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Studies on styrene concentration in drinking water and hot beverages in some settings

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Water bottles and cups composed of polystyrene also contain non-polymerized styrene. Styrene's toxicological profile is associated with several health issues for humans. Mainly, the central and peripheral nervous systems are highly disturbed by styrene ingestion. Styrene is also considered to be a carcinogenic agent and has been linked to cancer. The HPLC method was validated through prepared QC samples. The HPLC method validated over the range (0.2 - 50 ng) with good linearity r^2=0.9998. The validation data proved on average 97.5% accuracy with this method. The analysis further depicted that both sources of water contained styrene; 2.2 and 3.2 ng/mL for fresh and stored water, respectively. Styrene was released in larger quantities in boiled water than in cold water. In fresh water, the styrene level was raised by 50% and by 100% for the stored water. On the average, a person may be exposed up to 7 µg/day for cold water and up to 13 µg/day for hot water. Consequently, the effect of sugar on bottled water, which showed a 180 and 250% increase on cold and boiled water respectively was also studied. Caffeine was found to increase the leachability of styrene; 150% in case of fresh water and 170% in stored water.

Key words: Styrene, water, hot beverages, high-performance liquid chromatography, fluorescent detector (HPLC-FD).

INTRODUCTION

Polystyrene (PS) is used worldwide as a food packaging material. The non-polymerized styrene monomer migrates from packaging material into our food and beverages every day. There are several known health impacts in connection to exposure to styrene (Muratak et al., 1991; Varner and Berede, 1981; Varner et al., 1983). Styrene, also known as vinylbenzene or ethynylbenzene (Figure 1), is a naturally occurring, colorless liquid that

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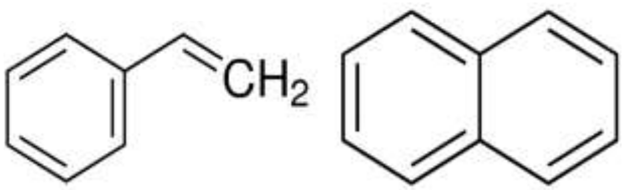


Styrene Naphthalene

Figure 1. Styrene and Naphthalene chemical structure.

easily evaporates with an unpleasant smell, similar to gasoline. Recycled PS materials leach greater amounts of styrene than virgin PS (Qin-Baol et al., 2017). A recent study urged the need for a policy to specifically address the problems of PS plastic contamination in ocean water environments (Kwon et al., 2018). Exposure to styrene vapors may cause irritation to the throat, eyes, nose and skin. Styrene also has a toxic effect on the liver, and is thought to cause depression on the central nervous system as well as cause neurological impairment (Cohen et al., 2002). Chronic effects of styrene and styrene epoxide (metabolite of styrene) are traced to chromosomal aberrations in lymphocytes, which damage the human liver and nervous system. In recent years, studies of the toxic effect of styrene have given widespread concern on the hematopoietic, central and peripheral nervous systems, ingestion, reproductive organs, and the lymphatic system (Sherrington and Routledge, 2001; Brown et al., 2000).

Early in 1993, the International Agency for Research on Cancer (IARC, 1994) classified styrene as a ‘group 2B’ carcinogen (possibly carcinogenic to humans) (WHO and IARC, 1993). The carcinogenicity of styrene is not clearly proven, but there are many similar volatile organic chemicals, which serve as carcinogenic agents. The National Toxicology Program (NTP) listed styrene as an anticipated carcinogenic agent to humans. Most of the genotoxic effects associated with exposure to styrene are thought to be caused by 7, 8-oxide (SO). This compound is considered to be a human carcinogen based on sufficient evidence of carcinogenicity in multiple animal species at multiple tissue sites (WHO and IARC, 1993; IARC, 2002). According to a study (ASTDR, 1992), styrene was detected in adipose tissues as well as blood. There is great importance in knowing the toxic effect of styrene and its leachability in food materials and water from PS.

Water is an essential resource that is vitally important for humans and should be consumed while clean and uncontaminated (Cabejskova, 2016). It is needed in everyday life and strongly affects the well-being of each individual. Global consumption of bottled water increased drastically over the last few decades (Cabejskova, 2016). The FDA has determined that the styrene concentration in bottled drinking water should not exceed 0.1 ppm (FDA, 2007). Abdominal discomfort was observed in humans exposed to elevated levels of styrene in drinking water (Arnedo-Pena et al., 2003). Tap water is seen to be safer than bottled water (Ashton, 2014), because polystyrene (PS) and Styrofoam leach styrene into the bottled water containers (Maqbool and Ahmed, 2007). The presence of styrene has been confirmed in drinking water containers made of PS (FDA, 2003; Health Canada, 1993). There are many factors affecting the rate of styrene migration such as quality of plastic, storage time and temperature. The concentration of styrene steadily increased to 69.53 µg/L after one-year of storage (Maqbool and Ahmed, 2007).

Modern high-performance liquid chromatography (HPLC) with UV detection (Bourque et al., 1994; Fujii et al., 1999; Inoue et al., 1991), gas chromatography (GC) with flame ionization detection (Chakroum et al., 2008; Kataoka et al., 1991), and high-performance chromatography with mass spectrometry (Pacenti et al., 2008; Marais and Laurens, 2005) were performed for evaluating styrene. The level of styrene present in food and drinks depend on many factors such as heat, pH, fats, and the time of storage. It is important to accurately evaluate the amount of styrene in drinking water for safe human consumption. Since bottled water is used every day for drink and food preparation in many countries around the world, it is necessary to evaluate how much styrene is consumed on a daily basis and to what extent health might be affected.

Studies on the use of Styrofoam and PS cups (Khaksar and Ghazi-Khansari, 2009) for water revealed that the water was in fact, contaminated with styrene. Those researchers have determined the migration of monomer styrene from GPPS (general purpose polystyrene) and HIPS (high impact polystyrene) cups in hot drinks. It was observed that temperature plays a major role in the leaching of a styrene monomer from GPPS and HIPS as well with a minor difference in the amount measured. Hot caffeinated beverages contain caffeine and sugar; thus, these two factors might be affecting the leachability of styrene from PS containers into cold or hot drinks. This research hypothesized that caffeine and sugar might increase styrene level inside beverages that are served in polystyrene or plastic cubs. These two factors have not yet been investigated. Here, the effect of storage time and heat factors concerning bottled water is also being observed. In addition, we will evaluate the additional contamination of styrene in bottled water versus that served in PS cubs. Furthermore, we will explore the effects of caffeine and sugar in hot drinks.

MATERIALS AND METHODS

Styrene (purity 99%) and naphthalene (purity 99%) analytical grade was obtained from Sigma Aldrich (Steinheim, Germany). High performance liquid chromatography water and methanol were obtained from Sigma Aldrich (Seelze, Germany). Caffeine (purity 99%) of analytical grade was obtained from Sigma Aldrich (Seelze, Germany). Water packed in PS bottles and Styrofoam, PS and paper cups (size 250-ml) and sugar were collected from local market in Madinah, Saudi Arabia.

A Shimadzu ultra-high-performance liquid chromatography (UHPLC, purchased 2016) system (Japan, Kyoto) consisted of Shimadzu Prominence LC equipped with LC-20AD quaternary gradient pump, a Prominence RF-20A fluorescence and Prominence SPD-M-20A Diode Array detector, CBM-20A communication bus module, CTO-20A column oven, a SIL-20AP auto sampler, and Shimadzu LC solution software (ver. 1.21 SP1 from Shimadzu, Japan) was used. All samples and standards were filtered through 0.2 µm (Millipore) filters.

Analytical column

Compounds were separated isocratically on Thermo BDS Hypersil C18 column (150 mm × 4.6 mm, 5 µm). Separation was maintained at ambient temperature (25±2°C).

Mobile phase

This involves a mixture of methanol and water (30:70, v/v). The flow rate was 1.0 mL min^{-1} and detection was adjusted at wavelength λ= 270 nm. The mobile phase was filtered and degassed by sonication using the ultrasonic cleaner (Ultrasons-HD) from Selecta S.A. (Barcelona, Spain). The flow rate was set at 1.0 mL min^{-1} and the HPLC chromatograms was monitored at emission wavelength (λem= 310 nm) after excitation at (λex= 250 nm).

Calibration curve standards and quality controls samples

A stock solution for styrene was prepared in HPLC grade 50/50 methanol/water (styrene free) solution at concentration of 1 mg/mL. Styrene calibration curve solutions were made by diluting the stock solution to six different concentrations (0.2, 1, 5, 10, 25 and 50 ng/mL). Quality control samples were prepared at 0.25, 2.0 and 20 ng/mL from a separate stock solution. Internal standard of the method was naphthalene. Naphthalene solution (1 mg/mL) was prepared and diluted in HPLC grade 50/50 methanol/water (styrene free). This stock solution was further diluted in HPLC grade water to the final concentration working solutions.

Sample collection and preparation

All water samples in PS bottles were collected fresh from the local market and were analyzed on the same day. All samples were assayed for styrene monomer contents in cold and boiled water. Before analysis, pH of bottled water was measured 7.2 to 7.4. Cold and boiled water were directly added to (paper and PS) cups. The boiler, steering rod and HPLC tubes were all made of glass in order to avoid any additional contamination of styrene.

Cups were labeled ahead of time. Cold water and boiled water were transferred to cups (PS and paper) at the same time. Using a stop-watch, solutions in all cups were stirred with the glass rod for exactly 10 min. The samples were then transferred to the HPLC tube and analyzed on the same day. The temperature of the HPLC autosampler was adjusted to 4°C. The amount of sugar used to study its effect was 12 g per cup. The caffeine working solution was prepared in three different concentrations (25, 50 and 100 g).

RESULTS

Chromatographic separation and choosing the proper detector

The fluorescent detector (FD) was used in our experiment due to its high sensitivity to styrene at excitation wavelength (250 nm) and emission wavelength (310 nm) at a concentration range of 0.2 to 50 ng/ml. Also, ultraviolet Diode Array (DAD) wavelength (254 nm) in parallel with FD was used. Diode array detector (DAD) appeared less sensitive and was unable to detect styrene at low concentration ranges levels (Figure 2a and b).

HPLC calibration curve data

A calibration curve was constructed using the six concentrations of standard styrene that ranged from 0.2 – 50 ng/mL. The curve drawn was shown between styrene concentrations versus the measure peak area ratio. Another calibration curve has been drawn between reciprocal of standard styrene concentration on the x-axis and the ratio between peak areas to standard styrene concentration on the y-axis. This curve was found to be more accurate in its use for calculations. Styrene concentrations were calculated by the equation: Y=0.4107+0.234, and r^2=0.999 for determination of styrene concentration.

Limits of quantification and detection

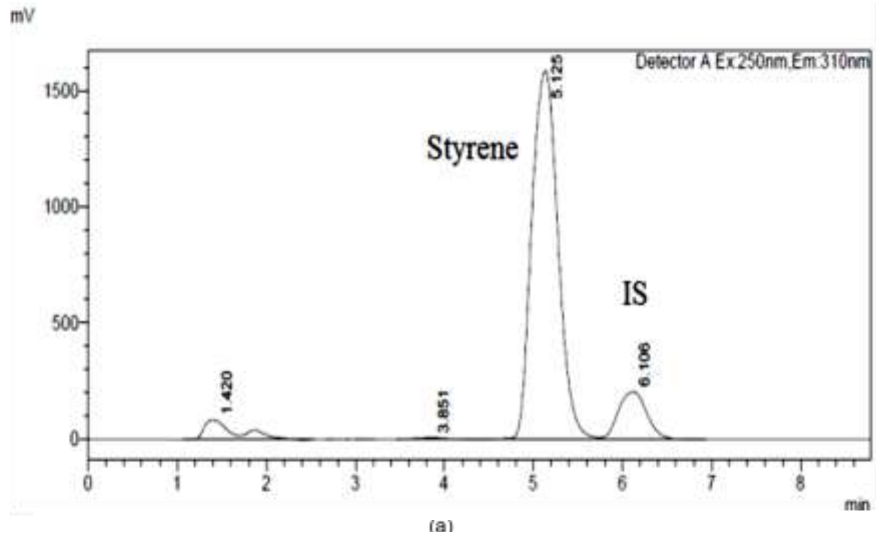
The lower limit of quantification (LLOQ) was defined as the concentration for which both the relative standard deviation (CV%) and the percent deviation from the nominal concentration (dev%) were less than 20%. The upper limit of quantification (ULQ) was defined as the concentration for which both the relative standard deviation and the percent of deviation from the nominal concentration were less than 15% (USFDA, 2001). The detection limit was defined as the signal-to-noise ratio of 3:1.

Accuracy and precision

The results of the method validation are shown in the Table 1. All observed data for inter assay precision were at or below 15%, and in accordance with the FDA guidelines (USFDA, 2001). The method showed good accuracy that ranged from 99.9 to 100.6%. The deviation from nominal concentration ranged from -1.8 to 2.0% for all QC samples. The method’s precision was always within 8.6%.

Concentration of styrene in water samples

Table 2 represents the type of water sample in the first column. The amount of styrene monomer released in 1 mL of water was represented in the second column. Column three illustrates the amount of styrene in 200 mL of water (one cup). Sugar increases the release of



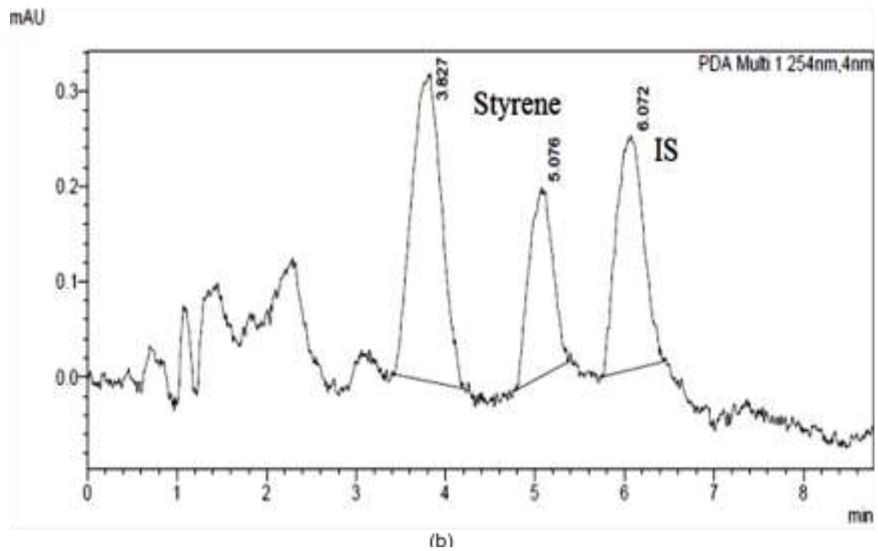


Figure 2. Chromatogram of (a) styrene standard (20 ng/ml) (tR=5.1) and naphthalene internal standard (100 ng/ml) (tR=6.1) using fluorescent detector (λex 250 nm, λem 310 nm) (b) styrene standard (20 ng/ml) (tR=5.07) and naphthalene internal standard (100 ng/ml) (tR=6.07) using UV detector (λ=254).

Table 1. Inter-assay accuracy and reproducibility (n=6).

Nominal concentration (ng/mL)	Back calculated (ng/mL)	Deviation (%)	R.S.D. (%)	Accuracy (%)
0.25	0.2514	2.0	4.0	100.6
2.00	2.0650	0.2	8.6	103.3
20.00	19.9798	-1.8	1.9	99.9

styrene by 150%.

Comparison of styrene level for all samples

A histogram was plotted to represent the comparison between the styrene concentration in all water samples. Figure 3 shows the calculated data for all water samples; stored (old) and fresh, hot and cold, with and without sugar.

Effect of heat and contact time

Heat's effect on styrene concentrations is presented in

Table 2. Concentration of styrene in different water samples.

Water sample	Styrene concentration (n=3) (ng/ml)	Styrene concentration (ng/cup)
Cold fresh water	2.20	440
Boiled fresh water	3.28	656
Cold fresh water with sugar	3.96	792
Boiled fresh water with sugar	8.29	1658
Cold stored water	3.22	644
Boiled stored water	6.47	1294

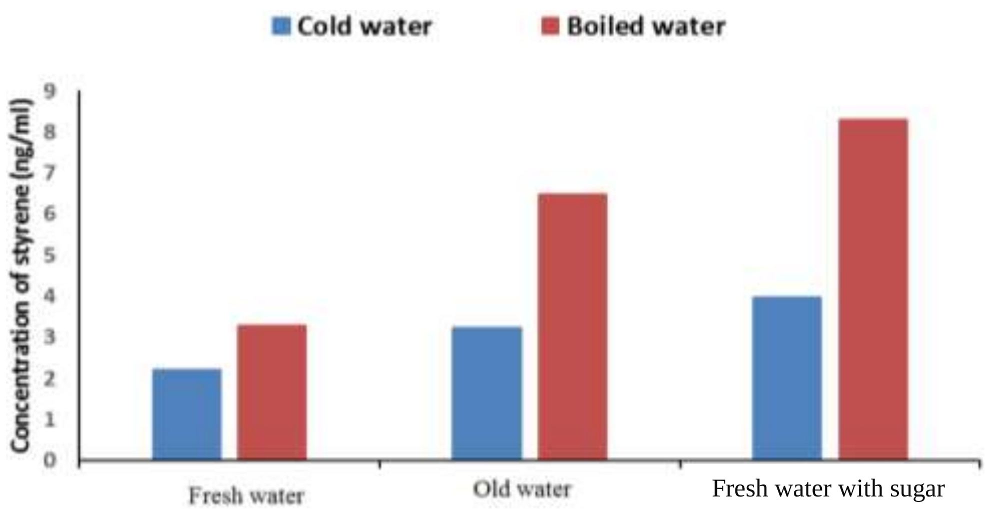


Figure 3. Styrene concentration in different water samples.

Table 3. Heat effect % for both stored and fresh water.

Sample type	Cold water (n=3) (ng/mL)	Boiled water (n=3) (ng/mL)	Difference (ng/mL)	Effect (%)
Fresh water	2.20	3.28	1.08	49.09
Stored water	3.22	6.47	3.25	100.93
Fresh water with sugar	3.96	8.29	4.33	109.34

Table 4. Daily styrene consumption from drinking bottled water.

Sample type	Styrene (n=3) (ng/mL)	Volume of water per (mL/day)	Styrene (ng/day)
Fresh water	2.20	2000	4400
Stored water	3.22	2000	6440

Table 3. The effect of adding sugar on styrene migration in the cold and boiled fresh water is also present. Styrene migration on boiled water increased by about 50% for fresh and 101% for long stored water compared with amount migrated on the cold water for both. Comparing sugar effect on cold and boiled water, styrene migration increases 109% of the cold. This was observed only for fresh water.

The daily consumed styrene from cold and boiled drinking water

The calculated amount of styrene that can be consumed by an individual per day is represented in Table 4. A person who drinks 2 L of cold water per day can absorb styrene up to 4,400 ng of fresh water, and up to 6,440 ng of stored water in polystyrene bottles.

Table 5. Consumed styrene from drinking one cup of hot water.

Sample type	Styrene (n=3) (ng/mL)	Size of one cup (mL)	Styrene (ng/cup)
Fresh water	3.28	200	656
Stored water	6.47	200	1294

Table 6. Consumed styrene from drinking one cup of hot beverage prepared from fresh and old (stored) water.

Caffeine (mg)	Styrene (fresh water) (n=5) (ng/ml)	Amount of styrene/cup (ng)	Styrene (Stored water) (n=5) (ng/ml)	Amount of styrene/cup (ng)
0	3.27	654	6.27	1,254
25	4.68	936	8.31	1,662
50	5.10	1020	9.80	1,960
100	5.57	1,114	11.25	2,486

As shown in Table 5, hot drinks, which were prepared using boiled water, contain more total styrene. Styrene is calculated as ng/cup.

Effect of caffeine

Consumed styrene from drinking one cup of hot beverage prepared from fresh and old (stored) water is illustrated in Table 6. Caffeine clearly increases the amount of leached styrene from cups into the water. Water samples from paper cups containing caffeine were analyzed as well. There was no detected styrene in the samples which proves that no interreference occurred. A caffeine beak was eluted early at 2.1 min (Figure 2a and b). Caffeine increasingly releases styrene by 170% if prepared from fresh bottled and 180% if prepared from stored water.

DISCUSSION

Styrene's toxicological profile is an indication that it poses several health issues for humans. The world's plastic production is consequentially increasing every year (Jambeck et al., 2015). The total global consumption of styrene is approximately 25 million metric tons annually, which estimates about 30 billion in the USD market (Lian et al., 2016). About 1.5 million tons of plastic is produced yearly by the bottled water industry alone. Recently, a Micro-Fourier infrared spectroscopy analysis was carried out on the water of Bohai Sea, China. This study showed that polystyrene was among the main microplastic contaminants in the water (Zhang et al., 2017). The non-plasticizer styrene migration to water starts from day one of the destruction process to the aging of plastics. The presence of unbound low molecular mass compounds considerably increases migration levels (Chakroun et al., 2008). Styrene monomers exist in drinking bottles and cups and other "food-use" items. In most countries around the world, drinking water is delivered to homes through metal pipes. Metal pipes do not leach styrene into the water source. In Madinah, KSA, and in almost all cities in the Gulf Cooperation Council (GCC) countries and around the world, drinking water is packed in plastic containers. The public should be advised to adapt to drinking tap water instead of bottled water (Saylor et al., 2011). It is also observed that all food and drinks prepared in some homes comes from bottled water as well. Moreover, hot drinks are served in polystyrene cups. The extent to which migration occurs depends upon factors such as the contact area, type of plastic, temperature, contact time, solution pH, fat contents and food additives (FDA-Food and Drug Administration).

Because drinking water is packed in polystyrene containers and consumed out of polystyrene cups in some settings, it was hypothesized that leached styrene from the big containers and small cups could be doubling the amount of styrene in our drinks; cold or hot. It is important to evaluate the amount of styrene present in drinking water. In this study, styrene is measured in hot and cold water, as well as fresh and stored water. This work aimed to determine how much total styrene is released in water. To the authors' knowledge, there are no published reports that have calculated the total amount of released styrene in the final consumed drinking water for individual handling water in multiple PS containers. This is the first work of literature that focuses on finding the amount of styrene in bottled water consumed per day by an average person in communities that heavily depend on bottled water. Furthermore, the effect of caffeine and sugar as new factors affecting styrene migration was studied.

The powerful technique of using high performance liquid chromatography (HPLC) has become increasingly popular over the last thirty or forty years. We have chosen to use RP-chromatography separation in our work and fluorescence detector (FD). As indicated in the experimental section, the methods were well validated assuring styrene measurements with an excellent accuracy. The standard curve linearity over a wide range (0.2 - 50 ng/m) is convenient for the sample analysis. Back calculated accuracy of the three QC's 0.25, 2.0 and 20.0 was 100.4, 100.6 and 91.5% with average 98%. The UHLPC with fluoresce detector in this research proved to have more accuracy and sensitivity than Khakstar and Ghazi-Khanari method whom used UV detector (Khaksar and Ghazi-Khansari, 2009).

The generated results from this study showed that styrene monomer is found in relatively fresh and stored water packed in PS bottles. There is significant increase in the amount of styrene contamination over longer contact periods with the water container. This finding is in agreement with Maqbool and Ahmed (2007). Moreover, additional amounts of styrene were observed when water was boiled and decanted in Styrofoam cups as reported by Khakstar and Ghazi-Khanari (Khaksar and Ghazi-Khansari, 2009).

Data analysis shows that both sources of water contain styrene; 2.2 and 3.2 ng/mL for fresh and stored water, respectively. These values are exceeding the permitted level of styrene set by FDA guidelines (FDA, 2007). Through literature, it was documented that the highest rate of migrated styrene in boiled water was done over the first 10 min of exposure. Styrene was released in a higher amount in boiled water than in cold water. This study's data also indicates that in fresh boiled water, styrene levels rose 50 to 100% for the long time stored boiled water.

Studying hot drinks, time of water contact with polystyrene cups was set to be 10 min. Water temperature was also adjusted by boiling for 10 min before transferring to cups containing sugar alone or caffeine alone. When sugar was added to hot or cold drinks, styrene migration increased. Hot drinks containing sugar presented the highest amount of styrene migration in PS cups. Also, normal person daily consumption of SM from hot drinks prepared or served in PS bottled water was calculated. Caffeine doubles the amount of released styrene at 100°C. Finally, the average person can be exposed up to 7 µg/day for cold drink and up to 13 µg/day for hot drink. More research work is needed to study other beverages with different additives and under different conditions.

Conclusion

Successfully, this research was able to measure the heat and time effects on drinking water, as well as study the effect of caffeine and sugar for the first time in releasing styrene from PS cups into our hot beverages. Consequently, sugar showed positive effect in increasing released styrene from PS cups. Coffee is the world's number one drink served with many different additives which could release even more styrene from cups. Caffeine is also found to increase styrene leachability in hot beverages served in PS cubs. Styrene water content is accurately measured using the HPLC method. This research data relied on an accurate and sensitive UHPLC with FD analytical method which was fully validated for ensuring accurate data. The research findings here are important for the public's awareness of using tap water instead of PS cups for hot drinks, especially caffeinated drinks.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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