ORIGINAL RESEARCH PAPER

PARTICULARITY ON LIQUID CHROMATOGRAPHIC SEPARATION FOR AN EQUIMOLAR MIXTURE OF 2,4-DINITROPHENYLHYDRAZONES PROVIDED BY ACETALDEHYDE AND DIACETYL♦

Gheorghe Zgherea[1]*, Cristina Stoian[1], Sandu Peretz[2]

[1]*University "Dunărea de Jos" of Galati, Faculty of Sciences, Department of Chemistry, 111 Domneasca Street, 800201 Galati, Romania*
[2]*Institute of Physical Chemistry "I. Murgulescu", Department of Colloids, 202 spl. Independentei, 060021, Bucharest, Romania*

Corresponding author: gzgherea@ugal.ro

Abstract: An equimolar mixture of 2,4-dinitrophenylhidrazones (2,4-DNPH-ones) providing by acetaldehyde and diacetyl has been analyzed by liquid-chromatographic separation, using the mechanism of partition with reverse phase. As mobile phase are utilized various binary mixtures, containing water (0 – 45%) and methanol. By the experimental studies were identified four domains of behavior and two optimal binary mixtures, containing 25 and 45% water respectively. The peaks are characterized by values of retention time and by position. The separation processes were appreciated by difference between the retention times of peaks; if the percent of water increase, the values of retention times is higher, heaving a maxim value when the percent of water is 25%. For a mixture of mobile phase with 35% water and 65% methanol, the difference between retention time's values became null; the behavior is comparable with those of pure methanol, but for higher values of retention times. When the percent of water is 45%, the difference between the retention times is also maxim, associated with a change of peaks positions.

♦ Paper presented at the 6th edition of *Colloque Franco-Roumain de Chimie Appliquée*, **COFrRoCA 2010**, 7-10 July 2010, Orléans, France

INTRODUCTION

Any foods obtained by fermentation have small quantities of carbonyl compounds. They have a very important contribution to the flavor and the fragrance; between these compounds there are acetaldehyde and diacetyl. On consider that the diacetyl is the vicinal diketone with a very important contribution at the sensorial properties of beer. In addition, its concentration in beer $(0.01 - 0.2$ mg.$L^{-1})$ is a reference values for the level of oxidative process during preparative operations. That explains the major preoccupations on the physical-chemistry methods to identification and to dose beer's diacetyl. Between these methods there is the distillation of carbonyl compounds and transfer in a strong acid solution of 2,4-dinitrophenylhidrazine (2,4-DNPH); the mixture of insoluble precipitates of 2,4-DNPH-ones is separated by filtration, washed with bi distilled water, dried and solved in organic solvent (methanol, acetonitrile, THF); the analytical process is finished by an appropriate liquid-chromatographic separation.

The described analytical method may be use for any mixture of 2,4-DNPH-ones. The 2,4-DNPH-ones are solid substances, yellow, soluble in organic solvents [1]. The literature doesn't have specific reference about their polarity [2, 3]; may be compared only the polarity values for carbonyl compounds providers.

After their behavior, the mixtures of 2,4-DNPH-ones may be analyzed by HPLC, using the mechanism of partition with reverse phase; may be used a slightly polar stationary phase (octadecysilan) and a mixture of mobile phase containing water (strongly polar solvent) with a slightly polar organic solvent [4 – 7]. The non polar stationary phase assures a strongly retaining of slightly polar molecules of 2,4-DNPH-ones. The non polar mobile phase provides a better solubilization of 2,4-DNPH-ones, assuring a better mass transfer between the mobile and stationary phase; thus, the peaks are symmetrically, narrow and with small values of retention times. The polar mobile phase provides a small solubilization and a hard mass transfer between the phases; the peaks are asymmetrically and with higher values of retention times. Therefore, a higher resolution between peaks is assured only by optimal mixture of mobile phase, when the difference between retention time values is maxim, for the adjacent peaks.

In the upper case, analytical difficulties are generating by the similar behavior of 2,4-DNPH-ones providing by diacetyl (2,4-DNPD) and acetaldehyde (2,4-DNPHAA). It has to be established the optimal mixture of mobile phase (with water and methanol) to assure the best separation for an equimolar mixture (a model synthetic mixture) of the two 2,4-DNPH-ones.

MATERIALS AND METHODS

Etalons, equimolar mixture, solvents, mobile phase and liquid chromatographic separations

The 2,4-DNPH-ones etalons, provided by acetaldehyde and diacetyl, were synthesized in our laboratory, starting from pure carbonyl compounds (Merck) and solution of 2,4-DNPH (LOBA-Chemie-Austranal), by simply mixing at room temperature; the

preparative process has a good yield, being perfectly reproducible. The 2,4-DNPH-one is water insoluble and forms an insoluble precipitate; this must be separated by filtration, washed with bi distilled water until the effluent is neutral, and dried. In acetonitrile (Merck) was obtained a standard solution 10^{-4} M, for each 2,4-DNPH-one. The equimolar mixture was obtained by mixing the two standard solutions in ratio 1:1 (v:v); in the equimolar mixture each 2,4-DNPH-one has $5 \cdot 10^{-5}$ M concentration. The bi distilled water (2 $\mu S.cm^{-1}$) was prepared in our laboratory with GFL Glass Water Stills type 2302.

The mobile phases are methanol and nine binary mixtures containing bi distilled water and methanol (Merck, for liquid chromatography); the percent of water has the value 0-45%, controlled by LC gradient programmer module.

Apparatus

For the separations was used a LC-XPD Pye Unicam Philips liquid chromatograph which includes: solvent metering system (two reservoirs for mobile phases, the module of controlled mixing type LC gradient programmer, high pressure pump type Spectra Physics 100A), Rheodyne valve 7125 (for the mixture sample), liquid chromatographic column (Spherisorb 5ODS with gradient of stationary phase, 25 cm length and 4.6 mm inner diameter) conditioned with a module of thermostatation with a water recirculation bath, LC-UV detector, electronic integrator for chromatographic peak (type DP101) and potentiometer recorder (type PM8251).

Experimental conditions

The equimolar mixture of 2,4-DNPH-ones was liquid-chromatographic separated, under the following conditions:

- 10 μL, volume of mixture;
- 37.5 ºC, temperature of separation column, considering that the higher values of temperature has a benefic action on the mass transfer process of 2,4-DNPH-ones between the two immiscible phases;
- $\lambda = 365$ nm, incident optical signal;
- 1 $mL.min^{-1}$, flow rate of mobile phase.

Liquid chromatographic separations

Identical volumes of equimolar mixture were separated by HPLC, using the binary mixtures of mobile phases.

RESULTS AND DISCUSSIONS

Ten chromatograms were obtained. Each chromatogram has two distinctly chromatographic peaks; the identity of signals was verified by addition method.

Results

The behavior of eluent (methanol or mixtures of mobile phase) was appreciate by difference between the values of retention times of 2,4-DNPH-ones, defined as:

$$\Delta RT = (RT_{2,4DNFHAA} - RT_{2,4DNFHD}) \ [s] \tag{1}$$

The ΔRT [s] values depend on the molecular structure of 2,4-DNPH-ones and on the phenomena that are produced during the liquid chromatographic separation process, being an expression of the relation between their partition coefficients values.

The ΔRT [s] values are graphically represented against the mobile phase polarity, defined by the percent of water value in the mixture of mobile phases. On obtain the diagram from Figure 1, with delimitation of four special domains behavior of eluent mixtures.
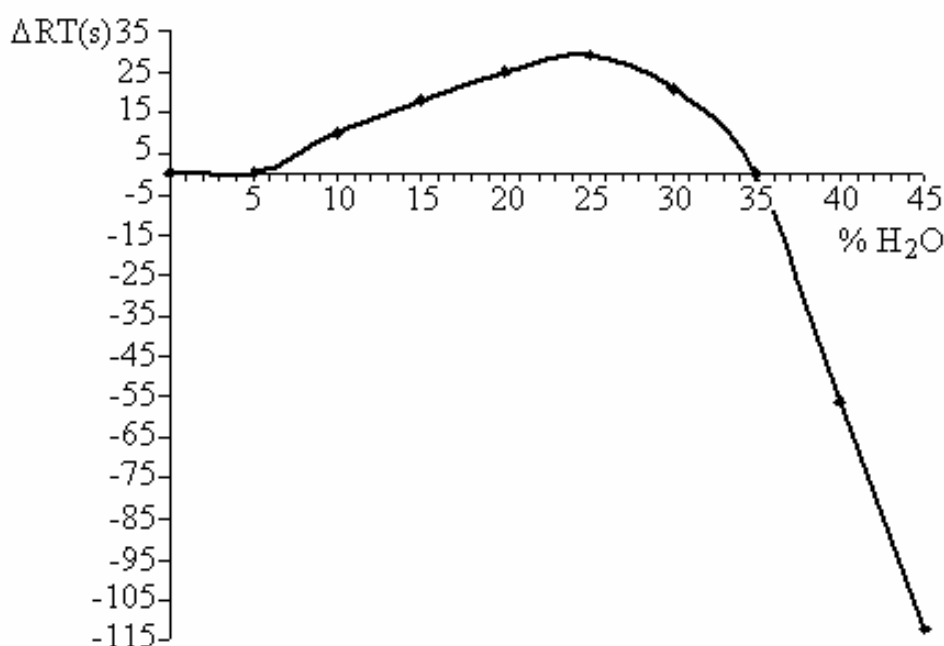

Figure 1. *Diagram of ΔRT (s) values against the polarity of mobile phase mixture*

Need to explain the structure and behavior of two 2,4-DNPH-ones, beginning from structure of provider's carbonyl compounds. To explain the difference between behaviors, need to consider physical behavior of two species of molecules: mass, volume and polarity. Can be considerate that the asymmetric molecule of acetaldehyde provide an asymmetric 2,4-DNPH-one (2,4-DNPHAA, $M_{2,4\text{-DNPHAA}}$ = 224 g.mol^{-1}); that

are slowly more polar than the biggest and symmetric molecules of 2,4-DNPHD ($M_{2,4\text{-DNFHD}}$= 446 g.mol^{-1}), considering that the diacetyl has a symmetrically molecule.

To explain the four distinct behaviors of the mobile phase mixtures, need to considerate the contributions of the essential processes which there are on the stationary phase: the zone of concentration, for each 2,4-DNPH-one, is in extension. This process affects the retention time values, thus and the ΔRT values.

On the stationary phase there are, simultaneously, three distinct phenomena: the longitudinal diffusion, the turbulent diffusion and the local non equilibrium (resistance at the mass transfer process, between the two immiscible phases). On may be considered that only two distinct phenomena have the most important contribution at the concentrated zone extension. The stationary phase have only spherically and equal particles and the mobile phase flow at the high pressure (> 100 bar); in this instance, the contribution of turbulent diffusion is insignificant for the general process.

Discussions

Follow-up are presented the behavior of mobile phase, against the water percent in the mixture. Because the water molecule is more polar than methanol molecule, can be considered that the mobile phase mixture is more polar as the water percentage is higher; many important and diverse applications, using mixtures that have another organic compounds miscible with water, as dioxan, acetonitrile and THF, are mentioned [8 – 10].

The domain 0-5% water (the mobile and stationary phase are non polar too)

The polarity of mixture is very low, as the stationary phase as. The interaction between the 2,4-DNPH-one's molecules and the stationary phase must be higher, but the most important part of molecules is dissolved in the non polar liquid mobile phase. Local non equilibrium is completely absent, but the phenomenon of longitudinal diffusion is very weak, generating a simultaneously elution for the molecules of the two 2,4-DNPH-ones. The two molecular species are characterized by identically and low partition coefficients and retention time values, thus ΔRT = 0 s, for low values of retention times.

The domain 5-25% water (the molecules' behavior is explained by their dimensions)

Increase the polarity of the eluent by water percent; this value produces an increase of interaction between the 2,4-DNPH-one's molecules and non polar stationary phase. Simultaneously, increase the intensity of local non equilibrium. The smaller and asymmetrically molecules of 2,4-DNPHAA, weak polar, are characterized by lower values of diffusion coefficient from the stationary phase to mobile phase, being retained more intense. The lower diffusion process in mobile phase produces an intense process of longitudinal diffusion, thus an extension of zone; the 2,4-DNPHAA molecules generate large peaks, symmetrically, but with high values of retention time. The bigger and symmetrically molecules of 2,4-DNPHD, non polar or slightly polar, are characterized by higher values of the diffusion coefficient from the stationary to mobile

phase. Due to their physical dimension, these molecules have lower values of the longitudinal diffusion coefficient in the mobile phase, generating narrow and symmetrically peaks, but with low values for the retention time. Like the different behavior, the relation between the partition coefficients is:

$$k_{2,4-\text{DNPHAA}} > k_{2,4-\text{DNPHD}} \qquad (2)$$

The retention time values there are in the same relation, thus the ΔRT values are positive; the mobile phase with 25% water represents the binary eluent mixture that generates two chromatographic peaks with a maxim value of difference between retention time values, $\Delta RT = 29$ s.

The domain 25-35% water (the molecules' behavior is explained by their low polarity)

For higher values of water percent, the polar eluent produces a higher interaction of molecules with the particles of stationary phase, increasing the values of retention times. The phenomenon of longitudinal diffusion becomes important. In addition, becames very intense the process of local non equilibrium (resistance at mass transfer from the stationary phase to the mobile phase); the eluent mixture with a high percent of water undertakes very hard the organic molecules of 2,4-DNPH-ones. Conclusively, it increases the dimensions of the concentration zone and the values of the retention times. The values of retention times have an unequal increasing, with the tendencies to become identically (at higher values of water percent), thus the molecular species have the same behavior; the ΔRT values subside. If the mobile phase has 30% water, the corresponding value $\Delta RT = 18$ s.

For the upper limit of this domain, 35% water, the two 2,4-DNPH-ones are characterized by identical values of partition coefficients:

$$k_{2,4-\text{DNPHAA}} = k_{2,4-\text{DNPHD}} \qquad (3)$$

Consequently, are generated equal and higher values of retention times, and ΔRT values became null.

In all the mixtures of mobile phase with 25-35% water (plus polar mixtures) the peak's resolution subsides.

The domain 35-45% water (large value of mobile phase polarity)

The eluent mixtures become very polar and produce an intense interaction between the non polar molecules and the particles of stationary phase, non polar too; the behavior of molecules may be justified again by the slowly different values of polarity. In this case, the values of partition coefficients increase very much; for the phenomenon of mass transfer from stationary phase to mobile phase there is a similarly evolution, extending the zones of concentration. Simultaneously, the retention times values increase with proper rate. On observe that the relation between the values of partition coefficients becomes:

$$k_{2,4-\text{DNPHAA}} < k_{2,4-\text{DNPHD}} \qquad (4)$$

A similar relation exists between retention times values, thus ΔRT values become negative. Because the water percent is very high, the 2,4-DNPH-ones molecules transport is affected by local non equilibrium and by high values of coefficient of longitudinal diffusion. The symmetric and non polar molecules of 2,4-DNPHD are strongly retained on stationary phase particles. The asymmetric and weak polar molecules of 2,4-DNPHAA passes soft from the particles of stationary phase in the liquid polar mobile phase. The polar mixture mobile phase provides an inversion of zones position on stationary phase, thus a position's inversion of the peaks in chromatogram. The eluent mixture with 40% water assures a separation process characterized by a good resolution between peak, ΔRT = - 56 s; if the percent of water becames 45%, the process of chromatographic separation has a better resolution, ΔRT = -112 s.

CONCLUSIONS

On the experimental results may be formulated the following conclusions:
1. Using a binary isocratic mixture, containing methanol and water (0-45%, v/v), the equimolar mixture of 2,4-DNPHAA and 2,4-DNPHD may be separated by liquid-chromatography with reverse phase; the resolution of chromatographic peaks depends by the polarity of the mobile phase.
2. The similarly behavior of 2,4-DNPH-ones may be described by the structural particularity of the carbonyl compounds provider: dimension, symmetry, solubility in solvents and, especially, polarity.
3. The succession of concentrated zones on the stationary phase, thus the positions of peaks in the chromatogram is dependent of the mobile phase polarity; the retention time values of two 2,4-DNPH-ones are higher for larger values of the mobile phase polarity.
4. Higher values of the mobile phase polarity provide a change of the chromatographic signal position, thus the inversion of peaks positions in the chromatogram.

REFERENCES

1. Zgherea, G.: Synthesis and Comparative Characterization of Eight 2,4-dinitrophenylhydrazones, *The Annals of „Dunărea de Jos" University of Galaţi*, Romania, **1999**, **VI**, 4-9;
2. Lide, D.R. ed.: *CRC Handbook of Chemistry and Physics,* Internet Version, **2005***,* CRC Press, Boca Raton, FL, available at: http://www.hbcpnetbase.com;
3. Zahn, C.T.: *The Dielectric Constant of Dichlorethane, Dibromethane, Chlorobromethane, and Diacetyl and the Phenomenon of Free Rotation*, The American Physical Society, **1932**, available at: http://link.aps.org/abstract/PR/v40/p2;
4. Liteanu, C., Gocan, S., Hodisan, T., Nascu, H.: *Cromatografia de lichide* (*Liquid Chromatography*, in Romanian), Ed. Ştiinţifică, Bucharest, **1974**, 156-157;
5. Skoog, D.A.: *Principles of Instrumental Analysis*, 3rd ed., Saunders College Publishing, **1985**, 801-815;

6. Gocan, S.: *Cromatografie de înaltă performanţă, Partea a II-a, Cromatografia de lichide pe coloane* (*High Performance Chromatography, Part II. Column Liquid Chromatography*, in Romanian), Ed. Risoprint, Cluj-Napoca, Romania, **2002**, 173-183;
7. Scott, R.P.W.: *Liquid Chromatography*, 55-62, http: //www.library4science.com/eula.html;
8. Risner, C.H., Martin, P.: Quantitation of Formaldehyde, Acetaldeyde and Acetone in Sidestream Cigarette Smoke by High-Performance Liquid Chromatography, *Journal of Chromatographic Science*, **1994**, **32**, 76-82;
9. Whittle, P.J., Rennie, P.J.: Determination of Formaldehyde in River Water by High-performance Liquid Chromatography, *Analyst*, **1988**, **113**, 665-666;
10. Renon, P., Biondi, P.A., Malandra, R.: HPLC determination of formaldehyde in frozen crustaceans, *Italian Journal of Food Science*, **1991**, **III** (I), 59-62.