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Technical Note

Direct Analysis of Plasticizers in Aqueous Samples by Atmospheric Pressure Chemical Ionization-Tandem Mass Spectrometry (APCI-MS–MS)

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Introduction

The widespread manufacture of plastics requires the similarly ubiquitous use of plasticizers. Plasticizers such as bis(2-ethylhexyl) adipate (DOA) and bis(2-ethylhexyl) phthalate (DOP) enhance polymer strength and flexibility and are found in polymeric products such as cosmetics, detergents, and building and storage products (1). However, these additives are not bound to the polymer matrix and are subject to leaching. A recent Health Canada report warned that DOP may leach from medical devices and cause harm to infants, young boys, pregnant women, and nursing mothers (2). The United States Environmental Protection Agency (U.S. EPA) estimates that over 450,000 pounds of DOA were released to land and water during the period of 1987–1993 (3).

Several methods exist for the determination of plasticizers in aqueous samples. For example, U.S. EPA methods 506 and 525.1 may be used to analyze drinking water for DOA and DOP, among other organic compounds (4,5). Extraction of the analytes from the water matrix is achieved by either liquid–liquid extraction or by passing the sample through a solid-phase extraction disk. Extracts are analyzed by gas chromatography (GC) with either photoionization (method 506) or mass spectrometric detection (MS) (method 525.1). Recently, a liquid chromatography–mass spectrometry (LC–MS) method for the analysis of plasticizers in water was reported (6). Regardless of the instrumental method employed, all of these methods require sample volumes ranging from 200 to 1000 mL in addition to lengthy liquid–liquid or solid-phase extraction procedures. Furthermore, both soluble and immiscible analytes are partitioned into the organic phase and quantitated as though the entire quantity were completely soluble in the sample. Not only does the extractionless method reported here reduce the sample volume required for analysis, but it also drastically reduces the labor required to prepare the samples.

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Experimental

Methanol (HPLC Grade) and acetic acid were used to prepare the mobile phase (Fisher Scientific, Fair Lawn, NJ). The aqueous portion of the mobile phase was 1% acetic acid in water (v/v). The organic portion was 100% methanol. Concentrated DOP and DOA standards (Aldrich Chemical, Milwaukee, WI) were prepared in methanol. Working standard solutions were prepared in 20:80 methanol/water by volume.

Mobile phase was delivered with a binary pump (HP1100, Agilent Technologies, Palo Alto, CA). The pump was modified by removing the mixing column and placing a mobile phase contaminant trap [4-mm i.d. Deltabond octadecylsilane guard column (DB-ODS), Keystone Scientific, Bellefonte, PA] immediately prior to the injection valve of the autosampler (Figure 1). The autosampler was equipped with a 900-mL metering valve and large-volume loop (HP1100). The needle wash feature of the autosampler was employed to minimize sample carryover. A column switching valve (HP1100 Thermostatted Column Compartment) directed flow to either the column or waste (Figure 1). The analytical column (4-mm i.d. DB-ODS guard column identical to the mobile phase trap column) was placed between the column switching valve and the ion-trap tandem MS equipped with a waste valve and an atmospheric pressure chemical ionization (APCI) source (LCQ, Thermo Finnigan Corp., San Jose, CA).

Standard and sample solutions were loaded onto the analytical column with a mobile phase of 40% organic (60% aqueous). This mobile phase was allowed to sweep the entire contents of the sample loop onto the analytical column for 5 min (Figures 1 and 2). The detector waste valve directed flow to waste at this time. At 5.0 min, the mobile phase was quickly ramped to 90% methanol and the injection and column switching valves directed flow away from the loop (injection valve) and toward waste (column switching valve). At 6.5 min, the column switching and LCQ waste valves directed flow to the column and detector for 1.5 min for the elution of the analytes. At 8.0 min, the LCQ waste valve directed flow to waste. The MS collected data only during these 1.5 min. The original mobile phase con-

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ditions were restored at 8.0 min, and the injection valve directed flow to the sample loop at the end of the run (10.5 min; Figures 1 and 2).

The MS was operated in the positive ion mode for both analytes. The vaporizer temperature was 475°C; sheath gas flow 80%; auxiliary gas 0%; corona discharge current 4 mA; and the heated capillary temperature was 200°C. Detection of DOA was achieved by tandem MS isolation of the *m/z* 371 parent ion followed by helium-induced fragmentation (28% collision energy). The collision products were scanned from *m/z* 100 to 375, and the chromatographic response resulted from the sum of two extracted ions (*m/z* 259 and 273). The *m/z* 391 parent ion of DOP was similarly subjected to collision (25% collision energy) and the products were scanned from *m/z* 105 to 400. The chromatographic response was generated from the sum of two extracted ions (*m/z* 261 and 279).

Detector response linearity was assessed by injecting varying volumes of either a 5.20-ng/mL solution of DOA or a 9.87-ng/mL solution of DOP into the instrument. Triplicate injections were made at six different mass levels. The mass range of DOA was 130 to 3120 pg, and the DOP range evaluated was 98.7 to 7900 pg. Bias and recovery were assessed from HPLC-grade water (Fisher Scientific) fortified with either DOA or DOP. Bottled HPLC-grade water was also used for control samples. Eight replicate fortifications and three controls were prepared for each analyte in 50-mL screw-cap culture tubes. Analyte concentrations of the 25 mL solutions were 464 pg DOA/mL water or 852 pg DOP/mL water. Freshly prepared fortified samples and controls were subjected to centrifugation (6000 × *g*) to remove particulates before injecting 900 mL into the instrument.

The method limit of detection (MLOD) and method limit of quantitation (MLOQ) were evaluated from the responses obtained for fortified water samples. The MLOD for each analyte was defined as the concentration of analyte required to produce a chromatographic response equal to two times the peak-to-peak noise observed in the blank samples. The method limit of quantitation (MLOQ) was defined as the concentration of analyte required to produce a chromatographic response equal to 10 times the noise.

The method was compared to a modified USEPA method 525.1 (employing solid-phase extraction with surrogate and internal standards) for the analysis of DOA in two hard-water solutions from an aqueous toxicity study. Four 400-mL aliquots were obtained for each solution and held in 1.0-L amber glass solvent bottles. Three complete aliquots from each solution were extracted according to the modified 525.1 methodology and the mean concentrations determined to be 1.53 and 3.54 ng/mL. The remaining aliquot from each solution was analyzed by placing approximately 1.5 mL of sample in autosampler vials and injecting 900 mL into the chromatographic system as described. Each aliquot was analyzed in duplicate by allowing them to sit at room temperature for 44 h prior to collecting 1.5 mL for analysis. Following this collection, the 400-mL solutions were vigorously shaken by hand and sampled in duplicate for analysis.

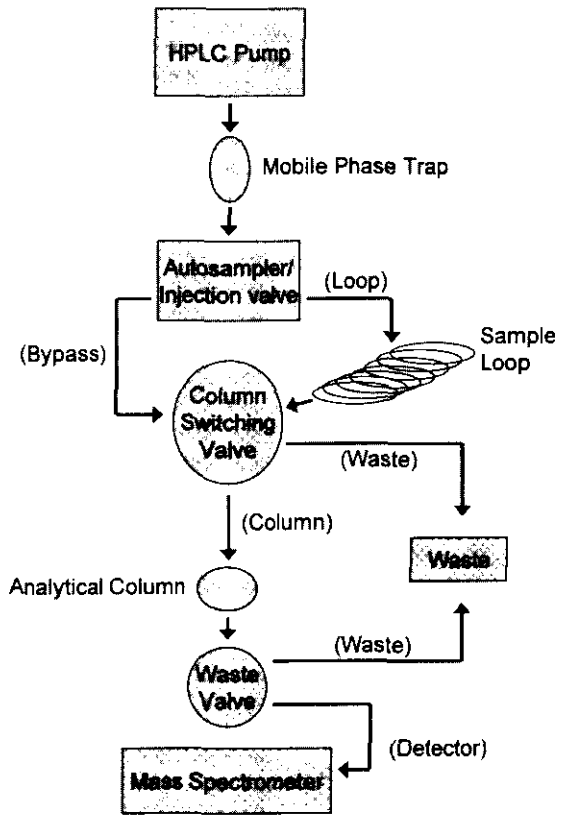

Figure 1. Schematic of the HPLC system employed for the analyses of DOA and DOP. Three valves were used to direct flow between either the sample loop or loop bypass (injection valve); the analytical column or waste (switching valve); and between the MS or waste (waste valve).

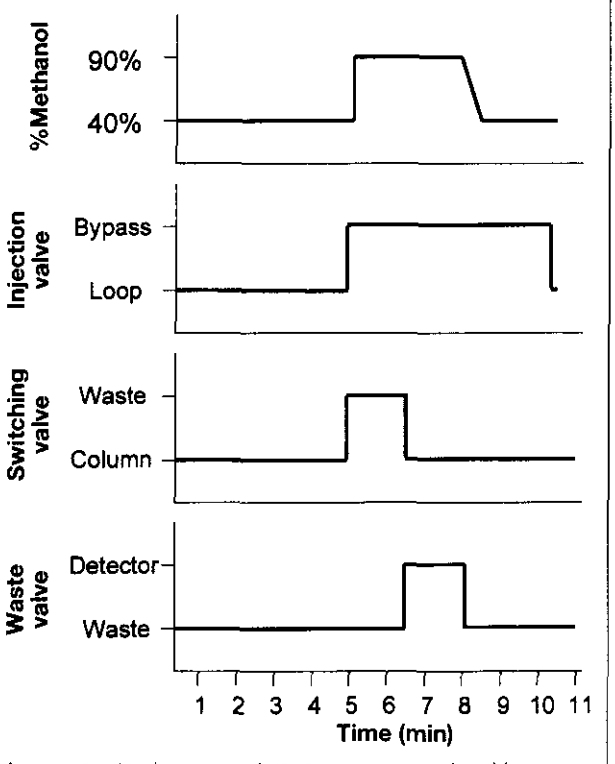

Figure 2. Graphical summary of HPLC parameters employed for an analytical run. The mobile phase composition and flow path at each valve (see Figure 1) is provided for any given time during the 10.5-min run. The analytes are eluted at approximately 7.0 min.

Results and Discussion

An analytical method employing column focusing for the analysis of a hydrophobic drug was recently reported (7). Trapping the analyte onto an analytical guard column combined with tandem MS provided excellent selectivity and low limits of detection. It was anticipated that a similar strategy could be applied to the analysis of aqueous samples containing analytes with large octanol/water partition coefficients. Evaluation of stationary phases indicated that the DB-ODS phase not only trapped and concentrated the analytes from the water matrix, but also produced excellent chromatographic peaks. Unfortunately, the narrow peaks produced by this technique precluded simultaneous monitoring of co-eluting DOA and DOP responses. In order to ensure that chromatographic peaks were described by at least eight data points, separate chromatographic analyses were made for each analyte.

Early in method development, it was noted that trace amounts of DOA present in the mobile phase were trapped on the analytical column under the loading conditions (40% methanol). The chromatographic peak resulting from elution with 90% methanol was directly proportional to the volume of mobile phase that passed through the column. This problem was solved by adding a mobile phase contaminant trap (DB-ODS) and directing the eluted peak to waste—prior to analyte elution from the analytical column (Figures 1 and 2).

Statistical analysis of the DOA response data indicated that response was linear and directly proportional to the mass of DOA injected. The linear model yielded a coefficient of determination (R^2) of 0.996, a significant slope ($p < 0.0001$), and an intercept that was not significantly different from zero ($p = 0.565$), indicating that single-point calibration was justified over the range of 130 to 3120 pg injected. Similar analysis of DOP response data also indicated that single-point calibration was valid over the range investigated (98.7 to 7900 pg); a coefficient of determination (R^2) of 0.993, a significant slope ($p < 0.0001$), and an intercept that was not significantly different from zero ($p = 0.482$) were obtained. Thus, fortified samples were quantitated using a single-point calibration.

Excellent analyte recovery from fortified water samples was observed for both compounds. The bias and repeatability study for DOA (464 pg/mL) yielded 96.1% recovery with low relative standard deviation (RSD; 7.1%). Recovery of DOP exhibited greater variability (115%; RSD = 14.4%). Control samples did not produce detectable analyte responses. The MLOD for DOA was determined to be 40.5 pg DOA/mL water, and the MLOQ was 202 pg DOA/mL water. For DOP, an MLOD of 115 pg DOP/mL water and an MLOQ of 577 (pg/mL) were observed.

Analysis of freshly prepared fortified samples was an important feature of method validation and sample handling. During development, seemingly spurious results were later found to be functions of time elapsed from sample fortification to analysis. A subsequent comparison of recently agitated fortified samples (after sitting undisturbed for 4 h) versus samples sitting undisturbed for 4 h, demonstrated that recovery was negatively impacted when fortified water samples sat undisturbed. After four hours, DOA recovery was 57.9% ($n = 3$) in the undisturbed samples. However, when fortified samples were agitated by vortex mixing after the 4-h period, recovery was 96.0% ($n = 3$).

Similar results were obtained from the hard-water samples. Sub-samples collected after sitting at room temperature for 44 h yielded DOA values of 0.531 pg/mL and 0.441 pg/mL (as compared to 1.53 ng/mL as determined by the modified 525.1 method). This same solution was determined to be 1.19 and 1.14 ng/mL in duplicate sub-samples after vigorous shaking of the original container. For the solution determined to be 3.54 ng/mL by the modified 525.1 method, the concentrations observed at 44 h were 1.40 and 1.18 ng/mL. However, the concentrations of the duplicate sub-samples were 3.71 and 3.68 ng/mL after vigorous shaking.

These results indicate that concentrations of hydrophobic compounds in aqueous samples may be overstated when liquid–liquid or solid-phase extractions are employed for quantitative analysis. In fact, the solubility of DOA may actually be much lower than the enforceable Maximum Contaminant Level of 400 ng/mL set by U.S. EPA (3). Problems associated with the determination of water solubility have recently been discussed for highly hydrophobic compounds (8). Using DDT as a case study, the variability of solubility data reported in the literature was found to span 2–4 orders of magnitude. Analytical methods for determining solubility (as opposed to indirect or computational) contributed greatly to this variation (8). Methods employing liquid–liquid or solid-phase extraction may contribute to this variability by extracting both insoluble and soluble analyte associated with the sample. Reported variability and our observations raise serious questions regarding the actual concentration of hydrophobic analytes in water samples when liquid–liquid or solid-phase extractions are employed.

The method reported here has several distinct advantages over traditional extraction methodologies. First and foremost, the sample volume and sample preparation time required for analysis are drastically reduced. Second, potential analyte contamination from other sources is minimized because non-polar solvents (in which DOA and DOP are highly soluble) are not employed. Finally, this method measures only that proportion of analyte that is actually soluble in the matrix.

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