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Determination of Bromate and Iodate from Bread and Flour by Ion Chromatography

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Abstract: ***An accurate, simple, reproducible, and sensitive method for the determination of Bromate and Iodate was developed and validated. Bromate and Iodate were separated using a high capacity anion exchange column by isocratic elution with a flow rate of 1.0mL/min. Acidified Promethazine was added as post-column derivatization reagent (PCR). The mobile phase composition was 20mM Sodium Hydroxide and Ion chroamtographic PCR UV detection technique was carried out. The linearlity of method has been tested in the range of 0.03mg/l to 2.0mg/l for Bromate and 0.05 to 5.0mg/l for Iodate. Correlation cofficient (R^2) of more than 0.999 was observed for both Bromate and Iodate. The method was shown excellent reproducible, linear, specific, sensitivity, rugged. The Limits of Detection and Quantification have been also established for Bromate as 0.01mg/l & 0.03mg/l and for Iodate as 0.03mg/l & 0.05mg/l respectively. Hence, the validated method is easy to adapt for regular analysis.***

Keywords: Bread, Flour, Ion Chromatography, UV, Post Column Derivatization, Bromate, Iodate

1. Introduction

Bread is generally prepared using flour dough and water through baking process. It is one of the oldest man made food and it is consumed in almost entire world. Commercially available breads were manufactured using additives to improve its texture, flavor, nutrition, shelf life, color and most importantly ease of production [1].

Potassium bromate, or basically called bromate, is an oxidizer used to fortify mixture and improve its flexibility. This prepares uniform and whitened bread. Typically potassium bromate of around 15-30 parts per million (ppm) is added to dough or its similar products. Regularly, heating changes its compound structure and renders it innocuous, leaving no trace in the completed item. Be that as it may, if a lot of the additive is utilized, or the bread isn't prepared sufficiently long or at a sufficiently high temperature, at that point a leftover sum will remain. So also, Potassium Iodate was likewise added to in flour/batter to make bread as it is quick acting mixture oxidizing specialist that works in blender to completely build up the dough. For the most part, it is utilized around 75ppm at dough blender [2].

In 1964, a specialist board of trustees controlled by the World Health Organization and Food and Agriculture Organization began assessing potassium bromate. In 1983, it incidentally acknowledged a limit of 75 ppm gave there are almost no residues at end product, on the understanding that all bromate gets changed over into bromide amid preparing. This limit was later diminished to 60 ppm. After long haul thinks about, potassium bromate/iodate was considered a 'genotoxic cancer-causing agent'. In 1992, the advisory group chose that utilizing potassium bromate as a flour treatment operator might have been "not suitable", likewise considering there were options. In 1999, the International Agency for Research on Cancer (IARC) grouped potassium bromate as perhaps cancer-causing to people. In 2012, the Codex Alimentarius, a worldwide sustenance wellbeing reference office kept running by the WHO and FAO, formally pulled back particulars of potassium bromate in accordance with the master advisory committee view [2].

The European Union, Peru, Columbia, Sri Lanka, Australia, China, South Korea, Brazil and Canada have banned the use of potassium bromate as a flour treatment agent. Potassium Iodate is also banned in the EU as well. USA continue to allow use of potassium bromate in permissible limits. FSSAI India currently banned usage of potassium bromate in bread [3]. But usage of iodate up to 50 ppm on flour mass basis is regulated .USA allows it up to 75 ppm and manufacturers must list the ingredient on food labels. However, the USA administration requests bakers not to use potassium bromate and potassium iodate and if it is used they need to put as warning label Therefore, many USA bread and bakery manufacturers have voluntarily stopped usage of bromate and iodate[2].

Ion exchange chromatography is a liquid chromatographic technique, in which ionic and strongly polar species can be well separated and detected. It can be used to detect the presence of Iodate and Bromate from bread and flour by making it specific by using acidfied promethazine (reduces Iodate and Bromate) as post column derivatization solution and detected at 515nm. This method provides excellent separation between Iodate and Bromate when compared to other conventional methods and allows detection of them at low mg/l(ppm) level. The developed methodology has been validated and it is highly effective to estimate Bromate and Iodate from bread and flour matrices.

2. Experimental

2.1 Reagents and Chemicals

All chemicals used for preparation of reagents, standards and mobile phase were of analytical grade. Ultrapure deionized water (18.2 MΩ cm, Milli-Q system) was used for the preparation of mobile phase, standards and samples. Sodium Bromate (AR grade) and Potassium Iodate (AR grade) was used for the preparation of Bromate and Iodate standards

respectively, 50% Sodium Hydroxide (Sigma Aldrich) was used to prepare eluent of 20mM Sodium Hydroxide and 37% HCl (Trace Metal Grade) and Promethazine Hydrochloride (TCI, Tokyo) was used for the preparation of post column derivatization solutions. Samples includes brown bread, white bread, pizza bread, pav, maida flour, regular cold dough and donut bread. All samples were purchased from local market.

2.2 Apparatus

The equipment used was Thermo Fisher Dionex Ion Chromatography system with VWD detector having autosampler with a 25µL loop, IonPac AS19 column (4 x 250mm inner diameter) and IonPac AG19 guard (4 x 50mm inner diameter) was used as separator column. The experiment was conducted using a pre-degassed eluent 20mM Sodium Hydroxide (NaOH) at a flow rate of 1.0ml/min. Post column reagent (PCR) solution of 10mM Promethazine Hydrochloride was prepared in 5M Hydrochloric acid. Dionex PC10 module is used to deliver flow 0.5ml/min of PCR. Column outlet and PCR outlet was connected to mixing tee, outlet of mixing tee was connected to 375µL reaction coil and it was further connected to VWD (UV-Vis) detector. Software used for data acquisition was Thermo Fisher Dionex Chromeleon (version: 6.80 SP2). Chromatograms were monitored simultaneously during analysis.

2.3 Procedure

Preparation of 20mM Sodium Hydroxide (Eluent):- 2.1mL of 50% NaOH solution was taken in 2000ml volumetric flask containing 500ml of ultrapure deionized water. It was swirled for 1 minutes and made up to the mark with ultrapure deionized water. It was then filtered through 0.2µ nylon membrane filter.

Preparation of Post Column Reagent (PCR):- 5.68g of Promethazine Hydrochloride was taken in 2000ml flask. 500ml of ultrapure deionized water was added to it. 880ml of 37% HCl was added to it. Solution was sonicated to dissolve and make up to mark with ultrapure deionized water.

Preparation of standard solutions:-
Certified Sodium Bromate and Potassium Iodate salts was procured from sigma Aldrich. From these salts, a 1000mg/l separate standard solutions were prepared from it. From this 1000mg/l standard solution, mixture of 0.03, 0.10, 0.20, 0.50, 1.00 and 2.00 mg/l of Bromate and 0.05, 0.10, 0.50, 1.00, 2.50 and 5.00mg/l of Iodate was prepared for the Linearity study, and mixture of 0.5mg/l of Iodate and 0.20mg/l of Bromate was was prepared for the precision study. Mixture of 0.03mg/l of Iodate and 0.01mg/l of Bromate solution was prepared for Limit of Detection (LOD) and Mixture 0.05mg/l of Iodate and 0.03mg/l of Bromate were prepared for Limit of Quantification (LOQ).
Sample preparation: - Samples need to dry at 75°C for 1hrs on hot air oven and grind sample by mixer before weighing. Around 0.2 g of ground Bread or Flour sample was weighed in 50ml polyethylene bottle with polyethylene cap, 10ml of ultra-pure deionized water was added to it. It was then mixed well after closing with cap. Samples were sonicated samples for 15mins and transferred it to centrifuge machine. It was centrifuged at 5000rpm for 10mins. Supernatant was filtered through 0.2u Nylon membrane filter. Samples were passed through OnGuard II RP (PN 057084) to remove organic matrices. It was collected in auto sampler vial. This procedure was repeated for each sample along with recovery samples and diluent.

An Autosampler (Dionex AS-AP) was used to inject solutions containing Iodate and Bromate into the ion chromatography system. Subsequently, the standard solution in the sample loop was transferred onto the separator column, on which Bromate and Iodate was separated. After separation on the column, Iodate and Bromate was detected by VWD detector at 515nm after reacting it with post column reagent solution. A sequence containing the blank, standards, samples and recovery samples were run and results were then interpreted. Following is diagram which shows connections with various assembly of Ion Chromatography system

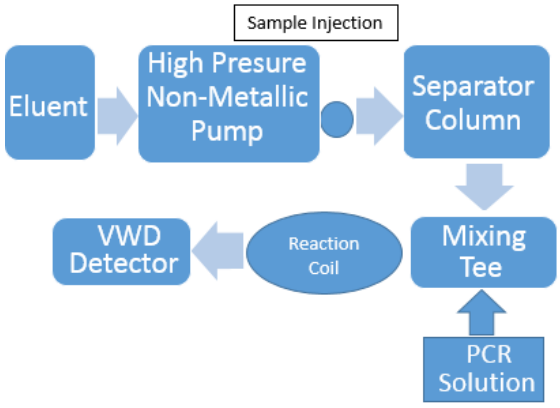


Figure 1: Ion Chromatography system schematic diagram for Bromate and Iodate analysis

3. Results and Discussions

Limit of Detection (LOD) for Iodate 0.03mg/l and it was injected (n) six times and observed average signal to noise ratio (S/N) was 5.5. Similarly, LOD for Bromate was 0.01mg/l and it was injected (n) six times and observed average signal to noise ratio (S/N) was 3.7. Limit of Quantification (LOQ) for Iodate was 0.05mg/l, it was injected (n) six times and observed signal to noise ratio (S/N) was 12.3. Similarly, LOQ for Bromate was 0.03mg/l, it was injected (n) six times and observed signal to noise ratio (S/N) was 11.0. Table 1 shows results for LOD and LOQ of Iodate and Bromate.

Table 1: LOD and LOQ data for Iodate and Bromate

Iodate	*Amount, mg/l*	*S/N*	*% RSD (n=6)*
LOD	0.03	5.5	0.41
LOQ	0.05	12.3	1.11

Bromate)	*Amount, mg/l*	*S/N*	*% RSD (n=6)*
LOD	0.30	3.7	1.32
LOQ	0.50	11.0	1.67

The response of Iodate was linear over the range of 0.05 to 5.0mg/l and of Bromate was linear over the range of 0.03 to 2.0mg/l. Calibration curve fits well and that is significantly linear having correlation coefficient of 0.99993 for Iodate and 0.99986 for Bromate (figure 2). This linearity study was performed for the concentration range of 0.05, 0.10, 0.50, 1.00, 2.50 and 5.00mg/l for Iodate and 0.03, 0.10, 0.20, 0.50, 1.00 and 2.00 of Bromate. Each standard injection was repeated thrice. Therefore, number of calibration points (n) for linearity study was 18. Its data had been shown in table 2.

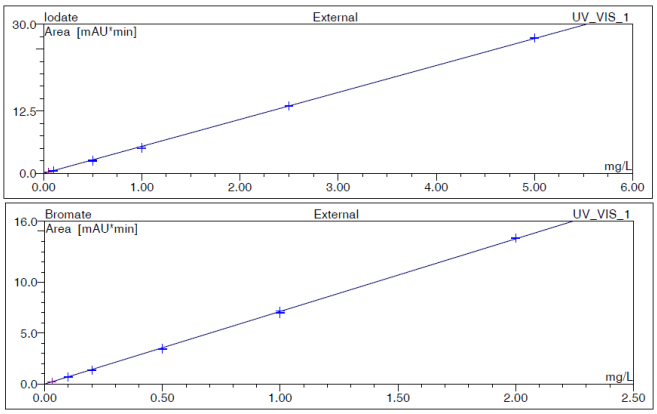
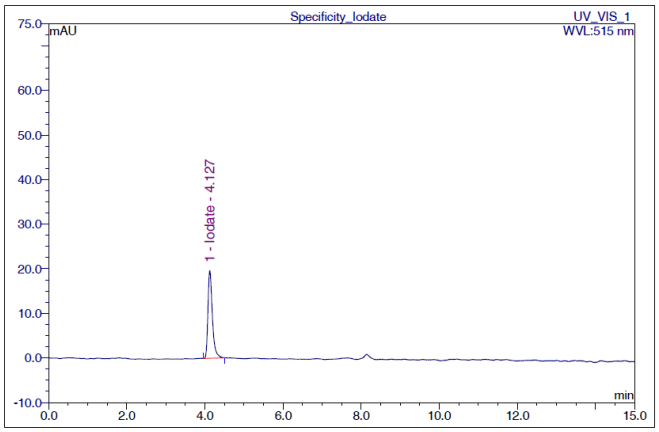


Figure 2: Linearity plot for Iodate and Bromate

Table 2: Linearity data for Iodate and Bromate

Analyte	*Points*	*Corr. Coeff.*	*Offset*	*Slope*
Iodate	18	0.99993	0	5.43
Bromate	18	0.99986	0	7.13

Method specificity was also done with separate injection of Bromate (0.5mg/l) and Iodate (0.5mg/l). Its chromatograms was shown in figure 3.



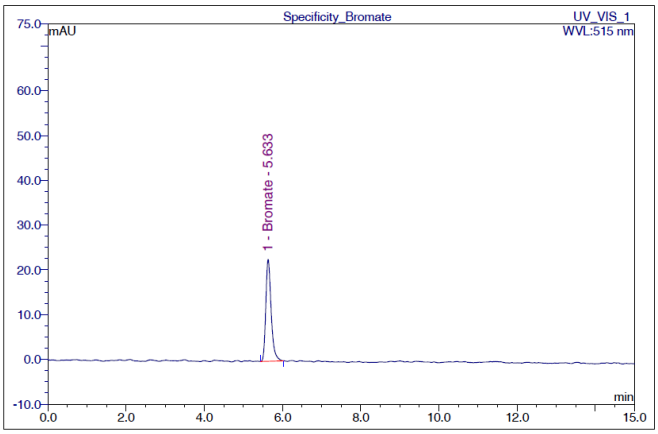


Figure 3: Specificity chromatograms for Iodate (0.5mg/l) and Bromate (0.5mg/l)

Replicate injections of Iodate and Bromate were done and their percent relative standard deviation for peak area was 0.68% and 0.73% respectively. Table 3 shows results for its precision study.

Table 3: Precision data for Iodate and Bromate

Analyte	*Amount, mg/l*	*% RSD (n=6)*
Iodate	0.50	0.68
Bromate	0.20	0.73

hromatogram for Iodate and Bromate standard mixture for six consecutive injections is shown in figure 4.

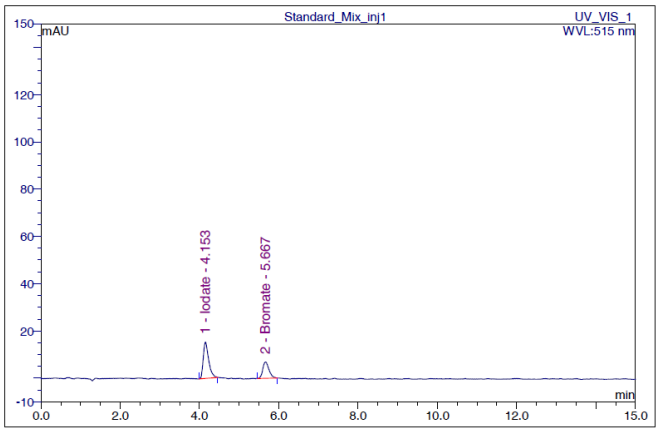


Figure 4: Standard chromatogram for mixture of Iodate (0.5mg/l) and Bromate(0.2mg/l)

Sample results: - Samples were analysed using the linearity calibration method. Replicate injections of same sample was also done. Its results and routine analysis sample results were shown in table 4 and table 5.

Table 4: Sample precision

Analyte	*Sample*	*Number of preparations*	*Results, mg/Kg*
Iodate	Brown Bread	10.0	Not Detected
Bromate	Brown Bread	10.0	Not Detected

Table 5: Routine sample analysis results

Sample.	*Iodate mg/Kg*	*Bromate* mg/Kg
Brown Bread	Not Detected	Not Detected
White Bread	Not Detected	Not Detected
Pizza Bread	Not Detected	Not Detected
Pav	12.5	9.05
Maida Flour	Not Detected	Not Detected
White Bread	12.09	10.79
Regular Cold Dough	Not Detected	Not Detected
Donut Bread	Not Detected	Not Detected

Iodate and Bromate was not detected in most of samples, but some samples shows presence of Iodate and Bromate which gives confirmation that iodate and bromate was added during their manufacturing/production process. Intraday analysis of Samples was done for seven consecutive days for which sample results were observed to be similar as given in table 5. Sample Chromatogram was shown is figure 3.

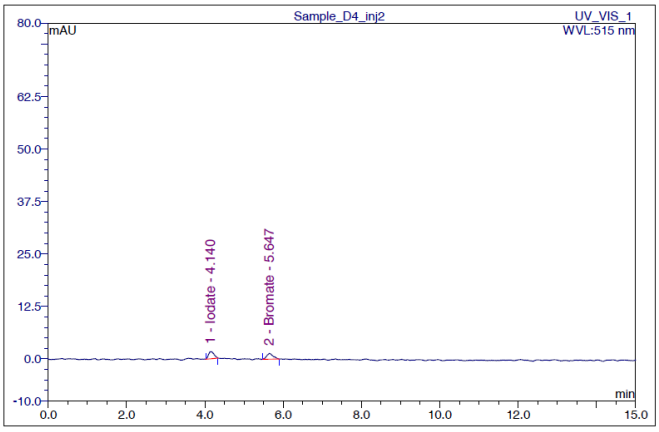


Figure 5: Sample chromatogram (White Bread) for estimation of Iodate and Bromate

Recovery: - The sample used for recovery study was Brown Bread (average concentration was taken for calculation). Recovery test solutions were injected in triplicate Also for recovery study, known concentrations of amount was added to sample at three different levels as shown in table 6.

Table 6: Recovery study (Iodate and Bromate) for sample (Brown Bread) (n = 3)

Analyte	*Level*	*Amount Added mg/l*	*Amount Recovered* mg/l	*% Recovery*
Iodate	1	0.050	0.051	100.40
	2	0.500	0.466	93.20
	3	1.000	0.974	97.40
	4	1.500	1.435	95.67

Analyte	*Level*	*Amount Added* mg/l	*Amount Recovered* mg/l	*% Recovery*
Bromate	1	0.030	0.031	103.33
	2	0.250	0.263	105.20
	3	0.500	0.491	98.20
	4	0.750	0.694	92.53

The same method has been used on another Ion chromatography model like ICS 5000+, Aquion and Integrion with different lot of AS 19 column as a part of ruggedness study, for which there is no significant variations of sample results were observed

4. Conclusions

Ion Chromarography – PCR – UV-Vis detection gives specific, sensitive and precise method for estimation of Iodate and Bromate. This present method was used for analysis of Bread and flour samples for Iodate and Bromate content without any much pretreatment. The detection limits for Iodate was 0.03mg/l and for Bromate was 0.01mg/l. This technique is cost-effective with respect to analysis required for keeping a check on the limits of Iodate and Bromate in bread and flour as per FSSAI (India) regulations and other regulatory bodies.

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Author Profile

Chetan Chavan had completed the B.Sc (Chemistry). and M.Sc (Chemistry) degrees from Mumbai University and completed his ph.D in year, 2018., He had joined Dionex India Pvt. Limited, which is now Thermo Fisher Scientific Pvt. Ltd., as Applications Manger for Ion Chromatography (IC) and HPLC and currently working as Product Manager – IC/SP. His role is to provide technical support coordination with factory for India team requirements, provide guidance to sales, application and service team for IC and HPLC.

Chanakya Thaker received the B. Sc (Chemistry). and M.Sc (Analytical Chemistry) degrees from Mumbai University in 2006 and 2008, respectively. From 2009, he had joined Dionex India Pvt. Limited, which is now Thermo Fisher Scientific Pvt. Ltd., as Applications Specialist for Ion Chromatography (IC) and HPLC and currently working as Applications Manager – IC/SP. His role is to manage application team, provide customer training, sample analysis, technical presentations, method development and troubleshooting on IC and HPLC systems.

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