Stability of the Secondary Antioxidant Bis(2,4-di-*tert*-butylphenyl)pentaerythritol Diphosphite in Food Simulants

C. Pérez-Lamela,[†] R. Rijk,[‡] and J. Simal-Gándara*[,§]

Nutrition and Bromatology Group, Department of Analytical Chemistry, Nutrition and Bromatology, Pharmacy Faculty, Campus of Santiago de Compostela, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain; Packaging Research Group, Analytical Sciences Division, TNO-Nutrition and Food Research Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands; and Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Food Science and Technology Faculty, Campus of Ourense, University of Vigo, 32004 Ourense, Spain

To establish the stability of Ultranox 626 (an antioxidant added to plastics) in food simulants under migration conditions, migrations tests have been performed. A method has been developed for the determination of Ultranox 626 in the aqueous food simulants distilled water, 3% (w/v) acetic acid, and 15% (v/v) ethanol and in the fatty food simulants 95% (v/v) ethanol and isooctane. The method uses reversed-phase high-performance liquid chromatography with ultraviolet detection at 230 nm, is fast, and can be run automatically. To determine the stability of Ultranox 626, it was heated in each of the listed food simulants under the conditions stipulated in EU regulations for testing for compliance with migration limits. These experiments showed that this additive had acceptable stability in water, 15% and 95% (v/v) ethanol, and isooctane but that it decomposed completely in 3% (w/v) acetic acid. Migration testing with 3% acetic acid is of no use, since by the end of the testing regime the additive will have undergone substantial or total decomposition, and the level detected will not reflect the true level of migration. The EU Commission should replace 3% acetic acid with 15% ethanol as an appropriate test simulant for the determination of Ultranox 626 in all types of acid- and alcohol-containing foodstuffs. A number of experiments were carried out to develop a suitable method for the determination of Ultranox in fat simulants such as olive oil and HB 307. It appeared not possible, within the scope of this project, to obtain a method suitable to establish the stability of Ultranox 626 in fat simulants. Best results were obtained by freezing out the fat at −80 °C, but recovery was limited to 50%, which was insufficient for the intended purpose. Further experiments are required to establish the stability of Ultranox 626 in fat simulants such as olive oil and HB 307.

Keywords: *Ultranox 626; phosphite secondary antioxidants; food safety; liquid chromatography with ultraviolet detection; stability and migration tests*

INTRODUCTION

Article 2 of the European Economic Community (EEC) Directive 89/109/EEC (OJEC, 1989) states that 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 framework directive, other specific directives have been written specifying the quantity of a specific substituent or the total of all substituents in the food contact article that are allowed to transfer or migrate to the food (see Article 3). Monomers, starting compounds, and some additives allowed in food contact articles are published in a so-called positive list [Directive 90/128/EEC (OJEC, 1990) and its amendments 92/39/EEC (OJEC, 1992), 93/9/EEC (OJEC, 1993), 95/9/EC (OJEC, 1995), and 96/11/EC (OJEC, 1996)] and are, to a large extent, assigned limits for residual levels in the polymer and for specific migration in the food. The limits are determined from toxicological evaluation of the substance. The EC Member States are obliged to implement EC directives and thus to carry out tests to ensure that food contact materials comply with their requirements. Besides tests using real food, EC Directive 97/48/EC (OJEC, 1997) prescribes four food simulants for testing plastics: distilled water, 3% (w/v) acetic acid, 15% (v/v) ethanol, and rectified olive oil, synthetic fat HB 307, sunflower oil, or substitute fatty food simulants [95% (v/v) ethanol and isooctane]. However, if it is demonstrated that one of the substitute fatty food simulants is not appropriate for the component or polymer under investigation, then that substitute shall not be used. Alternatively, other substitute test media can be used when the suitability of that medium has been demonstrated. The indicated simulants are intended to cover all types of foodstuffs and to represent a worst case situation.

At present, a large number of monomers with specific migration limits are listed in EU directives. In future

* Author to whom correspondence should be addressed (fax +34-88-387001; e-mail jsimal@uvigo.es).
[†] University of Santiago de Compostela.
[‡] TNO-Nutrition and Food Research Institute.
[§] University of Vigo.

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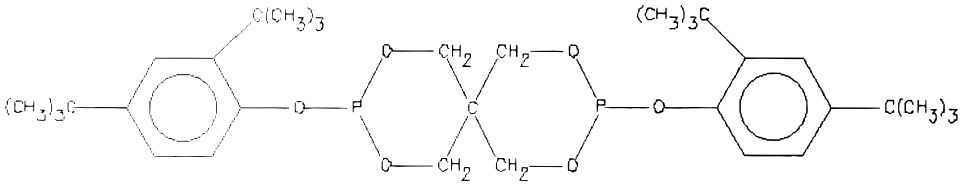

Figure 1. Molecular structure of Ultranox 626.

directives, when the positive list of additives is completed, the number of specific migration or residual content limits may increase to many hundreds of substances. The introduction of food consumption factors (FCF) and plastic use factors (PUF) may reduce the number of restrictions significantly. When PUF are introduced, the advantages of general use of monomers and additives in food contact materials will necessarily be reduced. As a consequence, the number of petitions for the use of substances in specified food contact materials, to be prepared by industry, will increase significantly.

In the various EU Directives restrictions are set to the intentionally added monomers and, in the future, to additives. With a few exceptions, migration of decomposition products as well as impurities of the subject substance are only briefly considered in the toxicological evaluation of the substance by the Scientific Committee for Food (SCF). In general, this will not be a problem as impurities and decomposition of the substance during manufacturing of the food contact material are limited. Recently the SCF has included the oxidation product of a phosphite type antioxidant in the migration limit. This may be the start of a new philosophy in setting migration limits for substances that are added with the explicit intention to decompose to protect the polymer from thermal oxidation.

The migration of impurities and decomposition products is a matter of the SCF and the legislator. In compliance testing and enforcement of directives the substance may decompose in the food simulant. If a substance is sensitive to decomposition due to reaction or hydrolysis with the food simulant, then the migration in food simulants by definition will be very low. As decomposition in real food may deviate from decomposition in migration tests, the consumer may be exposed to potential toxic substances, while in compliance or enforcement testing the material fulfills the requirements. This is an undesirable situation. The regulations assume stability under migration testing conditiona and make no allowance for possible loss during migration testing or the fact that if there are losses, then reaction products may be formed which may deviate in toxicity from the parent substance.

Expensive development and validation of a method for a substance that appears to be unstable under migration conditions would be a waste of valuable time and money. Therefore, in the scope of the AAIR (Agriculture and Agro-Industrial Research) program of EU Directorate General XII, a study was initiated to determine the stability in food simulants of certain additives, for which a specific migration limit is foreseen in a future EU directive. The antioxidant Ultranox 626 [bis(2,4-di-*tert*-butylphenyl)pentaerythritol diphosphite] is an additive for which an SML of 0.6 mg/kg of food is indicated in a draft document (Synoptic Document No. 7, 1994).

The antioxidants, added to polymers to reduce the effects of thermal oxidation, can be divided into primary and secondary antioxidants. Primary antioxidants (PAOs), also known as chain-breaking antioxidants, protect the polymer by trapping the free radicals produced by direct oxygen attack (Schwarzenbach, 1987). The hindered phenolics are PAOs.

Secondary antioxidants (SAOs), also known as synergistic or preventive antioxidants, prevent oxidation by reacting with hydroperoxides formed by thermal oxidation among other reactions (Schwetlick et al., 1986; Schwarzenbach, 1987). Examples of SAOs are the phosphites

$$\mathrm{ROOH + P(OR)_3 \rightarrow ROH + O{=}P(OR)_3} \quad (1)$$

and phosphonites

$$\mathrm{ROOH + R'P(OR)_2 \rightarrow ROH + O{=}PR'(OR)_2} \quad (2)$$

Ultranox 626 (Figure 1) is a phosphite-type stabilizer that is often added to polyolefins such as polypropylene (PP) or high-density polyethylene (HDPE). The present work reports our efforts at the development of a method for the analysis of Ultranox 626. As far as we know, there is currently no such a method available in the scientific literature. Likewise, we did not find any work examining the stability of this additive. Inspection of its structure suggests that it may be prone to oxidation and/or hydrolysis under testing conditions which involve heating in an aqueous or acidic environment:

$$\mathrm{P(OR)_3 \xrightarrow{O_2} O{=}P(OR)_3} \quad (3)$$

$$\mathrm{P(OR)_3 \xrightarrow{H^+/H_2O} (RO)_2P(OH) \longleftrightarrow (RO)_2\overset{H}{\underset{|}{P}}{=}O \longrightarrow (RO)P(OH)_2} \quad (4)$$

The question to be answered by the present work is whether Ultranox 626 is stable in food simulants under the most frequently applied test conditions of 10 days at 40 °C, as well as under the most severe test conditions of 1 h at reflux temperature with aqueous food simulants and 2 h at 175 °C with fat. Wasteful method development and method validation, where stability data indicate that the survival of the migrating additive is improbable, should be avoided. In this respect it was considered that a decrease of >50% of the initially added amount of Ultranox 626 in a food simulant should lead to the conclusion that the substance is not stable in that food simulant using a specified test condition.

If the substance is not stable in any of the food simulants, then the EU authorities should consider the conclusion of the study and change the type of restriction in a future directive. When the substance appeared to be unstable in only one food simulant, then the use of that simulant should be excluded from compliance testing.

EXPERIMENTAL PROCEDURES

Materials. *Reagents* included analytical grade water, acetic acid, absolute ethanol, isooctane, aceton, and the fatty food simulants olive oil and HB307 (a synthetic mixture of triglycerides).

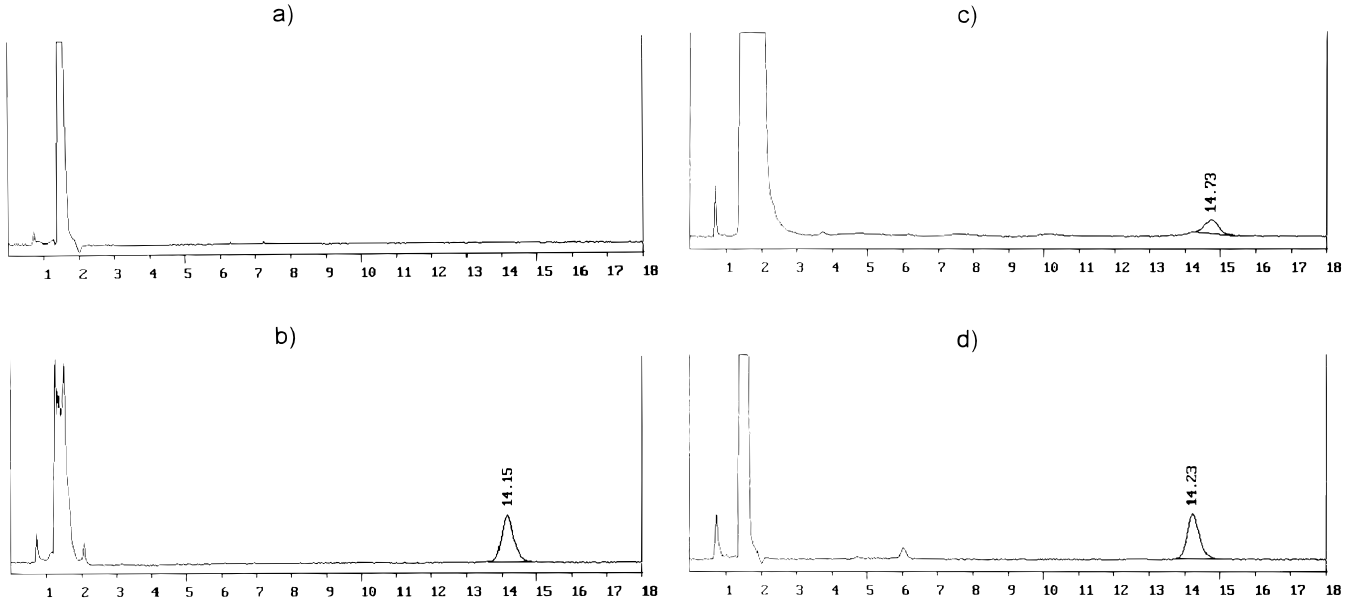

Figure 2. Chromatograms of Ultranox 626 in 95% (v/v) ethanol: (a) blank; (b) standard; (c) after 10 days at 40 °C; and (d) after 1 h at reflux.

Standards. A stock solution of ~0.5 mg/mL Ultranox 626 [bis(2,4-di-*tert*-butylphenyl)pentaerythritol diphosphite, PM (packaging material) ref 38820, CAS (Chemical Abstracts Service) Registry No. 26741-53-7, and EINECS (European Inventory of Existing Commercial Chemical Substances) No. 247-952-2] was made up in aceton.

Apparatus. *General equipment* included glassware; an ultraviolet−visible spectrophotometer; 2-mL HPLC injection vials, provided with twist caps and PTFE-lined butyl rubber septa; 22-mL headspace vials, provided with crimp caps and PTFE-lined butyl rubber septa; an oven; a hotplate used for reflux; a N_2 supply used for evaporations to dryness; and an ultrasonic bath used for preparing solutions.

The high-performance liquid chromatographic (HPLC) system was equipped with an autosampler, a 50-mL injection loop, and an ultraviolet detector linked to a personal computer running Winner On Windows software for data acquisition and processing. The mobile phase was continuously degassed by means of a degasser.

HPLC Conditions. *Injection Procedure.* Duplicate determinations were always assayed. *The column* was a 25 cm × 3.9 mm i.d. stainless steel column packed with 5 mm Hypersil ODS. *The elution program* provided 18 min of isocratic elution with 95:5 acetonitrile/water. Flow rate was 2 mL/min throughout. Ultranox 626 eluted at ≈14 min. *Ultraviolet detection* was at $\lambda = 230$ nm; output, 1 V; range, 0.002; offset, 5%; rise time, 0.5 s.

Calibration. Figure 2 includes chromatograms for a blank and the standard. Calibrations were performed as described below.

Aqueous Simulants and 95% (v/v) Ethanol. The Ultranox 626 stock solution was diluted 1 to 100 times with the simulant [water, 3% (w/v) acetic acid, 15% (v/v) ethanol, or 95% (v/v) ethanol]. Diluted Ultranox 626 solution (1, 3, 6, 10, or 15 mL) was pipetted into a series of 50-mL volumetric flasks, which then were filled to the mark with the corresponding simulant. Calibration lines for Ultranox 626 in each simulant were constructed by plotting HPLC peak area against concentration for the five standard solutions, which contained approximately 0.1, 0.3, 0.6, 1.0, and 1.5 μg of Ultranox 626/mL of simulant, and for blanks comprising the simulant alone.

Isooctane. Since isooctane could not be injected into the HPLC column, for samples in isooctane, solvent was eliminated along with any volatile contaminants before analysis. Thus, by means of a syringe, 2, 6, 12, 20, and 30 μL of Ultranox 626 stock solution were transferred into a series of 22-mL headspace vials containing 10.0 mL of isooctane, affording standard solutions containing approximately 0.1, 0.3, 0.6, 1.0, or 1.5 μg of Ultranox 626/mL of isooctane. The isooctane was then evaporated using a gentle stream of N_2, and the residue was dissolved in 5.0 mL of aceton, diluted with 5.0 mL of distilled water, and carefully mixed. The calibration line was obtained as above using these five solutions and a similar solution derived from an isooctane blank.

Detector response over the range of 0.1−1.5 μg of Ultranox 626/mL appeared to be linear. Correlation coefficients better than 0.996 were obtained.

Table 1. Chromatographic Standard Deviations and Repeatabilities for Six Simulant Solutions Spiked with 0.6 mg of Ultranox 626/L[a]

simulant	mean ± $SD_{(n-1)}$[b] (%)	repeatability[c] (%)
distilled water	96.7 ± 4.4	12.5
3% acetic acid	73.7 ± 8.0	22.6
15% ethanol	95.9 ± 1.9	5.4
95% ethanol	96.3 ± 2.0	5.7
isooctane	102.3 ± 3.3	9.3

[a] For isooctane, this variability check also includes the step of evaporation to dryness and residue reconstitution, prior to HPLC analysis. [b] Standard deviation [$SD_{(n-1)}$]. [c] Repeatability = 2.83 × $SD_{(n-1)}$.

Detection Limit (DL). The DL, following DIN 32645 (Deutsches Institut für Normung, 1994), was interpolated from the calibration line as the smallest concentration detectable at a 95% level of confidence (the concentration corresponding to the peak area at which the upper 95% confidence limit boundary cuts the ordinate axis). The DL for Ultranox 626 was 0.07 mg/L in distilled water, 0.34 mg/L in 3% (w/v) acetic acid, 0.1 mg/L in 15% (v/v) ethanol, 0.08 mg/L in 95% (v/v) ethanol, and 0.05 mg/L in isooctane.

Repeatability. For each simulant, six replicate, freshly prepared solutions and one blank were used to estimate chromatographic repeatability, in the case of aqueous simulants and 95% (v/v) ethanol, and recovery repeatability, for isooctane (Table 1), following ISO-3534-1 (The International Organization for Standardization, 1993). For the aqueous simulants and 95% (v/v) ethanol, 1.2 mL of an ≈5 μg/mL Ultranox 626 solution was diluted to 10 mL in a volumetric flask. For isooctane, 12 μL of the stock solution (≈0.5 mg of Ultranox 626/mL of aceton) was diluted to 10 mL. The solutions thus obtained contained ~0.6 μg of Ultranox 626/mL of simulant (the specific migration limit expected to be applied by the EU to migration of Ultranox 626 into foods and food simulants).

Table 2. Results of the Stability Experiments Carried out for Ultranox 626 in the Food Simulants

simulant	test conditions (time/temp)	recovery (%) mean	recovery (%) $SD_{(n-1)}$
distilled water	10 days at 40 °C	72	9
	1 h at reflux	98	3
3% acetic acid	10 days at 40 °C	0	0
	1 h at reflux	0	0
15% ethanol	10 days at 40 °C	80	10
	1 h at reflux	88	5
95% ethanol	10 days at 40 °C	63	6
	1 h at reflux	91	1
isooctane	2 days at 20 °C	100	2
	3 h at 60 °C	96	6

Stability Experiments. Three replicate simulant solutions spiked with Ultranox 626 and two simulant blanks were subjected to each set of conditions given in Table 2. For the isooctane solutions, the spiked simulants were prepared as described under Repeatability. For the aqueous simulants and 95% (v/v) ethanol, the spiked simulant solutions were prepared as follows.

For the conditions "10 days at 40 °C", the spiked simulant solutions were prepared as described under Repeatability, and then these solutions and two blanks were poured into separate 22-mL headspace vials (the liquid/headspace volume ratio was thus 1:1) and stored for 10 days in an oven at 40 °C.

For the conditions "1 h at reflux", a stock solution of ≈50 mg of Ultranox 626 in 100 mL of aceton was made up and diluted 1 to 100 with each simulant. Test solutions were prepared by further diluting 3 mL of the resulting solution to 25 mL in a volumetric flask. These solutions and 25 mL of two blank solutions contained in 50-mL conical flasks were fitted to reflux condensers, placed on a hot plate, and gently refluxed for 1 h.

After the exposure time, they were allowed to cool to room temperature and then analyzed by reversed-phase HPLC for the recovery of Ultranox 626.

RESULTS AND DISCUSSION

The results of the stability tests in each simulant are presented in Table 2. In stability testing, >50% of the Ultranox 626 added should be recovered after the exposure time. The results show that in water and 15% and 95% (v/v) ethanol, Ultranox 626 has—at least—acceptable stability for up to 10 days at 40 °C and high stability for up to 1 h at reflux temperature. In isooctane, Ultranox 626 appeared to be stable for—at least—up to 2 days at 20 °C and up to 3 h at 60 °C. According to Directive 97/48/EC these test conditions correspond to 10 days at 40 °C and 1 h at 100 °C with olive oil.

In 3% acetic acid, Ultranox 626 appeared to decompose completely, recovery being zero after 1 h at reflux or after 10 days at 40 °C. This indicates that this additive has greatest lability under acid conditions.

The determination of Ultranox 626 in aqueous food simulants, 95% ethanol, or isooctane was straightforward using reversed-phase HPLC with UV detection. However, the determination of Ultranox 626 in olive oil or HB307 proved difficult by this method. The best result obtained was a recovery of ≈50% for HB307 (see below for method); however, the coefficient of variation was rather high (23%), and so this method was not suitable for use in the stability tests.

Initial experiments suggested that olive oil contained many interfering components, which were not present in the fat simulant HB307, and so the latter was used as the fatty food simulant in all subsequent experiments. Various attempts were made to determine Ultranox 626 in HB307 by the reversed-phase HPLC method that had proved to be suitable in the case of the aqueous food simulants. When this failed, the reversed-phase column, composition of the mobile phase, or detection wavelength was varied. However, none of the sets of conditions tested proved suitable. In most cases, the chromatogram was of poor quality due to the presence of peaks due to HB307. Attempts to remove the fat simulant by liquid−liquid extraction were fruitless due to the high solubility of Ultranox 626 in many organic solvents. The use of normal-phase HPLC with silica gel or aluminum oxide columns was likewise unsuccessful due to the presence of interfering components from the simulant. Isolation of the Ultranox 626 on a 2-cm-diameter column of silica gel, and subsequent elution followed by analysis of the eluate, was similarly marred by the presence of HB307. Finally, we attempted freezing out of the HB307 at −80 °C as follows.

Standard mixtures of Ultranox 626 in HB307 were prepared and dissolved in aceton. These solutions were cooled to −80 °C to crystallize the triglycerides, and the solvent was filtered off at −80 °C and then evaporated to dryness. The residue was redissolved in a suitable solvent, and the Ultranox 626 in the resulting solution was determined. Recovery of Ultranox 626 varied from 20% to 52%, too low to allow meaningful results to be obtained in the stability studies. At this point, attempts at the development of a method for determination of Ultranox 626 in the fatty food simulant HB307 were ceased, and attention was focused on the alternative simulants 95% ethanol and isooctane.

CONCLUSIONS

The stability of Ultranox 626 could not be determined in olive oil or HB307 as no suitable analytical method was available. Until a suitable method becomes available, alternative tests should be used for the determination of the specific migration of Ultranox 626 into fatty food simulants.

The method for the determination of Ultranox 626 in aqueous and substitute fatty [95% (v/v) ethanol and isooctane] simulants gave consistent results across the entire calibration range studied. This method is fast and can be run automatically, and it has a detection limit that is low enough to permit its use in migration tests. It is therefore recommended for determination of Ultranox 626 in such tests involving the indicated aqueous and fatty simulants.

The results of the stability tests justify the use of substitute fatty food simulants. However, this does not imply that the substitute test media are suitable for the determination of the migration in real fatty simulants. Additional investments may be required to establish the suitability of the substitute test media in migration testing.

The results of the stability tests with aqueous and fatty simulants show that in water and 15% and 95% (v/v) ethanol, Ultranox 626 has acceptable stability, by criterion of—at least—50% recovery, for up to 10 days at 40 °C and high stability for up to 1 h at reflux temperature. In isooctane, Ultranox 626 appeared to be stable for up to 2 days at 20 °C and for up to 3 h at 60 °C. The latter conditions are selected to replace 10 days at 40 °C and 1 h at 175 °C with fatty simulant.

By contrast, Ultranox 626 decomposes rapidly in 3% acetic acid, recovery being not detectable after 10 days at 40 °C or after 1 h at reflux. This means that,

irrespective of the extent of migration of Ultranox 626 into this simulant, it is unlikely to be detected at levels exceeding the statutory limit set for this simulant by the EU.

Two issues arise as a result of this work:

Some assessment of the stability of an additive in a foodstuff and/or food simulant should be made before time is invested in the development of a validated method for the determination of the additive in that food and/or simulant.

Provisions should be made by the European Commission to insert a remark, on the SML to be set for Ultranox 626, that instructs investigators to replace 3% acetic acid by, e.g., 15% ethanol as a simulant for all acid- and alcohol-containing foodstuffs.

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