Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 2, pp. 255–264, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0731-7085/94 $6.00 + 0.00

Determination of 2′-deoxy-3′-thiacytidine (3TC) in human urine by liquid chromatography: direct injection with column switching*

D.M. MORRIS† and K. SELINGER

Department of Clinical Pharmacology, Glaxo Inc. Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709, USA

Abstract: 3TC (GR109714X) is a cytidine dideoxynucleoside analogue which has been shown to have *in vitro* activity against a variety of strains of HIV-1 and is currently being investigated in clinical trials as a treatment for HIV infection. An HPLC method for the determination of 3TC in human urine has been developed and validated. The method allows direct injection of urine (10 μl) using HPLC column switching followed by UV detection. On-line extraction is performed using a Spherisorb-SCX (5 μm, 20 × 4.0 mm) eluted with deionized water at 1 ml min^{-1}. 3TC is retained while the bulk of urine constituents are eluted to waste. The SCX column is then backflushed to a BDS-Hypersil-C_{18} (5 μm, 250 × 4.6 mm) and eluted with 100 mM acetate pH 4.5–methanol (95:5, v/v) for final separation. 3TC is detected by UV absorbance at λ = 285. The quantitation range of the assay was 0.5–500 μg ml^{-1}. The method has demonstrated sufficient ruggedness to be used in support of 3TC clinical trials. Application to other cytidine analogues including DDC has been demonstrated.

Keywords: *3TC; nucleoside; urine; HPLC; direct injection; column switching.*

Introduction

3TC (Fig. 1), (GR109714X), (2*R*,*cis*)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, is a cytidine dideoxynucleoside analogue which has been shown to have *in vitro* activity against a variety of strains of human immunodeficiency virus HIV-1 and HIV-2 including zidovudine-resistant strains [1]. 3TC is currently under evaluation in clinical trials to establish the potential therapeutic use for treatment of patients infected with HIV.

A method was desired that would have sufficient sensitivity and ruggedness to support clinical trials and allow a minimum risk for those performing the method. Initial efforts with direct injection on HPLC of urine spiked with 3TC yielded usable chromatography but proved to be variable in terms of sensitivity. To support clinical trials a more rugged method was required which would use the same advantages of direct injection but with on-line (automated) sample pretreatment to enhance the sensitivity and reproducibility of the assay. HPLC column switching proved to allow the requirements above to be fulfilled.

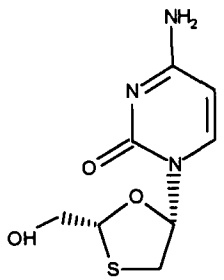

Figure 1
Structure of 3TC.

The concepts and utility of column switching and the use of multiport values to accomplish the switching have been described [2–5]. Application of these techniques have also been demonstrated for determination of compounds, including other nucleoside analogues, from biological fluids [6–8]. We describe an automated method for the determination of 3TC in human urine using two six-port column switching valves to perform sample pretreatment and final quantitative chromatography. The valve configuration allowed rapid method development and simple automation. The method allowed direct injection of urine with

*Presented at the 'Fourth International Symposium on Pharmaceutical and Biomedical Analysis', April 1993, Baltimore, MD, USA.

†Author to whom correspondence should be addressed.

UV detection and was linear over the range of 0.5–500 μg ml^{-1}. Validation of the method demonstrated acceptable reproducibility, sample throughput, and freedom of interference from other nucleoside analogues. 3TC is recovered at >85% and has been shown to be stable under the conditions of the method.

Experimental

Instrumentation

The HPLC system consisted of Waters 600 controller and fluidics unit (Millipore) modified to accommodate a Waters column heater and two, Model 7000, six-port valves with pneumatic actuators and solenoid valves (Rheodyne). The Waters 600 controller served to perform the column switching through programmed external events. The Waters 600 fluidics unit was designated pump 1 for pumping mobile phase 1 (deionized water). A Waters 510 pump (Millipore) was designated as pump 2 for pumping mobile phase 2 (100 mM acetate buffer pH 4.5–methanol, 95:5, v/v). Injection were made by a Waters model 715 Ultra-WISP (Millipore). A Waters model 484 UV absorbance detector (Millipore) was set to a wavelength of 285 nm for all analysis. Data was acquired via an HP35900A, A/D interface and converter to an HP 1000 model A900 computer (Hewlett–Packard). All chromatography was analysed using HP3350 Laboratory Automation System (LAS; rev.D.00.01) (Hewlett–Packard). Chromatograms which required reintegration were processed through automatic or manual definition using the HP19471A Advanced Graphic Chromatogram Processor (Hewlett–Packard). Linear regression calculations were made in the LAS resident Post-Run Analysis of Bioanalytical Samples software (PRANBAS; rev.02.03.00) (Glaxo Inc.). Linear regression analysis from peak area counts with $1/x^2$ weighting was used to derive the external standard curves from analyte area counts.

Chemicals

2′-deoxy-3′-thiacytidine (3TC), (GR109714X) and (Carbovir), (GR90352X) were synthesized by Glaxo Group Research (Greenford, UK). 2′,3′-dideoxycytidine (DDC), 3′-O-acetyl-2′-deoxycytidine (ADC), acycloguanosine (Acyclovir), 3′-azido-3′-deoxythymidine (AZT) was available from Sigma (St Louis, MO). Ammonium acetate and glacial acetic acid were A.R. grade from Mallinckrodt (Paris, KY). Methanol was high purity grade from Burdick & Jackson (Muskegon, MI). Deionized water was prepared by MilliQ system from Millipore (Marlborough, MA). Drug-free control urine was obtained from willing, healthy volunteers. Sparge gas was helium, high purity grade from National Specialty Gases (Durham, NC).

Preparation of mobile phase

Mobile phase 1: filtered, deionized water. Mobile phase 2 (100 mM acetate buffer pH 4.5–methanol, 95:5, v/v): 7.7 g of ammonium acetate was dissolved with 1800 ml of deionized water and 7.0 ml of glacial acetic acid. The pH was adjusted to 4.5 with glacial acetic acid and the volume adjusted to 2 l with deionized water. Acetate buffer (2000 ml) was mixed with methanol (105 ml) then filtered (0.45 μm) before use.

Preparation of 3TC urine calibration standards

A 1.0 mg ml^{-1} stock solution was made by dissolving 5.0 mg of 3TC in 5.0 ml of drug-free control urine. Calibration standard samples were prepared on the day of analysis from the 1.0 mg ml^{-1} urine stock by diluting with drug free control urine. The concentrations used for the standard curve were 0.5, 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 μg ml^{-1}.

Preparation of 3TC quality control (QC) samples

Quality control sample sets (A, B and C) were prepared in bulk (50 ml) by two chemists from independently prepared 1.0 mg ml^{-1} urine stock solutions. QC set A and B were prepared by chemist 1 and set C was prepared by chemist 2. The concentrations of each QC set were 1.5, 175 and 300 μg ml^{-1}. All QC samples were separated into 400 μl aliquots and stored at ≤−30°C until the day of analysis.

Preparation of 3TC standards in water

Calibration standards were prepared in water to be used as comparators for the determination of 3TC recovery from the method. Standards were prepared from a 1.0 mg ml^{-1} stock solution in water using the same dilutions as in the preparation of 3TC urine calibration standards.

Preparation of nucleoside analogues

A solution of 3TC and other nucleosides

(DDC, ADC, AZT, Acyclovir and Carbovir) was prepared by dissolving 100 μg of each in a single 1.0 ml of urine.

Method description

The system used the six-port valves to direct the sample flow between two HPLC columns [schematically represented in Fig. 2(A–C)]. Urine (10 μl) was injected directly onto Column 1 (Spherisorb-SCX, 5 μm, 20 × 4.0 mm). Column 1 was eluted with mobile phase 1 (deionized H_2O) at 1.0 ml min^{-1} using Pump 1. 3TC was retained while other urine components eluted through the UV detector to waste [Fig. 2(A)]. At 8 min, after the UV absorbance has returned to baseline levels, the column switching valves were actuated and 3TC was backflushed from column 1 to column 2 with mobile phase 2 (100 mM acetate buffer pH 4.5–methanol, 90:5), at 1.5 ml min^{-1} using Pump 2 [Fig. 2(B)]. Final chromatography was performed on column 2 (BDS-Hypersil, C_{18}, 5 μm, 250 × 4.6 mm) [Fig. 2(C)]. 3TC was eluted in a run time of

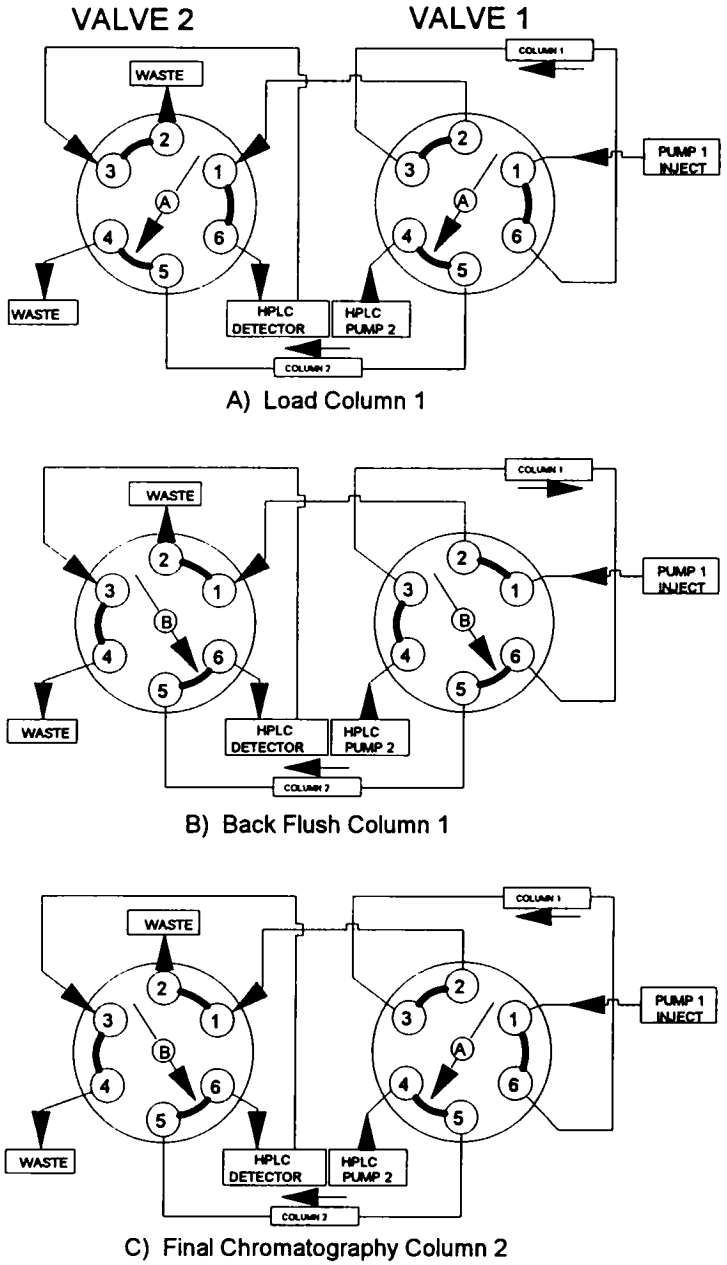

Figure 2
Schematic of configuration of the column switching valves during the stages of operation.

approximately 17 min (total run time = 20 min) with mobile phase 2 (100 mM acetate buffer pH 4.5–methanol, 90:5), at 1.5 ml min^{-1}, 45°C, with UV detection at λ = 285 nm.

Four analytical runs generated on four separate days were included in the validation. Analytical runs consisted of calibration standards (0.5, 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 μg ml^{-1}) in duplicate and two sets of quality control samples (1.5, 175.0 and 375.0 μg ml^{-1}) in duplicate. Additional urine samples were included in each analytical run. Analytical runs consisted of 96 total samples. One set of the calibration standards was analysed at the beginning of a run and a second set at the end of a run. Quality control samples and stability samples were interspersed throughout the analytical run to monitor method performance. Stability of 3TC in urine was determined using 175 and 375 μg ml^{-1} QC samples. A total of six samples of each concentration were exposed at each of the following conditions: (a) 58°C for 5 h; (b) room temperature (RT) for 4 days; (c) RT for 24 days; (d) +4°C for 24 days; and (e) −30°C for 24 days. The QC samples were analysed after exposure to the different temperatures and time periods and the area of the 3TC peaks compared to the peak areas of control QC samples from the same QC set analysed on the day in which they were prepared (day 0). Stability was expressed as a percentage difference from the control.

The recovery of 3TC from urine during the course of the column switching was tested by comparing a set of standards prepared in deionized water and injected directly onto column 2 without column switching with a set of standards in urine injected using the column switching method above. This recovery quantification provide an arbitrary index of the efficiency of the extraction of GR109714X from column 1 (SCX) to the analytical column 2 (C_{18}).

The chromatograph and controller were modified to allow a linear methanol gradient (0–15% methanol in 100 mM acetate buffer pH 4.5, v/v) to be run on the C_{18} analytical column. The solution of urine containing 100 μg ml^{-1} each of 3TC, DDC, ADC, AZT, Acyclovir and Carbovir was analysed by the same method except using the gradient for final chromatography.

Results and Discussion

Precision and accuracy

Peak area responses were measured for the quantitation of 3TC. Representative chromatography is shown in Fig. 3(a–d). The 0.5 μg ml^{-1} 3TC peak was well resolved from all endogenous peaks. Retention time for 3TC was approximately 17 min. A weighted linear regression ($1/x^2$) was used for the calibration and quantitation. Daily calibration data demonstrated acceptable precision with all calibrators yielding interpolated concentrations at >90% of the expected nominal concentration at all levels (Table 1). The calibrator concentration groups had %RSD ranging from 1.3 to 5.2 over the concentration span. All standard curves for each of the four validation runs exceeded the target correlation coefficient requirement for linearity of 0.99 with the lowest r value being 0.9984. Table 2 shows the slope, intercept and correlation coefficients for each analytical run in the validation.

All QC samples yielded interpolated concentrations within 10% of the expected values at each level however, the low (1.5 μg ml^{-1}) QC sample prepared by chemist 2 was noted to be −18% different from nominal by the end of Run 2. An additional low (1.5 μg ml^{-1}) QC sample was prepared by chemist 2 as above for QC set C and included in Run 3 and 4 as QC set D. Excluding the low (1.5 μg ml^{-1}) QC set C, all QC samples for all sets (A, B, C and D) showed interpolated concentrations at >90% of the nominal concentrations (Table 3).

Table 1
Standard curve samples

	Calibrator conc. (μg ml^{-1})						
	0.50	1.00	5.00	10.00	50.00	100.00	500.00
Mean	0.498	0.996	5.132	10.298	50.507	100.035	469.670
SD	0.0260	0.0420	0.1019	0.1461	1.2166	0.8616	5.9056
%RSD	5.2	4.2	2.0	1.4	2.4	0.9	1.3
n	7	8	8	8	8	8	8
%Nominal	99.7	99.6	102.6	103.0	101.0	100.0	93.9

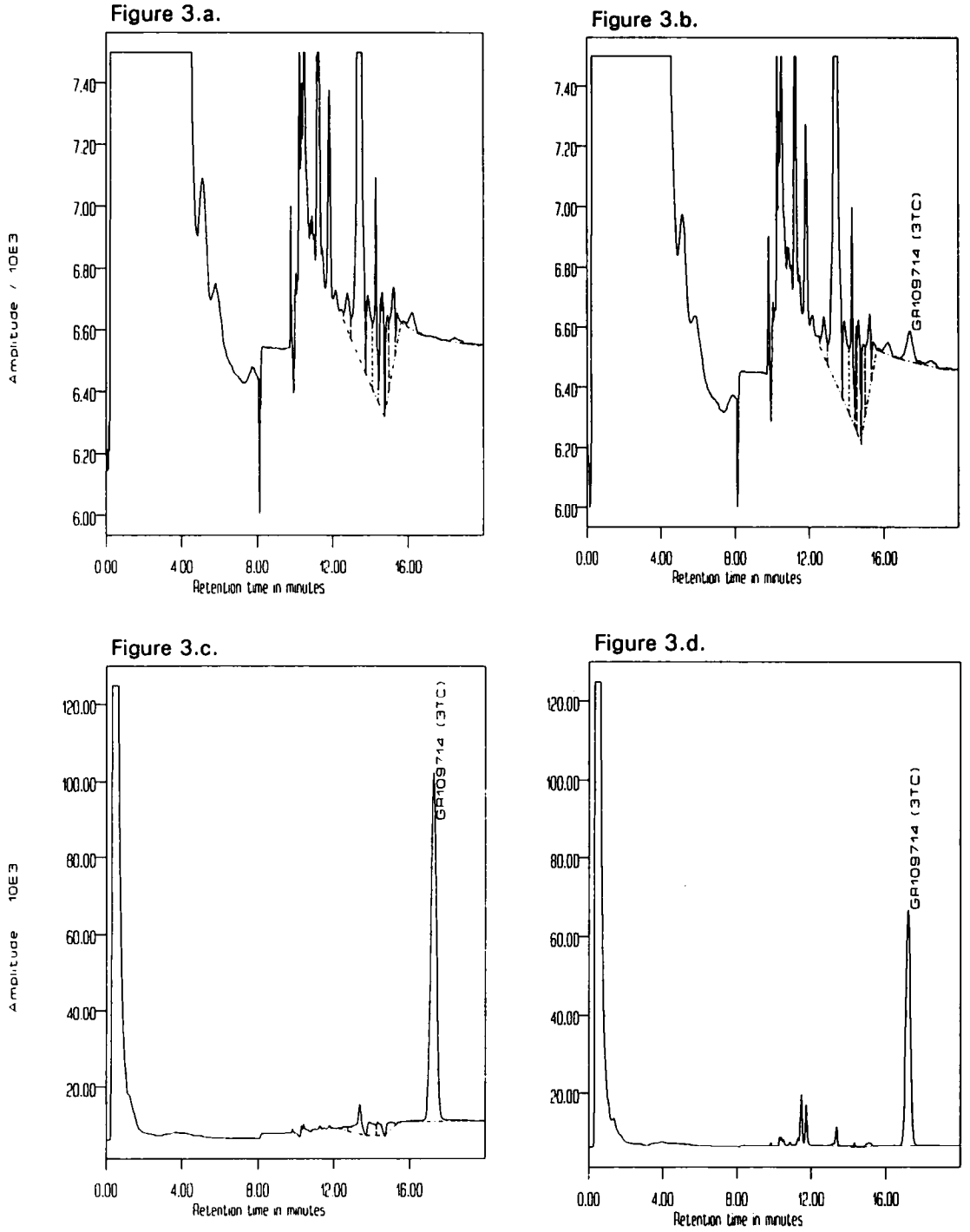

Figure 3
Representative chromatography. (a) Separation of drug-free control urine. (b) Separation of 0.5 μg ml^{-1} of 3TC in control urine. (c) Separation of 375 μg ml^{-1} of 3TC in control urine. (d) Separation of 3TC from HIV positive patient urine. Sample volume was 10 μl. Full chromatographic conditions are described in the text. Column switch from column 1 (SCX) to column 2 (C_{18}) occurred at 8.0 min. Chromatography from 0–8 min is the elution of column 1 (SCX) at 1 ml min^{-1} with deionized water. Chromatography from 8 to 20 min is the elution of column 2 (C_{18}) with 100 mM acetate buffer pH 4.5–methanol (95:5, v/v) at 1.5 ml min^{-1}.

Stability

3TC was shown to be stable in urine with <5% loss in peak area over the range of temperature and time exposures except at the extreme of 24 days at room temperature (Table 4). Run time for a full 96 sample analytical run was approximately 32 h. Loss of 3TC at room temperature was not deemed to present a problem as on-system exposure time is well under the 4 day time period. Exposure of 3TC

Table 2
Calibration curve regression ($1/x^2$ weighting)

Run no.	Slope (area μg^{-1})	Intercept (area)	R
1	9786.04	2743.80	0.99918
2	9910.59	1011.86	0.99919
3	9640.98	712.70	0.99893
4	9867.70	956.51	0.99840

urine samples to room temperature for 4–24 days is not recommended. Refrigeration of the autosampler was not used for these exercises, but could prove beneficial in the event of inadvertent extensions of the on-system run time. Stability of 3TC in urine at 58°C was important to determine as all HIV positive clinical samples are heat inactivated for 5 h at 58 ± 2°C prior to analysis to reduce the risk of exposure to the virus [9, 10].

Recovery

A set of calibration standards were prepared in deionized water and 10 μl of each sample was injected onto column 2 (BDS-Hypersil-C_{18}). The system was eluted with mobile phase 2. A regression analysis ($1/x^2$) was performed using the peak areas of these standards in water. A set of urine calibration standards was then analysed with column switching as described above. Concentrations of the urine calibration standards were interpolated from the regression of the standards in water. The interpolated concentrations for the urine standards were compared with the corresponding nominal concentrations and reported as per cent recovered. Recoveries ranged from 89% at 0.5 μg ml^{-1} to 105% at 50 μg ml^{-1} (Table 5). This measurement of the recovery involved altering the plumbing of the valves in order to run the standards prepared in water on the C_{18} alone and then replumbing for the urine standards. The replumbing of the system with the accompanying peak-shape differences between direct injection to column 2 and direct injection with column switching from column 1 to column 2, did not appear to effect the recovery determination.

Selectivity

Prior to validation, drug-free control urine samples were screened for possible interferences at the elution time of 3TC. Urine samples from four normal donor samples and from six clinical subject predose samples were analysed by the method. Chromatography at the elution time of 3TC was free fo any interferences which could be misinterpreted as drug. It should be noted that analysis of 3TC in urine from HIV positive subjects occasionally revealed a different chromatographic profile with more peaks than in urine analysed from normal, healthy subjects. In an effort to reduce the potential for interfences from these other minor peaks, the UV wavelength λ = 285 nm was chosen. Previously, the UV absorbance maxima for 3TC of λ = 270 nm has been used for the analysis of 3TC in serum [11]. It is not clear whether these occasional peaks from HIV positive samples are a result of concomitant drug therapy or a result of the disease state in general, but the use of a slightly longer wavelength seemed to improve selectivity for 3TC.

Analysis of the solution of nucleoside analogues revealed no interferences. Figure 4(b) shows the elution profile of the mixed nucleoside solution. Acyclovir and AZT were eluted in the solvent front with urine constituents from the SCX column so are not named in the chromatogram. Using the SCX as column 1 for sample pretreatment provides an effective filter for compound which cannot interact with the anion support and greatly reduces the interference from other urine constituents.

The use of the cation exchanger as automated sample pretreatment for 3TC and other cytosine nucleoside structures is effective. Ideally, the sample pretreatment step should proceed in a binary fashion. It was found in prevalidation exercises that the volume of urine injected onto the SCX column had an effect on the recovery and on the peak shape of the recovered peak. Additionally, similar poor recovery and/or poor peak shape was noted when using some urine but not others. It appeared that the ionic strength of the urine and/or the volume injected, and hence the overall ionic burden on the SCX column, had an important effect on the retention of 3TC during sample pretreatment. As injection volume increased, it is speculated that, the retention of 3TC during the sample pretreatment step becomes less of a binary cation exchange mechanism and more of a mixed-mode mechanism. Urine injection volumes up to 100 μl have been used successfully, however 10 μl was chosen to allow for the variation which is expected in urine samples from clinical

Table 3
Assay precision, quality control concentrations

	Set A			Set B			Set C			Set D
Run no.	1.50	175.00	375.00	1.50	175.00	350.00	1.50	175.00	375.00	1.50
1	1.48	169.64	359.33	1.52	170.06	359.60	1.18	167.73	355.02	NS
	1.38	174.70	365.07	1.54	174.65	368.44	1.18	172.44	364.34	NS
2	1.48	173.74	358.95	1.55	173.16	365.79	1.28	170.94	363.27	NS
	1.47	171.29	362.21	1.56	170.98	365.41	1.30	170.23	359.66	NS
3	1.51	176.22	368.56	1.56	171.72	370.69	1.30	174.41	364.09	1.55
	1.50	175.57	368.17	1.52	174.68	366.86	1.32	171.50	362.25	1.46
4	1.44	171.32	360.21	1.55	168.89	359.88	1.32	167.68	353.46	1.59
	1.42	168.79	357.96	1.51	171.24	362.72	1.28	168.20	357.83	1.45
Mean	1.459	172.659	362.557	1.538	171.924	346.924	1.271	170.391	359.990	1.515
SD	0.0423	2.7835	4.2091	0.0177	2.0897	3.9455	0.0571	2.4244	4.2030	0.0647
%RSD	2.9	1.6	1.2	1.2	1.2	1.1	4.5	1.4	1.2	4.3
n	8	8	8	8	8	8	8	8	8	4
%Nominal	97.3	98.7	96.7	102.5	98.2	97.3	84.7	97.4	96.0	101.0

NS = no sample. Quality control (QC) samples were analysed in each of four validation runs. QC sample sets (A, B, C and D) were prepared in bulk (50 ml) by two chemists from independently prepared 1.0 μg ml^{-1} 3TC-urine stock solutions and stored frozen until analysed.

Table 4
Stability of 3TC in human urine

Condition		Conc. (μg ml^{-1}) 175.0	375.0
Control	Mean	1665852.33	3531476.00
(Pk area)	SD	11309.312	13594.498
	%RSD	0.7	0.4
	n	6	6
58°C	Mean	1674632.8	3537728.7
5 h	SD	10439.6	22047.0
(Pk area)	%RSD	0.6	0.6
	n	6	6
	%Difference	0.5	0.2
Room temp.	Mean	1590322.5	3384745.2
4 days	SD	12480.7	14198.6
(Pk area)	%RSD	0.8	0.4
	n	6.0	6.0
	%Difference	−4.5	−4.2
Room temp.	Mean	1321817.8	2777131.7
24 days	SD	14497.6	29842.0
(Pk area)	%RSD	1.1	1.1
	n	6.0	6.0
	%Difference	−21.1	−21.9
4°C	Mean	1675732.7	3556702.8
24 days	SD	5193.3	37541.1
(Pk area)	%RSD	0.3	1.1
	n	6.0	6.0
	%Difference	0.0	0.1
−30°C	Mean	1655736.7	3490794.3
24 days	SD	6339.3	20226.9
(Pk area)	%RSD	0.4	0.6
	n	6.0	6.0
	%Difference	−1.2	−1.8

Quality control samples were analysed after exposure to the conditions indicated. The peak areas of 3TC from the exposed QC samples were compared with the 3TC peak areas from control QC samples. Stability is expressed as the % difference as compared to stability control samples (day 0). A % difference of ±5% was considered as acceptable stability.

Table 5
Recovery of 3TC from urine during sample pretreatment, $n = 4$

Urine standard conc.	%Recovery Mean	SD	%RSD
0.5 (μg ml^{-1})	88.9	6.6530	7.5
1.0 (μg ml^{-1})	97.0	3.6954	3.8
5.0 (μg ml^{-1})	95.5	1.1961	1.3
10.0 (μg ml^{-1})	104.9	0.4522	0.4
50.0 (μg ml^{-1})	99.9	0.5550	0.6
100.0 (μg ml^{-1})	103.8	0.3245	0.3
500.0 (μg ml^{-1})	99.2	0.4359	0.4

Recovery of 3TC from urine during the course of the column switching was tested by comparing a set of standards prepared in deionized water and injected directly onto column 2 without column switching with a set of standards in urine injected using the column switching method above.

studies, while still allowing sufficient volume for sensitivity. Further experiments are necessary to fully understand the retention mechanisms of 3TC on the cation exchange column.

Safety considerations

Column switching offered the advantage that sample pretreatment could be performed on-line. This allowed a significant reduction in the risk of exposure to HIV when compared to methods which might be more manual handling intensive. HIV positive samples were handled with necessary Universal Precautions [12] at all time, but exposure risk for this assay was limited to a single step transfer of an aliquot of the sample from the shipping tube to the autosampler vial following the 5 h heat inactivation period note above.

Figure 4.a.

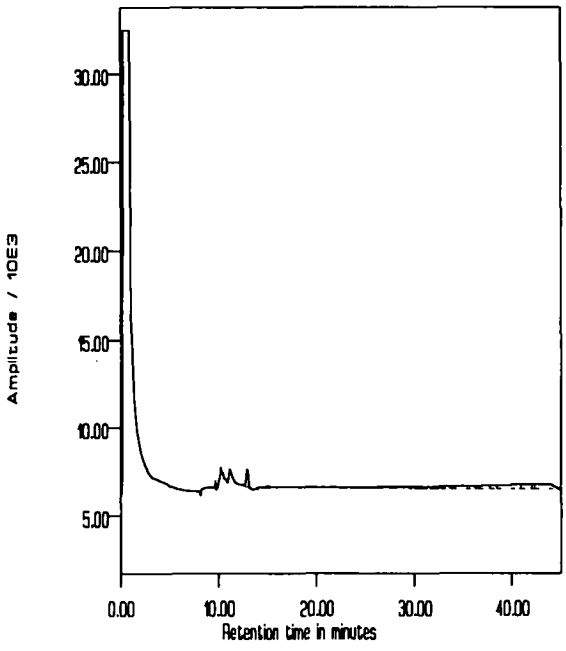

Figure 4.b.

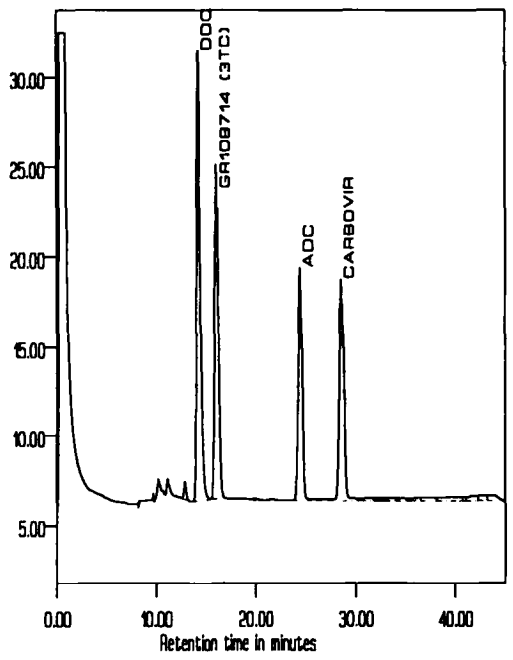

Figure 4
Separation of a nucleoside mixture. (a) Separation of drug-free control urine. (b) Separation of control urine containing 100 μg ml^{-1} each of 3TC, DDC, ADC, AZT, Acyclovir and Carbovir. Sample volume was 10 μl. Full chromatographic conditions are described in the text. Column switch from column 1 (SCX) to column 2 (C_{18}) occurred at 8.0 min. Chromatography from 0 to 8 min is the elution of column 1 (SCX) at 1 ml min^{-1} with deionized water. Chromatography from 8 to 20 min is the elution of column 2 (C_{18}) with a linear methanol gradient (0–15% methanol in 100 mM acetate buffer pH 4.5, v/v) at 1.5 ml min^{-1}. AZT and Acyclovir were not retained by column 1 (SCX) and are not identified in the chromatogram.

Clinical study utility

This automated method, has been subsequently used in the analysis of 3TC in urine from samples for a phase-I, single center, open-label, cross-over study to determine the pharmacokinetics of GR109714X (3TC) in asymptomatic, HIV positive patients following oral doses (50 mg) with and without food. Sample collection intervals were predose, 0–4, 4–8, 8–12, 12–16 and 16–24 h. Concentrations in urine samples from this study were over a range of 0–148 μg ml^{-1}. Results of this study have determined that the amount of 3TC excreted in the urine remains unchanged with food, A_e (% of dose) = 69 ± 11% vs 70 ± 10% (n = 10, P = 0.2267) [13].

Conclusions

An assay for the determination of 3TC in human urine has been validated which uses direct on column injection and column switching. The method is linear over the concentration range of 0.5–500 μg ml^{-1}. The assay has been shown to be rugged and sensitive enough to support clinical studies. To date, >800 urine samples have been analysed from HIV positive subjects in other ongoing clinical studies for 3TC using this method.

The utility of column switching in automated analysis of compounds in biological fluid is quite powerful. In the configuration described, column switching offers: (1) rapid assay development by virtue that the chemist may observe the elution of both columns by the same detector; (2) increased life of the analytical column 2 by virtue that the sample is pretreated by column 1; (3) greater selectivity by virtue of the different chemistry which can be used for column 1 and column 2; and (4) and offers reduced risk when analysing samples of biohazardous nature by virtue of the freedom from manual manipulations.

Acknowledgements — The authors gratefully acknowledge the assistance of Scott P. Depee for his technical assistance in the laboratory and of James Williams for his efforts with the graphics design of the valve figure.

References

[1] J.A.V. Coates, N. Cammack, H.J. Jenkinson, I.M. Mutton, B.A. Pearson, R. Storer, J.M. Cameron and C.R. Penn, *Antimicrob. Agents Chemother.* **36**, 202–205 (1992).
[2] M.C. Harvey and S.D. Sterns, in *Liquid Chromatography in Environmental Analysis* (J.F. Lawrence, Ed.), pp. 301–340. Humana Press, Clifton, New Jersey (1983).
[3] C.J. Little and O. Stahel, *Chromatographia* **19**, 322–326 (1984).
[4] K.A. Ramsteiner, *J. Chromatogr.* **456**, 3–20 (1988).
[5] M.J. Koenigbauer and R.E. Majors, *LC–GC* **8**, 510–514.
[6] S.R. Binder, M. Regalia, M. Biaggi-McEachern and M. Mazhar, *J. Chromatogr.* **473**, 325–341 (1989).
[7] B. Kuo, A. Mandagere, D.R. Osborne and K. Hwang, *Pharm. Research* **7**, 1257–1261 (1990).
[8] L. Mathes, *J. Chromatogr.* **432**, 346–351 (1988).
[9] J.S. McDougal, L.S. Martin, S.P. Cort, M. Mozen, C.M. Heldebrant and B.L. Evatt, *J. Clin. Invest.* **76**, 875–877 (1992).
[10] L. Resnick, K. Veren, S.Z. Salahuddin, S. Tondreau and P.D. Markham, *JAMA* **255**, 1887–1891 (1986).
[11] A. Harker, G.L. Evans, D.M. Morris and A.E. Hawley, Published Abstract, VIII International Conference on AIDS/III STD World Congress, Amsterdam.
[12] National Committee for Clinical Laboratory Standards (NCCLS), Protection Of Laboratory Workers From Infectious Disease Transmitted By Blood, Body Fluids, And Tissue, NCCLS Document M29-T2, 11(14) (1991).
[13] J.B. Angel, E.K. Hussey, S.T. Hall, K.H. Donn, D.M. Morris, I.P. McCormack, J.S.G. Montaner and J. Ruedy, *AIDS*, in press.

[Received for review 19 April 1993; revised manuscript received 15 July 1993]