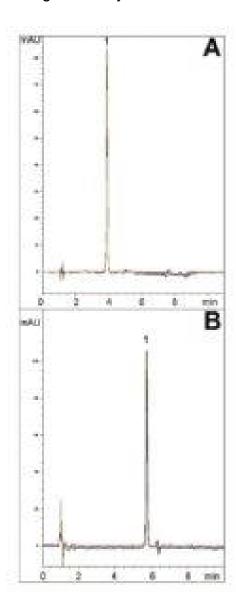


Fluoxetine Orthogonal Assay Method - AppNote

Simple Methods Without the Use of Ion-Pairing Agents

Unlike the USP assay method, Ion Pair Agents are not needed in this application. Both figures show how good 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.



Peak:

Fluoxetine

Method Conditions

Columns:

Fig. A: Cogent Phenyl Hydride™, 4µm, 100Å

Fig. B: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.:

Fig. A: 69020-7.5P

Fig. B: 70000.7.5P

Dimensions:

Fig. A: 4.6 x 75 mm

Fig. B: 4.6 x 75 mm

Mobile Phase:

A: DI Water / 0.1% Formic Acid

B: 97% Acetonitrile / 3% DI Water / 0.1% Formic Acid

Gradient:

Figure A.

1 19 - 1 - 1	
Time (minutes)	%В
0	10
6	90
7	10

Figure B.

Time (minutes)	%В
0	95
6	60
7	95

Temperature:

Fig. A: 35°C

Fig. B: 25°C

Injection vol.: 10µL

Flow rate: 1.0 mL / minute Detection: UV @ 228nm

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 minutes, sonicated 5 minutes, then filtered through a 0.45µm Nylon Syringe Filter (MicroSolv Technology Corp.)

Syringe Filter (MicroSolv Technology Corp.).

Working Solution: 100µL stock diluted with 900µL 50:50 Solvent A: Solvent B

Notes: Fluoxetine is a widely prescribed anti-depressant 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.



Attachment

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