix of interest. Therefore, some published data may misrepresent the true precision and accuracy of the method as it pertains to the application for analysis of tissues or biofluids.

Consistent with specimen collection and handling, the reported stability of drugs and metabolites in processed and/or stored samples was low (<10%) and offered little support for storage of tissue and biofluids collected during current clinical studies. This is concerning since most of these specimens are analyzed after all have been collected and specimens may be stored for a year or more. Therefore, there is a need for generation of data to support the valid use of clinical samples under various conditions of exposure.

Special considerations for biofluids & tissues

Normalizing tissue and biofluid concentration values may help to provide better modeling outcomes when considering PK and PD relationships. Very few methods reported using such procedures, but the actual application of results was not known. Since these specimens may vary in content and chemical and/or physiological properties or have the potential to be easily contaminated, more guidance should be sought. Biofluids such as saliva, semen and CVF, may vary in viscosity, pH and cellular debris, as well as contain environmental contaminants such as food, cells or blood. Biofluids from the female genital tract may also vary as they are subject to menstrual cycle changes. Breast milk contents vary throughout the feeding time with the fat content increasing toward the end of breastfeeding; the nutritional status of the mother may also cause variation of breast milk contents. Tissues vary in vascular content and their disease states may alter their capacity to function effectively. Even biofluids such as CSF can vary in protein content effectively causing greater variation in drug measurements [11]. Little detail has been provided for normalizing concentration results by appropriate parameters of the specimen. As researchers collect and analyze these uncommon specimens, much information is needed to maintain best practices and include quality assurance.

Current ARV method data are scarce

Most of the references provided information for older ARV, and the current ARV biofluid and tissue method data in newer areas, such as **HIV prevention research**, are scarce. For example, the use of tenofovir and emtricitabine to prevent HIV transmission will generate a demand for the measurement of these compounds and their metabolites with precision and

accuracy at lower limits of concentrations in various biofluids and tissues. The literature searches were completed in 2011 and at that time very few bioanalytical methods had been published for the measurement of tenofovir in biofluids and tissues of interest. Even fewer had been published for measurement of emtricitabine as well as other new ARVs. In the past year, more data have been published, but the details of collection, stabilization, stability, and assay precision and accuracy are lacking [215–228]. There is an important need or gap for data from bioanalytical validations for current ARVs used for treatment and/or prevention. The publishing of such information would serve to expedite the research processes.

Future perspective

Nontraditional specimen types, other than those commonly required for bioanalysis, introduce challenges for the collection, storage, processing and bioanalysis of their contents. As ARV research expands to include treatment of HIV and co-infections, prevention strategies, nanotechnology formulations and eradication from reservoirs, specimens of utmost interest will include many tissue types such as epithelial, connective, nerve, muscle and adipose tissues as well as biofluids such as saliva, breast milk, genital tract samples and CSF. Published ARV method validations for the bioanalysis of tissue and biofluid specimens are available. However, key information, such as specimen collection and handling and ARV stability, is limited and confidence in the reliability of historical published data should be contemplated. Given the importance of reducing the rate of transmission, the goal of eradicating HIV, as well as global economic impact, research in this area would greatly benefit from validated bioanalytical assay reports to facilitate quality, standardized procedures for specimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Key Terms

HIV treatment and adherence	Patient adherence is critical to success of HIV replication reduction and the measurement of antiretroviral in hair has been shown to correlate with long-term adherence.
HIV eradication	As formulations of drugs begin to target organ specific sites and HIV sanctuaries, the concentration of drug in tissues (e.g., gut-associated lymphoid tissue, brain tissue) becomes more relevant than that in central compartments.
HIV maternal-to-child transmission	As the number of patients receiving antiretroviral as therapy or prevention increases, more unborn children require protection from acquiring HIV. The knowledge of transmission of antiretrovirals through amniotic fluid and placental tissue to the fetal tissues during pregnancy, as well as postpartum through breast milk, is important to obtain.
HIV prevention research	Use of oral and topical antiretrovirals to prevent HIV transmission, as well as treatment as prevention of transmission, has precipitated the collection of many genital compartment specimens.

References

Papers of special note have been highlighted as:

of interest

of considerable interest

1. Gandhi M, Greenblatt RM. Hair it is: the long and short of monitoring antiretroviral treatment. *Ann. Intern. Med.* 2002; 137(8):696–697. [PubMed: 12379072] Older method article addressing the measurement of antiretroviral (ARV) in hair. ARV measurement in hair is gaining popularity for adherence applications.
2. Bernard L, Peytavin G, Vuagnat A, De Truchis P, Perronne C. Indinavir concentrations in hair from patients receiving highly active antiretroviral therapy. *Lancet.* 1998; 352(9142):1757–1758. [PubMed: 9848361]
3. Duval X, Peytavin G, Breton G, et al. Hair versus plasma concentrations as indicator of indinavir exposure in HIV-1-infected patients treated with indinavir/ritonavir combination. *AIDS.* 2007; 21(1):106–108. [PubMed: 17148976]
4. Gandhi M, Ameli N, Bacchetti P, et al. Protease inhibitor levels in hair strongly predict virologic response to treatment. *AIDS.* 2009; 23(4):471–478. [PubMed: 19165084]
5. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, Messenkoff N. Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 2008; 22(21):3401–3409. [PubMed: 18837069]
6. Van Heeswijk RP, Veldkamp AI, Mulder JW, et al. Saliva as an alternative body fluid for therapeutic drug monitoring of the nonnucleoside reverse transcription inhibitor nevirapine. *Ther. Drug Monit.* 2001; 23(3):255–258. [PubMed: 11360034]
7. Wintergerst U, Kurowski M, Rolinski B, et al. Use of saliva specimens for monitoring indinavir therapy in human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* 2000; 44(9):2572–2574. [PubMed: 10952622]
8. Letendre S. Central nervous system complications in HIV disease: HIV-associated neurocognitive disorder. *Top. Antivir. Med.* 2011; 19(4):137–142. [PubMed: 22156215]
9. Hendrix, C.; Minnis, A.; Guddera, V., et al. MTN-001: a phase 2 cross-over study of daily oral and vaginal TFV in healthy, sexually active women results in significantly different product acceptability and vaginal tissue drug concentrations; Presented at: 18th Conference on Retroviruses and Opportunistic Infections; Boston, MA, USA. 27 February-2 March 2011;
10. Kashuba, AD.; Karim, SSA.; Kraft, E., et al. Do systemic and genital tract tenofovir concentrations predict HIV seroconversion in the CAPRISA 004 tenofovir gel trial?; Presented at: XVIII International AIDS Conference; Vienna, Austria. 18-23 July 2010;
11. Dicenzo R, DiFrancesco R, Cruttenden K, Donnelly J, Schifitto G. Lopinavir cerebrospinal fluid steady-state trough concentrations in HIV-infected adults. *Ann. Pharmacother.* 2009; 43(12):1972–1977. [PubMed: 19934383]
12. Robbins BL, Nelson SR, Fletcher CV. A novel ultrasensitive LC-MS/MS assay for quantification of intracellular raltegravir in human cell extracts. *J. Pharm. Biomed. Anal.* 2012; 70:378–387. [PubMed: 22727807]
13. Anderson PL, Zheng JH, King T, et al. Concentrations of zidovudine- and lamivudine-triphosphate according to cell type in HIV-seronegative adults. *AIDS.* 2007; 21(14):1849–1854. [PubMed: 17721092]
14. US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Center for Veterinary Medicine. Guidance for Industry: Bioanalytical Method Validation. FDA; Rockville, MD, USA: 2001.
15. Estrela Rde C, Ribeiro FS, Barroso PF, et al. ABCB1 polymorphisms and the concentrations of lopinavir and ritonavir in blood, semen and saliva of HIV-infected men under antiretroviral therapy. *Pharmacogenomics.* 2009; 10(2):311–318. [PubMed: 19207033]
16. Ghosn J, Chaix ML, Peytavin G, et al. Absence of HIV-1 shedding in male genital tract after 1 year of first-line lopinavir/ritonavir alone or in combination with zidovudine/lamivudine. *J. Antimicrob. Chemother.* 2008; 61(6):1344–1347. [PubMed: 18343806]

17. Rolinski B, Bogner JR, Sadri I, Wintergerst U, Goebel FD. Absorption and elimination kinetics of zidovudine in the cerebrospinal fluid in HIV-1-infected patients. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 1997; 15(3):192–197. [PubMed: 9257653]
18. Alcorn J, Mcnamara PJ. Acyclovir, ganciclovir, and zidovudine transfer into rat milk. *Antimicrob. Agents Chemother.* 2002; 46(6):1831–1836. [PubMed: 12019097]
19. Granda BW, Giancarlo GM, Von Moltke LL, Greenblatt DJ. Analysis of ritonavir in plasma/serum and tissues by high-performance liquid chromatography. *J. Pharmacol. Toxicol. Methods.* 1998; 40(4):235–239. [PubMed: 10465159]
20. Mirochnick M, Thomas T, Capparelli E, et al. Antiretroviral concentrations in breastfeeding infants of mothers receiving highly active antiretroviral therapy. *Antimicrob. Agents Chemother.* 2009; 53(3):1170–1176. [PubMed: 19114673]
21. Shapiro RL, Holland DT, Capparelli E, et al. Antiretroviral concentrations in breastfeeding infants of women in Botswana receiving antiretroviral treatment. *J. Infect. Dis.* 2005; 192(5):720–727. [PubMed: 16088821]
22. Kwaro A, Delong A, Rezk N, et al. Antiretroviral drug concentrations and HIV RNA in the genital tract of HIV-infected women receiving long-term highly active antiretroviral therapy. *Clin. Infect. Dis.* 2008; 46(5):719–725. [PubMed: 18220480]
23. Taylor S, Back DJ, Drake SM, et al. Antiretroviral drug concentrations in semen of HIV-infected men: differential penetration of indinavir, ritonavir and saquinavir. *J. Antimicrob. Chemother.* 2001; 48(3):351–354. [PubMed: 11532998]
24. Dumond JB, Yeh RF, Patterson KB, et al. Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis. *AIDS.* 2007; 21(14):1899–1907. [PubMed: 17721097]
25. Burger DM, Rosing H, Ten Napel CH, et al. Application of a radioimmunoassay for determination of levels of zalcitabine (ddC) in human plasma, urine, and cerebrospinal fluid. *Antimicrob. Agents Chemother.* 1994; 38(12):2763–2767. [PubMed: 7695259]
26. Gandhi M, Ameli N, Bacchetti P, et al. Atazanavir concentration in hair is the strongest predictor of outcomes on antiretroviral therapy. *Clin. Infect. Dis.* 2011; 52(10):1267–1275. [PubMed: 21507924]
27. Chu CK, Bhadti VS, Doshi KJ, et al. Brain targeting of anti-HIV nucleosides: synthesis and *in vitro* and *in vivo* studies of dihydropyridine derivatives of 3 -azido-2 ,3 -dideoxyuridine and 3 azido-3 -deoxythymidine. *J. Med. Chem.* 1990; 33(8):2188–2192. [PubMed: 2374145]
28. Hamidi M. Central nervous system distribution kinetics of indinavir in rats. *J. Pharm. Pharmacol.* 2007; 59(8):1077–1085. [PubMed: 17725849]
29. Yilmaz A, Stahle L, Hagberg L, Svennerholm B, Fuchs D, Gisslen M. Cerebrospinal fluid and plasma HIV-1 RNA levels and lopinavir concentrations following lopinavir/ritonavir regimen. *Scand. J. Infect. Dis.* 2004; 36(11-12):823–828. [PubMed: 15764168]
30. Gisolf EH, Enting RH, Jurriaans S, et al. Cerebrospinal fluid HIV-1 RNA during treatment with ritonavir/saquinavir or ritonavir/saquinavir/stavudine. *AIDS.* 2000; 14(11):1583–1589. [PubMed: 10983645]
31. Yilmaz A, Fuchs D, Hagberg L, et al. Cerebrospinal fluid HIV-1 RNA, intrathecal immunoactivation, and drug concentrations after treatment with a combination of saquinavir, nelfinavir, and two nucleoside analogues: the M61022 study. *BMC Infect. Dis.* 2006; 6:63. [PubMed: 16566834]
32. Kravcik S, Gallicano K, Roth V, et al. Cerebrospinal fluid HIV RNA and drug levels with combination ritonavir and saquinavir. *J. Acquir. Immune Defic. Syndr.* 1999; 21(5):371–375. [PubMed: 10458617]
33. Tashima KT, Caliendo AM, Ahmad M, et al. Cerebrospinal fluid human immunodeficiency virus type 1 (HIV-1) suppression and efavirenz drug concentrations in HIV-1-infected patients receiving combination therapy. *J. Infect. Dis.* 1999; 180(3):862–864. [PubMed: 10438381]
34. Yilmaz A, Watson V, Else L, Gisslen M. Cerebrospinal fluid maraviroc concentrations in HIV-1 infected patients. *AIDS.* 2009; 23(18):2537–2540. [PubMed: 19855252]

35. Doshi KJ, Gallo JM, Boudinot FD, Schinazi RF, Chu CK. Comparative pharmacokinetics of 3 - azido-3 -deoxythymidine (AZT) and 3 -azido-2 ,3 -dideoxyuridine (AZddU) in mice. *Drug Metab. Dispos.* 1989; 17(6):590–594. [PubMed: 2575492]
36. Lupia RH, Ferencz N, Lertora JJ, Aggarwal SK, George WJ, Agrawal KC. Comparative pharmacokinetics of two prodrugs of zidovudine in rabbits: enhanced levels of zidovudine in brain tissue. *Antimicrob. Agents Chemother.* 1993; 37(4):818–824. [PubMed: 8494380]
37. Taylor S, Van Heeswijk RP, Hoetelmans RM, et al. Concentrations of nevirapine, lamivudine and stavudine in semen of HIV-1-infected men. *AIDS.* 2000; 14(13):1979–1984. [PubMed: 10997403]
38. Chan DJ, Ray JE, McNally L, Batterham M, Smith DE. Correlation between HIV-1 RNA load in blood and seminal plasma depending on antiretroviral treatment status, regimen and penetration of semen by antiretroviral drugs. *Curr. HIV Res.* 2008; 6(5):477–484. [PubMed: 18855660]
39. Taylor S, Jayasuriya AN, Berry A, et al. Darunavir concentrations exceed the protein-corrected EC(5)(0) for wild-type HIV in the semen of HIV-1-infected men. *AIDS.* 2010; 24(16):2583–2587. [PubMed: 20736813]
40. Yilmaz A, Izadkhashti A, Price RW, et al. Darunavir concentrations in cerebrospinal fluid and blood in HIV-1-infected individuals. *AIDS Res. Hum. Retroviruses.* 2009; 25(4):457–461. [PubMed: 19320601]
41. Patterson K, Jennings S, Falcon R, Mrus J, Kashuba A. Darunavir, ritonavir, and etravirine pharmacokinetics in the cervicovaginal fluid and blood plasma of HIV-infected women. *Antimicrob. Agents Chemother.* 2011; 55(3):1120–1122. [PubMed: 21173188]
42. Anderson BD, May MJ, Jordan S, Song L, Roberts MJ, Leggas M. Dependence of nelfinavir brain uptake on dose and tissue concentrations of the selective P-glycoprotein inhibitor zosuquidar in rats. *Drug Metab. Dispos.* 2006; 34(4):653–659. [PubMed: 16434546]
43. Ding Y, Williamson LN, White CA, Bartlett MG. Determination of 2 ,3 -dideoxycytidine in maternal plasma, amniotic fluid, placental and fetal tissues by high-performance liquid chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004; 811(2):183–189.
44. Clark TN, White CA, Chu CK, Bartlett MG. Determination of 3 -azido-2 ,3 -dideoxyuridine in maternal plasma, amniotic fluid, fetal and placental tissues by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* 2001; 755(1-2):165–172. [PubMed: 11393701]
45. Clark TN, White CA, Bartlett MG. Determination of Abacavir in maternal plasma, amniotic fluid, fetal and placental tissues by a polarity switching liquid chromatography/tandem mass spectrometry method. *Rapid Commun. Mass Spectrom.* 2004; 18(4):405–411. [PubMed: 14966847]
46. Pereira AS, Kenney KB, Cohen MS, Eron JJ, Tidwell RR, Dunn JA. Determination of amprenavir, a HIV-1 protease inhibitor, in human seminal plasma using high-performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2002; 766(2):307–317.
47. Clark TN, White CA, Bartlett MG. Determination of didanosine in maternal plasma, amniotic fluid, fetal and placental tissues by high-performance liquid chromatography-tandem mass spectrometry. *Biomed. Chromatogr.* 2006; 20(6-7):605–611. [PubMed: 16779780]
48. Zhong L, Yeh KC. Determination of indinavir in human cerebrospinal fluid and plasma by solid-phase extraction and high-performance liquid chromatography with column switching. *J. Chromatogr. B Biomed. Sci. Appl.* 1999; 734(1):63–71. [PubMed: 10574191]
49. Alnouti Y, White CA, Bartlett MG. Determination of lamivudine in plasma, amniotic fluid, and rat tissues by liquid chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004; 803(2):279–284.
50. Estrela RC, Ribeiro FS, Seixas BV, Suarez-Kurtz G. Determination of lopinavir and ritonavir in blood plasma, seminal plasma, saliva and plasma ultra-filtrate by liquid chromatography/tandem mass spectrometry detection. *Rapid Commun. Mass Spectrom.* 2008; 22(5):657–664. [PubMed: 18257112]
51. DiFrancesco R, Diczienzo R, Vicente G, et al. Determination of lopinavir cerebral spinal fluid and plasma ultrafiltrate concentrations by liquid chromatography coupled to tandem mass

- spectrometry. *J. Pharm. Biomed. Anal.* 2007; 44(5):1139–1146. [PubMed: 17601694] Example article that normalized biofluid (cerebrospinal fluid) results using protein content of the sample.
52. Theron A, Cromarty D, Rheeders M, Viljoen M. Determination of salivary efavirenz by liquid chromatography coupled with tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2010; 878(28):2886–2890.
 53. Hoetelmans RM, Van Essenberg M, Meenhorst PL, Mulder JW, Beijnen JH. Determination of saquinavir in human plasma, saliva, and cerebrospinal fluid by ion-pair high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B Biomed. Sci. Appl.* 1997; 698(1-2): 235–241. [PubMed: 9367213]
 54. Rezk NL, Abdel-Megeed MF, Kashuba A. Development of a highly efficient extraction technique and specific multiplex assay for measuring antiretroviral drug concentrations in breast milk. *Ther. Drug Monit.* 2007; 29(4):429–436. [PubMed: 17667797]
 55. Choi SO, Rezk N, Kim JS, Kashuba AD. Development of an LC-MS method for measuring TNF in human vaginal tissue. *J. Chromatogr. Sci.* 2010; 48(3):219–223. [PubMed: 20223089]
 56. Deremer M, D'ambrosio R, Morse GD. Didanosine measurement by radioimmunoassay. *Antimicrob. Agents Chemother.* 1996; 40(6):1331–1334. [PubMed: 8725995]
 57. Lafeuillade A, Solas C, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Differences in the detection of three HIV-1 protease inhibitors in non-blood compartments: clinical correlations. *HIV Clin. Trials.* 2002; 3(1):27–35. [PubMed: 11819183]
 58. Launay O, Tod M, Louchahi K, et al. Differential diffusions of indinavir and lopinavir in genital secretions of human immunodeficiency virus-infected women. *Antimicrob. Agents Chemother.* 2004; 48(2):632–634. [PubMed: 14742224]
 59. Dumond JB, Reddy YS, Troiani L, et al. Differential extracellular and intracellular concentrations of zidovudine and lamivudine in semen and plasma of HIV-1-infected men. *J. Acquir. Immune Defic. Syndr.* 2008; 48(2):156–162. [PubMed: 18360288]
 60. Yajima T, Juni K, Saneyoshi M, Hasegawa T, Kawaguchi T. Direct transport of 2,3 -didehydro-3 -deoxythymidine (D4T) and its ester derivatives to the cerebrospinal fluid via the nasal mucous membrane in rats. *Biol. Pharm. Bull.* 1998; 21(3):272–277. [PubMed: 9556159]
 61. Lorello G, La Porte C, Pilon R, Zhang G, Karnauchow T, Macpherson P. Discordance in HIV-1 viral loads and antiretroviral drug concentrations comparing semen and blood plasma. *HIV Med.* 2009; 10(9):548–554. [PubMed: 19515092]
 62. Solas C, Lafeuillade A, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* 2003; 47(1):238–243. [PubMed: 12499197] Describes the measurement of ARV concentrations in biofluids and tissues and relates these concentrations to HIV viral changes. The same approach will be followed as eradication and new ARV site-targeted formulations are researched.
 63. Washington CB, Wiltshire HR, Man M, et al. The disposition of saquinavir in normal and P-glycoprotein deficient mice, rats, and in cultured cells. *Drug Metab. Dispos.* 2000; 28(9):1058–1062. [PubMed: 10950849]
 64. Takasawa K, Terasaki T, Suzuki H, Ooie T, Sugiyama Y. Distributed model analysis of 3 -azido-3 -deoxythymidine and 2,3 -dideoxyinosine distribution in brain tissue and cerebrospinal fluid. *J. Pharmacol. Exp. Ther.* 1997; 282(3):1509–1517. [PubMed: 9316866]
 65. Wong SL, Van Belle K, Sawchuk RJ. Distributional transport kinetics of zidovudine between plasma and brain extracellular fluid/cerebrospinal fluid in the rabbit: investigation of the inhibitory effect of probenecid utilizing microdialysis. *J. Pharmacol. Exp. Ther.* 1993; 264(2):899–909. [PubMed: 8437131]
 66. Jordan HL, Pereira AS, Cohen MS, Kashuba AD. Domestic cat model for predicting human nucleoside analogue pharmacokinetics in blood and seminal plasma. *Antimicrob. Agents Chemother.* 2001; 45(7):2173–2176. [PubMed: 11408248]
 67. Hoesterey BL, Galinsky RE, Anderson BD. Dose dependence in the plasma pharmacokinetics and uptake kinetics of 2,3 -dideoxyinosine into brain and cerebrospinal fluid of rats. *Drug Metab. Dispos.* 1991; 19(5):907–912. [PubMed: 1686234]

68. Best BM, Koopmans PP, Letendre SL, et al. Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. *J. Antimicrob. Chemother.* 2011; 66(2):354–357. [PubMed: 21098541]
69. Colebunders R, Hodossy B, Burger D, et al. The effect of highly active antiretroviral treatment on viral load and antiretroviral drug levels in breast milk. *AIDS.* 2005; 19(16):1912–1915. [PubMed: 16227801]
70. Khaliq Y, Gallicano K, Venance S, Kravcik S, Cameron DW. Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and cerebrospinal fluid from patients infected with human immunodeficiency virus. *Clin. Pharmacol. Ther.* 2000; 68(6):637–646. [PubMed: 11180024]
71. Tuntland T, Nosbisch C, Unadkat JD. Effect of pregnancy, mode of administration and neonatal age on the pharmacokinetics of zalcitabine (2', 3'-dideoxycytidine) in the pigtailed macaque (*Macaca nemestrina*). *J. Antimicrob. Chemother.* 1997; 40(5):687–693. [PubMed: 9421317]
72. Cao YJ, Ndovi TT, Parsons TL, Guidos AM, Caffo B, Hendrix CW. Effect of semen sampling frequency on seminal antiretroviral drug concentration. *Clin. Pharmacol. Ther.* 2008; 83(6):848–856. [PubMed: 17914441]
73. Pereira CM, Nosbisch C, Baughman WL, Unadkat JD. Effect of zidovudine on transplacental pharmacokinetics of ddI in the pigtailed macaque (*Macaca nemestrina*). *Antimicrob. Agents Chemother.* 1995; 39(2):343–345. [PubMed: 7726494]
74. Chaudry NI, Eron JJ, Naderer OJ, et al. Effects of formulation and dosing strategy on amprenavir concentrations in the seminal plasma of human immunodeficiency virus type 1-infected men. *Clin. Infect. Dis.* 2002; 35(6):760–762. [PubMed: 12203175]
75. Haas DW, Johnson B, Nicotera J, et al. Effects of ritonavir on indinavir pharmacokinetics in cerebrospinal fluid and plasma. *Antimicrob. Agents Chemother.* 2003; 47(7):2131–2137. [PubMed: 12821458]
76. Antinori A, Perno CF, Giancola ML, et al. Efficacy of cerebrospinal fluid (CSF)-penetrating antiretroviral drugs against HIV in the neurological compartment: different patterns of phenotypic resistance in CSF and plasma. *Clin. Infect. Dis.* 2005; 41(12):1787–1793. [PubMed: 16288405]
77. Karlstrom O, Stahle L, Perrin L, Tegude H, Sonnerborg A. Efficacy of nelfinavir-based treatment in the central nervous system of HIV-1 infected patients. *Scand. J. Infect. Dis.* 2006; 38(5):371–374. [PubMed: 16709540]
78. Gehrig AK, Mikus G, Haefeli WE, Burhenne J. Electrospray tandem mass spectroscopic characterisation of 18 antiretroviral drugs and simultaneous quantification of 12 antiretrovirals in plasma. *Rapid Commun. Mass Spectrom.* 2007; 21(16):2704–2716. [PubMed: 17639576]
79. Hudelson SE, McConnell MS, Bagenda D, et al. Emergence and persistence of nevirapine resistance in breast milk after single-dose nevirapine administration. *AIDS.* 2010; 24(4):557–561. [PubMed: 20057308]
80. Van Praag RM, Weverling GJ, Portegies P, et al. Enhanced penetration of indinavir in cerebrospinal fluid and semen after the addition of low-dose ritonavir. *AIDS.* 2000; 14(9):1187–1194. [PubMed: 10894283]
81. Brewster ME, Anderson WR, Webb AI, et al. Evaluation of a brain-targeting zidovudine chemical delivery system in dogs. *Antimicrob. Agents Chemother.* 1997; 41(1):122–128. [PubMed: 8980767]
82. Rolinski B, Wintergerst U, Matuschke A, et al. Evaluation of saliva as a specimen for monitoring zidovudine therapy in HIV-infected patients. *AIDS.* 1991; 5(7):885–888. [PubMed: 1892595]
83. Aweeka F, Jayewardene A, Staprans S, et al. Failure to detect nelfinavir in the cerebrospinal fluid of HIV-1-infected patients with and without AIDS dementia complex. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 1999; 20(1):39–43. [PubMed: 9928728]
84. Edwards JE, Brouwer KR, Mcnamara PJ. GF120918, a P-glycoprotein modulator, increases the concentration of unbound amprenavir in the central nervous system in rats. *Antimicrob. Agents Chemother.* 2002; 46(7):2284–2286. [PubMed: 12069991]
85. Salado-Rasmussen K, Theilgaard ZP, Chiduo M, Pedersen C, Gerstoft J, Katzenstein TL. Good performance of an immunoassay based method for nevirapine measurements in human breast milk. *Clin. Chem. Lab. Med.* 2011; 49(7):1171–1175. [PubMed: 21504374]

86. Barau C, Delaugerre C, Braun J, et al. High concentration of raltegravir in semen of HIV-infected men: results from a substudy of the EASIER-ANRS 138 trial. *Antimicrob. Agents Chemother.* 2010; 54(2):937–939. [PubMed: 19995925]
87. Burhenne J, Riedel KD, Martin-Facklam M, Mikus G, Haefeli WE. Highly sensitive determination of saquinavir in biological samples using liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2003; 784(2):233–242.
88. Avery LB, Parsons TL, Meyers DJ, Hubbard WC. A highly sensitive ultra performance liquid chromatography–tandem mass spectrometric (UPLC–MS/MS) technique for quantitation of protein free and bound efavirenz (EFV) in human seminal and blood plasma. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2010; 878(31):3217–3224.
89. Hoetelmans RM, Van Essenberg M, Profijt M, Meenhorst PL, Mulder JW, Beijnen JH. High-performance liquid chromatographic determination of ritonavir in human plasma, cerebrospinal fluid and saliva. *J. Chromatogr. B Biomed. Sci. Appl.* 1998; 705(1):119–126. [PubMed: 9498678]
90. Lafeuillade A, Solas C, Chadapaud S, Hittinger G, Poggi C, Lacarelle B. HIV-1 RNA levels, resistance, and drug diffusion in semen versus blood in patients receiving a lopinavir-containing regimen. *J. Acquir. Immune Defic. Syndr.* 2003; 32(4):462–464. [PubMed: 12640207]
91. Mudigonda K, Jukanti R, Apte SS, et al. HPLC quantification of the HIV-1 protease inhibitor saquinavir in brain and testis of mice. *Biomed. Chromatogr.* 2006; 20(10):1028–1032. [PubMed: 16506264]
92. Pav JW, Rowland LS, Korpalski DJ. HPLC–UV method for the quantitation of nevirapine in biological matrices following solid phase extraction. *J. Pharm. Biomed. Anal.* 1999; 20(1-2):91–98. [PubMed: 10704012]
93. Salama NN, Kelly EJ, Bui T, Ho RJ. The impact of pharmacologic and genetic knockout of P-glycoprotein on nelfinavir levels in the brain and other tissues in mice. *J. Pharm. Sci.* 2005; 94(6):1216–1225. [PubMed: 15858856]
94. Namane A, Gouyette C, Fillion MP, Fillion G, Huynh-Dinh T. Improved brain delivery of AZT using a glycosyl phosphotriester prodrug. *J. Med. Chem.* 1992; 35(16):3039–3044. [PubMed: 1501232]
95. Tuntland T, Odinecs A, Pereira CM, Nosbisch C, Unadkat JD. *In vitro* models to predict the *in vivo* mechanism, rate, and extent of placental transfer of dideoxynucleoside drugs against human immunodeficiency virus. *Am. J. Obstet. Gynecol.* 1999; 180(1 Pt 1):198–206. [PubMed: 9914604]
96. Ndesendo VM, Pillay V, Choonara YE, et al. *In vivo* evaluation of the release of zidovudine and polystyrene sulfonate from a dual intravaginal bioadhesive polymeric device in the pig model. *J. Pharm. Sci.* 2011; 100(4):1416–1435. [PubMed: 20960571]
97. Takasawa K, Terasaki T, Suzuki H, Sugiyama Y. *In vivo* evidence for carrier-mediated efflux transport of 3 -azido-3 -deoxythymidine and 2 ,3 -dideoxyinosine across the blood-brain barrier via a probenecid-sensitive transport system. *J. Pharmacol. Exp. Ther.* 1997; 281(1):369–375. [PubMed: 9103519]
98. Odinecs A, Nosbisch C, Keller RD, Baughman WL, Unadkat JD. *In vivo* maternal–fetal pharmacokinetics of stavudine (2 ,3 -didehydro-3 -deoxythymidine) in pigtailed macaques (*Macaca nemestrina*). *Antimicrob. Agents Chemother.* 1996; 40(1):196–202. [PubMed: 8787905]
99. Tuntland T, Odinecs A, Nosbisch C, Unadkat JD. *In vivo* maternal–fetal–amniotic fluid pharmacokinetics of zidovudine in the pigtailed macaque: comparison of steady-state and single-dose regimens. *J. Pharmacol. Exp. Ther.* 1998; 285(1):54–62. [PubMed: 9535994]
100. Chow HH, Li P, Brookshier G, Tang Y. *In vivo* tissue disposition of 3 -azido-3 -deoxythymidine and its anabolites in control and retrovirus-infected mice. *Drug Metab. Dispos.* 1997; 25(4):412–422. [PubMed: 9107539]
101. Poirier MC, Patterson TA, Slikker W Jr, Olivero OA. Incorporation of 3 -azido-3 -deoxythymidine (AZT) into fetal DNA and fetal tissue distribution of drug after infusion of pregnant late-term rhesus macaques with a human-equivalent AZT dose. *J. Acquir. Immune Defic. Syndr.* 1999; 22(5):477–483. [PubMed: 10961609]
102. Stahle L, Martin C, Svensson JO, Sonnerborg A. Indinavir in cerebrospinal fluid of HIV-1-infected patients. *Lancet.* 1997; 350(9094):1823. [PubMed: 9428261]

103. Letendre SL, Capparelli EV, Ellis RJ, Mccutchan JA. Indinavir population pharmacokinetics in plasma and cerebrospinal fluid. The HIV Neurobehavioral Research Center Group. *Antimicrob. Agents Chemother.* 2000; 44(8):2173–2175. [PubMed: 10898694]
104. Martin C, Sonnerborg A, Svensson JO, Stahle L. Indinavir-based treatment of HIV-1 infected patients: efficacy in the central nervous system. *AIDS.* 1999; 13(10):1227–1232. [PubMed: 10416527]
105. Kaddoumi A, Choi SU, Kinman L, et al. Inhibition of P-glycoprotein activity at the primate blood-brain barrier increases the distribution of nelfinavir into the brain but not into the cerebrospinal fluid. *Drug Metab. Dispos.* 2007; 35(9):1459–1462. [PubMed: 17591677]
106. Giri N, Shaik N, Pan G, et al. Investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) on pharmacokinetics and central nervous system penetration of abacavir and zidovudine in the mouse. *Drug Metab. Dispos.* 2008; 36(8):1476–1484. [PubMed: 18443033]
107. Lewis LL, Venzon D, Church J, et al. Lamivudine in children with human immunodeficiency virus infection: a Phase I/II study. The National Cancer Institute Pediatric Branch-Human Immunodeficiency Virus Working Group. *J. Infect. Dis.* 1996; 174(1):16–25. [PubMed: 8655986]
108. Brewer E, Felix T, Clarke P, Edgington A, Muirhead D. An LC–MS/MS method for quantitative determination of maraviroc (UK-427,857) in human plasma, urine and cerebrospinal fluid. *Biomed. Chromatogr.* 2010; 24(12):1316–1323. [PubMed: 21077250]
109. Vergara TR, Estrela RC, Suarez-Kurtz G, Schechter M, Cerbino-Neto J, Barroso PF. Limited penetration of lopinavir and ritonavir in the genital tract of men infected with HIV-1 in Brazil. *Ther. Drug Monit.* 2006; 28(2):175–179. [PubMed: 16628127]
110. Sankatsing SU, Droste J, Burger D, et al. Limited penetration of lopinavir into seminal plasma of HIV-1-infected men. *AIDS.* 2002; 16(12):1698–1700. [PubMed: 12172099]
111. Kinman L, Brodie SJ, Tsai CC, et al. Lipid-drug association enhanced HIV-1 protease inhibitor indinavir localization in lymphoid tissues and viral load reduction: a proof of concept study in HIV-2287-infected macaques. *J. Acquir. Immune Defic. Syndr.* 2003; 34(4):387–397. [PubMed: 14615656]
112. Huang Y, Zurlinden E, Lin E, et al. Liquid chromatographic–tandem mass spectrometric assay for the simultaneous determination of didanosine and stavudine in human plasma, bronchoalveolar lavage fluid, alveolar cells, peripheral blood mononuclear cells, seminal plasma, cerebrospinal fluid and tonsil tissue. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004; 799(1):51–61.
113. Capparelli EV, Holland D, Okamoto C, et al. Lopinavir concentrations in cerebrospinal fluid exceed the 50% inhibitory concentration for HIV. *AIDS.* 2005; 19(9):949–952. [PubMed: 15905676]
114. Isaac A, Taylor S, Cane P, et al. Lopinavir/ritonavir combined with twice-daily 400 mg indinavir: pharmacokinetics and pharmacodynamics in blood, CSF and semen. *J. Antimicrob. Chemother.* 2004; 54(2):498–502. [PubMed: 15254024]
115. Van Zyl GU, Van Mens TE, McIlleron H, et al. Low lopinavir plasma or hair concentrations explain second-line protease inhibitor failures in a resource-limited setting. *J. Acquir. Immune Defic. Syndr.* 2011; 56(4):333–339. [PubMed: 21239995]
116. Desormeaux A, Bergeron MG. Lymphoid tissue targeting of anti-HIV drugs using liposomes. *Meth. Enzymol.* 2005; 391:330–351. [PubMed: 15721390]
117. Zhang L, Price R, Aweeka F, Bellibas SE, Sheiner LB. Making the most of sparse clinical data by using a predictive-model-based analysis, illustrated with a stavudine pharmacokinetic study. *Eur. J. Pharm. Sci.* 2001; 12(4):377–385. [PubMed: 11231104]
118. Dumond JB, Patterson KB, Pecha AL, et al. Maraviroc concentrates in the cervicovaginal fluid and vaginal tissue of HIV-negative women. *J. Acquir. Immune Defic. Syndr.* 2009; 51(5):546–553. [PubMed: 19546811]
119. Tiraboschi JM, Niubo J, Curto J, Podzamczar D. Maraviroc concentrations in cerebrospinal fluid in HIV-infected patients. *J. Acquir. Immune Defic. Syndr.* 2010; 55(5):606–609. [PubMed: 20706127]
120. Huang CS, Boudinot FD, Feldman S. Maternal–fetal pharmacokinetics of zidovudine in rats. *J. Pharm. Sci.* 1996; 85(9):965–970. [PubMed: 8877888]

121. Mandelbrot L, Peytavin G, Firtion G, Farinotti R. Maternal–fetal transfer and amniotic fluid accumulation of lamivudine in human immunodeficiency virus-infected pregnant women. *Am. J. Obstet. Gynecol.* 2001; 184(2):153–158. [PubMed: 11174495]
122. Chappuy H, Treluyer JM, Jullien V, et al. Maternal–fetal transfer and amniotic fluid accumulation of nucleoside analogue reverse transcriptase inhibitors in human immunodeficiency virus-infected pregnant women. *Antimicrob. Agents Chemother.* 2004; 48(11):4332–4336. [PubMed: 15504861]
123. Sadler BM, Chittick GE, Polk RE, et al. Metabolic disposition and pharmacokinetics of [14C]-amprenavir, a human immunodeficiency virus type 1 (HIV-1) protease inhibitor, administered as a single oral dose to healthy male subjects. *J. Clin. Pharmacol.* 2001; 41(4):386–396. [PubMed: 11304895]
124. Yang Z, Huang Y, Gan G, Sawchuk RJ. Microdialysis evaluation of the brain distribution of stavudine following intranasal and intravenous administration to rats. *J. Pharm. Sci.* 2005; 94(7):1577–1588. [PubMed: 15920773]
125. Yang Z, Brundage RC, Barbhuiya RH, Sawchuk RJ. Microdialysis studies of the distribution of stavudine into the central nervous system in the freely-moving rat. *Pharm. Res.* 1997; 14(7):865–872. [PubMed: 9244142]
126. McDowell JA, Lou Y, Symonds WS, Stein DS. Multiple-dose pharmacokinetics and pharmacodynamics of abacavir alone and in combination with zidovudine in human immunodeficiency virus-infected adults. *Antimicrob. Agents Chemother.* 2000; 44(8):2061–2067. [PubMed: 10898676]
127. Seki T, Sato N, Hasegawa T, Kawaguchi T, Juni K. Nasal absorption of zidovudine and its transport to cerebrospinal fluid in rats. *Biol. Pharm. Bull.* 1994; 17(8):1135–1137. [PubMed: 7820125]
128. Yarchoan R, Mitsuya H, Pluda JM, et al. The National Cancer Institute Phase I study of 2,3 - dideoxyinosine administration in adults with AIDS or AIDS-related complex: analysis of activity and toxicity profiles. *Rev. Infect. Dis.* 1990; 12(Suppl. 5):S522–S533. [PubMed: 1974724]
129. Rakhmanina NY, Capparelli EV, Van Den Anker JN, et al. Nevirapine concentration in nonstimulated saliva: an alternative to plasma sampling in children with human immunodeficiency virus infection. *Ther. Drug Monit.* 2007; 29(1):110–117. [PubMed: 17304158]
130. Bennetto-Hood C, Johnson VA, King JR, Hoesley CJ, Acosta EP. Novel methodology for antiretroviral quantitation in the female genital tract. *HIV Clin. Trials.* 2009; 10(3):193–199. [PubMed: 19632958]
131. Pereira AS, Kashuba AD, Fiscus SA, et al. Nucleoside analogues achieve high concentrations in seminal plasma: relationship between drug concentration and virus burden. *J. Infect. Dis.* 1999; 180(6):2039–2043. [PubMed: 10558966]
132. Kinman L, Bui T, Larsen K, et al. Optimization of lipid-indinavir complexes for localization in lymphoid tissues of HIV-infected macaques. *J. Acquir. Immune Defic. Syndr.* 2006; 42(2):155–161. [PubMed: 16760797]
133. Hoetelmans RM, Kraaijeveld CL, Meenhorst PL, et al. Penetration of 3 -amino-3 - deoxythymidine, a cytotoxic metabolite of zidovudine, into the cerebrospinal fluid of HIV-1-infected patients. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 1997; 15(2):131–136. [PubMed: 9241112]
134. Van Leeuwen E, Ter Heine R, Van Der Veen F, Repping S, Beijnen JH, Prins JM. Penetration of atazanavir in seminal plasma of men infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* 2007; 51(1):335–337. [PubMed: 17074793]
135. Cruciani M, Liuzzi G, Chirianni A, et al. Penetration of didanosine in semen of HIV-1-infected men. *J. Antimicrob. Chemother.* 2006; 57(6):1244–1247. [PubMed: 16556633]
136. Taylor S, Reynolds H, Sabin CA, et al. Penetration of efavirenz into the male genital tract: drug concentrations and antiviral activity in semen and blood of HIV-1-infected men. *AIDS.* 2001; 15(15):2051–2053. [PubMed: 11600838]

137. Ghosn J, Chaix ML, Peytavin G, et al. Penetration of enfuvirtide, tenofovir, efavirenz, and protease inhibitors in the genital tract of HIV-1-infected men. *AIDS*. 2004; 18(14):1958–1961. [PubMed: 15353984]
138. Van Praag RM, Van Heeswijk RP, Jurriaans S, Lange JM, Hoetelmans RM, Prins JM. Penetration of the nucleoside analogue abacavir into the genital tract of men infected with human immunodeficiency virus type 1. *Clin. Infect. Dis*. 2001; 33(8):e91–e92. [PubMed: 11565093]
139. Ljungdahl-Stahle E, Guzenda E, Bottiger D, Wahren B, Oberg B, Stahle L. Penetration of zidovudine and 3 -fluoro-3 -deoxythymidine into the brain, muscle tissue, and veins in cynomolgus monkeys: relation to antiviral action. *Antimicrob. Agents Chemother*. 1992; 36(11):2418–2422. [PubMed: 1489185]
140. Burger DM, Kraaijeveld CL, Meenhorst PL, et al. Penetration of zidovudine into the cerebrospinal fluid of patients infected with HIV. *AIDS*. 1993; 7(12):1581–1587. [PubMed: 8286068]
141. Kunz A, Frank M, Mugenyi K, et al. Persistence of nevirapine in breast milk and plasma of mothers and their children after single-dose administration. *J. Antimicrob. Chemother*. 2009; 63(1):170–177. [PubMed: 18974161]
142. Shaik N, Giri N, Pan G, Elmquist WF. P-glycoprotein-mediated active efflux of the anti-HIV1 nucleoside abacavir limits cellular accumulation and brain distribution. *Drug Metab. Dispos*. 2007; 35(11):2076–2085. [PubMed: 17709369]
143. Reddy YS, Gotzkowsky SK, Eron JJ, et al. Pharmacokinetic and pharmacodynamic investigation of efavirenz in the semen and blood of human immunodeficiency virus type 1-infected men. *J. Infect. Dis*. 2002; 186(9):1339–1343. [PubMed: 12402205]
144. Boal JH, Plessinger MA, Van Den Reydt C, Miller RK. Pharmacokinetic and toxicity studies of AZT (zidovudine) following perfusion of human term placenta for 14 hours. *Toxicol. Appl. Pharmacol*. 1997; 143(1):13–21. [PubMed: 9073587]
145. Hirt D, Urien S, Jullien V, et al. Pharmacokinetic modelling of the placental transfer of nelfinavir and its M8 metabolite: a population study using 75 maternal-cord plasma samples. *Br. J. Clin. Pharmacol*. 2007; 64(5):634–644. [PubMed: 17892516]
146. Van Praag RM, Repping S, De Vries JW, Lange JM, Hoetelmans RM, Prins JM. Pharmacokinetic profiles of nevirapine and indinavir in various fractions of seminal plasma. *Antimicrob. Agents Chemother*. 2001; 45(10):2902–2907. [PubMed: 11557488]
147. Moodley J, Moodley D, Pillay K, et al. Pharmacokinetics and antiretroviral activity of lamivudine alone or when coadministered with zidovudine in human immunodeficiency virus type 1-infected pregnant women and their offspring. *J. Infect. Dis*. 1998; 178(5):1327–1333. [PubMed: 9780252]
148. Borg N, Stahle L. Pharmacokinetics and distribution over the blood-brain barrier of zalcitabine (2,3 -dideoxycytidine) and BEA005 (2,3 -dideoxy-3 -hydroxy-methylcytidine) in rats, studied by microdialysis. *Antimicrob. Agents Chemother*. 1998; 42(9):2174–2177. [PubMed: 9736530]
149. Schinazi RF, Boudinot FD, Ibrahim SS, Manning C, Mcclure HM, Liotta DC. Pharmacokinetics and metabolism of racemic 2,3 -dideoxy-5-fluoro-3 -thiacytidine in rhesus monkeys. *Antimicrob. Agents Chemother*. 1992; 36(11):2432–2438. [PubMed: 1336946]
150. Jin SX, Bi DZ, Wang J, Wang YZ, Hu HG, Deng YH. Pharmacokinetics and tissue distribution of zidovudine in rats following intravenous administration of zidovudine myristate loaded liposomes. *Pharmazie*. 2005; 60(11):840–843. [PubMed: 16320946]
151. Hartman NR, Yarchoan R, Pluda JM, et al. Pharmacokinetics of 2,3 -dideoxyadenosine and 2,3 -dideoxyinosine in patients with severe human immunodeficiency virus infection. *Clin. Pharmacol. Ther*. 1990; 47(5):647–654. [PubMed: 2111751]
152. Pereira AS, Smeaton LM, Gerber JG, et al. The pharmacokinetics of amprenavir, zidovudine, and lamivudine in the genital tracts of men infected with human immunodeficiency virus type 1 (AIDS clinical trials group study 850). *J. Infect. Dis*. 2002; 186(2):198–204. [PubMed: 12134255]
153. Hawkins ME, Mitsuya H, McCully CM, et al. Pharmacokinetics of dideoxypurine nucleoside analogs in plasma and cerebrospinal fluid of rhesus monkeys. *Antimicrob. Agents Chemother*. 1995; 39(6):1259–1264. [PubMed: 7574512]

154. Sugioka N, Haraya K, Maeda Y, Fukushima K, Takada K. Pharmacokinetics of human immunodeficiency virus protease inhibitor, nelfinavir, in poloxamer 407-induced hyperlipidemic model rats. *Biol. Pharm. Bull.* 2009; 32(2):269–275. [PubMed: 19182388]
155. Brown SD, Bartlett MG, White CA. Pharmacokinetics of intravenous acyclovir, zidovudine, and acyclovir-zidovudine in pregnant rats. *Antimicrob. Agents Chemother.* 2003; 47(3):991–996. [PubMed: 12604532]
156. Blaney SM, Daniel MJ, Harker AJ, Godwin K, Balis FM. Pharmacokinetics of lamivudine and BCH-189 in plasma and cerebrospinal fluid of nonhuman primates. *Antimicrob. Agents Chemother.* 1995; 39(12):2779–2782. [PubMed: 8593019]
157. Mirochnick M, Fenton T, Gagnier P, et al. Pharmacokinetics of nevirapine in human immunodeficiency virus type 1-infected pregnant women and their neonates. *Pediatric AIDS Clinical Trials Group Protocol 250 Team. J. Infect. Dis.* 1998; 178(2):368–374. [PubMed: 9697716]
158. Van Rompay KK, Hamilton M, Kearney B, Bischofberger N. Pharmacokinetics of tenofovir in breast milk of lactating rhesus macaques. *Antimicrob. Agents Chemother.* 2005; 49(5):2093–2094. [PubMed: 15855535]
159. Balis FM, Pizzo PA, Murphy RF, et al. The pharmacokinetics of zidovudine administered by continuous infusion in children. *Ann. Intern. Med.* 1989; 110(4):279–285. [PubMed: 2643914]
160. Kline MW, Dunkle LM, Church JA, et al. A Phase I/II evaluation of stavudine (d4T) in children with human immunodeficiency virus infection. *Pediatrics.* 1995; 96(2 Pt 1):247–252. [PubMed: 7630678]
161. Musoke P, Guay LA, Bagenda D, et al. A Phase I/II study of the safety and pharmacokinetics of nevirapine in HIV-1-infected pregnant Ugandan women and their neonates (HIVNET 006). *AIDS.* 1999; 13(4):479–486. [PubMed: 10197376]
162. Gingelmaier A, Kurowski M, Kastner R, et al. Placental transfer and pharmacokinetics of lopinavir and other protease inhibitors in combination with nevirapine at delivery. *AIDS.* 2006; 20(13):1737–1743. [PubMed: 16931938]
163. Siu SS, Yeung JH, Pang MW, Chiu PY, Lau TK. Placental transfer of Zidovudine in first trimester of pregnancy. *Obstet. Gynecol.* 2005; 106(4):824–827. [PubMed: 16199642]
164. Moyle GJ, Sadler M, Buss N. Plasma and cerebrospinal fluid saquinavir concentrations in patients receiving combination antiretroviral therapy. *Clin. Infect. Dis.* 1999; 28(2):403–404. [PubMed: 10064262]
165. Zhou XJ, Havlir DV, Richman DD, et al. Plasma population pharmacokinetics and penetration into cerebrospinal fluid of indinavir in combination with zidovudine and lamivudine in HIV-1-infected patients. *AIDS.* 2000; 14(18):2869–2876. [PubMed: 11153668]
166. Ved PM, Kim K. Poly(ethylene oxide/propylene oxide) copolymer thermo-reversible gelling system for the enhancement of intranasal zidovudine delivery to the brain. *Int. J. Pharm.* 2011; 411(1-2):1–9. [PubMed: 21356294]
167. Taylor S, Back DJ, Workman J, et al. Poor penetration of the male genital tract by HIV-1 protease inhibitors. *AIDS.* 1999; 13(7):859–860. [PubMed: 10357387]
168. Walker DK, Bowers SJ, Mitchell RJ, Potchoiba MJ, Schroeder CM, Small HF. Preclinical assessment of the distribution of maraviroc to potential human immunodeficiency virus (HIV) sanctuary sites in the central nervous system (CNS) and gut-associated lymphoid tissue (GALT). *Xenobiotica.* 2008; 38(10):1330–1339. [PubMed: 18853388]
169. Galinsky RE, Flaharty KK, Hoesterey BL, Anderson BD. Probenecid enhances central nervous system uptake of 2',3'-dideoxyinosine by inhibiting cerebrospinal fluid efflux. *J. Pharmacol. Exp. Ther.* 1991; 257(3):972–978. [PubMed: 1904495]
170. Talameh JA, Rezk NL, Kashuba AD. Quantifying the HIV-1 integrase inhibitor raltegravir in female genital tract secretions using high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2010; 878(1):92–96.
171. Hoetelmans RM, Profijt M, Mennhorst PL, Mulder JW, Beijnen JH. Quantitative determination of (–)-2'-deoxy-3'-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B Biomed. Sci. Appl.* 1998; 713(2):387–394. [PubMed: 9746254]

172. Huang CS, Boudinot FD, Feldman S. Radioimmunoassay for zidovudine in rat placenta and fetus. *J. Pharm. Biomed. Anal.* 1996; 14(7):855–860. [PubMed: 8809710]
173. Yilmaz A, Gisslen M, Spudich S, et al. Raltegravir cerebrospinal fluid concentrations in HIV-1 infection. *PLoS ONE*. 2009; 4(9):e6877. [PubMed: 19721718]
174. Clavel C, Peytavin G, Tubiana R, et al. Raltegravir concentrations in the genital tract of HIV-1-infected women treated with a raltegravir-containing regimen (DIVA 01 study). *Antimicrob. Agents Chemother.* 2011; 55(6):3018–3021. [PubMed: 21444705]
175. Calcagno A, Bonora S, D'avolio A, et al. Raltegravir penetration in seminal plasma of healthy volunteers. *Antimicrob. Agents Chemother.* 2010; 54(6):2744–2745. [PubMed: 20308369]
176. Calcagno A, Bonora S, Bertucci R, Lucchini A, D'avolio A, Di Perri G. Raltegravir penetration in the cerebrospinal fluid of HIV-positive patients. *AIDS*. 2010; 24(6):931–932. [PubMed: 20234194]
177. Keller MJ, Madan RP, Torres NM, et al. A randomized trial to assess anti-HIV activity in female genital tract secretions and soluble mucosal immunity following application of 1% tenofovir gel. *PLoS ONE*. 2011; 6(1):e16475. [PubMed: 21283552]
178. Svensson JO, Sonnerborg A, Stahle L. Rapid and simple determination of indinavir in serum, urine, and cerebrospinal fluid using high-performance liquid chromatography. *Ther. Drug Monit.* 2000; 22(5):626–629. [PubMed: 11034271]
179. Bernard L, Vuagnat A, Peytavin G, et al. Relationship between levels of indinavir in hair and virologic response to highly active antiretroviral therapy. *Ann. Intern. Med.* 2002; 137(8):656–659. [PubMed: 12379065]
180. Vernazza P, Daneel S, Schiffer V, et al. The role of compartment penetration in PI-monotherapy: the Atazanavir-Ritonavir Monomaintenance (ATARITMO) trial. *AIDS*. 2007; 21(10):1309–1315. [PubMed: 17545707]
181. Hugen PW, Burger DM, De Graaff M, et al. Saliva as a specimen for monitoring compliance but not for predicting plasma concentrations in patients with HIV treated with indinavir. *Ther. Drug Monit.* 2000; 22(4):437–445. [PubMed: 10942185]
182. Anderson PL, Noormohamed SE, Henry K, Brundage RC, Balfour HH Jr, Fletcher CV. Semen and serum pharmacokinetics of zidovudine and zidovudine-glucuronide in men with HIV-1 infection. *Pharmacotherapy*. 2000; 20(8):917–922. [PubMed: 10939552]
183. Lowe SH, Van Leeuwen E, Droste JA, et al. Semen quality and drug concentrations in seminal plasma of patients using a didanosine or didanosine plus tenofovir containing antiretroviral regimen. *Ther. Drug Monit.* 2007; 29(5):566–570. [PubMed: 17898645]
184. Sparidans RW, Hoetelmans RM, Beijnen JH. Sensitive liquid chromatographic assay for amprenavir, a human immunodeficiency virus protease inhibitor, in human plasma, cerebrospinal fluid and semen. *J. Chromatogr. B Biomed. Sci. Appl.* 2000; 742(1):185–192. [PubMed: 10892597]
185. Mueller BU, Lewis LL, Yuen GJ, et al. Serum and cerebrospinal fluid pharmacokinetics of intravenous and oral lamivudine in human immunodeficiency virus-infected children. *Antimicrob. Agents Chemother.* 1998; 42(12):3187–3192. [PubMed: 9835513]
186. Hamidi M. Simple and sensitive high-performance liquid chromatography method for the quantitation of indinavir in rat plasma and central nervous system. *J. Sep. Sci.* 2006; 29(5):620–627. [PubMed: 16605079]
187. Lewis SR, White CA, Bartlett MG. Simultaneous determination of abacavir and zidovudine from rat tissues using HPLC with ultraviolet detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2007; 850(1-2):45–52.
188. Pereira AS, Kenney KB, Cohen MS, et al. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.* 2000; 742(1):173–183. [PubMed: 10892596]
189. Alnouti Y, White CA, Bartlett MG. Simultaneous determination of zidovudine and lamivudine from rat plasma, amniotic fluid and tissues by HPLC. *Biomed. Chromatogr.* 2004; 18(9):641–647. [PubMed: 15386504]

190. Alnouti Y, Lewis SR, White CA, Bartlett MG. Simultaneous determination of zidovudine and lamivudine from rat tissues by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 2005; 19(4):503–508. [PubMed: 15678520]
191. Alnouti Y, White CA, Bartlett MG. Simultaneous quantitation of zidovudine and zidovudine monophosphate from plasma, amniotic fluid and tissues by micellar capillary electrophoresis. *Biomed. Chromatogr.* 2004; 18(8):523–531. [PubMed: 15386521]
192. Brown KC, Patterson KB, Malone SA, et al. Single and multiple dose pharmacokinetics of maraviroc in saliva, semen, and rectal tissue of healthy HIV-negative men. *J. Infect. Dis.* 2011; 203(10):1484–1490. [PubMed: 21502084]
193. Haworth SJ, Christofalo B, Anderson RD, Dunkle LM. A single-dose study to assess the penetration of stavudine into human cerebrospinal fluid in adults. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 1998; 17(3):235–238. [PubMed: 9495223]
194. Van Praag RM, Van Weert EC, Van Heeswijk RP, et al. Stable concentrations of zidovudine, stavudine, lamivudine, abacavir, and nevirapine in serum and cerebrospinal fluid during 2 years of therapy. *Antimicrob. Agents Chemother.* 2002; 46(3):896–899. [PubMed: 11850283]
195. Brady KA, Boston RC, Aldrich JL, Macgregor RR. Stavudine entry into cerebrospinal fluid after single and multiple doses in patients infected with human immunodeficiency virus. *Pharmacotherapy.* 2005; 25(1):10–17. [PubMed: 15767215]
196. Haas DW, Stone J, Clough LA, et al. Steady-state pharmacokinetics of indinavir in cerebrospinal fluid and plasma among adults with human immunodeficiency virus type 1 infection. *Clin. Pharmacol. Ther.* 2000; 68(4):367–374. [PubMed: 11061576]
197. Rezk NL, White N, Bridges AS, et al. Studies on antiretroviral drug concentrations in breast milk: validation of a liquid chromatography–tandem mass spectrometric method for the determination of 7 anti-human immunodeficiency virus medications. *Ther. Drug Monit.* 2008; 30(5):611–619. [PubMed: 18758393]
198. L’homme RF, Muro EP, Droste JA, et al. Therapeutic drug monitoring of nevirapine in resource-limited settings. *Clin. Infect. Dis.* 2008; 47(10):1339–1344. [PubMed: 18922070]
199. Van Lelyveld SF, Nijhuis M, Baatz F, et al. Therapy failure following selection of enfuvirtide-resistant HIV-1 in cerebrospinal fluid. *Clin. Infect. Dis.* 2010; 50(3):387–390. [PubMed: 20047481]
200. Pereira De Oliveira M, Garcion E, Venisse N, Benoit JP, Couet W, Olivier JC. Tissue distribution of indinavir administered as solid lipid nanocapsule formulation in *mdr1a* (+/+) and *mdr1a* (–/–) CF-1 mice. *Pharm. Res.* 2005; 22(11):1898–1905. [PubMed: 16132350]
201. Else LJ, Lyons F, O’shea S, et al. Total and unbound lopinavir concentrations in the female genital tract of HIV-1 infected women during pregnancy. *AIDS.* 2011; 25(5):722–725. [PubMed: 21368593]
202. Croteau D, Letendre S, Best BM, et al. Total raltegravir concentrations in cerebrospinal fluid exceed the 50-percent inhibitory concentration for wild-type HIV-1. *Antimicrob. Agents Chemother.* 2010; 54(12):5156–5160. [PubMed: 20876368]
203. Patterson TA, Binienda ZK, Newport GD, et al. Transplacental pharmacokinetics and fetal distribution of 2,3-didehydro-3-deoxythymidine (d4T) and its metabolites in late-term rhesus macaques. *Teratology.* 2000; 62(2):93–99. [PubMed: 10931506]
204. Patterson TA, Binienda ZK, Lipe GW, Gillam MP, Slikker W Jr, Sandberg JA. Transplacental pharmacokinetics and fetal distribution of azidothymidine, its glucuronide, and phosphorylated metabolites in late-term rhesus macaques after maternal infusion. *Drug Metab. Dispos.* 1997; 25(4):453–459. [PubMed: 9107545]
205. Pereira CM, Nobsch C, Winter HR, Baughman WL, Unadkat JD. Transplacental pharmacokinetics of dideoxyinosine in pigtailed macaques. *Antimicrob. Agents Chemother.* 1994; 38(4):781–86. [PubMed: 8031046]
206. Anderson BD, Hoesterey BL, Baker DC, Galinsky RE. Uptake kinetics of 2,3-dideoxyinosine into brain and cerebrospinal fluid of rats: intravenous infusion studies. *J. Pharmacol. Exp. Ther.* 1990; 253(1):113–118. [PubMed: 2109797]

207. Vuong Le T, Ruckle JL, Blood AB, et al. Use of accelerator mass spectrometry to measure the pharmacokinetics and peripheral blood mononuclear cell concentrations of zidovudine. *J. Pharm. Sci.* 2008; 97(7):2833–2843. [PubMed: 17854048]
208. Akula SK, Rege AB, Dreisbach AW, DeJace PM, Lertora JJ. Valproic acid increases cerebrospinal fluid zidovudine levels in a patient with AIDS. *Am. J. Med. Sci.* 1997; 313(4):244–246. [PubMed: 9099156]
209. Fox E, Bungay PM, Bacher J, McCully CL, Dedrick RL, Balis FM. Zidovudine concentration in brain extracellular fluid measured by microdialysis: steady-state and transient results in rhesus monkey. *J. Pharmacol. Exp. Ther.* 2002; 301(3):1003–1011. [PubMed: 12023531]
210. Odinecs A, Nosbisch C, Unadkat JD. Zidovudine does not affect transplacental transfer or systemic clearance of stavudine (2',3'-didehydro-3'-deoxythymidine) in the pigtailed macaque (*Macaca nemestrina*). *Antimicrob. Agents Chemother.* 1996; 40(6):1569–1571. [PubMed: 8726043]
211. Bennetto-Hood C, Bryson YJ, Stek A, King JR, Mirochnick M, Acosta EP. Zidovudine, lamivudine, and nelfinavir concentrations in amniotic fluid and maternal serum. *HIV Clin. Trials.* 2009; 10(1):41–47. [PubMed: 19362995]
212. Yeh RF, Rezk NL, Kashuba AD, et al. Genital tract, cord blood, and amniotic fluid exposures of seven antiretroviral drugs during and after pregnancy in human immunodeficiency virus type 1-infected women. *Antimicrob. Agents Chemother.* 2009; 53(6):2367–2374. [PubMed: 19307360]
213. Lowe SH, Wensing AM, Droste JA, et al. No virological failure in semen during properly suppressive antiretroviral therapy despite subtherapeutic local drug concentrations. *HIV Clin. Trials.* 2006; 7(6):285–290. [PubMed: 17197376] Excellent review of male and female genital tract PKs with some method information.
214. Else LJ, Taylor S, Back DJ, Khoo SH. Pharmacokinetics of antiretroviral drugs in anatomical sanctuary sites: the male and female genital tract. *Antivir. Ther.* 2011; 16(8):1149–1167. [PubMed: 22155899]
215. Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. *Sci. Transl. Med.* 2011; 3(112):112re4.
216. Schwartz JL, Rountree W, Kashuba AD, et al. A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. *PLoS ONE.* 2011; 6(10):e25974. [PubMed: 22039430]
217. Nuttall J, Kashuba A, Wang R, et al. Pharmacokinetics of tenofovir following intravaginal and intrarectal administration of tenofovir gel to rhesus macaques. *Antimicrob. Agents Chemother.* 2012; 56(1):103–109. [PubMed: 21986823]
218. Beigi R, Noguchi L, Parsons T, et al. Pharmacokinetics and placental transfer of single-dose tenofovir 1% vaginal gel in term pregnancy. *J. Infect. Dis.* 2011; 204(10):1527–1531. [PubMed: 21930612]
219. Dumond JB, Nicol MR, Kendrick RN, et al. Pharmacokinetic modelling of efavirenz, atazanavir, lamivudine and tenofovir in the female genital tract of HIV-infected pre-menopausal women. *Clin. Pharmacokinet.* 2012; 51(12):809–822. [PubMed: 23044523]
220. Anton PA, Cranston RD, Kashuba A, et al. RMP-02/MTN-006: a phase 1 rectal safety, acceptability, pharmacokinetic, and pharmacodynamic study of tenofovir 1% gel compared with oral tenofovir disoproxil fumarate. *AIDS Res. Hum. Retroviruses.* 2012; 28(11):1412–1421. [PubMed: 22943559]
221. Tiraboschi JM, Niubo J, Ferrer E, et al. Etravirine concentrations in seminal plasma in HIV-infected patients. *J. Antimicrob. Chemother.* 2013; 68(1):184–187. [PubMed: 22954493]
222. Palombi L, Pirillo MF, Andreotti M, et al. Antiretroviral prophylaxis for breastfeeding transmission in Malawi: drug concentrations, virological efficacy and safety. *Antivir. Ther.* 2012; 17(8):1511–1519. [PubMed: 22910456]
223. Nirogi R, Bhyrapuneni G, Kandikere V, et al. Pharmacokinetic profiling of efavirenz-emtricitabine-tenofovir fixed dose combination in pregnant and non-pregnant rats. *Biopharm. Drug Dispos.* 2012; 33(5):265–277. [PubMed: 22610784]

224. Tiraboschi JM, Niubo J, Vila A, Perez-Pujol S, Podzamczar D. Etravirine concentrations in CSF in HIV-infected patients. *J. Antimicrob. Chemother.* 2012; 67(6):1446–1448. [PubMed: 22357802]
225. Croteau D, Letendre S, Best BM, et al. Therapeutic amprenavir concentrations in cerebrospinal fluid. *Antimicrob. Agents Chemother.* 2012; 56(4):1985–1989. [PubMed: 22290964]
226. Moss JA, Baum MM, Malone AM, et al. Tenofovir and tenofovir disoproxil fumarate pharmacokinetics from intravaginal rings. *AIDS.* 2012; 26(6):707–710. [PubMed: 22210639]
227. Clark MR, Johnson TJ, McCabe RT, et al. A hot-melt extruded intravaginal ring for the sustained delivery of the antiretroviral microbicide UC781. *J. Pharm. Sci.* 2012; 101(2):576–587. [PubMed: 21976110]
228. Brown KC, Patterson KB, Jennings SH, et al. Single- and multiple-dose pharmacokinetics of darunavir plus ritonavir and etravirine in semen and rectal tissue of HIV-negative men. *J. Acquir. Immune Defic. Syndr.* 2012; 61(2):138–144. [PubMed: 22614898]

Executive summary

Standardized specimen collection, handling and storage for biofluids and tissue to be analyzed for antiretrovirals (ARVs) is warranted. Due to the nature of these specimens, stability from collection to analysis for each ARV of interest is needed. Intra-individual variation ought to be characterized.

Bioanalytical methods used to quantitate ARV in biofluids and tissues should be fully validated if concentration results are to be applied as outcomes or to future research.

Characterization of results should be strongly considered in order to reduce or explain some of the variation seen in data within and between individuals and/or specimen types.

Publications Reporting Arv Concentrations Should Provide Key Information For The Robustness Of The Collections, Handling And Storage Of Specimens, The BioanaLysis Method(S) And The Interpretation Of The Concentration Data.

All of these recommendations underscore the importance of sharing scientific information to advance the decline and ultimate termination of HIV infections more rapidly.

Figure 1. Number of biofluids and tissues methods shown by areas of HIV research
(A) Hair and saliva as antiretroviral treatment adherence specimens. **(B)** CSF and internal tissue as eradication specimens. **(C)** CVF and semen/seminal plasma as prevention specimens. **(D)** Fetal and placental tissues, amniotic fluid, and breast milk as maternal-to-child transmission. 3TC: Lamivudine; ABC: Abacavir; APV: Amprenavir; ATV: Atazanavir; CSF: Cerebrospinal fluid; CVF: Cervicovaginal fluid; D4T: Didanosine; DDC: Dideoxycytidine; DDI: Didanosine; DRV: Darunavir; ECF: Extracellular cerebral fluid; EFV: Efavirenz; ETR: Etravirine; FTC: Emtricitabine; IDV: Indinavir; LPV: Lopinavir;

MVC: Maraviroc; NFV: Nelfinavir; NVP: Nevirapine; RAL: Raltegravir; RTV: Ritonavir; SQV: Saquinavir; TFV: Tenofovir; TPV: Tipranavir; ZDV: Zidovudine.

Table 1

Summary of methods used to analyze antiretrovirals in biofluids and tissues[†].

Fluid type	Total number of methods	HPLC–UV (n)	HPLC–MS (n)	HPLC–MS/MS (n)	All ligand binding [‡] (n)	Other assay (n)
Amniotic fluid	33	24	1	4	3	1 [§]
Breast milk	19	9	1	5	3	0
Cervicovaginal fluid	13	4	4	4	0	1 [¶]
CSF and ECF	85	50	6	18	9	3 [#]
Saliva	15	6	1	5	0	1
Male seminal plasma/semen	45	21	1	18	1	4 [#]
Total	210	114	14	54	16	10
Tissue type	Total number of methods	HPLC–UV (n)	HPLC–MS (n)	HPLC–MS/MS (n)	Radioimmunoassay (n)	Other assay (n)
Fetal	15	9	0	3	2	1 [§]
Hair	6	2	0	4	0	0
Placenta	12	7	0	3	2	0
Internal	38	24	8	2	1	3 ^{††}
Total	71	42	8	12	5	4

CSF: Cerebrospinal fluid; ECF: Extracellular cerebral fluid.

[†] For specific references see Supplementary Table 1.

[‡] Includes all ligand-binding assays (e.g., radioimmunoassay and enzyme immunoassay).

[§] Capillary electrophoresis–UV.

[¶] UPLC–MS/MS.

[#] HPLC–fluorescence detection, UPLC–MS.

^{††} UPLC evaporative light scattering, fluorescence microscopy, UPLC–MS.

Table 2

Summary of available antiretroviral stability data in biofluid and tissue specimens.

Matrix	Drug(s)	Stability (time)	Storage temperature (°C)	Ref.
Tissues				
Internal tissue: brain	Nevirapine	48 h	Ambient	[186]
Internal tissue: brain, liver	Ritonavir	1 week	−20	[19]
Internal tissue: brain, kidneys, muscle, spleen, liver, heart, lung, lymph node, thymus	Zidovudine, zidovudine phosphorylated metabolites	4 months	−20	[100]
Internal tissue: alveolar cells, tonsil tissue	Didanosine, stavudine	3 months	−80	[112]
Internal tissue: vaginal tissue	Tenofovir	2 months	−70	[55]
Hair	Lopinavir, ritonavir, efavirenz	5 days	4	[5]
		4 months	Ambient	
Fetal tissue	Dideoxycytidine	22 h	Ambient	[43]
	Abacavir	11 h	Ambient	[45]
	Abacavir, zidovudine	Chromatography samples: 24 h	Ambient [†]	[187]
		Three freeze–thaw cycles	Freeze–thaw conditions	
Placental tissue	Dideoxycytidinelamivudine	Chromatography samples: 22 h	Ambient [†]	[43]
		Three freeze–thaw cycles	−20	
	Abacavir	11 h	Ambient	[45]
	Abacavir, zidovudine	Chromotography samples: 24 h	Ambient [†]	[187]
		Three freeze–thaw cycles	−20	
Fluids				
Amniotic fluid	Dideoxycytidine, lamivudine	22 h	Ambient	[43]
		11 h	Ambient	
	Abacavir, zidovudine	Chromatography samples: 24 h	Ambient [†]	[187]
		Three freeze–thaw cycles	Freeze–thaw condition	
Breast milk	Nevirapine	4 h	Ambient	[85]
		Three freeze–thaw cycles	Freeze–thaw at −80	
	Lamivudine, stavudine, zidovudine, nevirapine, nelfinavir, ritonavir, lopinavir	24 h	Ambient	[197]
		72 h	4	
CSF and ECF	Didanosine, stavudine	3 months	−80	[112]

Matrix	Drug(s)	Stability (time)	Storage temperature (°C)	Ref.
	Lopinavir	Test samples: 18h	−70	[51]
		Chromatography samples: 96 h	4	
	Amprenavir	1 h	60	[184]
		24 h	25	
		7 days	2	
		43 days	−20	
	Maraviroc	6 h	Ambient (+ light)	[108]
		Three freeze–thaw cycles	−20	
		Chromatography samples: 79 h	Ambient (+ light)	
Saliva	Nevirapine	17 h	Ambient	[198]
		2 months	−40	
	Efavirenz	48 h	4	[52]
Semen and seminal plasma	Didanosine, stavudine	3 months	−80	[112]
	Lamivudine, zidovudine	18 months	−70	[131]
	Amprenavir	1 h	60	[184]
		24 h	25	
		7 days	2	
		43 days	−20	
	Efavirenz	6 months	−80	[88]
		Three freeze–thaw cycles	−80	
		Three freeze–thaw cycles	−20	

CSF: Cerebrospinal fluid; ECF: Extracellular cerebral fluid.

[†] Assumed since no autosampler temperature was specified.