m-Xylylenediamine Determination in the Official EU Aqueous Food Simulants

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Abstract

European Union directive 90/128/EEC prescribes a specific migration limit of 0.05 mg/kg for the aliphatic diamine *m*-xylylenediamine (*m*-XDA) into food or food simulants, but there is no generally accepted method of analysis available for compliance testing with the given restriction. A method is described for the determination of *m*-XDA monomer in the following food simulants: distilled water, 3% (w/v) acetic acid, and 15% (v/v) ethanol. The method is appropriate for the quantitative determination of *m*-XDA at a minimum level of 0.020 mg/kg in these food simulants. Detection limits are in the range of 0.004 to 0.010 mg *m*-XDA per kilogram food simulant (depending on the type of food simulant). The method should also be applicable to other aqueous food simulants. *m*-XDA in aqueous simulant test samples is determined by high-performance liquid chromatography with fluorescence detection following derivatization with fluorescamine. Quantitation is relative to external standards. The identity of *m*-XDA may be confirmed by the presence of a second peak in the chromatograms obtained from samples derivatized with less fluorescamine or by comparison with authentic samples.

Introduction

The scientific activities carried out by the European Union (known as the EU R&D Framework Programme) attempt to provide even coverage of those scientific areas judged to be important to the Union as a whole. One of these areas is that of measurement and testing. This work is part of a series of analytical methods to test plastic materials and articles in contact with foodstuffs. These test methods are concerned with the determination of specific migration times of plastic constituents into foodstuffs and food simulants or the determination of residual plastic constituents in the finished plastic article.

m-Xylylenediamine (*m*-XDA), 1,3-benzenedimethanamine, 3-(aminomethyl)-benzylamine, and $C_8H_{12}N_2$ (PM reference number 13000, CAS number 1477-55-0) are used in the synthesis of polyamides (for bags, barrier packs, etc.) and as hardeners for epoxy resin coatings intended for certain food containers (large metal or concrete vats or tanks for water, fruit juices, wine, oil, etc.). After manufacture, residual *m*-XDA monomer can remain in the polymer and may migrate into foodstuffs coming into contact with the plastic material or article. According to Article 2 of the EU directive 89/109/EEC (1), articles intended to come into contact with foodstuffs should not, under normal or foreseeable conditions of use, transfer their substituents to foodstuffs in quantities which could endanger human health or bring about unacceptable changes in the foodstuffs. To fulfill this broad directive framework, more specific directives have been introduced that specify the quantity of a given substituent or the sum of all substituents in the article which are allowed to transfer or migrate to the food. Because of toxicological reasons (2), legal limits on *m*-XDA migration into food and food simulants have been proposed (3) or enacted (4).

Several chromatographic methods based mainly on high-performance liquid chromatography (HPLC) (5–7) and also capillary gas chromatography (GC) (8–10) have been developed to allow the analysis of diamines and polyamines in foods. The majority of these methods are based on the derivatization of amines using various derivatizating agents. Compared to GC, liquid chromatography (LC) presents numerous advantages; no complex clean-up is necessary, reducing the likely sources of error and increasing absolute recoveries, and there is no need to use "appropriate" internal standards to correct for poor recovery and/or instrumental errors due to injection discrimination. Some of the derivatization reagents used for the LC analysis of diamines are 1-naphthyl isocyanate (11), 1-naphtylisothiourea (12), polymeric anhydride (13), *o*-phthalaldehyde-sulfite (14), benzoyl chloride (15), 9-fluorenylmethyloxycarbonyl (16), dansyl chloride (17), polymeric benzotriazole (18), 3-(7-methoxycoumarin-3-carbonyl)- and 3-(7-dimethylaminocoumarin-3-carbonyl)-2-oxazolones (19), coumarin-6-sulphonyl chloride (20), and lumarins 1 and 2 (21), among others.

Previous studies carried out by us (22–24) showed that the use of fluorescamine as a derivatizating agent is particularly favorable because it provides a simple and rapid derivatization reaction yielding derivatives which can be easily analyzed by HPLC

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with fluorescence detection (25), allowing its determination at very low levels (26). Fluorescamine (4-phenylspiro[furan-2(3H), 1'-phthalan]-3,3'-dione) became a reagent used for detection of other primary amines at ppb concentrations (27). Fluorescamine is not a fluorescent itself (and neither are its degradation products), but in aqueous media at room temperature, it reacts with amines almost instantaneously to yield highly fluorescent derivatives (28).

The objective of this work was to develop a simple and rapid HPLC method for the quantitative determination of *m*-XDA as its difluorescamine derivative in the three official EU aqueous food simulants: distilled water, 3% (w/v) acetic acid in water, and 15% (v/v) ethanol in water. The aim of this method development was to present a method to be forwarded to CEN TC 194/SC 1 for further validation and publication as a standard CEN method.

Experimental

Chemicals, solvents, solutions, and disposables

Reagents such as fluorescamine (98% purity), disodium tetraborate decahydrate, and sodium hydroxyde; solvents such as acetone; and standards such as *m*-XDA (99% purity) were of analytical quality. Solvents for the HPLC mobile phase and samples, such as distilled water and methanol, were HPLC grade.

The following solutions were prepared with these chemicals. Fluorescamine solution (2 mg/mL) was prepared by placing 10 mg fluorescamine into a 5-mL volumetric flask and filling the flask with acetone. This solution may be stored for up to 1 week in darkness at 5°C. Sodium hydroxide (5M) was prepared by placing 20.0 g sodium hydroxide into a 100-mL volumetric flask and filling the flask with water. Borate buffer (0.15M, pH 9.2) was prepared by adding 14.3 g disodium tetraborate 10-hydrate into a 250-mL volumetric flask and filling the flask with water. Any precipitate caused by a fall in temperature must be totally redissolved before the solution is used. Borate buffer (0.02M, pH 9.2) was prepared by transferring 133 mL of 0.15M borate buffer into a 1-L volumetric flask and filling the flask with water. The mobile phase for HPLC consisted of borate buffer–water–methanol (18:37:45) prepared by mixing 180 mL of 0.02M borate buffer with 370 mL of water and 450 mL of methanol. Solutions of *m*-XDA in the official EU food simulants were prepared from a standard stock solution of *m*-XDA in water at a known concentration of approximately 1000 mg/L. Under a nitrogen atmosphere, 100 mg (± 2) of *m*-XDA was weighed and placed into a 100-mL volumetric flask with an accuracy of 0.1 mg. The flask was filled with water, stoppered, and mixed thoroughly. This solution may be stored for up to 3 months in darkness at 5°C.

Intermediate standard diluted stock solutions of *m*-XDA at a known concentration of approximately 2 mg/L were used for the preparation of aqueous food simulant calibration samples. These solutions were prepared by pipetting 2 mL of the standard stock solution of *m*-XDA into a 100-mL volumetric flask and diluting to volume with the appropriate aqueous food simulant, thus obtaining a diluted stock solution of *m*-XDA of approx. 20 mg/L. Ten milliliters of this solution was then pipetted into a 100-mL volumetric flask and diluted to volume with the same simulant, thus obtaining a diluted stock solution of *m*-XDA of approximately 2 mg/L. These solutions may be stored in darkness at 5°C for up to 1 month (water as food simulant) or 3 months (other simulants). Microfilters (PTFE membrane, 13-mm diameter, 0.5-µm pore size) were used for filtering samples before chromatographic analysis.

Apparatus and operating conditions

Glass migration cells as described by Simal-Gándara et al. (24) were used for the migration experiments. Microfilters were used for filtering samples prior to analysis by HPLC and a fluorescence detector connected to a strip chart recorder or integrator.

A Spherisorb ODS 2 octadecylsilane column (150 mm × 4.6-mm i.d., 5-µm spherical packing with 12% carbon loading) from Sugelabor (Madrid, Spain) was found to be capable of fully resolving derivatized *m*-XDA from interferences arising from injection media. The following operating conditions were suitable for this column: injection volume, 50-µL total loop fill; mobile phase composition, borate buffer–water–methanol (18:37:45); flow rate, 1 mL/min; fluorescence detection; excitation wavelength, 394 nm; emission wavelength, 480 nm. Under these condition, the retention time of derivatized *m*-XDA was determined to be 5.0 min.

A method was adopted for *m*-XDA determination (as a basis for further development) consisting of derivatization with 20 µL of 2 mg/mL fluorescamine solution per 2.4 mL of test solution, followed by HPLC with fluorescence detection (excitation, 394 nm; emission, 480 nm) and a calibration range from 50 ppb, the specific migration limit (SML) of *m*-XDA, to 1000 ppb (22–24). Chromatograms obtained under these conditions feature two peaks, one for mono-fluorescamine-*m*-XDA and the other for difluorescamine-*m*-XDA. With the intent of improving the reproducibility of the chromatographic conditions and the sensitivity and precision of the method (to comply with the general recommendation that calibration ranges cover at least the 0.1–2.0 SML range), the following modifications have since been introduced: the concentration of fluorescamine has been increased from 20 to 300 µL per 2.4 mL of test solution so as to ensure that chromatograms feature a single peak corresponding

Table I. Linear Regression Equation ($y = a \cdot x + b$) Obtained from the Calibration Curve of Derivatized *m*-XDA in the Four Food Simulants*

Food simulant	Linear equation coefficients: *a* (L/µg)	*b* (counts)	Correlation coefficient *r*	Standard error of calibration Sr(*x*)	Detection limit (µg/L)
Distilled water	2199	3886	0.999	1.3	3.9
3% (w/v) Aqueous acetic acid	2144	–2866	0.993	3.3	9.6
15% (V/V) Aqueous ethanol	2032	–5211	0.993	3.2	9.3

* 10 calibration points: two sets of solutions at the levels of 20, 40, 60, 80, and 100 µg/L.

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In order to optimize peak shape and minimize retention time, the 1.15M phosphate buffer (pH 8) used for the derivatization reaction and the HPLC mobile phase have been replaced with 0.15M borate buffer (pH 9.2) for the derivatization reaction and 0.02M borate buffer (pH 9.2) for the mobile phase. The proportions of buffer, water, and methanol in the mobile phase have been modified accordingly. It should be noted that under these conditions, the pH exceeds the limit of pH 8 recommended for ODS2 columns; as a result, the column life may be reduced and the stationary phase may undergo minor alterations that make it unusable for other purposes. Nevertheless, pH 9.2 is recommend.

Table II. Recovery % (Mean) and Precision % (Standard Deviation) Obtained for *m*-XDA in the Three Aqueous Food Simulants*

Food simulant	Nominal concentration (µg/L)	Recovery (%)	Precision (%)
Distilled water	50	88.0	5.5
3% (w/v) Aqueous acetic acid	46	99.9	3.5
15% (v/v) Aqueous ethanol	46	102.8	2.9

* Five degrees of freedom ($n - 1$).

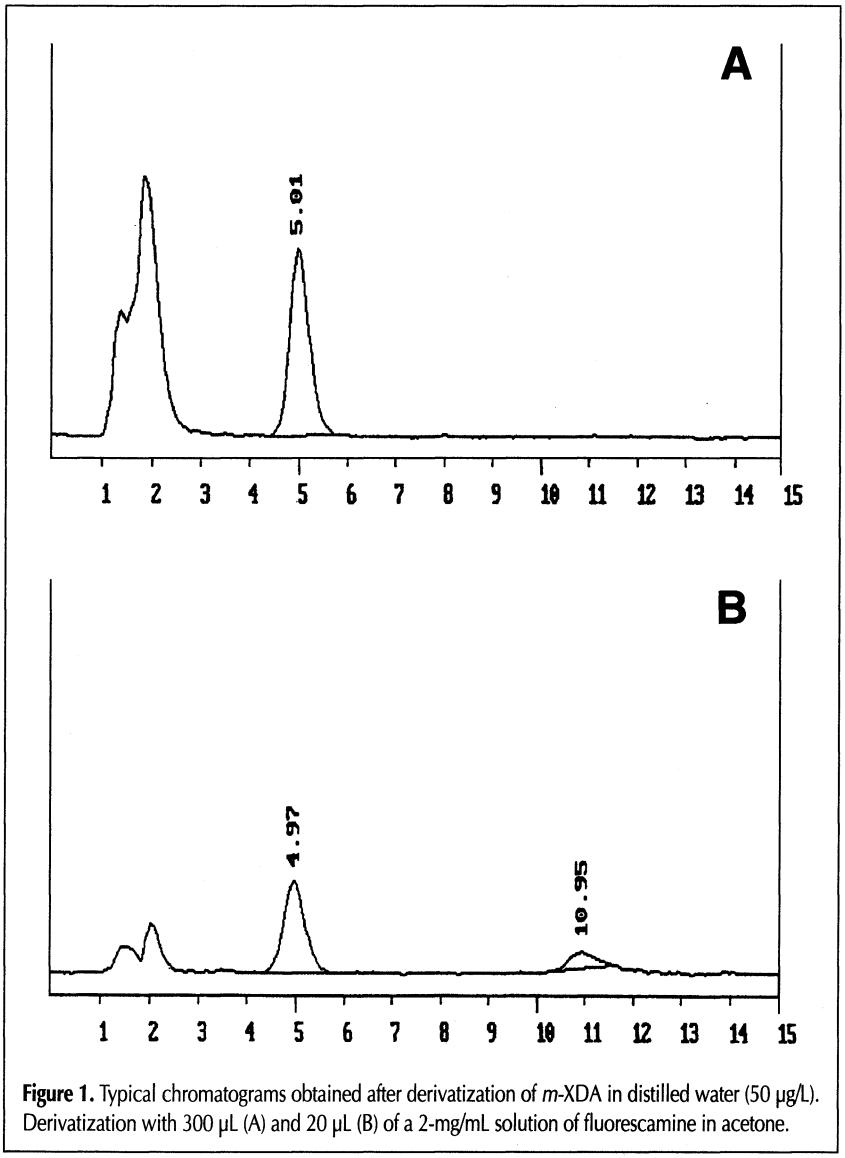

Figure 1. Typical chromatograms obtained after derivatization of *m*-XDA in distilled water (50 µg/L). Derivatization with 300 µL (A) and 20 µL (B) of a 2-mg/mL solution of fluorescamine in acetone.

Preparation of standard calibration solutions and calibration

For each of the food simulants, duplicate sets of calibration samples were prepared, and each was run in triplicate. Aliquots of 1, 2, 3, 4, and 5 mL of the intermediate standard diluted stock solution of *m*-XDA at a concentration of approximately 2.0 mg/L were pipetted into a series of 100-mL volumetric flasks. The flasks were then filled to the 100-mL mark with the same aqueous food simulant as used in its preparation to give approximately 0.02, 0.04, 0.06, 0.08, and 0.1 mg per 1-L *m*-XDA solutions. The procedure was repeated using a second 2.0-mg/L intermediate standard diluted stock solution.

Experiments to determine the performance of the method for the measurement of *m*-XDA samples with concentrations greater than 100 µg/L showed that linearity was maintained (and the calibration line was therefore usable) up to the concentration at which the fluorescence detector became saturated (approximately 700 µg *m*-XDA per L). Non-linearity occurs at approximately 1 ppm because of insufficient fluorescamine.

The details of the calibration line calculation are listed in Table I. The essential data and regression results are summarized together with the detection limit that was calculated from the calibration line in accordance with DIN 32645. All calibration lines had a correlation coefficient r no smaller than 0.993, and standard errors $Sr(x)$ of less than 3.5 µg *m*-XDA per liter. Detection limits were in the range of 0.004 to 0.010 mg *m*-XDA per kilogram food simulant (depending on the type of food simulant). To verify the effective detection at the limit calculated from the calibration lines, *m*-XDA solutions were run in triplicate at those levels. In all cases, the signal-to-noise ratio of the *m*-XDA peak was approximately 5. Noise was measured as the maximum amplitude of the chromatogram of a blank between 5 and 6 min. This criterion for noise is both simpler and stricter than its calculation as three times the standard deviation of the mean blank.

Derivatization of standards and samples

The derivatization reaction before HPLC analysis depended on the food simulant type.

Water and 15% (v/v) ethanol food simulants

A 2-mL aliquot of test sample or blank sample was pipetted into a 5-mL glass tube, 0.4 mL of 0.15M borate buffer was added, the solution was mixed thoroughly, and 300 µL of the fluorescamine solution was injected by syringe. The solution was stirred for 1 min and allow to stand for 10 min.

3% (w/v) Acetic acid food simulant

A 10-mL aliquot of test sample or blank sample was pipetted into a 25-mL glass beaker. The volume of 5M NaOH solution necessary to bring the pH to 9.2 was determined to within ± 0.01 mL using a microburette. Another 10 mL of the test sample was pipetted into a 25-mL glass beaker, the volume of 5M NaOH was added (± 0.01 mL), and the solution was mixed thoroughly. The pH of this mixture should be in the range of pH 8.0–9.5. A 2-mL aliquot was pipetted into a 5-mL glass tube, 0.4 mL of 0.15M borate buffer was added by pipette, the solution was mixed thoroughly, and a 300-µL aliquot of fluorescamine solution was added by syringe. The mixture was stirred for 1 min and allow to stand for 10 min.

Once the set of calibration samples have been processed and derivatized as described, they were injected in duplicate. the areas of the derivatized *m*-XDA peaks were measured. The calibration line was constructed by plotting mean derivatized *m*-XDA peak area against the concentration of *m*-XDA in the calibration samples (mg/L).

Stability of standard stock and diluted stock solutions

m-XDA stock solutions containing 1000 mg of *m*-XDA per liter water were stored for three months at –20°C, 0–5°C, and room temperature and then determined after dilution to 100 µg/L with distilled water. No change in *m*-XDA concentration was detected in any of these solutions. The recommend storage temperature is 0–5°C.

Diluted *m*-XDA stock solutions containing 1000 µg *m*-XDA per liter of aqueous food simulant were similarly stored for three months at –20°C, 0–5°C, and room temperature and were then determined after dilution to 100 µg/L with simulant. The solutions in 3% (w/v) acetic acid and 15% (v/v) ethanol underwent no significant reduction in concentration as a result of storage except for a 15% reduction in the 15% ethanol solution stored at –20°C. The solutions in distilled water, on the other hand, underwent reductions in concentration ranging from approximately 15% at –20°C to 50% at 0–5°C (although a sample stored at 0–5°C for one month showed no reduction in concentration).

Note that *m*-XDA is unstable in air, probably because of oxidation and carbonation reactions. It should always be handled under nitrogen. *m*-XDA is stable in the stock solution in water and the 1-ppm diluted stock solutions in 3% acetic acid and 15% ethanol, but there appear to be problems with the diluted solution in water. Although the concentrations of oxygen and CO_2 in water are not sufficient enough to cause a relatively significant change in the concentration of *m*-XDA in the 1000-ppm stock solution, they might change the concentration of the diluted solution. The stability of the *m*-XDA in the diluted stock solutions in 3% acetic acid and 15% ethanol may be due to the relative acidity of these media protecting the amine from oxidation and carbonation ($-NH_3^+$ being more stable than $-NH_2$).

Method recovery and precision (including derivatization and chromatography)

Because of the SML of *m*-XDA (50 µg/L), six *m*-XDA samples at a concentration of approximately 50 µg *m*-XDA per 1 simulant were prepared for each food simulant. These solutions and the corresponding blanks were processed and run in duplicate.

The nominal concentrations applied to the EU food simulants in the recovery experiments, together with the corresponding recovery figures, are listed in Table II. For all three aqueous food simulants, recoveries greater than 88% were achieved with a standard deviations of less than 5.5%.

Food simulant sample processing

All laboratory samples of food simulant to be analyzed can be kept frozen at 4°C in closed containers and in the absence of light for up to three months. If necessary, a sample of the aqueous food simulant obtained from the migration experiment can be filtered through a microfilter. The derivatized sample can be injected. The preparation of blank samples is the same as previously stated

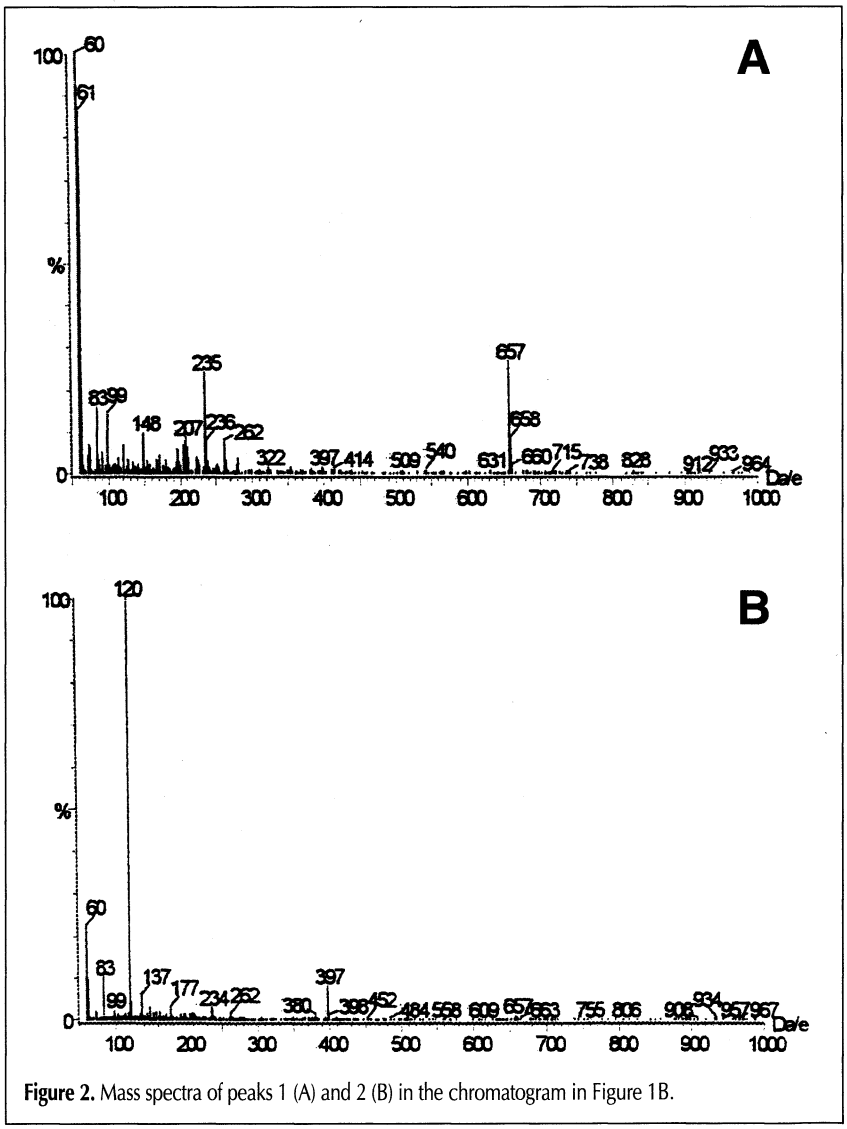

Figure 2. Mass spectra of peaks 1 (A) and 2 (B) in the chromatogram in Figure 1B.

using an *m*-XDA-free food simulant.

Confirmation of *m*-XDA identity

The confirmation of analyte identity by formation of a second *m*-XDA derivative is as follows: the calibration sample with the *m*-XDA concentration closest to the estimated *m*-XDA concentration in the test sample is processed and derivatized, and 20 µL of the fluorescamine solution is to be used instead of 300 µL. HPLC of the derivatized sample should yield two peaks, one at 5.0 min and the other at 11.0 min. This process is repeated using the sample instead of the calibration solution. The retention times obtained for the sample should not differ by more than ± 5% from the retention times obtained for the calibration solution. Typical chromatograms are shown in Figure 1. The two relevant peaks in chromatogram B were confirmed by mass spectrometry as corresponding to di- and mono-fluorescamine-*m*-XDA in the order of elution (Figure 2).

It should be noted that the order in which the mono- and difluorescamine-*m*-XDA peaks appear is the reverse of the order in which they appeared in the initial method, which used a phosphate buffer of pH 8 in the mobile phase (22–24). This might be explained by the influence of ionic strength or the salt effect.

Results and Discussion

Specific migration from the polymer after 10 days at 40°C

The polymer coating that was tested was an epoxy paint based on a biphenol A diglycidyl ether epoxy resin with *m*-XDA as a curing agent. Its manufacture also involves the addition of benzyl alcohol (to lower the glass transition temperature) and other products such as filler and pigments to improve water resistance and color. It is intended for use as a coating on food storage vessels.

Two glass discs (total surface area 2.7 dm^2) were painted with the polymer coating on one side, cured for 24 h at room temperature, painted on the other side, cured at room temperature for 5 days, and then immersed in 150 mL of food simulant in a glass cell specially designed for testing epoxy coating migration into food simulants (24). The surface-to-volume ratio, 1.8 dm^2 per 100 mL of simulant, was not significantly different from the 2 dm^2/100 mL usually used in migration testing.

Migration tests were carried out in triplicate. The polymer sample was left in the food simulant for 10 days at 40°C before duplicate determination of *m*-XDA in the migration solution. Blanks consisted of simulant stored for 10 days at 40°C. Since migration into the aqueous food simulants resulted in *m*-XDA concentrations much higher than the upper limit of the method calibration range, the migration solutions were diluted prior to *m*-XDA determination (200-fold for water, 500-fold for 3% acetic acid, and 250-fold for 15% ethanol). The main results obtained are summarized in Table III. Migration into aqueous food simulants in the migration test cell resulted in *m*-XDA concentrations much higher than the SML of *m*-XDA. Nevertheless, it should be pointed out that much lower concentrations would result from migration of the same amount of *m*-XDA per unit surface area of coating into the liquid contents of the large tanks for which the polymer coating is intended; as a result, *m*-XDA would comply with EU norms after application of the corrections scheduled in Points 1 and 5 of the Annex to EU Commission Directive 90/128 (4).

m-XDA stability in fortified simulants after 10 days at 40°C

The stability of *m*-XDA in the food simulants at the migration testing conditions was further investigated by determining the *m*-XDA in simulant that had been fortified with *m*-XDA to a concentration of 50 µg/L and then stored for 10 days at 40°C. The main results are compared in Table IV. Recoveries for the three aqueous simulants are 91.7–114.5% with standard deviations less than 14%; thus, the amine was stable in the three aqueous simulants.

The results obtained with distilled water in this and the previous stability study appear to show, somewhat surprisingly, that *m*-XDA is stable for 10 days at 40°C and for 1 month at 0–5°C, but not for 3 months at 0–5°C. If the cause of the alteration observed at this temperature is, as supposed, a reaction with dissolved 0_2 and CO_2, the peculiar variation of stability with temperature may be attributed to both gases being less soluble in water at 40°C than at 0–5°C.

Table III. Specific Migration Levels of *m*-XDA from the Polymer Sample after 10 Days at 40°C*

	Measured concentration (mg/L)	
Food simulant	Mean	Standard deviation
Distilled water	7.8	1.8
3% (w/v) Aqueous acetic acid	34.7	3.6
15% (v/v) Aqueous ethanol	21.2	2.1

* Two degrees of freedom ($n - 1$).

Table IV. Recovery % (Mean) and Precision % (Standard Deviation) Obtained for *m*-XDA in the Three Aqueous Food Simulants After 10 Days at 40°C*

Food simulant	Nominal concentration (µg/L)	Recovery (%)	Precision (%)
Distilled water	46	91.7	11.0
3% (w/v) Aqueous acetic acid	46	100.0	0.8
15% (v/v) Aqueous ethanol	46	114.5	14.0

* Two degrees of freedom ($n - 1$).

Conclusion

The proposed HPLC method for the quantitative determination of *m*-XDA performs well in all three food simulants, exhibiting satisfactory analytical quality in terms of sensitivity, linearity, repeatability, and recovery. No problems of interference have been detected in either simulants or

real foodstuff samples, probably due to the specificity of the derivatization reaction. The only sources of interference that seem at all likely would be other primary amines with fluorescamine derivatives behaving like those of *m*-XDA under HPLC. This suggests the possibility of developing a single method for joint determination of primary amines in food simulants.

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