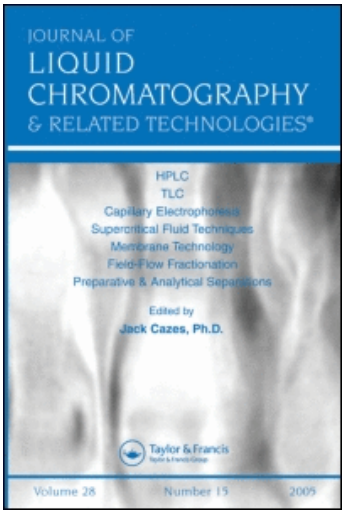


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF ZIDOVUDINE AND LEVOFLOXACIN IN HUMAN PLASMA BY REVERSED PHASE HPLC AND SOLID PHASE EXTRACTION

William V. Caufield[a]; James T. Stewart[a]
[a] Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA, U.S.A.

Online publication date: 24 July 2002

To cite this Article Caufield, William V. and Stewart, James T.(2002) 'DETERMINATION OF ZIDOVUDINE AND LEVOFLOXACIN IN HUMAN PLASMA BY REVERSED PHASE HPLC AND SOLID PHASE EXTRACTION', Journal of Liquid Chromatography & Related Technologies, 25: 12, 1791 — 1805
To link to this Article: DOI: 10.1081/JLC-120005874
URL: http://dx.doi.org/10.1081/JLC-120005874


MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
Vol. 25, No. 12, pp. 1791–1805, 2002

DETERMINATION OF ZIDOVUDINE AND LEVOFLOXACIN IN HUMAN PLASMA BY REVERSED PHASE HPLC AND SOLID PHASE EXTRACTION

William V. Caufield and James T. Stewart*

Department of Pharmaceutical and Biomedical Sciences,
College of Pharmacy, The University of Georgia, Athens,
GA 30602-2352, USA

ABSTRACT

A new high performance liquid chromatography (HPLC) assay was developed for the simultaneous determination of zidovudine (AZT) and levofloxacin in human plasma. Plasma samples were treated with a solid-phase extraction procedure. The compounds were separated using a mobile phase of 86 : 14 v/v 25 mM sodium phosphate monobasic monohydrate and 0.1% trifluoroacetic acid (pH 2.4) – acetonitrile on an octadecylsilane column (150 × 4.6 mm i.d.) with UV detection at 266 nm. Ciprofloxacin was used as the internal standard (IS). The method was validated over the range of 26.3–2600 ng/mL for AZT, and 51.2–5069 ng/mL for levofloxacin. The method proved to be accurate (percent bias for all calibration samples varied from −6.2 to 5.6%) and precise (within-run precision ranged from 0.9 to 9.7% and between-run precision ranged from 1.3 to 7.5%).

*Corresponding author. E-mail: jstewart@merc.rx.uga.edu

DOI: 10.1081/JLC-120005874
1082-6076 (Print): 1520-572X (Online)

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

The mean absolute recoveries were 94.1% for AZT, 91.2% for levofloxacin, and 84.7% for the internal standard. The assay should be suitable for use in pharmacokinetic studies and routine plasma monitoring of these drugs in HIV infected patients.

INTRODUCTION

At the end of 2001, there had been an estimated 3 million deaths worldwide due to human immunodeficiency virus (HIV) infections and 40 million people were currently living with HIV.[1] Although, several drugs have been developed to combat this epidemic, zidovudine (3′-azido-3′-deoxythymidine; AZT) continues to be one of the first-line therapeutic agents in treating HIV. AZT is a nucleoside reverse transcriptase inhibitor with antiviral activity against HIV-1, HIV-2, human *T* lymphotrophic virus, and other retroviruses. It is anabolized intracellularly to a triphosphate metabolite by cellular enzymes to produce the active drug.[2] Side effects that have been associated with AZT therapy include gastrointestinal intolerance, bone marrow toxicity, and myelosuppression.[3] Long-term exposure to AZT has also been shown to result in the development of AZT resistant strains of HIV-1.[4] It has been demonstrated, that dose-related toxicities can be reduced and patient outcomes can be improved when a specific concentration range of AZT is maintained.[5] It has also been suggested, that HIV patients might benefit from a pharmacokinetic approach to AZT therapy.[6] Therapeutic drug monitoring of antiviral drugs such as AZT is also necessary to avoid or delay resistance from the virus, to monitor adherence, and to monitor drug-drug and drug–food interactions.

Many human immunodeficiency virus (HIV)-infected patients develop secondary bacterial infections because of their compromised immune systems. Coinfection with mycobacterium species, especially Mycobacterium avium complex (MAC) and mycobacterium tuberculosis, is often treated with multiple antibacterial agents, including levofloxacin.[7–9] Levofloxacin is a chiral fluorinated carboxyquinolone and is the *L*-isomer of the racemate ofloxacin. It is a broad-spectrum antibacterial agent with activity against a wide range of gram-positive, gram-negative, and anaerobic bacteria. Levofloxacin has been found in vitro to be generally twice as active as ofloxacin against many of these organisms.[10] The bactericidal activity of levofloxacin is maximized when the ratios of peak plasma drug concentrations (C_{max}): minimum inhibitory concentrations (MIC) or area under the concentration-time curve (AUC): MIC exceed certain threshold levels.[11] Therefore, therapeutic drug monitoring of levofloxacin plasma levels would make it possible to administer the correct dose of the drug to the patient at the appropriate interval. Monitoring of the C_{max} to MIC ratio is particularly important in patients at risk for malabsorption, such as those infected with HIV.[12]

Analytical methods have been described to quantify the individual drugs in biological media,[13–16] but no methods have been reported for the simultaneous determination of AZT and levofloxacin in human plasma. This paper describes the development and validation of an assay that is both rapid and sensitive for determining AZT and levofloxacin in human plasma. For sample pre-treatment, the method utilizes solid phase extraction that does not require an evaporation step. Elution is performed isocratically with 266 nm UV detection.

EXPERIMENTAL

Chemicals

Zidovudine (AZT) was obtained from the United States Pharmacopeial Convention Inc. (Rockville, MD, USA). Levofloxacin was kindly provided by R. W. Johnson Pharmaceutical Research Institute (Spring House, PA 19477). Ciprofloxacin Hydrochloride was purchased from Serologicals Proteins Inc. (Kankakee, IL 60901). Monobasic sodium phosphate, phosphoric acid, HPLC grade methanol, and acetonitrile were from J.T. Baker Inc. (Phillipsburg, NJ 08865). Trifluoroacetic acid was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI 53201). Water was purified by a cartridge system (Continental Water System, Roswell, GA, USA). Drug free human plasma was obtained from Bioreclamation Inc. (Hicksville, NY 11801).

Instrumentation

The chromatographic separations were performed on a Model 1090 HPLC system (Hewlett Packard Co., Palo Alto, CA, USA). This system included a pump, an autosampler equipped with a 250 μL loop, and a Model 117 variable wavelength UV detector (Gilson, Middleton, WI, USA). 0.010″ I.D. tubing was used before and after the column and was kept at a minimum length. Turbochrom (Perkin Elmer, Norwalk, CT, USA) chromatography software was used for data integration. Separations were performed on a 150 mm × 4.6 mm I.D. octadecylsilane (C_{18}) column (5 μm particle size, and 100 Å pore size, Luna, Phenomenex, Torrance, CA 90501).

Chromatographic Conditions

The mobile phase consisted of 86 : 14 v/v 25 mM sodium phosphate monobasic monohydrate and 0.1% trifluoroacetic acid (pH 2.4) – acetonitrile.

The mobile phase was filtered through a 0.22 μm nylon-66 filter (MSI, Westborough, MA, USA) and degassed in an ultrasonic bath for 15 min before use. The HPLC pump flow rate was 1.5 mL/min and all analyses were conducted at ambient temperature. The injection volume was 50 μL and the UV detector was operated at 266 nm.

Preparation of Standard Stock Solutions

Standard stock solutions of zidovudine (AZT) and levofloxacin were prepared by dissolving appropriate amounts of each drug in pooled human plasma to obtain final drug concentrations of 503 and 980 μg/mL, respectively. Working solutions were prepared by further diluting these stock solutions with pooled human plasma. The internal standard (ciprofloxacin) stock solution was prepared by dissolving an appropriate amount of the drug in methanol to obtain a final concentration of 540 μg/mL. A working internal standard solution was prepared by further diluting this stock solution with 25 mM sodium phosphate buffer solution to yield a concentration of 540 ng/100 μL.

Sample Preparation Procedure

Calibration standards and quality control samples were prepared by making appropriate dilutions of the working standard solution with pooled human plasma. Solid phase extraction cartridges (Varian Inc., Bond Elut C_{18}, 1 cc 100 mg) were placed on a vacuum elution manifold (Alltech, Deerfield, IL 60015) and rinsed with 1 mL of methanol, followed by 1 mL of purified water and 1 mL of 25 mM sodium phosphate monobasic monohydrate, containing 0.1% trifluoroacetic acid (pH 2.4). Care was taken that the cartridges did not run dry. 1.1 mL of each standard or sample was transferred to a 1.5 mL polypropylene microcentrifuge tube and centrifuged at 13,000 rpm for 15 min. Following centrifugation, 1 mL of each standard or sample was transferred to a 1.5 mL polypropylene microcentrifuge tube and mixed with 100 μL of internal standard. The entire spiked plasma samples were then transferred to the SPE cartridges. The microcentrifuge tubes were then rinsed with 250 μL of 25 mM sodium phosphate monobasic monohydrate containing 0.1% trifluoroacetic acid (pH 2.4), and the rinses were transferred to the cartridge.

Vacuum was then applied to obtain a flow through the cartridges of 1–2 mL/min. The cartridges were then washed with two 1-mL aliquots of 95 : 5 v/v 25 mM sodium phosphate monobasic monohydrate containing 0.1% trifluoroacetic acid (pH 2.4)–methanol, followed by vacuum suction for one min. The analytes were eluted from the cartridges with two 250 μL aliquots of

80 : 20 v/v 25 mM sodium phosphate monobasic monohydrate containing 0.1% trifluoroacetic acid (pH 2.4)–acetonitrile, followed by vacuum suction for one min. Extracts were collected directly into 1.5 mL autosampler vials, vortex mixed and 50 μL was then injected onto the liquid chromatograph.

Specificity

The specificity of the assay was checked by analyzing four independent blank human plasma samples. The chromatograms of these blank plasma samples were compared with chromatograms obtained by analyzing human plasma samples spiked with the analytes.

The specificity was also assessed for other compounds that could reasonably be expected to be present in the plasma of HIV infected patients.

Linearity

Calibration plots for the analytes in plasma were prepared by diluting stock solutions with pooled human plasma to yield concentrations of 26.3–2600 ng/mL (26.3, 138, 261, 1325, and 2600 ng/mL) for AZT and 51.2–5069 ng/mL (51.2, 268, 509, 2582, and 5068 ng/mL) for levofloxacin. Calibration standards at each concentration were extracted and analyzed in triplicate. Calibration curves were constructed using ratios of the observed analyte peak area to internal standard versus nominal concentrations of analyte. Linear regression analysis of the data gave slope, intercept, and correlation coefficient data. From this data, a first order polynomial model was selected for each analyte. To confirm that the chosen linear model was correct, a statistical lack of fit test was performed.

Benchtop Stability

The stability of the processed sample in the sample compartment of the HPLC was also assessed. Prepared samples at two concentrations (80.3 and 2062 ng/mL for AZT, 156 and 4019 ng/mL for levofloxacin) from day 1 of the precision and accuracy assessment were pooled and injected every two hr. over a 24 hr period. During this time, the samples were protected from light at ambient temperature. The peak area ratio of analyte to internal standard was plotted verses time and was used for least squares regression analysis. The regression data was used to determine if a significant change in analyte concentration occurred over the course of the 24 hr period. For the purposes of this validation, a change in concentration was considered significant if it exceeded 10%.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Precision and Accuracy

The within-run and between-run accuracy and precision of the assay in plasma were determined by assaying four quality control samples in triplicate over a period of three days. The concentrations represented the entire range of the calibration curves. The lowest level was at the expected LOQ for each analyte (26.3 ng/mL for AZT and 51.2 ng/mL for levofloxacin). The second level was at three times the LOQ (80.3 ng/mL for AZT and 156 ng/mL for levofloxacin) and the third level was at the mid-point of the calibration curves (261 ng/mL for AZT and 509 ng/mL for levofloxacin). The fourth level was at 80% of the upper boundary of the calibration curves (2062 ng/mL for AZT and 4019 ng/mL for levofloxacin). Calibration curves were prepared and analyzed daily and linear models were used to determine concentrations in the quality control samples. The nine measured concentrations per concentration level (triplicates from three runs) were subjected to analysis of variance (ANOVA) to estimate the within-run and between run precision. Percent accuracy was determined (using the data from the precision assessment) as the closeness of spiked samples to the nominal value of in-house standards. Precision was reported as percent relative standard deviation (%RSD).

Limit of Detection and Limit of Quantification

Decreasing concentrations of the analytes were prepared by diluting stock solutions with pooled human plasma, and then analyzed. The limit of detection (LOD) was defined as the concentration that yields a signal-to-noise ratio of 3. The limit of quantification (LOQ) was calculated to be the lowest analyte concentration in plasma that could be measured with a between run relative standard deviation (RSD) of $<20\%$ and an accuracy between 80 and 120%.

Recovery

The absolute recoveries of AZT and levofloxacin from plasma were assessed at two concentrations (80.3 and 2062 ng/mL for AZT, and 156 and 4019 ng/mL for levofloxacin). The recovery of the internal standard from plasma was assessed at the working concentration of 540 ng/mL. For each level, three samples were extracted and analyzed in triplicate. Three replicates of each concentration, prepared in the eluent, were directly injected. The assay absolute recovery for each compound, at each concentration, was computed using the following equation: absolute recovery = (peak area in extract)/(mean peak area direct injection) $\times$ 100.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Freezing and Thawing Stability

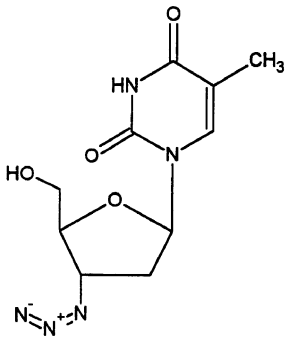
The stability of plasma samples after three freeze–thaw cycles was also examined. The two concentrations used in the benchtop stability study were assayed in triplicate over a period of three days. After each analysis, the samples were re-frozen until the next day. After three freeze–thaw cycles, the results were compared to the initial fresh unfrozen samples from the accuracy and precision assessment. The unpaired *t*-test (two tailed) was used to determine if the means from each level were significantly ($\alpha = 0.05$) different. An *f*-test was also used to determine if the variances were significantly different.

RESULTS AND DISCUSSION

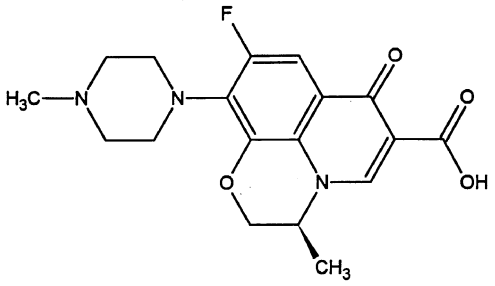
The chemical structures for AZT, levofloxacin, and the internal standard ciprofloxacin are shown in Fig. 1. The goals in developing this method were low ng/mL sensitivity, a run time of less than ten min, and a simple extraction method that could be easily automated. The large difference in lipophilicity between AZT and levofloxacin posed the greatest challenge in the development of the separation. The more hydrophilic AZT tended to elute with endogenous substances in the plasma extract, whereas levofloxacin and the internal standard tended to elute much later in the run. Initially, a series of reversed phase columns were investigated including C_8, C_{16}-amide, halogen specific C_{18}, and conventional C_{18}. A gradient separation was not desirable due to the additional time required for the column to re-equilibrate to the initial conditions. Ultimately, a 150×4.6 mm conventional C_{18} column and an isocratic run were selected with a mobile phase of 86 : 14 v/v 25 mM sodium phosphate monobasic monohydrate and 0.1% trifluoroacetic acid (pH 2.4)–acetonitrile. Trifluoroacetic acid was added to lower the mobile phase pH and to minimize the retention of endogenous sample components. These conditions were found to give good selectivity and sensitivity in a 10 min run.

The primary objectives in the development of the extraction method were to minimize interfering endogenous sample components, while at the same time providing high recoveries of the analytes. Liquid-liquid extraction and several protein precipitation techniques were evaluated but were not as effective as solid phase extraction (SPE) in the removal of endogenous sample components. During development of the solid-phase extraction method, a series of different extraction cartridges were investigated, such as C_{18}, C_8, phenyl, Oasis™, and Absolute™ cartridges. The 1 cc Bond Elut C_{18} cartridge was found to give the highest recoveries, while at the same time removing endogenous interferences. 95 : 5 v/v 25 mM sodium phosphate monobasic monohydrate and 0.1% trifluoroacetic acid (pH 2.4) – methanol was used to wash the cartridges after loading spiked plasma

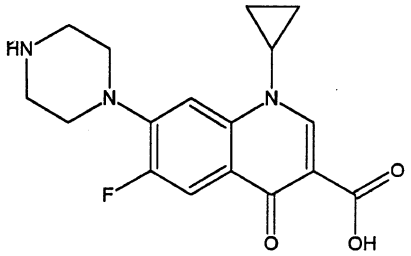
MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016



Zidovudine



Levofloxacin



Ciprofloxacin

Figure 1. The chemical structures of the analytes.

to help retain the hydrophilic analytes. Cleaner extracts were observed using 5% methanol-buffer washes than buffer washes alone. An 80 : 20 v/v 25 mM sodium phosphate monobasic monohydrate and 0.1% trifluoroacetic acid (pH 2.4)–acetonitrile solution was strong enough to elute all of the analytes including the quinolones, and leave most of the highly hydrophobic plasma interferences on the SPE cartridges. Good recoveries were obtained after the addition of 1 mL of spiked plasma by elution with only 0.5 mL of the SPE eluent. This resulted in a two-fold sample concentration and avoided the necessity of a long extract evaporation step.

Specificity

AZT and levofloxacin were well separated under the HPLC conditions applied. Retention times were 4.9 min. for AZT and 8.0 min. for levofloxacin. The internal standard (ciprofloxacin) was well resolved from levofloxacin with a retention time of 9.4 min. No interferences were observed in drug free human plasma samples. Figures 2 and 3 show chromatograms of a blank plasma sample and a calibration sample, respectively. Since AZT is often prescribed with other

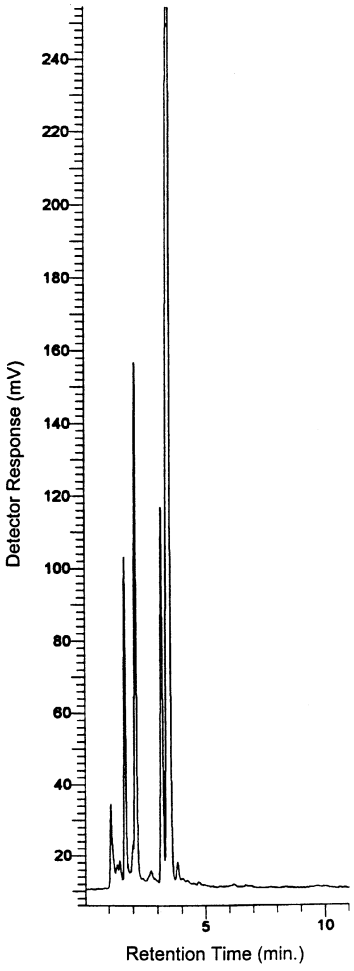


Figure 2. Chromatogram of blank pooled human plasma.

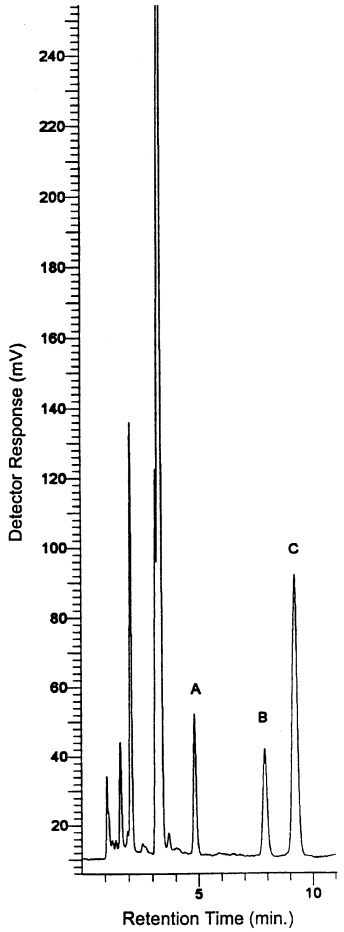


Figure 3. Chromatogram of pooled human plasma spiked with (A) 261 ng/mL AZT, (B) 509 ng/mL levofloxacin, and (C) 540 ng/mL internal standard (ciprofloxacin).

antiviral agents, the specificity was assessed with regard to several HIV drugs, as well as drugs that may be used to treat opportunistic infections. Several non-prescription drugs were also evaluated as potential interferences. Table 1 shows the retention factors (k) of these drugs in order of ascending k value. As can be seen, none of the evaluated drugs interfere with AZT or levofloxacin. However, guafenesin was found to have a k very close to that of the internal standard. Therefore, this method would not be suitable for individuals who have recently used guafenesin.

Table 1. HPLC Retention Data for Compounds Evaluated as Possible Interferents

Compound	Retention Factor, k
Zalcitabine	0.25
Lamivudine	0.38
Theophylline	1.13
Acetaminophen	1.22
Caffeine	1.72
Pseudoephedrine	1.92
Zidovudine (*AZT*)	3.61
Trimethoprim	4.57
Levofloxacin	6.66
Guafenesin	7.89
Ciprofloxacin	7.96
Acetylsalacylic acid	>9.38
Ibuprofen	>9.38
Indinavir	>9.38
Naproxen	>9.38
Nevirapine	>9.38
Pyrimethamine	>9.38
Saquinavir	>9.38
Sulfamethoxazole	>9.38

Linearity

The calibration curves showed good linearity in the range of 26.3–2600 ng/mL for AZT and 51.2–5069 ng/mL for levofloxacin. The correlation coefficients (r) of calibration curves of each drug were higher than 0.996 as determined by least squares analysis. The test for lack of fit ($\alpha = 0.05$) indicated that the linear models are appropriate for establishing a relationship between the concentration and the response. No significant lack of fit was observed.

Benchtop Stability

The benchtop stability assessment showed a relatively small change in the concentrations of both drugs, with the greatest change being less than 5%. This indicates that an autosampler can be loaded with enough samples to span a 24 hr time period, with very little change in sample composition between the beginning and the end of the run. The results of the benchtop stability experiments are presented in Table 2.

Table 2. Benchtop Stability (24 hr) of AZT and Levofloxacin at Low and High Concentrations

Drug	Nominal Concentration (ng/mL)	Area Ratio at $t = 0$ (ng/mL)	Area Ratio at $t = 24$ hr (ng/mL)	Concentration Change (%)
AZT	80.3	0.1066	0.1016	−4.8
	2062	2.350	2.292	−2.5
Levofloxacin	156	0.1102	0.1100	−0.2
	4019	2.728	2.654	−2.7

Precision and Accuracy

A summary of the accuracy and precision results is given in Table 3. The method proved to be accurate (percent bias for all calibration samples varied from −6.2 to 5.6%) and precise (within-run precision ranged from 0.9 to 9.7% and between-run precision ranged from 1.3 to 7.5%). The acceptance criteria (within-run and between run %RSD's of <15% and an accuracy between 85 and 115%) were met in all cases.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD, as defined in the Experimental section, was 8.8 ng/mL for AZT and 25.0 ng/mL for levofloxacin. The lowest concentration of each calibration

Table 3. Within-Run and Between-Run Accuracy and Precision for the Analysis of AZT and Levofloxacin in Human Plasma ($n = 9$)

Drug	Nominal Concentration (ng/mL)	Measured Concentration (ng/mL)	Bias (%)	Within-Run RSD (%)	Between-Run RSD (%)
AZT	26.3	24.6	−6.2	7.5	7.5
	80.3	75.5	−6.0	6.6	7.1
	261	256	−1.9	2.5	1.3
	2062	2072	0.5	0.9	2.1
Levofloxacin	51.2	50.6	−1.2	9.7	3.1
	156	165	5.6	4.3	5.6
	509	520	2.2	2.0	5.3
	4019	4055	0.9	0.9	5.5

Table 4. Range of Calibration Curves, Limits of Detection (LOD) and Limits of Quantitation (LOQ) of AZT and Levofloxacin in Spiked Human Plasma

Drug	Range of Calibration Curves (ng/mL)	Limit of Detection (LOD) (ng/mL)[a]	Limit of Quantitation (LOQ) (ng/mL)[b]
AZT	26.3–2600	8.8	26.3
Levofloxacin	51.2–5069	25.0	51.2

[a]S/N = 3
[b]S/N = 10

graph was 26.3 ng/mL for AZT and 51.2 ng/mL for levofloxacin, which was therefore, the LOQ. LOD and LOQ data are shown in Table 4. Accuracy and precision data for the LOQ were also acceptable and are reported in Table 3.

Recovery

The results of the recovery experiments were satisfactory. The mean absolute recoveries were 94.1% for AZT, 91.2% for levofloxacin, and 84.7% for the internal standard.

Freezing and Thawing Stability

The mean of the measured concentrations after three freezing and thawing cycles were not significantly different from the data obtained in the precision and accuracy assessment. Also, the variances of the freezing and thawing data were not significantly different from equivalent levels of the precision and accuracy data. No significant deterioration was observed after three freezing and thawing cycles.

CONCLUSION

A method has been developed and validated for the determination of zidovudine (AZT) and levofloxacin in human plasma. The method combines a solid phase extraction procedure with a fast and sensitive isocratic reversed phase HPLC analysis with UV detection. The method is suitable for monitoring drug concentrations in human plasma and for pharmacokinetic studies.

REFERENCES

1. World Health Organization, HIV/AIDS/STI Surveillance. World Health Organization, Geneva, **2001**, Available at http://www.who.int/emc/diseases/hiv/index.html, (accessed January 2002).
2. Hayden, F.G. Antimicrobial Agents: Antiviral Agents. *In Goodman & Gilman's the Pharmacological Basis of Therapeutics*; Hardman, J.G., Limbard, L.E., Eds.; McGraw-Hill, Health Professions Division: New York, 1996; 1191–1223.
3. World Health Organization, Safety and Tolerability of Zidovudine. World Health Organization, Geneva, **2000**, Available at http://www.unaids.org/publications/documents/mtct/index.html, (accessed January 2002).
4. Larder, B.A.; Darby, G.; Richman, D.D. HIV with Reduced Sensitivity to Zidovudine (AZT) Isolated During Prolonged Therapy. Science **1989**, *243*, 1731–1734.
5. Fletcher, C.V.; Acosta, E.P.; Henry, K.; Page, L.M.; Gross, C.R.; Kawle, S.P.; Remmel, R.P.; Erice, A.; Balfour, H.H., Jr., Concentration-Controlled Zidovudine Therapy. Clin. Pharmacol. Ther. **1998**, *64*, 331–338.
6. Morse, G.D.; Shelton, M.J.; O'Donnell, A.M. Comparative Pharmacokinetics of Antiviral Nucleoside Analogues. Clin. Pharmacokinet. **1993**, *24*, 101–123.
7. Cohn, D.L. Prevention Strategies for Mycobacterium Avium-Intracellulare Complex (MAC) Infection. A Review of Recent Studies in Patients with AIDS. Drugs **1997**, *54*, Suppl 2, 8–15.
8. From the Centers for Disease Control and Prevention. Tuberculosis Morbidity—United States, 1996. JAMA **1997**, *278*, 1309.
9. Alangaden, G.J.; Lerner, S.A. The Clinical Use of Fluoroquinolones for the Treatment of Mycobacterial Diseases. Clin. Infect. Dis. **1997**, *25*, 1213–1221.
10. Fu, K.P.; Lafredo, S.C.; Foleno, B.; Isaacson, D.M.; Barrett, J.F.; Tobia, A.J.; Rosenthale, M.E. In Vitro and In Vivo Antibacterial Activities of Levofloxacin (l-Ofloxacin), An Optically Active Ofloxacin. Antimicrob. Agents Chemother. **1992**, *36*, 860–866.
11. Aminimanizani, A.; Beringer, P.; Jelliffe, R. Comparative Pharmacokinetics and Pharmacodynamics of the Newer Fluoroquinolone Antibacterials. Clin. Pharmacokinet. **2001**, *40*, 169–187.
12. Berning, S.E. The Role of Fluoroquinolones in Tuberculosis Today. Drugs **2001**, *61*, 9–18.
13. Macek, J.; Ptacek, P. Determination of Ofloxacin in Human Plasma Using High-Performance Liquid Chromatography and Fluorescence Detection. J. Chromatogr. B Biomed. Appl. **1995**, *673*, 316–319.

14. Wong, F.A.; Juzwin, S.J.; Flor, S.C. Rapid Stereospecific High-Performance Liquid Chromatographic Determination of Levofloxacin in Human Plasma and Urine. J. Pharm. Biomed. Anal. **1997**, *15*, 765–771.
15. Nadal, T.; Ortuno, J.; Pascual, J.A. Rapid and Sensitive Determination of Zidovudine and Zidovudine Glucuronide in Human Plasma by Ion-Pair High-Performance Liquid Chromatography. J. Chromatogr. A **1996**, *721*, 127–137.
16. Schrive, I.; Plasse, J.C. Quantification of Zidovudine and One of its Metabolites in Plasma and Urine by Solid-Phase Extraction and High-Performance Liquid Chromatography. J. Chromatogr. B, Biomed. Appl. **1994**, *657*, 233–237.

Received February 22, 2002
Accepted March 21, 2002
Manuscript 5793