Direct estimation of ethanol as a negative peak from alcoholic beverages and fermentation broths by reversed phase-HPLC

M.Nisha[a], Mukund Shankar[b], Nagasathiya Krishnan[c], Lilly M. Saleena[a], Mathur Rajesh[b], M. Vairamani[*]

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[a]Department of Bioinformatics, School of Bioengineering, SRM University, Kattankulathur, Tamil Nadu, India.

[b]Department of Chemical Engineering, School of Bioengineering, SRM University, Kattankulathur, Tamil Nadu, India.

[c]Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur ,Tamil Nadu, India.

*Corresponding author, School of Bioengineering, SRM University, Kattankulathur-603203, Kancheepuram District, Tamil Nadu, India. Phone: +91-44-27417812, dean.biotech@ktr.srmuniv.ac.in.

Standard methanol quantification and analysis

Standard methanol in water were prepared from 0.1 to 100 % (v/v) and serially injected with the protocol followed for ethanol quantification. Analysis of methanol in ethanol was attempted with three approaches involving ternary mixtures of the two concerned alcohols in water. Approach 1 was performed with 5% alcohol mixtures varying in proportions of 0-5, 0.5-4.5, 1.25-3.75, 2.5-2.5, 3.75-1.25, 5-0 % (v/v) of ethanol-methanol and remaining 95% was water. Approach 2 was performed with 1:1 percentage ratio of ethanol-methanol mixtures with remaining volume made up with water. Approach 3 involved solvent mixtures of 95-5, 90-10, 75-25, 50-50, 40-60, 30-70, 25-75 % (v/v) of absolute ethanol-methanol respectively. A negative peak at 3.11 ± 0.04 min and a positive peak at 2.77 ± 0.01min were observed across all standard methanol concentrations and varied proportionately. The negative peak was similar to the water peak as observed in ethanol analysis. Both positive and negative peaks gave R^2 of 0.99.

Table S.1 Summary of methanol peak trend.

Peak polarities	Positive	Positive	Negative	Negative
Retention time (min)	2.77 ± 0.01	3.09 ± 0.05	3.11 ± 0.04	3.46 ± 0.07
Approach 1				
5M : 0E : 95W	*	-	*	-
4.9M : 0.1E : 95W	*	-	*	*
4.5M : 0.5E : 95W	*	-	*	*
3.75M : 1.25E : 95W	*	-	*	*
2.5M : 2.5E : 95W	*	-	*	*
1.25M : 3.75E : 95W	*	-	*	*
0M : 5E : 95W	-	*	-	*
Approach 2				
50M : 50E : 0W	*	*	-	*
45M : 45E : 10W	*	*	-	*
40M : 40E : 20W	*	*	-	*
35M : 35E : 30W	*	*	-	*
30M : 30E : 40W	*	*	-	*
25M : 25E : 50W	*	*	-	*
20M : 20E : 60W	*	*	-	*
10M : 10E : 80W	*	*	-	*
5M : 5E : 90W	*	*	-	*
Approach 3				
0M : 100E : 0W	-	*	-	*
5M : 95E : 0W	*	*	-	*
10M : 90E: 0W	*	*	-	*
25M : 75E : 0W	*	*	-	*
50M : 50E: 0W	*	*	-	*
60M : 40E: 0W	*	*	-	*
70M : 30E : 0W	*	*	-	-
74M : 26E : 0W	*	*	-	-
75M : 25E : 0W	*	-	*	*
100M: 0E : 0W	*	-	*	*

(Row label axis: % Methanol : %Ethanol : %Water)

*Peak detected, - Peak not detected