Post-Column Derivatization and Quantitative Determination of Vanillin in Ice cream and Custard Powder by High Performance Liquid Chromatography Technique (HPLC)

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ABSTRACT

An analytical method for quantitative determination of vanillin in ice cream and custard powder was established. The milk proteins were removed by precipitation and the vanillin content of the food products was extracted by liquid-liquid extraction (LLE). The separation of the analyte was achieved in an ODS hypersil C_{18} column maintained at 30^{o}C. Acetonitrile/10 % Acetic acid in distilled water 55:45 (v/v) was used as the mobile phase. The measurement was made with a UV/fluorescent detector at 280 nm and a flow rate of 1.0 mL/min. Direct injection of the extract into the HPLC proceeded without interference from excess solvent before post-column derivatization and the retention time was 2:18 min, thus allowing this method to be applied for routine analysis of vanillin in food products. The calibration curve was linear over the concentration range of 2-10 μgmL^{-1}. A correlation regression of the order 0.99 was obtained and the vanillin concentration in the food products ranged from 8.09 ± 0.18 ↔ 14.61±0.06 μgmL^{-1} in ice cream and11.11 ± 0.01 ↔ 15.23±0.15 μgmL^{-1} in custard powder respectively. The respective limit of detection and quantification of the vanillin in the food products was 0.05 $\mu gmL^{-1}$and 0.17 μgmL^{-1}. The relative standard deviation which is a measure of precision was less than 3% (n = 10). The percentage recovery of the analyte was 87 %, all the validated data obtained in this study was higher than the acceptable daily intake for the vanillin in the food samples analyzed except for the Golden scoop ice cream. This method is well suited for the simultaneous and convenient analysis of vanillin in a vanilla enriched ice cream and custard powder to evaluate the quality of the vanilla extract and determine the quality of the food products for human consumptions.

Keywords: Precipitation, Interference, Quantification and Validated

INTRODUCTION

Food is the basic necessity of life. One works hard and earns to satisfy hunger. But at the end of the day, many of us are not sure of what we eat. We may be eating dangerous flavors and dyes. Often, we invite diseases rather than good health. Aromatic aldehyde (Vanillin in ice cream and custard powder) is one of the food additives that can be adulterated. If its consumption is above the acceptable daily intake, it has serious adverse health implication. Most artificial food dyes are made from petroleum oil and coal tar, and this is one of the widest spread and critically important groups of Pollutants[1]. Food adulteration is an act of internationally debasing the quality of food

offered for sale by admixture of inferior artificial food dyes and additives[2]. Adulteration frequently involves the replacement of high cost ingredients with cheaper substitutes. Although adulteration is done for economic reasons, the action affects the chemical composition and quality parameters of food. Adulteration of food can pose serious risk to health in some cases[3]. For example; hyper kinesis and Learning Disabilities (H, LD) is linked to the ingestion of Artificial Food Colors and Flavors[4].

Flavor is one of the most important aspects of consumer acceptance of any food product, and aroma is the most important aspect of perceived flavor[5]. In order for aroma compounds to be perceived they must first be released from the food. The flavor intensity then, is dependent on the amount of flavor released from the food matrix[6]. Thus, flavor-food interactions become important as flavor compounds bound to food ingredients are no longer available for perception. Manufacturers use color additives to cover up an absence of natural color. If manufacturers wanted to add color to a food or candy it was done primarily with natural plant and vegetable based compounds. Because of its desirable flavor characteristics and aroma properties, vanillin is the world's most popular and widely used flavoring material in confectionery, food, and beverages[7, 8]. The high demand for natural vanillin far exceeds the supply from all sources. The low supply/demand ratio and the high cost of natural vanillin, compared with that of synthetic vanillin, are the primary economic incentives for misrepresenting synthetic vanillin, or a mixture of synthetic and natural vanillin flavors, as the pure natural flavoring material[9].

Most countries have regulations to control the content of synthetic vanilla products and by-products such as the US Code of Federal Regulations from 1988, which requires that beverage alcohol products are labeled, if synthetic flavors are used[10]. An average sized person would probably need to eat over 75 grams (2.5 ounces) of pure vanillin for it to have a toxic effect. The established "safe" daily intake of vanilla is 10 mg per kg[10]. The production of vanilla beans is quite expensive, since it is a very labor intensive process and harvesting takes place two to three years after planting. There is no difference between the chemistry of natural and artificial food flavoring, but adverse health effects are believed to come from the artificial flavoring[11].

There are some potential problems with synthetic food dyes, specifically: Carcinogenicity – leading to cancer development, Genotoxicity – leading to mutations or damaging chromosomes, Neurotoxicity – leading to the damage of nerve tissues.

Cancer still remains a major threat to human health, representing the second leading cause of death worldwide[12]. Therefore, the aim of this

present study was to develop an HPLC method to evaluate the vanillin content used as additives in ice cream and custard powder in Nigerian market in order to ascertain the levels and compare it to the acceptable daily intake by Food and Agricultural Organization (FAO). The new method was developed for the determination of vanillin, which can provide technical support for food products quality and safety monitoring and inspection.

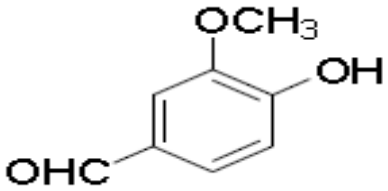


Fig. 1: Chemical structure of vanillin (4-hydroxy-3-methoxybenzaldehyde)

MATERIALS AND METHODS

Reagent and Chemicals

Reagents and Chemicals used in this study include vanillin (99 % BDH), 10 % Acetic Acid (Sigma Aldrich), Methanol (Sigma Aldrich), Sodium carbonate, n-hexane, HCl, MIBK, Sodium hydroxide, Copper sulphate and double distilled water. All the solvent used for high performance liquid chromatography (HPLC) analyses were of the HPLC grade. All other chemicals were of the analytical grade.

Standard and Sample Preparation

Standard Stock Solution (99 % purity BDH) were prepared by dissolving 10 mg of vanillin in 100 mL of methanol. This solution was stored in a light resistant bottle and this was further used to furnish working standard to give a calibration ranges between 2-10 mg/L of vanillin.

Vanillin from the two samples (vanilla flavoured ice cream and custard powder) was extracted with methanol; 0.5 g of sample (Ice cream and custard powder) was extracted by adding 10 mL of methanol and sonicated for 15 min after the addition of 1 mL of saturated copper sulphate solution to precipitate the milk protein in the sample. The resulting solution was filtered using whatmann filter paper No. 42. The filtrate of the ice cream samples was treated with 5 mL 15 % (w/v) aqueous sodium carbonate, extracted once with 20 mL hexane and acidified with 10 mL 6M HCl. This acidic solution of ice cream and custard powder were extracted with three portions of 10 mL methylisobutyl ketone (MIBK)[13]. The extract were combined and vanillin was extracted into aqueous medium with two portion of 5 mL 0.1M sodium hydroxide and the volume of the combined extracts was reduced to 1 mL through evaporation prior to injection into an HPLC.

Instrumentation and Chromatographic Condition

The Chromatographic apparatus consisted of a Cecil 4300 series high performance liquid chromatography. The analytical column was ODS hypersil C_{18}, 5 µm particle size in 300 mm x 3.9

mm I.D stainless steel column. The mobile phase consisted of acetonitrile/10 % Acetic acid in distilled water 55:45 v/v. the flow rate was 1.0 mL/min and the column temperature was maintained 30^{o}C with detection at 280 nm using UV/Fluorescence detection. In all cases, 5 µL of the prepared sample was injected into the HPLC column for satisfactory separation. Thin Layer chromatography plates were developed with a mobile phase of Methanol/water/glacial acetic acid (20:05:02; v/v).

RESULTS AND DISCUSSION

Although ice cream consists largely of water (about 60 % w/w), other components such as milkfat (10-16 % w/w), proteins and carbohydrates (12-16 % w/w), sweeteners, sucrose and glucose based corn syrup (12-16 % w/w) add up to a quite complex mixture[13]. Vanillin is mostly added to the ice cream as synthetic vanillin because of the cost implication of the natural vanillin which is in the form of extracts or macerated vanilla beans. Table 1 below shows the concentration range of vanillin in the sampled vanilla ice cream and custard powder.

Table 1: Concentration of Vanillin in Ice cream and Custard Powder

Samples	Peak Area (mAs)	Concentration Range (μgmL^{-1})	Mean Conc. ±Sdev (μgmL^{-1})	Relative Standard Dev. (%)
FR	1253.4-1258.8	12.5431-12.6545	12.5988±0.0788	0.6254
SL	1434.6-1442.8	14.5676-14.6578	14.6127±0.0638	0.4366
FC	1005.4-1008.2	10.4784-10.5002	10.4893±0.0154	0.1468
SM	1338.7-1342.5	12.7654-12.8291	12.7973±0.0450	0.3516
GS	847.7-857.5	07.9625-08.2184	08.0905±0.1810	2.2372
SOJ	1121.4-1123.2	11.1021-11.1232	11.1127±0.0149	0.1341
BR	1142.2-1148.6	11.4278-11.5930	11.5104±0.1168	1.0147
EB	1221.5-1132.3	11.9171-11.9915	11.9543±0.0526	0.4400
GD	1543.6-1602.6	15.2107-15.3521	15.2814±0.0999	0.6537
UN	1534.3-1564.6	15.1244-15.3341	15.2293±0.1483	0.9737

Ice Cream: FN- Fan Royale, SL-Smyle, FC- Fanice, SM-Supreme, and GS-Golden Scoop
Custard Powder: SOJ, BR-Bolero, EB-Egg Banana, GD-Glad and UN-Unique.

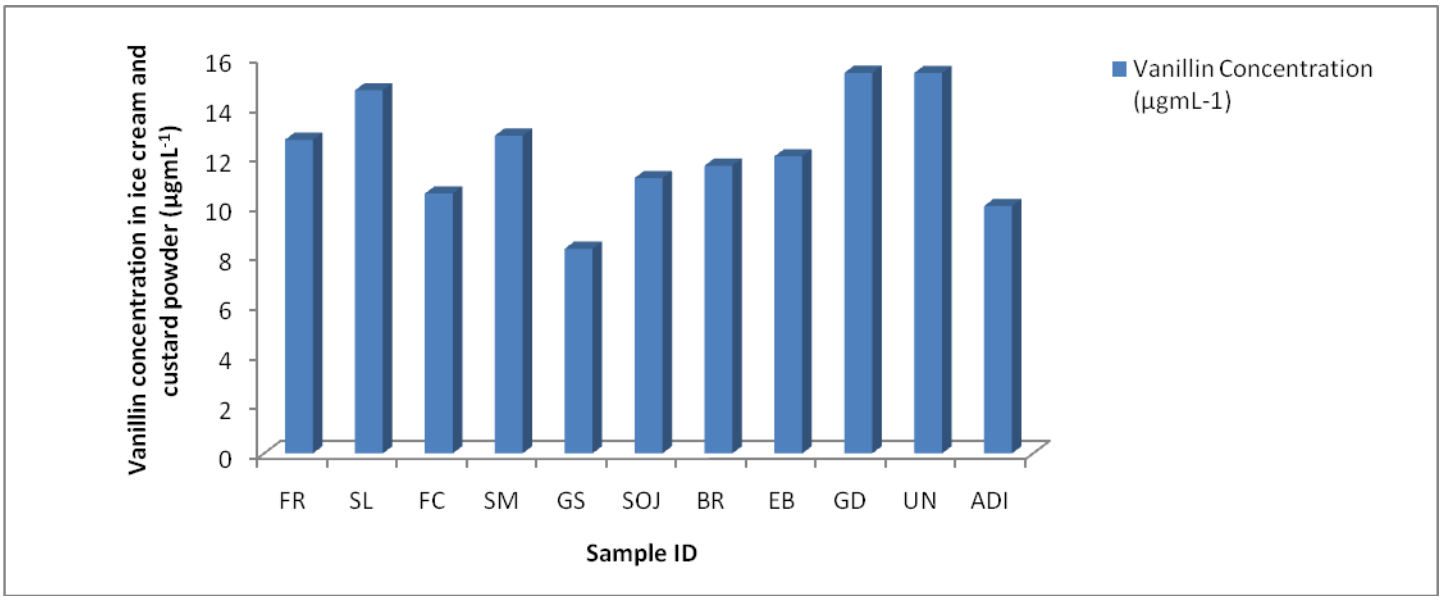
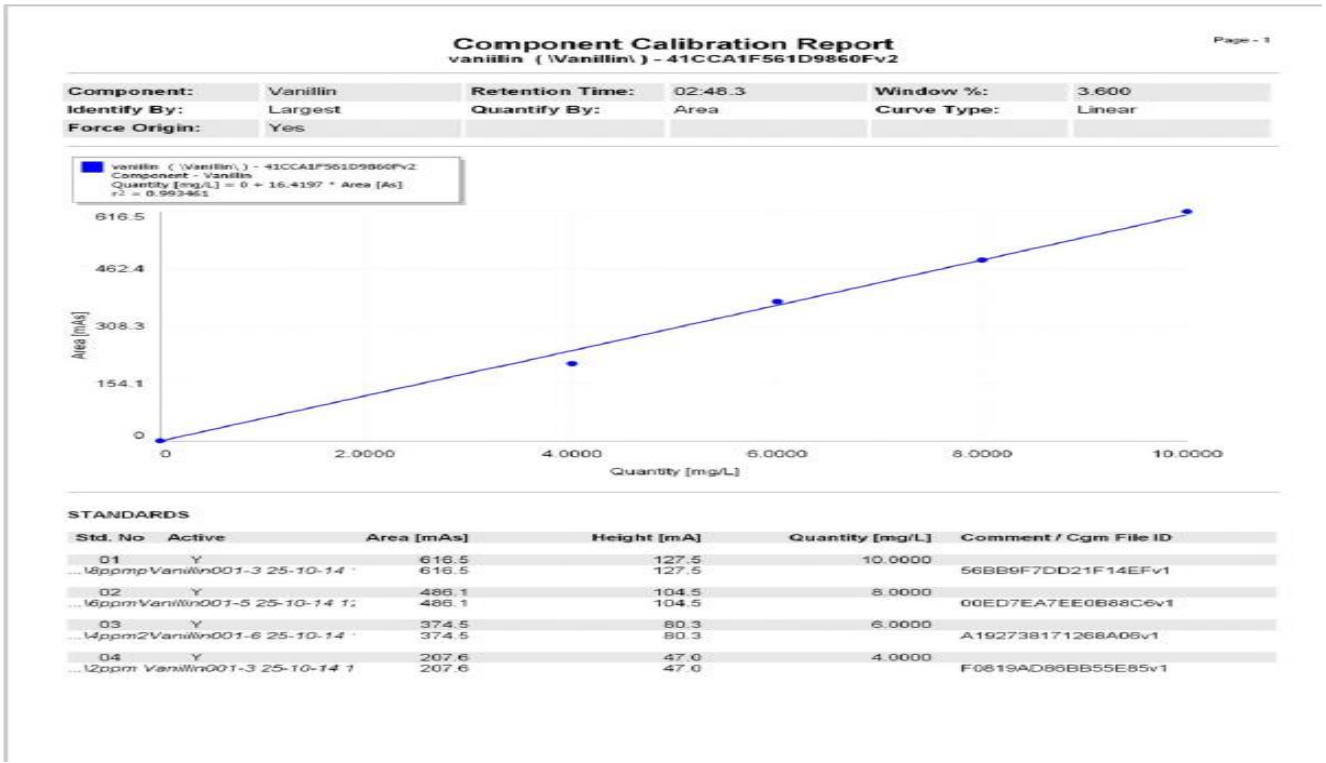


Figure 2: Showing the Concentration of Vanillin in Ice cream and Custard Powder with the Acceptable Daily Intake by FAO.ADI = Acceptable Daily Intake (ADI).



Component Calibration Report

vaniillin (\Vanillin\ ) - 41CCA1F561D9860Fv2

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Component:	Vanillin	**Retention Time:**	02:48.3	**Window %:**	3.600
Identify By:	Largest	**Quantify By:**	Area	**Curve Type:**	Linear
Force Origin:	Yes				

vaniillin (\Vanillin\ ) - 41CCA1F561D9860Fv2
Component - Vanillin
Quantity [mg/L] = 0 + 16.4197 * Area [As]
r2 = 0.9934&1

616.5
462.4
308.3
154.1
0
Area [mAs]
0
2.0000
4.0000
6.0000
8.0000
10.0000
Quantity [mg/L]

STANDARDS

Std. No	Active	Area [mAs]	Height [mA]	Quantity [mg/L]	Comment / Cgm File ID
01	Y	616.5	127.5	10.0000	
...\8ppmpVanillin001-3 25-10-14		616.5	127.5		56BB9F7DD21F14EFv1
02	Y	486.1	104.5	8.0000	
...\6ppmVanillin001-5 25-10-14 1:		486.1	104.5		00ED7EA7EE0B88C6v1
03	Y	374.5	80.3	6.0000	
...\4ppm2Vanillin001-6 25-10-14		374.5	80.3		A19273817126BA06v1
04	Y	207.6	47.0	4.0000	
...\2ppm Vanillin001-3 25-10-14 1		207.6	47.0		F0619AD86BB55E85v1

Figure 3: Standard graph of vanillin by HPLC method

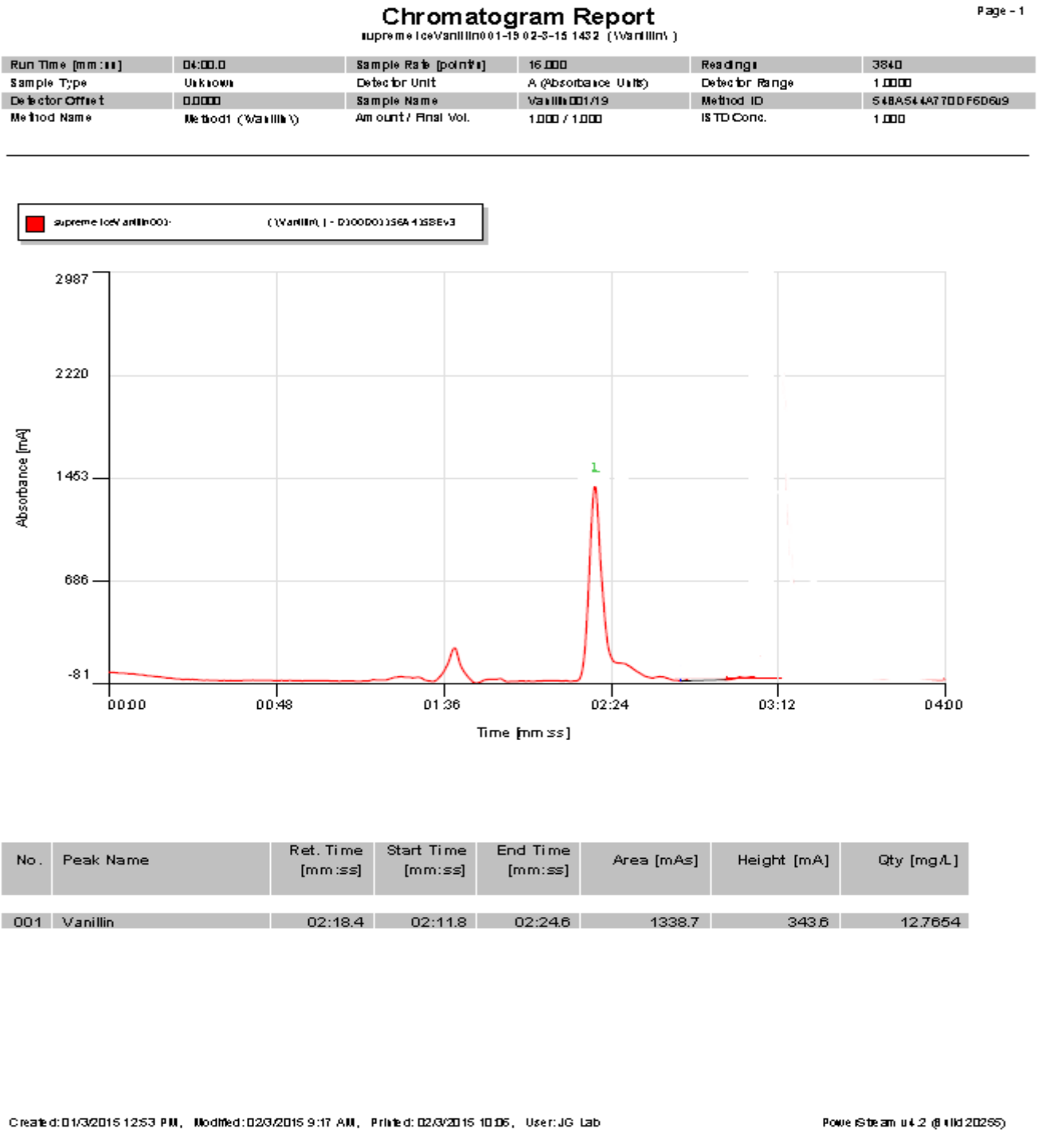

Fig. 4: Representative chromatogram of the separation of ice cream extracts containing synthetic vanillin.

Table 2: Calibration Parameters and Sensitivity of Data for Vanillin by the HPLC/UV-Fluorescence Detector

Analyte	Linearity Range (mg/L)	Slope	Intercept	r^2	LOD (mg/L)	LOQ (mg/L)
Vanillin	10.00 – 2.00	16.4197	0.0	0.9935	0.05	0.17

Table 3: Recovery Study of Vanillin in Ice Cream

Analyte	Sample Concentration (mg/L)	Standard Concentration (mg/L)	Expected Conc. of Spiked sample (mg/L)	Actual Conc. of Spiked Sample mg/L	%R
Vanillin	2.00	1.00	3.00	2.87	87

$$\% \, Recovery = \frac{Vanillin\ concentration\ in\ spiked\ sample - Vanillin\ concentration\ in\ unspiked\ sample}{Conc.of\ vanillin\ standard\ added} \quad (1)$$

HPLC Determination of Vanillin in Ice Cream and Custard Powder

Chromatography separation of vanillin was achieved under isocratic elution on a C_{18} column set at 30^{o}C with UV/Fl wavelength at 280 nm. A mobile phase consisting of acetonitrile, distilled water in 10 % acetic acid (55:45) at a flow rate of 1.0 ml/min was found to be suitable. A standard reference graph of vanillin was prepared at a detector wavelength of 280 nm by injecting 20 µL of vanillin dissolved in acetonitrile at the concentration range of 2.00 mg/L to 10 mg/L respectively. The peaks are well resolved, with a baseline separation, which allows low level detection/quantification of the analyte. This chromatogram revealed that the reagent and solvent did not interfere with the analysis (Fig. 4). The method gave a good recovery of 87 % (Table 3). The HPLC method was reproducible with relative standard deviation which is a measure of precision < 3.00. The calibration curve exhibit a good precision and sensitivity with a vanillin standard. The curve was linear with a correlation coefficient (r) 0.993 (Fig. 3). A similar trend was observed with a correlation coefficient of 0.999 but with a retention time of 30 minutes which is far above that obtained in this study[9]. LOD and LOQ was determined after the injection of the lower concentration of the vanillin standard and

the standard concentration of the peak area was calculated (Table 2). The LOD and LOQ were calculated using the following equations[14].

$$LOD\left(\frac{mg}{L}\right) = \frac{3\ x\ Standard\ deviation}{Slope\ of\ the\ calibration\ curve} \quad (1)$$

$$LOQ\left(\frac{mg}{L}\right) = \frac{10\ x\ Standard\ deviation}{Slope\ of\ the\ calibration\ curve} \quad (2)$$

Using the two formulas above, the calculated LOD and LOQ of vanillin in this procedure was 0.05 mg/L and 0.17 mg/L respectively. The relative standard deviation was also found to be < 3 % (The smaller the coefficient of variation or RSD, the more precise is a set of measurements). Therefore, this method exhibit good precision and sensitivity for vanillin separation and detection.

The vanillin contained in the five commercial ice creams and custard powder was determined using the developed method. The results are shown in Table 1. The amount of vanillin in the samples examined ranged from 8.09±0.18 ↔ 14.610.06 μgmL^{-1} in ice cream and11.11±0.01 ↔ 15.23±0.15 μgmL^{-1} in custard powder respectively. This value is far above the acceptable daily intake (ADI) of 10 mg/kg[10] of the artificial vanillin added to the food sample as flavouring agents to enhanced the taste and quality[13]. Among the ice cream and custard powder analyzed, Golden scoop ice cream and SOJ custard powder were found to contain the least amount of the flavours. Though this obtained value was in-line with similar study carried[15] using derivatization procedure to enhance the detection. The recovery of vanillin added to was 87 % and the relative standard deviation which is < 3% was obtained.

CONCLUSION

The concentration of vanillin obtained from this food samples (Ice cream and custard powder is far above the acceptable daily intake, thereby causing a lot of havoc to human health through their consumption. This developed method for the determination of vanillin in the food products is accurate, precise, sensitive and eco-friendly. Therefore, this method is therefore useful for both quantitative and routine analysis of the analyte (vanillin) in food products by the food and beverages industry.

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