Application Note 1003

CACO-2 Permeability Coefficient Determination in LDTD-MS/MS

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Overview

- High-throughput determination of permeability coefficients in early drug discovery development
- CACO-2 / TC-7 model
- Validation against UPLC-MS/MS using 11 commercial compounds

Instrumentation

- Phytronix Technologies LDTD ion source (model T-960); Operate with a generic method
- Thermo Fisher Scientific TSQ® Vantage™ mass spectrometer.

LDTD ionization process

The LDTD ion source uses an infrared laser diode to desorb sample that have been dried onto a well of a LazWell™ (96-well plate). The desorbed gas phase molecules are carried into a corona discharge region to undergo APCI, and then they are transferred directly into the mass spectrometer for detection.

Incubation (Figure 1)

Apical solution : Hank's balanced Salt Solution (HBSS) pH 7.4 with 0.5 % of Bovine Serum Albumine (BSA) with test compound at 20µM

Basal solution : HBSS pH 7.4 with 5 % of BSA

Incubation performed at 37^0C for 2 hours including a pre-incubation of 30 minutes

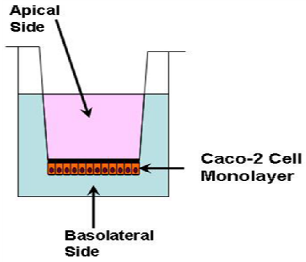

Figure 1 Incubation model

Sample preparation

Following the incubation period, the Apical solution sample was diluted 1/20 with a HBSS solution at pH 7.4 containing 5 % of BSA. No dilution was performed on the Basal solution sample. The incubation was "stopped" by protein precipitation adding one part of acetonitrile for one part of Basal or Apical solution followed by a centrifugation at 3000 rpm. A calibration curve, ranging from 0.01 to 1 µM was performed into HBSS.

The UPLC-MS/MS analysis was performed on the supernatant while a dilution (50/150) with a solution of methanol/water (75/25) containing clomiphene (internal standard) at 50ng/mL was performed for the LDTD-MS/MS analysis.

The permeability coefficients were calculated using the following equation :

$$P_t = C_{Basal} \times V_{Basal} / S \times C_{t=o} \times t$$

Where S = Surface, t the incubation time.

LDTD-MS/MS analysis

The analysis was performed using a 2 µL sample spotted into a 96 LazWell plate. The solvent was evaporated at room temperature.

The carrier gas flow was set at 3 L/min and the laser desorption pattern was the following :

2 seconds at 0 % of Laser power
2 seconds ramping the Laser power up to 45 %
3 seconds plateau at 45 % of Laser power
Laser power shut down to 0 % in 0.01 seconds

Results and Discussion

LDTD-MS/MS validation with UPLC-MS/MS

11 commercial compounds with a wide range of chemical properties and molecular weights were incubated for permeability study and the permeability coefficient for each compound was obtained following the analysis in UPLC-MS/MS and in LDTD-MS/MS (**Table 1**).

Table 1 Permeability coefficient (nM/sec) of 11 commercial compounds obtained in LDTD-MS/MS and UPLC-MS/MS

		LDTD		UPLC	
Compound	**Formula**	**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	$C_{22}H_{26}N_2O_4S$	185.92	6.65	176.23	8.37
Alfusozin	$C_{19}H_{27}N_5O_4$	1.55	0.50	2.30	0.40
Hydroxyzine	$C_{21}H_{27}ClN_2O_2$	227.71	21.08	212.71	13.06
Desloratadine	$C_{19}H_{19}ClN_2$	11.79	0.94	14.51	1.02
Atenolol	$C_{14}H_{22}N_2O_3$	3.00	4.20	0.80	0.00
Nadolol	$C_{17}H_{27}NO_4$	0.40	0.00	0.20	0.00
Sulpiride	$C_{15}H_{23}N_3O_4S$	0.40	0.30	0.40	0.20
Encatropine	$C_{17}H_{25}NO_3$	71.11	3.51	70.60	2.48

The results obtained showed an excellent correlation between both analytical systems with a slope of 1.0225 and r^2 of 0.9862 (**Figure 2**). Moreover, the variability obtained with LDTD-MS/MS is comparable to the one obtained in UPLC-MS/MS.

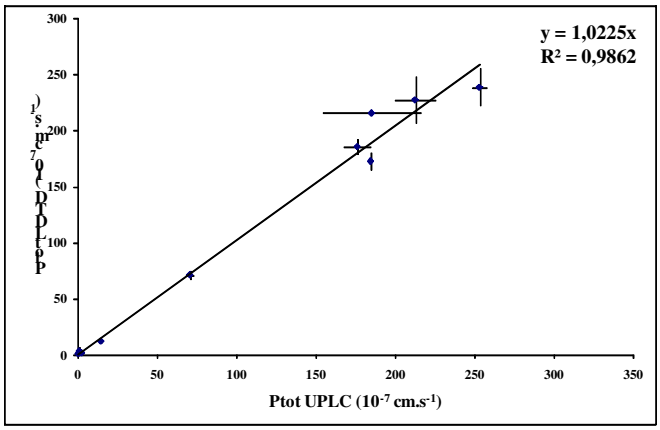

Figure 2 Correlation between LDTD-MS/MS and UPLC-MS/MS for permeability coefficient determination.

High throughput analysis using LDTD-MS/MS

The LDTD-MS/MS sample-to-sample run time is 25 seconds. This time includes the thermal desorption of the LDTD (10 seconds) and a waiting time coming from the mass spectrometer to be ready for the next sample (15 seconds).

Running the same sample using an UPLC-MS/MS system will takes 4 minutes. **Therefore, switching to a LDTD-MS/MS system will increase the sample throughput by a factor of 4.4**

Conclusions

The LDTD-MS/MS allows determining accurate and reproducible permeability coefficient as confirmed by a cross-validation against an UPLC-MS/MS system performed on 11 commercial compounds.

Moreover, the LDTD-MS/MS system allows you to run your samples 4.4 times faster than an UPLC-MS/MS system.

High-throughput analysis with accuracy and precision can be achieved using LDTD as ion source in mass spectrometry.