

Is Your Paclitaxel Method HIV Positive?

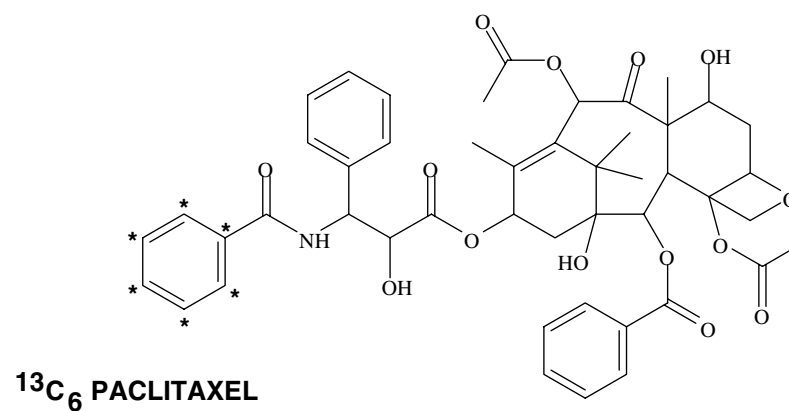
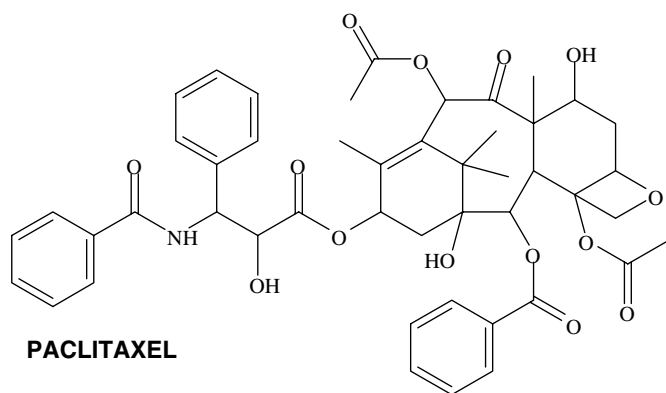
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Introduction and Purpose

BASi has analyzed thousands of samples for paclitaxel with a limit of quantification of 0.1 ng/mL and lower. This assay is performed using LC-MS/MS to detect paclitaxel and a high-purity isotopically-substituted $^{13}\text{C}_6$ -paclitaxel as the internal standard.

This study was conducted to verify non-interference of common HIV and over-the-counter drugs in the sensitive LC-MS/MS method for paclitaxel in human plasma. Also, the study investigated the stability of paclitaxel to heat treatment to deactivate the HIV virus.

Structures of Paclitaxel and Internal Standard



Method

The method of analysis used for these tests was previously reported¹. In short, a 0.400 mL plasma sample is fortified with a stable-label internal standard, extracted into MTBE, evaporated and reconstituted. The HPLC conditions consist of a 2.1 x 50 mm SB-C₁₈ column maintained at 50°C with a flow rate of 0.2 mL/min. The pseudo-molecular ions of thirteen HIV drugs (amprenavir, delavirdine, efavirenz, indinavir, lopiravir, naltiravir, nevirapine, saquinavir, D4T, 3TC, abacavir, zidovudine, and tipranavir) and a panel of OTC drugs or their major metabolites (acetaminophen, caffeine, cotinine, nicotine, salicylic acid, ibuprofen, ketoprofen and naproxen) were monitored under the conditions of the assay. Additionally, plasma samples containing 0, 0.3 or 100 ng/mL paclitaxel were heat-treated at 56°C for 30 min prior to being extracted. This procedure has been reported to inactivate the HIV virus.

¹ Alexander, M.S., Kiser M.M., Culley T., Kern J.R., Dolan J.W., McChesney J.D, Zygmunt J., Bannister S.J. (2002). Measurement of paclitaxel in biological matrices: high-throughput liquid chromatographic-tandem mass spectrometric quantification of paclitaxel and metabolites in human and dog plasma. *Journal of Chromatography B* (785, 253-261).

MS/MS

Mass spectrometric analysis was performed on a MicroMass Quattro Ultima triple quadrupole mass spectrometer using an electrospray interface. The MS was operated in positive ion mode. Detection was by multiple reaction monitoring observing the following transitions:

Paclitaxel	854 > 286 amu
$^{13}\text{C}_6$ Paclitaxel	860 > 292 amu

Results

None of the HIV or OTC drugs tested eluted at the retention time of interest. No interferences were seen in the analyte or internal standard mass spectral channels. Heat treatment of the plasma resulted in no interferences in the blanks and acceptable accuracy (deviation from nominal <6%) and precision (CV<10%).

Paclitaxel Validation Sample Results

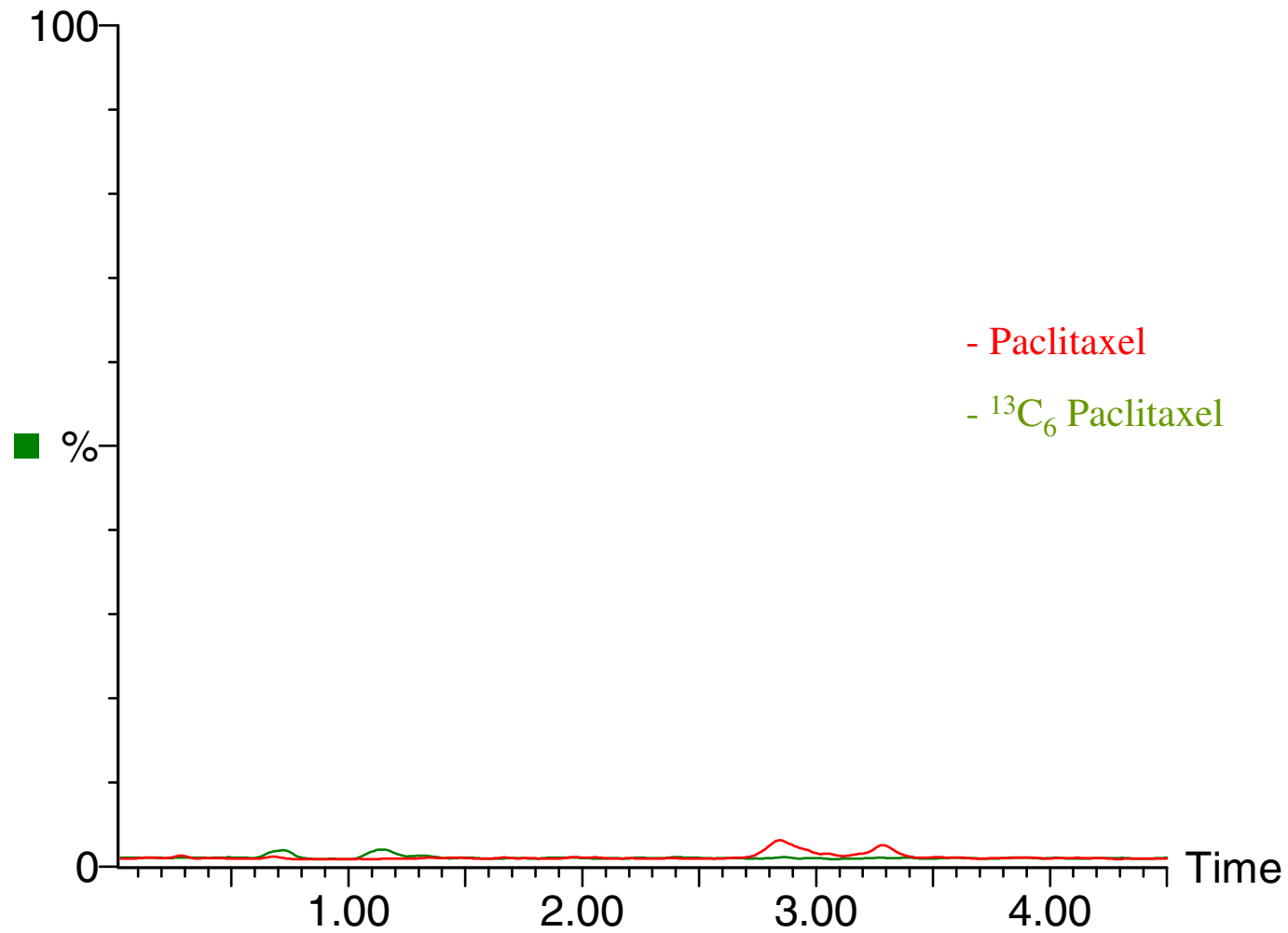
Nominal Concentration (ng/mL)	0	0.1	0.3	30	100
Calculated Concentration (ng/mL)	nd	0.101	0.294	32.5	100
Run 1 (030514K1)	nd	0.103	0.323	29.8	101
	nd	0.103	0.266	32.6	95.6
	nd	0.147*	0.294	30.2	99.8
	nd	0.0996	0.315	32.7	100
	nd	0.102	0.300	31.8	95.7
Within-run Statistics					
N	6	5	6	6	6
Average (ng/mL)	na	0.102	0.299	31.6	98.7
SD (ng/mL)	na	0.00150	0.0195	1.28	2.36
Deviation (%)	na	1.7	-0.43	5.4	-1.3
CV (%)	na	1.5	6.5	4.0	2.4

nd = no peak detected or peak detected <20% of LLOQ

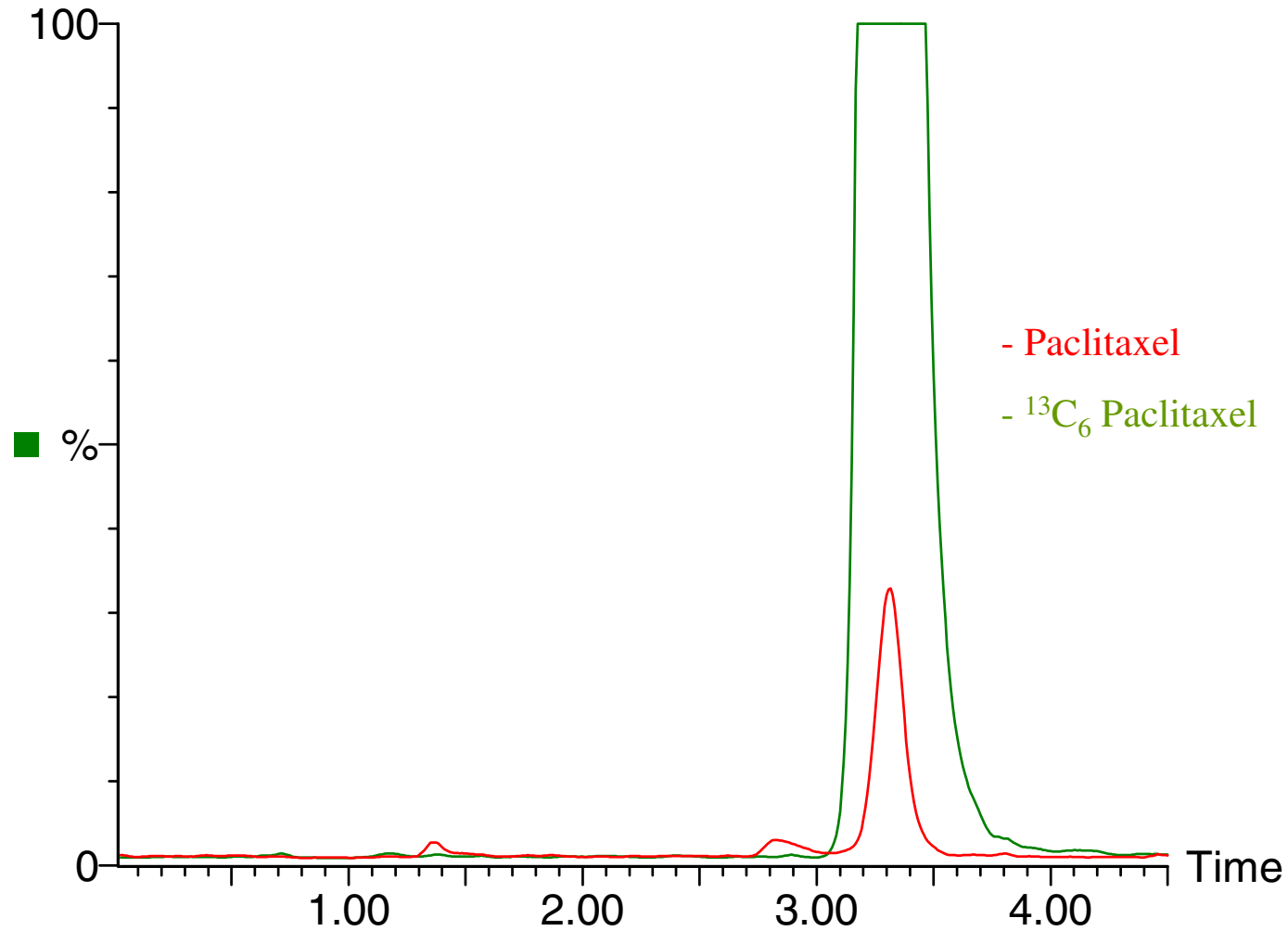
na = not applicable

* Sample excluded based on Q-Test, not used in statistics

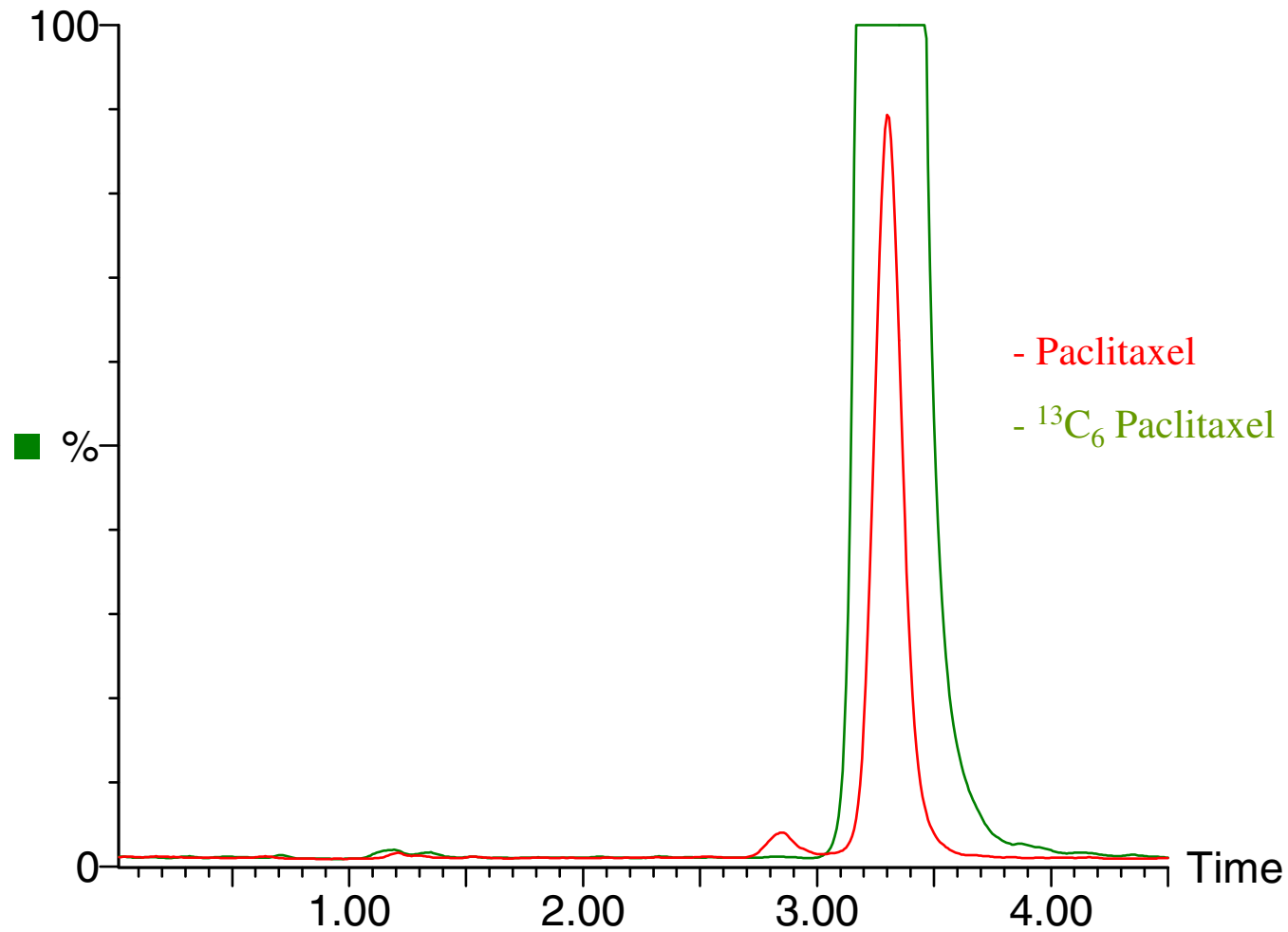
Typical Blank MRM Chromatogram



Typical LLOQ MRM Chromatogram, 0.1 ng/mL



Typical Low QC Chromatogram, 0.3 ng/mL



Drugs Tested for Interference

Drug Tested	Concentration Tested (µg/mL)	Retention Time (Minutes)
Paclitaxel and IS	NA	3.34
Acetaminophen	50	<2.00
Caffeine	50	<2.00
Cotinine	50	<2.00
Nicotine	0.05	<2.00
Salicylic Acid	50	<2.00
Ibuprofen	10	<2.00
Ketoprofen	0.02	1.85
Naproxen	50	<2.00
Amprenavir	1	1.78
Delavirdine	1	1.38
Efavirene	1	4.21
Indinavir	1	0.93
Lopiravir	1	4.24
Naltiravir	1	1.27
Nevirapine	1	1.03
Saquinavir	1	1.20
D4T	1	2.02
3TC	1	0.73
Abacavir	1	0.73
Zidovudine	1	1.07

Heat Treatment Stability of Paclitaxel in Human KEDTA Plasma

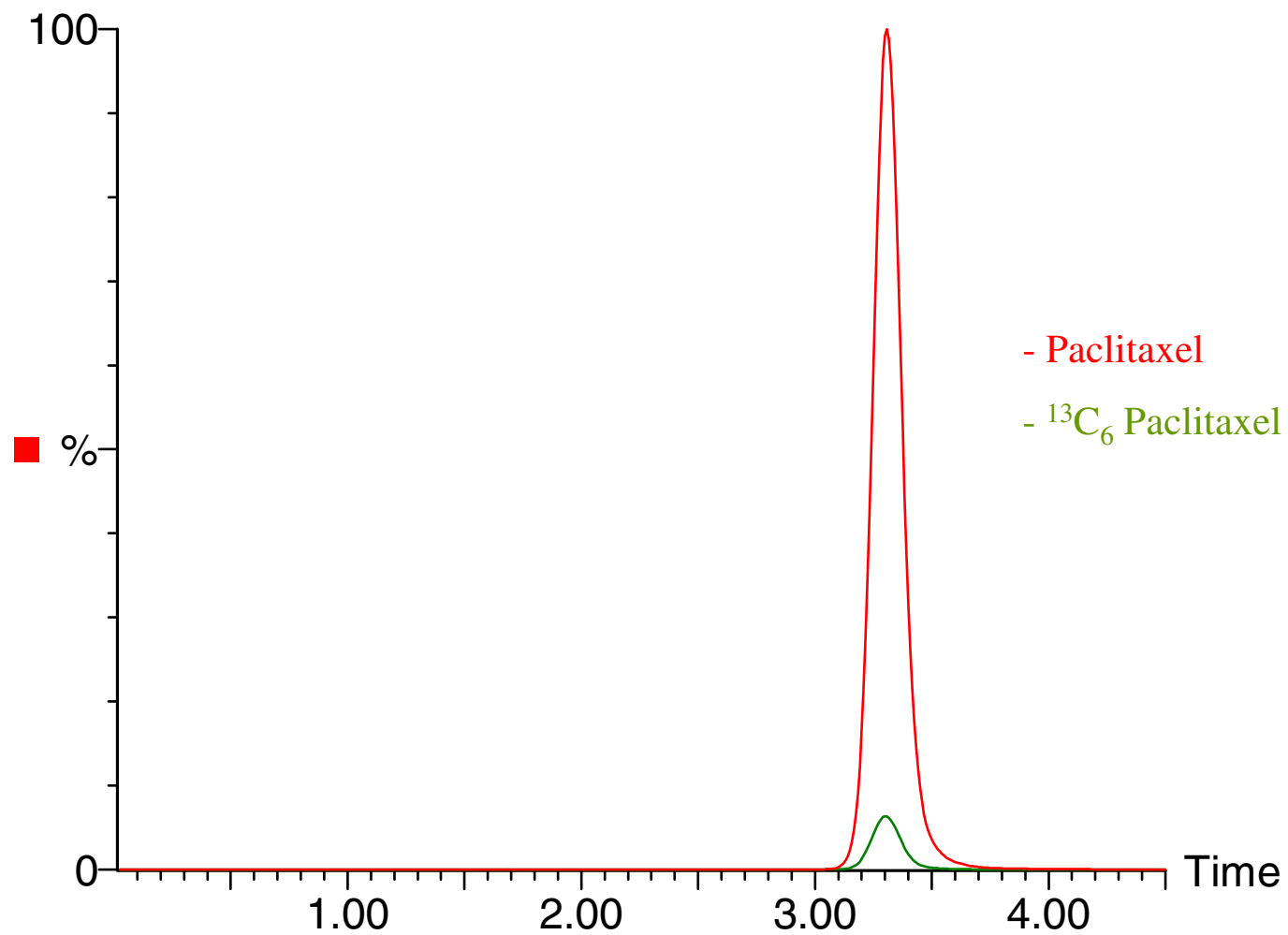
Nominal Concentration (ng/mL)	0	0.3	100
Calculated Concentration (ng/mL)	nd	0.273	93.0
Run 1 (030514K1)	nd	0.276	93.0
	nd	0.331	102
	nd	0.327	90.3
	a	0.282	98.2
	nd	0.275	91.2
Summary Statistics			
N	6	6	6
Average (ng/mL)	na	0.294	94.6
SD (ng/mL)	na	0.0271	4.59
Deviation (%)	na	-2.0	-5.4
CV (%)	na	9.2	4.8

nd = no peak detected or peak detected <20% of LLOQ

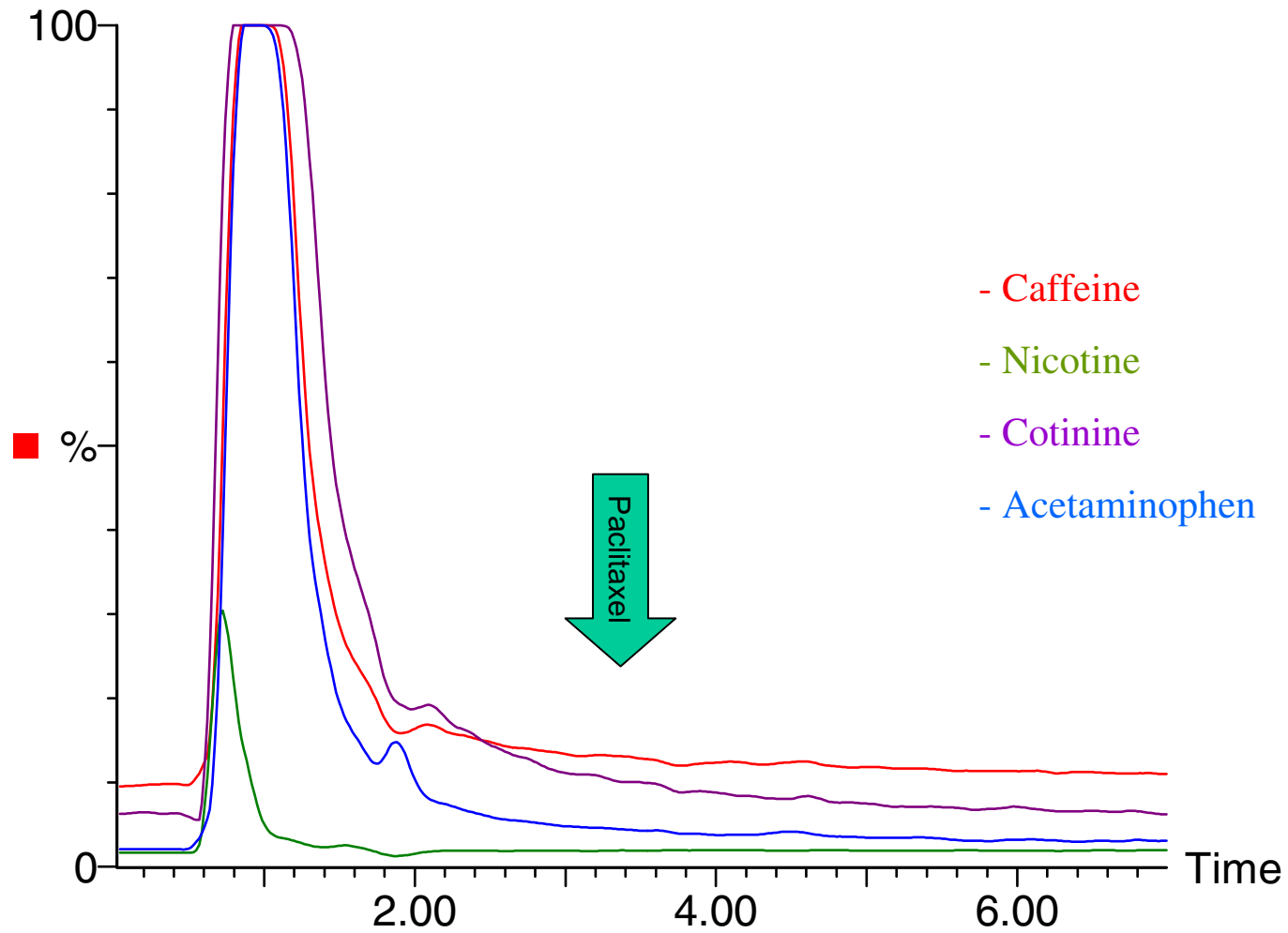
na = not applicable

a = peak detected > 20% of LLOQ

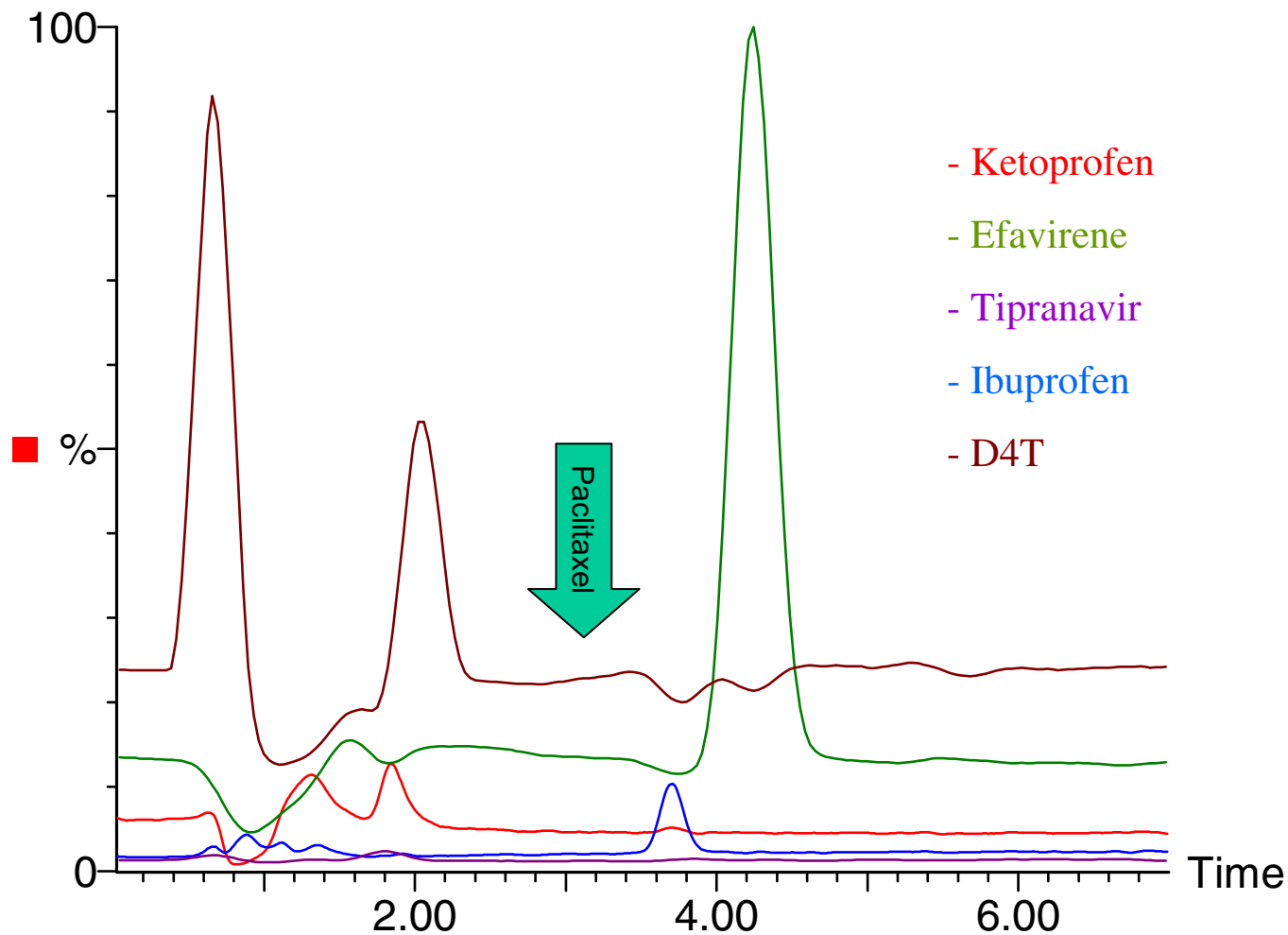
Typical High QC Chromatogram, 100 ng/mL



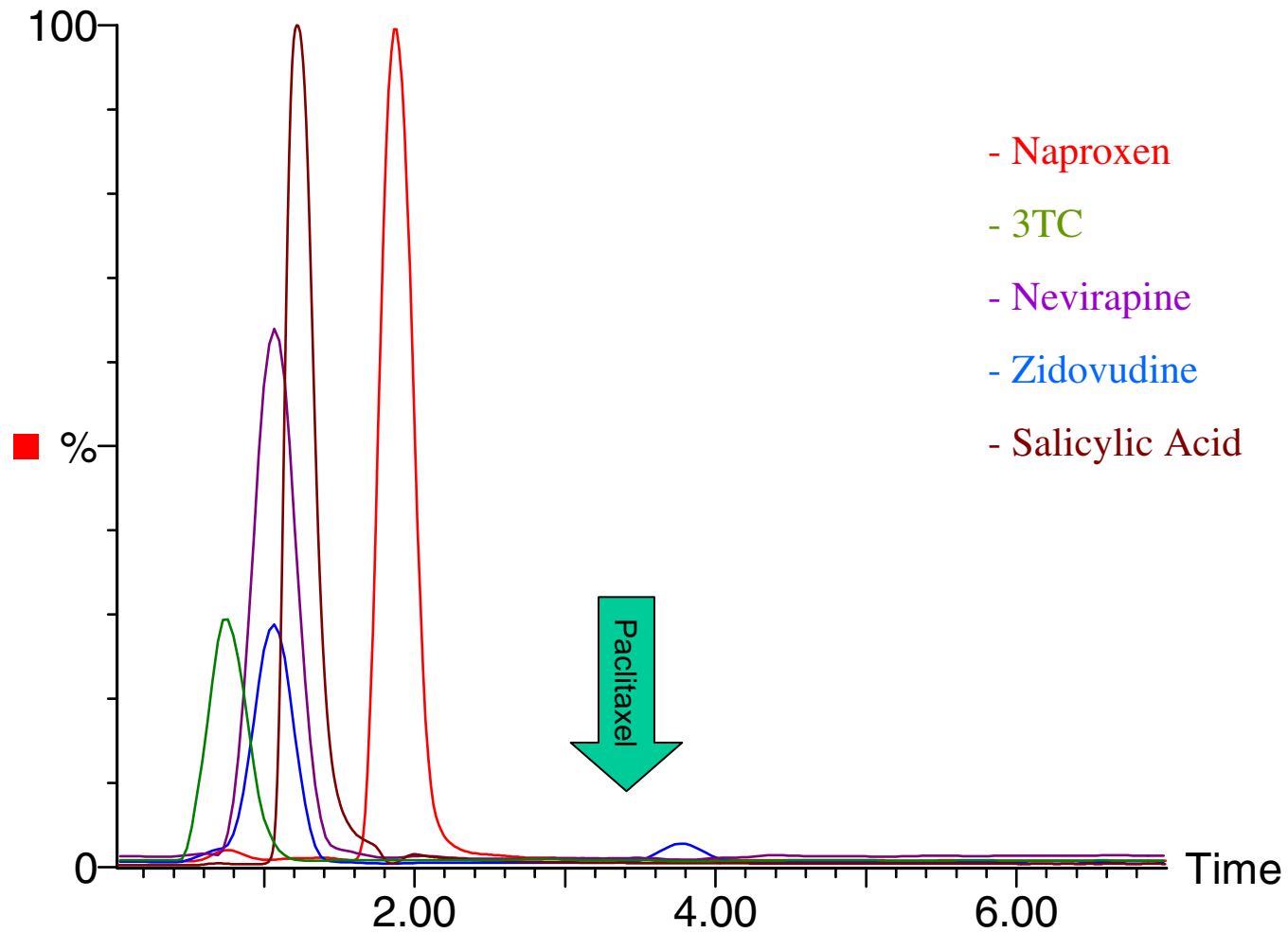
Interference Testing Results



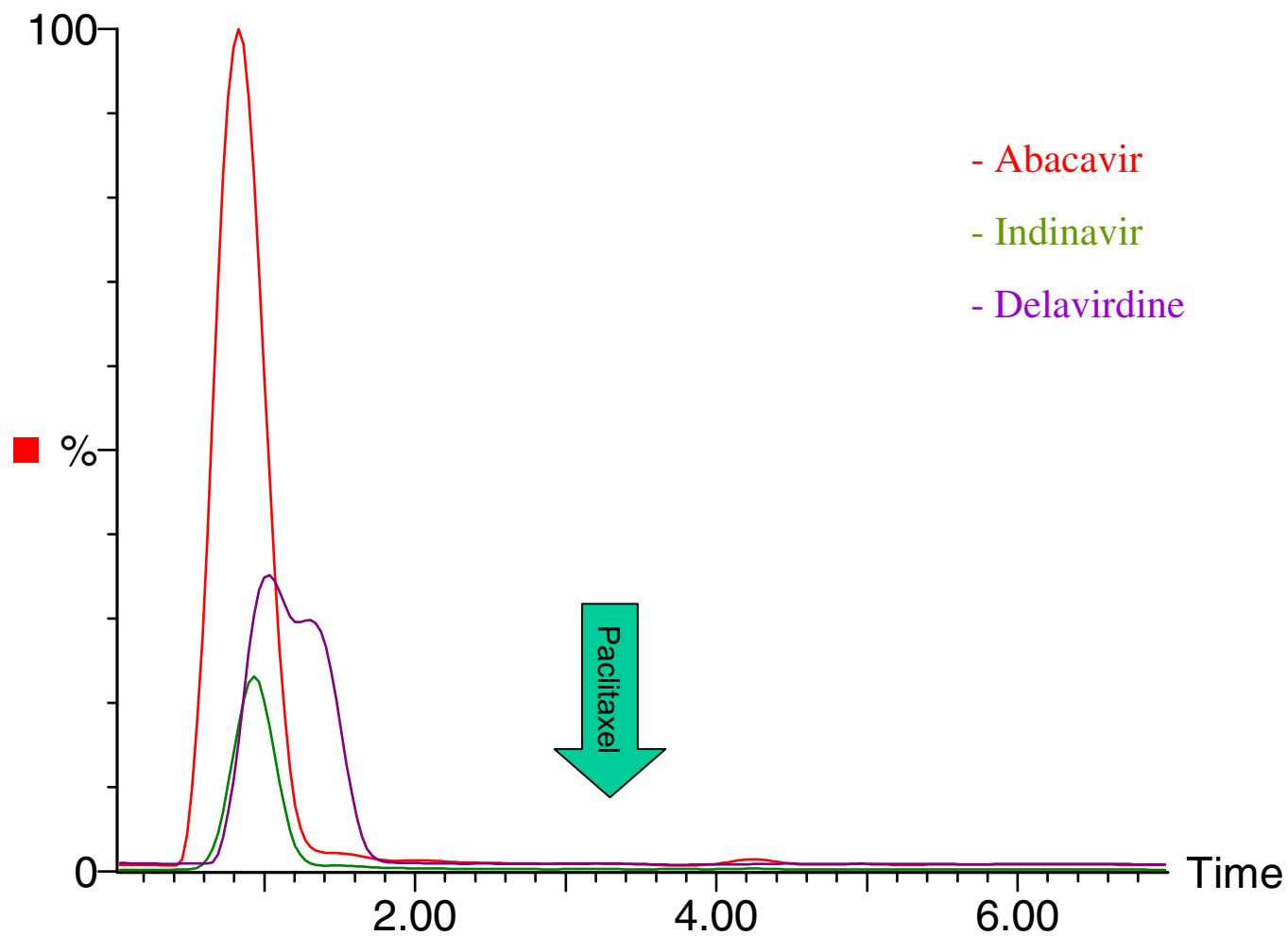
Interference Testing Results



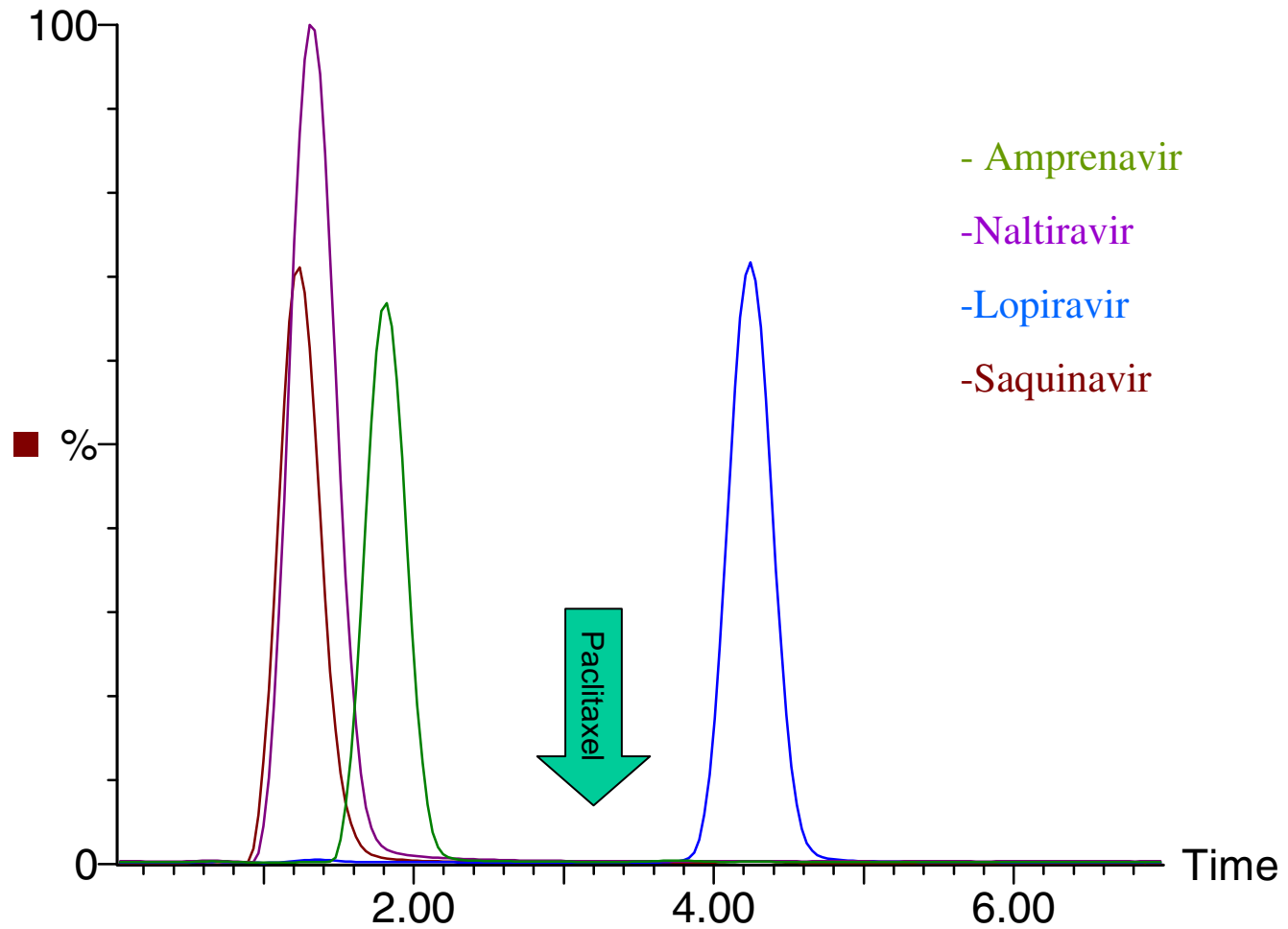
Interference Testing Results



Interference Testing Results



Interference Testing Results



Conclusions

The presence of physiologically relevant concentrations of HIV or OTC drugs did not interfere with a sensitive LC-MS/MS assay for paclitaxel in human plasma. The assay has a validated LLOQ of 0.1 ng/mL.

Treatment of samples with heat to inactivate the HIV virus prior to sample analysis also did not interfere with the quantification of paclitaxel in these samples. This method has been used to analyze samples from clinical studies.