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High-performance liquid chromatographic determination of 2-furaldehyde in spirits

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ABSTRACT

Official methods for the determination of 2-furaldehyde in spirits involve for a spectrophotometric evaluation, which is characterized by poor specificity. Gas chromatographic evaluations have also been proposed, which offer a much higher sensitivity, particularly when capillary columns are used. In this paper a high-performance liquid chromatographic (HPLC) method based on the formation of the 2,4-dinitrophenylhydrazones of carbonyl compounds and subsequent reversed-phase separation of these derivatives is described. Derivatization is carried out by utilizing an acidic solution of 2,4-dinitrophenylhydrazine in acetonitrile. Precipitation of the derivatives is avoided, and direct injection of the sample into the HPLC system is allowed. The determination offers a high specificity and a detection limit of the order of 10^{-8} mol/l. Accuracy and reproducibility data are presented.

INTRODUCTION

The determination of 2-furaldehyde (F) in alcoholic beverages represents an interesting goal not only in analytical respects, but also with regard to regulatory implications. Current Italian regulations, for example, fix very strict limits for the presence of F in spirits. They state that F must be absent, or present only in trace amounts, in spirits of both domestic production and import [1]. On the contrary, French regulations state that the lower limit of F allowed in genuine cognac must be 1 g per 100 l of ethanol, corresponding to 4 mg/l of sample [2]. However, F is produced by pentoses during wine and cereal distillation, and also during barrel storage of spirits such as whisky and cognac, and therefore, F is certainly present in these types of products.

The classical method for the determination of F in alcoholic beverages is based on a spectrophotometric evaluation, the only one provided by the official AOAC methods [3]. In this procedure, the UV absorbance of the solution obtained by steam distillation of the sample is measured. This procedure

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is characterized by poor specificity, as other carbonyl compounds present in the sample may contribute to the absorbance [2].

Gas chromatographic procedures have also been proposed; they offer a much higher sensitivity, particularly when capillary columns are used [4,5].

In recent years, high performance liquid chromatographic (HPLC) methods have also been developed based on the determination of F either without derivatization [2,6] or after preparation of a derivative which allows the sensitivity of the method to be improved [7].

In this paper an HPLC method is described that is based on the formation of the 2,4-dinitrophenylhydrazones (DNPH-ones) of carbonyl compounds by addition of an excess of an acidic solution of 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile. The DNPH-ones obtained are then separated by reversed-phase HPLC and determined with spectrophotometric detection.

EXPERIMENTAL

Standards and reagents

2-Furaldehyde (Prolabo) was doubly distilled and kept in a refrigerator at 0–4°C. 2,4-Dinitrophenylhydrazine (Prolabo) was purified by successive crystallizations with HPLC-grade methanol and kept in a refrigerator at 0–4°C. Perchloric acid (70%) was obtained from Prolabo and acetonitrile and absolute ethanol (HPLC-grade) from Carlo Erba. Water was distilled, deionized and then further purified with a Milli-Q system (Millipore).

2,4-Dinitrophenylhydrazine solution. Reagent solution containing 2.5×10^{-3} mol/l of 2,4-dinitrophenylhydrazine was prepared in acetonitrile.

2-Furaldehyde standard solutions. A stock standard solution containing 10^{-2} mol/l of F was prepared in ethanol–water (40:60, v/v). By successive dilutions with the ethanol–water mixture, working standard solutions containing down to 10^{-7} mol/l of F were prepared.

Calibration graph

A 5-ml volume of F working standard solution and 4 ml of DNPH solution were transferred into a 10-ml glass-stoppered volumetric flask, a few drops of perchloric acid were added to pH 2 and the volume was made up to the mark with DNPH solution. The solution was kept on a magnetic stirrer at room temperature for at least 25 min, then 10 μl of the solution were immediately injected into the HPLC system.

Sample preparation

The same procedure described under *Calibration graph* was applied to a 5-ml volume of sample instead of F working standard solution.

Determination of recoveries of 2-furaldehyde

To 2.5 ml of a sample of brandy were added 2.5 ml of F working standard solution containing from 2×10^{-3} down to 2×10^{-7} mol/l of F. The solution obtained was processed as described under *Sample preparation.*

High-performance liquid chromatography

A Spectra-Physics Model 8700 high-performance liquid chromatograph, equipped with a Knauer Model 8700 variable-wavelength spectrophotometric detector and a 10-μl loop, was used. A Supelcosil LC-18 stainless-steel column (250 × 4.6 mm I.D.; film thickness 5 μm) was employed.

Analyses were carried out isocratically at room temperature with acetonitrile–water (65:35, v/v) as eluent at a flow-rate of 1 ml/min. The spectrophotometric detector was set at 388 nm. Peak areas were determined by means of a Spectra-Physics Model 4270 integrator.

RESULTS AND DISCUSSION

Optimization of the derivatization step

In recent years, HPLC has been employed to determine F in several real samples, particularly fruit juices [8–11] and spirits [2,6,7]. The determination may be carried out without preliminary derivatization, but usually the preparation of a derivative is to be preferred. In fact, the selectivity and sensitivity of the method may be enhanced by introducing a suitable chromophore into the molecule [7]. The most widely utilized derivative is the corresponding DNPH-one; it is usually obtained by adding an excess of DNPH aqueous solution in the presence of hydrochloric acid. Nevertheless, the utilization of an acetonitrile DNPH solution offers the advantage of obtaining a solution of the derivative that may be injected directly into the HPLC system [12]. Long

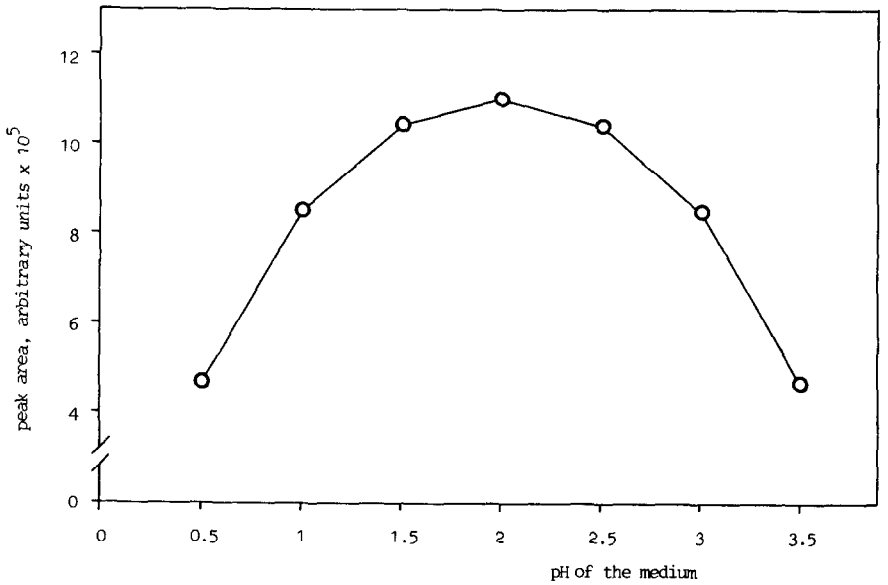

Fig. 1. Conversion of 2-furaldehyde to its 2,4-dinitrophenylhydrazone as a function of the acidity of the medium. 2,4-Dinitrophenylhydrazine-to-2-furaldehyde molar ratio = 2.5; reaction time = 30 min.

and tedious steps, such as filtration and washing of the derivatives obtained in aqueous solution and preparation of a derivative solution in a suitable solvent before the HPLC determination, may therefore be avoided [12]. The use of perchloric acid instead of hydrochloric acid is due to its higher solubility in acetonitrile [12].

In our case, the derivatization step was optimized at room temperature with respect to the DNPH to F molar ratio, the perchloric acid concentration of the medium and the reaction time. For this purpose, the amount of the derivative obtained was evaluated on F standard solutions. The F standard solutions were prepared by employing ethanol–water

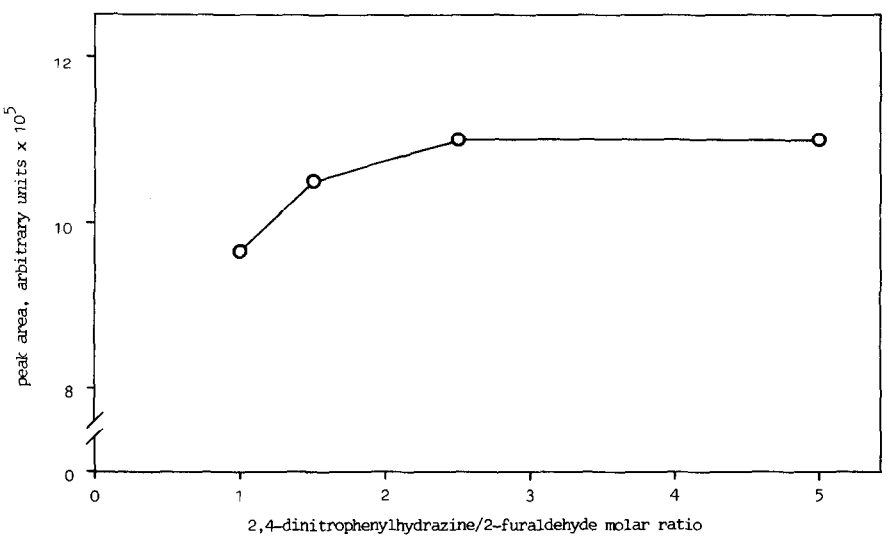

Fig. 2. Conversion of 2-furaldehyde to its 2,4-dinitrophenylhydrazone as a function of the 2,4-dinitrophenylhydrazine-to-2-furaldehyde molar ratio. pH of the medium = 2; reaction time = 30 min.

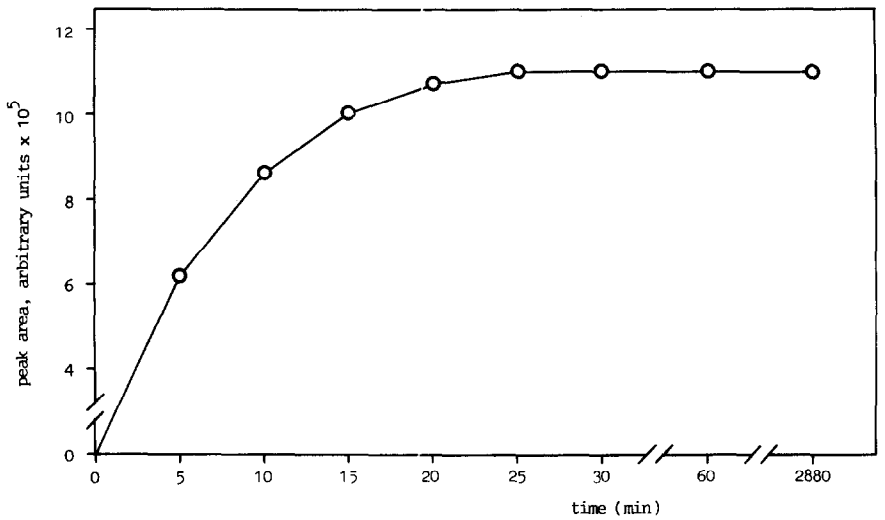

Fig. 3. Conversion of 2-furaldehyde to its 2,4-dinitrophenylhydrazone as a function of reaction time. 2,4-Dinitrophenylhydrazine-to-2-furaldehyde molar ratio = 2.5; pH of the medium = 2.

(40:60, v/v). This mixture simulates natural matrices as much as possible. The results obtained are shown in Figs. 1–3.

As can be seen, the reaction is quantitative when the reagent to analyte ratio is at least 2.5:1 and the acidity of the medium, as evaluated with a pH meter, is about 2. Under these conditions, F is quantitatively converted into its DNPH-one within 25 min. A similar result was obtained previously by Selim [13] for the derivatization of propionaldehyde. The derivative obtained is stable at room temperature for at least 48 h.

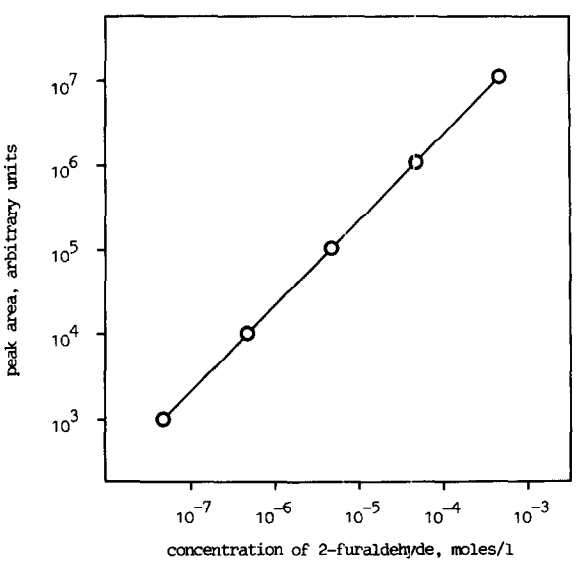

Fig. 4. Calibration graph of peak area of the 2,4-dinitrophenylhydrazone of 2-furaldehyde *versus* concentration of 2-furaldehyde. 2,4-Dinitrophenylhydrazine-to-2-furaldehyde molar ratio = 2.5; pH of the medium = 2; reaction time = 30 min.

Calibration

The calibration graph was obtained by employing standard solutions of F under optimum experimental conditions as described in the preceding section, and is shown in Fig. 4. A straight line was obtained over a wide range of examined concentrations, which represent values typically found in real samples. By setting the detector wavelength at the maximum absorbance of the F derivative, it is possible to determine the detection limit as $3\sigma/S$ [14], where S is the sensitivity, which is $2.23 \cdot 10^{10}$ as obtained from the calibration graph, and σ is the peak threshold of the integrator, which was set by us at 100. The detection limit is therefore $1.3 \cdot 10^{-8}$ *M*.

Specificity, recovery and reproducibility

The method shows a high specificity because, under the described conditions, the F derivative is well separated with respect to the other carbonyl compounds present in the sample under examination. As an example, Fig. 5 shows a typical separation

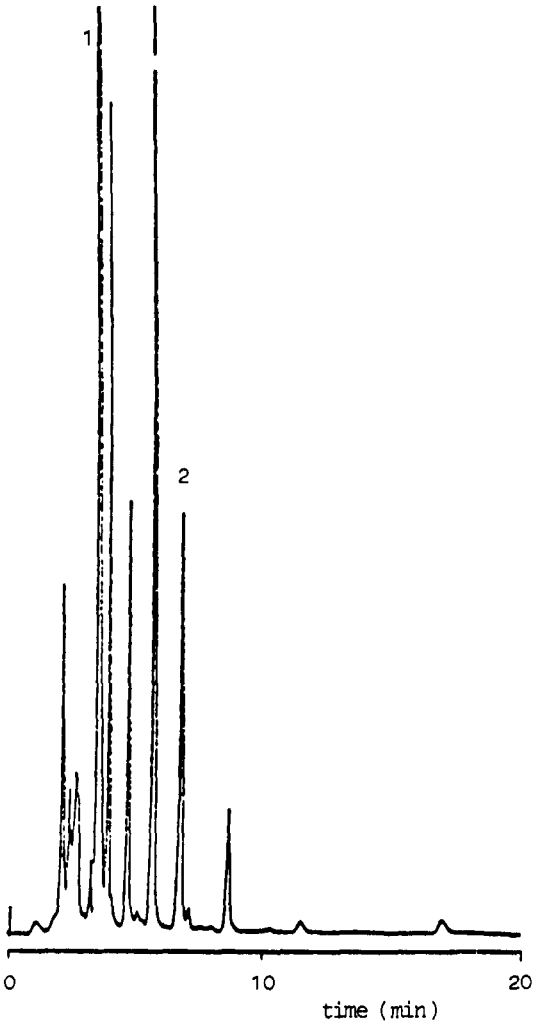

Fig. 5. HPLC separation of the 2,4-dinitrophenylhydrazones of carbonyl compounds from a sample of whisky. For conditions, see Experimental. Peaks: 1 = 2,4-dinitrophenylhydrazine; 2 = 2,4-dinitrophenylhydrazone of 2-furaldehyde.

obtained on a sample of a 12-year-old whisky. DNPH must be at least 20 times more concentrated than the analyte to be determined in the analyses of real samples, as an aliquot of the reagent is employed in the derivatization of the other carbonyl compounds present, in particular of acetaldehyde. In all the samples so far examined, a 1:20 ratio between F and DNPH was sufficient, as a large peak of the DNPH excess appears in the chromatogram.

Recovery was evaluated by adding different levels of F to a sample of brandy. The sample was selected on the basis of its low F content ($4.4 \cdot 10^{-7}$ mol/l), one of the lowest levels among those which we found in real samples. The results obtained are shown in Table I. Recoveries ranged from 98 to 100%.

TABLE I

RECOVERY OF 2-FURALDEHYDE ADDED TO BRANDY

Concentration of 2-furaldehyde (mol/l)			
Originally present	Added	Found	Recovery (%)
$2.2 \cdot 10^{-7}$	$1.0 \cdot 10^{-7}$	$3.2 \cdot 10^{-7}$	100
$2.2 \cdot 10^{-7}$	$1.0 \cdot 10^{-6}$	$1.21 \cdot 10^{-6}$	99
$2.2 \cdot 10^{-7}$	$1.0 \cdot 10^{-5}$	$1.00 \cdot 10^{-5}$	98
$2.2 \cdot 10^{-7}$	$1.0 \cdot 10^{-4}$	$9.91 \cdot 10^{-5}$	99
$2.2 \cdot 10^{-7}$	$1.0 \cdot 10^{-3}$	$9.92 \cdot 10^{-4}$	99

Reproducibility was evaluated by carrying out the determination six times on the same sample of whisky over a period of 48 h. The average concentration of F was $5.6 \cdot 10^{-5}$ mol/l, with a standard deviation of $1.6 \cdot 10^{-6}$ mol/l and a relative standard deviation of 2.78%.

Application

The procedure was applied to the determination of F in different spirits and the results are summarized in Table II. F was found in higher concentrations in samples such as cognac and whisky, that is, samples usually stored in barrels.

TABLE II

CONCENTRATIONS OF 2-FURALDEHYDE FOUND IN SOME SPIRITS

Spirit	Concentration of 2-furaldehyde (mol/l)	Spirit	Concentration of 2-furaldehyde (mol/l)
Cognac 1	$1.2 \cdot 10^{-5}$	Brandy 2	$4.4 \cdot 10^{-7}$
Cognac 2	$1.5 \cdot 10^{-5}$	Vodka 1	$8.9 \cdot 10^{-7}$
Cognac 3	$6.3 \cdot 10^{-5}$	Vodka 2	$1.1 \cdot 10^{-6}$
Cognac 4	$3.9 \cdot 10^{-5}$	Whisky 1	$5.6 \cdot 10^{-5}$
Brandy 1	$2.8 \cdot 10^{-6}$	Whisky 2	$7.9 \cdot 10^{-5}$

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