

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

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Overview

- High throughput analysis of CYP inhibition samples in **4.8 seconds** sample to sample;
- Drug probe : Midazolam, Tolbutamide, Bufurolool, S-mephenytoin, Buproprion, Resorufin and Coumarin ;
- A protein precipitation + salting out extraction procedure is 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, Carbamazepine, 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 Triple TOF™ 5600 System

Introduction

For ADME analysis, the compound optimization is time-consuming. With the new TripleTOF™ 5600 System, we can avoid this step and operate the system in High Resolution MS and extract the signal of every interested compound into a sample. We also propose to use the LDTD ion source instead of the traditional LC-ESI system to even get more analytical speed.

Samples Preparation (CYP inhibition assays)

Protein precipitation + Salting out extraction

- 40 µL microsome solution in buffer
- 120 µL Internal standard solution (Clomiphene in Acetonitrile)
 - Vortex
- 40 µL NaCl (saturated solution in water)
 - Vortex and centrifuge (14000rpm/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 (14000rpm/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 s

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, QCs, samples and internal standard peaks are extracted using MultiQuant™ 2.0 companion software (Figure 2).

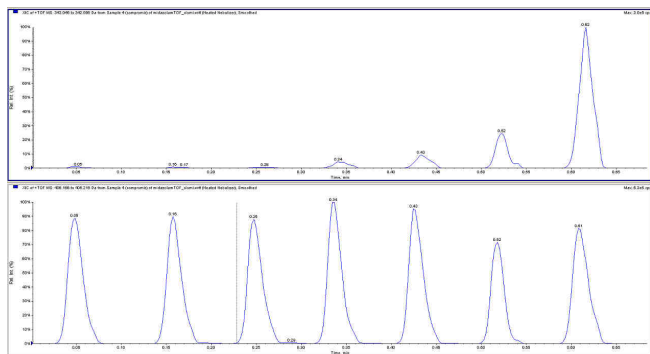


Figure 2 Peak extraction and integration using MultiQuant™ software.

CYP extraction sample analysis

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

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

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

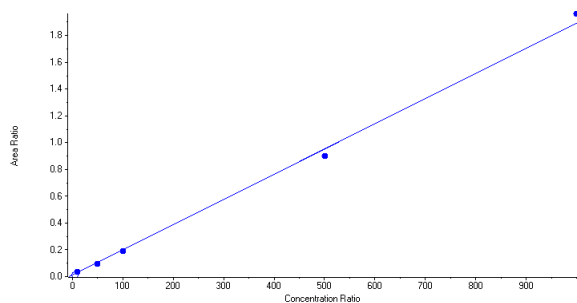


Fig 3 Typical standard curve of probe CYP (OH-Midazolam)

Caco-2 extraction sample analysis

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

Table 2 Calibration curve parameters for the different commercial compounds evaluated into the CACO-2 medium.

| 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 |

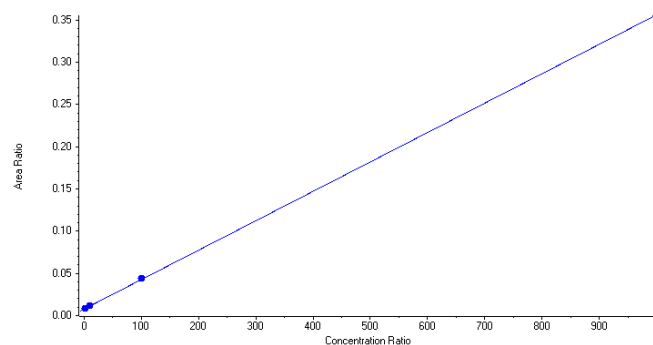


Figure 4 Typical standard curve of Caco-2 sample extract (Carbamazepine)

Conclusion

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

The results show that at this speed, accurate results can be obtained. The LDTD-TripleTOF™ 5600 System shows good sensitivity and excellent linearity for samples in complex matrix such as CYP450 and CACO-2 medium.

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