DETERMINATION AND VALIDATION OF INORGANIC BROMIDE BY GAS CHROMATOGRAPHY IN SEVERAL FOODS

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ABSTRACT

An analytical method for determination of inorganic bromide in cereals, herbs, fruits and vegetables had been validated. The comminuted samples are suspended in an acidified aqueous solution of propylene oxide, with bromide being simultaneously extracted. Organic bromide is converted to mixture of 1-bromo-2-propanol and 2-bromo-1-propanol and partitioned by ethyl acetate and then determined by GC-ECD without further cleanup, the analytical parameters of the method such as limit of quantification and linearity have been investigated. The limit of quantification is found to be 2 mg/kg for dry samples and 0.2mg/kg for fresh samples. The method showed to be linear up to 100 mg/kg. The average recoveries at different concentration levels on the fresh samples and dry samples ranged from 84 to113%. The reproducibility expressed as relative standard deviation was less than 12.8 % for dry samples and 11.6 % for fresh samples. The measurement uncertainty expressed as expanded uncertainity in terms of relative standard deviation at 95 % confidence level was found to be within the range ±33%

Keywords: validation, inorganic bromide.

INTRODUCTION

Fumigants containing bromine, mainly methyl bromide, are used for soil disinfection as well as post harvest treatment of plant products. As a degradation product of these fumigants, bromide may be absorbed by plants from treated soils or it may be contained in fumigated products. Over the last 30 years the use of methyl bromide as a fumigant has become widespread in Europe. Because of the environmental problems related to the use of methyl bromide, global controls have been established in numerous countries (Thomas, 1996; FAO, 1999).

Methyl bromide affects human health both directly and indirectly. EPA classified methyl bromide as a category I acute toxin. It is toxic primarily to the central nervous system and damage lungs, kidneys, eyes and skin. Workers involved in the manufacture and use of methyl bromide run the greatest risk of toxic exposure and resulting injury.Crops growing in a soil fumigated with methyl bromide contain much more inorganic bromide than those produced in an environment free from this plaguicide (Mino and Yukita, 2005). A contribution to this inorganic bromide is coming from the breakdown product of methyl bromide and brominated fumigants (Di Narda *et al.,* 2001). The presence of elevated dietary levels (above the acceptable dietary intake 1 mg kg–1 body weight/day) of bromide has been described as yielding a casual replacement of iodide by bromide during the biosynthesis of thyroid hormones, affecting the status of the thyroid gland (Mishra *et al.,* 2001; Mino

and Yukita, 2005). For these reasons, around 168 countries have agreed to gradually reduce methyl bromide production and use, and to phase out its use in agriculture by the year 2005..

Bromide quantification is of great interest in the foodstuffs for monitoring agricultural sources. Determination of low levels of bromide has traditionally been a difficult task, because of the strong dependence on the nature of the sample and interference caused by other species present in the matrix. Different methods have been reported for the determination of bromide in food: X-ray fluorescence spectrometry (Mino and Yukita, 2005), polarography (Vallon *et al.,* 1980; Di Narda *et al.,* 2003), flow injection analysis (Freeman *et al.,* 1993), ion chromatography (Miyara and Saito, 1994), inductive coupled plasma mass spectrometry (Di Narda *et al.,* 2001; Di Narda *et al.,* 2003), and gas-chromatography mass spectrometry (Mishra *et al.,* 2001). However, spectrophotometric methods have been the most use in government laboratories for quality control of bromide in water (normalized methods of analysis, 1992) because of their low cost and wide availability. Several authors (Basel *et al.,* 1982; Dobolyi, 1984; Jones, 1993; Di Narda, 2001; Di Narda, 2003) have described the use of spectrophotometry for the determination of bromide in water and mushrooms.

Validity is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data (ISO17025, 2005). Validation of analytical method is recognized as a potentially weak link in the quality chain of laboratories. The validation procedure needs to be considered the context of the fitness for the purpose and cost benefit criteria. Validation of analytical methods is the measurement of performance characteristics such as accuracy, precision, specificity, linearity and range, limits of detection and quantitation, intra-laboratory variations (robustness), and inter-laboratory variations (ruggedness)**.** In the present study, a simple, rapid, and reliable method for determination of inorganic bromide on cereals, herbs, fruits and vegetables using acidified aqueous solution of propylene oxide, with bromide being simultaneously extracted and dervatized into 1-bromopropanol-2 and 2-bromopropanol- The derivatives are partitioned into ethyl acetate before GC-ECD determination.

The aims of this validation method study were to confirm that the analytical procedure employed for a specific test is suitable for its intended use validate the analytical method for food analysis and results from the method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

MATERIALS AND METHODS

Chemical and reagents:

Potassium bromide anhydrous, Anhydrous Sodium Sulphate, and ammonium sulphate were purchased from (Riedel-deHaen) (assay 99.5%). Ethyl acetate was obtained from (LAB-SCAN) (PESTISCAN) or (HPLC).). Propylene oxide was obtained from (Fluka). H_2SO_4 (Conc. 96 %) was

purchased from (Riedel-deHaen) to prepare 6N. H_2SO_4 aqueous solution (33.3 ml of conc. H_2SO_4/ 100 ml of water).

Standard preparation:

Stock solution of 1000 µg/ml can be prepared by dissolving 149 mg of potassium bromide in 100 ml deionized water. Stock solution must be prepared every two years. Prepare appropriate dilutions of the bromide stock solution which contain 2, 10, 25, 50 and 100 µg of bromide per milliliter.

Propylene oxide solution (5%):

Fill 100 ml volumetric flask with ice cold dist. Water, then remove 5 ml of water and fill up to the volume with propylene oxide.

Note: Use ice cool distilled water for preparation of propylene oxide reagent. Propylene oxide must be kept in freezer at (-5 °C) and fresh solution made daily

Standard solution (calibration curve)

Prepare appropriate dilutions of the bromide stock solution which contain 2,10,25,50 and 100 ug of bromide per milliliter. To 1, 00 ml each of the bromide stander solution, add 9 ml of water, 10 ml of propylene oxide solution and 2 ml sulfuric acid**.** Allow to stand for 1 h at room temperature and follow the procedure, inject equal volumes of the Standerd solution (calibration curve) derivatization by propylene oxide (bromopropanol) .plot a calibration curve from the peak areas obtained for the higher one of the two bromopropanol peaks against the mass of bromide added.

Equipments:

Oven, approx. 80°C with ventilation, Conical flasks (100 ml) ,- Pipettes (50, 10 and 1 ml). Volumetric flasks (100 ml). Test tubes (10 ml) and Gas Chromatograph equipped with electron capture detector (HP5890).

Apparatus and GC analysis conditions.

GC system: HP 5890 Gas Chromatograph were equipped with electron capture detector (ECD) Hewlett Packard, California, USA..

GC conditions. Column , Agilent Technologies: HP-WAX(Column ID: 0.53 mm, Film thickness: 1.0 um , Column length: 30 m) .

Temperatures: Injector temp, 225 °C**;** Detector temp**,** 300°C**;** column oven**,** Initial temp, 80 °C **(2** min hold **) :** Rate (20°C/min) to 150 Temp (°C) .

Flow rate of nitrogen: 1.3 ml/min carrier gas, total flow (carrier + makeup): 55 ml/m Total Run Time: 21.83 min.

Sample preparation:

1g of dry sample was weighed with 10ml of water , while in the case of fresh samples 10g was weighed only into 100 ml conical flask then both were homogenized with 2ml 6N.H_2SO_4 and 10 ml Propylene oxide reagent. The dry sample and fresh sample were swirled for 1 min and then were leaved for 1h at room temperature, after that 4 g ammonium sulphate and 50 ml ethyl acetate were added and then were shaked vigorously for 1 min by hand and were let stand for 20 min (occasionally shaking), 10 ml of ethyl acetate layer was taken into 10 ml test tube, then 2 g anhydrous sodium sulphate was added, stoppered the test tube and then tube was shaked vigorously, then injected volumes of the sample test solution derived into the gas chromatograph.

Calculation of the result

Read off the mass of bromide present in the injection volume from the calibration curve .calculation the mass fraction *W* of bromide, in milligrams per kilogram, using this equation.

$W = X/ms$

X is the mass of bromide read off from the calibration curve. In micrograms;

ms is the mass of the test portion , in grams.

RESULTS AND DISCUSSION

The method validation:

The selected parameters for the verification were mainly taken from Eurachem guidelines (1998).

Limit of quantitation (LOQ):

The limit of quantitation is the minimum concentration of analyte in the test sample that can be determined with acceptable precision and recovery under the stated conditions of the test. The lowest practical limit of quantification was estimated by using repeated spiked samples at about the expected lowest level that is 2 mg/kg, on rice, majoram, and cotton samples as well as 0.2 mg/kg on potatoes sample. Recovery and relative standard deviation are shown in Table 1.

Table 1: Limit of quantitation (LOQ) of inorganic bromide in dry and fresh samples:

Matrix	Limit of quantitation (LOQ) (mg/kg)	Number of replicates (n)	Mean Recovery (%)	CV%
Rice	2	6	91	12.8
Majoram	2	6	106	8.1
Cotton	2	6	106	15.1
Potatoes	0.2	6	105	6.9

Recovery tests:

The recovery tests for inorganic bromide were made by using four types of commodities at three spiking levels. The average recoveries and relative standard deviation on each level was calculated as shown in Table 2

Table 2: Recovery tests at different concentration levels.

Matrix	Spiking level (mg/kg)	Number of replicates (n)	Mean Recovery (%)	CV%
Rice	2	6	91	12.8
	25	6	84	12.3
	50	6	94	6.8
Majoram	2	6	106	8.1
	25	6	93	11.6
	50	6	99	5.2
Cotton	2	6	106	15.1
	25	6	113	10.3
	50	6	97	7.7
Potatoes	0.2	6	105	6.9
	2.5	6	86	11.6
	5	6	96	8.6

Trueness:

The trueness of a method is an expression of how close the mean of a set of results (produced by the method) is to the true value. To check trueness of the method, spiked samples are used at different levels on tested samples. The table 2 shows accepted recovery for the different three levels. The lab also has participated in certified reference material. The results of the certified reference material are shown in the (table 3).

Table 3. Certified Reference Material Evaluation (CRM)

Compounds	Accepted range	Assigned value	found	REC%
Br	(31.28-46.92)	39.1	33.5	85.7
	(31.28-46.92)	39.1	39.19559	100.2

Measurement Uncertainty

Parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measured. The parameter may be, for example, a standard deviation (or a given multiple of it), or the width of a confidence interval.

In estimating the overall uncertainty, it may be necessary to take each source of uncertainty and treat it separately to obtain the contribution of each source.

Each of the separate contributions to uncertainty is referred to as an uncertainty component. When expressed as relative standard deviation an uncertainty component is known as relative standard uncertainty. The total uncertainty, combined standard uncertainty, equal to the positive square root of the sum of the squares of the individual uncertainty components. For most purposes in analytical chemistry, an expanded uncertainty, should be used. The expanded uncertainty provides an interval within which the value of the measured is believed to lie a higher level of confidence. Expanded uncertainty is obtained by multiplying the combined uncertainty, by a coverage factor k, for confidence level of 95% k is 2.

Relative Standard Uncertainty

The random effects were estimated as the relative standard deviation of repeated spike samples at different concentration levels.

The following equations are used for relative standard uncertainty calculations in;

$$S = \sqrt{\frac{\sum \left(x_i - \bar{x}\right)^2}{n-1}} \qquad RSd\% = \frac{S}{\bar{x}} \times 100$$

S, is the standard deviation

RSd%, relative standard deviation $\bar{x}$, the average of n samples

Precision

The precision was estimated over an extended time period 2011 and chosen to allow natural variation of all factors affecting the result. The precision was estimated using results arise from the daily analyzed control

samples; in this case the variation due to sample processing must be accounted for.

This gives a value for the relative standard uncertainty due to run to run variation of the overall analytical process. Relative standard uncertainty due to precision ($U_{recision}$) comes from spike samples was found to be 16.0 %. In this case uncertainty due to sample processing should be accounted for

Bias

The bias of the analytical procedure was investigated from recovery data using spiked samples. The recovery (90%) was observed with standard deviation s = 14.4 and n= 33

The standard uncertainty was calculated as the standard deviation of the mean

Standard Uncertainty $= \frac{s}{\sqrt{n}}$ = 2.5 % Relative Standard Uncertainty (UBias) = 2.5%

A significance test was applied to test if the recovery is significantly different from 100 %. For 32 degrees of freedom t_{tab} = 2.04 and t_{calc}=4.11 The relative standard uncertainty (2.5/90)=2.8%

In this case (since t_{calc} is greater than t_{tab}) the recovery is statistically significantly different from 100%, but in the normal application of the method no correction is applied. The uncertainty must be increased to take account of the fact that the recovery has not been corrected for.

Other sources

All balances and the important volumetric measuring devices are under regular control. Precision and recovery studies take into account the influence of the calibration of the different volumetric measuring devices because during the investigation various volumetric flasks and pipettes have been used.

The uncertainty due to reference standard preparation was estimated by accounting for reference standard purity tolerance, balance, volumetric flask and pipettes. The uncertainty component due to reference standard preparation was found to be 0.7 %.

Combined Uncertainty (U_C)

Combined uncertainty, is the positive square root of the sum of the squares of different uncertainty components, was found to be less than 16.3 %.

The following equation is used for combined uncertainty calculations

$$U_C = \sqrt{(U_p)^2 + (U_{\mathrm{Re}c})^2 + U_{\mathrm{Re}f}}$$

Expanded Uncertainty

Expanded uncertainty is obtained by multiplying the combined uncertainty, by a coverage factor k, for confidence level of 95% k is 2.
The expanded uncertainty (at 95 % confidence level) was found to be less than 34 %. The table (4) summarizes the uncertainty calculations;

Table 4. Estimation of measurement uncertainty

	1-Precision (Recovery feb-sep, 2009)		
	RSD($U_{recision}$) Br	16%	
	2-Bias (Br)		
n		33	
Mean Rec.		90%	
S		14.4%	
Standard Uncertainty		2.5%	
Relative Standard Uncertainty (Ubias)		2.8%	
Correction for recovery (t-test)			
deg. Freed.	32		
t(calc)=	4.11	Recovery is significantly different from 100%	
t(tab)=	2.04		
3-Type B			
1-Stock Solution Preparation			
Due to reference standard purity assuming rectangular distribution =			0.0058
(± 1.0 %)			
Due to volumetric flask (100ml) assuming triangle distribution =			0.0004
(100 ± 0.1 ml)			
2- Intermediate Solution Preparation			
Due to volumetric flask (50ml) assuming triangle distribution =			0.0005
(50 ± 0.06 ml)			
Due to pipette 5.0 ml assuming triangle distribution			0.0018
(5 ± 0.022 ml)			
3- Calibration Solution Preparation			
Due to volumetric flask (100ml) assuming triangle distribution =			0.0004
(100 ± 0.1 ml)			
Due to pipette 2.0 ml assuming triangle distribution			0.0020
(2 ± 0.01 ml)			
Due to pipette 1.0 ml assuming triangle distribution			0.0029
(1 ± 0.007 ml)			
Balance (210g ± 0.0006g, K=2)			0.000001
Uref =			0.7%
Combined Uncertainty (Ucom)=			16.3%
Expanded Uncertainty (Uexp)=			33%

CONCLUSIONS

This proposed method, the comminuted samples are suspended in an acidified aqueous solution of propylene oxide, The derivatives are partitioned into ethyl acetate and determined by GC-ECD without further clean up is simple, rapid and reliable. Satisfactory recoveries and repeatability were observed. The described method requires little amount of solvents and sample.

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تقدير وتقييم طريقة للبروميد غير العضوى بجهاز التحليل الكروماتوجرافى فى جميع أنواع الأغذية

على على محمود ، جودة رمضان و لمياء رياض
المعمل المركزى لتحليل متبقيات المبيدات و العناصر الثقيلة فى الأغذية
مركز البحوث الزراعية- وزارة الزراعة

تم تثبيت طريقة لتقدير البروميد غير العضوى فى الحبوب والأعشاب والفاكهة والخضر وقد تم استخلاص البروميد عن طريق محلول حمض البروبولين اوكسيد. يتم تحويل البروميد العضوى الى خليط من١- بروموـ ٢- بروبانول و ٢- بروموـ ١- بروبانول ويتم استخلاص المركب بواسطة مذيب خلات الايثيل والتقدير باستخدام جهاز الغاز الكروماتوجرافى . وتم اختبار كفاءة الطريقة عن طريق اختبار العناصر المختلفة للطريقة منها حدود التقدير الكمى للبروميد غير العضوى المختبرة وتوكيد خطية الطريقة المستخدمة. وكانت حدود التقدير الكمى هى ٢ مج/ كجم للعينات الجافة و ٠.٢ مج/ كجم للعينات الطازجة كما اثبتت ان الطريقة خطية عند التركيزات المختلفة من حدود التقدير الكمى حتى ١٠٠ مج / كجم . وقد وجد أن متوسط معدل الأسترجاع للطريقة مابين ٨٤- ١١٣٪ وكان معامل الانحراف المعيارى أقل من ١٢.٨٪ للعينات الجافة و١١.٦٪ للعينات الطازجة . وكانت قياسات اللأيقين عند مستوى ثقة ٩٥٪ مابين ± ٣٣٪.