

# Determination of Fluconazole in Human CSF Using DBS Extraction and HPLC/MS/MS

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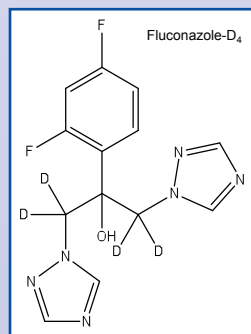
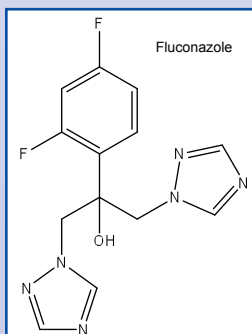
## Overview

- ▶ **Purpose** – Develop a DMS extraction procedure and an HPLC/MS/MS method to determine concentrations of fluconazole in human CSF.
- ▶ **Methods** – DMS methanol extraction and HPLC/MS/MS (API5000).
- ▶ **Results** – Range from 1 – 500 ng/mL with accuracies and precision better than  $\pm 15\%$  using HPLC/MS/MS, and ambient stability of up to 5 days, see Tables 1 - 3 for data.

## Introduction

Fluconazole is a triazole antifungal agent used to combat secondary fungal infections such as cryptococcal meningitis, which persists in the membranes surrounding the brain and spinal cord.<sup>1</sup> A lumbar puncture may only yield small volumes of cerebral spinal fluid (CSF) for performing diagnostics or determining exposure of fluconazole during treatment. Dried blood spot (DBS) or in this case dried matrix spot (DMS) analysis, was ideal for the small sample volume analysis (< 20  $\mu$ L) of CSF. This DMS method, coupled with HPLC/MS/MS analysis, was developed for determining the fluconazole concentration in CSF samples. Additionally, we discovered that application of a color-indicating dye prior to sample spotting allowed a visual confirmation of the dried sample location, see Figure 1.

Here we demonstrate a novel use of DBS for other biological fluids that expands the potential applications of this established technique.



## Methods

### Extraction

- ▶ Fluconazole extracted from human CSF using a DMS extraction procedure
- ▶ Card type: FTA DMPK-C
- ▶ 15  $\mu$ L sample volume
- ▶ Punch diameter: 6 mm
- ▶ Extraction solvents: 100  $\mu$ L water + 1% formic acid (5 minutes) then 400  $\mu$ L methanol

### HPLC

- ▶ Gradient HPLC using acetonitrile and water with 1% formic acid
- ▶ Flow rate = 0.7 mL/minute
- ▶ HSC18 2.1x50 mm (Supelco)
- ▶ Column heated to 50°C

### Mass Spectrometry

- ▶ Sciex API5000 operating in MRM mode
- ▶ ESI
- ▶ Positive ion mode
- ▶ MRM transitions for fluconazole and fluconazole-D4 respectively  
307.0  $\rightarrow$  237.7  
311.4  $\rightarrow$  242.0

**Table 1.** Intra-day accuracy (%) and precision ( $\pm$  %) for the HPLC/MS/MS analysis of fluconazole in human CSF reported at five QC levels.

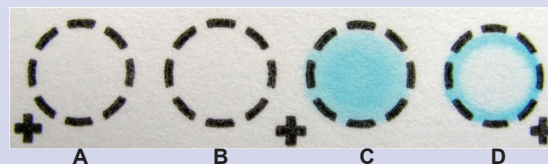
QC Levels (ng/mL)				
1	3	25	400	500
98.7 $\pm$ 5.6	99.2 $\pm$ 4.1	98.1 $\pm$ 3.9	95.0 $\pm$ 2.4	94.1 $\pm$ 3.6

**Table 2.** Recovery (%) for both analyte and internal standard reported for the low, mid, and high QC levels. IS normalized matrix factor at the low QC level.

QC Levels (ng/mL)			
	3	25	400
Analyte Recovery	80.1	89.1	83.2
IS Recovery	89.8	90.6	92.6
Matrix Factor	1.1	–	–

**Table 3.** Stability of fluconazole spotted onto card and stored at ambient for five days (% Bias from nominal concentration) reported for the low, mid, and high QC levels.

QC Level (ng/mL)	3	25	400
% Bias	8.1	10.8	-0.8

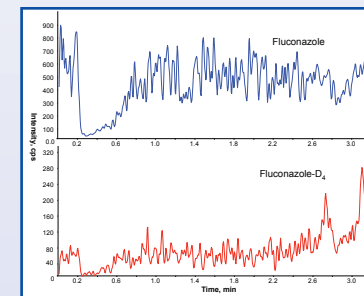


**Figure 1.** FTA DMPK-C Card.

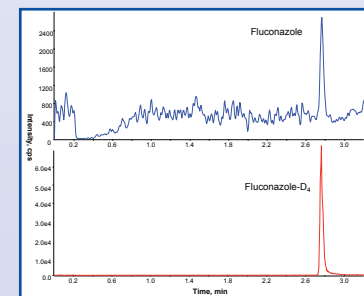
- (A) No color-indicating dye or CSF sample spot
- (B) No color-indicating dye with CSF sample spot
- (C) Color-indicating dye with no CSF sample spot
- (D) Color-indicating dye with CSF sample spot

## Conclusions

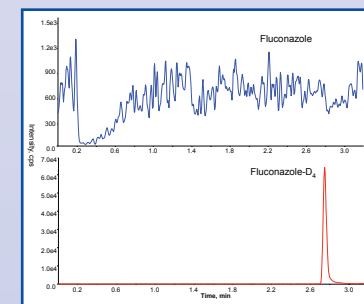
- ▶ Developed a method using DMS and HPLC/MS/MS to quantify fluconazole in human CSF.
- ▶ This method is ideal for fluids such as CSF where only limited sample volumes are available (<30  $\mu$ L).
- ▶ For colorless matrices, exact sample spot location can be visually identified with application of color-indicating dye prior to sample spotting.



HPLC/MS/MS Chromatogram from the Analysis of Blank Human CSF



HPLC/MS/MS Chromatogram from the Analysis of Human CSF Fortified with 1 ng/mL of Fluconazole



HPLC/MS/MS Chromatogram from the Analysis of Human CSF Containing Internal Standard Fluconazole D4

## References

1. Hillis, et al. *Chromatographia*. 2004, 59:S203-S207.