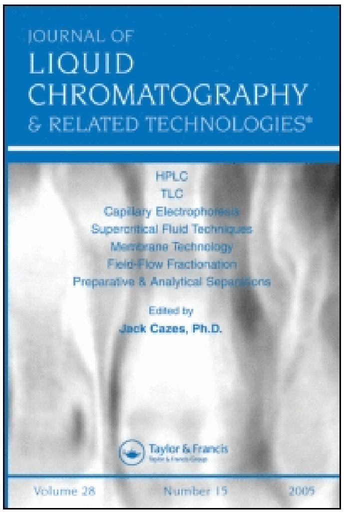

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF FURANIC COMPOUNDS IN COMMERCIAL BRANDIES AND CARAMELS

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ABSTRACT

A technique using high performance liquid chromatography to determine the furanic aldehydes: furfural and 5-hidroxymethil furfural, in aged brandies and hydroalcoholic solutions of commercial caramels, was developed. Separation and quantification of these substances can be carried out by injecting the samples directly into the chromatograph, the relative error presented under this method is 0.52% for furfural and 0.29% for 5-HMF. The high concentrations of 5-hidroxymethilfurfural found in 100% of the brandy samples analysed is due to the caramel added as a colouring agent.

INTRODUCTION

The chemical compositions of brandies aged in oak barrels and caramel contain the furanic compounds furfural and 5-hidroxymethilfurfural.

These compounds may have two different origins: caramel added to the brandies as a colouring agent to intensify the golden colour acquired during the aging process(1) or the thermic reduction of the celuloses and hemiceluloses of the wooden barrel. When the staves(2) are bound to give the barrel it's shape, the wood is burnt so as to avoid breakage. this treatement involves the partial reduction of the celulose which generates 5-hidroxymethilfurfural whilst furfural is produced by the hemicelulose.

These furanic aldehydes can be determined by various techniques ranging from spectrophometric methods described in the AOAC(3), and accepted by our food legislation as analytic techniques relating to brandies(4), to the latest chromagraphic techniques in liquid phase, not to mention gas chromatography widely used in the last decade to determine volatile constituents in brandy and other alcoholic drinks.

Diez, J. et al(5); Baumes, R et al(6); and Boidron, J.N. et al(7) and others, used gas chromatography to analyse the volatile constituents in aged brandies and wines, among them furfural, extracted from the drinks with organic solvents.

Puech, et al(8) also used gas chromatography when studying the evolutions of the furanic and phenolic compounds presents in cognac aged in French oak barrels from Limousin and they introduced the novelty of injecting the samples directly into the chromatograph, not having extracted them beforehand, which somewhat simplifies the analysis.

However, during the last few years high performance liquid chromatography has begun replaced all the other techniques in the chemical analysis field(9) due to it's rapidity, simplicity and accuracy.

This study aims to bring up date a high performance liquid chromatography method, by means of direct injection of the samples, which allows and exact separation and quantification of the furanic aldehydes, furfural and 5-HMF, in aged brandies and synthetic solutions of commercial caramels.

The second part of this study concerns the ratio between furfural and 5-HMF which depends on the brandy's aging process and the addition of caramel as acolouring agent.

MATERIALS AND METHODS

1.- Experimental conditions

HPLC instrumentation consisted of a Konik model KNK-500A chromatograph, with a Konik injector model 7125. The column used was a Lichrospher Merck CH-18, 10cms long and with an interior diametre of 4.7mm, the mobile phase consisted of a mixture of methanol and water (10-90 V/V), with a flow rate 1ml/min. A Konik spectrophotometre detector model U/VIS 200 was used with a wavelength of 280nm the experiments were carried out at room temperature and the injection volume was 15μl.

2.- Solutions standard

Increasing volumes of a solution of 200mg/l of 5-HMF and 50mg/l of furfural in ethanol of 40^{0} G.L. were taken, from which we prepared hydroalcoholic solutions 40% V/V of concentrations ranging from 0.3mg/l to 25mg/l for furfural. These solutions were injected into the chromatograph directly under the aforementioned conditions.

3.- Preparation of samples

4 sorts of commercial caramels supplied by Merck, were analysed, we identified them under the following names:

-caramel TS
-caramel TPS
-caramel TR
-caramel 42005

The samples were firstly diluted in distilled water until they reached the concentration required for linearity, then they were injected directly into the chromatograph, after passing through Millipore filters with pore diametres of 0.45mm.

4.- Commercial brandies

38 brandy samples, chosen from different commercial centres were analysed. The quality of these brandies varied but they were all guaranteed to be genuine. These samples were divided into 2 groups according to the aging process followed:

- brandies aged along the dynamic solera system
- brandies aged along the traditional static system

These samples were not extracted, but directly injected into the chromatograph after filtration through millipore filters with pore diametres of 4.5mm.

RESULTS AND DISCUSSION

1.- Reproducibility

We studied the reproducibility of this method from 10 injections of a hydroalcoholic mixture at 40% V/V which contained concentrations of furfural and 5-HMF, 10 mg/l and 14mg/l respectively, under identical experimental conditions.

The results appear in Table 1.

The accuracy of this method for each aldehyde is expressed by the coefficient variant Z, calculated from Student's factor $t_{0.95}$.

$$Z = \frac{\sigma \times t}{\text{average area}} \times 100$$

Figure 1 shows the chromatograph obtained from injecting the hydroalcoholic mixture directly into the chromatograph under the conditions previously described.

2.- Calibration curves

To determine the intervals in which the analytic method presents lineal proportionality between the furanic aldehydes'concentration and the area of the peak height found, we prepared hydroalcoholic mixtures of increasing concentrations in both furanic compounds and these were directly injected into the HPLC under identical conditions. Figure 2 shows the curves we obtained. The way in which these lines progress gives us an absolute response factor for each aldehyde(10). The response factor is calculated by dividing each constituent's concentration by its area found when analysing the calibration mixture.

$$\text{absolute RF} = \frac{\text{concentration (mg/l)}}{\text{absolute area of peak height}}$$

TABLE 1: Study of Reproducebility in the Determination of Furfural and 5-HMF by H.P.L.C.

	Furfural Area	5-HMF Area
	320820	357020
	322610	356500
	321340	355900
	322980	356990
	322700	357120
	321450	356020
	321710	356460
	322110	356280
	320990	357020
	321560	356240
Mean Area	321827	356555
σ_{N-1}	743	453
Z Variat.Coef.	0.52	0.29

Under these conditions, furfural presents linearity between 0.5mg/l and 20mg/l (figure 2) and its response factor has a constant value of 3.10670 x 10^{-5} (table 2). The response factor for 5-HMF is 3.92482 x 10^{-5} and it presents linearity between 0.5mg/l and 60mg/l. Proportionality between each aldehydes area and its correponding concentration does not exist outside the intervals.

3.- Additions

To confirm the exactness of the determinations carried out, we added known quantities of furfural and 5-HMF to 6 types of brandy whose concentrations of both furanic aldehydes were known.

TABLE 2: Determination of the Average Response Factor from the Solutions Standard of increasing concentrations.

Furfural (mg/l)	Area x 10^4	Response Factors	5-HMF (mg/l)	Area x 10^5	Response Factors
0.5	1.6093	3.10681 X 10^{-5}	0.5	0.1290	3.87626 X 10^{-5}
1	3.2412	3.08527 X 10^{-5}	1	0.2540	3.93700 X 10^{-5}
2	6.4856	3.08375 X 10^{-5}	2	0.5090	3.92927 X 10^{-5}
4	12.8512	3.11254 X 10^{-5}	2.5	0.6255	3.99680 X 10^{-5}
6	19.4569	3.08373 X 10^{-5}	5	1.2726	3.92911 X 10^{-5}
8	26.0130	3.07538 X 10^{-5}	10	2.5486	3.92379 X 10^{-5}
10	32.4280	3.08375 X 10^{-5}	15	3.8277	3.91885 X 10^{-5}
13	40.2570	3.22925 X 10^{-5}	20	5.1017	3.92026 X 10^{-5}
15	48.3766	3.10067 X 10^{-5}	30	7.6359	3.92880 X 10^{-5}
17	54.7188	3.10679 X 10^{-5}	40	10.2345	3.90834 X 10^{-5}
20	64.3759	3.10675 X 10^{-5}	50	12.8020	3.90563 X 10^{-5}
			60	15.2917	3.92370 X 10^{-5}
Mean Response Factor 3.10679 X 10^{-5}			Mean Response Factor 3.92482 X 10^{-5}		

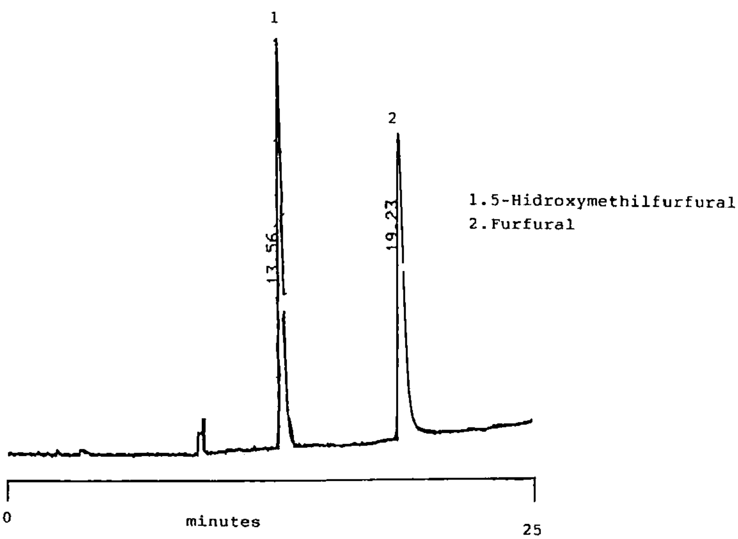

FIGURE 1: Chromatogram of a Mixture of Furfural (10mg/l) and 5-Hidroxymethilfurfural (14mg/l).

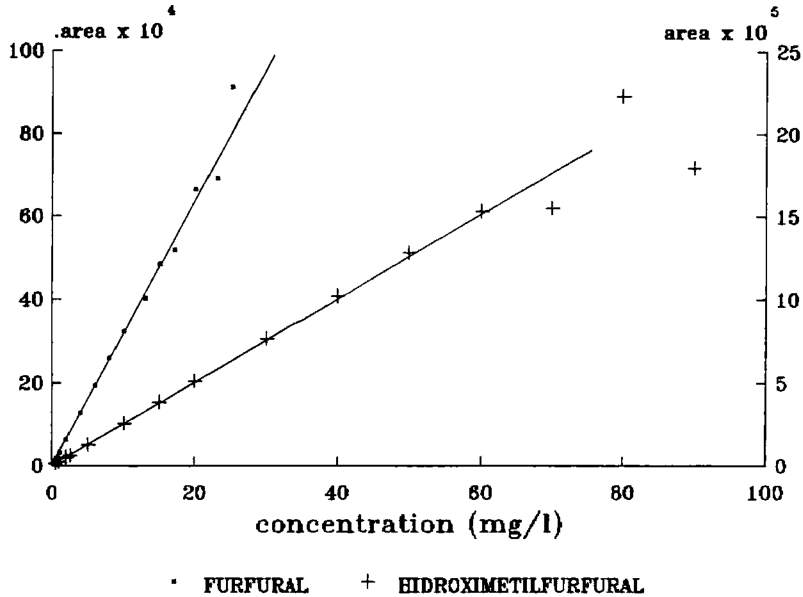

FIGURE 2: Calibration Curves of the Furanic Aldehydes.

The concentrations of these aldehydes in the samples analysed were calculated from the absolute areas of each peak height in the chromatogram and from the absolute response factor of each aldehyde previously calculated.

concentration (i) mg/l = peak area x absolute RF

The results obtained are shown in Table 3. The concordance between the quantities added and the quantities discovered are expressed as a percentage of recovery.

4.- Study of furanic aldehydes in commercial caramels and brandies.

Commercial caramels.

The concentrations of furfural and 5-HMF found in the different types of commercial caramels analysed are shown in figure 3. As far as 5-HMF was concerned, these concentrations varied from

TABLE 3: Recovery (%) of Furfural in Brandy samples

Brandy	Initial Amount (mg/l)	Added (mg/l)	Found (mg/l)	Total (mg/l)	Recovery %	σ
1	0	0.5	0.49	0.5	98.00	0.085
		5	4.81	5	96.20	
2	0	0.5	0.48	0.5	96.00	0.068
		5	4.86	5	97.20	
3	0.29	0.5	0.67	0.79	84.80	0.065
		5	5.03	5.29	95.10	
4	0.63	0.5	1.06	1.13	93.80	0.17
		5	5.23	5.63	92.90	
5	3.44	0.5	3.81	3.94	96.70	0.065
		5	8.39	8.44	99.40	
6	1.01	0.5	1.51	1.51	100.00	0.055
		5	5.90	6.01	98.20	

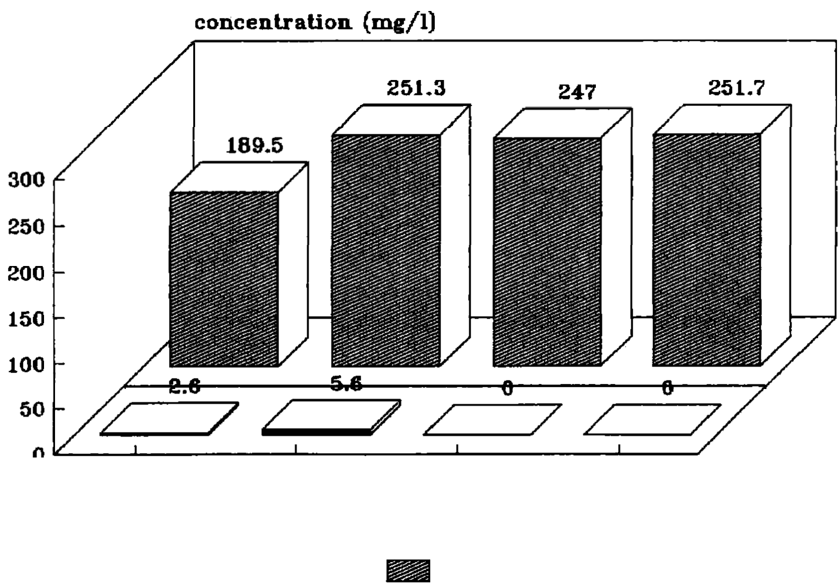

TABLE 4: Recovery (%) of 5-HMF in Brandy samples.

Brandy	Initial Amount (mg/l)	Added (mg/l)	Found (mg/l)	Total (mg/l)	Recovery %	σ
1	23.59	10	32.69	33.59	97.32	0.800
		80	102.08	103.59	98.54	
2	40.01	10	49.19	50.01	98.36	0.355
		80	119.66	120.01	99.70	
3	8.77	10	17.85	18.77	95.10	1.470
		80	88.05	88.77	99.20	
4	100.66	10	109.45	110.66	98.91	1.775
		80	178.40	180.66	98.75	
5	18.83	10	28.61	28.83	99.24	0.515
		80	97.80	98.83	98.96	
6	22.62	10	32.62	32.62	100.00	1.165
		80	101.10	102.62	98.52	

189.5mg/l in caramel TS to 251.7mg/l in caramel TR, whilst furfural was found in much lower concentrations, 2.6mg/l in caramel TS and 5.6mg/l in caramel 42005. The latter aldehyde was not detected in the samples of caramels TPS and TR.

This study has enabled us to observe that the major constituent in the caramel samples analysed (and used as a colouring agent in the alcohol industry) is 5-HMF, representing 99.9% of the total furanic aldehydes.

The ratio furfural/5-HMF for these caramels is below unity with values ranging from 0 to 0.02.

Commercial brandies.

The concentrations of furanic aldehydes in the brandies aged along the soleras method and along the traditional method, are show is figures 4 and 5, respectively.

The samples of brandy also showed much lower levels of furfural than 5-HMF. In general, the soleras aged brandies had an average concentration in 5-HMF of 35.56mg/l, very similar to the

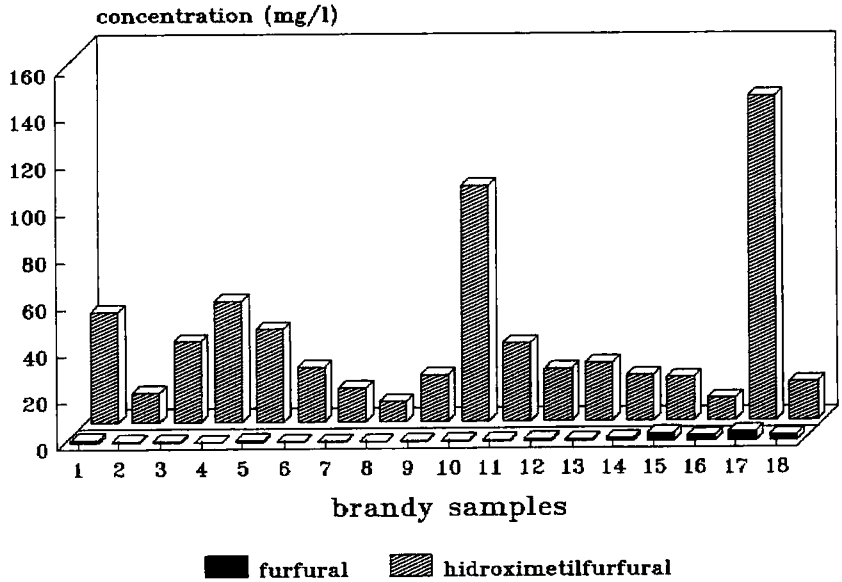

FIGURE 4: Furanic Compounds in Commercial Samples of Soleras aged Brandies.

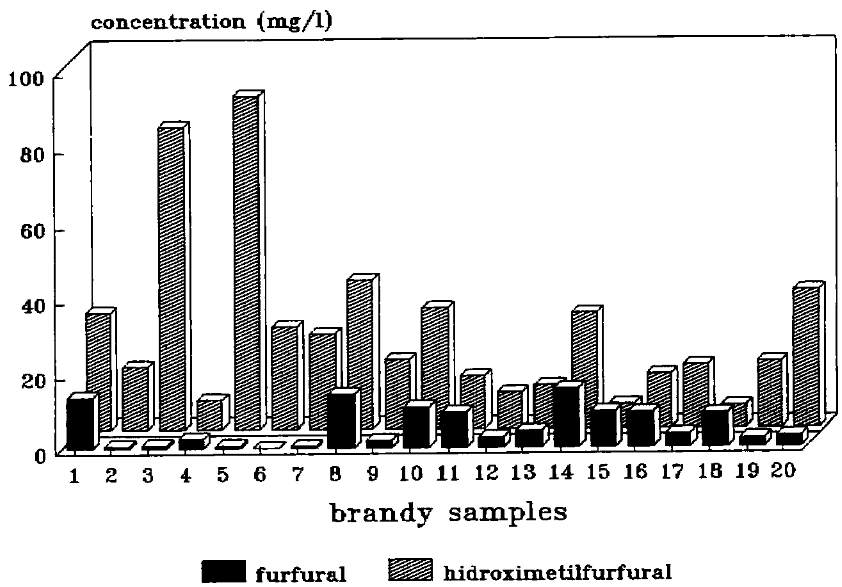

FIGURE 5: Furanic Compounds in Commercial Samples of Traditionally aged Brandies.

traditionally aged brandies which had an average concentration of 26.53mg/l.

It's possible that the traditionally aged brandy goes through a longer natural aging process which explains the higher content of furfural in these samples. However, the addition of caramel to brandies aged along both methods to give them the golden colour that they should acquire after a natural aging process in oak barrels, conditions the high concentration of 5-HMF in all the samples analysed.

The ratio furfural/5-HMF varied within the limits of 0 - 0.04 for soleras aged brandies and 0.02 - 0.21 for traditionally aged brandies. This variation is fundamentally due to the addition of caramel to these drinks.

It was proved that the samples with a higher content of 5-HMF were the ones with a higher concentration of caramel. This compound was quantified using Buhrer's (11) technique. The results have not been included as they only serve as a guide in stablishing the furfural/5-HMF ratio.

In any case. in both the caramel and brandy samples, the ratio is always maintained below unuty. These data are different from those obtained by Puech, J.L. et al.(12) when they studied samples of cognac and armagnac. They controlled the aging process themselves wich meant they could gurantee the absence of caramel in the samples.

The discordance between the values Puech, J.L. found and our values can be put down to the high content of caramel found in our samples.

CONCLUSION

The use of HPLC to determinate furfural and 5-HMF has proved to be a rapid and precise tecnique as it dispences with the extraction process and allows a direct injection of the samples to be analysed into the chromatograph.

As far as furanic aldehydes are concerned, the aging process followed does nos establish any significant differences in the value of the furfural/5-HMF ratio.

In accordance with the bibliography consulted, this ratio is only maintained above unity when the brandy is aged naturally which means it is kept in an oak barrel for (a more or less) prolonged period of time.

In this study, we have observed that the addition of caramel to these drinks, to give them an aged character, inverts the value of this ratio as 5-HMF is the major constituent in caramel.

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