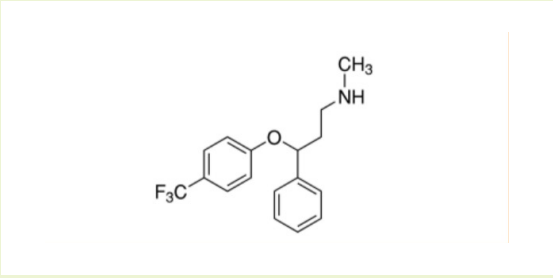
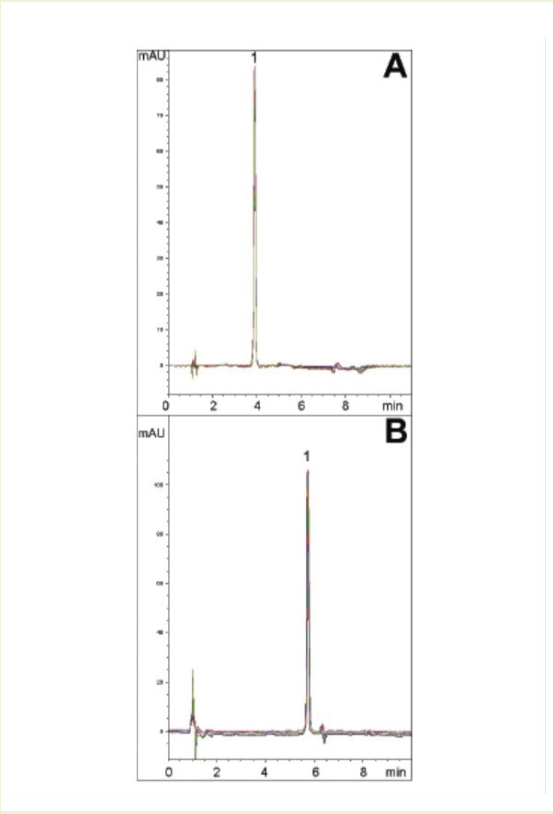
Orthogonal Assay for Fluoxetine

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: A: **Phenyl Hydride™**, 4µm, 100Å
B: **Diamond Hydride™**, 4µm, 100Å

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

Dimensions: 4.6 x 75 mm

Solvents: A: DI H_2O/ 0.1% formic acid
B: 97% Acetonitrile/ 3% DI H_2O/ 0.1% formic acid

Gradient A:

time (min.)	%B
0	10
6	90
7	10

Gradient B:

time (min.)	%B
0	95
6	60
7	95

Temperature: A: 35°C B: 25°C

Injection vol: 10µL

Flow rate: 1.0 mL/min

Detection: UV 228 nm

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 0.45µm nylon membrane (MicroSolv Technology Corp.). Working Solution: 100µL stock diluted with 900µL 50:50 A:B.

Peak: 1. Fluoxetine

Discussion

Unlike the USP assay method, ion pair agents are not needed in this application. 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.

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