

Supplementary S1: LC/MS/MS instrumentation method and analysis parameters to measure cytochrome P450 activity levels of HepaRG cells.

Instrumentation

Mass Spectrometer	API 5000 (SCIEX, Concord, Ontario, Canada) Triple Quadrupole Mass Spectrometer with Turboion Spray source (Cobalt)
HPLC	Waters (Milford, MA) Acquity UPLC with autosampler and column compartment

Chromatography Conditions

Column	Phenomenex (Torrance, CA) Luna C18(2) (30 x 3 mm, 5 µm) with C18 guard cartridge
Injection Volume	10 microliters
Mobile Phase	A: 0.05% Formic Acid in 5 mM Ammonium Formate, B: 0.05% Formic Acid in Acetonitrile:Methanol (95:5)
Flow Rate	0.7 mL/min
Gradient	Initially 2% B, change linearly to 5% B over 0.5 min. Change linearly to 71% B over 2 min, then return to initial conditions over 0.5 min

MS Parameters: Positive TurboSpray Ionization

Parameter	MS	MS	MS Setting_1-
	Setting_Acetaminophen	Setting_Hydroxybupropion	hydroxymidazolam
Polarity	Positive	Positive	Positive
Ion Source	TurboSpray	TurboSpray	TurboSpray
Resolution	Unit	Unit	Unit
Curtain Gas	10	10	10
Gas 1	50	50	50
Gas 2	50	50	50
IonSpray Voltage	2500.00	2500.00	2500.00
Source Temperature	650	650	650
Collision Gas (CAD)	8	8	8
Entrance Potential	10.00	10.00	10.00
Declustering Potential	41	46	76
Collision Cell Exit Potential	18	16	31
Collision Energy	23	17	24
Analyte MRM	151.95 → 110.0	256.12 → 238.0	342.07 → 324.0
Internal Standard MRM	154.95 → 111.0	262.10 → 243.8	346.00 → 328.0

Analytes and Stable Label Internal Standards

Analyte or ISTD	Manufacturer	Catalog #	Lot #
Acetaminophen	Sigma (St. Louis, MO)	A7085	053K0040
¹³ C ₂ - ¹⁵ N-Acetaminophen	Cambridge Isotope Labs, Inc. (Tewksbury, MA)	CNLM-3726-1.2	SDED-003
Hydroxybupropion	BD Gentest (Corning, NY)	451711	00081
Hydroxybupropion-d ₆	BD Gentest	451003	78308
1'-Hydroxymidazolam	BD Gentest	451038	51142
1'-Hydroxymidazolam-d ₄	Cerilliant (Round Rock, TX)	61-546	FN092807-02