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TREATMENT STRATEGY AND BIO ANALYTICAL METHODS FOR ESTIMATION OF ANTI-VIRAL DRUGS IN THEIR BIOLOGICAL SAMPLES –A REVIEW

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ABSTRACT

This paper describes treatment strategy for viral infections and the various anti-viral drug estimation methods reported for quantitative estimation in the biological samples of in vivo systems. These methods are said to be used for IVIVC studies and for toxicity studies. The various extraction procedures adopted for avoiding the matrix effect in various methods were described. Quantification of active drug moiety in the presence of the metabolites and other biological products is typical task for the analyst, but in spite of costly extraction procedures, it's mandatory for therapeutic drug monitoring in order to assess the toxicity and to adjust the dose and it's frequency to maintain the therapeutic window and subsequently in the pharmacokinetic profiling.

1. INTRODUCTION

DEFINITION OF ANTIVIRAL DRUGS

Antiviral drugs are a class of medication used specifically for treating viral infections rather than bacterial ones. Most antivirals are used for specific viral infections, while a broad-spectrum antiviral is effective against a wide range of viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development.

TYPES OF VIRAL DISEASES

Respiratory viral diseases: Examples include flu, common cold, respiratory syncytial viral infection, adenovirus infection, parainfluenza virus infection, severe acute respiratory syndrome (SARS).

Treatment: Respiratory viral diseases usually heal on their own. But over-the-counter (OTC) medications, including nasal decongestants, cough suppressants, and pain relievers, can help to reduce symptoms. In addition, Tamiflu, an antiviral drug, is sometimes prescribed if someone is in the very early stages of developing the flu.

Gastrointestinal viral diseases: Examples include norovirus infection, rotavirus infection, some adenovirus infections, astrovirus infection.

Treatment: There aren't any treatments for gastrointestinal viral diseases. In many cases, they resolve on their own within a day or two. In the meantime, drink plenty of fluids to replace those lost from diarrhea or vomiting.

Exanthematous viral disease: Examples include measles, rubella, chicken pox, roseola, fifth disease, small pox, chickungunya viral infection.

Treatment: Treating exanthematous viral diseases focuses on managing symptoms. Fever-reducing medications, such as acetaminophen, can help with some of the more bothersome symptoms. Antiviral drugs, such as acyclovir, may be given for chickenpox or shingles.

Hepatic viral diseases: Examples include hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E.

Treatment: Hepatitis A: Hepatitis A usually doesn't require treatment because it is a short-term illness. Bed rest may be recommended if symptoms cause a great deal of discomfort. If you experience vomiting or diarrhea, follow your doctor's orders for hydration and nutrition. The hepatitis A vaccine is available to prevent this infection. Most children begin vaccination between ages 12 and 18 months. It's a series of two vaccines. Vaccination for hepatitis A is also available for adults and can be combined with the hepatitis B vaccine.

Hepatitis B: Chronic hepatitis B is treated with antiviral medications. This form of treatment can be costly because it must be continued for several months or years.

Treatment for chronic hepatitis B also requires regular medical evaluations and monitoring to determine if the virus is responding to treatment. Hepatitis B can be prevented with vaccination. The CDC recommends hepatitis B vaccinations for all newborns. The series of

three vaccines is typically completed over the first six months of childhood. The vaccine is also recommended for all healthcare and medical personnel.

Hepatitis C: Antiviral medications are used to treat both acute and chronic forms of hepatitis C. People who develop chronic hepatitis C are typically treated with a combination of antiviral drug therapies. They may also need further testing to determine the best form of treatment. People who develop cirrhosis (scarring of the liver) or liver disease as a result of chronic hepatitis C may be candidates for a liver transplant. Currently, there is no vaccination for hepatitis C.

Hepatitis D: No antiviral medications exist for the treatment of hepatitis D at this time. According to a 2013 study, a drug called alpha interferon can be used to treat hepatitis D, but it only shows improvement in about 25 to 30 percent of people. Hepatitis D can be prevented by getting the vaccination for hepatitis B, as infection with hepatitis B is necessary for hepatitis D to develop.

Hepatitis E: Currently, no specific medical therapies are available to treat hepatitis E. Because the infection is often acute, it typically resolves on its own. People with this type of infection are often advised to get adequate rest, drink plenty of fluids, get enough nutrients, and avoid alcohol. However, pregnant women who develop this infection require close monitoring and care.

Cutaneous viral diseases: Examples include warts including genital warts, oral herpes, genital herpes, molluscumcontagiosum.

Hemorrhagic viral diseases: Examples include Ebola, yellow fever, Lassa fever, dengue fever, Marburg hemorrhagic fever, Crimean Congo hemorrhagic fever.

Treatment: There’s no specific treatment for hemorrhagic viral diseases. It is important to stay hydrated if you have a viral hemorrhagic disease. Some people may need intravenous (IV) fluids to maintain electrolyte balance. Supportive care to maintain hydration and electrolyte balance is essential. In some cases, the antiviral drug ribavirin may be given.

Neurologic viral diseases: Examples include polio, viral meningitis, viral encephalitis, rabies.

Treatment: There's no specific treatment for people with mild viral meningitis or encephalitis. Getting plenty of rest, staying hydrated, and taking OTC anti-inflammatories to ease pain or headaches can all help. In some cases, antiviral medication may be prescribed. Polio or severe cases of meningitis or encephalitis may require additional treatment, such as breathing assistance or IV fluids. If an animal that's suspected to have the rabies virus bites you, you'll be given a series of shots to help prevent the rabies virus from infecting you.

2. DESCRIPTION

2.1: ACYCLOVIR AND VALACYCLOVIR

Kanneti, R. et al. reported LC–MS–MS Method for the Simultaneous Quantitation of Acyclovir and Valacyclovir in Human Plasma with the title "An LC–MS–MS Method for the Simultaneous Quantitation of Acyclovir and Valacyclovir in Human Plasma" is described as follows: A simple, sensitive and selective LC–MS–MS method has been developed for the simultaneous determination of acyclovir and valacyclovir in human plasma. Acyclovir and valacyclovir in plasma were concentrated by solid phase extraction and chromatographed on a C18 column using a mobile phase of 0.1% formic acid: methanol (30:70% *v/v*). The method was validated over a linear range of 47–10,255 and 5–1,075 ng mL^{-1} for acyclovir and valacyclovir respectively. The LOQs were 47.6 and 5.0 ng mL^{-1}. The validated method was applied for the quantitation of acyclovir and valacyclovir from plasma samples in a pharmacokinetic study.

2.2: ACYCLOVIR, VALACYCLOVIR AND VAL-VALACYCLOVIR

Dias, C. et al. reported Ocular penetration of acyclovir and its peptide prodrugs valacyclovir and val-valacyclovir following systemic administration in rabbits: An evaluation using ocular microdialysis and LC-MS with the title "Ocular penetration of acyclovir and its peptide prodrugs valacyclovir and val-valacyclovir following systemic administration in rabbits: An evaluation using ocular microdialysis and LC-MS" is described as follows: To investigate the ocular penetration of acyclovir and its prodrugs following systemic administration and to elucidate the mechanism of penetration. Hydrophilic peptide prodrugs of acyclovir were infused intravenously in New Zealand albino rabbits over 45 min at a dose equivalent to 30 mmoles/kg acyclovir. Aqueous and vitreous humor samples were obtained utilizing ocular microdialysis and blood samples were obtained from the mid ear vein using a cannula.

2.3: CIDOFOVIR

Breddemann, A. et al. reported Quantification of cidofovir in human serum by LC-MS/MS for children with the title "Quantification of cidofovir in human serum by LC-MS/MS for children" is described as follows: A new method for the quantification of cidofovir (CDV), an acyclic nucleotide analogue of cytosine with antiviral activity against a broad-spectrum of DNA viruses, in human serum, using high-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has been developed. A strong anion exchange (SAX) solid-phase extraction procedure was applied for the sample preparation. The tandem mass spectrometer was tuned in the multiple reaction monitoring mode to monitor the *m*/*z* 278.1 → 234.9 and the *m*/*z* 288.1 → 133.1 transitions for CDV and the internal standard 9-(2-phosphonylmethoxyethyl) guanine (PMEG), respectively, using negative electrospray ionization. The MS/MS response was linear over the concentration range from 78.125 ng/ml to 10,000 ng/ml, with a lower limit of quantification of 78.125 ng/ml. The intra- and inter-day precisions (relative standard deviation (%)) for CDV were less than 7.8% and the accuracies (% of deviation from nominal level) were within ±12.1% for quality controls. The novel LC–MS/MS method allowed a specific, sensitive and reliable determination of CDV in human serum and was applied to investigate the yet unknown pharmacokinetic properties of CDV in pediatric cancer patient.

2.4: AMANTADINE

2.4.1. DI, Bin et al. reported LC-MS/MS determination of pharmacokinetics and bioavailability of compound paracetamol and amantadine capsules in Chinese healthy volunteers with the title "LC-MS/MS determination of pharmacokinetics and bioavailability of compound paracetamol and amantadine capsules in Chinese healthy volunteers" is described as follows: To establish an accurate and sensitive LC-MS/MS method for the simultaneous determination of paracetamol, amantadinehydrochloride, caffeine, chlorphenamine maleate in human plasma and to evaluate the pharmacokinetics and bioavailability in healthy male volunteers after a single oral dose of compound paracetamol and amantadine capsules. 24 healthy male volunteers were divided into a two-way cross-over design. A single oral dose of a compound paracetamol and amantadine test or reference capsule (each containing paracetamol 250 mg, amantadine hydrochloride 100 mg, caffeine 15 mg and chlorphenamine maleate 2 mg) was given to each volunteer according to an open randomized crossover study. The concentrations of paracetamol, amantadinehydrochloride, caffeine and chlorphenamine maleate in plasma were determined by LC-ESI-MS/MS method

with positive ion SRM detection using clenbuterol hydrochloride as internal standard. The pharmacokinetics and bioavailability were compared.

2.4.2. Abhaysingh Bhadoriya et al. reported Sensitive and rapid determination of amantadine without derivatization in human plasma by LC–MS/MS for a bioequivalence study with the title "Sensitive and rapid determination of amantadine without derivatization in human plasma by LC–MS/MS for a bioequivalence study" is described as follows: A highly sensitive, rapid and rugged liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) method was developed for reliable estimation of amantadine (AMD), an antiviral drug in human plasma. The analyte and internal standard (IS), amantadine-d6 (AMD-d6), were extracted from 200 µL plasma by solid phase extraction on Phenomenex Strata-X-C 33 µ cartridges. Chromatography was performed on Synergi™ Hydro-RP C18 (150 mm × 4.6 mm, 4 µm) analytical column using a mixture of acetonitrile and 10 mM ammonium formate, pH 3.0 (80:20, v/v) as the mobile phase. Detection and quantitation wasdone by multiple reaction monitoring in the positive ionization mode for AMD (m/z 152.1 → 135.1) and IS (m/z 158.0 → 141.1) on a triple quadrupole mass spectrometer. The assay was linear in the concentration range of 0.50–500 ng/mL with correlation coefficient (r2) ≥ 0.9969. The limit of detection of the method was 0.18 ng/mL. The intra-batch and inter-batch precisions were ≤ 5.42% and the accuracy varied from 98.47% to 105.72%. The extraction recovery of amantadine was precise and quantitative in the range of 97.89%–100.28%. IS-normalized matrix factors for amantadine varied from 0.981 to 1.012. The stability of AMD in whole blood and plasma was evaluated under different conditions. The developed method was successfully applied for a bioequivalence study with 100 mg of AMD in 32 healthy volunteers. The reproducibility of the assay was determined by reanalysis of 134 subject samples.

2.5: OSELTAMIVIR

Ajay Gupta et al. reported Simultaneous quantification of prodrugoseltamivir and its metabolite oseltamivircarboxylate in human plasma by LC-MS/MS to support a bioequivalence study with the title "Simultaneous quantification of prodrugoseltamivir and its metabolite oseltamivircarboxylate in human plasma by LC-MS/MS to support a bioequivalence study" is described as follows: A simple, precise and rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the simultaneous determination of oseltamivir and oseltamivircarboxylate, a

neuraminidase inhibitor, using their deuterated analogs as internal standards (ISs). The method involved solid phase extraction of the analytes and ISs from 200Î¼L human plasma with no reconstitution and drying steps. The chromatographic separation was achieved on a Symmetry C18 (100mmÃ—4.6mm, 5Î¼m) column using 10mM ammonium formate and acetonitrile (30:70, v/v) as the mobile phase in a run time of 2.0min. Quantitation of analytes and ISs were done by multiple reaction monitoring on a triple quadrupole mass spectrometer in the positive ionization mode. The linearity of the method was established in the concentration range of 0.5â€“200ng/mL and 2.0â€“800ng/mL for oseltamivir and oseltamivircarboxylate respectively. The mean extraction recovery for oseltamivir (94.4%) and oseltamivircarboxylate (92.7%) from spiked plasma samples was consistent and reproducible. The application of this method was demonstrated by a bioequivalence study in 42 healthy Indian subjects with 75mg oseltamivir phosphate capsules. The assay reproducibility was established by reanalysis of 151 incurred subject samples.

2.6: ZIDOVUDINE

2.6.1. MU, Ling-li et al. reported Determination of zidovudine triphosphate concentrations in human peripheral blood mononuclear cells by SPE-LC-MS/MS with the title "Determination of zidovudine triphosphate concentrations in human peripheral blood mononuclear cells by SPE-LC-MS/MS" is described as follows: To develop a method for the determination of zidovudine triphosphate(ZDV-TP) in human peripheral blood mononuclear cells (hPBMCs) using solid phase extraction(SPE) and liquid chromatography-tandem mass spectroscopy (LC-MS/MS), and apply it to quantitate ZDV-TP concentrations in hPBMCs. Methods: By performing a salt gradient anion exchange SPE, ZDV-TP was isolated from zidovudine (ZDV), zidovudine monophosphate (ZDV-MP), and zidovudine diphosphate(ZDV-DP). Isolated ZDV-TP was dephosphorylated with acid phosphatase to its parent drug from ZDV, which was then desalted by HLB SPE cartridges. The separation of the analytes was achieved on a Phenomenex Gemini-C18 column with 0.2% formic acid solution and methanol as the mobile phase. ZDV was detected by an electrospray ionization tandem mass spectroscopy in the multiple-reaction-monitoring positive mode. The precursor-product ion transit ions monitored were m/z 267.9→126.8 for ZDV and m/z 247.9→120.8 fortinidazole (internal standard).

2.6.2. Prashant S. Devrukhakar et al. reported A stability-indicating LC–MS/MS method for zidovudine: Identification, characterization and toxicity prediction of two major acid

degradation products with the title "A stability-indicating LC–MS/MS method for zidovudine: Identification, characterization and toxicity prediction of two major acid degradation products" is described as follows: Zidovudine (AZT) is a nucleoside analogue reverse transcriptase inhibitor (NRTI), a class of anti-retroviral drug. A stability-indicating assay method for AZT was developed in line with ICH guideline. Successful separation of AZT and its degradation products was achieved by gradient elution mode on reverse phase C18 column using 10 mM ammonium acetate: acetonitrile as the mobile phase at 0.8 mL/min flow rate, 25 µL injection volume, 30 °C column temperature and 285 nm detection wavelength. Two major acid degradation products were identified and characterized by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI/MS/MS) and accurate mass measurements. The probable mechanisms for the formation of degradation products were identified based on a comparison of the fragmentation pattern of the [M + H] + ions of AZT and its degradation products. One of the degradation products, DP-1, was isolated by semi-preparative high-performance liquid chromatography (HPLC) using Waters XBridge Prep C18 (250 mm×10 mm, 5 µm). Degradation products showed higher toxicity compared to the drug in some models assessed by TOPKAT software. The method validation was performed with respect to robustness, specificity, linearity, precision and accuracy as per ICH guideline Q2 (R1).

2.7: ZIDOVUDINE, LAMIVUDINE AND ABACAVIR

Robbins, B. L et al. reported Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid phase extraction and LC-MS/MS with the title "Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid phase extraction and LC-MS/MS" is described as follows: All nucleoside reverse transcriptase inhibitors (NRTI) must first be metabolized to their triphosphate forms in order to be active against HIV. Zidovudine (ZDV), abacavir (ABC) and lamivudine (3TC) have proven to be an efficacious combination. In order simultaneously to measure intracellular levels of the triphosphates (-TP) of ZDV, ABC (carbovir, CBV) and 3TC, either together or individually, we have developed a cartridge-LC–MS/MS method. The quantitation range was 2.5–250 pg/µl for 3TC-TP, 0.1–10.0 pg/µl for ZDV-TP and 0.05–5.00 pg/µl for CBV-TP. This corresponds to 0.1–11.0 pmol 3TC-TP per million cells, 4–375 fmol ZDV-TP per million cells and 2–200 fmol CBV-TP per million cells, extracted from 10

million cells. Patient samples demonstrated measured levels in the middle regions of our standard curves both at pre-dose and 4 h post-dose times.

2.8: DIDANOSINE

Cahours, X. et al. reported Analysis of intracellular didanosinetriphosphate at sub-ppb level using LC-MS/MS with the title "Analysis of intracellular didanosinetriphosphate at sub-ppb level using LC-MS/MS" is described as follows: An analytical procedure has been developed for the analysis of intracellular didanosinetriphosphate (ddATP). An electrospray ionization tandem mass spectrometer (ESI-MS) was interfaced to liquid chromatography (LC) using a mobile phase CH3OH/H2O (25/75) containing 1% formic acid for the analysis of the 5′-triphosphate metabolite of the antiviral didanosine. In this procedure, ddATP was extracted from CEM-T4 cells, isolated using an exchange anion solid phase extraction procedure, enzymatically dephosphorylated and then analyzed by LC-MS/MS within a 1 min run time. The influence of several parameters (electrospray ionization interface, acidic modifiers of the mobile phase) has been studied. A calibration curve was generated and the linear regression analysis yielded a regression coefficient (r2) greater than 0.999. Using LC-MS/MS detection in single reaction monitoring mode (SRM), the limit of quantitation of ddA in CEM-T4 cells was 0.02 ng ml−1. Furthermore, this procedure could be used to perform simultaneous detection of five nucleoside reverse transcriptase inhibitors, such as AZT, 3TC, ddA, ddC and d4T and make LC-MS/MS a method of choice for Therapeutic Drug Monitoring (TDM) in a clinical environment.

2.9: STAVUDINE

Mistri, H. N. et al. reported High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma with the title "High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudineand nevirapine in human plasma" is described as follows: A selective and high throughput liquid chromatography/tandem mass spectrometry (LC–MS/MS) method has been developed and validated to separate, detect and simultaneously quantify lamivudine (3TC), stavudine (d4T) and nevirapine (NVP) in human plasma using metaxalone as internal standard (IS). After solid phase extraction (SPE), the analytes and the IS were chromatographed on a Symmetry C18 (150 mm × 3.9 mm i.d., 5 μm particle size) column using 5 μL injection volume with a run time of 4.5 min. An isocratic mobile phase consisting of 0.5% glacial acetic acid in water:acetonitrile (20:80, v/v) was used to separate all these drugs. The precursor and product

ions of these drugs were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring mode (MRM) without polarity switch. The method was validated over the range of 25–3000 ng/mL for 3TC, 20–2000 ng/mL for d4T and 50–5000 ng/mL for NVP. The absolute recoveries for analytes (≥86%) and IS (98.12%) achieved from spiked plasma samples were consistent and reproducible. Inter-batch and intra-batch precision (%CV) across four validation runs (LLOQ, LQC, MQC and HQC) was less than 10. The accuracy determined at these levels was within ±8% in terms of relative error. The method was successfully applied to a pivotal bioequivalence study of [60 (3TC) + 12 (d4T) + 100 (NVP)] mg dispersible tablets in 60 healthy human subjects under fasting condition.

2.10: LAMIVUDINE

ZHENG et al. reported Establishment of LC-MS/MS Method for Determination of Lamivudine with the title "Establishment of LC-MS/MS Method for Determination of Lamivudine" is described as follows: A sensitive and fast liquid chromatography-tandem mass spectrometry LC-MS/MS method was developed for the determination of lamivudine in human plasma. The separation was performed on a J'sphere ODS C18column. 30% a mixture of methanol and 70% 5 mmol·L-1 ammonium acetate (pH 4.2) was used as mobile phase. Flow rate was 0.2 mL·min-1. Detection was performed on a tandem mass spectrometry in multiple reaction monitoring (MRM) mode. The running time was 4 min.

2.11: LAMIVUDINE AND ABACAVIR TRIPHOSPHATE

Gautam, Nagsen et al. reported Simultaneous quantification of intracellular lamivudine and abacavirtriphosphate metabolites by LC–MS/MS with the title "Simultaneous quantification of intracellular lamivudine and abacavirtriphosphate metabolites by LC–MS/MS" is described as follows: The combination of lamivudine (3TC) and abacavir (ABC) is one of the first lines for HIV therapy. Therefore, a sensitive, selective, accurate, and precise LC–MS/MS method was developed and validated for the simultaneous quantification of 3TC- and ABC-TP metabolites in mouse blood and tissues. Calibration curves were linear over the range of 10–100,000 pg/ml for 3TC-TP and 4–40,000 pg/ml for carbovir-TP (CBV-TP; phosphorylated metabolite of ABC). This corresponds to 2.1–21,322 fmol/106 cells for 3TC-TP and 0.8–8000 fmol/106 cells for CBV-TP. Accuracy and precision were less than 15% for all quality control sample (QCs), and absolute extraction recovery of were >65% for 3TC-TP and >90% for CBV-TP. The method was optimized to ensure stability of TP samples and standards during sample collection, preparation, analysis, and storage conditions. This

method has enhanced sensitivity and requires smaller amounts of blood and tissue samples compared to previous LC–MS/MS methods for 3TC- and CBV-TP quantification. The developed method was successfully applied to characterize the pharmacokinetic profile of TP metabolites in mouse peripheral blood mononuclear cells (PBMCs), spleen, lymph nodes, and liver cells. In addition, another direct, simple, and high-throughput method for the quantification of TP standards was developed and used for the analysis of stability samples.

2.12: LAMIVUDINE, STAVUDINE AND NEVIRAPINE

Mistri, H. N. et al. reported High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma with the title "High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudineand nevirapine in human plasma" is described as follows: A selective and high throughput liquid chromatography/tandem mass spectrometry (LC–MS/MS) method has been developed and validated to separate, detect and simultaneously quantify lamivudine (3TC), stavudine (d4T) and nevirapine (NVP) in human plasma using metaxalone as internal standard (IS). After solid phase extraction (SPE), the analytes and the IS were chromatographed on a Symmetry C18 (150 mm × 3.9 mm i.d., 5 µm particle size) column using 5 µL injection volume with a run time of 4.5 min. An isocratic mobile phase consisting of 0.5% glacial acetic acid in water:acetonitrile (20:80, v/v) was used to separate all these drugs. The precursor and product ions of these drugs were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring mode (MRM) without polarity switch. The method was validated over the range of 25–3000 ng/mL for 3TC, 20–2000 ng/mL for d4T and 50–5000 ng/mL for NVP. The absolute recoveries for analytes (≥86%) and IS (98.12%) achieved from spiked plasma samples were consistent and reproducible. Inter-batch and intra-batch precision (%CV) across four validation runs (LLOQ, LQC, MQC and HQC) was less than 10. The accuracy determined at these levels was within ±8% in terms of relative error. The method was successfully applied to a pivotal bioequivalence study of [60 (3TC) + 12 (d4T) + 100 (NVP)] mg dispersible tablets in 60 healthy human subjects under fasting condition.

2.13: LAMIVUDINE, EMTRICITABINE AND TENOFOVIR:

Waitt, Catriona et al. reported Development, validation and clinical application of a method for the simultaneous quantification of lamivudine, emtricitabine and tenofovir in dried blood and dried breast milk spots using LC–MS/MS withthe title "Development, validation and clinical application of a method for the simultaneous quantification of lamivudine,

emtricitabine and tenofovir in dried blood and dried breast milk spots using LC–MS/MS" is described as follows: DBS and DBMS were prepared from 50 and 30 µL of drug-spiked whole blood and human breast milk, respectively. Following extraction with acetonitrile and water, chromatographic separation utilised a Synergi polar column with a gradient mobile phase program consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Detection and quantification was performed using a TSQ Quantum Ultra triple quadrupole mass spectrometer. The analytical method was used to evaluate NRTI drug levels in HIV-positive nursing mothers-infant pairs.

2.14: LAMIVUDINE, ZIDOVUDINE AND ABACAVIR

Robbins, B. L. et al. reported Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid phase extraction and LC-MS/MS with the title "Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid phase extraction and LC-MS/MS" is described as follows: All nucleoside reverse transcriptase inhibitors (NRTI) must first be metabolized to their triphosphate forms in order to be active against HIV. Zidovudine (ZDV), abacavir (ABC) and lamivudine (3TC) have proven to be an efficacious combination. In order simultaneously to measure intracellular levels of the triphosphates (-TP) of ZDV, ABC (carbovir, CBV) and 3TC, either together or individually, we have developed a cartridge-LC–MS/MS method. The quantitation range was 2.5–250 pg/µl for 3TC-TP, 0.1–10.0 pg/µl for ZDV-TP and 0.05–5.00 pg/µl for CBV-TP. This corresponds to 0.1–11.0 pmol 3TC-TP per million cells, 4–375 fmol ZDV-TP per million cells and 2–200 fmol CBV-TP per million cells, extracted from 10 million cells. Patient samples demonstrated measured levels in the middle regions of our standard curves both at pre-dose and 4 h post-dose times.

2.15: ABACAVIR

Rao, R. N et al. reported Separation and characterization of forced degradation products of abacavirsulphate by LC-MS/MS with the title "Separation and characterization of forced degradation products ofabacavirsulphate by LC-MS/MS" is described as follows: Abacavirsulphate was subjected to forced degradation under the conditions of hydrolysis (acid, alkali and neutral), oxidation, photolysis and thermal stress as prescribed by ICH. Eight degradation products were formed and their separation was accomplished on Waters XTerra

C18 (250 mm × 4.6 mm, 5 µm) column using 20 mM ammonium acetate:acetonitrile as a mobile phase in gradient elution mode by LC. The degradation products were characterized by LC–MS/MS and its fragmentation pathways were proposed.

2.16: ABACAVIR AND LAMIVUDINE

Gomes, N. A et al. reported LC–MS–MS Method for Simultaneous Analysis of Abacavir and Lamivudine in Human Plasma, and Its Application to a Bioequivalence Study with the title "LC–MS–MS Method for Simultaneous Analysis of Abacavir and Lamivudine in Human Plasma, and Its Application to a Bioequivalence Study" is described as follows: A rapid and sensitive LC–MS–MS method has been developed and validated for simultaneous analysis of abacavir (ABA) and lamivudine (LAM) in human plasma. The analytes were extracted from human plasma by SPE. Nelfinavir (NEL) and emtricitabine (EMT) were used as the internal standards for ABA and LAM, respectively. An RP18 column enabled chromatographic separation of the analytes. The method involves simple isocratic chromatography and MS detection in positive-ionization mode. Validation of the method showed response was a linear function of concentration in the ranges 100.0–7000.0 ng mL−1 for ABA and 80.0–5000.0 ng mL−1 for LAM. At the LOQ levels, inter-run and intra-run precision were within 5.80 and 3.51%, respectively, for ABA and within 4.68 and 3.16%, respectively, for LAM. Overall recovery for ABA and LAM was 59.32 and 105.18%, respectively. Total elution time was 2 min only, which enabled quantification of more than 200 plasma samples per day. This validated method was used successfully for analysis of plasma samples from a bioequivalence study.

2.17: TENOFOVIR

Delahunty, T et al. reported Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS with the title "Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS" is described as follows: An LC/MS/MS assay for the determination of tenofovir (TNF) was developed and validated for use with the EDTA anticoagulated human plasma matrix. Heparin-treated plasma and serum matrices were also validated. After addition of adefovir as an internal standard, trifluoroacetic acid was used to produce a protein-free extract. Chromatographic separation was achieved with a Polar-RP Synergi, 2.0 mm × 150 mm, reversed-phase analytical column. The mobile phase was 3% acetonitrile/1% acetic acid, aq. Detection of TNF and the internal standard was achieved by ESI MS/MS in the positive ion mode using 288/176 and 274/162 transitions, respectively.

The method was linear from 10 to 750 ng/ml with a minimum quantifiable limit of 10 ng/ml when 250 µl aliquots were analyzed. The usefulness of this LC/MS/MS method to routinely monitor plasma concentrations of TNF was demonstrated along with its ability to assist in the performance of pharmacokinetic studies.

2.18: TENOFOVIR AND EMTRICITABINE

Zheng, J. H. et al. reported Quantitation of tenofovir and emtricitabine in dried blood spots (DBS) with LC-MS/MS with the title "Quantitation of tenofovir and emtricitabine in dried blood spots (DBS) with LC-MS/MS" is described as follows: A reversed-phase high performance liquid chromatographic (LC), tandem mass spectrometry (MS/MS) assay for the determination of tenofovir (TFV) and emtricitabine (FTC) in dried blood spots (DBS) from human whole blood was developed and validated. Whole blood samples were spotted, dried, and a 3 mm punch was extracted with methanol for analysis by LC–MS/MS utilizing stable isotope labeled internal standards. The assay was validated over the range of 2.5–1000 ng/mL for TFV and 2.5–5000 ng/mL for FTC. The method was accurate (within ±15% of control) and precise (coefficient of variation ≤15%) for hematocrit concentrations ranging from 25% to 76%; using edge punches vs. center punches; and spot volumes of 10–50 µL. Analytes were stable for five freeze/thaw cycles and up to 6 days at room temperature, whereas long-term storage required −20 °C or −80 °C. Comparison of TFV and FTC in DBS vs. plasma yielded r2 ≥ 0.96, indicating that DBS can be used as a plasma alternative for pharmacokinetic analyses in vivo.

2.19: TENOFOVIR, EMTRICITABINE, RILPIVIRINE, ELVITEGRAVIR AND DOLUTEGRAVIR

Prathipati et al. reported Simultaneous quantification of tenofovir, emtricitabine, rilpivirine, elvitegravir and dolutegravir in mouse biological matrices by LC–MS/MS and its application to a pharmacokinetic study with the title "Simultaneous quantification of tenofovir, emtricitabine, rilpivirine, elvitegravir and dolutegravir in mouse biological matrices by LC–MS/MS and its application to a pharmacokinetic study" is described as follows:A precise method is required to quantify the drug concentration in biological matrices to study pharmacokinetic behavior and tissue distribution profile in animals and/or humans. We have developed and validated a sensitive and precise liquid chromatography–tandem mass spectrometry method for simultaneous quantification of selected antiretroviral drugs, tenofovir (TNF), emtricitabine (FTC), rilpivirine (RPV), dolutegravir (DTG) and elvitegravir

(EVG) in mouse biological matrices. This method involves a solid phase extraction, simple isocratic chromatographic separation using Restek Pinnacle DB BiPh column (50 mm × 2.1 mm, 5 μm) and mass spectrometric detection by an API 3200 Q Trap instrument. The total run time for each sample was 6 min. The method was validated in the concentration range of 5–2000 ng/mL for FTC, RPV, DTG, EVG and 10–4000 ng/mL for TNF respectively with correlation coefficients (r2) higher than 0.9976. The results of intra and inter-run assay precision and accuracy were within acceptance limits for all the five analytes.

2.20: TENOFOVIR, LAMIVUDINE AND EMTRICITABINE

Waitt, Catriona et al. reported Development, validation and clinical application of a method for the simultaneous quantification of lamivudine, emtricitabine and tenofovir in dried blood and dried breast milk spots using LC–MS/MS with the title "Development, validation and clinical application of a method for the simultaneous quantification of lamivudine, emtricitabine and tenofovir in dried blood and dried breast milk spots using LC–MS/MS" is described as follows: DBS and DBMS were prepared from 50 and 30 μL of drug-spiked whole blood and human breast milk, respectively. Following extraction with acetonitrile and water, chromatographic separation utilised a Synergi polar column with a gradient mobile phase program consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Detection and quantification was performed using a TSQ Quantum Ultra triple quadrupole mass spectrometer. The analytical method was used to evaluate NRTI drug levels in HIV-positive nursing mothers-infant pairs.

2.21: NEVIRAPINE

Chi, J. et al. reportedLC-MS-MS method for the determination of nevirapine, a non-nucleoside reverse transcriptase inhibitor, in human plasma with the title "An LC-MS-MS method for the determination of nevirapine, a non-nucleoside reverse transcriptase inhibitor, in human plasma" is described as follows: A sensitive and rapid liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been developed to measure the levels of the HIV-1 non-nucleoside reverse transcriptase inhibitor nevirapine (NVP) in human plasma. The analyte and internal standard (IS) are isolated from plasma by a simple perchloric acid precipitation of plasma proteins followed by centrifugation. LC-MS-MS in positive mode used pairs of ions at m/z of 267/226 for NVP and 628/421 for the IS, respectively. Two linear calibration curves were established for quantitation of NVP with the low curve ranging from 25 to 1000 ng/ml and the high curve ranging from 1000 to 10 000 ng/ml. Mean inter- and

intra-assay coefficients of variation (CVs) over the ranges of the two standard curves were less than 10%. The overall recovery of NVP was 92.4%.

2.22: NEVIRAPINE, STAVUDINE AND LAMIVUDINE

Mistri, H. N. et al. reported High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma with the title "High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudineand nevirapine in human plasma" is described as follows: A selective and high throughput liquid chromatography/tandem mass spectrometry (LC–MS/MS) method has been developed and validated to separate, detect and simultaneously quantify lamivudine (3TC), stavudine (d4T) and nevirapine (NVP) in human plasma using metaxalone as internal standard (IS). After solid phase extraction (SPE), the analytes and the IS were chromatographed on a Symmetry C18 (150 mm × 3.9 mm i.d., 5 μm particle size) column using 5 μL injection volume with a run time of 4.5 min. An isocratic mobile phase consisting of 0.5% glacial acetic acid in water:acetonitrile (20:80, v/v) was used to separate all these drugs. The precursor and product ions of these drugs were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring mode (MRM) without polarity switch. The method was validated over the range of 25–3000 ng/mL for 3TC, 20–2000 ng/mL for d4T and 50–5000 ng/mL for NVP. The absolute recoveries for analytes (≥86%) and IS (98.12%) achieved from spiked plasma samples were consistent and reproducible. Inter-batch and intra-batch precision (%CV) across four validation runs (LLOQ, LQC, MQC and HQC) was less than 10. The accuracy determined at these levels was within ±8% in terms of relative error. The method was successfully applied to a pivotal bioequivalence study of [60 (3TC) + 12 (d4T) + 100 (NVP)] mg dispersible tablets in 60 healthy human subjects under fasting condition.

2.23: EFAVIRENZ

Danilo Cesar Galindo Bedor et al. reported a sensitive and robust lc-ms/ms method with monolithic column and electrospray ionization for the quantitation of efavirenz in human plasma: application to a bioequivalence study with the title "A sensitive and robust lc-ms/ms method with monolithic column and electrospray ionization for the quantitation of efavirenz in human plasma: application to a bioequivalence study" is described as follows: An LC-MS/MS method has been developed for the determination of efavirenz (EFZ) in human plasma using hydrochlorothiazide as internal standard (I.S.). An ESI negative mode with multiple reaction-monitoring was used monitoring the transitions m/z 313.88→69.24 (EFZ)

and 296.02→204.76 (I.S.). Samples were extracted using liquid-liquid extraction. The total run time was 2.0 min. The separation was achieved with HPLC-RP using a monolithic column. The assay was linear in the concentration range of 100 - 5000 ng mL-1. The mean recovery was 83%. Intra- and inter-day precision were < 9.5% and < 8.9%, respectively and accuracy was in the range ± 8.33%. The method was successfully applied to a bioequivalence study.

2.24: EFAVIRENZ, 8-HYDROXYFAVIRENZ AND 8,14 DIHYDROXYFAVIRENZ

Kim, K. B. etal. reported Rapid and Simultaneous Determination of Efavirenz, 8-Hydroxyefavirenz, and 8,14-Dihydroxyefavirenz Using LC–MS–MS in Human Plasma and Application to Pharmacokinetics in Healthy Volunteers with the title "Rapid and Simultaneous Determination of Efavirenz, 8-Hydroxyefavirenz, and 8,14-Dihydroxyefavirenz Using LC–MS–MS in Human Plasma and Application to Pharmacokinetics in Healthy Volunteers" is described as follows: We developed a rapid and sensitive method for determining efavirenz, 8-hydroxyefavirenz, and 8,14-dihydroxyefavirenz in human plasma simultaneously using liquid chromatography-tandem mass spectrometry (LC-MS-MS). Three compounds and ritonavir, an internal standard, were extracted from plasma using ethyl acetate in the presence of 0.1 M sodium carbonate after incubation of β-glucuronidase (500 U). After drying the organic layer, the residue was reconstituted in mobile phase (acetonitrile:20 mM ammonium acetate, 90:10, v/v) and injected onto a reversed-phase C18 column. The isocratic mobile phase was eluted at 0.2 mL min-1. The ion transitions monitored in multiple reaction-monitoring mode were m/z 314 → 244, 330 → 258, 346 → 262, and 721 → 296 for efavirenz, 8-hydroxyefavirenz, 8,14-dihydroxyefavirenz, and ritonavir, respectively. The retention time is 1.93, 1.70, 1.52, and 1.82 min for efavirenz, 8-hydroxyefavirenz, 8,14-dihydroxyefavirenz, and ritonavir, respectively. The coefficients of variation of the assay precision were less than 10.7%, and the accuracy was 90-111%. The lower limits of quantification (LLOQ) were 5 ng mL-1 for efavirenz and 8-hydroxyefavirenz. This method was used to measure the plasma concentrations of efavirenz and its metabolites from healthy volunteers after a single 600 mg oral dose of efavirenz. This analytical method is a very rapid, sensitive, and accurate to determine the pharmacokinetic profiles of efavirenz including its metabolites.

2.25: ENFUVIRTIDE

2.25.1. Chang, D et al. reported Bioanalytical method development and validation for a large peptide HIV fusion inhibitor (Enfuvirtide, T-20) and its metabolite in human plasma using LC-MS/MS with the title "Bioanalytical method development and validation for a large peptide HIV fusion inhibitor (Enfuvirtide, T-20) and its metabolite in human plasma using LC-MS/MS" is described as follows: A method for measuring a human immunodeficiency virus (HIV) cell membrane fusion inhibitor (T-20/Ro 29-9800) and its metabolite (M-20/Ro 50-6343) in human plasma by liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed. The relatively large peptide analytes and their corresponding deuterated (d(10)) peptides used as internal standard were isolated from plasma by protein precipitation with two volumes of acetonitrile to plasma. A large pore size reversed-phase C(18) column was employed to elute the peptides. A triple quadrupole mass spectrometer with electrospray interface operating in positive ion and multiple reaction monitoring modes with transitions m/z 1124-->1343 for both T-20 and M-20 was utilized for peak detection. The advantages of the method were a simple sample preparation, specific and sensitive MS/MS detection, and a wide dynamic range of 10-2000 ng/ml for T-20. The method was validated and used for analyzing samples from clinical studies to provide pharmacokinetic profiles of the HIV fusion inhibitor peptide drug and its metabolite.

2.25.2. Van den Broek, I. et al. reported Enzymatic digestion as a tool for the LC-MS/MS quantification of large peptides in biological matrices: Measurement of chymotryptic fragments from the HIV-1 fusion inhibitor enfuvirtide and its metabolite M-20 in human plasma with the title "Enzymatic digestion as a tool for the LC-MS/MS quantification of large peptides in biological matrices: Measurement of chymotryptic fragments from the HIV-1 fusion inhibitor enfuvirtide and its metabolite M-20 in human plasma" is described as follows: The use of enzymatic digests of the peptide HIV-1 fusion inhibitor enfuvirtide as a tool for the absolute quantification of this polypeptide (MW4492Da) in human plasma by LC–MS/MS has been evaluated. Two different methods applying digestion of enfuvirtide with chymotrypsin aftersolid phase extraction (SPE) of the plasma samples have therefore been developed and validated. One method used a stable isotopically labeledanalog of the complete peptide (d60-enfuvirtide) as internal standard (IS) and could use as much as four different chymotryptic fragments forthe quantification of enfuvirtide in a range of 100–10,000ng/ml. Intra- and inter-assay precisions and deviations from the nominal concentrationsvaried for the different fragments, but were below 9% when the four results

were averaged. The other method used a stable isotopically labeledchymotryptic fragment of the peptide (d10-ASLW) as IS. Although this IS does not correct for variations in digestion recovery, it allows theselective quantification of enfuvirtide (100–10,000ng/ml), besides the quantification of the sum of enfuvirtide and its de-amidated metaboliteM-20 (120–12,000ng/ml). Both methods were suitable for the absolute quantification of enfuvirtide and M-20 in plasma, but proper selection of the fragment(s) used for the quantification appeared crucial when the deuterated fragment was used as IS.

2.26: INTERFERON

2.26.1. Stigter, E. C. et al. reported Development of an on-line SPR-digestion-nano LC-MS/MS system for the quantification and identification of interferon-g in plasma with the title "Development of an on-line SPR-digestion-nano LC-MS/MS system for the quantification and identification of interferon-g in plasma" is described as follows: An automated, on-line system for protein quantification and identification, employing Surface Plasmon Resonance (SPR), enzymatic protein digestion, nanoLC and tandem-MS (MS/MS), has been developed. For the experiments recombinant human interferon-gamma (rhIFN-gamma) in buffer or diluted bovine plasma was used as a model protein. Upon injecting 90muL of a 1 mugmL(-1) solution of rhIFN-gamma in diluted plasma at a flow rate of 10muLmin(-1), 320fmol of protein was reproducibly bound to the sensor surface. After desorption of the isolated protein from the SPR surface using 10mM glycine pH 1.3, on-line digestion, nanoLC and MS/MS analysis, rhIFN-gamma could be identified on basis of peptide masses and MS/MS fragmentation data. A sequence recovery of 66% was found when a pepsin micro reactor was used. For a trypsin microreactor the sequence recovery was 50%. In the latter case, the desorbed protein solution was pH-tuned with a TRIS buffer for optimal enzyme activity. With the identified trypsin- and pepsin-produced peptides and because parts of their amino acid sequences overlap, the protein sequence can be largely elucidated showing the potential for the analysis of unknown proteins. The SPR-digestion-nanoLC-MS/MS platform provides unattended analysis of a sample within 60min.

2.26.2. Yang, Z. etal. reported A sensitive and high-throughput LC-MS/MS method for the quantification of pegylated-interferon-a2a in human serum using monolithic C18 solid phase extraction for enrichment with the title "A sensitive and high-throughput LC-MS/MS method for the quantification of pegylated-interferon-a2a in human serum using monolithic C18 solid phase extraction for enrichment" is described as follows: The analysis of pegylated-

interferon-alpha(2a) in patient serum samples is of high interest for clinic research trials, as this therapeutic protein has become an important antiviral treatment. In this study, an LC-MS/MS method for the absolute quantification of pegylated-interferon-alpha(2a) in human serum was developed. The assay achieved a lower limit of quantification of 3.6 ng/mL (60 pM) with the use of a monolithic C (18) solid phase extraction to enrich the target protein. The linear range of the assay was defined up to 54 ng/mL to measure the typical clinical pegylated-interferon-alpha(2a) levels, and within this range, the precision and accuracy were found to be within +/-20%. The method was applied to a clinical study and found suitable for high-throughput analysis of pegylated-interferon-alpha(2a) in human serum. In addition, further investigations suggested the enrichment step may have general application to the sensitive analysis of other low molecular weight proteins.

2.27: RIBAVARIN

2.27.1. Zironi, E. etal. reported Development of a rapid LC-MS/MS method for ribavirin determination in rat brain with the title "Development of a rapid LC-MS/MS method for ribavirin determination in rat brain" is described as follows: A rapid and specific liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of ribavirin (RBV) in rat brain was developed. Sample preparation required only two centrifuge steps before LC-MS/MS analysis and the chromatographic separation was achieved in isocratic conditions using an Atlantis T3 column with a nearly totally aqueous (95%) mobile phase. The method showed a good linearity over a concentration range of 5-1000ppb and satisfactory results in terms of accuracy.

2.27.2. Danso, D. etal. reported LC-MS/MS quantitation of ribavirin in serum and identification of endogenous isobaric interferences with the title "LC-MS/MS quantitation of ribavirin in serum and identification of endogenous isobaric interferences" is described as follows: To facilitate therapeutic drug monitoring, a liquid chromatography-tandem mass spectrometry method was validated for quantitation of ribavirin in serum. After protein precipitation, ribavirin is quantitated using a (13)C(5)-ribavirin internal standard, on a Hypercarb analytical column designed for retention of polar analytes.

2.28: RIBAVARIN AND VIRAMIDINE

Yeh, L. T. etal. reported LC-MS/MS method for simultaneous determination of viramidine and ribavirin levels in monkey red blood cells with the title "LC-MS/MS method for simultaneous determination of viramidine and ribavirin levels in monkey red blood cells" is

described as follows: A high performance liquid chromatography-tandem mass spectrometry (LC–MS/MS) method has been developed for the simultaneous determinations of total viramidine (viramidine, viramidine monophosphate, viramidine diphosphate, and viramidine triphosphate) and total ribavirin (ribavirin, ribavirin monophosphate, ribavirin diphosphate, and ribavirin triphosphate) in monkey red blood cells (RBC). The method involves the addition of internal standards and perchloric acid, conversion of viramidine or ribavirin phosphorylated metabolites to viramidine or ribavirin, purification with an aminopropyl (NH2) solid phase extraction (SPE) cartridge, and LC–MS/MS analysis. The MS/MS is selected to monitor m/z 245 → 113, 250 → 113, 244 → 112, and 249 → 112 for ribavirin, [13C] ribavirin, viramidine, and [13C] viramidine, respectively, using positive electrospray ionization. The calibration curves are linear over a concentration range of 100–10,000 ng/mL (0.412–41.2 μM) with a lower limit of quantification (LLOQ) of 100 ng/mL for both compounds. Mean inter-assay recoveries for ribavirin are 101%, 98.9%, and 96.0%, with coefficient of variance (%CV) values between 1.95 and 4.50% for 100, 1000, and 10,000 ng/mL quality control (QC) samples, respectively. Mean inter-assay recoveries for viramidine are 96.3%, 101%, and 102%, with coefficient of variation (%CV) values between 3.61 and 7.22%, for 100, 1000, and 10,000 ng/mL QC samples, respectively. Over-curve dilution QC at 400 μg/mL (1639 μM) for both viramidine and ribavirin are used to ensure the dilution accuracy (25 X dilutions) for monkey samples. The method has been used to simultaneously determine the total concentrations of ribavirin and viramidine in monkey RBC following 5, 15, and 36 weeks dosing of viramidine or ribavirin (60 mg/kg). The concentrations of total ribavirin following ribavirin dosing are 1242 μM at week 5, 1257 μM at week 15, and 1146 μM at week 36. The concentrations of total ribavirin following viramidine dosing are 634 μM at week 5, 716 μM at week 15, and 683 μM at week 36. Only small amounts of viramidine are detected in RBC following viramidine dosing, 7.80 μM at week 5, 6.63 μM at week 15, and 10.4 μM at week 36. The results suggest that ribavirin levels in RBC were at steady state at week 5 of ribavirin or viramidine dosing. At steady state, ribavirin levels in RBC are approximately 2× after ribavirin dosing than viramidine dosing. The relatively small percentage of viramidine in RBC suggests that viramidine either poorly penetrated into RBC or was extensively converted to ribavirin following entry into RBC.

2.29: VIRAMIDINE

Liu, Y. etal. reported Sensitive and specific LC-MS/MS method for the simultaneous measurements of viramidine and ribavirin in human plasma with the title “Sensitive and

specific LC-MS/MS method for the simultaneous measurements of viramidine and ribavirin in human plasma" is described as follows: To facilitate pharmacokinetics studies of viramidine in man, a sensitive and specific LC-MS/MS method for the simultaneous analyses of viramidine and ribavirin in human plasma was developed and validated. The method involved the addition of [13C] viramidine and [13C] ribavirin as internal standards, protein precipitation with acetonitrile, HPLC separation, and quantification by MS/MS system using positive electrospray ionization in the multiple reaction monitoring mode (MRM). The precursor-->product ion transitions were monitored at 245-->113, 250-->113, 244-->112, and 249-->112 for ribavirin, [13C] ribavirin, viramidine, and [13C] viramidine, respectively. The calibration curves for viramidine and ribavirin were linear over a concentration range of 1-1000 ng/mL. For both viramidine and ribavirin, the lower limit of quantification (LLOQ) was 1 ng/mL. For viramidine, intra- and inter-day analyses of QC samples at 1, 5, 250, and 1000 ng/mL indicated good precision (%CV between 1.0 and 7.0%) and accuracy (%bias between -4.3 and 5.2%). For ribavirin, intra- and inter-day analyses of QC samples at 1, 5, 250, and 1000 ng/mL indicated similar precision (%CV between 0.8 and 8.3%) and accuracy (%bias between -5.8 and 9.4%). Both viramidine and ribavirin were stable in human plasma stored at room temperature for at least 3 h, 4 degrees C for at least 6 h, and for at least three freeze-thaw cycles. This accurate and highly specific assay provides a useful method for evaluating the pharmacokinetics of viramidine and ribavirin in man following administration of viramidine.

2.30: SAQUINAVIR

Chi, J. et al. reported Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS with the title "Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS" is described as follows: A sensitive and rapid liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been developed to measure the levels of five HIV protease inhibitors nelfinavir (NFV), indinavir (IDV), ritonavir (RTV), saquinavir (SQV) and amprenavir (APV) in human plasma. The analytes and internal standard are isolated from plasma by a simple acetonitrile precipitation of plasma proteins followed by centrifugation. LC-MS-MS in positive mode used pairs of ions at m/z of 568.4/330.0, 614.3/421.2, 720.9/296.0, 671.1/570.2 and 505.9/245.0 for NFV, IDV, RTV, SQV and APV, respectively and 628/421 for the internal standard. Two 1/x weighted linear calibration curves for each analyte were established for

quantitation with the low curve ranging from 5 to 1000 ng/ml and while the high curve ranging from 1000 to 10,000 ng/ml. Mean inter- and intra-assay coefficients of variation (CVs) over the ranges of the standard curves were less than 10%. The overall recovery of NFV, IDV, RTV, SQV and APV were 88.4, 91.4, 92.2, 88.9 and 87.6%, respectively.

2.31: INDINAVIR

2.31.1. Jayewardene, A. L. et al. reported LC-MS-MS method for the determination of indinavir, an HIV-1 protease inhibitor, in human plasma with the title "LC-MS-MS method for the determination of indinavir, an HIV-1 protease inhibitor, in human plasma" is described as follows: The analyte and internal standard are isolated from plasma by a simple acetonitrile precipitation of plasma proteins followed by centrifugation. LC-tandem mass spectrometry in positive ion, multiple reaction monitoring mode used pairs of ions at m/z of 614/421 for indinavir and 628/421 for internal standard, respectively. The calibration curve had a linear range from 3.0 to 12320 ng/ml when linear least square regression weighing 1/x was applied to the concentration versus peak area plot. The advantages of this method are the fast sample preparation, wide dynamic assay range and quick analysis taking only 5 min for each sample run. The robust nature of this assay has been further verified during routine use over several months involving multiple analysts.

2.31.2. Pereira de Oliveira et al. reported LC-MS/MS determination of the HIV-1 protease inhibitor indinavir in brain and testis of mice with the title "LC-MS/MS determination of the HIV-1 protease inhibitor indinavir in brain and testis of mice" is described as follows: Indinavir and the internal standard (IS) amprenavir were isolated from homogenized tissue matrices using a mixed-mode solid-phase extraction (SPE) procedure and were then analyzed by reversed-phase liquid chromatography/tandem mass spectrometry (LC-MS/MS). The mass spectrometer in the positive-ion multiple reaction monitoring mode used pairs of ions at m/z of 614.1/421.3 for indinavir and of 506.1/245.3 for IS. The calibration curves were linear over the range 0.0012-0.0390 micromol/kg for brain and 0.39-12.50 micromol/kg for testis. Linearity, repeatability and accuracy were validated. The applicability of the method was demonstrated by assessing indinavir in brain and testis of three mice dosed with intravenous bolus administration of indinavir (16.3 micromol/kg).

2.32: INDINAVIR, NELFINAVIR, RITONAVIR, SAQUINAVIR AND AMPRENAVIR:

Chi, J. et al. reported Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS with the title "Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS" is described as follows: A sensitive and rapid liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been developed to measure the levels of five HIV protease inhibitors nelfinavir (NFV), indinavir (IDV), ritonavir (RTV), saquinavir (SQV) and amprenavir (APV) in human plasma. The analytes and internal standard are isolated from plasma by a simple acetonitrile precipitation of plasma proteins followed by centrifugation. LC-MS-MS in positive mode used pairs of ions at m/z of 568.4/330.0, 614.3/421.2, 720.9/296.0, 671.1/570.2 and 505.9/245.0 for NFV, IDV, RTV, SQV and APV, respectively and 628/421 for the internal standard. Two 1/x weighted linear calibration curves for each analyte were established for quantitation with the low curve ranging from 5 to 1000 ng/ml and while the high curve ranging from 1000 to 10,000 ng/ml. Mean inter- and intra-assay coefficients of variation (CVs) over the ranges of the standard curves were less than 10%. The overall recovery of NFV, IDV, RTV, SQV and APV were 88.4, 91.4, 92.2, 88.9 and 87.6%, respectively.

2.33: ATAZANAVIR

Cheng, Changfu et al. reported Revealing the metabolic sites of atazanavir in human by parallel administrations of D-atazanavir analogs with the title "Revealing the metabolic sites of atazanavir in human by parallel administrations of D-atazanavir analogs" is described as follows: Atazanavir (Reyataz®) is an important member of the HIV protease inhibitor class. Because of the complexity of its chemical structure, metabolite identification and structural elucidation face serious challenges. So far, only seven non-conjugated metabolites in human plasma have been reported, and their structural elucidation is not complete, especially for the major metabolites produced by oxidations. To probe the exact sites of metabolism and to elucidate the relationship among in vivo metabolites of atazanavir, we designed and performed two sets of experiments. The first set of experiments was to determine atazanavir metabolites in human plasma by LC-MS, from which more than a dozen metabolites were discovered, including seven new ones that have not been reported. The second set involved deuterium labeling on potential metabolic sites to generate D-atazanavir analogs. D-atazanavir analogs were dosed to human in parallel with atazanavir. Metabolites of

D-atazanavir were identified by the same LC-MS method, and the results were compared with those of atazanavir. A metabolite structure can be readily elucidated by comparing the results of the analogs and the pathway by which the metabolite is formed can be proposed with confidence. Experimental results demonstrated that oxidation is the most common metabolic pathway of atazanavir, resulting in the formation of six metabolites of mono oxidation (M1, M2, M7, M8, M13, and M14) and four of di-oxidation (M15, M16, M17, and M18). The second metabolic pathway is hydrolysis, and the third is N-dealkylation. Metabolites produced by hydrolysis include M3, M4, and M19. Metabolites formed by N-dealkylation are M5, M6a, and M6b.

2.34: RITONAVIR

Hendrikx, Jeroen J. M. A. at.al reported Combined quantification of paclitaxel, docetaxel and ritonavir in human feces and urine using LC-MS/MS with the title "Combined quantification of paclitaxel, docetaxel and ritonavir in human feces and urine using LC-MS/MS" is described as follows: A combined assay for the determination of paclitaxel, docetaxel and ritonavir in human feces and urine is described. The drugs were extracted from 200 µL urine or 50 mg feces followed by high-performance liquid chromatography analysis coupled with positive ionization electrospray tandem mass spectrometry. The validation program included calibration model, accuracy and precision, carry-over, dilution test, specificity and selectivity, matrix effect, recovery and stability. Acceptance criteria were according to US Food and Drug Administration guidelines on bioanalytical method validation. The validated range was 0.5–500 ng/mL for paclitaxel and docetaxel, 2–2000 ng/mL for ritonavir in urine, 2–2000 ng/mg for paclitaxel and docetaxel, and 8–8000 ng/mg for ritonavir in feces. Inter-assay accuracy and precision were tested for all analytes at four concentration levels and were within 8.5% and <10.2%, respectively, in both matrices. Recovery at three concentration levels was between 77 and 94% in feces samples and between 69 and 85% in urine samples. Method development, including feces homogenization and spiking blank urine samples, are discussed. We demonstrated that each of the applied drugs could be quantified successfully in urine and feces using the described assay. The method was successfully applied for quantification of the analytes in feces and urine samples of patients.

2.35: RITONAVIR AND LOPINAVIR

2.35.1. Damaramadugu et al. reported Simultaneous Determination of Ritonavir and Lopinavir in Human Plasma after Protein Precipitation and LC-MS-MS with the title

"Simultaneous Determination of Ritonavir and Lopinavir in Human Plasma after Protein Precipitation and LC-MS-MS" is described as follows: A simple, rapid, specific and sensitive method has been developed and validated for simultaneous determination of lopinavir and ritonavir in human plasma. The analytes and internal standard are isolated from plasma by a simple acetonitrile precipitation of plasma proteins followed by centrifugation. Separation was carried out on an Inertsil ODS column using a mobile phase consisting of acetonitrile and 5 mM ammonium acetate buffer. The total run time of analysis was 2.0 min. A linear response function was established for the range of concentrations 50.67–10,008.82 ng mL−1 for lopinavir and 5.066–1,000.693 ng mL−1 for ritonavir. The method was successfully applied to an oral bioequivalence study in humans.

2.35.2 Ehrhardt etal. reported Monitoring of lopinavir and ritonavir in peripheral blood mononuclear cells, plasma, and ultrafiltrate using a selective and highly sensitive LC/MS/MS assay with the title "Monitoring of lopinavir and ritonavir in peripheral blood mononuclear cells, plasma, and ultrafiltrate using a selective and highly sensitive LC/MS/MS assay" is described as follows: For the determination of the HIV protease inhibitors lopinavir and ritonavir in human plasma, plasma ultrafiltrate, and peripheral blood mononuclear cells (PBMCs) a highly sensitive and selective method has been developed, validated, and applied to samples of a healthy volunteer. BD Vacutainer CPT and Amicon Centriplus centrifugal filter devices were used for separation of PBMCs and for ultrafiltrate generation, respectively. After liquid/liquid-extraction extracts were chromatographed isocratically within 6 min on a Jupiter Proteo column. The drugs were quantified using 2H5-saquinavir as internal standard and electrospray tandem mass spectrometry in the selected reaction monitoring mode. Limits of quantification for both analytes were 4.0 ng/mL in plasma, 0.2 ng/mL in ultrafiltrate, and 0.1 ng/cell pellet (approximately 3 x 10(6) cells) in PBMCs. The calibration ranges were linear over more than three logs with an over-all accuracy varying between 98.7% and 111.5% and an over-all precision ranging from 6.2% to 14.0% (SD batch-to-batch). After a regular oral dose of Kaletra (400 mg lopinavir, 100 mg ritonavir) analyte concentrations were detectable over a full dosing interval in plasma, ultrafiltrate, and PBMCs. The method is well suited for monitoring of free and total plasma, and intracellular lopinavir/ritonavir concentrations in samples from clinical trials.

2.36: RITONAVIR AND ELVITEGRAVIR

Rao, R. N et al. reported Diatomaceous earth supported liquid extraction and LC-MS/MS determination of elvitegravir and ritonavir in rat plasma: application to a pharmacokinetic study with the title "Diatomaceous earth supported liquid extraction and LC-MS/MS determination of elvitegravir and ritonavir in rat plasma: application to a pharmacokinetic study" is described as follows: A rapid, sensitive and selective bio-analytical method was developed for determination of elvitegravir and ritonavir in rat plasma by high-performance liquid chromatography–tandem mass spectrometry. Sample preparation was based on diatomaceous earth supported liquid extraction using dichloromethane to extract elvitegravir and ritonavir from rat plasma. Chromatographic separation was performed on a Waters Symmetry C18 column (4.6 × 250 mm, 5 μm) using 20 mM ammonium formate containing 0.05% trifluoroacetic acid and acetonitrile (35:65 v/v) for isocratic elution and mass detection. Calibration curves were linear with regression coefficients greater than 0.997 over the concentration range of 2–5000 ng mL−1 for elvitegravir and ritonavir. Absolute mean recoveries were in the range of 87.31 to 92.18% and no significant interferences were observed at the retention times of the elvitegravir, ritonavir and internal standard. The method exhibited good intra- and inter-day performance in terms of 1.55–7.47% precision and 0–5% accuracy. The developed method has been successfully applied to the pharmacokinetic study of elvitegravir in healthy rats following a single oral administration of a 15 mg kg−1 dose boosted with 5 mg kg−1 ritonavir.

2.37: NELFIVAVIR

Tiwari, R. N et al. reported ted LC, LC-MS/TOF and MS^n studies for the identification and characterization of degradation products of nelfinavir mesylate with the title "LC, LC-MS/TOF and MS^n studies for the identification and characterization of degradation products of nelfinavir mesylate" is described as follows: The objective of the present investigation was to separate, identify and characterize the major degradation products (DPs) of nelfinavir mesylate generated under hydrolytic, oxidative, photolytic and thermal stress conditions as advised in International Conference on Harmonization (ICH) guideline Q1A(R2). The drug was found to degrade under acidic, basic, oxidative and photolytic stress, while it was stable in neutral and thermal stress conditions. A total of three degradation products were formed, which were separated on a C-18 column employing a gradient HPLC method. A complete mass fragmentation pathway of the drug was first established with the help of multi-stage (MS(n)) and MS/TOF accurate mass studies. Then stressed samples were subjected to LC-

MS/TOF studies, which provided their fragmentation pattern and accurate masses. The mass spectral data were employed to characterize the DPs and assign structures to them. The total information was also used to establish the degradation pathway of the drug. The degradation products were identified as 3-hydroxy-N-((2R,3R)-3-hydroxy-1-(phenylthio)butan-2-yl)-2-methylbenzamide(3S,4aS,8aS)-N-tert-butyl-2-((2R,3R)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfinyl)butyl)decahydroisoquinoline-3-carboxamide.

2.38: AMPRENAVIR

Chi, J et al. reported Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS with the title "Simultaneous determination of five HIV protease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS" is described as follows: A sensitive and rapid liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been developed to measure the levels of five HIV protease inhibitors nelfinavir (NFV), indinavir (IDV), ritonavir (RTV), saquinavir (SQV) and amprenavir (APV) in human plasma. The analytes and internal standard are isolated from plasma by a simple acetonitrile precipitation of plasma proteins followed by centrifugation. LC-MS-MS in positive mode used pairs of ions at m/z of 568.4/330.0, 614.3/421.2, 720.9/296.0, 671.1/570.2 and 505.9/245.0 for NFV, IDV, RTV, SQV and APV, respectively and 628/421 for the internal standard. Two 1/x weighted linear calibration curves for each analyte were established for quantitation with the low curve ranging from 5 to 1000 ng/ml and while the high curve ranging from 1000 to 10,000 ng/ml. Mean inter- and intra-assay coefficients of variation (CVs) over the ranges of the standard curves were less than 10%. The overall recovery of NFV, IDV, RTV, SQV and APV were 88.4, 91.4, 92.2, 88.9 and 87.6%, respectively.

2.39: LOPINAVIR

Jaiswal, S. etal. reported Simultaneous LC–MS-MS Determination of Lopinavir and Rifabutin in Human Plasma with the title "Simultaneous LC–MS-MS Determination of Lopinavir and Rifabutin in Human Plasma" is described as follows: A simple and specific liquid chromatography tandem mass spectrometry method was developed for quantification of rifabutin (RBT) and lopinavir (LPV) simultaneously in human plasma. Following extraction using 60% n-hexane in ethyl acetate, the processed samples were chromatographed on a Discovery HS C18 column (5 µm, 50 × 4.6 mm, id) using mobile phase [85% acetonitrile in ammonium acetate buffer (10 mM, pH 4.5)] at a flow rate of 0.7 mL/min. Mass

spectrometric detection was performed in positive electrospray ionization mode using multiple reaction monitoring (RBT, m/z 847.7 → 815.4; LPV, m/z 629.6 → 447.4). Raloxifene and phenacetin were used as internal standards for RBT and LPV, respectively. Linearity was established in the range of 1-1,000 ng/mL and 0.5-10 µg/mL ($R2 \geq 0.99$) for RBT and LPV, respectively. The recovery of LPV and RBT were always >90 and >50%, respectively. The precisions and accuracies were well within the acceptable limits of variation.

2.40: LOPINAVIR AND RITONAVIR

2.40.1. Kakadiya, P. R. et al. reported Low level determinations of methyl methane sulfonate and ethyl methane sulfonate impurities in Lopinavir and Ritonavir Active pharmaceutical ingredients by LC/MS/MS using electrospray ionization with the title "Low level determinations of methyl methane sulfonate and ethyl methane sulfonate impurities in Lopinavir and Ritonavir Active pharmaceutical ingredients by LC/MS/MS using electrospray ionization" is described as follows: A sensitive LC/MS/MS method is developed and validated for the determination of MMS and EMS impurities in both Lopinavir and Ritonavir Active pharmaceutical ingredient. Method utilizes, Atlantis T3 column with electrospray ionization in multiple reactions monitoring (MRM) mode for quantitation of impurities. The proposed method is specific, linear, accurate and precise. The calibration curves show good linearity over the concentration range of 0.01–0.23 µg/mL for MMS and 0.005–0.23 µg/mL for EMS. The correlation coefficient obtained is >0.99 in each case. Method has very low limit of detection (LOD) and quantification (LOQ). LOD and LOQ of MMS and EMS are as low as ~0.002 µg/mL and ~0.01 µg/mL respectively. Method has accuracy within 80–120% for both the analytes. This method is a good quality control tool for quantitation of MMS and EMS impurities at very low levels in Lopinavir and Ritonavir.

2.40.2. Myasein, F. et al. reported Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC-MS/MS with the title "Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC-MS/MS" is described as follows: With stacked protein precipitations and SALLE, the sample preparation for a 96-well plate can be completed within 20 min by an automated pipette. Due to the unique cleanliness of SALLE extracts post double protein precipitations, the extracts were injected

into an ultrafast liquid chromatography and tandem mass spectrometry system (LC-MS/MS) after simple dilution. An Agilent Zobax Extend-C18 Rapid resolution HT column (1.8 micron, 2.1 mm x 30 mm) was used for the separation. A mixture of acetonitrile:water (55:45, v/v) with 0.1% formic acid was used as the mobile phase. LC ran for approximately 48 s at a flow rate of 0.5 mL min (-1), tandem mass spectrometric data collection started at 15 s and lasts for 30 s. The method was validated with reference to Industry Guidance for Bioanalytical Method Validation and then used for clinical samples. The method is ultrafast, and robust. Results of incurred samples demonstrated excellent method of reproducibility. This ultrafast analysis speed did not compromise with the data quality. To our knowledge, this is the fastest analytical method for simultaneous determination of lopinavir and ritonavir.

3. CONCLUSION

In the quantification of Acyclovir and Valacyclovir in plasma were concentrated by solid phase extraction and chromatographed. In the quantification of Cidofovir, a strong anion exchange (sax) solid-phase extraction procedure was applied for the sample preparation. In the quantification of Amantadine, the analyte and internal standard (IS), amantadine-d6 (AMD-d6), were extracted from 200 µL plasma by solid phase extraction on Phenomenex Strata-X-C 33 µ cartridges. In quantification of prodrugoseltamivir and its metabolite oseltamivircarboxylate in human plasma, the method involved solid phase extraction of the analytes and ISs from 200Î¼L human plasma with no reconstitution and drying steps. In determination of Zidovudine triphosphate concentrations in human peripheral blood mononuclear cells by SPE-LC-MS, salt gradient anion exchange Solid Phase Extraction was used.In Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir in human peripheral blood mononuclear cells Combined anion exchange solid phase extraction was used Intracellular Didanosine triphosphate was extracted from CEM-T4 cells, isolated using an exchange anion solid phase extraction procedure. In determining plasma Tenofovir concentrations**,** after addition of adefovir as an internal standard, trifluoroacetic acid was used to produce a protein-free extract. For the determination of nevirapine, the analyte and internal standard (IS) are isolated from plasma by a simple perchloric acid precipitation of plasma proteins followed by centrifugation. Simultaneous Determination of Efavirenz, 8-Hydroxyefavirenz, and 8,14-Dihydroxyefavirenz, three compounds and Ritonavir, an internal standard, were extracted from plasma using ethyl acetate in the presence of 0.1 M sodium carbonate after incubation

of β-glucuronidase (500 U). Overall Solid phase Extraction is adopted widely for extraction of anti viral drugs from biological matrices.

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