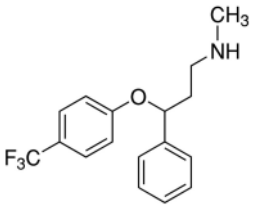
APPLICATION NOTES

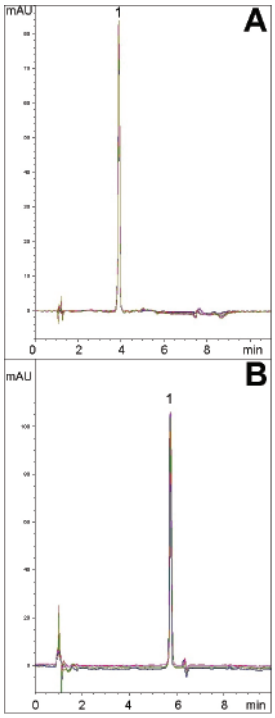
Cogent™ Phenyl Hydride

HPLC
RP/ANP

Orthogonal Assay for Fluoxetine

Simple methods without the use of ion-pairing agents





Notes:
Fluoxetine is a widely prescribed antidepressant which acts by selective inhibition of presynaptic serotonin reuptake. In addition, fluoxetine can also act as a non-competitive antagonist of nicotinic acetylcholine receptors. Sold as a racemic mixture, fluoxetine's *R* and *S* forms show similar efficacy *in vivo*, and its binding affinity has been shown to be largely stereo-independent.

Method Conditions

Column: **Fig. A:** Phenyl Hydride™, 4µm, 100A
Fig. B: Diamond Hydride™, 4µm, 100A

Catalog No.: **Fig. A:** 69020-7.5P **Fig. B:** 70000-7.5P

Dimensions: 4.6 x 75 mm

Solvents: A: DI water/ 0.1% formic acid
B: 97% Acetonitrile/ 3% DI water/ 0.1% formic acid

Gradient:

Fig. A		Fig. B	
time (min.)	%B	time (min.)	%B
0	10	0	95
6	90	6	60
7	10	7	95

Flow rate: 1.0 mL/min

Detection: UV 228 nm

Injection volume: 10µL

Temperature: **Fig. A:** 35°C **Fig. B:** 25°C

Sample: Fluoxetine capsule extract

Stock Solution: 20 mg strength capsule contents added to 100 mL volumetric flask, diluted to mark with 50:50 A:B, Vortexed 5 min, sonicated 5 min, then filtered through a 0.45 µm nylon membrane (MicroSolv Technology Corp. Eatontown, NJ, USA). Working Solution: 100 µL stock diluted with 900 µL 50:50 A:B.

Peak: 1. Fluoxetine

Discussion

The USP assay methods for fluoxetine use ion-pairing agents in the mobile phase in order to reduce analyte peak tailing due to silanolic interactions. However, this peak tailing is not an issue with this method since these silanols are mostly replaced with Si–H groups. A disadvantage of ion-pairing agents is their slow uptake and release from the HPLC column, resulting in long equilibration times and poor repeatability. Both figures show how excellent peak symmetry can be achieved either in the reversed phase (RP, Figure A) or aqueous normal phase (ANP, Figure B) mode with only formic acid as the mobile phase additive. The ability to perform the assay in either the RP or ANP mode is highly beneficial for development of orthogonal analytical methods. Chromatograms show 5 runs overlayed to highlight precision.

For more information visit www.MTC-USA.com

Cat. No.	Description
69020-7.5P	Phenyl Hydride™ HPLC Column, 100A, 4µm, 4.6mm x 75mm
70000-7.5P	Diamond Hydride™ HPLC Column, 100A, 4µm, 4.6mm x 75mm

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