

# Miniaturization of enzymatic assay based on the LOV-sequential injection system: application to the determination of ethanol in alcoholic beverages



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A LOV system was developed for the enzymatic assay of ethanol in beverages, based on the conversion of ethanol to acetaldehyde by alcohol dehydrogenase. The quantification of the analyte was based on two different approaches, peak height and initial rate measurement.

## Flow Manifold

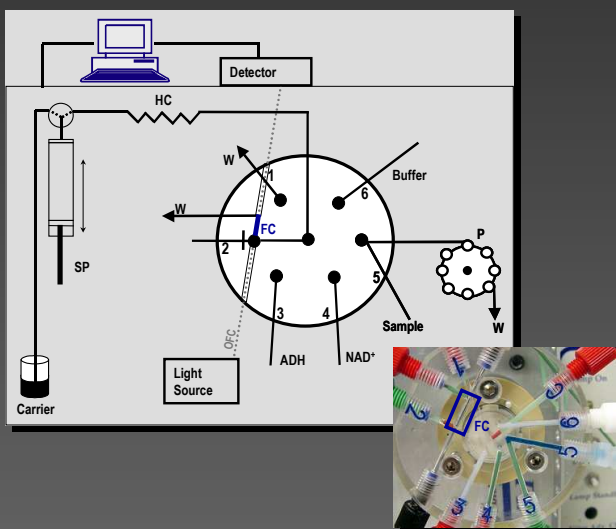
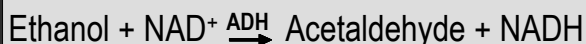


Fig. 1- SI-LOV manifold for the determination of ethanol; Carrier, water; SP, syringe pump (2.5 mL); HC, Holding coil; FC, Flow cell; OFC, optical fibre cable; Detector, Diode array spectrophotometer; ADH, Alcohol dehydrogenase; NAD<sup>+</sup>, cofactor; Buffer, phosphate buffer pH 9.5; W, waste.



## Flow Protocol Sequence

Description	Volume (μL)	Flow rate (μL/s)	Port
Aspirate carrier to HC	1000	100	-
Aspirate buffer to HC	50	80	6
Aspirate sample to HC	15	25	5
Aspirate enzyme (ADH) to HC	5	25	3
Aspirate cofactor (NAD <sup>+</sup> ) to HC	5	25	4
Aspirate buffer to HC	100	25	6
Initial rate measurement			
Dispense portion of stacked zones	100	15	2
Stop period (15 s), data acquisition	-	-	-
System washing, SP empty	-	100	2
Peak height measurement			
Reverse flow, reference scan	10	15	2
Stop period (30 s)	-	-	-
Dispense HC content, data acquisition	450	15	2
System washing, SP empty	-	100	2

## Figures of merit for initial rate and peak height measurement

Parameter	Initial rate measurement	Peak height measurement
Reagent consumption per assay		
Enzyme (ADH)	0.12 U	0.12 U
Cofactor (NAD <sup>+</sup> )	0.066 mg	0.066 mg
Sample solution	15 μL	15 μL
Buffer	150 μL	150 μL
Waste production per assay		
	1.2 mL	1.2 mL
Application range	Up to 0.04% (v/v)	Up to 0.04% (v/v)
Determination rate	37 h <sup>-1</sup>	27 h <sup>-1</sup>
LOD	0.004% (v/v)	0.003% (v/v)
LOQ	0.01% (v/v)	0.009% (v/v)
Repeatability (RSD)	< 5.0%	< 1.0%

## Application to beverage samples

Sample	Red table wine <sup>a</sup>	Red table wine <sup>a</sup>	Red table wine <sup>a</sup>	White table wine <sup>a</sup>	Beer 1 <sup>b</sup>	Beer 2 <sup>b</sup>	Beer 3 <sup>b</sup>	Spirit 1 <sup>c</sup>	Spirit 2 <sup>c</sup>	Spirit 3 <sup>c</sup>	Certified CRM 653 <sup>d</sup>
Ref. Meth.	9.3 (± 0.1)	12.4 (± 0.1)	11.1 (± 0.1)	10.2 (± 0.1)	5.2 (± 0.1)	4.6 (± 0.1)	4.7 (± 0.1)	36.7 (± 0.1)	36.6 (± 0.1)	39.2 (± 0.1)	0.539 (± 0.0095)
% ethanol v/v											
Initial rate meas.	9.7 (± 0.6)	12.3 (± 0.2)	10.4 (± 2.9)	10.4 (± 0.1)	5.1 (± 1.2)	4.8 (± 0.5)	4.7 (± 0.2)	36.7 (± 3.7)	36.3 (± 2.9)	39.8 (± 2.0)	0.548 (± 0.026)
% ethanol v/v											
Peak height meas.	9.1 (± 0.1)	12.3 (± 0.9)	11.1 (± 0.1)	10.4 (± 0.1)	-	-	-	-	-	-	0.537 (± 0.028)
% ethanol v/v											

<sup>a</sup> dilution factor 350; <sup>b</sup> dilution factor 400; <sup>c</sup> dilution factor 2500; <sup>d</sup> dilution factor 20.

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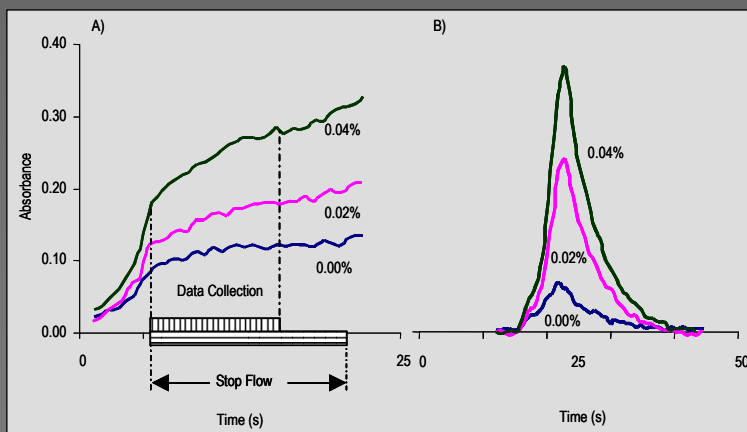


Fig. 2- Variation of the absorbance with the increase of concentration of ethanol by (A) initial rate and (B) peak height measurements.