Salt Assisted Liquid-Liquid Extraction (SALLE)[1] technique In CYP inhibition Using LDTD-TripleTOF™ 5600 System

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Overview

- Salt-induced phase separation applied to CYP inhibition analysis giving a cleaner and more concentrated extract.
- High-Throughput analysis of CYP inhibition samples in **5 seconds** sample to sample;
- Drug probe : Diclofenac, Dextromethorphan and Midazolam
- Analyzed metabolite: 4-Hydroxy-Diclofenac, Dextrorphan and 1-Hydroxy-Midazolam

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 CYP analysis, a cocktail containing phosphate buffer, HEPES, microsomes and cofactors is used as reactional medium. The inhibition process is stopped by the addition of Acetonitrile. Usual sample preparation is achieved through a dilution with Acetonitrile in a 1 to 4 v/v ratio. In order to enhance the signal, a fast and easy Salt Assisted Liquid-Liquid Extraction (SALLE) technique is used for CYP metabolite analysis. The dilution factor is reduced by 2.5 times and as the extract is cleaner, 4µl are spotted instead of 2µl.

Samples Preparation (CYP inhibition assays)

Protein precipitation + Salt-induced phase separation

- 40 µL Microsome solution in buffer
- 40 µL Internal standard (in Acetonitrile)
  - Vortex
- 40 µL NaCl (saturated solution in water)
  - Vortex and centrifuge (14000rpm/2min)
- 40 µL of Acetonitrile
- Spot: 4 µL of the upper layer on 96 LazWell plate

HR-MS Parameters

Mode	APCI (+)
Accumulation time	0.05 sec

Source/Gas

GS1	0
GS2	0
CUR	10
TEM	0
NC	3

Drug	Prod ion	CE	DP
4-Hydroxy-Diclofenac	312 -> 230.0134	30	100
4-Hydroxy-Diclofenac-$^{13}C_6$	318 -> 236.0333	30	100
Dextrorphan	258 -> 157.0648	30	80
Dextrorphan-d_3	261 -> 157.0648	30	80
1-Hydroxy-Midazolam	342 -> 203.0371	30	80
1-Hydroxy-Midazolam-$^{13}C_6$	348 -> 209.0573	30	80

LDTD Parameters

Laser power pattern: 0 to 45% in 3.0 sec and maintain 2 seconds at 45% before dropping to 0%.
Carrier gas flow: 3 L/min (Air)

Results and Discussion

Figure 2 shows a desorption peak signal using SALLE technique. Acquiring all the samples in a single file optimizes analysis time. From this file, the standard curve calibration points, QCs, samples and internal standard peaks are extracted using MultiQuant™ 2.0 companion software.

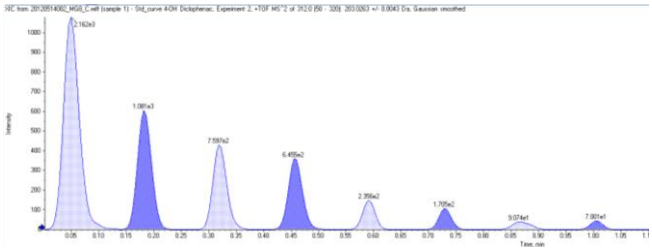

Figure 2a Peak extraction and integration of analyte.

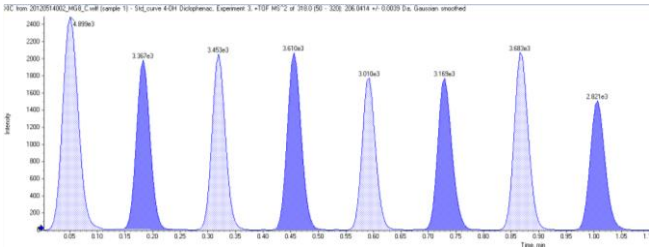

Figure 2b. Peak extraction and integration of internal standard

CYP extraction sample analysis

Using the product ion mode, each drug probe metabolites were extracted and standard curve is generated (e.g. **Figure 3, 4 and 5**). 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	r	Range (nM)	Accuracy (%)
4-Hydroxy-Diclofenac	0.9939	25-426	82-119
Dextrorphan	0.9975	30-516	83-112
1-Hydroxy-Midazolam	0.9938	23-389	81-115

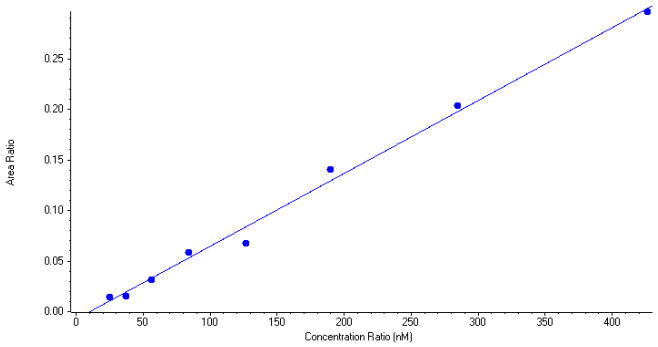

Fig 3. Standard curve of probe CYP (OH-DIclofenac)

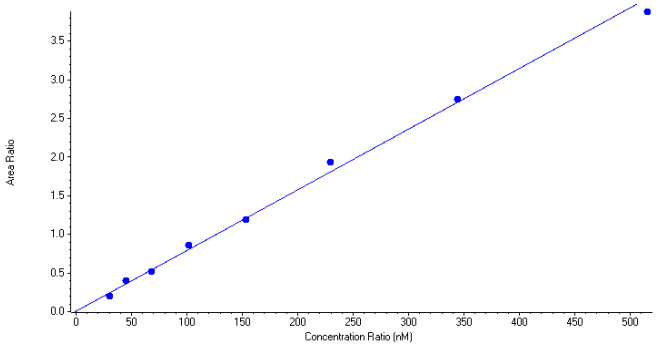

Fig. 4 Standard curve of probe CYP (Dextrorphan)

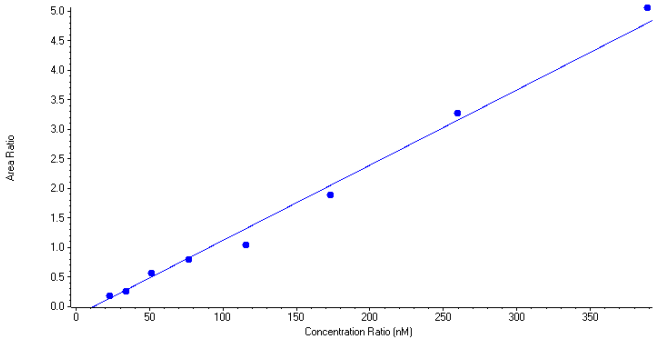

Fig. 5 Standard curve of probe CYP (OH-Midazolam)

Conclusion

The results clearly demonstrate that using Salt Assisted Liquid-Liquid Extraction (SALLE) technique gives much more sensitive and accurate results. The on-plate deposit amount of material is 5 times higher than traditional dilution approach. This sample preparation method combined with the LDTD-TripleTOF™5600 system gives an exceptional high-throughput analytical technique for the CYP450 assays.

1) Reference: Majors, R.E., Salting-out Liquid-Liquid Extraction (SALLE), LCGC North America, Jul., 2009

