Application Note 20513

HPLC-UV Method for the Determination of Alkaloids Using a Syncronis aQ Column

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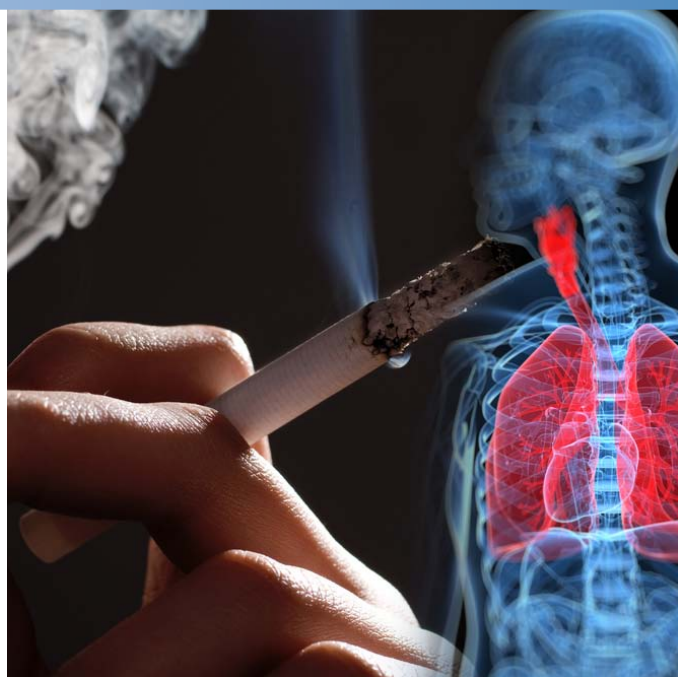
Key Words
Nicotine, anabasine, cotinine, syncronis aQ column, stimulants in mammals, alkaloid

Abstract
This application note demonstrates the use of the Thermo Scientific Syncronis aQ Column for the determination of alkaloids by HPLC-UV.



Introduction
The sensitive and specific detection of nicotine, its metabolites and the tobacco alkaloid anabasine is useful in evaluating the success of smoking cessation treatments and detecting tobacco use, passive exposure and nontobacco nicotine exposure in potential transplant and elective surgical patients.

One of the key goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained. The Syncronis™ column range has been engineered to provide exceptional reproducibility due to its highly pure, high surface area silica, dense bonding and double endcapping, all controlled and characterized through the use of rigorous testing.

This application note demonstrates the successful retention and separation of nicotine, anabasine and cotinine using Syncronis aQ 5 µm column. The polar endcapping used in Syncronis aQ provides a controlled mechanism for retention of these polar compounds.

Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade ammonium acetate	A/3446/50
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Alkaloids purchased from Sigma Aldrich	

Sample Handling Equipment	Part Number
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

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Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela UHPLC system	
Column:	Syncronis aQ 5 µm, 150 x 2.1 mm	97305-152130
Mobile phase:	90:10 (v/v) 20 mM ammonium acetate/acetonitrile	
Flow rate:	1.0 mL/min	
Column temperature:	40 °C	
Injection details:	2 µL partial loop	
Injection wash solvent:	90:10 (v/v) 20 mM ammonium acetate/acetonitrile	
UV detector wavelength:	260 nm	
Backpressure:	Approximately 220 bar	

Solutions
Working standard contained 100 µg/mL of nicotine, anabasine and cotinine in mobile phase.

Results

The analysis was performed on a Syncronis aQ 5 µm, 150 x 2.1 mm column. As shown in Figure 1, nicotine, anabasine and cotinine were analyzed in less than 5 minutes. Table 1 shows the results from six replicate injections.

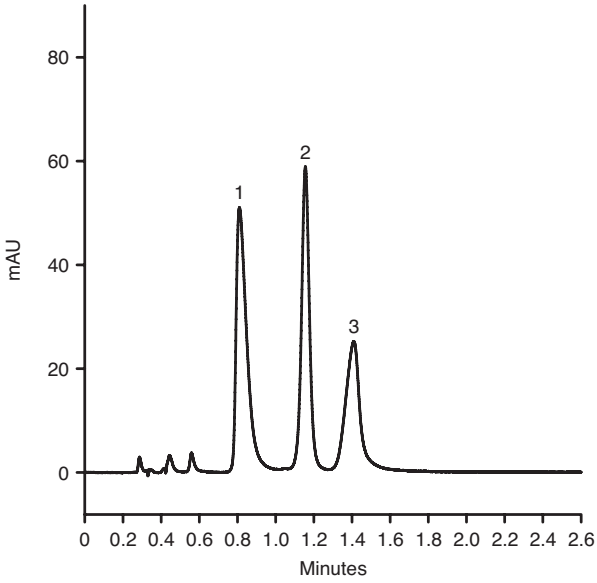

Figure 1: Chromatogram of alkaloids analyzed using a Syncronis aQ 5 µm, 150 x 2.1 mm column 1. Anabasine 2. Cotinine 3. Nicotine

	Anabasine	Cotinine	Nicotine
Retention time (minutes)	0.81	1.16	1.41
%RSD on retention time	0.00	0.35	0.39
Area	197049	169593	130437
%RSD on area	0.41	0.23	0.51

Table 1: Retention time and area results for anabasine, cotinine and nicotine

Conclusion

Replicate injections of alkaloids showed that Syncronis aQ produced stable and reproducible results. This demonstrates that Syncronis aQ is an excellent choice of column for the rapid analysis of these nicotine related alkaloids.

References

Specific detection of Anabasine, Nicotine, and Nicotine Metabolites in Urine by Liquid Chromatography-Tandem Mass Spectrometry.

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