MIXTURES WITH GRADIENT OF MOBILE PHASES UTILIZED IN HPLC SEPARATIONS OF 2.4-DINITROPHENYLHIDRAZONES PROVIDED BY INFERIOR CARBONYL COMPOUNDS

Gheorghe ZGHEREA[1], Cristina STOIAN[1], Sandu PERETZ[2]

[1]University "Dunarea de Jos" of Galati, Department of Chemistry,
111 Domneasca Street, 800201 Galati, Romania, gzgherea@chem.ugal.ro, cstoian@ugal.ro,
[2] Institute of Physical Chemistry "I. G. Murgulescu", Department of Colloids,
202 Spl. Independentei, 060021, Bucharest, Romania, peretz@icf.ro

Abstract. *Many mixtures of small quantities of carbonyl compounds are present in foods, concerning sensorial qualities (aroma and fragrance). The inferior carbonyl compounds (C_2-C_4, boiling point <100°C) – mono and dicarbonyl – can be identified and their concentrations can be measured, after being separated by distillation on water bath. They are transferred into a strongly acid solution of 2.4-dinitrophenylhidrazine (2.4-DNPH), generating a mixture of insoluble 2.4-dinitrophenylhidrazones (2.4-DNPH-ones). The 2.4-DNPH-ones are organic compounds with weak polarity, solids, crystallized, yellow and water insoluble, but soluble in organic solvents. The mixture of 2.4-DNPH-ones may be separated by liquid chromatography, using HPLC the reverse phase mechanism [1-3]. This paper contains experimental and theoretical considerations on the means of separation through liquid chromatography of two models and a natural mixture containing 2.4-DNPH-ones provided by inferior carbonyl compounds; to obtain decisive results, in the model mixtures 2.4-DNPH-ones provided by carbonyl compounds having three (acetone and propanal) and four atoms of carbon (isobutylaldehyde) were introduced.*

Keywords: *acetaldehyde, diacetyl, 2.4-dinitrophenylhidrazone, reverse phase, low polarity, gradient of mobile phase*

Introduction

In many cases, for the foods obtained by fermentation, it is very important to know the concentration of diacetyl and acetaldehyde. According to literature, beer contains diacetyl and acetaldehyde in the ratio 1:100, in mass units. The interest on diacetyl concentration requires especially analyticcal conditions. It is possible to solve that problem by HPLC for the mixtures of 2.4-DNPH-ones provided by inferior carbonyl compounds. HPLC can make a good separation only for the model mixtures of 2.4-DNPH-ones; the molecules of inferior carbonyl compounds have properly physical and chemical behavior, assuring a good separation [4]. The difficulty appears for the natural mixtures concerning acetaldehyde and diacetyl; the two carbonyl compounds – and 2.4-DNPH-ones – have similar physical behavior. In addition, their mass ratio creates great problems in liquid-chromatographic separation. To have a very good analytical performance, it is necessary to use liquid chromatography separation with gradient of mobile phase [5]; in addition, separation columns with gradient of stationary phase are used in this paper.

Materials and methods

Solvents and mixtures of mobile phases

The acetonitrile was utilized to solve the pure 2.4-DNPH-ones. For liquid-chromatographic separations, two similar mixtures with gradient of mobile phase, according with the programs abbreviated $MGMP_I$ and $MGMP_{II}$, containing bidistillated water (2 □S·cm^{-1}) and methanol pro chromatography (Merck) were utilized.

The model mixtures of 2.4-DNPH-ones

The pure 2.4-DNPH-ones – yellow powders – were obtained in our laboratory, using a strongly acid solution of 2.4-DNPH and chemical pure carbonyl compound; by synthesizing: 2.4-DNPHAA (acetaldehyde), 2.4-DNPHD (diacetyl), 2.4-DNPHA (acetone) and 2.4-DNPHiBA (isobutylaldehyde). In acetonitrile (Merck, pro liquid chromatography), $5 \cdot 10^{-4}$ M solutions were obtained. By controlled mixing we obtained two model mixtures, abbreviated MM_I and MM_{II}, considered as approximate models of natural mixtures; the ratio between the quantities 2.4-DNPHAA and 2.4-DNPHD is higher to one, in each mixture.

The beer's mixtures of 2.4-DNPH-ones

The carbonyl compounds from beer were separated by distillation on water bath and transferred into a strongly acid solution of 2.4-DNPH; the precipitates form a natural mixture of 2.4-DNPH-ones. They are isolated by filtration, washed with pure water, dried and solved in acetonitrile.

Apparatus

For the separations we utilized Pye Unicam Philips liquid chromatograph, equipped with: an installation for degasing of mobile phase (by refluxing) [7], gradient programmer for mobile phase (type LC-XPD, able to mix two different liquids), separation columns with gradient of stationary phase, installation for column thermostat control, electronic integrator (type DP101, Spectra-Physics) and potentiometer recorder (type PM8251, Philips). The mixing program of liquids has nine segments of time, g=1-9; each timing segment has independent dimension of 0-99 minutes. Every moment of analytical separation, the value of B, the percent of the second component in the mixture of mobile phase (A + B=100%), is

$$\%B = k \cdot t^n \qquad (1)$$

where:

- t – dimension of timing segment (minutes),
- k – slope of curve (describes the evolution of B value on the t segment),
- n – exponent, with values 0.0-9.9 (describes the geometry of mixing curve).

Conditions of chromatographic separations

- sample volume: 10 □L;
- separation column: L = 25 cm, □□= 4.6□ mm;
- stationary phase: Spherisorb 5 ODS, with gradient of stationary phase;
- 37.5ºC, the temperature of separation column:
- LC UV detector, □ = 365 nm;
- flow rate: 1mL·min^{-1};
- eluate: a controlled mixture of A-methanol and B-water, accordingly to two programs – $MGMP_I$ and $MGMP_{II}$ – achieved by the LC-XPD chromatographic module.

The liquid-chromatographic separations

Both categories of liquid-chromatographic separations were carried out on the same column, at the same temperature, changing only the program of mixing liquids in binary mixture of mobile phase.

Results and Discussion

The model mixtures (MM_I and MM_{II}) and a natural mixture from beer were separated by liquid-chromatography, using a mechanism of reverse phase.

The mixtures of mobile phases have the same initially composition, with 45% water. In this instance, the 2.4-DNPHAA will be eluted before the 2.4-DNPHD. In this way we guaranteed the maximum difference between the values of retention times for the mentioned 2.4-DNPH-ones and a preliminary separation of the 2.4-DNPH-ones provided by aliphatic carbonyl compounds with three and four atoms of carbon. The model mixture MM_I contains four 2.4-DNPH-ones, in the following ratio of volume:

2.4-DNPHAA: 2.4-DNPHD: 2.4-DNPHA: 2.4-DNPHiBA = 2:1:1:1.

The first program of binary mobile phase, $MGMP_I$ (32 minutes, figure 1), contains four time segments; figure 2 shows the chromatogram of model mixture MM_I, obtained with $MGMP_I$, with the retention times (seconds, in brackets).

According to $MGMP_I$, the separation begins with a mobile phase having 45% water; the mixture of mobile phase has a higher polarity. This mixture is hold during the first time segment, g = 1 (t = 12 minutes), assuring a better resolution between the peak of 2.4-DNPHAA (844 s) and the peak of 2.4-DNPHD (918 s). The weak polar molecules of two 2.4-DNPH-ones are strongly retained at the no polar stationary phase. Therefore, the longitudinal diffusion of concentrated zone is higher.

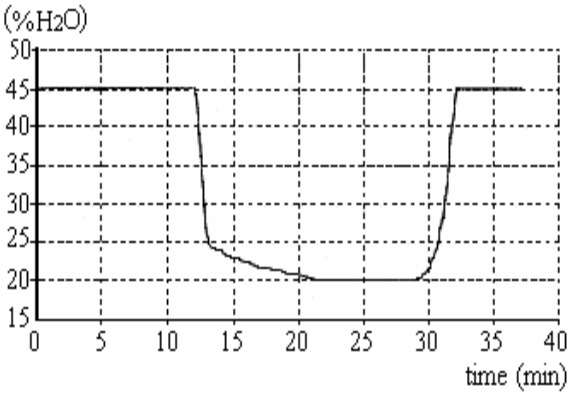

Figure 1. The program of $MGMP_I$

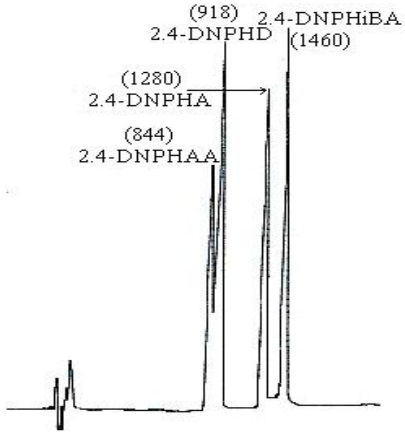

Figure 2. The chromatogram of model

Simultaneously, in the stationary phase 2.4-DNPH-ones provided by acetone and isobuthanal are strongly retained.

On the second timing segment, g = 2 (t = 10 min, n = 0.1), the percent of water will be reduced at 20%, thus the polarity of mobile phase subsides; the 2.4-DNPHAA and 2.4-DNPHD (more soluble in organic solvent) diffuse in the mobile phase and will be transported to the end of separation

column. On the third timing segment, g = 3 (t = 5 min, n = 1), the percent of water is constant (20%), to assure the best separation of 2.4-DNPHA and 2.4-DNPHiBA. The last timing segment, g = 9 (t = 5 min, n = 5.5), is meant to recondition stationary phase, for a new separation; the intermediate timing segments, g = 4-8, are inactive.

The second program of binary mobile phase, $MGMP_{II}$ (40 minutes, figure 3), contains four time segments too; figure 4 shows the chromatogram of model mixture MM_{II}, obtained with $MGMP_{II}$. The

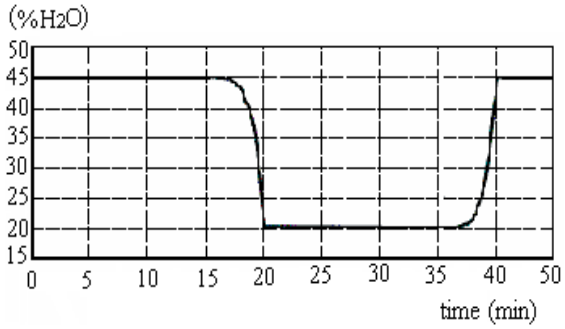

Figure 3. The program of $MGMP_{II}$

As in the previous case, the first timing segment, g = 1 (t = 15 min), the mixing program assures an eluate with 45% water, for the best separation between 2.4-DNPHAA and 2.4-DNPHD; the polarity of mobile phase is higher (the molecules of 2.4-DNPH-ones are strongly retained on the slow polar stationary phase).

The second timing segment, g = 2 (t = 10 min, n = 9.9), the percent of water is reduced at 20%; this value is constant and during the third timing segment, g = 3 (t = 15 min). Subside of mobile phase polarity produces a desorptive process in the stationary phase.

The timing segments, g = 4-8 have not got a specific content. In the last timing segment, g = 9 (t = 5 min, n = 0.1) the initial mixture of mobile phase is quickly rebuilt; at cessation, the analytical system is completely ready for a new separation.

By comparing the chromatograms from figures 2 and 4 we have drawn the following conclusions: the $MGMP_I$ assures

model mixture MM_{II} contains the same 2.4-DNPH-ones as MM_I, in the ratio of volume:

2.4-DNFHAA: 2.4-DNFHD:2.4-DNFHA: 2.4-DNFHiBA = 4:1:5:5.

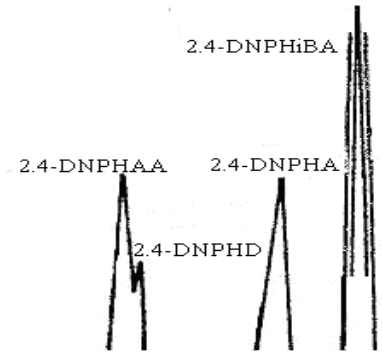

Figure 4. The chromatogram of model mixture abbreviated MM_{II}

a better separation for the 2.4-DNPHAA and 2.4-DNPHD; for the 2.4-DNPHA and 2.4-DNPHiBA, the resolutions being comparable.

Figure 5 shoes a chromatogram of a natural mixture of 2.4-DNPH-ones, for the inferior carbonyl compounds of beer. The liquid-chromatographic separation is on the same column and the eluting program is $MGMP_I$. The peaks for 2.4-DNPHAA and 2.4-DNPHD are in the central zone of chromatogram.

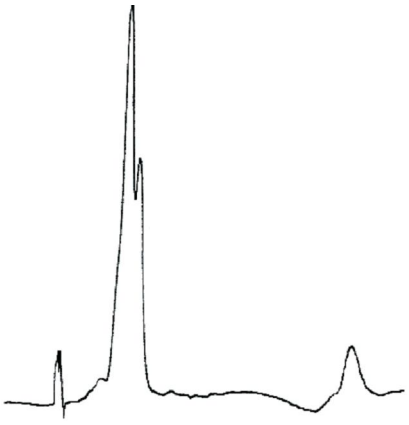

Figure 5. The chromatogram of natural mixture of 2.4-DNFH-ones

Using the external standard quantitative method, for the chromatographic peak of 2.4-DNPHAA, we obtained 14.75 $mg \cdot L^{-1}$ as concentration of acetaldehyde in beer; the value complies with literature data [6], assuring a higher satisfaction to the analyst operator.

Using the same quantitative method, the surface of chromatographic peak of 2.4-DNPHD gives a value of 2.5 $mg \cdot L^{-1}$ as concentration of diacetyl in beer; this value is higher, bringing no satisfaction to the analyst operator. In beer, the normal value of diacetyl concentration is 0.01-0.2 $mg \cdot L^{-1}$; 0.15 $mg \cdot L^{-1}$ is the threshold value [6].

Conclusions

On the basis of these experiments, the following conclusions may be drawn:

1. We established two optimal programs for providing mixtures of binary eluate; according to them, two mixtures of binary phase that assure liquid-chromatographic separation, with a good resolution are obtained for the etalon mixtures of 2.4-DNPH-ones provided by the aliphatic carbonyl compounds with a number of 2-4 atoms of carbon.
2. The binary mixtures of mobile phase, obtained by the programs $MGMP_I$ and $MGMP_{II}$, assure a better separation of model mixtures which contain derivate compounds of inferior carbonyl compounds; in these model mixtures the ratio between 2.4-DNPHAA and 2.4-DNPHD is higher than one (in mass units), but very low.
3. In the case of natural mixtures of 2.4-DNPH-ones, similarly with model mixtures, the binary mixtures of mobile phase offer only partially analytical satisfaction. Thus, the figure 5 shows obviously the dominant peak of 2.4-DNPHAA. By using the surface value for quantitative appreciation by external standard method, we obtained experimental values accordingly to literature, namely 15 $mg \cdot L^{-1}$ acetaldehyde [6]. In the same chromatogram, the peak of 2.4-DNPHD is the second, but it is on the tailing peak of the first one. As for the natural mixture of 2.4-DNPH-ones one may notice the following aspect: if literature offers real concentration values for the two carbonyl compounds, the accuracy of analytical system will be justified by high value, ~100, of the ratio between the quantities of acetaldehyde and diacetyl. In this instance, the low peak of 2.4-DNPHD appears as a tailing peak on the high peak of 2.4-DNPHAA, thus, its surface is higher than the normal one; the mistake value of surface chromatographic peak became a source of mistake for concentration value.
4. Each chromatogram – figures 2, 4, and 5 – contains any peaks with lower values of retention time; it is the peaks for solvent.

References

1. SIMION GOCAN, Cromatografie de Înaltă Performantă, Partea a II-a, Cromatografia de Lichide pe Coloane, Editura RISOPRINT, Cluj-Napoca, Romania, 2002, 173-183 pp
2. DOUGLAS A. SKOOG, Principles of Instrumental Analysis, Third edition, Saunders College Publishing, 1985, 801-815 pp
3. CANDIN LITEANU; SIMION GOCAN; T. HODISAN; H. NASCU, Cromatografia de Lichide, Editura Stiintifică, Bucuresti, Romania, 1974, 156-157 pp
4. RADU BACALOGLU; CAROL CSUNDERLIK; LIVIUS COTARCĂ; HANS-HORST GLATT, Structura si Proprietătile Compusilor Organici, vol I, Editura Tehnică, Bucuresti, Romania, 1985, 116-127 pp
5. GORT S.M., HOGENDOORN E.A.; DIJKMAN E.; VAN ZOONEN P.; R. HOOGERBRUGGE, The Optimisation of Step-Gradient Elution Conditions in Liquid Chromatography, Chromatographia, vol. 42, No ½, ian 96, 17-24 pp
6. BIERANALYSE 9.11 Bestimmung der Gärungsneben produkte in Bier, 246-250 pp
7. GHEORGHE ZGHEREA, Installation to Continuously Degassation by Refluxing of Mobile Phases for HPLC, Annals of West University of Timisoara, Romania, series Chemistry 12 (3) (2003) 1157-1160 pp