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INTRODUCTION

Laser Diode Thermal Desorption (LDTD) is a high throughput ionization method for mass spectrometry (MS). There is no chromatographic separation and the system requires no solvents. This innovative technology needs only 10-20 s/sample to record data. Early *in vitro* ADME studies are performed in routine analysis by UHPLC-MS/MS with an analytical time about 3 min/sample. In order to reduce the analytical time, LDTD-APCI performance was tested, and successful assays removing the UHPLC part using LDTD-APCI-MS/MS for Caco-2/TC7 permeability model were reported (1). Actually, when this model is used in primary screening, a huge number of compounds are analyzed and the SRM optimizations are still time-consuming. The Exactive Orbitrap which delivers high resolution accurate mass measurement was coupled to the LDTD system to eliminate the MS method development step. The aim of this study is to evaluate the performance of the LDTD-APCI-Orbitrap Exactive system in the Caco-2/TC7 permeability model.

EXPERIMENTAL

The LDTD uses a Laser Diode to produce and control indirect thermal desorption (Figure 1). The energy (Diode Laser, 980 nm, 20 W) is transferred through the sample holder to the dry sample which vaporizes prior to be carried by a gas (air) in an APCI region for ionization (Figure 2). The LDTD source was coupled with a ThermoFisher Scientific Exactive Orbitrap analyzer operating in full scan mode (Figures 3 & 4).

Permeability Assay using the Caco-2/TC-7 human intestinal absorption model for the determination of the permeability coefficient (Figure 5).

Apical medium: Hank' balanced salt solution (HBSS), pH 6.5 with 0.5 % of Bovine Serum Albumin (BSA).

Basal Medium: HBSS, pH 7.4 with 5% BSA.

Test compound: 20 μM added in the apical compartment.

Incubation: performed at 37°C for 2 hours under agitation.

Sample preparation:

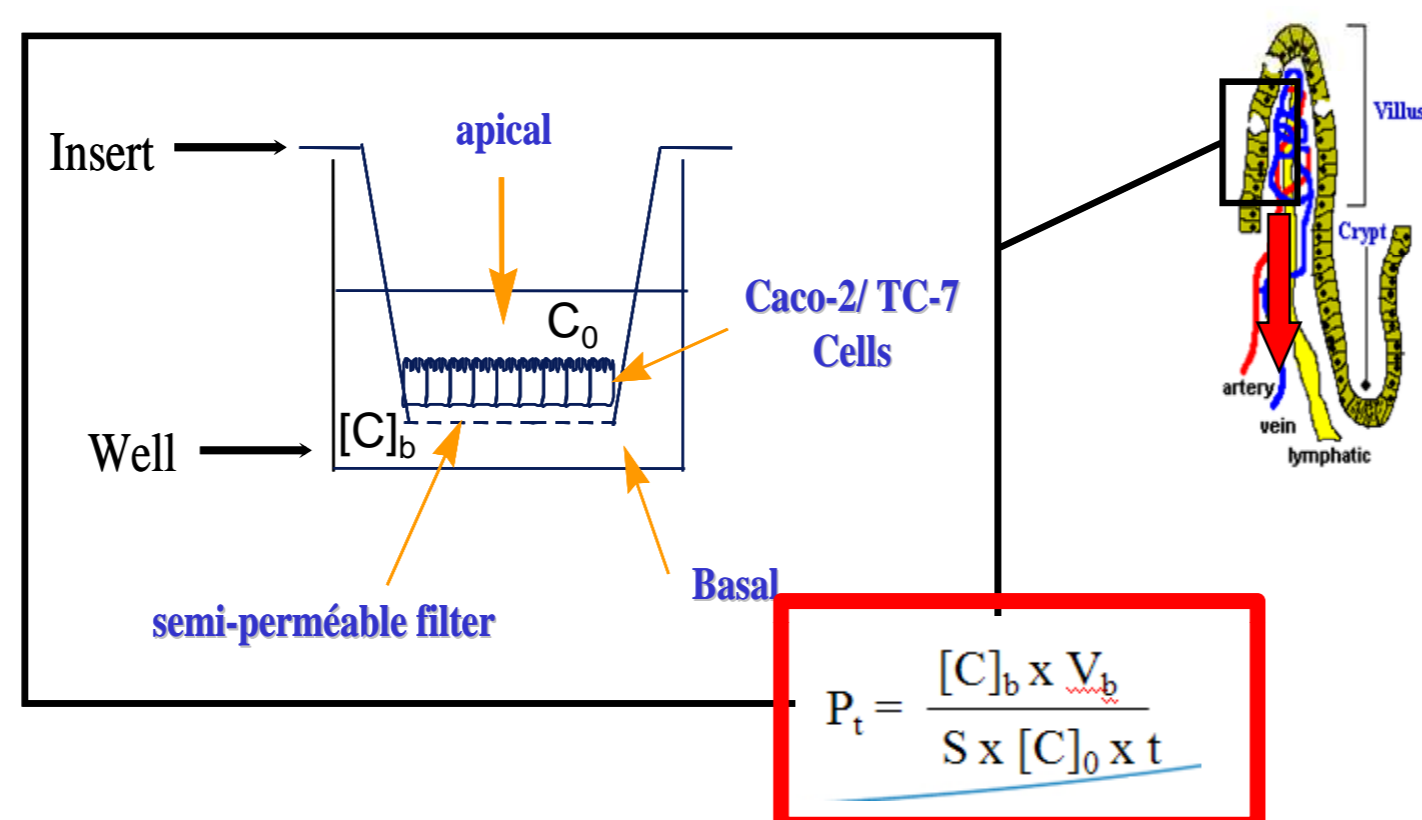
- Apical : Dilution 1/20 with HBSS pH 7.4 with 5 % BSA
- Basal : No dilution
- Protein precipitation 1:1 with acetonitrile (both Apical and Basal media) followed by centrifugation (1800 g for 10 min at 10°C).

UHPLC analysis:

5 μL of the supernatant were injected
ISTD : Clomiphene (25 ng.mL⁻¹)
Multiple column used (C₁₈, CN, HILIC)

LDTD analysis:

- Supernatant was diluted 1/3 with a methanol/water mixture (75/25, v/v) containing Clomiphene (ISTD) at 25 ng/mL.
- 2 μL of the diluted mixture was spotted onto the Lazwell plate using a HAMILTON Robot equipped with a 96-head.
- Solvent was evaporated at room temperature before analysis (less than 30 minutes).



P_t or P_{tot} = Permeability coefficient (cm.s⁻¹)

Figure 5: Caco-2/TC7 human intestinal absorption model

MS EXPERIMENTAL CONDITIONS:

UHPLC:

Kinetex, XB-C₁₈, 1.7 μm, 50 x 2.1 mm, Phenomenex
Flow rate: 500 μL/min, Mobile phase:

A: water/Acetonitrile/ammonium acetate/Formic acid (900/100/0.25/1, v/v/w/v)
B: water/Acetonitrile/ammonium acetate/Formic acid (100/900/0.25/1, v/v/w/v)

Rapid gradient from 0 up to 100 % of B in 1 minute

LDTD:

Gas Flow: 3 L/min

Laser desorption pattern: up to 45 % in 2 seconds

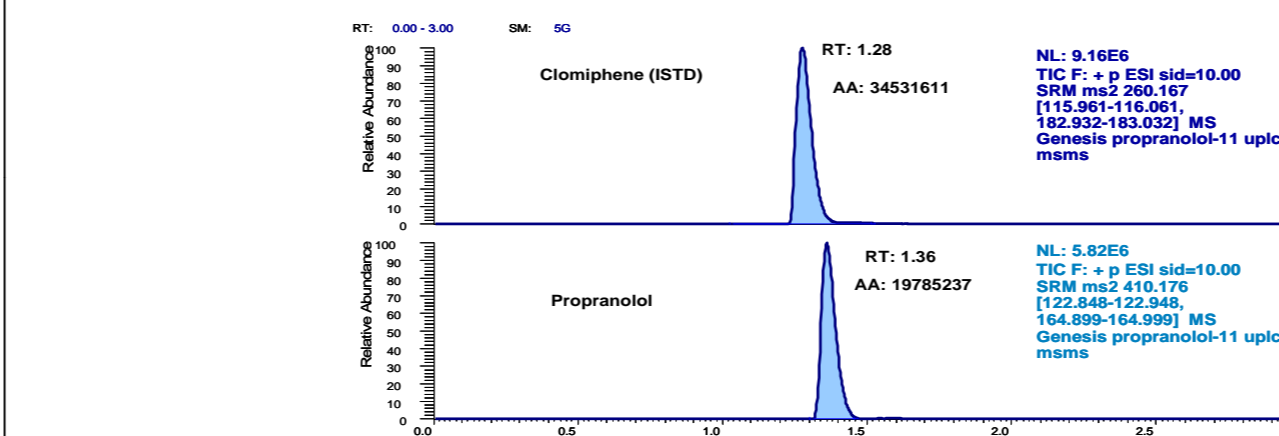


Figure 8: Typical UHPLC-MS/MS chromatogram

MS/MS (TSQ Quantum Ultra, ThermoFisher Scientific):

HESI (UHPLC) or APCI (LDTD) ionization, positive or negative

Spray voltage: 4000 V (HESI)

Corona current: 3 μA

Standard gas values (UPLC)

No gas (LDTD)

Vaporized temperature: 250°C

Capillary temperature: 380°C (UPLC), 250°C (LDTD)

For each component, Tube Lens voltage and Collision Energy were optimized using automatic QuickQuan software for SRM analysis

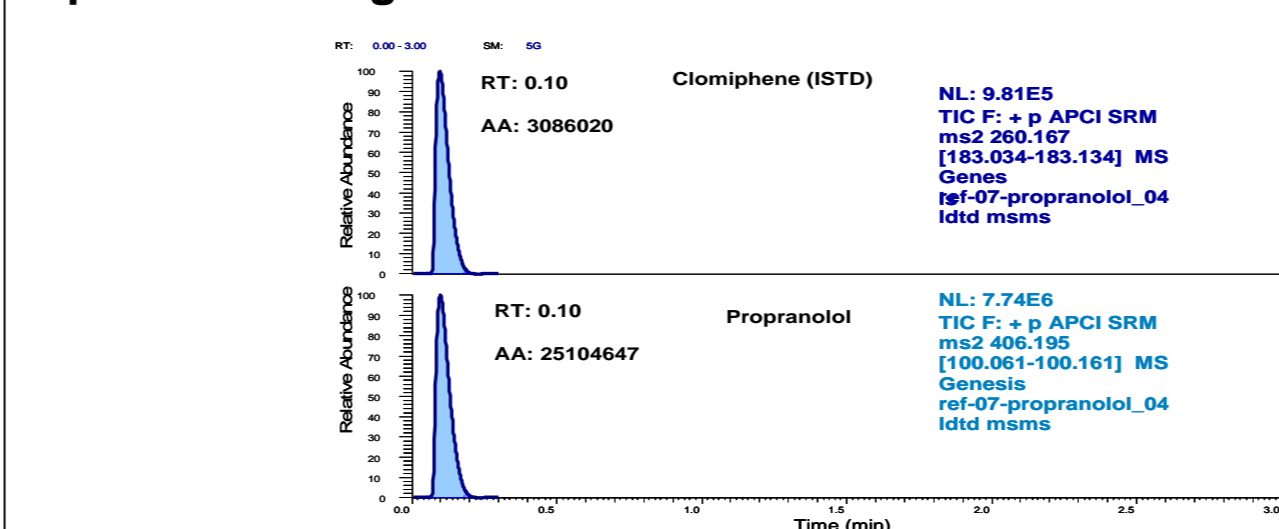


Figure 9: Typical LDTD-APCI-MS/MS signal.

Exactive, ThermoFisher Scientific:

FT resolution: 25 000 at m/z 200

AGC target: 1E⁶

Injection time: 20 ms

Tube lens voltage: 120 V

Scan range: 150 to 700 m/z

No specific optimization was needed, exact mass of the different compounds was directly extracted from the full scan analysis.

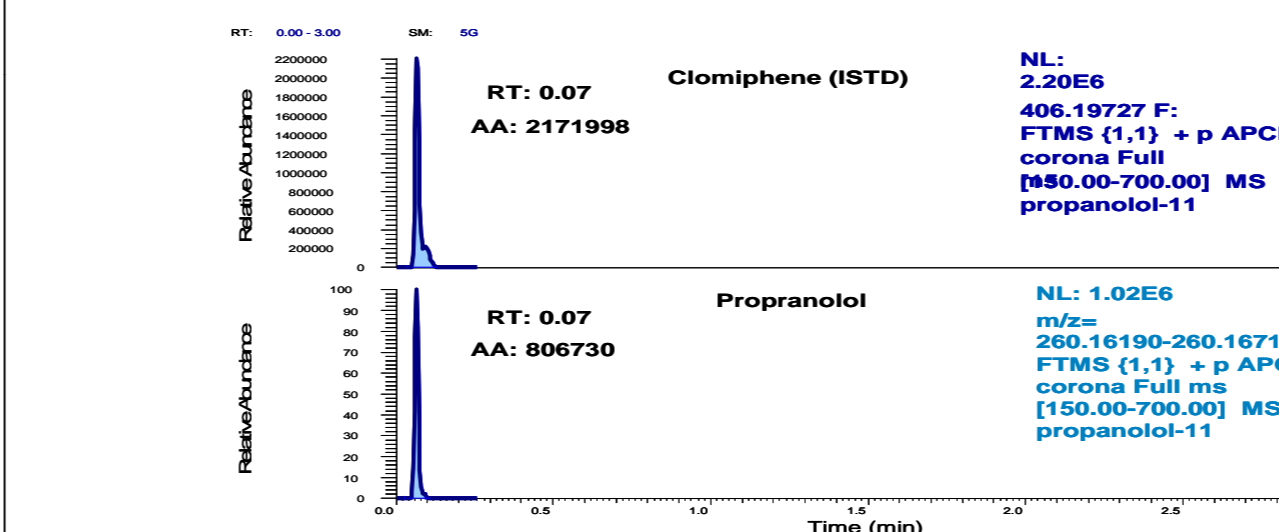


Figure 10: Typical LDTD-APCI-Orbitrap Exactive signal

RESULTS AND DISCUSSION

Table 1: Molecular weight and recorded permeability (P_{tot}) values of tested drugs for LDTD-APCI-MS/MS and LDTD-APCI-Orbitrap Exactive analysis.

	MW	P _{tot} (10 ⁻⁷ cm.s ⁻¹)	
		Quantum ultra	Exactive
Alfuzozin	389,4	4,3	2,2
Amiodarone	645,3	20,3	22,7
Antipyrine	188,2	272,0	338,4
Carbamazepine	236,2	478,5	310,3
Desloratadine	310,8	85,0	87,7
Dextromethorphan	271,4	200,8	177,2
Disopyramide	339,4	0,7	0,0
Lanzoprazole	369,3	91,6	48,1
Metoprolol	267,3	173,1	174,9
Nadolol	309,4	0	0
Phenacetin	179,2	516,0	416,1
Propafenone	341,4	139,9	116,5
Propranolol	259,3	169,2	231,7
Rimonabant	463,8	86,4	154,1
Saredutant	552,5	0,2	1,3
Trimethobenzamide	388,4	6,9	10,8
Verapamil	454,6	198,6	216,4
Xaliproden	381,4	15,2	18,6

Our strategy was to perform a direct comparison of permeability data recorded for 18 commercial compounds using either UHPLC-HESI-MS/MS (gold standard), or LDTD-APCI-MS/MS (already validated) or LDTD-APCI-Orbitrap Exactive.

We were able to achieve our experiments with targeted sensitivity (0.01 μM). A good correlation was obtained for permeability data between LDTD-APCI-Orbitrap Exactive analysis versus UHPLC-HESI-MS/MS (Figure 11) and LDTD-APCI-MS/MS versus UHPLC-HESI-MS/MS (Figure 12). The permeability data recorded on marketed drugs are shown in Table 1.

Using our standard thresholds of < 20.10⁻⁷ cm.s⁻¹ for differentiating poor and high permeability compounds, no discordant pairs were identified comparing LDTD-APCI-Orbitrap Exactive versus LDTD-APCI-MS/MS. Therefore, the ranking of chosen marketed drugs were similar using these two different approaches.

Moreover, these permeability data were in accordance with published data. Nevertheless it has to be emphasized that based on our overall knowledge based on hundreds of new chemical entities from Sanofi-Aventis library which were chosen to represent a large chemical diversity, success rate for LDTD-MS/MS versus UHPLC-MS/MS was 85 % versus 97 % respectively (2, 3).

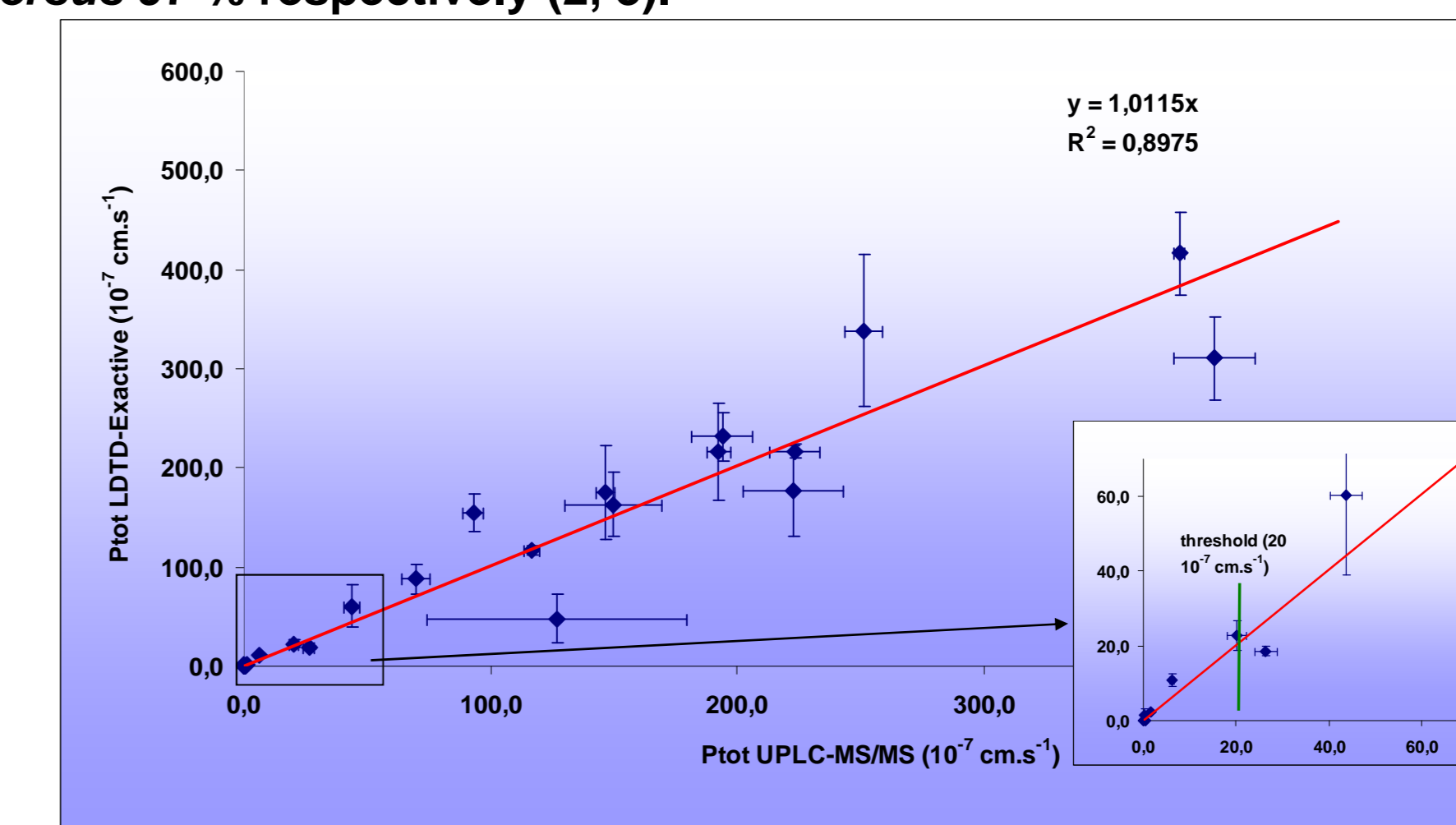


Figure 11: LDTD-APCI-Orbitrap Exactive vs UHPLC-HESI-MS/MS with a focus at threshold 20.10⁻⁷cm.s⁻¹

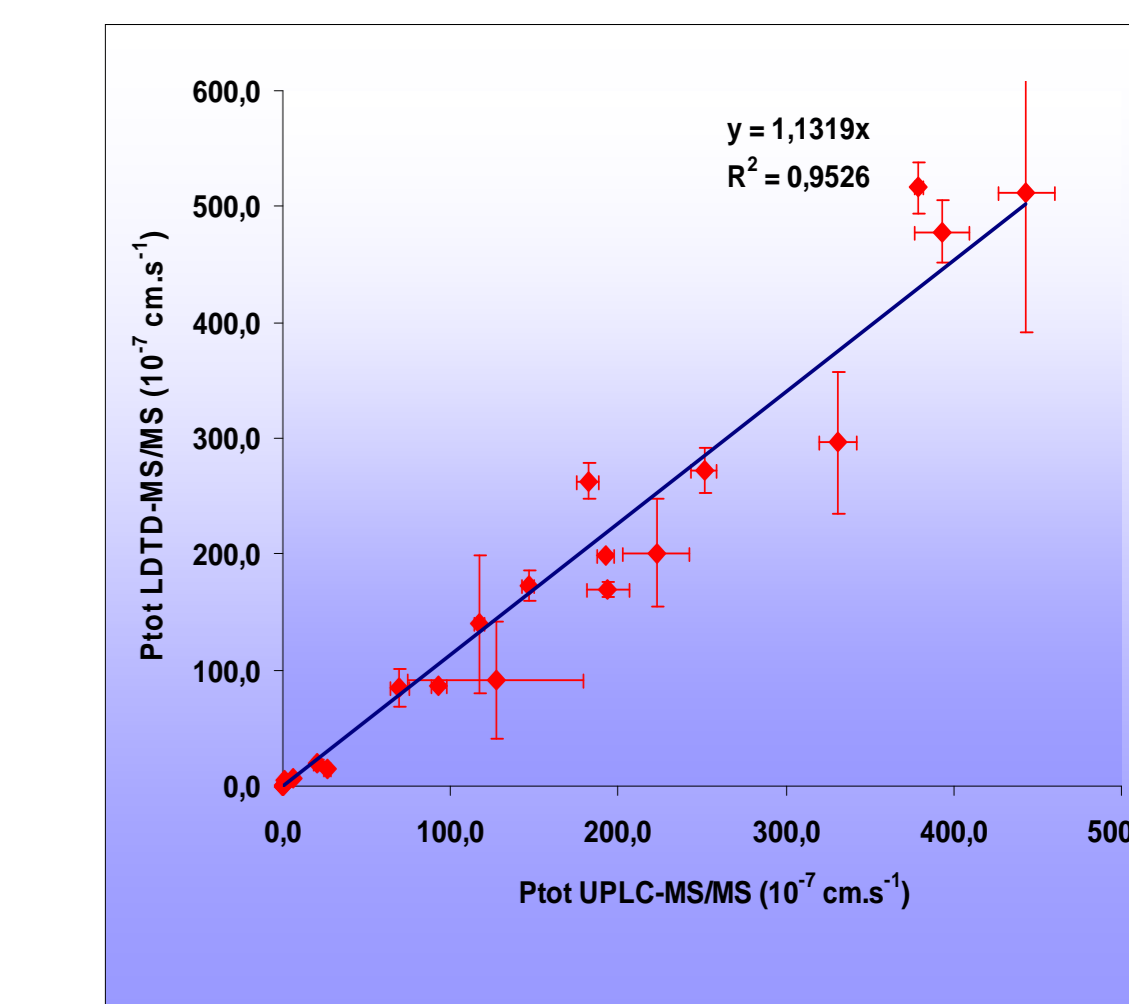


Figure 12: LDTD-APCI-MS/MS vs UHPLC-HESI-MS/MS

QUANTIFICATION

18 Commercial compounds were selected (Table 1)

For each compound, the calibration curve was prepared with 3 levels: 1, 0.1 and 0.01 μM. Regression was quadratic (UHPLC) or linear (LDTD), not forced through origin. The figures 6 and 7 show experimental signal and calibration curve recorded for Propranolol (internal standard: Clomiphene)

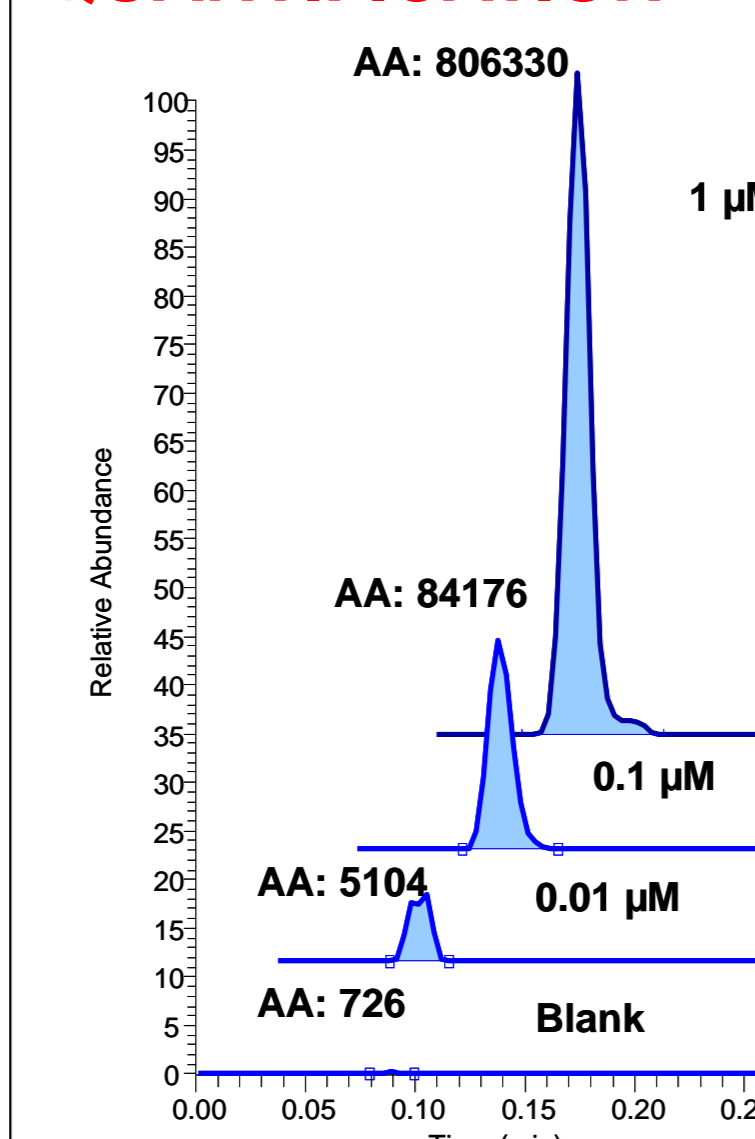


Figure 6: Chromatograms obtained for the different levels used for the calibration curve (e.g. propranolol).

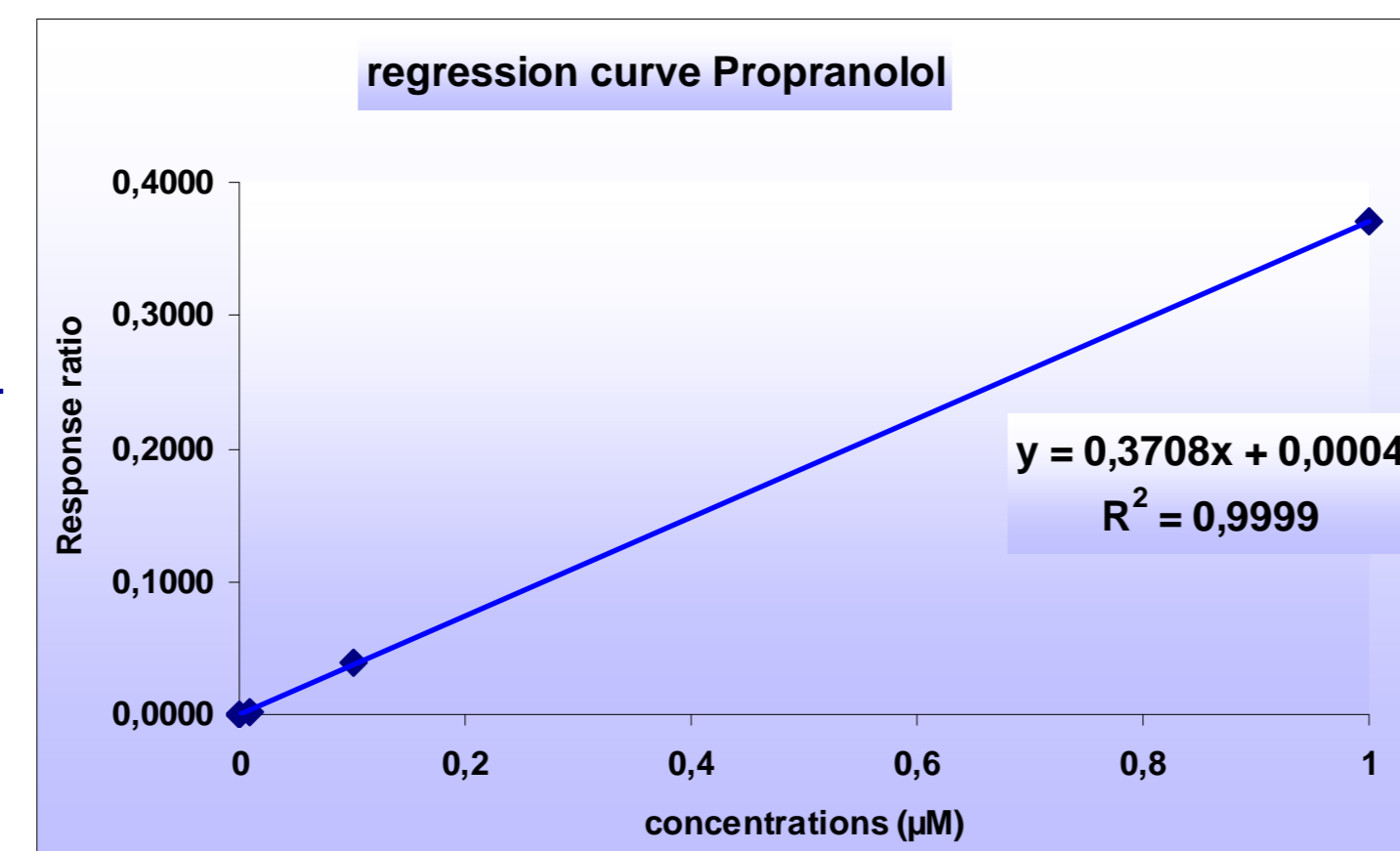
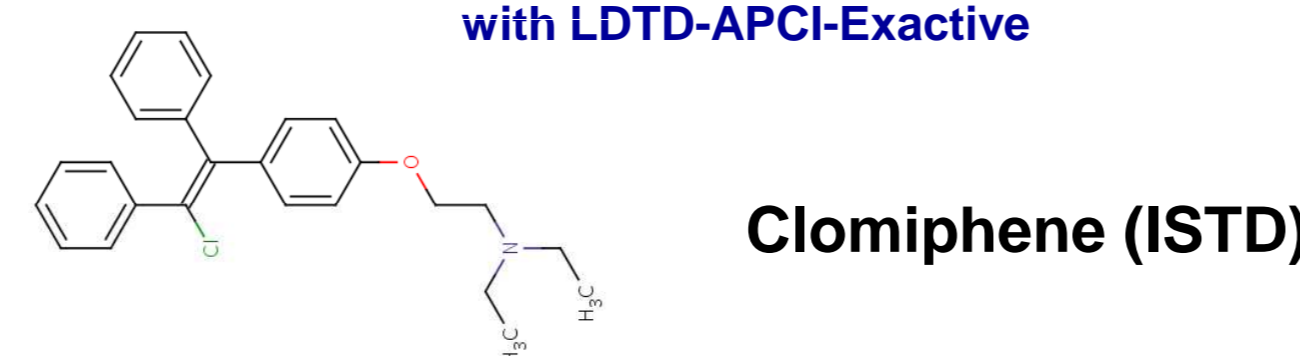
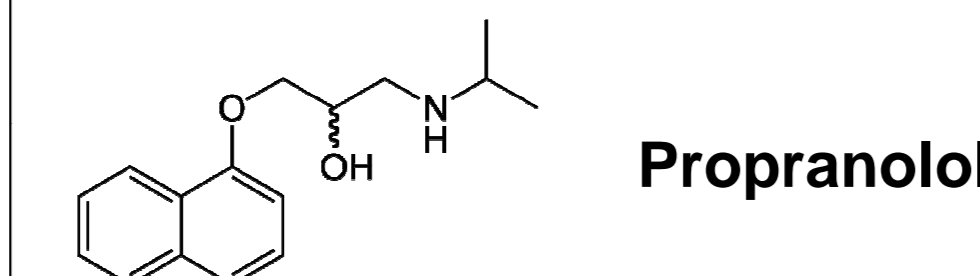


Figure 7: Propranolol calibration curve obtained with LDTD-APCI-Exactive



CONCLUSION

For early *in vitro* ADME studies, the Orbitrap Exactive environment simplifies drastically MS method development leading to an improved analytical throughput with high specificity. Indeed, in our experience, while two hours are necessary for SRM transitions optimization of 100 compounds using the automatic optimization software QuickQuan in LDTD-APCI-MS/MS environment, no time is required when using the LDTD-Exactive system which needs only the exact mass of the compounds for data processing after acquisition. We were also able to achieve our experiments with targeted sensitivity (0.01 μM) and a good correlation for permeability data was achieved using LDTD-APCI-Orbitrap Exactive versus UHPLC-HESI-MS/MS (Gold standard). However, as 85 % of the molecules show ionization using LDTD-APCI, we need to use LDTD-UHPLC to have a success rate in the Caco-2/TC7 model close to 100%. This is the reason why this study is being currently extended to UHPLC-Orbitrap Exactive, and also to a large number of NCE from Sanofi-Aventis.