Extraction of acetaldehyde from mineral water using 2,4- dinitrophenylhydrazine as derivatizing agent

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ABSTRACT
Acetaldehyde generated, when PET bottles of water are deposited at high temperature for long period of time. The acetaldehyde produced, transferred into the mineral water and alter the organoleptic properties of water. Acetaldehyde is also a possible carcinogen and mutagen. In the present research work, acetaldehyde in mineral water was derivatized with 2, 4-dinitrophenyl hydrazine (DNPH), extracted by solid phase extraction (SPE) using octadecyl silane oxide (C$_{18}$) and finally investigated by gas chromatography (GC). Acetaldehyde-2, 4-dinitrophyl hydrazone (A-DNPH) formed after derivatization which were adsorbed on the surface of C$_{18}$. Solvent acetonitrile was used for the elution of A-DNPH from the solid phase extraction (SPE) column. Effect of storage time and light on the migration of acetaldehyde was investigated and found the mineral water stored for longer period of time in sun light change their organoleptic properties due to migration of acetaldehyde into water.

Detection limit of this method was 9.8µg/L while the efficiency of the method was 87.6%, which was higher than solid phase micro-extraction. The concentration of acetaldehyde determined by this method was in the range of 9.8 to 61 µg/L. The results indicate that C$_{18}$ packed in column had good efficiency to hold the acetaldehyde-DNPH, hence it is good adsorbent for acetaldehyde extraction from bottled mineral water. Thus, it has been concluded that bottled water should not be stored at elevated temperature for longer period of time. High temperature and long storage duration increase the acetaldehyde concentration in bottled water which changes the organoleptic properties of water. Water with acetaldehyde concentration greater than 20 g/L have changed organoleptic properties but is acceptable for consumption.

Key words. Acetaldehyde, Octadecyl silane oxide, Acetonitrile, Poly (ethylene terephthalate), Gas chromatography

1. INTRODUCTION
Polyethylene terephthalate (PET) formed by ongoing reaction of terephthalic acid and ethylene glycol or by reaction of dimethyl terephthalate with ethylene glycol. The reaction of terephthalic acid and ethylene glycol proceeds at 240-260 °C while reaction of dimethyl terephthalate and ethylene glycol carried out at 140-220 °C, both the reactions produced bis (hydroxyethyl) terephthalate (BHET) [1-5].

Generally high temperature is compulsory in processing of bottles from the PET material. This high temperature consequences in thermal degradation of polymer and contributes to the production of acetaldehyde in polymer structure [6, 7]. Shukla, Lofgren [8] investigated that by raising the processing temperature by 10 °C the acetaldehyde concentration increase to double within the PET. Acetaldehyde has specific taste and odor and has low threshold value (10-20 ppb). Acetaldehyde is the thinkable carcinogen, mutagen and vagaries the organoleptic properties of product. As consumption of mineral water is rising in developing country since various chemicals and biological pollutants are added into tap water repeatedly. So it is essential to measure the acetaldehyde concentration in drinking water [9-12].

Bao, Pantani [13] investigated aldehydic compound compounds by using SPME and gas chromatography-electron capture detector (GC-ECD). The samples were first derivatized with O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) and then removed by SPME and finally examined by GC-ECD. Splitless injection mode was used while injector and detector temperature were kept at 250 and 300 °C respectively. Limit of detection of SPME-GC-ECD method was 0.02 µg/L while that of head space-solid phase microextraction-gas chromatography-electron capture detector (HS-SPME-GC-ECD) was 0.03 µg/L. The acetaldehyde concentration investigated by liquid SPME-GC-ECD and liquid-liquid extraction gas chromatography electron capture detector (LLE-GC-ECD) were 4.30 and 4.4 µg/L respectively [14-19].
Acetaldehyde in drinking water was determined by using headspace solid phase micro extraction (HS-SPME) coupled with gas chromatography and GC-ECD. As acetaldehyde is greatly unstable, therefore, PFBHA was used as derivatizing agent for derivatization of acetaldehyde. Cancho et al. [20] reported that HS-SPME-GC-ECD procedure is very valuable in determining the acetaldehyde concentration in water at ppb ranks. The derivatizing agent PFBHA produced corresponding oxime derivates which are unstable and less polar and for the withdrawal of these oxime derivates, poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fibers are appropriate.

Tsai and Chang [21] used SPME with on-fiber derivatization. Helium was used as carrier gas with the flow rate of 1 mL/min. Injection mode was in split and its temperature was kept at 250. Water samples were treated with SPME with on-fiber derivatization and agitated by HS to extract aldehydes. Aldehydes obtained were then examined by gas chromatography/mass spectrometry (GC/MS). Recoveries of all aldehydes were 100 ± 15% while the finding limit of this method was in the range of 0.12-0.34 g/L.

Low-molecular weight aldehyde was investigated by using HS-SPME. Pentafluorophenylhydrazine (PFPH) and PFBHA as in-fiber derivatizing agent by Wang, O’Reilly [22]. The samples were first derivatized with in-fiber derivatizing agent monitored by GC-FID. Fused silica capillary column Rtx-5MS of 30 m 0.25 mm inner diameter was used. For PFPH derivatization technique the injector temperature was kept at 260 while for PFBHA derivatization technique it was kept at 250. The flow rate of hydrogen, nitrogen and air were 30, 25 and 300 mL/min respectively. Finding boundary of this process with PFPH derivatizing agent was 5 µg/L while detection border with PFBHA derivatizing agent was 0.5 µg/L. The results show that PFBHA in-fiber derivatization used in HS-SPME and GC-FID gave improved results as compare to PFPH.

Hill, Lipert [23] determined aldehyde in water by means of microgravity-compatible method including colorimetric-solid phase extraction (C-SPE). The samples collected were passed through C-SPE cartridges followed by stirring at the revolution of 400 rpm. The concentrated creation was then analyzed on-disk by diffuse reflectance spectroscopy and linked with calibration curve. Finding limit of this method was in the variety of 0.08-20 ppm.

Aldehydic compounds were derivatized with PFBHA followed by SPME and GC-FID by Valtierra, Ciprés [24]. In GC detector temperature was kept at 250 and injector temperature was retained at 260 while the length of capillary column was 30 m with 0.25 mm inner diameter. Two oxime peaks were formed on chromatogram by GC-FID which shows that two oxime derivatives were attained after derivatization of acetaldehyde. Valtierra used PDMS/DVB adsorbent for the extraction of PFBHA from water. Maximum concentration of acetaldehyde determined was 154 µg/mL.

Ye, Zheng [25] investigated aldehyde in water samples by using PFBHA as derivatizing agent tracked by ultrasound-assist dispersive liquid-liquid micro extraction (UDLLME) and lastly studied by GC/MS. Helium was used as mover gas in this technique with the stream rate of 1 mL/min. The transfer line temperature, injector temperature, MS source temperature and quadrupole temperature were 280, 250, 230 and 150 °C respectively. Initially the samples were derivatized with PFBHA and then extracted obtained by UDLLME and lastly investigated by GC/MS. The comparative recovery of this technique was in the range 85-105 % while border of finding was in the range 0.16-0.23 µg/L. In this process derivatization, removal and concentration complete in one step. The acetaldehyde studied was in the range of 0.8-120 µg/L.

Numerous procedures were used for determination of acetaldehyde in mineral water. SPME followed by GC and HS-GC have low range of recovery. The aim of the purposed study was to study the effect of different solvents on the extraction of acetaldehyde and to develop simple, sensitive and low-cost technique for determination of acetaldehyde in mineral water by means of octadecyl silane oxide adsorbent followed by GC-FID.

2. EXPERIMENTAL

2.1 Materials

For solution preparation double, distilled water was used obtained from Millipore system. Acetonitrile (CH$_3$COCN), methanol (CH$_3$OH), n-hexane, ethanol, acetone, hydrochloric acid, sodium chloride and 2,4-DNPH of reagent grade were bought from Scharlau (Spain). Acetaldehyde (CH$_3$COH) and sulphuric acid (H$_2$SO$_4$) were obtained from Sigma Aldrich (Switzerland). All the volumetric flask, beakers, graduated cylinders, funnel, conical flasks, and other glass ware used in experiments were made of Pyrex.

2.2 Gas chromatograph

For analysis of samples gas chromatograph (GC-2010 Plus series, Shimadzu) with FID and auto sampler were used. The capillary column used was TRB-1 with the measurement of 25 m and 0.25 mm inner diameter. Splitless injection mode was maintained while injection volume was 1 µL. Temperatures of detector and injector were maintained at 320 and 300
respectively. The oven temperature was enlarged from 100 to 290 at the rate of 10/min and kept at this temperature for 10 min. The column stream was maintained at 2.03 mL/min while pressure was 70.7 kPa. The make-up flow and purge flow were kept at 30 and 3 mL/min respectively.

2.3 Solid Phase Extraction columns

For the removal of acetaldehyde from water samples SPE columns (CNWBOND HS-C$_{18}$) were used. The systematic name of C$_{18}$ is octadecyl silane oxide SPE columns were purchased from CNW technologies. The columns have polymeric structures called octadecyl silane oxide (as shown in the figure 1) which adsorbed ethanal-2, 4-dinitrophenylhyrazones which were eluted with acetonitrile and analyzed by GC.

![Figure 1: Structure of C$_{18}$ used in SPE column for the extraction of acetaldehyde from water samples.](image)

2.4 Sample collection

Fifteen samples of 1.5 L of bottle mineral water of 5 different brands with same production date were collected from the local market in Peshawar (Pakistan) and labelled as A, B, C, D and E. These samples were stored at three different controlled temperatures in laboratory at 20°C, 30°C and 40°C for 90 days.

2.5 Sample preparation

Different samples were analyzed at different regular time interval of 10 days. From each sample 100 mL was taken, 6 mL of NaCl saturated solution was mixed with it and its pH was kept at 2 with the assistance of HCl solution. Later on 6 mL of Brady’s reagent was added to it and retained at hot and stirrer plate at 550 rpm for one hour. The sample was then passed through C$_{18}$ column and eluted with acetonitrile and lastly analyzed by GC-FID.

2.6 Retention time of acetaldehyde-DNPH

The retention time of ethanal-DNPH was investigated by running standard solution of Acetaldehyde-2, 4-dinitrophenyl hydrazone (A-DNPH) on GC-FID. After analyzing the standard acetaldehyde-DNPH two peaks were detected in GC chromatograms. First peak was positioned at 2.00 minute while the second peak was situated at 5.86 minute. The peak which was positioned at 2.00 minute was that of acetonitrile while the peak at 5.86 minute was that of A-DNPH as shown in the figure 2.
2.7 Extraction of acetaldehyde from mineral water

Mineral water was mixed with DNPH, and the extracted with the octadecyl silane oxide followed by washing and finally analyzed by GC as shown in the figure 3. The whole process consists of five steps. First step is the washing of C\(_{18}\) columns, second is the introducing sample into C\(_{18}\) columns, third step is the extraction of sample with acetonitrile solvent, fourth step consist of collection of samples into vials and the last step is the introduction of sample into GC. The GC chromatogram shows that there were two compounds, first acetaldehyde and the second was A-DNPH.

3. RESULTS AND DISCUSSION

Acetaldehyde is extremely volatile that’s why it is derivatized with DNPH in mineral water which form A-DNPH which is then hold by octadecyl silane oxide adsorbent. The validation of the method was required for the analysis of aldehyde is presented according to NF-XP 70-210, standard technique. Numerous solvents were used for the withdrawal of A-DNPH from octadecyl silane oxide, but acetonitrile provided good results. Using acetonitrile, the extraction efficiency was increased up to 87.6 %, whereas, no other solvent gave such a high efficiency of extraction. Octadecyl silane oxide has good capacity to hold the A-DNPH, therefore, it is good adsorbent for the removal of A-DNPH from water. Using C\(_{18}\) columns have high capacity to absorb DNPH compared to other absorbents. Due to its elongated structure large amount of DNPH adhere to the chain of octadecyl silane oxide. So, for the analysis of water small amount of sample is required due to high efficiency of C\(_{18}\) columns.

Various samples of bottled water were collected from local market, were stored at three different temperatures for investigation of acetaldehyde from PET bottles to the water. Acetaldehyde was determined in all these samples using...
DNPH as derivatizing agent, which indicates that the all the samples of bottled mineral water are contaminated with acetaldehyde due to degradation of PET bottles. This degradation of the polymer bottles increases with elevation of temperature. Kim and Jabarin [26] examined that acetaldehyde present in all samples in Japan examined by them. Acetaldehyde produced during the processing of PET bottles, it also generates when water is stored in these PET bottles at elevated temperature for longer period of time.

Acetaldehyde produced from PET by various pathways as shown in the figure 4. These various pathways indicate the thermal degradation of PET polymer and confirms the production of acetaldehyde. The acetaldehyde generates from PET then transfer to PET content kept in it, this is also reported by number of researchers. Hence our results are in good agreement with the reported literature [27-29].

![Figure 4: Mechanism of acetaldehyde production from PET polymer.](image)

### 3.1 Effect of solvent on extraction of acetaldehyde from mineral water

Figure 5 indicates withdrawal of acetaldehyde from the bottled water by using various solvents. This shows that the removal of acetaldehyde with ethanol is 71% and with acetone it is 76.5%. Acetonitrile provided 87.6% effectiveness while n-hexane indicates 84.2% efficiency. A-DNPH were removed with various solvents. After extraction of A-DNPH from the bottled water of various brands, they were studied by gas chromatography with FID. The results show that the acetonitrile gave good results for the withdrawal of acetaldehyde from the bottled water, since, it indicates highest efficiency as related to the other solvents. It indicates maximum extraction of A-DNPH for sample “E”. [30, 31].

![Figure 5: Effect of solvent on extraction of acetaldehyde from mineral water](image)
3.2 Efficiency of the method
The effectiveness of the technique was studied by preparing 5, 10 and 15 ppm solution of A-DNPH in 100 mL of double distilled water. The acetaldehyde was then treated with DNPH derivatizing agent and analyzed by GC-FID coupled with TRB-1 capillary column.

As there are number of methods were used for determination of acetaldehyde. Various factors such as solvent, temperature, pH, derivatizing agent, and the equipment effect the recovery and efficiency of method. The average recovery of the method calculated was 87.6%, as given in the table 1, which shows that this analytical technique is good for the extraction of acetaldehyde from the water. Researcher reported the recovery of acetaldehyde in water using solid phase microextraction to be 100 15 %, which show a close agreement with our results [21].

| S. No. | STANDARD SOLUTIONS (µg/L) | RECOVERY (%) | AVERAGE RECOVERY (%) |
|-------|---------------------------|--------------|----------------------|
| 1     | 5                         | 88.3         |                      |
| 2     | 10                        | 79.1         |                      |
| 3     | 15                        | 95.5         |                      |

3.3 Effect of time on extraction of acetaldehyde
Figure 6 indicates the removal of acetaldehyde from bottled water using acetonitrile as solvent. This shows that the acetaldehyde amount in water increased with the storage time. After 90 days the sample “E” has highest amount (61.7 µg/L) of acetaldehyde, whereas the sample “D” has lowest amount (49.8 µg/L) of acetaldehyde. The results show that there was excessive rise in acetaldehyde concentration within 30 days, and after 60 days this rise became very slow. Within 30 days the acetaldehyde amount rises in the range of 22.6 to 27 µg/L. With the passage of time the amount of acetaldehyde amplified which alters the organoleptic properties of water. The other cause for rise in acetaldehyde amount is the thermal degradation of PET. At high temperature the degradation of PET is higher. Polymer degradation is highly favored by elevation of temperature. With temperature elevation long chain polymers are broken down into smaller compounds, these compounds then release in to container or environment. At low temperature only weak bonds are broken, strong bonds remain intact, however, with rise in temperature and passage of time stronger linkages starts to break down with the release of smaller compounds. One the smallest compound that release by degradation of PET polymer is acetaldehyde. That’s is the reason production of acetaldehyde increase with temperature and storage duration. [9, 26, 32].

PET products hold acetaldehyde, through storage the acetaldehyde transfer to the bottled water and rise the amount of acetaldehyde in water. The results indicate that the amount of acetaldehyde in bottled mineral water is not constant and show variation with passage of time as shown in the figure 6. Degradation is slow and steady process, which increases with the elevation of temperature. So, the samples which were stored at constant temperature for longer period of time show greater amount of acetaldehyde compared to sample stored at same temperature for short period of time.

Biscardi, Monarca [33] investigated the migration of mutagenic and carcinogenic compounds from PET bottles into mineral water. Plant mutagenicity test was used in which the gas evolved was collected and analyzed by GC/MS. The samples stored in PET bottles up to 12 months and analyzed at different time intervals. It has been concluded the not only the storage time but the storage material and water pipeline also contributed to the migration of mutagenic and carcinogenic compounds in to water [33-36].
4. CONCLUSION

Acetaldehyde generated from PET material transferred to the bottled water was investigated by different techniques. Various solvents and adsorbent were used by the investigators to find out the amount of acetaldehyde in water. In proposed analytical method the acetaldehyde in bottled water was derivatized with DNPH and removed with octadecyl silane oxide adsorbent by means of acetonitrile as solvent. DNPH have high capacity to hold the acetaldehyde making A-DNPH. A-DNPH was extracted from C$_{18}$ columns using acetonitrile as solvent. These samples were then analyzed by GC coupled with FID and TRB-1 capillary column. By using octadecyl silane oxide adsorbent packed in column increase the range of recovery of acetaldehyde from bottled mineral water. The competence of this technique was 87.6% with correlation coefficient of calibration curve of A-DNPH was 0.997.

The results show that the acetonitrile is the good solvent for the extraction of acetaldehyde from bottled water. The acetaldehyde investigated by this technique was in the range of 9.88 to 61.7 µg/L. The results show that the concentration of acetaldehyde significantly rise in the first 30 days while after 60 days there was very minute increase in its amount. The sample “E” has highest amount (61.7 µg/L) and the sample “D” has lowermost concentration (49.8 µg/L) of acetaldehyde subsequently 90 days extracted by octadecyl silane oxide. The results indicate the range of recovery and efficiency of recovery has greatly been increased by using octadecyl silane oxide (C$_{18}$) as adsorbent. The results also show that the proposed analytical technique is a simple, low cost and efficient technique for the extraction of acetaldehyde in trace amount from mineral water.

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