Development and Validation of Chromatographic Methods for the Identification and Quantitation of Organic Compounds Leached from a Laminated Polyolefin Material

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Abstract

Chromatographic methods for the identification of organic compounds leached from a plastic material used in solution containers in the pharmaceutical industry are described. Based on a set of compounds identified in extracts of a multilayered polyolefin film, targeted leachables are delineated for accumulation assessments, and methods to perform target quantitation are developed and validated.

Introduction

Plastic materials are widely used in medical applications, such as solution containers, transfusion sets, transfer tubing, and devices. Although an important performance feature of plastics used in medical applications is chemical inertness, interactions between a plastic material and a contacted pharmaceutical product are well documented. Such interactions may include leaching, the release of plastic material components to the product. In the case of leaching, both the identities of the leached substances and their accumulation levels may impact the ultimate viability of the product.

Numerous strategies can be envisioned and utilized to assess the impact that the accumulation of leached substances has on pharmaceutical products contacted by a plastic material during their manufacture, storage, or use (or both). Such strategies share two fundamental components: the identification of the leached substances and the measurement of the actual or probable accumulation levels of the identified substances. The process of establishing the identity of the leachables can be an extensive exercise in investigative analysis. Such an exercise is accomplished via a comprehensive process of utilizing sensitive and information-rich scouting analytical methods whose dual purposes are to first reveal the leachables and then provide relevant information (e.g., formula and structure), which leads to their identification. The dual requirements of sensitivity and universality typically dictate the use of chromatographic methods. The utilization of various chromatographic methods to either identify or quantitate (or both) plastic material leachables is well documented in the analytical and pharmaceutical literature (1–11).

In regulated industries, such as the pharmaceutical industry, analytical methods used to demonstrate the safety and efficacy of products must be validated; that is, the methods must be capable of adhering to strict performance guidelines including, but not necessarily limited to, accuracy, precision, sensitivity, selectivity, linearity, ruggedness, and robustness. Validation guidelines have been provided by various official agencies (e.g., 12,13).

In this manuscript, chromatographic methods used for the identification of extracted compounds from a plastic material used in medical product solution storage applications (e.g., container systems) are described. Methods used for the quantitation of specific (or targeted) leachables were then developed and validated.

Experimental

Container material

The container material is a commercially-available, multilayer polyolefin laminate. The laminate contains a medical-grade, very-low-density polyethylene as the fluid contact layer, a layer of ethyl-vinyl-alcohol and a polyurethane-type adhesive. The test containers, when filled with 250 mL of buffer, had an estimated contact surface area to solution volume ratio of 1.9 cm^2/mL. This surface area to solution volume ratio falls within the range anticipated for field-use containers with a capacity of 20–600 L. The containers were sterilized in a manner consistent with their intended biopharmaceutical application (gamma irradiation, dose range 27.2–35.8 kGy).

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Extraction media

The following buffer media are representative of solutions used in the biopharmaceutical industry and thus the developed assays were validated for application with these solutions: (I) 0.06M ammonium sulfate, 0.022 M4-morpholinesulfonic acid (MES), 0.0024M potassium phosphate, pH 5.4; (II) 0.05M tromethamine (TRIS), 0.15M sodium chloride, pH 7.2; (III) 2.0M TRIS base; (IV) 0.02 M TRIS-hydrochloride, 0.05M sodium chloride, pH 8.0; (V) 0.02M sodium phosphate, 0.15 M Sodium Chloride, 0.02% Tween-80, pH 5.5; (VI) 1% Tween-80; (VII) 6.0M guanidine-hydrochloride; and (VIII) 0.15M sodium chloride, pH 5.4.

The media were prepared in presterilized glass bottles using commercially-available, reagent-grade chemicals. Adjustments to pH were made with sodium hydroxide or hydrochloric acid as appropriate.

Test samples and controls: identification of target extractables

Identification of target extractables is facilitated if the concentration of the extractables is relatively high and the extraction matrix is analytically simple. Although the conditions used to produce samples for leachables identification should meet these objectives, the conditions and the extraction medium should also simulate intended product use. Otherwise, the extractables profile obtained during product use may be different from that obtained from simulated use or exaggerated conditions. Thus, unbuffered water was used as the extraction solution and the extraction conditions were 70°C for approximately 3 days. Use of these extraction conditions allows one to simulate material/solution contact for extended periods of time at near ambient conditions (typical product use for storage of drug products) without subjecting the plastic to the extreme temperatures of terminal sterilization (autoclaving). Two bags and two blanks (glass bottles) were filled with 250 mL water and stored under such conditions.

Analysis and analytical methods: identification of target extractables

Water extracts and controls were subjected to several screening tests to establish the general chemical properties of the extractables and search for specific extracted compounds. The screening methods included pH, UV absorbance, and total organic carbon (TOC). Several chromatographic methods were used to search for specific extracted substances, including gas (GC) and liquid (LC) chromatography with mass spectrometric (MS) detection and ion exclusion chromatography (IEC) with suppressed conductivity detection. Operational details associated with these methodologies were the following.

IEC

Column, Dionex (Sunnyvale, CA) HPICE-AS1; mobile phase, 1mN HCl at 0.8 mL/min; regenerant, 5mM tetrabutylammonium hydroxide at approximately 1.5 mL/min; sample size, 50 µL; and sample preparation, none (direct injection).

GC–MS

Instrument, Hewlett-Packard (Wilmington, DE) 5890 GC and HP-5971 MSD; column, J&W (Folsom, CA) DB-5Ht fused-silica capillary (30 m × 0.25 mm, 0.1-µm film); oven program, start at 40°C and ramp at 10°C/min to 325°C, hold for 5 min; carrier gas, He at a head pressure of 5 psi; injector temperature, 325°C; transfer line temperature, 325°C; injection, 2 µL (splitless); MS operating range, 35–650 amu; MS ionization, EI (+) 70 eV; solvent delay, 4 min for underivatized samples and 7 min with derivatized samples; sample preparation, samples analyzed as is or after acidification to approximately pH 2 with HCl. Samples were extracted and concentrated (~ 200 mL to 1 mL) via solid-phase extraction (Empore C18 extraction disk). Concentrated samples were analyzed by direct injection or, for the acidified sample, after derivatization with a mixture of bis(trimethylsilyl)trifluoroacetamide and dichlorotrimethylsilicate.

LC–MS (method A)

Instrument, HP 1100 LC/MSD; column, Agilent (Palo Alto, CA) Zorbax Eclipse C8 (150 × 4.6 mm, 5-µm particles); mobile phase gradient, component A of 10mM ammonium acetate and component B of methanol (for gradient profile, see Table I); mobile phase flow rate, 0.5 mL/min; detection (UV), at 200 and 210 nm; detection (MS), atomospheric pressure ionization–electrospray (API–ES) (positive ion); and mass range, 55–1000 amu (other conditions: gas temperature, 300°C; fragmentor, 65 V; drying gas, 10.0 L/min; nebulizer pressure, 25 psig; Vcap, 5000 V); sample size, 100 µL; column temperature, 45°C; sample preparation, none (direct injection).

LC–MS (method B)

Instrument, HP 1100 LC/MSD; column, Phenomenex (Torrance, CA) Prodigy C8 (150 × 4.6 mm, 5-µm particles); mobile phase gradient, component A of 0.025% formic acid and component B of methanol (for gradient profile, see Table II); mobile phase flow rate, 0.6 mL/min; detection (UV), UV at 210 and 230 nm; detection (MS), API–ES (positive ion); and mass range of 50–1000 amu (other conditions: gas temperature, 325°C; frag-

Table I. Gradient Timetable (Method A)

Elapsed time (min)	Proportion A	Proportion B
0.00	90	10
1.50	90	10
8.00	60	40
15.00	15	85
28.00	2	98
30.00	2	98
32.00	90	10

Table II. Gradient Timetable (Method B)

Elapsed time (min)	Proportion A	Proportion B
0.00	95	5
20.00	15	85
28.00	5	95
37.00	5	95
37.50	95	5
40.00	95	5

mentor, 65 V; drying gas, 11.0 L/min; nebulizer pressure, 35 psig; Vcap, 5000 V); sample size, 100 µL; column temperature, not controlled; and sample preparation, none (direct injection).

Optimized methods for leachables quantitation

Assays used for analyte identification must be sensitive, selective, and broad in scope. Even though these characteristics may also be relevant for assays used for analyte quantitation, such assays need to be less broad in scope (as the analytes are known). More importantly, the quantitative assays must be accurate, precise, and have a response that can be correlated (preferably via a well-defined linear function) to analyte concentration. Thus, methods used for identification may not always be optimal for quantitation.

Based on the delineation of the targeted leachables, chromatographic methods were optimized to perform the quantitative analysis. Performance characteristics of these assays are as follows.

Table III. Gradient Timetable

Elapsed time (min)	Proportion (10mM NH_4OH)	Proportion (methanol)
0.00	95	5
1.40	95	5
1.50	50	50
15.00	5	95
20.00	5	95
20.10	95	5
25.00	95	5

Table IV. Aqueous Leachables Profile*,†

Compound identification information			Estimated extracted level (mg/L)‡	
Name	Chemical name/ empirical formula	CAS RN	by GC–MS	by LC–MS
A1§,**	$C_{12}H_{20}O_4$	–	< 0.2	< 0.2
A2§,**	$C_{12}H_{20}O_4$	–	< 0.2	< 0.2
B**	$C_{15}H_{26}O_4$	–	< 0.2	< 0.2
G**	$C_{11}C_{18}O_4$	–	< 0.2	< 0.2
Caprolactam	2-Oxohexamethyleneimine	105-60-2	NA††	< 0.2
Erucamide	Cis-13-docosenoamide	112-84-5	NA	< 0.2
I‡‡	$C_{17}H_{24}O_3$	–	NQ§§	< 0.2
C‡‡	$C_{17}H_{26}O_3$	–	NP***	< 0.2
H‡‡	$C_{17}H_{26}O_4$	–	NP	< 0.2
PTS	*p*-Toluenesulfonamide	70-55-3	NA	0.2
Caproic Acid	Hexanoic acid	142-62-1	< 0.2	< 0.2
CaprylicAcid	Octanoic acid	124-07-2	NQ	< 0.2

* In addition to the compounds found in this list, formic acid (CAS RN 64-18-6) and acetic acid (CAS RN 64-19--7) were measured in the extracts at levels greater than 0.2 mg/L each.
† Although additional leached substances were observed in the various chromatograms, these leachables (e.g., D, E, and F in Figure 1) were not present in sufficient quantities to allow for their confirmed identification.
‡ These concentrations are specific for the extraction geometry used.
§ Structural isomers.
** These compounds are all associated with the polyurethane adhesive.
†† NA = not applicable. The method as implemented in this study is not suited for this compound.
‡‡ These compounds are hydrolysis and degradation products of a hindered phenolic antioxidant commonly used in polyolefin materials.
§§ NQ = not quantitated.
*** NP = not present at detectable levels.

IEC (for acetate and formate)

Column, Dionex HPICE-AS1; mobile phase, 1mM (or mN) HCl at 0.9 mL/min; regenerant, 5mM tetrabutylammonium hydroxide at approximately 1.0–1.5 mL/min; sample size, 10-µL; and sample preparation, none (direct injection) except for the 6M Guanidine formulation (matrix VII), which was diluted 1 to 20 with water because of the large injection response.

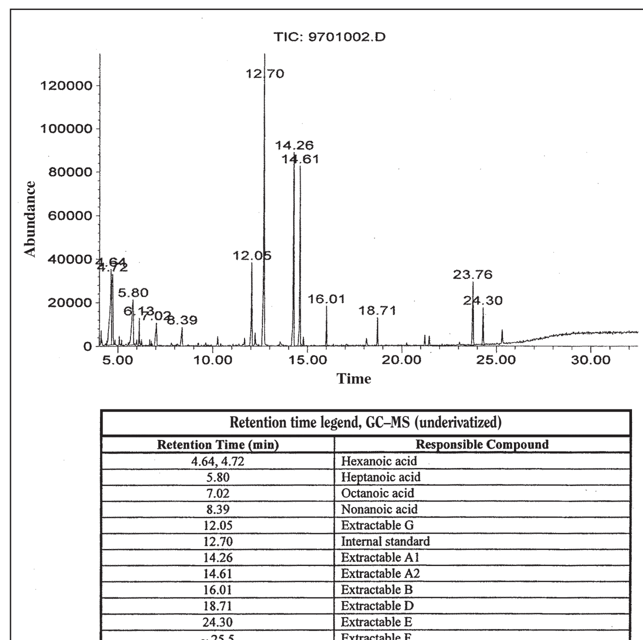
LC–MS

Instrument, HP 1100 LC/MSD; column, Phenomenex Prodigy C8 (150 × 4.6 mm, 5-µm particles); and mobile phase gradient, components were 10mM ammonium acetate and methanol (for gradient profile, see Table III); mobile phase flow rate, 0.6 mL/min. Detection strategies: UV at 230 nm. API-ES (positive ion): gas temp, 325°C; fragmentor, 65 V; drying gas, 11.0 L/min; nebulizer pressure, 35 psig; and Vcap, 5000 V. Specific compounds monitored (and their respective target ion) included caprolactam (114); A1 and A2 (229); C (296); and B (271). API-ES (negative ion): gas temp, 325°C; fragmentor, 65 V; drying gas, 11.0 L/min; nebulizer pressure, 35 psig; and Vcap, 5000 V. Specific compounds monitored (and their respective target ion) included hexanoic acid (115); C (277); and stearic acid (283). Sample size: 100 µL. Sample preparation: none (direct injection). However, an inline trap column was used to eliminate injection of matrix salts into the analytical system. The entire injection volume of the sample was passed through the trap column with the effluent going to waste. After a certain flush time (1–1.5 min), the trap column was backflushed, with the effluent containing the target analytes being eluted into the analytical system. Trap columns used included an Alltech (Deerfield, IL) C18 precolumn [5-µm particles (used during method evaluation)] and an Alltech Altima C18 column [30 × 4.6 mm, 5-µm particles (used in the analysis of the 6 week test samples)]. It is noted that the analysis of the ethanol-containing samples was performed with direct injection (no trap column).

Holistic method validation

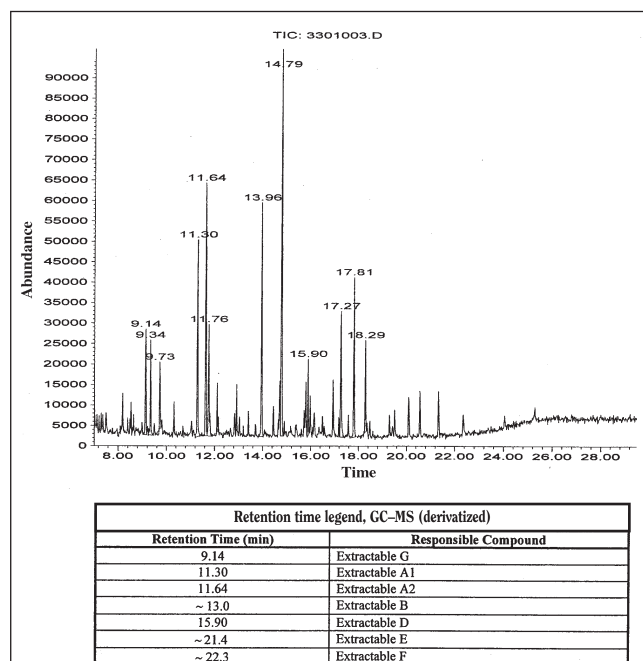
The holistic validation included an assessment of accuracy, precision, response linearity, sensitivity, and specificity. To assess accuracy, fortified (spiked) and unfortified test samples were analyzed. The acceptance criterion was that the mean percent recovery of the spike be 70–130%, which is appropriate performance for analytes present at trace levels (< 0.5 ppm) in analytically challenging matrices. Precision was assessed by replicate (n = 3) analysis of the spiked test sample. The acceptance criterion for precision was that the percent relative standard deviation (%RSD) for the triplicate analyses be not more than 10%. Linearity was assessed by generating response versus concentration data for standard solutions that bracket

the concentrations anticipated in the extracting solvents. At least three standards bracketing such a concentration range were used. Each standard was analyzed at least in duplicate. Concentration versus response profiles were obtained for the standards. Such profiles allowed for a correlation to be established between response and concentration. The acceptance criterion was that the coefficient of determination (r^2) associated with such a profile be not less than 0.99. Sensitivity, expressed as the limit of detection (LOD), was determined at 3 times the signal-to-noise ratio and had an acceptance criterion of LOD not more than 50 ppb. Specificity was established by examining the integrity of all analytical responses. This process included visual examination of matrix blank chromatograms and, in the cases of MS and UV detection, spectral evaluation of any coeluting peaks.



Retention time legend, GC–MS (underivatized)	
Retention Time (min)	Responsible Compound
4.64, 4.72	Hexanoic acid
5.80	Heptanoic acid
7.02	Octanoic acid
8.39	Nonanoic acid
12.05	Extractable G
12.70	Internal standard
14.26	Extractable A1
14.61	Extractable A2
16.01	Extractable B
18.71	Extractable D
24.30	Extractable E
~ 25.5	Extractable F

Figure 1. Total ion current GC–MS chromatogram of the underivatized water extract (70°C for ~ 3 days). Although some of the chromatographic peaks observed in the extract chromatograms were also present in chromatograms for the extraction blanks, the peaks assigned to specific extracted compounds were not present in the extraction blanks.



Retention time legend, GC–MS (derivatized)	
Retention Time (min)	Responsible Compound
9.14	Extractable G
11.30	Extractable A1
11.64	Extractable A2
~ 13.0	Extractable B
15.90	Extractable D
~21.4	Extractable E
~ 22.3	Extractable F

Figure 2. Total ion current GC–MS chromatogram of the TMS-derivatized water extract (70°C for ~ 3 days). Although some of the chromatographic peaks observed in the extract chromatograms were also present in chromatograms for the extraction blanks, the peaks assigned to specific extracted compounds were not present in the extraction blanks.

Reference materials

Reference materials used in this study were either synthesized internally or purchased from external vendors. Specifically, leachables A1, A2, B and C (see Table IV) were synthesized, purified and characterized internally. Other reference materials, including caprolactam, stearic acid, hexanoic acid, sodium acetate (anhydrous), and sodium formate were obtained from Aldrich Chemical (Milwaukee, WI).

Results and Discussion

Identification of extracted substances

Water solutions stored in the containers and glass controls (250-mL fill, 70°C for 3 days) were analyzed for their general chemical properties such as pH, UV absorbance, and TOC. These analyses provide qualitative information related to the amount and chemical nature of accumulated organic leachables. For example, the pH of the water stored in the containers ranged from 4.67 to 4.71, and the solutions stored in the inert glass controls had pH values of 6.03 and 6.18. Thus, one concludes that some of the leachables have acidic functional groups. The UV absorbance of the extracted compounds was very low over the wavelength range of 200–300 nm and the absorption spectra contained no significant peaks or features. Net extracted absorbances (sample – control) were 0.026 AU at 220 nm, 0.012 AU at 240 nm, and 0.007 AU at 250 nm. This information implies that the extracted compounds do not contain UV chromophoric functional groups and are predominately nonaromatic. The extracted TOC associated with these samples (sample – control) was 1.72 mg/L. Thus, the total amount of extracted carbon was small.

To identify extracted leachables, water extracts and controls were analyzed by several chromatographic methods whose elution and detection conditions were such that they could potentially respond to a large number of analytes with a wide range of chemical characteristics. As the qualitative data suggested that the leachables included one or more acids, an IEC method was used to screen the extracts for low-molecular-weight organic acids. Consistent with the pH and UV data, such analyses indicated that formate and acetate were present at measurable levels (> 0.2 mg/L) in the container extracts (but not in the glass controls). Acetic and formic acids are commonly associated with irradiated polyolefins and other plastics (e.g., reference 14). It was estimated that formate and acetate combined accounted for nearly 30% of the extracted TOC and accounted for the pH values measured. Thus it was logical that these analytes become targets

to be quantitated in buffer solutions stored in containers of the material of interest.

GC–MS and LC–UV–MS chromatograms obtained from the analysis of the container extracts were characterized by numerous peaks (as is illustrated in Figures 1–4). The identities of the compounds responsible for the observed chromatographic responses were ascertained by several methods including searching of spectral libraries and interpretation of MS fragmentation patterns and confirmed by analyzing standards containing known quantities of specific compounds whose chromatographic characteristics were known. A compound identification assigned to a peak observed in the extract chromatogram was confirmed if two criteria were met. The first criterion was a retention time match between the known peak in the standard chromatogram and the unknown peak in the extract chromatogram. The second criterion was a match in the mass spectra between the known peak in the standard and the unknown peak in the extracts chromatograms. Typically this mass spectral match involved a comparison of the indicated molecular ions (for LC–MS data), whereas in the GC–MS applications fragmentation patterns were also compared.

The list of definitively identified leachables is compiled in Table IV. When acetate and formate are added, this list is the container's aqueous leachables profile. The likely genesis of these leachables is as follows.

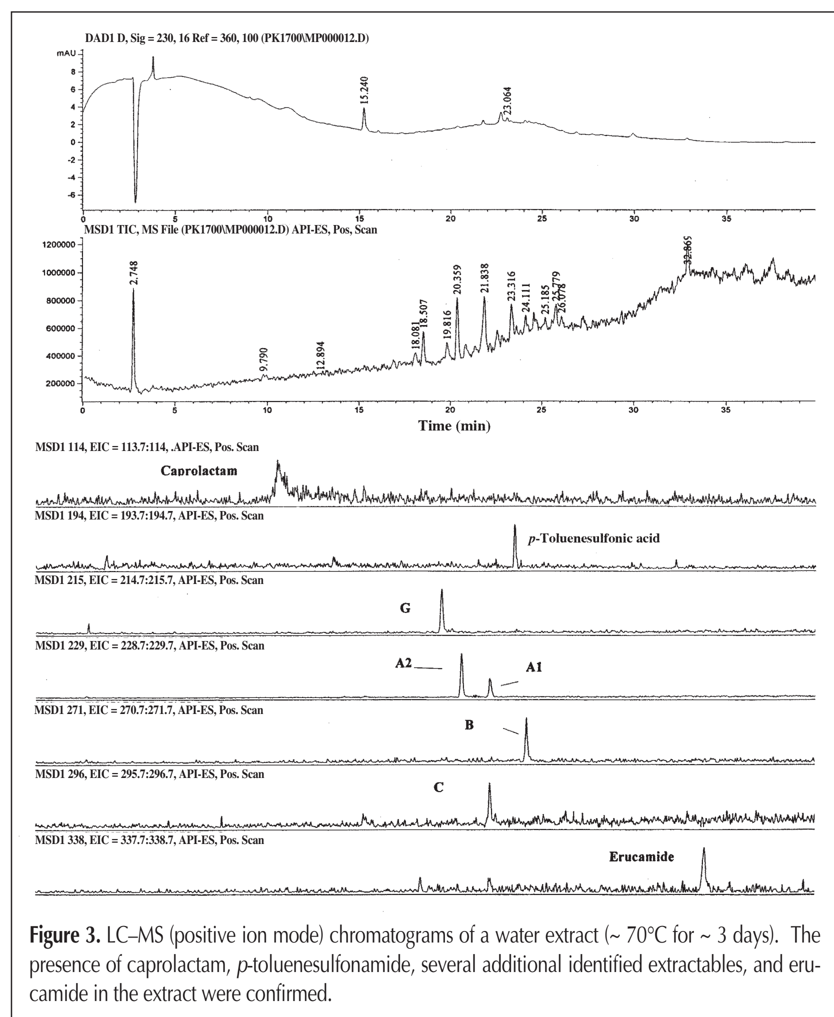


Figure 3. LC–MS (positive ion mode) chromatograms of a water extract (~ 70°C for ~ 3 days). The presence of caprolactam, *p*-toluenesulfonamide, several additional identified extractables, and erucamide in the extract were confirmed.

Caprolactam, component of the polyurethane adhesive; compounds A1, A2, B, G, formed during the manufacturing of the polyurethane prepolymers; erucamide, common slip agent; stearic acid (and related lower molecular weight acids), secondary plasticizer or lubricant and its common impurities or decomposition products (or both); *p*-toluenesulfonamide, related to printing inks (14); and compounds C, H, I, decomposition products of the material's antioxidants.

In addition to the compounds listed in Table IV, the chromatographic methods suggested that several other compounds were responsible for small peaks observed. Such compounds included higher molecular weight esters (e.g., extractables D–F in Figures 1 and 2) and additional organic acids (range from C6 to C18). Because the peaks associated with these compounds were small and, in many cases, authentic reference standards were not available for the proposed compounds, these identifications could not be unilaterally confirmed. Finally, there were several peaks in the chromatograms, especially for LC–MS, with corresponding compounds that could not be identified. In all cases, however, such peaks were small relative to the major identified peaks.

The chromatographic methods are not only valuable for the identification of compounds that produce chromatographic responses. Alternatively, the same methods can also establish the absence of compounds from a given sample. This capability can be particularly useful in the situation in which the composition of the material or container system being evaluated is not completely known, and one uses the identified leachables to "reverse engineer" the material.

Delineation of target leachables

There are at least two reasons why it is appropriate to monitor the levels of target leachables as opposed to monitoring each individual member of the leachables profile. The first reason is that many of the identified members of the leachables profile accumulate at only very low levels. Even though such low levels may be measurable in the water solution used in the identification experiments, they may not be measurable (or even detectable) in the more complex buffer solutions. The second reason is strictly practical and reflects the level of effort required to quantitate each member of the profile.

Thus, the set of target leachables was delineated based on four characteristics of the compounds. The first consideration is absolute concentration; the list of target leachables should include those compounds that make up a large portion of the total extracted carbon. The second consideration is the chemical nature of the compounds. Targets should be chosen that effectively represent the types of functionalities present in the entire profile. Third, the targets should be chosen so that they represent all of the container components that are suspected to contribute leachables. Finally, any member of the leachables profile that may have a known or suspected impact on

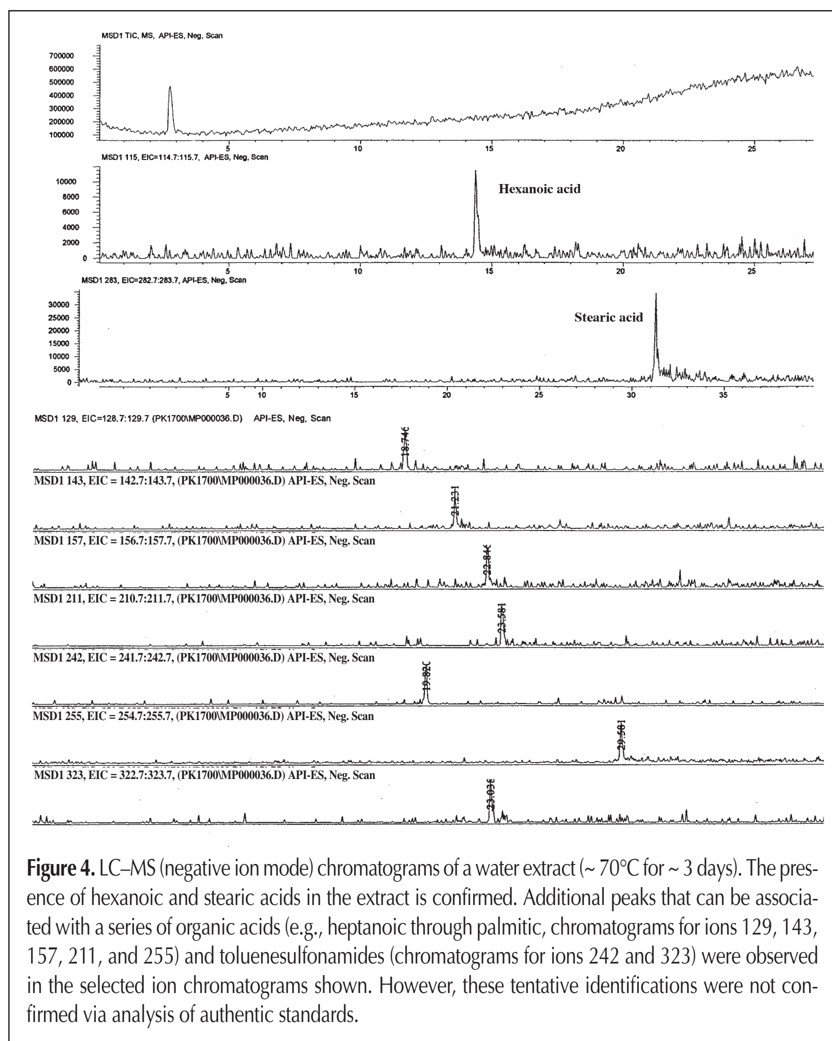


Figure 4. LC–MS (negative ion mode) chromatograms of a water extract (~ 70°C for ~ 3 days). The presence of hexanoic and stearic acids in the extract is confirmed. Additional peaks that can be associated with a series of organic acids (e.g., heptanoic through palmitic, chromatograms for ions 129, 143, 157, 211, and 255) and toluenesulfonamides (chromatograms for ions 242 and 323) were observed in the selected ion chromatograms shown. However, these tentative identifications were not confirmed via analysis of authentic standards.

Table V. Accuracy and Precision of the IEC Method for Acetate and Formate Quantitation

Matrix identification		Formate		Acetate	
Code	Composition	Accuracy*	Precision†	Accuracy*	Precision†
–	Water	90.2	8.6	105	4.7
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	126	4.9	108	2.1
II	0.05M Tris. 0.15M NaCl, pH 7.2	124	3.0	96.6	3.6
III	2.0M TRIS	149	7.4	107	1.5
IV	0.02M Tris-HCl, 0.05M NaCl, pH 8.0	127	2.0	112	6.9
V	Sodium phosphate, sodium chloride, Tween, pH 5.5	104, 93.6‡	2.4	89.9, 87.6‡	3.8
VI	0.1% Tween	104	3.3	99.2	2.6
VII	6M Guanidine	–§	–§	–§	–§
VIII	0.15M NaCl, pH 5.4	102	5.6	95.5	3.6
Acceptance criterion:		70–130%	NMT 10%	70–130%	NMT 10%

* As % recovery of an approximate 1 mg/L spike.
† %RSD of 3 injections of the spiked sample.
‡ Duplicate assessments were performed for this matrix.
§ Accuracy and precision could not be assessed in this matrix because the undiluted matrix produced a void response which overwhelmed the formate and acetate signals. Accuracy and precision with a 1/20 sample dilution were within the acceptance criteria.
** NMT = not more than.

product safety or efficacy should be a target leachable. Additionally, the list of target leachables must be consistent with the ability to effectively analyze the test samples. To this end, the following leachables were chosen as targets: acetate and formate; leachables A2, B, and C; hexanoic and stearic acids; and caprolactam.

Method development and validation

Acetate and formate by IEC

The IEC (suppressed conductivity detection) method examined for the quantitation of acetate and formate is well suited for the analysis of buffer solutions that consist primarily of inorganic salts and organic entities that are not retained under the chromatographic conditions used. However, the high concentration of such formulation additives, relative to the anticipated low levels of acetate and formate extracted from the container, results in chromatograms with large void volume responses. To minimize this effect, a small injection volume (10 mL) was used, and thus acetate and formate produced recognizable chromatographic responses in all of the buffer matrices except 6M guanidine. This buffer solution produced such a large void response that it needed to be diluted by a factor of 20 before acetate and formate peaks could be recognized in this matrix. Despite the presence of the large void volume response, the specificity of the IEC method was adequate for this study because no interfering peaks were observed in the chromatograms associated with the buffer controls.

Linearity was assessed over two ranges: approximately 1–10 and 0.3–3 mg/L. This assessment was performed by injecting three standards (~ 1, 3, and 10 mg/L or 0.3, 1.0, and 3.0 mg/L) 2 or 3 times each over the course of extended analytical runs and subjecting the resulting concentration versus response data to linear regression analysis. The coefficient of determination (r^2) was the assessment parameter used. Coefficients of 0.9989 and 0.9999 were obtained over the range of 1–10 mg/L and of 0.9937 and 0.9982 over the range of 0.3–3.0 mg/L for formate and acetate, respectively. Both of these results met the acceptance criterion of not less than (NLT) 0.99.

Accuracy and precision were assessed by replicate (n = 3) analysis of formulation controls spiked to contain approximately 1 mg/L of each analyte. Accuracy was calculated as the recovery of this spiked amount, and precision was calculated as the %RSD of the triplicate injections.

The results of the accuracy and precision assessments are summarized in Table V. A dilution by a factor of 20 was subsequently adopted for the 6M guanidine matrix, and accuracy was assessed with this dilution. The performance for

acetate was acceptable in all the other sample matrices. Although precision for formate was acceptable in all matrices, its recovery was poor in a number of matrices. The performance for formate was poorer than that of acetate because formate is the earlier eluting compound and is, therefore, more strongly influenced by the large void response. Thus, it was concluded that the IEC method for acetate and formate is valid in the eight matrices studied.

The nature of the evaluation experiments allowed for the assessment of sensitivity and response stability. Detection limits (DL), three times the baseline signal to noise, were 0.029 and 0.053 mg/L for formate and acetate, respectively. Response stability was assessed as the percent change in response between replicate injections of the standards made at the beginning and the end of an analytical run that took approximately 12 h to complete. The largest percent change observed was 9.5% for the 1 mg/L formate standard.

Other target leachtables by LC–MS

The LC–MS method evaluated for the quantitation of target leachables was similar to the methods used for target identification. However, the high concentration of formulation additives such as inorganic salts and organic entities relative to the antici-

Table VI. Linearity Assessment, Organic Extractables by LC–MS

Target extractable	Coefficient of determination (r^2)*
Positive ion mode	
Caprolactam	0.9944
Extractable A1	0.9995
Extractable C	0.9983
Extractable B	0.9979
Negative ion mode	
Hexanoic acid	0.9975
Extractable C	0.9985
Stearic acid	0.9910
Acceptance criterion	NLT 0.99

* Obtained over a concentration range of approximately 0.2 to 1.2 mg/L.
† NLT = not less than.

Table VII. Accuracy, Organic Extractables by LC–MS

	Matrix	Caprolactam	A1	C	B	Hexanoic acid	C	Stearic acid
		Accuracy*						
		Positive ion mode				Negative ion mode		
I	Ammonium sulfate, MES, potassium phosphate (pH 5.4)	73.2	75.1	77.8	77.0	97.2	88.7	54.9
II	0.05 M Tris. 0.15M NaCl (pH 7.2)	65.2	76.8	77.7	80.2	95.8	85.9	59.9
III	2.0 M TRIS	78.9	72.8	92.2	84.6	94.5	95.1	97.8
IV	0.02 M Tris-HCl, 0.05M NaCl (pH 8.0)	72.8	81.7	88.4	84.3	103	89.4	77.8
V	Sodium phosphate, sodium chloride, Tween (pH 5.5)	41.2	86.8	91.9	89.6	100	92.1	95.8
VI	0.1% Tween	26.6	79.2	89.6	93.2	95.7	94.7	108
VII	6M Guanidine	22.9	64.2	56.4	63.7	70.0	55.4	27.3
VIII	0.15 M NaCl (pH 5.4)	74.8	85.1	88.1	88.2	99.0	88.3	78.4
Acceptance criterion		%recovery 70–130%						

* %Recovery of approximately 1 mg/L spike.

Table VIII. Precision, Organic Extractables by LC–MS

	Matrix	Caprolactam	A1	C	B	Hexanoic acid	C	Stearic acid
		Precision*						
		Positive ion mode				Negative ion mode		
I	Ammonium sulfate, MES, potassium phosphate (pH 5.4)	1.7	1.3	1.2	2.4	0.4	0.9	5.5
II	0.05 M Tris. 0.15 M NaCl (pH 7.2)	2.7	1.4	1.5	1.6	2.7	0.6	5.2
III	2.0 M TRIS	9.1	9.3	3.1	6.3	< 10	< 10	< 10
IV	0.02 M Tris-HCl, 0.05 M NaCl (pH 8.0)	1.7	1.8	6.0	1.7	4.7	0.4	4.7
V	Sodium phosphate, sodium chloride, Tween (pH 5.5)	1.5	0.9	2.9	1.4	2.2	0.9	1.7
VI	0.1% Tween	0.5	4.7	0.8	1.0	9.7	0.1	0.9
VII	6 M Guanidine (diluted 1/20)	3.0	1.5	2.7	2.0	4.5	1.6	8.7
VIII	0.15 M NaCl (pH 5.4)	2.9	1.4	5.2	1.8	4.8	0.5	3.7
Acceptance criterion		%RSD NMT 10%						

* %RSD of three injections of a control spiked with approximately 1 mg/L of each analyte.

pated low levels of the compounds extracted from the container resulted in operational and performance problems when direct sample injection was used. In order to minimize such effects, the chromatographic system was equipped with a trap column between the injector and the analytical column. Although this strategy did not completely eliminate these issues, chromatograms containing all the target extractables could be reproducibly obtained for all formulation matrices. Despite the presence of these potentially interfering matrix components, the specificity of the LC–MS method was adequate for this study. No interfering peaks were observed in the chromatograms obtained from any of the buffer controls when single ion monitoring was used.

Linearity was assessed over the range of approximately 0.2–1.2 mg/L by injecting four standards (~ 0.2, 0.4, 0.8, and 1.2 mg/L) in duplicate, each over the course of an extended analytical run and subjecting the resulting concentration versus response data to linear regression analysis. Coefficients of determination obtained are summarized in Table VI and met the acceptance criterion of NLT 0.99 per the validation protocol.

The accuracy data summarized in Table VII identify several instances of poor performance. First, the performance in the 6M guanidine matrix is poor for all analytes. Because the 6M guanidine matrix has the largest dissolved solids content and also is high in pH, these poor recoveries are readily attributed to a matrix effect that the trap column could not completely counter. It is also noted that caprolactam recoveries tended to be low in the high organic matrices (Tween and guanidine). Although the recoveries for the other analytes and matrices do not always fall within the acceptance range, they are adequate for analytes present in test samples at levels of 0.1 ppm (100 ppb) or lower. The precision data summarized in Table VIII all meet the acceptance criterion. Sensitivity, expressed as the DL, varied somewhat from analyte to analyte but was generally 0.020 ppm (20 ppb) or lower.

Conclusion

A comprehensive analytical strategy has been used to discover and identify organic substances that can be leached from a polyolefin plastic material by buffer and media solutions. Specific analytical methods have been developed and validated for their use in quantitating the levels to which selected leachables accumulate in such solutions. The strategies and method used in this study are generally applicable to leachables investigations across material types and industrial applications.

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