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Article

Development of Gas Chromatographic Method for the Analysis of Trihalomethanes (THMS) in Drinking Water

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Abstract

The Environmental Protection Agency (EPA) requires that Disinfection by-products (DBPS) levels must be determined as part of the current regulatory testing; the trihalomethanes (THMs) are the indicator chemicals for the other DBPs. The long-used established USEPA method 551.1 has been employed by many researches: it involves a Liquid-liquid Extraction and analysis using Gas chromatography. This study makes some important modifications in the method of the Gas Chromatographic analysis for the determination of the trihalomethanes (THMs) levels in the drinking water of four Water Treatment Plants (WTPs) in Lagos and Ogun States of Southwest Nigeria. A total of One hundred and four raw and processed water samples were collected and analyzed between January and May, using HP-1ms Ultra Inert Agilent 7890A Gas Chromatograph (GC) with Autosampler Agilent 7683B equipped with an Electron Capture Detector (ECD). The modified split ratio reduced the level of contaminants in the GC column. The signal rate reduced to 5HZ enhanced better flow and separation of peaks. The multi-level calibration helped in obtaining accurate quantitative results. The results of the recovery test validate the method accuracy.

Keywords: TriHalomethanes, Gas Chromatography, Calibration, Recovery test.

1. Introduction

Drinking water disinfection by-products (DBPs) are unintended consequences of using chemical disinfectants to kill harmful pathogens in water [1-3]. DBPs are formed by the reaction of disinfectants with naturally-occuring organic matter, anthropogenic contaminants, bromide and iodide [4]. Disinfectants are added to water after filtering out the bigger particles of other pollutants, to kill the remaining deadly pathogens including viruses and bacteria such as *Salmonella*, *Campylobacter* and *Shigella*, and protozoa such as *Giardia lamblia* and other *cryptosporidia* [5]. The major chemical disinfection agents are chlorine, chloramine, chlorine dioxide, ozone and ultraviolet light. Chlorine is the mostly used of these disinfectants and the process is referred to as chlorination [6-8]. The sodium hypochlorite solution is actually used instead of the toxic chlorine gas; it is cheaper and on dissolution in water, free chlorine is released which is very rapid in killing the pathogens [9-11]. However, the use of any form of chlorine from any source produces the carcinogenic chlorination by-products trihalomethanes (THMs) and haloacetic acids (HAAs) that pose threats to human health [2,4,12].

Several epidemiological studies have reported the relationship between disinfection by-products (DBPs) and different health outcomes such as cancers and re-productive outcomes [13-16]. A review of the various researches on DBPs established that eleven (11) of them are currently regulated by the USEPA while seventy four (74) are regarded as emerging and not yet regulated

because their occurrence and toxic nature are considered to be moderate [17]. The trihalomethanes (THMs), a set of the regulated DBPs produced by the action of chlorine and chloramine on organic and inorganic atters in water include bromodichloromethane (BDCM), dibromochloromethane(DBCM), bromoform (TBM) and chloroform(TCM) [3,17,18].

The requirement of the safe drinking water act is that the environmental protection agency should carry out periodic review of the national primary drinking water regulation for each contaminant and disinfection by-product; and also do appropriate revision of the regulation using new scientific data [1, 19,20,21]. Different analytical methods have been developed to analyze trihalomethanes in drinking water [22]. The most widely used are based on Gas chromatography (GC) with Electron-capture or Mass spectrometry detection after extraction with organic solvents such as pentane and hexane or purge- and-trap techniques [22-25].

2. Materials and Methods

2.1 Description of study sites

Selected for the study were two potable water treatment plants (WTPs) in Ado-odo/Ota local government area of Ogun State including one public water treatment plant (Ogun Water Works) and one private water treatment plant (Hebron Waters, Canaanland); also two WTPs in Agbado/Oke-odo local government area of Lagos state including one public Water treatment plant (Lagos Water Works) and one private water treatment plant (Sigma Waters, Abule egba). Each of them employs sone of the two main treatment processes (Chlorine-Chlorine, Chlorine-UV) [3].

2.2 Chemicals/Reagents

All the reagents and chemicals used in this work are of HPLC grade and of highest purity; they include: n-Pentane and n-Hexane both from Scharlau Chemie S.A, Spain; Dichloromethane from Sigma Aldrich, U.S.A., Methanol and Ascorbic acid both from Tedia Company Incorporated, U.S.A. and commercial standards of Trihalomethanes Mix supplied with certificate of analysis from Accu Standard Incorporated, U.S.A.

2.3 Sample collection and Pretreatment

Samples were taken from the source (untreated) water, at the primary disinfection stage (after the sedimentation tanks), and at the secondary disinfection stage (the distribution system) of each WTP from the month of January to May, 2015. Measurements of the physicochemical parameters were done at the different WTPs and the data were collected monthly during every sample collection.

For the analysis of THMs, samples were taken in 40mL glass vials with screw-caps lined with Teflum-faced septa; filled to overflowing, ensuring that there are no air bubbles. 25mg of ascorbic acid was added to each vial as a reducing agent to quench the further production of disinfection by-products (DBPs). Vials were then sealed and samples stored at $4^{o}C$ prior to analyses.

2.4 Preparation of calibration standards

Using the stock standard, nine calibration standards were prepared. The calibration standards' concentrations were calculated as follows:

Given: Conc of standard is 2.0mg/mL

This is equal to $\frac{2.0mg}{}$

$10^{-3}L$ = 2,000mg/L

= 2,000ppm

That is, C_1 = 2,000ppm

Thus, using $C_1V_1 = C_2V_2$

$V_1 = \frac{C_2 \times V_2}{}$

2,000ppm

Therefore, for volume V_2= 1000µL (1mL)

Conc. 10ppm, that is, C_2 = 10ppm:

V_{10ppm} = 10ppm x 1,000µL

2000ppm

= 5µL of standard into 1000µL of Methanol (HPLC grade).

For 20ppm; V_{20ppm} = 20ppm x 1,000µL

2,000ppm

=10µL of standard into 1000µL of Methanol (HPLC grade).

For 30ppm; V_{30ppm}= 30ppm x 1,000µL

2000ppm

=15µL of standard into 1000µL of Methanol (HPLC grade).

For 40ppm; $V40_{ppm}$= 40ppm x 1000µL

2000ppm

= 20µL of standard into 1000µL of Methanol (HPLC grade).

From the 10ppm concentration, lower concentrations of the standards were prepared as follows:

1ppm = 1000ppb

10ppm =10,000ppb; that is, C_1 = 10,000ppb

Thus, using $C_1V_1 = C_2V_2$:

For 20ppb; V_{20ppb} = 20 x 1000

10,000

= 2 µL of 10ppm standard into 1000µL of methanol

For 40ppb; V_{40ppb} = 40 x 1000

10,000

= 4µL of 10ppm standard into 1000µL of methanol.

For 60ppb; V_{60ppb} = 60 x 1000

10,000

= 6µL of 10ppm standard standard into 1000µL of methanol.

For 80ppb and 100ppb: 8µL and 10µL respectively of 10ppm standard into 1000µL of methanol.

2.5 Preparation of internal standard

According to Benson et al, [3], the internal standard was prepared by dissolving 5µL dichloromethane in 10mL hexane and mixed well by hand-shaking. 50µL of this solution was added to 50mL of pentane before the pentane was added to the sample to be extracted.

2.6 Extraction of Trihalomethanes

In extracting the trihalomethanes, the USEPA, 1998. METHOD 551.1-Liquid-liquid extraction was used(Benson et al, 2017).The samples were prepared by opening the screw top vial and removing 5 mL of the solution. The vial was recapped and weighed to the nearest ±0.1 mg. 2.00 mL of pentane (with the internal standard) was added to each vial and shaken vigorously for one minute (1 min). The two phases were allowed to separate for two minutes (2 min) and a glass pipette was then used to transfer at least 1 mL of the pentane (the upper phase) to a 1.8-mL screw top sample vial with a TFE septum, and stored at 4^{o}C until ready to inject into the GC for t**he Gas chromatographic analysis.** The instrument- Gas chromatograph HP-1ms ultra inert, Agilent

7890AGC was used with Autosampler Agilent 7683B equipped with an Electron capture detector (ECD) in the chemistry department of the Covenant University, Ota, Nigeria.

Based on the EPA method 551.1, a new method was developed by adjusting the equilibration time to three (3) minutes, max temperature to 260 degrees centigrade with the slow fan on. The oven program was adjusted to 4 degrees centigrade for 10minutes,

then 5°C/min. to 70°C for 0min, then 10°C/min to 200°C for 1 min. and Run time

to 30 minutes. The back injector's Injection volume was 0.5µl, Solvent A washes (PreInj)-2, Solvent A washes (PostInj) - 2, Solvent B washes (PreInj)- 2, Solvent B washes (PostInj)-2. Back SS Inlet Helium mode was split, Heater - 250°C,Pressure - 5psi, Total flow - 52.779 ml/min, Septum Purge flow - 3ml/min, Split ratio - 50: 1, Split flow - 48.803ml/min.

Column:

Agilent 19091J – 413HP-5 5% Phenyls Methyl Siloxan

325°C: 30m x 320µm x 0.25µm

In: Back SS Inlet He

Out: Back Detector µECD

(Initial)	-------	40°C
Pressure	-------	5psi
Flow	-------	0.97606ml/min
Average velocity	-------	18.086cm/sec
Holdup Time	-------	2.7646min
Pressure program	-------	On 5psi for 0min
Run Time	-------	30min

Back Detector µECD:

Heater	----- 300°C
Anode Flow	----- Off
Makeup Flow	----- 60ml/min
Electrometer	----- On
Back signal	-----Save on 5Hz

2.7. Recovery Test

The recovery test for the method's calibration was done after running all the samples, by spiking 10ppm, 20ppm, 30ppm and 40ppm of the standard each in 1000µL of methanol. These were run on the same method and calibration and the percentage recovery for each DBP was calculated.

$$\text{Percentage recovery} = \frac{\text{Yield}}{\text{Input}} \times 100$$

For 10ppm: Chloroform = $\frac{10.61591}{10} \times 100 = 106\%$

Bromodichloromethane (BDCM) = 109.21%, Dibromochloromethane = 113.32% and Bromoform = 119.18%.

For 20ppm: Chloroform = $\frac{19.04025}{20} \times 100 = 95.20\%$

BDCM= 99.80%, DBCM = 104.42%, and Bromoform = 110.13%.

For 30ppm: Chloroform = $\frac{29.47203}{30}$ x 100 = 98.24%.

BDCM = 103.85%, DBCM = 110.11% and Bromoform = 115.82%

For 40ppm: Chloroform = $\frac{38.95727}{40}$ x 100 =97.44%

BDCM = 103.88%, DBCM = 110.57% and Bromoform = 115.84%

3. Results and Discussions

The multi-level calibration table is shown below (Figure 1), followed by the calibration curves (Figure 2); chromatograms of the different calibration standard levels (Figure 3) and their signal overlay (Figure 4). Table 1 shows a summary of the recoveries of the trihalomethanes at the different spiked levels.

Calibration Table

Calib. Data Modified : 5/12/2015 7:55:45 PM

Rel. Reference Window :	5.000 %
Abs. Reference Window :	0.000 min
Rel. Non-ref. Window :	5.000 %
Abs. Non-ref. Window :	0.000 min
Uncalibrated Peaks :	not reported
Partial Calibration :	Yes, identified peaks are recalibrated
Correct All Ret. Times:	No, only for identified peaks
Curve Type :	Linear
Origin :	Included
Weight :	Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: ECD2 B, Back Signal

RetTime [min]	Sig	Lvl	Amount [ppm]	Area	Amt/Area	Ref Grp	Name
3.931	1	1	10.00000	3905.12280	2.56074e-3		Chloroform
		2	20.00000	7553.61621	2.64774e-3		
		3	30.00000	1.30928e4	2.29133e-3		
		4	40.00000	1.53444e4	2.60682e-3		
5.197	1	1	10.00000	1.66886e4	5.99211e-4		Dichlorobromomethane
		2	20.00000	3.58829e4	5.57369e-4		
		3	30.00000	6.49954e4	4.61571e-4		
		4	40.00000	7.74843e4	5.16233e-4		
8.015	1	1	10.00000	1.17828e4	8.48693e-4		Dibromochloromethane
		2	20.00000	2.57380e4	7.77061e-4		
		3	30.00000	4.52469e4	6.63028e-4		
		4	40.00000	5.43334e4	7.36196e-4		
13.198	1	1	10.00000	3706.38599	2.69805e-3		Bromoform
		2	20.00000	8092.94775	2.47129e-3		
		3	30.00000	1.41097e4	2.12619e-3		
		4	40.00000	1.70360e4	2.34797e-3		

Peak Sum Table

No Entries in table

Figure 1: The multi- level calibration table.

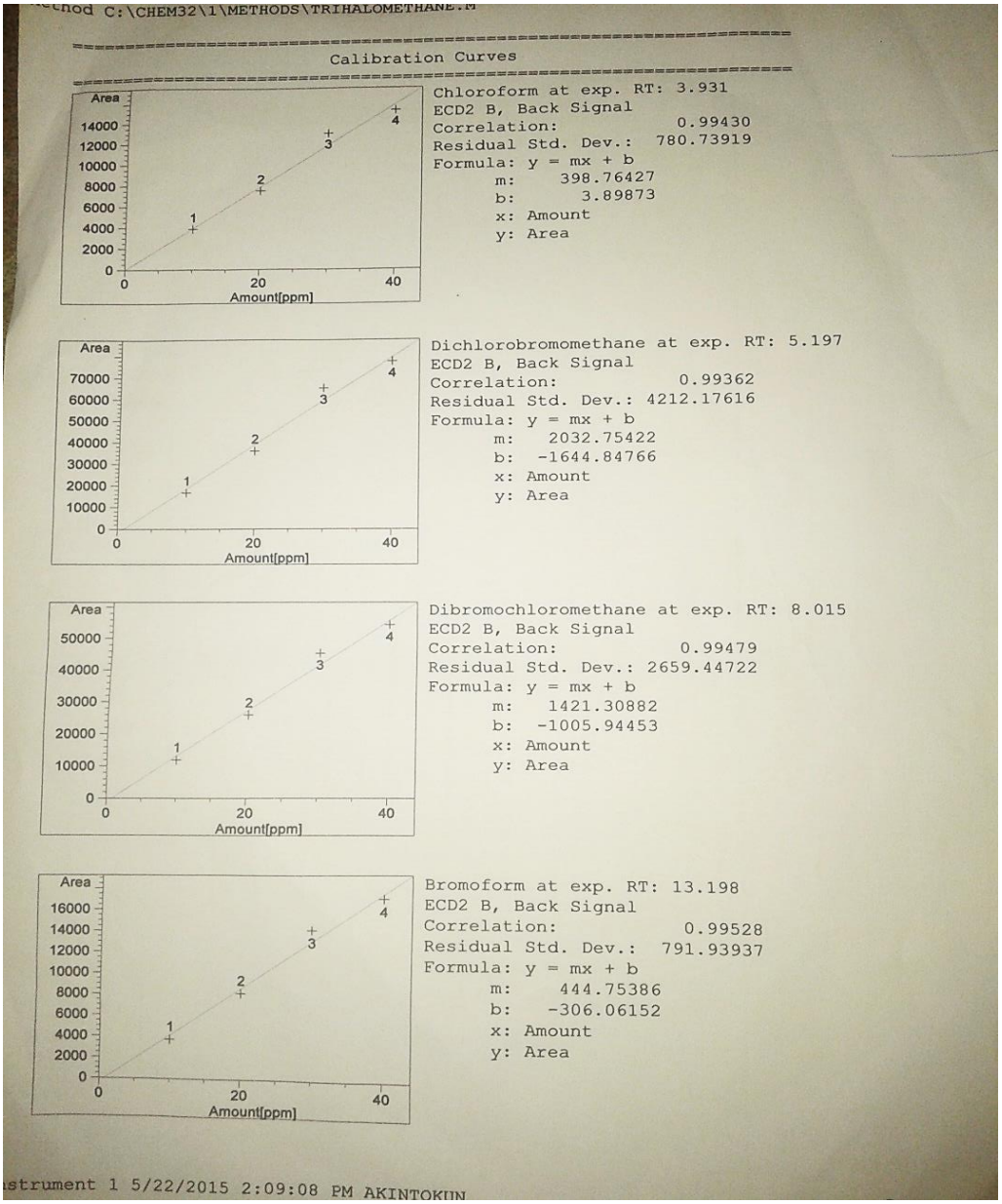

Figure 2: The calibration curves

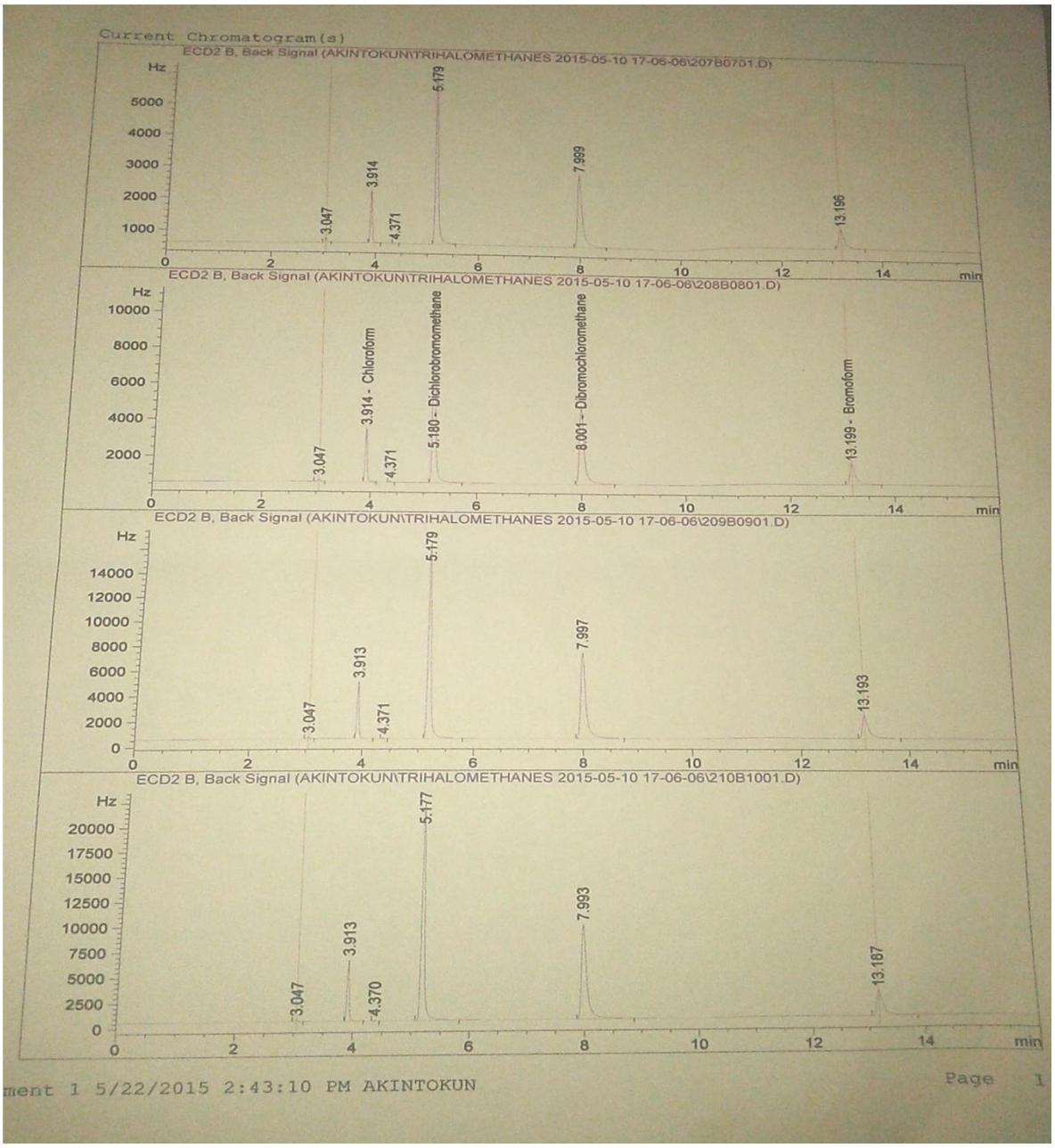

Figure 3: The Calibration Chromatograms.

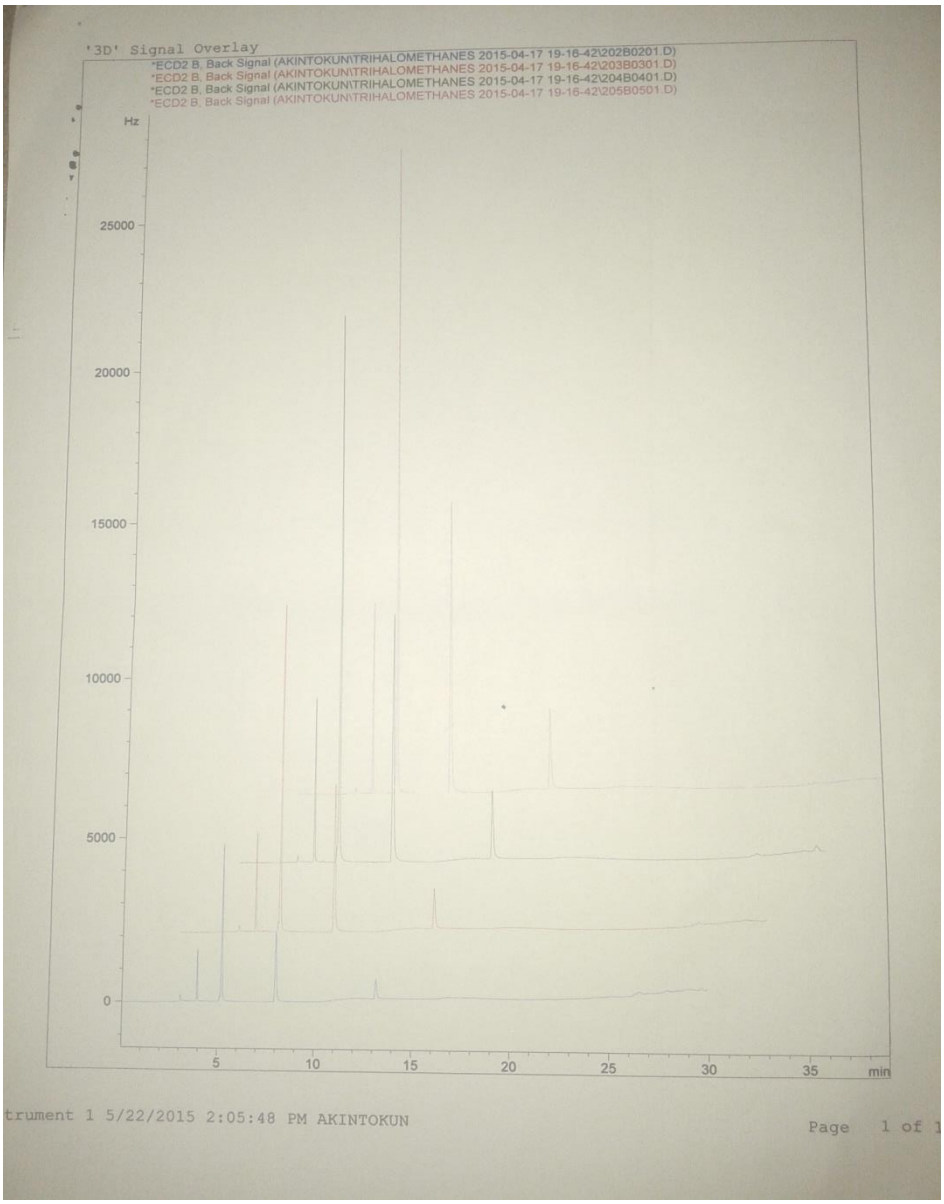

Figure 4: Overlaid Chromatograms of standard solutions at 10, 20, 30, and 40ppm respectively.

Table 1: Recovery Test results

SPIKED	**RECOVERY (%)**			
STD. LEVEL (ppm)	**TCM**	**BDCM**	**DBCM**	**TBM**
10	106.00	109.21	113.32	119.18
20	95.20	99.80	104.42	110.13
30	98.24	103.85	110.11	115.82
40	97.44	103.88	110.57	115.84

The ability of procedures and instruments for the determination of THMs levels was tested by the calibration of working standard solution. The linear regression of response (area) versus concentration of THMs was used to assess the linearity of calibration. From the result indicated by the calibration curves (Figure 1), it is seen that procedures and instruments had good ability to separate the THMs components. Response of THMs was linear for four working standard solutions at concentrations 10, 20, 30, and 40ppm (For Chloroform, Correlation R^2= 0.994, n= 4; for bromodichloromethane, R^2= 0.993, n= 4; for dibromochloromethane, R^2= 0.995, n= 4 and for bromoform, R^2= 0.995, n= 4. Figure 2 shows the chromatograms of the working standard solutions. The overlaid chromatograms (figure 4) clearly points out the peak height and retention time of each standard solution; average retention time of TCM was 3.931, of BDCM - 5.197, DBCM – 8.015 and TBM – 13.198. The run time of standard solutions was 30minutes and the recoveries are stated in table 1 above.

4. Conclusion

The procedures and Gas Chromatography instruments used were acceptable in determining the levels of trihalomethanes (THMs) in drinking water as indicated by calibration curves (Correlation R^2= 0.994, n= 4; R^2=0.993, n= 4; R^2= 0.995, n= 4 and R^2= 0. 995, n= 4). The modified split ratio reduced the level of contaminants in the GC column. The signal rate reduced to 5HZ enhanced better flow and separation of peaks. The multilevel calibration helped in obtaining accurate quantitative results. The results of the recovery test validated the method accuracy. The improved method will be more efficient in the evaluation of THMs levels in drinking water easily and accurately.

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