4.8 Seconds Sample to Sample Analysis In ADME Using LDTD-TripleTOF™ 5600 System

Pierre Picard[1], Serge Auger[1], Patrice Tremblay[1] and Alex Birsan[1]
[1]Phytronix Technologies, Quebec, Canada

Overview

- High throughput analysis of CYP inhibition samples in **4.8 seconds** sample to sample
- Drug probe : Midazolam, Tolbutamide, Bufurolol, S-mephenytoin, Buproprion, Resorufin and Coumarin
- A protein precipitation + salting out extraction procedure are used to prepare the CYP sample
- High throughput analysis of commercial compounds into typical Caco-2 buffer solution in **4.8 seconds** sample to sample
- Commercial compounds: Amiodarone, Carba-mazepine, Dextromethorphan, Metoprolol and Ticlopidine
- A sample dilution procedure is used to prepare the Caco-2 samples

Instrumentation (Figure 1)

- Phytronix Technologies LDTD ion source (model S-960)
- AB SCIEX TripleTOF™ 5600 System

Fig. 1 LDTD- AB SCIEX TripleTOF™ 5600 System

Introduction

For ADME analysis, compound optimization is generally considered a time-consuming step. With the new TripleTOF™ 5600 system we can avoid this step and operate the system in High Resolution MS in order to extract the signal of every compound of interest from a sample. We propose the use of the LDTD ion source instead of the traditional LC-ESI system for an increase in sample analysis speed hence allowing for a robust high-throughput analytical solution.

Samples Preparation (CYP inhibition assays)

Protein precipitation + Salting out extraction:

- 40 µL microsome solution in buffer
- 120 µL Internal standard (Clomiphene in Acetonitrile)
  - Vortex
- 40 µL NaCl (saturated solution in water)
  - Vortex and centrifuge (14000 RPM/2min)
- Spot: 4 µL on 96 LazWell plate

Samples Preparation (Caco-2 assays)

Sample dilution:

- 10 µL sample in buffer with 5 %BSA
- 10 µL Internal standard solution (Clomiphene in Acetonitrile)
  - Vortex
- 60 µL MeOH:Water (75:25)
  - Vortex and centrifuge (14000 RPM/2min)
- Spot: 4 µL on 96 LazWell plate

HR-MS Parameters (Generic method)

Mode	APCI (+)
Scan time	0.1 sec
Needle current	3 µA
Mass range	100 → 900 amu
Mass window	10 ppm

LDTD Parameters

Laser power pattern: 0 to 45% in 3.0 sec
Carrier gas flow: 3 L/min (Air)

Results and Discussion

The 4.8 seconds sample to sample analysis time is reached by acquiring only one file containing all the samples. From this file, the standard curve calibration points, QC, samples and internal standard peaks are extracted using MultiQuant™ 2.0 companion software (**Figure 2**).

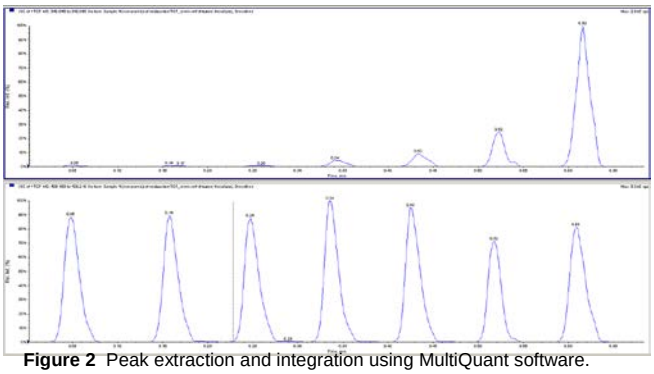

Figure 2 Peak extraction and integration using MultiQuant software.

CYP extraction sample analysis

The exact mass of each drug probe metabolites were extracted and standard curve is generated over the concentration range of 5 to 1000 nM (e.g. **Figure 3**). As shown in **Table 1** a good sensitivity and excellent linearity was reached for all metabolites.

Table 1: Calibration curve parameters for the different drug metabolites evaluated.

Drug metabolites	Exact Mass	Mode	r	Range (nM)	Accuracy (%)
OH-Midazolam	340.06584	(M-H)	0.9986	10-1000	88-119
4OH-Tolbutamide	285.09145	(M-H)	0.9964	5-1000	91-111
1OH-Bufurolol	276.16052	(M-H)	0.9960	5-1000	87-115
4OH-mephenytoin	235.10772	(M+H)	0.9982	10-100	70-116
OH-Buproprion	256.10998	(M-H)	0.9975	5-1000	85-112
Resorufin	214.04987	(M+H)	0.9980	5-1000	94-110
7OH-Coumarin	161.02442	(M-H)	0.9998	10-1000	93-103

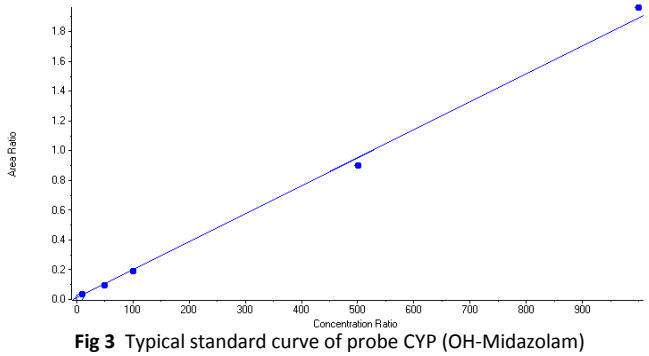

Fig 3 Typical standard curve of probe CYP (OH-Midazolam)

Caco-2 extraction sample analysis

The exact mass of each tested commercial compound, extracted from the BSA/buffer solution, was obtained and the standard curves are generated over the concentration range of 1 to 1000 nM (e.g. **Figure 4**). As shown in **Table 2,** good sensitivity as well as excellent linearity was reached for all compounds. The dynamic range obtained is equivalent to that of a triple quadrupole running in MS/MS mode.

Table 2: Calibration curve parameters for the different commercial compounds evaluated in CACO-2 buffer.

Probe	Exact Mass (M+H)	r	Range (nM)	Accuracy (%)
Amiodarone	646.03096	0.9991	10-1000	87-111
Carbamazepine	237.10224	0.9999	1-1000	98-103
Dextromethorphan	272.20089	0.9995	10-1000	91-109
Metoprolol	268.19072	0.9999	1-1000	95-105
Ticlopidine	264.06082	0.9985	1-1000	95-117

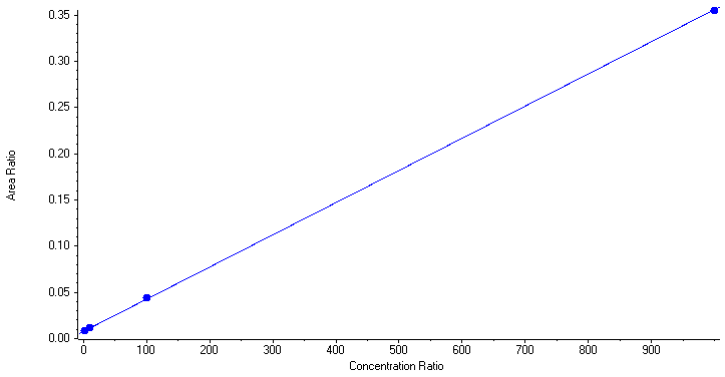

Fig 4: Typical standard curve of Caco-2 sample extract (Carbamazepine)

Conclusion

ADME sample analysis can be performed in HR-MS which avoids the need for a compound optimization step. Moreover, operating the TripleTOF™ 5600 coupled with an LDTD™ ion source allows for an outstanding analytical speed of **4.8 seconds** per sample.

The results clearly demonstrate that accurate results can be obtained using this high-throughput analytical approach. The LDTD-TripleTOF™ 5600 system exhibits good sensitivity and excellent linearity for samples in complex matrix such as CYP450 and CACO-2 medium.

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
www.absciex.com

Phytronix Technologies
Parc technologique du Québec métropolitan
4535, boulevard Wilfrid-Hamel, suite 120, Québec (Qc) Canada G1P 2J7
www.phytronix.com