

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Study of in-vitro metabolism of selected antibiotic drugs in human liver microsomes by liquid chromatography coupled with tandem mass spectrometry

Malgorzata Szultka-Mlynska, Boguslaw Buszewski

Table S1 Characteristic of isoenzymes used during the study

Enzyme	Enzymatic activity [pmol/(mg×min)]
<i>Total P450</i>	250 pmole/mg
<i>Cyt. b₅</i>	460 pmole/mg
<i>CYP1A2</i>	820
<i>CYP2A6</i>	1200
<i>CYP2B6</i>	28
<i>CYP2C8</i>	190
<i>CYP2C9</i>	3000
<i>CYP2C19</i>	53
<i>CYP2D6</i>	99
<i>CYP2E1</i>	1900
<i>CYP3A4</i>	4900
<i>CYP4A11</i>	3500
<i>FMO</i>	680
<i>UGT1A1</i>	920
<i>UGT1A4</i>	890
<i>UGT1A9</i>	2400

Table S2 Intra-day accuracy and precision of target compounds in human liver microsomes

Compound	Theoretical concentration [mg/ml]	Observed concentration [mean±SD]	Accuracy [%]	RSD
CEF (n=10)	1	1.1±0.3	110.0	6.83
	3	3.3±0.1	110.0	1.66
	5	5.3±0.7	106.0	6.25
	7	6.9±0.4	98.5	0.24
	10	9.5±0.4	95.0	3.10
	15	15.2±0.3	101.3	3.26
	20	20.1±0.4	100.5	2.44
	30	28.9±0.1	96.3	2.90
	50	47.3±0.6	94.6	2.76
CIP (n=10)	1	1.1±0.3	110.0	7.03
	3	2.9±0.1	96.6	1.70
	5	5.1±0.7	102.0	6.43
	7	6.7±0.4	95.7	0.24
	10	9.8±0.4	98.0	3.19
	15	15.1±0.3	100.6	3.35
	20	20.2±0.4	101.0	2.51
	30	29.3±0.1	97.6	2.98
	50	48.2±0.6	96.4	2.84
FLU (n=10)	1	1.1±0.3	110.0	6.69
	3	3.1±0.1	103.3	1.62
	5	5.2±0.7	104.0	6.12
	7	7.1±0.4	101.4	0.23
	10	9.9±0.4	99.0	3.03
	15	15.2±0.3	101.3	3.19
	20	19.9±0.4	99.5	2.39
	30	30.1±0.1	100.3	2.84
	50	49.4±0.6	98.8	2.70
GEN (n=10)	1	1.1±0.3	110.0	6.48
	3	3.1±0.1	103.3	1.57
	5	5.1±0.7	102.0	5.93
	7	7.2±0.4	102.8	0.22
	10	9.8±0.4	98.0	2.94
	15	15.1±0.3	100.6	3.09
	20	20.3±0.4	101.5	2.31
	30	29.5±0.1	98.3	2.75
	50	48.6±0.6	97.2	2.62
KLI (n=10)	1	0.9±0.3	90.0	6.89
	3	3.2±0.1	106.6	1.67
	5	5.1±0.7	102.0	6.31
	7	7.1±0.4	101.4	0.24
	10	9.7±0.4	97.0	3.13
	15	15.1±0.3	100.6	3.29
	20	20.3±0.4	101.5	2.46
	30	29.7±0.1	99.0	2.92
	50	49.2±0.6	98.4	2.78
LIN (n=10)	1	0.9±0.3	90.0	7.01
	3	2.8±0.1	93.3	1.70
	5	5.1±0.7	102.0	6.40
	7	7.3±0.4	104.2	0.24
	10	9.9±0.4	99.0	3.17
	15	15.1±0.3	100.6	3.34
	20	20.4±0.4	102.0	2.50
	30	30.2±0.1	100.6	2.97
	50	50.2±0.6	100.4	2.82
MET	1	1.1±0.3	110.0	6.97

(n=10)	3	3.1±0.1	103.3	1.69
	5	5.1±0.7	102.0	6.38
	7	7.2±0.4	102.8	0.24
	10	9.8±0.4	98.0	3.16
	15	14.8±0.3	98.6	3.32
	20	19.9±0.4	99.5	2.49
	30	29.3±0.1	97.6	2.96
	50	49.5±0.6	99.0	2.81

Table S3 Accuracy and precision results

Compound	Nominal concentration (µg/ml)		Intraday (n=4)		Interday (n=3)	
			%Bias	%CV	%Bias	%CV
Cefotaxime	LQC	1	-2.5	10.8	-10.7	12.6
	MQC	20	-0.5	11.7	-5.0	7.9
	HQC	50	-2.8	6.1	-6.9	5.6
Ciprofloxacin	LQC	1	-1.1	12.4	-9.6	11.8
	MQC	20	-0.9	10.3	-5.8	6.5
	HQC	50	-3.1	7.2	-3.5	3.9
Fluconazole	LQC	1	-0.8	10.8	-10.3	11.5
	MQC	20	-0.9	5.9	-7.4	7.5
	HQC	50	-3.5	2.9	-3.9	4.9
Gentamicin	LQC	1	-1.2	9.6	-9.4	12.3
	MQC	20	-0.8	6.8	-6.4	10.6
	HQC	50	-4.4	2.7	-2.9	8.5
Clindamycin	LQC	1	-1.3	12.1	-9.4	12.2
	MQC	20	-0.9	9.6	-8.3	8.9
	HQC	50	-4.2	3.8	-3.7	5.4
Linezolid	LQC	1	-1.2	10.6	-10.2	11.4
	MQC	20	-1.1	5.9	-5.9	9.6
	HQC	50	-3.9	3.7	-3.8	6.8
Metronidazole	LQC	1	-1.1	9.5	-9.5	10.5
	MQC	20	-0.9	5.8	-8.3	9.7
	HQC	50	-4.4	3.4	-4.2	3.5

Table S4 The stability of target compounds in human liver microsomes under different conditions

Antibiotic drug	Amount added	Stability			
		Short-term stability [12 h/20°C]	Long-term stability [30 days/-20°C]	Freeze-thaw stability [-20°C to room temperature]	Post-preparative stability [24 h/10°C]
		Amount found [mean±SD]			
CEF	1 µg/ml	1.2±0.82	1.3±1.11	1.2±0.48	1.1±0.36
	7 µg/ml	6.9±0.39	7.2±1.29	7.3±2.11	7.1±0.94
	15 µg/ml	15.1±1.21	15.2±4.21	14.9±1.34	15.3±0.87
CIP	1 µg/ml	1.1±0.68	1.2±1.13	1.1±0.49	1.2±0.39
	7 µg/ml	7.1±0.42	7.1±1.49	7.2±2.18	7.4±0.99
	15 µg/ml	15.3±1.18	15.1±3.21	15.2±1.35	15.1±0.57
FLU	1 µg/ml	1.1±0.72	1.3±1.17	1.1±0.98	1.2±0.76
	7 µg/ml	7.3±0.69	7.2±1.25	7.3±2.61	7.1±0.95
	15 µg/ml	15.2±1.51	15.1±4.71	15.4±1.36	15.1±1.17
GEN	1 µg/ml	1.1±0.89	1.1±1.15	1.1±0.58	1.2±0.85
	7 µg/ml	7.2±0.69	7.1±1.69	7.1±3.15	7.2±2.58
	15 µg/ml	15.1±1.69	15.2±2.38	14.8±1.35	15.1±0.59
KLI	1 µg/ml	1.2±0.69	1.1±1.19	1.1±0.98	1.2±0.74
	7 µg/ml	7.1±0.45	7.2±1.35	7.1±2.72	7.2±0.97
	15 µg/ml	15.2±1.89	15.1±3.28	15.1±1.59	15.2±1.49
LIN	1 µg/ml	1.1±3.85	1.1±1.19	1.2±1.38	1.2±0.58
	7 µg/ml	6.9±0.59	7.1±2.28	7.2±4.18	7.1±2.38
	15 µg/ml	15.2±1.39	15.1±4.28	14.9±1.28	15.1±0.84
MET	1 µg/ml	1.1±2.88	1.2±1.15	1.1±2.92	1.2±1.38
	7 µg/ml	6.9±0.39	7.2±1.29	7.3±2.15	7.3±0.29
	15 µg/ml	15.1±1.21	15.2±4.21	14.9±1.3	15.3±0.87

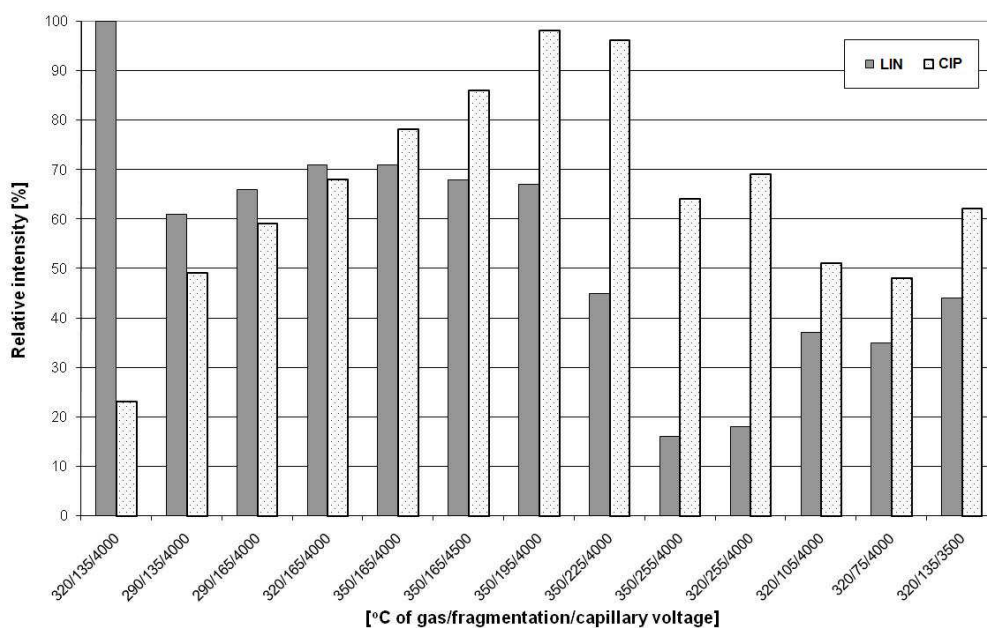


Fig. S1 Comparison of the relative intensity at $m/z=338$ $[M+H]^+$ for LIN and $m/z=332$ $[M+H]^+$ for CIP using different values of parameters: drying gas temperature (DGT), fragmentation (F), capillary voltage (CV)