SPECTROPHOTOMETRIC AND HIGH PERFORMANCE LIQUID CHROMATOGRAHIC DETERMINATION OF SODIUM BENZOATE AND POTASSIUM SORBATE IN SOME SOFT DRINKS

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ABSTRACT
Sodium benzoate and potassium sorbate are preservatives commonly used in drinks and their concentration is of importance due to their health implications. This research is aimed at determining the concentration of sodium benzoate and potassium sorbate in some drinks sold in Lagos, Nigeria using high performance liquid chromatography (HPLC) and UV-visible spectrometry. Thirty samples were collected from different super markets in Lagos State and the pH, as well as the concentration of sodium benzoate and potassium sorbate were determined. A significant difference was observed between the HPLC and the UV methods at 95% confidence limit. The concentration of sodium benzoate in the different drinks analysed, ranged from ND to 1040 mg/L and that of potassium sorbate from ND to 499 mg/L. It was observed that about 40 % of the drinks were above the limit of 150 mg/L for benzoate, while 43 % were found to contain concentrations of sorbate above the permissible limits of 250 and/or 300mg/L. The acceptable daily intake (ADI) per kg of body weight of some consumers was exceeded, and this could expose the consumers to the adverse effect of continuous consumption of these drinks.

Keywords: Drinks, HPLC, potassium sorbate, sodium benzoate, UV-Visible spectrometry

INTRODUCTION
Preservation which is an important practice in food technology is used to delay the growth of microorganisms, maintain the nutritional value and other properties of food products and prolong the shelf life and quality of foods (Ding et al., 2015). This practice has gone on the rise due to the increase in the production of processed and convenience products (Khosrokhavar et al., 2010). Sodium benzoate (E211) and potassium benzoate (E202) are one of the most commonly used food chemical preservatives.

Although these preservatives are legally used in foods, they can be harmful if the uptake by a body is higher than the permitted limits (Gören et al., 2015). Sodium benzoate is a salt derived from benzoic acid though benzoic acid is widely used due to its low toxicity and taste in addition to its antimicrobial capabilities. Sodium benzoate is most effective in an acidic environment (pH $\leq$4.5) and is not recommended for use at higher pH. Sodium benzoate aggravates asthma, worsens hyperactivity in children, and it is a suspected neurotoxin (Pandey and Upadhyay, 2012).The US Food and Drug Administration (FDA) and the UK Food Standard Agency (FSA) in 2006 conducted a research on the determination of benzene in soft drinks available in UK and USA, and found out that benzene was found in beverages that had synthetic or naturally occurring benzoates and ascorbic acid in them. However, the research concluded that the level of benzene in these drinks were lower than their limits of 0.1% and, therefore, posed no safety concern to consumers (FDA, 2006; FSA, 2006).

Similarly, sorbates have been observed to be more active in acidic media. Sorbates are inactive at a pH of 7.0 and above but they are at optimum activity at pH below 6.0 although their efficiency increases with increased acidity (Tfouni and Toledo, 2002; Qi et al., 2009). Sorbic acid and its calcium, sodium and potassium salts (collectively referred to as sorbates) are permitted for use in a range of foods including cheese and cheese products, preserved fruit and vegetables, confectionery, bakery products, preserved meat and fish products, fruit and vegetable juices, soft drinks, wine, fruit wine, dairy and fat based desserts and dips, sauces among many others. Potassium sorbate is internationally recognized as a safe and non-poisonous food preservative as it does not accumulate in the human body. It undergoes normal fat metabolism in human body and oxidizes into carbon dioxide and water. Allergic reaction to potassium sorbate is rare, although sorbic acid is a skin irritant that can cause rashes, asthma, and hyperactivity (Gören et al., 2015).

Various methods have been used for the analysis of benzoates and sorbates in drinks. Some of them include thin layer chromatography, UV spectroscopy, high performance liquid chromatography (HPLC) and gas chromatography. Nowadays, HPLC is the most common analytical procedure for the detection and quantification of these preservatives in foods and beverages (Tfouni, and Toledo, 2002). A research conducted using the reverse phase high performance liquid chromatography (HPLC) method at a wavelength of 254 nm on the determination of preservatives (sodium benzoate and potassium sorbate) in soft drinks and herbal extracts in Iran, concluded that the concentration of sodium benzoate as preservative in the samples ranged from not detected to 2477 mg/L. The study also showed that the concentration of sodium benzoate in soft drink samples were also higher than the ADI (Acceptable Daily Intake) of normal consumers (Khosrokhavar *et al.*, 2010). Also, the assessment of the intake of for benzoates in different subgroups of the Flemish population showed that the median estimated daily intake is, 2.0 (1.0–3.2) mg/kg body weight (bw), 1.7 (1.1–2.7) mg/kg bw and 1.92 (1.3–3.0) mg/kg bw respectively for preschool children, adolescents, and adult women (Bilau et al., 2008).

To the best of our knowledge, no data seems to be available in the literature about the exposure of Nigerians to benzoates and sorbates. This research therefore aims at determining the concentration of sodium benzoate and/or potassium sorbate in some selected flavoured drinks in Lagos Nigeria, and comparing two different methods for the quantification of these preservatives.

MATERIALS AND METHODS

Sample Collection

Thirty (30) flavoured drink samples were purchased from different supermarkets in Lagos, Nigeria and were selected based on products that declared the presence of sodium benzoate and/or potassium sorbate preservatives on their labels. Selections were also made based on their affordability, attractiveness (attractive packaging especially for children) and accessibility to consumers (drinks commonly found and consumed). The samples were appropriately labelled and coded.

Sample Extraction and Analysis using UV-Visible Spectrometry

The UV method used was as described in the ISO nos. 5518 and 6560 procedures (ISO 1978; ISO 1983).

An aliquot of 0.4 mL of 6 M hydrochloric acid was added to 5 mL of each of the sample and thereafter extracted with 45 mL of petroleum ether.

For the calibration, working standards solutions of 0.5 mg/L, 2 mg/L, 5 mg/L10 mg/Land 12 mg/L of both sodium benzoate and potassium sorbate were prepared from stock solutions of 1000 mg/L of sodium benzoate and potassium sorbate respectively. 5 mL each of the working standard solutions prepared was extracted with 0.4 mL of 6 M hydrochloric acid and 45 ml of petroleum ether and the UV absorbance measurements were used to generate the calibration curves.

Quantification was done using the T90+ UV/Visible Spectrophotometer PG Instrument Limited at 226 nm and 250 nm for sodium benzoate and potassium sorbate respectively.

Sample Extraction and Analysis using HPLC

The HPLC method used was a modification of the Khosrokhavar *et al.*, (2010) method. Samples were centrifuged using a Uniscope SM800B Model Centrifuge at 3000rpm for 5mins. An aliquot of 1 ml of the supernatant was diluted with 9 mL of distilled water. Working solutions containing mixed standards of 60 mg/L, 40 mg/L, 20 mg/L, 5 mg/L, 2 mg/L, 1 mg/L and 0 mg/L (blank) each of sodium benzoate and potassium sorbate were prepared from stock solutions.

HPLC analysis was conducted on a system consisting of an agilent 1100 chromatograph coupled to a uv detector. Separation was performed with a reversed phase analytical column (C18 polymeric 100 mm, 3.0 mm ID, 3.5 µm) maintained at 30^0C.The UV detector was set at 254 nm for analyzing sodium benzoate and potassium sorbate with 5 µl injection volume. Isocratic elution was used with 85% phosphate buffer and 15 % acetonitrile at a flow rate of 0.5 mL/min.

RESULTS

The reproducibility of the HPLC method was determined by analyzing standard solution-40 mg/L sodium benzoate and 4 mg/L potassium sorbate six times to determine their precision from the measurement of the % RSD. The % RSD for the area of sodium benzoate was found to be 0.4% and that of potassium sorbate was 0.1 %. This implies that the HPLC method with respect to the instrumental conditions is precise for the analysis of sodium benzoate and potassium sorbate. The reproducibility of the UV method, though was not as good as the HPLC method, still had a % RSD below 5 % and this is acceptable

An optimal separation of sodium benzoate and potassium sorbate at retention times of 5.97 min and 7.56 mins respectively was achieved with a pH of the phosphate buffer at 4.4. The flow rate and other HPLC conditions used also enhanced proper separation.

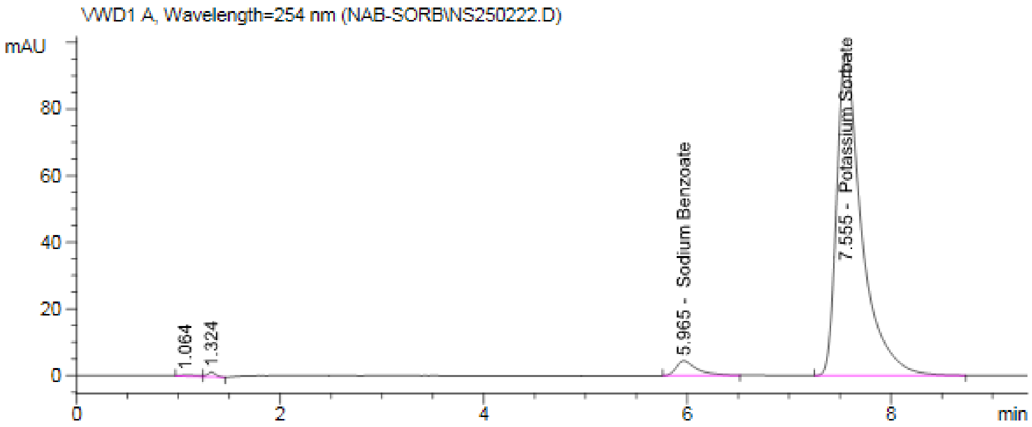


Fig. 1: Chromatogram of sodium benzoate and potassium sorbate

Table 1: pH, sodium benzoate and potassium sorbate concentrations in drink samples using HPLC analysis

Sample code	pH	Sodium benzoate (mg/L)	Potassium sorbate (mg/L)
TD	3.8	15 ± 1	1.15 ± 0.02
SB	2.5	56.8 ± 1.2	82.7 ± 0.1
FF	3.4	77.1 ± 1.0	55.5 ± 0.1
VD	3.5	93.5 ± 3.1	ND
MC	2.7	111± 1	67.5 ± 0.1
RF	3.0	141± 1	**277** ± 1
OD	3.4	143± 2	**290** ± 1
CD	3.2	145 ± 2	**279** ± 1
SC	2.9	148 ± 2	6.14 ± 0.06
FD	3.1	**152** ± 2	**277** ± 1
LC	2.6	**154** ± 5	ND
VT	3.9	**156** ± 1	114 ± 1
MI	2.8	**170** ± 2	81.9 ± 0.1
CO	3.3	**170** ± 1	211 ± 1
MD	3.8	**185** ± 1	**406** ± 1
FD	2.9	**220** ± 1	148 ± 1
FO	2.8	**220** ± 3	ND
TB	2.5	**220** ± 2	ND
PD	2.7	**234** ± 2	ND
MM	3.7	**273** ± 2	311 ± 1

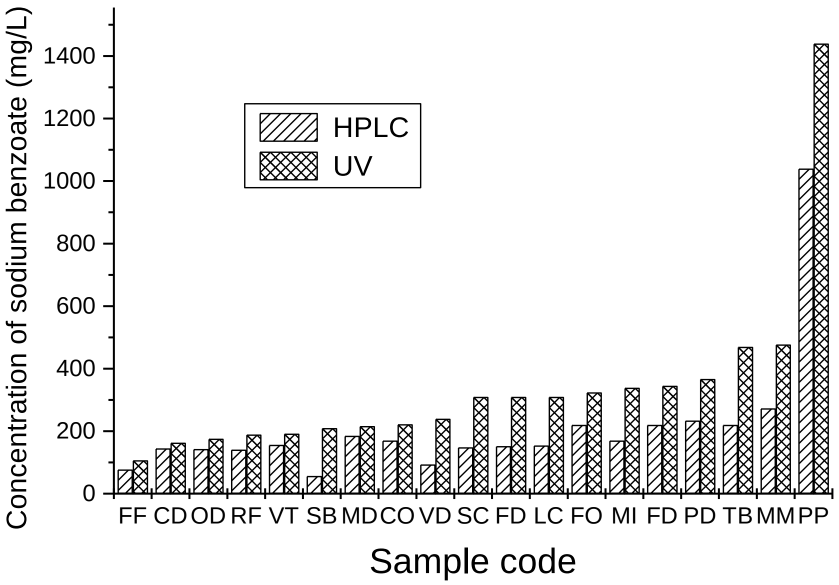
PP	3.3	**1040** ± 4	ND
TG	4.2	ND	200 ± 1
VM	4.0	ND	**316** ± 1
BA	4.2	ND	**343** ± 1
NP	3.8	ND	**355** ± 1
RB	2.9	ND	**366** ± 1
BB	3.9	ND	**370** ± 2
NA	3.8	ND	**378** ± 1
DM	4.1	ND	**388** ± 1
CC	4.2	ND	**499** ± 1
Permissible limit		150	300/250*

ND- not detected: Values in bold are above the permissible limits
* Permissible concentration when present with another preservative

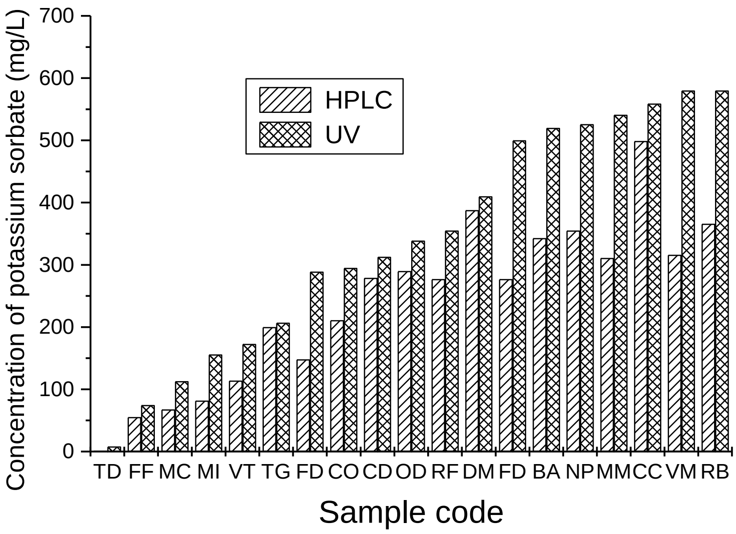
Table 2: Acceptable Daily Intake (ADI) for sodium benzoate and potassium sorbate

Weight of consumer(kg)	ADI for sodium benzoate(mg/bw)	Number of Exceeded Drinks	ADI for potassium sorbate (mg/bw)	Number of Exceeded Drinks
10	50	19	250	14
20	100	17	500	Nil
30	150	12	750	Nil
40	200	7	1000	Nil
50	250	2	1250	Nil
60	300	1	1500	Nil
70	350	1	1750	Nil

Calculation was based on the assumption that 1 litre is taken eachday
bw = bodyweight



(a)



(b)

Fig. 2: **Graphs showing the comparisom of HPLC and UV methods for the determination of sodium benzoate (a) and potassium sorbate (b)**

DISCUSSION

Table1 shows that the pH values of the drink samples ranged from 2.5-4.2 which were below the threshold for tooth demineralization. It has been reported that although the saliva helps to restore the natural acid content of the mouth, prolonged exposure to drinks with low pH (pH below 5.5) may result in irreversible damage to the tooth enamel (Jain et al., 2007). The pH range is however, suitable for the stomach, as the stomach can withstand much lower pH of about 1.5. The lower the pH of a beverage the higher the calcium released from the enamel (Dhanker *et al*., 2013). From this study it can be concluded that two of the sampled drinks-SB and TB had more calcium releasing potential compared to other fruit drinks studied.

It was observed while sampling that the preservatives contained in each drink were not stated on the labels in most cases while those that had the preservatives stated did not declare the concentrations. A limit of 150 mg/L and 300 mg/L have been set for benzoate (benzoic acid) and of sorbate (sorbic acid) respectively, if they are found separately in packaged drinks, a limit of 150 and 250 mg/L respectively, if they are found together in a particular drink (JECFA, 1996; WHO, 2000). It was observed that some drinks that did not declare the presence of sodium benzoate and potassium sorbate on their labels had them within the range of 15-111 mg/L and 6-83 mg/L respectively which are within the WHO limit. Our research showed that benzoates and sorbates in a number of drinks sampled were above the permissible limits. Out of the 30 drinks investigated, 12 (40 %) of the samples exceeded the WHO limit of benzoate, while 13 (43 %) exceeded the limits for sorbate.

According to JECFA (Joint FAO/WHO Expert Committee on Food Additives), the acceptable daily intake (ADI) of sodium benzoate food preservative is 5 mg/kg of body weight and 25 mg/kg of body weight for potassium sorbate (WHO, 1996). From Table 2, for sodium benzoate, it was observed that 19 of the drinks exceeded the limit of daily consumption for a 10 kg weight consumer, and 17 drinks exceeded the ADI for a 20 kg weight consumer, while the ADI for potassium sorbate was exceeded by 14 of the drinks for a 10 kg weight consumer only, based on consumption of 1 liter.

Comparing the results of the HPLC and the UV (Fig 2), it was observed that the concentrations obtained from the UV were higher and this could be as a result of some compounds (chromophores) that probably interfere with the UV absorbance of sodium benzoate and potassium sorbate. This is similar to findings by Marjan et al., (2011) who compared UV and HPLC methods for the analysis of sodium benzoate and potassium sorbate. The researchers observed that compounds such as vanilla, and flavouring agents added to drinks and foods could interfere in the UV absorbance. Statistical analysis of our data using SPSS (version 13.0) showed a statistically significant difference ($P < 0.05$) between the results obtained from the two methods. Based on the findings of this research, the HPLC method may be a more suitable method for the determination of sodium benzoate and potassium sorbate as possible interferences may be resolved and avoided.

CONCLUSION

Based on the current findings it could be concluded that high performance liquid Chromatograhic method is a better method for the determination of sodium benzoate and potassium sorbate in some soft drinks. Findings also showed that non-alcoholic flavoured drinks are an important source of benzoate intake. It is, therefore, important for the soft drink industry to lower the use of benzoates in soft drinks to the acceptable limits especially since benzene, a known carcinogen, has been detected at low levels in some soft drinks, as a result of the interaction between sodium benzoate and ascorbic acid(vitamin C) (BfR Expert Opinion, 2006). It is, therefore, recommended that the Standard Organization of Nigeria and other relevant agencies set acceptable limits for the use of these additives in Nigeria, setting strict guidelines and doing proper monitoring, as this will limit consumer ingestion of additives beyond the acceptable daily intake thereby preventing possible allergies and illnesses that can emanate from their ingestion.

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