Laser Diode Thermal Desorption (LDTD) -MS/MS Analysis of Chloroquine, Doxycycline, Proguanil and Mefloquine in Human Urine

Tandem LABS™
A LabCorp Company

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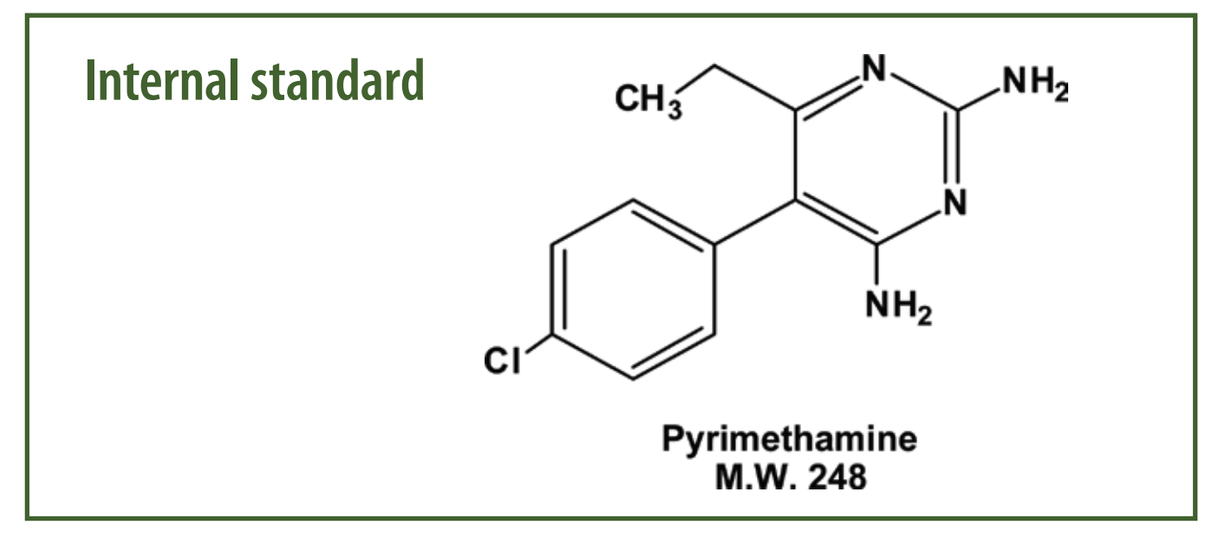
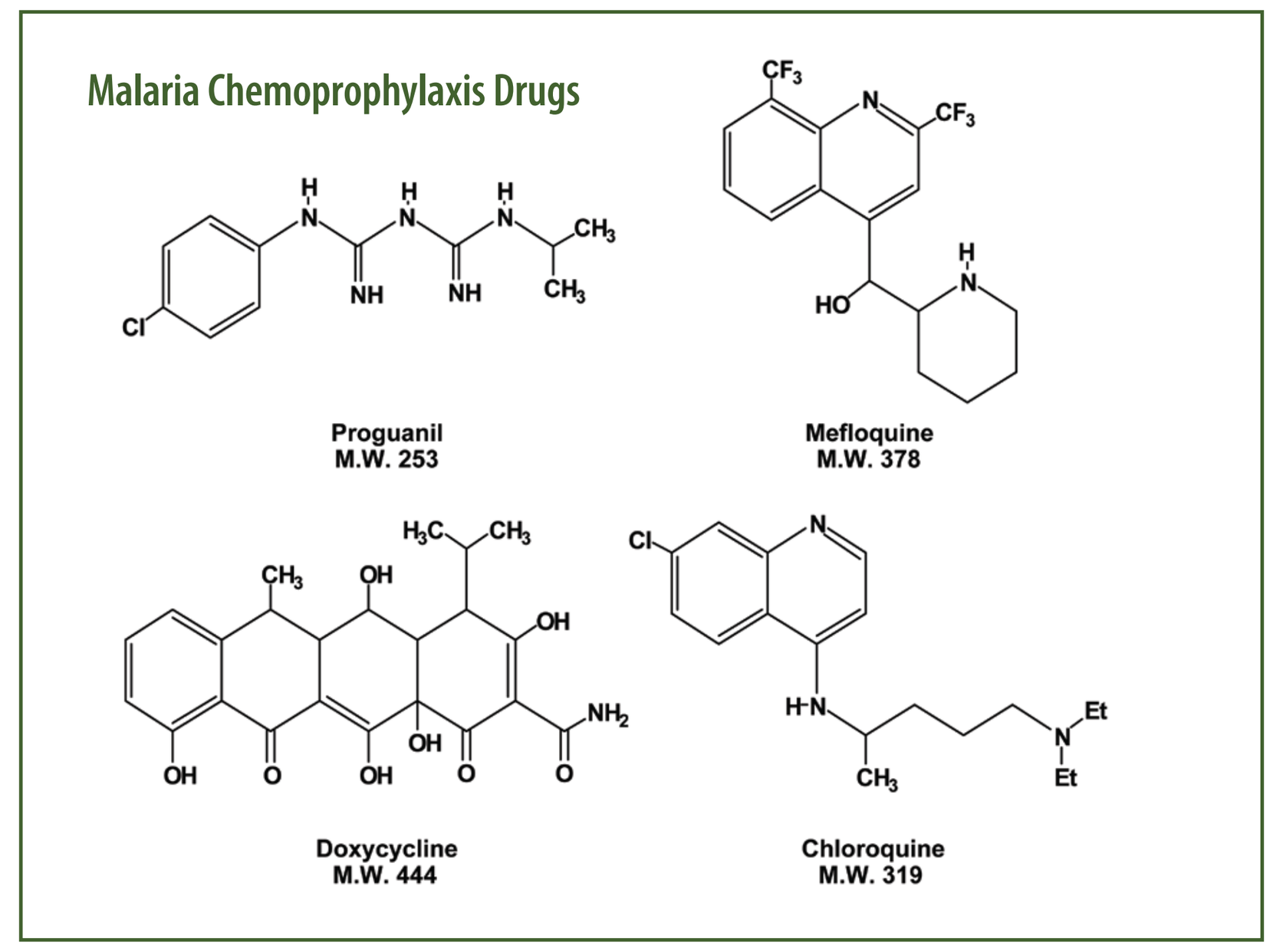
Introduction

The analysis for the anti-malarial drugs Chloroquine, Doxycycline, Proguanil and Mefloquine in human urine is currently performed by Liquid Chromatography-MS/MS analysis. The limitations of this analysis are lengthy run-time, persistent carryover from autosampler and LC column, and numerous dilution requirements. We propose to use the Laser Diode Thermal Desorption (LDTD) ion source coupled with MS/MS instead of the traditional LC/ Electrospray Ionization Source (ESI) combined with tandem mass spectrometry. Because the LDTD does not require traditional chromatography, there is no true autosampler or LC system, thus eliminating carryover. In addition, sample run-time is reduced to less than 20 seconds per sample, vastly increasing sample throughput.

Two sets of human urine quality control (QC) samples (n=23) were analyzed with the two different methods, LDTD/MS/MS and LC/ESI-MS/MS, to evaluate the feasibility of switching from the chromatographic/ESI analysis method to an LDTD-MS/MS method for a specific non-GLP project. Because this analysis is semi-quantitative in nature and reports results above and below a pre-determined cut-off, the results of this evaluation were also compared primarily for determination of positive or negative relative to the cut-off threshold, and secondarily for accuracy of the quantitative concentration. These QC samples had been pre-prepared to contain different levels of the 4 common anti-malarial compounds: Chloroquine, Doxycycline, Proguanil and Mefloquine. Pyrimethamine is used as internal standard (IS) in both analyses.

Analyte and IS Structures





Sample Preparation for LDTD-MS/MS Analysis

CHLOROQUINE, PROGUANIL AND MEFOQUINE

1. Aliquot 25 µL sample
2. Dilute 1:1 with 25 µL Methanol/Water(75/25)
3. Vortex 10 seconds
4. Centrifuge 5 minute
5. Analyze 2 µL

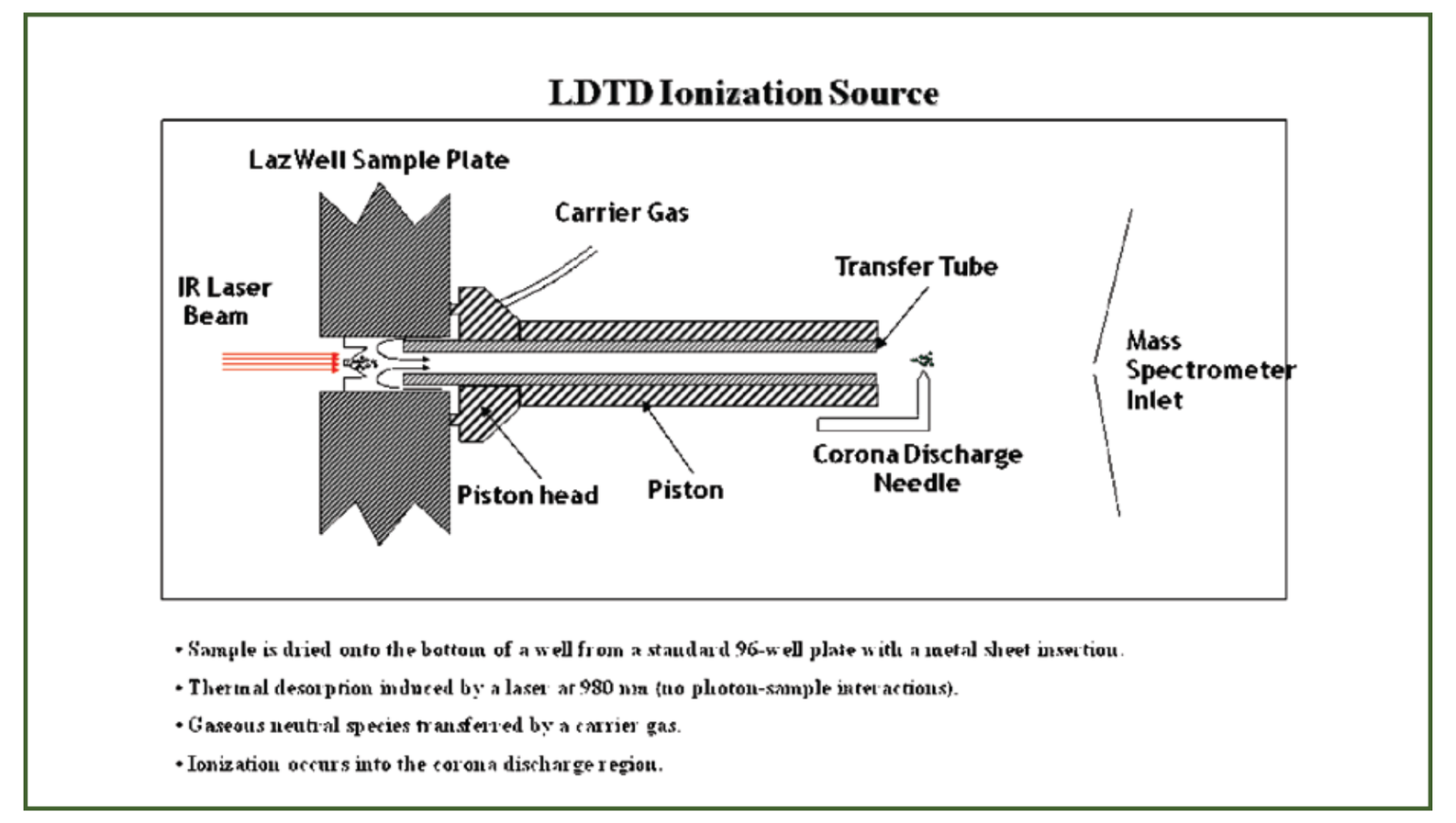
DOXYCYCLINE

1. Aliquot 25 µL of sample
2. Dilute 1:1 with 25 µL commercial Buffer at pH7(Potassium Phosphate Monobasic – Sodium Hydroxide Buffer 0.05 M from Fisher Chemical SB108-500)
3. Vortex 5 seconds
4. Add 150uL of Ethyl Acetate
5. Vortex 5 seconds
6. Transfer 25 µL of organic layer to a clean test tube
7. Add 25 µL of Methanol/Water (75/25) with EDTA at 400ng/mL
8. Vortex 5 seconds
9. Analyze 2 µL

Instrument

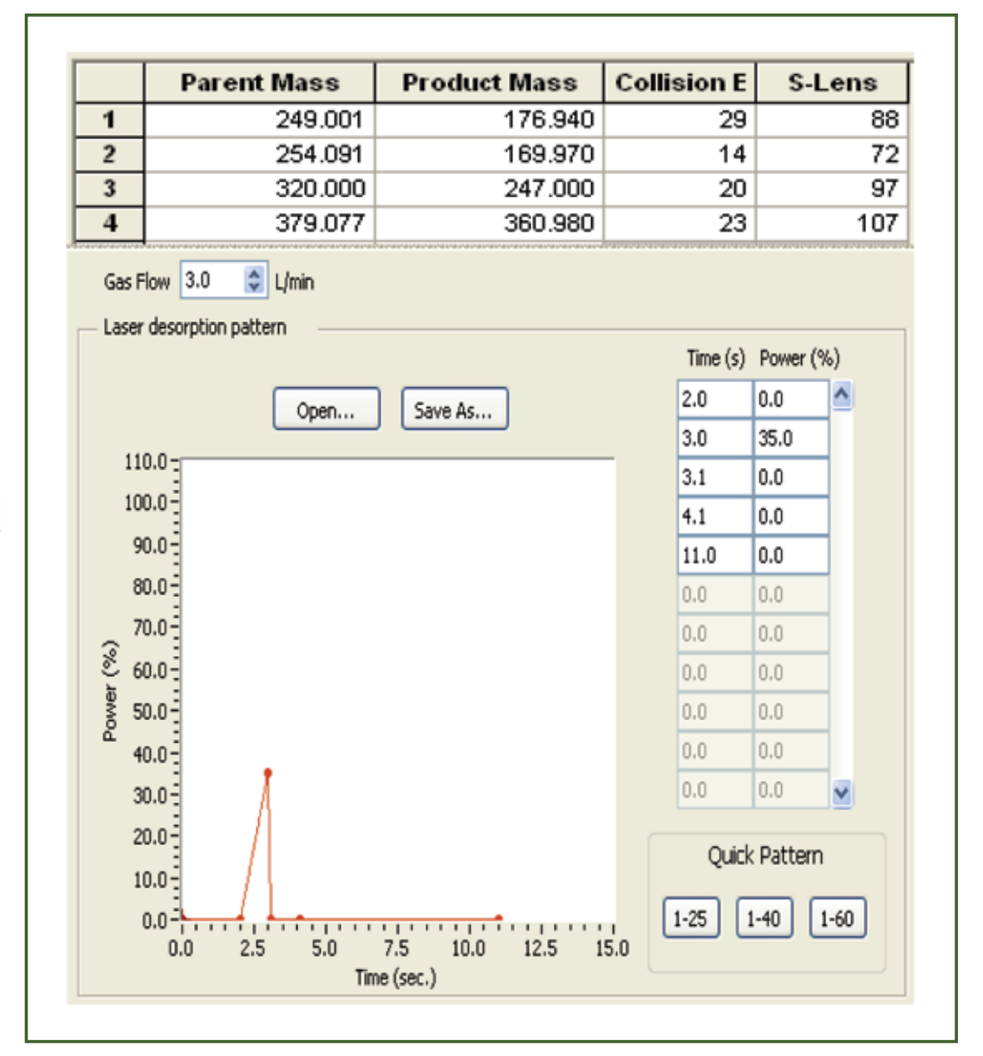
Thermo Vantage with LDTD model T-960

LDTD Source



Chloroquine, Mefloquine and Proquanil Analysis - LDTD/MS/MS

A two-point calibration curve was generated in human urine at concentrations of 100 and 500 ng/mL for Chloroquine, Mefloquine and Proquanil. Pyrimethamine was used as internal standard at 50 ng/mL.



Doxycycline Analysis – LDTD/MS/MS

A two-point calibration curve was generated in human urine at concentrations of 100 and 500 ng/mL for Doxycycline. Pyrimethamine was used as internal standard at 50 ng/mL.

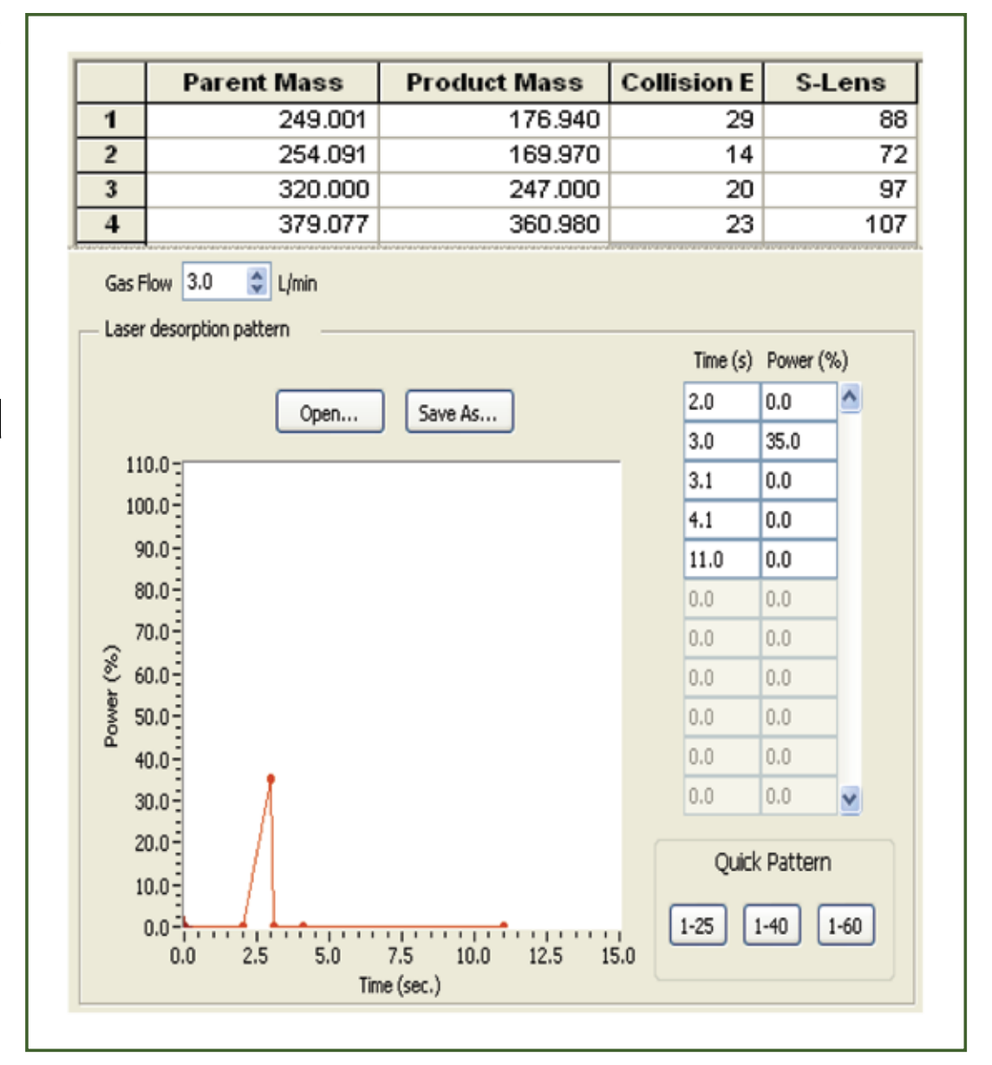
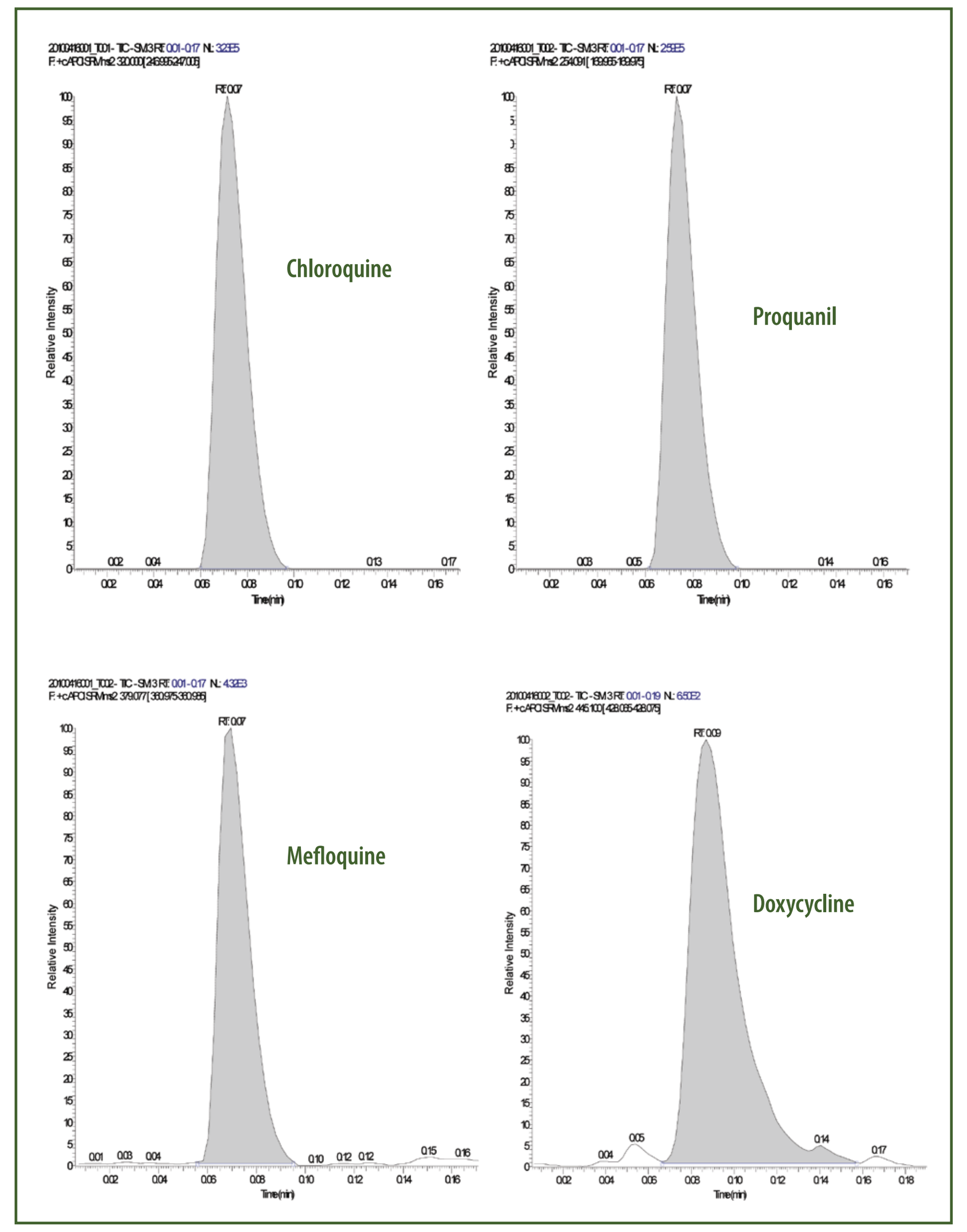


FIGURE 1: Thermal Desorption Signal (100ng/mL)



Chloroquine, Doxycycline, Proguanil and Mefloquine Analysis – LC/ESI-MS/MS

A two-point calibration curve was generated in human urine at concentrations of 100 and 500 ng/mL for Chloroquine, Doxycycline, Proguanil and Mefloquine. Pyrimethamine is used as internal standard at 50 ng/mL. LC/ESI-MS/MS analysis used a Sciex API4000 mass spectrometer with Shimadzu LC pumps and a Leap CTC autosampler. Sample preparation is directly dilution of urine sample with internal standard under positive mode using ESI source.

FIGURE 2: Representative LC-ESI/MS/MS Chromatograms (100ng/mL):

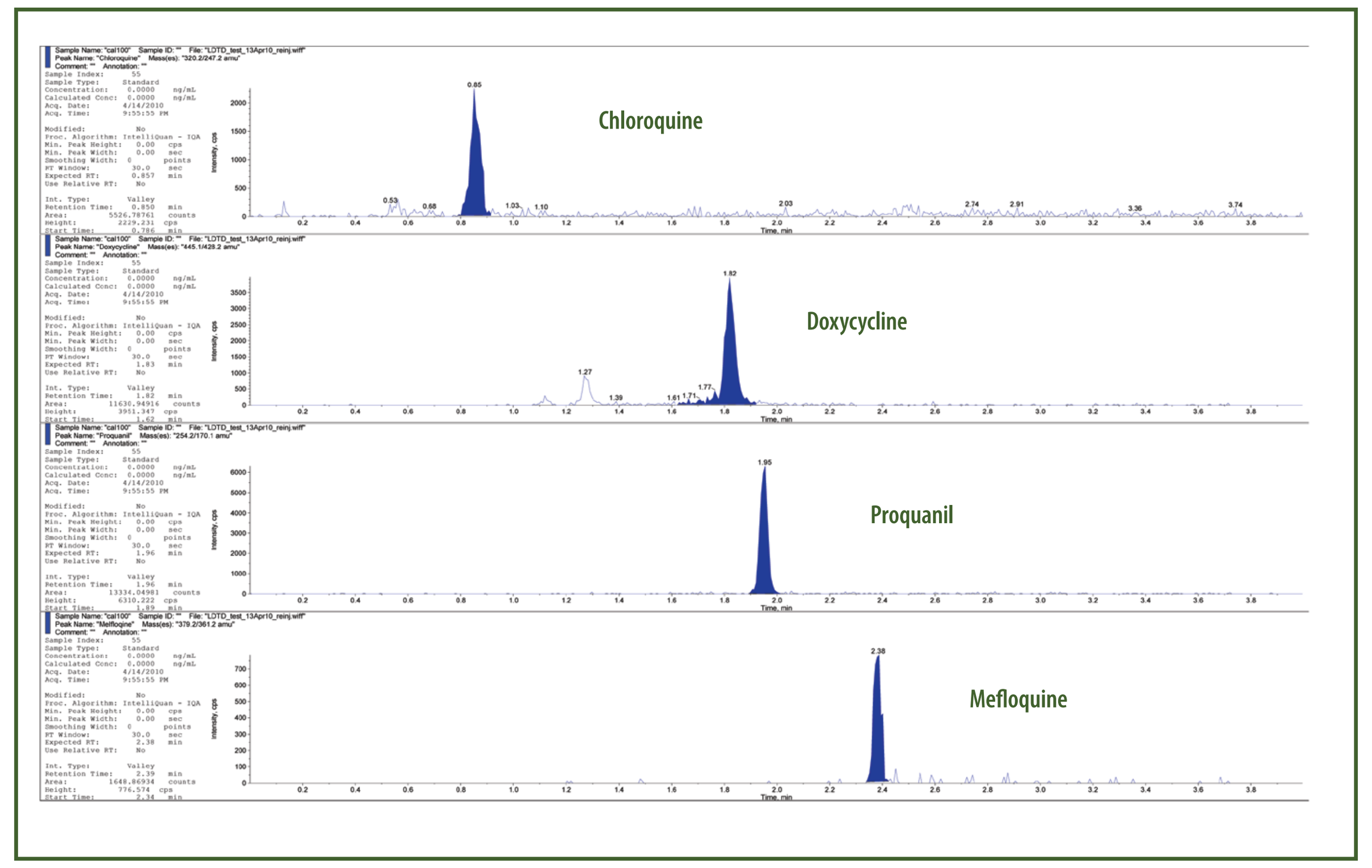


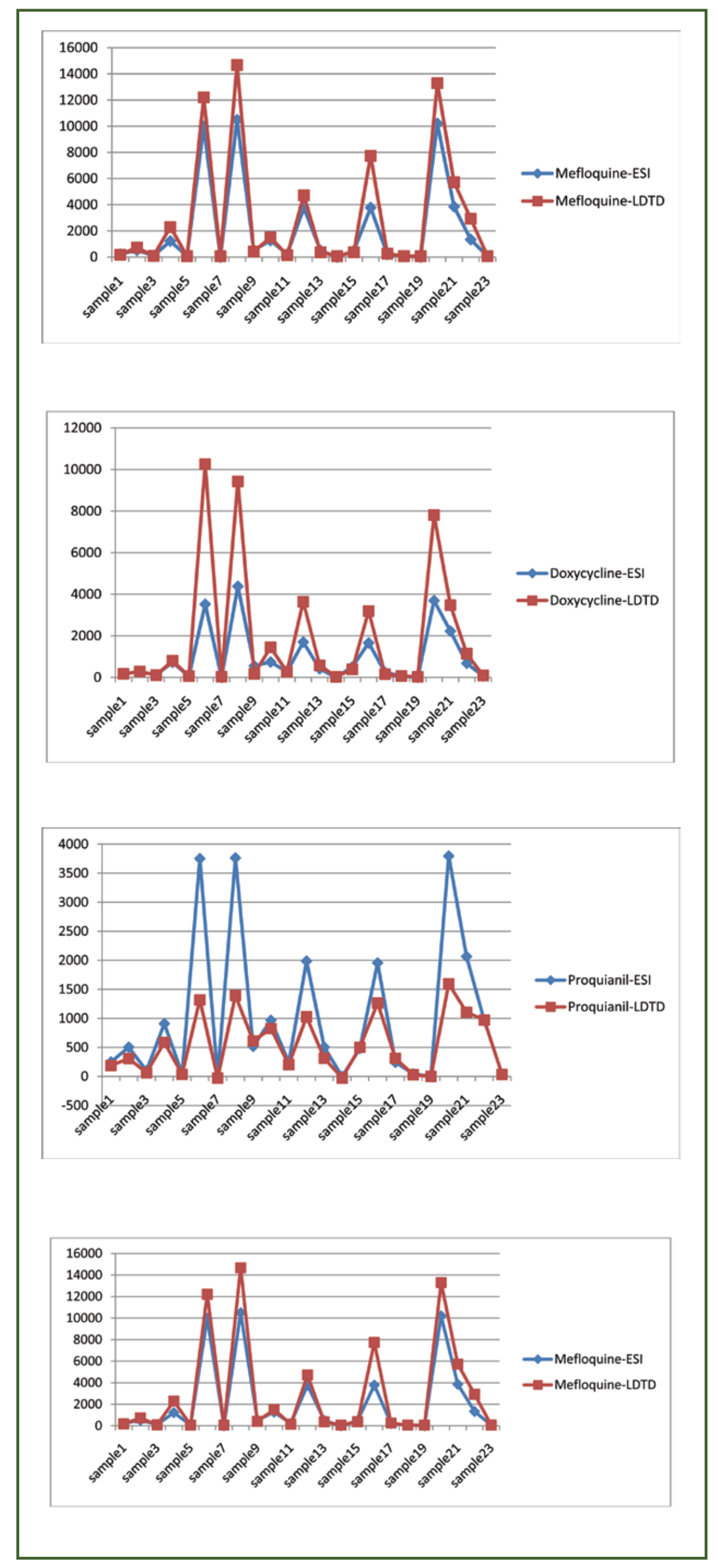
TABLE 1
Chloroquine and Doxycline Results vs. Cutoff

	ESI		LDTD		ESI		LDTD	
Sample Name	Chloroquine	Positive or Negative	Chloroquine	Positive or Negative	Doxycycline	Positive or Negative	Doxycycline	Positive or Negative
sample1	236	+	193	+	182	+	169	+
sample2	415	+	314	+	276	+	280	+
sample3	122	+	88	-	116	+	108	+
sample4	956	+	1067	+	726	+	803	+
sample5	67.0	-	52	-	49	-	59	-
sample6	4247	+	2085	+	3519	+	10253	+
sample7	0	-	8	-	0	-	37	-
sample8	4102	+	1937	+	4381	+	9427	+
sample9	524	+	420	+	552	+	163	+
sample10	1051	+	852	+	740	+	1449	+
sample11	251	+	252	+	263	+	264	+
sample12	2118	+	1400	+	1700	+	3640	+
sample13	518	+	384	+	418	+	578	+
sample14	0	-	8	-	0	-	30	-
sample15	476	+	500	+	506	+	389	+
sample16	2143	+	1711	+	1657	+	3188	+
sample17	242	+	239	+	242	+	154	+
sample18	68.6	-	61	-	66	-	65	-
sample19	0	-	8	-	0	-	32	-
sample20	4114	+	1892	+	3694	+	7810	+
sample21	2111	+	1442	+	2226	+	3476	+
sample22	992	+	1002	+	679	+	1139	+
sample23	70.9	-	62	-	61	-	93	-

TABLE 2
Proguanil and Mefloquine Results vs. Cutoff

	ESI		LDTD		ESI		LDTD	
Sample Name	Proguanil	Positive or Negative	Proguanil	Positive or Negative	Mefloquine	Positive or Negative	Mefloquine	Positive or Negative
sample1	248	+	187	+	220	+	181	+
sample2	504	+	308	+	520	+	723	+
sample3	99	-	64	-	102	+	87	-
sample4	909	+	586	+	1219	+	2293	+
sample5	43	-	36	-	74	-	67	-
sample6	3751	+	1317	+	9981	+	12211	+
sample7	0	-	-28	-	0	-	62	-
sample8	3764	+	1393	+	10490	+	14670	+
sample9	517	+	611	+	525	+	415	+
sample10	967	+	824	+	1266	+	1500	+
sample11	241	+	203	+	217	+	152	+
sample12	1987	+	1027	+	3767	+	4718	+
sample13	504	+	313	+	491	+	366	+
sample14	0	-	-28	-	0	-	64	-
sample15	481	+	501	+	476	+	372	+
sample16	1956	+	1264	+	3779	+	7737	+
sample17	243	+	311	+	211	+	254	+
sample18	46	-	30	-	76	-	66	-
sample19	0	-	0	-	0	-	62	-
sample20	3796	+	1593	+	10206	+	13297	+
sample21	2065	+	1105	+	3853	+	5723	+
sample22	958	+	971	+	1333	+	2931	+
sample23	41	-	34	-	74	-	70	-

FIGURE 3: Quantitative Comparison of LDTD vs. LC/ESI



Discussions:

1. The results of the LDTD-MS/MS analysis demonstrated excellent agreement with the LC/ESI-MS/MS analysis for the four anti-malaria drugs based on the acceptance criteria (positive or negative determination) for a particular non-GLP study (Table 1 & 2). An increased quantitative discrepancy between the two methods was observed in the higher concentration samples due to the fact that their concentrations are significantly above the upper limit of quantitation for this semi-quantitative assay, and also because the two different methods (and MS systems) likely have different linear ranges (Fig. 3). These quantitative discrepancies are not significant for this assay with regard to reporting unknown samples as either positive or negative relative to the cut-off threshold of 100 ng/mL.
2. The LDTD analysis time is less than 20 seconds per sample, compared to ~4 min per sample for the LC method (See Figures 1 & 2). The significant reduction in analysis time using the LDTD technology dramatically increases the potential for sample throughput in the laboratory.
3. Carryover is not observed with the LDTD analysis because there is no autosampler or LC system. Because the current LC method requires the use of ~25 extra control blank samples per run to provide sufficient "wash-out" after high concentration Doxycycline samples, the lack of carryover also significantly decreases the number of samples necessary for an analytical run. In addition, because there is no carryover, it is not necessary to reassay positive Doxycycline samples that may be positive only as a result of carryover from other truly positive Doxycycline samples.
4. The only obvious disadvantage for the LDTD/MS/MS analysis compared to the existing LC/ESI-MS/MS method is that a second sample preparation procedure is required for the analysis of all 4 compounds. Doxycycline cannot be analyzed by LDTD using a simple "dilute and shoot" procedure, and therefore requires additional liquid/liquid sample clean-up. However, the significant reduction in per-sample run time and in the number of samples required for analysis should more than make up for any additional sample preparation time, much of which can be automated with advanced liquid handling devices.