SUPPORTING INFORMATION

Analysis of antiretrovirals in single hair strands for evaluation of drug adherence with IR-MALDESI MSI

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Supporting Data

Figure S-1: Evaluation of IR-MALDESI analyte extraction efficiency from incubated hair strands.

Table S-1: Physicochemical properties of HIV antiretrovirals.

Table S-2**:** Summary of ARV response in incubated hair strands from LC-MS/MS and IR-MALDESI analysis.

Table S-3: Linear regression estimates of IR-MALDESI calibration data.

Figure S-2: Calibration of IR-MALDESI signal abundance to LC-MS/MS response for ARVs in incubated hair strands.

Figure S-3: Calibration of IR-MALDESI signal abundance to LC-MS/MS response for EFV in dosed hair strands.

Figure S-4: IR-MALDESI ion map of PTCA response from strands of three dosed patients after oxidation of hair melanin by H_2O_2.

Figure S-5: Mass excess plots of all hair-specific peaks identified by IR-MALDESI and comparison to lipid classes.

Figure S-1. IR-MALDESI depth profiling of EFV in incubated hair strands. Re-analysis of a hair strand (right panel) that has already undergone laser desorption by IR-MALDESI (left panel) indicated a reduction of a 61% reduction in average EFV signal abundance. Under the assumption that all EFV is removed from the strand during re-analysis, this suggests that ~80% (4.9×10^4/ $(4.9+1.9) \times 10^4$) of EFV is extracted from an incubated strand during initial IR-MALDESI analysis. This value represents an upper bound on analyte extraction efficiency.

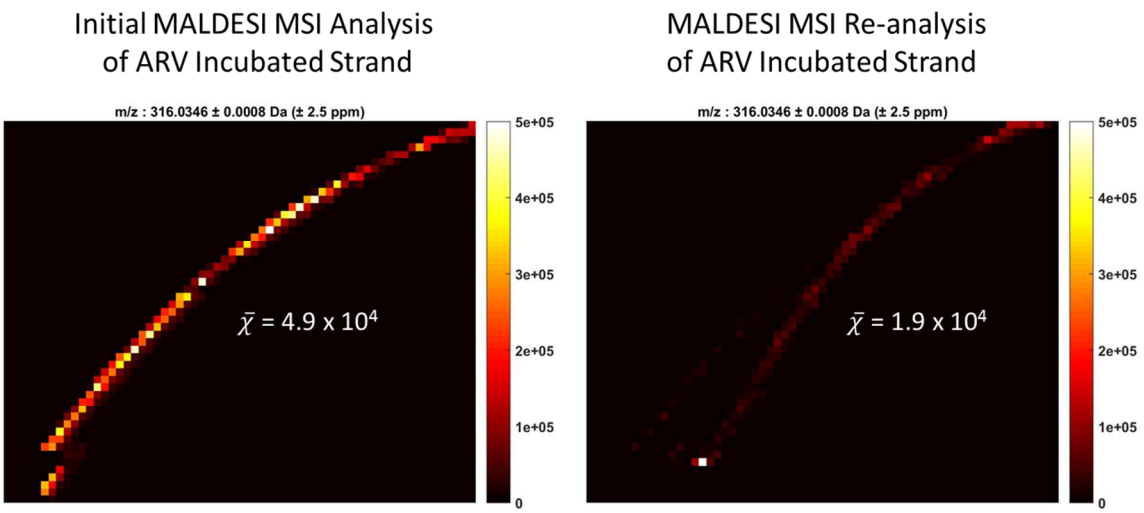


Table S-1. Physicochemical properties of selected ARVs for hair strand incubation and IR-MALDESI calibration.

Antiretroviral		Physicochemical Properties		
		pKa		***log P****
NONNUCLEOSIDE RT INHIBITOR				
Efavirenz	*EFV*	10.2[†]		4.5
INTEGRASE INHIBITOR				
Dolutegravir	*DTG*	8.2[‡]		1.7
Raltegravir	*RAL*	6.7[•]		-0.4
PROTEASE INHIBITOR				
		Strongest Basic	*Strongest Acidic*	
Darunavir	*DRV*	2.4*	13.6*	2.8
ENTRY INHIBITOR				
Maraviroc	*MRV*	7.3[◊]		3.6
NUCLEOSIDE ANALOGUE RT INHIBITOR				
Lamivudine	*3TC*	4.3[□]		-1.1

*Properties predicted based on structure[1].

[†]Kashuba *et al*[2]

[‡]Dolutegravir prescribing information[3]

[•]Moss *et al*[4]

[◊]Abel *et al*[5]

[□]Shalaeva *et al*[6]

Table S-2. Summary of ARV response in incubated hair strands from LC-MS/MS and IR-MALDESI analysis.

	LC-MS/MS (ng/mg hair)				IR-MALDESI (signal abundance)				
HIV	*Blank*	*Stock concentration (ng/ml)*			*Blank*		*Stock concentration (ng/ml)*		
ARVs		*Low - 1000*	*Med - 10000*	*High - 100000*	$\bar{x}$	σ	*Low - 1000*	*Med - 10000*	*High - 100000*
3TC	0.00	1.00±0.21	6.78±0.21	61.76±1.56	$3.66x10^2$	$9.78x10^2$	$4.87±0.54x10^3$	$1.75±0.20x10^4$	$1.25±0.60x10^5$
MVC	0.00	1.23±0.35	6.20±1.19	69.77±3.11	$5.42x10^2$	$2.66x10^3$	$2.14±0.28x10^4$	$2.70±0.66x10^5$	$2.64±1.44x10^6$
DRV	0.00	2.45±0.31	15.29±0.56	136.24±22.45	$3.48x10^1$	$2.35x10^2$	$5.44±3.20x10^3$	$2.88±0.33x10^4$	$1.19±0.71x10^5$
RAL	0.00	1.38±0.09	24.25±2.9	337.86±2.22*	0.00	0.00	$7.88±1.08x10^3$	$2.32±0.75x10^5$	$9.94±0.54x10^5$
DTG	0.00	8.60±0.76	264.34±22.19	900.43±51.11*	$6.63x10^1$	$4.45x10^2$	$1.03±0.34x10^5$	$3.73±1.17x10^6$	$3.85±0.95x10^6$
EFV	0.00	24.34±1.39	165.02±20.41	1772.58±44.45*	$4.12x10^1$	$1.73x10^2$	$4.49±0.93x10^4$	$3.79±0.46x10^5$	$1.32±0.56x10^6$

*** Shaded values represent incubated hair concentrations for the highest point in the calibration range that exceed 150 ng/mg hair.**

Table S-3. Linear regression estimates of IR-MALDESI calibration data.

	All Calibration Data				Excluding High Calibration Data > 150 ng/mg hair			
HIV	Linear Regression of Calibration			Estimated LOD*	Linear Regression of Calibration			Estimated LOD*
ARVs	*Slope*	*Intercept*	R^2	*(ng/mg hair)*	*Slope*	*Intercept*	R^2	*(ng/mg hair)*
EFV	$6.99x10^2$	$9.22x10^4$**	0.96226	0.81	$2.33x10^3$	$-5.34x10^3$	0.99919	0.24
DRV	$8.38x10^2$	$6.11x10^3$	0.98448	0.92	$8.38x10^2$	$6.11x10^3$	0.98448	0.92
3TC	$1.99x10^3$	$2.38x10^3$	0.99931	1.63	$1.99x10^3$	$2.38x10^3$	0.99931	1.63
RAL	$2.81x10^3$	$5.35x10^4$**	0.97330	N/A	$9.69x10^3$	$-2.68x10^3$	0.99956	N/A
DTG	$4.10x10^3$	$7.18x10^5$**	0.64508	0.36	$1.41x10^4$	$-8.98x10^3$	0.99998	0.10
MRV	$3.78x10^4$	$3.12x10^3$	0.99961	0.23	$3.78x10^4$	$3.12x10^3$	0.99961	0.23

$*Limit\ of\ Detection\ (LOD) = 3.3\sigma_{Blank}/Slope_{Calibration}$

** Intercept values derived from fits including hair concentration data for the highest point in the calibration range that exceed 150 ng/mg hair.

Figure S-2. Calibration of IR-MALDESI signal abundance to LC-MS/MS response for ARVs in incubated hair strands. For ARVs in which the highest incubated concentrations exceed 150 ng/mg hair, linear estimates are shown using all data (black lines) and excluding the highest data points (red lines).

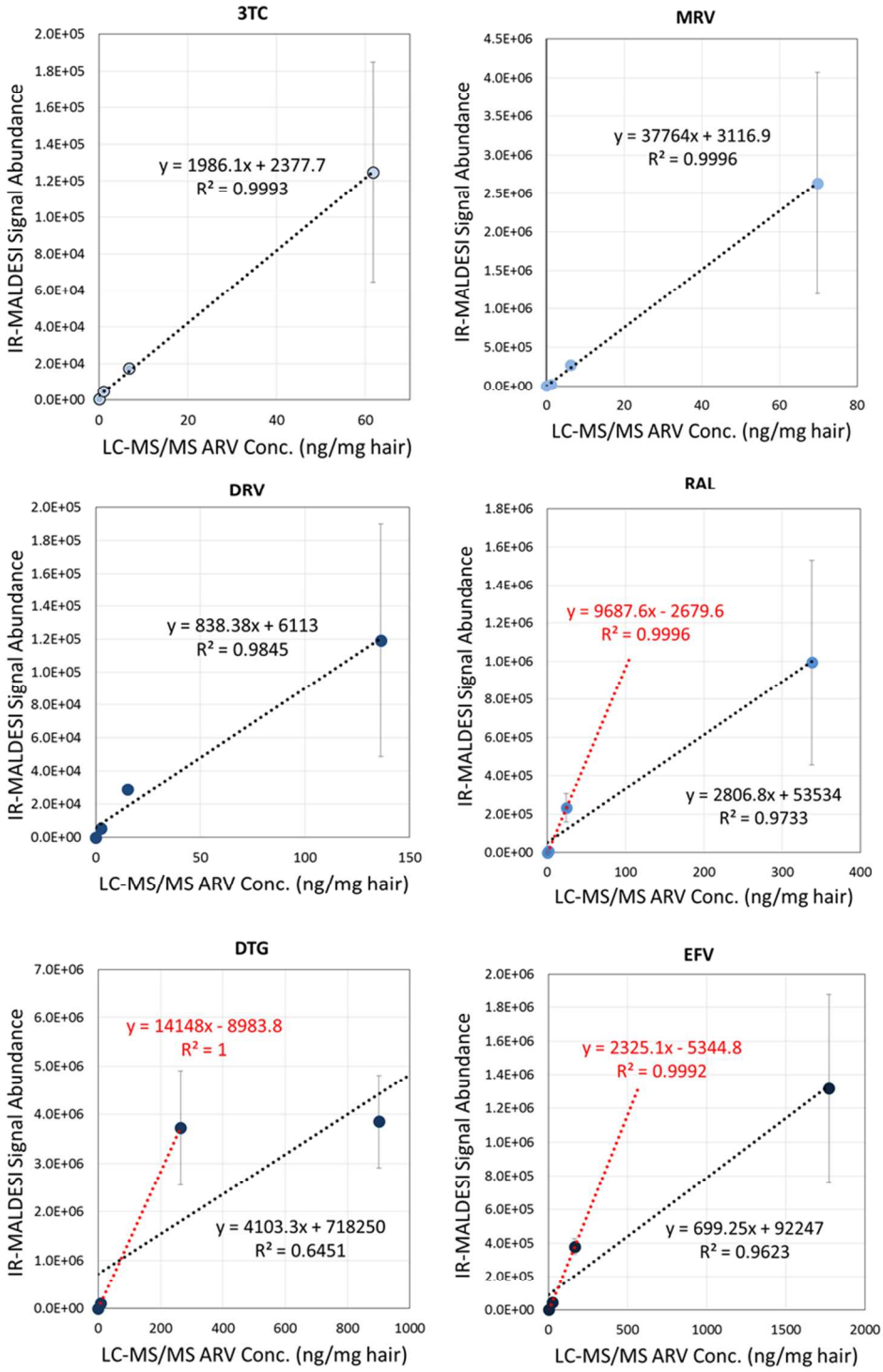


Figure S-3. Calibration of IR-MALDESI signal abundance to LC-MS/MS response for EFV in dosed hair strands.

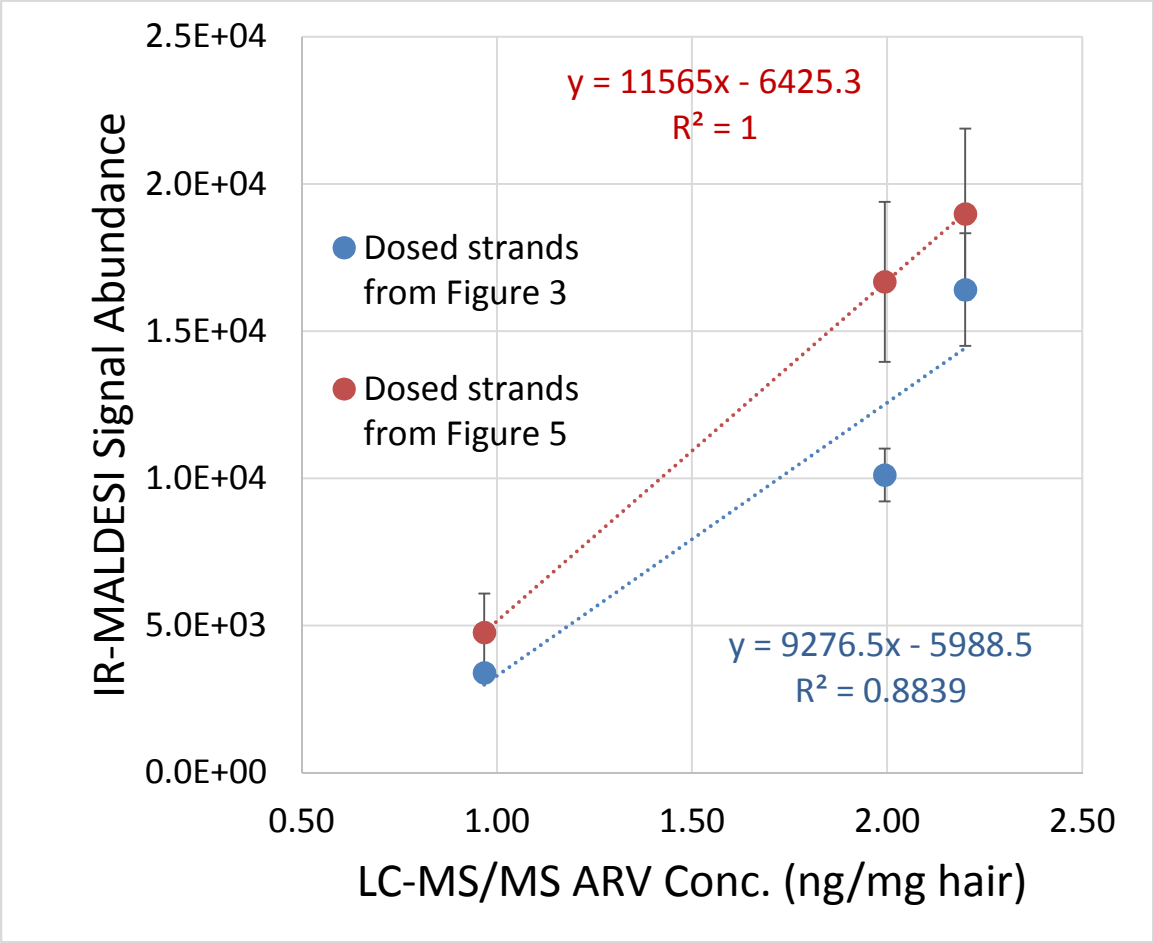


Figure S-4. IR-MALDESI ion map of PTCA response from strands (n=4) of three dosed patients after oxidation of hair melanin by H_2O_2.

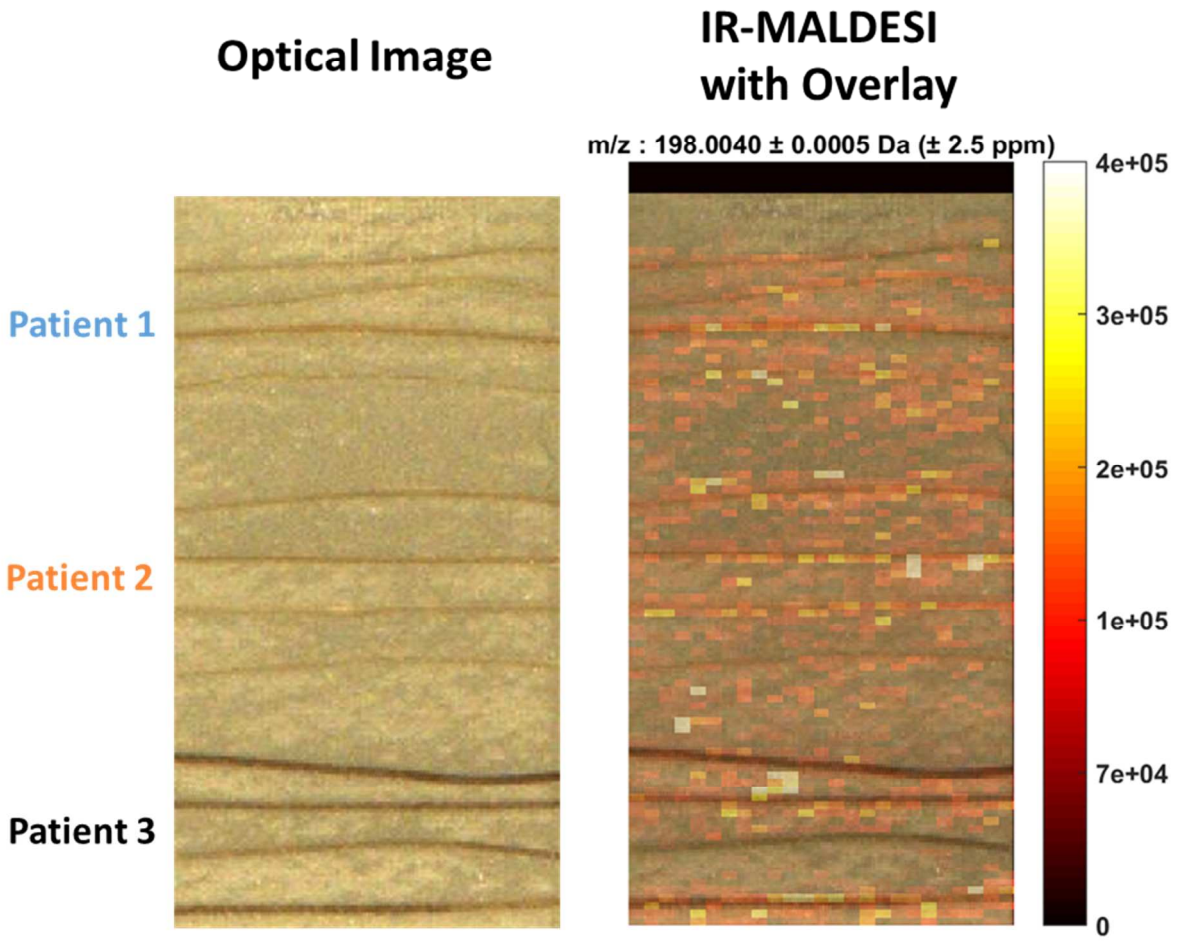
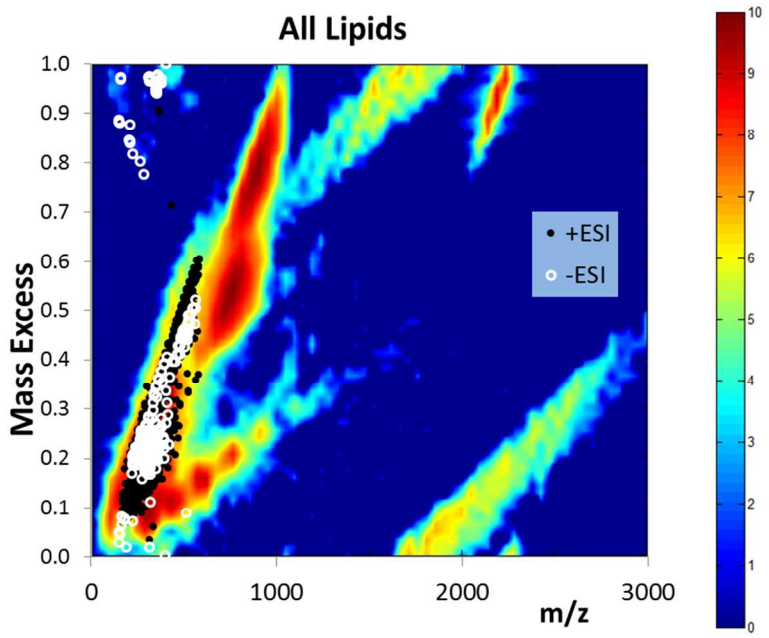
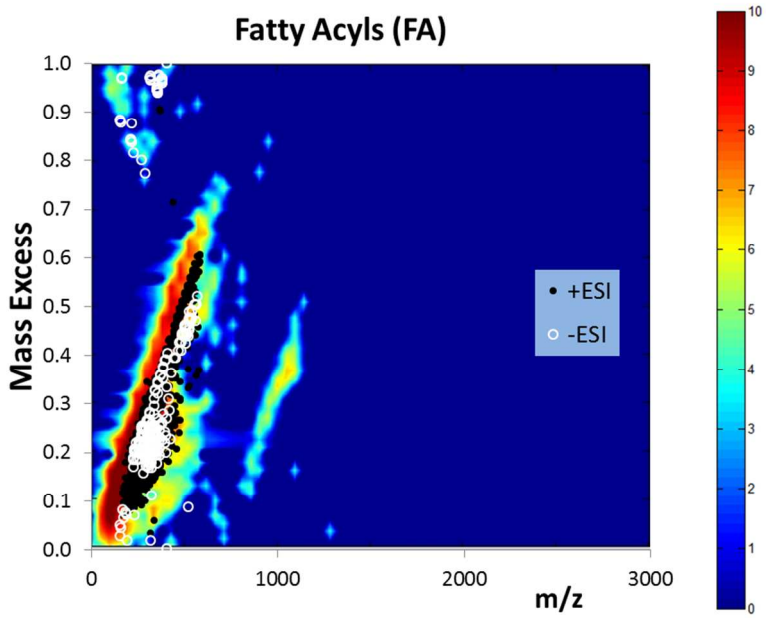


Figure S-5. Mass excess of all hair-specific peaks (+ESI, black dots; -ESI, white dots) overlaid on mass excess distribution of lipids generated from LIPIDMAPS Structure Database[7]. The plots indicate that all of the hair-specific peaks correspond to lipids across different classes, particularly fatty acyls.





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