Mass spectrometry imaging of cassette-dosed drugs for higher throughput pharmacokinetic and biodistribution analysis

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1. Supplementary Methods

1.1 Bioanalytical Stock Solution Preparation. Stock solutions of test compounds were prepared in DMSO to give a final concentration of 2 mM. Compounds were mixed into a cassette to a final concentration of 0.2 mM and were subsequently serially diluted to form working solutions for use in calibration curve construction. Samples for the standard curves and quality controls were prepared from different solutions by spiking control rat plasma with the appropriate working solution. Calibration standards (50 µL) were prepared in control rat plasma at 1, 5, 10, 20, 50, 100, 500, 1000, 2000, 5000 and 10000 nM. Duplicate quality controls (50 µL) were spiked at 10, 100 and 2000 nM in control rat plasma and were interspersed at regular intervals throughout the analytical batches. The calibration standards were injected at the beginning and end of the analytical run and were subsequently used to construct a calibration curve and quantify the unknowns and quality controls. Analytical batches were considered successful if calibration standards and quality controls (a minimum of 2/3) were within 25% of the nominal concentration. 50 µL of plasma was protein precipitated with 200 µL of chilled acetonitrile containing internal standard (generic from the AZ compound library). The samples were then vortex mixed and centrifuged at 4500g for 10 mins. 50 µL of supernatant was then mixed with 300 µL of distilled water prior to analysis by LC-MS/MS.

1.2 Bioanalytical Method. A TSQ Quantum Vantage (Thermo Fisher Scientific, San Jose, CA, USA) mass spectrometer operating with a heated electrospray ionization interface was used for the LC-MS/MS determination of the compounds dosed discretly and in cassettes. The mass transitions monitored for compounds dosed orally were *m/z* 472.3>436.4, 313.1>256.1, 394.1>278.1, 402.1>384.3 for terfenadine, olanzapine, erlotinib and moxifloxacin, corresponding collision energies were 25, 22, 31 and 21 eV respectively. The sheath gas and

auxiliary gas were set at 80 and 40 (Arb) respectively. An Agilent 1200SL HPLC pump (Agilent, Stockport, UK) and a CTC Analytics HTC PAL autosampler (Presearch Ltd., Basingstoke, UK) were used to introduce the samples to the mass spectrometer. Chromatography was performed on a Eclipse plus (50mm×2.1mm ID, 5µm) HPLC column (Agilent, Cheadle, UK) with mobile phase consisting of eluent (A) water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid. The linear gradient used was (T = minutes): at T = 0.0, 95%A:5%B, T = 3.0, 5%A:95%B, T = 4.0, 5%A:95%B, T = 4.1, 95%A:5%B, T = 5.0, 95%A:5%B. The flow rate used was 750 µL/min. Concentration-time data was processed using QuickCalc/GMSU (Gubbs Inc, Alpharetta, Georgia, USA) to quantify peak areas. Pharmacokinetic parameters were calculated using non-compartmental analysis performed in WinNonlin 5.2.1 (Pharsight Corp., Mountain View, California, USA).

Figure S-1. Plasma concentrations (ng/mL) of moxifloxacin and olanzapine after oral dosing at 25 and 10 mg/kg, respectively, as part of a cassette dose and a discrete dose. Plasma concentration/time profiles for cassette and disctretly dosed compounds can be seen to be identical for moxifloxacin (black) and olanzapine (red) after oral dosing.

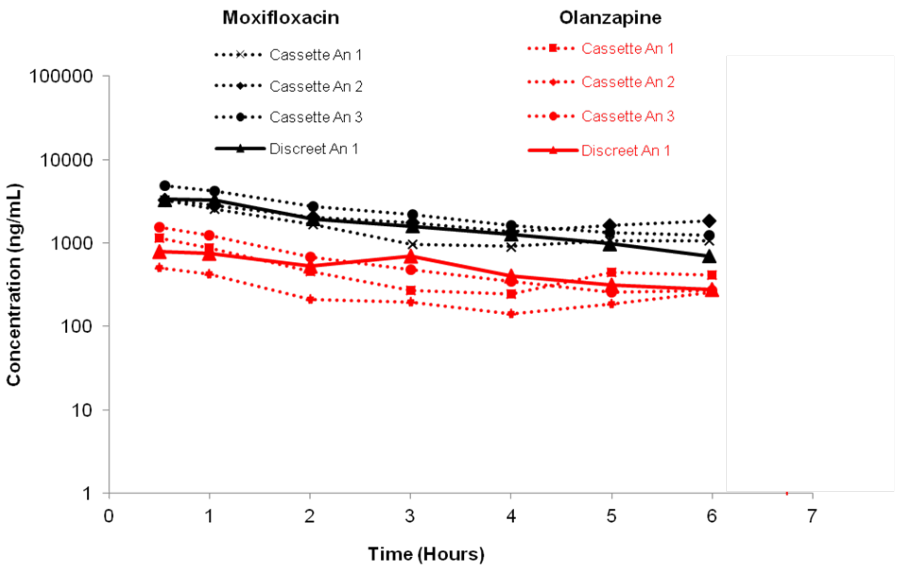

Table S-1: Pharmacokinetic parameters of pharmaceutical compounds after oral cassette or discrete dosing to male Hans Wistar rats.

	Erlotinib			Moxifloxacin			
Parameter	Cassette 1	Cassette 2	Cassette 3	Cassette 1	Cassette 2	Cassette 3	Discreet 1
Dose (mg/kg)	10	10	10	25	25	25	25
Cmax (ng/mL)	4354	3081	5576	3746	3787	5657	3777
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Half-life (h)	9.5	13.1	5.6	3.2	5.6	2.6	2.3
AUC0-t (hr*ng/mL)	16711	13160	21701	9719	12898	16045	11480
AUCinf (hr*ng/mL)	55064	54488	46179	15129	29806	21099	13991

	Terfenadine			Olanzapine			
Parameter	Cassette 1	Cassette 2	Cassette 3	Cassette 1	Cassette 2	Cassette 3	Discreet 1
Dose (mg/kg)	25	25	25	10	10	10	10
Cmax (ng/mL)	43	32	79	1162	507	1562	791
Tmax (h)	1.0	0.5	2.0	0.5	0.5	0.5	0.5
Half-life (h)	3.5	2.4	1.4	3.8	4.7	2.0	3.7
AUC0-t (hr*ng/mL)	155	71	261	2879	1438	3618	3032
AUCinf (hr*ng/mL)	245	102	288	5131	3193	4418	4530

Figure S-2 Sample spectra confirming exposure in lung tissue of A) moxifloxacin, B) olanzapine, C) erlotinib and D) terfenadine by accurate mass and comparison of each with a vehicle dosed control sample. The mass accuracy of each analyte confirms the response obtained can only be from the compounds dosed.