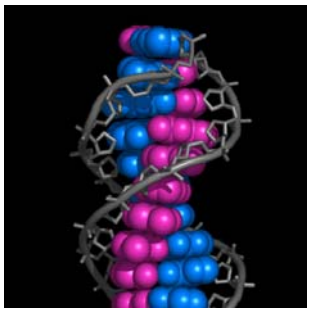
KNAUER

Application Note

► Online UHPLC-MS with liquid beam laser desorption for the quantitative determination of bio- and organic molecules

Category	bioanalytical, environment, quality monitoring
Matrix	aqueous phase
Method	Online UHPLC-MS
Keywords	Online UHPLC, bioanalytics, environment
Analytes	nucleotides and bases
ID	**VBS3**, 05/10



Summary

Do you really want to know which substances could be hidden behind your HPLC peaks? In this application we show the power of UHPLC, coupled online to high performance time-of-flight mass spectrometry (TOF MS). The TOF MS is equipped with a liquid beam laser desorption source.[1] Here we show the separation of nucleosides and bases in aqueous solution. The linearity of the detection and buffer tolerance is very promising and competitive.[1] The present liquid phase MS detection in combination with KNAUER's ultra high performance PLATINblue technology allows powerful separation and safe quantification of complex mixtures in environmental science, bioanalytics, and quality monitoring. The advantages are easy sample preparation, better detection limits, and competitive performance when compared with other MS techniques.

Introduction

UHPLC is a powerful established method for the efficient separation of complex samples. Further separation power and sensitivity is achieved if UHPLC is coupled to mass spectrometry (MS). Liquid beam laser desorption MS is a relatively new technology providing an alternative to classical electrospray- and MALDI-MS. It allows for online coupling of MS and UHPLC with good linearity over several orders of magnitude of concentrations. This is relevant for quantitative measurements, low matrix interference and improved salt and buffer tolerance.[1]

The technology is ready to address your most challenging separation problems. At KNAUER's laboratories numerous separation problems in the field of bioanalytics, environmental sciences, and quality control in production lines have been addressed. Here we show a few results for the separation of a mixture of nucleosides and bases.

Experimental: Sample preparation

The preparation of the standard solution for the present application is quite easy. When all material is dissolved, the solution is ready to use. In the experiment only diluted stock solutions have been employed.

UHPLC method parameters

Column	100 x 2 mm Blue Orchid PFP 1.8 µm
Eluent A	20 mM NHAC pH 3.5
Eluent B	A/MeOH 90:10 (v/v)
Gradient	isocratic 90% A / 10% B
Column temperature	30 °C
System pressure	approx. 500 bar
Ionization source	Liquid phase laser desorption ionization[1]
Detection	Waters Micromass Q-TOF2 MS
Flow rate	500 µl/min
Injection volume	10 µl

MS method parameters and sample

U_{cone}	25 V
U_{MCP}	2200 V
$T_{source\ block}$	80°C
$Q_{cone\ gas}$	< 50 L/hour
t_{scan}	1 s
f_{laser}	20 Hz
E_{laser}	1 mJ
λ_{laser}	2900 nm

	M_w g/mol	Conc. ng/µL	Col.-load ng
Cytosine	111.10	6.13	61.3
Uracil	112.09	6.40	64.0
Thymine	126.04	6.93	69.3
Adenine	135.13	6.20	62.0
Guanine	151.13	6.47	64.7
Thymidine	242.23	-	-
Cytidine	243.22	-	-
Uridine	244.20	6.60	66.0
Adenosine	267.24	6.13	61.3
Guanosine	283.23	6.40	64.0

Results

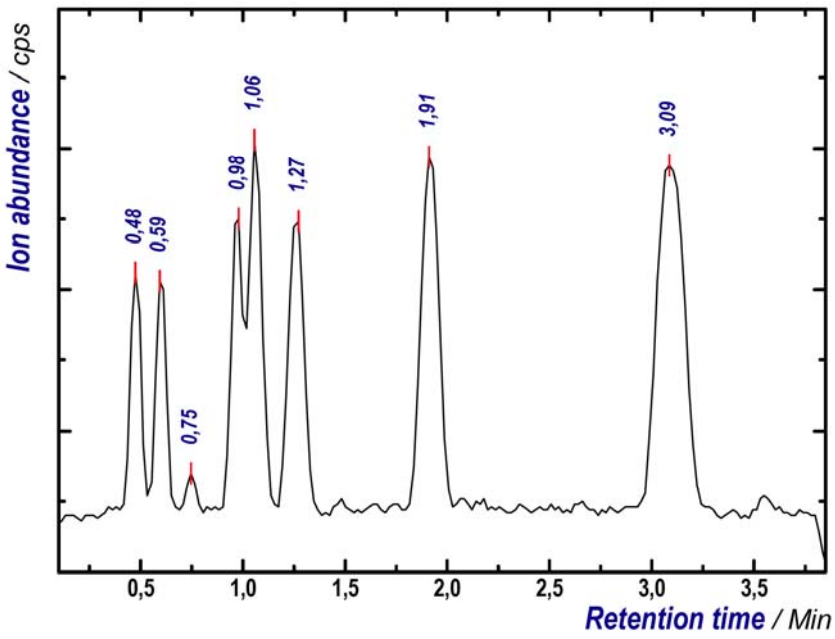


Figure 1
Mass chromatogram:
Total ion current (base peak ion chromatogram) as a function of time

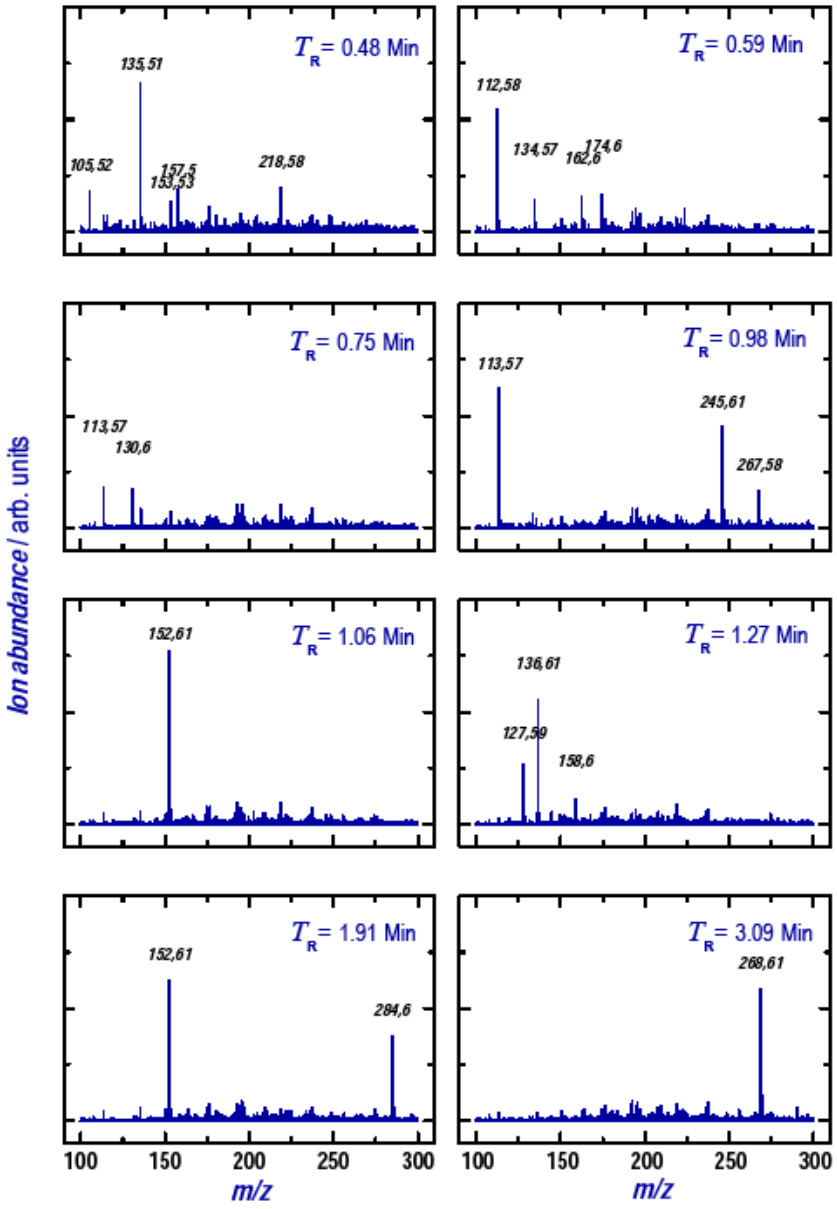


Figure 2
Mass chromatogram: Mass spectra corresponding to the maximum of the single elution peaks

The figures 1 and 2 show preliminary data for the separation of the nucleoside standards by UHPLC followed by Q-TOF MS detection. The ionization of the nucleoside standard mixture by liquid beam laser in highly aqueous solution allows identification of all 8 nucleoside standards (figures 1 and 2). Poor separation of two nucleosides, uridine and guanine at a retention time of 0.98 min and 1.06 min, respectively, as well as co-elution of some nucleosides (thymine and adenine at a retention time of 1.27 min) could be overcome by using longer UHPLC columns. In opposite to ionization by liquid beam laser the use of a conventional electrospray ionization method (ESI) for the ionization of the nucleoside standard mixture in highly aqueous solution resulted in lower signal intensity and detection of only five to six nucleosides (data not shown).

Method performance

Limit of detection	~ 10 ng/µl (not optimized here)
MS linearity range	several orders of magnitude [1]

For evaluation of the analytical results we recommend comparison with KNAUER's detector performance (PDA-1, 210 nm, and 10mm 2 µl flow cell) and measurements with a standard electrospray source.

Conclusion

In the present application we showed the first successful combination of liquid phase laser desorption MS detection with the KNAUER PLATINblue UHPLC technology. The ionization of the nucleoside and bases mixture in a highly aqueous solution by liquid beam laser desorption ionization allows better separation and detection in comparison with the conventional electrospray ionization on the bases of different MS platforms (data not shown). The presented data set shows a potential for the powerful separation and safe quantification of complex mixtures with competitive performance when compared with other MS techniques.

Reference

1. E. Rapp, A. Charvat, A. Beinsen, U. Plessmann, U. Reichl, A. Seidel-Morgenstern, H. Urlaub and B. Abel, *Atmospheric Pressure Free Liquid Infrared MALDI Mass Spectrometry: Toward a combined ESI/MALDI-Liquid Chromatography Interface*, Anal. Chem., 81, 443–452 (2009).

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Physical properties of recommended column

BlueOrchid PFP is an endcapped phase for alternative C18 selectivity especially developed for the analysis of fluorinated or other halogenated polar compounds with π – double bonds like halogenated phenols, taxanes and polar compounds with hydroxyl, carbonyl, nitro or other polar groups. Due to the narrow particle size distribution, the column back pressure of all BlueOrchid columns is lower than other high speed column materials on the market.

Stationary phase	BlueOrchid PFP
USP code	L43
Particle size	1.8 µm
Form	spherical
% C	8
Endcapping	yes
Dimensions	100 x 2 mm
Order number	10BI057BOE

Recommended instrumentation

This application requires a PLATINblue UHPLC system and a high performance mass spectrometer (e.g., Q-TOF) equipped with a liquid beam desorption source. Other configurations are also available. Please contact KNAUER to configure a system that is perfect for your needs.

Description	Order No.
PLATINblue HPG system	A69400MS
PLATINblue Pump P-1, incl. 5ml pump head	
PLATINblue Pump-P1, incl. 5ml pump head and degasser	
HPG SmartMix 100	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1	
PLATINblue stainless steel capillary kit	
PLATINblue UHPLC method converter	

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