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Determination of primary aromatic amines in water food simulant using solid-phase analytical derivatization followed by gas chromatography coupled with mass spectrometry

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Abstract

Solid phase analytical derivatization with trifluoroacetic anhydride has been introduced as sample preparation for the determination of primary aromatic amines in water by gas chromatography coupled with mass spectrometry. Water was used as a food simulant for testing migration from laminated flexible food packaging materials. The method was evaluated for 8 primary aromatic amines in 200 ml water samples, which resulted in detection limits in the 0.1–0.4 μg/l range, relative standard deviations in the 4–17% range and acceptable linearity (R^2=0.997–1.000). Detectable levels of 2,4-diaminotoluene, 2,6-diaminotoluene and 4,4′-methylenedianiline were found in water food simulant from some of the investigated food packaging materials.

Keywords: Food packaging; Solid phase analytical derivatization; Migration; Derivatization, GC; Amines, aromatic; Primary aromatic amines

1. Introduction

Primary aromatic amines (PAA) are substances that can be transferred from food packaging materials into foodstuffs [1–3]. In production of multilayer plastic materials it is common to use reactive adhesive mixtures containing aromatic isocyanate monomers. In cases of incomplete curing, residues of the aromatic isocyanates react with water to produce primary aromatic amines. Some primary aromatic amines, including 2,4-diaminotoluene and 4,4′-methylenedianiline, are classified as 'possibly carcinogenic to humans' by the International Agency for Research on Cancer, and thus their appearance in foodstuffs should be prevented. According to European legislation, the total concentration of PAA migrated into foodstuffs or food simulants should not be detectable using an analytical method with detection limit of 20 μg/kg foodstuff. The current method applied for testing migration of PAA is based on spectrophotometry [4]. After migration exposure, where the food packaging material is in contact with an aqueous food simulant, the total PAA concentration level is determined in the food simulant by measurement of absorbance of colored PAA species produced by reaction with a coupling reagent. The result is then reported as aniline equivalents, by

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using aniline as calibration substance. However, the possibility exists that other migrated substances react with the coupling reagent and contribute to the absorbance level. Thus, if positive samples are encountered by use of the spectrophotometry method, an additional analysis will be required to confirm the presence and concentration level of each non-approved PAA compound.

The confirmation method should be able to determine PAA's in the food simulant below the 20 μg/l concentration level. Due to the possibility of other interfering substances at this low level, a chromatographic method of high resolution and selectivity offers some advantage. Gas chromatography coupled with mass spectrometry (GC–MS) has previously been reported as a suitable method for trace level determination of aromatic amines in water samples [5–11]. Either liquid–liquid extraction (LLE) or solid-phase extraction (SPE) can be used for sample preparation [12]. The latter is usually preferred due to higher extraction yields, speed and reduced solvent consumption. In order to avoid peak tailing and loss of signal due to strong interactions of the amines in the gas chromatography column, derivatization of the amines can be utilized, e.g., with trifluoroacetic anhydride, to produce volatile and less polar trifluoroacetamides [13].

A new GC–MS method has been developed for the determination of primary aromatic amines in water samples, using solid-phase analytical derivatization (SPAD) for the sample preparation. SPAD is a relatively new sample preparation technique and has recently been reviewed [14]. The present work extends the range of SPAD applications by introducing the extraction of PAA from water to a polymeric adsorbent followed by derivatization on the solid-phase with trifluoroacetic anhydride as reagent. The method is evaluated for the determination of PAA in water food simulant and utilized for the migration testing of flexible multilayer food packaging materials on the Norwegian market.

2. Experimental

2.1. Chemicals and solvents

The following solvents were of analytical grade: Ethylacetate, diethylether, methanol (VWR, Germany), acetone (SDS, France), and methyl-tert.-butyl-ether (Rathburn, UK). The water was highly purified by a Milli-Q gradient system (Millipore, France). Cleaning of glass-ware and tubing was done by pure technical grade acetone (VWR) followed by water. Trifluoroacetic anhydride (Fluorochem, UK) and anhydrous K_2HPO_4 (VWR) were both of analytical grades. The following compounds were of analytical or high purity grade and were used as model compounds: Aniline (Fluka, Switzerland), toluene-2,4-diamine (Aldrich, USA), 4,4′-diaminodiphenylmethane (Sigma, USA), toluene-2,6-diamine, 1,5-naphthalene-diamine, 4,4′-oxydianiline, 3,3′-dimethylbenzidine and 1,3-phenylenediamine (kindly donated by Chemisches Landes- und Staatliches Veterinäruntersuchungsamt, Germany). The internal standard was 3-chloro-4-fluoro-aniline of analytical grade (Acros, USA).

2.2. Migration to water

Flexible multilayer food packaging materials were subjected to single surface testing using pouches filled with water as food simulant. Material samples were cut into squares of 18×18 cm and then heat-sealed 1.95 cm from edges. After heat-sealing three sides, the pouches were filled with 200 ml degassed water, followed by heat-sealing of the fourth side. Thus, the pouches had a total inner area of 4 dm^2 and had exactly the same simulant volume to area ratio as specified in the European prestandard EN 13130-1. All remaining material outside the seams was cut off. The pouches were tightly fitted between two 1 mm thick aluminium plates, and placed upright in a rack. The rack was kept in an oven with a combination of time and temperature chosen according to the intended use of the food packaging material.

2.3. Water extraction

Solid phase extraction (SPE) columns were easily prepared by transfer of 0.100 g polymeric adsorbent into empty 3 ml polypropylene tubes (IST, UK) fitted with a polyethylene frit at the exit (IST). Another PE frit was press-fitted at the top of the adsorbent. The following polymeric adsorbents were

evaluated in this work: Oasis HLB (Waters, USA), abselut NEXUS (Varian, USA), ENVI-CHROM P (Supelco, USA) and ISOLUTE ENV+ (IST). Prior to use, the SPE columns were placed on a Visiprep 12 port SPE station (Supelco) and conditioned with 3 ml of each of ethyl acteate, acetone and methanol, followed by brief drying by vacuum. The SPE columns were fitted with tube adapters and Teflon tubing and then inserted into syringe needles penetrating a rubber cork fitted on a 5 l vacuum flask. Ten samples were handled simultaneously. By applying a slight vacuum, the 200 ml water samples from the pouches were transferred to the polymeric adsorbent at 10–15 ml/min. Three ml of 0.5 *M* $K_2HPO_{4(aq)}$ were added to the samples before the extraction to increase the pH to pH 10. An internal standard, 2 μg of 3-chloro-4-fluoro-aniline, was added to all water samples. After the extraction, any remaining K_2HPO_4 was removed from the columns by washing twice with 3 ml water.

2.4. Derivatization

After water extraction, all tubes were dried inside with cotton sticks followed by complete removal of the remaining water by applying a flow of 99.999% nitrogen (Hydrogass, Norway) at 80–100 ml/min while heating the columns at 55 °C for 25 min. Before adjustment of the gas flow-rate, a series of three short pressure pulses was found useful for removing most of the water in the columns. One hundred μl of a 1+1 mixture of trifluoroacetic anhydride and diethylether was added onto the top frit of each SPE column and was allowed to be drained into the dry adsorbent material. The SPE columns were dried for another 15 min by applying a flow of 99.999% nitrogen at 80–100 ml/min while heating the columns at 55 °C. Remaining reagent and reaction by-product (trifluoroacetic acid) were removed completely by washing the SPE columns with 1 ml of 0.5 *M* K_2HPO_4 (aq) and then twice with 3 ml of water. The SPE columns were dried with a cotton stick, a series of pressure pulses and then with a nitrogen flow at 55 °C for 25 min. Finally, 2 ml of methyl-tert.-butyl ether was applied for elution, followed by evaporation of the extracts to approximately 100 μl.

2.5. Gas chromatography coupled with mass spectrometry

The gas chromatograph was a Hewlett-Packard model 5890 series II (Agilent, USA). One μl of each sample extract was injected in the split-less mode with a Hewlett-Packard model 7673 autoinjector. The GC capillary column was a 25 m and 0.25 mm I.D. Zebron ZB-5 (Phenomenex, USA) with a 5% phenyl- and 95% methylsiloxane stationary phase of 0.25 μm thickness. The carrier gas was 99.9999% helium (Hydrogass). Temperature program: 5 min at 40 °C, then programmed 10 °C/min to 320 °C and hold for 15 min. Pressure program: 1 min at 4 p.s.i. during the time of injection, then programmed simultaneously with the temperature from 1 p.s.i. to 10 p.s.i. at 0.33 p.s.i./min with 15 min hold. Injector temperature: 250 °C. Detector temperature: 280 °C. The mass spectrometer was a Hewlett-Packard model 5972 Mass Selective Detector operated by the ChemStation software G1701BA version B.01.00. Mass spectra were recorded in the full scan mode (m/z 33–700) at a scan speed of 1.16 scan/s by using 70 eV electron impact ionization. Reconstructed ion chromatograms (RIC) were made afterwards by using ions of high abundance in the mass spectra for the PAA trifluoroacetamide compounds. The peak area corresponding to the PAA on the respective RIC was used for quantification. The scan mode was chosen instead of selected ion monitoring (SIM) in order to utilize mass spectrum recognition as an additional identification parameter.

3. Results and discussion

3.1. Extraction

Polymeric adsorbents have some advantages compared to silica-based adsorbents for the extraction of water samples, such as higher extraction yields for polar compounds and avoiding loss of analyte in cases where the adsorbent run dry during extraction. Seven polymeric adsorbents based on a styrene–divinylbenzene copolymer have previously been shown to give high yield for the extraction of 33 aromatic amines from drinking water [10]. In the present work four polymeric adsorbents were evalu-

ated using aniline, 2,4-diaminotoluene and 4,4′-methylenedianiline as model substances. It was possible to recover these PAA from the water using any of the adsorbents (Fig. 1). However, some variation in the relative recovery was found compared to what was previous reported. For instance, significantly more aniline and 2,4-diamino-toluene was recovered using ENVI-CHROM P compared to ISOLUTE ENV+.

pH was adjusted to 10 by addition of K_2HPO_4 to the water samples prior to extraction. This increased the extraction yield of the three amines with 30–80%. No unwanted formation of colloid precipitates was observed in any water sample, as previously reported to happen at pH 12 [10].

3.2. Derivatization

The reagent mixture consisted of trifluoroacetic anhydride and diethylether (1:1). By adding only 100 μl of the mixture to the SPE column it was possible to avoid elution of the analytes that were retained on 100 mg of adsorbent. Furthermore, the reagent mixture was highly volatile and quickly evaporated into the particles of the SPE column. Preliminary experiments revealed that the reagent was retained in the adsorbent for several minutes during the flushing with nitrogen gas at elevated temperature. Hence, sufficient time and temperature conditions were

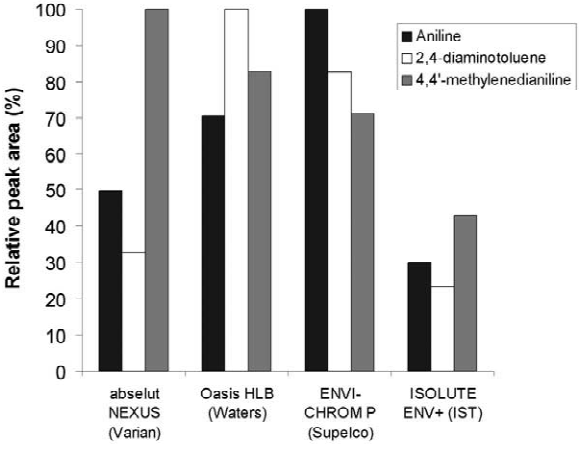

Fig. 1. Relative responses for the determination of three primary aromatic amines by GC–MS with the use of solid-phase analytical derivatization on four different adsorbents.

obtained for the reaction with the PAA compounds on the solid support, as evidenced by no trace of free PAA in the GC–MS chromatograms.

Trifluoroacetic anhydride reacts violently with water to produce trifluoroacetic acid. It was, therefore, important to dry the adsorbent properly before addition of the reagent, in order to avoid insufficient reactivity. The acid by-product is also produced in the reactions with the PAA compounds and has to be removed from the extract prior to the GC–MS analysis, in order to avoid damage to the GC column [13]. Previously, acid has been removed by liquid–liquid extraction with a slightly basic buffer solution [5]. However, this approach implements another time-consuming step in the sample preparation procedure. In the present work, the acid was washed out with a hydrogenphosphate solution, while the PAA derivatives were still retained on the solid-phase.

Two ml of methyl-tert.-butyl-ether was sufficient for the elution of the PAA derivatives. This final sample extract was reduced to 100 μl in order to increase the concentration and, thus, lower the detection limits of the method. However, care was taken not to evaporate the extract to dryness, due to the risk of loosing the most volatile derivatives (e.g. trifluoroacetamide of aniline).

Unwanted reactions occurred between the trifluoroacetic anhydride and the ISOLUTE ENV+ adsorbent material. This became apparent by observing a change in color of the adsorbent from yellow to grey and also by observing several additional peaks in the chromatogram. ISOLUTE ENV+ is a hydroxylated polystyrene–divinylbenzene copolymer, and it was likely that the trifluoroacetic anhydride had reacted with phenol-groups on the adsorbent. These reactions might have competed with the PAA derivatization reactions and may explain the low relative recovery when using ISOLUTE ENV+. For the other three adsorbent materials, no additional peaks in the chromatograms were found to indicate unwanted reactions. ENVI-CHROM P is a neat polystyrene–divinylbenzene copolymer and was chosen for further development of the method.

3.3. Analytical characteristics

Calibration curves were constructed by applying 200 ml water samples containing the model com-

Table 1
Detection limits and relative standard deviations for the determination of primary aromatic amines in 200 ml water samples

Compound	RIC at m/z	Detection limit[a] (μg/l)	Relative standard deviation (%) from 10 water samples containing 5 μg/l of each PAA
Aniline	189	0.1	4
1,3-phenylenediamine	300	0.4	17
2,6-diaminotoluene	314	0.2	13
2,4-diaminotoluene	314	0.2	10
1,5-diaminonaphthalene	350	0.1	4
4,4′-oxydianiline	392	0.1	4
4,4′-methylenedianiline	390	0.1	4
3,3′-dimethylbenzidine	404	0.1	4

[a] Defined as the concentration to give $S/N=2$.

pounds at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 5.0, 10, 15 and 20 μg/l. The detection limits for the 8 PAA model substances ranged from 0.1 to 0.4 μg/l (Table 1). Furthermore, ten water samples containing 5 μg/l of each PAA gave relative standard deviations in the 4–17% range, which is acceptable for such a low concentration level.

All calibration curves were linear ($R^2=0.997$–1.000) from the limit of detection and up to 10 μg/l. Non-linearity for some of the compounds above this level was due to overloading of the GC column, which resulted in poor peak-shapes and difficulties in the peak integration. The problem might be dealt with by either diluting the final sample extract or by using a more polar GC stationary phase with a higher film thickness.

3.4. Food packaging materials

Initially, 50 flexible multilayer food packaging materials were tested with respect to migration of PAA to water. Total levels of PAA above 1 μg/l were monitored spectrophotometrically in water from 15 of the materials that were intended for high temperature use, i.e., for applications where the packaging is not removed before heating the food.

Table 2
Concentration levels of primary aromatic amines determined in water food simulant used in migration testing of 15 flexible food packaging materials

Material number	Time (min)	Temp. (°C)	2,4-diaminotoluene (μg/l)	2,6-diaminotoluene (μg/l)	4,4′-methylenedianiline (μg/l)
1	60	90	<0.2	<0.2	<0.1
2	60	90	<0.2	<0.2	<0.1
3	60	90	0.29±0.06	<0.2	0.62±0.16
4	60	90	0.49±0.10	<0.2	0.56±0.14
5	60	100	<0.2	<0.2	0.13±0.04
6	60	100	<0.2	<0.2	0.21±0.05
7	60	100	<0.2	<0.2	0.16±0.04
8	60	100	<0.2	<0.2	<0.1
9	60	100	<0.2	<0.2	0.40±0.10
9*	120	100	<0.2	<0.2	1.30±0.30
10	60	100	<0.2	<0.2	<0.1
11	120	100	<0.2	<0.2	0.43±0.11
12	120	100	<0.2	<0.2	0.41±0.10
13	120	100	<0.2	<0.2	0.51±0.13
14	120	100	0.20±0.04	<0.2	3.01±0.21
15**	120	100	0.23±0.04	0.24±0.05	3.23±0.23

Time and temperature are given for the migration exposure. *Six and **four samples respectively, one sample for all the other.

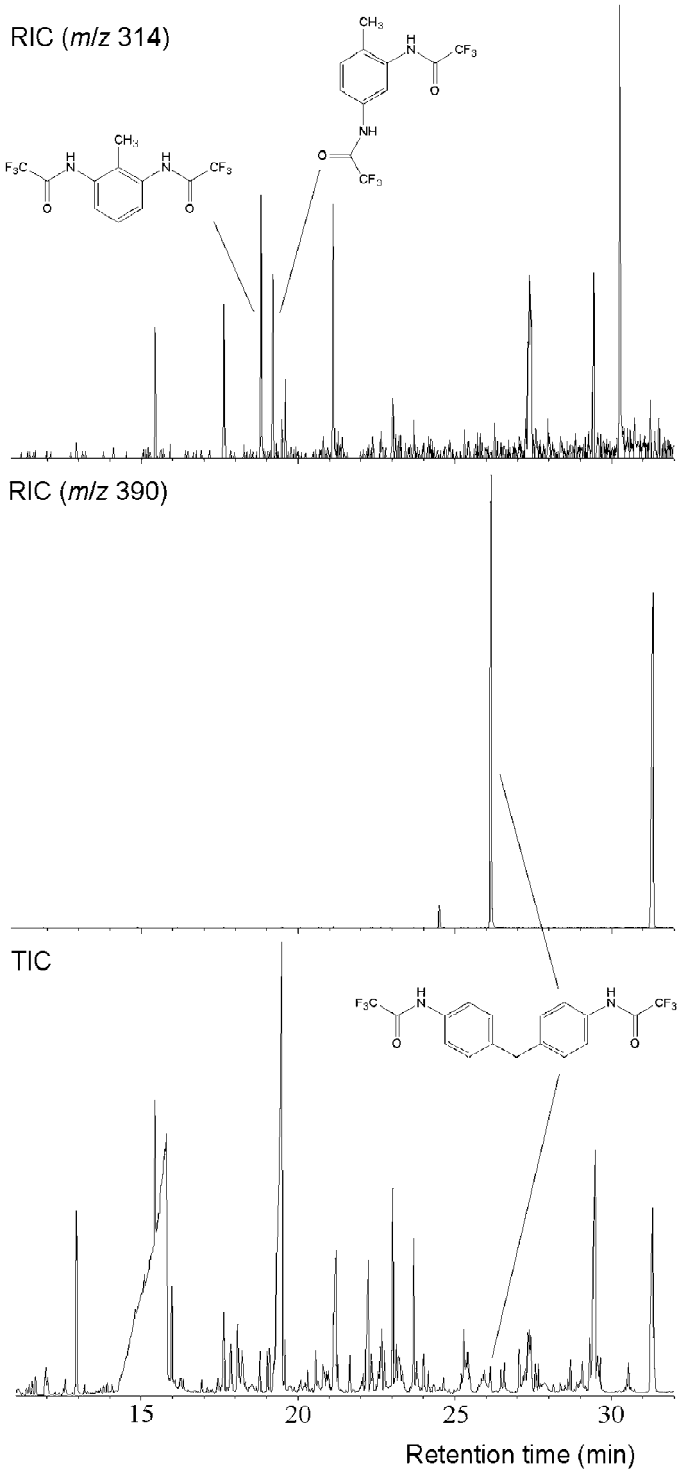

Fig. 2. Total ion chromatogram and reconstructed ion chromatograms for the analysis of water food simulant from food packaging material number 15. Different scales of abundances.

Despite the fact that none of these food packaging materials produced PAA levels above the EU limit (20 μg/l), further migration testing were conducted in order to determine the concentrations of single PAA compounds by using the method developed in the present work. 2,4-diaminotoluene, 2,6-diaminotoluene and 4,4′-methylenedianiline were detected in the water from some of the food packaging materials (Table 2). The use of reconstructed ion chromatograms (RIC) based on abundant ions for the PAA derivatives was essential to avoid interference from other migrated substances. This is illustrated by comparing the total ion chromatogram (TIC) with RIC's from a water simulant sample from food packaging material number 15 (Fig. 2). The RIC at m/z 314 shows the presence of the trifluoroacetamide derivatives of 2,6-diaminotoluene and 2,4-diaminotoluene respectively, while the RIC at m/z 390 shows the presence of the derivative of 4,4′-methylenedianiline.

Furthermore, it was possible to extract the background-corrected mass spectra of each PAA derivative detected in the simulant samples. Acceptable correlations were found by comparison with previously recorded mass spectra of the PAA derivatives, which then provided an additional identification parameter.

Finally, it was possible to establish a precision level for the entire migration testing procedure by applying several samples of the same food packaging material. Six samples of material number 9 resulted in the determination of 4,4′-methylenedianiline at the 1.30 μg/l level with a relative standard deviation of 25%. Four samples of material number 15 resulted in the determination of 4,4′-methylenedianiline at the 3.23 μg/l level with a relative standard deviation of 7% and the determination of 2,4-diaminotoluene and 2,6-diaminotoluene at the 0.2 μg/l level with relative standard deviations of 20%. The relative standard deviations established for these two materials at different concentration levels were used to estimate standard deviations for the testing of the other materials. There was indication of a slight increase in standard deviation for the entire migration testing procedure compared to the analysis of spiked water samples. This was expected and found to be acceptable, because uncertainty exists in both the migration exposure and in the analytical method.

4. Conclusion

Solid phase analytical derivatization has been applied as sample preparation for the GC–MS determination of primary aromatic amines in water. Extraction and derivatization supported on a neat polystyrene–divinylbenzene copolymer adsorbent material provided several advantages over conventional sample preparation techniques, including less solvent consumption, less time-consuming steps in the method, low detection limits, excellent repeatability and no loss of volatiles. The method was suitable for testing migration of primary aromatic amines from food packaging materials into water food simulant. Future work with the method will include the possible determination of additional aromatic amines and the use of 3% acetic acid as food simulant for acidic foodstuffs.

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