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TECHNICAL NOTE

Comparison of spectrophotometric and chromatographic methods of determination of furanic aldehydes in wine distillates

J. Quesada Granados, M. Villalón Mir, H. López García Serrana & M. C. López Martínez

Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, 18012–Granada, Spain

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A comparison of two methods of determination of furanic aldehydes in commercial brandies was carried out. The brandy samples were analysed using the spectrophotometric technique recommended by the Spanish Ministry of Health and Consumption, and by another HPLC technique. The results were subjected to appropriate statistical treatment and both techniques were found to be suitable, as there were no significant statistical differences between them, although the chromatographic technique is somewhat faster and easier to use than the spectrophotometric method.

INTRODUCTION

Furfural and 5-hydroxymethyl furfural are two furanic aldehydes found in wine distillates subjected to ageing in oak barrels and make up part of the so-called 'non-alcoholic content' of these beverages.

In report no. 869 of the OIV, Cantagrel *et al.* (1992) emphasised the convenience of comparing existing methods for determination of all those elements affecting the quality of distilled beverages. Analytical methods could then be harmonised and definite conclusions reached regarding a single, rapid method of analysis, that would above all be reliable when testing the quality of an alcoholic beverage as regards the non-alcoholic content. This is desirable because of the diversity of techniques the analyst can use when attempting to determine the so-called 'non-alcoholic content' in distillates and their constitutive elements (Dehove, 1984).

In Spain, as in many other EC countries, the recommended method for furfural determination (Ministerio de Sanidad y Consumo, 1985) is based on steam distillation followed by spectrophotometric measurement of its absorption at 277 nm.

Apart from this classic method, other procedures have been developed, such as gas-phase chromatography (Guymon & Crowell, 1972) and, more recently, high-performance liquid chromatography (Jeurings & Kuppers, 1980). However, there is no agreement about which of these is the better analytic technique.

The aim of this paper is to compare the spectrophotometric method recommended by the Spanish Ministry (Ministerio de Sanidad y Consumo, 1985) and an HPLC technique designed by the authors in order to examine the relative merits of both methods as regards both their analytical properties and their speed, efficiency and cost for an analytical laboratory involved in quality evaluation of distilled beverages.

MATERIALS AND METHODS

The authors analysed 38 samples of commercial brandies, divided into two groups according to the technique used for maturation: dynamic ageing by solera and traditional static ageing.

The furanic aldehyde concentrations in the samples were determined using the following methods.

Spectrophotometric method

Furfural was determined using the technique recommended by the Spanish Ministry of Health and Consumption (Ministerio de Sanidad y Consumo, 1985) and AOAC (1990), consisting of steam distillation of the brandy, followed by spectrophotometric measurement of the distillate at 277 nm. Determination of 5-hydroxymethyl furfural was carried out using the technique developed by Villalón Mir *et al.* (1987), which

determines the aldehyde by the difference between the total furanic aldehyde concentrations measured directly by spectrophotometry in the brandy at 280 nm, and the previously determined furfural content.

Chromatographic method

The HPLC technique developed by Villalón Mir *et al.* (1992) was used to determine furanic aldehydes. The most significant characteristic of this technique is direct injection of the samples, after filtering through 0·45 micra pore-size Millipore® membranes.

RESULTS AND DISCUSSION

In order to study the concentration of furanic aldehydes (furfural and 5-HMF) in samples of commercial brandies, a model system was prepared reproducing the conditions to be found when determining the furanic compounds. This model system consisted of a hydro-alcohol solution of 2 mg/litre furfural and 35 mg/litre 5-HMF in 40° GL ethanol, thus simulating concentrations similar to those found in brandy.

Determination of furfural and 5-HMF in the model system

The determinations of furfural and 5-HMF in the model system were carried out using the spectrophotometric and HPLC methods described above. The precision and accuracy of the analytic techniques were tested by applying the statistical method of Martin and Luna del Castillo (1988) to the model system. The accuracy expressed as percentage recovery and the percentages found are shown in Tables 1 and 2. The concentration of furfural and 5-HMF found by each

Table 1. Precision and accuracy of the spectrophotometric method applied to the model system

	Furfural	50-Hmf
Real amount (mg/litre)	2	35
Found (mg/litre)	1·98	31·8
Recovery (%)	99·0	90·9
SD	0·07	0·18
CV (%)	3·3	0·6
Standard error	0·02	0·06
Relative error (%)	2·4	0·4
$X_m \pm S_m \times t$	1·98 ± 0·05	31·8 ± 0·1

Table 2. Precision and accuracy of the chromatographic method applied to the model system

	Furfural	50-Hmf
Real amount (mg/litre)	2	35
Found (mg/litre)	1·9	34·6
Recovery (%)	94·0	98·9
SD	0·02	0·22
CV (%)	0·9	0·6
Standard error	50.6×10^{-3}	0·07
Relative error (%)	0·61	0·46
$X_m \pm S_m \times t$	1·88 ± 0.01	34·6 ± 0·2

Table 3. Comparison of different analytical methods for determination of furfural and 5-hydroxymethyl furfural in commercial brandies (mg/litre)

Brandy	Traditionally-aged				Solera-aged			
	Furfural		5-HMF		Furfural		5-HMF	
	ESP UV[a]	HPLC[b]	ESP UV[c]	HPLC[b]	ESP UV[a]	HPLC[b]	ESP UV[c]	HPLC[b]
1	16·72	13·47	35·39	31·19	1·61	1·35	59·60	47·62
2	1·31	0·55	18·62	16·75	1·10	0·53	15·50	12·90
3	0·98	0·74	83·19	80·29	0·97	0·58	45·10	34·83
4	1·34	1·30	12·79	7·85	0·98	—	53·92	52·05
5	1·18	0·62	94·47	88·27	0·49	—	48·20	40·01
6	0·79	—	31·16	27·28	0·62	—	26·70	23·59
7	0·39	—	30·23	25·26	—	—	19·45	14·64
8	13·84	13·50	43·71	39·66	—	—	11·32	8·77
9	1·48	1·30	25·66	18·48	0·79	0·52	22·57	20·00
10	11·81	10·64	35·25	32·14	1·25	0·63	108·36	100·66
11	11·74	9·30	18·15	14·03	0·93	0·62	39·68	33·68
12	4·57	2·71	15·49	9·55	0·45	—	27·62	22·62
13	5·06	4·28	14·36	11·54	0·72	0·70	27·08	25·08
14	16·15	13·45	35·89	30·77	0·60	0·55	23·77	19·77
15	10·04	9·33	10·47	6·35	3·97	3·44	21·83	18·83
16	11·29	9·10	17·95	14·37	3·02	2·30	11·96	9·74
17	4·53	3·43	20·83	16·75	5·09	4·03	145·30	138·50
18	10·13	8·81	12·15	5·87	3·53	2·50	20·70	16·70
19	3·07	2·29	21·76	17·58				
20	4·58	2·87	40·10	36·70				

[a]Method recommended by the Ministerio de Sanidad y Consumo (1985).
[b]HPLC method by Villalón Mir *et al.*(1992).
[c]UV spectrophotometry based on the technique developed by Villalón Mir *et al.* (1987)

Table 4. Examination of known quantities (mg/litre) of furfural and 5-HMF added to brandy samples by UV spectrophotometry

	Brandy	Initial amount	Added	Found	Total	Recovery
Furfural						
	1	0·79	0·5	1·287	1·29	99·8
			5	5·755	5·79	99·4
	2	0·39	0·5	0·886	0·89	99·6
			5	5·363	5·39	99·5
	3	0	0·5	0·498	0·5	99·6
			5	4·988	5	99·7
	4	0	0·5	0·499	0·5	99·8
			5	4·995	5	99·9
	5	0·45	0·5	0·945	0·95	99·5
			5	5·412	5·45	99·3
5-HMF						
	1	31·16	10	36·921	41·16	89·7
			80	101·378	111·16	91·2
	2	30·23	10	36·328	40·23	90·3
			80	100·200	110·23	90·9
	3	19·45	10	26·480	29·45	89·9
			80	90·500	99·45	91·0
	4	11·32	10	19·081	21·32	89·5
			80	82·371	91·32	90·2
	5	27·62	10	34·046	37·62	90·5
			80	96·750	107·62	86·9

technique represents the average of 10 determinations made on the model system.

Determination of furfural and 5-HMF in commercial brandies

Table 3 shows the results obtained for determination of furfural and 5-HMF by both methods. The brandies with no detectable concentrations of furfural and 5-HMF were used as a matrix to determine the accuracy of the spectrophotometric and chromatographic methods by addition of quantities of pure furfural and 5-HMF. The accuracy of the methods was expressed as percentage recovery and the results are shown in Tables 4 and 5.

The results of these two determinations (spectrophotometric and HPLC methods) were used to construct Figs 1 and 2. The linear regression of the HPLC results is graphically represented as a function of the spectrophotometric results for furfural and 5-HMF in the different brandy samples. The correlation coefficient between the two measurements is also represented.

Table 5. Examination of known quantities (mg/litre) of furfural and 5-HMF added to brandy samples by HPLC

	Brandy	Initial amount	Added	Found	Total	Recovery
Furfural						
	1	0	0·5	0·464	0·5	92·8
			5	4·670	5	93·4
	2	0	0·5	0·469	0·5	93·8
			5	4·675	5	93·5
	3	13·50	0·5	13·076	14	93·4
			5	17·390	18·5	94·0
	4	0	0·5	0·471	0·5	94·1
			5	4·660	5	93·2
	5	0	0·5	0·465	0·5	93·0
			5	4·695	5	93·9
5-HMF						
	1	27·28	10	36·721	37·28	98·5
			80	105·993	107·28	98·8
	2	25·26	10	34·837	35·26	98·8
			80	104·628	105·26	99·4
	3	39·66	10	49·461	49·66	99·6
			80	118·104	119·66	98·7
	4	52·05	10	61·677	62·05	99·4
			80	130·597	132·05	98·9
	5	40·01	10	49·60	50·01	99·2
			80	118·929	120·01	99·1

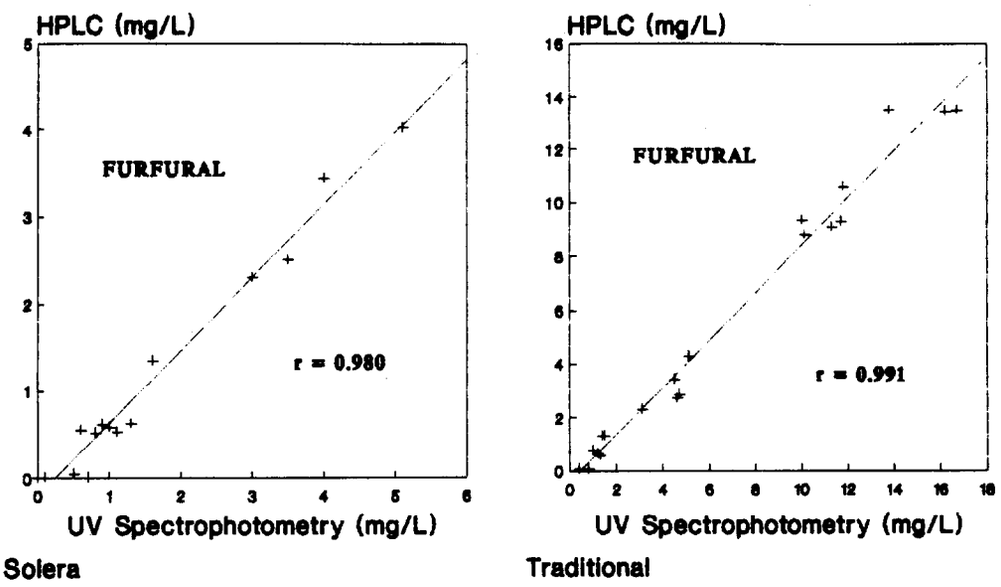

Fig. 1. Correlation between HPLC and spectrophotometric methods of determination of furfural in commercial samples of brandy.

The spectrophotometric method of furfural determination invariably gave slightly higher results than the chromatographic method. In the solera-aged brandies, the mean concentration of furfural was found to be 1·45 mg/litre by the first method and 0·99 mg/litre using the second, whereas for brandies aged by the traditional method, the mean concentrations were determined as 6·55 mg/litre and 5·39 mg/litre, respectively.

Figure 1 shows good correlation between the two series of measurements in both maturation systems. For brandies aged using the traditional system the correlation coefficient is 0·991 and the slope of the linear regression (very close to one) is 0·883. For solera-aged brandies the linear regression gave a correlation coefficient of 0·980 and a slope of 0·840.

The results obtained by spectrophotometry were also slightly higher than those obtained by chromatography in determination of 5-HMF: in traditionally aged brandies, the mean concentration of this aldehyde was determined as 30·9 mg/litre by the former method and 26·5 mg/litre by the latter, and in solera-aged brandies the mean concentration was determined as 40·5 mg/litre and 35·6 mg/litre, respectively.

The linear regressions obtained for 5-HMF are shown in Fig. 2, where we can observe very good correlation between both methods for the samples of traditionally aged brandies, with a correlation coefficient of 0·998 and a slope of 0·998, whereas the corresponding values for the solera-aged brandies were 0·997 and 0·956, which reveals the similarity in results obtained by the two different methods.

Statistic treatment

In order to specify the differences obtained between each pair of results and definitely establish whether the

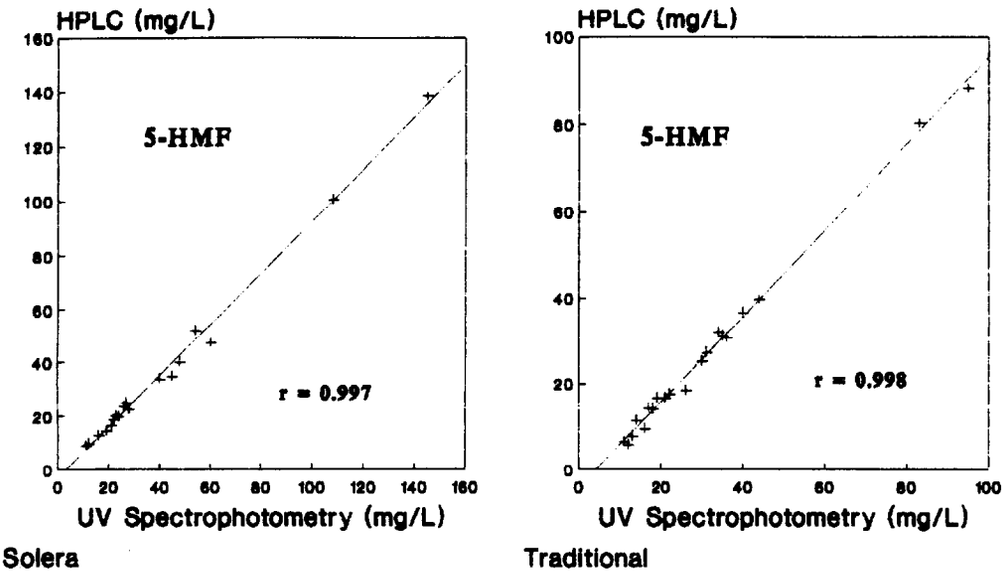

Fig. 2. Correlation between HPLC and spectrophotometric methods of determination of 5-hydroxymethyl furfural in commercial samples of brandy.

techniques for determination of furanic aldehydes are equivalent, a statistical treatment was applied to the results in Table 1. This consisted of a comparison of two independent variables (*t*-test) using the STATGRAPHICS 5·0 statistics package at a 95% confidence limit.

Before applying the STATGRAPHICS 5·0 package, the normal distribution of sample populations was tested by the Kolmogorov–Smirnov method, with a 95% level of confidence, and then a parametric comparison test of two independent samples (*t*-test) (Porretta & Sandei, 1991) was carried out. The results shown in Tables 6, 7, 8, and 9 were then obtained.

All the tables show significancy levels of over 0·05 required by the 95% confidence level. This means that, despite obtaining higher concentrations of furfural and 5-HMF by the spectrophotometric method, there are, however, no significant statistical differences between the different sample populations analysed as regards the analytical method applied for determination of furfural and 5-HMF in commercial brandies.

Table 6. *t*-test comparison of furfural concentrations obtained by methods A and B in samples of solera-aged brandy

Statistics	Method A[a]	Method B[b]	Pooled
n	18	18	36
Mean	1·45	0·99	1·22
Variance	2·11	1·54	1·82
SD	1·45	1·24	1·35
Median	0·95	0·57	0·67
Hypothesis test for H_0			
Diff = 0	Statistics t = 1·02		
vs Alt: NE	Significance level = 0·32		
at Alpha = 0·05	So do not reject H_0		

[a]UV spectrophotometry (Ministerio de Sanidad y Consumo, 1985).
[b]HPLC (Villalón Mir *et al.*, 1992).

Table 7. *t*-test comparison of 5-HMF concentrations obtained by methods B and C in samples of solera-aged brandy

Statistics	Method B[a]	Method C[b]	Pooled
n	18	18	36
Mean	35·56	40·48	38·01
Variance	1127	1225	1176
SD	33·57	35·00	34·29
Median	23·11	26·89	24·43
Hypothesis test for H_0			
Diff = 0	Statistics t = 0·43		
vs Alt: NE	Significance level = 0·67		
at Alpha = 0·05	So do not reject H_0		

[a]HPLC (Villalón Mir *et al.*, 1992).
[b]UV spectrophotometry (Villalón Mir *et al.*, 1987).

Table 8. *t*-test comparison of furfural concentrations obtained by methods A and B in samples of traditionally aged brandy

Statistics	Method A[a]	Method B[b]	Pooled
n	20	20	40
Mean	6·55	5·39	5·97
Variance	30·96	24·55	27·75
SD	5·57	4·95	5·27
Median	4·58	3·15	4·41
Hypothesis test for H_0			
Diff = 0	Statistics t = 0·70		
vs Alt: NE	Significance level = 0·49		
at Alpha = 0·05	So do not reject H_0		

[a]UV spectrophotometry (Ministerio de Sanidad y Consumo, 1985).
[b]HPLC (Villalón Mir *et al.*, 1992).

Table 9. *t*-test comparison of 5-HMF concentrations obtained by methods B and C in samples of traditionally aged brandy

Statistics	Method B[a]	Method C[b]	Pooled
n	20	20	40
Mean	26·53	30·88	28·7
Variance	494	494	494·145
SD	22·23	22·23	22·2294
Median	18·03	23·71	21·295
Hypothesis test for H_0			
Diff = 0	Statistics t = 0·62		
vs Alt: NE	Significance level = 0·54		
at Alpha = 0·05	So do not reject H_0		

[a]HPLC (Villalón Mir *et al.*, 1992).
[b]UV spectrophotometry (Villalón Mir *et al.*, 1987).

CONCLUSION

In view of these results, it can be said that the spectrophotometric technique for determination of furanic aldehydes in commercial brandies is highly suitable. However, the high-performance liquid chromatography method may be included as an alternative analytical procedure, as there are no significant statistical differences between the methods and this technique is faster than the spectrophotometric method.

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