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Determination of a small molecule drug in rat brain by acoustic homogenisation and UPLC-MS

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Abstract

A method was developed for the determination of a small molecule drug in rat brains using acoustic homogenisation for the sample preparation of the rat brains. Compared to traditional mechanical homogenisation using a metal blade, the method used was less time consuming and with no risk of cross contamination between the samples. The homogenised brain tissue samples were precipitated by acidic solvent and centrifuged. The supernatants were taken to dryness and re-dissolved in a small volume of solvent. The samples were analysed by UPLC-MS. The small molecule drug and its metabolites were detected in brains from dosed rats but not in brains from control rats.

Introduction

Focused acoustics technology was investigated for homogenisation of rat brains. The principle of acoustic homogenisation is based on computer generated acoustic shockwaves that are focused on the tissue samples, leading to effective disruption and homogenisation. Each sample is subjected to an identical process method within a closed vessel providing an uniform processing of each sample.

Method

A rat brain was put in a Tissue CryoPrep bag (Figure 1) and the bag holder was placed in liquid nitrogen for 5min. The frozen rat brain was pulverised using a Covaris Cryoprep (Figure 2). This single use, non-contact and closed-vessel system eliminates clean-up and sample contamination, and the CryoPrep system enables a uniform impact force to be applied to each tissue sample.

By increasing the tissue surface area the extraction efficiency of the target drug molecules should be improved. Pulverised tissue (Figure 3), 0.5-1g, is easily transfered to a homogenisation tube as a closed system. 1mL of Ringer buffer (pH 7.4) was added to the homogenisation tube (Figure 4).

Figure 1. Covaris CryoPrep bag and a Cryobag sealed to a homogenisation tube.

Figure 2. Covaris CryoPrep CP-02 System.

Figure 3. Pulverised rat brain obtained from cryofrozen brain

Figure 4. 1g pulverised rat tissue in 1mL of Ringer buffer.

Figure 5. Covaris S-2 acoustic homogeniser with laptop control pc.

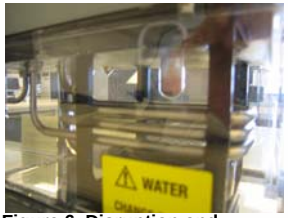

Figure 6. Disruption and homogenisation of rat brain tissue.

Figure 7. Homogenised rat brain sample.

Method (continued)

The homogenisation tube was placed in the acoustic homogeniser (Figure 5). The instrument is controlled by Covaris SonoLAB software. The system generates adaptive focused acoustic energy that cause disruption and homogenisation of the tissue (Figure 6). After 2min process time, a smooth homogenate was obtained (Figure 7).

Two mL of acetonitrile/glacial acetic acid 96/4 (v/v) was added to the homogenisation tube in order to precipitate the proteins and the tube was shaken for 10min using a Heidolph Multi Reax at maximum speed. The test tube was centrifuged at 3,600xg for at +8˚C for 15min. The resulting supernatant was evaporated to dryness in a SpeedVac. The residue was re-dissolved in water/acetonitrile 7/3 (v/v) containing 0.05% (v/v) TFA. The samples were analysed by UPLC-MS. The small molecule drug and its metabolites were detected in brain extracts from rats dosed with the drug (Figure 8) but not in extracts of brains from control rats (Figure 9), i.e. rats dosed with formulation vehicle.

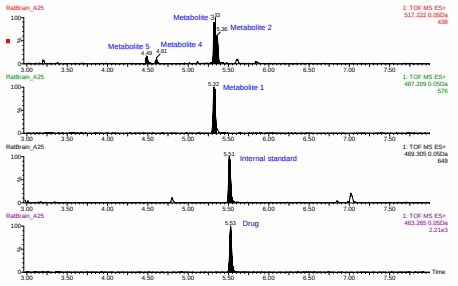

Figure 8. Extracted ion chromatograms illustrating the detection of a small molecule drug and its metabolites in a extract of rat brain from a dosed rat.

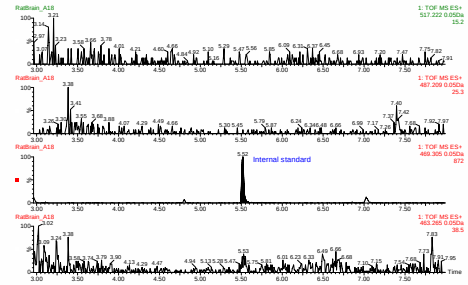

Figure 9. The same ions extracted as in Figure 8 from the UPLC-MS analysis of a rat brain extract from a control rat. Only the added internal standard is detected.

Conclusion

- A UPLC-MS method was developed for the determination of a small molecule drug and its metabolites in rat brain tissue.
- The Covaris system with the CryoPrep unit and the acoustic homogeniser was very efficient for homogenisation of the rat brain tissue samples. Sample processing was fast. No cross-contamination between samples was seen. No need for clean-up and rinsing was needed as always is the case when using a metal blender homogeniser for tissue preparation.
- The extraction efficiency of spiked control brains was 95-105% for concentrations 10, 100 and 1000nM.