Supplementary Information

Quantitative Mass Spectrometry Imaging of Drugs and Metabolites: a Multiplatform Comparison

AUTHORS

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Table S1. Intensity ratio and standard deviation of calibration points in multiplatform comparison

		Drug candidate A					
		Xevo Q-ToF	Synapt Q-ToF			Xevo QqQ	
Calibration level	Concentration (µg/g)	MS scan	MS scan	HDMS	HDMS^E	MS scan	MRM
C0	0.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1	12.50	0.01 ± 0.00	<LOD	<LOD	<LOD	<LOD	<LOD
C2	24.99	0.01 ± 0.00	<LOD	<LOD	<LOD	<LOD	<LOD
C3	124.97	0.02 ± 0.01	0.04 ± 0.02	<LOD	<LOD	<LOD	0.04 ± 0.02
C4	249.96	0.04 ± 0.02	0.09 ± 0.07	0.31 ± 0.36	<LOD	<LOD	0.19 ± 0.14
C5	500.11	0.15 ± 0.12	0.34 ± 0.30	1.26 ± 0.42	1.56 ± 0.66	0.13 ± 0.03	0.43 ± 0.20
C6	1250.08	0.53 ± 0.05	0.98 ± 0.36	3.34 ± 0.32	2.89 ± 0.06	0.26 ± 0.05	1.90 ± 0.51
C7	2500.14	1.19 ± 0.09	1.56 ± 0.28	8.46 ± 2.98	7.43 ± 2.97	0.55 ± 0.11	3.40 ± 0.27
		n=3	*n=3*	*n=3*	*n=3*	*n=3*	*n=3*

		Drug candidate B					
		Xevo Q-ToF	Synapt Q-ToF			Xevo QqQ	
Calibration level	Concentration (µg/g)	MS scan	MS scan	HDMS	HDMS^E	MS scan	MRM
C0	0.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1	12.50	0.02 ± 0.01	0.07 ± 0.02	0.22 ± 0.36	<LOD	<LOD	0.49 ± 0.04
C2	24.99	0.04 ± 0.02	0.30 ± 0.32	0.52 ± 0.56	<LOD	<LOD	2.09 ± 1.55
C3	124.97	0.14 ± 0.07	0.30 ± 0.27	0.71 ± 0.53	1.30 ± 1.08	0.23 ± 0.09	7.90 ± 6.00
C4	249.96	0.33 ± 0.12	0.69 ± 0.28	2.02 ± 1.20	3.78 ± 1.64	0.21 ± 0.16	13.30 ± 7.19
C5	500.11	1.26 ± 0.88	8.59 ± 1.06	67.13 ± 24.30	46.85 ± 48.27	0.41 ± 0.27	28.98 ± 5.52
C6	1250.08	6.20 ± 0.51	16.04 ± 3.09	146.86 ± 97.52	162.61 ± 56.86	1.24 ± 0.32	73.34 ± 10.34
C7	2500.14	13.45 ± 0.72	29.00 ± 12.03	318.08 ± 6.31	372.50 ± 175.60	2.59 ± 0.25	143.67 ± 19.12
		n=3	*n=3*	*n=3*	*n=3*	*n=3*	*n=3*

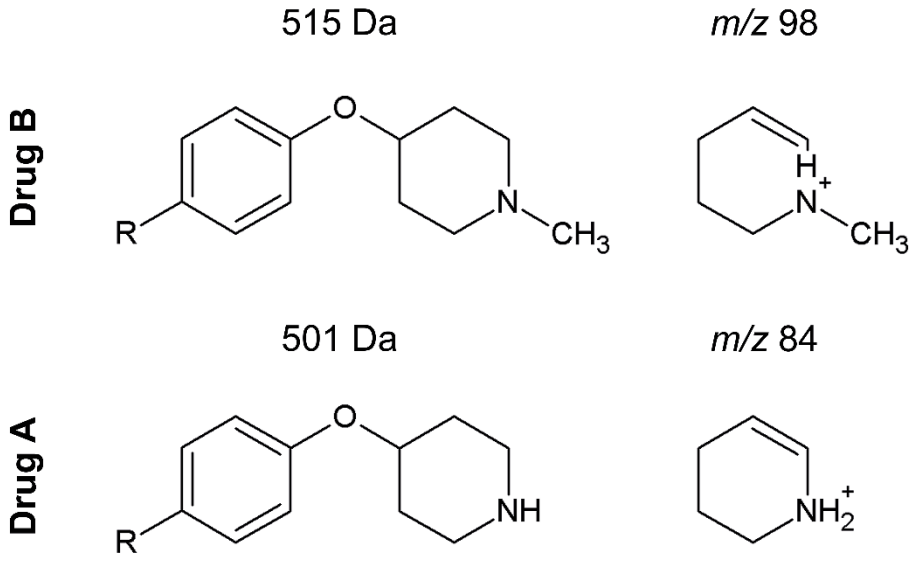

Fig. S1. *Partial molecular structures of drug candidate A and B at, respectively, 501 and 515 dalton. Two most-likely fragment ions that are used in the MRM transitions are shown at m/z 98 and m/z 84.*

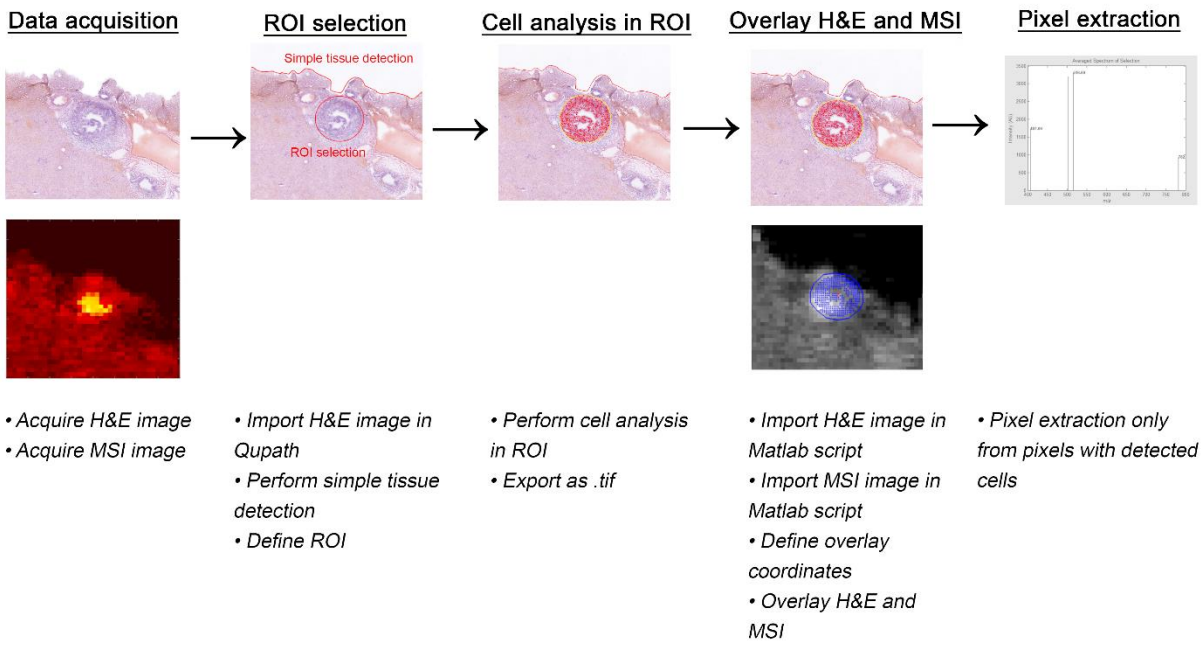

Fig. S2. *Data analysis workflow consists of a 5-step procedure. Step 1: MSI and H&E images are acquired. Step 2: ROI selection in Qupath. Step 3: Cell analysis in Qupath to detect cells. Step 4: Overlay H&E image (with detected cells marked) and MSI image in in-house written Matlab script. Step 5: MSI pixel selection and extraction in in-house written Matlab script. From these MS spectra, intensity ratios between the drug candidates and endogenous lipid are used.*

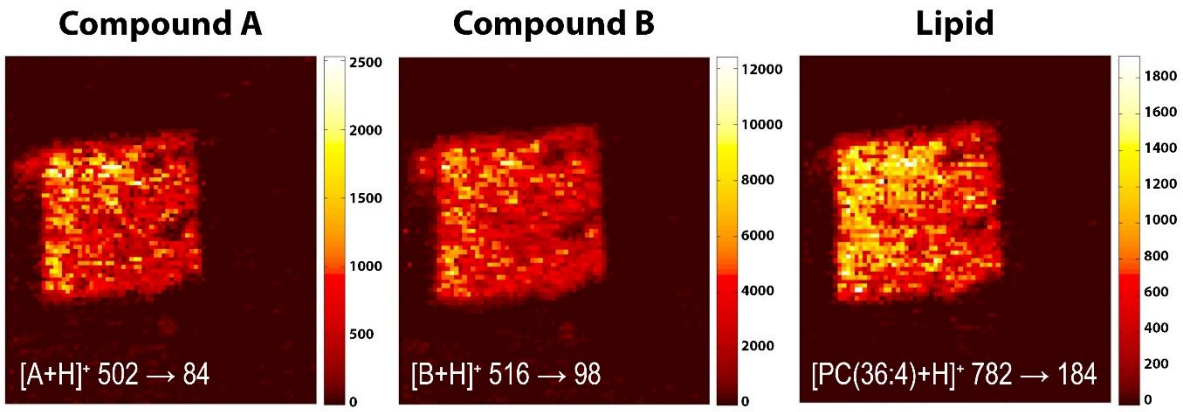

Fig. S3. *Diffusion of drug candidates into gelatin is shown by DESI-MRM images of a tissue homogenate pillar for drug candidate A, drug candidate B, and the endogenous lipid (used as a correction factor). The MRM images were acquired in positive ion mode at a spatial resolution of 50 µm. Dwell times were 0.247 s/pixel.*

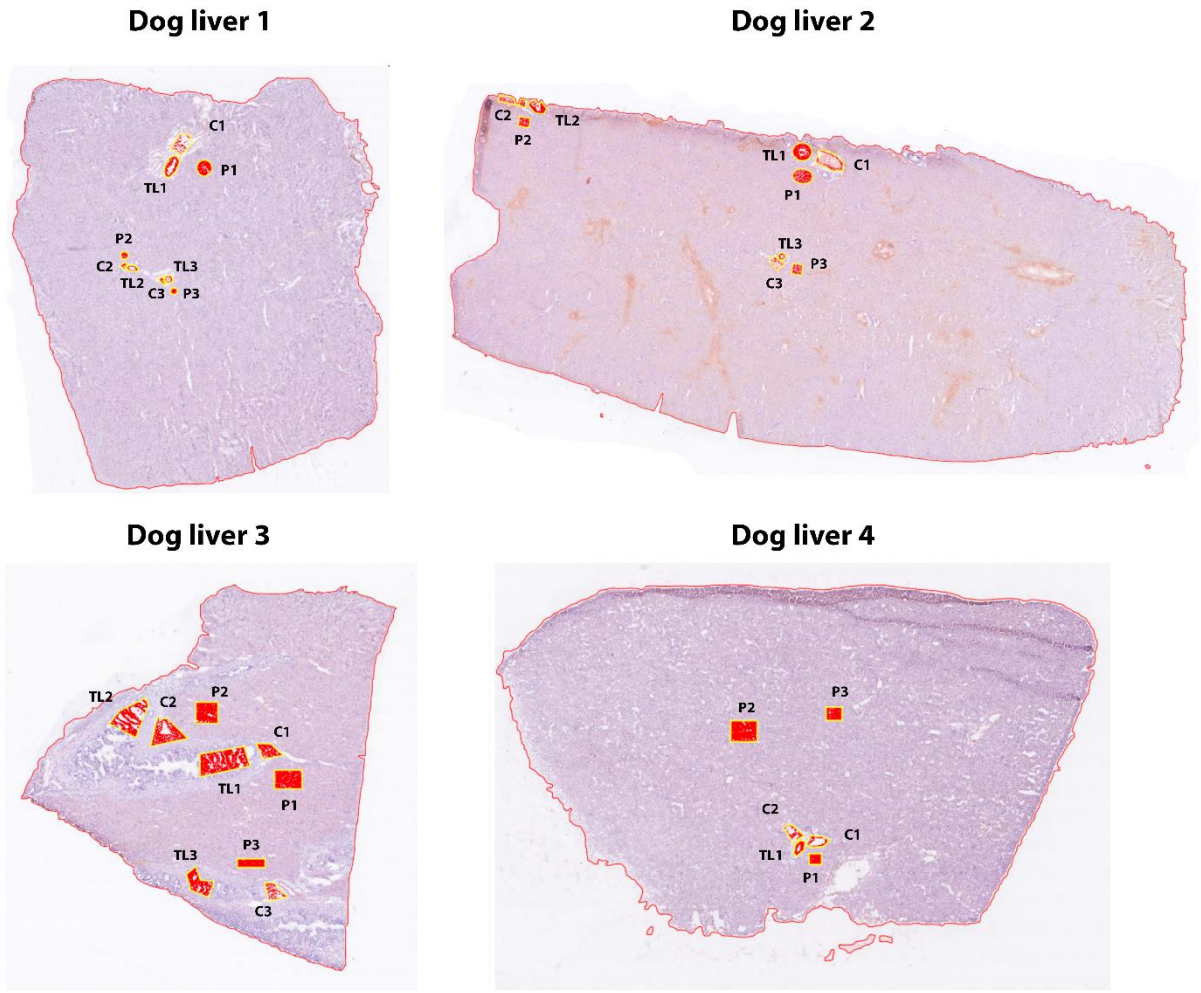

Fig. S4. *ROI selection in four dog liver tissues in their H&E images (Qupath software). For each dog liver tissue, the tissue is annotated by a pathologist and classified as tissue lesion (TL), connective tissue (C), and parenchyma (P). Where possible for each dog liver, ROIs of all tissue types are extracted in triplicate (f.e. TL1, TL2, and TL3). These H&E images were overlaid with the MSI images as described in Fig. S1.*

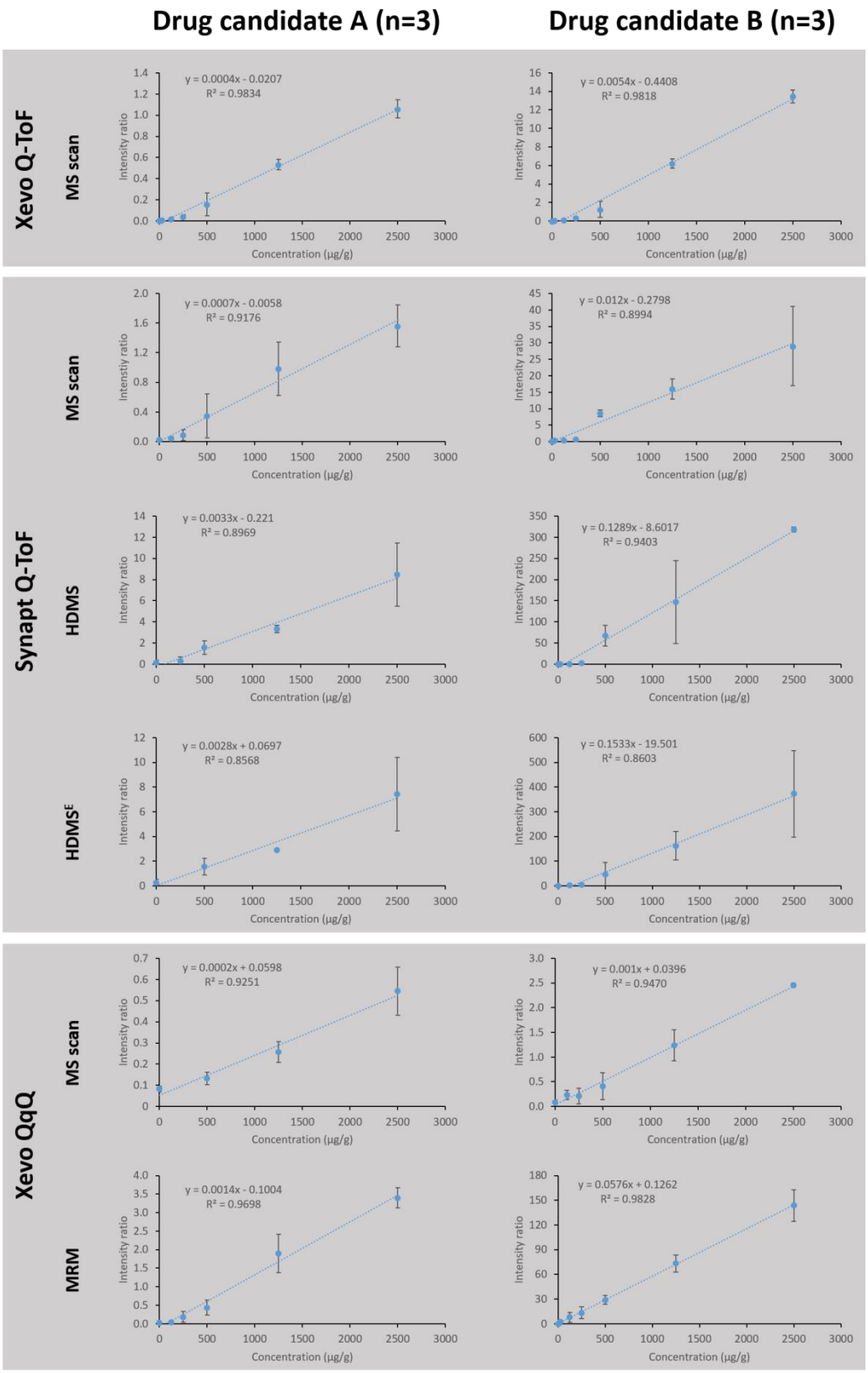

Fig. S5*. Calibration curves obtained in the multiplatform comparison for drug candidates A (left column) and B (right column). The multiplatform comparison includes calibration lines achieved on the Xevo Q-ToF (MS scan), Synapt Q-ToF (MS scan, HDMS, and HDMS^E), and Xevo QqQ (MS scan and MRM). Error bars show the standard deviation of the intensity ratio for each calibration level. This figure complements Table 1 and Table S1.*