Development of an Automated Homogenizer –Autogizer and Its Application in Brain Uptake Studies

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INTRODUCTION

BACKGROUND

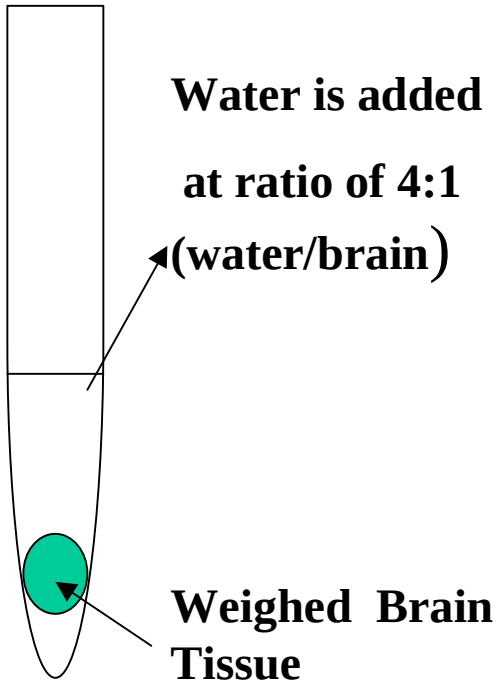
- **The evaluation of brain uptake of drug candidates is essential in drug discovery.**
- **Sample preparation for brain tissue sample is labor intensive and limits the turnover time of the analysis.**
- **Long-term exposure to noise from manual homogenization of tissue samples is a health hazard.**

OBJECTIVE

- **To design an automated homogenizer to process brain samples at much faster rate**
- **To improve the working environment**

Picture of Autogizer

Method: Brain Sample Homogenization



15-mL centrifuge tube

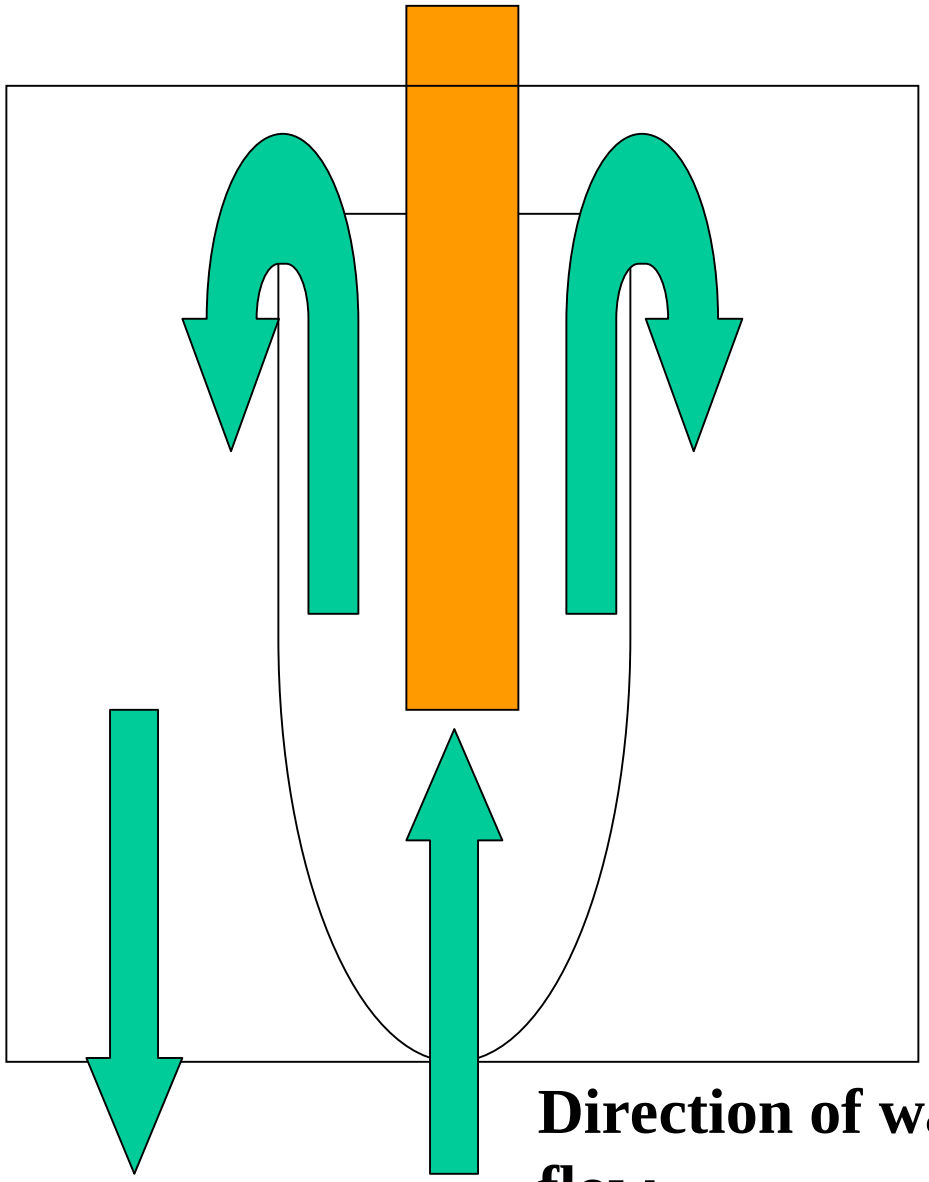
Homogenize
use Autogizer

Parameters setting

- OSC speed: 4
- cycle: 3
- cycle time: 45 sec.
- 3 washes. each wash 35 sec.

Total time: 2.5 min/5 samples

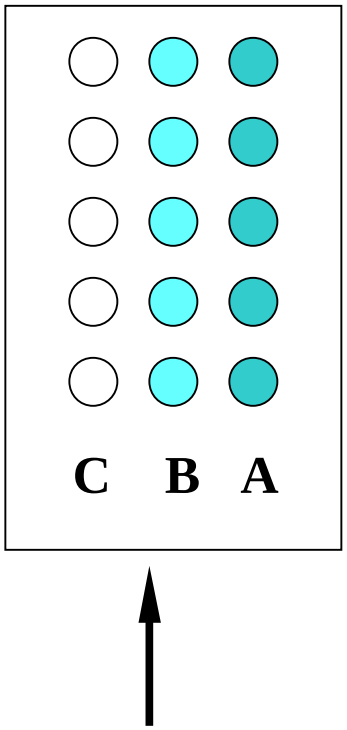
Design for Washing Reservoir

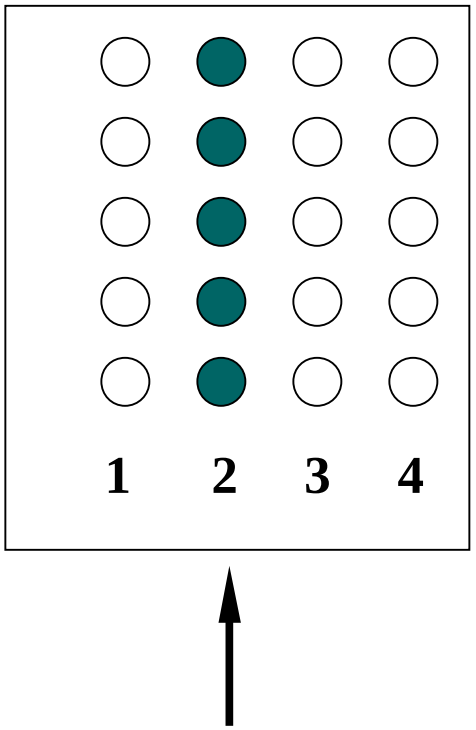


Direction of water flow

Using continuously up-flowing water to rinse the probe is much more efficient than rinsing the tube in static water

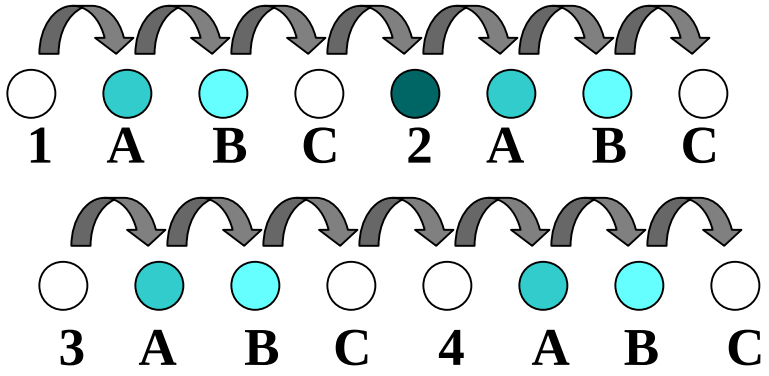
Experiment Design: Carry-Over of Autogizer





Procedures:

Autogenizer was programmed to move with the following order:



Aliquots from 1 , 3, and 4 were taken for LC/MS analysis.

Washing reservoir:

Tubes in Column A, B, C were filled with continuously flowing water from bottom

Sample Processing Tray:

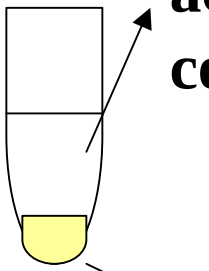
Column 1: Blank brain tissue

Column 2: Brain tissue spiked with 5000 ng/g of 4 Standards

Column 3: Blank brain tissue

Column 4: Blank brain tissue

Brain Homogenate Preparation for LC/MS Analysis



200 µL of acetonitrile/methanol containing IS

Brain homogenates 40 µL

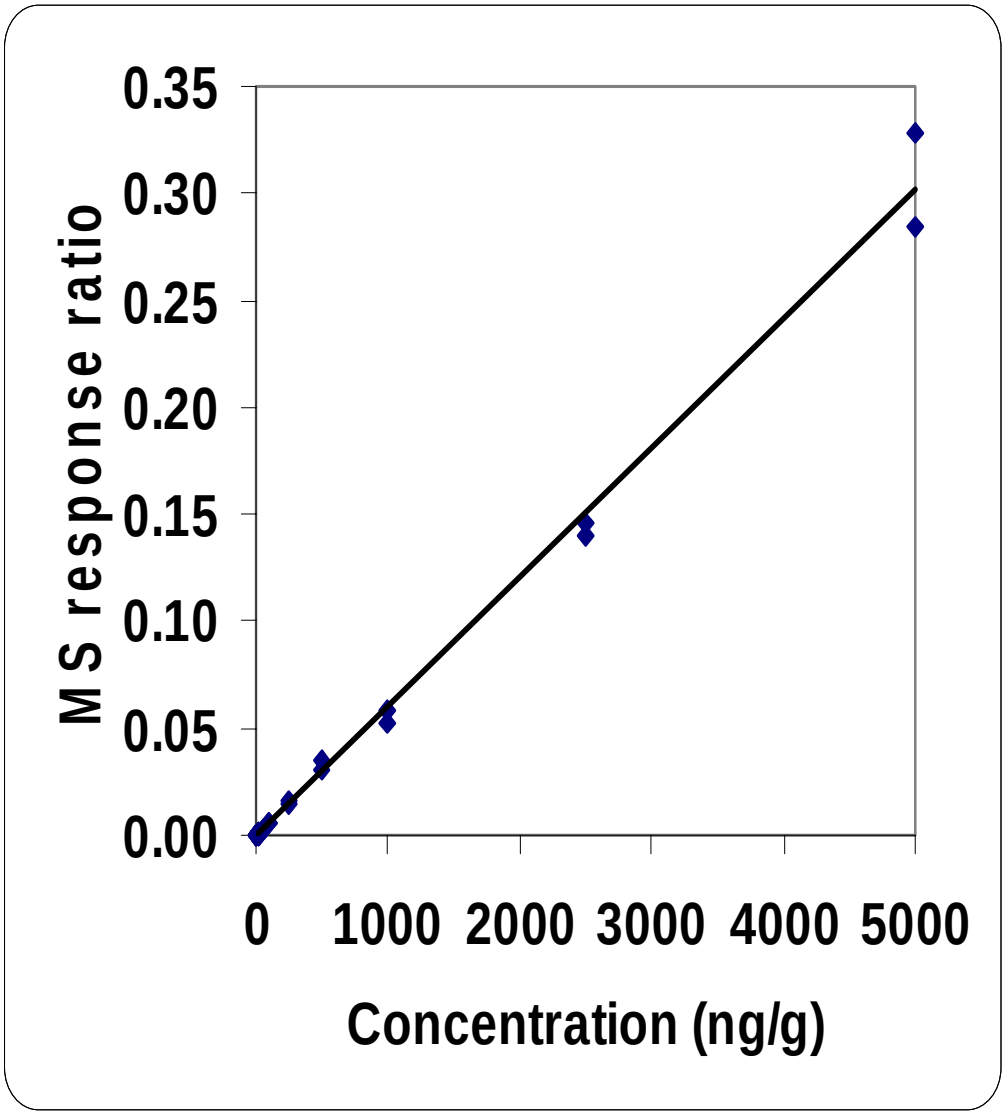
- **Vortex**
- **Centrifuge**

10-20µL injected for LC/MS/MS analysis

Result: Carry-over of Autogizer after Processing 5000 ng/g Brain Standards

	Concentration (ng/g)			
	CMPD_A	CMPD_B	CMPD_C	CMPD_D
Column 1 (before processing 5000 ng/g standards)				
tube 1	0	0	0	0
tube 2	0	1	0	0
tube 3	0	1	0	0
tube 4	0	1	1	0
tube 5	0	0	0	0
Column 3 (after processing 5000 ng/g standards and 3 washes with water)				
tube 1	**1**	**0**	**0**	**3**
tube 2	**0**	**0**	**0**	**1**
tube 3	**1**	**0**	**1**	**3**
tube 4	**1**	**0**	**0**	**1**
tube 5	**1**	**0**	**0**	**1**
Column 4 (after Column 3)				
tube 1	0	0	0	1
tube 2	0	0	0	0
tube 3	0	0	0	1
tube 4	0	0	0	1
tube 5	0	0	0	1

Results: Calibration Curve of Brain Standards

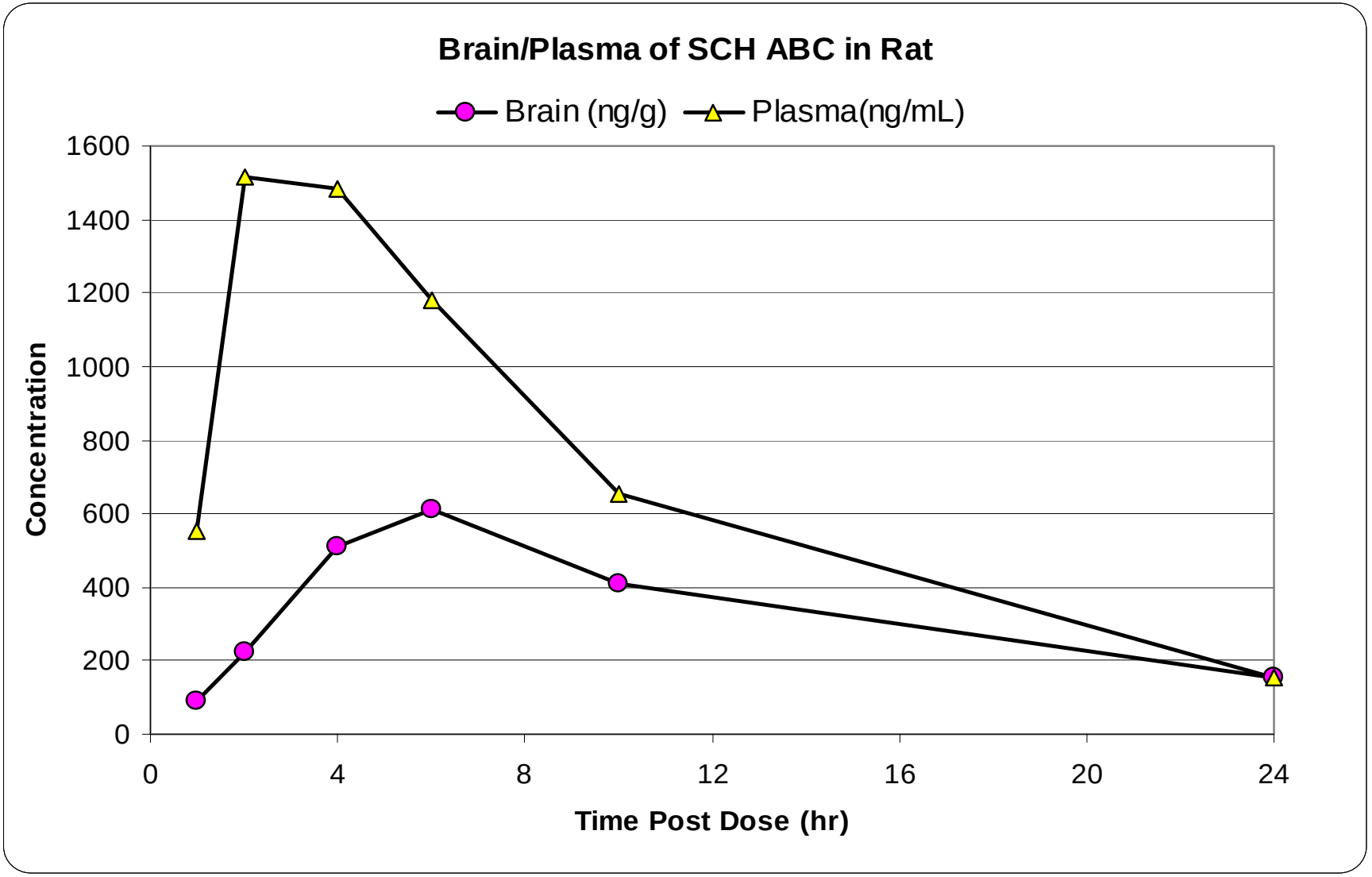


	Front Standards		Back Standards	
Conc.ng/g	Back-calc.	%DEV	Back-calc.	%DEV
1	1	20	0.9	-9.7
2.5	2	-2.2	2	-12
5	6	9.2	6	24
10	10	-0.6	11	12
25	24	-3.9	23	-9.2
50	60	20	56	13
100	106	5.8	95	-5.5
250	257	2.9	266	6.5
500	600	20	514	2.9
1000	875	-13	989	-1.1
2500	2457	-1.7	2367	-5.3
5000	4794	-4.1	5538	11

Results: Within-run Reproducibility in Brain Tissue

	Conc. (ng/g)	Calculated Conc. (ng/g)	Mean (N=6) (ng/g)	CV %	Accuracy
1	5	5.5; 5.9; 4.6; 4.6; 6.1; 5	5	12	106%
2	50	53; 55; 60; 49; 38; 64	53	18	106%
3	500	453; 436; 531; 499; 468; 524	485	8	97%

Results: An Example of Brain Uptake Study



Results: Comparison of Manual Homogenization and Auto Homogenization (based on 20 samples)

	Manual	Auto
Total Time	100 min	10 min
Labor Time	100 min for a set of brains	~0. No labor needed. The robot did the job
Noise Hazard	Yes. Person exposed to noise for 40 min	No. The robot was set in a separated room.
Safety Compliance	Person used his/her hand holding the vibrating probe for 40 min, prone to accidents	The robot did the job without human intervention
Solvent Consumption	20L of water and 2L of MEOH	4L of water

CONCLUSIONS

- The designed Autogizer can process brain samples at least 10 times faster than manual homogenization.
- The precision and accuracy are very good and meet our acceptance criteria for drug discovery sample analysis
- It has < 0.1% carry-over and consumes no organic solvent and uses 5 times less water to rinse the probe.
- Because it is automated and can be left alone, it is noise hazard free and more safety compliant.