Final Report S-2014-03653 AMi

SET UP AND VALIDATION OF A TITRIMETRIC METHOD FOR THE QUANTIFICATION OF AVAILABLE CHLORINE RESIDUES IN FRESH FOOD AND CHEMICAL ANALYSIS ON A 3% SOLUTION OF THE TEST ITEM "VIRES5" AFTER SIMULTED USE

Study program: S-2014-03653 AMi

Contract n: PPR120120160-04

Sponsor: VIRES5 Bvba
Oudaenstraat 1, 2960 Brecht
BELGIUM

Test item: VIRES5

Study Director: (Marta Lanata) Marta Lanata Released on: May 6th 2015

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development, Paris 1998.
- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE e 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).
- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments.
- Certification N. 038/2013 released by the Italian Ministry of Health on November 19th 2013 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affected the quality or integrity of the study.

Marta Lanata

Study Director
(Marta Lanata)

May 6th 2015

Date

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved study program and the Standard Operating Procedures of Eurofins Biolab Srl.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

QAU INSPECTIONS	
PHASE	**DATE**
Experimentation:	
-Audit process-based	March 02nd-09th, 2015
-Audit study-based	February 26th, 2015
Documentation:	
- Study program	February 10th, 2015
- Raw data	May 6th, 2015
- Final report	May 6th, 2015

This report accurately reflects the raw data.

Alessandra Scarabelli
QA GLP
Alessandra Scarabelli

May 6th 2015
DATE

SUMMARY

The aim of the study was to set up and validate a titrimetric method for the quantification of available chlorine residues in fresh food sample surface. The red radicchio salad has been used for this purpose.

Once developed, the method was validated according to the SANCO/3029/99 rev.4 guidelines. The following parameters were determined:

Specificity: was tested against the blank solution (MilliQ water) and the blank containg a suitable amount of red radicchio salad.

LOD/LOQ: was theoretically estimated on diluted solutions.

Linearity: was tested in 5 points corresponding to experimental LOQ (experimental), 4LOQ, 8LOQ, 15LOQ, 30LOQ, concentrations.

Precision: was performed on the same reconstitued samples (sample added to salad surface) prepared for the Accuracy test. RSD% has been calculated.

Accuracy: was performed on 10 reconstitued samples (sample added to salad surface): 5 at LOQ (experimental) and 5 at 30LOQ concentrations. Recovery % has been calculated.

The method proved to be specific, linear, precise and accurate.

The following chemical determinations on a 3% solution of the test item after simulated use (salad washing) have been also performed:

- Total chlorides
- Available chlorine

Results section reports the values obtained in detailed tables.

INTRODUCTION

On behalf of VIRES5 Bvba a study aimed to set up and validate (according to SANCO/3029/99 rev.4 guidelines) a titrimetric method for the quantification of available chlorine residues in fresh food sample surface was performed. In addition some chemical determinations on a 3% solution of the test item after simulated use (salad washing) have been also performed.

The study was performed at the Test Facility Eurofins Biolab S.r.l of Vimodrone (MI) – via B. Buozzi n. 2 (Italy).

EXPERIMENTATIONS	*START*	*END*	*RESEARCHER*
Set up and validation of a titrimetric method for the quantification of available chlorine residues in fresh food	February 11th 2015	February 17th 2015	A.Bonetti, F.Abbiati
Chemical analysis on a 3% solution of the test item after simulated use	February 26th 2015	February 27th 2015	F.Abbiati

BIBLIOGRAPHY

1) Guidance on Regulation (EU) No 528/2012 guidelines concerning the marking available on the market and use of Biocidal Product (BPR) Version 1.0 July 2013.
2) SANCO/3029/99 rev. 4: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414.

3) APAT IRSA-CNR "Metodi analitici per le acque", Volume secondo, Paragrafo 4090 "Cloruro (titolazione argentometrica, mercurimetrica e potenziometrica), Metodo B.
4) Metrohm, Application bulletin n. 249/1e "Titrimetric determination of total and residual chlorine in drinking and process water"

FILING

The study program, all raw data are filed in the archives of Eurofins Biolab S.r.l. for ten years after the issuing of the final report.
At the end of the study residual sample will be kept until November 25th, 2017, the expiry date provided by the Sponsor.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the GLP Test Facility Eurofins Biolab S.r.l.

TEST ITEM IDENTIFICATION

NAME:	VIRES 5
CODE:	V5
PRODUCT TYPE AND USE:	Biocide
STABILITY:	more than 30 months
STORAGE:	between 3-18°C, protected from light
RISK PHRASES:	R20, R21, R22, R41

COMPOSITION OF SAMPLE DECLARED BY THE SPONSOR:

Ingredient	%w/w
H_2O	99.69
NaCl	0.26
HClO+OCl	<0.05

Storage: the tested samples were stored according to the storage information until the beginning of the study.

Waste procedure: the tested samples will be discarded after the analytical tests according to local current laws.

ANALYSED SAMPLE

The test sample, representative of the test item, consists of a transparent liquid contained in a plastic bottle closed by a screw cap.

Name	VIRES 5
Batch number	23032
Manufacturing date	September 25th, 2014
Expiry date	November 25th, 2017
Receiving	EUITVI-55928
Date	November 27th, 2014
#ID	ACE-2014-00148684

The test item and the information concerning the test item were provided by the Sponsor.
All data related to the test item are under the responsibility of the Sponsor and have not been verified by the test facility.

TITRANT

A titrant solution has been prepared using the following Phenylarsine oxide. Its normality has been determined during the study (see addendum 1)

Name	Phenylarsine oxide
Supplier	Sigma-Aldrich
Code	P3075
Batch	MKBS0847V
Assay	99%

Experimentation: S-2014-03653 AM: Set up and validation of a titrimetric method for the quantification of available chlorine residues in fresh food

TEST METHOD
Titrimetric.

EXPERIMENTAL DESIGN
Parameters under investigation

Specificity

This is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present in the test item. Specificity was assessed demonstrating that blank (MilliQ water) and blank containing red radicchio salad did not consume any titrant.

Linearity

Linearity refers to the ability of a detection system to produce an acceptable correlation between the consume of titrant and the weight of the sample. The linearity of the method was assessed on 5 different amounts of the sample corresponding to experimental LOQ, 4LOQ, 8LOQ, 15LOQ, 30LOQ, concentrations. Each concentration level was prepared at least twice. The titrant volume was plotted against the sample weight. For the resulting interpolation line, parameters like correlation factor, slope, intercept and confidence limits were calculated. The confidence interval at 95% for the intercept was calculated.

Accuracy

Accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted as a conventional true value and the value found with the method applied. Accuracy was calculated by the percentage recovery of the assay obtained adding known amounts of sample to the salad surface (reconstituted sample).
Accuracy was performed on 10 reconstitued samples: 5 at experimental LOQ and 5 at 30 experimental LOQ concentrations.

Precision

Precision of an analytical procedure refers to the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment, within short intervals of time. Precision was performed on the same reconstitued samples (sample added to salad surface) prepared for the Accuracy test. RSD% has been calculated.

Acceptance criteria

Parameters	*Measurement Unit*	*Acceptability criteria*
Specificity	Titrant volume	No titrant consumed by the blank and blank containing red radicchio salad. Any interference < 30% of LOQ can be neglected.
Linearity	R	R>0.99 and/or The confidence interval at 95% for the intercept contains zero
Accuracy	% Recovery and/or Confidence range	% Recovery = 70%-110% and/or the confidence interval at 95% for the recovery contains 100%
Precision	RSD%	RSD% ≤ 20%

Reagents
- Water, MilliQ grade (produced in situ)

- Potassium Iodide (Sigma-Aldrich, code 30315, batch SZBE0920V)
- Sulfuric acid 98% (Sigma Aldrich, code 84716, batch BCBJ3985V)
- Sodium hydroxide 0.5M (Fluka, code 31950, batch SHBC8952V)

Standard
- Potassium iodate solution 1/60M (0.1N) (Sigma Aldrich, code 34273, batch SZBD2520V) (see addendum 2)

The validity of standard and reagents have been assessed before starting the analyses.
The traceability of batches used during analytical activity is assured by internal procedures.

Equipment
- Automatic titrator, Crison titromatic 1S
- Electrode Mettler Toledo DM 140-SC
- Ordinary laboratory equipment

The calibration and the status of equipment have been assessed before starting the analyses. Maintenance, calibration and/or qualification reports are available on relative logbook following internal procedures.

Analysis
In the amperometric determination of available chlorine, chlorine is titrated with a standard reducing agent such as phenylarsine oxide (PAO). During the course of titration, chlorine is reduced at the cathode to chloride (Cl^-) from the reaction with PAO. PAO is oxidized from the +3 to the +5 oxidation state at the anode:

$$PhAsO\ (PAO) + Cl_2 + 2H_2O \longrightarrow PhAsO\ (OH)_2 + 2Cl^- + 2H^+$$

As long the oxidant (chlorine) is present in the titrated sample, a current flows through the cell. When all of the oxidant is reacted, the rate of current change is zero, signaling the end point of the titration. After the end point is reached the solution cannot conduct current even though excess PAO is added. The amount of PAO used at the titration end point is proportional to the chlorine concentration in the sample.
In the case of chloramine determination, the pH is lowered to 4 and potassium iodide is added to convert the chloramine species to an equivalent amount of triiodide ion.

$$NH_2Cl + 3I^- + H_2O + H^+ \longrightarrow NH_4OH + Cl^- + I_3^-$$

$$NHCl_2 + 3I^- + H_2O + 2H^+ \longrightarrow NH_4OH + 2Cl^- + I_3^-$$

The triiodide is titrated with PAO with the current change measured amperometrically

$$PhAsO\ (PAO) + I_3^- + 2H_2O \longrightarrow PhAsO\ (OH)_2 + 3I^- + 2H^+$$

SET UP
During this phase also the specificity was evaluated.

Working solutions preparation
Blank 1
MilliQ water.

Blank 2
MilliQ water containing red radicchio salad.

Potassium iodate working solution 0.005 N
5.00 ml of Potassium iodate solution 0.1 N have been diluted to 100 ml with MilliQ water.

Sulfuric acid 1:4
200 ml of Concentrated Sulfuric acid (98%) have been carefully added to 800 ml of MilliQ water with stirring.

Phenylarsine oxide (PAO) solution (theoretical assay 0.00094 N) (double titration)
About 160 mg of Phenylarsine oxide have been exactly weighed into a 1000 ml and dissolved in a small amount of MilliQ water and 2 ml of Sodium hydroxide 0.5 M. The flask has been filled to the mark with MilliQ water and its content has been mixed.

Phenylarsine oxide solution titration: about 1.5 g of Potassium iodide have been dissolved in 100 ml of MilliQ water in a beaker. After addition of 5 ml Sulphuric acid 1:4 and 2 ml of Potassium iodate 0.005 N the solution has been placed in the dark for 5 minutes. 100 ml of MilliQ water have been added and the titration was performed with Phenylarsine oxide.

Phenylarsine oxide normality = 2 x Potassium iodate normality / Phenylarsine oxide volume (ml)

Phenylarsine oxide normality has to be determined every day, before the analytical session.

Sample solution at 3%
About 6 g of the test item have been exactly weighed into a 200 ml volumetric flask and made to volume with MilliQ water.

Titration process
200 ml of solution (blank 1, blank 2 or the sample solution at 3%) have been immediately titrated with standardized Phenylarsine oxide solution.
For the calculation:
According to Metrohm bulletin 1 ml of 0.00564 N solution corresponds to 0.2 mg Chlorine.
Using our PAO dilution value results that: 1 ml of 0.00094 N PAO solution corresponds to 0.033 mg Chlorine.

Such conditions are to be considered as the method set up starting point. It is likely to be changed during the study.

No amounts of titrant were consumed by blank1 and 2.

For this reason it was decided to proceed with method validation.

VALIDATION

Specificity

See SET UP section.

Linearity

Level 1 (about 0.00013 mg/ml Chlorine, five preparative)

About 0.2 g of test sample were exactly weighed into a 200 ml volumetric flask and brought to volume with MilliQ water.

Level 2 (about 0.0005 mg/ml Chlorine, double preparative)

About 0.8 g of test sample were exactly weighed into a 200 ml volumetric flask and brought to volume with MilliQ water.

Level 3 (about 0.001 mg/ml Chlorine, double preparative)

About 1.6 g of test sample were exactly weighed into a 200 ml volumetric flask and brought to volume with MilliQ water.

Level 4 (about 0.002 mg/ml Chlorine, double preparative)

About 3 g of test sample were exactly weighed into a 200 ml volumetric flask and brought to volume with MilliQ water.

Level 5 (about 0.004 mg/ml Chlorine, double preparative)

About 6 g of test sample were exactly weighed into a 200 ml volumetric flask and brought to volume with MilliQ water.

Titration process

Each solution have been immediately titrated with Phenylarsine oxide solution (theoretical assay 0.00094 N).

According to Metrohm bulletin 1 ml of 0.00564 N solution corresponds to 0.2 mg Chlorine.

Phenylarsine oxide normality has to be determined every day, before the analytical session.

LOD and LOQ

LOQ and LOD were estimated in the following way, from the linearity line at low concentration (level 1):

$$LOQ = \frac{10 \times SD}{b}$$

$$LOD = \frac{3 \times SD}{b}$$

Where:

b = slope of the linearity line

SD = standard deviation of five analysis on the lower linearity level (Level 1)

It was decided not to insert a sample having the theoretical LOQ concentration because the titration volume necessary to reach the end point would be too low respect to the volume added by the titrator: consequently the associated error would grow.

So it was decided to consider the concentration corresponding to Level 1 of linearity (about 0.00013 mg/ml) as experimental LOQ related to this kind of analysis.

Accuracy
Process description:
washed red radicchio salad was put in a 250 ml becker, the test sample was weighted on it. After a contact time of 1 minute 100 ml of MilliQ water have been added. The salad was then removed from the becker, raised on the becker and washed with 100 ml of MilliQ water. The resulting 200 ml of solution were then titrated.

Experimental LOQ level (about 0.00013 mg/ml Chlorine, five preparatives)
About 0.2 g of test sample exactly weighed were subjected to the process described above

Level 5 (about 0.004 mg/ml Chlorine, five preparations)
About 6 g of test sample exactly weighed were subjected to the process described above

Precision
Precision was performed on the same reconstitued samples prepared for the Accuracy test.
RSD% has been calculated.

CALCULATIONS
Calculate the content of **available Chlorine** (Cl_2) as follows:

$$\% \text{ Available chlorine (w/w)} = \left[\frac{V_{PAO} \times N_{PAO} \times 0.2}{0.00564 \times W_{Sample} \times 1000} \right] \times 100$$

$$\text{Available chlorine (mg/L)} = \frac{V_{PAO} \times N_{PAO} \times 0.2}{0.00564 \times 0.2}$$

The first formula is related to the % of available Chlorine present in the product
The second one is related to the available Chlorine in the analysed solution.

Where:
According to Metrohm bulletin 1 ml of 0.00564 N solution corresponds to 0.2 mg Chlorine.
V_{PAO} = PAO volume (ml)
N_{PAO}= PAO normality
W_{Sample} = sample weight (g)

RESULTS
Specificity
The method proved to be specific because no amount of titrant was consumed by blank1 and blank 2: the automatic titrator gave a result "out of the criteria" because it was impossible to define any equivalence point.

Linearity
The method proved to be linear. The linearity was proved on 5 different concentration levels (minimum double preparation) between solutions having a concentration of 0.00013 mg/ml (experimental LOQ) and solution having a concentration of 0.004 mg/ml (about 30 times the experimental LOQ).
All acceptance criteria were satisfied (see addendum 3).

Linearity	
R	1.0000
Slope	2.1454
Intercept	-0.0304
Confidence interval at 95% for the intercept	[-0.0174;0.0178]

LOD and LOQ

LOD and LOQ were calculated and the results are the following:

	Chlorine µg/ml
Theoretical LOD	0.01
Theoretical LOQ	0.03

Accuracy

Accuracy was calculated by the percentage recovery of the assay obtained adding known amounts of sample according to the process described in page 12.

Accuracy was performed on 10 reconstitued samples: 5 having a concentration of 0.00013 mg/ml (experimental LOQ) and 5 having a concentration of 0.004 mg/ml (about 30 times the experimental LOQ).

Accuracy				
	Recovery %	**Average Recovery %**	**95% confidence interval lower limit**	**95% confidence interval upper limit**
Reconstituted sample at experimental LOQ	82.1	88.9	82.8%	95.0%
Reconstituted sample at experimental 30 LOQ	95.7			

All acceptance criteria were satisfied (see addendum 4): the data show that the 95% confidence interval do not contain 100%, but all recovery values belong to the range 70%-110%.

Precision

Precision was performed on the same reconstitued samples prepared for the Accuracy test.

RSD% has been calculated.

Acceptance criteria were satisfied (see addendum 4).

Precision	
	RSD %
Reconstituted sample at experimental LOQ	8.89
Reconstituted sample at experimental 30 LOQ	1.57

Experimentation: S-2014-03653 AM: Chemical analysis on a 3% dilution of the test item after simulated use

TEST METHOD
Titrimetric

EXPERIMENTAL DESIGN
Parameters under investigation

A suitable amount of red radicchio salad has been washed for 1 minute immersing it in a test product solution at 3% v/v (Solution A), then the cleaned salad has been transferred into a becker containing 200ml of Milli Q water (Solution B). The solution has been shaken for 30 seconds and then the salad has been removed. The solution B was analysed.

Also absolute density of the test item has been determined in order to express the dilution as %(w/v), %(w/w) or %(v/v).

Reagents:
- Water Milli Q (produced in situ)
- Silver nitrate 0.1N (Fluka, code 35375, batch SZB02840V)

Standard
Phenylarsine oxide (PAO) (Sigma-Aldrich, code P3075, batch MKBS0847V) see addendum 1
Sodium chloride (Sigma Aldrich, code S7653, batch SZBD2910V) see addendum 2

Instruments:
- Automatic titrator, Crison titromatic
- Electrode Mettler Toledo DM 141-SC
- Electrode Mettler Toledo DM 140-SC
- Ordinary laboratory equipment

Blank 1
MilliQ water

Blank 2
MilliQ water containing red radicchio salad

Sample at 3% (v/v)
30 ml of test sample were exactly transferred into a 1000 ml volumetric flask and brought to volume with MilliQ water.

Silver nitrate 0.01N
100 ml of Silver nitrate 0.1N have been transferred to a 100 ml volumetric flask and brought to volume with MilliQ water. The title of the obtained solution has been determined using a solution of sodium chloride.

Sodium chloride solution
About 200 mg of sodium chloride have been exactly weighted in a 100 ml volumetric flask and brought to volume with MilliQ water.

Silver nitrate titration
1.5 g of sodium chloride solution have been exactly weighted ad titrated with silver nitrate 0.01N.

Procedure:

1_Total chlorides measurement has been performed potentiometrically after simulated use on red radicchio salad: the solution B has been transferred to a flask and titrated with 0.01N Silver Nitrate until the potentiometric end-point.

2_Available chlorine assay has been performed potentiometrically after simulated use on red radicchio salad: the solution B has been titrated following the validated method (named S-2014-03653 AMi MdP).

RESULTS

The following results were obtained on the red radicchio salad after washing with a 3% (v/v) solution (see addendum 5)

Parameters	Results
Total chlorides	<0.4 mg/l*
Available chlorine	<0.13 mg/l (experimental LOQ)

*0.4 mg/l is the value obtained titrating Blank 2 sample. The obtained chlorides values on solutions B are lower: however the titration volumes corresponding to potential variation are too low to be reliable. For this reason the total chlorides value is expressed as worst case considering the higher value obtained (blank 2 solution).

DEVIATION

No deviation has been recorded from study program.

CONCLUSIONS

The method described identified as S-2014-03653 AMi-MdP (see addendum 6) proved to be specific, linear, precise and accurate and was successfully validated.
The results obtained after simulated use demonstrates that the available chlorine assay is lower than experimental LOQ and that total chlorides assay is comparable to the blank value.

ADDENDA

ADDENDUM	TITLE	NUMBER OF PAGES
1	Phenylarsine oxide CoA	1
2	Potassium iodate solution 1/60M (0.1N) CoA Sodium chloride CoA	1+2
3	EXCEL SHEET LINEARITY	1
4	EXCEL SHEET ACCURACY-PRECISION	1
5	EXCEL SHEETS CHLORINE AND CHLORIDE	2
6	S-2014-03653 AMI-MDP	2

ADDENDUM 1

SIGMA-ALDRICH®

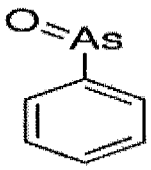
sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Phenylarsine oxide - ≥97%, powder

Product Number:	**P3075**
Batch Number:	**MKBS0847V**
Brand:	SIGMA
CAS Number:	637-03-6
MDL Number:	MFCD00001990
Formula:	C6H5AsO
Formula Weight:	168.02 g/mol
Quality Release Date:	01 AUG 2014



Test	Specification	Result
Appearance (Color)	White to Off-White	White
Appearance (Form)	Powder	Powder
Solubility (Color)	Very Faint Yellow to Yellow	Very Faint Yellow
Solubility (Turbidity) 100 mg/mL, CHCl3	Clear to Turbid	Slightly Hazy
Solubility (Color)	Very Faint Yellow to Yellow	Very Faint Yellow
Solubility (Turbidity) 50 mg/mL, DMSO	Clear to Slightly Hazy	Clear
Proton NMR Spectrum	Conforms to Structure	Conforms
Purity (TLC)	≥ 97 %	99 %

ali ataei

Ali Ataei, Manager
Quality Control
Milwaukee, WI US

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	Potassium iodate solution volumetric, KIO_3 <FRAC Type=regular>1/60</FRAC>M 0.1N
Product Number:	34273
Batch Number:	SZBD2520V
Brand:	Fluka
CAS Number:	
Formula:	KIO_3
Formula Weight:	214.00

TEST	SPECIFICATION	RESULT
factor	1.000±0.002	1.000
Production Date	09.Sep.13	
Expiry Date	30.Aug.15	
QC Release Date	27.Sep.13	

Katja Vogler

Katja Vogler
Quality Management
Seelze, Germany

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	Sodium chloride BioXtra, >= 99.5 % AT
Product Number:	S7653
Batch Number:	SZBD2910V
Brand:	Sigma-Aldrich
CAS Number:	7647-14-5
Formula:	NaCl
Formula Weight:	58.44

TEST	SPECIFICATION	RESULT
assay (argentometric)	≥ 99.5	99.6 %
pH (1 M, 20°C)	5.0-8.0	6.3
solution 1 M in H20	complying	complying
insoluble matter	complying	complying
filter test	complying	complying
aluminium (Al)	≤ 0.0005	< 0.0005 %
arsenic (As)	≤ 0.0001	< 0.0001 %
barium (Ba)	≤ 0.0005	< 0.0005 %
bismuth (Bi)	≤ 0.0005	< 0.0005 %
calcium (Ca)	≤ 0.002	< 0.002 %
cadmium (Cd)	≤ 0.0005	< 0.0005 %
chromium (Cr)	≤ 0.0005	< 0.0005 %
cobalt (Co)	≤ 0.0005	< 0.0005 %
copper (Cu)	≤ 0.0005	< 0.0005 %
iron (Fe)	≤ 0.0001	< 0.0001 %
potassium (K)	≤ 0.005	< 0.005 %
lithium (Li)	≤ 0.0005	< 0.0005 %
magnesium (Mg)	≤ 0.0005	< 0.0005 %
manganese (Mn)	≤ 0.0005	< 0.0005 %
molybdenum (Mo)	≤ 0.0005	< 0.0005 %
nickel (Ni)	≤ 0.0005	< 0.0005 %
lead (Pb)	≤ 0.0005	< 0.0005 %
strontium (Sr)	≤ 0.0005	< 0.0005 %
zinc (Zn)	≤ 0.0005	< 0.0005 %
bromide (Br)	≤ 0.01	< 0.01 %

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Certificate of Analysis

Iodide (I)	≤ 0.001	< 0.001 %
phosphate (PO4)	≤ 0.0005	< 0.0005 %
sulfate (SO4)	≤ 0.05	< 0.05 %
absorbance at 260 nm	≤ 0.01	< 0.01
absorbance 280 nm	≤ 0.01	< 0.01
Solubility (Color): colorless		
Solubility (Turbidity): clear; method: 1M at 20°C		
Production Date	18.Oct.13	
Rec. Retest Date	22.Sep.18	
QC Release Date	01.Nov.13	

Katja Vogler

Katja Vogler
Quality Management
Seelze, Germany

S-2014-03653 AM

Titer determination of the titrant (phenylarsine oxide)

KIO3 normality 0,005

phenylarsine oxide consumed (ml)	phenylarsine oxide titer (N)	Average	RSD%
5,908	0,00169	**0,00170**	1,8
6,001	0,00167		
5,786	0,00173		

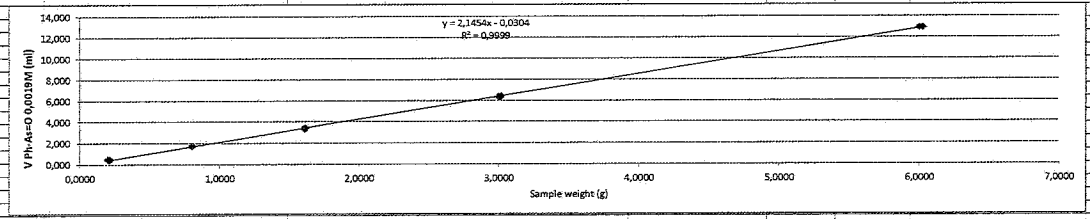
phenylarsine oxide titer (N)	correction factor
0,00170	0,30063

1ml phenylarsine oxide 0,00564N corresponds to 0,2mg Cl

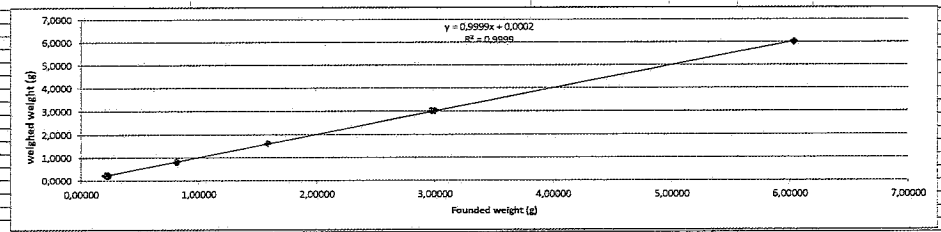
linearity	weight (g)	titrant volume (ml)	titrant volume corrected (ml)	mg Cl	% w/w on sample	ppm sol	Average	RSD%-agreement%
level 1	0,2175	0,438	0,132	0,03	0,012108	0,13	0,13	7,07
	0,2160	0,427	0,128	0,03	0,011886	0,13		
	0,2029	0,468	0,141	0,03	0,013869	0,14		
	0,2164	0,431	0,130	0,03	0,011975	0,13		
	0,2167	0,478	0,144	0,03	0,013263	0,14		
level 2	0,8070	1,727	0,519	0,10	0,012867	0,52	0,52	0,44
	0,8035	1,712	0,515	0,10	0,012811	0,51		
level 3	1,6102	3,376	1,015	0,20	0,012606	1,01	1,02	0,46
	1,6191	3,379	1,016	0,20	0,012548	1,02		
level 4	3,0209	6,406	1,926	0,39	0,012750	1,93	1,92	0,18
	3,0012	6,348	1,908	0,38	0,012718	1,91		
level 5	6,0283	12,914	3,882	0,78	0,012881	3,88	3,88	0,28
	6,0013	12,907	3,880	0,78	0,012931	3,88		
				Avg	0,012709			
				RSD%	4,18			

		0,0069	SD LOQ
Theoretical LOQ ppm	Theoretical LOD ppm		
0,032	0,010		

Straight line parameters	
Slope	2,1454
Intercept	-0,0304
R	1,0000
R^2	0,9999



Sample weight founded (g)
0,21832
0,21319
0,23230
0,21506
0,23696
0,81914
0,81215
1,58776
1,58916
3,00008
2,97305
6,03355
6,03029



Slope (a)	0,9999	0,0002	intercept (b)
dev.st (a)	0,0029	0,0080	dev.st (b)
r2	0,9999	0,0213	dev.st (y)
F	116714,9364	11,0000	df
SSreg	53,0602	0,0050	SSresid
		2,201	t (n-2 degrees of freedom)

Confidence interval of b (95%)	
L+	0,0178
L-	-0,0174

ADDENDUM 3

Visto 3 tabelle excel
13/2/15 MC

13/02/15 FA

1/3

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	S-2014-03653 AM														
2															
3															
4	Titer determination of the titrant (phenylarsine oxide)														
5															
6	KIO3 normality	0,005													
7															
8	phenylarsine oxide consumed (ml)	phenylarsine oxide titer (N)	Average	Agreement%											
9	5.741	0.00174	0.00174	0.3											
10	5.726	0.00175													
11															
12	phenylarsine oxide titer (N)		correction factor		1ml phenylarsine oxide 0.00564N corresponds to 0.2mg Cl										
13	0,00174		0,30927												
14															
15															
16	Straight line parameters														
17	Slope	2,1454													
18	Intercept	-0,0304													
19	R	1,0000													
20	R^2	0,9999													
21															
22															
23															
24	Acc-Prec	weight (g)	titrant volume (ml)	titrant volume corrected (ml)	mg Cl	% w/w on sample	ppm sol	Average	RSD%-agreement%	Sample weight founded (g)	Recovery %	Avg level	Average		
25		0,2181	0,368	0,114	0,02	0,010437	0,11			0,1857	85,1				
26		0,2192	0,371	0,115	0,02	0,010489	0,11			0,1871	85,4				
27	level LOQ	0,2066	0,294	0,091	0,02	0,008802	0,09	0,11	8,89	0,1512	73,2	82,1			
28		0,2113	0,375	0,116	0,02	0,010977	0,12			0,1860	88,4				
29		0,2121	0,322	0,100	0,02	0,009390	0,10			0,1622	77,4		86,92		
30		6,0491	12,161	3,761	0,75	0,012454	3,76			5,5626	94,1				
31		6,0254	12,455	3,852	0,77	0,012786	3,85			5,8196	96,6				
32	level 5	5,9968	12,081	3,736	0,75	0,012461	3,74	3,82	1,57	5,8453	94,1	95,7			
33		6,0266	12,530	3,875	0,78	0,012850	3,88			5,8546	97,1				
34		6,0255	12,472	3,857	0,77	0,012803	3,86			5,8275	96,7				
35											Global recovery %				
36											88,92				
37											global rsd%				
38											9,5				
39											SD		t student 95%	Interval lower limit	Interval upper limit
40											8,5		2,262	82,85	94,99
41															

ADDENDUM 4

Viste 2 tabelle excel
17/2/15 MC

17/02/15 RA

1/2

	A	B	C	D	E
1	**S-2014-03653 AM**				
2					
3					
4	Titer determination of the titrant (phenylarsine oxide)				
5					
6	KIO3 normality	0,005			
7					
8	phenylarsine oxide consumed (ml)	phenylarsine oxide titer (N)	**Average**	Agreement%	
9	5,875	0,00170	**0,00172**	2,5	
10	5,729	0,00175			
11					
12	phenylarsine oxide titer (N)		correction factor		1ml phenylarsine oxide 0,00564N corresponds to 0,2mg Cl
13	0,00172		0,30564		
14					
15	**Sample**	**weight (g)**	**titrant volume (ml)**	**titrant volume corrected (ml)**	**mg Cl**
16	**1**	200	ND	ND	<0,02
17	**2**	200	ND	ND	<0,02
18	**3**	200	ND	ND	<0,02

ADDENDUM 5 1/2

Vista 2 Tabelle excel
26/2/15 MC

26/02/15 FA
1/2

	A	B	C	D	E	F	G	H	I	J	K
1	**CHLORIDE**										
2				**Weight (g)**	**Assay % (w/w)**	**ppm**			**MW NaCl**	**MW Cl**	**absolute density**
3			**NaCl**	0,2005	99,6	1992,5			58,44	35,45	1,001
4			**H2O**	100,0251							
5											
6	**AgNO3 titration**		**Weight (g)**	**Cl mmol**	**V AgNO3 0,01 M**	**AgNO3 assay (M)**	**Avg**	**RSD %**			
7		1	1,5271	0,052	5,287	0,0098	0,0099	0,82			
8		2	1,5105	0,051	5,164	0,0100					
9		3	1,5444	0,053	5,362	0,0098					
10											
11	**Chloride titration**										
12			**sample weight (g)**	**sample volume (ml)**	**tirant volume (ml)**	**Chloride (mmol)**	**mg Chloride**	**Chloride mg/Kg**	**Chloride mg/L**	**Avg**	**RSD %**
13		1	199,9955	199,7957	0,107	0,001	0,04	0,19	0,19	0,17	7,70
14		2	199,9953	199,7955	0,095	0,001	0,03	0,17	0,17		
15		3	199,9952	199,7954	0,093	0,001	0,03	0,16	0,16		
16											
17	**BLANK + salad**		200	200	0,247	0,002	0,09	0,43	0,43		

ADDENDUM 5 2/2

Viste e tabelle excel
27/2/'15 ML

27/02/15 FA

ADDENDUM 6

TITRIMETRIC METHOD FOR THE QUANTIFICATION OF AVAILABLE CHLORINE RESIDUE IN FRESH FOOD

Aim: Analytical method for the quantification of the available chlorine residue in fresh food (red radicchio salad)

Range: Available chlorine: 0.03 mg ÷ 0.8 mg

Reagent
- Water, MilliQ grade
- Potassium Iodide
- Sulfuric acid 98%
- Sodium hydroxide 0.5M

Standard
- Potassium iodate solution 1/60M (0.1N)
- Phenylarsine oxide

Apparatus
- Automatic titrator, Crison titromatic
- Electrode Mettler Toledo DM 140-SC
- Ordinary laboratory equipment

Blank 1
MilliQ water.

Blank 2
MilliQ water containing red radicchio salad.

Potassium iodate working solution 0.005 N
5.00 ml of Potassium iodate solution 0.1 N diluted to 100 ml with MilliQ water.

Sulfuric acid 1:4
200 ml of Concentrated Sulfuric acid (98%) added to 800 ml of MilliQ water with stirring.

Phenylarsine oxide (PAO) solution (theoretical assay 0.00094 N) (double titration)
About 160 mg of Phenylarsine oxide have to be exactly weighed into a 1000 ml and dissolved in a small amount of MilliQ water and 2 ml of Sodium hydroxide 0.5 M. The flask has to be filled to the mark with MilliQ water and its content has to be mixed.

Phenylarsine oxide solution titration: about 1.5 g of Potassium iodide have to be dissolved in 100 ml of MilliQ water in a beaker. After addition of 5 ml Sulphuric acid 1:4 and 2 ml of Potassium iodate 0.005 N the solution has to be placed in the dark for 5 minutes. 100 ml of MilliQ water have to be added and the titration performed with Phenylarsine oxide.

Phenylarsine oxide normality = 2 x Potassium iodate normality / Phenylarsine oxide volume (ml)

Phenylarsine oxide normality has to be determined every day, before the analytical session.
At least two determination should be performed

Sample solution
200 ml of red radicchio salad washing solution in MilliQ water.

ADDENDUM 6

METHOD

200 ml of solution (sample solution) have to be immediately titrated with standardized Phenylarsine oxide solution.
For the calculation:
According to Metrohm bulletin 1 ml of 0.00564 N solution corresponds to 0.2 mg Chlorine.

At least three determination should be performed

CALCULATIONS

Calculate the content of **available chlorine** (Cl_2) as follows:

$$\textit{Available chlorine (mg/L)} = \frac{V_{PAO} \times N_{PAO} \times 0.2}{0.00564 \times 0.2}$$

Where:

According to Metrohm bulletin 1 ml of 0.00564 N solution corresponds to 0.2 mg Chlorine.
V_{PAO} = PAO volume (ml)
N_{PAO}= PAO normality

Method prepared and approved by:

Marta Lanata

Marta Lanata (Study Director)

Date:

February 27th 2015

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