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Application Note

Ostro Sample Preparation Products: Crosstalk

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief highlights ostro sample preparation products.

Introduction

One measure of a microplate's performance is the amount of crosstalk, or cross-contamination, that occurs between adjacent wells. The influence of adjacent samples upon each other may cause errors in analyte quantitation which may effect the evaluation of a plate's performance. This potential influence on the final results should be evaluated and taken into account if necessary. As part of the evaluation of the Ostro plate, specific experiments were conducted in order to ensure that detrimental crosstalk effects were not observed.

Experimental

Samples

- Fortified plasma – labeled "o": ketoprofen
  - 20 mg/mL: final concentration
- Blank plasma – labeled "x"
- Standard sample solution: 1 µg/mL
  - 1 µg/mL: 0.005% of fortified plasma concentration
- Sample positions:

	1	2	3	4	5	6	7	8	9	10	11	12
A	o	x	o	x	o	x	o	x	o	x	o	x
B	x	o	x	o	x	o	x	o	x	o	x	o
C	o	x	o	x	o	x	o	x	o	x	o	x
D	x	o	x	o	x	o	x	o	x	o	x	o
E	o	x	o	x	o	x	o	x	o	x	o	x
F	x	o	x	o	x	o	x	o	x	o	x	o
G	o	x	o	x	o	x	o	x	o	x	o	x
H	x	o	x	o	x	o	x	o	x	o	x	o

96-Well Plate Diagram

Protocol

- Load 200 µL fortified plasma into “o” wells.
- Load 200 µL blank plasma into “x” wells.
- Vigorously add 600 µL 1% FA in ACN to all the wells.
- Aspirate samples 3X using an autopipette set to 600 µL.
- Using a 2 mL sample collection plate, apply 15-20” Hg vacuum for 5 minutes.
- Evaporate the whole plate under 50 °C with N2 flow until all the wells are dry.
- Reconstitute all “x” wells with 200 µL 80% (v/v) MeOH.
- Cover plate with cap mat.
- Inject and analyze all “x” well samples and compare to standard at 1 µg/mL. The levels in the “x” wells should be <1 µg/mL (<0.005% of the fortified plasma).
- Detection wavelength: PDA at 250-260 nm.

Results and Discussion

Plate	Solvent Blank Injection Area	n = 48 Blank Plasma “x“ Wells	n = 8 1µg/mL Standard Average Area
		Average Area	
1	41	41.7	2277
2	41	140	1668

In both plates, the average area counts for the blank plasma "x" wells were at least an order of magnitude lower than the standard which represents 0.005% of the fortified plasma, indicating zero to negligible cross-talk.

Crosstalk Ketoprofen Area Count Results

Conclusion

Based on these data, there is no significant cross-talk observed when using the Ostro plate.

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