The mixtures 2,4-dinitrophenylhidrazones from acetic aldehyde and diacetyl and their separation through liquid chromatography with ternary gradient of mobile phases

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Abstract
Carbonyl compounds from foods products obtained by fermentation confer them fragrance and aroma and even more, the identity and concentration of carbonyl compounds characterize the capacity of the raw material to be transformed. The highest carbonyl compounds are formed during specific processes of fermentation; they confer characteristic sensorial features to the aliment. It is given careful consideration to those lower carbonyl compounds which are the result of transformation or union of acetyl fragments generated in the process of fermentation; the simplest compounds from this category are acetaldehyde and diacetyl.

Keywords: acetaldehyde, diacetyl, 2,4-dinitrofenilhidrazone, liquid chromatography, mechanism of separation with reverse phase, ternary gradient of mobile phases, isomers

1. Introduction

Carbonyl compounds are liquid substances which do not show very specific characteristics. The qualitative and quantitative study of these compounds has to be preceded by a hard preparatory stage. The role of this stage is to separate carbonyl compounds from natural mixtures and to transform them into derivatives accessible to known physical-chemical investigation methods.

It is preferable the transformation of carbonyl compounds with 2,4-dinitrophenylhidrazine (2,4-DNPH) in a strong acid medium; heating or the presence of another catalyze compound is not necessary. The precipitates 2,4-dinitrophenylhidrazones (2,4-DNPH-ones) crystallize from distilled water, they are separated through filtration them and washed with water (on filter paper) until the effluents has a neutral reaction (Zgherea, 1998).

The mixtures of 2,4-DNPH-onesdisolves itself very well in tetrahydrofuran or dioxan. The yellow solution that is obtained is separated through chromatography, using the mechanism of reverse phase, according to the nature of carbonyl compounds that generates the mixtures of 2,4-DNPH-ones, it was to be identified the experimental conditions to assure their separation.

This paper contains experimental and theoretical considerations to the means of separation through liquid chromatography of synthetically mixtures that contain 2,4-DNPH-ones provided by acetaldehyde (2,4-DNPHAA) and diacetyl (2,2-DNPHD), using a ternary mixture of liquid mobile phases.

2. Materials and methods

They were interpreted liquid-chromatograms of two synthetically mixtures that contain 2,4-DNPH-ones got from acetaldehyde and diacetyl.

Solvents and mobile phases

In order to dissolve the standard 2,4-DNPH-ones it was used acetonitrile Merck. To chromatographic separations of 2,4-DNPH-ones mixtures it was used a ternary mixture gradient mobile phase. The ternary mixture is got by mixing two binary solutions that contain acetonitrile, tetrahydrofuran (THF) (Merck) and double distilled water prepared by

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General Chemistry Laboratory from our department.

Synthetically mixtures of 2,4-DNPH-ones

Using 2,4-DNPHAA and 2,2-DNPHD with confirmed purity (Zgherea, 1999) are prepared standard solutions with concentration $5 \cdot 10^{-4}$ molar. Through controlled mixing then ware got to synthetically mixtures, SMI and SMII, where the ratio between the 2,4-DNPHAA and 2,2-DNPHD has different values.

Apparatus

For separations them were used the liquid chromatograph type LC-XPD, equipped with: installation for liquid degassing by refluxation (Zgherea, 2003), gradient programmer for mixing two mobile phases, column of separation (type Pye Unicam) which assure a gradient stationary phase, installation for column thermostatation, electronic integrator (Spectra Physics, type DP101) for chromatographic signal of UV detector and potentiometric recorder (type Philips PM 8251).

3. Results and discussion

The synthetically mixtures of 2,4-DNPH-ones was separated by liquid-chromatography, using a reverse phase mechanism (Liteanu *et al*., 1976), in the following conditions:

- Volume of synthetically mixtures: 10 μL;
- Column of separation: Spherisorb 5 ODS (L = 25 cm, Φ = 4.6 mm);
- Temperature of separation column: 40ºC;
- Wavelength of incidental radiation of UV detector: λ = 355 nm;
- Each mobile phase represents a mixture of two pure liquids, such as:
- A-water: tetrahidrofuran = (80:20) (v/v)
- B-acetonitril: tetrahidrofuran = (80:20) (v/v)
- Flow of ternary mixture of mobile phase: 1 mL/min.

Liquid-chromatographic separations

In diagram 1 is represented the program of ternary gradient of mobile phase and in the details from diagram 2 are put the liquid-chromatograms of the two synthetically mixtures. The program of mixing of two mobile phases is represented by the equation that is used to calculate the percentage of mobile phase B, in every moment of the fragment of time

$$\%B = 100 - \%A = k \cdot t^p \quad (1)$$

where: k is the slope of the curve which $\%B$ follows, t represents the duration of every fragment of the program (expressed in minute) and p represent the exponent.

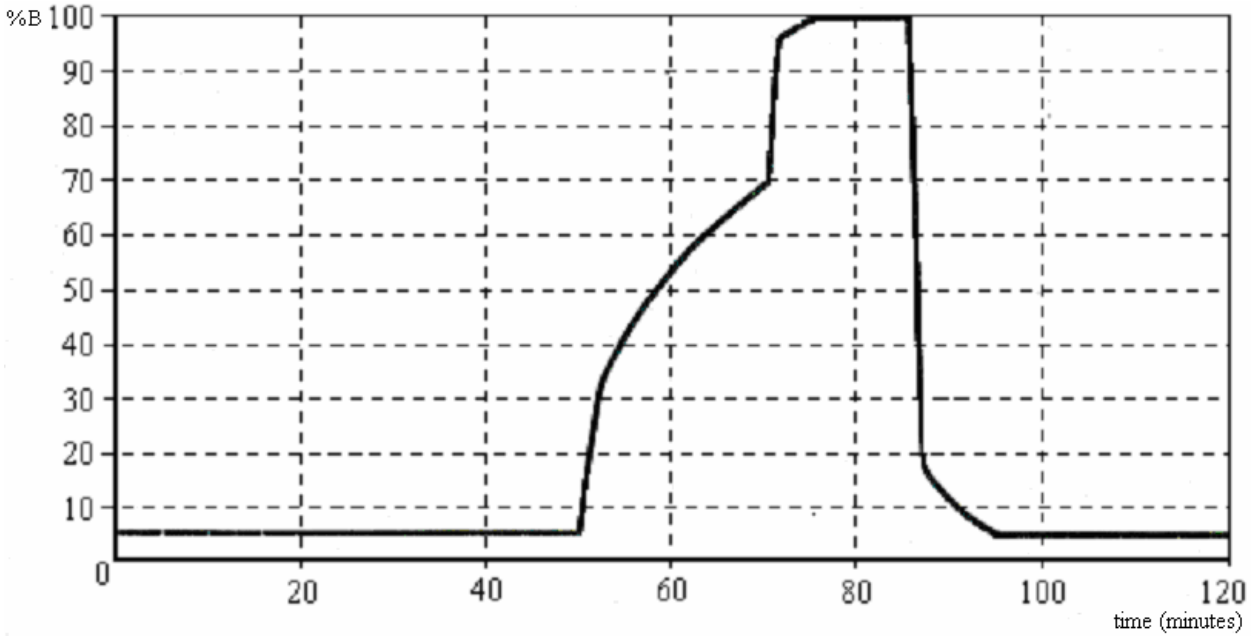

Figure 1. The program of the ternary mixture of the gradient mobile phase

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The synthetically mixture MSI contain the standard solutions of the 2,4-DNPH-ones mentioned, in proportion with the volume 1:1 (2,4-DNPHAA: 2,4-DNPHD), the proportion of two mass substances being 1:2.

Following simultaneously the diagram presented in figure 1 and chromatogram a) from figure 2, considerations upon the successive stages of separation can be formulated.

The fragment 1 of the gradient program of mobile phase (50 min, %*B* = 5%) has the role to provide concentration of components in partially superpose zones on the stationary phases layer from the separation column. To assure the increase the efficiency of this separation process, the following four experimental variants were verified.

1) The substitution of the mobile phase mixture marked A, with pure water. There are obtained the chromatograms with deformed signals; the geometry of these signals are due to the difficult transfer of the 2,4-DNPH-ones molecules from nonpolar stationary phase to polar mobile phase.
2) The utilization of mobile phase marked A, in witch water and THF are presented in another proportion. The utilization of particular parameters was necessary for the next fragment time, but this was not followed by an improving of separation. So the initial mixture of water and THF (80:20, v/v) may be considered the best both from point of view of efficiency separation and the diminution of the degraphitisation process, too; the seals of the piston are made by graphitized teflon, and the mobile phase which contain a high percentage of water favor the process of degraphitisation.
3) The modification of mobile phase *p*H. The mobile phase A was brought to *p*H = 2 (using 80% formic acid solution) or to *p*H = 7.6 (with 10^{-2}m K_2HPO_4 solution). Each of these two experimental variants had the same results: the diminishing of the resolution efficiency. It was formulated the conclusion that the A mobile phase which contains 20% THF in water – with *p*H = 5.6 – offer the most efficient preliminary separation of two 2,4-DNPH-ones.

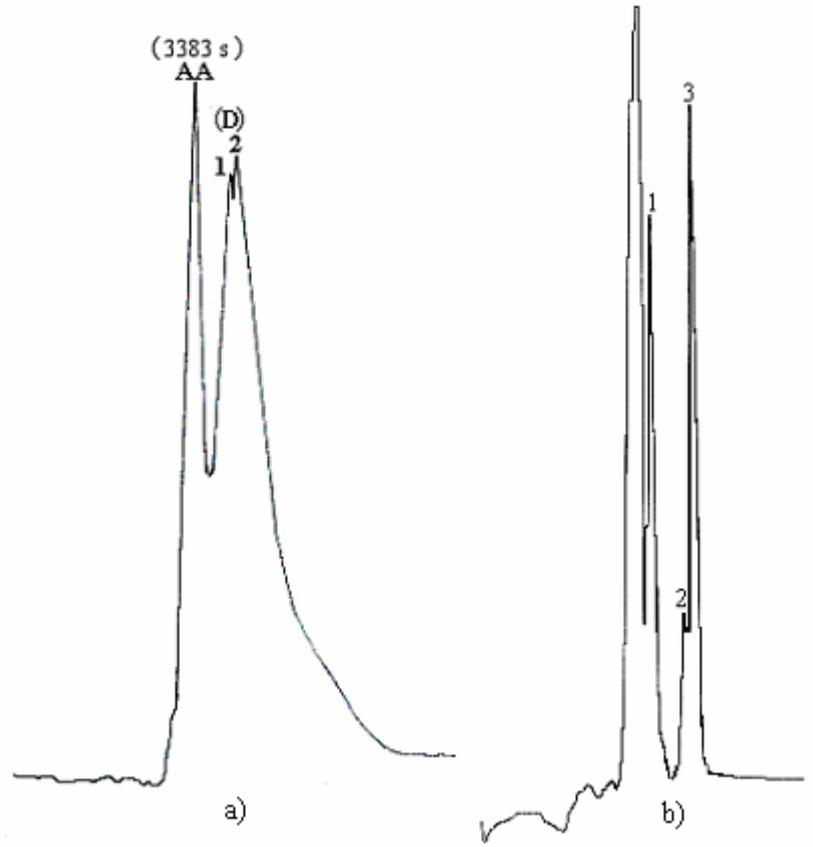

Figure 2. The chromatograms of synthetically mixtures
a) synthetically mixture SM_I; b) synthetically mixture SM_{II}

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4) The complete elimination of fragment 1 of gradient program of elution. After a conditioner of the stationary phase, which correspond to zero fragment, then were tested the variants which the percentage of B mobile phase can have other values and other profile, with the proper size of fragment; the result were unsatisfied, the need of fragment 1 being imposed by the nature and behavior of 2,4-DNPH-ones mixtures.

The fragment 2 (20 min, %B = 5→70, p = 0.4) has the roll to reduce dimension of the concentration zones of the components on the stationary phase layer to provide chromatographic signals individualization. The increase of mobile phase B percentage has as the effect to provide a preponderant mobile phase mixture. As a result, the mass transfer of the components – from the nonpolar stationary phase into a mobile phase with reduced polarization – it is easily produced, the negative roll of local transfer being canceled (Gocan, 2002).

The fragment 3 (5 min, %B = 70→100, p = 0.1) has the roll to generate a mobile phase containing only organic liquid (acetonitrile and tetrahidrofuran).

The fragment 4 (10 min, %B = 100) to fully remove of all the molecular species retained on the stationary phase.

The fragment 5 (15 min, %B = 100→5%, p = 0.1) represents the interval of time during which the composition of mobile phase is remade corresponding the moment of starting program.

In chromatogram a) from diagram 2, the signal for 2,4-DNPHAA is very well individualized. In the same chromatogram, the signal corresponding to 2,4-DNPHD is divided in into two signals that have very similar values of retention time. Excluding the possibility that the 2,4-DNPHD is impure, two aspects of the process of doubling a signal can be taken into consideration.

1) The doubling of the signal due to the difficult mass transfer from the nonpolar stationary phase into a polar mobile phase. This is not the best justification, because the obtained signals have pointed tops, so they characterize two well defined and different concentration zones. In addition, to the difficult mass transfer correspond to flat chromatographic signals, which the integrator can't adequately appreciate.

2) The doubling of signals due to the deficient taking of the mixture volume in the mobile phase circuit (a large time period). This behavior is characteristic to the gas chromatographic separation practiced in extreme conditions (vaporizer at the high temperature, when some chemical species decompose due to the catalytic action of metallic pipes of transport circuit (Piringer and Tătaru, 1969); the valves with multiple ways – used in liquid-chromatography – don't generate such behavior.

Taking in consideration that in beer the two carbonyl compounds – diacetyl and acetaldehyde - are in mass proportion of 1:100 (Berzescu et al., 1981), the synthetically mixture named SM_{II}, similar to this natural model, has been realized. In the detail b) from diagram 2 there is the chromatogram of this synthetically mixture, obtained with the same ternary mixture of the gradient mobile phase. In the chromatogram from the detail b), is very well individualized the signal of 2,4-DNHFAA.

This is followed by three signals; two of them are typical and sensible equal as areas, and one is 3-4 times smaller, and flanked by the other two. The conclusion that (in the chromatogram of the mixture which contains small quantities of 2,4-DNHFD) can be formulate: the three signals correspond to his isomer structures that we know, as in figure 3.

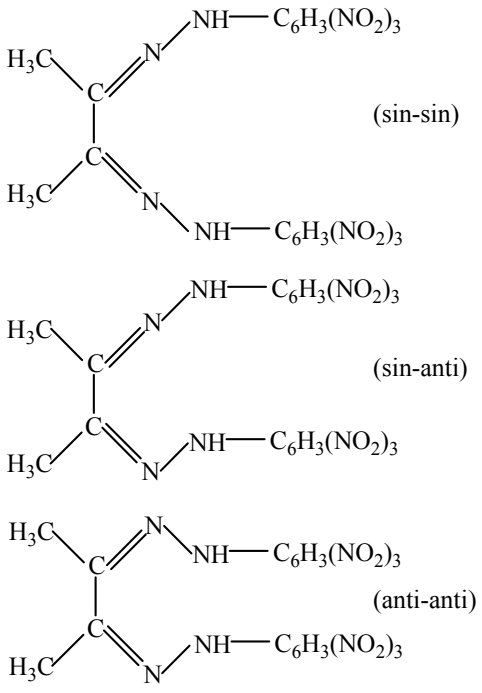

Figure 3. The possible isomer structures of 2,4-DNHFD

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The level of actual endearment does not allow to fix the succession in which are eluted the three isomer structures corresponding to 2,4-DNHFD. This instance, it is not possible to specify the identity of the three signals. Moreover, it is not possible to make quantitative appreciations referring to the relation between the areas of the three signals and the quantity of 2,4-DNHFD which generate them.

4. Conclusions

The using of the gradient ternary mobile phase offers:

- The individualization of the signal for 2,4-DNPHAA, that might be dosed, using this method;
- The separation of the isomer structures of 2,4-DNFHD;
- The two possible isomer structure (sin and anti) of 2,4-DNFHAA do not separate, being simultaneously eluted.

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