

Consistent High-throughput Metabolic Stability Screening Across Mass Spectrometry Platforms

Michelle V. Romm, Nikunj Parikh, Vaughn Miller, William A. LaMarr, Selena Larkin, Can C. Ozbal
BioTrove, Inc., Woburn, MA, <http://www.biotrove.com>

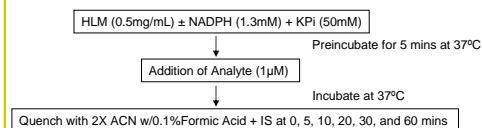


Abstract

The metabolic half-life or stability of a drug candidate has important pharmacokinetic and clinical significance *in vivo*, because it influences both oral bioavailability and plasma concentration of a compound, which in turn, affect its efficacy. Therefore, a high-throughput metabolic stability assay is a valuable tool in the go/no-go decision-making process in drug discovery. However, conventional LC-MS requires several minutes per sample processing time. Using the RapidFire system, we have developed analytical techniques which lower analysis times to only a few seconds per sample, allowing metabolic stability assays to be performed at throughputs consistent with other drug discovery screening platforms. In the present study we evaluated the compatibility of the RapidFire system with 3 MS platforms (ABI/Sciex, ThermoFisher, and Agilent) by measuring half lives using conventional assay methods. The values measured across all MS platforms tested were within a 2-fold window. This data shows that the RapidFire system produces consistent, high-throughput, metabolic stability data across multiple MS platforms enabling performance of these critical assays in a typical drug discovery environment.

Assay Conditions

Human Liver Microsome Incubations



Analysis

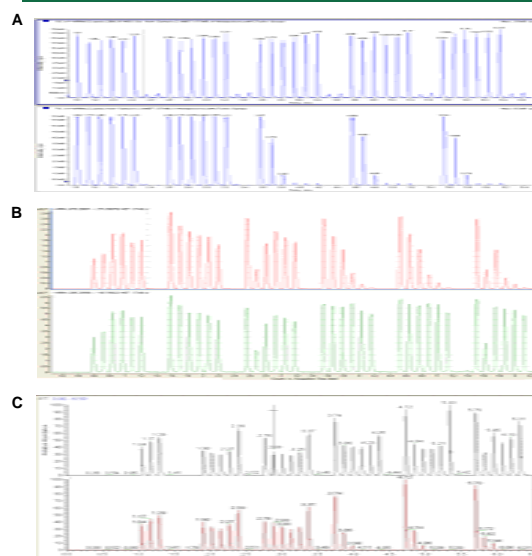
All assays were monitored using the RapidFire high-throughput mass spectrometry system software interfaced to an Agilent 6410, an Applied Biosystems Sciex API4000, and a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer.

RapidFire Conditions

- Buffer A = Water with 0.09% formic acid, 0.01% TFA; 1.5 mL/min flow rate
- Buffer B = 100% acetonitrile with 0.09% formic acid, 0.01% TFA; 1.0 mL/min flow rate
- SPE Column A (reversed-phase C_{18} chemistry)

The compound and its internal standard (Bupivacaine) were monitored simultaneously in all experiments

Rapidfire system



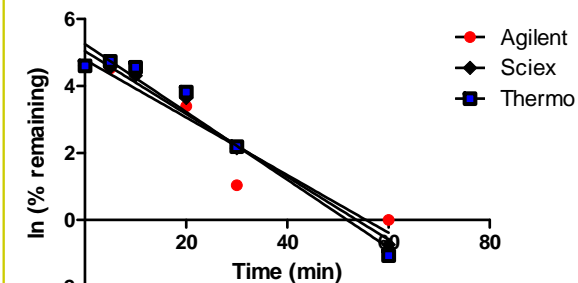
- (A) The EIC of Nefazodone and Bupivacaine (IS) from a half-life plate for Nefazodone using a RapidFire connected to an Agilent 6410.
(B) The EIC of Nefazodone and Bupivacaine (IS) from a half-life plate for Nefazodone using a RapidFire connected to an ABI4000.
(C) The EIC of Nefazodone and Bupivacaine (IS) from a half-life plate for Nefazodone using a RapidFire connected to a TSQ Quantum Ultra.

Results

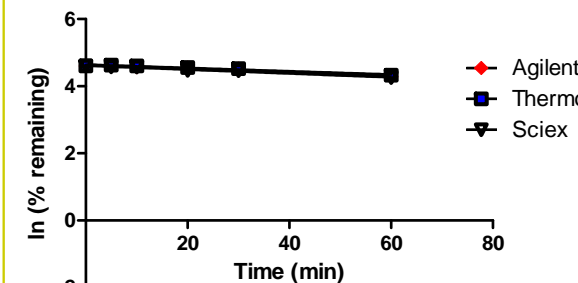
Compound	XLogP3	Mol Formula	MW	H-bond Donor	H-bond Acceptor	Half Lives		
						Agilent	Sciex	Thermo
High clearance (<20)								
Nefazodone	4.3	C ₂₅ H ₃₂ ClN ₂ O ₂	470.00688	0	5	1.2	3.2	2.4
Nimodipine	3.1	C ₂₁ H ₂₈ N ₂ O ₇	418.44034	1	8	7.7	10.5	15.6
Nicardipine	3.8	C ₂₀ H ₂₈ N ₂ O ₆	479.52496	1	8	2.7	4.4	3
Midazolam	2.5	C ₁₉ H ₁₃ ClFN ₃	325.767323	0	3	5.9	8.49	8.46
Intermediate clearance (20-60)								
Propafenone	3.3	C ₂₁ H ₂₇ NO ₃	341.44398	2	4	39.1	46.7	41.7
Terfenadine	6.6	C ₂₀ H ₂₁ NO ₂	471.67344	2	3	43	71	51.8
Buspirone	2.6	C ₁₂ H ₁₅ N ₂ O ₂	385.50314	0	6	28.2	25.4	33
Ticlopidine	3.6	C ₁₄ H ₁₄ CIN ₃ S	263.78566	0	1	26.2	26.5	38.7
Verapamil	3.8	C ₂₇ H ₃₈ N ₂ O ₄	454.60162	0	6	39	76	45.9
Fluphenazine	4.4	C ₂₂ H ₂₈ F ₃ N ₂ OS	437.52155	1	7	30.6	96	41
Chlorpromazine	5.2	C ₁₇ H ₁₉ ClN ₂ S	318.86416	0	2	54.9	71	53
Thioridazine	5.9	C ₂₁ H ₂₆ N ₂ S ₂	370.57454	0	2	55	96.9	75.8
Low clearance (>60)								
Imipramine	4.8	C ₁₉ H ₂₃ N ₂	280.40726	0	2	>60	>60	>60
Tolbutamide	2.3	C ₁₂ H ₁₈ N ₂ O ₃ S	270.34792	2	3	>60	>60	>60
Desipramine	4.9	C ₁₈ H ₂₂ N ₂	266.38068	1	2	>60	>60	>60
Diphenhydramine	3.3	C ₁₇ H ₂₁ NO	255.35474	0	2	>60	>60	>60
Carbamazepine	2.5	C ₁₅ H ₁₂ N ₂ O	236.26858	1	1	>60	>60	>60
Nizatidine	1.6	C ₁₂ H ₂₁ N ₅ O ₂ S ₂	331.45744	2	6	>60	>60	>60
Promazine	4.5	C ₁₇ H ₂₀ N ₂ S	284.4191	0	2	>60	>60	>60
Chlorpheniramine	3.4	C ₁₈ H ₁₉ ClN ₂	274.78846	0	2	>60	>60	>60
Cinnarizine	5.8	C ₂₀ H ₂₈ N ₂	368.51392	0	2	>60	>60	>60
Norfloxacin	-1	C ₁₈ H ₁₈ FN ₃ O ₃	319.330823	2	7	>60	>60	>60
Haloperidol	3.2	C ₂₁ H ₂₅ ClFN ₂ O	375.864223	1	4	>60	>60	>60
Fluvoxamine	2.6	C ₁₂ H ₂₁ F ₃ N ₂ O ₂	318.33465	1	7	>60	>60	>60
Diltiazem	3.1	C ₂₂ H ₂₈ N ₂ O ₄ S	414.51784	0	5	>60	>60	>60
Propranolol	3	C ₁₆ H ₂₁ NO ₂	259.34344	2	3	>60	>60	>60
Promethazine	4.8	C ₁₇ H ₂₀ N ₂ S	284.4191	0	2	>60	>60	>60
Dextromethorphan	3.4	C ₁₈ H ₂₅ NO	271.3972	0	2	>60	>60	>60
Lansoprazole	2.8	C ₁₆ H ₁₄ F ₃ N ₂ O ₂ S	369.36147	1	7	>60	>60	>60
Tripolidine	NA	NA	NA	NA	NA	>60	>60	>60
Tamoxifen	7.1	C ₂₆ H ₃₂ NO	371.51456	0	2	>60	>60	>60
Amoxapine	2.6	C ₁₇ H ₁₆ ClN ₃ O	313.78144	1	4	>60	>60	>60
Amitriptyline	5	C ₂₀ H ₂₃ N	277.40332	0	1	>60	>60	>60
Metoprolol	1.9	C ₁₅ H ₂₅ NO ₃	267.3639	2	4	>60	>60	>60
Nadolol	0.7	C ₁₇ H ₂₇ NO ₄	309.40058	4	5	>60	>60	>60
Clozapine	3.2	C ₁₈ H ₁₉ ClN ₄	326.82326	1	4	>60	>60	>60
Quinidine	2.9	C ₂₀ H ₂₂ N ₂ O ₂	324.41676	1	4	>60	>60	>60

*XLogP3 values are from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov>). These database values were calculated using XLogP3 software (*J. Chem. Inf. Model.* 2007, **47**, 2140-2148; <http://www.sioc-ccbq.ac.cn/software/xlogp3/>).

Results



Example of a high clearance metabolite, Midazolam. The half-life was calculated based on first order linear kinetics.



Example of a low clearance metabolite, Tolbutamide. The half-life was calculated based on first order linear kinetics.

Conclusions

- Multiple MS Vendors gave the same results
 - Corresponding half-life values were within 2-Fold
- RapidFire 300 sustained throughputs of 6-8 seconds/sample
- Generic SPE conditions were effective for the analysis of a diverse set of compounds (xlogP3 -1 to 7.1)