

LDTD384-MS/MS

for in vitro assays

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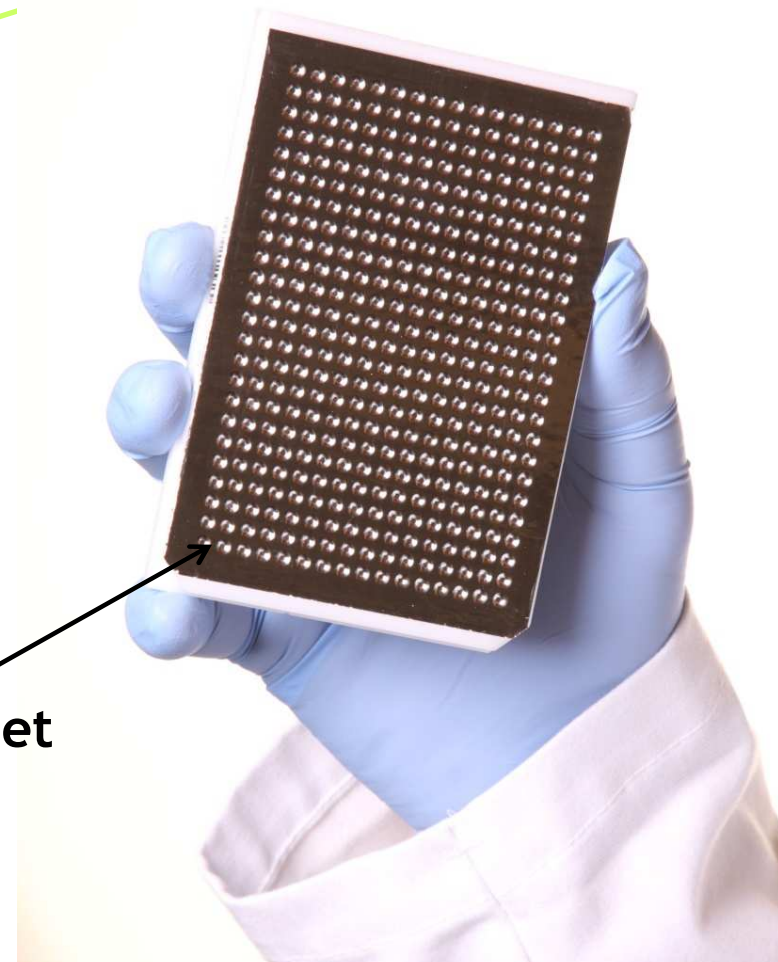
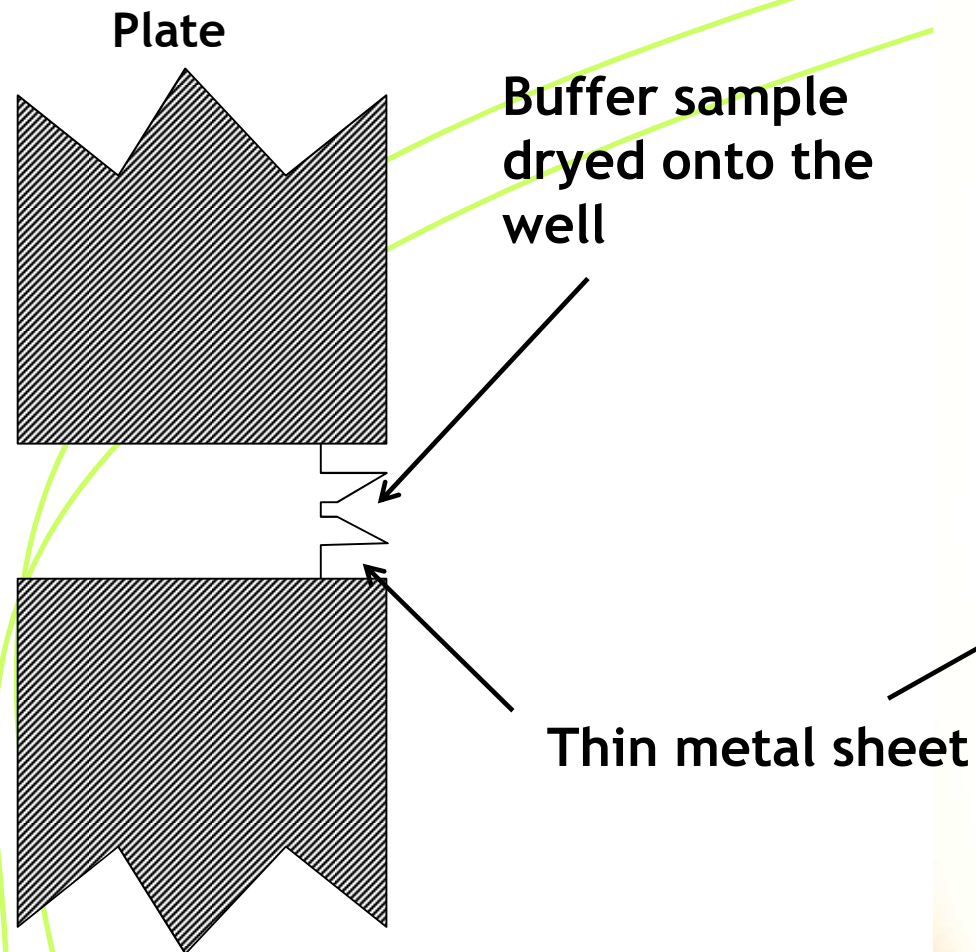
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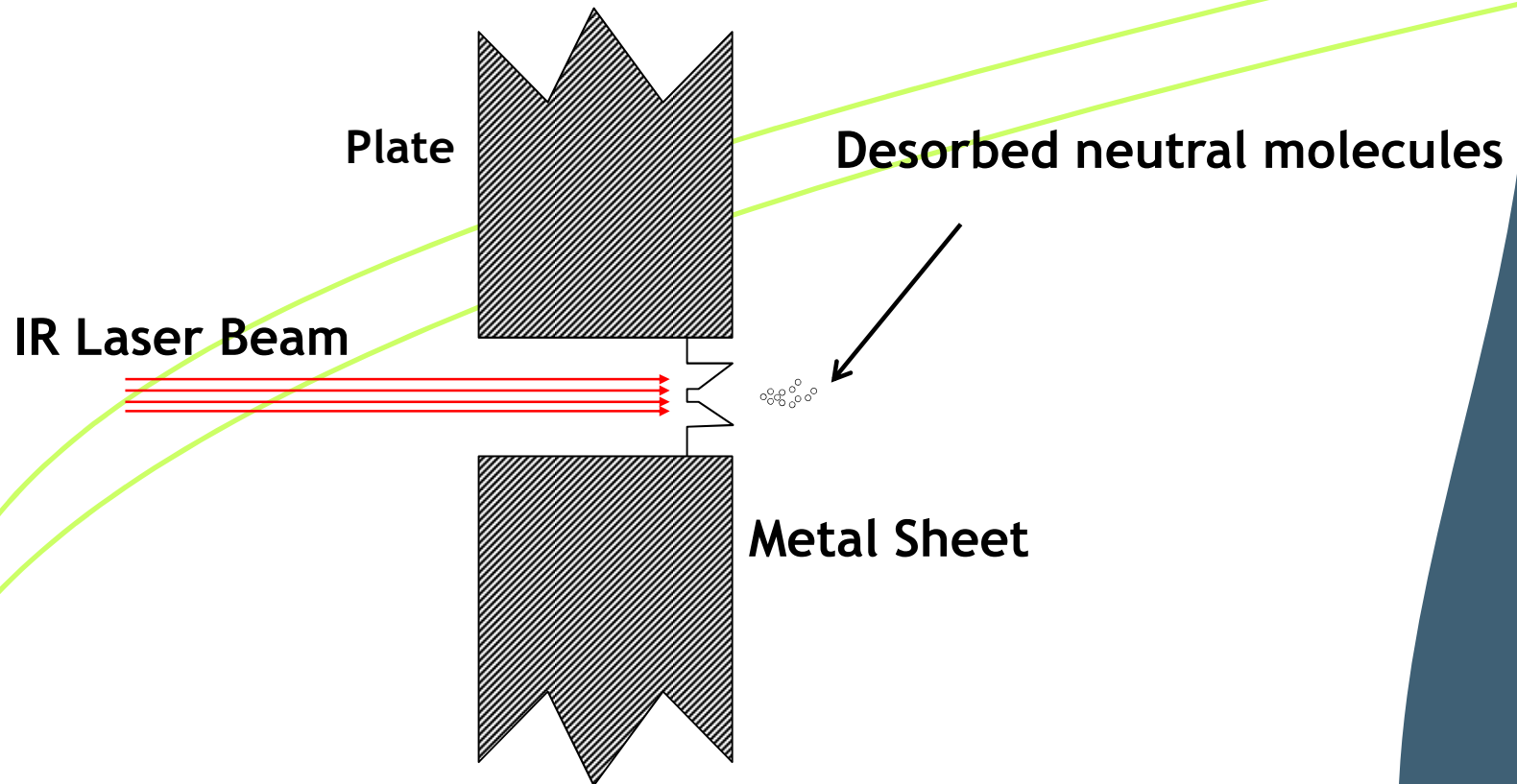
Because health matters

What is Laser Diode Thermal Desorption ?

- The 386-Well plate



What is Laser Diode Thermal Desorption ?



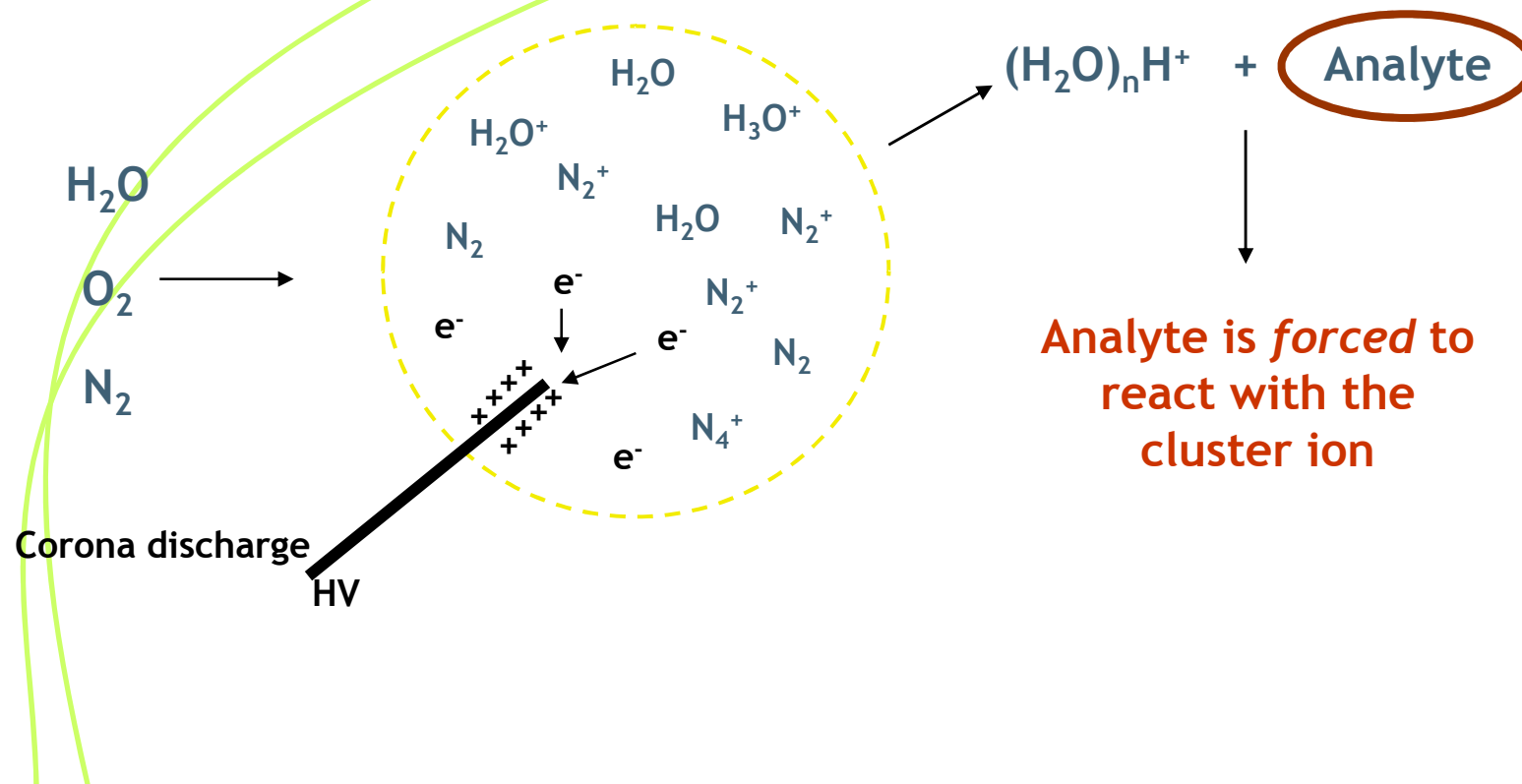
- No photon interaction with the sample
- Ultra fast heat transfer
- Quantitative sample desorption

LDTD-APCI

LDTD-APCI : Ionization Source

Gas-phase atmospheric pressure chemical ionization

Dry sample, no solvent and no salt or acid/base added



LDTD-APCI Ionization Source

384 well plates (also available in 96 format)

High throughput analysis as the thermal desorption process takes few seconds

Ten 384 well plates capacity loader

Same MS interface attachment



LDTD-APCI in Early Drug Development

- Wet chemistry labs generate many drug candidates / week
- Fast and Accurate *in vitro* data needs to be generated
 - Compound solubility in physiologic buffer
 - Cell membrane permeability
- Looking for efficient generic method
- Bottleneck : UPLC-MS/MS analysis (1-3 min / sample)
- Proposed solution : LDTD-MS/MS (10 sec / sample)

In Vitro Studies

- Can the LDTD operate in buffered environment ?
- Solution tested :
 - Solubility Assay
 - 0.1 M K_2HPO_4 pH = 7.4
 - Permeability Assay
 - Corneal Epithelial Model Differentiation Medium permeability buffer (CEMDM)
 - Dulbecco's Modified Eagle Medium (DMEM)
 - Hank's Balanced Salt Solution (HBSS) with HEPES and BSA

Evaluation

- Generate calibration curves for each solution
 - Range : 1 to 10 000 nM
- Evaluation performed on 18 commercial compounds
 - Limit of quantification (LLOQ) and Upper limit of linearity (ULOL) and the Linearity
- Real life samples from a pharmaceutical company
 - Cross-validation between LDTD (96-well) and UPLC
 - High Throughput comparison

Generic LDTD Conditions

Instrumentation

TSQ Vantage (Canada)/Ultra(France)

LDTD T-3840

QuickQuan software



Sample preparation (dilute and shoot)

Sample 20 μL (1 to 10 000 nM) + Water 60 μL

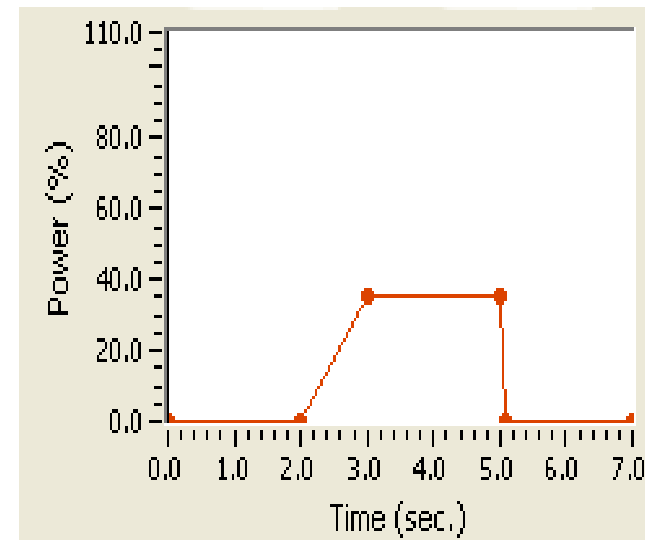
1 μL « injected »

Laser

Laser power at 0 % for 3 s

Laser power from 0 % to 35 % in 1 s

Laser power back to 0 % in 0.01 s



Results

	Phosphate			HBSS			DMEM			CEMDM		
	r ²	LLOQ	ULOL	r ²	LLOQ	ULOL	r ²	LLOQ	ULOL	r ²	LLOQ	ULOL
Cetirizine	0.98	10	1000	0.99	1	1000				0.99	50	1000
Diazepam	0.97	10	1000	0.99	5	10000	0.99	10	10000	0.99	5	10000
Diltiazem	0.99	1	10000	0.99	1	10000	0.99	5	10000	0.99	5	10000
Eucatropine	0.98	5	1000	0.99	1	10000	0.98	1	50	0.99	1	100
Hydroxyzine	0.98	1	1000	0.99	1	10000	0.99	1	1000	0.99	1	1000
Nortriptyline	0.99	50	1000	0.99	10	1000	0.99	10	10000	0.99	50	10000
5-OH-Propafenone				0.97	1	1000				0.99	50	10000
Propafenone	0.99	5	1000	0.99	1	10000	0.99	1	10000	0.99	5	10000
Propantheline	0.99	10	1000	0.99	5	10000				0.98	50	10000
Propranolol	0.99	5	1000	0.99	10	1000	0.99	10	10000	0.99	5	1000
Desipramine	0.99	50	1000	0.99	10	10000	0.99	50	10000	0.99	50	10000
Desloratadine	0.98	10	1000	0.99	10	1000	0.99	10	10000	0.99	50	10000
Imipramine	0.99	1	10000	0.99	10	10000	0.99	100	10000	0.98	10	10000
Loratadine	0.99	50	10000	0.99	10	10000	0.98	50	1000	0.98	50	10000
Terfenadine	0.98	10	10000	0.99	10	10000	0.99	5	1000	0.98	10	10000
Dextromethorphan	0.99	10	10000	0.99	10	10000	0.99	50	10000	0.99	50	10000
Fluxetine	0.99	50	1000	0.99	10	1000	0.96	50	10000	0.98	50	1000
Verapamil	0.99	10	1000	0.99	1	10000	0.99	10	1000	0.99	10	10000

LLOQ ≤ 10 nM

72 %

72 %

50 %

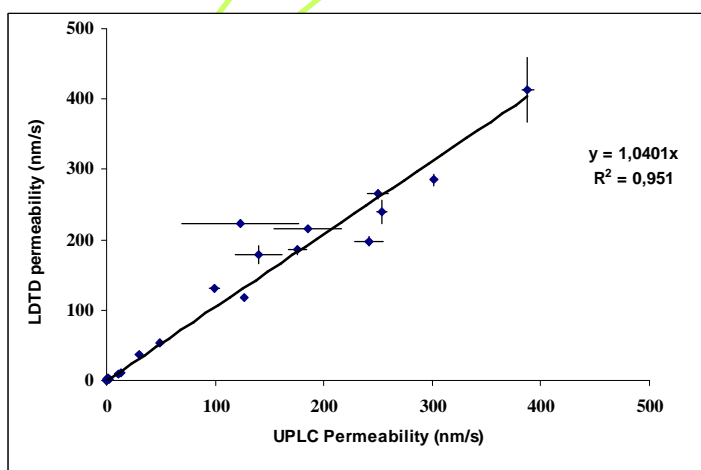
Results

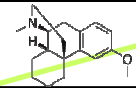
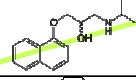
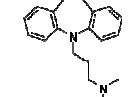
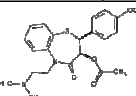
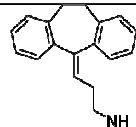
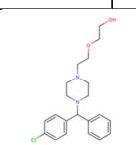
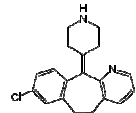
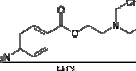
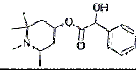
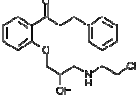
	Phosphate			HBSS			DMEM		CEMDM		
	r ²	LLOQ	ULOL	r ²	LLOQ	ULOL	LLOQ	ULOL	r ²	LLOQ	ULOL
Cetirizine	0.98	10	1000	0.99	1	1000			0.99	50	1000
Diazepam	0.97	10	1000	0.99	1	10000	10	10000	0.99	5	10000
Diltiazem	0.99	1	10000	0.99	1	10000	5	10000	0.99	5	10000
Eucatropine	0.98	5	1000	0.99	1	10000	1	50	0.99	1	100
Hydroxyzine	0.98	1	1000				1	1000	0.99	1	1000
Nortriptyline	0.99	50	1000	0.99	10	1000	10	10000	0.99	50	10000
5-OH-Propafenone				0.97	1	1000			0.99	50	10000
Propafenone	0.99	5	1000	0.99	1	10000	1	10000	0.99	5	10000
Propantheline	0.99	10	1000						0.98	50	10000
Propranolol	0.99	5	1000	0.99	5	10000	10	10000	0.99	5	1000
Desipramine	0.99	50	1000	0.99	10	1000	50	10000	0.99	50	10000
Desloratadine	0.98	10	1000				10	10000	0.99	50	10000
Imipramine	0.99	1	10000	0.99	10	10000	100	10000	0.98	10	10000
Loratadine	0.99	50	10000	0.99	10	1000	50	1000	0.98	50	10000
Terfenadine	0.98	10	10000	0.99	10	10000	5	1000	0.98	10	10000
Dextromethorphan	0.99	10	10000				50	10000	0.99	50	10000
Fluxetine	0.99	50	1000	0.99	10	10000	50	10000	0.98	50	1000
Verapamil	0.99	10	1000	0.99	10	10000	10	1000	0.99	10	10000
				0.99	10	10000					
				0.99	10	1000					
				0.99	1	10000					

LLOQ ≤ 10 nM 100 %

Permeability results : HBSS Buffer

- Comparable permeability results
- Comparable repeatability
- Wide chemical structure tested



Compound	Formula	structure	LDTD		UPLC	
			Ptot	SD	Ptot	SD
Dextromethorphan	C ₁₈ H ₂₅ NO		172.88	7.97	184.76	0.7
Propranolol	C ₁₆ H ₂₁ NO ₂		216.06	0.00	185.30	30.90
Imipramine	C ₁₉ H ₂₄ N ₂		239.12	16.70	253.30	4.62
Diltiazem	C ₂₂ H ₂₆ N ₂ O ₄ S		185.92	6.65	176.23	8.37
Nortriptyline	C ₁₉ H ₂₁ N		289.74	13.29	185.62	5.98
Hydroxyzine	C ₂₁ H ₂₇ ClN ₂ O ₂		227.71	21.08	212.71	13.06
Desloratadine	C ₁₉ H ₁₉ ClN ₂		11.79	0.94	14.51	1.02
Procaine	C ₁₃ H ₂₀ N ₂ O ₂		167.94	1.53		
Encatropine	C ₁₇ H ₂₅ NO ₃		71.11	3.51	70.60	2.48
Propafenone	C ₂₁ H ₂₇ NO ₃		268.53	36.84	155.23	10.26

Note : LDTD data provided by Sanofi-Aventis ran on a T-960

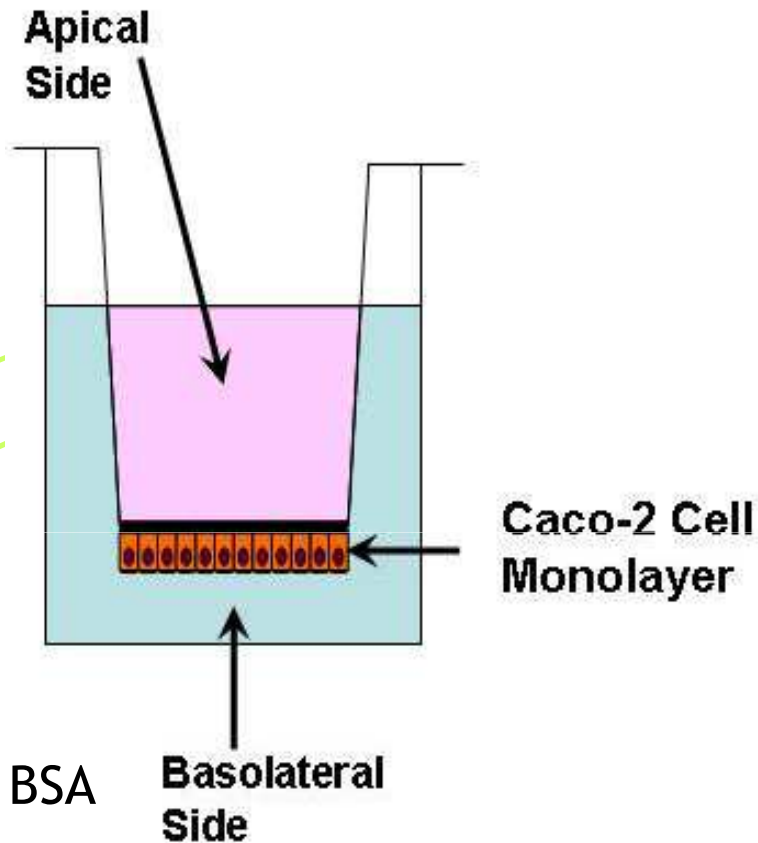
LDTD in high throughput environment

Case Study for Early Drug
Discovery using HBSS buffer

Caco-2 / TC-7 model

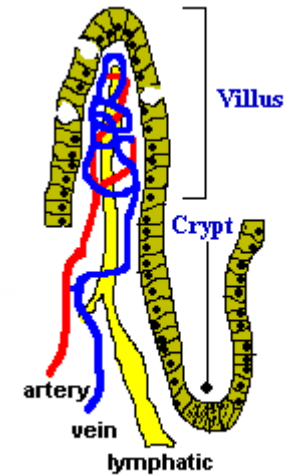
HBSS pH 7.4 with
0.5 % BSA

Test compound at 20 μM



HBSS pH 7.4 with 5 % BSA

Incubation at 37°C for 2 hours



Blank (3), Apical T-0h (2), Apical T-2h (2), Basal T-2h (2), 3 standards

Sample Preparation for Analysis

Apical : Dilution 1/20 with HBSS pH 7.4 with 5 % BSA

Basal : No dilution

Protein precipitation 1:1 with acetonitrile (both Apical and Basal sides) followed by centrifugation 3000 rpm

UPLC-MS/MS analysis

- 2 mass spectrometers
- Multiple columns

Apical and Basal dilution

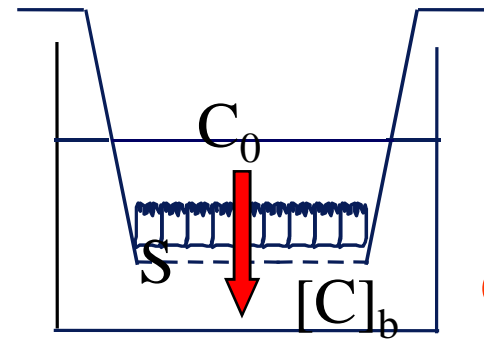
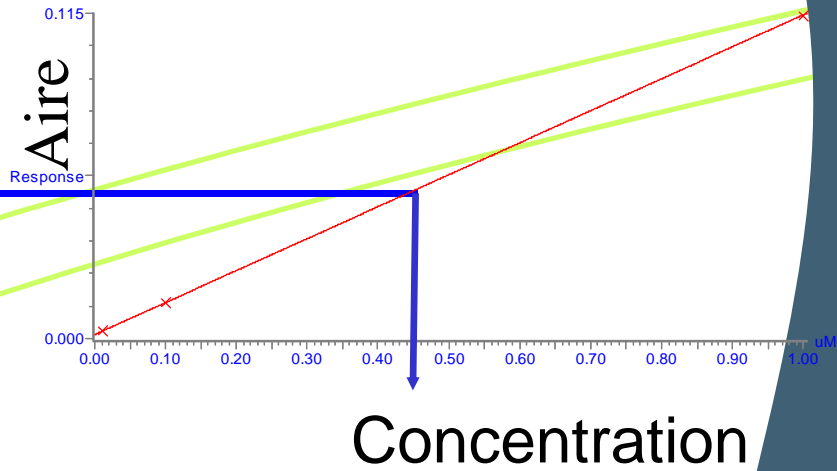
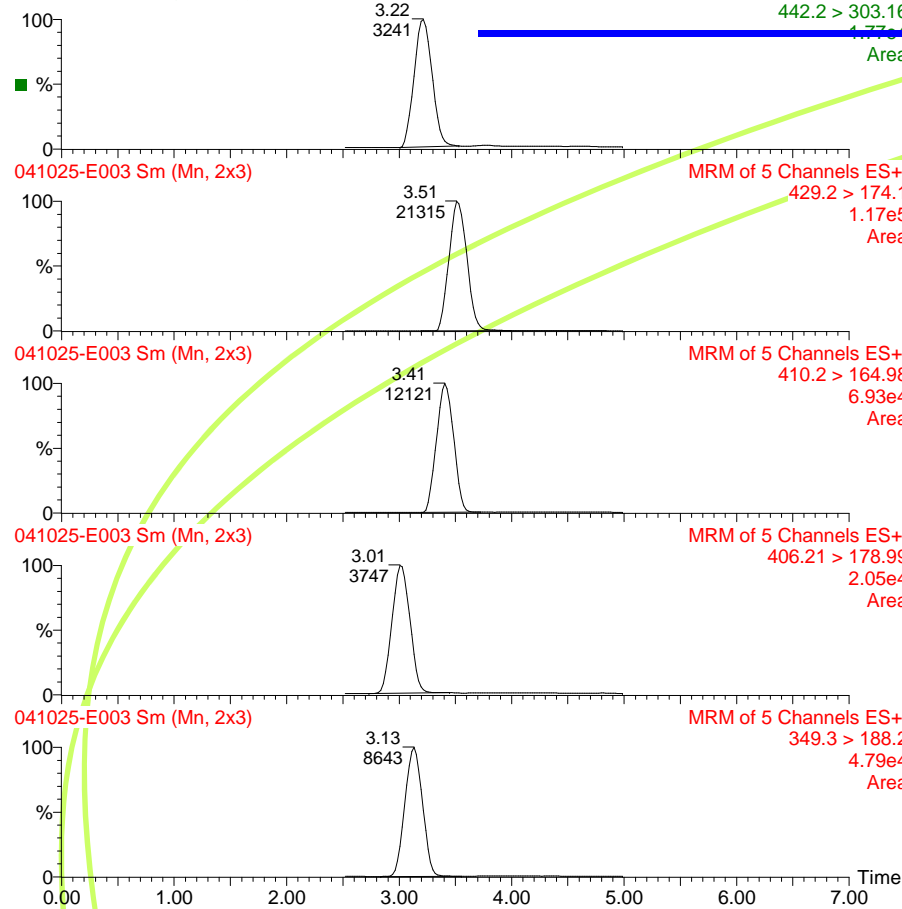
- 1:3 with a methanol/water (75:25) solution with ISTD at 50ng/mL
- 2 μ L spotted on LazWell for analysis

↓
**LDTD-MS/MS (96-well) on a
TSQ Ultra
using a generic method**

Permeability Coefficient

B4 mel1

041025-E003 Sm (Mn, 2x3)



Permeability coefficient determination

$$P_t = \frac{[C]_b \times V_b}{S \times [C]_0 \times t}$$

Analysis Setup

12 compounds

	LDTD-MS/MS	UPLC-MS/MS
Compound optimization (min)	15	90
Incubation Caco-2 (min)	150	150
Sample preparation (min)	82	55
Sample Analysis (12 samples)	60	600
Total time for 12 samples	5.1 h	15 h

- **Compound optimization**

- UPLC using infusion including chromatographic separation optimization
- LDTD using QuickQuan

- **Sample analysis**

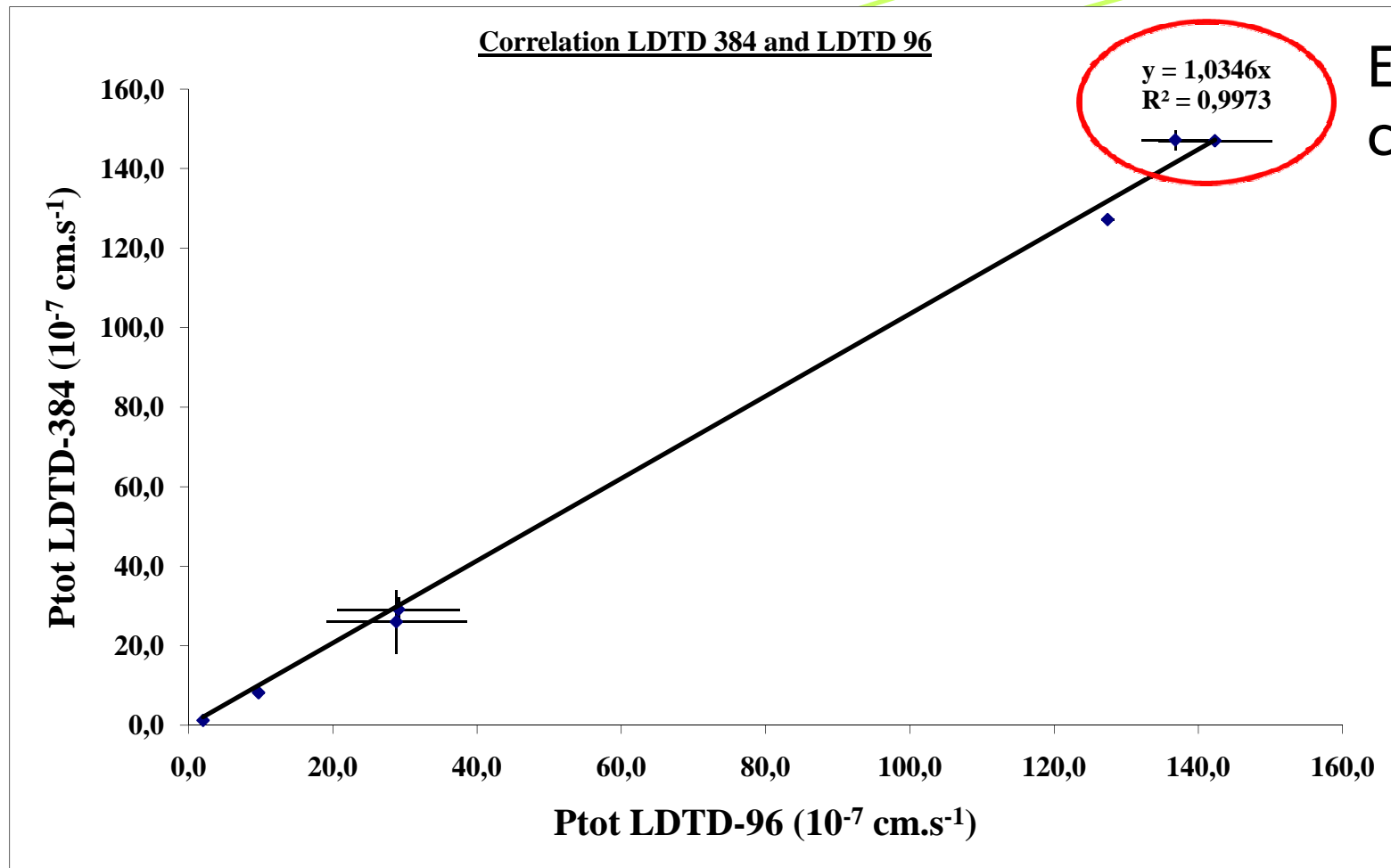
- UPLC/MS/MS : 4 mins / run
- LDTD-MS/MS : 25 seconds / run
 - LDTD desorption 10 seconds
 - MS to be ready : 15 seconds

Drug Candidate Analysis

- 125 new drugs evaluated (SARXXXXXX)
- Results following the **compound optimization** :
 - LDTD (107/125) attrition : 85 %
 - UPLC (121/125) attrition : 97
- LDTD T-3840 (Phytonix) versus LDTD T-960 (Sanofi-Aventis)

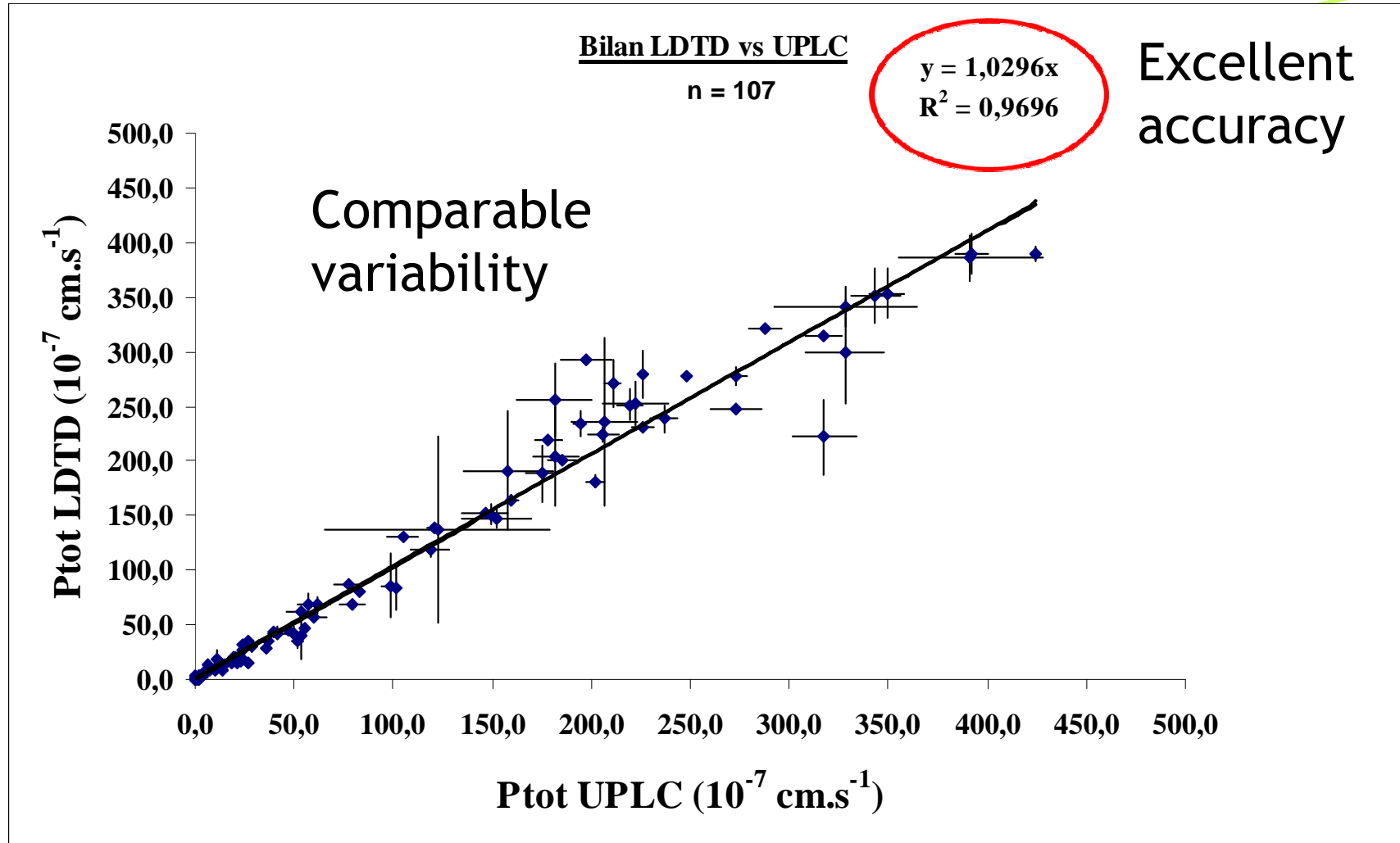
Correlation LDTD-96 and LDTD-384

- 7 SARXXX used
- Covers a wide range of permeability values

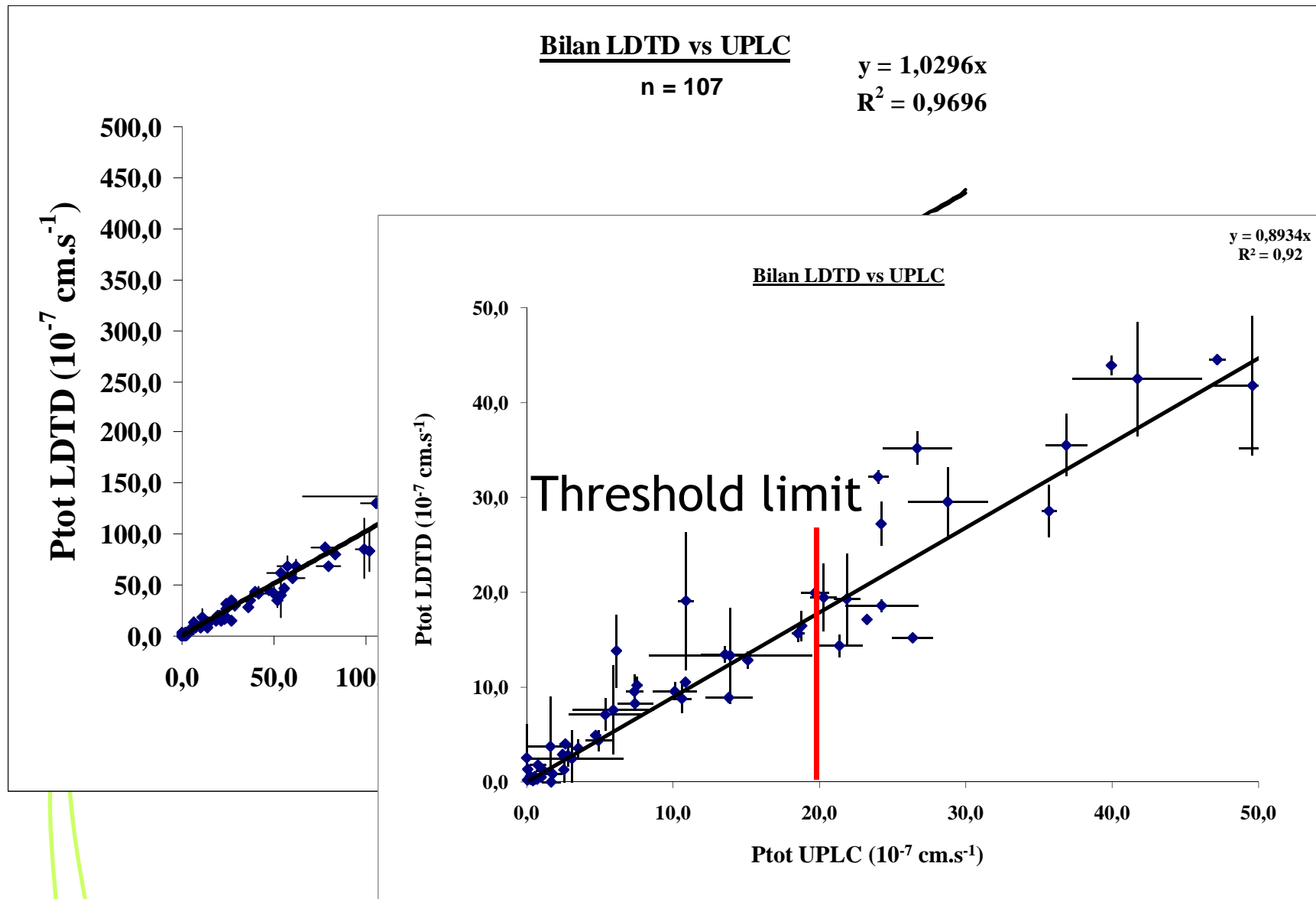


Excellent
correlation

Drug Candidate Analysis



Drug Candidate Analysis



Drug Candidate Analysis

- Excellent correlation between LDTD and UPLC permeability coefficients for all values over 4 orders of magnitude (0.05 to 500 nM/sec)
- Good rate of success using the generic LDTD method
- Validation for standard screening and correlation *in vitro* and *in vivo* in human

Model Validation using LDTD-MS/MS

Chemicals selected to get a wide range of physico-chemistry parameters (LogP, MW, Chemical structures)

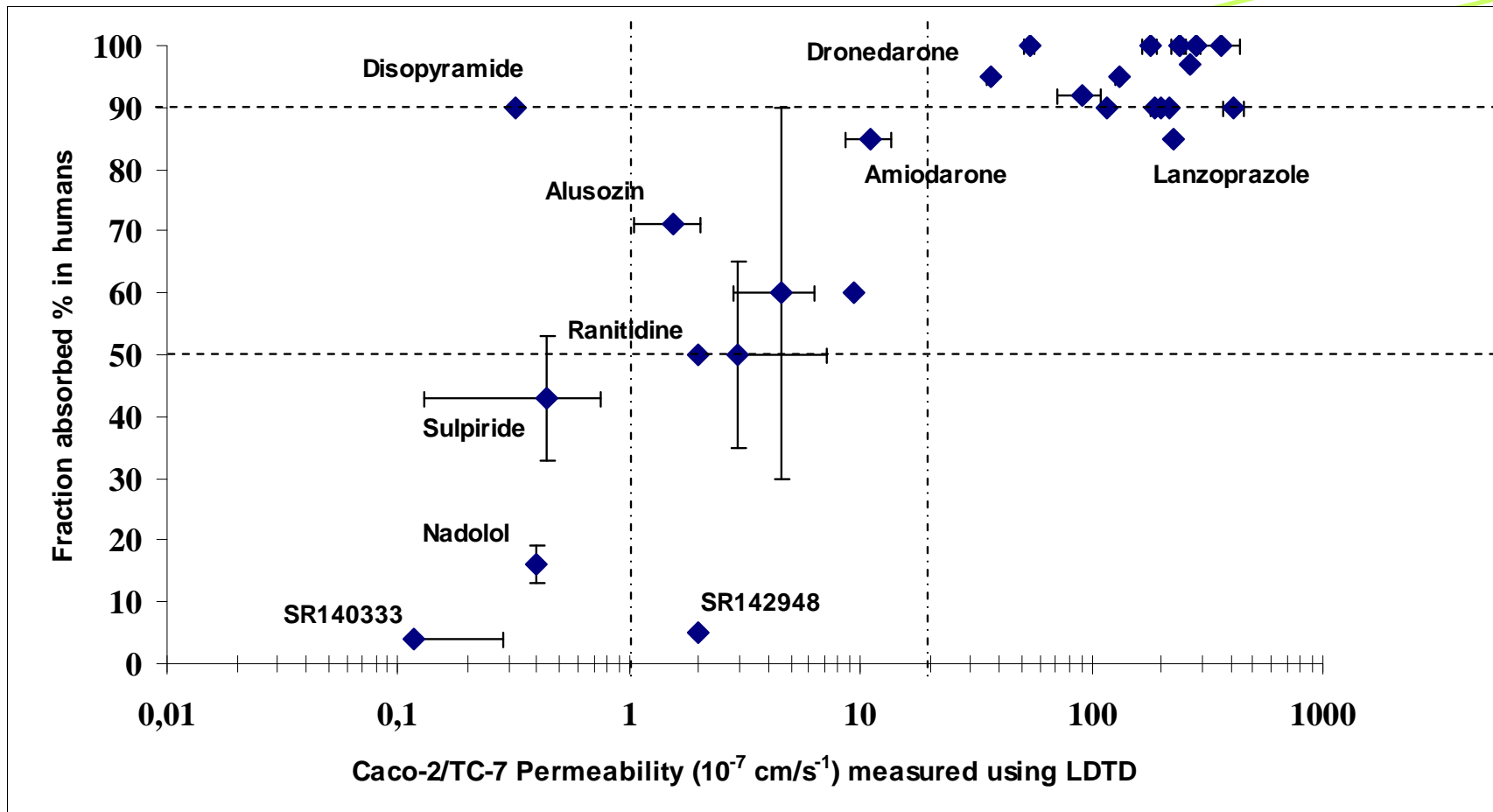
	Compound	Caco-2/TC-7 Permeability \pm SD		Fraction absorbed in humans %
		LDTD	UPLC/MS/MS	
1	Alfuzozin	1.5 \pm 0.5	2.3 \pm 0.4	71 ^b
2	Amiodarone	11.2 \pm 2.5	12.9 \pm 2.1	85 ^b
3	Amoxicilline ^a	No result		100 ^{c,e}
4	Antipyrine ^a	265.3 \pm 6.4	249.6 \pm 9.5	97 ^{c,e}
5	Atenolol ^a	3.0 \pm 4.2	0.8 \pm 0.0	40-70, 50 ^{c,e}
6	Caffeine ^a	362.1 \pm 77.3	No result	100 ^{c,e}
7	Carbamazepine ^a	285.2 \pm 88.0	301.0 \pm 2.4	> 96 ^{d,h}
8	Diclofenac	53.9 \pm 3.1	48.7 \pm 0.9	100 ^e
9	Diltiazem	185.9 \pm 67.0	176.2 \pm 8.4	90 ^h
10	Disopyramide	0.3 \pm 0.0	0.5 \pm 0.2	> 80 ^{d,h}
11	Dronedarone	36.5 \pm 1.2	30.1 \pm 0.0	95 ^c
12	Famotidine	No result	1.7 \pm 0.3	38 ^g
13	Fluvastatin ^a	No result		98 ^e
14	Hydrochlorothiazide ^a	No result	2.6 \pm 0.4	69 ^e
15	Imipramine	239.1 \pm 16.7	254.0 \pm 4.6	100 ^e
16	Ketoprofen ^a	90.4 \pm 19.7	No result	92 ^e
17	Lansoprazole	223.5 \pm 0.8	123.3 \pm 54.2	> 85 ^g
18	Metoprolol ^a	131.2 \pm 4.0	99.2 \pm 5.0	95 ^c
19	Nadolol	0.4 \pm 0.0	0.2 \pm 0.0	14-18 ^e
20	Naproxen ^a	No result		99 ^e
21	Nifedipine	413.1 \pm 45.7	388.1 \pm 5.4	90 ^h
22	Probucol	No result		10 ⁱ
23	Propranolol ^a	216.0 \pm 0.0	185.3 \pm 30.9	90 ^c
24	Ranitidine ^a	> 2.0 [*]	2.5 \pm 1.0	50 ^j
25	Rimonabant	117.4 \pm 2.4	126.6 \pm 1.4	90 ^b
26	RU44403	No result	0.1 \pm 0.2	5 ^b
27	SR140333	0.1 \pm 0.2	0.1 \pm 0.0	4 ^b
28	SR142948	> 2.0 [*]	0.3 \pm 0.0	5 ^b
29	Sulpiride	0.4 \pm 0.3	0.4 \pm 0.2	35-50 ^e
30	Terbutaline	4.6 \pm 1.7	No result	25-80, 73 ^c
31	Theophylline ^a	242.1 \pm 6.3	No result	100 ^e
32	Ticlopidine	198.3 \pm 7.1	241.6 \pm 13.5	90 ^e
33	Tiludronate	No result		5 ^e
34	Trimethobenzamide	9.5 \pm 0.2	10.9 \pm 0.9	60 ^e
35	Verapamil ^a	179.3 \pm 12.5	139.9 \pm 21.7	100 ^e

Correlation *in vitro* / *in vivo*

Low Permeability
Low Absorption

Grey Zone

High Permeability
High Absorption



Same predictivity for *in vivo* model using
the LDTD-MS/MS

LDTD-MS/MS and UPLC-MS/MS

- Analysis setup :
 - 85 % using the LDTD (107 compounds)
 - 15 % using UPLC (18 compounds)

	LDTD (107)	UPLC (18)	=	LDTD / UPLC (125)
Compound optimization (hour)	2.6*	2.3	=	4.9
Incubation Caco-2 (hour)	22.3	3.8	=	26.1
Sample preparation (hour)	12.1	1.4	=	13.5
Sample Analysis (hour)	8.9	15.0	=	23.9

* For 125 compounds. The compounds that failed to be optimized are runned in UPLC

What is the real increase in throughput ?

LDTD-MS/MS and UPLC-MS/MS

- 85 % (LDTD) :15 % (UPLC) setup

	LDTD / UPLC (125)	UPLC (125)	Improvement
Compound optimization (hour)	4.9	15.6	X 3.2
Incubation Caco-2 (hour)	26.1	26.0	=
Sample preparation (hour)	13.5	9.5	Less X 1.4
Sample Analysis (hour)	23.9	104.2	X 4.4

- UPLC-MS/MS is the bottleneck in the overall process
- LDTD-MS/MS can improve the throughput

Conclusions

- LDTD can be used to analyze buffer solutions
- HBSS is more compatible with the LDTD
- Easy sample preparation : dilute and shoot
- Easy to operate and low optimization required
- High rate of success in using a generic method
- Excellent correlation between LDTD and UPLC for validated standard screening compounds
- Good *in vivo* prediction from *in vitro* data obtained with standard screening compounds
- Same predictivity for the *in vivo* model using the LDTD-MS/MS *in vitro results*

Questions

