

# Sensitive LC-MS Analyses of Basic Compounds Using Discovery HS F5

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## Abstract

Sensitive analyses of basic analytes are of great importance to the pharmaceutical, life science and other industries. In this report, the use of a fluorinated stationary phase, Discovery HS F5, is shown to provide enhanced retention for basic analytes in mobile phases with very high organic content. These relatively volatile mobile phases are easily desolvated and thus result in enhanced LC-MS sensitivity relative to traditional reversed-phase chromatography on C18 stationary phases.

## Introduction

The analyses of basic compounds continue to pose significant problems for the analytical chemist. This is especially evident for bases with low hydrophobic character that are difficult to retain on traditional alkyl stationary phases. Needham (1) has shown that fluorinated stationary phases, such as the Discovery HS F5, can be used to retain basic analytes at high organic modifier content. The advantages of retention in this realm compared to the use of low organic modifier content on C18 stationary phases include increased LC-MS sensitivity, alternative selectivity and the potential for high-speed analyses.

In this study the retention and LC-MS response of pergolide (an antiparkinsonian) and sumatriptan (an antimigraine therapeutic) using Discovery HS F5 is compared to results obtained using a traditional C18 column. Pergolide and sumatriptan represent relatively hydrophobic and hydrophilic basic analytes, respectively. The enhanced MS sensitivity observed using the fluorinated stationary phase is discussed.

## Experimental

Pergolide (Cat. No. P8828) and ammonium acetate (Cat. No. 73594) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Sumatriptan was obtained from Kemprotec Limited (Middlesbrough, UK). Acetonitrile (Cat. No. 92679) and water (Cat. No. 39253) were of LC-MS grade from Reidel-de Haën (Seelze, Germany). Acquisitions were made using a Waters (Milford, MA USA) 2690 HPLC system interfaced with a Waters/Micromass ZQ single quadrupole mass spectrometer via an electrospray ionization (ESI) source. Chromatographic conditions are provided in the respective figures. Octanol-water partition coefficients (LogP) were estimated using an on-line prediction tool ([www.syrres.com/esc/est\\_kowdemo.htm](http://www.syrres.com/esc/est_kowdemo.htm)).

## Results and Discussion

Figure A shows the structures of the study analytes and Figures B and C present the chromatograms obtained for pergolide and sumatriptan, respectively, on both Discovery HS F5 and Discovery HS C18. Of immediate note is the increase in MS

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Figure B. LC-MS Analysis of Pergolide on (I) Discovery HS F5 and (II) Discovery HS C18

Column I: Discovery HS F5, 3.3cm x 2.1mm ID, 3µm particles  
Cat. No.: 567501-U  
Column II: Discovery HS C18, 3.3cm x 2.1mm ID, 3µm particles  
Cat. No.: Custom  
Mobile Phase I: 4mM ammonium acetate in 10:90, water:CH<sub>3</sub>CN  
Mobile Phase II: 4mM ammonium acetate in 55:45, water:CH<sub>3</sub>CN  
Flow Rate: 0.2mL/min  
Temp.: 35°C  
Det.: MS ESI+  
Inj.: 2µL  
Sample: 1µg/mL in mobile phase

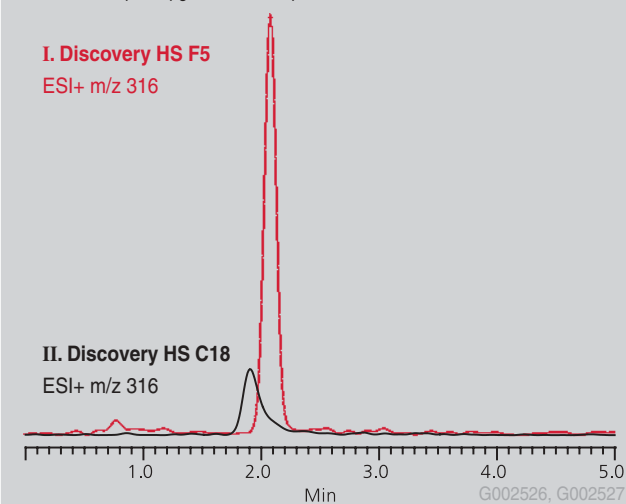
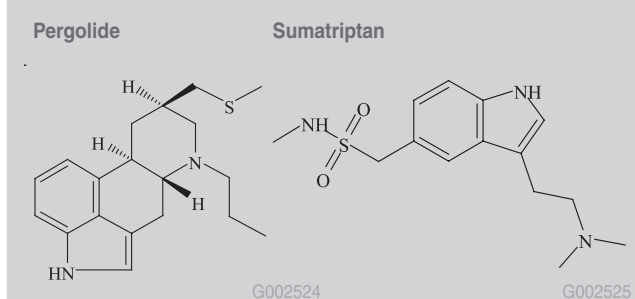


Figure A. Structures of Pergolide and Sumatriptan



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response using the fluorinated phase as compared to the C18 phase. For pergolide, a relatively hydrophobic base ( $\text{LogP} = 4.02$ ), suitable retention was obtained on the fluorinated phase using 90% acetonitrile. To achieve similar retention on the C18 phase, 45% acetonitrile was required. It is generally accepted that the increase in MS response is due to facilitated desolvation in the ESI source using the more volatile (90% acetonitrile) mobile phase. In this case, an approximately 350% increase in area response was observed using the Discovery HS F5.

Sumatriptan is a relatively hydrophilic ( $\text{LogP} = 1.05$ ) analyte and is thus poorly retained on traditional C18 stationary phases. As shown in Figure C, this analyte is well retained using the fluorinated phase at 90% acetonitrile. On the C18 phase, however, 10% acetonitrile had to be used to obtain similar retention. The high water content of the C18 mobile phase results in poor MS sensitivity. An increase in area response of approximately 600% is observed in this case.

In addition to the LC-MS sensitivity advantage, the chromatograms in Figures B and C show excellent peak shapes for the basic analytes. The use of short analytical columns also provides for fast run times making this approach suitable for high-throughput analyses.

## Conclusions

The retention of basic molecules is often enhanced relative to alkyl phases using fluorinated stationary phases. This retention is due to alternative mechanisms of interaction not available from alkyl stationary phases. In this study the retention of pergolide and sumatriptan using mobile phases rich in organic modifier has been demonstrated on Discovery HS F5. Under these conditions, MS response is shown to significantly improve due to the relatively volatile mobile phases. The increase in sensitivity is especially apparent for the hydrophilic analyte, sumatriptan, which is poorly retained on traditional C18 stationary phases. Excellent peak shapes and fast analysis times make this approach suitable for sensitive high-throughput applications.

## References

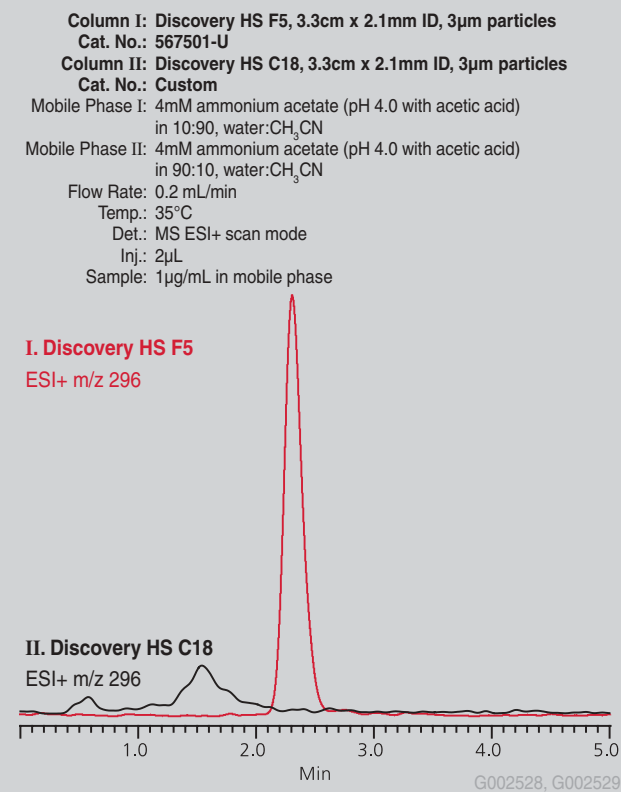
1. S.R. Needham, P.R. Brown, K. Duff, D. Bell, J. Chromatogr. A 869 (2000) 159.



## Related Information

For more information request *Discovery LC-MS Columns*, T404073 (HBL) and *CHROMASOLV LC-MS Solvents*, T304179 (GND), or visit [sigma-aldrich.com/discovery-lcms](http://sigma-aldrich.com/discovery-lcms) for a full listing of Discovery LC-MS literature and applications.

Figure C. LC-MS Analysis of Sumatriptan on (I) Discovery HS F5 and (II) Discovery HS C18



## Related Products

## Discovery LC-MS Columns

Phase	ID (mm)	Length (cm)	Cat. No.
5 $\mu$ m Discovery C8 Columns	2.1	2	577501-U
	2.1	3	577502-U
	3.0	2	577503-U
	3.0	3	577504-U
	4.6	2	577505-U
	4.6	3	577506-U
5 $\mu$ m Discovery C18 Columns	2.1	2	577507-U
	2.1	3	577508-U
	3.0	2	577509-U
	3.0	3	577510-U
	4.6	2	577511-U
	4.6	3	577512-U
5 $\mu$ m Discovery Cyano Columns	2.1	2	577513-U
	2.1	3	577514-U
	3.0	2	577515-U
	3.0	3	577516-U
	4.6	2	577517-U
	4.6	3	577518-U
<b>NEW!</b> 3 $\mu$ m Discovery HS F5 Columns	2.1	3.3	567501-U
	3.0	3.3	567505-U
	4.6	3.3	567509-U

For a complete listing of all Sigma-Aldrich products, log on to our website: [sigma-aldrich.com](http://sigma-aldrich.com)