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SELECTIVE SEPARATION OF POLAR UNSATURATED ORGANICS USING A WATER STATIONARY PHASE IN GAS CHROMATOGRAPHY

by

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ABSTRACT

A water stationary phase is explored as a novel means of selectively separating unsaturated analytes in gas chromatography. Several unsaturated/saturated analyte pairs consisting mainly of carboxylic acids and alcohols were examined using both a 30 m conventional non-polar HP-5 column and an 11 m water phase column. For most investigated on the HP-5 column, analytes often eluted very close to each other (~6-12 seconds apart) with poor resolution. By comparison, the shorter water phase column well separated each of the analogue pairs by about 3-8 minutes or more. As well on the water phase, analytes with a triple bond were much more retained than those with two double bonds, which in turn were much more retained than those with one double bond. Conversely, on the HP-5 column these were poorly separated if at all. Additionally, cis/trans isomers were baseline resolved on the water phase but co-eluted on a 30 m conventional polar Carbowax column. Similarly, positional isomers varying the location of the double bond were found to separate with a selectivity value near 1.1 on the Carbowax column, whereas on the water phase column they yielded a value of 1.3 and eluted in the reverse order. Addition of various metal ion salts to the water phase were explored. While Ca^{2+} ion produced modest increases in selectivity, the addition of Ag^{+} ion was most influential and further increased the original water phase selectivity by a factor of 2.3. The mechanistic implications of OH---pi bonding in the water phase was discussed as a potential origin for the selectivity observed. The method was applied to gasoline, essential oil, and food stuff analysis. Results indicate that this method could be a very useful means of selectively separating such unsaturated analytes.

Keywords: unsaturated analytes; isomer separation; water; stationary phase; gas chromatography

INTRODUCTION

Gas chromatography (GC) is a prominent separation technique employed in the analysis of volatile organic compounds [1]. Accordingly, GC is relied upon as an analytical tool in a number of important areas such as environmental monitoring, forensic analysis, industrial quality control, and oil exploration [2-5]. Owing to its superior performance in these applications, on-going efforts are being made to further advance GC and expand its field of use.

Stationary phase development is very important in GC since it can greatly improve separations. For example, non-polar phases such as dimethylpolysiloxane or polar phases such as polyethylene glycol are often conventionally used to coat fused silica capillary GC columns for use [6, 7]. However, novel GC stationary phases that can offer greater analyte selectivity and other complimentary properties are increasingly of interest. For example, various phases have reported advantages in GC separations such as those composed of ionic liquids [8], microporous organic networks [9], and triphenylene-grafted polysiloxanes [10].

The analysis of unsaturated organics is very important in many areas. For example, these include light olefins in industrial chemical production [11, 12], fatty acid lipids in food quality [13, 14], small carboxylic acids in resin and plastics manufacturing [15], phenolic compounds in fuel stability [16], and small alcohols in medicine, agriculture, perfumes, and flavors [17], to list a few. Although GC is often used in the analysis of unsaturates, such separations can be quite difficult since many of these analytes exist in matrices containing structurally similar saturated molecules that possess near identical boiling points, polarity, and hence, retention properties.

Accordingly, methods to help overcome this and differentiate such analytes are important. For instance, alumina-based porous layer open tubular (PLOT) columns have often been used in GC separations of light unsaturated analytes. While useful for this, they can occasionally be limited by their sample capacity, chemical inhomogeneity, and high operating temperatures [11].

Alternately, argentation chromatography has also been widely used for separating unsaturated analytes [11, 14, 18]. This technique offers enhanced separation selectivity for unsaturates through reversible pi-bond complexation with silver ions present in the system [13, 18, 19]. However, instability in these coatings and the difficulty in controlling ion concentrations on column can hamper separation reproducibility [13, 14, 18]. Therefore, investigating alternate ways to selectively analyze unsaturated organic analytes is of continued interest.

In recent years, we have been developing the use of water as a novel stationary phase in capillary GC [20-24]. In this method, water is coated on the inner wall of a stainless-steel capillary and is used in the GC analysis of both aqueous and organic samples. The technique offers efficient, reliable performance, and provides notable retention for polar analytes but little affinity for non-polar ones [20-24], which is mainly influenced by water solubility rather than boiling point. Given these unique properties, further exploration and development of this method would be beneficial.

In working with the water stationary phase system an interesting observation was recently made. Specifically, a few closely related analyte pairs were found to separate with unusually large selectivity [22]. In each case the analogues were nearly identical in molecular structure and otherwise should have eluted very closely to one another. However, they differed only by the presence of a double bond in one analyte and were instead very well separated. While this suggests that the water phase could perhaps offer unique selectivity toward unsaturated organics, this phenomenon has yet to be examined. Here we explore the novel use of a water stationary phase to selectively separate unsaturated analytes in capillary GC. The general operating parameters, separation characteristics, and analyte selectivity of the method are presented. Finally, it is applied to the direct analysis of various samples in order to gauge its effectiveness.

EXPERIMENTAL

Instrumentation

The instrumental setup used here has been detailed previously [21, 22]. Briefly, experiments were performed using an HP-5890 Series II GC (Agilent, Palo Alto, CA, USA). High purity nitrogen (Air Liquide, Calgary, Canada) was used as a carrier gas. An ISCO model 100DX syringe pump (Teledyne ISCO, Lincoln, NE, USA) supplied water to saturate the carrier gas and keep the column hydrated at elevated temperatures. The nitrogen carrier gas and water pump were connected via a 1/16" stainless steel (SS) Valco zero dead volume tee (Vici-Valco, Houston, TX, USA) situated inside the GC oven. The outlet of this tee was joined to a 1 m SS preheating coil (1/16" O.D. x 250 µm I.D.; Chromatographic Specialties, Brockville, ON, Canada) that led into the injector, which was operated at 220 °C.

The capillary column used for separations was an 11 m length of 316 SS tubing (1/16" O.D. x 250 µm I.D.; Chromatographic Specialties) coated with the water stationary phase. It was typically operated with a carrier gas velocity of 36 cm/s and a 5:1 split ratio. A 2 m length of the same was also employed as a column in certain experiments exploring the addition of various salts to the phase. A restrictor was connected to the outlet of the column via a zero dead volume union (Vici-Valco) to maintain backpressure and help stabilize the stationary phase at elevated temperatures. Both 316 SS (620 µm O.D. x 75 µm I.D. x 25 cm) and bare fused silica (365 µm O.D. x 100 µm I.D. x 30 cm) capillary restrictors were used for this. A flame ionization detector (FID), operated at 220 °C, was used for the detection of analytes. The end of the restrictor was placed inside the FID jet at the burner surface where analytes were directly deposited into the flame. High purity hydrogen and medical grade air (Air Liquide), with respective flows of 90 and 350 mL/min, were used to support the flame. A conventional DB-5 column (95% methyl/5%

phenyl polysiloxane; 320 µm I.D. x 30 m; 0.25 µm thick; Agilent), Carbowax column (polyethylene glycol; 530 µm I.D. x 30 m; 1 µm thick; Restek, Bellefonte, PA, USA), and PLOT column (Al_2O_3/Na_2SO_4; 530 µm I.D. x 50 m; 10 µm thick; Varian, Palo Alto, CA, USA) were also used for comparison work.

Stationary Phase Materials

HPLC grade water (Honeywell Burdick & Jackson, Muskegon, USA) was used for all stationary phase experiments. A stock solution of 20 mM sulfamic acid was prepared as described previously [22] and used as an acidified water phase for all acid separations. Most salt solutions including $Ca(NO_3)_2$ (99.8%; Fisher Scientific, Hampton, NH, USA), $AgNO_3$ (99%; VWR Chemicals BDH; Mississauga, Canada), $LiNO_3$, $RbNO_3$, $Ni(NO_3)_2$, $NiCl_2$, $NaNO_3$, and KNO_3 (all ≥98%; Sigma-Aldrich, Oakville, Canada) were made directly in water. CuCl, $PdCl_2$, and $PtCl_2$ (all ≥98%; Sigma-Aldrich) solutions were made in 0.2 M HCl. The salt concentrations were varied as specified in the text. Solutions were carefully coated onto the SS capillary column as described previously [20] and it was then connected inside the GC oven for use in separations.

Chemicals and Reagents

All analyte solutions were normally prepared to a final concentration of about 10 µg/µL. Alcohols including cyclohexanol (>95% J.T. Baker, Phillipsburg, NJ, USA), phenol (>95%; Anachemia Science, Richmond, Canada), 1-propanol, 2-propen-1-ol, 1-butanol, 2-buten-1-ol, 2,3-butadien-1-ol, 3-butyn-1-ol, 1-pentanol, cis-2-penten-1-ol, trans-2-penten-1-ol, 4-penten-1-ol and 2-cyclohexen-1-ol (all ≥95%; Sigma-Aldrich) were prepared in hexane (98.5%; Sigma-Aldrich). Carboxylic acids including propanoic acid (99.5%; Fluka, Germany), cyclohexane carboxylic acid, benzoic acid, pentanoic acid, 3-pentenoic acid, butanoic acid, trans-2-butenoic acid, and propenoic acid (all ≥97%; Sigma-Aldrich) were also prepared in hexane. Gasoline was purchased from a

local vendor and spiked with 10 µg/µL of phenol. Essential oil was purchased from a local vendor and diluted in hexane to 10 µg/µL. Balsamic vinegar and apple juice were purchased from local vendors and spiked with 5 µg/µL each of propanoic acid, sorbic acid (99%; Sigma-Aldrich) and benzoic acid. A small amount of ethanol (99%; Sigma-Aldrich; already native to the samples) was added to aid dissolution. All other details are outlined in the text.

RESULTS AND DISCUSSION

General Retention Characteristics

Initial work was aimed at establishing if the water stationary phase could indeed selectively retain unsaturated analytes over their saturated analogues. For this, a wide variety of analytes were examined on the water phase and also a conventional non-polar HP-5 column for comparison. Experiments consistently showed that the water phase yielded relatively substantial selectivity towards the unsaturated analytes. Fig. 1 illustrates this for a series of C_3 to C_5 alkyl carboxylic acids examined on both columns, where each pair differs structurally by only one double bond. As seen in fig. 1a, on the 30 m HP-5 column at 70 ^oC, the saturated and unsaturated acid pairs largely co-elute (in fact the C_3 pair perfectly co-elute, obscuring the data point) and are retained primarily according to their boiling point. By contrast, in fig. 1b these same analyte pairs each separate by about 4 to 8 minutes on the 11 m water phase column at 110 ^oC and elute with increasing polarity [20].

Table 1 displays the retention times for all the analytes investigated on both the water phase and HP-5 columns. These are mainly comprised of organic alcohols and acids, and the table lists each group roughly by molecular size. Each saturated analyte is followed by unsaturated analogues examined for comparison. Boiling points are also included as a reference. As seen again, overall

analyte retention on the HP-5 column generally increases with analyte boiling point, while on the water stationary phase it more dominantly increases with analyte polarity (and hence water solubility). Even more, the HP-5 column showed little separation of unsaturated and saturated analyte pairs. For example, the retention difference between a saturate and its analogue with one double bond is less than 1 minute across the compounds, with many eluting 6 to 12 seconds apart. This is somewhat expected and demonstrates the challenge in separating such analytes since they share similar boiling points and molecular weights. Conversely however, the water stationary phase often retained unsaturates nearly twice as long as their saturated analogues, with most eluting 3 to 8 minutes apart. This is further notable since the water phase column was about 1/3 the length and often operated at a much higher temperature. One exception is the unsaturated isomers cis-2-penten-1-ol and 4-penten-1-ol, which separated slightly more (0.4 minutes retention difference) on the HP-5 column (at 50 ^{o}C) than on the water phase (0.2 minutes retention difference). In this case, the higher 100 ^{o}C operating temperature of the latter impacted the separation seen. For instance, separations of these analytes at lower temperatures of 65 ^{o}C provided the same differences in retention of 0.4 minutes. Results were also quite reproducible as replicate injections yielded analyte retention times that varied within 1% RSD (n=3) from run to run, 3% RSD (n=7) over 24 hr periods in day to day trials, and 4% RSD (n=2) over a one year period. This is consistent with earlier reports that showed external carrier gas hydration to aid analyte retention stability over time [21]. Specifically, only a few µL/min of water added to the carrier gas stream can yield retention times that differ by only 1% RSD over about a 5 hour span examined at 70 ^{o}C [21]. Therefore, the water stationary phase appears to offer substantial selectivity towards unsaturated analytes.

Also interesting in Table 1 is that di-enes are much more retained than mono-enes on the water stationary phase. Of note, 2,3-butadien-1-ol was nearly 2 times more retained than 2-buten-1-ol, which in turn was roughly 2 times more retained than butanol. By comparison, on the HP-5 column these analytes eluted within 6 to 18 seconds of one another. This effect also further increased for a triply bonded analyte. Specifically, 3-butyn-1-ol was slightly less retained than the other C_4 alcohols on the HP-5 column, whereas it eluted almost 4 times later than 2-buten-1-ol on the water phase, marking a 15 minute span between it and the saturated analogue butanol. This trend is also seen in the cyclic organics. For instance, 2-cyclohexen-1-ol and phenol were respectively 2 and 6 times more retained than cyclohexanol on the water phase. Comparatively, they were little separated on the HP-5 column, except for phenol which eluted later due to its higher boiling point. This is also true for the saturate cyclohexane carboxylic acid and its aromatic analogue benzoic acid, which separated by only 1.3 minutes on the HP-5 column and over 22 minutes on the shorter water phase column at the same temperature. Therefore, multiple double bonds further increase retention on the water stationary phase. Fig. 2 demonstrates typical separations for some of the analytes in Table 1. As seen in fig. 2a, the C_4 alcohols elute very close to each other just after the hexane solvent on the HP-5 column, while they are well separated and highly retained on the water phase. So much so that 3-butyn-1-ol is nearly 5 times more retained and as a result is also more broadened in appearance and nearer to the baseline noise that can be seen slightly on its peak profile. Fig. 2b also shows this for the C_5 acids, which almost entirely co-elute on the HP-5 column but separate by nearly 6 minutes on the water phase.

For better context, results were also compared with other conventional columns. For instance, 1-butanol and 2-buten-1-ol were analyzed on a 30 m polar Carbowax column and displayed good peak shape with a selectivity value near 1.6. Conversely, on the 11 m water phase

column these analytes yielded a favorable selectivity near 2.5. A conventional Al_2O_3 PLOT column was also examined but the alcohols did not elute given its polarity.

Addition of Salts to the Water Phase

As mentioned, certain metal ions such as Ag^+ and others are well known to strongly interact with olefins and have been used to invoke separations of saturated and unsaturated organics [18]. Since they were easy to add to the water stationary phase, several metal ion salts were also examined here to see if they could further improve separations. For this, the selectivity of 2-buten-1-ol over 1-butanol was compared between a pure water stationary phase and one loaded with salt, using a simple 2 m column at ambient temperature to facilitate the process. Concentrations up to 0.1 M for most salts were well tolerated by the system, but higher levels were often impractical and caused restrictor plugging and system instability. As such, thorough water rinsing between runs helped to offset these problems, as others have reported [19].

Fig. 3 shows the results as a ratio plot of the change in selectivity between the alcohols after/before salt addition. In this way, a value of 1 indicates no change is observed by the addition of metal ion to the water phase, while values greater than 1 indicate a further improvement in the separation. All the salts were either nitrates or chlorides (Ni^{2+} was examined as both). As can be seen, some metal ions did not elicit any difference while others further improved selectivity by increasing retention of the unsaturated analyte. For instance, addition of Li^+, Rb^+, Cu^+, or Ni^{2+} (nitrate and chloride) to the water phase did not significantly alter the selectivity between 1-butanol and 2-buten-1-ol, producing ratios near 1.0. By comparison, additions of Na^+, K^+, and Pt^{2+} showed a moderate increase with ratios around 1.1. Interestingly, Ca^{2+} demonstrated a notable increase in selectivity with a ratio of about 1.3, but was limited to a 0.1 M loading since larger amounts caused

system issues. To our knowledge, such Ca^{2+} based separations have not been reported and may be useful to investigate in the future.

Perhaps not surprisingly, Ag^+ was found to greatly increase the selectivity of this analyte pair with a ratio near 2.3. Coincidentally, this occurred for a salt loading of 0.5 M since Ag^+ was the only ion that could be used up to this level before issues arose. For instance, Ag^+ concentrations of 0.01, 0.05, 0.1, and 0.5 M produced selectivity increases of 1.02, 1.04, 1.23, and 2.34 times respectively. Thus, since selectivity was found to increase with metal ion concentration here, the superior performance of Ag^+ in this regard may be partly attributed to its higher concentration in the phase. For instance, at 0.1 M both Ca^{2+} and Ag^+ produced near equivalent increases in separation selectivity. Fig. 4 illustrates the selectivity improvement observed between 2-buten-1-ol and 1-butanol by adding 0.5 M Ag^+ to the water phase. As seen in fig. 4a, while the analytes are already well separated by about 4 minutes on this 2 m column, the addition of Ag^+ (fig. 4b) greatly increases this to nearly 20 minutes.

In terms of loading, the results here compare favorably to others. For example, after using a 1.2 M Ag^+ solution for loading, a packed HPLC column was reported to contain 80 mg of Ag^+ and yielded a typical separation selectivity of about 2 for a mono-unsaturate/saturate pair [25, 26]. By comparison, the typical water phase volume is about 5 µL/m [27]. As such, the short 2 m column here coated with 0.5 M Ag^+ solution contains about 1 mg of Ag^+ and produces a mono-unsaturate/saturate selectivity near 4 (fig. 4b). Thus, with only a moderate Ag^+ loading, the selectivity of the water stationary phase can be even further enhanced to provide better separations. Further, the ion loading here should also be quite stable given that homogenous stock solutions are used for coating and the stationary phase coverage for such SS columns has previously been shown to be very reproducible [27]. This is also consistent with the data here. For example, run to run

trials using 0.5 M $AgNO_3$ produced saturate/unsaturate analyte pair selectivity values that agreed within 6% RSD (n=4).

It should be noted that other salts such as $PdCl_2$ and $AuCl_3$ were also investigated but could not be used as they were found to be potentially corrosive to the system. As well, mixed salt systems were explored using $AgNO_3$ and $CaNO_3$ solutions combined at near 0.1 M each, but they did not notably improve the selectivity further. This is another useful facet of the water stationary phase as it readily allows the exploration of various additives for their impact on such separations. While it is currently unknown why certain metal ions have greater impact on separation than others, it may be due to their ionic radius and ability to interact with analyte pi-bonds as discussed later in the text. Regarding the different concentrations of salts that could be utilized, it might be that properties such as the melting point of the salt could impact their ability to traverse the restrictor without plugging it. For instance, $AgNO_3$ has one of the lowest melting points of the salts listed in fig. 3 and also has the highest concentration limit. Regardless, further investigation would be needed to better establish this. Finally, it should be noted that the trends observed above were largely based on the 2-buten-1-ol and 1-butanol analyte pair investigated. Still, it is worth mentioning that others showed stark improvements as well. For example, the selectivity of the cis-2-penten-1-ol/pentanol pair increased from 2.6 to 5.5 when switching from water to 0.5 M $AgNO_3$. Similarly, the trans-2-penten-1-ol/pentanol pair also showed a similar increase from 2.2 to 4.5.

Separation of Unsaturated Pairs

The separation of certain unsaturated analytes was also explored. The first of these were cis/trans isomers. For this, a mixture of cis-2-penten-1-ol and trans-2-penten-1-ol was analyzed on both the 11 m pure water stationary phase column and a 30 m conventional Carbowax column each at 100 ^{o}C. The results are shown in fig. 5a. As seen, the cis/trans analyte pair separates well

on the water stationary phase, with baseline resolution and good peak shape. Comparatively, on the longer Carbowax column at the same temperature these analytes co-elute almost entirely. Further, each column retains the cis isomer more than the trans, which has been attributed to reduced steric hinderance and stronger stationary phase interactions of the cis isomer [11, 14, 18]. Interestingly, this same separation attempted with a 0.5 M Ag^+ coating did not yield any further increase in selectivity for the cis/trans pair.

Positional isomers were also investigated since these species, where the double bond changes its position along the hydrocarbon backbone, have been difficult to separate (if at all) previously due to their similar physicochemical properties [19, 28]. Such separations are also important in many fields as the double bond position can impact a variety of molecular properties [29-30]. For this, a mixture of trans-2-penten-1-ol and 4-penten-1-ol was analyzed on the 11 m water stationary phase column and again on the 30 m Carbowax column. The results are displayed in fig. 5b. As seen, the water phase well separates these isomers, retaining the 4-position analyte longer than the 2-position one with a selectivity value of 1.3. By comparison, the longer Carbowax column separated these isomers with a selectivity value near 1.1 and in the opposite elution order, suggesting a different separation mechanism on this column. As above, a 0.5 M Ag^+ phase did not further improve this separation. Incidentally, the water phase column also partially separated cis-2-penten-1-ol from 4-penten-1-ol (in that order, resolution ~0.9). Therefore, the water stationary phase can separate both cis/trans and positional isomers of certain unsaturates efficiently.

Mechanism

To firmly establish the chemical interactions leading to the above water stationary phase retention properties, a deeper investigation beyond the scope of the current work is required. Still, some relevant points are useful to discuss. For instance, the strong affinity for unsaturated organics

may involve non-covalent pi interactions between the analyte and water molecules, which are well known [31]. In particular, OH---pi interactions are dominant and relatively stable in systems where unsaturated organics are in aqueous media [32, 33]. If so, the increased retention observed above for diene and triply bonded analytes may arise from increased points of interaction with the solutes.

Coincidentally, also helpful to consider here is analyte water solubility, which is known to directly impact retention on this phase [33]. For instance, many of the saturate/unsaturate pairs here have similar structures and boiling points, but often differ in their water solubility. For example, at 20 °C the water solubility of 1-pentanol is about 0.24 mol/L while the that of 4-penten-1-ol is near 0.65 mol/L [34]. Similarly, cyclohexanol and phenol have respective water solubilities of about 0.36 and 0.78 mol/L [34]. As a result, the unsaturated analytes are more water soluble and thus more retained on that basis. Taken together, it could be that the OH---pi interactions of unsaturated analytes lead to greater water solubility for them and larger retention.

This is also true for non-polar unsaturated analytes. For example, at 25 °C pentane and 1-pentene have respective water solubilities near 0.00052 and 0.0021 mol/L [34]. Compared to above, these values differ from each other by a similar degree but are smaller in absolute magnitude by several hundred times. As a result, such analytes should be very little retained and/or separated on the water phase. Indeed, unsaturated non-polar analytes such as benzene and toluene were found here to be completely unretained by the water stationary phase. This agrees with reports that OH---pi bonding in non-polar molecules is relatively weaker than OH---O bonding in water itself [35] but can be strengthened by the presence of polar groups such as alcohols on the analyte molecule [36]. Therefore, such interactions may hold implications for the separation mechanism here.

Applications

To gain context of the analytical utility of this method, it was applied to the analysis of various samples. The first of these was gasoline containing phenol. Phenols are common components of fuels and can cause sediments to form that damage engines [16]. As a result, they are routinely monitored using GC, which is effective but separations can often be complex [16].

To test this here, a neat gasoline sample containing phenol was first analyzed using a conventional HP-5 column, as shown in fig. 6a (left). As seen, the fuel sample contains a multitude of hydrocarbons, and phenol (denoted by *) elutes near 7 minutes amidst numerous other unresolved peaks making it difficult to analyze. By comparison, the same sample on the water stationary phase (fig. 6a right) is much simpler as the bulk of the non-polar fuel components elute unretained near the void volume while the polar unsaturated phenol analyte is well retained and elutes after about 20 minutes without interference. Incidentally, phenol was also similarly analyzed directly from aqueous samples. This may be potentially useful for wastewater analysis since extensive sample preparation is often required to transfer organic analytes out of the water matrix first, which can be time consuming and error prone [37-39].

Next examined were essential oils, which are commonly used in the pharmaceutical, cosmetic and food industries [40]. However, since certain components can offer medicinal benefit while others can induce allergic reaction it is important to monitor their content [41]. GC is commonly used for this but the large number of sample components can obscure the resolution of primary target analytes when using conventional columns [42]. Fig. 6b (left) shows an example of the analysis of lavender oil on the conventional HP-5 column. As seen, the oil contains numerous analyte peaks owing to the complex hydrocarbon matrix. In particular, the main component of interest linalool (denoted by *) elutes amongst a great many other species on this column, complicating its potential isolation and analysis. Comparatively, when this sample is analyzed on

the water stationary phase (fig. 6b right) the chromatogram is simplified. For instance, most of the non-polar hydrocarbon peaks again elute unretained near the void volume. Conversely, linalool (a diene-ol terpene species) is well retained and elutes several minutes after the primary matrix components under the temperature program used. Given that many essential oils contain such unsaturated alcohols, the water stationary phase could thus be potentially useful in their analysis.

The final samples investigated were food stuffs. Benzoic acid, sorbic acid and propionic acid are naturally occurring preservatives which are found in a large variety of foods [43]. However, at higher levels they can cause health issues and need to be regularly monitored [44-45]. Conventionally, this has required two separate methods for this analysis (i.e. HPLC and GC) [43-44], or more recently a single GC method that employs additional sample preparation steps [44]. Here, several food stuffs containing the preservatives were analyzed directly on the water stationary phase. Fig. 6c (left) shows the analysis of a neat balsamic vinegar sample (containing benzoic, propanoic, and sorbic acid) directly injected on the water stationary phase. As seen, the separation produces well separated peaks for the various preservatives and others for the dominant acetic acid and minor ethanol presence as well. Fig. 6c (right) shows the same for an apple juice sample. Again, apart from some minor flavor components and an ethanol peak eluting early [46], the main preservative peaks are well separated from each other. Thus, these components can be analyzed by a single method and without sample preparation since the water stationary phase can readily accommodate aqueous samples such as vinegars and juices.

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397

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403 **REFERENCES**

404 [1] Dewulf J, Van Langenhove H (2002) Analysis of volatile organic compounds using gas
405 chromatography. Trac-Trend Anal Chem 21:637-646

406 [2] Poster DL, Schantz MM, Sander LC, Wise SA (2006) Analysis of polycyclic aromatic
407 hydrocarbons (PAHs) in environmental samples: a critical review of gas chromatographic (GC)
408 methods. Anal Bioanal Chem 386:859-881

409 [3] Medeiros PM, Simoneit BRT (2007) Gas chromatography coupled to mass spectrometry for
410 analysis of organic compounds and biomarkers as tracers for geological, environmental, and
411 forensic research. J Sep Sci 30:1516-1536

412 [4] Anderson HE, Santos IC, Hildenbrand ZL, Schug KA (2019) A review of the analytical
413 methods used for beer ingredient and finished product analysis and quality control. Anal Chim
414 Acta 1085:1-20

415 [5] Wang Y, Han Y, Hu W, Fu D, Wang G (2020) Analytical strategies for chemical
416 characterization of bio-oil. J Sep Sci 43:360-371

417 [6] de Zeeuw J, Luong J (2002) Developments in stationary phase technology for gas
418 chromatography. Trac-Trend Anal Chem 21:594-607

419 [7] Mansour FR, Zhou L, Danielson ND (2015) Applications of poly(ethylene)glycol (PEG) in
420 separation science. Chromatographia 78:1427-1442

421 [8] Poole CF, Poole SK (2011) Ionic liquid stationary phases for gas chromatography. J Sep Sci
422 34:888-900

[9] Li X, Cui Y-Y, Yang C-X (2021) Covalent coupling fabrication of microporous organic network bonded capillary columns for gas chromatographic separation. Talanta 224:121914

[10] Xu L, Bai J, Du A, Yang Z, Wu B (2020) 1,4-Diphenyltriphenylene grafted polysiloxane as a stationary phase for gas chromatography. New J Chem 44:695-703

[11] Nan H, Zhang C, Venkatesh A, Rossini AJ, Anderson JL (2017) Argentation gas chromatography revisited: Separation of light olefin/paraffin mixtures using silver-based ionic liquid stationary phases. J Chromatogr A 1523:316-320

[12] Anselmi C, Centini M, Fedeli P, Paoli ML, Sega A, Scesa C, Pelosi P (2000) Unsaturated hydrocarbons with fruity and floral odors. J Agric Food Chem 48:1285:1289

[13] Aponte JC, Dillon JT, Tarozo R, Huang Y (2012) Separation of unsaturated organic compounds using silver-thiolate chromatographic material. J Chromatogr A 1240:83-89

[14] Nikolava-Damyanova B (2009) Retention of lipids in silver ion high-performance liquid chromatography: Facts and assumptions. J Chromatogr A 1216:1815-1824

[15] Ushikubo T, Nakamura H, Koyasu Y, Wajiki S (1995) Method for producing an unsaturated carboxylic acid. US Patent US5380933 A

[16] Farajzadeh MA, Yadeghari A, Khoshmaram L, Ghorbanpour H (2014) Gas chromatographic determination of some phenolic compounds in fuels and engine oil after simultaneous derivatization and microextraction. J Sep Sci 37:2966-2973

[17] Sato S, Sato F, Gotoh H, Yamada Y (2013) Selective dehydration of alkanediols into unsaturated alcohols over rare earth oxide catalysts. ACS Catal 3:721-734

443 [18] Momchilova S, Nikolova-Damyanova B (2003) Stationary phases for silver ion
444 chromatography of lipids: Preparation and properties. J Sep Sci 26:261-270

445 [19] Correa RA, Ferraz V, Medvedovici A, Sandra P, Cerne K, David F (1999) Positional and
446 configurational separation of fatty acid isomers by micro reversed-phase liquid chromatography
447 with an Ag^+ -containing mobile phase. J Chromatogr A 848:83-93

448 [20] Gallant JA, Thurbide KB (2014) Properties of water as a novel stationary phase in capillary
449 gas chromatography. J Chromatogr A 1359:247-254

450 [21] Darko E, Thurbide KB (2016) Capillary gas chromatographic separation of organic bases
451 using a pH-adjusted basic water stationary phase. J Chromatogr A 1465:184-189

452 [22] Darko E, Thurbide KB (2017) Capillary gas chromatographic separation of carboxylic acids
453 using an acidic water stationary phase. Chromatographia 80:1225-1232

454 [23] Darko E, Thurbide KB (2019) Dynamic control of gas chromatographic selectivity during the
455 analysis of organic bases. Anal Chem 91:6682-6688

456 [24] Darko E, Thurbide KB (2020) Active control of selectivity in organic acid analysis by gas
457 chromatography. Anal Chim Acta 1106:216:223

458 [25] Christie WW (1987) A stable silver-loaded column for the separation of lipids by high
459 performance liquid chromatography. J High Res Chromatogr 10:148-150

460 [26] Christie WW (1995) In: Sebedio J-L, Perkins EG (ed) New trends in lipid and lipoprotein
461 analysis. Champaign, Illinois

462 [27] Murakami JN, Thurbide KB (2015) Coating properties of a novel water stationary phase in
463 capillary supercritical fluid chromatography. J Sep Sci 38:1618-1624

[28] Huie CW (2003) Effects of organic solvents on sample pretreatment and separation performances in capillary electrophoresis. Electrophoresis 24:1508-1529

[29] Christie WW, Dobson G, Adlof RO (2007) A practical guide to the isolation, analysis and identification of conjugated linoleic acid. Lipids 42:1073-1084

[30] Quehenberger O, Armando AM, Dennis EA (2011) High sensitivity quantitative lipidomics analysis of fatty acids in biological samples by gas chromatography-mass spectrometry. Biochim Biophys Acta 1811:648-656

[31] Anslyn EV, Dougherty DA (2005) Modern physical organic chemistry. Sausalito, California

[32] Jain A, Ramanathan V, Sankararamakrishnan R (2009) Lone pair center dot center dot center dot pi interactions between water oxygens and aromatic residues: Quantum chemical studies based on high-resolution protein structures and model compounds. Protein Sci 18:595-605

[33] Andersen J, Heimdal, Nelander B, Larsen RW (2017) Competition between weak OH···π and CH···O hydrogen bonds: THz spectroscopy of the C_2H_2-H_2O and C_2H_4-H_2O complexes. J Chem Phys 146:194302

[34] Yalkowsky SH, He Y (2003) Handbook of aqueous solubility data. Boca Raton, Florida

[35] Gierszal KP, Davis JG, Hands MD, Wilcox DS, Slipchenko LV, Ben-Amotz D (2011) , π-hydrogen bonding in liquid water. J Phys Chem Lett 2:2930-2933

[36] DuPre DB, Yappert MC (2002) Cooperative hydrogen- and pi H-bonded interactions involving water and the ethylenic double bond. J Phys Chem A 106:567-574

[37] Zhang T, Chen X, Liang P, Liu C (2006) Determination of phenolic compounds in wastewater by liquid-phase microextraction coupled with gas chromatography. J Chromatogr Sci 44:619-624

485 [38] Koe LCC, Shen W (1997) High resolution GC-MS analysis of VOCs in wastewater and
486 sludge. Environ Monit Assess 44:549-561

487 [39] Nigiz FU, Hilmioglu ND (2017) Removal of acetone from wastewater by POSS loaded PDMS
488 membrane. Period Polytech Chem 61:163-170

489 [40] Turkmenoglu A, Ozmen D (2021) Allergenic components, biocides, and analysis techniques
490 of some essential oils used in food products. J Food Sci 86:2226-2241

491 [41] Do TKT, Hadji-Minaglou F, Antoniotti S, Fernandez X (2015) Authenticity of essential oils.
492 TRAC-Trend Anal Chem 66:146-157

493 [42] Marriott PJ, Shellie R, Cornwell C (2001) Gas chromatographic technologies for the analysis
494 of essential oils. J Chromatogr A 936:1-22

495 [43] Yun SS, Kim J, Lee SJ, So JS, Lee MY, Lee G, Lim HS, Kim M (2019) Naturally occurring
496 benzoic, sorbic, and propionic acid in vegetables. Food Addit Contam B 12:167-174

497 [44] Tungkijanansin N, Alahmad W, Nhujak T, Varanusupakul P (2020) Simultaneous
498 determination of benzoic acid, sorbic acid, and propionic acid in fermented food by headspace
499 solid-phase microextraction followed by GC-FID. Food Chem 329:127161

500 [45] List of permitted preservatives (2021) Government of Canada https://www.canada.ca.
501 Accessed 2 Sept 2021

502 [46] McKenzie DL (1988) Physical and chemical properties of apple juice and apple juice
503 particulate. MSc Thesis. Dept Food Sci Univ of British Columbia

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507 **Table 1: Retention time of various analytes on water phase and HP-5 columns**

	Saturated Analyte and Unsaturated Analogue(s)	b.p. (°C)	Water Phase[a] t_R (min)	HP-5 Column[b] t_R (min)
1.	Propanol	97	3.7	3.0
2.	2-Propen-1-ol	97	6.2	3.0
3.	Butanol	118	2.7	3.8
4.	2-Buten-1-ol	121	5.1	3.9
5.	2,3-Butadien-1-ol	53	9.7	4.2
6.	3-Butyn-1-ol	129	17.6	3.7
7.	Pentanol	138	2.0	5.8
8.	*cis*-2-Penten-1-ol	138	4.2	5.9
9.	4-Penten-1-ol	137	4.4	5.5
10.	Cyclohexanol	162	5.4	11.5
11.	2-Cyclohexen-1-ol	166	10.1	11.7
12.	Phenol	182	29.4	24.0
13.	Propanoic acid	141	13.6	3.2
14.	Propenoic acid	141	17.0	3.2
15.	Butanoic acid	164	8.2	4.1
16.	*trans*-2-Butenoic acid	185	16.0	4.9
17.	Pentanoic acid	186	5.9	6.0
18.	3-Pentenoic acid	194	11.9	6.3
19.	Cyclohexane carboxylic acid	233	11.6	5.9
20.	Benzoic acid	249	33.3	7.2
21.	Camphor	209	1.3	6.6
22.	Verbenone	228	5.7	8.5

508 a. 11 m Column; Temperature is 100 °C (analytes 1-12) and 110 °C (13-22)
509 b. 30 m Column; Temperature is 50 °C (analytes 1-12),70 °C (13-18), and 110 °C (19-22)

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511

FIGURE CAPTIONS

Fig. 1: Retention times for an n-alkyl series of C_3-C_5 saturated (●) and unsaturated (○) carboxylic acids as a function of their carbon chain length on a) a 30 m HP-5 column at 70 °C and b) an 11 m water stationary phase column at 110 °C. Analytes: propanoic, propenoic, butanoic, *trans*-2-butenoic, pentanoic, and 3-pentenoic acid.

Fig. 2: Separations of a) butanol (1), 2-buten-1-ol (2), and 3-butyn-1-ol (3) and b) pentanoic acid (1) and 3-pentenoic acid (2), each in hexane, on both a 30 m HP-5 (left) and an 11 m water stationary phase (right) column. Temperatures are a) 50 (left) and 100 (right) °C, and b) 70 (left) and 110 (right) °C.

Fig. 3: Change in selectivity of 2-buten-1-ol over 1-butanol for various metal ions added to the water stationary phase. Ratio shown is the value achieved at the highest salt concentration possible relative to that of pure water. All ions are nitrate salts except for a. which is a chloride salt. Salts were examined at their maximum possible concentration as follows: Ca^{2+}, K^+, Li^+, Ni^{2+}(nitrate) salts were 0.1 M; Rb^+, Ni^{2+}(chloride), Cu^+ salts were 0.05 M; Ag^+, Pt^{2+}, and Na^+ salts were 0.5, 0.06, and 0.08 M respectively. Separations were done on a 2 m column at 25 °C.

Fig. 4: Separation of 1) 1-butanol and 2) 2-buten-1-ol on a 2 m water stationary phase column coated with a) pure water and b) 0.5 M $AgNO_3$. Temperature is 25 °C.

Fig. 5: a) The separation of 1) trans-2-penten-1-ol and 2) cis-2-penten-1-ol on the 11 m water stationary phase column at 100°C (left) and the 30 m Carbowax column at 100°C (right). b) The separation of 1) trans-2-penten-1-ol and 3) 4-penten-1-ol on the 11m water stationary phase column at 110°C (left) and the 30 m Carbowax column at 100°C (right).

Fig. 6: a) Neat gasoline containing phenol (*) injected on (left) an HP-5 column (50°C then 4°C/min to 120°C) and (right) the water stationary phase at 100°C. b) Lavender oil sample containing linalool (*) injected on (left) an HP-5 column (50°C then 4°C/min to 280°C) and (right) the water stationary phase (40°C for 2 min then 60°C for 2 min then 70°C); full scale shown inset. c) Neat balsamic vinegar (left) and neat apple juice (right) each containing 1) propanoic acid, 2) sorbic acid and 3) benzoic acid injected on the water stationary phase at 100 °C (left) and 110 °C (right). Other analytes identified are 4) ethanol and 5) acetic acid.

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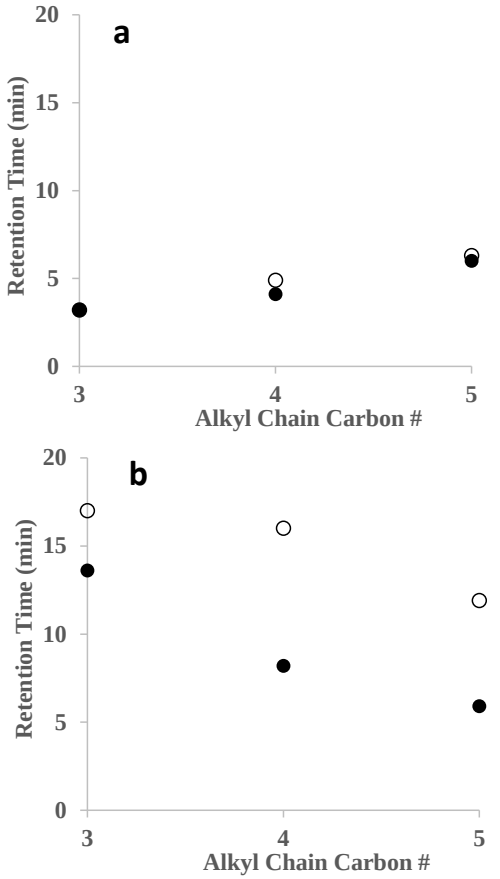

Marno
Thurbide
Fig. 1

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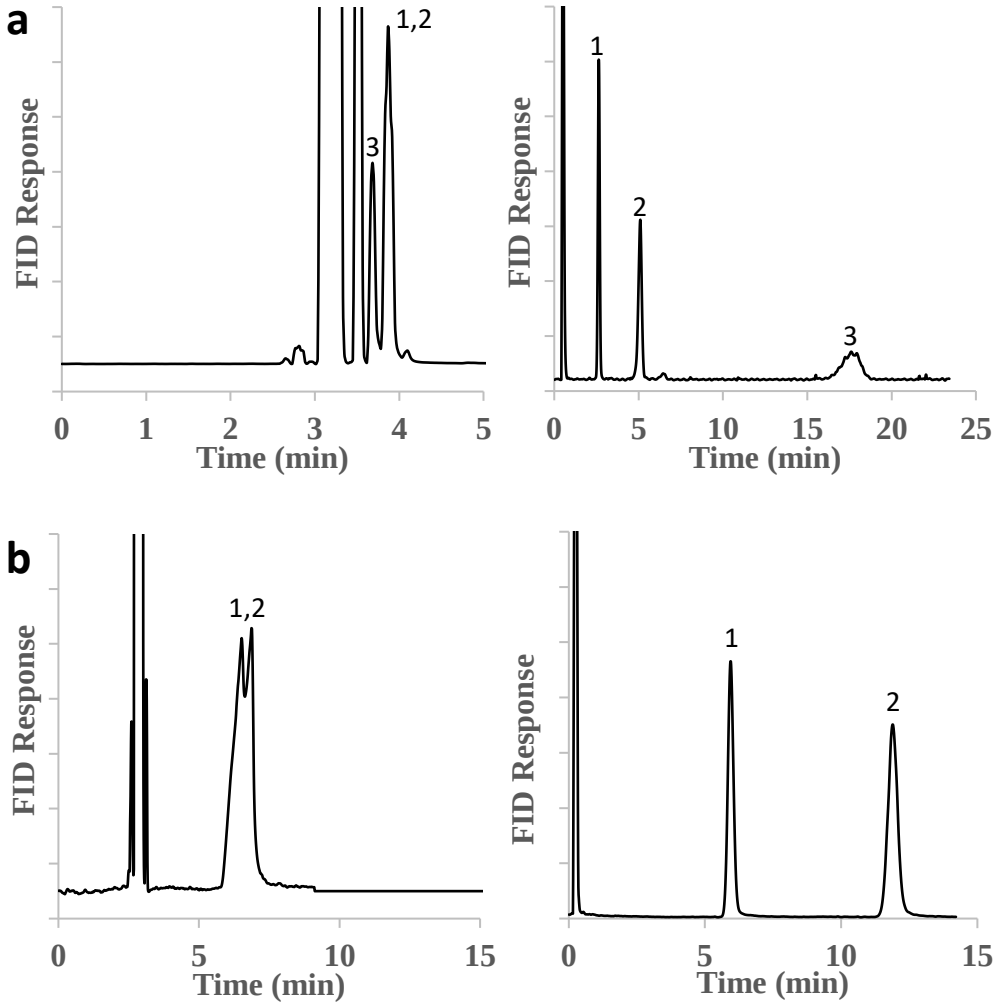

Marno
Thurbide
Fig. 2

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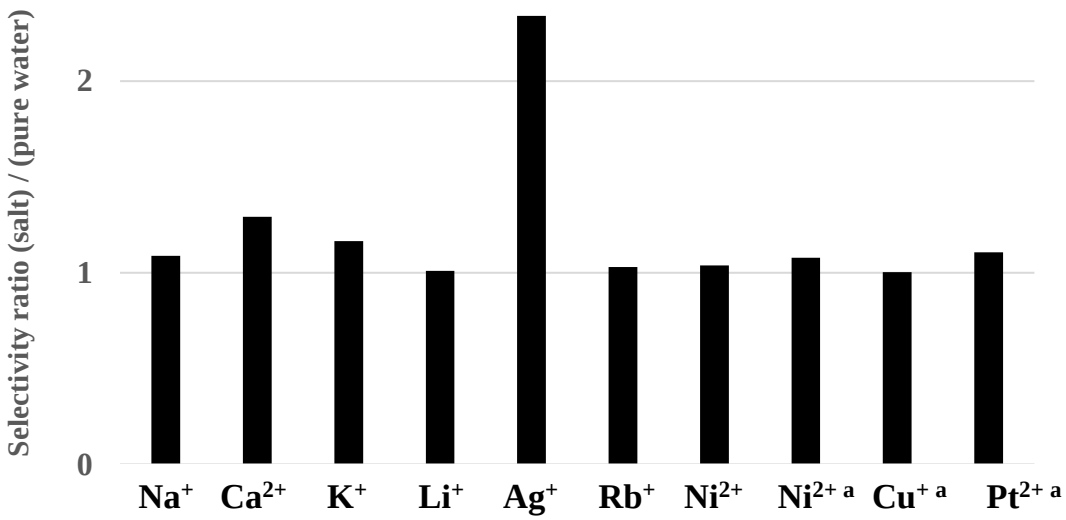

Marno
Thurbide
Fig. 3

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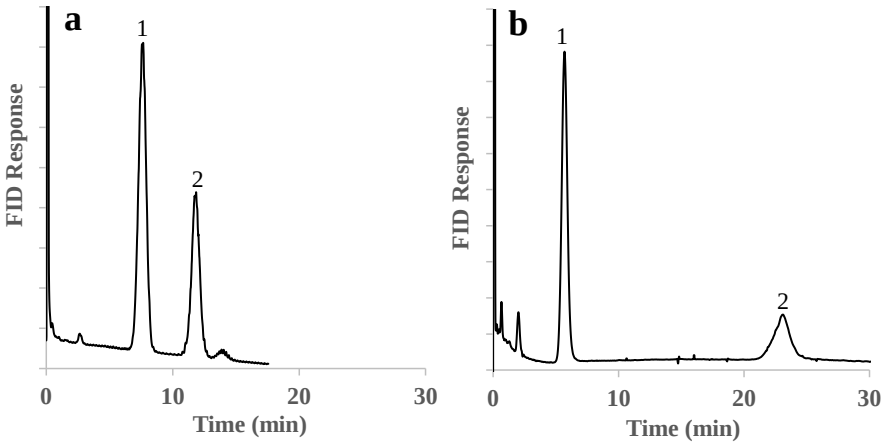

Marno
Thurbide
Fig. 4

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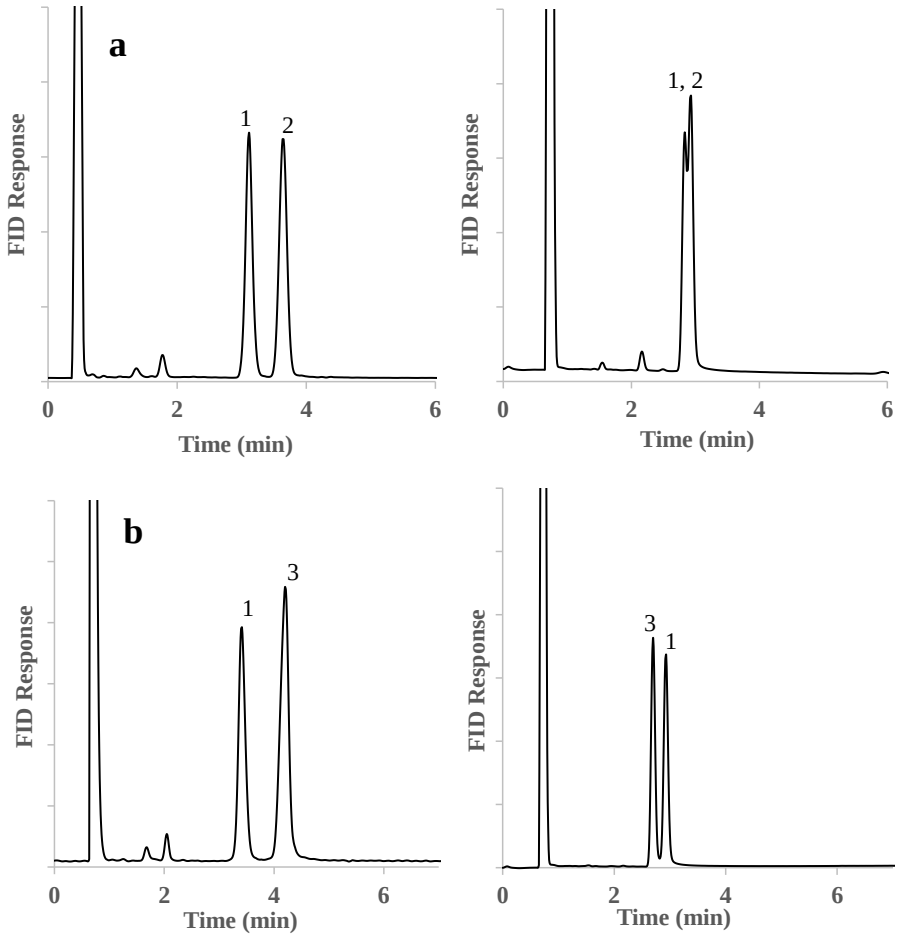

Marno
Thurbide
Fig. 5

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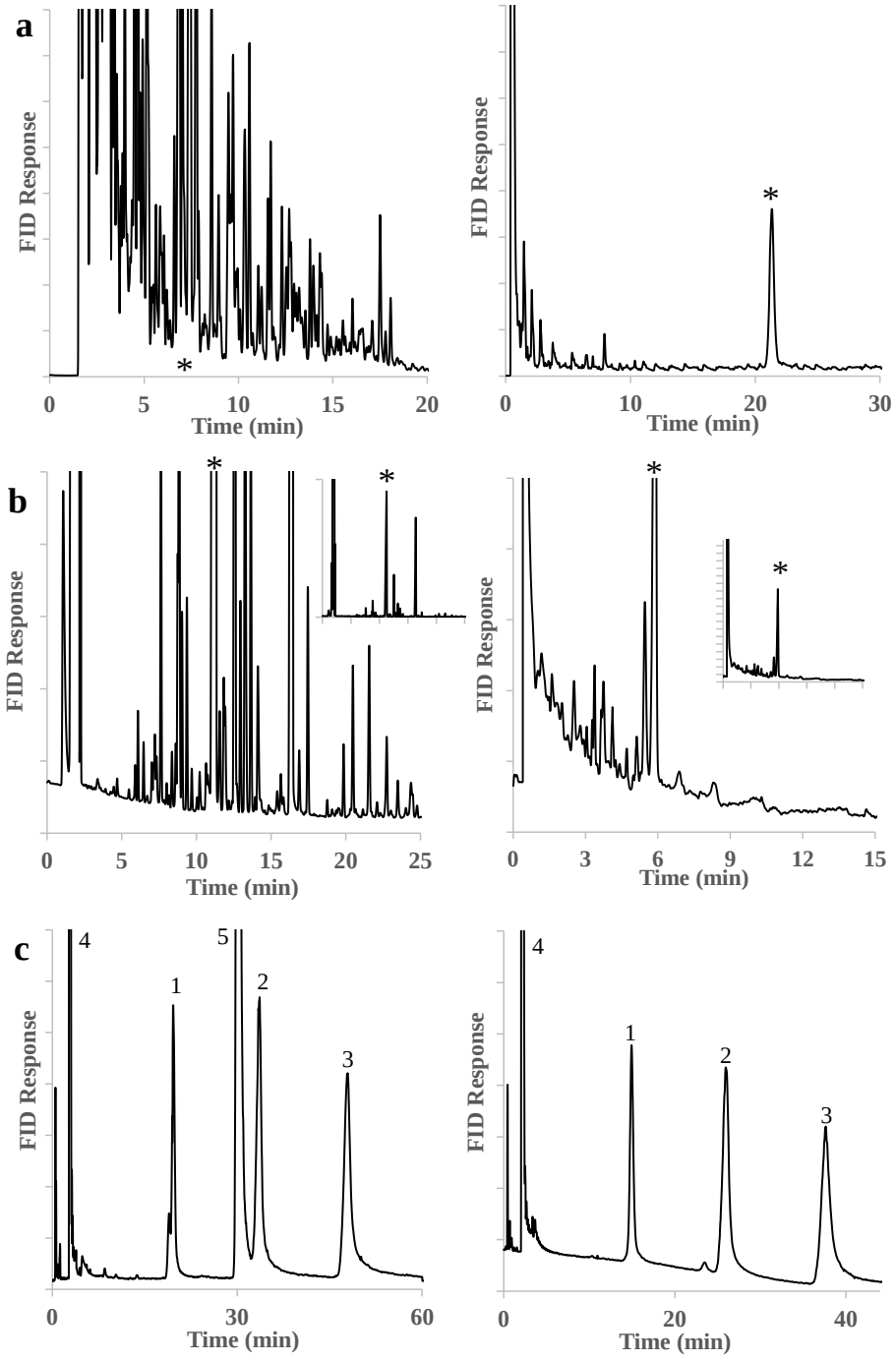

Marno
Thurbide
Fig. 6