A simple and rapid method for spectrophotometric determination of bromate in bread

J. El harti [1]*, Y. Rahali [2], A. Benmoussa [1], M. Ansar [1], H. Benziane [1], J. Lamsaouri [1], M. O.B. Idrissi [3], M. Draoui [3], A. Zahidi [1], J. Taoufik [1]

[1]*Laboratory of Medicinal chemistry, Faculty of Medicine and Pharmacy, University Mohammed V, Souissi Rabat, Morocco*
[2]*Laboratory of Pharmaceutics and Pharmaceutical Technology, Faculty of Medicine and Pharmacy, University Mohammed V, Souissi Rabat, Morocco*
[3]*Laboratory of Analytical chemistry, Faculty of Medicine and Pharmacy, University Mohammed V, Souissi Rabat, Morocco*

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**Corresponding Author Email: harti.jaouad@gmail.com*

Abstract

The bromate is used in bread making as agent of maturation, however it has a high potential toxic. A rapid and reliable spectrophotometric method was validated to determine the level of bromate in bread; this method is based on the red-ox reaction between bromate and promethazin in acidic medium. This produced a red-pink product with maximum absorption at 515nm. The calibration curve was linear (r = 0.9989) over the range 0.5 µg/ml – 4.5 µg/ml of bromate, the proposed method has been successfully applied to determination of bromate in commercial bread.

Keywords : Bromate, bread, analysis, spectrophotometry, validation, control

1. Introduction

Bromate is widely used as a part of the bread-making process for the maturation of flour and is, therefore, a good additive. It is also used in the production of fish paste, fermented beverages, and in cold wave hair lotion [1].
Acute effect of bromate toxicity includes nausea, vomiting, abdominal pain, peripheral neuropathy, anemia, hypotension, renal insufficiency or failure and central nervous system dysfunction including seizures [2-9].The international agency for research on cancer (IARC) has classified $KBrO_3$ as a 2B (a possible human carcinogen) based on sufficient evidence that $KBrO_3$ induces cancer in experimental animals [5,10].
Potassium bromate has been the oxidant of choice for a long time [11], 2005; [12], it is believed to act by oxidizing thiol groups to disulfide linkages, thus strengthening the protein network [13]. Consequently an increase in dough expansion capacity and improvement of the bread appearance.
The FDA and China permit the use of potassium bromate up to a maximum level in bread of 50 mg/kg of flour mass, but Japan permits only 10 mg/Kg of flour [14-15].

Many analytical methods have been developed for the determination of bromate, such as the colorimetric method [16], spectrofluometric method [17], capillary electrophoresis [18], flow injection analysis [19-20] and ion chromatography [21]. However the methods mentioned above suffered for more or less time consuming procedures and complicated instrumentation.
In this work, we report a very simple and validated method for direct determination of bromate, that has been based on a red-pink product with maximum absorption at 515nm produced by the reaction of bromate and promethazin in acid medium, this method has been used in determination of bromate in commercial bread.

2. Materials and methods

2.1 Apparatus
A model Jasko V-530 spectrophotometer was used in the measurement of the absorbance and using a 1cm quartz cell.

2.2 Reagents and chemicals
Promethazin hydrochloride (PTZ) was offered graciously by Rhone Poulenc RORER, all other reagents were of analytical grade and water was always distilled.
Stock of bromate potassium ($KBrO_3$) solution 50 mg/l was preparedin distilled water. An accurate weighted quantity of PTZ was transferred into 50 ml volumetric flask, dissolved in water, sonicated to obtain stock solution of 10^{-2}M of PTZ.

2.3 Preparation of standard solutions
Aliquots of 100µl, 200µl, 400µl, 600µl and 800µl from the primary stock solution of $KBrO_3$ were placed in 20ml capacity tubes, and an aliquot of 10^{-2}M PTZ were added. Mixtures were diluted with distilled water up to 10ml to obtain final concentration of bromate in the range of 0.5 µg/ml to 5 µg/ml, 200µl HCl 12Mwere added. Mixtures were well shaken for 1min and the absorbances were measured at 515nm against a blank reagent, results were used to plot the calibration curve and calculate the equation of the linear regression.

2.4 Preparation of samples
A sample of 10g of bread was cuted and triturated into 200ml of distillated water with magnetic stirrer and then filtered through a Wathman no 41. A measured volume of the filtrate solution (8.8ml) was transferred into a 15ml volumetric tube and mixed with 1ml of PTZ 10^{-2}M. 200µl of HCl 12M was added and the final mixture was shaked well for 1min. The colored solution obtained was measured spectrophotometrically at 515nm. The unknown concentration was calculated from the linear regression curve obtained from the standard solutions of bromate as mentioned in the previous section.
A preliminary qualitative test can be effected directly on a portion of bread with 2ml of PTZ 10^{-2}M and 600µl HCl 12M.

3. Results and discussions

The heterocyclic centered radical cations in general produce red-pink color [22], subsequent oxidation such as 4-hydroxy- 3- oxo- 3H- phenothiazine -5 -oxide [22-23], thus promethazin oxided with bromate to produce an red-pink product measured at 515 nm (Fig. 1).
A preliminary test was carried to show if bread contains more than 50 mg/Kg of bromate. For this a four samples of bread were made with flour without broamte, 50 mg/Kg, 100 mg/Kg and 250 mg/Kg. It found that three samples made with bromate show a coloration of red-pink with 2ml of PTZ 10^{-2}M and 600µl HCl 12M. In addition the intensity of coloration is less than which obtained in aqueous solution at 50 mg/l of bromate, that can be explained by the effect of temperature of cooking to oxide bromate [1].
To aim to establish of the optimal conditions for this reaction, series of experiments were carried, all parameters which affect the intensity of product were studied by altering each variable in turn, while keeping others constant.
The effect of PTZ concentration was investigated at concentration of 10^{-4}M of bromate and by varying the concentration of PTZ from 10^{-4}M until 10^{-2}M, the maximum absorbance was obtained with PTZ at 10^{-3}M. However, in this study, the variation of concentration of HCl into 0.1M to 1M shows that about 0.25M HCl

concentration was sufficient to gave the maximum effect on the absorbance. The complete reaction required 15 min and the coloration was stable about 60min at 25°C (Fig. 2). The optimum conditions were applied in further experiments.

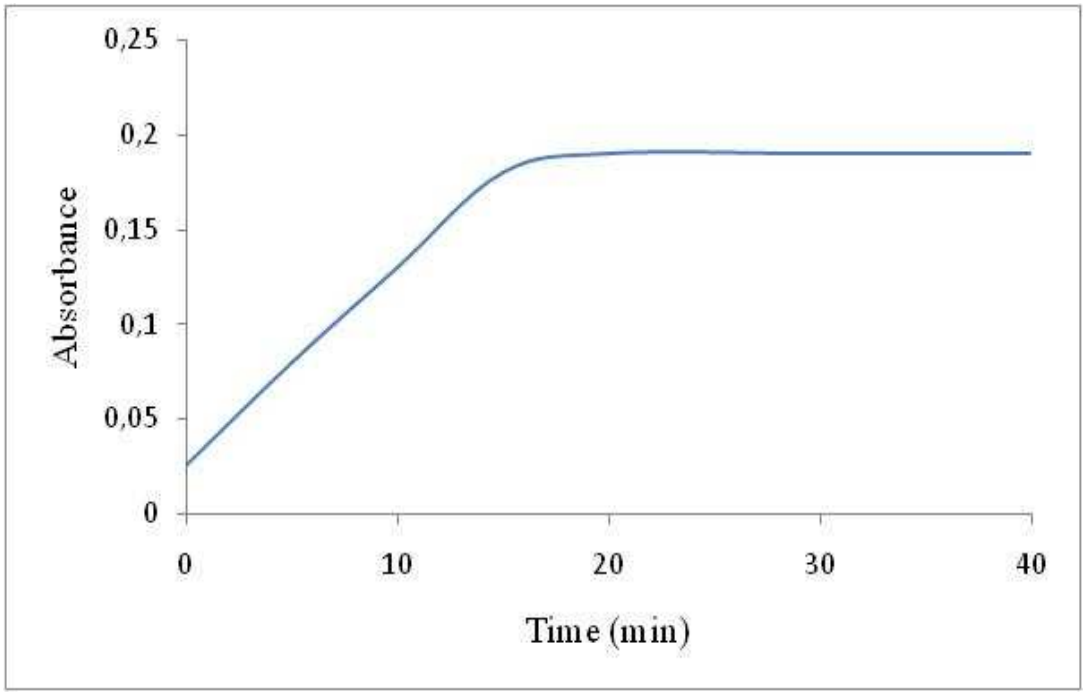

Fig. 1: Absorption spectra of oxidizing product between PTZ 10^{-3}M and bromate [a] at 0,5 µg/ml, [b] 2 µg/ml and [c] 4 µg/ml

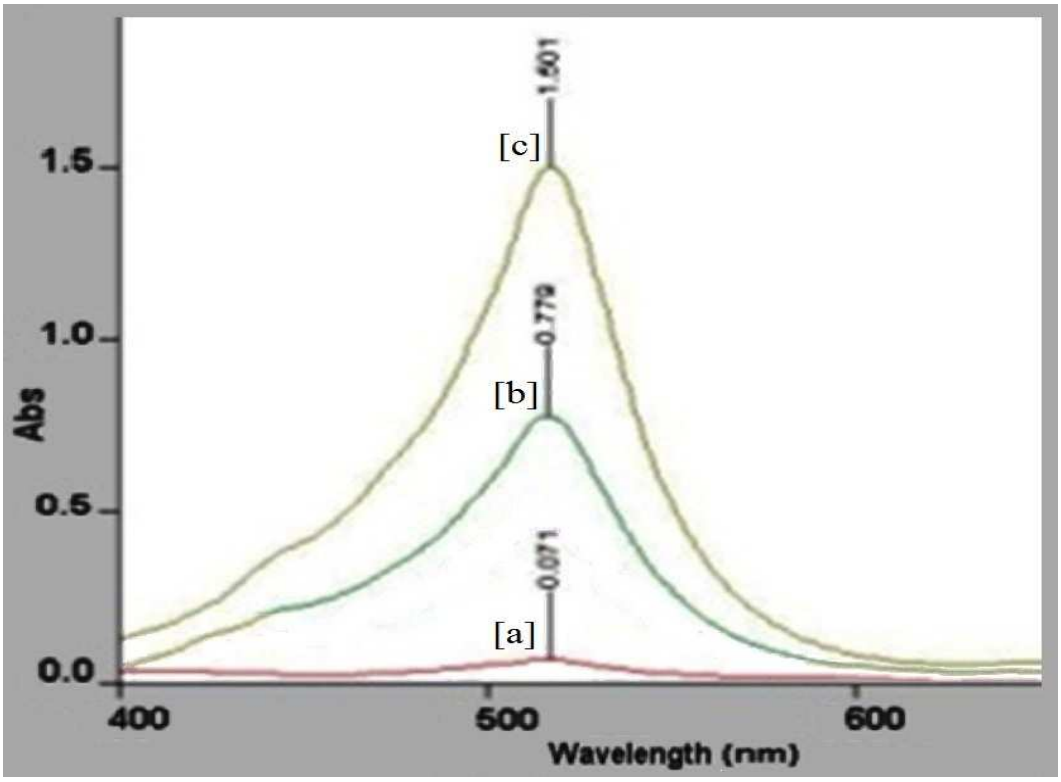

Fig. 2: Optimization the time of reaction; PTZ 10^{-3}M and bromate 1µg/ml

The validation of the proposed method consists to determine the following parameters: selectivity, linearity, accuracy, repeatability, intermediate precision, lowest limit of detection (LOD) and lowest limit of quantification (LOQ) following ref. [24].
The flour is a complex matrix, the following anions were studied: Cl^-, Br^-, I^-, NO_3^- and NO_2^-. No interference was observed, in addition the aqueous extract of bread without bromate did not show any coloration compared to aqueous extract of bread with 50 mg/Kg of bromate.
The Beer's law was obeyed and the standard calibration curve was linear over the concentration range of 0.5 µg/ml – 5 µg/ml. the regression equation of calibration plot was calculated by the least squares methods with Y (Abs) = 0,0825x + 0,0207 (n=3, r = 0.9989) (Fig. 3). The student test of the data indicated that the correlation coefficient r was found to be real and significant at the 5% level ($\rho \neq 0$; ρ value < 0,05) [24].
Lowest limit of detection (LOD) and lowest limit of quantification (LOQ) were calculated using the following equations [25]: LOD = 3.S/b and LOQ = 10.S/b, where S is the standard deviation of the blank performed by analyzing an appropriate number of blank samples and calculating the standard deviation of responses, and b is the slope for the calibration curve. In accordance with the formula, the LOD and the LOQ were found to be equal to 0.027µg/ml and 0.069µg/ml, respectively.
The accuracy was evaluated by calculating the mean percentage recovery of three different concentrations taken within the calibration range (three replicates). Repeatability was verified by measuring the absorbance of the medium concentration in the interval range during the same experimental conditions (intra-day assay) (six replicates).

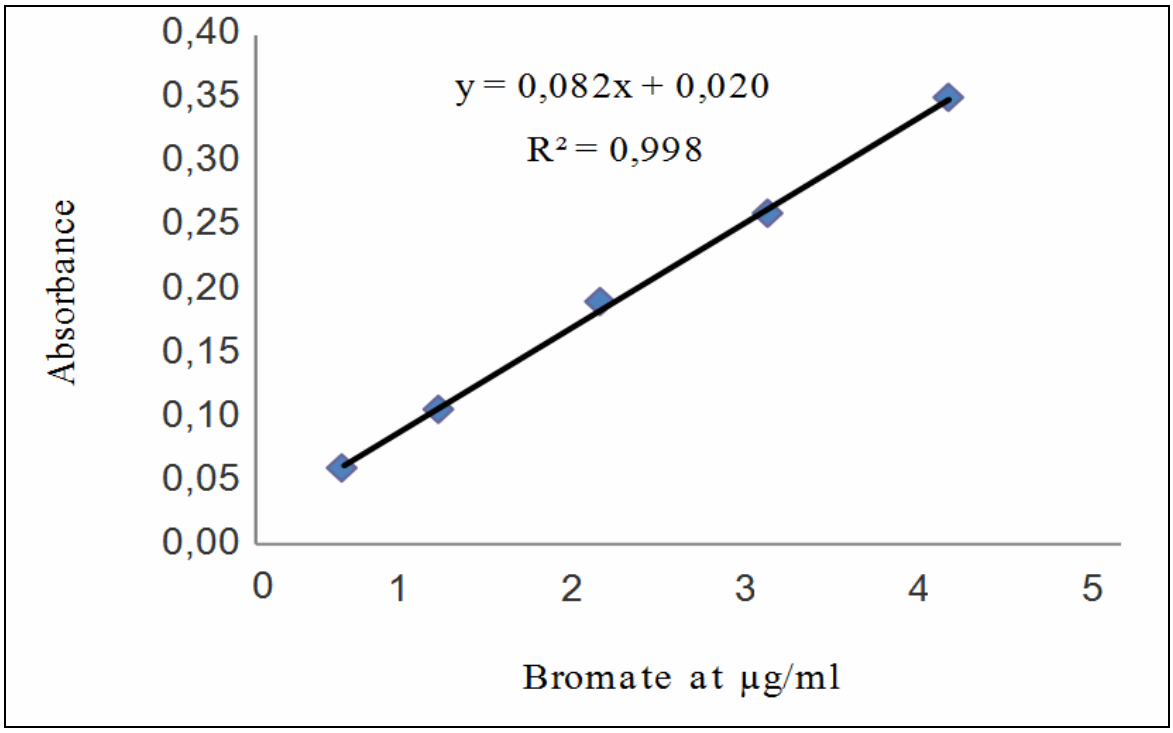

Fig. 3: Calibration curve and spectrophotomtric response of PTZ to bromate

The intermediate precision was evaluated by comparing the absorbance of standard solutions of the calibration curve prepared on three different days (three replicates) (inter-day assay).
The intra-day and inter-day precision were determined as the relative standard deviation and accuracy was determined as percentage recovery. Results of precision and accuracy summarized in (Table 1), demonstrate good precision and accuracy over the concentration ranges selected.
The proposed method was used to investigate the level of bromate in bread done by some bakers in different regions in Rabat. For this a sufficiently samples of bread were recuperated, in parallel a bread without bromate and with 50 mg/Kg of bromate were prepared as blank and witness of this investigation.
The results of 12 samples from different bakeries showed that only 2 samples contain about 5 mg/kg of bromate, which are not over the norm of FDA [15].

Table 1 Precision and accuracy data for bromate obtained by the proposed method

Accuracy ; n = 3

Expected concentration (µg/ml)	Concentration found (µg/ml)	% Recovery
0,5	0.484	96.81
2	2.098	104.95
4	4.103	102.58

Intraday precision; n = 6

Expected concentration (µg/ml)	Mean found (µg/ml)	RSD %
2	2.17	1.19

Intermediate precision (inter-day); n=3

Expected concentration (µg/ml)	Concentration found (µg/ml)	RSD %
0.5	0.503	3.86
1.0	1.112	3.88
2.0	2.123	4.51
3.0	2.994	0.69
4.0	3.930	1.62

4. Conclusion

The present work describes a simple, rapid and validated spectrophotometric method of searching bromate, hence it can be recommended for the routine control of bromate in bread. This method will be an alternative for the laboratories not equipped with expensive materials in the aim to preserve the security of consumers, especially in the developing countries.

5. References

1. AIRC Some naturally occurring and synthetic food components, furocumarins and ultraviolet radiation. IARC Monogr. Eval. Carcinog. *Risk Chem. Hum.* 40 (1986) 207.
2. Crofton, K. M. Bromate : Concern for developmental neurotoxicity ?. *Toxicology*. 221 (2006) 212.
3. De Vriese, A., Vanholder, R., and Lamiere, N. Several acute renal failure due to bromate intoxication. *Nephrol. Dial. Transplant.* 12 (1976) 204.
4. Deshimaru, M., Miyagawa, T., Sumiyoshi, S., and Normura, Y. Peripheral nerve disease associated with acute renal failure due to bromate poisoning. *No To Shinkei.* 28 (8) (1976) 807.
5. Fawell, J., and Walker, M. Approaches to regulatory values for carcinogens with particular reference to bromate. *Toxicology.* 221 (2006) 149.
6. Gradus, D. B., Rhoads, M., Bergstrom, L. B., and Jordan, S. C. Acute bromate poisoning associated with renal failure and deafness presenting as hemolytic uremic syndrome. *Am. J. Nephrol*. 4 (1984) 188.
7. Kathleen, C. M. Bromate induced toxicity. *Toxicology.* 221 (2006) 205.
8. Matsumoto, I., Morizono, T., and Paparella, M. Hearing loss following potassium bromate : two case reports. Otolaryngol. *Head Neck. Surg.* 88 (1980) 625.

9. Quick, C. A., Chole, R. A., and Mauer, M. Deafness and renal failure due to potassium bromate poisoning. *Arch. Otolaryngol.* 101 (1975) 494.
10. Martha, M. M., and Tao, C. Mutagenicity of bromate: Implications for risk assessment. *Toxicology*. 221 (2006) 190.
11. Gandikota, S., and MacRitchie, F. Expansion capacity of doughs : methodology and applications. *J. Cereal. Sci.* 42 (2005) 157.
12. Dupuis, B. The chemistry and toxicology of potassium bromate. *Cereal F.W.* 42 (1997) 171.
13. Mehmet, H., and David Schofield, J. Heat and additive induced biochemical transitions in gluten from good and poor breadmaking quality wheats. *J. Cereal Sci.* 42 (2005) 245.
14. Kurokawa, Y., Maekawa, A., Takahashi, M., and Hayashi, Y. Toxicity and carcinogenicity of potassium bromate a new renal carcinogen. *Environ. Health Perspect.* 87 (1990) 309.
15. U.S. Food and Drug Administration, U.S. Code Fed. Regul, (1984).
16. Boschserrat, F. Colorimetric determination of bromates in flours with previous extraction in N,N-dimethylformamide. *Quimica Anal.* 16 (1997) 47.
17. Gahr, A., Huber, N., and Niessner, R. Fluorimetric determination of bromate by ion exchange separation and post-column derivatization. *Microch. Acta.* 129 (1998) 281.
18. Doble, P., Macka, M., and Haddad, P. R. Use of dyes as indirect detection probes by capillary electrophoresis. *J. Chromatog. A.* 804 (1998) 327.
19. Xingguo, C., Xingwei, Z., Zhide, H., and Zongyan, K. Determination of BrO_3^- by flow injection analysis with 5-Br-PADAP and SCN^-. *Anal. Lett.* 23 (1990) 119.
20. Ketai, W., Huitao, L., Jian, H., Xingguo, C., and Zhide, H. Determination of bromate in bread additives and flours by flow injection analysis. *Food Chem.* 70 (2000) 509.
21. Cox, D., Harrison, G., Jandik, P., and Jones, W. Application of ion chromatography in the food and beverage industry. *Food Technol.* 7 (1985) 41.
22. Girard, Y., Hamel, P., Therien, M., Springer, P., Hirshfield, I. J. First synthesis of sulfoxides and sulfones in the 3H-phenothiazin-3-one and 5H-benzoa.phenothiazin-5-one ring systems. *Org. Chem.* 52 (1987) 4000.
23. Hanson, P., and Norman, R. O. C. Heterocyclic free radicals. Part IV. Some reactions of phenothiazine, two derived radicals, and phenothiazin-5-ium ion. *J. Chem. Sot. Perkin. Trans.* 2 (1973) 264.
24. ICH Guidelines Q2B. Federal Register 62 (1997).
25. Valleron, A. J. Introduction à la biostatistique. Collection évaluation et statistique. Masson Paris (1998).

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