

Short communication

# Changes in the order of elution of some organic compounds in high-resolution gas chromatography on polar columns depending on the injection method and water content in the sample

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## Abstract

During the GC analysis of apple aroma essence using a capillary column with polar stationary phases, i.e., bonded/crosslinked polyethylene glycol (HP-Innowax) and acid-modified polyethylene glycol (FFAP) the phenomenon of changing the elution order of some compounds was observed (“cross-over phenomenon”). The elution order within two pairs of closely eluting compounds: 2-methylbutyl acetate/pentan-3-ol (internal standard) and *trans*-2-hexen-1-al/2-methylbutan-1-ol differed between samples introduced onto the column with direct injection and with static headspace method. Experiments carried out on standard solutions of investigated compounds in water–acetone mixtures of gradually reduced water ratio (99:1 to 0:100) proved that changes of elution times leading first to co-elution and subsequently to reversion of elution order of compounds within both pairs depended on the amount of water in the injected sample. The same effect was observed while the amount of water was diminished by reducing the sample volume introduced into the column.

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## 1. Introduction

The high-resolution gas chromatography (HRGC) analysis of polar or medium polar compounds on medium or highly polar stationary phases shows the various interactions of analyzed substances with stationary phase. These interactions may play a crucial role in the separation of components of many mixtures [1]. Some of these interactions, e.g., hydro-

gen bonds [2] are known well enough to predict retention of many chemical compounds on polar or medium polar phases. The overlap theory [3] or partial least-squares modeling [4] are used for that purpose.

Gas chromatography using capillary column with bonded/crosslinked stationary phases made possible the repeated injections of water samples. In consequence, in the analysis of many natural food products the solvent extraction step can be avoided. One such foodstuff is commercial fruit essence, which is the ethanol–water mixture of organic volatile com-

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pounds, evaporated from the fruit juice and concentrated.

During the GC analysis of apple aroma essence using a polar capillary column we have observed the phenomenon of changing the elution order of some compounds (“cross-over phenomenon”). The elution order of the compounds differed when the samples were introduced on the column applying direct injection or static headspace method.

Changes in retention times of certain substances depending on the amount of water transferred to the column has been previously reported [5,6]. It has been explained as a consequence of increased column polarity caused by dissolution of water in the column coating. One of the effects of this alteration of stationary phase polarity was accelerated elution of apolar substances and delayed elution of polar substances [6]. Temperature dependent change of elution sequence of certain compounds had also been observed and was explained by the changes in solute–stationary phase interactions [7]. This work is a further attempt to document this phenomenon, taking into account the influence of water on the separation properties of polar stationary phases.

## 2. Experimental

### 2.1. Materials

Commercial apple essence was purchased from a local producer. Five standard solutions were prepared, each containing  $100 \mu\text{g ml}^{-1}$  of 2-methylbutyl acetate,  $100 \mu\text{g ml}^{-1}$  of *trans*-2-hexen-1-al,  $200 \mu\text{g ml}^{-1}$  of pentan-3-ol and  $200 \mu\text{g ml}^{-1}$  of 2-methylbutan-1-ol in the following acetone–water mixtures: 100:0 (a); 90:10 (b); 75:25 (c); 50:50 (d), and 1:99 (e).

### 2.2. Methods

HRGC analyses were carried out using a HP-Innowax (30 m×0.25 mm,  $d_r=0.25 \mu\text{m}$ ) capillary column, the stationary phase being bonded/cross-linked polyethylene glycol or a capillary column with crosslinked FFAP (acid-modified polyethylene glycol) stationary phase.

In all experiments the following column tempera-

ture program was used: 5 min isothermal at  $45 \text{ }^\circ\text{C}$ , increase at a rate of  $2 \text{ }^\circ\text{C min}^{-1}$  up to  $60 \text{ }^\circ\text{C}$  and at a rate of  $6 \text{ }^\circ\text{C min}^{-1}$  up to  $200 \text{ }^\circ\text{C}$ . Helium was used as a carrier gas.

Experiment I:  $3 \mu\text{l}$  of apple essence with  $120 \mu\text{g ml}^{-1}$  of pentan-3-ol as internal standard was introduced into the column using splitter, split ratio was 1:35. Experiment II: the apple essence with internal standard ( $120 \mu\text{g ml}^{-1}$  of pentan-3-ol) was introduced into the chromatograph using the static headspace method. Experiment III:  $3 \mu\text{l}$  of five acetone–water solutions of standard compounds was successively injected into the column with the splitter, the split ratio was 1:35. The amount of water introduced with the sample was approx. 0; 0.008; 0.021; 0.042 and  $0.082 \mu\text{l}$  for solutions (a), (b), (c), (d) and (e), respectively. Experiment IV:  $3 \mu\text{l}$  of solution (e) was injected into the column through the splitter. The five split values were successively set at: 1:700; 1:350; 1:140; 1:70 and 1:35. The amount of water introduced into the column was approx. 0.004; 0.008; 0.021; 0.042 and  $0.082 \mu\text{l}$ .

## 3. Results

During the gas chromatographic analysis of apple essence the different elution order of some compounds was observed, depending on the method of introducing the sample into the column.

The chromatogram obtained as a result of analysis carried out with the direct injection on the column of the essence, containing ca. 95% water, is shown in Fig. 1 (experiment I). Compounds of interest composed two pairs of closely eluting peaks, in the following order: 2-methylbutyl acetate and pentan-3-ol (first pair), and *trans*-2-hexen-1-al and 2-methylbutan-1-ol (second pair).

Fig. 2 shows the chromatogram obtained by introducing into the column the gaseous phase (the headspace) of essence, containing a significantly less amount of water than the essence (experiment II). In that case the elution order of compounds within the above mentioned two pairs of compounds was reversed and was as follows: pentan-3-ol and 2-methylbutyl acetate (first pair) and 2-methylbutan-1-ol and *trans*-2-hexen-1-al (second pair). The rest of

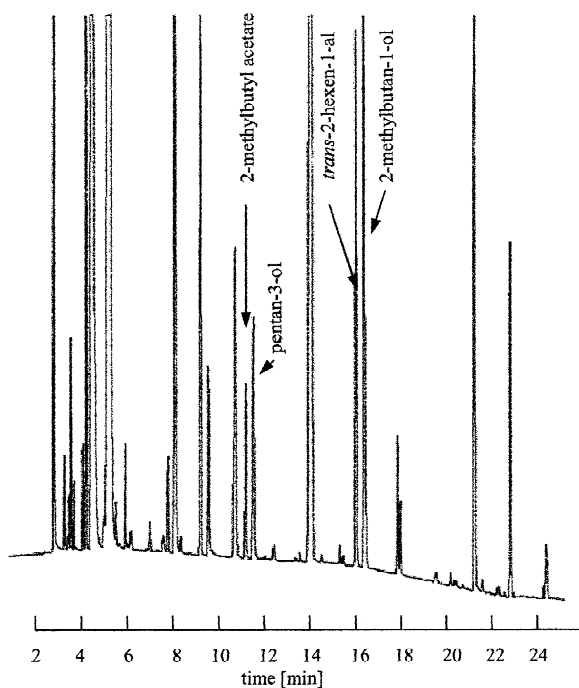


Fig. 1. Chromatogram of apple essence (with pentan-3-ol as internal standard) obtained by direct injection in split mode—experiment I.

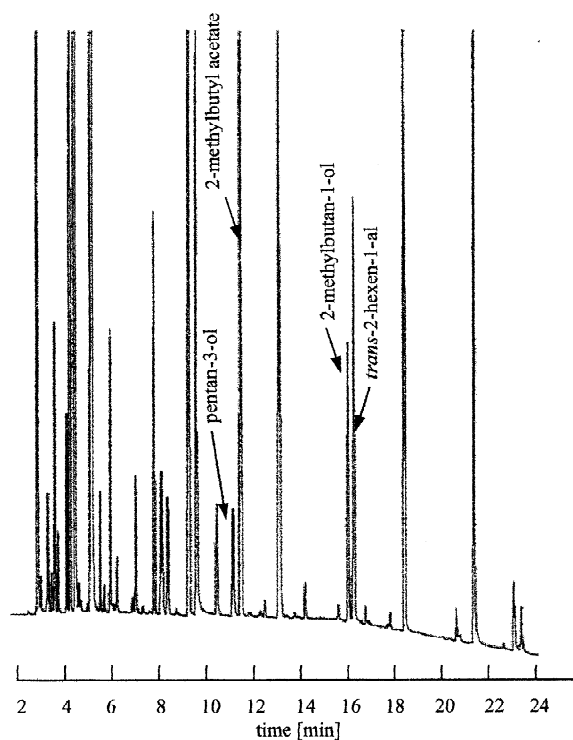


Fig. 2. Chromatogram of apple essence (with pentan-3-ol as internal standard) obtained by the head space method—experiment II.

identified compounds eluted in the same order as in experiment I.

The elution order of standard compounds dissolved in acetone (Fig. 3A) was identical as in the apple essence analyzed with headspace method and opposite than in the case of standards dissolved in acetone–water (1:99) mixture (Fig. 3B), which was the same as in the essence directly injected into the column. In experiment III the standard solutions of different acetone–water ratios, 100:0; 90:10; 75:25; 50:50 and 1:99 were analyzed under the same conditions (experiment III). The results of this experiment enabled to establish the ratio of retention times ( $t_R$ ) within two pairs of investigated compounds, i.e.,  $t_R$  of 2-methylbutyl acetate to  $t_R$  of pentan-3-ol and  $t_R$  of 2-methylbutan-1-ol to  $t_R$  of *trans*-2-hexenal, as a function of water amount in the sample (Fig. 4). In that figure the progressive change of relative retention times of compounds within these two pairs, leading to final changing of their order of elution is shown. In the case of the ratio value 1 the retention times of both compounds are equal and the

co-elution occurs. For the first pair of compounds the change of order happened at a lower amount of water in the injected sample (ca. 0.01  $\mu$ l) than in the second pair (ca. 0.018  $\mu$ l). A similar effect was observed while the water amount of injected sample had been controlled by introducing 3  $\mu$ l of solution (e), containing ca. 99% water, and successively increasing the split value from 1:35 to 1:700 (experiment IV) (Fig. 5).

The observed change of elution order of chemical compounds set in pairs (2-methylbutyl acetate/pentan-3-ol, *trans*-2-hexen-1-al/2-methylbutan-1-ol) is in agreement with the assumption [6] that water injected into the polar column increases the stationary phase polarity, and thereby moderately delays the elution of more polar substances (in this case 2-methylbutyl acetate and *trans*-2-hexen-1-al, dipole moments of which are 4.11 and 3.28 D, respectively) and greatly accelerates the elution of less polar substances (in that case pentan-3-ol and 2-

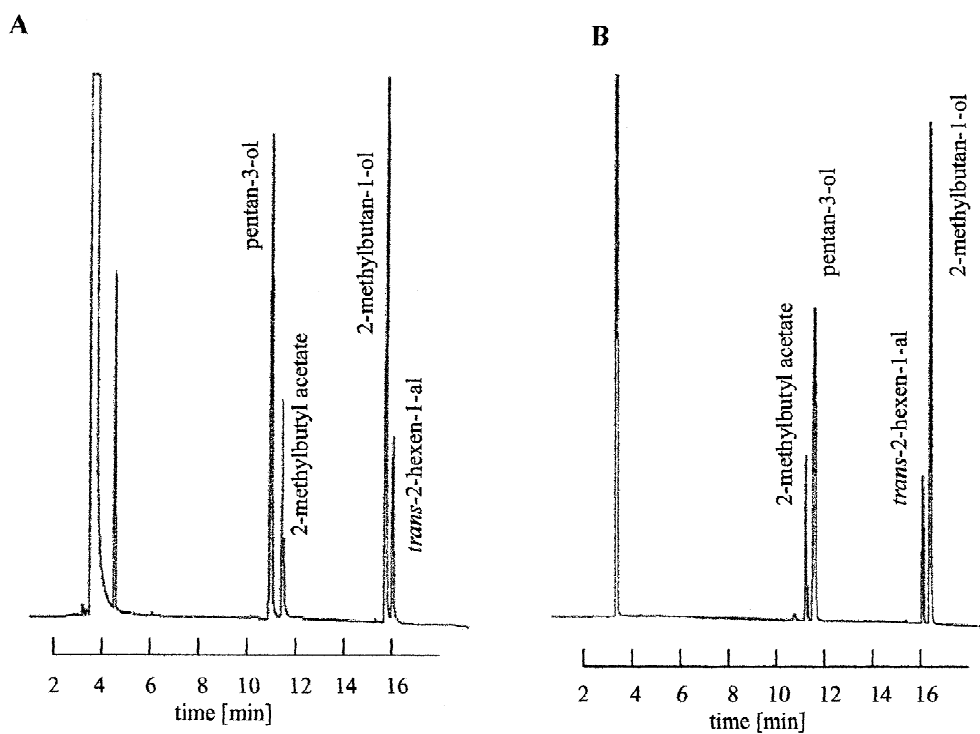


Fig. 3. Chromatograms of standard solutions: (A) solution (a) in acetone, (B) solution (e) in acetone–water (1:99) (3  $\mu$ l injections, split 1:35).

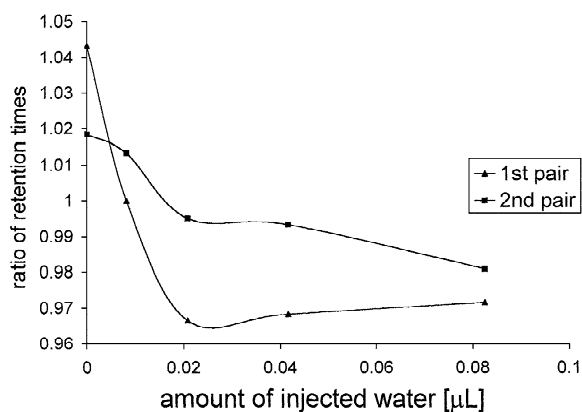


Fig. 4. Effect of injected water amount, controlled by the water–acetone ratio of the solution, on the relative retention times within two pairs of separated compounds (first pair, 2-methylbutyl acetate/pentan-3-ol; second pair, 2-methylbutan-1-ol/*trans*-2-hexen-1-al, 3  $\mu$ l injections, split 1:35)—experiment III.

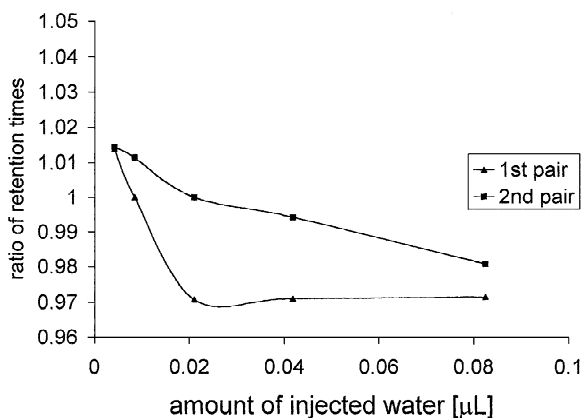


Fig. 5. Effect of injected water amount, controlled by the split value, on the relative retention times within two pairs of separated compounds [first pair, 2-methylbutyl acetate/pentan-3-ol; second pair, 2-methylbutan-1-ol/*trans*-2-hexenal, solution (e), 3  $\mu$ l injections)—experiment IV.

methylbutan-1-ol characterized by dipole moments 1.64 and 1.37 D, respectively). No elution sequence changes among studied compounds were observed when apolar column (OV-1) was used under the same temperature conditions.

The presented work leads to the practical conclusion that when carrying out GC analysis of mixtures containing water on a polar column the differences in elution order of certain compounds may occur. The differences may be caused by different methods of introducing the sample into the column and in fact, by the presence of different amounts of water in the analyzed samples. Water may seriously affect the resolution properties of the polar column and in certain conditions may lead to even a total lack of separation of the components of analysed mixtures.

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