Evaluation of Chemical Stability and Bioanalytical Assay Quality Using Adaptive Focused Acoustics Tissue Homogenization and LC-MS/MS Analysis

Hang Zeng, Kristen MacFarland, Joe Gallagher, Ping-Ping Wang and Daksha Desai-Krieger

Bioanalytical/DMPK/GPCD, Johnson & Johnson Pharmaceutical R&D LLC, Spring House, PA

In the past, a popular method for tissue homogenization prior bioanalysis was to use rotary blade devices. This technology presents challenges in laboratory safety, sample throughput/efficiency, and potential for carryover/cross-contamination. Recently, a novel technology has been introduced in this field to overcome these problems. Adaptive Focused Acoustics (AFA) energy is designed to use for tissue homogenization as an alternative approach to extract pharmaceutical compounds from tissue samples prior to LC-MS/MS analysis. AFA devices provide improvements for automated robotic sample handling and increased recovery and reproducibility in tissue assay. The most appealing advantage is "Non-contact" sample processing – therefore NO cleaning (rotary blade) required and eliminates cross-contamination potential. In this presentation, analyte chemical stability and assay quality of discovery compounds were evaluated using AFA process coupled with LC-MS/MS analysis.

The sample preparation was performed in a Costar 96-DW cluster plate. For standard and QCs: 50 µL of control homogenate + 25 µL of internal standard (IS) solution + 50 µL of standard spiking solution; for samples: 50 µL of sample + 25 µL of IS + 50 µL of solvent. All sample mixture were then extracted with either LLE/PP or PP/plus online extraction.

Sample on-line analysis conditions
Autosampler: CTC HTS PAL units with multiple injection valves
HPLC system: Rheos 2000 binary pumps
Extraction column: Cyclone, 0.5x50mm, 30µ
Mobile phase: A) dI-H_2O + 0.1% Formic acid; B) Acetonitrile + 0.1% FA
Flow rate: 1.5 mL/min, step gradient; Injection volume: 10 µL

Sample separation conditions
HPLC system: Rheos 2000 binary pumps
HPLC columns: Aglient Zorbax SB C-18 column, 4.6 x 50 mm, 3.5 µ
Mobile phase: A) dI-H_2O + 0.1% Formic acid; B) Acetonitrile + 0.1% FA
Flow rate: 1.0 mL/min, linear gradient

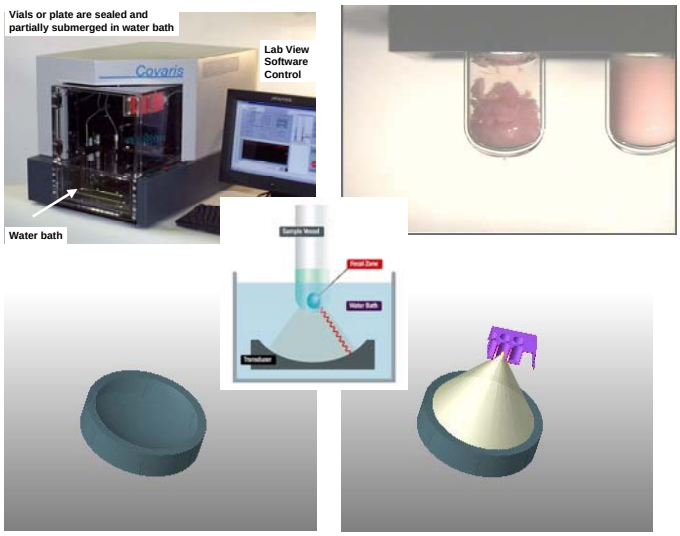

Fig. 1 AFA Instrument and work principle scheme

Mass spectrometer conditions (API-4000 MS)
Turbo-ionspray, Positive ion, MRM; CAD: 7;
CUR: 10; GS1: 45; GS2: 65; IS: 5000; TEM: 550; EP: 10; Tdwell: 100 ms.

Table 1 Summary of Chemical Stability of Generic Analytes in Rat Brain Homogenate

Generic Analyte	Percent Different in Responses of AFA Treated Sample vs. without AFA (N=5)*						Ave. %Diff
	0.1 ug/mL	0.4 ug/mL	1 ug/mL	2 ug/mL	5 ug/mL	10 ug/mL	
Benzoylecgonine	-0.9	1.6	1.5	1.6	0.5	-3.1	0.2
Fenfluramine	-7.0	2.6	-1.7	3.2	5.6	-1.6	0.2
Haloperidol	0.8	-0.1	-0.8	0.7	1.4	1	0.5
Oxazepam	4.2	-1.7	-1.5	1.1	1.6	-2.7	0.2
Puromycin	-5.6	-0.3	-1.6	-2.9	0.1	-0.5	-1.8

Table 2 Summary of Chemical Stability of Discovery Analytes in Rat Brain Homogenate

NME Analyte	Percent Different in Responses of AFA Treated Sample vs. without AFA (N=5)						Ave. %Diff
	0.1 ug/mL	0.4 ug/mL	1 ug/mL	2 ug/mL	5 ug/mL	10 ug/mL	
Compound-A	0.5	0.3	-0.3	0.4	-0.1	2.3	0.5
Compound-B	3.9	-0.3	-0.2	-0.4	0.3	2.4	1.0
Compound-C	0.9	0.8	-0.3	1.8	1.1	2	1.0
Compound-D	0.6	1.5	-0.7	-1.3	0.1	1.0	0.2
Compound-E	1.1	1.3	-0.6	-0.2	-0.4	0.1	0.2
Compound-F	-0.1	-0.4	-0.2	-0.2	0.4	0.2	-0.1

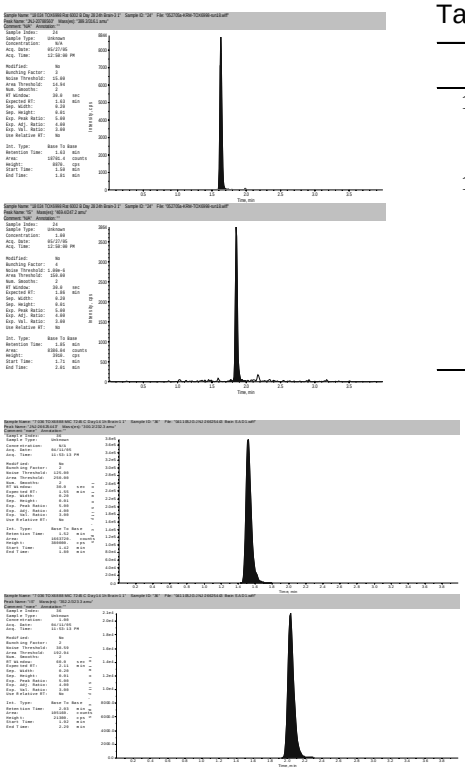

Table 3 Summary of QCs for Compound B in Sample Analysis

Run Date	Curve Number	QC-10.0 ng/mL	QC-400 ng/mL	QC-1000 ng/mL
11-Apr-05	7	9.53	373	967
		8.63	383	964
		9.16	332	755a
12-Apr-05	8	9.23	426	997
		9.16	364	982
Mean		9.14	376	978
S.D.		0.324	34	15.2
%C.V.		3.5	9	1.6
%Bias		-8.6	-6	-2.2
N		5	5	4

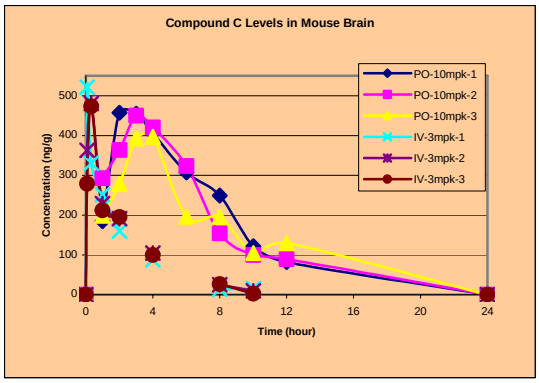

Fig. 2. Chromatograms of Compound A&B In Tissue Assay

Fig. 3. Brain Concentration - Time Profile of Compound C From a PK Study After IV and PO Doses in Mice

- The analytes tested in this work are stable after the AFA energy processes; there was no significant impact on chemical stability (Ave. % Diff. < 1.8%). Therefore, the AFA system is suitable for tissue sample analysis in pharmaceutical development
- The new tissue homogenization technique has been successfully applied in authors' laboratory to support many preclinical studies from different species for several NMEs
- This novel technology using Adaptive Focused Acoustics Energy increases sample throughput by reducing process time; the non-contact closed vessel process eliminates carryover/cross-contamination between samples and provides safety work environment
- Covaris instrument (E200 and S2) can be easily operated; E200 offers complete automation for 12/24 samples/load; standardized AFAE transfer ensures uniform sample process with high recovery and efficiency.