

## Liquid Chromatography

## Authors:

Ross Birch

Kathryn Lawson-Wood

PerkinElmer, Inc.

Seer Green, UK

## HPLC Analysis of Fluoxetine Hydrochloride Using a Quasar C8 Column in Accordance with the United States Pharmacopeia

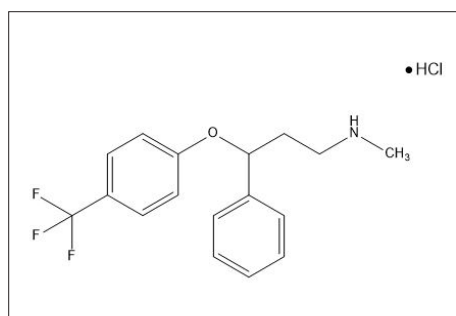


Figure 1. Structure of fluoxetine hydrochloride.

### Introduction

Fluoxetine hydrochloride is an antidepressant belonging to a class of drugs known as selective serotonin reuptake inhibitors. It was notably marketed as Prozac.<sup>1</sup> Fluoxetine is also

effective at treating obsessive compulsive disorder and bulimia. It blocks the serotonin reuptake transporter in the presynaptic terminal which results in increased levels of serotonin in certain areas of the brain.<sup>2</sup> Fluoxetine is favorable in comparison with older antidepressants as it is a selective inhibitor for serotonin, has weak affinity for noradrenaline receptors and no affinity for dopamine receptors, giving it a far more desirable adverse effect profile.

This application brief describes the use of a Quasar™ C8 column for the analysis of fluoxetine hydrochloride (Figure 1) in accordance with the official Fluoxetine Hydrochloride USP monograph.<sup>3</sup>

## Experimental Conditions

### Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters.

Instrument	PerkinElmer Flexar™ with PDA Plus™ Detector			
Quasar C8	250 mm	4.6 mm	5 µm	N9308879
Mobile Phase	A: Buffer (Triethylamine and Water (1:98), pH 6.0 with Phosphoric acid) B: Methanol C: Tetrahydrofuran 60% A 10% B 30% C			
Flow Rate	1.0 mL/min			
Temp	Ambient			
Wavelength	227 nm			
Injection Volume	10 µL			
Analyte	Fluoxetine Hydrochloride			

### Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.45 µm nylon filter 13 mm, P/N 02542903.

A standard solution of USP fluoxetine hydrochloride (0.11 mg/mL) was prepared using pre-mixed mobile phase as diluent.

The mobile phase buffer solution was prepared by dissolving 10 mL triethylamine in 980 mL water. The pH was then adjusted to pH 6.0 by adding concentrated phosphoric acid dropwise.

## Results and Discussion

The USP monograph specifies the use of an L7 column (250 x 4.6 mm, 5 µm). This is defined as octyl silane chemically bonded to porous silica particles (1.5 – 10 µm in diameter). The Quasar C8 column complies with the USP monograph and is ideal for the separation of fluoxetine hydrochloride as can be seen in the results, Table 2.

The analysis of fluoxetine hydrochloride was carried out using a Quasar C8 (250 x 4.6 mm, 5 µm) column (P/N: N9308879) in under 15 minutes (11.6 minutes), Figure 2. The USP monograph states that the run time must be no less than 1.2 times the retention time of fluoxetine hydrochloride. The run was continued until 15 minutes to ensure compliance with the monograph. The USP monograph requires that the relative standard deviation (RSD) of five replicate injections be no more than 0.73 % and that the tailing factor be no more than 2.0. The Quasar C8 column met both requirements (Table 2) and gave an efficient separation (11,179 N, calculated using the tangential method). This is due to Quasar's ultra-high purity silica and its optimized ligand bonding technology.

Table 2. Results Summary. RSD calculated from five successive injections.

Suitability Parameter	Quasar	USP Requirement
Peak Area RSD (%)	0.65	< 0.73
Tailing Factor (5% Peak Height)	1.16	< 2.0

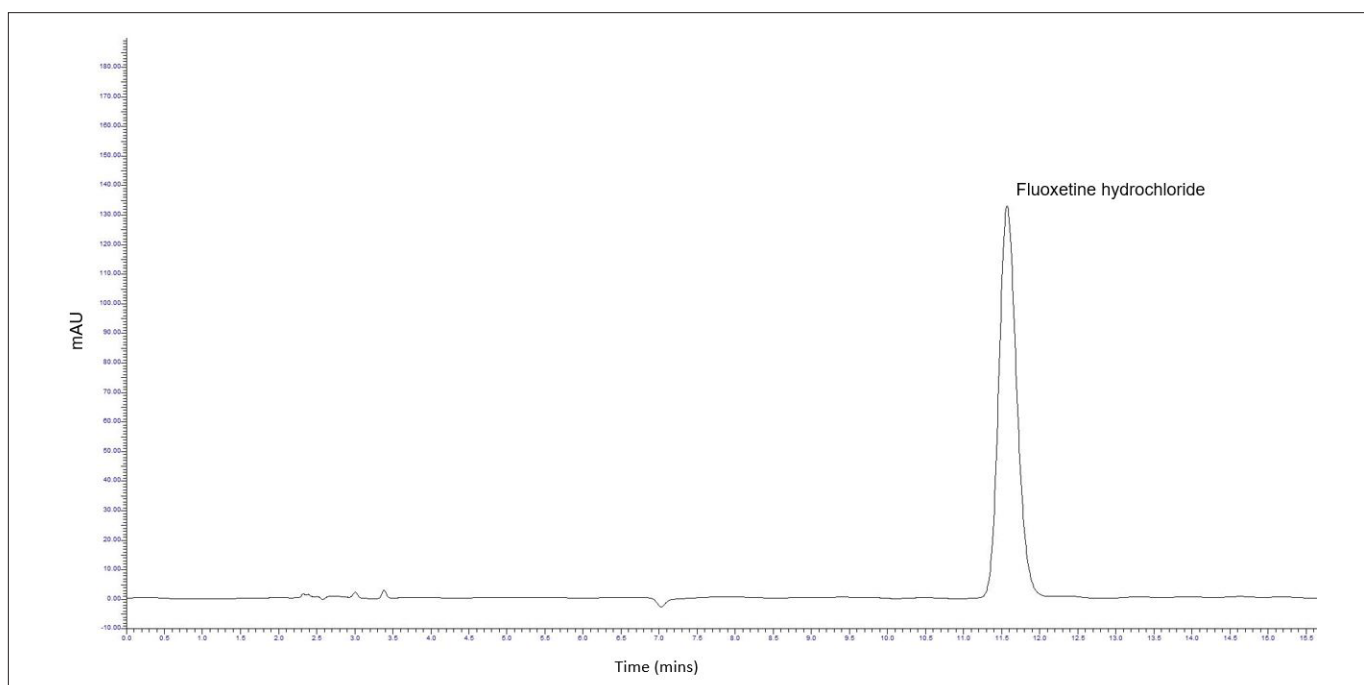


Figure 2. Analysis of fluoxetine hydrochloride using a Quasar C8 column (250 x 4.6 mm, 5 µm).

## Conclusion

- Rapid analysis of fluoxetine hydrochloride was carried out on a Quasar C8 column (250 x 4.6 mm, 5 µm) in under 15 minutes.
- The Quasar C8 column provides efficient and repeatable separations, meeting all USP requirements.
- Run time could be reduced by using a shorter Quasar C8 column (150 x 4.6 mm, 3 µm) which would allow quicker analysis and shorter equilibration times. The USP allows changes so that the ratio of length to particle size is within -25% and 50% of the parameters prescribed in the monograph.

## References

1. US Food and Drug Administration, [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2000/18936558LBL.PDF](https://www.accessdata.fda.gov/drugsatfda_docs/label/2000/18936558LBL.PDF), (accessed 17/07/20).
2. Drug Bank database, <https://www.drugbank.ca/drugs/DB00472>, (accessed 11/08/20).
3. USP monograph fluoxetine hydrochloride, [https://online.uspnf.com/uspnf/document/1\\_GUID-79F0CBCD-8DBE-44CB-B606-C38EE2932B17\\_5\\_en-US?source=Search%20Results&highlight=fluoxetine%20hydrochloride%23%5D](https://online.uspnf.com/uspnf/document/1_GUID-79F0CBCD-8DBE-44CB-B606-C38EE2932B17_5_en-US?source=Search%20Results&highlight=fluoxetine%20hydrochloride%23%5D), (accessed 11/08/20).

## Consumables Used

Component	Description	Part Number
Column	Quasar C8 (250 x 4.6 mm, 5 µm)	N9308879
HPLC Vials	2 mL Amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (polypropylene) Cap with PTFE/Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable, Pack of 100	02542890
Syringe Filters	0.45 µm Nylon Filter, 13 mm	02542903
PEEK Fittings	Finger-tight for 1/16" OD PEEK Tubing	09920513
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	N9306301