

Appendix 1

Plasma sample analysis

Patient plasma samples were stored at -80°C . Samples were prepared for analysis by thawing on ice followed by a centrifugation step to remove insoluble plasma components, 50 μL of plasma was removed from the sample and deproteinated using an equal volume of ice cold acetonitrile. The deproteinated sample was diluted with 100 μL of 0.1% formic acid in water and the sample centrifuged at 15K rpm to remove all insoluble materials. The sample was analyzed for curcumin using a Waters Xevo TQS mass spectrometer coupled to a Waters Acquity UPLC with a Phenomenex Kinetex PFP 2.6 μm 50 \times 2 mm analytical column. Curcumin was detected using electrospray positive ionization in selected reaction monitoring mode (m/z 369.1 \rightarrow 177.05). Quantification was performed by comparing the sample curcumin peak area to a regression curve prepared from peak area of known concentrations of curcumin in protein precipitated human plasma to match the patient plasma sample matrix.

Mass spectrometry instrument conditions

MS conditions

Capillary voltage: 2.43 kV;

Cone voltage: 44 V;

Collision energy: 22 eV;

Source temperature: 150 $^{\circ}\text{C}$;

Desolvation temperature: 200 $^{\circ}\text{C}$.

LC conditions

Mobile phase A: 0.1% formic acid in water;

Mobile phase B: 80:20 (acetonitrile: methanol);

Sample temperature: 10 $^{\circ}\text{C}$;

Column temperature: 60 $^{\circ}\text{C}$;

Injection volume: 10 μL ;

Gradient (linear): see [Table S1](#);

Curcumin retention time: 2.22 \pm 0.1 minutes.

Tissue extraction procedure

The tissue extraction procedure was similar to previous reports (51). Samples were reconstituted and then injected into an Agilent 6460 triple quadrupole liquid chromatograph/mass spectrometer for quantitative analysis. Curcumin was separated on a Phenomenex Luna C18 column (3 μM , 2 \times 100 mm). Curcumin was eluted with mobile phase A 0.1% formic acid in water and mobile phase B 0.1% formic acid in acetonitrile using a gradient, delivered at a flow rate of 0.3 mL/min. The total run time was 15 min (31). Ionization was determined using electrospray in the positive ionization mode. Curcumin was monitored in the multiple reaction monitoring (MRM) mode. The MRM transition for curcumin was m/z 369 \rightarrow 285. Concentrations of metabolites were quantified using an authentic standard curve and normalized to the protein concentration of the homogenate.

References

51. Ramalingam P, Ko YT. A validated LC-MS/MS method for quantitative analysis of curcumin in mouse plasma and brain tissue and its application in pharmacokinetic and brain distribution studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 2014;969:101-8.

Table S1 Gradient (linear)

Time (min)	Flow rate (mL/min)	%A	%B
Initial	0.350	75	25
0.25	0.350	75	25
1.50	0.350	2	98
3.00	0.350	2	98
3.50	0.350	75	25
5.00	0.350	75	25