High Throughput LDTD-MS/MS IC_{50} Determination of CYP Inhibition in HLM

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Introduction

In early drug discovery, CYP inhibition assays are an important step to identify potential drug-drug interactions from new candidates. LC-MS/MS methods used for this analysis are time consuming and represent the bottleneck in a process that often requires high-throughput. We offer a Laser-Diode Thermal Desorption (LDTD) high throughput solution for CYP inhibition assays.

The LDTD Ion Source uses an infrared laser diode to desorb samples that have been previously dried onto a 96-wells LazWell™ plate after sample preparation extraction. The rapid desorption produces neutral species which are carried into a corona discharge region to undergo an efficient protonation and are subsequently transferred directly into the mass spectrometer for detection.

LDTD-MS/MS System

Figure 1: LDTD system on AB SCIEX 5500 Qtrap Mass Spectrometer

Sample Method

Extraction Procedure

- 40 µL microsome solution buffer
- 120 µL IS (50 ng/mL clomiphene in acetonitrile)
    - Vortex
- 40 µL NaCl (saturated solution in water)
    - Vortex and Centrifuge (2min / 14000 rpm)
- Spot 5 µL of organic phase on 96 LazWell™ Plate

LDTD-MS/MS Parameters

Laser power pattern :

- Increase power from 0% to 45% in 3 sec
- Hold at 45% for 2 sec then back to 0%

Carier gas flow: 3.0 L/min (Air)

MS/MS transitions:

Table 1: MS/MS transition of monitored metabolites

CYP	Probe substrate	Monitored metabolite	APCI	Q1	Q3
1A2	Phenacetin**	Acetaminophen	-	150	107
1A2	Ethoxy-Resorufin	Resorufin	+	214	186
2A6	Coumarin	OH-Coumarin	+	163	107
2B6	Bupropion	OH-Bupropion	+	256	131
2C8	Paclitaxel	OH-Paclitaxel	+	870	286
2C9	Diclofenac	4-OH-diclofenac	+	312	231
2C9	Tolbutamide	4-OH-Tolbutamide	+	287	89
2C19	S-Mephenytoin	4-OH-Mephenytoin	+	235	150
2D6	Bufurolol	1-OH-Bufurolol	+	278	186
2D6	Dextromethorphan	Dextrorphan	+	258	133
3A4	Midazolam	1-OH-Midazolam	+	342	203
3A4	Testosterone	6β-OH-Testosterone	+	305	269
3A4	Nifedipine	Dehydro-nifedipine	+	345	284

** Phenacetin concentration has to be lower than 20 µM

Results and Discussion

Linearity Results

Figure 2 demonstrates excellent linearity (r > 0.99) for standard curves obtained for quantification range from 1 to 1000 nM.

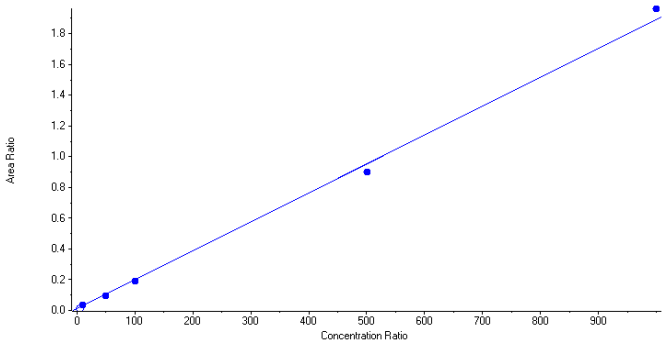

Figure 2: Typical standard curve for OH-Midazolam.

Method specificity

Operating without chromatographic separation in APCI and no liquid mobile phase, the possibility of in-source fragmentation occurring with the LDTD needs to be evaluated. Each probe was analyzed in matrix at the working concentration and the corresponding metabolites were monitored.

In (+)APCI, signal of Acetaminophen (MW 151.2 g mol^{-1}) SRM was observed when a blank containing 50 µM of Phenacetin (MW 179.2 g mol^{-1}) was analyzed. The contribution of Phenacetin as it converts into Acetaminophen was measured at 1.5 %, thus affecting the IC_{50} determination. The same analysis was performed in (-)APCI and under these conditions the observed interfering signal was 10-times lower. Fragmentation pattern of Phenacetin in negative LDTD-APCI does not form deprotonated acetaminophen[1]. The IC_{50} was calculated and the value obtained was comparable to the LC-MS/MS method. ***Therefore, in LDTD-MS/MS, Acetaminophen probe should be monitored in negative APCI to obtain accurate IC_{50} determination.***

Accuracy and Precision

Examples of calculated IC_{50} values are presented in Table 2. Comparison between LC-MS/MS and LDTD-MS/MS results shows equivalent performance of both systems.

Table 2: IC_{50} values obtained in LC-MS/MS vs LDTD-MS/MS

CYP	Monitored metabolite	IC_{50} LC-MS/MS	IC_{50} LDTD
1A2	Acetaminophen	1.630	1.287
2C9	4-OH-diclofenac	0.585	0.555
2D6	Dextrorphan	0.025	0.031
3A4	1-OH-Midazolam	0.093	0.095

IC50 Shift example

Mechanism-based inhibition example is presented in the figure 3 as the IC_{50} shift for Acetaminophen with aminobentriazole. Equivalent results are obtained with LC-MS/MS and LDTD-MS/MS.

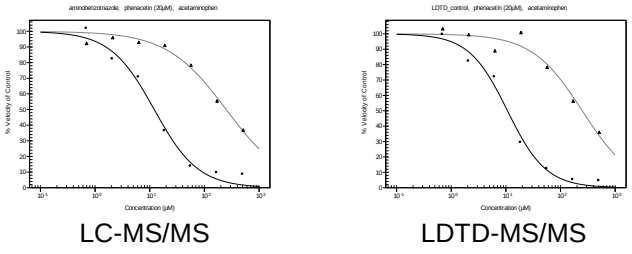

Figure 3: IC50 shift of acetaminophen analyzed in LC-MS/MS and LDTD-MS/MS.

Conclusions

LDTD-MS/MS system performs with an incredible sample-to-sample analysis time of **8 seconds**. The analytical speed provided by the LDTD increases the CYP inhibition assay throughput without compromising the accuracy of IC_{50} results.

[1] **Laycock, 60th ASMS, 24-May-2012**