

# Determination of Amino Acid Profiles of Grapes and Wines by HPLC-MS

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## Introduction

Nitrogen is an important nutrient for yeast. To ensure a good alcoholic fermentation, grape juice should contain more than 200 mg N/l in form of ammonium and primary amino acids. The amino acid part of this, so called yeast available nitrogen (YAN) can be measured by spectrophotometric method using OPA derivatization [1]. However this method give only the molar concentration of primary amines and not the real quantity of available nitrogen. To determine the real value of YAN, the amino acid profile of grape jus is also required. The goal of this work is to develop and evaluate a method for the rapid determination of this profile by HPLC-MS.



## Validation of method

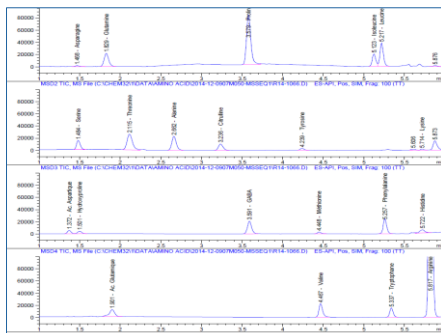
The quantification of 24 free amino acids is achieved in grape juice and wine samples using single ion monitoring (SIM) mode.

**The linearity** of the method was good for all compounds ( $R^2 > 0.996$ ) over the tested range (table 1), which corresponds to the amount of the amino acids in the jus and wine samples.

**The recovery**, determined by spiking grape juice with amino acids, were between 85% and 114%.

**The repeatability** was determined with a red wine, introduced in the sequence after each tenth injection as a control. The coefficient of variation of 25 analyses, was equal or lower than 12% over a period of 5 days.

Figure 1: Chromatogram of grape jus



## Methods

Agilent 6100 Series Quadrupole LC-MS was used throughout this study. The separation was done on a Zorbax Eclipse AAA column (150 x 3mm; 3.5µm, Agilent). The mobile phase (1.0 ml/min) was composed of water (A) and acetonitrile (B), both containing 0.5% of formic acid and 0.1% of perfluoroheptanoic acid (PFHA) as ion-pairing agent. A linear gradient is applied from 100% A (0-2 min) to 100% B (16 min).

**MS conditions:** ESI ionization in positive mode using 3500V, 12 l/min drying gas, 350°C, nebulizer pressure 40 psi.

**Samples** are diluted 20 times with the mobile phase A before injection.

Table 1: Validation results

AA	RT	SIM	Range	Recovery	CV
	[min]	Amu/z	[mg N/l]	%	%
Asp	1.36	134	0.05-1.0	114.2	9
Asn	1.45	133	0.05-1.0	-	9
Ser	1.47	106	0.09-1.8	92.4	12
Hpro	1.48	132	0.07-1.1	-	7
Gly	1.63	76	0.08-1.6	112.9	7
Glu	1.81	147	0.05-0.5	-	-
Glu	1.89	148	0.05-0.8	96.9	8
Cys	1.99	122	0.05-1.0	110.3	-
Thr	2.11	120	0.06-1.3	103.5	10
Ala	2.65	90	0.06-1.3	107.9	6
Citrulline	3.21	176	0.04-0.3	-	6
Pro	3.55	116	0.25-5.2	85.5	6
GABA	3.55	104	0.08-1.5	-	6
Tyr	4.19	182	0.04-0.8	100.7	5
Met	4.38	150	0.05-1.0	116.6	7
Val	4.44	118	0.05-1.0	109.0	6
Ileu	5.13	132	0.06-1.3	110.6	9
Leu	5.24	132	0.05-1.1	108.1	8
Phe	5.29	166	0.04-0.8	108.1	6
Cystine	5.33	241	0.03-0.7	103.9	-
Try	5.36	205	0.03-0.7	-	5
Ornithine	5.71	133	0.04-0.9	-	-
Lys	5.74	147	0.03-0.7	110.2	6
Arg	5.86	175	0.15-3.2	113.1	6

## Results

Comparing the new HPLC-MS method to the earlier used HPLC-DAD method (OPA derivatization [2]), a good correlation ( $R^2 > 0.92$ ) is observed for the total primary amino acids (PAA) contain (Fig. 2). The profiles determined by the two methods are not significantly different (Fig. 3). The HPLC-MS method allowed the quantification of two more AA: citrulline and ornithine. These amino acids play a role in the urea cycle.

Figure 2: Totale PAA contain [mg N/l] in grape jus.

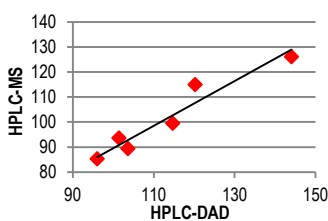


Figure 3: PAA profile of grape jus expressed in percentage of total PAA value.

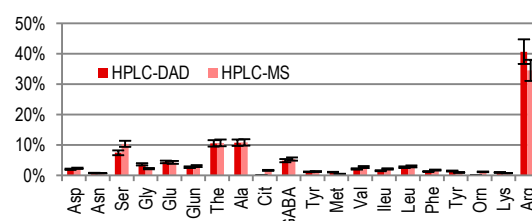
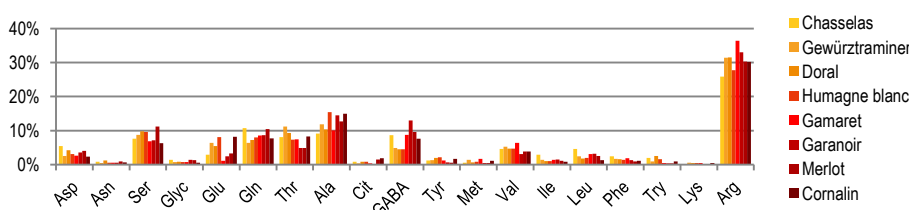


Figure 4: PAA profile of 8 grapes varieties measured by HPLC-MS, expressed in percentage of total PAA value.



## References

- Dukes, B.C., and C.E. Butzke. Am. J. Enol. Vitic. 49: 125-134 (1998)
- Agilent ZORBAX Eclipse AAA Instruction for Use (Agilent Technologie)

## Conclusion

This HPLC-MS method allows a rapid and reliable determination of amino acid profiles in grapes and wines. The comparison of the amino acid profiles of grape varieties cultivated in Switzerland is currently carried out to find explication for atypical behavior of some varieties during the fermentation.