

Technical University of Denmark



Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware

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Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Simoneau, C., Hoekstra, E., Bradley, E., Bustos, J., Golja, V., Kappenstein, O., ... Vanhee, F. (2011). Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware. Luxembourg: European Commission. (Uden navn; No. EUR 24815 EN 2011).

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Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware

1st edition 2011

[in support of Commission Regulation 284/2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from People's Republic of China and Hong Kong Special Administrative Region, China]



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The mission of the JRC-IHCP is to protect the interests and health of the consumer in the framework of EU legislation on chemicals, food, and consumer products by providing scientific and technical support including risk-benefit assessment and analysis of traceability.

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JRC 64903

EUR 24815 EN 2011

ISBN 978-92-79-20201-8 (print)

ISBN 978-92-79-20202-5 (pdf)

ISSN 1018-5593 (print)

ISSN 1831-9424 (online)

doi:10.2788/19565

Luxembourg: Office for Official Publications of the European Union

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Printed in Italy

Executive Summary

Comparability of results is an important feature of the measurements carried out for official controls purposes. In the area of food contact materials and articles comparability of results is dependent on the availability of samples representative of a consignment, the type of exposure and the test conditions used as well as on the performance of the method of analysis.

These guidelines contain practical information on sampling, migration testing and methodologies for the analytical determination of primary aromatic amines and of formaldehyde. These guidelines were developed specifically in the context of the Regulation (EU) No 284/2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from [the] People's Republic of China and Hong Kong Special Administrative Region, China.

These guidelines have been prepared by the European Union Reference Laboratory in collaboration with its EU official Network of National Reference Laboratories and have been endorsed by the European Commission competent service DG Health and Consumers (DG SANCO) and its network of Member State Competent Authorities. They are primarily addressed to official control laboratories, national reference laboratories and third party laboratories. The sampling strategy is addressed to the points of first introduction of import goods in the EU.

Acknowledgment: This work was performed under the EU-RL mandate SANCO/2011/foodsafety/063-food contact materials

Note: the guidelines have been developed in support of the above mentioned specific regulation, but this does not exclude their application to other context

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1 CONTEXT

Under Regulation EC No 882/2004 the EU Reference Laboratory (EU-RL) and National Reference Laboratories (NRLs) have the duty to develop a harmonised approach for sampling and the selection of test conditions when these are not explicitly established or detailed in existing EU legislation.

These guidelines are developed in the context of the Commission Regulation (EU) No 284/2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from [the] People's Republic of China and Hong Kong Special Administrative Region, China. The EU-RL NRL Network has already developed guidelines on testing conditions for kitchenware (EUR 23814, 2009). These guidelines were used as a reference and basis for this document.

This is the first time that guidance on sampling of such heterogeneous items and different types of consignments has been set up. It is extremely relevant for the harmonisation of sampling and its effect on reliability of results and therefore ultimately for the trust in legal decisions for non-compliant articles both under the Regulation on official feed and food controls (EC) No 882/2004 and under Commission Regulation (EU) No 284/2011.

2 SCOPE AND OBJECTIVE

The objective of these guidelines is to develop unified sampling and testing procedures for kitchenware made of polyamide and/or melamine.

These guidelines aim to address the sampling strategy, treatment of the test specimen(s), exposure (e.g. exposure type/time/temperature, simulant) and the interpretation of results for the testing of the migration of primary aromatic amines (PAAs) from polyamide kitchenware and of formaldehyde (FA) from melamine kitchenware.

It is necessary to fix general criteria for compliance in order to ensure that laboratories, in charge of official control, use methods of analysis with comparable levels of performance. It is also of major importance that analytical results are reported and interpreted in a uniform way in order to ensure a harmonised enforcement approach across the EU.

3 QUALITY CONTROL AND UPDATES

The EU-RL and its NRL Network for food contact materials (FCMs) was charged with the development of these technical guidelines and will update them when new scientific and technical knowledge is available. DG JRC under its mandate as EU-RL drafted the guidelines with contributions and consensus of the NRLs. The European Commission, DG SANCO and Member States Competent Authorities approved and endorsed them.

4 LEGISLATIVE CONTEXT

Commission Regulation (EU) No 284/2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from [the] People's Republic of China and Hong Kong Special Administrative Region, China

Regulation (EC) No 1935/2004 states that food contact materials shall be safe. They shall not transfer their constituents into the food in quantities that could endanger human health, change the composition of the food in an unacceptable way or deteriorate the organoleptic properties of foodstuffs.

Commission Regulation (EU) No 10/2011 is a specific measure of Regulation (EC) No 1935/2004 and establishes the specific rules for plastic materials and articles to be applied for their safe use. This Regulation is applicable as from 1 May 2011.

Commission Directive 2002/72/EC is repealed by Regulation EC No 10/2011 as from 1 May 2011. The provisions on verification of compliance with migration limits for repeated use articles (Article 23) should remain applicable until 31/12/2012.

Regulation (EC) No 882/2004 includes quality standards for laboratories entrusted by the Member States with the official control of foodstuffs.

The GMP Regulation (EC) No 2023/2006 contains the basic principles of good manufacturing practice in the area of food contact materials.

Directives 93/8/EEC and 82/711/EEC containing the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs as well as 85/572/EEC containing the list of simulants remain applicable until 31/12/2012. As of 01/01/2013 they are replaced by Regulation (EU) No 10/2011.

5 EXPLANATORY DEFINITIONS

Plastic kitchenware means plastic materials as described in paragraphs 1 and 2 of Art 1 of Directive 2002/72/EC and falling within CN code ex 3924 10 00, as described in Regulation (EU) No 284/2011.

The **CN** is a means of designating goods and merchandise which was established to meet the requirements both of the Common Customs Tariff and of the external trade statistics of the Community. The abbreviation CN stands for Combined Nomenclature. This Combined Nomenclature contains the goods classification prescribed by the European Union for international trade statistics. The CN is an 8-digit classification consisting of a further specification of the 6-digit Harmonised System.

A **consignment** means a quantity of polyamide and/or of melamine plastic kitchenware, covered by the same document(s), conveyed by the same means of transport and coming from the same third country (Regulation (EU) No 284/2011). A consignment can be composed of one or more lots.

A **lot** means a stated portion of the consignment to be tested for the migration of primary aromatic amines or formaldehyde. A lot consists of a number of a given article or of a given set of articles with a corresponding reference or item number or code and a description in the delivery document.

A **batch** is a defined quantity of starting material, packaging material, or product

processed in a single process or series of processes so that it could be expected to be homogeneous. In the case of continuous manufacture, the batch must correspond to a defined fraction of the production, characterized by its homogeneity. A batch can be identified by a batch number.

An **article** (repeated use article) is as described in paragraphs 1 and 2 of Art 1 of Directive 2002/72/EC and Article 3 of Regulation (EU) No 10/2011. An article refers to an individual thing or element of a class; a particular object or item for sale; for kitchen articles that are the object of the Regulation (EU) No 284/2011, it refers to repeated use articles such as cup, plate spoon etc.

A **sample** is as described in Directive 2002/72/EC (Annex I). It means one or more articles (or parts of the article) selected in a prescribed or systematic manner from a lot, prepared for sending to the laboratory and intended for inspection or testing.

A **test specimen** means the portion of the sample on which the test is performed.

A **set** means a number of articles (same or different) that are sold together (e.g. in a box).



Figure 1: Examples of polyamide kitchenware; illustrations shown include articles such as cooking spatulas, slotted spoons, tongs and pasta tongs, whisks, etc.



Figure 2: Examples of melamine kitchenware; illustrations shown include articles such as picnic sets, children plates, bowls cups, ladles, spoons, etc.

Melamine: term used in Regulation (EU) No 284/2011 as melamine refers in technically correct terms to formaldehyde based resins with melamine or urea.

6 SAMPLING

6.1 Documentary checks of consignments

In accordance with Regulation (EU) No. 284/2011 documentary checks on all consignments should be carried out within two working days of arrival.

Each consignment should be accompanied by a declaration confirming that it meets the requirements of the Regulation and each declaration should be accompanied by a laboratory test report describing the results of migration tests of PAAs from polyamide kitchenware and of FA from melamine kitchenware. Laboratory test results should be provided for each lot in the consignment (i.e. a separate report should be provided for each article accompanied by a unique reference number, i.e. lot, and

where possible the corresponding batch number) or the laboratory report should clearly establish which articles it refers to.

6.2 Sampling approach

Sampling shall be performed at the point of first introduction in the EU.

In accordance with Regulation (EU) No. 284/2011 identity and physical checks, including laboratory analysis should be carried out on 10% of consignments. If a consignment consists of more than one lot of polyamide and/or melamine plastic kitchenware then each lot should be sampled.

Identity checks are a means a visual inspection to ensure that the documents accompanying the consignment tally with the contents of the consignment.

Samples for physical checks (i.e. laboratory analysis) should be taken according to the approaches described below.

A consignment may consist of one or more lots. Different lots may be produced by the same or different manufacturers. Each lot should be sampled.

6.2.1 General recommendations

Sampling should be performed by authorised personnel as designated by the Member State Competent Authority.

In the course of sampling and preparation of the samples, precautions should be taken to ensure the safety of the personnel taking the samples.

For lots consisting of a number of cartons or packing bags, sampling should be performed from a representative number of cartons/packing bags.

Each sample taken should be placed in a clean bag/container offering adequate protection from contamination and against damage during transport. All necessary precautions should be taken to avoid any change in or damage to the sample, which might arise during transport or storage.

Each sample taken for official use should be sealed at the place of sampling. A record should be kept of each sample taken, with clear reference to the consignment and lot identity and indicating the date and place of sampling together with any additional information likely to be of assistance for official controls following the rules of the Member State.

6.2.2 Sample size

The following sample size should be taken for each lot:

- 5 articles (or 5 sets of articles) as laboratory test specimens for enforcement purposes, and
- 5 articles (or 5 sets of articles) for trade purposes (defence, e.g. repeat analysis in case of dispute)

If required according to the rules of the Member State Competent Authority then a further:

- 5 articles (or 5 sets of articles) should be taken for reference (referee)

The samples for trade and reference purposes should be retained securely following the rules of the Member State, unless such a procedure conflicts with Member States' rules as regards the rights of the food business operator.

7 LABORATORY TESTING

7.1 Number of test specimens

5 test specimens (articles or sets) should be taken for enforcement purposes:

- 1 test specimen for the identification of the polymer type
- 1 test specimen for the surface area calculation
- 3 test specimens (individual articles or sets of articles) for the migration test

7.2 Identification of the polymer type

If doubts exist as to the identity of the material of which the articles are made then the polymer type should be determined. Different methods can be applied to determine polymer type such as Fourier transform infra-red spectroscopy (FT-IR) methods and/or pyrolysis methods.

The FT-IR spectrum of the polymer should be acquired and compared to the standard spectra for either polyamide or melamine. This can be done using Attenuated Total Reflectance (ATR), by Diffuse Reflectance Infrared Fourier Transform (DRIFT) or by preparation of potassium bromide (KBr) pellet.

Descriptions of tests by flame and by pyrolysis can be found detailed in: Von A. Krause, A. Lange and M. Ezrin; *Plastics analysis guide: chemical and instrumental methods*; ISBN 3-446-12587-1. Hanser Publ. (Munich), 1983, as well as D. Braun: *Simple Methods for Identification of Plastics*, with the plastics identification table, 4 Edition, Hanser (Munich), 1999.

If the identity of the polymer is confirmed for the article, then the migration testing described below should be carried out.

If the polymer type is not polyamide/melamine then this should be reported and no migration tests needs to be conducted.

7.3 Surface area calculation of a test specimen

In the case of kitchenware, the surface area in contact with foods for the test can be calculated from the measured dimensions of the test specimen. For the calculation of the surface area, the surface area can be seen as the sum of all the areas that make up the food contact surface of an article. Empirical methods can be used to calculate the surface area by placing the article on a piece of millimetric or plain paper of sufficient size. The paper is then wrapped around the contour of the article and cut to size. The contact area is then calculated by either counting the area based on millimetric measurements or by weighing the paper and converting the weight to the surface area.

Note: for articles tested by immersion the area of both sides should be included in the calculation of the contact area.

7.4 Rules on testing conditions

7.4.1 Type of contact: total immersion or article filling

According to the type of article the test should be performed by total immersion or by

article filling.

If the article is intended to be immersed partially or totally in the food (e.g. spatulas, spoons, ladles, etc), then the test is performed by immersion. The test specimens should be immersed in the simulant to cover entirely the functional part that comes into contact with the foodstuff.

[Note: the functional part means for example the round part of the spoon]

If the article is intended to be filled with food (e.g. plates, cups etc) then the test should be performed by article filling.

The surface area and simulant volume should be recorded.

7.4.2 Repeated use article testing

Until 31/12/2012 the rules for testing repeated use articles given in Directive 2002/72/EC should be applied. The provisions in Directive 2002/72/EC require three successive migration tests to be carried out for all articles intended for repeated use and the results of the **third test** are taken. Therefore for polyamide and melamine kitchenware three successive migration tests should be performed.

As of 01/01/2013 the rules under Regulation (EU) No 10/2011 will apply. These provisions include that:

- For primary aromatic amine migration from polyamide kitchenware only one migration test will be carried out (for non-detectable substances, its compliance will be checked on the basis of the level of the migration found in the **first** test).
- For formaldehyde migration from melamine kitchenware, three migration tests are carried out. Its compliance shall be checked on the basis of the level of the migration found in the **third test**.

7.4.3 Simulants

The test is conducted with simulant B 3% (w/v) acetic acid, as it has been demonstrated that this simulant represents the worst case for the migration of PAAs from polyamide kitchenware and for formaldehyde from melamine kitchenware.

7.4.4 Time-temperature exposure conditions

Time and temperature conditions should be in accordance with the relevant legislation (Directive 82/711/EEC, as amended, and Regulation (EU) No 10/2011), on the basis of the specific function of the kitchenware article(s).

Recommendations on time-temperature exposure conditions for kitchenware have been described in Guidelines on testing conditions for articles in contact with foodstuffs (EUR 23814 EN 2009). The test conditions defined in the aforementioned guidelines were selected based on the relevant Directives or Regulation for food contact materials and articles. These are harmonised for plastics at EU level.

Articles that could be used or foreseeably used during cooking, (e.g. ladles, spatulas, spoons) the test conditions should be 2 hours (– 0, + 5 minutes) at the boiling point of the simulant (tolerance is +/- 3°C at 100°C).

Articles that could not foreseeably be used for cooking, (e.g. bowls, cups, plates, potato mashers, tongs) the test conditions should be 2 hours (– 0, + 5 minutes) at 70°C measured in the simulant itself (+/- 2°C).

Other:

Testing conditions should be carefully chosen based on expert judgment and labelling to reflect the worst case scenario based on the use and function of the article. In addition, if a specific labelling information/instruction contains a maximum temperature for use, the temperature conditions will be chosen accordingly from the corresponding temperature exposures in the relevant legislation. In any case the minimum contact time for the migration test should be at least 30 minutes.¹

7.5 Preparation of laboratory samples

Complete traceability from the sample to the test result should be ensured.

Dust may be removed by wiping the sample with a lint-free cloth or brushing with a soft brush. If articles are labelled with an instruction that they should be cleaned before use then this instruction should be followed before testing.

7.6 Procedure for repeat use kitchenware – 3 successive migration tests

Note: Although Commission Directive 2002/72/EC is repealed by Regulation EC No 10/2011 as from 1 May 2011, the provisions on verification of compliance with migration limits for repeated use articles (Article 23) should be applied until 31/12/2012.

Depending on the article type the migration test is performed either by immersion or by filling.

The experimental set-up must limit the evaporation that occurs during the exposure phase. The volume or weight of the simulant should be recorded following the completion of the test.

7.6.1 Lot(s) of individual articles

Polyamide kitchenware articles that require testing by immersion

The three test specimens for the specific migration test are tested together in one beaker for the first exposure and the simulant is analysed for PAAs.

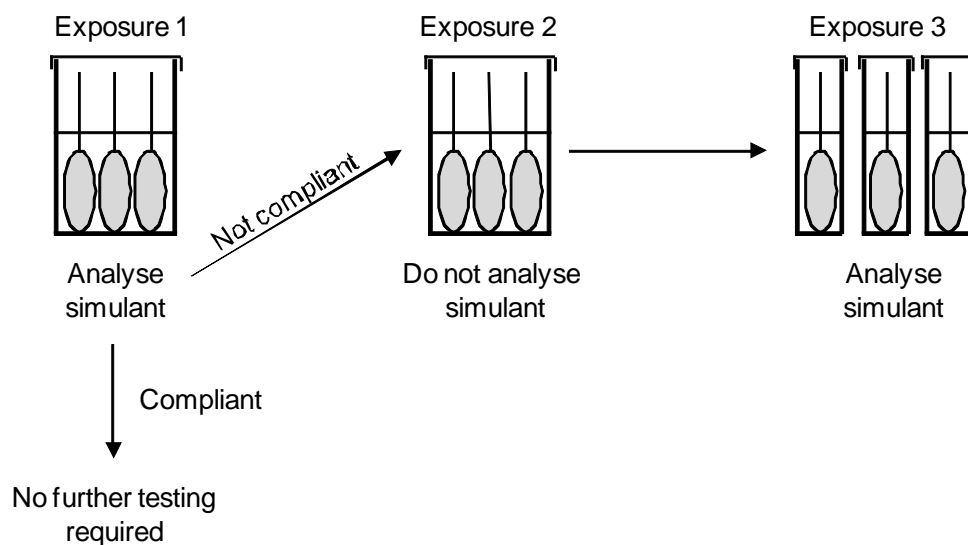
If the test specimens are compliant in this first test assuming that all of the PAAs measured in the simulant have migrated from the article with the smallest surface area (i.e. the worst case), then no further testing is required.

Note: Migration tests are normally conducted three times for repeat use articles, and the results of the third migration taken into account. However, the migration of PAAs is known to decrease after the first migration, therefore in this scheme the simulant from the 1st migration is analysed for PAAs and if results are compliant then no further testing is required.

If the samples are not compliant a second exposure should be carried out in the same way (i.e. all the three test specimens exposed together in one beaker).

¹ A minimum contact time should be 30 minutes because it cannot be demonstrated that home or catering use would always be less than 5 minutes.

The samples should then be subjected to the third migration test but individually (1 test specimen in 1 beaker) (see below).



The three portions of simulant derived from exposure 3 should be tested for PAAs.

Note: when data is available to demonstrate that the presence of cut edges does not have a significant effect on the migration results, then test specimens may be cut into a few pieces prior to migration testing.

For example, the following approach has been found to be suitable:

Materials:

3 beakers (cylindrical flask) of diameter as narrow as possible whilst accommodating the width of the article (usually 500 ml and 800 ml long necks have been found adequate), and 3 glass or tightly fitted aluminium foil covers. Hotplate (or oven or Bunsen burner), cutting implement, a timer, 10 anti-bumping granules/ beads
Solution of 3% w/v aqueous acetic acid in sufficient amount for the test (10 litres)

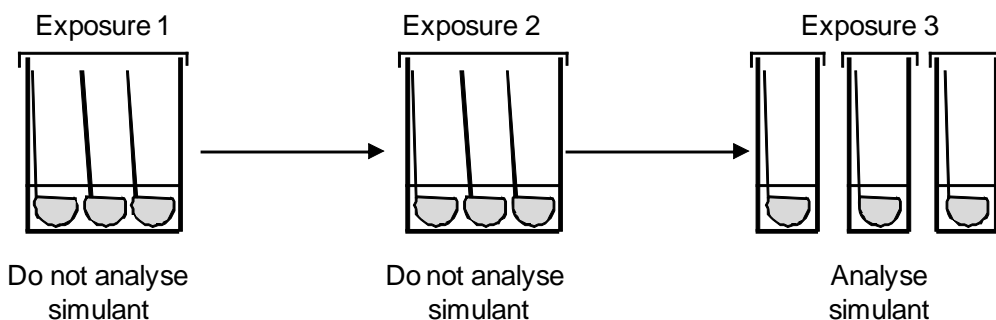
Test protocol:

Take one beaker (cylindrical flask) of diameter as narrow as possible whilst accommodating the width for containing 3 articles together fully exposed. Take three samples and cut for each the handle at the height of the beaker minus 1 cm. Prepare a solution of 3% w/v aqueous acetic acid and heat up a portion to the testing temperature. Fill the beaker with the simulant equilibrated at the test temperature to a volume able to submerge at least the entire "food contact surface area" of the test specimen (1cm of the handle can be included if desired). Add 2-3 anti-bumping granules to the beaker. Immerse all 3 articles together in one beaker of solution, covering the top of the beaker with a glass plate or hour glass or aluminium foil in order to reduce the loss of the simulant by evaporation. When temperature reaches testing temperature, start the timer to record time and set to 2 hrs. After 2 hrs, stop the timer. Remove the test specimens from the simulant. Determine the simulant volume/weight (or add simulant till original volume/weight) and analyse the exposed simulant for PAAs. If the results of this test are below the limit, then the set is considered to be compliant and no further testing is required. If the articles fail the test, then conduct a second migration in the same way (i.e. with all 3 test specimens exposed together in the same portion of fresh simulant). It is not necessary to analyse the simulant from this second exposure. Separate the articles. Proceed with the third migration test with fresh simulant (previously equilibrated at the test temperature). Immerse each test specimen in a separate beaker; cover the top of the beaker with a glass plate or hour glass in order to reduce the loss of the simulant by evaporation. When the temperature reaches testing temperature start the timer to record time and set to 2 hrs. After 2 hrs, stop the timer. Remove the test specimens from the simulant and allow the simulant to cool to room temperature before proceeding. Determine the simulant volume. Determine the concentration of PAAs in each of the three exposed simulant samples. Retain enough simulant to repeat the analysis if necessary.

Melamine kitchenware articles that require testing by immersion

The three test specimens for the migration test are tested together in one beaker for the first exposure. The simulant is removed and a second successive migration test performed in the same way (i.e. all three kitchenware articles immersed together).

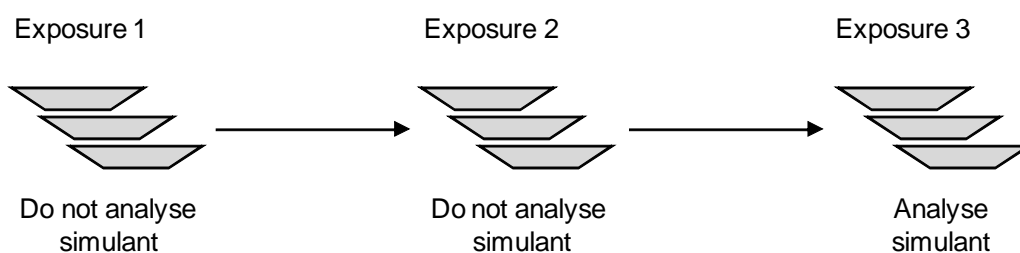
The simulant from the first and second test is discarded. The samples should then be subjected to the third migration test but individually (1 article in 1 beaker).



The three portions of simulant derived from exposure 3 should be tested for migration of formaldehyde.

Melamine kitchenware articles that require testing by filling

Each test specimen is filled to 0.5 cm below the rim and exposed to the simulant. The simulant is removed and a second and third successive migration tests performed in the same way. The simulant from the first and second test can be discarded.



The three portions of simulant derived from exposure 3 should be tested for formaldehyde.

7.6.2 Lot(s) of sets of articles

A lot of grouped articles sold in sets (e.g. with different shapes in one set)

Polyamide kitchenware articles that require testing by immersion

The three sets of test specimens for the migration test are tested together in one beaker for the first exposure as shown below and the simulant is analysed for PAAs.

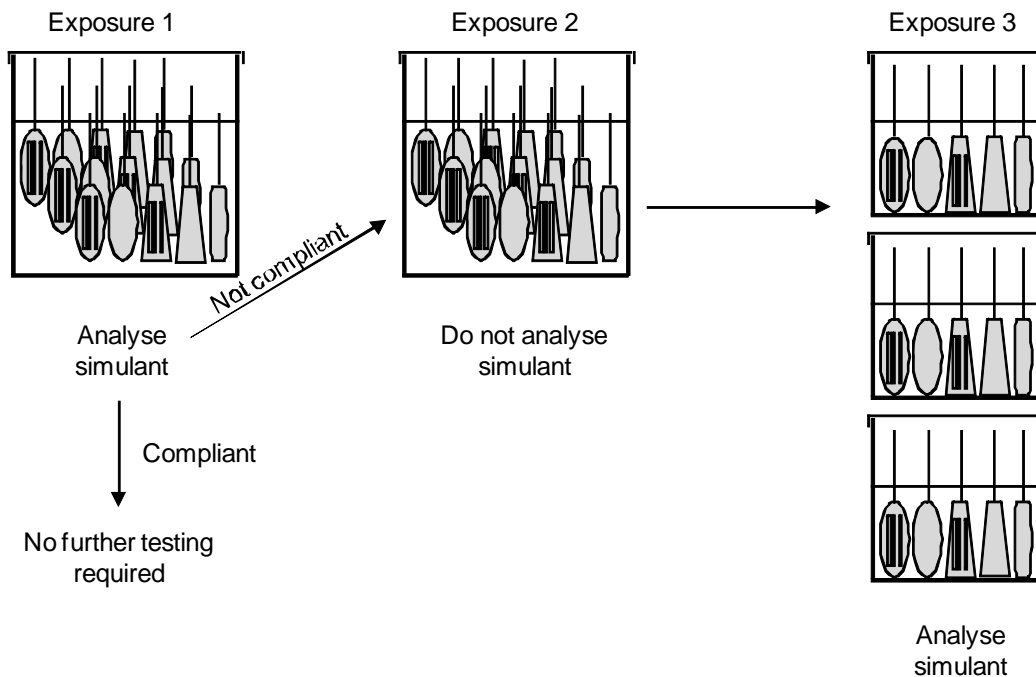
If the test specimens are compliant in this first test assuming that all of the PAAs measured in the simulant have migrated from the article with the smallest surface area (i.e. the worst case), then no further testing is required.

Note: Migration tests are normally conducted three times for repeat use articles, and the results of the third migration taken into account. However, the migration of PAAs is known to decrease after the first migration, therefore in this scheme the simulant from the 1st migration is analysed for PAAs and if results are compliant then no further testing is required. If the sample sets are not compliant a second exposure should be carried out in the same way (i.e. the three sets of test specimens exposed together in one beaker).

Note: The experimental design above assumes that all the migrating PAAs have come from one article which is the smallest article in order to give the worst case.

The sample sets should then be subjected to the third migration test but each set separately (1 set of test specimens in 1 beaker) (see below).

Note: This design considers that the consumer purchases the whole set, and therefore the set itself is considered an entity. This implies that each article in the set does not represent a test specimen, but the set as a whole.



The three portions of simulant derived from exposure 3 should be tested for PAAs.

Note: when data is available to demonstrate that the presence of cut edges does not have a significant effect on the migration results, then test specimens may be cut into a few pieces prior to migration testing.

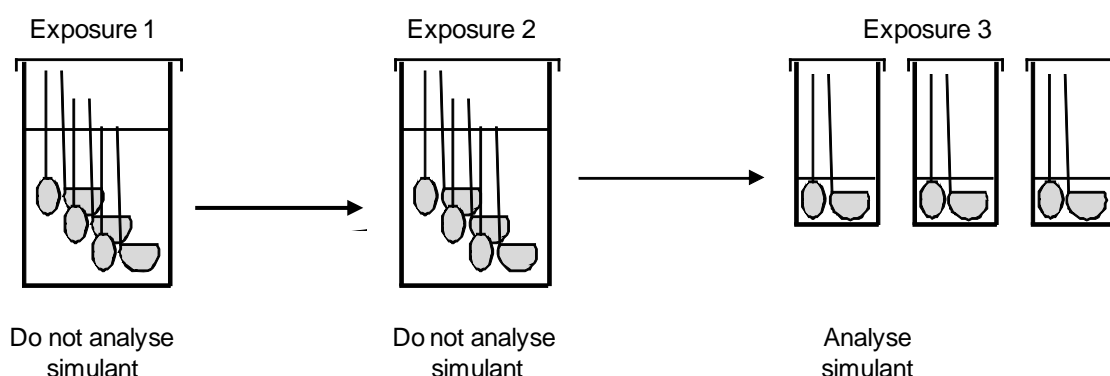
Note: if a set contains article types for which different time-temperature exposure conditions are specified in the Guidelines on testing conditions for articles in contact with foodstuffs (EUR 23814 EN 2009) then those test specimens to be tested at the same temperature should be grouped together in the exposures. For example if the set contained a slotted spoon, a turner, a potato masher and a whisk then the slotted spoon and the turner should be exposed together under one set of time/temperature conditions (i.e. tested under conditions appropriate for contact with foods at > 100°C) and the potato masher and whisk exposed under a second set of time/temperature conditions (i.e. tested under conditions appropriate for contact with foods at < 100°C).

Note: if a migration only mildly exceeding the limit is found in the first exposure, it is should be considered to split up at least one set in the second exposure and analyse the simulant in order to identify the migrating article in the set. The third test would then be performed separately only on the articles which were positive in the second test. The set is however considered one entity where each article is part of the same test specimen for compliance purposes.

Melamine kitchenware articles that require testing by immersion

The three sets of test specimens for the migration test are tested together in one beaker for the first exposure. The simulant is removed and a second successive migration test performed in the same way (i.e. all three sets of test specimens immersed together). The simulant from the first and second test can be discarded.

The sets of test specimens should then be subjected to the third migration test but each set of test specimens tested separately (1 set of test specimens in 1 beaker) (see below).



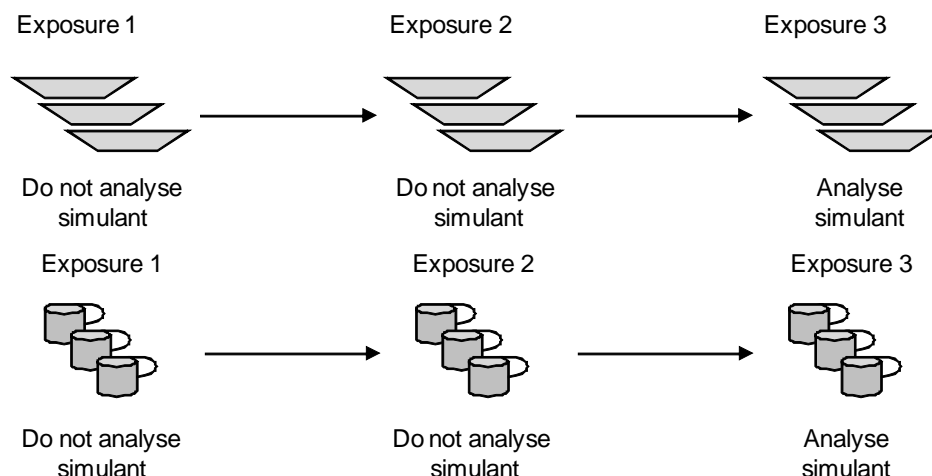
The three portions of simulant derived from exposure 3 should be tested for formaldehyde.

Note: if a set contains article types for which different time-temperature exposure conditions are specified in the Guidelines on testing conditions for articles in contact with foodstuffs (EUR 23814 EN 2009) then those test specimens to be tested at the same temperature should be grouped together in the exposures. For example if the set contained a slotted spoon, a turner, a potato masher and a whisk then the slotted spoon and the turner should be exposed together under one set of time/temperature conditions (i.e. tested under conditions appropriate for contact with foods at > 100°C) and the potato masher and whisk exposed under a second set of time/temperature conditions (i.e. tested under conditions appropriate for contact with foods at < 100°C).

Melamine kitchenware articles that require testing by filling

The three sets for the migration test are exposed to simulant in parallel.

Each test specimen is filled to 0.5 cm below the rim and exposed to the simulant. The simulant is removed and a second and third successive migration test performed in the same way. The simulant from the first and second test is discarded.



The three portions of simulant derived from exposure 3 for each article type in the set should be tested for formaldehyde.

Note: if a set is composed of articles requiring different test conditions (e.g. plate and spoon), the spoon will be tested by immersion and the plate by filling.

8 METHODS OF ANALYSIS

8.1 General requirements

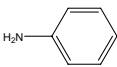
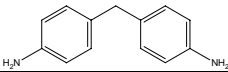
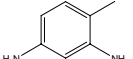
Methods of analysis used for food control purposes must comply with the provisions of Annex III (characterisation of methods of analysis), of Regulation (EC) No 882/2004. Laboratories performing the physical checks must fulfil the requirements of 1) being accredited laboratories (ISO 17025) and 2) using validated methods for PAAs and for FA according to the guidelines and criteria specifically set by the EU-RL-NRL Network (EUR 24105, 2009).

8.2 Primary aromatic amines

Examples of descriptions of methods using LC-MS and HPLC-DAD for the specific identification and quantification of PAAs can be found in Annex 1.

The substances that have generally been found to migrate at the highest levels from polyamide kitchenware are aniline, 4,4'-methylenedianiline (4,4'-MDA) and 2,4-toluenediamine (2,4-TDA).

Note: A spectrophotometric method is available for the determination of total PAAs (Brauer and Funke, 2002). This method gives result in units of μg aniline equivalents/kg simulant which does not correspond to the legislative requirements of Directive 2002/72/EC, as amended and Regulation (EU) No 10/2011). However, experimental data has demonstrated that 4,4'-MDA has a response 57% compared to aniline and 2,4-TDA has a response 58% compared to aniline. This method can be used for screening purposes and cannot be used to demonstrate non-compliance. The method may be used for compliance only when the calculated concentration in aniline equivalents has been recalculated as a total PAAs concentration. If the total PAA concentration is greater than 2 $\mu\text{g}/\text{kg}$ food simulant then a specific method of analysis should be used (e.g. LC-MS or HPLC-DAD as described in Annex 1).

Name	Abbreviation	CAS No.	Structure	MW
Aniline	ANL	62-53-3		93,1
4,4'-Methylenedianiline	4,4'-MDA	101-77-9		198,3
2,4-Toluenediamine	2,4-TDA	95-80-7		122,1

8.3 Formaldehyde

Examples of descriptions of methods can be found in Annex 2.

9 PRESENTATION OF RESULTS AND COMPLIANCE

9.1 Presentation of results

Regulation (EU) No 10/2011 will apply in the frame of Regulation (EU) No 284/2011 (from 1 May 2011). Following the migration test and analysis the concentration of the target substances (PAAs or FA) measured in the exposed simulant should be converted to a migration value in units of mg/kg.

For articles that:

- are fillable and have a volume in the range of 500 ml up to 10 l
- are (intended to be) in contact with food for infants and young children

the analytical result needs to be corrected for the difference in surface-to-volume ratio between the experiment and the real food contact conditions.

For articles that:

- fillable articles that have a volume below 500 ml or higher than 10 l.
- articles for which it is not practical to estimate the relationship between the surface of that material and the volume of food.

the analytical result needs to be corrected for the difference in surface-to-volume ratio between the experiment and the food contact conditions of 6 dm²/kg.

The analytical results have to be reported together with the expanded measurement uncertainty at the legislative limit, as referred in the EU-RL-NRL Network (EUR 24105, 2009).

9.2 Establishment of compliance

As regards polyamide kitchenware, the specific migration limit for PAAs is set as non-detectable except for those on the positive list of the relevant legislation (CAS 108-45-2, CAS 1477-55-0, CAS 106246-33-7). They should not release into foods or food simulants PAAs in a detectable quantity. The detection limit applies to the sum of primary aromatic amines. For the purpose of the analysis the detection limit for PAAs is set at 0.01 mg/kg food or food simulants.

As regards melamine kitchenware, the specific migration limit for formaldehyde with hexamethylenetetramine is 15 mg /kg food.

Compliance with maximum levels should be established on the basis of the levels determined in the laboratory samples.

The limit is exceeded when the analytical result is higher than the relevant specific migration limit (SML) taking into account measurement uncertainty.

9.3 Quality and safety

9.3.1 Internal quality control and proficiency

Official control laboratories must comply with Regulation (EC) No 882/2004 on the subject of additional measures concerning the official control of foodstuffs.

Laboratories should be able to demonstrate that they have internal quality control procedures in place. Examples of these are the "ISO/AOAC/IUPAC Guidelines on Internal Quality Control in Analytical Chemistry Laboratories".

Participation in appropriate proficiency testing schemes which comply with the "International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories" developed under the auspices of IUPAC/ISO/AOAC and ISO 17043.

9.3.2 Health and Safety

Suitable protective clothing, eye protection and gloves should be worn at all times.

Refer to the Material Safety Data Sheets for all relevant chemicals. Some chemicals are toxic compounds and/or suspected human carcinogens. All solids, standard solutions and samples should be treated with the utmost care.

Appropriate laboratory care and precautions should be exercised at all times.

Appropriate disposal of exposed simulants, solvents and used kitchenware must be exercised.

10 TEST REPORT

The test report should be at minimum provided in the English language

The test report should contain, as a minimum, the following:

Identification of the sample (i.e. consignment number, lot number, sample number);

Date and method of obtaining the laboratory sample;

Name of laboratory; Name of person responsible for analysis; Date of report; Analyte(s); A reference to the method(s) used; Performance characteristics of the method(s) used as in Regulation (EC) No 882/2004 and EUR 24105, 2009. The parameters must include limit of detection [for each substance in the case of PAAs, limit of quantification (for formaldehyde), and measurement uncertainty.

All information necessary for complete identification of the sample, e.g. chemical type, trade mark, grade, batch number, dimensions, shape etc – pictures should be included together with a ruler

The type of the migration test (i.e. immersion or article fill, number of exposures);

The duration and temperature;

The surface area exposed and volume of food simulant used;

Any departures from the standard methods presented here, reasons for the departures;

Any relevant comments on the test results;

Details of any confirmation procedure(s), if any.

11 REFERENCES

Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC.

Commission Regulation (EC) No 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food

Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29

April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs.

Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs.

Council Directive 85/572/EEC of 19 December 1985 laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs.

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EN 13130-1 Materials and articles in contact with foodstuffs: Plastics substances subject to limitation Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants.

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Dietrich Braun: Simple Methods for Identification of Plastics, with the plastics identification table, 4 Edition, Hanser Munich, 1999

BfR Opinion Nr. 012/2011, 09 March 2011, Release of melamine and formaldehyde from dishes and kitchen utensils, www.bfr.bund.de

12 ANNEXES

Annex 1:

Examples of methods for Primary aromatic amines from polyamide kitchenware

Annex 2:

Examples of methods for formaldehyde from melamine kitchenware

Annex 1 – Primary aromatic amines

This annex presents three HPLC-MS based methods and one HPLC-DAD method for the determination of primary aromatic amines (PAA) in the food simulants distilled water and 3 % acetic acid.

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1 PROTOCOL A BY LC-MS

1.1 Principle

This method was elaborated and validated for those PAA's being derived from isocyanates listed in Directive 2002/72/EC and the following Directives. The method can be extended to amines of any other origin. This protocol has been validated by interlaboratory comparisons between Danish laboratories (Mortensen et al, 2005) and an EU validation organized by BfR (report: 2009)

1.2 Materials and Chemicals

NOTE: All reagents should be of recognised analytical quality unless otherwise stated.

1.2.1 Analytes and chemicals

1.2.1.1 Methanol (pro analysis)

1.2.1.2 Ethanol (absolute, pro analysis)

1.2.1.3 Glass distilled water or purified water

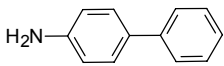

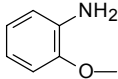
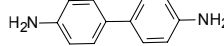
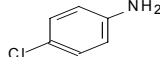
1.2.1.4 Pentafluoropropionic acid (PFPA), e.g. 97 %

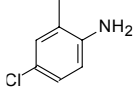
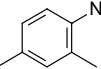

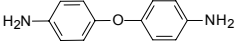
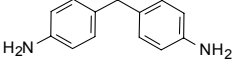
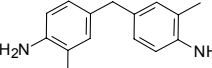
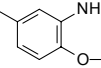
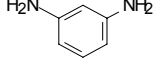
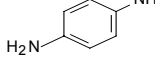
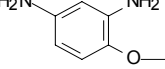
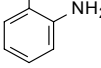

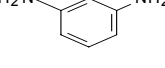
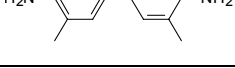
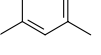
1.2.1.5 Glacial acetic acid, 100 % (pro analysis)

1.2.1.6 Acetone

1.2.1.7 Analytical standards of the 20 PAA

Table 1: Analytical grade of the standards of the 20 PAA

Name (Abbreviation)	CAS No.	Structure	MW	Purity
4-Aminobiphenyl (4-ABP)	92-67-1		169.2	98.4
Aniline (ANL)	62-53-3		93.1	99
o-Anisidine (o-ASD)	90-04-0		123.2	99+
Benzidine (BNZ)	92-87-5		184.2	99.5
4-Chloro-Aniline (4-CA)	106-47-8		127.6	98

4-Chloro-o-Toluidine (4-CoT)	95-69-2		141.6	98
2,4-Dimethylaniline (2,4-DMA)	95-68-1		121.2	99
2,6-Dimethylaniline (2,6-DMA)	87-62-7		121.2	99
4,4-Diaminodiphenylether (4,4'-DPE)	101-80-4		200.2	98
4,4'-Methylenedianiline (4,4'-MDA)	101-77-9		198.3	97
4,4-Methylenedi-o-toluidine (4,4'-MDoT)	838-88-0		226.3	95+
2-Methoxy-5-Methylaniline (2-M-5-MA)	120-71-8		137.2	95
m-Phenylenediamine (m-PDA)	108-45-2		108.1	99+
p-Phenylenediamine (p-PDA)	106-50-3		108.1	99+
4-Methoxy-m-phenylenediamine (4-M-mPDA)	615-05-4		138.2	-
o-Toluidine (o-T)	95-53-4		107.2	99.5+
2,4-Toluenediamine (2,4-TDA)	95-80-7		122.1	99+
2,6-Toluenediamine (2,6-TDA)	823-40-5		122.1	99.9
3,3-Dimethylbenzidine (3,3-DMB)	119-93-7		212.3	99
2,4,5-Trimethylaniline (2,4,5-TMA)	137-17-7		135.2	99

1.2.2 Solutions

1.2.2.1 Elution solution

Mobil phase A: Prepare 4.7 mM PFPA in methanol by adding 0.504 ml PFPA to 1

litre of methanol.

Mobil phase B: Prepare 4.7 mM PFPA in distilled water by adding 0.504 ml PFPA to 1 litre of Milli-Q water.

1.2.2.2 Stock solutions

Stock solutions of each compound at 0.5 mg/ml is made by weighing 10.0 mg of each of the analytes exactly into 20 ml ethanol (for 4,4-diaminodiphenylether into 20 ml acetone). Mix carefully. If any precipitate, ultrasonic may be used to facilitate the dissolution.

NOTE: The stock solutions may be stored in a refrigerator at 4 °C up to 1 year. p-PDA stock solution is only stable in a refrigerator up to a month.

1.2.2.3 Diluted stock solutions

A multistandard with 5µg/ml of each PAA is prepared by diluting the stock solutions with 3 % acetic acid or distilled water. The p-PDA is prepared as an independent solution.

NOTE: The multistandard may be stored in a refrigerator at 4 °C up to 5 weeks. The p-PDA must only be stored for 3 days at 4 °C.

1.2.2.4 Calibration solutions

Prepare 4 standard solutions at the concentration of 2, 10, 25 and 50 µg/l.

NOTE: The calibration standards are stable in a refrigerator at 4 °C for 3 days. Many PAA's are sensitive to light. Therefore storage of the PAA solutions should be done with exclusion of light either by the use of dark glass bottles or by covering the containers with aluminium foil.

1.2.2.5 Check sample

Second stock solutions are made of the two PAA's: 4,4 MDA and o-ASD. From these stock solutions a check sample containing 10 µg/l of 4,4 MDA and 10 µg/l of o-ASD is prepared by dilution with distilled water. This sample is stable in a refrigerator at 4 °C up to 6 month.

1.2.3 Apparatus

1.2.3.1 High performance liquid chromatograph, HPLC with a column heater

1.2.3.2 Triple quadruple mass spectrometer with electrospray interface.

1.2.3.3 Balance

1.2.3.4 Ultrasonic bath

1.2.3.5 LC-MS/MS quantification software

1.2.3.6 HPLC column:

The following column has been found suitable: Pre-column filter: 0.5 µm stainless filter, Column: Zorbax SB-C3, (2.1mm* 150mm, 5 µm)

1.3 Procedures

1.3.1 Analysis

Specific determination of PAA is performed on aqueous migration solutions of

distilled water or 3 % acetic acid from migration tests.

1.3.2 HPLC conditions

Injection volume: 3 µl Column temperature: 40 °C

Mobil phase A: 4.7 mM PFPA in methanol

Mobil phase B: 4.7 mM PFPA in distilled water (5.3.2.3)

Gradient:

Time	% A	% B	Flow (ml/min)
0	5	95	0.2
3	20	80	0.2
7.5	80	20	0.5
8.0	80	20	0.5
11.0	5	95	0.5
14.0	5	95	0.2

Mass spectrometer conditions

Ion mode:	ESI+	Capillary voltage (kV):	1.0
Cone (V):	20	Hex 1 (V):	20
Aperture (V):	0.2	Hex 2 (V):	0.2
Source temperature (°C):	130	Desolvation temperature (°C)	400
Cone gas flow (l/h)	40	Desolvation gas flow (l/h):	775
LM 1 and LM 2 resolution:	13	HM 1 and LM 2 resolution:	13
Ion energy 1 and 2:	1.0	Multiplier (V):	650

Table 2: MS/MS parameters for the specific PAA's:

PAA	MW	MRM	Retention time* (min)	Ret. window (min)	Collision Energy (eV)	Dwell time
m-PDA	108.1	109.1 > 92.2	3,0	1.5 – 4.8	22	0.5
2,6-TDA	122.1	123.1 > 108.3	3,3	1.5 – 4.8	18	0.5
2,4-TDA	122.1	123.1 > 108.3	3,5	1.5 – 4.8	18	0.5
ANL	93.1	94.0 > 77.2	4,0	1.5 – 4.8	18	0.5
4,4'-DPE	200.2	201.1 > 108.3	5,4	4.8 – 6.3	18	0.2
4,4'-MDA	198.3	199.1 > 106.2	5,6	4.8 – 6.3	22	0.2
3,3'-DMB	212.3	212.1 > 196.0	8,0	6.2 – 7.0	25	0.2

*) Observed retention times with given column.

1.3.3 HPLC calibration

In every analytical series the quantitative response of a check sample of two PAA's are determined. The check sample is injected 4 times to receive a stable response.

Note: The response of the 4th injection of a check sample must be constant across the analytical series. This is checked by the use of a control card.

The calibration solutions at 4 concentrations are then injected to perform the calibration curve. The response of each calibration standard is determined twice by repeating the analysis of the calibrations standards for every 10-15 samples.

A series of 10 -15 unknown samples are injected in addition to a blank sample. In every case the samples are determined by double injections.

1.3.4 Quantification of analyte level

The calculation of analyte levels in the samples is done by the use of external standard curves generated from the analysis of the standard solutions at 4 different levels. For every PAA each calibration standard is determined twice and the response of the two calibration standards is used for the calibration curve. Linear regression of calibration data is made using a weighted least squares method (weight 1/x).

NOTE: The calibration curves should be linear and the correlation coefficient should be 0.99 or better. Moreover the linearity of each PAA can be assessed by examining the plot of the residuals.

Samples with a PAA concentration above 50 µg/l are diluted with distilled water or 3 % acetic acid to a level < 50 µg/l and the sample is analysed again.

1.3.5 Confirmation of identity

If a PAA is detected, the identity of the PAA is verified by the detection of a supplementary daughter ion (see table 3). Verification is done by comparing the ratio of the signal of the supplementary daughter ion to those detected in the calibration standards.

Table 3: Additional daughter ions for verification of analyte identity

PAA	Retention time (min)*	Mother-ion > Daughter-ion
m-PDA	3,0	109,1 > 65,4
2,6-TDA	3,3	123,1 > 106,2
2,4-TDA	3,5	123,1 > 106,2
ANL	4,0	94,0 > 51,3
4,4'-DPE	5,4	201,1 > 184,0
4,4'-MDA	5,6	199,1 > 77,2
3,3'-DMB	8,0	212,1 > 211,1

*) Observed retention times with given column

1.4 Precision data

1.4.1 Validation

S_r: 4 – 19 % (RSD) at the level of 2 µg/l

S_{R (in house)}: 5 – 26 % (RSD) at the level of 2 µg/l

S_r: 2 – 12 % (RSD) at the level of 25 µg/l

S_{R (in house)}: 3 – 17 % (RSD) at the level of 25 µg/l

Detailed information on precision data from in-house validation is available for every PAA if needed.

1.4.2 Detection limits

Table 4: Detection limits on LC-MS/MS

LOD				
PAA	µg PAA/l		µg Aniline/l	
	Water	Acid	Water	Acid
m-PDA	0.6	0.9	0.6	0.8
2,6-TDA	0.3	1.1	0.2	0.9
2,4-TDA	0.5	1.0	0.4	0.7
ANL	0.5	0.8	0.5	0.8
4,4'-DPE	0.5	0.9	0.2	0.4
4,4'-MDA	0.9	0.7	0.4	0.3
3,3'-DMB	0.7	1.0	0.3	0.4

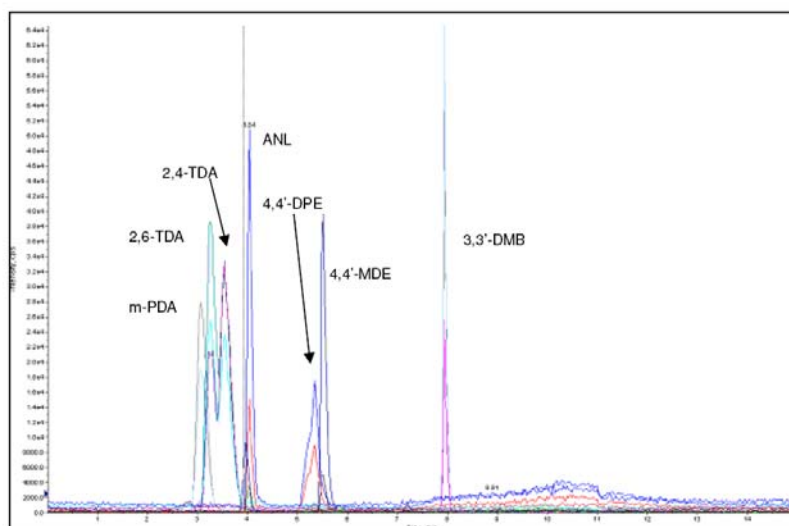
$$\text{LOD} = \text{blank} + 3 \cdot s_R$$

s_R is the internal reproducibility for samples with a level of 2 µg PAA/L.

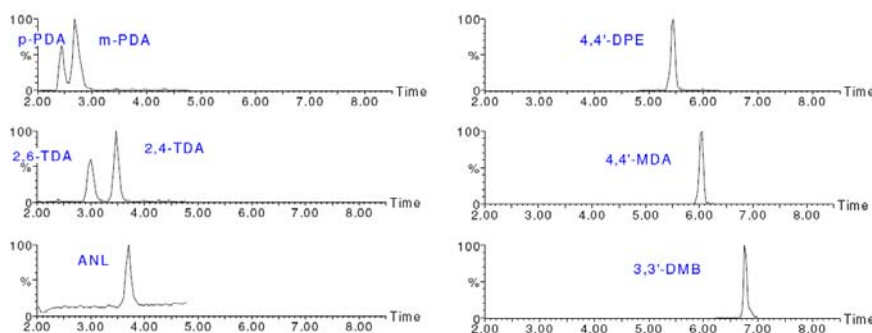
1.4.3 Recovery

Recoveries of the 20 PAA in distilled water or 3 % acetic acid (from spiking at the concentrations of 2-25 µg/l) was 89-107 %

Determination of primary aromatic amines with HPLC – standard chromatogram


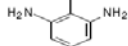
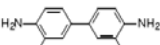
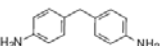
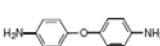




Chromatograms of 7 PAA detected with LC-MS/MS



Chromatograms obtained with a 10 µg/l multi-standard solution in 3 % acetic acid. Data acquisition was performed in the MRM mode using the primary MH^+ or M^+ precursor ions listed in Table 2.

Primary Aromatic Amines (PAA) in the method

Name	Abbreviation	CAS No.	Structure	MW [g/mol]
2,4-Toluenediamine	2,4-TDA	095-80-7		122,2
2,6-Toluenediamine	2,6-TDA	823-40-5		122,2
3,3'-Dimethylbenzidine	3,3'-DMB	119-93-7		212,3
4,4'-Methylenedianiline (= 4,4'-methylenebis-benzendiamine)	4,4'-MDA	101-77-9		198,3
4,4'-Diaminodiphenylether (= 4,4'-Oxydianiline)	4,4'-DPE	101-80-4		200,2
Aniline	ANL	062-53-3		93,1
m-Phenylenediamine	m-PDA	108-45-2		108,1

2 PROTOCOL B BY LC-MS

This procedure describes a method for the quantitative determination of primary aromatic amines (PAAs) in acidic aqueous food simulant 3% acetic acid, by liquid chromatography coupled to mass spectrometry (LC-MS/MS). The objective is the determination of PAAs in the specific migration tests of food contact materials. This protocol has been validated by a single laboratory validation by NRL-ES (Reference Sendon et al, 2010). The method has been validated for 8 PAAs, which are summarized in the table below, along with their corresponding working range in µg/kg simulant.

Amine	CAS	Range µg/kg simulant
m-Phenylenediamine (m-PDA)	108-45-2	≥2,5
2,6-Toluenediamine (2,6-TDA)	823-40-5	≥2,5
2,4-Toluenediamine (2,4-TDA)	95-80-7	≥2,5
1,5- Diaminenaphthalene (1,5-DAN)	2243-62-1	≥2,5
Aniline (ANL)	62-53-3	≥2,5
4,4'-Diaminodiphenylether (4,4'-DPE)	101-80-4	≥2,5
4,4'-Methylenedianiline (4,4'-MDA)	101-77-9	≥2,5
3,3'- Dimethylbenzidine (3,3-DMB)	119-93-7	≥2,5

2.1 Principle

The acidic food simulant obtained in the migration test is submitted to neutralization with ammonia before chromatography analysis. Individual PAAs are separated on a mixed-mode reverse phase C18 column and mass detection is performed using positive electrospray ionisation (ESI+), in multireaction monitoring mode (MRM).

2.2 Materials and chemicals

2.2.1 Analytes and chemicals

All reagents should be of analytical grade, unless otherwise specified.

2.2.1.1 Glacial acetic acid

2.2.1.2 Methanol, LC MS quality

2.2.1.3 Purified water

2.2.1.4 Ammonium acetate, 99%

2.2.1.5 Ammonium hydroxide 25%

2.2.1.6 m-phenylenediamine (m-PDA), CAS 00108-45-2,

2.2.1.7 2,6-Toluenediamine (2,6-TDA), CAS 00823-40-5

2.2.1.8 2,4-Toluenediamine (2,4-TDA), CAS 00095-80-7

2.2.1.9 Aniline (ANL), CAS 00062-53-3

2.2.1.10 1,5- Diaminonaphthalene (1,5-DAN), CAS 02243-62-1

2.2.1.11 4,4'-Diaminodiphenylether (4,4'-DPE), CAS 00101-80-4

2.2.1.12 4,4'-Methylenedianiline (4,4'-MDA), CAS 00101-77-9

2.2.1.13 3,3'- Dimethylbenzidine (3,3-DMB), CAS 00119-93-7

2.2.2 Solutions

100 mM ammonium acetate: weight 7.72 g, dissolve in water, adjust volume to 1L

10 mM ammonium acetate 10 mM: dilute 50 ml of 100mM ammonium acetate to 500 ml with water

2.2.2.1 PAAs individual stock solutions:

Weigh to the nearest 0.1 mg approximately 25 mg of each amine into individual 50 ml volumetric flasks, dissolve with methanol and make up to volume. Calculate the exact concentration in $\mu\text{g/ml}$ for each analyte.

NOTE: The stock solutions may be stored in a refrigerator (4-8°C) and protected from light up to 6 months.

2.2.2.2 PAAs Mix stock solution (5 $\mu\text{g/ml}$)

Transfer into a 10 ml volumetric flask 100 μl of each stock solution, dilute and make up to volume with methanol. Calculate the exact concentration in $\mu\text{g/ml}$ for each analyte.

NOTE: This mix solution may be stored in a refrigerator (4-8°C) and protected from light up to 6 months.

2.2.2.3 PAA's Diluted Mix solution (100 ng/ml)

Transfer into a 25 ml volumetric flask 500 µl of the mix stock solution, dilute and make up to volume with 3% acetic acid. Calculate the exact concentration in µg/ml for each analyte.

NOTE: This diluted mix solution may be stored in a refrigerator (4-8°C) and protected from light up to 2 weeks.

2.2.2.4 PAA's Calibration solutions (2-20 ng/ml)

Add 4 ml of simulant (3% acetic acid) subjected to the same migration conditions of time and temperature as samples, into a series of 5 ml volumetric flasks. Transfer into the flasks using micro syringes the following volumes of the diluted mix solution and make up to volume with 3% acetic acid. Neutralise the solutions by adding 250 µl of ammonium hydroxide and mix. Calculate the exact concentration in ng/ml for each analyte.

NOTE : check that the pH of the neutralized solution is basic.

NOTE: These calibration solutions must be prepared daily.

Volume of PAA's 100 ng/ml	Concentration ng/ml	Equivalent concentration in sample** µg/kg simulant
0 µl	0	0
100 µl	2	2.5
250 µl	5	6.25
500 µl	10	12.5
750 µl	15	18.75
1000 µl	20	25

** The dilution factor introduced in sample preparation has been taken into account

2.2.3 Apparatus

Analytical balance, Micro syringes: 100, 250, 500 ,1000 µl.,PTFE 0.2 µm sample filter

LC/MS-MS equipment:

- High performance liquid chromatograph, with a column heater
- Triple quadrupole mass spectrometer with electrospray ionization source
- Column: XTerra RP18, (150mm*4.6 mm* 5 µm) or equivalent
- Chromatography/data processing software

2.3 Procedures

2.3.1 Samples (3% acetic acid migration solutions)

Transfer 4 ml of the migration solution into a 5 ml volumetric flasks, make up to volume with 3% acetic acid. Neutralise the solutions by adding 250 µl of ammonium hydroxide and mix. Filter an aliquot for LC-MS analysis.

NOTE : check that the pH of the neutralized solution is basic.

2.3.2 HPLC conditions

Injection volume: 50 µl

Column temperature: 22 °C

Mobile phase A: 10 mM ammonium acetate Mobile phase B: methanol

Flow: 0.6 ml/min

Total runtime: 60 min

Gradient (linear):

Time	% A	% B
0.0 min	95	5
0.8 min	95	5
2.5 min	75	25
42.0 min	33	67
44.0 min	95	5

Mass spectrometer conditions

Ion mode:	ESI+
Capillary voltage (kV):	3
Source temperature (°C):	120
Desolvation temperature (°C):	400
Cone gas flow (l/h)	50
Desolvation gas flow (l/h):	700
LM 1 and HM 1 resolution:	14
LM 2 and HM 2 resolution:	15
Ion energy 1:	1.0
Entrance:	1
Exit:	2
Ion energy 2:	0.5
Gas collision flow (l/h)	0.3
Multiplier (V):	650

MS/MS parameters for the specific PAAs:

PAA	Segment (min)	MRM quantification MRM confirmation	Dwell (s)	Cone (V)	Collision Energy (V)	
m-PDA	0.0-7.9	108.9>91.9	0.2	20	15	
		108.9>64.8	0.2	20	15	
123.0>107.9		0.2	20	18		
123.0>105.9		0.2	20	18		
2,6-TDA		123.0>107.9	0.2	20	18	
		123.0>105.9	0.2	20	18	
2,4-TDA	8.0-13.9	158.9>143.1	0.2	25	20	
		158.9>114.8	0.2	25	20	
93.8>76.9		0.2	20	18		
93.8>51.0		0.2	20	30		
1,5 DAN		14.0-18.5	201.2>107.8	0.2	20	20
201.2>184.0			0.2	20	20	
ANL	18.0 -24.5	199.1>105.8	0.2	25	25	
		199.1>76.8	0.2	25	40	
4,4'-DPE	24.6-30.0	213.2>196.1	0.2	25	25	
		213.2>181.1	0.2	25	30	

2.3.3 HPLC Calibration

Calibration is performed by injecting 50 µl of the calibration solutions into the LC/MS under the conditions described. Because of the dilution step introduced in the sample preparation, the standard solutions represent migration solutions which are 1.25 fold higher concentrated (see table). For each PAA a linear regression calibration curve is obtained by plotting peak areas for the MRM quantification transition against the concentration values in µg/kg simulant ($y = mx + b$)

NOTE: The stability of the system should be checked by injecting several times a calibration solution until stability in the response is obtained.

NOTE: The calibration curves should be linear: $C_m = 100 \times (S_m/m) \leq 8\%$, and the correlation coefficient should be 0.99 or better. Residuals (%) should be ≤ 20 at the lowest concentration level and $\leq 15\%$ for the other concentrations of the calibration curve.

S_m = standard deviation of the slope

m = slope

Stability of the responses of calibration solutions should be checked by injecting every 5-6 injections a standard solution. Precision in terms of CV% should be $\leq 15\%$ for the lowest standard solution and $\leq 10\%$ for the other standard concentrations

2.3.4 Quantification of the analyte level

Inject 50 μl of each migration solution sample prepared as indicated, under the same conditions of the calibration solutions. If a series of samples is to be injected, a sequence should be followed so as to include a calibration solution every 4-5 sample injections. The concentration of each PAA in the sample is calculated using the external standard calibration curves obtained:

$$\text{Specific migration } (\mu\text{g/kg simulat}) = (y - b) / m$$

If the sample response is greater than that of the highest concentration standard in the calibration curve, dilute the migration solution with 3% acetic acid to a concentration level within the calibration range and neutralized as indicated. Reinject the diluted sample.

2.3.5 Confirmation of identity

The identity of the PAA is verified by:

- The retention time of the analyte shall be the same as that of the nearest calibration standard, within a margin of $\pm 5\%$
- MRM confirmation transition: a signal response detection should be obtained for the analyte in the sample, corresponding to the confirmation transition. The ion ratio (relative responses: MRM quantification / MRM confirmation) for the PAA in the sample should not differ in more than 30% with that of the nearest injected standard with a similar concentration.

2.4 Precision of the data

2.4.1 Validation

RSD (%) internal reproducibility on migration solutions from nylon utensils

PAAs	2 ng mL ⁻¹	5 ng mL ⁻¹	10 ng mL ⁻¹	20 ng mL ⁻¹
m-PDA	12.3	7.9	9.4	9.3
2,6-TDA	13.6	9.7	11.9	10.3
2,4-TDA	21.4	11.3	16.5	9.7
1,5 DAN	18.4	9.0	11.6	12.1
ANL	9.8	20.0	11.3	20.3
4,4'-DPE	12.2	6.8	10.3	5.6
4,4'-MDA	8.4	7.4	12.8	9.9
3,3'-DMB	15.2	8.9	13.1	13.1

Recoveries (%) on migration solutions from nylon cooking utensils

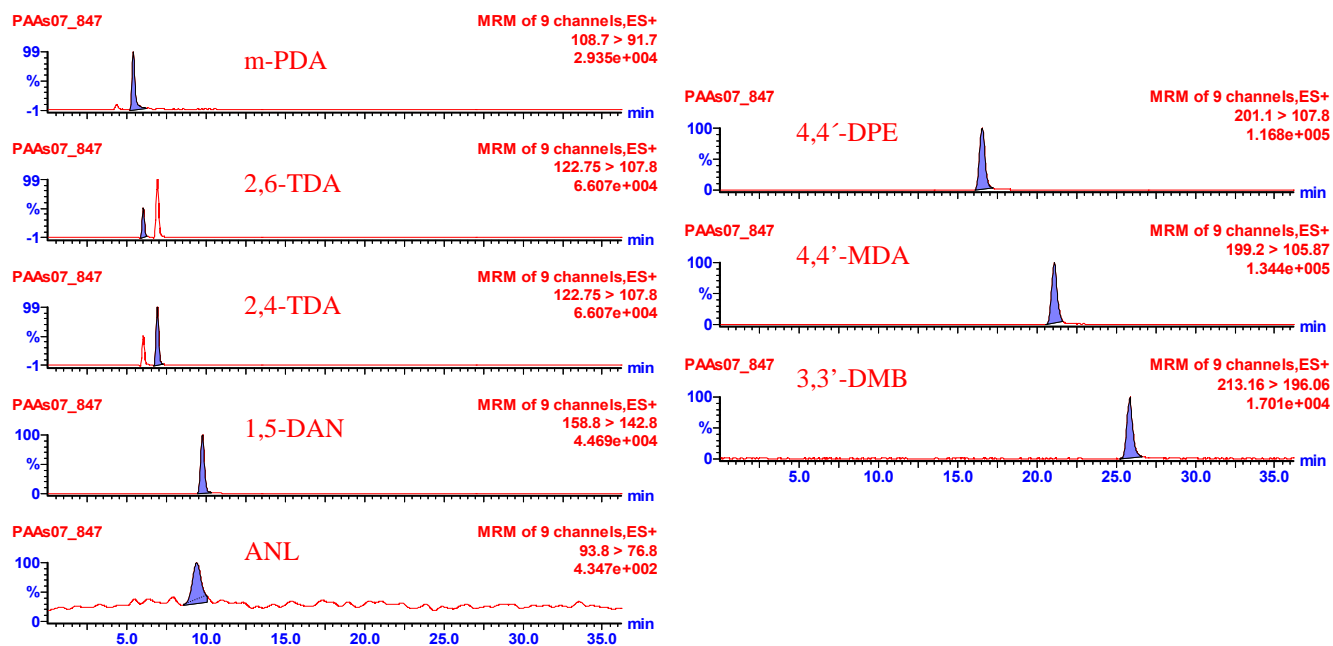
PAAs	2 ng mL ⁻¹	5 ng mL ⁻¹	10 ng mL ⁻¹	20 ng mL ⁻¹	Global mean
m-PDA	96.9	100.0	99.4	97.2	98.4
2,6-TDA	93.9	96.9	92.6	97.4	95.2
2,4-TDA	91.9	95.9	89.6	94.9	93.1
1,5 DAN	99.1	94.5	88.9	92.2	93.7
ANL	80.0	90.4	100.1	85.4	89.0
4,4'-DPE	104.0	101.0	97.0	95.1	99.3
4,4'-MDA	101.4	101.1	99.3	99.6	100.4
3,3'-DMB	99.5	99.9	96.6	93.4	97.4

2.4.2 Detection limits

DL estimated as S/N=3

PAA	µg L ⁻¹
m-PDA	0.5
2,6-TDA	0.5
2,4-TDA	0.5
1,5-DAN	1.0
ANL	1.0
4,4'-DPE	0.5
4,4'-MDA	0.5
3,3'-DMB	0.5

Appendix A: Chromatograms of 8 PAA with LC-MS/MS (solution at 10 µg / l)



Appendix B: Primary Aromatic Amines (PAAs) in the method

Name	Abbreviation	CAS N°	Structure	MW [g/mol]
m-Phenyldiamine	m-PDA	108-45-2		108,1
2,6-Toluenediamine	2,6-TDA	823-40-5		122,2
2,4-Toluenediamine	2,4-TDA	95-80-7		122,2
1,5-Diaminonaphthalene	1,5-DAN	2243-62-1		158,2
Aniline	ANL	62-53-3		93,1
4,4'-Diaminodiphenylether (= 4,4'-Oxydianiline)	4,4'-DPE	101-80-4		200,2
4,4'-Methylenedianiline (= 4,4'-Methylenebis-benzendiamine)	4,4'-MDA	101-77-9		198,3
3,3'-Dimethylbenzidine	3,3'-DMB	119-93-7		212,3

3 PROTOCOL C BY LC-MS

The method describes the qualitative procedure for the confirmation of the composition of the black nylon kitchen utensils by Fourier Transform-Infrared Spectroscopy (FT-IR) and the subsequent quantitative determination of the specific migration of primary aromatic amines (PAAs) from black nylon kitchen utensils by the analysis of their aqueous food simulant (3% w/v acetic acid) extracts by liquid chromatography-electrospray ionisation tandem mass spectrometry (UPLC-MS/MS). This method was in-house validated by NRL-IE For the purpose of this method, PAAs are described as those substances that have the same retention times and parent and daughter ion characteristics (MS/MS) as the standard PAAs used for the calibration under the conditions specified.

3.1 Principle

The method used involves boiling the black nylon kitchen utensils (100°C) in 3% w/v acetic acid for 2 h, 3 times on a hotplate. The final (third extract) solution is sampled and analysed by UPLC-MS/MS and the specific migration of the PAAs are determined by relating the results to the surface area of the utensil under analysis. The method used does not involve any pre-treatment steps of the extracts and the analytes of interest are resolved chromatographically using an Acquity BEH C₁₈ 1.7 μ m analytical column. Positive ion electrospray mass spectrometry (ESI+) is used to quantify and confirm the parent ion [M+H]⁺ and fragments for each target analyte. Prior to carrying out the specific migration tests a FT-IR spectrum of the article is acquired and compared to a standard to ensure that it is an appropriate article for the test.

3.2 Materials and Chemicals

3.2.1 Analytes and chemicals

- 3.2.1.1 Water (UPW) (HPLC grade).
- 3.2.1.2 Glacial acetic acid (analytical grade).
- 3.2.1.3 3% w/v Acetic acid solution
- 3.2.1.4 Anti-bumping granules
- 3.2.1.5 Methanol (MeOH), (HPLC grade).
- 3.2.1.6 Pentafluoropropionic acid (PFPA).
- 3.2.1.7 UPLC mobile phase A: 4.7 mM PFPA in methanol
- 3.2.1.8 UPLC mobile phase B: 4.7 mM PFPA in UPW
- 3.2.1.9 Doubly rectified absolute alcohol
- 3.2.1.10 2,4-Toluenediamine (2,4-TDA)
- 3.2.1.11 2,6-Toluenediamine (2,6-TDA)
- 3.2.1.12 Aniline (ANL)

3.2.1.13 4,4'-Methylenedianiline (4,4'-MDA)

3.2.1.14 o-Toluidine (o-T)

3.2.1.15 3,3'-Dimethylbenzidine (3,3'-DMB).

3.2.2 Solutions

3.2.2.1 Mixed stock standard solution (1,000 $\mu\text{g/ml}$).

To prepare a 1,000 $\mu\text{g/ml}$ stock standard solution, weigh to the nearest 0.001 g, 50 mg of each PAA into a 50 ml volumetric flask and make up to the mark with DRAA. This solution is stable for a year if stored at 2–8°C.

3.2.2.2 Intermediate mixed stock standard solution (10 $\mu\text{g/ml}$).

Make a 1/100 dilution of the above mixed stock standard solution in 3% acetic acid solution to give an intermediate mixed stock standard solution containing 10 $\mu\text{g/ml}$ of each analyte. The solution is stable for a month if stored at 2–8°C.

3.2.2.3 Working mixed stock standard solution (100 $\mu\text{g/l}$).

Make a 1/100 dilution of the above intermediate mixed stock standard solution in 3% acetic acid solution to give a working mixed stock standard solution containing 100 $\mu\text{g/l}$ of each analyte. The solution is stable for a week if stored at 2–8°C.

3.2.2.4 Working standards (25, 20, 10, 5, 2.5, 1.0 and 0.5 $\mu\text{g/l}$).

Make dilutions of the above working mixed stock standard solution in 3% acetic acid solution to give a working standard curve of the above concentrations. Store the solution at 2–8°C. Prepare this solution on the day of analysis.

3.2.3 Apparatus

3.2.3.1 UPLC system with mass spectrometer detector.

3.2.3.2 25, 100, 500 and 1,000 ml Class A graduated cylinders—uncalibrated.

3.2.3.3 Calibrated balances accurate to 0.01 g and 0.1 mg.

3.2.3.4 Calibrated electronic timer.

3.2.3.5 Calibrated temperature probe.

3.2.3.6 Hotplate.

3.2.3.7 2 l Glass beaker×3 (tall form).

3.2.3.8 Aluminium foil.

3.2.3.9 Retort stands and bossheads×3.

3.2.3.10 Calibrated pipettes (200±0.4 μl fixed volume; 1,000±0.3 μl fixed volume).

3.2.3.11 Volumetric flasks – 50/100/1,000

3.2.3.12 Automated diluter/dispenser

3.2.3.13 500 and 1,000 ml bottles

3.2.3.14 Normal laboratory glassware and apparatus.

3.3 Procedure

3.3.1 Analysis of test samples.

Samples are injected directly into the UPLC-MS/MS.

Note: In the event that an individual sample exceeds the range of the calibration curve appropriate dilutions can be made using 3% acetic acid solution (3.2.1.3) and the diluted sample re-injected. After analysis, the results are recorded and the specific migration calculated as outlined below.

3.3.2 HPLC Conditions

Set up the UPLC system according to the conditions described in Appendix and the mass spectrometer according to the conditions described in Appendix. Refer to the sample chromatogram illustrated in Appendix.

Note 1: Condition the UPLC column for 30 min. with mobile phase A (3.2.1.7) before commencing a run.

Note 2: Clean the head and cone of the mass spectrometer between batch runs to ensure optimal performance. Refer to SOP PALCS 0047 or SOP PALCS 0073 for the cleaning of the head and cone of the mass spectrometer, as appropriate.

Inject the specified aliquot of working standards and sample solutions onto the column. Ensure the correlation coefficient of the calibration curve (R^2) is at least 0.995. A correlation coefficient $R^2 \geq 0.995$ is acceptable for each PAA. If the correlation coefficient $R^2 < 0.995$ for any PAA perform a new calibration by injecting the standards again or preparing a new set of standards.

3.3.3 Quantification of the analyte level

Perform the standard calibration. The daughter ions monitored for the individual PAAs are given below:

PAA	Transitions monitored
2,4-TDA	122.9 → 108.0 and 122.9 → 106.0
2,6-TDA	122.9 → 108.1 and 122.9 → 106.0
ANL	94.0 → 76.8 and 94.0 → 51.1
4,4'-MDA	198.7 → 105.7 and 198.7 → 77.0
o-T	107.9 → 91.1 and 107.9 → 65.2
3,3'-DMB	211.6 → 196.4 and 211.6 → 180.6

Note: Use the first ion transition for quantification for all the PAAs except for 2,4-TDA where the second transition is used.

Calculation of the specific migration of the individual PAAs.

The concentration (C) of the PAA of interest in the test sample is given by:

$$\mu\text{g}/\text{dm}^2 = \mu\text{g}/\text{l PAA} \times \frac{v}{A}$$

where $\mu\text{g}/\text{l PAA}$ is the value obtained from the chromatogram for the individual amine; v is the volume of simulant in l from step; A is the surface area in dm^2 for the utensil being tested and calculated from step or step.

For samples received as 3% acetic acid solutions (e.g. FAPAS Proficiency Test

Samples) the concentration (C) of the PAA of interest in the test sample is given by:

$$\mu\text{g}/\text{kg} = \frac{\mu\text{g}/\text{l PAA}}{d}$$

where $\mu\text{g}/\text{l PAA}$ is the value obtained from the chromatogram for the individual amine and d is the density of the sample solution.

Results are expressed as $\mu\text{g}/\text{dm}^2$ to the nearest $0.1 \mu\text{g}/\text{dm}^2$.

Calculate the measurement uncertainty (MU) for the test parameter(s). For the individual PAAs the values are given

PAA	MU (%)
2,4-TDA	0.058
2,6-TDA	0.089
ANL	0.037
4,4'-MDA	0.060
o-T	0.035
3,3'-DMB	0.097

Ensure compliance with any legislation regarding measurement uncertainty which may govern the specific test parameters.

If the measurement uncertainty is to be applied to the analytical result, report it in the following format:

Result $100 \pm 10^*$

*The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%.

3.4 Precision of the data

3.4.1 Limit of detection and quantification

the limit of detection for the individual PAAs is given below:

PAA	L.O.D. (ppb)
2,4-TDA	0.19
2,6-TDA	0.13
ANL	0.04
4,4'-MDA	0.08
o-T	0.09
3,3'-DMB	0.25

Limit of quantitation—the limit of quantitation for the individual PAAs is given below:

PAA	L.O.Q. (ppb)
2,4-TDA	0.37
2,6-TDA	0.26
ANL	0.08
4,4'-MDA	0.17
o-T	0.18
3,3'-DMB	0.49

3.4.2 Bias and precision

Bias (WRm and WRs)–the values for the individual PAAs are given below:

PAA	WRm (%)	WRs (%)	Lower limits		Upper limits	
			Warning	Action	Warning	Action
2,4-TDA	100.80	3.872	93.06	89.18	108.54	112.42
2,6-TDA	101.61	4.673	92.26	87.59	110.96	115.63
ANL	100.64	3.086	94.47	91.38	106.81	109.90
4,4'-MDA	100.61	3.061	94.49	91.43	106.73	109.79
o-T	100.42	2.022	96.38	94.35	104.46	106.49
3,3'-DMB	98.945	6.702	85.54	78.84	112.35	119.05

Precision (Wp)–the values for the individual PAAs are below:

PAA	Wp (absolute)	Wp (relative)	Lower action limit	Upper action limit
	(ppb)	(%)		
2,4-TDA	0.244	4.828	-0.244	0.244
2,6-TDA	0.364	7.337	-0.364	0.364
ANL	0.146	2.898	-0.146	0.146
4,4'-MDA	0.281	5.620	-0.281	0.281
o-T	0.177	3.528	-0.177	0.177
3,3'-DMB	0.635	12.606	-0.635	0.635

3.4.3 Quality control.

UPLC/mass spectrometer instrument performance: Perform and record the following system suitability tests (based on the 10 µg/l working standard):

The area counts of the 10 µg/l aniline peak: The value should be >8,200. If the value is <8,200 investigate the reasons influencing this parameter (e.g. standard prepared incorrectly, problems relating to the probe (e.g. residue contamination, orientation, instrument tuning) or the injection system) and continue until the value for the standard is achieved.

The ion ratio of the 10 µg/l aniline peak: The value should be 12<x<18. If the value is not 12<x<18 investigate the reasons influencing this parameter (e.g. problems relating to the probe (e.g. residue contamination, orientation, instrument tuning) or the injection system) and continue until the value for the standard is achieved.

Blank determination: Determine a blank value for the recovery check matrix being used (3% acetic acid)

Repeatability check: The precision of the duplicate recovery check is recorded on the quality control chart. The value should conform to the parameters given in Section 3.4.2. If the value obtained does not conform re-inject the spiked samples or prepare a new set of spiked samples, as appropriate.

Recovery check: Analyse one sample of known PAA concentration in duplicate, i.e. a spiked sample. Suggested spiking level: 30 µl of the 100 ppb working standard (3.2.2.4) diluted (3.2.3.12) with 1170 µl 3% acetic acid solution (3.2.1.3).

Reference material check: Not applicable as reference material is not available.

External quality control: Proficiency schemes–FAPAS®, BfR.

Appendix A: UPLC Conditions

Column:

Analytical column: Waters Acquity BEH C₁₈ 1.7 µm 2.1×100 mm UPLC column, or equivalent
Guard column: Waters Assembly Frit S5 ODS2 2.1 mm 0.2 µm guard cartridge, or equivalent.
Column oven temperature Set at 45±5°C
Sample temperature: Set at 15±5°C.
Injection volume: Set at 20 µl.
Run time: Set at 6 min.
Spurge rate: N/A
Mobile phase: Mobile phase A: 4.7 mM PFPA in MeOH (3.2.1.7);
Mobile phase B: 4.7 mM PFPA in UPW (3.2.1.7).

Gradient

	Time	Flow	%A	%B	Curve
1	0.00	0.20	20.0	80.0	6
2	1.00	0.20	20.0	80.0	6
3	2.00	0.20	20.0	80.0	6
4	3.50	0.20	80.0	20.0	6
5	3.70	0.20	80.0	20.0	6
6	4.00	0.20	5.0	95.0	6
7	5.00	0.20	5.0	95.0	6
8	6.00	0.20	20.0	80.0	6

Flow rate: Set at 0.2 ml/min.
Weak wash: 5% MeOH (3.2.1.5) in UPW (3.2).
Strong wash: 95% MeOH (3.2.1.5) in UPW (3.2).
Seal wash: 5% MeOH (3.2.1.5) in UPW (3.2).

Integration parameters: Integration parameters are set having regard to the procedures outlined in the integrator manual.

Detector parameters:

Appendix below for the Mass Spectrometer Detector conditions.

Appendix B: Mass Spectrometer Detector Conditions

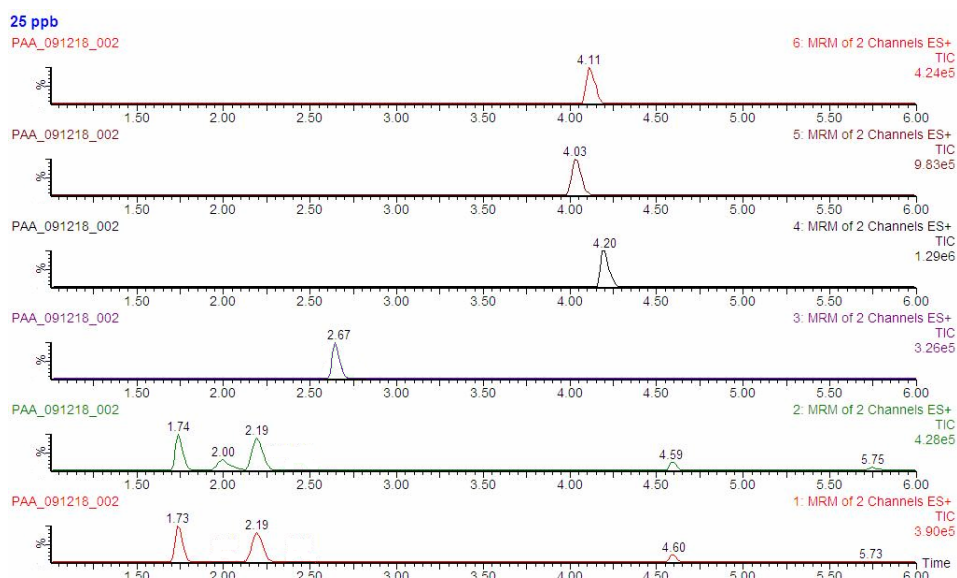
Tune Method

Source:	ESI+	ESI+
detector	MS-2	ESI+
Capillary (kV):	2.75	2.5
Cone (V):	35	35
RF Lens 1:	0	0.0
Aperture (V):	0	0.0
RF Lens 2:	0	0.0
Source temperature (°C):	130	130
Desolvation Gas temperature (°C):	450	450
Cone gas flow (l/h):	100	100.0
Desolvation gas flow (l/h):	800	800.0
Analyser:		
LM 1 Resolution:	12	10.0
HM 1 Resolution:	12	10.0
Ion energy 1:	0.8	0.7
Entrance:	-1	-1.0
Collision:	2	2.0
Exit:	1	3.0
Automatic gain control:	-	10.0
LM 2 Resolution:	12	10.0
HM 2 Resolution:	12	1.0
Ion energy 2:	1.5	630.0
Mutliplier:	650	

Scanning Method (MS1) and (MS2)

Channel	Parent (Da) MS1	Daughter (Da) MS1	Dwell (s) MS1	Cone (V) MS1	Coll (V) MS1	Delay (s) MS1	Channel	Parent (Da) MS2	Daughter (Da) MS2	Dwell (s) MS2	Coll. (V) MS	Delay (s) MS2
1	108.1	91.3 65.5	–	35	16 25	0.02	1	122.9	108.1 106.0	0.040	2 19 26	0.05
2	213.2	181.15 106.1	–	35	19 24	0.02	2	122.9	108.1 106.0	0.020	17 22	0.05
3	123.15	108.1 106.1	–	35	6 16	0.02	3	94.0	76.8 51.1	0.060	17 24	0.05
4	123.15	108.2 106.1	–	35	8 15	0.02	4	211.6	196.4 180.6	0.010	32 39	0.05
5	94.3	77.5 51.3	–	35	24 35	0.02	5	198.7	105.7 77.0	0.020	19 25	0.05
6	199.2	106.1 77.25	–	35	16 45	0.02	6	107.9	91.1 65.2	0.025	16 27	0.05

Appendix C: Sample Chromatogram



4 PROTOCOL D BY HPLC-DAD

This protocol has been validated by a single laboratory validation by the EURL (JRC) and an EU validation organized by NRL-DE (BfR)

4.1 Principle

The aqueous simulant test samples are submitted to a concentration step over a cation exchange column. The eluted solutions are analysed by HPLC with ultra violet (UV) detection. Low detection limits are achieved by using a large injection volume and concentration of this volume by a precolumn-backflush procedure. Confirmations of identity of the PAAs are carried out by diode array detection.

4.2 Materials and chemicals

NOTE: Reagents and solvents should be of analytical quality and appropriate for HPLC. If nothing else is described, aqueous solutions are ment.

4.2.1 Analytes and chemicals

1,3-Phenylendiamine, , CAS 00108-45-2, molecular weight, 108.14 g/mol

2,6-Toluenediamine (2,6 TDA), , CAS 823-40-5, MW: 122.2 g/mol

2,4-Toluenediamine (2,4 TDA), , CAS 95-80-7, MW 122.2 g/mol

1,5-Diaminonaphthalene, CAS 2243-62-1, MW 158.2 g/mol

Aniline, CAS 142-04-1, MW 129.6 g/mol

4,4'-Oxydianiline, CAS 101-80-4, MW 200.2 g/mol

4,4'-Diaminodiphenylmethane (4,4'-MDA), CAS 101-77-9, MW: 198.3 g/mol

3,3'-Dimethylbenzidine, CAS 00119-93-7

2,2'- Diaminodiphenylmethane (2,2'-MDA), CAS 6582-52-1

2,4'- Diaminodiphenylmethane (2,4'-MDA), CAS 1208-52-2

Methanol

Glacial acetic acid

Sodium acetate anhydrous

tri-Sodiumcitrate dihydrate, molecular weight: 294,2 g/mol

4.2.2 Solutions

4.2.2.1 Citrate buffer (c = 0,1 mol/l)

4.2.2.2 Elution solution

70 vol% 0,1 M citrate-buffer + 30 vol% methanol

3 % Acetic acid (w/v)

4.2.2.3 Stock solution

Weigh 3-4 mg of each of the analytes exactly into a 25 ml volumetric flask, dissolve with methanol, fill to the mark and mix carefully.

NOTE: The stocksolution may be stored in a refrigerator at 4°C up to 1 week

4.2.2.4 Diluted stock solution

Transfer 2 ml of the stock solution by a pipette into a 25 ml volumetric flask, fill to the mark with elution solution (12.2.2) and mix.

4.2.2.5 Standard solutions (10 – 250 µg/l)

Add 4 ml distilled water into a series of five 10 ml volumetric flasks. Transfer, using a micro syringe, 10, 30, 50, 100 and 250 µl of the diluted stock solution into the flasks, make up to the mark with elution solution (3.2.1.2) and mix.

4.2.3 Apparatus

PRS-Solid phase extraction columns (Propylsulfonic acid, 500 mg, Varian Bond Elut

High performance liquid chromatograph with a 100 µl injection loop, a variable UV detector and a precolumn-backflush equipment.

HPLC columns: The following has been found suitable

Pre- Column: Nucleosil 100- 5 C18 30*3 mm I.D.
 Column: Nucleodur C18, Gravity 3 µ, 250*3 mm I.D.

4.3 Procedures

4.3.1 Concentration step with PRS-column

Concentration procedure is carried out with 50 ml migration solution. Acetic acid (3 %) has a pH of 2,5, so acidic migration solution can directly be applied to the PRS-columns. Aqueous migration solution must be acidified by addition of 1,5 ml glacial acetic acid to 50 ml of the solution.

Equilibration of the PRS-columns is achieved with 1) 2 x 3 ml methanol and 2) 2 x 3 ml 3 % acetic acid. The columns are not allowed to get dry. By help of reservoirs the sample solutions are applied to the columns, beaker and reservoir are twice rinsed with 2 ml 3 % acetic acid. Then suck the columns dry for 30 seconds and elute the amines with 3 ml elution solution (12.2.2) slowly into a 5 ml volumetric flask. Do this in 1-ml-steps sucking the columns dry. Then dilute to the mark with bidistilled water (concentrated sample solution).

4.3.2 HPLC conditions

The following conditions have been found adequate

Injection volume 100 µl
 Solvent A 0,01 mol/l sodium acetate pH=7,2
 Solvent B methanol
 Column flow 0.300 ml/min
 Column temperature 30.0°C
 Wavelength: 230, 240, 280 nm

Time	Solvent B
0,00 min	0,0 %
2,50 min	21,1 %
42,00 min	65,3 %
43,00 min	100,0 %
50,00 min	100,0 %
53,00 min	0,0 %
59,90 min	0,0 %
No Post-time	
<u>Column Switching Valve:</u>	
Time	Solvent
0,00 min	Back-flushing off
0,80 min	Back-flushing on
53,00 min	Back-flushing off

Posttime 15.00 min

Dwell volume 0,9 ml

4.3.3 HPLC calibration

The calibration is performed by injecting the standard solutions into the HPLC under the conditions described in 5.2. Because of the enrichment step (5.1) the standard solutions represent migration solutions, which are 10fold lower concentrated.

For every amine a calibration curve is constructed, plotting peak areas at the wavelengths with the highest adsorption against concentration values (dimension $\mu\text{g/l}$).

NOTE: The calibration curves should be linear and the correlation coefficient should be 0.995 or better.

NOTE: Figure 1 shows a standard chromatogram with all analytes

NOTE: Figure 2 gives an overview about correlation coefficients and maximal wavelengths.

4.3.4 Confirmation of identity by diode array detection

The spectra of the peaks are taken over the range of 210-400 nm and compared with the standard spectra. If the overlaid spectral profiles of the peaks are identical with the spectra of the standards, the identity of the substance is confirmed

4.3.5 Evaluation of data

Calculation of analyte level

The calculation is carried out with the method of external standard using the peak areas. The areas of the standards and the concentrations in the migration solutions are subjected to a regression calculation. The result is a linear function:

$$\text{Specific migration } (\mu\text{g/l}) = A + B \times \text{Area}$$

4.4 Precision of the data

4.4.1 Validation

Repeatability and reproducibility- Standard deviations and recoveries

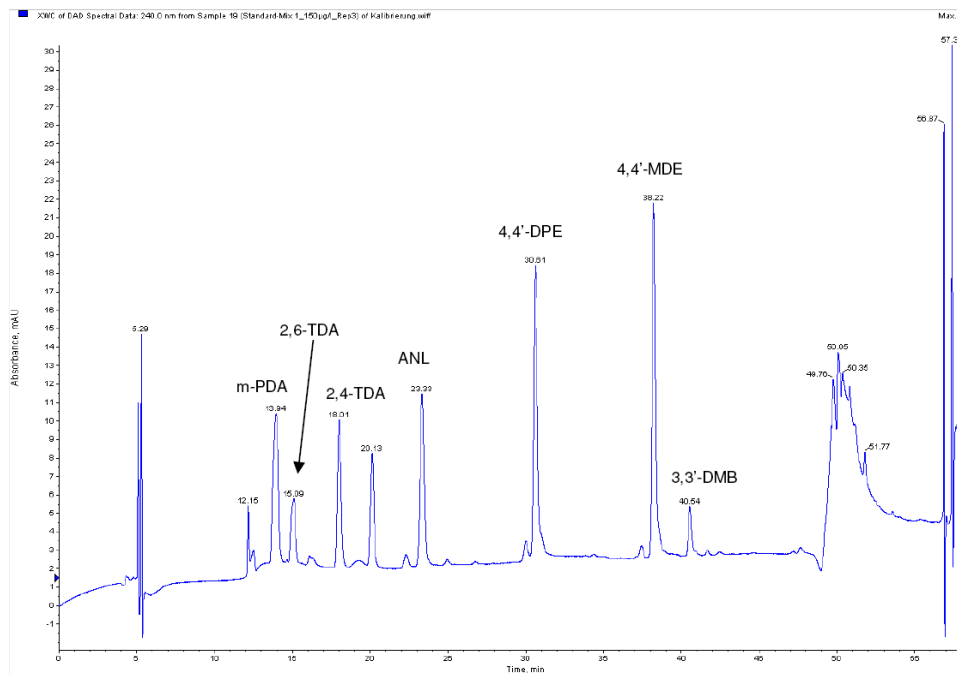
Acidic migration solutions of laminates were spiked with PAA standard solutions, worked up as described in 5.1 (concentrated sample solutions) and applied to HPLC according to 5.2. Calibration solutions with the same concentration of PAA as the concentrated sample solutions were chromatographed as external standards (ESTD) in the same way.

4.4.2 Detection limits

Aqueous migration solutions of laminates were spiked with PAA standard solutions and worked up according to 5.1. The detection limits were calculated by signal/noise ratio of 5:1, they are listed in figure 2.

Appendix A:

Figure 1: Determination of primary aromatic amines with HPLC – standard chromatogram (150 ppb, $\lambda = 240$ nm)




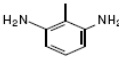
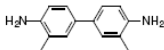
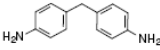
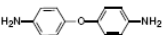


Appendix B:

Fig. 2: Determination of primary aromatic amines with HPLC - linearity, detection limits

No	Analyte	RT	Linear Concentration Range	Correlation Coefficient	Max. Wavelength	DL: S/N = 5:1
1	m-Phenylendiamin (m-PDA)	13,9 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9995	240 nm	0.26 µg/l
2	2,6-Toluenediamine (2,6-TDA)	15,1 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9998	240 nm	0.24 µg/l
3	2,4-Toluenediamine (2,4-TDA)	18,0 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9999	230 nm	0.27 µg/l
4	Aniline (ANL)	23,3 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9999	240 nm	0.19 µg/l
5	4,4'-Oxydianiline (= 4,4'-Diaminodiphenylether) (4,4'-DPE)	30,6 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9998	240 nm	0.17 µg/l
6	4,4'-Methylenedianiline (= 4,4' methylene-bisbenzendiamine) (4,4'-MDA)	38,2 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9999	240 nm	0.17 µg/l
7	3,3'-Dimethylbenzidine (3,3'-DMB)	40,5 min	10-250 ng/ml = 1 -25 µg/l Migrationsolv.	0.9998	280 nm	0.19 µg/l

Appendix C:

Primary Aromatic Amines (PAA) in the method

Name	Abbreviation	CAS No.	Structure	MW [g/mol]
2,4-Toluediamine	2,4-TDA	095-80-7		122,2
2,6-Toluediamine	2,6-TDA	823-40-5		122,2
3,3'-Dimethylbenzidine	3,3'-DMB	119-93-7		212,3
Diaminodiphenylmethane 4,4'-Methylenedianiline (= 4,4'-methylenebis- benzidine)	4,4'-MDA	101-77-9		198,3
4,4'-Diaminodiphenylether (= 4,4'-Oxydianiline)	4,4'-DPE	101-80-4		200,2
Aniline	ANL	062-53-3		93,1
m-Phenyldiamin	m-PDA	108-45-2		108,1

Annex 2 – Formaldehyde

This annex presents 2 methods for the determination of formaldehyde CH_2O , and hexamethylenetetramine (HMTA) $\text{C}_6\text{H}_{12}\text{N}_4$, in food simulant 3 % w/v aqueous acetic acid. The methods are appropriate for the quantitative determination of formaldehyde in approximate analyte concentration range of 3,0 mg to 30 mg formaldehyde per kilogram of food simulant, which corresponds to 2,3 mg to 23,3 mg hexamethylenetetramine per kilogram of food simulant. They are interchangeable for use as determination and confirmation. Both are spectrophotometric. One uses chromotropic acid in the presence of sulphuric acid, and the other pentane-2,4-dione in the presence of ammonium acetate.

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1 PROTOCOL A

1.1 Scope and principle

HMTA is treated with acid and heated to release formaldehyde. Formaldehyde in food simulant is determined by spectrophotometry. Formaldehyde reacts with chromotropic acid in the presence of sulphuric acid. This solution is measured in a spectrophotometer at the wavelength of 574 nm, with cells of an optical path length of 10 mm. Quantification is achieved using an external standard. The method has been pre-validated by collaborative trial with two laboratories.

1.2 Materials and chemicals

Reagents and solvents shall be of analytical quality.

1.2.1 Analytes and chemicals

- 1.2.1.1 Formaldehyde solution, CH_2O , minimum 37 % (w/v), stabilised with about 10 % methanol
- 1.2.1.2 Water deionised
- 1.2.1.3 Acetic acid glacial 100%
- 1.2.1.4 Ethanol absolute
- 1.2.1.5 Chromotropic acid disodium salt (dihydrate), 4,5-dihydroxy-2,7-naphthalene-disulphonic acid, $\text{C}_{10}\text{H}_6\text{Na}_2\text{O}_8\text{S}_2 \cdot 2\text{H}_2\text{O}$
- 1.2.1.6 Sulphuric acid, density 1.84 g/cm^3
- 1.2.1.7 Starch, soluble

1.2.2 Solutions

- 1.2.2.1 Stock solution of formaldehyde in water containing approximately 1,5 mg formaldehyde per millilitre

Weigh to the nearest 1 mg approximately 0,8 g formaldehyde into a 200 ml volumetric flask and make up to the mark with water.

Determine the strength of this solution as follows:

Pipette 10,0 ml formaldehyde stock solution into a beaker, add 25,0 ml of a 0,05 mol standard iodine solution and 10,0 ml of 1 mol sodium hydroxide solution to the beaker. Allow to stand for 5 min. Acidify with 11,0 ml of 1 mol hydrochloric acid and determine the excess iodine by titration with a 0,1 mol standard sodium thiosulphate solution using 0,1 ml of the starch solution as indicator.

Make a blank test on the corresponding amount of standard iodine solution.

1 ml of 0,1 N iodine consumed is equivalent to 1,5 mg formaldehyde.

Calculate the concentration in mg formaldehyde per millilitre solution.

Repeat the procedure to obtain a second stock solution.

Store the formaldehyde stock solution for up to 3 months in a refrigerator.

1.2.2.2 Diluted standard solutions of formaldehyde in aqueous food simulants

Pipette into a series of five 100 ml volumetric flasks, 100 μ l, 400 μ l, 700 μ l, 1000 μ l and 2000 μ l of the stock solution. Make up to the mark with the appropriate food simulant. The standard solutions thus obtained shall contain approximately 1,5 mg/l, 6,0 mg/l, 10,5 mg/l, 15 mg/l and 30 mg formaldehyde per litre.

Calculate the exact concentration in milligrams of formaldehyde per litre of solution corresponding to mg/kg food simulant.

Repeat the procedure using the second standard stock solution to obtain a second set of diluted standard solutions.

Store the solutions in well closed container for a maximum period of 3 months in a refrigerator

1.2.2.3 Iodine in aqueous solution, 0,05 M

1.2.2.4 Sodium hydroxide in aqueous solution solution, NaOH, 1 M.

1.2.2.5 Hydrochloric acid in aqueous solution solution, HCl, 1 M.

1.2.2.6 Sodium thiosulphate in aqueous solution solution, Na₂S₂O₃.5H₂O. 0.1 M..

1.2.2.7 0,5 % chromotropic acid disodium salt (dihydrate)

Weigh 500 mg of chromotropic acid into a 100 ml volumetric flask and make to the mark up with water. This solution shall be prepared freshly on the day of use.

1.2.2.8 Sulphuric acid solution

Measure 100 ml water and make up with sulphuric acid to 400 ml.

1.2.2.9 Sulphuric solution (4M)

Add carefully 222 ml sulphuric acid (1.2.1.6) to a flask containing approximately 700 ml of water. After cooling to room temperature, make up to 1 000 ml with water.

1.2.2.10 Starch solution

Dissolve 1 g soluble starch in 50 ml boiling water.

1.2.3 Apparatus

NOTE: An instrument or item of apparatus is listed only where it is special or made to a particular specification, usual laboratory glassware and equipment being assumed to be available.

Spectrophotometer, preferably with a double beam.

Water-bath

Micro syringe 0 -1.0 ml

Tubes, glass, stoppered

1.3 Procedure

1.3.1 Preparation of samples

1.3.1.1 test samples

1.3.1.1.1 For the determination of HMTA,

Take 25 ml of the aqueous food simulant into a 50 ml volumetric flask and make up to the mark with the diluted sulfuric acid (4.3.11). Immerse the flask in a water bath at 90 °C for 30 min. Shake while cooling. Transfer 1,0 ml of this solution into a 12 ml glass stoppered tube. Treat the sample as described in the section sample treatment.

1.3.1.1.2 For the determination of formaldehyde

Transfer 1,0 ml of food simulant obtained from the migration experiment into a 12 ml glass stoppered tube. Treat the sample as described in the section sample treatment.

1.3.1.2 Preparation of blank samples

Treat simulants which have not been in contact with packaging material in the same way.

1.3.2 Preparation of calibration samples

Transfer 1,0 ml of each of the diluted standard solutions into a 12 ml glass stoppered tube and treat the calibration samples as described in the section sample treatment.

1.3.3 Spectrophotometric analysis

The test samples, blanks and calibration samples are submitted to a reaction procedure with chromotropic acid. The absorption of reaction product is measured over the range of 650 nm to 450 nm. The absorption at 574 nm is used for quantitative calculations. Each solution must be analysed at least in duplicate

1.3.3.1 Sample treatment

Add to each of the vials 1 ml chromotropic acid solution and 8 ml sulphuric acid 75 %. Place the vials in a water-bath at 60°C for 20 min. Remove the vials from the water-bath and allow the vials to cool for one hour at room temperature.

Record the absorption curve of the solution obtained from 650 nm to 450 nm using cells with an optical path length of 10 mm and a reagent blank solution or zero level of the calibration curve as a reference. The absorption at 574 nm is used for quantitative calculations.

1.3.3.2 Calibration

Measure the absorption of the formaldehyde/chromotropic acid complex at 574 nm of the standard solutions. Plot the absorption against the concentration of formaldehyde in the calibration samples in milligrams per kilogram.

NOTE: Commission Directive 2002/72/EC [1] states that the specific gravity of all simulants should conventionally be assumed to be '1'. Milligrams of substance released per litre of simulant will thus correspond numerically to milligrams of substance released per kilogram of simulant and, taking into account of the provisions laid down in Directive 82/711/EEC [3], to milligrams of substance released per kilogram of foodstuff.

The calibration curves shall be rectilinear and the correlation coefficient should be 0,996 or better. The two sets of calibrant solutions made from independently

prepared stock solutions shall be cross-checked by generating two calibration curves which on the basis of absorption measurement should agree to $\pm 5\%$ of one another.

1.3.4 Evaluation of data

NOTE: The following calculations assume that for all measurements exactly the same weight or volume of food simulant has been used.

1.3.4.1 Spectroscopic interferences

Possible interferences due to background colour in the sample are eliminated by the use of a reference solution.

1. Calculation of analyte level

1.3.4.2 Graphical determination:

Measure the absorbance at 574 nm and calculate the average of absorption values obtained from the test samples according to the section sample treatment. Read the formaldehyde concentration of the test samples from the calibration graph.

1.3.4.3 Calculation from the regression parameters;

If the regression line equation is

$$y = a * x + b \quad (1)$$

where

y is the absorbance of formaldehyde/chromotropic acid complex

a is the slope of the regression line;

x is the concentration of formaldehyde in the food simulant (mg/kg)

b is the intercept of the regression line.

then

$$C_{For,fs} = (y-b)/a \quad (2)$$

where

$C_{For,fs}$ is concentration of formaldehyde in milligrams per kilogram of food simulant

Both procedures yield directly the formaldehyde concentration in the food simulant in milligrams of formaldehyde per kilogram of food simulant

The method applying calculation from the regression parameters shall be the preferred one.

1.3.4.4 Calculation of the specific formaldehyde migration

Depending on the fill volume of the test material and on the surface area/food simulant, the formaldehyde concentration in the test sample may need mathematical transformation to calculate the specific migration value to be compared to the restriction.

1.4 Precision

1.4.1 Validation

1.4.1.1 Determination of HMTA

This method was pre-evaluated by a collaborative trial with three laboratories. In each laboratory a within-laboratory precision experiment using the four official EU food simulants for establishment of precision data at the restriction criterion was carried out. Also within-laboratory migration tests were carried out with a polymer sample, manufactured using hexamethylenetetramine as a monomer, being in contact for 10 d at 40 °C with 15 % v/v aqueous ethanol and olive oil, respectively.

Evaluation of the within-laboratory precision experiment results according to ISO 5725, at a concentration of 11,7 mg HMTA per kilogram of food simulant, (corresponding to 15 milligrams of formaldehyde per kilogram for the 95 % probability level yielded the following performance characteristics (lab 1/lab 2/lab 3):

Repeatability:

$r = 0,2/0,8/1,0$ mg formaldehyde per kilogram in water;

$r = 0,8/0,7/1,9$ mg formaldehyde per kilogram in 3% w/v aqueous acetic acid;

$r = 0,6/0,6/2,6$ mg formaldehyde per kilogram in 15% v/v aqueous ethanol;

$r = 0,4/1,1/4,2$ mg formaldehyde per kilogram in olive oil.

1.4.1.2 Determination of formaldehyde

This method was pre-evaluated in 1993 by collaborative trial with two laboratories. In each lab a within-laboratory precision experiment using the four official EU food simulants for establishment of precision data at the restriction criterion was carried out as well as migration testing with formaldehyde containing samples in contact for 10 d at 40 °C, with 15 % v/v aqueous ethanol and olive oil, respectively.

Evaluation (ISO 5725) of the results of the two within-laboratory precision experiments at a concentration of 15 mg formaldehyde per kilogram of food yielded the following performance characteristics at the 95% probability levels (lab1/lab2):

Repeatability:

$r = 0,5/0,2$ mg formaldehyde per kilogram in water;

$r = 0,3/0,2$ mg formaldehyde per kilogram 3 % w/v aqueous acetic acid;

$r = 0,4/0,1$ mg formaldehyde per kilogram in 15 % v/v aqueous ethanol;

$r = 0,5/0,5$ mg formaldehyde per kilogram in olive oil.

1.4.2 Detection limit

1.4.2.1 Determination of HTMA

The within-laboratory detection limits (WDL), based on the calibration curve method according to DIN 32645, were found to be in the range of 0,8 mg/kg to 3,0 mg/kg food simulant, depending on the type of food simulant. Thus the method is capable of quantitative detection of HMTA, as formaldehyde at a minimum level of 3,0 mg per kilogram food simulant.

1.4.2.2 Determination of Formaldehyde

The within-laboratory detection limits (WDL), based on the calibration curve method according to DIN 32645, were found to be in the range of 0,5 mg/kg to 3,0 mg/kg

food simulant, depending on the type of food simulant. Thus the method is capable of quantitative detection of formaldehyde at a minimum level of 3,0 mg/kg food simulant.

2 PROTOCOL B

In cases where the specific migration of formaldehyde exceeds the restriction, e.g. a specific migration of 15 mg/kg, the result of the determination shall be confirmed. The confirmation procedure is quantitative. In collaborative trials with three laboratories comparable results using the chromotropic acid method and the acetyl acetone method were found for the detection limits, repeatability, recovery and migration of formaldehyde. Therefore the method of determination and the confirmation procedure are interchangeable.

2.1 Principle

Formaldehyde is reacted with pentane-2,4-dione in the presence of ammonium acetate to form 3,5-diacetyl-1,4-dihydropyridine. The absorbance of this complex is measured at 410 nm with a spectrophotometer.

2.2 Materials and chemicals

NOTE: Only additional chemicals required for the confirmation procedure are mentioned

2.2.1 Analytes and Chemicals

2.2.1.1 Ammonium acetate, anhydrous

2.2.1.2 Pentane-2,4-dione (acetyl acetone), $C_5H_8O_2$, distilled

2.2.2 Solutions

2.2.2.1 Pentane-2,4-dione reagent

This reagent shall be freshly prepared on the day of use.

Dissolve 15 g ammonium acetate in a 100 ml volumetric flask containing approximately 75 ml of water. Add 0,2 ml pentane-2,4-dione and 0,3 ml acetic acid. Make up to 100 ml with water (pH of solution about 6,4)

2.2.2.2 Reagent without pentane-2,4-dione

Prepare the reagent solution while omitting the addition of pentane-2,4-dione.

2.3 Procedure

2.3.1 Preparation of test samples

2.3.1.1 For the determination of HMTA

Transfer into a 50 ml flask 10,0 ml of aqueous food simulant, add 5 ml of sodium hydroxide solution (1.2.2.4) and 15 ml of water. Check the pH of the solution, and if necessary adjust to pH 4 to pH 6 using a few drops of sodium hydroxide solution

(1.2.2.4) or sulfuric acid solution (1.2.2.9), as appropriate. Add 5 ml of pentane-2,4-dione reagent (2.2.1.2) and continue as described in sample treatment.

2.3.1.2 For the determination of formaldehyde

Transfer into a 50 ml flask 5,0 ml of aqueous food simulant, add 20,0 ml water and 5,0 ml of pentane-2,4-dione reagent and continue as described in sample treatment.

2.3.2 Preparation of blank samples

Treat simulants that have not been in contact with packaging material, as described in sample preparation.

2.3.3 Preparation of calibration samples

Transfer into a 50 ml flask 5 ml of diluted standard solutions, 20 ml water and add 5.0 ml of pentane-2,4-dione reagent.

2.3.4 Reference solution

Transfer into a 50 ml flask 5,0 ml aqueous food simulant, 20 ml water and 5,0 ml of the reagent without pentane-2,4-dione.

NOTE: Possible interferences due to background colour in the test sample are eliminated by the use of this reference solution.

2.3.5 Sample treatment

Shake the mixtures prepared. Immerse the flasks in a waterbath at 60 °C for exactly 10 min. Allow to cool for 2 min in a bath of iced water. Within 25 min from the moment when the flasks are placed in the water-bath measure the absorbance at 410 nm of the sample solution with the reference solution in the reference cell.

2.4 Evaluation of data

Evaluate the data following the procedure given for protocol A.

European Commission

EUR 24815 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware - 1st edition 2011

Author(s): Simoneau C, Hoekstra E, Bradley E, Bustos J, Golja V, Kappenstein O, Kalsbeek D, Keegan J, Milana M, Cwiek-Ludwicka K, Petersen J, Polz M, Sauvegrain P, Vanhee F, authors Simoneau C, editor.

Luxembourg: Publications Office of the European Union

2011 – 58 pp. – 21 x 29 cm

EUR – Scientific and Technical Research series –

ISSN 1018-5593 (print)

ISSN 1831-9424 (online)

ISBN 978-92-79-20201-8 (print)

ISBN 978-92-79-20202-5 (pdf)

doi:10.2788/19565

Abstract

Comparability of results is an important feature of the measurements carried out for official controls purposes. In the area of food contact materials and articles comparability of results is dependent on the availability of samples representative of the consignment, the type of exposure and the test conditions used as well as on the performance of the method of analysis.

These guidelines contain practical information on sampling, migration testing and methodologies for the analytical determination of primary aromatic amines and of formaldehyde.

These guidelines were developed specifically in the context of the Regulation 284/2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from [the] People's Republic of China and Hong Kong Special Administrative Region, China.

These guidelines have been prepared by the European Union Reference Laboratory in collaboration with its EU official Network of National Reference Laboratories and have been endorsed by the European Commission competent service DG Health and Consumers (DG SANCO) and its network of Member State Competent Authorities. They are primarily addressed to official control laboratories, national reference laboratories and third party laboratories for providing certificates of compliance. The sampling strategy is addressed to the points of first introduction of import goods in the EU.

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ISBN 978-92-79-20202-5

