COMPARISON OF TWO IMMOBILE PHASES USED IN HPLC ANALYSIS OF ORGANIC ACIDS IN WINE

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Abstract: Organic acids are one of the most important substances in wine, which make major contributions to the composition, stability and organoleptic qualities of wines, responsible of wines' microbiological and physicochemical stability. For searching of optimal conditions for HPLC analysis of organic acids in wine there were investigated two immobile phases, such as: modified C18 type and column, in which separation of the analytes is based on ion exclusion and ion exchange effects. Thus NUCLEODUR C18 Pyramid (*Macherey-Nagel*) is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. The NUCLEOGEL SUGAR 810 H (*Macherey-Nagel*) is packed with a sulphonated spherical polymer of gel type in the H^+ form. It is specifically designed for the separation of numerous polar organic compounds like organic acids, alcohols, sugar alcohols and sugars.
Key-words: HPLC, separation of polar compounds, organic acids, reversed phase

Introduction

The success of wines produced depends on careful attention in controlling the vinification process. Organic acids make major contributions to the composition, stability and organoleptic qualities of wines. Their preservative properties also enhance wines' microbiological and physicochemical stability [1].

Obviously, it's necessary to have a reliable and simple method of identification of some important wine's indexes, which will help to improve and intensify the control over the wines quality during the making process and storage.

Modern scientific methods of analysis and control are needed to achieve this purpose.

High performance liquid chromatography (HPLC) is an important analytical tool for separating and quantifying components in complex liquid mixtures. A variety of liquids and stationary phases can be used in liquid chromatographic systems. Thus by choosing the appropriate equipment (i.e. column and detector), this method is applicable to samples with components ranging from small organic and inorganic molecules and ions to polymers and proteins with high molecular weights [2].

Method and Materials

The popularity of chromatography as a method of quantitative analysis is due to the fact that it combines the 2 processes at once. Above all, this is a separation of a mixture of substances, and if the sensitivity of the detector is known, quantification of the separated in the column of individual substances. Thus, in contrast to other analytical methods, chromatography is not necessary that the method of detection was specific to the substance or the class of substances. This method allows to quantify the content of each component without any treatment or difficult pre-sampling process of the test mixture. The advantage

of liquid chromatography consists in the fact that it allows to define the substance at ambient temperature, while the gas-chromatography requires high temperatures at which some substances may be disintegrated, and also allows to determine non-volatile components.

For searching of optimal conditions for HPLC analysis of organic acids in wine there were investigated two immobile phases, such as: modified C18 type and column, in which separation of the analytes is based on ion exclusion and ion exchange effects.

Thus NUCLEODUR C18 Pyramid (*Macherey-Nagel*) is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. It has the next *technical characteristics*: 250x4 mm; special phase with polar endcapping; pore size 100Å; particle sizes 5 μm; carbon content 14 %; pH stability 1–9 [3].

NUCLEOGEL SUGAR 810H (*Macherey-Nagel*) is packed with a sulphonated spherical polymer of gel type in the H^+ form with the next *technical characteristics*: 300x7.8 mm; strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form; particle sizes 7 μm. It is specifically designed for the separation of numerous polar organic compounds like organic acids, alcohols, sugar alcohols and sugars [4]. The separation of the analytes is based on a combination of different mechanism. Ion exclusion and ion exchange effects are dominated, due to the charge of sulfonic acid groups.

For searching of separating possibilities of these phases there was used liquid chromatograph LC-20AD by *Shimadzu* with spectrophotometer SPD-20AV on the wave length 210nm.

Results and Discussion

The columns NUCLEODUR C18 Pyramid (1) (*Macherey-Nagel*) and NUCLEOGEL SUGAR 810H (2) (*Macherey-Nagel*) were used for separating standard mixture of organic acids and real samples of wines.

For the first system there was used 0.2% solution of H_3PO_4 as eluent with flow rate 0.5ml/min. For the second system – 10mM H_2SO_4 with the same flow rate 0.5ml/min.

Chromatograms of standard solutions of the main organic acids are shown on the fig.1,2.

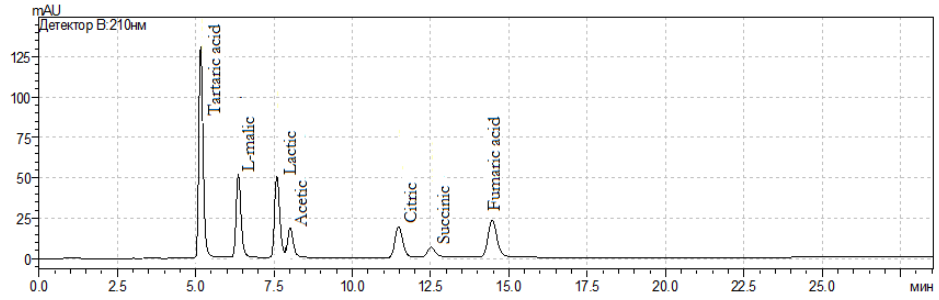

Fig. 1. Chromatogram of the standard solution of organic acids. Nucleodur C18 Pyramid, 250x4,0mm; 0.2% H_3PO_4; 0.5ml/min; 35°C; 210nm.

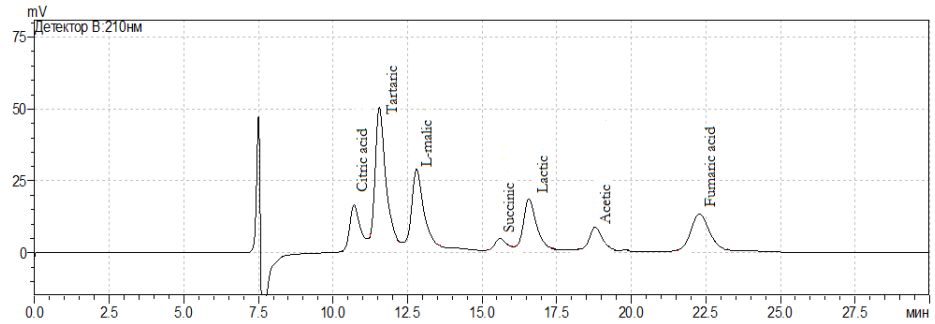

Fig. 2. Chromatogram of the standard solution of organic acids. Nucleogel SUGAR 810H, 300x7,8mm; 20mM H_2SO_4; 0.5ml/min; 40°C; 210nm.

In addition chromatographic column Nucleogel SUGAR 810H has good separating properties for "wine's sugars" like fructose, glucose, sucrose.

Analysis of real samples of wines were done in the same conditions described above. Unfortunately column Nucleogel SUGAR 810H is not suitable for this purpose, especially for analysis of semisweet and sweet wines, because of the close retention times of *Fructose* and *L-malic* acid. In the case of Nucleodur C18 Pyramid *Fructose*'s peak has its signal earlier than main wine's acids.

The comparative characteristics of signals obtained on these two immobile phases at the analysis of standard solutions of organic acids are shown in the Table 1. There are such characteristics like: ratio between height of the peak and its band width, resolution factor (Rs – fig.3).

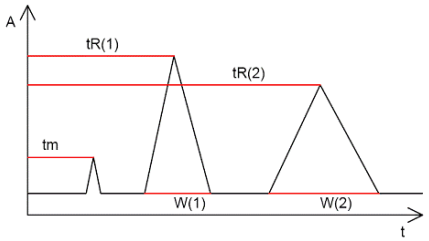

Fig. 3. Calculation scheme of resolution factor for two consecutive chromatographic peaks. $\Delta t = t_{R1} - t_{R2}$. $Rs = 2\,\Delta t / (\omega_1 + \omega_2)$.

If the difference in retention times of two peaks is relatively large, and the width of the bases ($\omega_1+\omega_2$) is small, then the resolution of the peaks is good. The two substances can be identified if their Rs=0,5. For a satisfactory separation the Rs factor should be equal to 1,0. In the case of a quantitative analysis Rs need to be from 1,2 to 1,5 [5].

As it's shown in chromatograms (fig.1 and 2) for column with modified C18 phase there are two close peaks (lactic and acetic acids) with resolution factor 1,0, when for the second chromatographic system there are two problem couples – citric acid plus tartaric acid and succinic acid with lactic acid with resolution factors respectively 0,9 and 1,0.

An important factor in the chromatography is an efficiency of the chromatographic system. Talking about the efficiency of chromatographic system it means the ability of this system to prevent the peaks broadening. Let the factor of efficiency (F) would be taken the

ratio of the peak height to its base width. It's obviously that if this factor is bigger, then the chromatographic system works effective.

Table 1. *The comparative characteristics of signals obtained on two immobile phases at the analysis of standard solutions of organic acids.*

Analyte	*F* (*F=h/w*) (Nucleodur C18 Pyramid, 250x4.0mm)	*F* (*F=h/w*) (Nucleogel SUGAR 810H, 300x7.8mm)
Tartaric acid	10,7	3,3
L-malic acid	4,3	1,8
Lactic acid	6,0	1,2
Acetic acid	2,0	0,7
Citric acid	1,7	1,2
Succinic acid	0,7	0,5
Fumaric acid	1,5	0,6

As it's shown in *Table 1* chromatographic system with column Nucleodur C18 Pyramid (*system 1*) is more effective than Nucleogel SUGAR 810H (*system 2*) for all analytes. For example for tartaric acid with concentration in aqueous solution 0.29g/dm^3 height of its chromatographic peak in *system 1* is 130mAU, when in *system 2* it's only 40.3mAU (3.25 times less).

Conclusions

Taking into account obtained data it may be noted that chromatographic system with column Nucleodur C18 Pyramid is more effective than compared Nucleogel SUGAR 810H. Thus peaks of the main organic acids of wine in the *system 1* have bigger value of their heights, while their base width are less than the same characteristics of peaks obtained in *system 2*. Besides, in *system 1* there is only one couple of chromatographic peaks with resolution factor 1,0, that talks about satisfactory separation of these peaks, but is not perfect for the quantitative analysis; when there are two problem couples of peaks in the *second system* with resolution factors 1,0 and less.

Consequently, chromatographic system with immobile phase Nucleodur C18 Pyramid in conditions: column parameters – 250x4.0mm, 100Å, 5 μm; eluent – 0.2% H_3PO_4; flow rate – 0.5ml/min; oven temperature – 35^{o}C; detection – 210nm – this system is effective for HPLC analysis of organic acids in wine.

References

[1]. Jacobson J.L. 2006: Introduction to Wine Laboratory. Practices and Procedures, p.119.
[2]. Raymond P. W. Scott. LIQUID CHROMATOGRAPHY. Chrom-Ed Book Series. 2003
[3]. http://www.mn-net.com/tabid/7121/default.aspx
[4]. http://www.mn-net.com/HPLCStart/SpecialHPLCphases/Sugars/NUCLEOGEL SUGAR810/tabid/10328/language/en-US/Default.aspx
[5]. ВВЕДЕНИЕ В ХРОМАТОГРАФИЧЕСКИЕ МЕТОДЫ АНАЛИЗА. Крылов В.А., Сергеев Г.М., Елипашева Е.В. Методические разработки к курсу лекций «Хроматографические методы анализа». Нижний Новгород: Нижегородский госуниверситет, 2010. p.16