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CAPILLARY GAS CHROMATOGRAPHIC SEPARATION OF ORGANIC BASES USING A pH-ADJUSTED BASIC WATER STATIONARY PHASE

by

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ABSTRACT

The use of a pH-adjusted water stationary phase for analyzing organic bases in capillary gas chromatography (GC) is demonstrated. Through modifying the phase to typical values near pH 11.5, it is found that various organic bases are readily eluted and separated. Conversely, at the normal pH 7 operating level, they are not. Sodium hydroxide is found to be a much more stable base than ammonium hydroxide for altering the pH due to the higher volatility and evaporation of the latter. In the basic condition, such analytes are not ionized and are observed to produce good peak shapes even for injected masses down to about 20 ng. By comparison, analyses on a conventional non-polar capillary GC column yield more peak tailing and only analyte masses of 1 µg or higher are normally observed. Through carefully altering the pH, it is also found that the selectivity between analytes can be potentially further enhanced if their respective pKa values differ sufficiently. The analysis of different pharmaceutical and petroleum samples containing organic bases is demonstrated. Results indicate that this approach can potentially offer unique and beneficial selectivity in such analyses.

Keywords: gas chromatography; water; stationary phase; basic; organic bases

1. INTRODUCTION

Gas chromatography (GC) is an important, widely used separation technique [1, 2] that serves an essential role in the analysis of volatile organic compounds [3]. Since its early introduction, GC has been involved in a variety of important areas such as industrial quality control, environmental monitoring, forensic analysis, and oil exploration [4-8]. Given its good sensitivity and reliable performance in such applications, further advancements in GC are continuously being developed in order to further improve its field of use.

Stationary phase development continues to be a very important area in GC since it can produce improved separation capabilities. While routinely used conventional fused silica capillary GC columns are often coated with non-polar phases such as dimethylpolysiloxane, or polar phases such as polyethylene glycol [9, 10], many efforts have been put forth to create novel columns that provide more thermal stability and/or greater analyte selectivity. For instance, in recent years, a diverse range of GC stationary phase coatings have been explored that have very useful separation characteristics, such as those composed of ionic liquids [11-14], metal-organic frameworks [15, 16], and dithienyl benzothiadiazoles [17].

We have recently introduced the use of water as a novel stationary phase in capillary GC [18]. In this technique, water is coated on the interior walls of a stainless steel capillary and can be used successfully for GC separations in the direct analysis of various compounds in both aqueous and organic samples. The method demonstrates good efficiency under optimum conditions and provides reliable performance over a wide range of operational temperatures [18]. Further, the water stationary phase displays notable selectivity towards many polar analytes and very little affinity for non-polar analytes. In this regard, retention time on the stationary phase also appears to be considerably dependent upon analyte water solubility, but much less so upon

analyte volatility. Therefore, given its interesting properties, further exploration and development of this technique would be potentially beneficial.

In working with the water stationary phase, one difficulty frequently noted involves the GC analysis of ionizable analytes. For example, many organic bases do not appear to elute from the system under regular operating conditions. This is presumably because many are largely ionized in the typical neutral pH water coating, and therefore are heavily partitioned into the phase in their charged state. As a result, such organic bases are very difficult to elute and observe with this technique.

Indeed the analysis of organic bases is very important in conventional GC. For example, such compounds are frequently of great concern in many important areas like oil and gas development [19, 20] and pharmaceutical production [21-23]. However, their GC analysis is often difficult due to the fact that they can engage in unfavorable interactions with column active sites, which can lead to poor peak shapes [24]. As a result, such bases are often first derivatized to relatively non-polar moieties to overcome this obstacle [25]. However, while this is effective, it can be time consuming, laborious, and a frequent source of error [25]. As a result, many efforts have been focused on trying to improve the separation of organic bases, including the use of ionic liquid coatings, graphitized carbon or porous polymer packings, and various derivitization and column deactivation schemes [26,27]. Therefore, continued exploration of new ways to analyze such bases more directly and simply in GC is of great interest.

Here we describe a novel method to separate and analyze organic bases in capillary GC by using a pH-adjusted water stationary phase. By carefully adjusting the pH of the water phase, and hence controlling analyte ionization *in-situ*, many organic bases can be readily separated and directly analyzed by this approach. The general operating parameters of the method are reviewed

and its separation characteristics and selectivity for several such analytes are explored. Finally, this technique is applied to the direct analysis of various organic bases in different samples in order to gauge its effectiveness.

2. EXPERIMENTAL

2.1 Instrumentation and Operation

A schematic diagram of the instrumental setup used here is shown in figure 1 and has been described previously [18]. An HP 5890-Series II GC instrument (Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) was employed. High purity Helium (Praxair, Calgary, Canada) was used as the carrier gas and was saturated with water vapour using an ISCO model 100DX syringe pump (Teledyne ISCO, Lincoln, NE, USA) that supplied water through a valco zero dead volume tee union (Vici-Valco, Houston, TX, USA). The outlet of the tee union was connected to a 1 m stainless steel (SS) pre-heating coil (1/16" O.D. x 250 µm I.D.; Chromatographic Specialties, Brockville, ON, CAN) and both were kept inside the oven. The coil outlet led into the injector. This setup differs from the passive hydrator used previously [18] and further prevented evaporation of the water stationary phase downstream during separations. For instance, in contrast to the slow phase erosion noted earlier [18] it was found here that actively directing 1-3 µL/min of water to the column carrier gas provided stable analyte retention times that only differed by about 1% RSD over 4-5 hours of operation at elevated temperatures.

The separation column was connected to the injector and typically operated with 22 cm/s of carrier gas and a 10:1 split ratio. A 10 m length of 316 SS capillary tubing (1/16" O.D. x 100 µm I.D.; Chromatographic Specialties) was normally used for separations. The column outlet was joined to a 1 m SS capillary restrictor (1/16" O.D. x 75µm I.D.) via a zero dead volume union (Vici-Valco, Houston, TX, USA). This restrictor provided system backpressure and

promoted phase stability at higher temperatures. It was positioned inside the FID jet at the burner surface where effluent was deposited directly into the detector flame. An injector/detector temperature of around 220 ^{o}C was maintained during the experiments. High purity Hydrogen and Air (Praxair) were used to support the detector flame at respective flows 90 and 350 mL/min. In some comparisons, a conventional DB-5 GC column (95% methyl/5% phenyl polysiloxane stationary phase; 250 µm I.D. x 30 m x 0.25 µm thick; J&W Scientific Inc., USA) was also used.

2.2 Stationary Phase Preparation

A 1 M base stock solution (ammonium or sodium hydroxide) was prepared using HPLC-grade water and used to adjust the pH of the water supply to be employed as a stationary phase. Using a pH meter, after calibration with an appropriate buffer, 500 mL of the water supply was stirred while the base stock solution was added dropwise until the desired pH was achieved. This pH-adjusted solution was then used immediately to coat the SS capillary column as described previously [18]. Once finished, the column was mounted inside the GC oven for separation use.

2.3 Chemicals and Reagents

Solvents included dichloromethane, hexane, heptane, acetone, ethanol (≥ 99% each; Sigma−Aldrich, Canada), and HPLC-grade water (Honeywell Burdick & Jackson, Muskegon, USA), which was also used to prepare coating solutions. Analytes, including methylamine (70% in water; Arkema Inc, Canada), benzylamine (Kodak, USA), diethylamine (Fisher Scientific, USA), butylamine, pentylamine, hexylamine, octylamine and piperidine (Sigma Aldrich, Canada) were all ≥ 98% purity and used to prepare standards (10 µg/µL) in heptane. A mixture (10 µg/µL each) of pyridine (≥ 99.0%; MERCK KGaA, Germany), aniline (≥ 99.0%; BDH Inc, Canada), quinoline (≥ 99.0%; Sigma−Aldrich, Canada) and indole (≥ 99.0%; Sigma−Aldrich, Canada) was also prepared in automotive fuel purchased from a local vendor. Standards (10

154 µg/µL) of fluoxetine HCl, benzydamine HCl, and caffeine (each 99.9%; Sigma−Aldrich,
155 Canada) were prepared in acetone or dichloromethane. A commercial benzydamine mouthwash
156 was purchased from a local supplier. All other variations are described in the text.

157 **3. RESULTS AND DISCUSSION**

158 **3.1 General Operating Characteristics**

159 Initial experiments were aimed at exploring if organic bases could be eluted from the
160 water stationary phase by adjusting its pH. Indeed, it was found that as the pH increased organic
161 bases could be readily analyzed in this way. Figure 2 demonstrates the typical results of this
162 approach with chromatograms for hexylamine at different water stationary phase pH levels.
163 Figure 2A illustrates the difficulty in analyzing organic bases on the pure water stationary phase.
164 As seen, no analyte peak is observed. This is presumably because hexylamine (pKa 10.5) should
165 be over 99.9% ionized under this neutral pH 7 condition, and therefore heavily partitioned to the
166 water stationary phase. In contrast to this, when ammonium hydroxide is used to increase the
167 phase pH to 10.5 (figure 2B), a clear hexylamine peak is readily observed near the 3.5 minute
168 mark. Under these conditions, about half of the analyte exists in the ionized form. Finally, in
169 figure 2C, the hexylamine peak is much shaper and more prominent when the pH is further
170 raised to 11.5 and only 9% of the analyte resides in the ionized form. Therefore, increasing the
171 pH of the water stationary phase can dramatically improve the ability of the system to analyze
172 organic bases. In general, it was found that a pH level 1 unit or higher than the analyte pKa
173 provided good peak shapes and so this was typically the aim of subsequent trials.

174 One aspect that was evident in the early experiments using ammonium hydroxide to alter
175 the stationary phase pH, was that over time the peak shape began to erode considerably. This is
176 because under the dynamic column conditions employed, the volatile ammonia substrate can

freely evaporate from the system and change the resulting pH since its boiling point is near 27^{o}C. Figure 3 shows an example of this for repeated injections of hexylamine using a pH 11.5 water stationary phase. As figures 3A, B, and C show, when using ammonium hydroxide to adjust the phase pH, the peak shape rapidly erodes after only a few minutes of operation until it is scarcely discernable after about a half hour due to the pH dropping over this period. Thus, sodium hydroxide was explored as a less volatile base for this same purpose. It was found to provide excellent performance, stability, and elicited no adverse detector attributes over long periods of operation. As seen in figure 3D, the analyte peak shape remains very good even after an hour of operation when using sodium hydroxide instead. To be clear, this occurs because the non-volatile sodium hydroxide remains dissolved within the intact water stationary phase that is maintained inside of the column during the separations and provides a stable pH as a result. This also provided stable elution properties, as repeat injections of hexylamine produced retention times that varied within 2% RSD (n=3) when using sodium hydroxide to establish the phase. Thus, given its superior performance, sodium hydroxide was used exclusively throughout the rest of the study.

3.2 Analyte Retention

In order to better understand the retention properties of the basic water stationary phase, a number of different organic base analytes were explored in the system. Table 1 displays the compounds examined, which include primary, secondary, and tertiary amines, an assortment of cyclic ring structures, as well as aromatic and aliphatic molecules. As can be seen from the analytes listed in increasing order of retention at a column temperature of 100 ^{o}C, a wide variety of compounds can be well retained and analyzed using this approach.

Findings reveal the column largely produces a normal phase retention pattern, where less polar analytes elute earlier than those more polar. For instance, octylamine elutes before

methylamine. This has been noted previously and partly ascribed to analyte water solubility [18]. Accordingly, octylamine is also near 1000-fold less water soluble than methylamine [28].

Also similar to previous studies [18], retention does not appear to correlate strongly with analyte boiling point (also included in table 1). For instance, highly volatile methylamine is greater retained than several other less volatile analytes, including benzydamine, which is the least volatile. Interestingly, the latter is also only sparingly soluble in water vs. methylamine [28]. Conversely, aniline and benzylamine possess similar volatility, yet benzylamine is more water soluble and much more retained [28]. Thus, in many cases it appears that more polar, water soluble compounds are greater retained on the basic water phase, irrespective of their volatility. This also agrees with earlier results [18].

Figure 4 further demonstrates the separation of various compounds from table 1. As can be seen, each of the various compounds (about 1 µg each) produce strong prominent peaks with reasonable shape indicating that the column can potentially work well for such analytes. In order to better place these results in context, a number of analytes were also examined on a conventional (unhydrated) DB-5 column and quite different results were obtained. Most notably, it was found that peak shapes were generally less favorable and signals could only be obtained above a certain analyte mass. For example, figure 5A demonstrates a typical chromatogram obtained for 1.5 µg of hexylamine on the DB-5 column. As can be seen, a clear peak is obtained, however it displays significant tailing and the baseline does not recover well after elution. This is understandable and somewhat expected since organic bases are known to produce poor peak shapes on conventional GC columns and are often first derivatized in efforts to avoid this [24,25].

Even more though, when smaller analyte masses below about 1 µg were introduced on the conventional column, peaks were often not observed under any condition, due to the column active sites present. By comparison, however, much smaller analyte masses could be readily observed on the basic water stationary phase. For instance, figure 5B shows a chromatogram obtained for 20 ng of hexylamine on the pH 11.5 water phase using a similar dimension column. As seen, a prominent peak with decent shape is still readily observed at these levels of analyte mass, which are near 2 orders of magnitude lower by comparison to figure 5A. As such, the basic water stationary phase approach may potentially provide beneficial sensitivity in such analyses.

3.3 Altering Selectivity

Since the pH of the water stationary phase can significantly impact the ability of organic bases to elute from the column, it was found that this could also be potentially employed to affect separation selectivity. For instance, where analytes possess sufficiently different pKa values, the water phase pH might be used to alter relative ionization and retention. Figure 6 presents an illustration of this with the separation of an aqueous mixture of pyridine and methylamine, which have respective pKa values of 5.25 and 10.63. As seen in figure 6A, when the stationary phase pH is 11.5 and above the pKa of each analyte, both neutral analytes readily elute with good peak shape. However, when the water phase is at pH 7 (figure 6B), the now ionized methylamine peak is highly retained and removed while the still neutral pyridine peak appears as before. Thus, through manipulating the water phase pH, it may be possible to adjust separation selectivity amongst different compounds or, alternatively, bracket the pKa values of various unknown analytes in mixtures. However, this requires additional study and was not further pursued here.

3.4 Applications

In order to gauge the potential ability of this approach to analyze organic bases, different applications were probed with it. The first of these was pharmaceutical analysis. Pharmaceuticals

247 frequently encompass a wide variety of chemical classes and often include organic bases.
248 Further, many such compounds can possess a relatively high molecular weight and/or multiple
249 functional groups, both of which pose challenges for separation using GC [24]. Therefore, they
250 were useful to examine with the pH-adjusted water stationary phase approach.

251 One of the first candidates to be explored was fluoxetine hydrochloride, an organic base
252 (pKa 10.1) which is the active pharmaceutical ingredient in Prozac, a commonly prescribed
253 antidepressant. Fluoxetine hydrochloride has a molecular weight of 345 g/mol and accordingly
254 HPLC is most widely used for its analysis [29, 30]. However, GC has also been employed for
255 this purpose but analyte derivitization is often required in advance [31, 32]. Figure 7A shows an
256 injection of a fluoxetine hydrochloride standard prepared in acetone using the pH 11.5 water
257 stationary phase. As seen, a sharp prominent peak appears for this analyte. Even more
258 remarkable, no derivitization was required and the fluoxetine eluted after about 30 minutes at a
259 column temperature of only 100 °C, despite the fact that its boiling point is several hundred
260 degrees higher than this. Thus, the basic water stationary phase may be used to readily and
261 directly analyze such compounds in GC. It should be noted that technically the conjugate acid
262 salt form of the analyte was injected and this could elicit a question of whether or not this
263 difference can impact separations. However, in experiments where either the acid salt or free
264 base forms were injected, the resulting peak and retention time realized was unchanged. This is
265 because upon contacting the basic phase, the salt analyte is also rendered into the free base form.

266 Another non-volatile pharmaceutical of interest was benzydamine hydrochloride (pKa
267 10.4), which is known for its analgesic and anti-inflammatory properties and is often
268 incorporated into oral rinse formulations. As such, an aqueous benzydamine hydrochloride
269 mouthwash was obtained from a local vendor and analyzed directly as a neat solution using the

basic water stationary phase approach. Figure 7B shows the result, which displays a clear peak for benzydamine near the 5 minute mark at a column temperature of 100 ^{o}C; again, well below its boiling point and without derivitization. Further, despite the neat injection, no significant interference from the matrix is observed. Of note, only ethanol and glycerol peaks appear later as they are major constituents of the formulation. However other excipients present, such as polysorbate and various flavor agents do not elute since they are very highly polar and remain strongly bound to the water stationary phase. This is further advantageous, though, since such samples can be directly injected without concern over potential column fouling effects because the phase can be discarded after usage. This behavior has been noted previously for other matrices [18] and indicates that certain pharmaceutical samples, aqueous or otherwise, can be directly analyzed with this system after little or no sample preparation.

A different application involved the analysis of nitrogen-containing compounds in petroleum products. This is an important issue since it is known that such molecules can reduce product quality, produce gum formation and engine deposits, and poison process catalysts [33-35]. Analytically, however, their determination poses significant challenges since petroleum comprises an extremely complex matrix with thousands of hydrocarbons that normally co-elute with target analytes and obscure their signal [33-35]. Further, some conventional methods also invoke analyte derivatization to improve adverse adsorption effects in the system, which can lead to long sample preparations and unwanted byproducts [25,36-38]. As such, methods that can promote the direct and highly selective analysis of these compounds are of great interest [33-35].

In order to investigate this, an automotive fuel was obtained from a local vendor, spiked with several nitrogen-containing analytes, and then analyzed directly as a neat injection on both the basic water stationary phase and a conventional DB-5 column. A similar reference standard

in heptane was also used to confirm the presence and retention time of each peak. Figure 8 shows the results, and first demonstrates the difficulty in analyzing such samples by conventional means using a regular non-polar capillary GC column (figure 8A). As seen, the fuel sample contains numerous peaks over the nearly 30 minute separation, making determination of the spiked target analytes very difficult due to the overlapping signals. In particular, the analytes were found to elute near 5 minutes (pyridine), 7 minutes (aniline), 15 minutes (quinolone), and 19 minutes (indole). However, as observed in the figure, the analyte peaks are obscured by the matrix and otherwise indiscernible on this column. Conversely, figure 8B demonstrates the same sample as analyzed on the pH 11.5 water stationary phase. In contrast to the conventional column, it can be seen that on the water stationary phase the bulk hydrocarbon matrix is essentially unretained due to its very low water solubility, which agrees with earlier findings [18]. However, the nitrogen-containing target analytes are well retained and separated and easily observed since there is no matrix interference to contend with. As a result, the basic water stationary phase can potentially allow for very selective analyses of such ionizable compounds in complex matrices of this nature.

4. CONCLUSION

Ionizable organic bases can be readily eluted and separated on a water stationary phase column after adjusting its pH appropriately. In this condition, such analytes are rendered neutral and can produce good peak shapes, even at low analyte levels. Through carefully adjusting the pH, analyte selectivity on the column can also be potentially further enhanced. Results indicate that this approach can offer beneficial properties in analyzing organic bases in a variety of matrices. Further application and exploration of this method would therefore be useful.

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FIGURE CAPTIONS

Figure 1: Schematic diagram of capillary GC water stationary phase system.

Figure 2: Chromatograms for hexylamine on a water stationary phase at pH A) 7.0, B) 10.5, and C) 11.5. Ammonium hydroxide is used to adjust the pH. The off-scale peak in each is the injection solvent.

Figure 3: Chromatograms for hexylamine on a water stationary phase adjusted to pH 11.5 with ammonium hydroxide A) immediately, B) 9 minutes, and C) 37 minutes after the column was installed. D presents the same following 1 hour of column operation after using sodium hydroxide to adjust the pH to 11.5.

Figure 4: Typical separation of about 1 µg each of various analytes on the basic water stationary phase (pH 11.5). Column temperature is 100 ^{o}C. Elution order is 1. heptane solvent, 2. octylamine, 3. diethylamine, 4. butylamine, 5. pyridine, 6. aniline, 7. quinoline, and 8. indole.

Figure 5: Chromatograms of hexylamine on A) a conventional DB-5 column, and B) a pH 11.5 water stationary phase column. Conditions: A) 1.5 µg analyte, 250 µm I.D. x 30 m column at 60 ^{o}C; B) 20 ng analyte, 250 µm I.D. x 10 m column at 100 ^{o}C.

Figure 6: Separation of an aqueous mixture of pyridine (1) and methylamine (2) on a water stationary phase at A) pH 11.5 and B) pH 7. Conditions: 250 µm I.D. x 10 m column at 110 ^{o}C.

Figure 7: Chromatograms of A) a 1 µg fluoxetine hydrochloride standard in acetone, and B) a neat injection of benzydamine hydrochloride mouthwash (1.5 mg/mL), each on a pH 11.5 water stationary phase column at 100°C. Elution order: A) 1. acetone, 2. fluoxetine, and B) 3. benzydamine, 4. ethanol, and 5. glycerol.

Figure 8: A mixture of nitrogen-containing analytes (1µg each) in automotive fuel as analyzed on A) a conventional DB-5 column and B) a pH 11.5 water stationary phase at 100 °C. The arrows in A indicate where the standards eluted on that column under the same conditions. Elution order for both trials: 1. pyridine, 2. aniline, 3. quinoline and 4. indole.

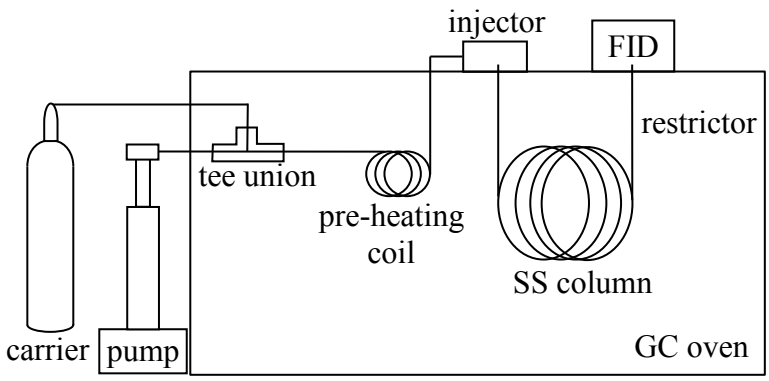
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Figure 1

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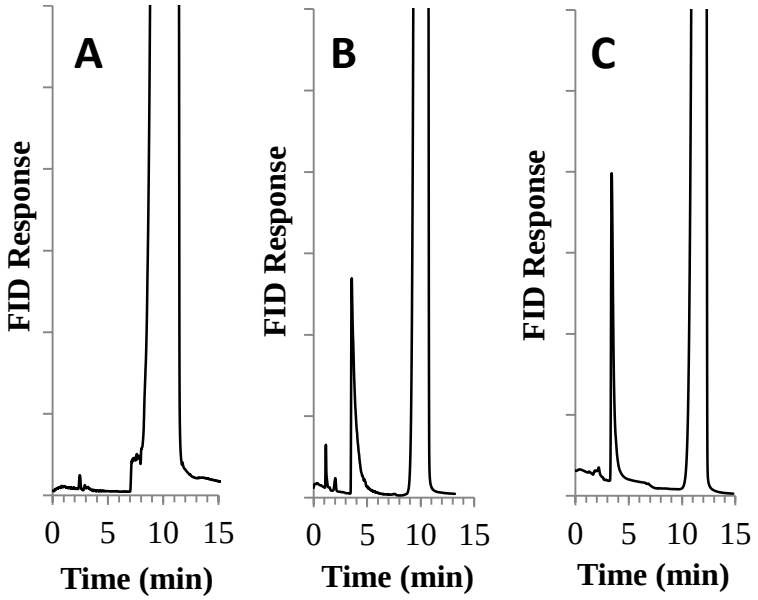

Figure 2

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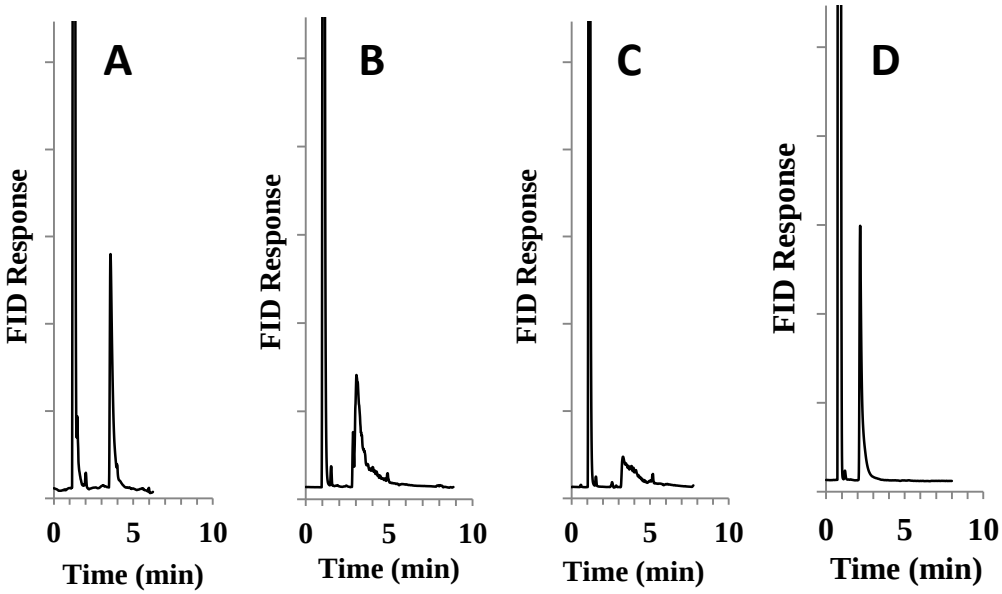

Figure 3

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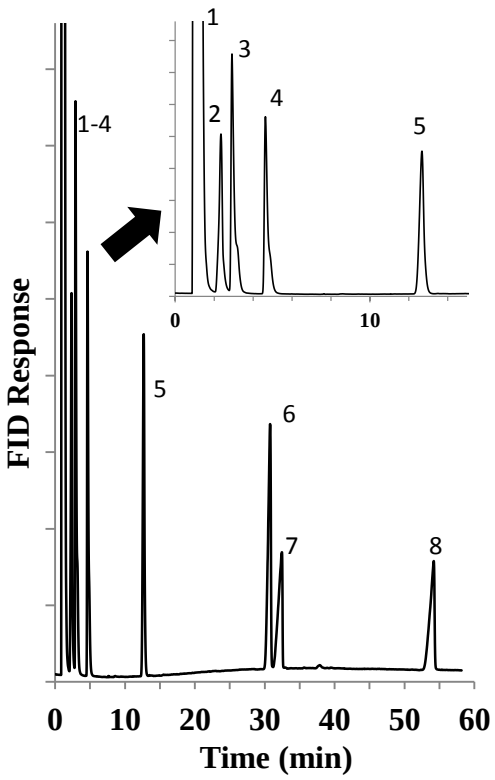

Figure 4

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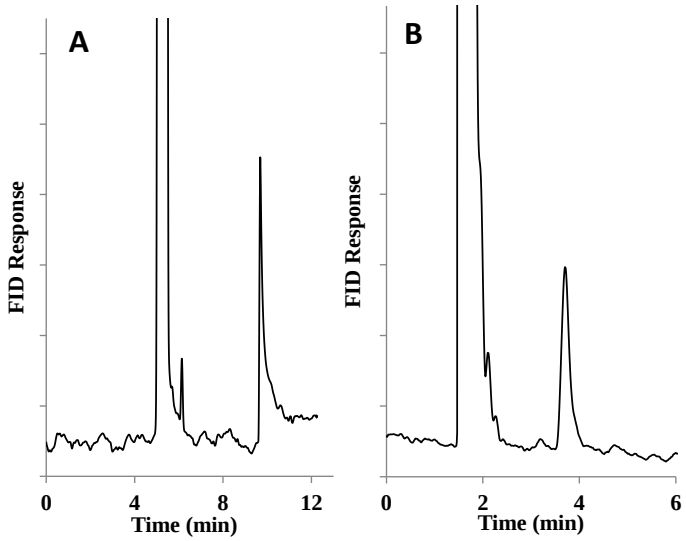

Figure 5

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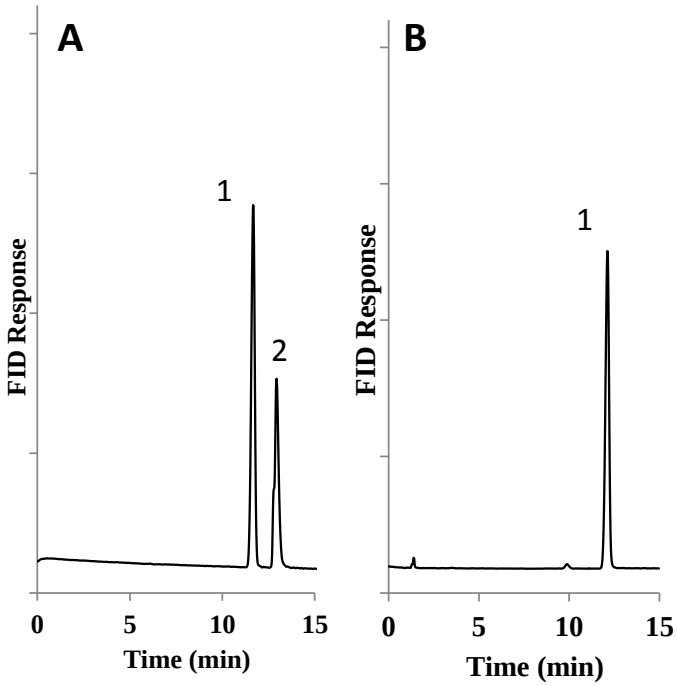

Figure 6

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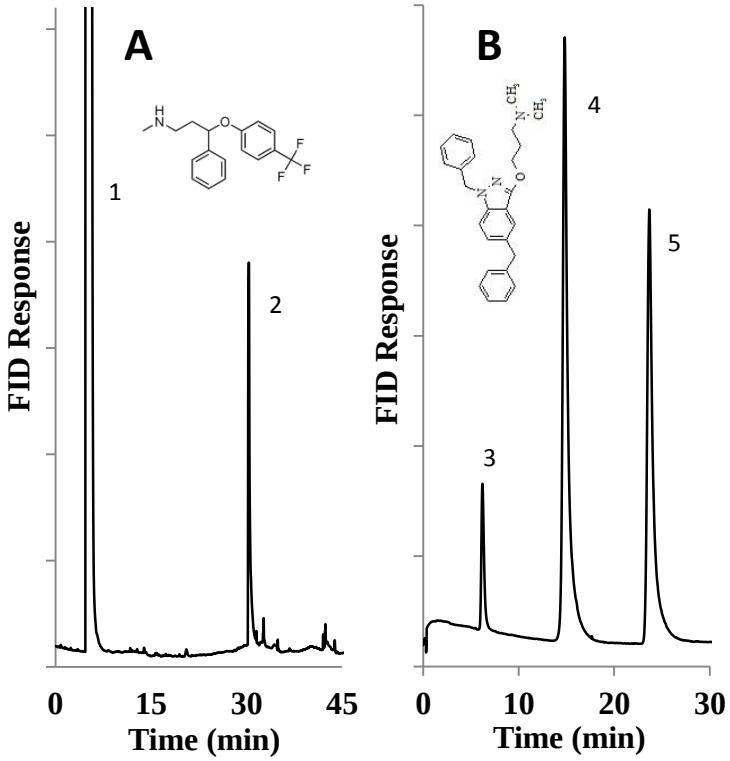

Figure 7

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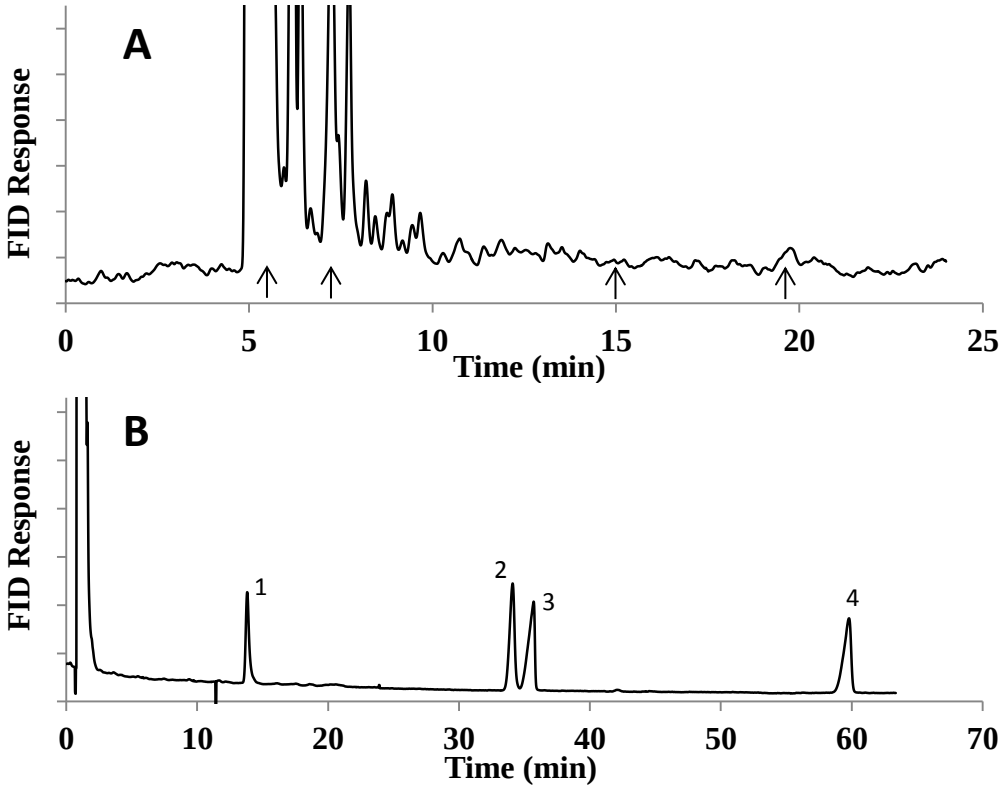

Figure 8

Table 1: Retention time of various analytes on the basic[a] water stationary phase

	Retention (min)	Boiling point (°C)
Triethylamine	1.0	90
Octylamine	2.1	175
Hexylamine	2.7	132
Diethylamine	2.9	55
Pentylamine	3.4	94
Butylamine	4.7	77
Benzydamine	5.1	474
Piperidine	10.6	106
Pyridine	12.7	115
Methylamine	15.0	-7
Caffeine	21.6	178[b]
Fluoxetine	30.4	395
Aniline	32.3	184
Quinoline	33.5	238
Benzylamine	54.1	185
Indole	56.8	254

a. pH = 11.5; b. sublimes
*100 µm I.D. x 10 m column at 100 ^{o}C; void time is 0.7 min.