LDTD-MS/MS for CYP 1A2 / 2C9 / 2D6 / 3A4 inhibition assays in 18 minutes

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Overview

- High throughput analysis of CYP 1A2 / 2C9 / 2D6 / 3A4 inhibition assays ;
- Probe : Testosterone, Dextromethorphan, Diclofenac and Phenacetin ;
- Metabolites : 6β-hydroxytestosterone, Dextrorphan, 4-hydroxydiclofenac and Acetaminophen ;
- IC_{50} comparable with LC-MS/MS and LDTD-MS/MS methods ;
- LC-MS/MS run time of more then 10 hours ;
- LDTD-MS/MS run time of 18 minutes.

Instrumentation

- LDTD ion source, S-960 (Phytronix Technologies) interface on a API 4000 triple quadrupole (Applied Biosystems/MDS Sciex) ;
- LC-MS/MS system including a PE Sciex API 2000 Triple quadrupole (Applied Biosystems/MDS Sciex) and a Shimadzu LC system (pumps, controller and autosampler).

Introduction

In early drug discovery, identifying potential drug candidates is a common practice. One important step in this discovery process is to identify drug-drug interactions. The most widespread procedure for this is to perform cytochrome P450 (CYP) inhibition assays using human liver microsomes (HLM). The most commonly used method for analyzing CYP inhibition assay samples is LC-MS/MS. However, this method is time-consuming and represents the bottleneck in this type of assay. To increase the throughput, we propose to use the LDTD as ionization source, which allows a sample desorption time of 6 seconds.

Samples Preparation

Incubation plates (final volume of 100 µL) are prepared by sequentially adding HLM, probe substrate, test compound and NADPH (concentrations reported in **Table 1**). The incubations are quenched with 100 µL of 40 % acetonitrile and 0.05 % formic acid in water. The samples are diluted with acetonitrile 12x for LC-MS/MS and 5x for LDTD-MS/MS analysis. One quenching solution is prepared for each CYP assays which contains the corresponding internal standard (IS) as follows : 1A2, 1µM [$^{13}C_2$,^{15}N]-acetaminophene; 2C9 15µM [dichlorophenol-U-$^{13}C_6$]-4'-hydroxydiclofenac; 2D6, 4µM [D_3]-dextrorphan and 3A4, 10µM [16,16,17-D_3]- 6β-hydroxytestosterone.

Metabolite calibration curves in protein precipitated HLM are prepared at concentrations ranging from 1.28 nM to 20 µM. The test compounds are listed in **Table 4**.

The optimized MS MRM are reported in **Table 2** and the operating conditions for the LDTD and the LC systems are reported in *Anal. Chem. 2007, 79 (12) 4657-4665*.

Results and Discussion

Linearity, Limit of detection, Reproducibility and Signal-to-noise ratio

The lower limit of linearity was found to be at least 1.28 nM for all metabolites. The limit of detection (LOD) was evaluated to be 1 nM for 6β-hydroxytestosterone, dextrorphan and 4-hydroxydiclofenac, and to be 6 nM for Acetaminophen.

Table 1 CYP inhibition assay materials and sequential solution addition procedure.

CYP / Metabolite	HLM conc. (mg/mL)	Probe substrate final conc. (µM)	Test compound final conc. (µM)	NADPH conc. (mM)	Incubation time at 37°C (min)
1A2 / Acetamimophen	0.5	100	0.03, 0.01, 0.3, 0.1, 3, 10, 30 and 100	1	10
2D6 / Dexthorphan	0.25	15			20
2C9 / 4'-hydroxydiclofenac		10			
3A4 / 6β-hydroxytestosterone		50			

Table 2 Metabolites MRM.

Compound	Q1 (m/z)	Q3 (m/z)
6β-hydroxytestosterone	305.3	269.1
[16,16,17-D_3]- 6β-hydroxytestosterone	308.4	272.1
dextrorphan	258.3	201.0
[D_3]-dextrorphan	261.0	201.0
4-hydroxydiclofenac	312.2	231.1
[dichlorophenol-U-$^{13}C_6$]-4'-hydroxydiclofenac	320.1	238.8
acetaminophen	152.0	110.2
[$^{13}C_2$,^{15}N]-acetaminophen	154.8	111.0

Results and Discussion

The linearity values (R^2) were for all metabolites greater than 0.99. No isobaric interference was observed with the selected MRM. The signal-to-noise ratio (S/N) were calculated for LDTD-APCI and also for LC-ESI and LC-APCI. In the overall, using the LDTD allows to reach higher S/N values (higher signal combined to lower background). The results show that LDTD is tolerant of complex matrices such as HLM without the need for LC separation unlike traditional LC-APCI or ESI (**Table 3**). Only dextrorphan shows a lower sensitivity in LDTD as compared to LC-ESI and APCI.

Table 3 Signal-to-noise ratio comparison between LDTD-APCI, LC-ESI and LC-APCI.

Compound	S/N *ESI*	*APCI*	*LDTD*
6β-hydroxytestosterone	78	600	54000
Dextrorphan	11000	27000	6600
4-hydroxydiclofenac	107	4500	27000
Acetaminophen	53	5400	12000

Table 4 CYP IC_{50} values comparison between LDTD-MS/MS and LC-MS/MS.

Compound	CYP	IC_{50}	
		LDTD-MS	*LC-MS*
miconazole	2C9	1.2	1.1
ketoconazole	3A4	0.03	0.03
nicardepine	2C9	1.7	1.5
nifedipine	2C9	4.2	4.2
mifepristone	2D6	9.4	9.6
clarithromicyn	2C9	> 100	> 100
α-naphtoflavone	1A2	0.06	0.07
quinidine	2D6	0.18	0.19
clozapine	2C9	51	68
imipramine	2D6	13	28
desipramine	2D6	11.5	9.4
bufurolol	2D6	20.7	23.8
sulfaphenazole	2C9	0.3	0.2
fluvoxamine	1A2	1.0	0.5
genistein	1A2	79	50

Complete results available at *Anal. Chem. 2007, 79 (12) 4657-4665*.

LDTD-MS versus LC-MS for CYP inhibition assays : Pooled samples

CYP inhibition assays were performed using fifteen (15) different commercial drugs. The samples obtained from CYP 1A2 / 2C9 / 2D6 / 3A4 were pooled before being run under LDTD-MS/MS or LC-MS/MS. The obtained IC_{50} values are reported in **Table 4**. All the IC_{50} values for all CYP isoforms obtained with the LDTD-MS/MS are in good agreement with the validated LC-MS/MS assay. An example of LDTD-MS/MS raw data is presented in **Figure 1**

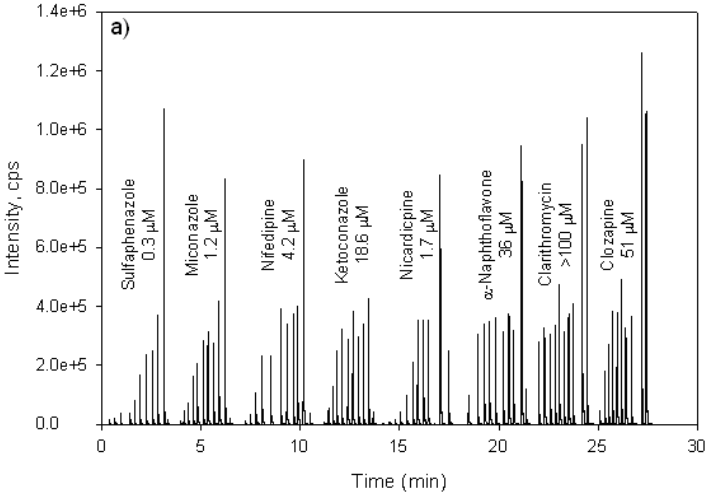

Figure 1 Raw LDTD-MS signal for several drug compound inhibition assays for CYP 2C9.

High throughput advantage of LDTD-MS/MS over LC-MS/MS

The sample run-time was 5.5 min. for the LC-MS/MS leading for more then 10 hours for all the assays. The same samples took 18 seconds to be run leading for less then 30 minutes for all the assays with the LDTD-MS/MS.

Conclusions

The LDTD-MS approach is 33-time faster than traditional LC-MS and 3- to 6-time than high throughput methods such UPLC-MS/MS without compromising the IC_{50} values accuracy and reproducibility, the analysis sensitivity and without carryover.

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