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

by

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41 **ABSTRACT**

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

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

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62 1. INTRODUCTION

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

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

77 We have recently introduced the use of water as a novel stationary phase in capillary GC
78 [18]. In this technique, water is coated on the interior walls of a stainless steel capillary and can
79 be used successfully for GC separations in the direct analysis of various compounds in both
80 aqueous and organic samples. The method demonstrates good efficiency under optimum
81 conditions and provides reliable performance over a wide range of operational temperatures [18].
82 Further, the water stationary phase displays notable selectivity towards many polar analytes and
83 very little affinity for non-polar analytes. In this regard, retention time on the stationary phase
84 also appears to be considerably dependent upon analyte water solubility, but much less so upon

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

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

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

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

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

111 **2. EXPERIMENTAL**

112 **2.1 Instrumentation and Operation**

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

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

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

137 **2.2 Stationary Phase Preparation**

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

144 **2.3 Chemicals and Reagents**

145 Solvents included dichloromethane, hexane, heptane, acetone, ethanol ($\geq 99\%$ each;
146 Sigma–Aldrich, Canada), and HPLC-grade water (Honeywell Burdick & Jackson, Muskegon,
147 USA), which was also used to prepare coating solutions. Analytes, including methylamine (70%
148 in water; Arkema Inc, Canada), benzylamine (Kodak, USA), diethylamine (Fisher Scientific,
149 USA), butylamine, pentylamine, hexylamine, octylamine and piperidine (Sigma Aldrich,
150 Canada) were all $\geq 98\%$ purity and used to prepare standards (10 µg/µL) in heptane. A mixture
151 (10 µg/µL each) of pyridine ($\geq 99.0\%$; MERCK KGaA, Germany), aniline ($\geq 99.0\%$; BDH Inc,
152 Canada), quinoline ($\geq 99.0\%$; Sigma–Aldrich, Canada) and indole ($\geq 99.0\%$; Sigma–Aldrich,
153 Canada) was also prepared in automotive fuel purchased from a local vendor. Standards (10

154 $\mu\text{g}/\mu\text{L}$) 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 (pK_a 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 pK_a
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

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

192 **3.2 Analyte Retention**

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

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

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

203 Also similar to previous studies [18], retention does not appear to correlate strongly with
204 analyte boiling point (also included in table 1). For instance, highly volatile methylamine is
205 greater retained than several other less volatile analytes, including benzydamine, which is the
206 least volatile. Interestingly, the latter is also only sparingly soluble in water vs. methylamine
207 [28]. Conversely, aniline and benzylamine possess similar volatility, yet benzylamine is more
208 water soluble and much more retained [28]. Thus, in many cases it appears that more polar,
209 water soluble compounds are greater retained on the basic water phase, irrespective of their
210 volatility. This also agrees with earlier results [18].

211 Figure 4 further demonstrates the separation of various compounds from table 1. As can
212 be seen, each of the various compounds (about 1 μg each) produce strong prominent peaks with
213 reasonable shape indicating that the column can potentially work well for such analytes. In order
214 to better place these results in context, a number of analytes were also examined on a
215 conventional (unhydrated) DB-5 column and quite different results were obtained. Most notably,
216 it was found that peak shapes were generally less favorable and signals could only be obtained
217 above a certain analyte mass. For example, figure 5A demonstrates a typical chromatogram
218 obtained for 1.5 μg of hexylamine on the DB-5 column. As can be seen, a clear peak is obtained,
219 however it displays significant tailing and the baseline does not recover well after elution. This is
220 understandable and somewhat expected since organic bases are known to produce poor peak
221 shapes on conventional GC columns and are often first derivatized in efforts to avoid this
222 [24,25].

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

231 **3.3 Altering Selectivity**

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

244 **3.4 Applications**

245 In order to gauge the potential ability of this approach to analyze organic bases, different
246 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

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

281 A different application involved the analysis of nitrogen-containing compounds in
282 petroleum products. This is an important issue since it is known that such molecules can reduce
283 product quality, produce gum formation and engine deposits, and poison process catalysts [33-
284 35]. Analytically, however, their determination poses significant challenges since petroleum
285 comprises an extremely complex matrix with thousands of hydrocarbons that normally co-elute
286 with target analytes and obscure their signal [33-35]. Further, some conventional methods also
287 invoke analyte derivatization to improve adverse adsorption effects in the system, which can lead
288 to long sample preparations and unwanted byproducts [25,36-38]. As such, methods that can
289 promote the direct and highly selective analysis of these compounds are of great interest [33-35].

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

293 in heptane was also used to confirm the presence and retention time of each peak. Figure 8 shows
294 the results, and first demonstrates the difficulty in analyzing such samples by conventional
295 means using a regular non-polar capillary GC column (figure 8A). As seen, the fuel sample
296 contains numerous peaks over the nearly 30 minute separation, making determination of the
297 spiked target analytes very difficult due to the overlapping signals. In particular, the analytes
298 were found to elute near 5 minutes (pyridine), 7 minutes (aniline), 15 minutes (quinolone), and
299 19 minutes (indole). However, as observed in the figure, the analyte peaks are obscured by the
300 matrix and otherwise indiscernible on this column. Conversely, figure 8B demonstrates the same
301 sample as analyzed on the pH 11.5 water stationary phase. In contrast to the conventional
302 column, it can be seen that on the water stationary phase the bulk hydrocarbon matrix is
303 essentially unretained due to its very low water solubility, which agrees with earlier findings
304 [18]. However, the nitrogen-containing target analytes are well retained and separated and easily
305 observed since there is no matrix interference to contend with. As a result, the basic water
306 stationary phase can potentially allow for very selective analyses of such ionizable compounds in
307 complex matrices of this nature.

308 **4. CONCLUSION**

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

315

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456 **FIGURE CAPTIONS**

- 457 **Figure 1:** Schematic diagram of capillary GC water stationary phase system.
- 458 **Figure 2:** Chromatograms for hexylamine on a water stationary phase at pH A) 7.0, B) 10.5,
459 and C) 11.5. Ammonium hydroxide is used to adjust the pH. The off-scale peak in
460 each is the injection solvent.
- 461 **Figure 3:** Chromatograms for hexylamine on a water stationary phase adjusted to pH 11.5
462 with ammonium hydroxide A) immediately, B) 9 minutes, and C) 37 minutes
463 after the column was installed. D presents the same following 1 hour of column
464 operation after using sodium hydroxide to adjust the pH to 11.5.
- 465 **Figure 4:** Typical separation of about 1 μg each of various analytes on the basic water
466 stationary phase (pH 11.5). Column temperature is 100 $^{\circ}\text{C}$. Elution order is 1.
467 heptane solvent, 2. octylamine, 3. diethylamine, 4. butylamine, 5. pyridine, 6.
468 aniline, 7. quinoline, and 8. indole.
- 469 **Figure 5:** Chromatograms of hexylamine on A) a conventional DB-5 column, and B) a pH
470 11.5 water stationary phase column. Conditions: A) 1.5 μg analyte, 250 μm I.D. x
471 30 m column at 60 $^{\circ}\text{C}$; B) 20 ng analyte, 250 μm I.D. x 10 m column at 100 $^{\circ}\text{C}$.
- 472 **Figure 6:** Separation of an aqueous mixture of pyridine (1) and methylamine (2) on a water
473 stationary phase at A) pH 11.5 and B) pH 7. Conditions: 250 μm I.D. x 10 m
474 column at 110 $^{\circ}\text{C}$.
- 475 **Figure 7:** Chromatograms of A) a 1 μg fluoxetine hydrochloride standard in acetone, and B)
476 a neat injection of benzydamine hydrochloride mouthwash (1.5 mg/mL), each on
477 a pH 11.5 water stationary phase column at 100 $^{\circ}\text{C}$. Elution order: A) 1. acetone,
478 2. fluoxetine, and B) 3. benzydamine, 4. ethanol, and 5. glycerol.
- 479 **Figure 8:** A mixture of nitrogen-containing analytes (1 μg each) in automotive fuel as
480 analyzed on A) a conventional DB-5 column and B) a pH 11.5 water stationary
481 phase at 100 $^{\circ}\text{C}$. The arrows in A indicate where the standards eluted on that
482 column under the same conditions. Elution order for both trials: 1. pyridine, 2.
483 aniline, 3. quinoline and 4. indole.
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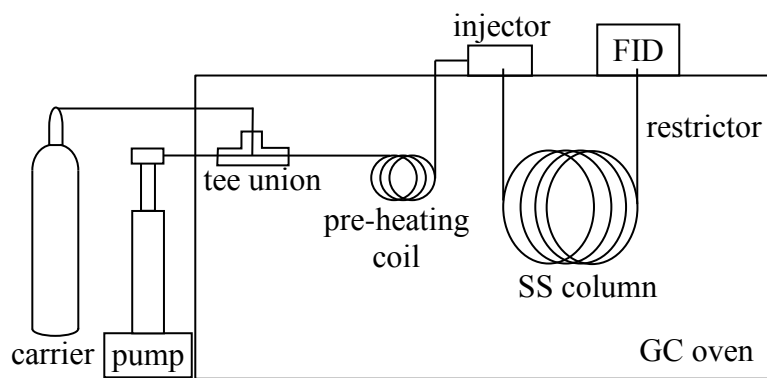


Figure 1

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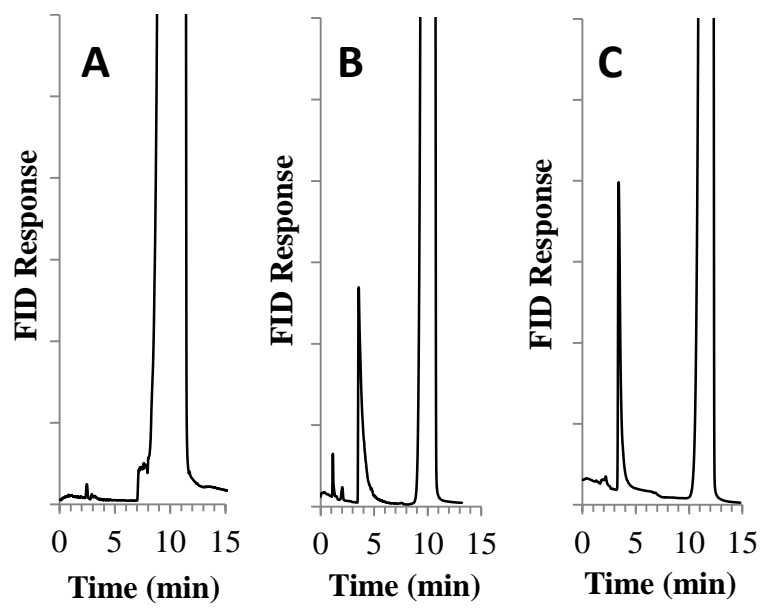


Figure 2

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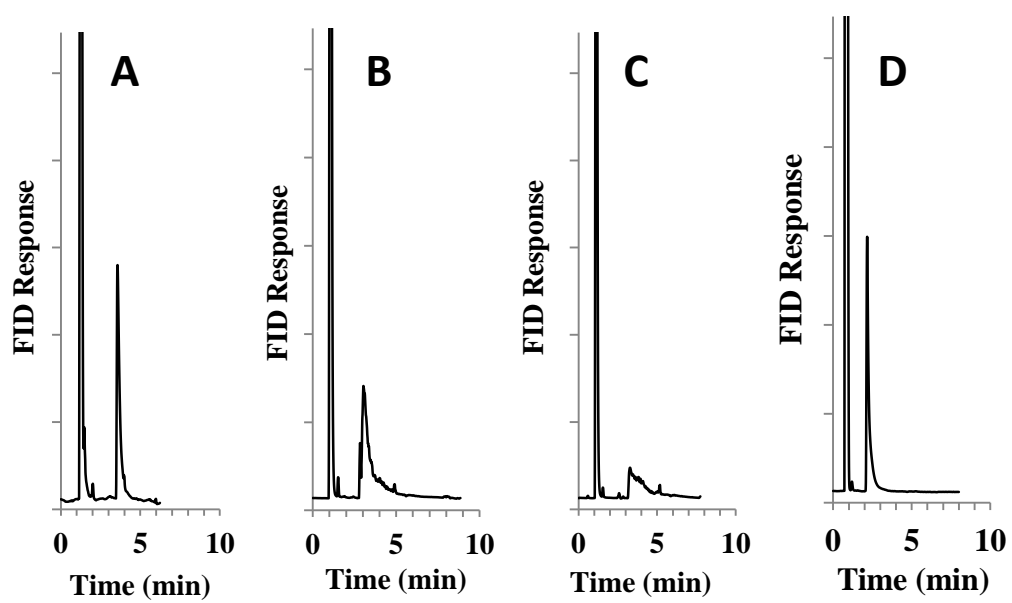


Figure 3

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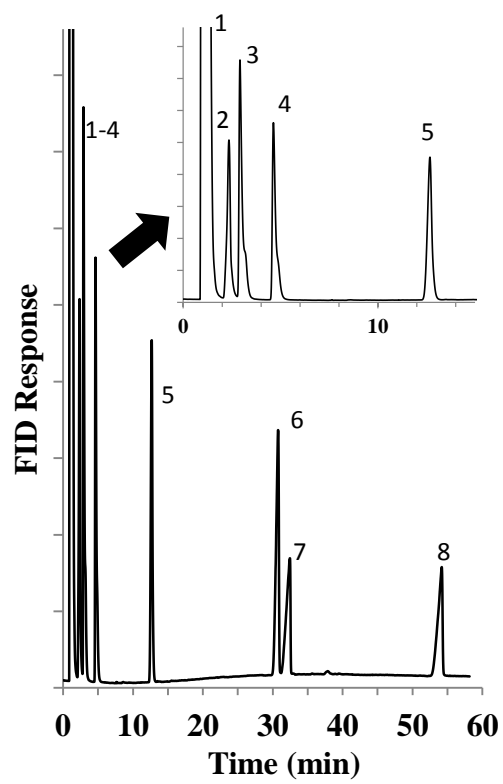


Figure 4

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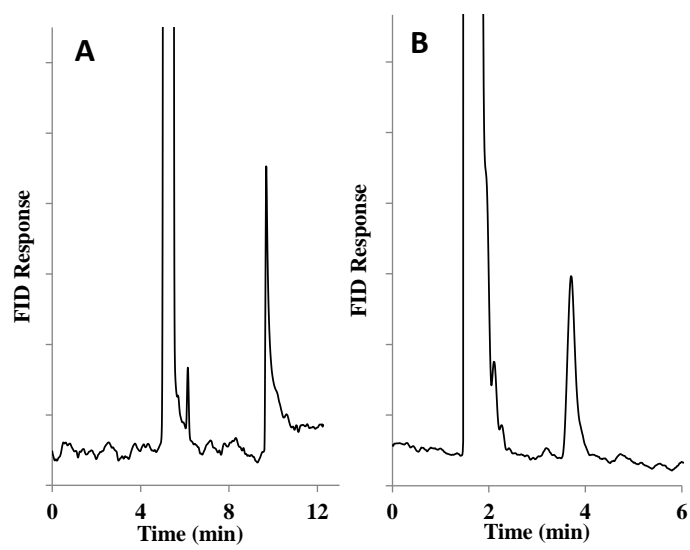


Figure 5

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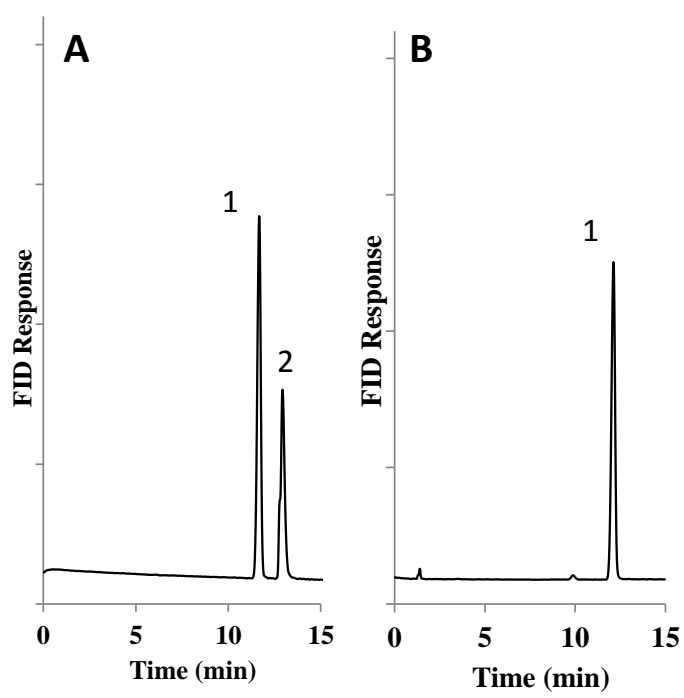


Figure 6

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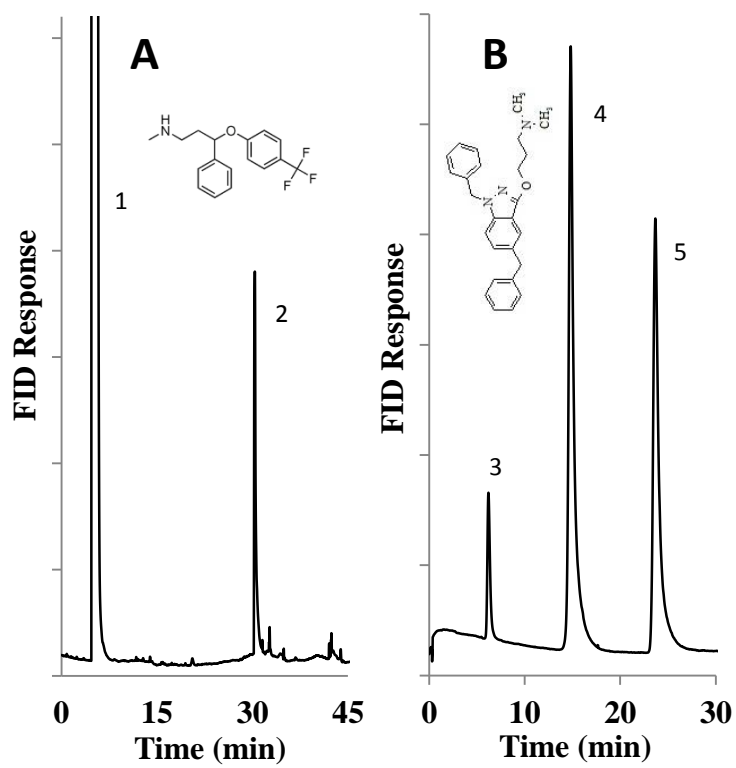


Figure 7

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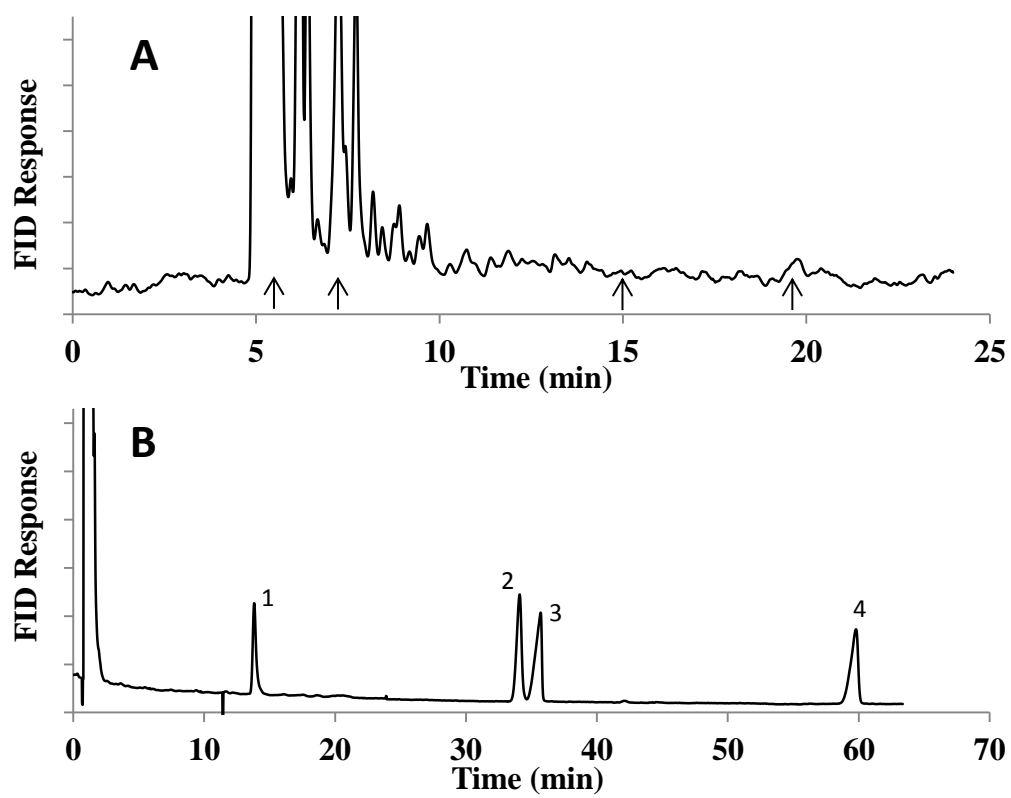


Figure 8

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527 **Table 1: Retention time of various analytes on the basic^a water stationary phase**

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	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 μ m I.D. x 10 m column at 100 °C; void time is 0.7 min.