Product information

YMC Europe GmbH

| YMC-Triart C18 | Allergen Detection in Wine with | Author: DE |
| Capillary Column | YMC-Triart C18 Capillary Column | Date: 08/23/2013 |

Introduction

The final steps in wine production require any cloudiness to be eliminated. For this, fining agents such as casein are stirred into wine barrels, where it forms agglomerates with the sediments and deposits them at the barrel bottom. After clarification the wine is racked to separate the sediment residue.

In response to a wine survey, where casein was found in trace amounts (<2 ppm), the European Food Safety Authority (EFSA) concluded, in 2011, that wine fined with casein, caseinate or milk products can cause adverse reactions in sensitive individuals [1]. In addition, a new EU legislation (concerning labelling) [2] pointed out that, if fining reagents such as casein, egg ovalbumin, etc are used in processing, methods for detection of these products in wine are needed.

A YMC-Triart C18 capillary column has been used in conjunction with the new microflow LC/MS/MS system to develop a method for the detection of casein in wine at sub ppm levels by AB SCIEX [3].

Results

The initial results for column selection show a typical sensitivity increase of between 4 and 12 fold in S/N-ratio when switching from high to micro flow. Figure 1 shows the separation of two peptides which were analysed by micro LC and compared to the separation at higher flow rates. The results clearly demonstrate an improvement in sensitivity when moving to micro LC which is not lost when the analyse time is further shortened to a runtime of 5.5 min to speed up the analysis (shortened run time gradient details: see table 2, page 3).
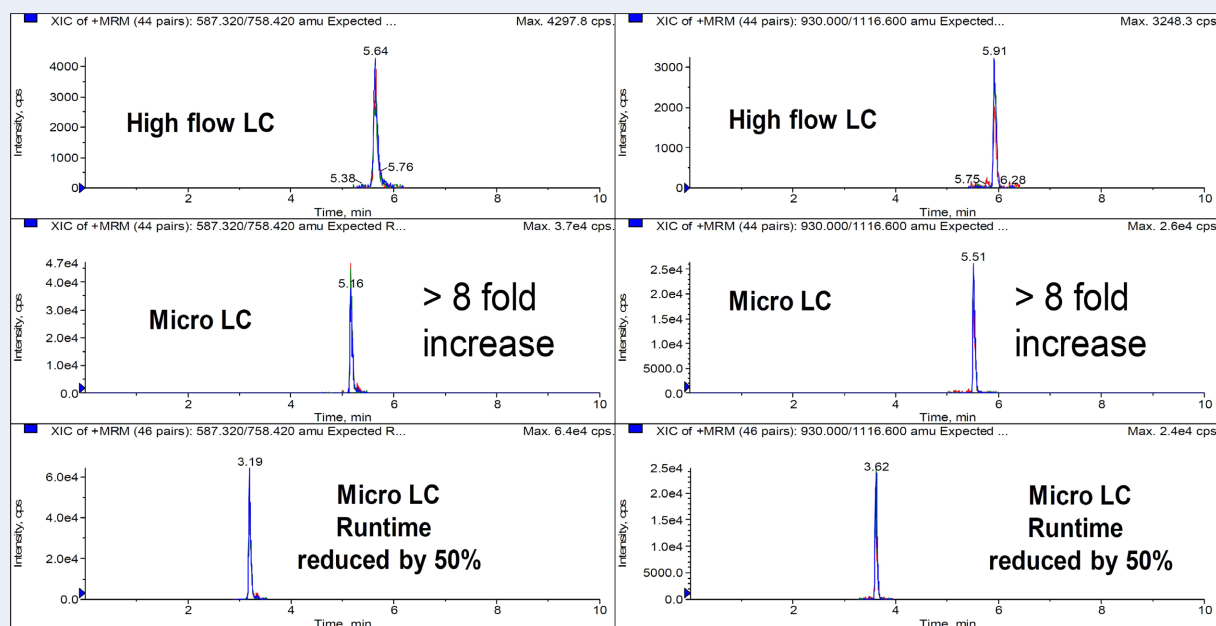
Figure 1: Comparison of high flow vs. micro LC using a white wine spiked with 1 ppm casein peptide (left) and an egg peptide (right).

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Allergen Detection in Wine with YMC-Triart C18 Capillary Column

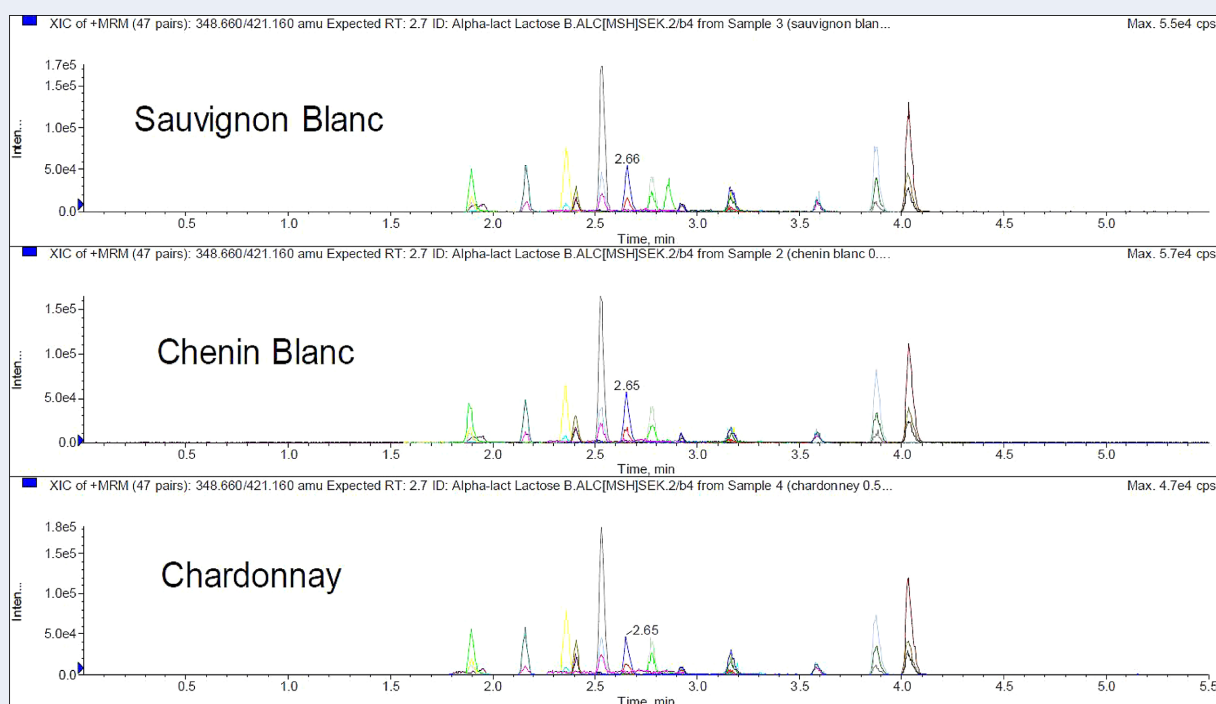**Author: DE**

Date: 08/23/2013

	High Flow	Micro Flow	
Column	Phenomenex Kinetex XB-C18 (2.6 µm, 10 nm, 50 x 2.1 mm ID)	YMC-Triart C18 (3 µm, 12 nm, 50 x 0.5 mm ID)	
HPLC	Shimadzu XP	Eksigent ekspert microLC 200	
Eluent	A) water (+ 0.1% FAC) B) acetonitrile (+ 0.1% FAC)		
Gradient	min A B 0 – 2 98 2 8 40 60 8.2 – 9 5 95 9.1 – 10 98 2		
Flow rate	300 µl/min	25 µl/min	
Temperature	40 °C		
Detection	AB SCIEX 5500 QTRAP, ESI		
Injection	10 µl		

Table 1: High flow and micro flow conditions for evaluation of casein and egg peptide.

The micro LC analysis was carried out using a further two wines and the results obtained showed the same sensitivity. Furthermore, it was possible to detect and identify several milk and egg proteins in one run (figure 2).

Figure 2: Micro LC-MS/MS analysis of 3 different white wines spiked with 0.5 ppm samples of milk and egg proteins.

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Column	YMC-Triart C18 (3 µm, 12 nm, 50 x 0.5 mm ID)		
Eluent	A) water (+ 0.1% FAC) B) acetonitrile (+ 0.1% FAC)		
Gradient	min	A	B
	0 – 0.3	98	2
	4	60	40
	4.1 – 4.3	5	95
	4.4 – 5.5	98	2
Flow rate	25 µl/min		
Temperature	40 °C		
Detection	AB SCIEX 5500 QTRAP, ESI		
Injection	10 µl		

Table 2: Shortened Micro LC-MS/MS method.

Linearity and sensitivity of this method is demonstrated in figure 3. A casein peptide is spiked into a Sauvignon Blanc (0.05 – 2 ppm). Linearity is provided without use of any internal standards. The inset chromatogram for 50 ppb spiked sample demonstrates highest sensitivity.

Calibration for milk protein 1 4_1: y = 4.65938e5 x + 6792.68001 (r = 0.99851) (weighting: None)
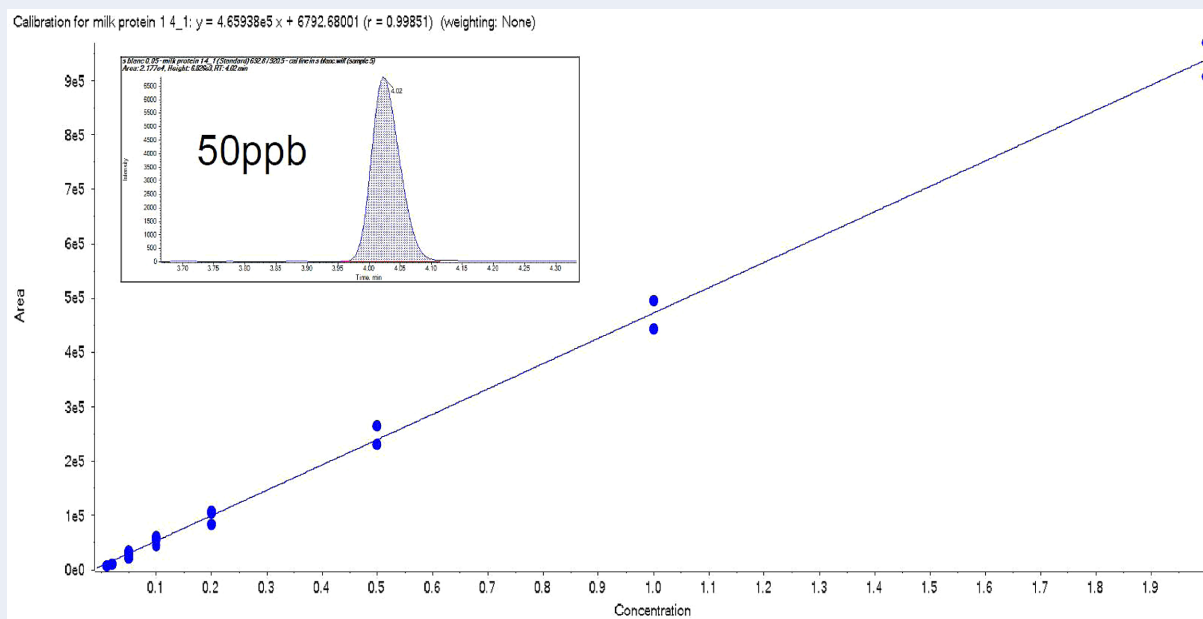
Figure 3: Calibration line from a peptide from casein, spiked into Sauvignon Blanc and chromatogram of 50 ppb spiked sample.

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Conclusions

Various milk and egg markers can be detected simultaneously in white wine using a YMC-Triart C18 capillary column in a micro LC-MS/MS system to allow reduction of the detection limit below 100 ppb, thereby meeting the requirements of detecting trace amounts.

Furthermore, analyse time can be halved with an increase in sensitivity by 10 whilst simultaneously reducing solvent consumption through the use of micro LC.

The LC-MS/MS approach has the additional advantage of being a potential multi allergen screen where different allergens, such as egg and milk, can be detected by a single method.

References

[1] Scientific Opinion related to a notification from the International Organization of Vine and Wine on casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids pursuant to Article 6, paragraph 11 of Directive 200/13/EC – for permanent exemption from labelling, EFSA Journal 2011, 9(10), 2384.

[2] Commission Regulation (EU) No 1266/2010 of 22 December 2010 amending Directive 2007/68/EC as regards labelling requirements for wines, 2010.

[3] Lock S, Allergen detection in wine by LC/MS/MS, Poster presented at the 127th AOAC International 2013 Annual Meeting & Exposition in Chicago, August 25 – August 28, 2013.

Trademarks

Kinetex is a trademark of Phenomenex
ekspert is a trademark of Eksigent