

## Liquid Chromatography

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# Comparison of Clotrimazole HPLC Analysis Using Quasar C18 and Inertsil ODS-3 Columns

### Introduction

Clotrimazole is an azole anti-fungal medication used primarily in the treatment of a wide range of fungal dermal infections. Examples include thrush, athlete's foot and ringworm. It has limited oral absorption and is commonly available in various topical preparations,

including creams, lotions, and solutions. As well as its anti-fungal activity, clotrimazole has become a drug of interest in the treatment of sickle cell disease, malaria and some cancers.<sup>1,2</sup> Clotrimazole has generic status and is thus globally produced and prescribed. It is on the World Health Organization's (WHO) list of essential medicines, which serves as a model of the safest and most effective medications needed by a health system.<sup>3</sup>

This application brief describes the use of a PerkinElmer Quasar™ C18 (150 x 4.6 mm, 5 μm) column in comparison with a GL Sciences Inertsil ODS-3 (150 x 4.6 mm, 5 μm) column for the analysis of clotrimazole, conducted by a pharmaceutical company in Chile.

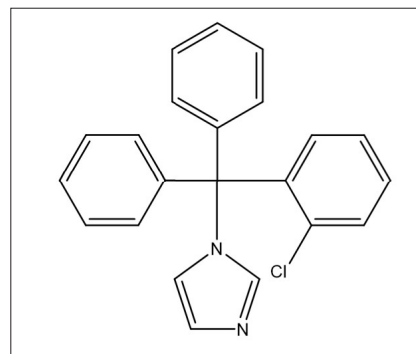


Figure 1. Chemical structure of clotrimazole.

## Experimental Conditions

### Method Parameters

Analysis was conducted by a Pharmaceutical CRO in Chile using a PerkinElmer Quasar C18 (150 x 4.6 mm, 5 µm) column and an Inertsil ODS-3 (150 x 4.6 mm, 5 µm) column. All HPLC method parameters are shown in Table 1. Mobile phase conditions were optimised during method development.

Table 1. HPLC method parameters for analysis.

Instrument	LC with PDA Detector			
Columns	150 mm	4.6 mm	5 µm	Quasar C18 (N9308802)
	150 mm	4.6 mm	5 µm	Inertsil ODS-3
Mobile phase	<b>A:</b> Methanol <b>B:</b> Buffer (K <sub>2</sub> HPO <sub>4</sub> ) 78 % <b>A</b> , 22 % <b>B</b>			
Flow rate	1.5 mL/min			
Column Temperature	30 °C			
Sample Temperature	25 °C			
Wavelength	220 nm			
Injection Volume	20 µL			
Analyte	Clotrimazole			

### Solvents and Samples

All solvents and reagents used were HPLC grade. Mobile phase buffer was prepared by dissolving 4.35 g of potassium phosphate dibasic (K<sub>2</sub>HPO<sub>4</sub>) in 1000 mL of water.

A 0.65 mg/mL stock solution of clotrimazole was prepared in methanol and sonicated. From this, a 0.13 mg/mL working standard solution of clotrimazole was prepared using mobile phase as the diluent. The working standard was injected in triplicate.

### Results and Discussion

The analysis of clotrimazole using the Quasar C18 and Inertsil ODS-3 columns (150 x 4.6 mm, 5 µm) is shown in Figure 2. Table 2 details the retention times, and system suitability parameters for clotrimazole using the two different columns. The Quasar C18 phase is ideally suited to the analysis of small molecules, providing higher efficiency (calculated using the USP tangential method) and better peak shape than the Inertsil ODS-3 column for the analysis of clotrimazole. This is due to Quasar's optimized ligand bonding technology and ultra-high purity silica base, which minimizes unwanted silanol interactions. Although Quasar demonstrated a slightly lower retention factor (k) in comparison with the Inertsil ODS-3 column, this does not impact overall performance. Quasar also provided greater resolution between the analyte and solvent peaks. This was a parameter calculated as part of the company's internal protocol.

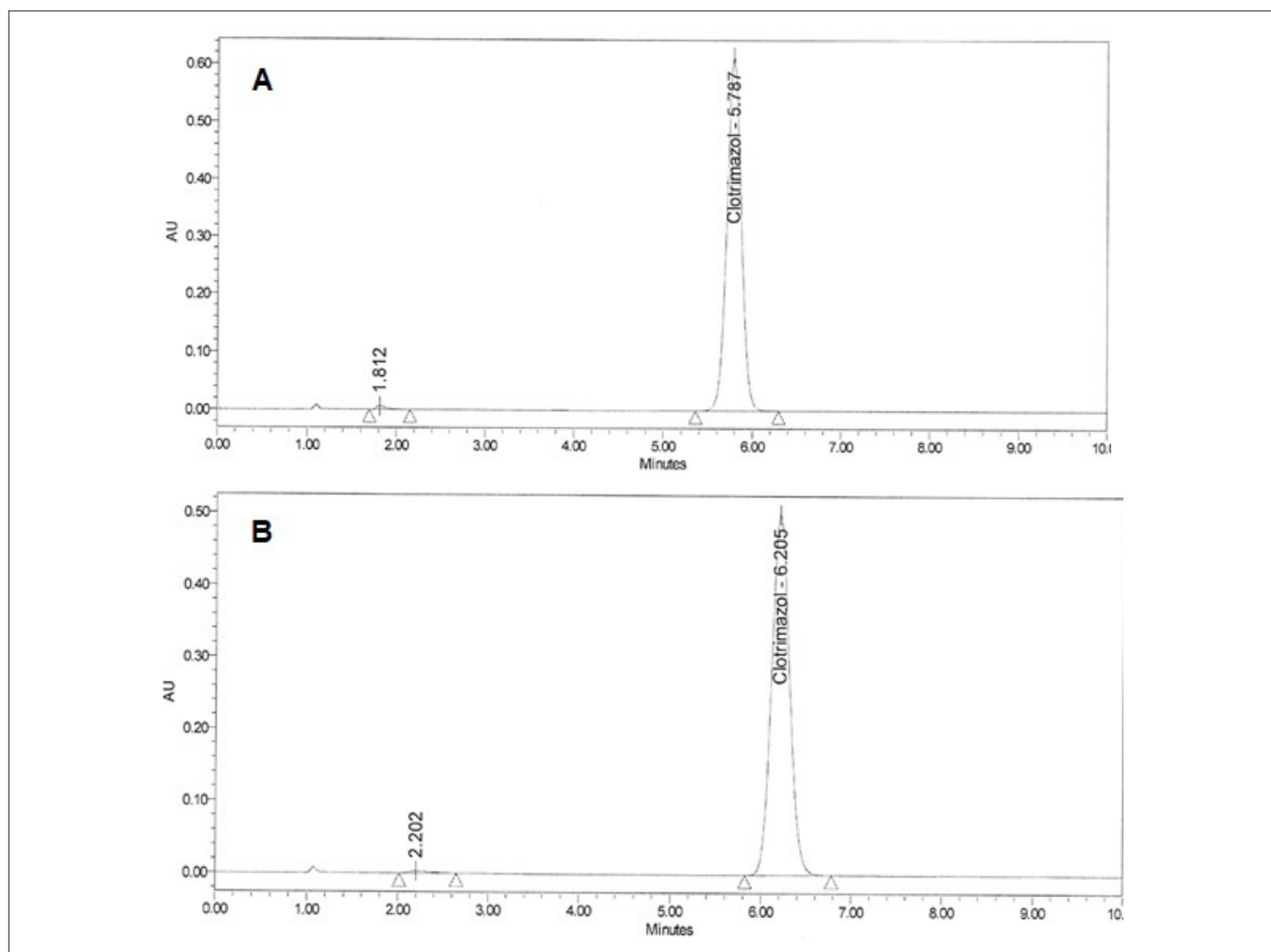


Figure 2. Analysis of clotrimazole using Quasar C18 150 x 4.6 mm, 5 µm (A) and Inertsil ODS-3 150 x 4.6 mm, 5 µm (B).

Table 2. System suitability results for the analysis of clotrimazole\* using the Quasar C18 and Inertsil ODS-3 columns (150 x 4.6 mm, 5 µm).

Column (150 x 4.6 mm, 5 µm)	Retention Time (min)	k	Resolution	Peakarea (µV*sec)	Peak Area RSD (%)	Peak Efficiency (Plates)	Tailing Factor (USP)
Quasar C18	5.78	4.46	16.57	6920431	0.02	6121	1.02
Inertsil ODS-3	6.20	4.86	10.80	6926178	0.06	4591	1.10

\*Taken from an average of 3 injections.

## Conclusion

- This application brief demonstrates the superior performance of a Quasar C18 column compared with an Inertsil ODS-3 column (150 x 4.6 mm, 5 µm).
- Quasar C18 offers greater efficiency for the separation of clotrimazole, and with greater resolution between analyte and solvent peaks.
- The ultra-high purity silica base and low residual silanol activity of the Quasar C18 yields excellent peak shape, with a lower tailing factor compared with the Inertsil ODS-3 phase.

## References

1. P.D. Crowley, and H.C. Gallagher, Clotrimazole as a pharmaceutical: past, present and future. *J Appl Microbiol.*, 2014, 117(3), 611-617.
2. P.K. Bolla, C.A. Meraz, V.A. Rodriguez, I. Deaguero, M. Singh, V. Kashyap Yellepeddi, and J. Renukuntla, Clotrimazole Loaded Ufosomes for Topical Delivery: Formulation Development and In-Vitro Studies, 2019, 24(17), 3139.
3. WHO Model List of Essential Medicines, <https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU 2019.06-eng.pdf?ua=1>, (accessed 11/06/2020).

## Consumables Used

Component	Description	Part Number
Column	Quasar C18 (150 x 4.6 mm, 5 µm)	N9308802
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
PEEK Fittings	Finger-tight for 1/16" OD PEEK Tubing	09920513