**Standard Operating Procedure (SOP)**

for drug metabolism studies by liver microsomes

04-07-2016

**Materials and reagent**

* 100 mM Phosphate buffer(pH 7.4), add 0.2M NaP to 0.2M NHP in proportion with 19:81, then dilute 0.2M PB using ddO.
* 20 mM NADPH, solubilized in 100 mM phosphate buffer.
* 100X stock of the test article solution
* 20 mg/mL microsomes, thaw microsomes slowly on ice immediately prior to experiment and adjust concentration to 20 mg/mL using phosphate buffer.
* 20 mg/mL heat-inactivated microsomes, 45℃ pretreatment for 30 min.
* testosterone, adjust concentration to the same with test article in the same solvent.
* ethyl acetate

**Protocol**

1. Add 183 μL of phosphate buffer, 2 μL of test article and 5 μL of microsomes to each PE tube.
2. Pre-incubate microsomes, buffer and test article in water bath at 37℃ for 5 min.
3. Add 10 μL of NADPH.
4. Incubate up to 60 min at 37℃ with gentle agitation.
5. Add 200 μL of ethyl acetate.
6. Vortex samples for 20 seconds and centrifuge at approximately 3000rpm for five minutes.
7. Withdraw the supernatant from the protein pellet to another PE tube.
8. Inject 10 μL to LC/MS/MS for analysis.

**Table 1 Experimental designs for drug metabolism studies by liver microsomes**

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| --- | --- | --- | --- | --- | --- | --- |
| Groups | Components added | | | | | Analysis points |
| Drug | PB | Microsomes | NADPH | ethyl acetate |
| Test | 2 μL test article | 183 μL | 5 μL | 10 μL | 200 μL | 60 min |
| Control 1 | 2 μL test article | 193 μL | 5 μL | 0 μL | 200 μL | 60 min |
| Control 2 | 2 μL test article | 183 μL | 5 μL (heat-inactivated) | 10 μL | 200 μL | 60 min |
| Control 3 | 2 μL test article | 183 μL | 5 μL | 10 μL | 200 μL | 0 min |
| Control 4 | 2 μL testosterone | 183 μL | 5 μL | 10 μL | 200 μL | 60 min |