



Evaluation of Drug Plasma Stability Using an Ultra-High Throughput Laser Diode Thermal Desorption (LDTD) Methodology

Authors

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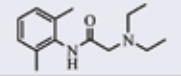
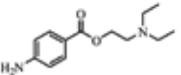
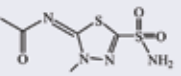
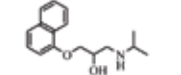
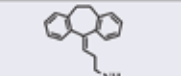
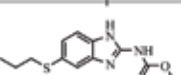
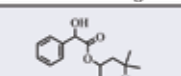
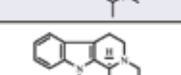
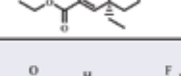
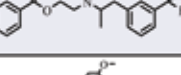
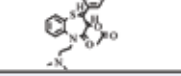
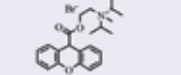
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Introduction

The Laser Diode Thermal Desorption (LDTD) interface with tandem mass spectrometry is a new technology allowing an ultra-high throughput sample analysis (approximately 10 seconds per sample). Unlike Matrix-assisted Laser Desorption/Ionization (MALDI), LDTD does not require enhancing matrix to assist compound ionization. The LDTD/MS/MS analysis does not utilize organic mobile phase and liquid chromatography. We performed plasma stability experiments to examine the applicability of this technology using twelve compounds with different chemical properties. Collected plasma stability samples were analyzed by LDTD/MS/MS and LC/MS/MS which was considered as a gold standard approach. The chemical structures and the half-lives of these compounds were evaluated by these two methodologies for a comparison.

Chemicals

Compound	Chemical Formula	Exact MW	Structure
Lidocaine	$C_{14}H_{22}N_2O$	234.17	
Procaine	$C_{13}H_{20}N_2O_2$	236.15	
Methazolamide	$C_5H_8N_4O_3S_2$	236.10	
Propranolol	$C_{16}H_{21}NO_2$	259.16	
Nortriptyline	$C_{19}H_{21}N$	263.17	
Albendazole	$C_{12}H_{15}N_3O_2S$	265.09	
Eucatropine	$C_{17}H_{25}NO_3$	291.18	
Vinpocetine	$C_{22}H_{26}N_2O_2$	350.20	
Benfluorex	$C_{19}H_{20}F_3NO_2$	351.14	
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.16	
Propantheline Bromide	$C_{23}H_{30}BrNO_3$	447.14	
Methotrexate	$C_{20}H_{22}N_8O_5$	454.17	

Experimental

- A group of 12 compounds were incubated with human and rat plasma at 5 μM for 0, 10, 30, 60 and 120 minutes at 37 $^{\circ}\text{C}$, respectively.
- At each time point, 100 μL of 5 μM plasma sample was taken and was added to 400 μL of 1 μM carbutamide/ACN solution in a 96-deep well plate.
- After protein precipitation, supernatants were evenly split for analysis.
- For LC/MS/MS analysis, supernatants were diluted with the mobile phase A (95/5 H₂O/ACN, 0.1% formic acid) prior to analysis.
- For LDTD/MS/MS analysis, 2 μL of supernatants were transferred into a 96-Lazwell plate. The solvent was allowed to evaporate at room temperature (less than 2 minutes) before the analysis.

LC/MS/MS

- Column: Phenomenex Hydro-RP C18 (50 x 2.0 mm)
- Column Heater: 40 \rightarrow $^{\circ}\text{C}$
- Mobile Phase A: 95/5 H₂O/ACN, 0.1% formic acid
- Mobile Phase B: 10/90 H₂O/ACN, 0.1% formic acid
- Injection Volume: 10 μL
- Gradient

Time (min)	% B	Flow (mL)
0.0	10	0.6
1.5	90	0.6
2.0	90	0.6
2.1	10	0.6
2.5	10	0.6

- API 5000 (Applied Biosystem/Sciex)
- MRM under an electrospray positive mode



Experimental continued

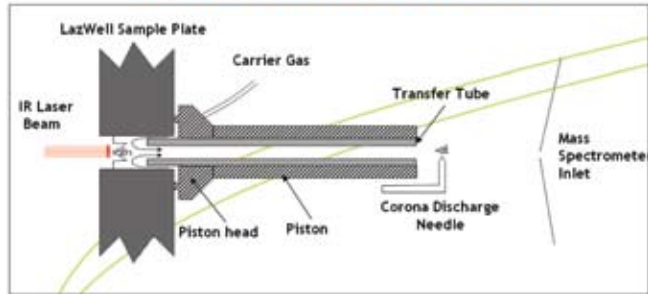
LDTD/MS/MS

- A Phytronix LDTD source (model T-960) on Thermo TSQ Vantage triple quadrupole mass spectrometer
- Two micro-liters of supernatants were deposited onto the LazWell plate.
- Dry the solvent on the LazWell plate prior to LDTD/MS/MS analysis

LDTD Source (T-960)



LDTD Ionization Source

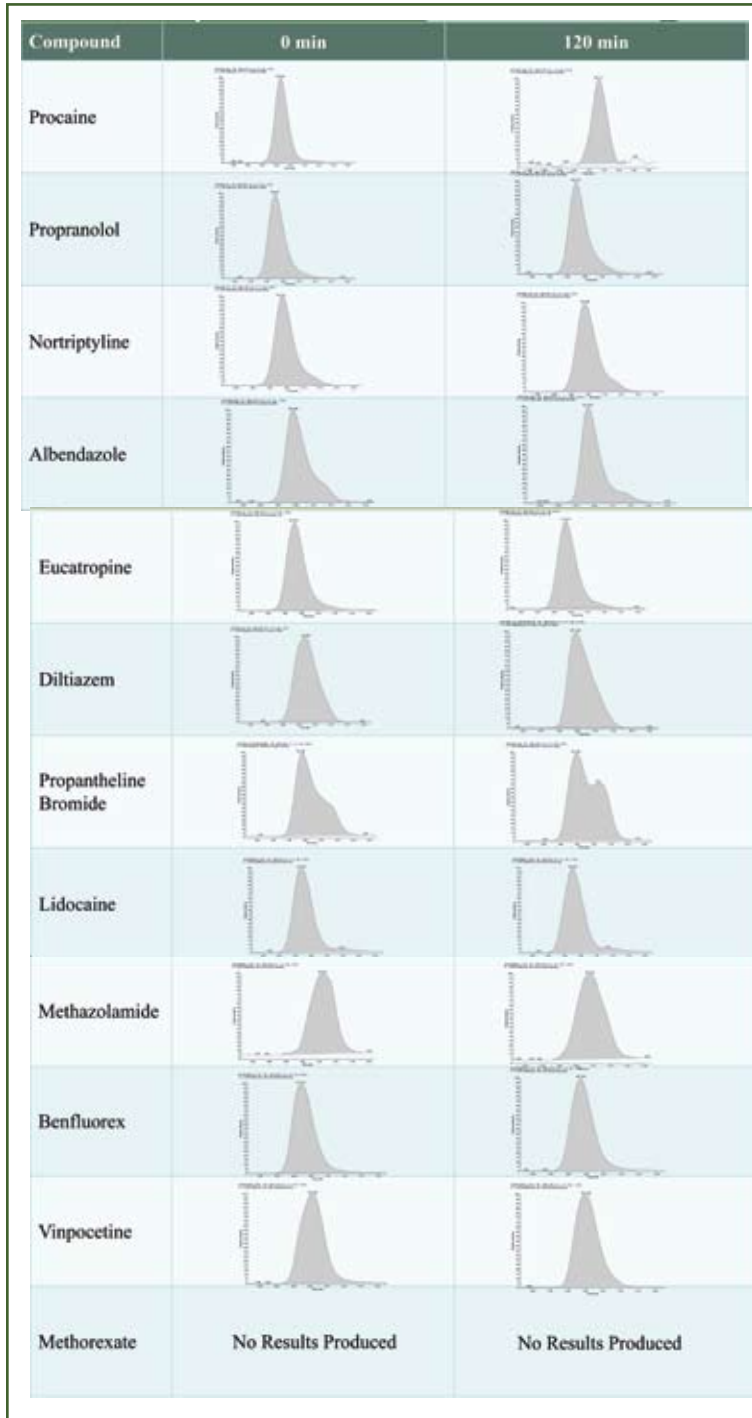


MRM TRANSITIONS

Compound	Precursor Ion (m/z)	Product Ion (m/z)	
		LC/MS/MS	LDTD/MS/MS
Albendazole	266.0	191.1	234.0
Benfluorex	352.5	230.2	230.4
Diltiazem	415.5	178.1	178.0
Eucaptopine	292.4	109.1	58.1
Lidocaine	235.2	86.2	86.1
Methazolamide	237.2	195.2	195.0
Methotrexate	454.9	308.4	NS*
Nortriptyline	264.4	233.2	233.1
Procaine	237.3	100.0	120.0
Proprantheline Bromide	368.2	181.0	181.0
Propranolol	260.3	116.1	183.0
Vinpocetine	351.2	280.3	322.1

Results and Discussion

REPRESENTATIVE LDTD/MS/MS THERMAL DESORPTION PEAKS



Human Plasma Stability Data (% Remaining)

Compound	Analysis	0 min	10 min	30 min	60 min	120 min	T _{1/2}
Procaine	LC/MS/MS	100	0.65	0.06	0.03	0.05	< 10 min
	LDTD/MS/MS	100	0.67	0.36	0.31	0.31	< 10 min
Propranolol	LC/MS/MS	100	107	79	91.7	85.8	> 4 hr
	LDTD/MS/MS	100	114	107	98.7	101	> 4 hr
Nortriptyline	LC/MS/MS	100	109	103	110	114	> 4 hr
	LDTD/MS/MS	100	106	95.6	105	110	> 4 hr
Albendazole	LC/MS/MS	100	77.9	109	94.1	108	> 4 hr
	LDTD/MS/MS	100	111	103	128	161	> 4 hr
Eucatropine	LC/MS/MS	100	71.6	51.4	33.9	9.68	34 min
	LDTD/MS/MS	100	83.9	48.2	27.5	6.79	31 min
Diltiazem	LC/MS/MS	100	78.9	89.5	78.3	87.7	> 4 hr
	LDTD/MS/MS	100	100	93.9	100	121	> 4 hr
Propantheline Bromide	LC/MS/MS	100	98.9	96.4	65.3	29.3	78min
	LDTD/MS/MS	100	79.0	75.2	73.5	34.3	82 min
Lidocaine	LC/MS/MS	100	93.3	106	104	105	> 4 hr
	LDTD/MS/MS	100	147	136	124	106	> 4 hr
Methazolamide	LC/MS/MS	100	104	111	102	107	> 4 hr
	LDTD/MS/MS	100	155	141	143	166	> 4 hr
Methotrexate	LC/MS/MS	100	126	108	103	167	> 4 hr
	LDTD/MS/MS	---	---	---	---	---	---
Benfluorex	LC/MS/MS	100	96.6	83.9	82.5	56.8	152 min
	LDTD/MS/MS	100	75.0	70.7	55.9	43.6	89 min
Vinpocetine	LC/MS/MS	100	102	107	130	127	> 4 hr
	LDTD/MS/MS	100	110	120	107	118	> 4 hr

Rat Plasma Stability Data (% Remaining)

Compound	Analysis	0 min	10 min	30 min	60 min	120 min	Half-Life
Procaine	LC/MS/MS	100	108	105.0	88.0	81.0	> 4 hr
	LDTD/MS/MS	100	119	109	116	88.9	> 4 hr
Propranolol	LC/MS/MS	100	84.2	85.2	90.7	102	> 4 hr
	LDTD/MS/MS	100	127	95.6	96.4	114	> 4 hr
Nortriptyline	LC/MS/MS	100	93.9	95.7	90.0	95.0	> 4 hr
	LDTD/MS/MS	100	121	126	128	107	> 4 hr
Albendazole	LC/MS/MS	100	114	117	128	159	> 4 hr
	LDTD/MS/MS	100	185	161	139	151	> 4 hr
Eucatropine	LC/MS/MS	100	100.4	83.3	99.8	89.5	> 4 hr
	LDTD/MS/MS	100	113	113	120	117	> 4 hr
Diltiazem	LC/MS/MS	100	98.2	86.0	78.6	48.7	122 min
	LDTD/MS/MS	100	118	87.4	87.3	41.5	107 min
Proprantheline Bromide	LC/MS/MS	100	108.1	103.2	104.7	95.3	> 4 hr
	LDTD/MS/MS	100	127	82.9	94.6	83.2	> 4 hr
Lidocaine	LC/MS/MS	100	93.4	85.4	89.3	82.3	> 4 hr
	LDTD/MS/MS	100	135	104	109	123	> 4 hr
Methazolamide	LC/MS/MS	100	92.5	90.4	91.5	97.2	> 4 hr
	LDTD/MS/MS	100	115	109	110	100	> 4 hr
Methotrexate	LC/MS/MS	100	140.9	149.8	139.4	123.7	> 4 hr
	LDTD/MS/MS	100	---	---	---	---	---
Benfluorex	LC/MS/MS	100	90.6	76.6	53.5	23.3	61 min
	LDTD/MS/MS	100	106	86.5	55.1	25.6	67 min
Vinpocetine	LC/MS/MS	100	86.4	81.3	67.2	34.5	83 min
	LDTD/MS/MS	100	96.8	90.2	71.0	32.1	82 min

Chemical Properties of Compounds Used for LDTD/MS/MS Analysis

Compound	MW	Aliphatic N	Hetero -Ar N	Ar N	OH	COOH	S	COOR	Amide	Ar	Hetero-Ar
Albendazole	265.09		2 X				X	X	X	X	X
Benfluorex	351.14	X, 2°						X, Ar		2X	
Diltiazem	414.16	X, 3°		X, 3°			X	X	X	2X	
Eucatropine	291.18	X, 3°			X, 2°			X		X	
Lidocaine	234.17	X, 3°							X		
Methazolamide	236.1	X, 3°	X				X		X		
Methorexate	454.17		2X	X		2X			X	X	2X
Nortriptyline	263.17	X, 2°								2X	
Procaine	236.15	X, 3°		X				X, Ar		X	
Propantheline Br	447.14	X, 4°						X		2X	X
Propranolol	259.16	X, 2°			X, 2°					2X-fused	
Vinpocetine	350.2	X, 3°	X					X		X	X



Results and Discussion

CHEMISTRY VS. IONIZATION: We found that the ionization of basic compounds, such as amine containing compounds are generally very good by using the LDTD/MS/MS method. The LDTD source produced strong signals for the hetero-cyclic compounds (e.g., Albendazole). Ionization of Albendazole was weak using an electrospray source on API 4000. However, for quaternary amines, the LC/MS/MS method has an advantage over the LDTD/MS/MS method. The presence of OH groups seemed to weaken the LDTD ionization. A signal could not be obtained when using the LDTD/MS/MS for compounds possessing a carboxylic acid functional group, e.g., Cerivastatin (data not shown) and Methotrexate.

THROUGHPUT: The total analysis time for 100 samples was less than 20 minutes by LDTD/MS/MS (10 seconds per sample) . It took approximately 5 hours using a 2.5-minute LC/MS/MS method.

PLASMA STABILITY: The plasma stability data generated from each method matched remarkably well. With each method, we were able to distinguish compounds with a long half-life (> 4hr) from those with a moderate half-life (within 2 hr) and a short half-life (< 10 min) in both human and rat plasma. We found that the stabilities of procaine, eucatropine, diltiazem, propantheline bromide and vinpocetin in rat plasma and in human plasma were different. The findings from both analytical methods also agreed with each other regarding the difference of half-lives of these compounds in these two species.

Conclusion

The LDTD/MS/MS technology can be applied as an ultra-high throughput screening method for the stability assessment of small molecules in plasma. The results over 12 compounds suggest a combination of the LDTD/MS/MS method (covering 90% of all screened compound) with the traditional LC/MS/MS method (covering the 10% uncovered by the LDTD/MS/MS) allows to increase the throughput on plasma stability screening of a factor of 6.2 times. The method development of the LDTD/MS/MS analysis of compounds with a carboxylic acid functional group is in progress using different solvents and/or the negative mode.