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Determination of aromatic amines in aqueous extracts of polyurethane foam using hydrophilic interaction liquid chromatography and mass spectrometry

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ABSTRACT

A method is presented for the determination of aromatic amines in aqueous extracts of polyurethane (PUR) foam. The method is based on the extraction of PUR foam using aqueous acetic acid (0.1%, w/v) followed by determination of extracted aromatic amines using hydrophilic interaction liquid chromatography (HILIC) and tandem mass spectrometry (MS/MS) with positive electrospray ionisation. The injections of volumes up to 5 μL of aqueous solutions were made possible by on-column focusing with partially filled loop injections. The fragmentation patterns for 2,4- and 2,6-toluene diamine (TDA) and 4,4′-methylene dianiline (MDA) were clarified by performing a hydrogen–deuterium exchange study.

TDA and MDA were determined using trideuterated 2,4- and 2,6-TDA and dideuterated 4,4′-MDA as internal standards. Linear calibration graphs were obtained over the range 0.025–0.5 μg mL^{-1} with correlation coefficients >0.996 and the instrumental detection limit for each compound was <50 fmol. The stability of the amines was influenced by the matrix, so their concentrations decreased over time.

Agreement was observed between the results of analyses of PUR foam extracts by HILIC–MS/MS and results obtained by ethyl chloroformate derivatisation and reversed phase (RP) liquid chromatography–mass spectrometry (LC–MS/MS).

TDA was observed to be unstable in extracts of foam but not in pure solutions.

1. Introduction

Polyurethane (PUR), which is produced by reacting diisocyanates with polyalcohols, is one of the most abundant plastics and its production volume is increasing. The most commonly used isocyanates in the production of PUR are toluene diisocyanate (TDI) and methylene bisphenyl diisocyanate (MDI). When isocyanates react with water an exothermic process occurs in which aromatic amines and carbon dioxide are formed, with the latter acting as an expansion agent.

Toluene diamine (TDA) and methylene dianiline (MDA) are hydrolysis products of the isocyanates named above. They have been determined in acidic, aqueous extracts of PUR-based products, which include pillows, mattresses, food packaging and medical devices [1–9]. 2,4-TDA and 4,4′-MDA have been identified as possible human carcinogens and are easily absorbed through the skin [10–12]. There are no threshold or limit values for the concentrations of extractable aromatic amines in consumer products, but an evaluation of the risk from exposure to TDA in PUR foam concluded that no adverse human health effects would be expected from exposure to products with a nominal TDA content of less than 1 ppm [13].

MDA and TDA have been determined as free amines using reverse phase high-performance liquid chromatography (RP-HPLC) [14,15]. Amines can be separated as uncharged molecules or as the protonated ions depending upon the pH (for example, by buffering of the mobile phase). However, for preferential retention on a RP column the target analyte should be unprotonated, because protonated amines of low molecular mass typically elute close to the solvent front and mobile phases containing buffers often have a negative influence on MS detection.

Aromatic amines are readily oxidised and are therefore frequently determined as chemical derivatives in order to improve stability, chromatographic separation, the selectivity and the limit of detection. Several derivatisation techniques, notably the formation of chloroformate esters [16] and perfluorofatty anhydrides [17], have been applied to aromatic amines. In a previous study [1], ethyl chloroformate and pentafluoropropionic anhydride (PFPA) as derivatisation reagents were investigated and when compared to each other the concentration of PFPA derivatised amines were typically higher and it was concluded that this reflected a fraction of free TDA and compounds that liberates TDA.

Hydrophilic interaction liquid chromatography (HILIC) has been developed as an alternative for separation of polar compounds. This

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separation technique was described in the 1950s and was developed for HPLC applications during the 1970s, but recently HILIC has matured as a practical alternative to RP-HPLC [18] because it is advantageous for the separation of small, basic analytes such as peptides, amino acids, oligonucleotides, hydrocarbons, proteins and saponins [18–22]. The elution order of analytes separated using HILIC are generally reversed in comparison to those obtained using RPLC.

The retention of analytes in HILIC is mediated by partitioning of the analytes between the water-rich layer of the hydrophilic stationary phase and the hydrophobic eluent, and also by weak electrostatic interactions [19,20]. The eluent typically consists of 5–40% water in acetonitrile.

The composition of the solution to be injected into the LC column is crucial and differences in eluotropic strength between the mobile phase and the injection solution affect the chromatography [23–26]. To achieve good performance for polar compounds on HILIC, the sample solvent should have an organic content greater than 50%, where the scale of relative solvent strengths is: acetone < acetonitrile < isopropanol < ethanol < methanol < water. The use of a high strength sample solvent will decrease the partitioning of solutes into the stationary phase, leading to reduced retention, lower chromatographic efficiency and poor separation, especially for weakly retained compounds and large injection volumes [27,28]. However, the use of weakly hydrophobic solvents for the extraction or dilution of samples is not always possible.

When using RP-HPLC–MS with electrospray ionisation, poor retention of the analyte on the column may result in insufficient resolution of the analyte and unfavourable matrix effects. A mobile phase with a high water content can also negatively affect the performance and stability of electrospray ionisation. The greater organic content of the mobile phase facilitated by HILIC can contribute to enhanced desolvation and ionisation under electrospray MS conditions, and consequently improved sensitivity [29].

The purpose of the present study was to develop a method for the determination of free aromatic amines and to apply it to the evaluation of their stability in PUR foam extracts. The possibility to analyse aqueous extracts containing free aromatic amines using HILIC was investigated. Electro spray tandem MS was investigated for TDA and MDA by hydrogen deuterium exchange.

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile, acetic acid, formic acid, toluene, potassium hydrogen phosphate and ammonia were obtained from Merck (Darmstadt, Germany). 2,4-TDA (>98% purity) was obtained from Merck-Schuchardt (Hohenbrumm, Germany), 2,6-TDA (98% purity) was obtained from TCI (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) and 4,4′-MDA was purchased from Acros Organics (New Jersey, USA). Trideuterated 2,4- and 2,6-TDA (d_3-TDA) and dideuterated 4,4′-MDA (d_2-MDA) were obtained from Sythelec (Lund, Sweden). PFPA was obtained from Pierce (Rockford, IL, USA), ethyl chloroformate from Merck-Schuchardt (Hohenbrumm, Germany), pyridine from Merck (Darmstadt, Germany), dideuterium oxide (D_2O) from Dr Glaser AG (Basel Switzerland) and formic acid (deuterium-labelled) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

2.2. Apparatus

2.2.1. HPLC–MS/MS

A Quattro-LC–MS/MS (Micromass, Altrincham, UK), operated in electrospray ionisation (ESI) mode, was connected to a LC10AdVP HPLC system (Shimadzu Corporation Kyoto, Japan). Partial filled loop injections, consisting of a central sample plug of 1–5 μL surrounded by 15–19 μL focusing liquid, in a 20 μL injection loop, were made using a LC-PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). The focusing liquid had a weaker eluent strength compared to the mobile phase (for HILIC operation high acetonitrile content and for RP operation high water content).

2.2.2. Free amines

For the determination of the free amines, positive ions were monitored at a cone voltage of 25 V, capillary voltage of 4.65 kV, collision energy of 15 eV for TDA and 25 eV for MDA, desolvation temperature of 200 °C and ion source temperature of 130 °C. Optimisation of the cone voltage and collision energy for the amines was performed by altering the cone voltage (10–40 V) and collision energy (9–35 eV) between chromatographic runs of standard solutions containing the different amines. Multiple reaction monitoring (MRM) was performed for $[M+H]^+ \rightarrow [M-17]^+$ for TDA and $[M+H]^+ \rightarrow [M-93]^+$ for MDA with a dwell time of 0.2 s. Quantification was performed by MRM of the free amines and of the corresponding deuterated internal standards, d_2-MDA and d_3-TDA. A linear elution gradient (from 70/30/0.01 to 50/50/0.01 acetonitrile/water/acetic acid, v/v/v) was applied over a period of 10 min at a flow rate of 50 μL min^{-1}. The HILIC column was then washed for 3 min with a mobile phase of 95/5/0.01 acetonitrile/water/acetic acid (v/v/v). A HILIC zwitterionic column (1.0 mm × 150 mm, 3.5 μm dp, 200 Å particles, SeQuant AB, Umeå, Sweden) was used with a HILIC zwitterionic pre-column (1.0 mm × 5 mm, 5.0 μm dp, 200 Å particles, SeQuant AB, Umeå, Sweden). 5.0 μL injections were made using 15 μL focusing liquid, which consisted of acetonitrile/water (95/5, v/v).

2.2.3. PFPA derivatives of the amines

For the determination of the PFPA derivatives of the amines, negative ions were monitored at a cone voltage of 20 V for TDA and 17 eV for MDA, capillary voltage of 3.5 kV, collision energy of 17 eV, desolvation temperature of 200 °C and ion source temperature of 130 °C. Optimisation of the cone voltage and collision energy for the amine-PFPA derivatives was performed by altering the cone voltage (5–40 V) and collision energy (10–35 eV) between chromatographic runs of standard solutions containing the different amine-PFPA derivatives. MRM was performed for $[M-H]^- \rightarrow [M-120]^-$ for MDA and TDA with a dwell time of 0.2 s. Quantification was performed by MRM of the amines-PFPA and of the corresponding deuterated internal standards amine-PFPA. Elution was performed by applying a linear gradient (from 40/60 to 63/37, acetonitrile/water, v/v) over 8 min at a flow rate of 70 μL min^{-1} to an Xterra C_{18} column (1.0 mm × 50 mm, 2.5 μm, Waters, Massachusetts, USA). 2.5 μL injections were introduced with 17.5 μL focusing liquid, which consisted of acetonitrile/water (5/95, v/v).

2.2.4. Ethyl chloroformate derivatives of the amines

For the determination of the ethyl chloroformate derivatives of the amines, positive ions were monitored at a cone voltage of 20 V, capillary voltage of 4.0 kV, collision energy of 10 eV for TDA and 15 eV for MDA, desolvation temperature of 200 °C and ion source temperature of 130 °C. Optimisation of the cone voltage and collision energy for the amine-ethyl chloroformate derivatives was performed by altering the cone voltage (10–40 V) and collision energy (9–35 eV) between chromatographic runs of standard solutions containing the different amine-ethyl chloroformate derivatives. MRM was performed for $[M+H]^+ \rightarrow [M-46]^+$ for TDA and $[M+H]^+ \rightarrow [M-165]^+$ for MDA with a dwell time of 0.2 s. Quantification was performed by MRM of the amines-ethyl chloroformate and of the corresponding deuterated internal standards