

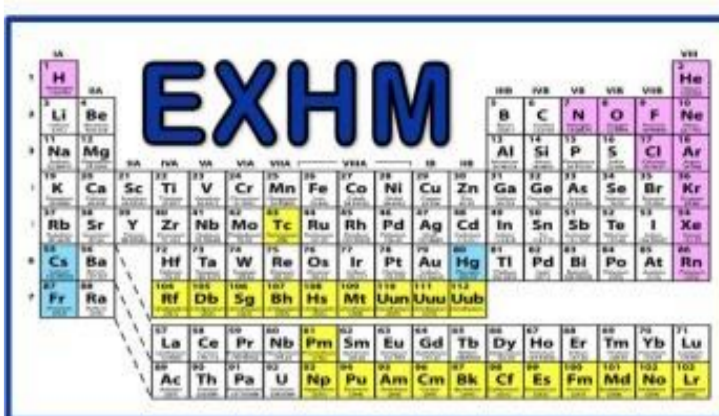
# ESTIMATION OF THE UNCERTAINTY IN SNIF-NMR MEASUREMENTS FOR THE DETERMINATION OF THE AUTHENTICITY OF WINES AND SPIRITS



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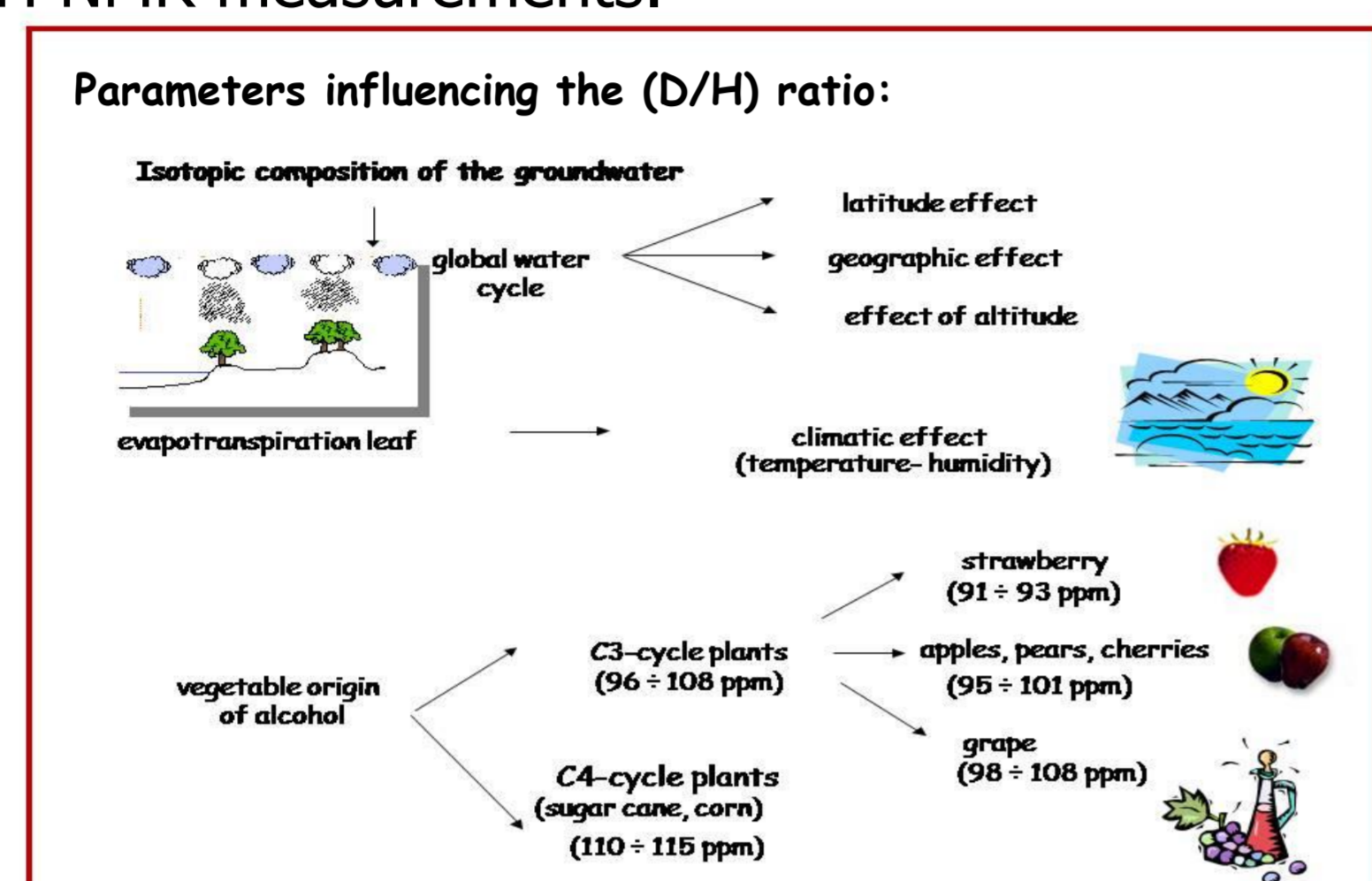
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## 1. Introduction

Stable isotope techniques provide powerful analytical tools that are regularly employed in the investigation of the origin and detection of food adulteration. The Site-specific Natural Isotope Fractionation - Nuclear Magnetic Resonance (SNIF-NMR) method [1] is the reference method for the determination of the origin of ethanol in wines (European Reg. 555/2008). The whole procedure has been officially endorsed by European Legislation and can be broken down into three basic stages: the condensation of the products to the preparation of a high-degree alcoholic distillate, the determination of its water content by the means of coulometric Karl Fisher titration, and finally the calculation of the deuterium ratios of the methyl (D/H)I and methylene (D/H)II groups of the ethanol in the distillate by Site-Specific Natural Isotope Fractionation Nuclear Magnetic Resonance Spectroscopy (SNIF-NMR). The purpose of the present communication is to reveal the factors affecting the uncertainty of the 2H NMR measurements.



## 2. Experimental procedure

ADCS: A homogeneous sample of a suitable volume V ml of the wine or the fermented product is introduced into the round bottom flask of the distillation apparatus. A ground conical flask is placed to receive the distillate. The product to be distilled is heated to obtain a constant reflux ratio at the level of the condenser. The collection of the distillate is started when a stable temperature of the vapors typical of the ethanol-water azeotrope (78 °C) is reached and stopped when the temperature increases. The collection of distillate should be continued until the ethanol water azeotrope is completely recovered.

KF titration: The weight mD of distillate collected is weighed to better than 0.1g. In order to avoid isotopic fractionation, the distillate should be kept in a tight vial preventing any evaporation until further use for determination of the alcoholic strength and preparation of the NMR tube. The water content of the distillate (p' g) can be determined by the Karl Fischer method using a sample of about 0.5 ml of alcohol of exactly known mass p' g. The alcohol strength by mass of the distillate is then given by:  
 $tm\ D\ \%\ w/w = 100\ (1-p')/p$

Yield of distillation:

The yield of distillation is estimated using the following formula:

Yield of dist.% =  $100\ tm\ DmD / (V.tv)$

Given the uncertainty on each term and especially on tv, the yield of distillation is estimated at ±0.5% (in the case of a wine of 10%v/v). When using the Cadiot column, no significant isotope fractionation effect is expected for yield of extraction higher than 96%.

NMR measurements: in a previously weighed bottle, 3.2 ml of distillate are collected and weighed to the nearest 0.1 mg; then 1.3 ml of the internal standard TMU are taken and weighed to the nearest 0.1 mg. Finally, 0.15 ml of hexafluorobenzene are collected and weighed to the nearest 0.1 mg as a field-frequency stabilization substance (lock).

## 3. Typical method performance data



sample distillation (ADCS)



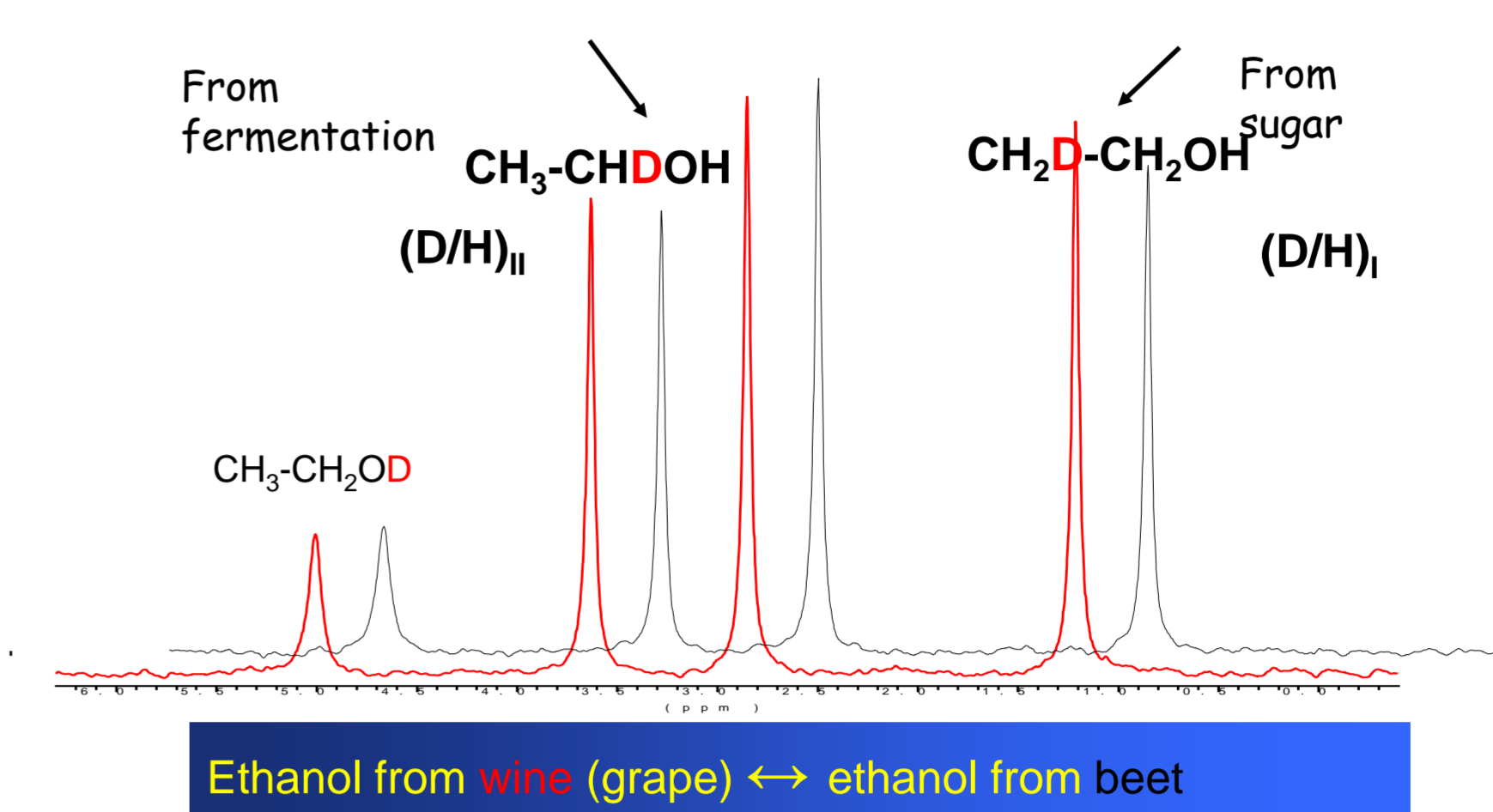
determination of ethanol content



2H NMR measurement

## 4. Results and discussion

### Typical 2H NMR Spectrum



### ANALYTICAL MODEL

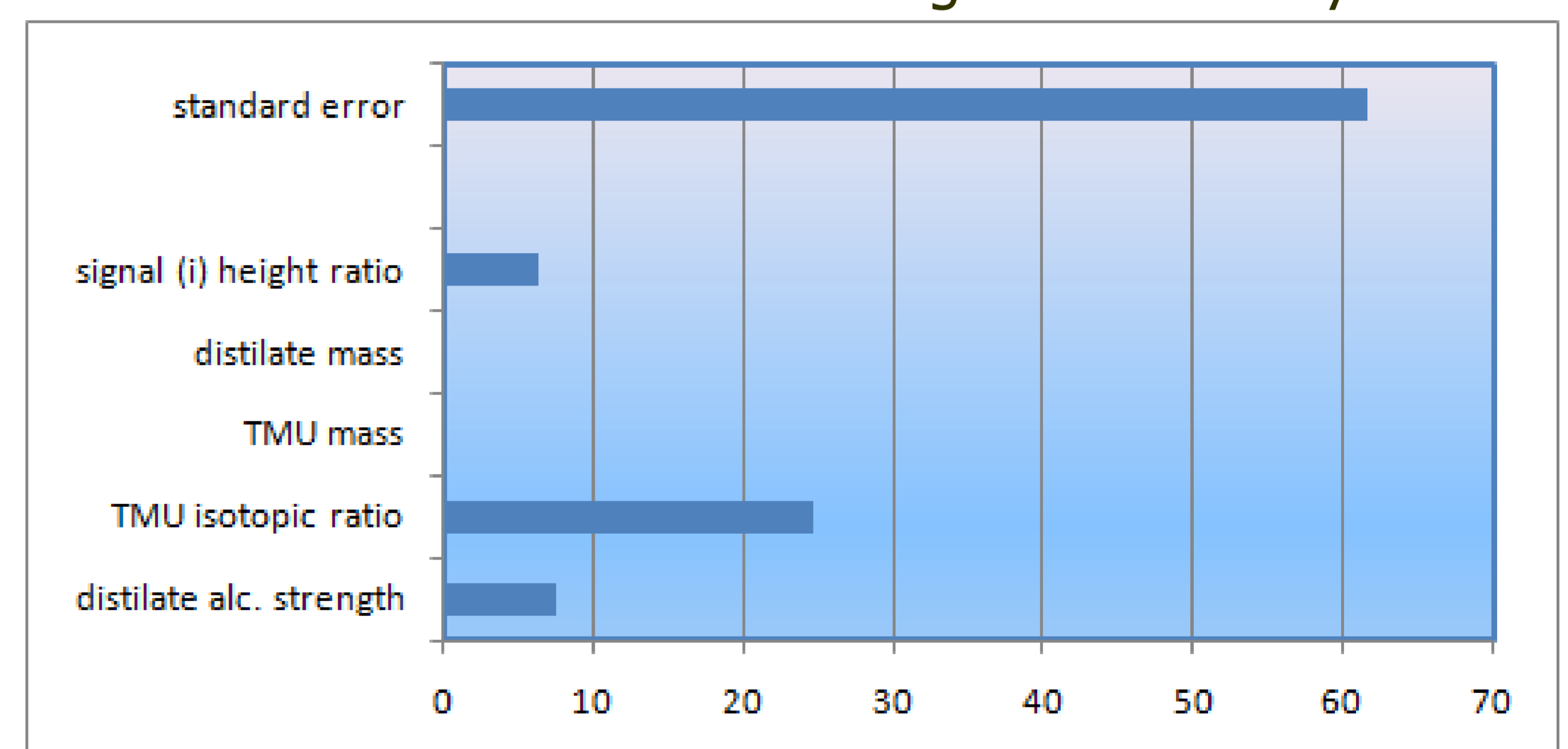
$$(D/H)_{I,II} = k_{I,II} \frac{I_{I,II\ sample}}{I_{TMU}} \frac{m_{TMU}}{m_{sample}} \frac{(D/H)_{TMU}}{t_m^D}$$

### UNCERTAINTY BUDGET

symbol	uncertainty component (xi)	value	unit	standard unc. ( $\Delta x_i$ )	relative unc. ( $\epsilon_{x_i}$ )	sensitivity coeff. (Ci)	$C_i \Delta x_i$	$(C_i \Delta x_i)^2$	relative contr.	degrees of freedom
	standard error	105,49		0,5288	0,0050	1,0000	0,5288	0,2796	61,48	9
$I_{sample}/I_{TMU}$	ratio of signal (i) height	88,64	-	0,1429	0,0016	1,190	0,1701	0,0289	6,36	10
$m_{sample}$	distillate mass	2,5558	g	0,0001	0,0000	-41,274	-0,0025	0,0000	0,00	∞
$m_{TMU}$	internal standard (TMU) mass	1,2306	g	0,0001	0,0001	85,720	0,0053	0,0000	0,01	∞
$(D/H)_{TMU}$	TMU isotopic ratio	141,9	ppm	0,4500	0,0032	0,743	0,3345	0,1119	24,61	∞
$t_m^D$	distillate alcoholic strength	91,09	% b.w.	0,1600	0,0018	-1,158	-0,1853	0,0343	7,55	∞
	(D/H) ratio	105,487	ppm							
	combined standard uncertainty	0,674	ppm							
	relative uncertainty	0,639	(%)							
	effective degrees of freedom	81								
	k (95% significance level)	2,000								
	expanded uncertainty	1,349	ppm							

$$\Delta(D/H)_{I,II} = \sqrt{(D/H)_{I,II}^2 \frac{RSD_R^2}{n} + \sum_{i=1}^m \frac{\partial(D/H)_{I,II}}{\partial x_i} \Delta x_i}$$

## 5. Factors contributing to uncertainty



From the chart above, it is obvious that the random errors are those that affect the determinations. Instrument reproducibility can be determined by the data collected from the analysis of reference ethanols (BCR-xxx), while the laboratory control sample provides the tool to determine overall reproducibility of the method. It is shown that the systematic biases introduced by weighing are negligible, while the TMU reference is contributing substantially to the uncertainty of the measurement. Finally, the strength of the distillate collected from the ADCS process is also quite significant.

## 6. Conclusions

The mathematical model of the analytical process is elaborated and the application of the procedure described in JCGM 100:2008 for the uncertainty estimation of the analysis is detailed. The uncertainty of the method is vital for the development of models that may differentiate wines that belong to different varieties or origins. Also, if D/H ratios are specified for a particular wine of origin (geographical or botanical), the uncertainty of the method should be taken into account to determine whether the product conforms to the specification. The approach described allows for the contribution of the individual parameters underlying the analytical processes to be revealed and discussed.

## 7. References

- G.J. Martin, C. Guillou, M.L. Martin, M.T. Cabanis, T. Yutthay and J. Aerny, Natural Factors of Isotope Fractionation and the Characterization of Wines, J. Agric. Food Chem., 36, 1988, 316-322.
- N. Ogrinc, K. Bat, I.J. Kosir, T. Golob and R.Kokkinofta, Characterization of Commercial Slovenian and Cypriot Fruit Juices Using Stable Isotopes, J. Agric. Food Chem., 57, 2009, 6764-6769.

