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THE CERTIFICATION OF A REFERENCE MATERIAL FOR THE DETERMINATION OF THE ALKALI LEACHING FROM PHARMACEUTICAL GLASS CONTAINERS

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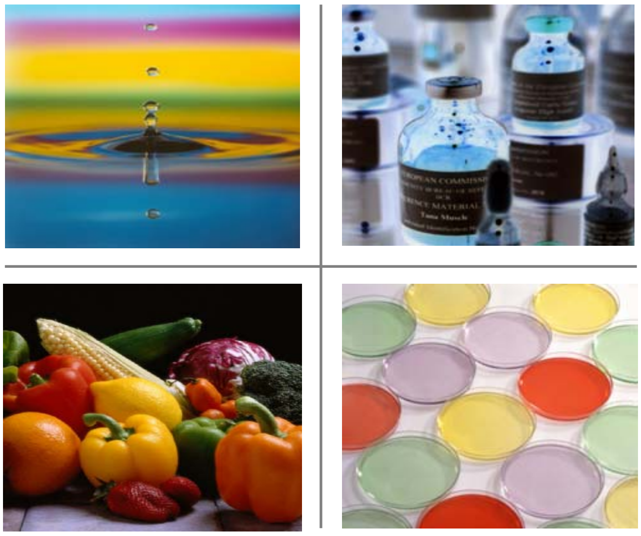
U. Wätjen, E. Guadagnino, A. Peters† and
S. Vandendriessche



Report EUR 22537 EN

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Contact information
U. Wätjen
European Commission
Directorate-General Joint Research Centre
Institute for Reference Materials and Measurements
Retieseweg 111
B-2440 Geel • Belgium

E-mail: uwe.waetjen@ec.europa.eu

Tel.: +32 (0)14 571 882
Fax: +32 (0)14 584 273

http://www.irmm.jrc.be
http://www.jrc.ec.europa.eu

Report EUR 22537 EN
ISBN 92-79-03798-6
ISSN 1018-5593
Luxembourg: Office for Official Publications of the European Communities

Printed in Belgium

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IRMM information
REFERENCE MATERIALS

THE CERTIFICATION OF A REFERENCE MATERIAL FOR THE DETERMINATION OF THE ALKALI LEACHING FROM PHARMACEUTICAL GLASS CONTAINERS

IRMM-435

U. Wätjen[1], E. Guadagnino[2], A. Peters[3]† and
S. Vandendriessche[4]

1) European Commission, DG Joint Research Centre,
Institute for Reference Materials and Measurements (IRMM),
Retieseweg 111, B-2440 Geel, Belgium

2) Stazione Sperimentale del Vetro, Venezia-Murano, Italy

3) Schott Glaswerke, Mainz, F.R. Germany

4) European Commission, DG for Research, B-1049 Brussels, Belgium

SUMMARY

This report describes the certification of the reference material IRMM-435 for the determination of the alkali release from the interior surface of glass containers for parenteral or injectable pharmaceutical solutions according to an adapted method based on the European Pharmacopoeia method and the International Standard ISO 4802.

Preliminary studies showed that an acceptable reproducibility between laboratories could only be achieved when taking some additional measures which are neither required by the European Pharmacopoeia nor the ISO method, such as:

- etching the vials prior to the autoclave procedure;
- capping the vials with closures of the design described in this report;
- measuring the temperature in the actual vials (not in the autoclave atmosphere around them) using a calibrated thermocouple;
- recording the rate of temperature rise vs. time;
- adding a spectral buffer to the extract solutions for flame spectrometric measurements;
- simulating the buffer additions to the leachate in the calibration solutions.

Consequently, a modified version of the ISO method was defined (see Annex I) and used for the subsequent certification work. When using this CRM in the laboratory for control purposes, the same modified procedure has to be applied – for the CRM only and **not** for other glass containers to be investigated - in order to establish comparable leaching (and measurement) conditions as during the certification measurements.

The reference material consists of a batch of 10,000 vials of approx. 19 mL, made of a semi-durable type of glass. The homogeneity of the batch was demonstrated. It was also demonstrated that the vials can be re-used at least 19 times, each time after applying a specific reconditioning procedure described in Annex II. The stability of a constant alkali release was verified over a period of 10 years.

Randomly selected vials were measured in 5 laboratories from 5 countries. On the basis of the results, the following alkali releases are certified (with expanded uncertainties U_{CRM} with a coverage factor $k = 2$, corresponding to a level of confidence of about 95 %).

Certified volume of titration solution per volume of leachate:

(0.38 ± 0.04) mL of 0.01 mol/L HCl per 50 mL of leachate.

The certified sodium release is:

[Na^+]: (1.41 ± 0.14) mg/L

which is equivalent to

(1.91 ± 0.19) mg of dissolved Na_2O per L of leachate.

Each unit of IRMM-435 consists of 20 vials with screw caps and includes details of the testing procedure applied for the certification measurements.

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GLOSSARY

Throughout the report the following abbreviations are used:

AAS	atomic absorption spectrometry
AES	atomic emission spectrometry
CRM	certified reference material
ECU	European currency unit
GUM	Guide to the Expression of Uncertainty in Measurement [1]
ISO	International Organization for Standardization
SI	Système International d'Unités, International System of Units
k	coverage factor according to GUM
s	standard deviation
s_{rel}	coefficient of variation (relative standard deviation)
s_{bb}	between-samples homogeneity (standard deviation)
s_{reus}	uncertainty in reusability (standard deviation)
$u_{(index)}$	combined standard uncertainty according to GUM
U	expanded uncertainty according to GUM
$\bar{x}$	mean value

1. INTRODUCTION

1.1 Need for a certified reference material

1.1.1 Technical background

Borosilicate glass has been and is, because of its inert surface, the most frequently used material for containers of pharmaceutical injectables. The only observed interaction between the glass and the contained liquid is the release of traces of alkaline substances (mainly NaOH) from the glass surface.
These traces are harmless from the health point of view, but many pharmaceuticals are at the same time dissolved in pure (unbuffered) water and very sensitive to the pH, so that these traces are sufficient to affect the properties of pharmaceuticals through a slight increase in pH. Therefore, the pharmacopoeias of most countries set limit values to the release of alkaline substances during a specified temperature cycling.

The method prescribed by both the European Pharmacopoeia [2] and by ISO 4802 [3] can be summarised as follows:

- fill the container with distilled water (ISO grade 2, i.e. in equilibrium with atmospheric CO_2, pH 5.5);

- heat to 100 °C; maintain this temperature for 10 minutes; heat to 121 °C; maintain this temperature for 1 hour; cool;

- determine either the OH^- content of the test water by titrating back to pH 5.5 (European Pharmacopoeia or ISO 4802 Part 1), or the Na content by atomic emission or absorption spectrometry (ISO 4802 Part 2).

The AES/AAS method allows testing of individual containers; the titration method requires the test water of several containers to be pooled to arrive at measurable quantities of OH^-.
The temperature cycling is performed in an autoclave and is usually referred to as the "autoclaving" step in the procedure.

The reproducibility of the test method is very poor for several reasons, which have been identified in the research project preceding the certification of the reference material described in this report (see chapter 3). Some of these reasons can be avoided by additional requirements for the autoclaving procedure, others require strict quality control supported by a certified reference material.

1.1.2 Economical background

The number of laboratories in Europe performing the test procedures routinely was estimated around 1990 at 150. The number of disputes in trade between manufacturers (glass industry) and users (pharmaceutical industry) is in fact very low because of the very strict batch rejection policy followed by the glass industry: batches are often discarded because the measurements do not convincingly show that the alkali release is below the agreed limits. However, it is believed that, in the large majority of the cases, the glass is in fact good, but the conclusions from the measurements are wrong because the method is so unreliable. The corresponding losses were estimated around 1990 at 50,000 ECU/year in Italy and at 80,000 ECU/year in Germany (1 ECU is nominally about 1 Euro).

The European glass industry has therefore proposed the certification of a reference material to improve the reliability of the measurements and hence to avoid the discarding of good glass containers.

1.2 Choice of the material

The alkali release rates, which are achieved with the highly resistant borosilicate glass used for most pharmaceutical purposes, are near the detection limit of the titration method. For the production of IRMM-435, preference was given to a type of glass of which the release rate is sufficiently high to allow certification using the titration method. Therefore, a semi-durable type of glass was selected, which is also used for pharmaceutical purposes.

The vials were produced from thick-walled tubing to enable repeated use after regeneration of the surface by etching with a hydrofluoric acid solution.

High vials were preferred in order to facilitate the autoclaving procedure.

1.3 The certification procedure

As it is not possible to prepare a batch of vials with a-priori known alkali release rates, the certification had to be done on the basis of measurements. Furthermore, the certified values of this reference material are operationally defined through the autoclaving procedure, to be followed during the leaching experiment.

Thus, the principle of certification applied in this case is to test samples of the candidate reference material in several independent laboratories, each of them adhering strictly to the defined autoclaving method, but preparing their own measurement calibrants traceable to the SI unit of amount of substance. This approach leads to a very small probability that all labs would make the same systematic errors. If then their results agree to within experimental uncertainty, the common result is considered accurate to within an uncertainty which can be estimated following the principles of the GUM [1].

The project consisted of the following steps:

1) The feasibility study

   Before starting the work on the batch of material, intended to serve as certified reference material, it had to be checked if the participating laboratories would achieve the level of experimental agreement required for a certification.

   This has actually been the longest part of the project, as several intercomparisons were necessary to achieve the required level of experimental agreement, and some parameters which were not fixed in the standard method had to be specified in a modified method.

The conclusions from this work, taking place from 1987 to 1991, are summarised in chapter 3.

2) The testing of the material

   - Homogeneity test: it was checked (by testing a representative number of units) whether all units of the CRM have the same alkali release rate (July 1992).

   - Reusability test: it was checked that the release rate was the same after repeated etching of the glass surface (July 1992).

   - Stability test: it was checked that - under the storage conditions applied - the release rate does not change significantly over the time scale in which the CRM is likely to be used. Beyond the formal demonstration of stability, obtained with tests carried out at Schott Glaswerke from 1992 to 1996, further evidence is given through verification measurements at Institut National du Verre between 1996 and 2002. Finally, the comparison of the certified values based on the certification round of 2002/03 with the results of the initial measurement round of 1992/93 can be considered as an extended stability study.

3) The certification measurements

   The autoclaving procedure (see Annex I) was defined in full detail (including details which are not specified in ISO 4802) and was strictly followed by all participants. On each of three different days, the complete testing procedure consisting of cleaning, etching and autoclaving was carried out.

   For the titration and AAS, each lab used its own well established procedure for which it performed adequately in terms of trueness (no significant bias) and of reproducibility (standard deviation consistent and explicable on the basis of random experimental uncertainties). The flame spectrometers were calibrated with gravimetrically prepared standards in order to ensure traceability of measurement results to the SI.

   A previous measurement campaign in 1992/93 was successfully concluded in technical-scientific terms, but nevertheless did not lead to a formal certification of the material. Since some of the basic certification principles have changed since then (especially on the requirements for traceability of measurement results, new ISO Guide 34 [4] and revised ISO Guide 35 [5]), a new certification measurement round was conducted in 2002/2003.

4) Formal certification

   Satisfactory consistency of results was achieved, such that the **certified values** with their **uncertainties** could be determined on the basis of a sound technical and statistical evaluation of the data.

1.4 Use of the reference material

The procedures for testing the quality of the inner surface of glass containers are highly complex, the prescriptions in the European Pharmacopoeia as well as in ISO 4802 are not sufficiently clear enough, and small deviations in the handling can yield larger deviations in the results. National and international collaborative work with the mentioned procedures proved that the between-laboratory comparison (reproducibility) is often not satisfying, although the within-laboratory repeatability can be reasonably good.

This certified reference material, when correctly used strictly following the autoclaving procedure documented in Annex I, allows

- to control the laboratory manipulations,
- to check the used apparatus, especially the whole autoclaving process , and
- to control the analytical technique used for the alkali determination.

The user should bear in mind that the property values of this reference material are operationally defined; the certified, constant alkali release is only obtained when especially the autoclaving procedure is strictly followed in order to establish comparable leaching (and measurement) conditions as during the certification measurements. Instructions for use are given in chapter 8.

Note – The procedure for use of this CRM, especially the pre-etching step (see Annex II), shall not be used as routine procedure for testing other glass containers for their alkali leaching properties.

2. PARTICIPANTS

2.1 Production of the material

The vials were produced by Glaskontor, Müllheim, F.R. Germany, from glass tubing supplied by Schott Rohrglas, Mitterteich, F.R. Germany.

2.2 Testing of the material

The homogeneity, reusability and stability testing were performed by Schott Glaswerke, Mainz, F.R.Germany.

2.3 Laboratories participating in the initial measurement campaign

The following laboratories participated in the preliminary studies leading to a previous attempt of certification (the name of the person representing each lab at the technical evaluation meeting is given in brackets):

- BSN - Centre de Recherche, Givors, France
- British Glass Manufacturers Confederation, Sheffield, United Kingdom (Mrs. P.V. Tingle)
- Forschungsgemeinschaft für technisches Glas, Wertheim, F.R.Germany (Dr. H.H. Fahrenkrog)
- Glasforskningsinstitutet, Växjö, Sweden (Dr. P. Sundberg)
- Glaskontor, Müllheim, F.R.Germany (Dr. H. Thoma)
- Institut National du Verre, Charleroi, Belgium (Dr. J. Simons)
- Kimble Italiana, Pisa, Italy (Dr. A. Cerino)
- Schott Glaswerke, Mainz, F.R.Germany (Dr. W. Heimerl)
- Schott Rohrglas, Mitterteich, F.R.Germany (Dr. P. Müller)
- Stazione Sperimentale del Vetro, Venezia-Murano, Italy (Dr. E. Guadagnino)
- Turkish Glass Research Centre, ŞIŞECAM, Istanbul, Turkey (Mr. O. Çorumluoğlu)
- Vetreria Parmense Bormioli Rocco, Parma, Italy (Dr. A. Biavati)

2.4 Laboratories participating in the final certification campaign

The following laboratories participated in the definitive certification campaign (the name of the person representing each lab at the certification meeting is given in brackets):

- Glasforskningsinstitutet (Glafo), Växjö, Sweden (Dr. P. Sundberg)
- Institut National Interuniversitaire des Silicates, Sols et Matériaux (INISMa), Mons, Belgium (Dr. J. Simons)
- Schott Glas, Mainz, F.R.Germany (Mrs. C. Strubel)
- Stazione Sperimentale del Vetro, Venezia-Murano, Italy (Dr. E. Guadagnino)
- Turkish Glass Research Centre, ŞIŞECAM, Istanbul, Turkey (Mr. O. Çorumluoğlu)

2.5 Coordination and management

The project was originally coordinated and managed jointly by:

- Dr. A. Peters, Schott Glaswerke, Mainz, F.R. Germany
- Dr. S. Vandendriessche, European Commission, Community Bureau of Reference

(BCR), Brussels, Belgium.

In its later phase leading to certification, it was coordinated by:

- Dr. E. Guadagnino, Stazione Sperimentale del Vetro, Venezia-Murano, Italy
- Dr. U. Wätjen, European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium

3. PRELIMINARY STUDIES

Several collaborative research projects - each one followed by a technical discussion meeting - were organised in the period from 1987 to 1991 to study the causes of the poor reproducibility between laboratories when applying the ISO method. The following causes were identified, and the following conclusions were drawn for the certification exercise:

- The main requirement for reference containers is that the inner surface is so homogeneous that the alkali release is reproducible. This is not necessarily the case for an original container surface as delivered, which may be affected by flame fluctuations during forming or by random interaction with water molecules from the ambient air after annealing rendering the containers slightly different from one another. Etching the surface with hydrofluoric acid produces a new surface corresponding to the chemical durability of the bulk, which is known to be homogeneous, and thus should yield identical leaching rate during autoclaving.
  Etching the vials prior to the autoclave procedure was therefore required.

- The autoclaves used in the studies were not equipped with calibrated thermometers, and errors up to 3 °C were detected in the participating laboratories. On the other hand, it was shown that small differences in temperature result in large differences in alkali release (e.g. ± 10 % release for ± 1 °C).
  The use of calibrated thermometers was therefore required.

- A heating rate of 1 °C/min from 100 °C up to 121 °C is prescribed by the standard methods in use, but laboratories do not follow rigorously this prescription on a routine basis. As variations in the heating rate may exert a not negligible influence, a careful recording of the entire heating cycle was therefore required in the modified protocol.
  (In order to decrease one factor contributing to variability also in routine measurements, it is recommended to rigorously control the heating rate in routine applications as well.)

- The temperature as measured in the actual vials was not always the same as that in the autoclave atmosphere around them, and did in fact not follow the temperature programme prescribed in the test procedure.
  The temperature was therefore measured (with a calibrated thermocouple) in the actual vials and was continuously recorded so as to prove that the test procedure was strictly applied (see Annex III).

- The autoclaves used in these studies could not be programmed to carry out the heating cycles prescribed in the pharmacopoeia, ISO 4802 or the modified protocol. Therefore, manual operation was required, often leading to poor application of the standard procedure.
  The autoclave cycle was therefore recorded as a temperature-time diagramme and carefully monitored in operation.

- Loosely capped containers, using Al foil for example, will evaporate some of their volume during the autoclave process. Measurements showed this loss may exceed 5 % depending on the opening width of the containers. Alternatively, the use of screw caps foreseen with a hole of 0.3 mm diameter, as developed in one of the feasibility studies, led to a volume loss of less than 1 %.

Whereas evaporation losses need to be accounted for when testing unknown containers for hydrolytic resistance, the loss of water during autoclaving is negligible, when using the reference containers with their perforated screw caps. Therefore, no evaporation corrections had to be applied in the certification exercise.

- The material of the screw caps must be inert and heat resistant. A material withstanding the autoclave temperatures up to 121 °C without deformation and not releasing alkali during the process was found. Traces of diluted acids, however, can be absorbed by the stopper material and later be released into the extraction water during autoclaving, leading to interference during the titration.

  Therefore, the closures must only be subjected to a mild cleaning process, even after drilling the venting holes.

- When measuring extraction solutions containing also other ions but alkali, spectral interferences and strong ionisation may occur. This can be overcome by acidifying the leachate with a spectrochemical buffer solution. Since the sodium signal in flame spectrometry is dependent on the ionisation degree and the pH value, an equivalent amount of buffer must be added to the reference solutions.

  Cesium chloride dissolved in hydrochloric acid was used as a spectral buffer. This buffer was added to the measuring solution (leachate) as well as the reference solutions used to establish calibration curves of the flame spectrometer. The dilution due to adding the buffer solutions had to be corrected for.

- Finally, the reproducibility of the titration method is poor because the person-to-person variation in endpoint detection (observation of the colour change) is considerable in comparison with the quantities of OH^- being determined. This uncertainty, however, cannot be reduced by a stricter protocol. In principle, results will differ by at least one drop of the titration acid, which corresponds to about 0.025 mL.

As result of the collaborative studies complete testing protocols (see Annexes I-III) were established.

4. PRODUCTION OF THE MATERIAL

4.1 Starting material

The starting material was commercial brown Illax glass tubing of (25.00 ± 0.25) mm diameter and (1.200 ± 0.005) mm wall thickness, supplied by Schott Rohrglas, Mitterteich, F.R. Germany.

4.2 Production of the vials

The certified reference material consists of a continuous batch of 10,000 vials produced from the above tubing in the normal production line of Glaskontor, Müllheim, F.R. Germany on 30 April 1992.

The height of the vials is (58.0 ± 0.5) mm. The average "brimful capacity" (= volume as determined by filling with water at (22 ± 2) °C) of the vials is 18.9 mL; the average "filling volume" (defined as 90 % of the "brimful capacity") is 17.0 mL.

During the production of the 10,000 vials, 50 vials were taken at regular intervals (one every 200) and set aside for the homogeneity, stability and reusability tests. These were numbered from 1 to 50 in the sequence of production.

4.3 Packaging

The produced vials were closed with the screw caps, selected in one of the preliminary studies, and immediately packed in parcels, each making up a unit of the future reference material. Each unit of IRMM-435 contains 20 vials. About 470 units of IRMM-435 remain available for sale.

5. TESTING OF THE MATERIAL

5.1 Homogeneity

In principle, glass from a single batch of production is one of the most homogeneous materials feasible. Even a very small inhomogeneity in the glass would interrupt the automatic production line for the vials running normally at a speed of 3 m/s. Therefore, the mere fact that the vials were obtained without technical difficulties is in fact an indication that the homogeneity of the batch is sufficient for all practical purposes. Nevertheless, modern certification principles require that the equality of the reference containers in their relevant properties (here the Na release) is demonstrated (homogeneity of the CRM).

50 vials, which were set aside for the homogeneity test as a representative sample of the whole batch of 10,000 vials (see chapter 4.2), were numbered by external engraving. They were submitted 3 times to the etching procedure described in Annex II, and then to the autoclaving procedure described in Annex I. The Na content of the resulting leachates was determined by AAS (10 of the vials in this first run had turned over during autoclaving and could not be measured). In a second run, 40 of the vials were again submitted first to the etching procedure (2 times), then the autoclaving procedure, and the Na content of the resulting leachate was determined by AAS again (run 2 performed on a different day). The results, expressed as mg/L Na_2O release and corrected for the 10 % dilution due to adding the buffer solution, are listed in Table 1 and Fig. 1 together with the raw data (Table 1 only). In conclusion, the coefficient of variation ("relative standard deviation") of the mean of the measurement results of each run is < 7 % and < 10 %, respectively, apparently largely determined by the reproducibility of leaching (cf. raw data of Table 1 with respect to an individual container). Since, however, the instructions for use (chapter 8) specify a minimum number of 9 or 10 vials for each test with titration or flame spectrometry, respectively, the coefficient of variation for individual vials must be divided by the square root of the number of vials in a test set. Within these limits, the

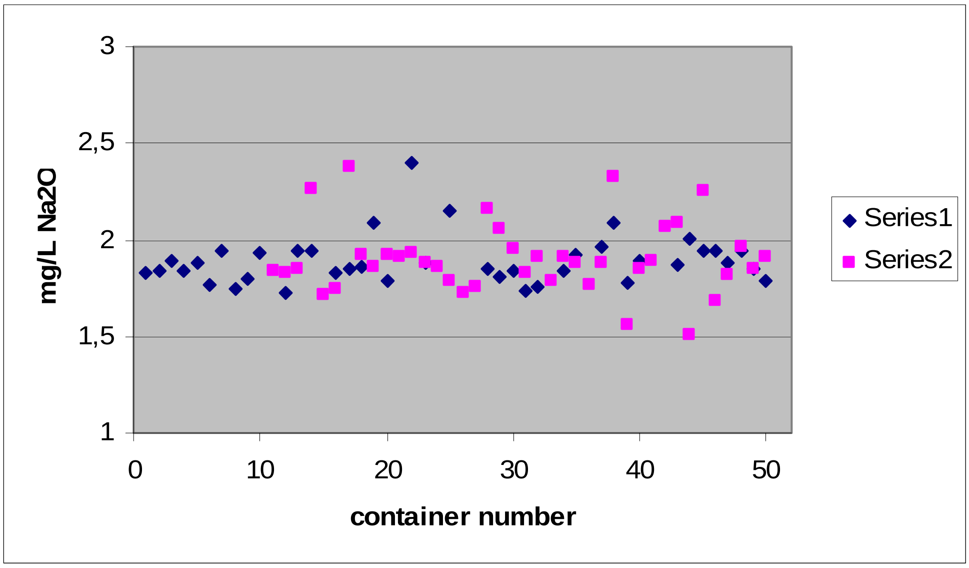


Fig. 1: Homogeneity measurements expressed as mg/L Na_2O release.

Table 1: Sodium release into extraction water (expressed as mg/L Na and $Na_2O^{dil\text{-}corr}$) measured with flame spectrometry after two autoclave runs on different days. Run 1: container # 1-10 measured 3x, rest 2x. Run 2: container # 11-19 measured 3x, rest 2x.

container number	single values mg/L Na			mean / mg/L Na	mean / mg/L Na_2O	single values mg/L Na			mean / mg/L Na	mean / mg/L Na_2O
1	1,21	1,24	1,25	1,23	1,83					
2	1,22	1,25	1,25	1,24	1,84					
3	1,26	1,28	1,29	1,28	1,89					
4	1,22	1,25	1,25	1,24	1,84					
5	1,25	1,27	1,28	1,27	1,88					
6	1,18	1,20	1,20	1,19	1,77					
7	1,28	1,33	1,31	1,31	1,94					
8	1,17	1,19	1,19	1,18	1,75					
9	1,21	1,22	1,22	1,22	1,80					
10	1,28	1,32	1,31	1,30	1,93					
11						1,24	1,23	1,25	1,24	1,84
12	1,16	1,18		1,17	1,73	1,23	1,24	1,24	1,24	1,83
13	1,31	1,31		1,31	1,94	1,25	1,25	1,25	1,25	1,85
14	1,30	1,32		1,31	1,94	1,51	1,52	1,54	1,52	2,26
15						1,15	1,15	1,17	1,16	1,72
16	1,23	1,24		1,24	1,83	1,19	1,17	1,19	1,18	1,75
17	1,25	1,24		1,25	1,85	1,59	1,60	1,62	1,60	2,38
18	1,25	1,26		1,26	1,86	1,30	1,33	1,25	1,29	1,92
19	1,41	1,41		1,41	2,09	1,23	1,24	1,30	1,26	1,86
20	1,21	1,21		1,21	1,79	1,29	1,30		1,30	1,92
21						1,29	1,28		1,29	1,91
22	1,62	1,62		1,62	2,40	1,30	1,30		1,30	1,93
23	1,27	1,26		1,27	1,88	1,29	1,25		1,27	1,88
24						1,26	1,25		1,26	1,86
25	1,45	1,45		1,45	2,15	1,21	1,20		1,21	1,79
26						1,17	1,17		1,17	1,73
27						1,19	1,18		1,19	1,76
28	1,24	1,25		1,25	1,85	1,47	1,45		1,46	2,16
29	1,22	1,22		1,22	1,81	1,40	1,38		1,39	2,06
30	1,24	1,24		1,24	1,84	1,32	1,31		1,32	1,95
31	1,16	1,19		1,18	1,74	1,23	1,24		1,24	1,83
32	1,19	1,18		1,19	1,76	1,28	1,29		1,29	1,91
33						1,21	1,21		1,21	1,79
34	1,24	1,24		1,24	1,84	1,29	1,28		1,29	1,91
35	1,29	1,30		1,30	1,92	1,27	1,26		1,27	1,88
36						1,20	1,19		1,20	1,77
37	1,32	1,33		1,33	1,96	1,26	1,27		1,27	1,88
38	1,40	1,42		1,41	2,09	1,57	1,57		1,57	2,33
39	1,20	1,20		1,20	1,78	1,06	1,05		1,06	1,56
40	1,28	1,27		1,28	1,89	1,25	1,25		1,25	1,85
41						1,28	1,27		1,28	1,89
42						1,40	1,39		1,40	2,07
43	1,26	1,26		1,26	1,87	1,41	1,41		1,41	2,09
44	1,35	1,36		1,36	2,01	1,02	1,02		1,02	1,51
45	1,31	1,30		1,31	1,94	1,52	1,52		1,52	2,25
46	1,30	1,31		1,31	1,94	1,14	1,13		1,14	1,68
47	1,26	1,27		1,27	1,88	1,23	1,23		1,23	1,82
48	1,30	1,31		1,31	1,94	1,32	1,32		1,32	1,96
49	1,23	1,26		1,25	1,85	1,25	1,24		1,25	1,85
50	1,21	1,20		1,21	1,79	1,29	1,29		1,29	1,91
mean					1,89					1,90
s					0,13					0,18
s_{rel} / %					6,6					9,7

estimate of inhomogeneity s_{bb} of the test sets (comprised of minimum 9 vials) with respect to leaching is found to be between 2.2 % and 3.2 %.

In conclusion, s_{bb} = 3.2 % (the larger of the two values) is adopted as uncertainty contribution for the inhomogeneity of leaching from the container walls between test sets containing minimum 9 vials.

5.2 Reusability

Leaching chemically alters the surface of glass containers. It is known that repeated etching yields every time a new surface comparable to the one existing before each leaching process, thus rendering alkali release essentially identical.

10 of the 50 containers used for the homogeneity test were submitted a total of 19 times to the regeneration procedure described in Annex II. On five occasions (after etching 3x, 5x, 9x, 14x and 19x) the containers were autoclaved following the procedure of Annex I, and the resulting sodium release determined by flame spectrometry (Table 2).

No technical reason could be found for the statistically outlying value of 2.33, therefore it was not discarded. The spectrometric measurements after etching 9 times, however, had to be discarded because of malfunctioning of the AAS spectrometer. The results are listed in Table 2 and Fig. 2, demonstrating that the sodium releases after 19 times etching are almost unchanged: less than 3 % deviation of the mean compared to the first measurement cycle, i.e. at the low end of the 2 % to 10 % range of the coefficients of variation for each measurement round. The relative standard deviation of the four mean values of each measurement cycle is 1.6 %.

Table 2: Reusability measurements of 10 containers, submitted to repeated etching. Sodium release after autoclaving measured with AAS, expressed as mg/L Na_2O (corrected for dilution with spectral buffer).

Container number	After etching for number of times			
	3 x	5 x	14 x	19 x
31	1,74	1,83	1,84	2,02
32	1,76	1,91	1,79	1,83
33		1,79	1,91	1,87
34	1,84	1,91	1,81	1,84
35	1,92	1,88	1,8	1,79
36		1,77	1,82	1,79
37	1,96	1,88	1,75	1,79
38	2,09	2,33	1,82	1,78
39	1,78	1,56	1,82	1,76
40	1,89	1,85	1,84	1,77
mean	1,87	1,87	1,82	1,82
s	0,12	0,19	0,04	0,08
s_{rel} / %	6,3	10	2,3	4,2

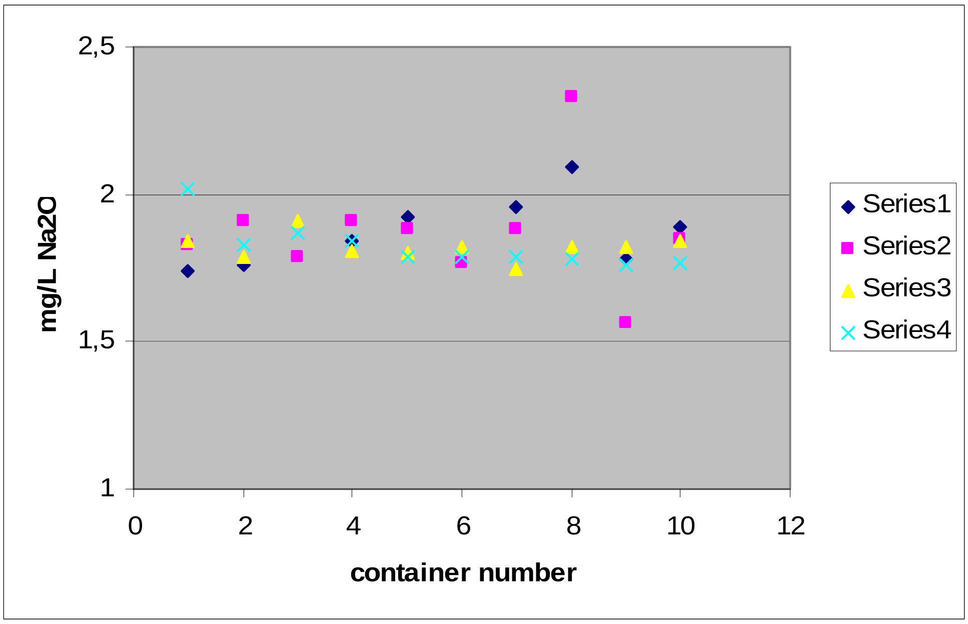


Fig. 2: Reusability measurements expressed as mg/L Na_2O release.

It is concluded that the containers – after removing the attacked surface by etching - can be reused at least 19 times as reference material with the certified property values of sodium and total alkali release. The uncertainty contribution for reusability is s_{reus} = 1.6 %.

5.3 Stability

The regeneration of a fresh surface – with the etching procedure of Annex II - prior to each leaching experiment will ensure the stability of the glass containers with respect to leaching rate over all practical time scales of storage. This is confirmed with the experiments described in the following.

5.3.1 Short and long term stability study

10 containers each were stored

- in “normal” atmosphere (office room temperature and humidity, all containers loosely covered by a common foil of cell wool)
- in humid atmosphere (water vapour saturated atmosphere, over water at ambient temperature in a big glass beaker)

and the sodium release determined with flame spectrometry after storage times of 1, 3, 9, 20 and 37 months, respectively. Results (**not** corrected for dilution with buffer solution) are given in Table 3 and Fig. 3. Since the stability test was not designed as isochronous study (i.e. Na_2O release from all storage periods would have been determined at the same time under repeatability conditions), the reproducibility of measurements taking place between August 1992 and September 1996 is not negligible. Nevertheless, it can be deduced from this study that within the limits of measurement reproducibility the sodium release rate from the reference glass containers is constant and independent of storage time. The coefficient of variation of mean values from all 10 measurement series is 3.2 %.

Table 3: Stability measurements, sodium release after autoclaving determined with AAS, expressed as mg/L Na_2O (**not** corrected for dilution with spectral buffer). 10 containers stored and measured in each period.

storage time	dry storage		wet storage	
months	mean	*s*	mean	*s*
1	1,76	0,02	1,81	0,02
3	1,63	0,05	1,72	0,02
9	1,74	0,03	1,66	0,02
20	1,71	0,04	1,71	0,04
37	1,67	0,04	1,65	0,03

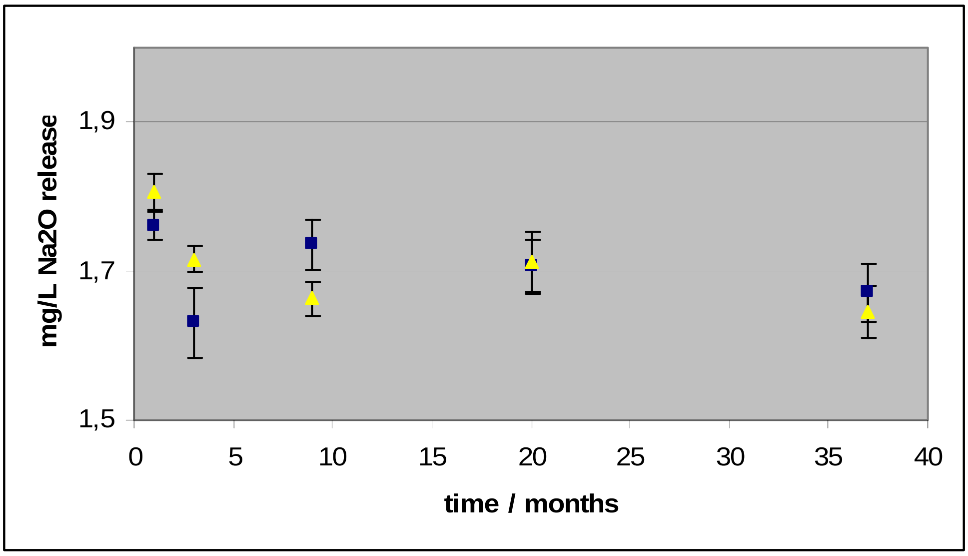


Fig. 3: Results of stability measurements, squares indicate dry storage, triangles wet storage.

5.3.2 Stability of alkali release during six years

Additional proof of stability of alkali release from the container surfaces is obtained from routine measurements performed at Institut National du Verre , Charleroi, Belgium between 1996 and 2002 [6]. These measurements are performed with an original unit of IRMM-435 and serve at INV to verify the correct operation of the autoclave procedure and alkali determination through titration. Table 4 presents the results given as titration volume of 0.01 mol/L HCl per 50 mL of extraction water. The mean value of 6 measurement series during 6 years is 0.407 mL, its standard deviation 0.012 mL and the coefficient of variation 2.9 %.

Table 4: Titration results for the leachates of a unit of IRMM-435 obtained during 6 years [6]. Given as volume of 0.01 mol/L HCl per 50 mL of extraction water.

time of measurement	26.06. 1996	20.08. 1998	14.01. 2000	08.09. 2000	28.06. 2001	09.07. 2002
volume/mL	0.42	0.415	0.413	0.39; 0.39	0.39; 0.40	0.41
s	0.02	0.013	0.011	0.01	0.01	0.01
mean (*n*=6)						0.407
s						0.012
s_{rel} / %						2.9

5.3.3 Comparison of certification measurements of 2003 with measurements of 1993

Proof of stability over a period of 10 years is finally obtained from a comparison of the certified values (see chapter 6) with the results of previous measurements performed in 1992/93 (see Annex IV, Tables 9 and 10).

Titration: the mean value and its standard deviation of 8 laboratory results in 1992/93 (after withdrawal of laboratory 11 and rejection of results from laboratory 12 for not strictly following the autoclaving procedure) was (0.40 ± 0.05) mL of 0.01 mol/L HCl per 50 mL of leachate. The certified value with its standard deviation of laboratory means (0.382 ± 0.014) mL per 50 mL of leachate is well within the rather large uncertainty of the old mean value.

Flame spectrometry: the mean value of 9 laboratory results (after withdrawal/rejection of those of laboratories 11 and 12) in 1992/93 was (1.94 ± 0.11) mg Na_2O per L leachate (now **corrected for dilution** with spectral buffer), the certified value with its standard deviation is (1.91 ± 0.09) mg/L Na_2O.

It can be concluded from a comparison of all experiments (chapters 5.3.1 to 5.3.3) used to demonstrate the short- and long-term stability that the reproducibility of the measurements is the limiting contribution independent of time of storage. It is, therefore, justified to adopt the method with the smaller standard deviation of laboratory means (flame spectrometry) in order to determine the uncertainty u_{lts} for long-term stability (time base of 10 years) from the combined uncertainties of the means in 1992/93 and 2002/2003:

$$\text{(relative) } u_{lts} = \sqrt{\left(\frac{s}{\sqrt{n}}\right)_{92}^{2} + \left(\frac{s}{\sqrt{n}}\right)_{02}^{2}} = 2.8\ \%.$$

This value is a conservative estimate, seen that it is considerably higher than the actual difference of mean values in 2002/2003 and 1992/93 for flame spectrometry (relative 1.6 %).

6. CERTIFICATION MEASUREMENTS

6.1 Analytical methods used

Each lab submitted the 20 vials from one set of IRMM-435 to the autoclaving procedure described in Annex I. One of the vials contained a calibrated thermocouple and was not used for analysis. Of the other 19 vials:

- the leachates of 5 were combined into a flask and the leachates of 4 were combined into another flask, and these 2 flasks were used for separate titrations;
- the remaining 10 vials were used for Na determination by AAS.

The above was done three times on different days, so that each laboratory obtained 6 results with the titration method and 30 with AAS.

The procedure for titration was the following:
50.0 mL of leachate are pipetted into a recipient, 4 drops of methyl red indicator solution are added. This solution is titrated with 0.01 mol/L HCl solution until the colour matches that of a solution obtained by adding the same amount of methyl red solution to 50 mL of water from the same (ISO grade 2) source as used for filling the vials prior to the autoclaving. The exact titre of the HCl solution was determined by titrating it with sodium hydroxide, which in turn was standardised against potassium hydrogen phthalate.

The procedure for AAS was as follows:
1.7 mL of Cs solution (spectral buffer) are added to each vial. The vials are swirled to mix the solution, which is then directly aspirated into the nebuliser of the flame spectrometer which is calibrated using gravimetrically prepared Na solutions containing the same concentration of Cs.

6.2 Presentation of the results

The individual results of the titration and the flame spectrometry method, respectively, are presented in Tables 5 and 6 for each laboratory together with its mean value and standard deviation. In order to avoid errors in the conversion of a large number of data, the data are reported here in the units which were used by the participants, i.e.

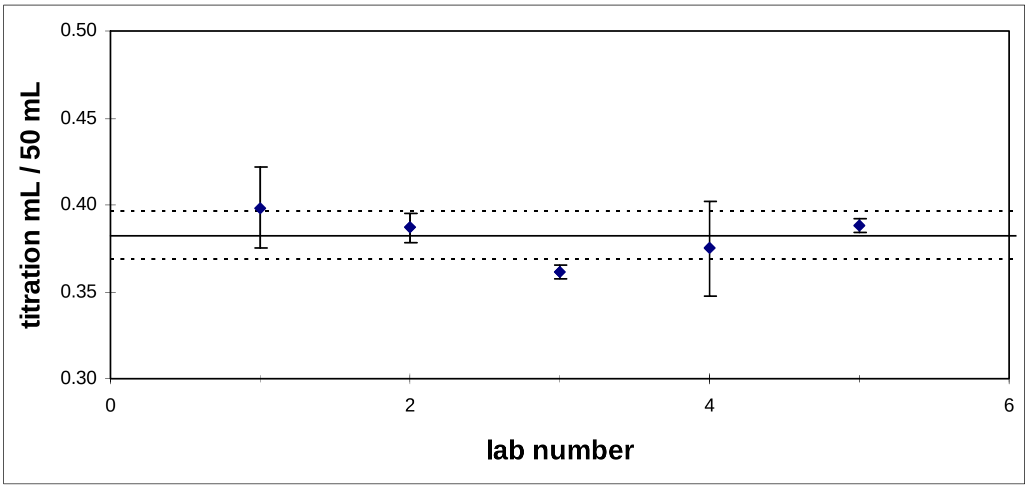
- for the titration method: mL of 0.01 mol/L HCl consumed by 50 mL of leachate;
- for flame spectrometry (AAS): mg of Na_2O per L of leachate, **not** corrected for the dilution with the spectral buffer.

The mean value of the 5 laboratory means and its standard deviation *s* is also given.

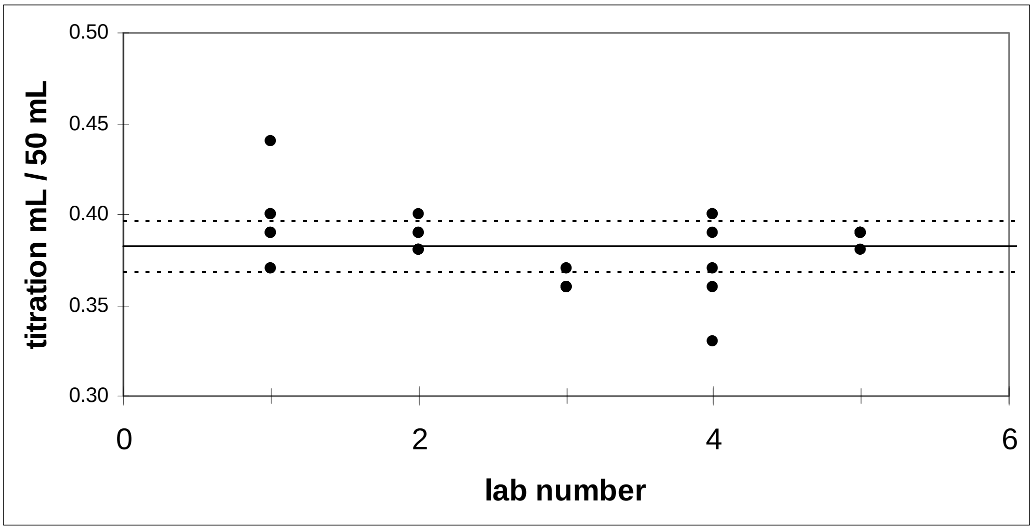
A graphical presentation of the corresponding data is given in Figures 4 and 5.

Table 5: Titration results, expressed in mL of 0.01 mol/L HCl solution needed to titrate 50 mL of leachate back to pH 5.5.

Lab Nr.	Replicate measurements / mL						mean / mL	*s* / mL
1	0.39	0.37	0.40	0.44	0.40	0.39	0.398	0.023
2	0.40	0.39	0.38	0.39	0.38	0.38	0.387	0.008
3	0.36	0.37	0.36	0.36	0.36	0.36	0.362	0.004
4	0.37	0.39	0.36	0.40	0.40	0.33	0.375	0.027
5	0.39	0.39	0.39	0.38	0.39	0.39	0.388	0.004
mean (*n*=5) and *s*							0.382	0.014
s_{rel} / %								3.7



a)



b)

Fig. 4: Titration results, expressed in mL of 0.01 mol/L HCl solution needed to titrate 50 mL of leachate back to pH 5.5. Solid line = mean value of all 5 laboratories, dashed line = standard deviation of the mean. **a)** Mean values and standard deviations *s* per laboratory, **b)** individual measurement results.

Table 6: AAS flame spectrometry results, expressed in mg Na_2O per L of leachate (**not** corrected for dilution of the leachate water with spectral buffer solution)**.**

Lab Nr.	Replicate measurements / mg/L	mean / mg/L	*s* / mg/L
1	1.70 1.65 1.58 1.63 1.67 1.79 1.75 1.73 1.76 1.79 1.74 1.76 1.74 1.86 1.68 1.92 1.87 1.77 1.86 1.79 1.75 1.77 1.72 1.66 1.64 1.72 1.75 1.69 1.77 1.71	1.74	0.08
2	1.705 1.658 1.692 1.638 1.658 1.719 1.719 1.719 1.678 1.685 1.631 1.618 1.638 1.651 1.685 1.672 1.665 1.624 1.624 1.638 1.766 1.658 1.631 1.604 1.725 1.591 1.564 1.569 1.591 1.672	1.66	0.05
3	1.70 1.70 1.70 1.71 1.73 1.69 1.79 1.70 1.75 1.77 1.70 1.69 1.70 1.74 1.70 1.73 1.73 1.71 1.75 1.67 1.67 1.69 1.73 1.67 1.69 1.70 1.70 1.69 1.66 1.70	1.71	0.03
4	1.545 1.727 1.745 1.719 1.706 1.722 1.732 1.683 1.834 1.707 1.687 1.516 1.519 1.857 1.670 1.646 1.805 1.673 1.610 1.737 1.643 1.725 1.716 1.686 1.688 1.817 1.746 1.881 1.614 1.555	1.70	0.09
5	1.88 1.78 1.78 1.89 1.76 1.87 1.84 1.89 1.84 1.72 1.89 1.92 1.47 1.88 1.77 1.97 1.91 1.88 2.03 1.85 2.01 2.02 1.86 1.86 1.81 1.96 1.91 1.99 1.88 1.89	1.87	0.11
mean (*n*=5) and *s*		1.74	0.08
s_{rel} / %			4.6

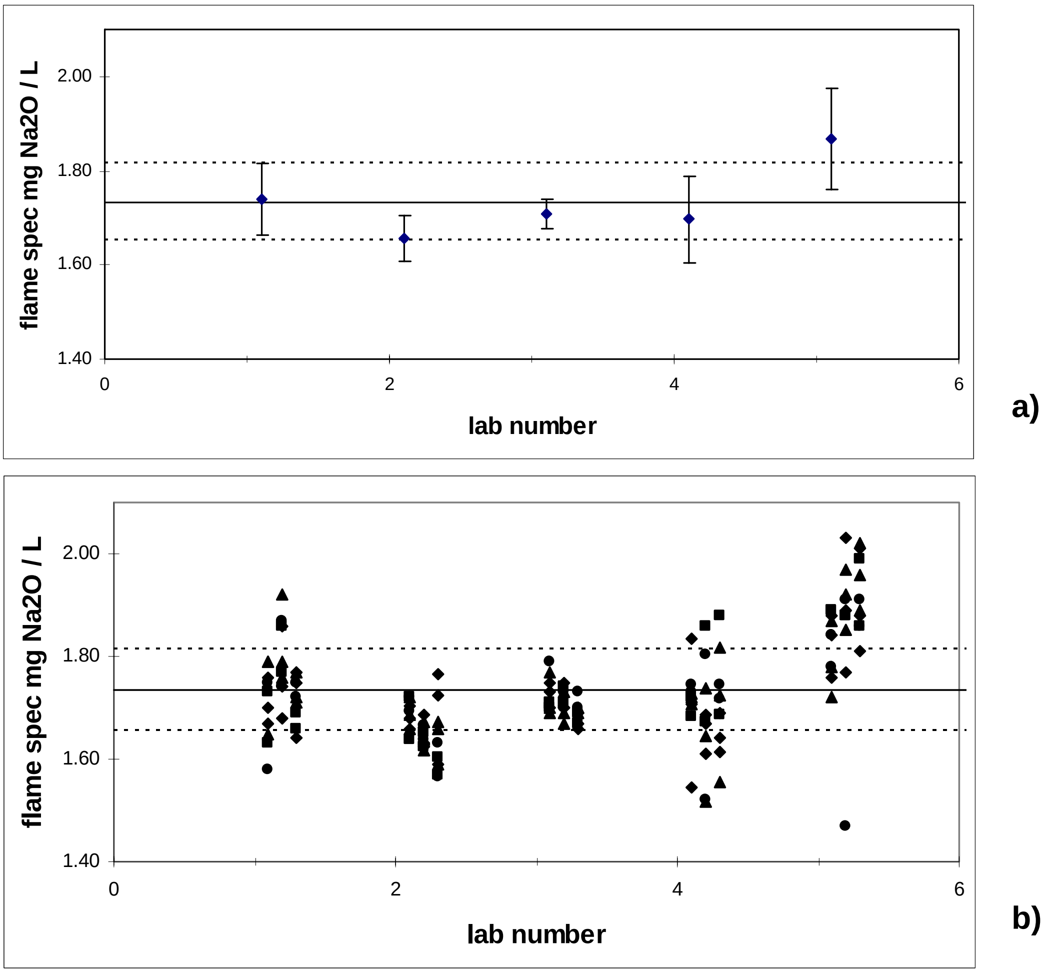


Fig. 5: AAS flame spectrometry results, expressed in mg Na_2O per L of leachate (**not** corrected for dilution). Solid line = mean value of all 5 laboratories, dashed line = standard deviation of the mean. **a)** Mean values and standard deviations *s* per laboratory, **b)** individual measurement results.

6.3 Technical discussion

6.3.1 Certification principles

All participants of the certification round were invited for a half day technical discussion on April 25, 2003 taking place on the premises of Schott Research Centre, Mainz, Germany on the occasion of the preceding meeting of Technical Committee 2 of the International Commission on Glass.

The discussions in this meeting focused for one part on the validity and usage of previous investigations and measurement results for various aspects of the present certification. Measurement results of earlier studies on the reusability of the glass containers and on the stability and homogeneity of the alkali leaching from these containers are still valid and can be used in the present certification. Furthermore, the initial measurement results of 1992/93, compared with the results of the present certification round, allow to demonstrate the stability of alkali-leaching from these containers on a very long time scale. For several reasons (new principles of certification with increased demands on traceability, direct availability of documentation, improved autoclave instrumentation), the characterisation of the property values themselves will be based solely on the recent results of the combined new certification and stability measurements of the year 2002.

To ensure the traceability of the property values, special attention was paid to the use of calibrants gravimetrically prepared from components of verified purity. In addition, since the property values are partly operationally defined, care was taken that all participating laboratories adhered strictly to the prescribed protocols (in etching for reconditioning of vials, in autoclaving and in determining the analytes).

6.3.2 Acceptance of data

All participants in the meeting, representing all 5 laboratories of the certification round, confirmed the use of gravimetrically prepared calibrants and the strict adherence to the preparation and measurement procedures (Annexes I to III).

The ten sets of measurement results were thoroughly discussed. Their individual values, laboratory means and standard deviations are presented in Tables 5 to 6 and graphically in Figures 4 and 5. The statistical outlier (an individual flame-spectrometric value of 1.47 from laboratory 5, not flagged, however, by the Nalimov test of *all* individual results) is kept in the ensemble, because no technical reason for its large deviation is known. Although differences in standard deviation appear to be systematic between different laboratories (generally smaller for laboratories 2 and 3, in the case of titration also for laboratory 5), reasons for this are somewhat speculative: A stricter temperature and time control of the autoclave procedure and of the handling of glass containers during etching, draining, drying and filling are possible reasons. Also the cleaning procedures for the AAS burners may vary from one laboratory to the other, as well as a contamination-free environment of the AAS equipment. It is known that memory effects after insufficient cleaning operations can influence subsequent measurements by increased variations, not necessarily by bias.

In the case of the titration method, however, there is a specific reason why the results of different laboratories do not agree within their random uncertainty. The colour change in the titration (from yellow through pink to red) takes 2 drops of titration solution, the volume of which contributes to the experimental uncertainty not reflected in the standard deviation. Each laboratory and each analyst will, however, read the colour always against the same background, under the same angle, and (as far as possible) under the same light conditions, and therefore obtain a smaller standard deviation. Moreover, two laboratories used automatic titrators.

In any case, the combined standard uncertainty of each laboratory's mean result is larger than the standard deviation shown in Figures 4 and 5, but none of the participating laboratories provided GUM-compliant uncertainty estimates. Whereas the laboratory mean values should overlap within their experimental uncertainty, a complete overlap of all values within the standard deviations alone cannot be expected.

It is concluded that *all obtained data are technically sound and they are all used to establish the certified values*.

It was also observed that the recent measurement round rendered more consistent results than the previous one (see Annex IV). This is attributed i.a. to the immense gain in experience of the laboratories involved, but for a part also to improvements in the construction of autoclaves.

6.4 Statistical evaluation

After technical scrutiny, the sets of accepted results (= all obtained results) were submitted to statistical tests in order to:

- demonstrate the level of agreement between the various sets of data accepted for certification;
- verify if all data sets can be considered as samples from a single population of data, and hence can be pooled;
- verify if the mean and standard deviation can be calculated assuming that the considered population of data is normally distributed.

The following statistical tests were applied:

- Kolmogorov-Smirnov-Lilliefors test for the assessment of the conformity of the distributions of individual results and of laboratory means with normal distribution.
- Nalimov test to detect statistically 'outlying' values in the population of individual results and in the population of laboratory means.
- Bartlett test to assess the overall consistency of the variance of values obtained in the participating laboratories.
- Cochran test to detect 'outlying' values in the laboratory variances (s_i^2).
- One way analysis of variance (F-test) to compare and estimate the between- and the within-laboratory components of the overall variance of all individual results.

For Cochran and Nalimov tests, a value is called 'outlying' when the hypothesis that it belongs to the population of considered results can be rejected with a 0.01 risk of error. For certification purposes, however, it is reasonable to adopt the additional criterion that an 'outlying' value of variance would lead to elimination of that laboratory's data set only in the case that the standard error of the mean ($s_i / \sqrt{n_i}$) of the set exceeds the standard deviation s of the distribution of all laboratory means.

The results of the tests are summarised in Table 7. The following conclusions can be drawn:

- Based on the additional criterion, explained above, none of the data sets for flame spectrometry is discarded although all of them are iteratively identified as having outlying variances according to the Cochran test.

- Owing to the inhomogeneity of the variances (Bartlett test), pooling of all data sets is not allowed. The laboratory means of the data sets are therefore treated as independent estimates of the values of the quantities to be certified.
- Gaussian statistics may be applied.

Table 7: Summary of statistical data for IRMM-435

	Titration	**AAS**
Number of accepted sets of results *p*	5	5
Number of accepted individual values	30	150
Outlying variances (Cochran test)	0	5 (2)
Homogeneity of variances (Bartlett test)	No	No
Normality of distribution of mean values (*)	Yes	Yes
Outlying mean values (Nalimov test)	0	0
Normality of distribution of individual values (*)	No	No
Outlying individual values (Nalimov test)	0	0
One-sided ANOVA (F-test):		
Mean of means (1)	0.382	1.734
Std.deviation of distribution of means (*s*) (1)	0.014	0.080
Within-labs std.deviation (s_w) (1)	0.017	0.076
Between-labs std.deviation (s_b) (1)	0.013	0.079
(*Kolmogorov-Smirnov-Lilliefors test)		

(1) expressed in *mL of 0.01 mol/L HCl consumed by 50 mL of leachate* and *mg of dissolved Na_2O per L of leachate*, respectively (not corrected for dilution)

(2) see explanation in text

7. CERTIFIED VALUES

The certified values for total alkali (determined by titration) and sodium release (determined by flame spectrometry) are calculated from the certification measurements after autoclaving as unweighted means of p accepted sets of results, each set being obtained in a different laboratory. The stated uncertainties represent the estimated expanded uncertainties U_{CRM} (with a coverage factor $k = 2$, corresponding to a level of confidence of about 95 %) as defined in the Guide to the Expression of Uncertainty in Measurement [1].

The expanded uncertainty of the CRM U_{CRM} is taking into account the uncertainty u_{char} of the characterization of the alkali and sodium release, the variability s_{bb} between individual test sets (containing minimum 9 vials) as determined in chapter 5.1, the reusability s_{reus} of the CRM after reconditioning (chapter 5.2) and the stability u_{lts} (chapter 5.3):

$$U_{CRM} = k \cdot \sqrt{u_{char}{}^2 + s_{bb}{}^2 + s_{reus}{}^2 + u_{lts}{}^2} \qquad (1)$$

A short-term stability study was carried out (cf. part of the data in chapter 5.3.1) with the sole purpose to fix transport conditions, more specific to demonstrate that there is no instability during transport (except for a total loss during transport due to breakage). Thus there is no uncertainty contribution from short-term stability u_{sts} in the uncertainty budget.

Due to the excellent stability of the material glass and the possibility of always reconditioning a fresh surface (with the corresponding uncertainty s_{reus}), the uncertainty from long-term storage, u_{lts}, could be demonstrated for a very long time base (10 years), but it is limited by the reproducibility of the leaching and measurement procedure.

The characterization uncertainty is estimated from equation (2)

$$u_{char} = \sqrt{u_c(I)^2 + u_c(II)^2 + u_c(III)^2} \qquad (2)$$

where $u_c(I)$ is the contribution from exclusively laboratory-dependent uncertainties and relates to the standard deviation of p laboratory means as given in equation (3)

$$u_c(I) = \frac{s}{\sqrt{p}} \qquad (3)$$

It has to be noted that this expression – the standard error of the mean of laboratory means - refers to completely independent observations, there are no common sources of uncertainty included in this. This equation implies that $u_c(I)$ only consists of random variation, systematic deviations are assumed to be averaged to zero.

$u_c(II)$ is an uncertainty contribution common to all laboratories. Although a common procedure is followed by all laboratories, this does not introduce an $u_c(II)$ component, because the certified values are operationally defined with this procedure.

$u_c(III)$ denotes combined standard uncertainties common to groups of laboratories. In our case it can be set to zero, there are no groups of laboratories using, for example, common calibrants.

In the certificate the uncertainties are given as expanded uncertainty with a coverage factor $k = 2$, corresponding to a level of confidence of approximately 95 %.

7.1 Total alkali leaching and release as determined by titration

The certified value with its expanded uncertainty U_{CRM} ($k = 2$) valid for a test set of minimum 9 vials is

(0.38 ± 0.04) mL of 0.01 mol/L HCl per 50 mL of leachate

7.2 Sodium leaching and release as determined by flame spectrometry

The certified value with its expanded uncertainty U_{CRM} ($k = 2$) valid for a test set of minimum 10 vials is (after correction for dilution of the leachate water with spectral buffer solution)

(1.91 ± 0.19) mg/L Na_2O

which is equivalent to a sodium release of

$[Na^+]$: (1.41 ± 0.14) mg/L

8. INSTRUCTIONS FOR USE

8.1 Precautions upon handling

For analysts familiar with the procedures following either European Pharmacopoeia [2] or ISO 4802, part 1 and/or 2 [3], attention must be drawn to the modifications to be observed when using this CRM. These modifications, detailed in the Annexes and justified in chapter 3, can be summarised as follows:

- the vials must be acid-etched just before testing,
- the cleaning (rinsing) and filling are different from ISO (but identical with European Pharmacopoeia),
- the vials are to be capped with perforated, cleaned and dried closures (before first use the screw caps must be foreseen with a small hole),
- the temperature must be measured inside a vial in the autoclave and be recorded with time; the temperature measurement system is to be calibrated,
- a spectroscopic buffer is to be added to the vials before flame spectrometric determination (similar to ISO 4802 Part 2).

8.2 Guidelines for the use of the CRM

Each unit of IRMM-435 consists of 20 vials with screw caps (holes to be drilled by the user) and includes details of the testing procedure applied for the certification measurements. Since the certified values of this CRM are operationally defined (see Annexes I to III for the testing protocol), these procedures must strictly be followed also in the normal usage of the CRM in order to establish comparable leaching and measurement conditions, under which the certified values should be reproduced within the measurement uncertainty. At least 10 vials are to be used when the sodium release determinations are carried out with flame spectrometry, at least 9 vials with titration for alkali release.

As laid down in Annex II and chapter 5.2, the vials can be reconditioned and re-used for the determination of sodium and alkali release at least 19 times without change in the release rate.

Since it was shown that small differences in autoclave temperature result in large differences in alkali release (see chapter 3), the use of calibrated thermometers is required. The temperature as measured in the actual vials is not always the same as that in the autoclave atmosphere around them, and does in principle follow the temperature programme of the test procedure only with a delay. It is, therefore, necessary to carry out temperature measurements in one of the actual vials (e.g. with a calibrated thermocouple - see Annex III) and to continuously record the temperature so as to verify that the conditions of the test procedure are fulfilled.

Note – The pre-etching step (Annex II or chapter 4 of Annex I) of the procedure for use of this CRM shall not be applied to the routine testing of unknown glass containers.

9. REFERENCES

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10. ACKNOWLEDGEMENTS

The authors would like to thank H. Schimmel and Th. Linsinger (IRMM) for the in-depth reviewing of the certification report, as well as the experts of the Certification Advisory Panel ‘Elements and inorganic ions’, O. Donard (Université de Pau et des Pays de l'Adour, FR), L. Jorhem (Livsmedelsverket, SE) and H. Muntau (Studio e Abiazione, IT) for their critical comments.

11. ANNEXES

Annex I THE AUTOCLAVING PROTOCOL

Full procedure for the determination of hydrolytic resistance of the interior surfaces of the reference glass containers IRMM-435

In principle, the testing methods comply with the International Standard ISO 4802 [3] for titration and flame spectrometry (titration also identical with the European Pharmacopoeia, Glass Monograph [2]) but are **deviating in some points**. Therefore, for the purpose of the certification of the test containers intended to serve as a certified reference material, the following procedure was **applied strictly**. Also in the normal usage of the CRM, this procedure must strictly be followed in order to establish comparable leaching and measurement conditions.

The deviations concern especially the closure of the vials, the traceability of standard solutions, the acidification of the extract solutions prior to the flame measurements, the accurate time-temperature control of the autoclave, and the exact documentation of all steps concerning calibration. Therefore, also clauses 3, 4 and 5 have to be applied strictly.

1. Reagents

Unless otherwise stated, use only reagents of recognized analytical grade.

1.1 **Test Water**

The water shall be double distilled, or de-ionized and then distilled (grade 2 water according to ISO 3696). This water shall be boiled for at least 15 minutes in a boiling flask of fused silica or borosilicate glass. The boiling flask shall be pretreated by subjecting it to the autoclave conditions described in clause 5.1, before it is used for the first time.

Such test water can normally be stored for 24 h in a stoppered flask without change of the pH value.

When tested immediately before use, the water shall be neutral to methyl red, i.e. it shall produce an orange-red (not a violet-red or yellow) colour corresponding to pH 5.5 ± 0.1 when four drops of the methyl red indicator solution (1.5) are added to 50 mL of the test water.

Note – The water, so coloured may also be used as the reference solution.

For flame spectrometric purposes only, also water of lower conductivity, e.g. grade 1 water according to ISO 3696, may be used. This water does not comply with the pH requirements for the waters as specified before, and shall therefore **not be used for determinations by titration**.

1.2 **Hydrochloric acid, standard volumetric solution,**

c(HCl) = 0.01 mol/L. This must be standardized against sodium hydroxide, which in turn is standardized against potassium hydrogen phthalate.

1.3 **Hydrochloric acid solution, c(HCl) ≈ 2 mol/L**

1.4 **Hydrofluoric acid, c(HF) ≈ 22 mol/L**
(i.e. = 400 g HF/L solution).

1.5 **Methyl red, indicator solution**
Dissolve 25 mg of the sodium salt of methyl red ($C_{15}H_{14}N_3NaO_2$) in 100 mL of the test water.

1.6 **Distilled water**
or water of equivalent purity (grade 3 water complying with the requirements specified in ISO 3696).

1.7 **Spectrochemical buffer solution (= “spec buffer”)**
Dissolve 10 g of cesium chloride CsCl in 0.01 mol/L HCl (1.2) contained in a 250 mL volumetric flask (2.5), make up the volume with 0.01 mol/L HCl and mix. Store the spec buffer solution in a pretreated borosilicate glass bottle.

1.8 **Stock solution**
Dry the chloride of sodium at (110 ± 5) °C for 2 h. Prepare gravimetrically an aqueous stock solution, using the test water (1.1) directly from the chloride such that the solution contains a concentration of 1000 mg/L, calculated as sodium oxide.

> **Note** – pay care, whether the concentration of the standard solution is given as Na or Na_2O for later calculations!

> **Note** – for the preparation of analytical solutions the test water (grade 2 water) can be used without further pre-boiling!

> **Note** – **In usage of the CRM:** A commercially available standard solution may also be used.

1.9 **Standard solution**
Prepare a standard solution by transferring 20 mL of the stock solution 1.8 into a 1 L volumetric flask and make up the volume with the test water (1.1, but see Note 2 above).

The concentration of Na_2O is 20 mg/L.

> **Note** – **In usage of the CRM:** A commercially available standard solution of appropriate concentration may also be used.

1.10 **Reference solutions and establishment of the calibration curve**
Transfer by means of a pipette (2.6) 10 mL of the spec buffer solution (1.7) and then by a burette of 10 mL capacity (2.2) the following volumes of the standard solution (1.9) into 100 mL volumetric flasks (2.5).
0 – 2 – 5 – 7.5 – 10 mL, corresponding to
0 – 0.4 – 1.0 – 1.5 – 2.0 mg/L Na_2O (after making up the volume with test water)

Measure the extinctions by spraying the solutions into the flame of the atomic absorption instrument, following the instructions of the producer’s manual. Start with the zero solution, then continue stepwise up to the 2.0 mg/L solution.

Measure each solution three times, calculate the mean and establish the calibration graph.

2. Apparatus

Ordinary laboratory apparatus, glassware – if not otherwise stated – made of borosilicate glass 3.3.

2.1 **Autoclave or steam sterilizer,**
capable of withstanding pressure of at least 2.5 x 10^5 N/m^2 (1) and of carrying out the heating cycle specified in 5.1. It should preferably by equipped with a constant-pressure regulator or other means of maintaining the temperature at (121 ± 1) °C. The vessel shall have an internal diameter of at least 300 mm and shall be equipped with a heating device, a possibility to lead a calibrated thermocouple (2.9) from the inner autoclave to an external measuring instrument (2.10), a pressure gauge, a pressure-release safety device, a vent-cock, and a rack for supporting the samples.

The autoclave vessel and ancillary equipment shall be thoroughly cleaned before use.

2.2 **Burettes, having a suitable capacity of 2 mL or 10 mL**
complying with the requirements specified for class A burettes in ISO 385/2 and made of glass of hydrolytic resistance grain class HGA 1 as specified in ISO 720(2).

2.3 **Beakers, having a suitable capacity**
and complying with the requirements specified in ISO 3819.
Before use, each new beaker shall be pretreated by subjecting it to the autoclaving conditions described in 5.1.

2.4 **Conical flasks, having a capacity of 100 mL or 250 mL**
and complying with the requirements of ISO 1773. Before use, each new flask shall be pretreated by subjecting it to the autoclaving conditions described in 5.1.

2.5 **One-mark volumetric flasks having a capacity of 100 mL and 1000 mL**
and complying with the requirements specified for class A one-mark volumetric flasks in ISO 1042.

2.6 **Pipettes, having a suitable capacity**
and complying with the requirements specified for class A pipettes in ISO 648.

2.7 **Water bath, capable of being heated to approximately 80 °C**

2.8 **Flame atomic absorption (AAS) or Flame atomic emission (AES)**
spectrometer.

AAS/AES instruments shall be equipped with a line source, gas supply and burner for measuring sodium.

AES instruments of older generation shall be equipped with air/propane gas supplies and burners for measuring sodium. Table 8 gives the conditions for measurement.

---

[1] 2.5 x 10^5 N/m^2 = 0.25 MPa = 2.5 bar

[2] Glass of hydrolytic resistance grain class ISO 719-HGB 1 adequately meets the requirements of class HGA 1 specified in ISO 720.

Table 8: Instrument conditions for measuring sodium (Na)

Element	Instrument	Usual optimum	Gas mixture	Detect. Line
Na	AES	Up to 10 µg/mL	Air/propane	Filters
Na	AAS/AES	Up to 3 µg/mL	Air/acetylene	589.0 nm

2.9 **Thermo couple of NiCr-Ni (Type K),**
or resistance thermometer integrated in the autoclave.

2.10 **Suitable measuring device (millivolt meter),**
with recorder for documentation. Calibrate the whole system thermocouple/resistance thermometer and measuring system, for instance millivolt meter and recorder (see Annex III).

2.11 **Pipette of suitable capacity,**
made of plastic.

3. Preparation of the closures (stoppers)

3.1 **Apply a small hole**
of maximum 0.3 mm diameter, e.g. by drilling or by penetrating with a needle. (Only applicable once before first use.)

3.2 **Cleaning of the closures**
Put the closures in a suitable glass beaker, fill with test water (1.1), boil for 5 minutes, empty, fill with water again and boil, empty and boil with water a last time as before. Then empty quantitatively, and allow to dry in an oven at about 110 °C, store in a desiccator, and **avoid any contamination** (also during later handling).

4. Preliminary etching

Mark 10 of the set of 20 containers with a number (scratching by glass writer for instance) and fill all containers up to the rim with a mixture of 1 volume of hydrofluoric acid (1.4) and 9 volumes of hydrochloric acid (1.3) using the pipette (2.11). Allow the filled samples to stand at ambient temperature for 10 minutes, then empty the solution very carefully (HF is dangerously etching!). Rinse the containers once with distilled water (1.6), then fill them again with the same mixture of acids, and allow to stand for further 10 minutes. Repeat the etching a third time. Then empty all solutions very carefully, rinse the containers 3 times with the distilled water (1.6), then at least twice with the test water (1.1). Dry in an oven at about 110 °C and store in a desiccator to cool.

5. Procedure

5.1 **Autoclave process**

After etching (4) fill all 20 dried containers by means of a burette (2.2) exactly with the prescribed volume = weight of the test water. Cap each container with a cleaned closure (3) (avoid contamination!).

Place all samples on the rack of the autoclave, containing distilled water (1.6) at ambient temperature, and ensure that they are held above the level of the water in the vessel. Insert into one of the filled containers the end of the thermocouple or resistance thermometer (2.9) through a hole of the closure of a not marked vial and lead it out of the autoclave. Connect it with the measuring device (2.10) as described in Annex III. Close the autoclave lid or door securely, but leave the vent-cock open. Start automatical plotting of the temperature on the recorder (2.10) versus time and heat the autoclave at a regular rate such that steam escapes vigorously from the vent-cock after 20 to 30 minutes, and maintain a vigorous evolution of steam for a further 10 minutes. Close the vent-cock and increase the temperature at a rate of 1 °C/min (= within 20 minutes) to (121 ± 1) °C, maintain this temperature for (60 ± 1) minutes from the time when the holding temperature is reached, then cool at a rate of 0.5 °C/min (= within 40 minutes) to 100 °C, venting to prevent formation of a vacuum. Open the autoclave carefully and note this point on the time-temperature record.

> **Note** – The rate of heating to (121 ± 1) °C and the rate of cooling to 100 °C are critical. Variations from the specified conditions can produce variable results even to the extent of invalidating them.

Remove the hot samples from the autoclave, place in the water bath (2.7) heated to about 80 °C, and run cold water into and out of the bath at a rate which will cool the samples to ambient temperature as quickly as possible (max 15 minutes).

> **Note** – Make sure that during cooling the edge of caps does not touch the cooling water, otherwise water could be sucked into the container and heavily contaminate the extraction solution.

The temperature-time process of the autoclave shall be from the start:

(autoclave open)	20-30 minutes from room temperature to steam escape 10 minutes of vigorous steaming
(autoclave closed)	20 minutes from 100 °C to 121 °C 60 minutes maintaining 121 °C 40 minutes decreasing from 121 °C to 100 °C
(autoclave possibly slightly opened)	
(autoclave open)	removing the containers as quickly as possible from the autoclave max 15 minutes cooling down from 100 °C to room temperature.

> **Note** – Recent safe operation practices (EN 61010-2-041 point 7.101.2) require that autoclaves are opened at 95 °C. With the necessary precautions against injuries due to burning with hot steam, however, all autoclaves *can be* opened at 100 °C. A collaborative study of TC2 of the International Commission on Glass has shown that the prolongation of the cooling time down to 95 °C increases the extracted alkali by measurable amounts [7]. The certified values of IRMM-435 are valid only for opening temperatures of the autoclave at 100 °C. This is in accordance with ISO 4802 and European Pharmacopoeia.

Carry out the analytical determinations immediately, i.e. the same day.

6. Analysis of the extraction solutions

6.1 By titration

Remove the closures from 9 unmarked containers, excluding the one with the thermometric device. Combine the extraction solutions of 5 or 4 containers, respectively, in two dry 250 mL conical flasks (2.4) and pipette by means of a pipette (2.6) a volume of 50.0 mL from each combined solution into new separate conical flasks (2.4) of 100 mL.

Prepare a reference solution by pipetting 50 mL of the test water into a third flask (2.4) of 100 mL.

Add four drops of methyl red indicator solution (1.5) to each flask, then titrate the extraction solutions with hydrochloric acid (1.2) until the colour matches exactly that of the coloured test water in the third conical flask.

Express the titration values to two decimal places.

6.2 By flame spectrometry

Remove the closures from the 10 marked containers. Add to each, by means of a 2-mL burette (2.2) 1.7 mL of the spec buffer (1.7) which is equal to 10 % of the filling volume. Shake. Aspirate the extraction solutions immediately from each container directly into the flame spectroscopic instrument (2.8) and determine the concentrations of **sodium oxide** by reference to the calibration graph, produced according to clause 1.10.

Express the measurement results to two decimal places in the unit mg/L Na_2O.

7. Repetition of the measurements

After having finished the first run of the tests, rinse the containers 2 times with test water, allow to drain and dry.

For a new test run start at another day (autoclave cooled down to room temperature), as described from clause 3.2 onwards with a new cleaning of the closures, new etching, followed by a new autoclave process and analyses. Repeating the whole procedure for a third time is usually not necessary.

Annex II THE ETCHING PROTOCOL FOR RECONDITIONING THE VIALS

Fill all containers up to the rim with a mixture of 1 volume of hydrofluoric acid (1.4) and 9 volumes of hydrochloric acid (1.3) using the pipette (2.11). Allow the filled samples to stand at ambient temperature for 10 minutes, then empty the solution very carefully (HF is dangerously etching!). Rinse the containers once with distilled water (1.6), then fill them again with the same mixture of acids, and allow to stand for further 10 minutes. Repeat the etching a third time. Then empty all solutions very carefully, rinse the containers 3 times with the distilled water (1.6), then at least twice with the test water (1.1). Dry in an oven at about 110 °C and store in a desiccator to cool.

> **Note** – Unknown containers, which are to be tested, shall not be etched, of course.

Annex III TEMPERATURE MEASUREMENTS WITHIN VIALS IN AN AUTOCLAVE

It is not sufficient to calibrate only the thermocouple, but the whole measuring system needs calibration. It is therefore essential to calibrate the temperature scale on the recordings using a thermometer, calibrated within ± 0.1°C and, for example, an oil bath in which the thermocouple/resistance thermometer is inserted.

Possible positions and feed-throughs of the thermometric devices in an autoclave vessel are shown in Figure 6. To avoid by-pass currents, the thermoelement should be insulated. A possible solution is shown in Fig. 7a. The connection from the thermocouple to the measuring device shall be made via cold-junction compensation (automatic compensation integrated in the measuring instrument) or by an external compensation facility (for instance an ice-water mixture) to obtain defined temperature measurements (Fig. 7).

These conditions must be identical during calibration and later measurements.

Some modern autoclaves are already equipped by the producers with thermometric devices, mostly resistance thermometers. Such measurement apparatus can also be used, but must be calibrated as well.

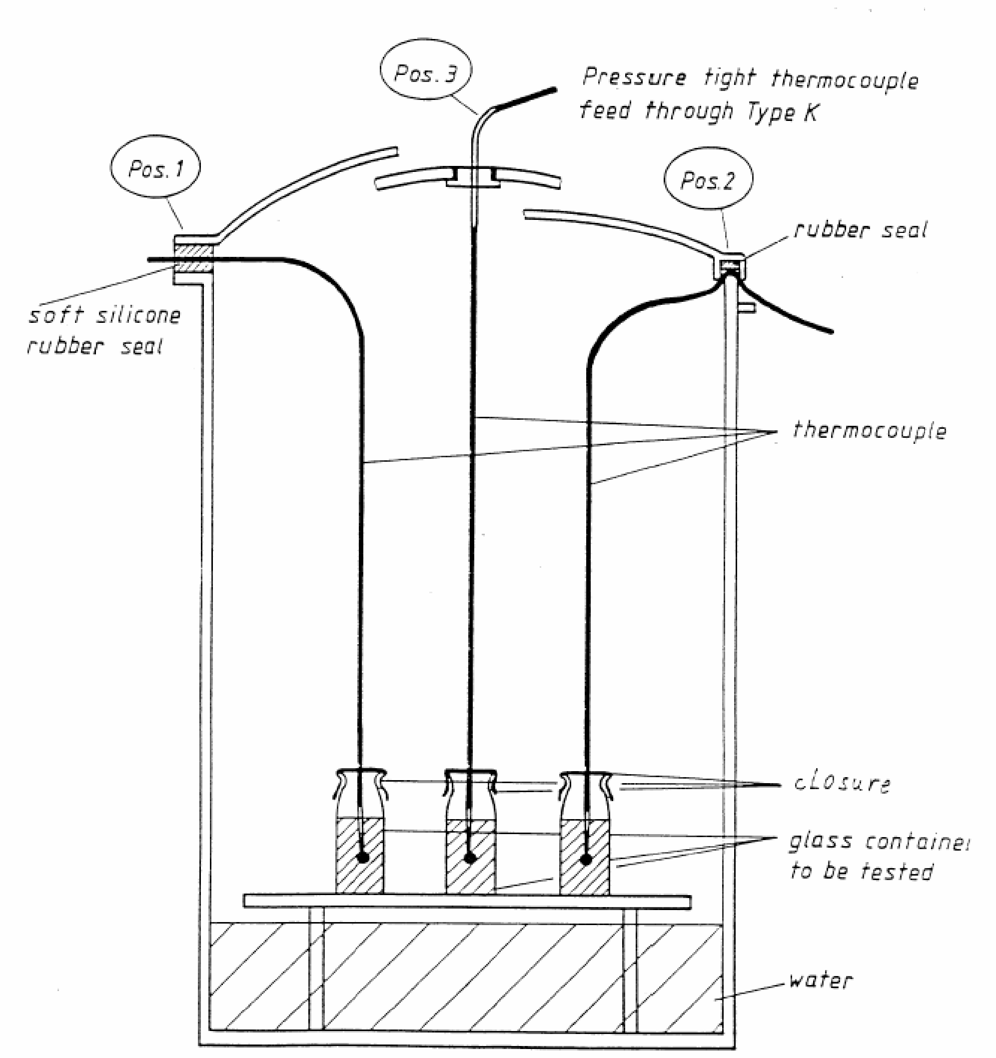

Fig. 6: Temperature measurement in an autoclave by thermocouples or resistance thermometers, using 3 different autoclave lid configurations.

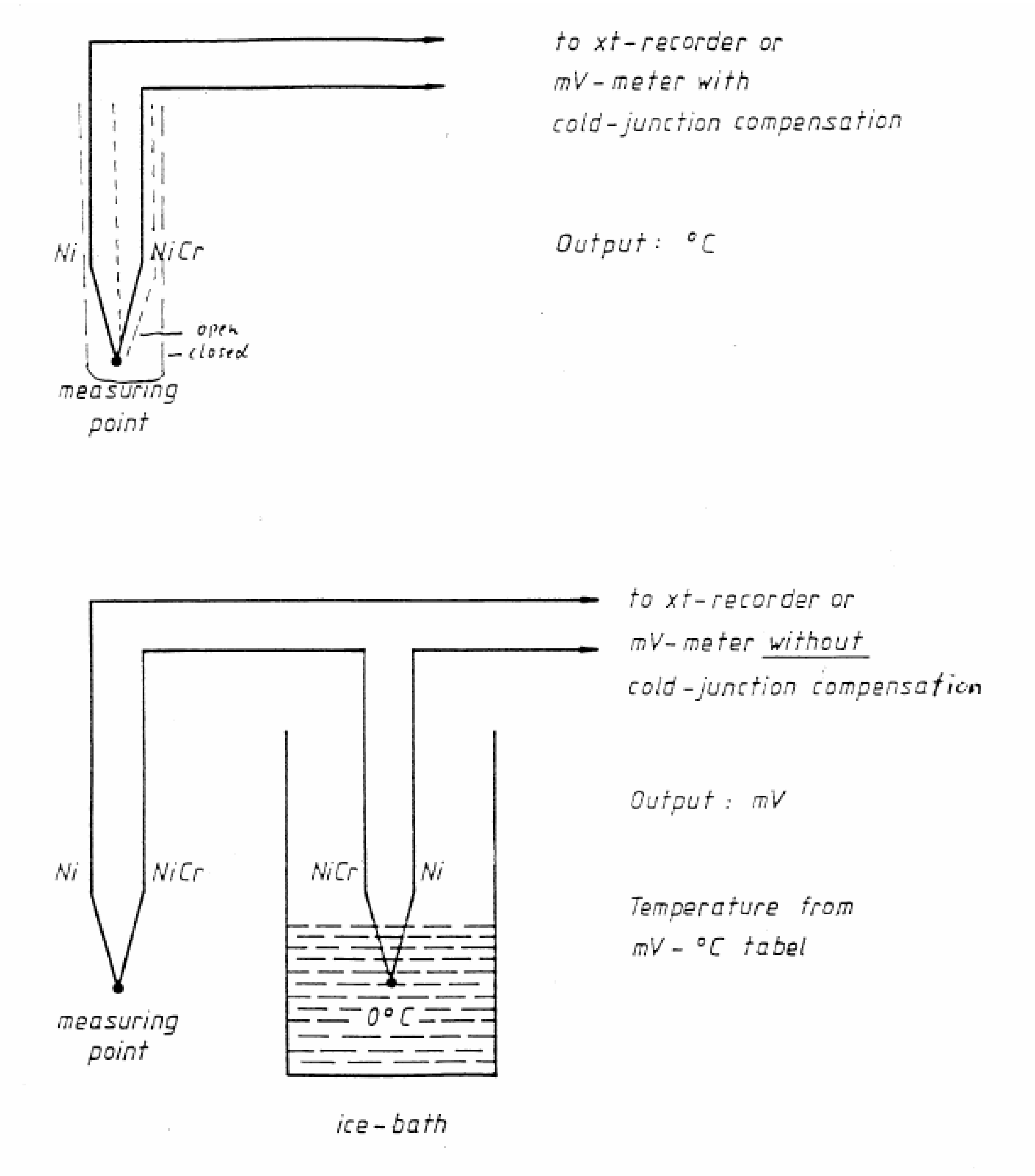

Fig.7: Connection of thermocouple with measuring instrument

a) with cold-junction compensation

b) with external compensation (ice-bath).

a) also shows the necessary insulation: open Al_2O_3 tube over one lead, both thermocouple leads in a closed Al_2O_3 tube.

Annex IV RESULTS OF THE INITIAL MEASUREMENT ROUND OF 1992/93

The results of the initial measurement campaign of 1992/93 are documented in Tables 9 and 10. See also chapter 5.3.3 under "Stability" for their usage.

Laboratory 11 withdrew its results and the results from laboratory 12 were rejected for not strictly following the autoclaving procedure. The mean value of the accepted laboratory means and its standard deviation *s* is also given.

Table 9: Titration results of initial measurement round, expressed in mL of 0.01 mol/L HCl solution needed to titrate 50 mL of leachate back to pH 5.5.
* withdrawn or rejected (see text)

Lab Nr.	Replicate measurements / mL	mean / mL	*s* / mL
01	0.37 0.38 0.38 0.39 0.39 0.40 0.39 0.41 0.36 0.38	0.385	0.015
02	0.43 0.43 0.43 0.44 0.45 0.45	0.438	0.010
03	0.36 0.36 0.36 0.37 0.35 0.36	0.360	0.007
05	0.43 0.46 0.42 0.42 0.38 0.39	0.417	0.029
06	0.46 0.47 0.46 0.46 0.46 0.45	0.460	0.007
07	0.35 0.35 0.34 0.34 0.37 0.36	0.352	0.012
08	0.44 0.44 0.50 0.48 0.47 0.46	0.465	0.024
09	0.33 0.34 0.34 0.35 0.34 0.35	0.342	0.008
*11	0.53 0.55 0.58 0.55 0.51 0.52	0.540	0.026
*12	0.30 0.30 0.31 0.30 0.29 0.30	0.300	0.007
mean (*n* = 8) and *s*		0.40	0.05
s_{rel} / %			12.5

Table 10: AAS/AES flame spectrometry results of initial measurement round, expressed in mg Na_2O per L of leachate (**not** corrected for dilution of the leachate water with spectral buffer solution)**.**
* withdrawn or rejected (see text)

Lab Nr.	Replicate measurements / mg/L	mean / mg/L	*s* / mg/L
01	1.89 1.87 1.99 1.85 1.90 1.86 1.99 1.87 1.95 1.83 1.87 1.80 1.83 1.78 1.82 1.82 1.81 1.76 1.81 1.89 1.83 1.83 1.76 1.88 1.80 1.81 1.78 1.77 1.87	1.846	0.061
02	1.65 1.68 1.68 1.68 1.66 1.76 1.70 1.80 1.68 1.71 1.69 1.73 1.75 1.71 1.78 1.75 1.80 1.82 1.75 1.78 1.72 1.75 1.75 1.80 1.78 1.80 1.80 1.79 1.78 1.80	1.744	0.050
03	1.60 1.68 1.46 1.59 1.65 1.58 1.67 1.69 1.61 1.56 1.62 1.71 1.61 1.68 1.57 1.62 1.59 1.67 1.58 1.70 1.55 1.61 1.62 1.60 1.58 1.62 1.60 1.59 1.58 1.63	1.614	0.052
05	1.86 1.80 1.79 1.84 1.80 1.85 1.80 1.76 1.81 1.84 1.76 1.77 1.76 1.77 1.74 1.80 1.77 1.75 1.76 1.79 1.80 1.74 1.74 1.70 1.80 1.80 1.78 1.69 1.78 1.82	1.782	0.040
06	1.81 1.89 1.84 1.84 1.84 1.87 1.91 1.83 1.85 1.90 1.78 1.82 1.89 1.73 1.76 1.84 1.87 1.81 1.82 1.78 1.88 1.84 1.87 1.94 1.82 1.77 1.82 1.85 1.90 1.89	1.842	0.048
07	1.76 1.75 1.73 1.75 1.74 1.73 1.74 1.74 1.72 1.73 1.55 1.58 1.60 1.62 1.58 1.40 1.62 1.60 1.55 1.55 1.67 1.69 1.68 1.65 1.71 1.69 1.70 1.62 1.66 1.67	1.659	0.083
08	1.93 1.86 1.88 2.01 1.98 1.86 1.88 1.93 1.90 1.90 2.31 1.94 2.18 2.12 1.90 1.88 1.82 1.92 1.69 1.94 1.90 2.05 1.87 1.79 1.83 1.69 1.95 2.00 1.87 1.93	1.92	0.13
09	1.74 1.77 1.59 1.78 1.51 1.70 1.70 1.76 1.79 1.66 1.68 1.64 1.72 1.67 1.64 1.57 1.66 1.65 1.51 1.66 1.82 1.77 1.79 1.76 1.82 1.79 1.78 1.77 1.71 1.81	1.707	0.087
10	1.70 1.75 1.80 1.79 1.75 1.77 1.77 1.79 1.84 1.85 1.95 1.86 1.84 1.82 1.74 1.70 1.74 1.70 1.65 1.74 1.78 1.74 1.74 1.74 1.79 1.72 1.75 1.75 1.72 1.75	1.768	0.060
*11	2.19 2.17 2.23 2.25 2.14 2.18 2.25 2.20 2.22 2.27 2.36 2.23 2.35 2.50 2.34 2.34 2.31 2.35 2.32 2.02 2.38 2.51 2.38 2.38 2.35 2.30 2.31 2.41 2.34 2.45	2.30	0.11
*12	1.53 1.51 1.49 1.55 1.51 1.50 1.48 1.54 1.49 1.51 1.59 1.52 1.56 1.54 1.53 1.56 1.54 1.43 1.52 1.50 1.43 1.48 1.44 1.44 1.38 1.45 1.43 1.42 1.39 1.40	1.489	0.056
mean (*n* = 9) and *s*		1.76	0.10
s_{rel} / %			5.5

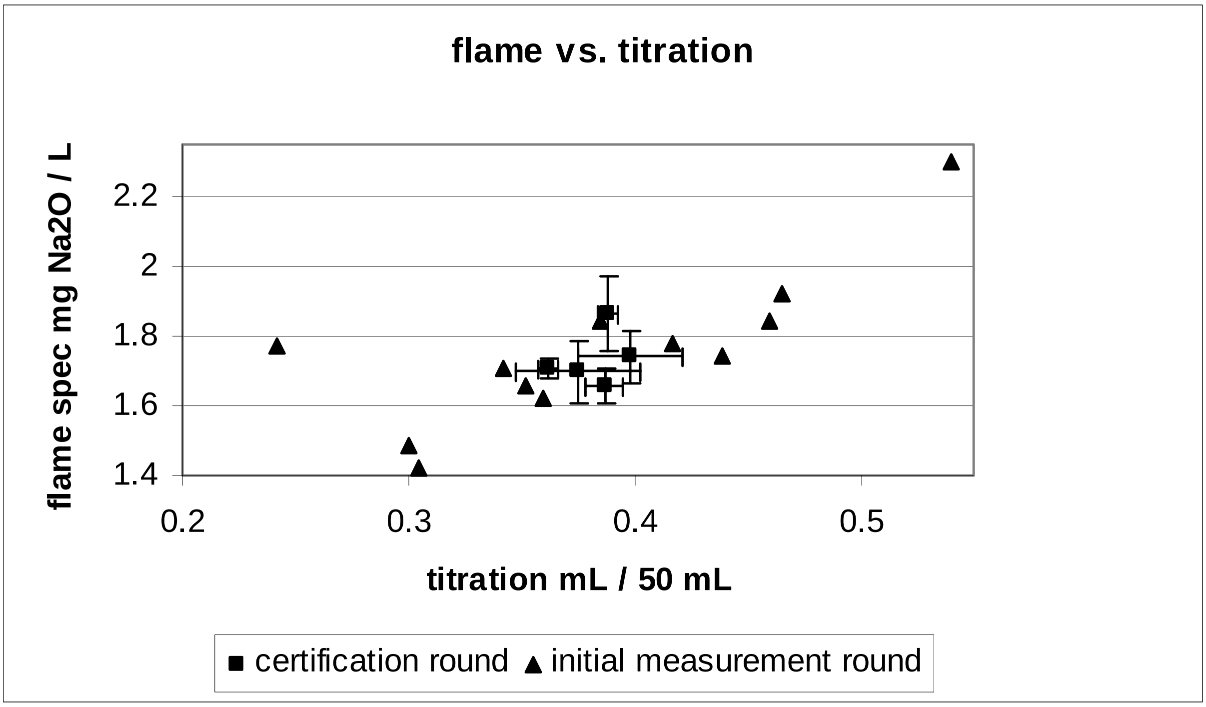

Fig. 8: Scatter plot of flame spectrometry versus titration measurement results (flame spectrometry values **not** corrected for dilution).

The consistency of measurement results from the final certification round of 2002/2003 compares very favourably with the large scatter of results from the initial measurement round of 1992/93 (see Fig. 8).

European Commission

EUR 22537 EN – DG Joint Research Centre, Institute for Reference Materials and Measurements –
THE CERTIFICATION OF A REFERENCE MATERIAL FOR THE DETERMINATION OF THE ALKALI LEACHING FROM PHARMACEUTICAL GLASS CONTAINERS - IRMM-435
Authors: U. Wätjen, E. Guadagnino, A. Peters† and S. Vandendriessche
Luxembourg: Office for Official Publications of the European Communities
2006 – 42 pp. – 21.0 x 29.7 cm
EUR - Scientific and Technical Research series; ISSN 1018-5593
ISBN 92-79-03798-6

Abstract

This report describes the certification of the reference material IRMM-435 for the determination of the alkali release from the interior surface of glass containers for parenteral or injectable pharmaceutical solutions according to an adapted method based on the European Pharmacopoeia method and the International Standard ISO 4802. The material has been certified for its alkali leaching and release determined by titration and for its sodium leaching and release determined by flame spectrometry.

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15 LA-NA-22537-EN-C

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ISBN 92-79-03798-
9 789279 037986