Gas-Diffusion Sequential Injection Enzymatic Determination of Ethanol in Wines: Study of Matrix Effects Derived From Direct Sample Introduction

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DETERMINATION OF ETHANOL

Determination of alcoholic degree

Precision of reference methods [1]:

on wine distillate : 0.04 -0.6% (v/v)

on non-distilled wine: 0.2% (v/v)

Fermentation monitoring

Precision of methods [2]:

on non-distilled wine: ≈ 2 % (v/v)

Enzymatic determination of ethanol

Alcohol dehydrogenase (ADH)

$$CH_3CH_2OH + NAD^+ \overset{ADH}{\leftrightarrow} CH_3CHO + NADH + H^+$$

- ☑ Higher stability
- ☑ Better selectivity
- ☒ Need of cofactor

Detection method
- •spectrophotometric
- •fluorimetric
- •amperometric

Alcohol oxidase (AOD)

$$CH_3CH_2OH + O_2 \overset{AOD}{\leftrightarrow} CH_3CHO + H_2O_2$$

- ☒ Lower stability
- ☑ Cofactor bounded to the enzyme

Detection method
- •chemiluminometric
- •amperometric
- •spectrophotometric

DEVELOPED SYSTEM

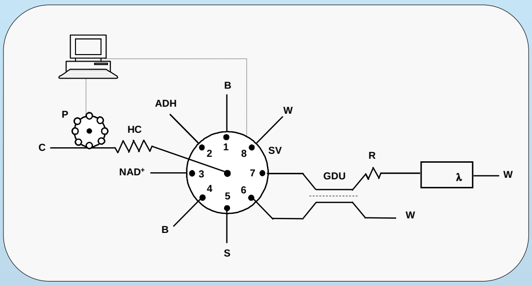

Figure 1. Sequential injection manifold for the enzymatic determination of ethanol in beverages, P, peristaltic pump; C, carrier, H_2O; B, buffer, pH 10; S, sample; R, reactor; HC, holding coil; SV, selection valve; ADH, alcohol dehydrogenase enzyme solution; GDU, gas diffusion unit;; λ, spectrophotometer, 340 nm; W, waste.

Flow protocol sequence

	SV position	Time (s)	Volume (µL)
Aspirate buffer solution	1	6	100
Aspirate enzyme solution	2	1.5	25
Aspirate NAD solution	3	1.5	25
Aspirate the buffer solution	4	6	100
Flow reversal and propel to GDU	7	12	192
Washing of HC	8	10	200
Aspirate the sample	5	2	30
Flow reversal and propelling sample through the donor channel of GDU	6	50	800
Propelling the reaction zone towards detection	7	80	2200

Analytical characteristics

Parameter	Value
Application zone	0-25% (v/v)
Repeatability RSD (n=10)	3.43% (0.5% (v/v)) 0.89% (12.5% (v/v))
LOD	0.2% (v/v)
LOQ	0.3% (v/v)
Determination rate	21 h^{-1}
Reagent consumption	
ADH	0.45 U/assay
NAD^+	1.3 mg/assay
Sample	32 µL/assay
Waste produced	3.4 mL/assay

ANALYSIS OF WINE SAMPLES

Results obtained in the analysis of certified reference materials

Samples	Concentration, % (v/v) Certified	Concentration, % (v/v) Found	RD, %	Recovery %
LGC 5405	15.04 ± 0.05	15.33 ± 0,07	2.0	101.9
BCR 613	0.539 ± 0.0095	0.576 ± 0,020	6.9	106.9

Results obtained in the analysis of wine samples of different origin

Samples	Concentration of ethanol, %(v/v) Ref. met.	SIA*	Dilution	R.D., %
White table wine	10.5 ± 0.1	10.3 ± 0.1	No	-1.9
White table wine	11.0 ± 0.1	11.1 ± 0.3	No	0.9
Red table wine	12.0 ± 0.1	10.9 ± 0.4	No	-9.2
Red table wine	12.5 ± 0.1	12.6 ± 0.3	No	0.8
White table wine	12.5 ± 0.1	12.5 ± 0.1	No	0
Red table wine	12.5 ± 0.1	12.5 ± 0.1	No	0
Port wine, White	19.0 ± 0.1	19.1 ± 0.5	3.5	0.5
Port wine, Tawny	20.2 ± 0.1	20.6 ± 0.4	3.5	2.0
Port wine, White	19.2 ± 0.1	19.1 ± 0.3	3.5	-0.5
Port wine, Ruby	19.0 ± 0.1	20.8 ± 0.5	3.5	9.5

* Mean and standard deviation (n=3); R.D., Relative deviation

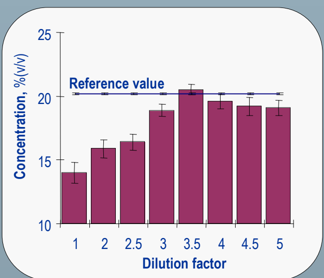

Figure 2. Effect of the dilution on the apparent concentration of a port wine sample.

Conclusions

❶ The complex matrix affects the gas diffusion process.

❷ The difficulties can be overcome by appropriate sample dilution.

❸ Achieved precision is more adequate for a screening method or fermentation monitoring.

References

[1] OIV, Recueil des methodes internationales d' analyse des vins et des mouts, *OIV* (2005).

[2] M. D. Luque de Castro, J. González-Rodríguez, P. Pérez-Juan, *Food Reviews International*, 21:231-265, 2005.

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