Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Nanocapillary sampling coupled to liquid chromatography mass spectrometry delivers single cell drug measurement and lipid fingerprints

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Table S1: The liquid chromatography gradient applied in this work, where mobile phase solvent A) 60:40 acetonitrile/water + 0.1% formic acid and mobile phase solvent B) 90:10 isopropanol/acetonitrile + 0.1% formic acid

Time (min)	% Solvent ***A***	% Solvent ***B***
0.0	60	40
1.0	50	50
3.6	31	69
12.0	12	88
14.0	60	40
16.0	60	40

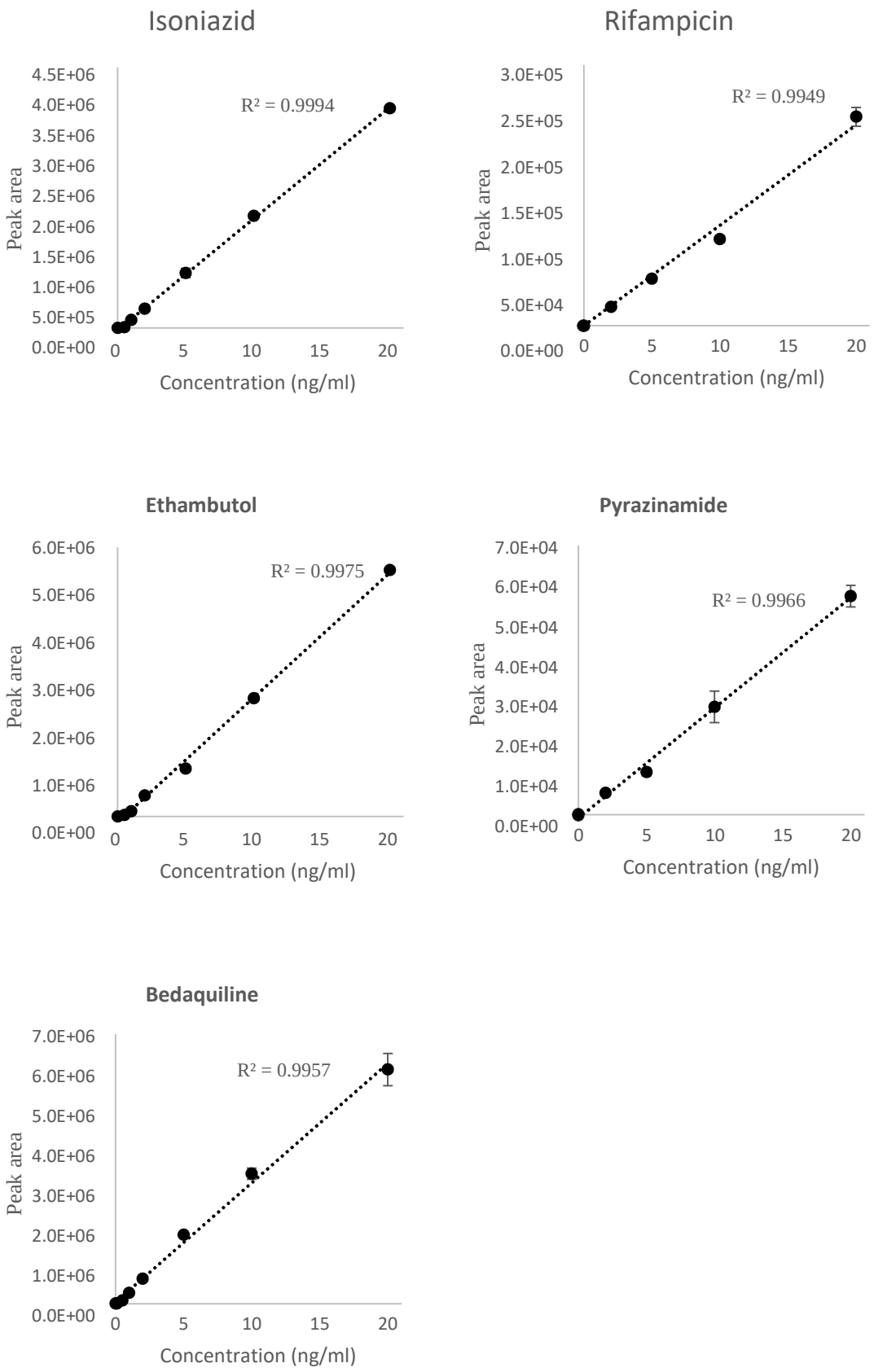


Figure S1: Calibration curves for isoniazid, rifampicin, ethambutol, pyrazinamide and bedaquiline and concentrations 0, 0.1, 0.5, 1, 2, 5, 10, and 20 ng/ml diluted in MeOH:EtOH using LC-MS method; (n=5) injections per sample where the error bars show the standard deviation between repeats.

Table S2. Limits of detection for the drug analytes in solution, determined by a method of infinite dilutions.

	Limit of detection (ng/ml)	Limit of detection (pg)
Isoniazid	1	5
Rifampicin	2	10
Ethambutol	0.5	2.5
Pyrazinamide	2	10
Bedaquiline	0.5	2.5

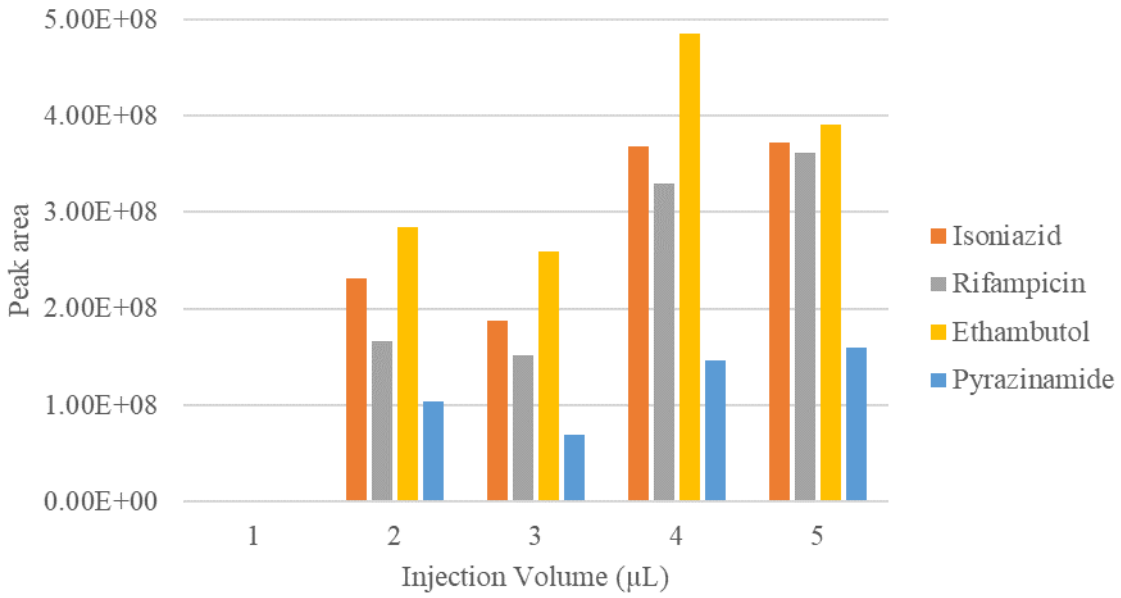


Figure S2: Signal intensity for isoniazid, rifampicin, ethambutol and pyrazinamide where the same mass (10 ng) of analyte is diluted in different injection volumes (n=1)

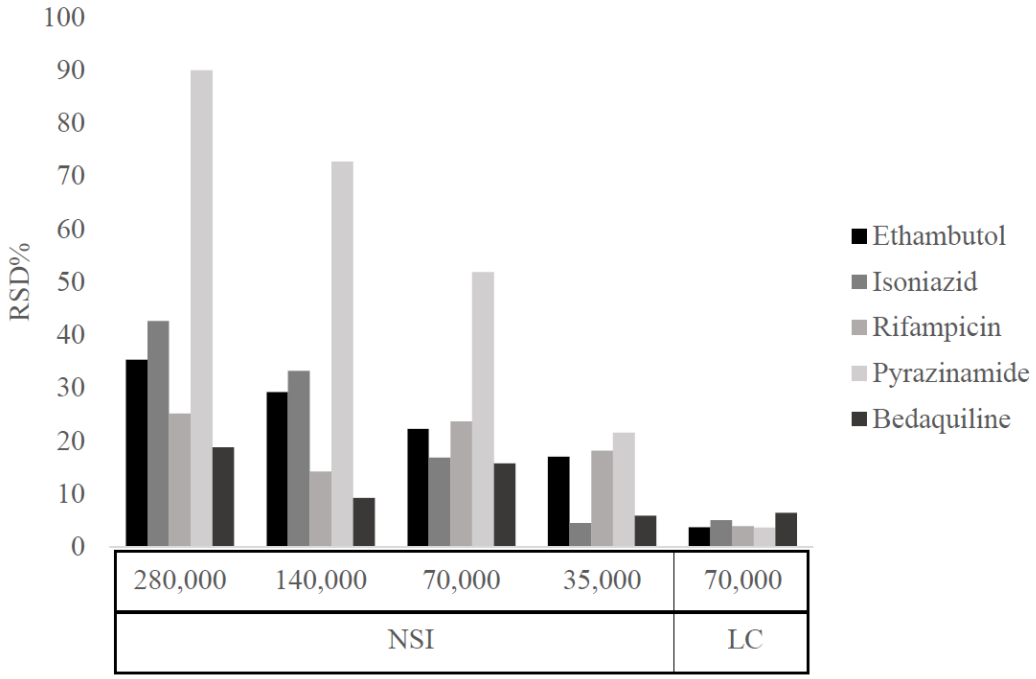


Figure S3: A graph to show the RSD% (n=5) of the [M+H]+ intensity of the five anti-TB drugs: ethambutol, isoniazid, rifampicin, pyrazinamide and bedaquiline (100 ng/ml), using NSI (3.5 minute acquisition) at four different mass resolutions: 280,000; 140,000; 70,000 and 35,000 and also LC at 70,000 (n=5) for comparison

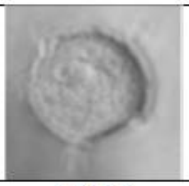
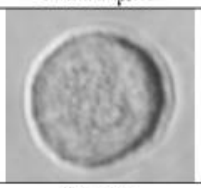
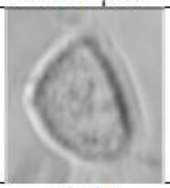
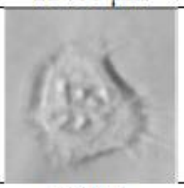
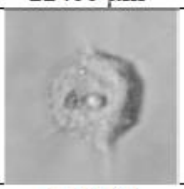
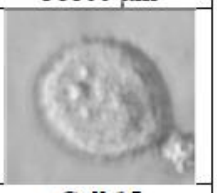
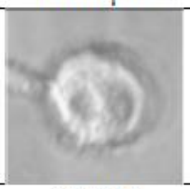
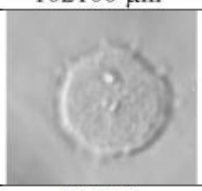
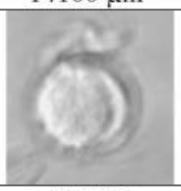
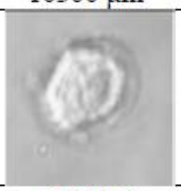
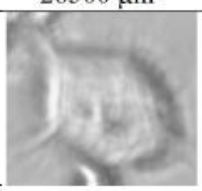
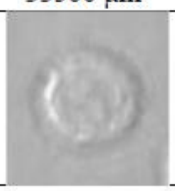
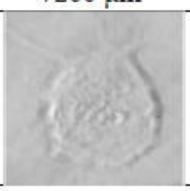
				
Cell 1	**Cell 2**	**Cell 3**	**Cell 4**	**Cell 5**
38 µm	27 µm	34 µm	35 µm	30 µm
28700 µm^2	10300 µm^2	20600 µm^2	22400 µm^2	14100 µm^2
				
Cell 6	**Cell 7**	**Cell 8**	**Cell 9**	**Cell 10**
20 µm	34 µm	22 µm	35 µm	42 µm
4200 µm^2	20600 µm^2	5600 µm^2	22400 µm^2	38800 µm^2
				
Cell 11	**Cell 12**	**Cell 13**	**Cell 14**	**Cell 15**
27 µm	31 µm	35 µm	27 µm	43 µm
10300 µm^2	15600 µm^2	22400 µm^2	10300 µm^2	41600 µm^2
				
Cell 16	**Cell 17**	**Cell 18**	**Cell 19**	**Cell 20**
58 µm	30 µm	33 µm	27 µm	31 µm
102100 µm^2	14100 µm^2	18800 µm^2	10300 µm^2	15600 µm^2
				
Cell 21	**Cell 22**	**Cell 23**	**Cell 24**	**Cell 25**
37 µm	32 µm	40 µm	24 µm	30 µm
26500 µm^2	17200 µm^2	33500 µm^2	7200 µm^2	14100 µm^2
				
Cell 26	**Cell 27**	**Cell 28**	**Cell 29**	**Cell 30**
41 µm	46 µm	32 µm	51 µm	32 µm
36100 µm^2	50900 µm^2	17200 µm^2	69500 µm^2	17200 µm^2

Figure S4. Microscope images of the 30 extracted cells before nanocapillary extraction alongside the corresponding cell diameters and volumes.

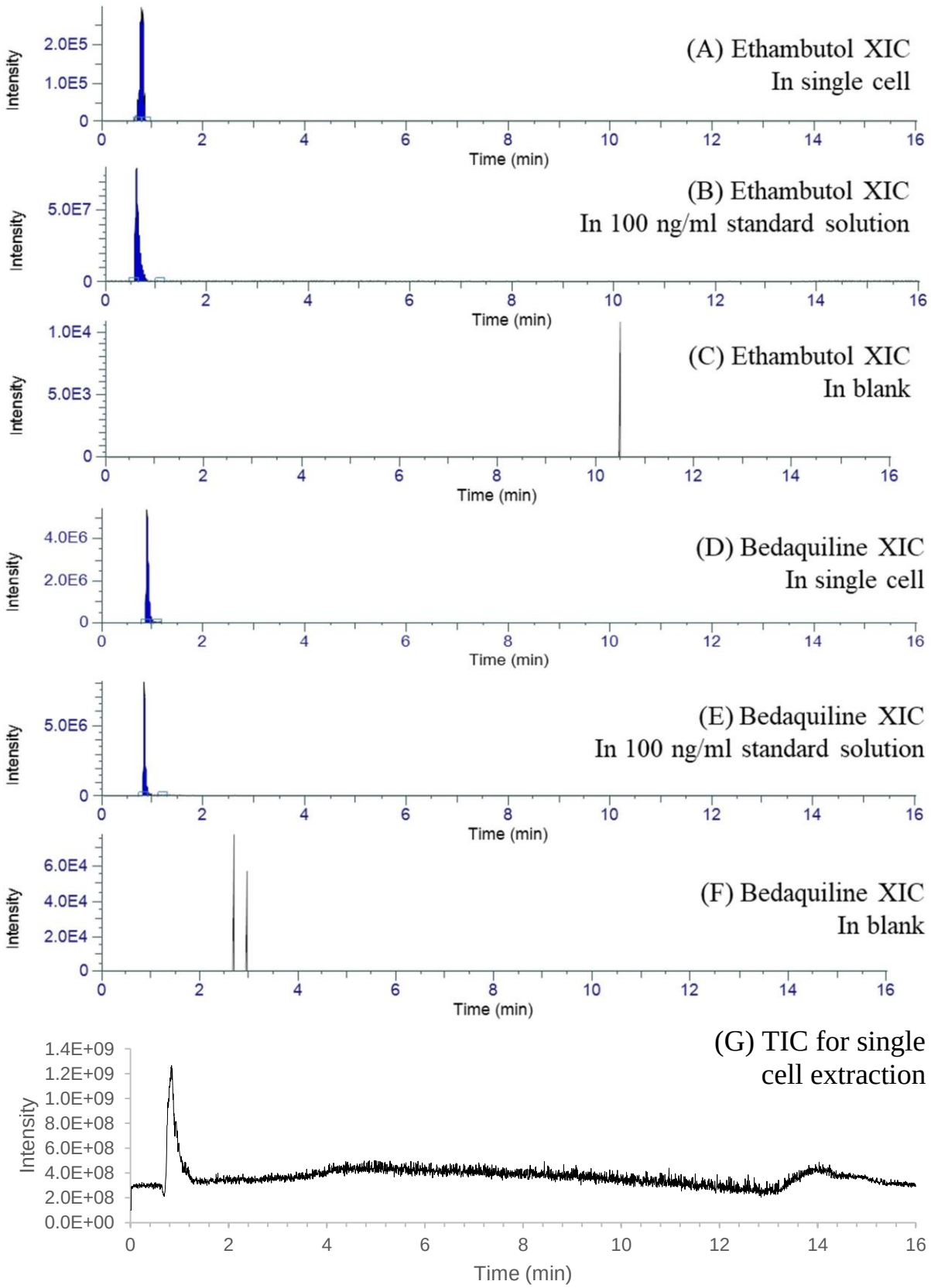


Figure S5: Extracted ion chromatograms for ethambutol (*m/z* 205.1911) in a single cell, in a 100 ng/ml standard solution and in a blank (PBS extracted using pressure injector diluted with 5µL 50:50 MeOH/EtOH solution) (A, B and C) and extracted ion chromatograms for bedaquiline (*m/z* 555.1642) in a single cell, in a 100 ng/ml standard solution and in a blank (PBS extracted using pressure injector diluted with 5µL 50:50 MeOH/EtOH solution) (D, E and F). The single cell Total Ion Chromatogram is also shown (G).

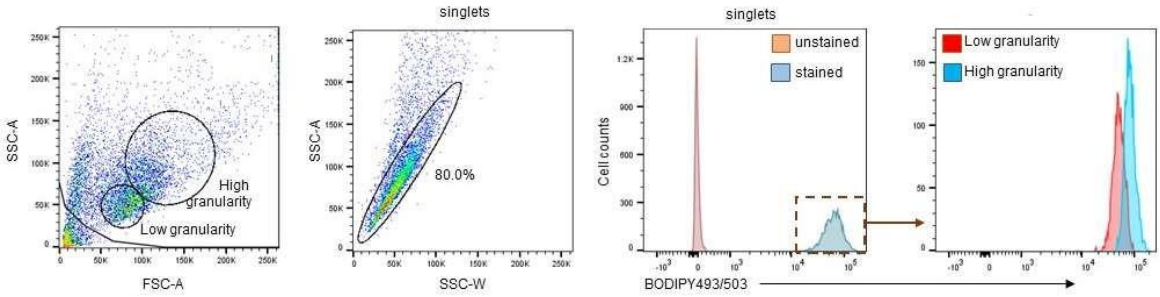


Figure S6: PANC-1 cells present high levels of lipid droplets as shown by the specific probe BODIPY 493/503.

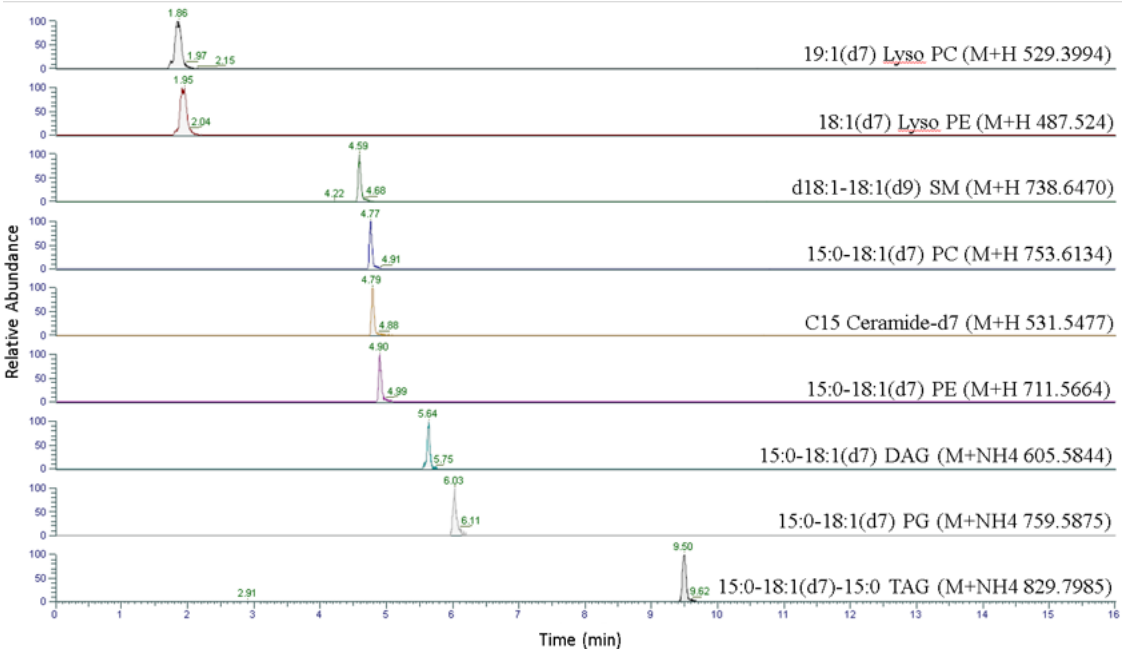


Figure S7: Extracted ion chromatograms of lipids analytes in the Avanti® Polar Lipids EquiSPLASH® diluted to 32 ng/mL dissolved in starting mobile phase, showing separation obtained using the chromatography method

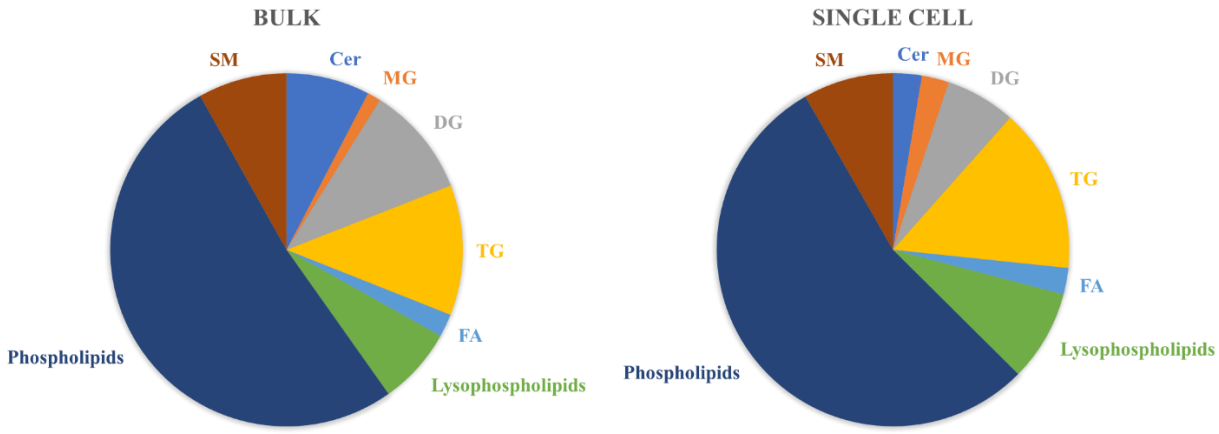


Figure S8: Charts showing proportion of assigned lipids detected (A) for cell suspension (B) averaged data for 12 single cells. Where SM: sphingolipids, Cer: ceramides, MG: monoglycerides, DG: diglycerides, TG: triglycerides, FA: fatty acids

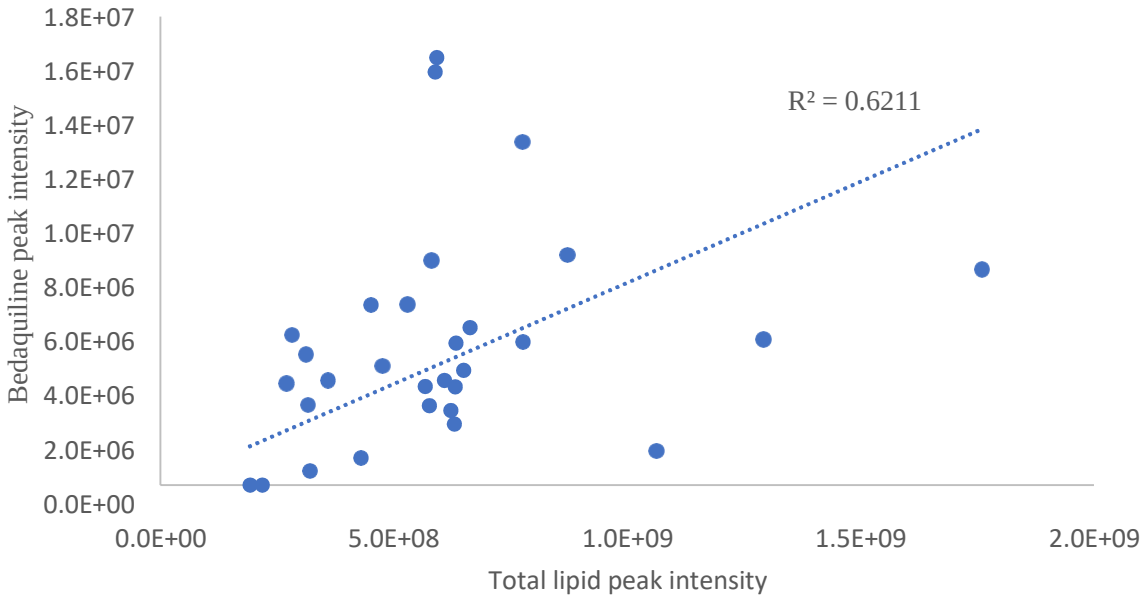
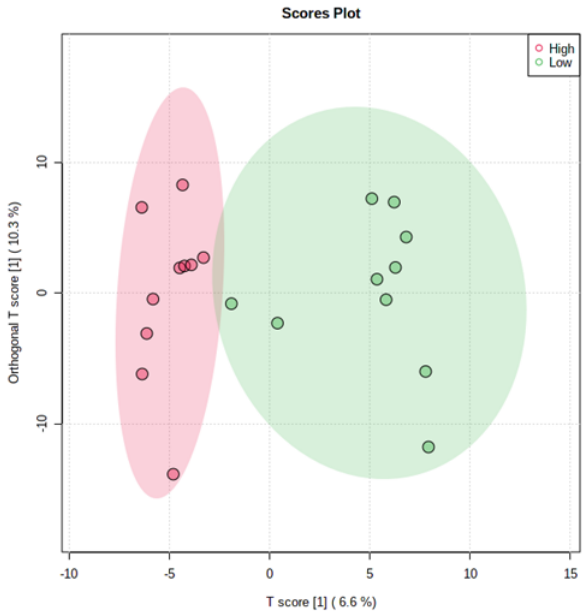


Figure S9: The total lipid peak intensity vs. the bedaquiline peak intensity for each extracted cell



m/z	Presumptive peak assignment	VIP score
843.7412	TG(14:0e_18:2_20:4)+Li	2.23
732.5150	PC(12:0_20:4)+Li	2.18
734.5119	PC(12:1e_22:6)+H	2.18
694.6133	Cer(d20:1_26:6)+H	2.12
950.7396	PS(24:1_23:0)+Li	2.07
775.7538	OAHFA(15:0_36:1)+H	2.06
894.7134	PS(20:0e_24:1)+Li	2.00
415.3030	DG(10:0_11:3)+Li	2.00

Figure S10: (A) Partial Least Squares Discriminant Analysis (PLS-DA) for the 10 cells with the highest measured ethambutol content and 10 cells with the lowest measured ethambutol content. (B) Top variable importance in projection scores.