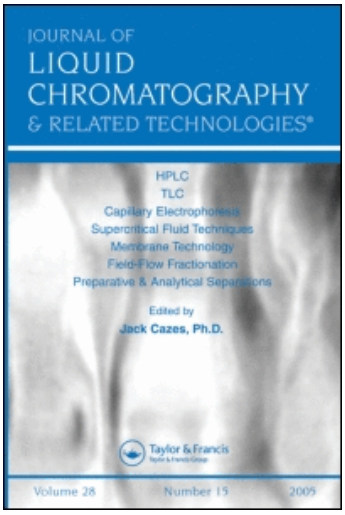

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HPLC and LC/MS Analysis of Pharmaceutical Container Closure System Leachables and Extractables

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Abstract: The term ''leachables'' refers to impurities in pharmaceutical products whose origin is the pharmaceutical container closure system in either direct or indirect contact with the formulation. Potential leachables identified through laboratory studies of pharmaceutical container closure system components are referred to as ''extractables.'' Extractables and leachables are most often chemical additives to plastic and elastomeric container closure system components, or organic residues on metal and glass surfaces. HPLC and LC/MS are used for the detection, identification and quantitation of extractables and leachables. This review presents and discusses the extractables/leachables issue within the contexts of both regulatory and analytical sciences. It also thoroughly reviews and describes the recent applications of both HPLC and LC/MS to this challenging and intriguing issue in pharmaceutical development.

Keywords: Extractables, leachables, HPLC, LC/MS

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INTRODUCTION AND BACKGROUND

Statement of the Problem

Pharmaceutical products are those products that produce a desirable therapeutic outcome when they are administered to a patient in order to address an issue related to the health of that patient. In order to produce the desired therapeutic outcome, pharmaceutical products must be manufactured, stored, and administered (i.e., delivered). Systems such as manufacturing suites, packaging/container closure systems, and medical devices have been developed to perform these functions.

Pharmaceutical products are formulated, and administration regimens developed, to maximize the therapeutic benefit derived from the product. Any action that modifies the formulation's composition can either directly or indirectly, adversely impact the derived benefit. One such action is the contact that occurs between the pharmaceutical product and its associated systems while the system is performing its function. Contact between the product and its associated system provides the opportunity for interactions to occur between the product and the system's materials of construction. One manifestation of such an interaction is the migration of substances from the system and into the pharmaceutical product. Such migrating substances become impurities in the drug product. These system-related impurities join impurities from other sources (e.g., the drug substance manufacturing process,[1] degradation of the drug substance,[2] the excipients and their manufacturing processes,[2] and reaction of the drug substance with an excipient[2]) to reflect the drug product's complete impurities profile.

As is the case with all impurities, those due to *leaching* of organic chemical entities from various components of the systems in general, but packaging systems in particular, can impact the suitability of the drug product for its intended use. The USFDA (US Food and Drug Administration)[3] has recognized the impact of leachable impurities, derived specifically from packaging (container closure) systems, on a drug product's suitability. These leachable impurities can interact with the drug product influencing its effect according to the route of administration (see Table 1).

Potential leachables from container closure system components are identified by extracting them from the particular component and analyzing the extract. These potential leachables are therefore referred to as *extractables*. Actual leachables are identified and quantified by analyzing the drug product itself. It is clear, therefore, that the ability to analyze extracts and drug products is the cornerstone of assessing the impact of drug product – container closure interactions on the suitability for use of the drug product. Interactions between drug products and container

Table 1. Examples of packaging concerns for common classes of drug products[3]

Degree of concern associated with the route of administration	Likelihood of packaging component-dosage form interaction		
	High	Medium	Low
Highest	Inhalation Aerosols and Solutions; Injections and Injectable Suspensions	Sterile Powders and Powders for Injection; Inhalation Powders	
High	Ophthalmic Solutions and Suspensions; Transdermal Ointments and Patches; Nasal Aerosols and Sprays		
Low	Topical Solutions and Suspensions; Topical and Lingual Aerosols; Oral Solutions and Suspensions	Topical Powders; Oral powders	Oral Tablets and Oral (Hard and Soft Gelatin) Capsules

closure systems have been a concern since such systems were first introduced in the early 20th Century. Early investigations were limited however to circumstantial observations and documentation of outcomes; this was due to the limitations of analytical chemistry to support a detailed, comprehensive and quantitative investigation and characterization of such phenomena. The development of wet chemical methods for the characterization of material extracts (such as the USP methods for Elastomeric Materials and Physiochemical Tests for Plastic Containers[4,5]) was a major step forward in the investigation and quantitation of potential interactions between a drug product and its container closure system. Such test methods provided insensitive general chemical information whose ability to address suitability for use issues was (and is) limited. The combination of the increasing complexity of container closure systems, the increasing diversity of especially plastic materials used in container closure systems, the increased complexity of drug product formulations, the increasing "rigor" of the conditions of contact between drug

products and their container closure systems, and the increased level of regulatory concern about suitability for use issues presented analytical chemistry with a challenge that was beyond its capabilities, even as late as the early 1960's.

The development, commercialization and routine adoption of spectroscopic and chromatographic methods allowed for the assessment of suitability for use of drug products. Hyphenation of chromatography with spectroscopy provided the tool to discover, identify and quantify (with a high degree of specificity and sensitivity) individual organic extractables or leachables.

This paper presents a review on the latest advances in the field of leachable determination using HPLC (High Performance Liquid Chromatography) hyphenated with different types of detection.

The Regulatory Environment for "High Concern" Drug Products

Analytical chemistry and trace organic analysis[6] have a significant role to play in the characterization of leachables and extractables, and regulatory authorities have come to expect that state-of-the-art instrumentation and technologies will be applied.

Regulatory requirements for leachables and extractables information, studies and methods/specifications are directly related to the degree of regulatory concern as expressed in Table 1. For inhalation products two USFDA regulatory guidance documents exist, one for MDIs and DPIs (in draft form),[7] and the other for Nasal Spray, Inhalation Solution and related drug product types.[8] In general, for inhalation drug product development, as well as for other relatively high concern drug product types such as injectables and opthalmics, USFDA suggests:[3,7–9]

- All available information regarding the composition, manufacturing, fabrication, and shipping/storage environment for each container closure system component deemed "critical" as that term is defined by regulatory guidance.[7,8,10]
- Controlled Extraction Study results for all critical components, including qualitative, structural, and quantitative analyses for all extractables above predetermined analytical thresholds.[10,12] These thresholds can be either safety based, as for inhalation drug products,[10–12] or technology based. Note that at the time of this writing safety based thresholds exist only for inhalation drug products.
- Drug product leachables studies including qualitative, structural, and quantitative analyses for all leachables above predetermined analytical thresholds[10–12] (see qualifying statements above). The leachables

information should be sufficient to allow for a leachables/extractables correlation, and to establish specifications/acceptance criteria for leachables.[10,12]
- Specifications/acceptance criteria for extractables sufficient to provide secondary control of drug product leachables.

The Analytical Challenge

The characterization of test components for extractables and leachables presents unique challenges to analytical scientists for the following reasons:

1. *Structural diversity*: Additives to rubber and plastic serve a variety of important functions within these materials and their manufacturing processes. These functions include: antioxidant, antistatic, antislip, curing agent, curing accelerator, UV-stabilizer, filler, lubricant, pigment, etc. Within an individual function there can be a significant diversity of chemical structure, and therefore in chemical and physical properties. For example, antioxidants can include phosphites, hindered phenols, aromatic amines, phenolic sulfides, thiophenols, sulphoxides, sulphones, metal dithiolates, organoboranes, etc.[13] Many of these can be present as leachables within the same drug product matrix.
2. *Sample diversity*: There are two main categories of rubber used for pharmaceutical container closure systems, sulfur cured and peroxide cured. Each category includes several different polymeric types with different applications. Also of interest are thermoplastic elastomers, which include a plastic such as low density polyethylene as a reinforcing agent in the rubber matrix. Each elastomer has different swelling properties during laboratory extraction and drug product leaching. Plastics behave differently than rubber during laboratory extraction studies. Extractables associated with metal and glass components are usually surface residues, and are often complex mixtures. Drug product matrices are also widely variable in composition and thus present a considerable analytical challenge for leachables assessments, particularly aqueous based formulations.
3. *Concentration levels*: Leachables can appear in drug products over a wide concentration range. For example, in MDI drug products primary rubber gasket additives can appear as leachables at the tens to several hundreds of μg/canister levels, polyaromatic hydrocarbons at the ng – low μg/canister levels, and N-nitrosamines at low ng/canister levels.[9,14] These levels are all considered "trace"[15] and require special considerations for analytical method development.[6,9]

These issues present obvious challenges for analytical methods specifically targeted at individual extractables/leachables, and even greater challenges for general methods designed to "profile" extractables/leachables from either a component or drug product. For the analytical scientist, the extractables/leachables issue more closely resembles problems in environmental trace organic analysis than a typical pharmaceutical impurity problem.

HPLC TECHNIQUES AND METHODOLOGIES

The Metered Dose Inhaler as an Example of a Container Closure System

Drug product container closure system components can be composed of a variety of materials including glass, metal (stainless steel and aluminum), plastics of various types, and elastomers (i.e., rubber) of various types. Each of these materials has the potential to leach organic chemical entities into a drug product. Consider for example, the Metered Dose Inhaler (MDI) shown in schematic form in Figure 1. The MDI is an Inhalation Aerosol drug product,[16] which consists of an active ingredient(s) and excipients either in solution or suspended in an organic propellant (i.e., chlorofluorocarbon or hydrofluorocarbon) under pressure in a metal canister. The drug product formulation is held in the canister and individual measured doses are delivered to the patient by a dose metering valve. This valve can be composed of metal and/or plastic components and includes rubber seals to secure it to the pressurized canister and maintain integrity around the valve stem. The valve components and metal canister inner surface are in contact with the drug product and therefore have the potential to leach organic chemical entities into the product. Other inhalation drug product types such as Dry Powder Inhalers (DPIs) have many rubber and plastic components, either in contact with the drug product formulation or the patient's mouth or nasal mucosa. Inhalation Solution unit doses can be contained in plastic vials, and Nasal Sprays are often contained in plastic bottles with plastic spray components and tubes. Injectable drug products can be contained in glass bottles or vials with rubber stopper seals, and intravenous drug products are contained in plastic bags.

Clearly, the simple gathering of information such as that shown in Table 2 is not sufficient to predict all extractables. Therefore, laboratory extraction studies designed to identify and quantitate such extractables are required, generally referred to as Controlled Extraction Studies.[10] These studies are designed and accomplished according to published "Best Practice" recommendations,[10,12] which include both best

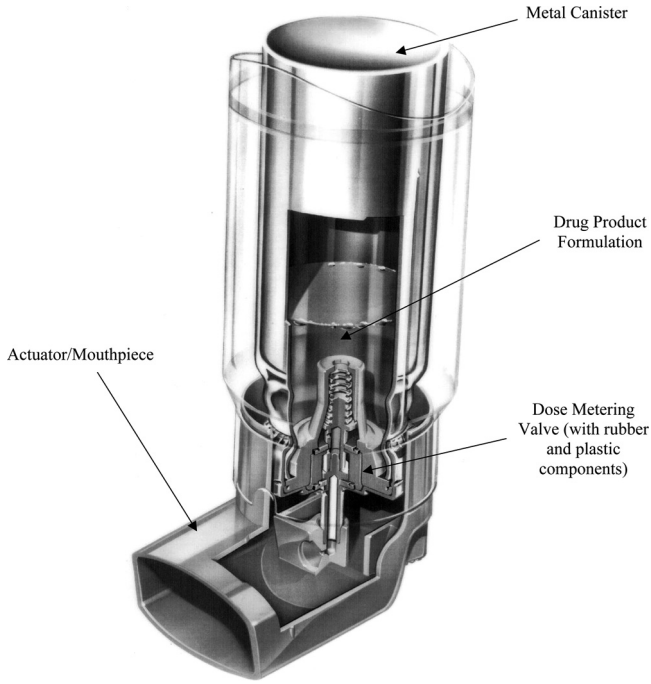


Figure 1. Schematic diagram of a Metered Dose Inhaler drug product. Note that "critical components" (valve components, canister, actuator/mouthpiece) are noted. This diagram is taken from the Bespak Image Resource Library.

demonstrated laboratory practices, as well as safety qualification and analytical thresholds for organic leachables in inhalation drug products which carry the highest risk for leachables issues as described above.

Table 2. Ingredients in a typical polypropylene[10]

Ingredient			
Chemical name	Registry #	Commercial name	Percent (w/w)
Tetrakis (methylene(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)) methane	6683-19-8	Irganox 1010 Anox 20	0.08
Bis(2,4-di-*tert*-butylphenyl) Pentaerythritol diphosphite	26741-53-7	Ultranox 626	0.05
Calcium Stearate	1592-23-0	N/A	0.03–0.4
Vegetable oil derived 90% alpha	31566-31-1	Pationic 901	0.3

General

Given the complexity of the analytical challenge faced by those performing extractables and leachables assessments, it is not surprising that success in such an endeavor is achieved via the utilization of a multitude of complementary, yet orthogonal, analytical methodologies. As noted in the Product Quality Research Institute's recent document proposing "Best Practice" recommendations for extractables and leachables studies in inhalation drug product development:[10]

"No single analytical technique will be sufficient to detect and/or identify all possible extractables (. . . *or leachables*) from any particular container/closure system component (. . . *or drug product matrix*), therefore, multiple broad spectrum techniques should be used to ensure complete evaluation of an extractables (. . . *or leachables*) profile."

In the case of organic extractables and leachables, many authorities have specifically recommended the use of Gas Chromatography (GC)/Flame Ionization Detection (FID), Gas Chromatography/Mass Spectrometry (GC/MS), and HPLC combined with either Mass Spectrometry (LC/MS) or UV detection (LC/UV). The separating power of capillary GC (Figure 2), combined with the universality, sensitivity and information content of its associated detection methods (e.g., FID and MS), makes this family of techniques highly applicable to extractables/leachables analysis.

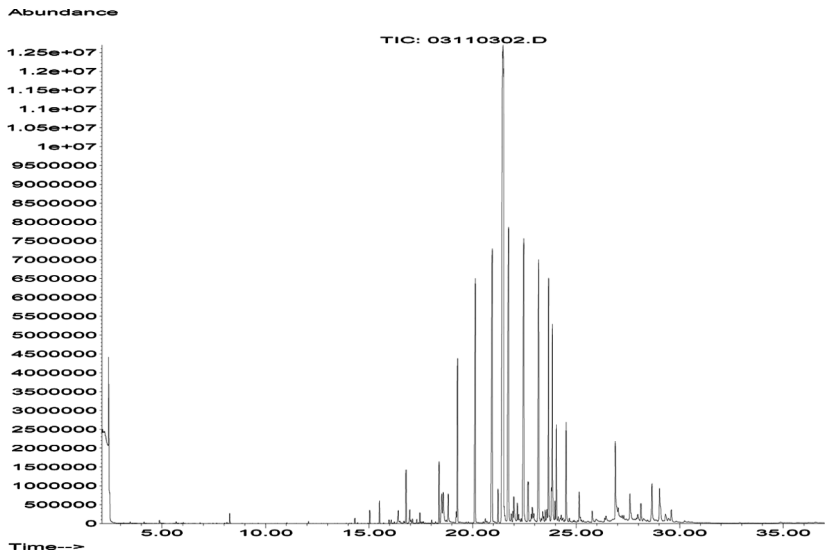


Figure 2. Total Ion Chromatogram (TIC) from a capillary Gas Chromatography/Mass Spectrometry (GC/MS) analysis of an elastomer extract (i.e., an extractables profile).

However, many extractables and leachables are not totally amenable to GC analysis, because of the lack of volatility, thermal instability, or chemical/physical interactions with the GC column or system. Under such circumstances, HPLC with different detection possibilities becomes the method of choice for a wide variety of leachables/extractables.

LC/UV

In a somewhat philosophical treatment of the fundamental principles of analytical chemistry, Valcárcel[15] distinguishes between three types of analysis:

- *Qualitative analysis* – "A type of chemical analysis by which the analyte or analytes in a sample are identified. The result is a YES/NO binary response."[15]
- *Quantitative analysis* – "A type of chemical analysis by which the proportion or amount of each analyte in a sample is determined. The result is a numerical response."[15]
- *Structural analysis* – "A type of chemical analysis by which the structure of a sample (*viz*. the spatial distribution of its constituents) or a pure analyte is established."[15]

Given these classifications in analytical chemistry, LC/UV is clearly capable of *qualitative* and *quantitative* analysis but limited in its capability for *structural* analysis. UV (and visible) spectrophotometry depends on an analyte molecule having the ability to absorb light at specific wavelengths in amounts directly proportional to the concentration of the analyte (i.e., Beer's Law). Certain structural features of analyte molecules (termed "chromophores") impart this light absorbing ability. These include aromatic and heteroaromatic rings, unsaturated carbon-carbon bonds, and various functional groups such as aldehyde, azo, carboxyl, ester, ketone, nitrile, nitro, sulfone, thiol, thioether, thioketone, etc. Many of the chemical entities that are additives to rubber and plastic, and can therefore appear as extractables and leachables, have chromophoric structural features. The variety and multitude of such structures dictates that the peak capacity of an HPLC system should be maximized. To obtain such a situation, the number of theoretical plates should be maximized and consequently the kinetics of analytes mass transfer. The latest in column technologies, such as fused core stationary phases allow for fast, selective and efficient separation of most leachables/extractables. Figure 3 shows an example of such separation.

The ability of photodiode array detectors (PDAs) to acquire UV spectra over defined wavelength ranges has given LC/UV the ability to

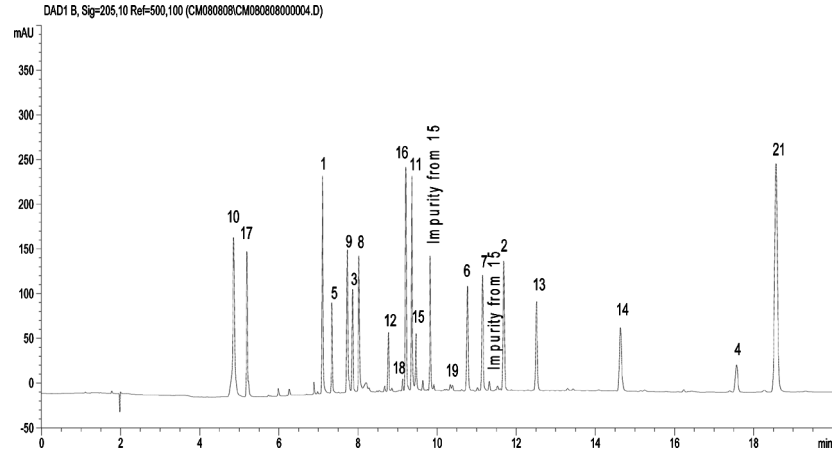


Figure 3. LC/UV chromatogram of a test mixture of extractables/leachables, including: 1) benzothiazole, 2) 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol), 3) tetramethylthiuram disulfide, 4) di-n-decyl phthalate, 5) tetramethylthiuram monosulfide, 6) 2,6-di-*tert*-butylphenol, 7) butylated hydroxytoluene, 8) 2,4-dihydroxybenzophenone, 9) 2-hydroxyacetophenone, 10) 3,5-dichlorobenzoic acid, 11) phenyl salicylate, 12) 3-*tert*-butyl-4-hydroxy-anisole, 13) 2,2′-methylenebis(6-*tert*-butyl-4-ethylphenol), 14) bis (2-ethylhexyl) phthalate, 15) N-phenyl-1-naphthylamine, 16) diphenylamine, 17) 2,4-diaminotoluene, 18) diphenyl-ethylenediamine, 19) Irganox 1010, 21) Irgafos 168. Platform: Halo C8, 150 × 4.6 mm, 2.7 µm particle size, Advanced Materials Technology, Wilmington, DE; Mobile Phase: A – Water/B-Acetonitrile; Gradient: 2% B for 0.5 min, to 90% B in 7 min, to 100% B in 6.5 min, hold at 100% B for 6 min; UV at 205 nm.

generate more structural information. However, this information is for the most part limited to compound class identification and not individual analyte structure elucidation. LC/UV is not, therefore, compound specific, that is "providing information unique to and characteristic of a particular chemical entity."[6]

LC/MS

Unlike spectrophotometry, mass spectrometry is compound specific. In general, mass spectrometers can produce the following information for organic chemical analytes (down to trace levels[15]):

- Molecular weight.
- Molecular formula (i.e., elemental composition).
- Fragmentation behavior according to defined chemical rules.

This compound specific information often allows for the *structural analysis* of trace organic chemical entities, including extractables and leachables, but not always. Mass spectrometry is limited in its ability to distinguish between isomers of various kinds, including geometric and structural. It is also limited in its ability to elucidate aromatic substitution patterns. The capability to elucidate these structural features for extractables/leachables must await the promise of LC/NMR (Nuclear Magnetic Resonance Spectroscopy). However, in spite of these limitations, mass spectrometry has become a primary technique for both *structural* and *qualitative analysis* of extractables and leachables. Further, due to its high selectivity and specificity relative to other detection techniques, mass spectrometry has also become a primary tool for high-sensitivity *quantitative analysis*.

APPLICATION OF LIQUID CHROMATOGRAPHY TO EXTRACTABLES AND LEACHABLES ASSESSMENT

General

Over the past decade, a number of comprehensive reviews on the application of HPLC, and other chromatographic techniques, to polymer characterization in general and extractables and leachables in particular, have been published. Jenke and co-workers have published several reviews (a) presenting insights and guidelines for the validation of chromatographic methods for quantitation of leachables and extractables in pharmaceutical solutions;[17,18] (b) identify reported extractables and leachables and their accumulation potential;[19,20] (c) provide details about chromatographic methods used for the identification and quantification of organic polymer additives[21] and of extractables/leachables from the packaging of different drug products.[22] Special emphasis is put on the extraction techniques and the method recoveries. A comprehensive review by Lau and Wong[23] addresses the use of chromatographic methodologies for the characterization of leachables, such as additives in polymeric packaging materials (plasticizers, thermal stabilizers, slip additives, light stabilizers and antioxidants), monomers and oligomers (styrene, vinyl chloride, bisphenol A, isocyanate, caprolactam and polyethylene terephtalate), and contaminants (decomposition products, benzene and other volatiles). Garcia and associates[24,25] have published reviews that discuss and compile analytical methods, including HPLC, for the quantitation of numerous migrants (including styrene, bisphenol A, 1-octene, limonene, diisopropylnapthalene, laurolactam, triacetin, tri-*n*-butyl citrate, butylated hydroxytoluene (BHT), Triclosan, Irganox 1076, Chimassorb 81,

caprolactam, diphenyl phthalate, Uvitex OB, benzophenone, bis-(2-ethylhexyl) adipate, diphenylbutadiene) from food packaging materials.

It is the nature of the rapidly changing analytical landscape that new techniques are constantly being developed, validated and utilized to solve "old" problems. As this is the case with the application of HPLC to extractables/leachables assessments, it is reasonable that these cited reviews are no longer complete nor current. The following review attempts to fill the gap between these cited reviews and the current knowledge base with respect to the application of HPLC to extractables and leachables assessment.

LC/UV Applications

Chromatographic methods are applied to extractables and leachables assessments to accomplish two objectives:

1. Discover and identify extractables/leachables (either *qualitative* or *structural analysis*), and
2. Measure extractables/leachables (*quantitative analysis*).

Due to their intrinsic nature and information contents as described above, the various detection methods are more or less better suited to facilitate these objectives. In general, UV detection is best suited to the discovery (*qualitative analysis*) and quantitation of extractables and leachables while mass spectrometric detection is better suited to extractables and leachables identification (*structural analysis*) and high-sensitivity measurement (*quantitative analysis*). Since it is envisioned that the readers of this review will be interested in experimental details, especially including HPLC platforms, Table 3 summarizes notable applications of LC/UV to extractables/leachables analysis published since, or not mentioned in, the aforementioned review articles. These references generally fall into two categories; those case studies that utilize more or less state-of-the-art methodology to accomplish a particular research objective (e.g., quantify extractables/leachables in a particular pharmaceutical product or packaging material) and those studies that document the use of innovative and/or emerging techniques to enhance the effectiveness and/or efficiency of such studies.

One example of the application of state-of-the-art methodologies/strategies to extractables/leachables investigations was documented by Zhang et al.[26] The purpose of this study was to identify the major extractables associated with rubber closures used for pre-filled semi-solid drug applicators. Exaggerated extracts were generated and characterized. Five extractables, including 4-(1,1-dimethyl-propyl)-phenol,

Table 3. Summary of LC/UV platforms and applications

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
26	4-(1,1-dimethylpropyl)-phenol sulfur 2,6-di-*t*-butyl-[1,4] benzoquinone furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol 2-bromo-4-(1,1-dimethylpropyl)-phenol	Rubber closures heated in Acetonitrile and refluxed for 8 h.	**Analytical reversed phase HPLC method:** Prodigy ODS (3), 3 μm, 150 mm × 3.2 mm, T = 40°C **Semi-preparative reversed phase HPLC method:** Prodigy ODS (3), 5 μm, 150 mm × 10 mm	**Analytical reversed phase HPLC method:** Binary gradient was used. Within the first minute, 70% Acetonitrile, 1–15 min, linear gradient to 90% Acetonitrile, 15–20 min, 90% Acetonitrile, and 20–25 min, linear gradient to 70% Acetonitrile. Flow rate = 0.6 mL/min. **Semi-preparative reversed phase HPLC method:** The binary gradient profile was the same as that for the analytical HPLC method Flow rate - = 5 mL/min. **Detection:** 220 nm
27	Compounds not identified	Rubber stoppers extracted for 2 days at 40°C with formulation placebo or Methylene Chloride Auto-sampler temperature is maintained between 2	**Experiment 1:** C8, 4.6 mm × 150 mm, 5 μm, 90 Å, T = 25°C **Experiment 2:** Phenyl Reverse Phase, 2.1 mm × 50 mm, 5 μm,	**Experiment 1:** Mobile phase A: 0.06% Trifluoroacetic Acid (TFA) in Water Mobile phase B 0.06% TFA in Acetonitrile Elution: isocratic at 5% B

	and 8°C.	120 Å, T = 15°C	for 5 min, then from 5% to 90% B for 85 min, hold at 90% B for 5 min, to 5% B over 1 min and equilibrated at 5% B for 15 min. Flow rate = 1 mL/min **Experiment 2:** Mobile phase A: 10% Methanol in aqueous 20 mM Ammonium Acetate Mobile phase B: 10% Water and 20% Acetonitrile in methanolic 10 mM Ammonium Acetate Elution: isocratic at 0% B for 3 min, then from 0% to 45% B for 12 min, then from 45% to 75% B for 50 min, then from 75% to 100% B for 20 min, hold at 100% B for 20 min, to 0% B over 5 min and equilibrated at 0% B for 20 min. Flow rate = 200 μL/min

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
28	1-methoxy-3-*o*-toyloxy-propan-2-ol	Analyzed drug stability sample (40 °C/20% relative humidity, 6 months) Extracted LDPE bottle, plug and cap, label with ink and varnish Extraction solvent: Water : Methanol 1:1 Injection volume ranged from 100 to 250 µL	Waters Symmetry™ C18, 4.6 mm × 150 mm, 3.5 µm, T = 30°C	**Detection:** 214 and 280 nm Mobile phase A: 90% Water/10% Acetonitrile/ 0.05% trifluoroacetic acid Mobile phase B: 10% Water/90% Acetonitrile/ 0.05% trifluoroacetic acid Gradient: 10% B at 0 min, increased to 100% B at 30.0 min, changed to 10% B at 30.1 min and held at 10% B for 10 min Flow rate = 1.0 mL/min **Detection:** UV–Vis range from 190–500 nm; monitor at 220 nm
29,30	**Reference Compounds:** 2,4-dichlorobenzoic acid 2-(2-butoxyethoxy) ethyl acetate DBAT 4-pentyl phenol BHA dibutyl phtalate butylated hydroxytoluene	O-Ring and Container leachables measured in unspecified products with unspecified contact conditions	Zorbax SB-C18, 4.6 mm × 150 mm, 3.5 µm, T = 40°C	Mobile Phase A: 0.1% Formic Acid in Water Mobile Phase B: 0.1% Formic Acid/90% Acetonitrile/10% Water Gradient: from 20% to 85% B in 29 min, to 100% B in 2 min, hold at 100% B for 10 min

	dioctyl phtalate **O-Ring Extractables:** Irganox 1076 3,5-bis(1,1-dimethyl)-4-hydroxybenzenepropanoic acid **High Density Polypropylene Container:** Palmitic acid Stearic Acid Oleamide			Flow rate = 1.0 mL/min **Detection:** UV, wavelength unspecified
31	zinc dithiocarbamate 2,6-di-*t*-butyl-*p*-cresol octylated diphenylamine antioxidant sulfur pentylphenol tetrakis(methylene(3,5-di-*t*-butyl-4-hydroxyhydrocinnamate))methane	Reference mixtures prepared in Ethanol; no extracts generated	**Method 1:** YMC-Pack Cyano, S-5 μm, 150 mm × 4.6 mm, Room Temperature **Method 2:** Waters XTerra™ MS C18, 50 mm × 4.6 mm, 5 μm **Method 3:** Waters XTerra™ MS C18, 50 mm × 4.6 mm, 5 μm	**Method 1:** Mobile phase A: 90% Water/10% Acetonitrile/0.05% TFA Mobile phase B: 0.05% TFA in Acetonitrile Gradient: from 0% to 50% B in 30 min, then 30–32 min linear gradient to 100% B, 32–35 min held at 100% B, 35–35.1 min, 100% A and finally, 35.1–40 min held at 100% A Flow rate = 1.0 mL/min **Method 2:** Mobile phase A: 75%

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
				Water/25% Acetonitrile Mobile phase B: Acetonitrile Gradient: 0–15 min, 50% B, 15–25 min, 55% B, 25–35 min, 65% B, 35–75 min, 100% B, 75–78 min, held at 100% B, and 78.1–85 min, 100% A Flow rate = 1.0 mL/min **Method 3:** Mobile phase A: 75% Water/25% Acetonitrile Mobile phases B: Acetonitrile Gradient: 0–15 min, 50% B, 15–25 min, 55% B, 25–28 min, 58% B, 28–30 min, 100% B, 30–35 min, held at 100% B, and 36–45 min, 100% A. Flow rate = 1.0 mL/min **Detection: Method 1 -** UV at 215 nm **Methods 2, 3 -** UV at 210 nm

32	First series cyclic trimer of polyethylene terephthalate	PET bottles cut up and extracted with 10 mL of Dichloromethane via a process of maceration (24 hrs) and sonication (1 hr). Extracts were filtered through 0.45 μm PTFE filters.	OmniSpher RP-C18 ; 20 mm × 4.6 mm, 5 μm	85% Methanol/15% Water **Detection:** UV at 254 nm
33,34	Diphenylbutadiene	LDPE extracts with food simulating solvents	Kromasil 100 C18, 150 mm × 4 mm, 5 μm, T = 30°C	Gradient: 0 – 2 min hold at 65% Acetonitrile/35% Water, increase to 100% Acetonitrile within 15 minutes. Flow rate = 1 mL/min **Detection:** UV with a PDA, wavelength unspecified
35	**Photoinitiators:** Irgacure 184 Irgacure 651 Irgacure 907 Quantacure ITX Quantacure EHA	Extraction of packaging: 1 gram in 10 mL Acetonitrile, 70°C for24 hr. Analysis of packaged product (infant formula)	Kromasil 100 C18, 150 mm × 4 mm, 5 μm, T = 30°C	Gradient: 0 – 2 min hold at 20% Acetonitrile/20% Water, increase to 80% Acetonitrile at 20 min, increase to 100% Acetonitrile at 23 min. Flow rate = 1 mL/min **Detection:** UV at multiple wavelengths
36	**Photoinitiators:** 1-benzoylcyclohexanol	Testing of tablets stored in labeled HDPE bottles	ODS or IB-Sil BDS, 250 mm × 4.6 mm	0.1% Formic Acid in Acetonitrile

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
	2-hydroxy-2-methylpropio-phenone			**Detection:** UV at 240 nm
37	2,4-di-*t*-butyl phenol	Containers filled with 4 model solvents (pH 3, Water, pH 10, 4/6 Ethanol/Water) and stored for 55°C for 1 week	Alltech Adsorbosphere HS C18, 100 mm × 4.6 mm, 3 μm, T = 40°C	Mobile phase A: 10 mM Ammonium Acetate in Water Mobile phase B = Methanol. Gradient: 0 min, 20% B, 12.0 min, 95% B, 15.0 min, 95% B, 18.0 min, 20% B, 21.0 min, 20% B Flow rate = 0.8 mL/min **Detection:** UV at 210, 230, 250 nm
38	4,4-methylene dianiline	20 grams of resin pellets extracted with 5% Ethanol in Water at 40°C for 72 hours	Alltima C18, 250 mm × 4.6 mm	Mobile phase: Acet-onitrile/Methanol/0.01 M M Sodium Acetate (27/11/62) **Detection:** UV at 248 nm
39	**Antioxidants:** AO2246 AO 425 BHA BHT	2 grams of LDPE film, extracted with 30 mL of dichloromethane at 55°C for 3 min; also extractions with food simulating	Waters Symmetry C18, 150 mm × 3.0 mm, 3.5 μm, T = 30°C	Mobile phase A = Methanol Mobile phase B = Water Gradient: 0 min, 50% B, 5.0 min, 0% B, 22.0 min,

	2,4-di-*t*-butyl phenol Ethanox 330 Irgafos 168 Irganox 1010 Irganox 1076	solvents		0% B, 23.0 min, 50% B, 25.0 min, 50% B Flow rate = 0.5 mL/min **Detection:** UV at 276 nm
40	butylated hydroxytoluene (BHT)	Various food items were solvent extracted and the extracts were appropriately processed for analysis.	Kromasil 100 C18, 150 mm × 4 mm, 5 μm	Mobile phase A = Acetonitrile Mobile phase B = Water Gradient: hold at 65% A/35% B for 2 min, raised to 100% A within 15 minutes, run for a total time of 30 min. **Detection:** UV at 205 and 280 nm
41	**Antioxidant-related Compounds:** 2,4-di-*t*-butyl-phenol 2,6-di-*t*-*p*-benzoquinone 3,5-di-*t*-butyl-4-hydroxy-phenylpropionic acid 2,6-di-*t*-butyl-4-methoxy-phenol 3,5-di-*t*-butyl-4-hydroxy-benzoic acid triphenyl phosphate	0.3 grams resin extracted with 14 mL 10% Ethanol in Water, various extraction times and temperatures	Discovery RP Amide C16, 150 mm × 4.6 mm, 5 μm	Mobile phase A = Acetonitrile Mobile phase B = Water Gradient: 0 min, 30% A, at 0.25 mL/min, 5.0 min, 30% A, at 0.25 mL/min, 45.0 min, 98% A at 0.25 mL/min, 50.0 min, 98% A at 0.40 mL/min, 70.0 min, 98% A at 0.25 mL/min, 75.0 min,

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
	tri-*p*-tolyl phosphate diphenyl phosphate			98% A at 0.25 mL/min, 80.0 min, 30% A at 0.25 mL/min, 85.0 min, 30% A at 0.25 mL/min **Detection:** UV at 220 nm
45	**Phthalate Plasticizers:** dipropyl phthalate dibutyl phthalate butyl benzyl phtalate dicyclohexyl phthalate dioctyl phthalate diisoamyl phthalate trioctyl trimelitate	Standard mixtures only	1) Diamondbond C18 (octadecylsilica) 2) Zirchrom PS (zirconia-based polystyrene) 3) Zirchrom PBD (zirconia-based polybutadiene)	**Column 1:** 70%, 75% and 80% Acetonitrile in Water Isothermal: 40°C, 70°C, 100°C Thermal gradient: 40°C–150°C at 5°C/min, 10°C and 15°C Flow rate: 2.0 mL/min **Columns 2 and 3:** 40% Acetonitrile in Water Thermal gradient: 40°C–150°C at 5°C/min, 10°C and 15°C Flow rate: 1.0 mL/min **Detection:** UV at 270 nm
45	**Antioxidants:** Irganox 245 Irganox 1098	Standard mixtures only	1) Diamondbond C18 (octadecylsilica) 2) Zirchrom PS (zirconia-	**Column 1:** 75% Acetonitrile in Water Thermal gradient: 40°C–

	Naugard XL-1 Irganox 1081 Irganox 1035		based polystyrene) 3) Zirchrom PBD (zirconia-based polybutadiene)	150°C at 5°C/min, 10°C and 15°C Flow rate: 1.0 mL/min **Column 2:** 40% Acetonitrile in Water Thermal gradient: 40°C–150°C at 5°C/min, 10°C and 15°C Flow rate: 1.0 mL/min **Column 3:** 48%, 52% and 55% Acetonitrile in Water Isothermal: 40°C, 70°C, 100°C Thermal gradient: 40°C–150°C at 5°C/min, 10°C and 15°C Flow rate: 1.0 mL/min **Detection:** UV at 225 nm
46	di-2-ethylhexyl phthalate di-*n*-butyl phthalate benzyl butyl phthalate Bisphenol A nonylphenol	In-tube solid-phase microextraction of pharmaceutical preparations	Hypersil ODS, 125 mm × 4.0 mm, 5 μm, T = 40°C	Mobile phases A: Acetonitrile Mobile phases B: Water Gradient: from 65 to 75% at 1.5 mL/min for a 5 min

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
	octylphenol diethyl phthalate di-*n*-propyl phthalate di-*n*-amyl phthalate di-*n*-hexyl phthalate di-*n*-octyl phthalate dicyclohexyl phthalate			run, from 75 to 95% at 1.5–2.0 mL/min for a 5 min run and hold 95% at 2.0 mL/min for 2 min.
47	dimethyl phthalate diethyl phthalate dibutyl phthalate	On-line microdialysis concentration of water extracts	Eurospher ODS-100, 250 mm × 4.6 mm, 5 μm	Mobile phases A: 40% Acetonitrile in Water, pH 6.0 Mobile phases B: 90% Acetonitrile in Water, pH 6.0 Gradient: 3 – 13 min = 45% Acetonitrile, 13 to 29 min = 90% Acetonitrile, after 29 min = 45% acetonitrile. Flow rates: 13 – 15 min - = 1.5 mL/min all other times = 1.0 mL/min
48	**Dyes:** Fat Brown RR, 95% Malachite Green Carbinol Base	Reference mixtures tested	UPLC: Acquity UPLC BEH C18, 50 mm × 2.1 mm, 1.7 μm, T = 30°C	Mobile phases A: Water Mobile phases B: Methanol Gradient: 0 min = 40% A/60% B, 0.8 min = 30%

	Dimethyl Yellow Sudan Orange G, 85% Sudan 1, 97% Solvent Blue 35, 98% Sudan II, 90% Sudan Black Sudan III Sudan IV **Antioxidants:** BHT Ethanox 330 AO 2246 Irgafos 168 Irganox 1010 Irganox 1076			A/70% B, 0.9 min = 10% A/90% B, 4.0 min = 0% A/100% B, 4.5 min = 0% A/100%, 5.0 min = 40% A/60% B, 6.0 min = 40% A/60% B Flow rate = 0.5 mL/min
49	2-mercaptobenzothiazole 2-(4-morpholinylmercapto) benzothiazole 2,2′-dibenzothiazyl disulfide N-cyclohexyl-2-benzothiazyl sulfonamide	Extraction with Acetone (side reactions with MMBT and CBS) and MTBE by ultrasonication. Filtration through Millipore filter.	Lichrospher RP 100 C18 column, 5 μm, 125 mm × 4.0 mm, Room temperature	35% – 45% Tetrahydrofuran in 0.1 M aqueous Sodium Acetate, pH = 6.0 with Acetic Acid Flow rate = 1.5 mL/min
50–52	2-mercaptobenzothiazole tetramethylthiuram monosulfide tetramethylthiuram disulfide	Extraction with Acetone, evaporation, then dilution in Acetonitrile and filtration before injection	Alltima C18, 4 mm, 150 mm × 4.6 mm, PEEK lined	Acetonitrile - Aqueous Zinc Sulfate (10^{-5} mol/L) at ratio of 50:50 for 5 min, then a linear gradient to 100%

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
	2-(4-morpholinylmercapto) benzothiazole zinc dimethyldithiocarbamate tetraethylthiuram disulfide dipentamethylenethiuram disulfide N-cyclohexyl-2-benzothiazyl sulfonamide zinc diethyldithiocarbamate zinc pentametylenedithiocarbamate 2,2′-dibenzothiazyl disulfide zinc dibenzyldithiocarbamate zinc dibutyldithiocarbamate N,N-dimethylthiocarbamyl-benzothiazole disulfide N,N-dimethylthiocarbamyl-benzothiazole sulfide 2-benzothiazolyl-N,N-diethyl-thiocarbamylsulfide			Acetonitrile for 35 min Flow rate = 1 mL/ min
53	2,4-dihydroxybenzophenone	Dilution in Ethanol and deionized Water (1/1,	Hypersil ODS Cas, 5 μm, 250 mm × 4.6 mm	Organic phase: Acetonitrile – Methanol

	2, 2′-4, 4′- tetrahydroxy-benzophenones 2-hydroxy-4-methoxyben-zophenone 2-hydroxy-4-methoxyben-zophenone-5-sulphonic acid 2–2′-dehydroxy-4,4′-dimethoxybenzophenone 2–2′-dihydroxy-4-methoxy-benzophenone) 2-hydroxy-4-methoxy-4′-methylbenzophenone	*v/v)* Injection Volume = 25 μL		Aqueous phase: Water with 1% Acetic Acid or without additives Isocratic elutions: 15% Acetonitrile/15% Methanol/70% Water 20% Acetonitrile/20% Methanol/60%Water 30% Acetonitrile/30% Methanol/40% Water Gradient elution: to 60 % organic phase in 40 min. Flow rate = 1.0 mL/min
54	thiourea ethylenethiourea dimetylthiourea diethylthiourea diphenylthiourea dibutylthiourea ethylbutylthiourea	Extraction with Acetone, evaporation, then dilution in Acetonitrile and filtration before injection	Alltima C18, 4 mm, 150 mm × 4.6 mm, PEEK lined	50% Acetonitrile/50% 10^{-5}mol/L Aqueous Zinc Sulfate for 5 min, then a linear gradient to 100% Acetonitrile for 35 min Flow rate = 1 mL/ min
55	*p*-benzoquinone dioxime *p*-nitrosophenol	0.06 mg/mL to 0.6 mg/mL in Methanol Injection Volume = 10 μL	Kromasil C18, 15 μm, 50 mm × 4.6 mm, T = 30°C	Isocratic elution of 30% Methanol/50% Water/20% 50 mM NH_4Ac-NH_3 solution, pH = 7.0

(*Continued*)

Table 3. Continued

Ref#	Studied compounds	Sample & preparation	Column	Elution conditions
				Flow rate = 1.0 mL/min
56	dimethyl phthalate diethyl phthalate di-*n*-butyl phthalate	Extraction with LPME apparatus. Solvents: *n*-Heptane, Cyclohexane, Toluene, Benzene, *n*-Octanol, Carbon tetrachloride, Chloroform, Dichloromethane, 1,2-Dichloroethane, Chloropropylene oxide	Venusil, XBP C18, 250 mm × 4.6 mm, 5 μm	Isocratic elution of 80% Methanol/20% Water Flow rate = 1.0 mL/min
57	dithiocarbamate thiram	Extraction with Ethylacetate and Cyclohexane by ultrasonication Filtration through sodium sulfate, decantation on Rotavapor, dilution in Ethylacetate and Cyclohexane (1 + 3, v/v) and filtration Injection Volume = 15 μL	Apex III Diol, 3 μm, 250 mm × 4.6 mm	**Normal phase HPLC** Mobile phases A: Cyclohexane Mobile phases B: 80% Cyclohexane/15% 2-Propanol/5% Methanol with 1 drop (10 μL) of 25% Ammonia per 250 mL solution Mobile phases C: 2-Propanol Gradient: from 95% A and 5% B to 54% A, 20% B and 26% C in 18 min Flow rate = 0.65 mL/min

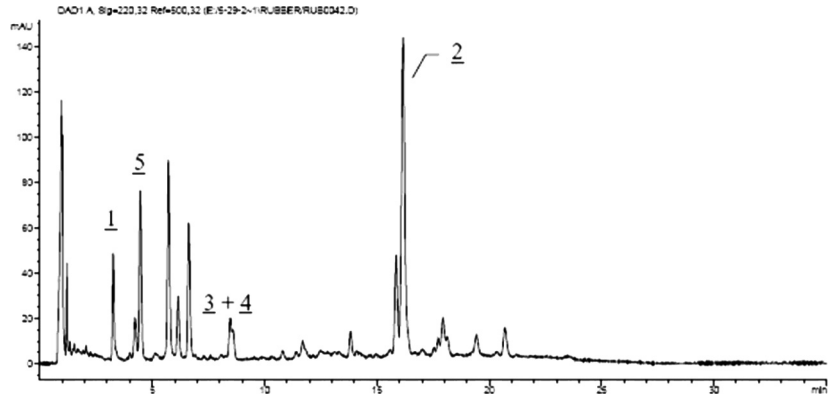


Figure 4. LC/UV chromatogram (220 nm) of a rubber closure extract; 4-(1,1-dimethylpropyl)phenol (**1**), sulfur (**2**), 2,6-di-*tert*-butyl-[1,4] benzoquinone (**3**), furan-2-yl-(5-hydroxymethyl-furan-2-yl)methanol (**4**), and 2-bromo-4-(1,1-dimethyl-propyl)phenol (**5**) (Reprinted from Ref. [26] with permission from Elsevier Limited).

sulfur, 2,6-di-*tert*-butyl-[1,4] benzoquinone, furan-2-yl-(5-hydroxy-methyl-furan-2-yl)-methanol, and 2-bromo-4-(1,1-dimethylpropyl)phenol were identified using high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), organic synthesis, and comparison with authentic compounds. Figure 4 shows the LC/UV separation of the compounds found in a rubber closure extract. This study is noteworthy in its strategy for addressing complicated extractables assessments, which was based on a combination of analytical and semi-preparative HPLC, GC/MS, and organic synthesis. It is in fact the general rule that comprehensive extractables/leachables assessments will use a battery of analytical techniques and methods.

Additional authors have provided perspectives on the strategic application of analytical methodologies to facilitate extractables assessments. DePaolis et al.[27] have documented an approach, based on two orthogonal HPLC separations (using C_8 and phenyl stationary phases) coupled with UV and MS detection, to screen biologic product placebos for rubber-related extractables. Pan and associates[28] have documented an analytical strategy, utilizing LC/UV, LC/MS and GC/MS, for discovering and identifying leachables in liquid ophthalmic dosage forms stored in Low Density Polyethylene (LDPE) bottles (see additional discussion below).

Establishing the identity of an extractable associated with a chromatographic peak is one of the significant challenges of an extractables/leachables investigation. Although information-rich, structure-indicating analytical methods (such as LC/MS and GC/MS) are typically used to

establish the compound's identity, Castner et al.[29,30] offer an alternate approach based on a compound's retention time. These researchers have correlated a compound's partitioning behavior (as reflected by log D) to its retention time in a specified HPLC method using model extractables. Once this correlation is established, it can be used to estimate the log D* value for an unknown compound based on its retention time. While the log D value is not particularly discriminating by itself (and thus is unlikely to be the sole basis for an extractable's identification), it can help establish a compound's probable identity among several candidate identities and may be used to support a particular identification (*qualitative* or *structural analysis*). Similarly, log D can be used to establish the presence/absence of specific leachables in a drug product formulation by establishing where a particular peak should "show up" in a chromatogram. Both situations are illustrated by cases studies that consider extractables from an o-ring (hydrolysis product of Irganox 1076), and a HDPE (high density polyethylene) container (oleamide). Figure 5 shows the chromatogram of the four reference compounds used in the study.

The recent literature contains several accounts of extractables/leachables investigations associated with a particular product application. For example, Xiao et al.[31] described the development and validation of LC/UV methods for the quantitation of several rubber stopper-related leachables (zinc dithiocarbamate, BHT, octylated diphenylamine antioxidant, sulfur, pentylphenol, Irganox 1010) in an experimental, surfactant-containing drug product vehicle containing polyoxyethylated Castor oil and ethanol. The validated method has quantitation limits between 1 and 10 ppm for the individual targeted

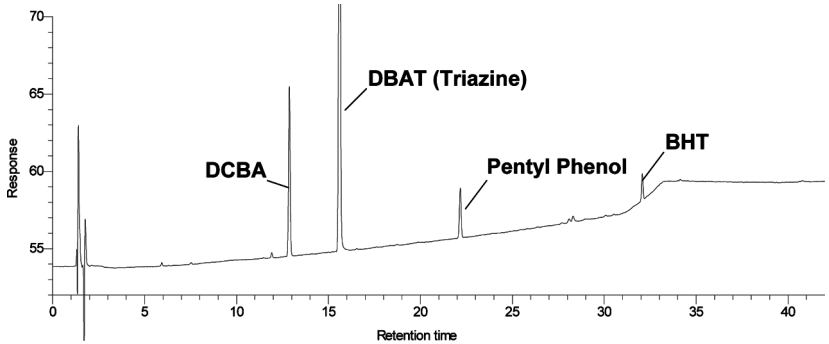


Figure 5. LC/UV chromatogram of reference compounds covering log D values from −0.34 to 5.3; DCBA -2,4-dichlorobenzoic Acid; DBAT – 6-(dibutylamino)-1,3,5-triazine-2,4-dithiol; BHT - butyated hydroxytoluene; pentyl phenol-4-pentyl phenol (Reprinted from Ref. [30] with permission from Russell Publishing – American Pharmaceutical Review).

leachables. Nasser et al.[32] have identified PET (polyethylene terephthalate) oligomers as extractables from PET bottles used for beverages and have validated an LC/UV method for the quantitation of the first series cyclic trimer in the bottles. Sanches-Silva et al.[33,34] have developed an LC/UV method for quantifying a model migrant from LDPE materials (diphenylbutadiene) and have used the method to determine the diffusion coefficients of this compound. These investigators also have reported an LC/UV method capable of measuring printing-related photoinitiators (including Irgacure 184, benzophenone, Irgacure 651, Irgacure 907, Quantacure ITX and Quantacure EHA) in milk with a detection limit of 0.1 mg/L.[35] Fang and associates[36] utilized LC/UV, in conjunction with other chromatographic methods to discover, identify and quantify several photoinitiators (1-benzoylcyclohexanol and 2-hydroxy-2-methylpropiophenone) found in a solid dosage form stored in labeled HDPE bottles. As these leachables were surfaced in the LC/UV method used for profiling the dosage form for impurities, the proper identification of these compounds as leachables (and not impurities) and their proper quantitation in the dosage form was necessary to establish their product impact. Jenke et al.[37] utilized several techniques, including LC/UV, to establish the impact of a change in a plastic material's additive package on the material's extractables profile. In this case, the addition of Irgafos 168 to the material's antioxidant package resulted in the identification of three new extractables: 1,3-bis(1,1-dimethylethyl) benzene, 2,4-di-*tert*-butyl phenol and 2,4-bis(1,1-dimethylethyl)-phenol phosphate. Mudumba et al.[38] presented an LC/UV method for the determination of methylene dianiline in aqueous extracts of polyurethane materials used in implantable medical devices. Dopico-Garcia et al.[39] described an LC/UV method for quantitating antioxidants, including BHA, BHT, AO 2246, Ethanox 330, Irganox 1010, Irganox 1076, Irgafos 168 (and its two degradation products), in food simulating extracts of polyethylene, polypropylene, polyvinyl chloride and polyethylene terephthalate. Sanches-Silva et al.[40] developed an LC/UV method for quantitating BHT in food samples. Burman et al.[41] coupled sample preparation by solid-phase extraction with LC/UV to measure antioxidant degradation products in 10% ethanol extracts of a polypropylene resin.

Additional discussions of the application of LC/UV to extractables/leachables investigations are included in several other manuscripts;[6,42–44] however, these manuscripts are general in nature and do not include details such as the specific compounds of interest and/or operating conditions.

In order to meet the analytical challenges posed by extractables/leachables investigations, analytical scientists move beyond the current state-of-the-art and develop and implement innovative techniques and methodologies. One particularly innovative technique, high-temperature

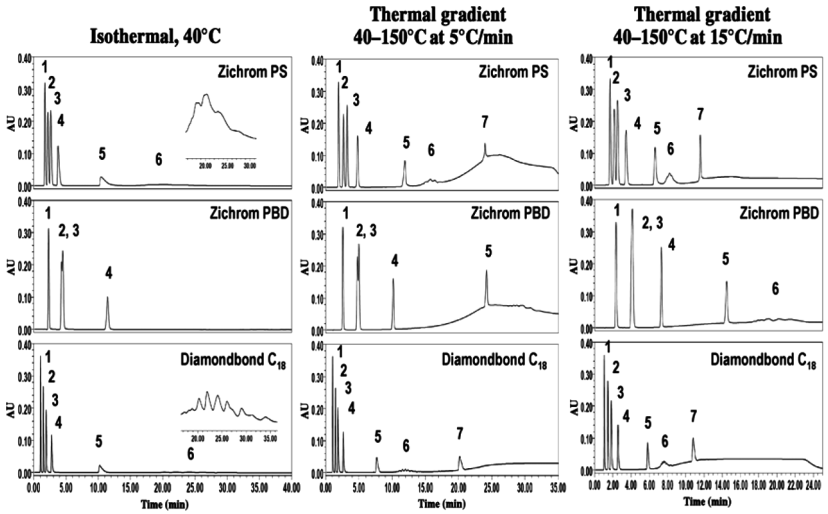


Figure 6. The effect of thermal gradients on the chromatographic profiles of a series of phthalate plasticizers. (Reprinted from Ref. [45] with permission from Preston Publications – Journal of Chromatographic Science).

liquid chromatography, was used by Kim et al.[45] for the separation of commercially available polymer additives. Separation efficiencies and elution behaviors for seven phthalate ester plasticizers (dipropyl phthalate, dibutyl phthalate, butyl benzyl phthalate, dicyclohexyl phthalate, dioctyl phthalate, diisoamyl phthalate, trioctyl trimelitate) and five antioxidants (Irganox 245, Irganox 1098, Naugard XL-1, Irganox 1081, Irganox 1035) were evaluated at elevated column temperatures and with a thermal gradient (see Figure 6). The use of high temperature liquid chromatography and zirconia based stationary phases was shown to enhance analyte resolution and overall analysis speed. The work demonstrated that at elevated column temperature and with a thermal gradient, the separation efficiency and the elution behaviors for these two analyte classes were enhanced.

A case study for analyzing contamination of endocrine disruptors in liquid medicines and intravenous injection solutions is described by Mitani et al.[46] Compounds such as bisphenol A, alkylphenols and phthalates were quantitated with a limit of quantification between 1 ng/mL and 10 ng/mL by on-line in-tube solid-phase microextraction coupled with high performance liquid chromatography (in-tube SPME/HPLC) with UV detection. The analytes in this study were: di-2-ethylhexyl phthalate, di-*n*-butyl phthalate, benzyl butyl phthalate, bisphenol A, nonylphenol, octylphenol, diethyl phthalate, di-*n*-propyl phthalate, di-*n*-amyl phthalate, di-*n*-hexyl phthalate, di-*n*-octyl phthalate

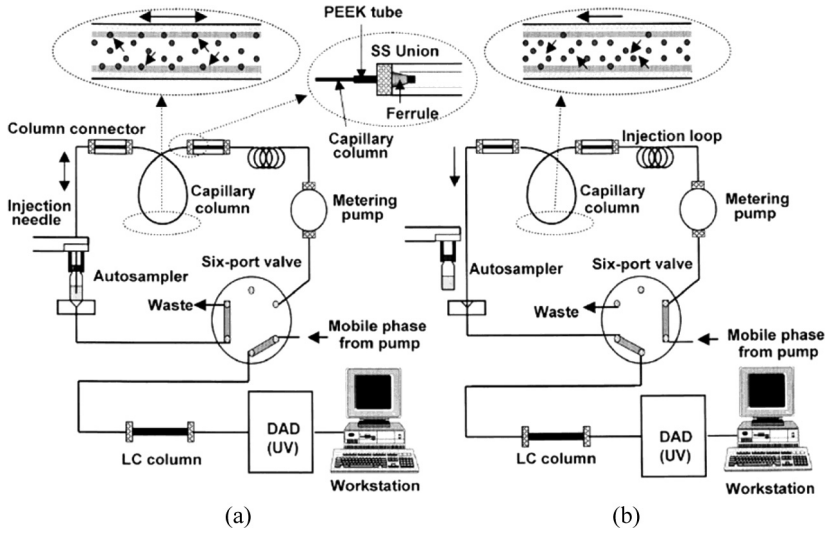


Figure 7. Schematic diagram of an automated in-tube SPME/HPLC-DAD system. (a) Extraction step, (b) desorption step (Reprinted from Ref. [46] with permission of Elsevier Limited).

and dicyclohexyl phthalate. The described method is simple, rapid, selective and sensitive. Figure 7 presents the diagram of the instrumentation used for the study. In Figure 8, the chromatograms from a standard solution and samples obtained by in-tube SPME/HPLC are shown. The described method was chosen because it is simple, rapid, selective and sensitive and can be applied for the screening and determination of a wide range of impurities in liquid medicines and intravenous injection solutions. The technique used for sample extraction, in-tube SPME, microextraction and preconcentration using an open tubular fused silica capillary with an inner surface coating, can be coupled on-line with HPLC and LC/MS and allows for the automation of the extraction process. This shortens the analysis time and provides better accuracy, precision, and sensitivity compared with off-line manual techniques. In a similar manner, Jen et al.[47] coupled on-line microdialysis with LC/UV to measure the levels of phthalate esters extracted from food-contacted plastics. These authors achieved detection limits of 4 μg/L or lower with this approach.

Considering chromatography in general, innovations on the separation side have been dominated by the development of small particle size, high efficiency packing materials and chromatographic hardware that can handle the high pressures that arise when columns packed with such materials are used. Given the chemical complexity of samples containing

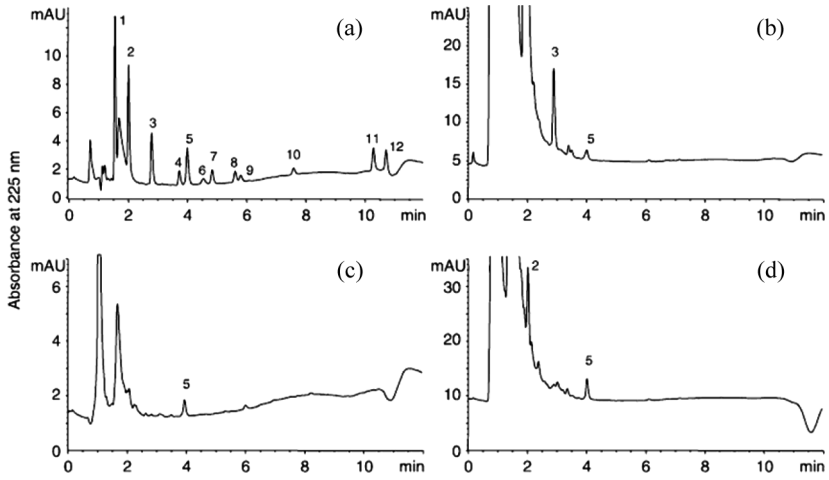


Figure 8. Chromatograms from a standard solution and samples by in-tube SPME/HPLC. (**a**) Standard solution (50–500 ng/mL), (**b**) intravenous injection solution, (**c**) syrup, (**d**) eye drops. Peaks: bisphenol A (**1**), diethyl phthalate (**2**), di-n-propyl phthalate (**3**), benzyl butyl phthalate (**4**), di-n-butyl phthalate (**5**), octylphenol (**6**), nonylphenol (**7**), di-n-amyl phthalate (**8**), dicyclohexyl phthalate (**9**), di-n-hexyl phthalate (**10**), di-2-ethylhexyl phthalate (**11**), di-n-octyl phthalate (**12**) (Reprinted from Ref. [46] with permission from Elsevier Limited).

extractables and leachables, it is reasonable to expect that Ultra Performance Liquid Chromatography (UPLC®) has the potential to facilitate extractables/leachables assessments. In fact, Noguerol-Cal and associates[48] have documented a UPLC® method for the determination of ten colorants and six antioxidants typically associated with polyolefin materials. Separation of the sixteen analytes is completed within 5 minutes and the detection limits for the individual compounds vary from approximately 0.1 to 0.6 mg/L.

As is the case with all fields of scientific endeavor, information relevant to one topic can often be found in the literature associated with a different, albeit related, topic. Thus, information on chromatographic separations of additives in, and extractables from, plastics can be found in papers that are unrelated to pharmaceutical products and to extractables and leachables. For example, Bergendorff and associates[49–51] have published HPLC methods for quantifying rubber allergens, including compounds with dithiocarbamate and benzothiazole structures, in various rubber containing entities (e.g., protective gloves used in healthcare, diving masks, etc.). In one study, these investigators studied the changes in composition of rubber materials that occur during vulcanization.[52] They observed, among other things, that thiuram disulfides, common rubber

additives, are converted to dithiocarbamates, or mercaptobenzothiazole addition products, during vulcanization.

Other studies that are related to extractables and leachables from pharmaceutical systems only indirectly (either due to the nature of the materials studied or the identity of the compounds of interest) are noted.[53–57] The operating details of these indirectly related methods are also summarized in Table 3.

LC/MS AND ITS APPLICATION TO EXTRACTABLES AND LEACHABLES ASSESSMENT

LC/MS – History and Background

The combination of HPLC with mass spectrometry in the so-called "hyphenated" technique of LC/MS allows the HPLC system to accomplish *structural analysis* (i.e., elucidation of the molecular structure of an analyte peak), as well as *qualitative analysis* (i.e., high sensitivity confirmation of the presence or absence of a target analyte), and high sensitivity *quantitative analysis*.[15] In this respect the mass spectrometer is a compound specific detector,[6,58] and comes very close to being a "universal" detector for HPLC. As stated by Norwood et al.[59] the fundamental problems confronting the interface of HPLC and MS are that the mass spectrometer operates at high vacuum in its mass analyzer and detector regions and the HPLC includes a liquid mobile phase, usually with relatively high concentrations of water (0.1 mL/min of liquid water equals 135 cm^3/min of gas at atmospheric pressure.[60]) Further, gas-phase ions must be produced for mass spectrometry to function.[60] The choice, therefore, is whether to attempt to separate the liquid mobile phase from analyte peaks prior to introduction of these analyte peaks into the mass spectrometer's high vacuum, or to affect ionization of analyte molecules in some other way without separating the liquid mobile phase. LC/MS interface systems of the first type, referred to as "transport devices,"[59] include the "moving belt" and "particle-beam" which have seen only limited application and are no longer in general use. LC/MS interface systems of the second type include "Thermospray" (TSP),[61] "Continuous-Flow Fast Atom Bombardment" (CF-FAB),[62] "Electrospray" (ESI),[63] and "Atmospheric Pressure Chemical Ionization" (APCI).[64] It was TSP and CF-FAB that allowed the general application of LC/MS to analytical and bioanalytical problems in the late 1980s and early 1990s. However, it is fair to state that it was ESI and APCI that have stimulated the explosive expansion and routine application of LC/MS in analytical and other laboratories and to a wide variety of applications, including rubber and plastic additives, extractables and leachables.

Those readers interested in greater detail about LC/MS, including its history and various applications, are referred to the treatises by Willoughby et al.,[58] de Hoffmann and Stroobant,[60] Boyd et al.,[65] and Gross.[66]

TSP, CF-FAB, ESI and APCI all incorporate unique ionization processes into LC/MS, and utilize the mobile phase as an integral part of the process. However, the ionization processes for both TSP and CF-FAB occur in or near the higher vacuum regions of the mass spectrometer, while both APCI and ESI ionization processes occur at atmospheric pressure with analyte ions "sampled" into the high vacuum mass analyzer region of the instrument via a differential pumping system. For APCI, the ionization process is relatively straightforward to understand since, as the name implies, it involves Chemical Ionization,[67] which relies on ion-molecule reactions between analyte molecules and a steady-state reactant ion plasma inside the ion source. In APCI LC/MS, eluent from the HPLC column passes through a heated probe (e.g., 500°C) and is volatilized with the assistance of inert gas flow (nitrogen) through the probe. The CI reactant gas is thus derived from volatilized HPLC mobile phase molecules, such as water, acetonitrile and methanol. Reactant gas ions are formed in the APCI source at atmospheric pressure via a corona discharge which results in a steady-state reactant ion population available for ion-molecule interactions with analytes eluting from the HPLC column. Positive molecular ions are formed via proton transfer and other reaction types:[66]

1. $M + H_3O^+ \rightarrow [M+H]^+ + H_2O$ (*proton transfer*)
2. $M + NH_4^+ \rightarrow [M+NH_4]^+$ (*adduct formation from ammonium acetate buffer*)
3. $M + Na^+ \rightarrow [M+Na]^+$ (*adduct formation from trace sodium*)
4. $M + X^+ \rightarrow [M - H]^+ + HX$ (*hydride ion abstraction*)
5. $M + X^+ \rightarrow M^+ + X$ (*charge exchange*)
6. $2M + H_3O^+ \rightarrow [2M+H]^+ + H_2O$ (*protonated dimer formation*)

Negative molecular ion formation typically occurs via proton abstraction or adduct ion formation:

1. $M + OH^- \rightarrow [M\text{-}H]^- + H_2O$ (*proton abstraction*)
2. $M + Cl^- \rightarrow [M+Cl]^-$ (*chloride ion attachment*)

The formation of both positive and negative molecular ions is governed by thermodynamic properties such as proton affinity and positive/negative ion stability. Clearly, the composition of the HPLC mobile phase has a strong influence on the APCI process.

ESI is less straightforward to understand since there is no heated probe or input of energy, as with the APCI probe and corona discharge. According to the discussion by de Hoffmann and Stroobant,[60] the ESI process results from the application of a strong electric field to a liquid (the HPLC column eluent) passing through a capillary tube at relatively low flow rates (1–10 μL/min). The electric field is formed by application of a voltage (~6 kV) between the capillary tip and a counter electrode. When the appropriate "onset voltage" is reached (~4 kV for water) charged droplets are emitted from the apex of a "Taylor Cone" of liquid formed at the capillary tip under the influence of the electric field. As the charged droplets move away from the capillary tip they break up and desolvate (assisted by inert gas flows) and their surface electric fields increase until desorption of ions from their surfaces occur (both analyte and mobile phase ions; positive and negative). Schematic representations of both the ESI and APCI processes, ion source designs and mass spectrometer differential pumping systems can be found in the previously mentioned mass spectrometry treatises.

Thus, unlike APCI in which analyte ionization occurs in the gas phase, ESI desorbs analyte ions from the liquid phase (i.e., the HPLC mobile phase) into the gas phase. As with APCI the analyte ions are "sampled" into the mass analyzer region of the mass spectrometer via a differential vacuum pumping system. In fact, ESI and APCI can be performed on the same basic atmospheric pressure ion source (with some differences between the two in probe, probe/source voltages, and inert gas flows) and most LC/MS systems come with combination ESI/APCI sources. Also like APCI, the HPLC mobile phase composition has an effect on the ESI process, in that ions in solution translate to ions in the gas phase.

The reader is advised that the explanations of APCI and ESI in this review are somewhat simplified, and the reader interested in additional details and theory is referred to the aforementioned comprehensive treatises. The reader is further advised that different LC/MS instruments and ion sources have different features and capabilities (e.g., ESI flow-rate capabilities, etc.) and that LC/MS instrumentation technology continues to advance.

Chromatography and LC/MS

Norwood et al.[59] have discussed in some detail the implications for HPLC methods and method development of using the mass spectrometer as a detector, as well as the general features of APCI and ESI that should be of concern to chromatographers. Willoughby et al.,[58] de Hoffmann

and Stroobant,[60] and Boyd et al.[65] also discuss features of LC/MS that impact chromatography, with Boyd et al. focusing in particular on trace quantitative analysis. Based on these discussions, the most significant features of LC/MS that impact HPLC and HPLC method development can be summarized as follows:

1. *ESI is fundamentally a solution phase process while APCI is a gas phase process*. Both ESI and APCI are affected by the composition of the HPLC mobile phase, but in different ways. In ESI the mobile phase conditions can either enhance analyte ion formation or suppress it. For example, lowering the pH of the mobile phase can enhance positive ion formation from certain analytes, and analytes with multiple ionizable sites can produce multiply charged ions.[60] However, highly ionizable buffers in the mobile phase (such as trifluoroacetic acid) can suppress desorption of analyte negative ions. Obviously, ESI is most effective for analytes which can be easily charged in solution, such as polar molecules. In APCI, the gas phase proton affinity of the mobile phase ions can affect sensitivity for certain analytes. Also, mobile phase ions can attach to analyte molecules to form adduct ions, either positive or negative.
2. *ESI and APCI are both "soft" ionization processes*. Analyte molecular ions from both ESI and APCI are formed under thermodynamically controlled processes and undergo numerous stabilizing collisions in the ion source prior to mass analysis and detection. This results in mass spectra which are dominated by molecular ions, although most ESI/APCI ion sources have features designed to enhance molecular ion fragmentation by collision induced dissociation processes should this be desired.
3. *ESI and APCI both prefer LC/MS "compatible" mobile phases*. Since both ESI and APCI require volatilization of the HPLC eluent in some fashion, involatile mobile phase constituents (e.g., phosphate, borate, sodium dodecylsulfate, etc.) are not desirable. As stated by Norwood et al.,[59] there are many volatile mobile phase additives, buffers, and ion-pairing reagents, including ammonium acetate, ammonium formate, acetic acid, formic acid, trifluoroacetic acid, heptafluorobutyric acid, etc. These authors also reference various reports and reviews regarding LC/MS compatible mobile phases.[58,68–74] There are, however, reports of the successful use of involatile mobile phase additives in LC/MS,[75] including the use of an innovative device for removing involatile buffers before the ion source,[76] however the practice is not generally employed for LC/MS.
4. *ESI and APCI both prefer reverse-phase HPLC*. Since ESI and APCI employ high temperatures, high voltages, strong electric

fields, etc. normal-phase HPLC systems are for the most part not desirable, and reverse-phase systems are highly compatible. This is fortuitous for pharmaceutical scientists, including those interested in extractables and leachables, since the vast majority of analytes of interest are most compatible with reverse-phase HPLC platforms.

5. *ESI tends to prefer relatively low HPLC flow-rates while APCI tends to prefer relatively high HPLC flow-rates*. Because of the fundamental nature of the ionization processes and the design and construction of typical ion sources, ESI tends to prefer lower HPLC flow-rates ($<0.5\,mL/min$;[59] $1–10\,\mu L/min$[60]) and APCI tends to prefer higher flow-rates ($0.5–1.5\,mL/min$[59]). This would seem to make ESI a good match for nano- to micro-scale HPLC, and APCI a good match for "analytical" HPLC.[59] The reader should again note that these are general statements and do not necessarily apply to all LC/MS instruments and source designs.

Capabilities of LC/MS

The capabilities of LC/MS for the analysis of extractables and leachables are the sum of the capabilities of HPLC (i.e., with regard to the analysis of relatively polar, involatile, and higher molecular weight analytes) and the compound specific detection capabilities of the mass spectrometer. These capabilities are useful for all three types of analysis,[15] but are most significant for *structural analysis* and high sensitivity *quantitative analysis*.

1. *Molecular weight determination*. Consider the positive ion APCI spectrum of diphenylamine (a commonly used antioxidant for rubber; MW 169) shown in Figure 9. Note the $[M+H]^+$ at m/z 170 confirmed by the $[M+H+acetonitrile]^+$ at m/z 211 (acetonitrile was in the mobile phase). The proton affinity of diphenylamine makes this analyte a good positive ion former, and its affinity for adduct ion formation with protonated acetonitrile allows for confirmation of molecular weight (if this were an unknown extractable/leachable). The positive ion ESI spectrum of tetramethylthiuram disulfide (a rubber vulcanization reagent; MW 240) is shown in Figure 10. Note the $[M+H]^+$ at m/z 241 with a clearly apparent sulfur isotope peak (m/z 243). Note also that some of the other significant ions in this spectrum appear not to come from the target analyte (e.g., m/z 201 clearly has no sulfur, based on its isotope pattern). The negative ion APCI spectrum of 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol) (a commonly used

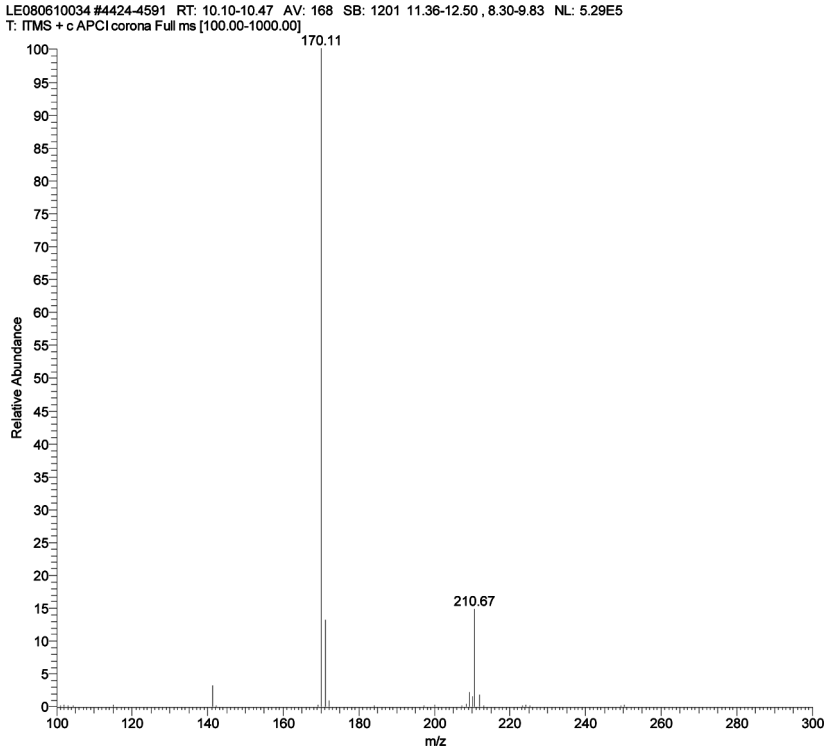


Figure 9. Positive ion Atmospheric Pressure Chemical Ionization (APCI) mass spectrum of diphenylamine. Note the $[M+H]^+$ at m/z 170 and the $[M+H+acetonitrile]^+$ at m/z 211.

hindered phenolic antioxidant; MW 340) presented in Figure 11 shows an $[M\text{-}H]^-$ at m/z 339. Note that the addition of a small amount of chloroacetonitrile to the HPLC mobile phase results in the chloride attachment ion $[M+Cl]^-$ at m/z 375 (Figure 12). The Cl^- for attachment is formed by corona induced fragmentation of the chloroacetonitrile. Note also the deuterium exchanged positive ion APCI spectrum of diphenylamine (Figure 13) which confirms the presence of one exchangeable (or active) hydrogen in this molecule ($[M+D+H]^+$ at m/z 172 and $[M+D+H+acetonitrile]^+$ at m/z 213). Clearly, HPLC mobile phase conditions and gas phase ion chemistry can be manipulated to great analytical advantage with LC/MS.

2. *Molecular formula (i.e., elemental composition)*. Several types of mass analyzers (magnetic sector, time-of-flight, ion cyclotron resonance) are capable of measuring the masses of ions with sufficient accuracy to

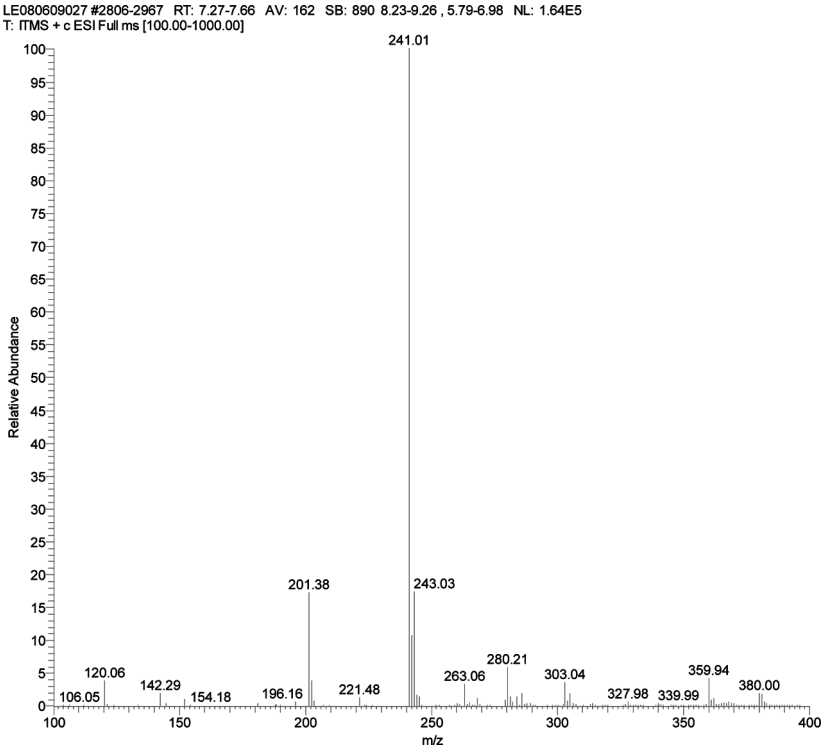


Figure 10. Positive ion Electrospray (ESI) mass spectrum of tetramethylthiuram disulfide. Note the $[M+H]^+$ at m/z 241 with a clearly apparent sulfur isotope peak (m/z 243). Note also that some of the other significant ions in this spectrum appear not to come from the target analyte (e.g., m/z 201 clearly has no sulfur, based on its isotope pattern).

allow molecular formulae to be determined. Consider the positive ion ESI spectrum of Irganox 1010 (MW 1176) shown in Figure 14 (molecular ion region only shown) acquired on a time-of-flight (TOF) LC/MS system. Note the $[M+NH_4]^+$ ion at m/z 1194 (ammonium acetate in the mobile phase) and the $[M+Na]^+$ ion (residual sodium in the HPLC system) at m/z 1199. The accurate mass of the $[M+Na]^+$ was determined to be 1199.7727 which indicated a molecular formula of $C_{73}H_{108}O_{12}Na$ (the correct answer) with an error of 1.0 ppm of mass. The utility of accurate mass measurements of molecular and fragment ions for structural analysis should be apparent.

3. *Fragmentation behavior according to defined chemical rules*. Fragment ions can be produced from molecular ions in ESI and APCI either in

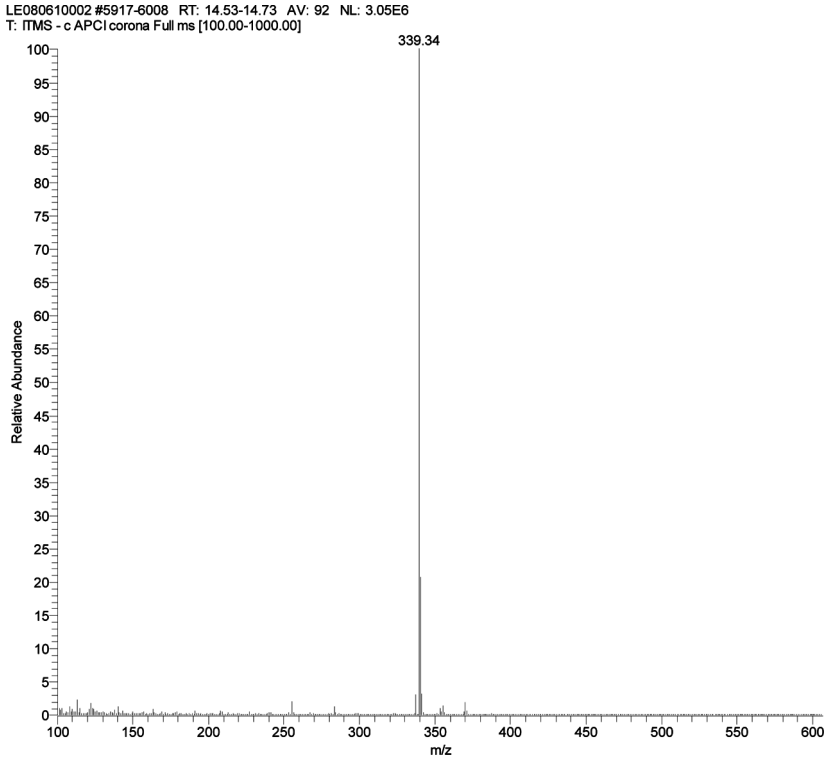
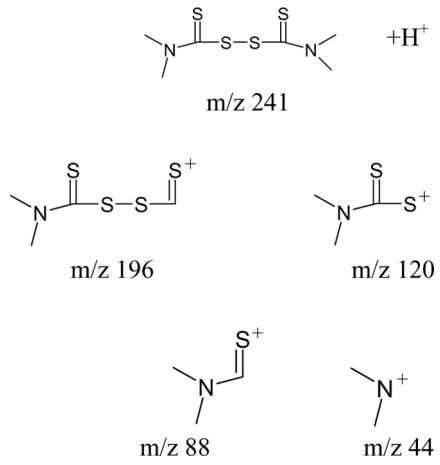


Figure 11. Negative ion Atmospheric Pressure Chemical Ionization (APCI) mass spectrum of 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol). Note the $[M\text{-}H]^-$ at m/z 339.

the ion source through unimolecular or collision induced processes or inside the mass spectrometer itself through collision induced dissociation. A triple quadrupole mass spectrometer has the ability to pass a molecular ion through its first quadrupole mass analyzer, fragment this ion via collision with inert gas molecules (e.g., Ar) in the second, and separate the resulting fragment ions into a mass spectrum in the third. Such a spectrum is referred to as a "product ion" spectrum, in that all of the fragments arise directly from the mass selected precursor ion, and the process is usually termed MS/MS or tandem mass spectrometry. Consider the product ion spectrum from the $[M+H]^+$ of tetramethylthiuram disulfide in Figure 15, noting the product ions. Based on decades of knowledge building regarding fragmentation mechanisms and processes,[77,78] the structures of these product ions can be assigned:



If this molecule were an unknown extractable/leachable, its structure could be hypothesized from the structures of these product ions.

MS/MS is also the basis for high sensitivity quantitative analysis by LC/MS. In the case of tetramethylthiuram disulfide above, the triple quadrupole LC/MS system can be configured to only monitor the

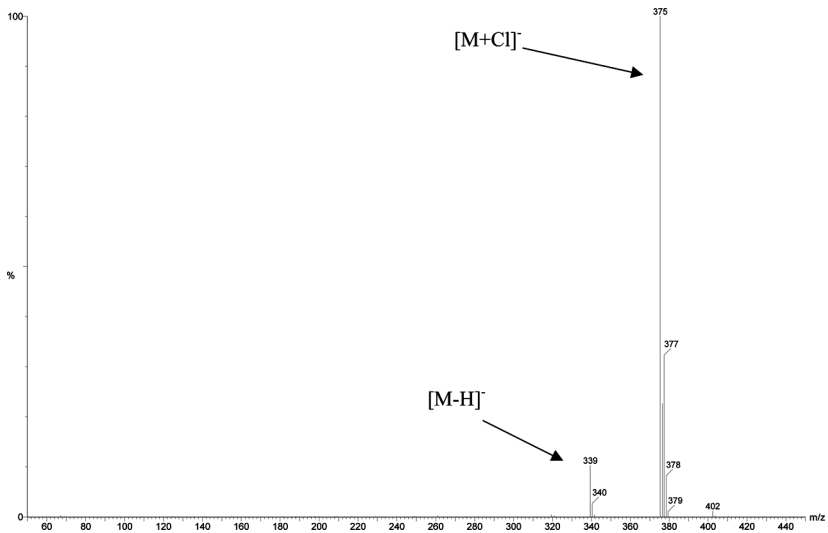


Figure 12. Negative ion chloride ion attachment Atmospheric Pressure Chemical Ionization (APCI) mass spectrum of 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol). Note the $[M+Cl]^-$ at m/z 375.

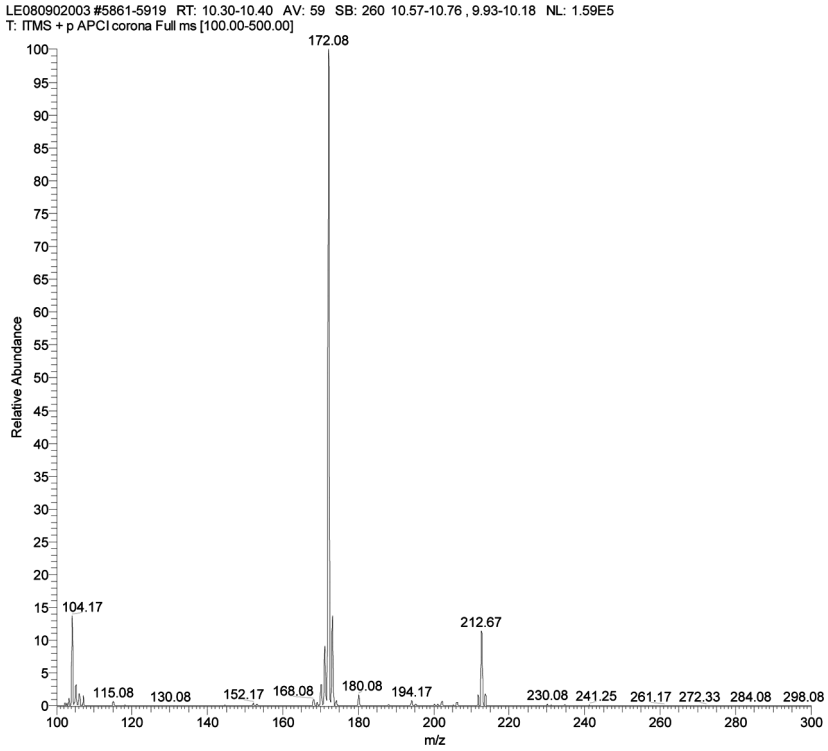


Figure 13. Deuterium exchanged positive ion Atmospheric Pressure Chemical Ionization (APCI) spectrum of diphenylamine, which confirms the presence of one exchangeable (or active) hydrogen in this molecule ($[M+D+H]^+$ at m/z 172 and $[M+D+H+acetonitrile]^+$ at m/z 213).

transition m/z 241 → m/z 88, referred to as "Selected Reaction Monitoring" or SRM. This selectivity advantage with LC/MS (based on SRM as well as "Selected Ion Monitoring" or SIM, in which only the target molecular ion(s) is monitored) is the basis for most high sensitivity quantitative LC/MS assays. For additional detail see Boyd et al.[65]

The capabilities of ESI and APCI LC/MS for profiling of extractables and leachables are clear from an examination of Figures 16–19.

LC/MS Applications

A search of the available scientific literature dating back to the advent of modern LC/MS failed to identify any comprehensive review articles on the application of LC/MS to the analysis of extractables and leachables from pharmaceutical container closure systems. In fact, few articles or

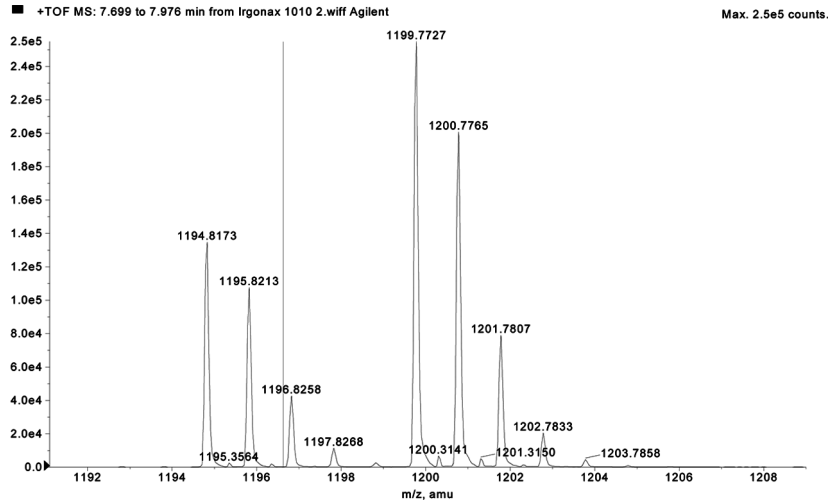


Figure 14. Positive ion Electrospray (ESI) mass spectrum of Irganox 1010 (MW 1176; molecular ion region only shown) acquired on a time-of-flight (TOF) LC/MS system. Note the $[M+NH_4]^+$ ion at m/z 1194 (ammonium acetate in the mobile phase) and the $[M+Na]^+$ ion (residual sodium in the HPLC system) at m/z 1199.

works of any kind have been published on this topic. It is no surprise, based on an examination of Figure 2, that the majority of applications were based on GC/MS rather than LC/MS. There are, however, numerous reported applications of LC/MS for structural and quantitative

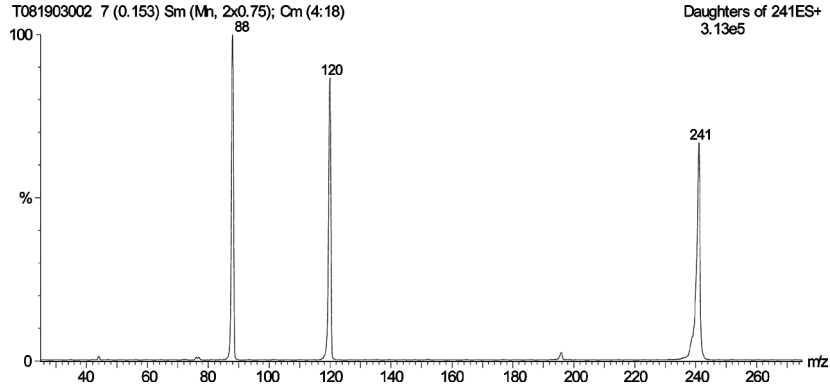


Figure 15. Electrospray (ESI) product ion spectrum from the $[M+H]^+$ of tetramethylthiuram disulfide (m/z 241). Note that the term "daughters" is an archaic expression found in older versions of software, and is no longer in general use. The term is not meant to imply that ions have gender.

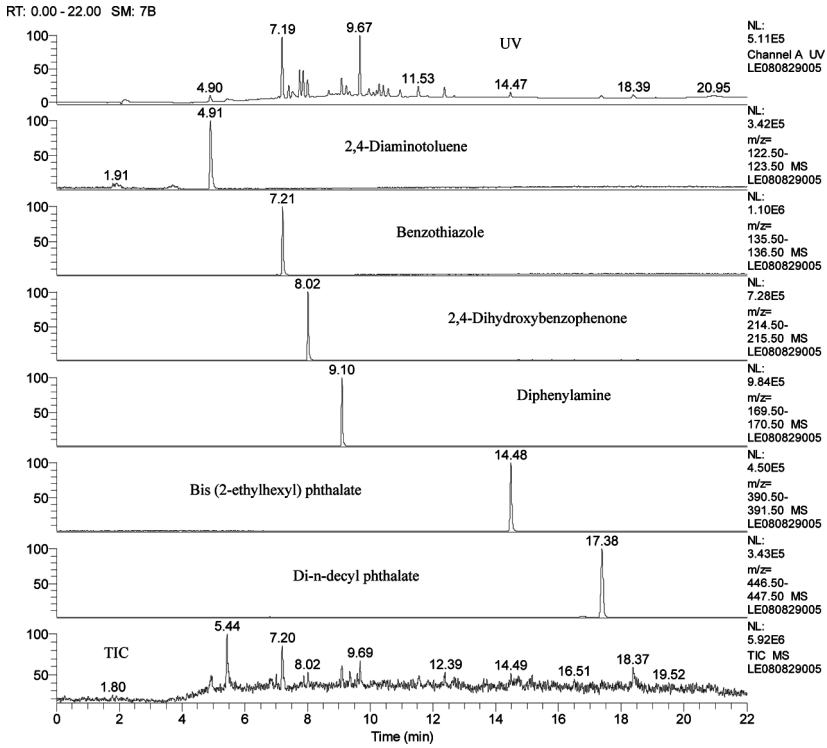


Figure 16. Positive ion Atmospheric Pressure Chemical Ionization (APCI) LC/MS analysis of a test mixture of extractables/leachables (see Figure 3 for the HPLC platform and analytes). Note that certain analytes are highlighted with mass chromatograms of their molecular ions. The on-line UV chromatogram is on the top and the Total Ion Chromatogram (TIC) is on the bottom.

analysis of plastic and rubber additives in environmental and other matrices, but this topic will be reserved for future consideration.

Of particular interest, however, are Marand et al.[79] who studied the extraction of aromatic amines (and their associated derivatives) from polyurethane foam with water, 0.1% acetic acids and simulated sweat. Extractables that were identified by LC/ESI-MS and GC/MS included the 2,4- and 2,6- isomers of toluenediamine (TDA), several isomers of methylene dianiline and dimers of TDA and toluene diisocyanate (TDI). These authors reported that the levels of extractable 2,4-TDA and 2,6-TDA depended on the specific polyurethane foam tested and the extraction conditions (temperature, duration, extraction medium). Ito et al.[80] measured the release of di-(2-ethylhexyl)phthalate (DEHP) and mono-(2-ethylhexyl)phthalate (MEHP) from PVC tubing using an

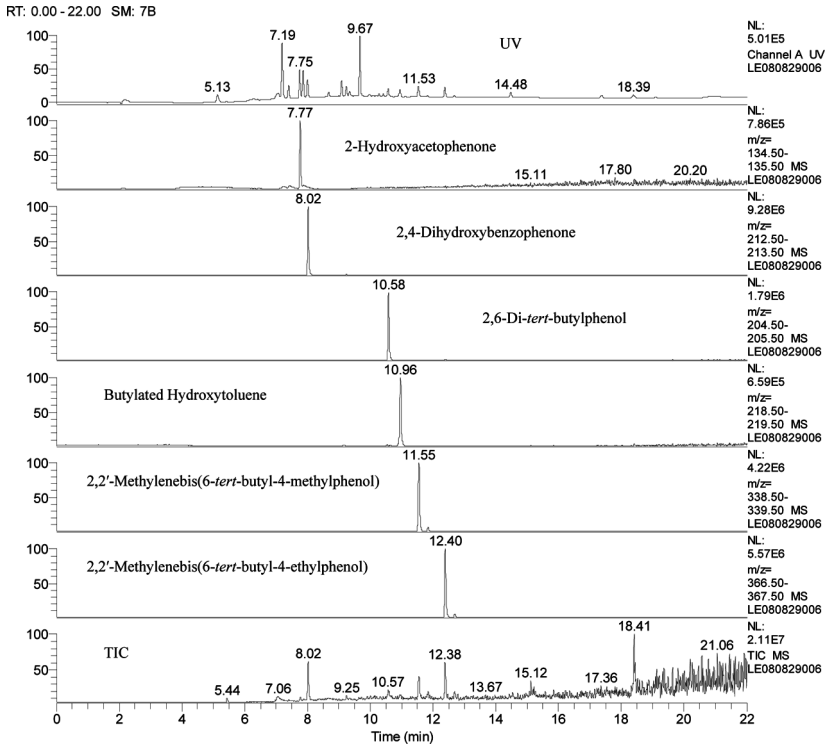


Figure 17. Negative ion Atmospheric Pressure Chemical Ionization (APCI) LC/MS analysis of a test mixture of extractables/leachables (see Figure 3 for the HPLC platform and analytes). Note that certain analytes are highlighted with mass chromatograms of their molecular ions. The on-line UV chromatogram is on the top and the Total Ion Chromatogram (TIC) is on the bottom.

on-line sample concentration process (column switching) coupled with LC-MS/MS. These analytes were quantitated in various drug solutions at low concentrations (LOQ = 2.5 and 0.8 ng/mL for DEHP and MEHP respectively) with no specificity issues. Fichtner et al.[81] have reported the development of an LC/MS methodology for the purpose of identifying and quantifying extractables from sterile-grade filtration cartridges. These authors document the optimization of separation and detection parameters, specifically for six ''well known'' extractables associated with polypropylene and polyethersulfones, including 4-hydroxybenzoic acid ethyl ester, diethylphthalate, bis-(4-chlorophenyl)sulfone, benzoic acid-*p*-tolylester, butylated hydroxytoluene and 1,3-di-*tert*-butylbenzene. Scott[82] has developed LC/MS methods to characterize dichloromethane extracts of acetal and polybutylene terephthalate (PBT) materials, and

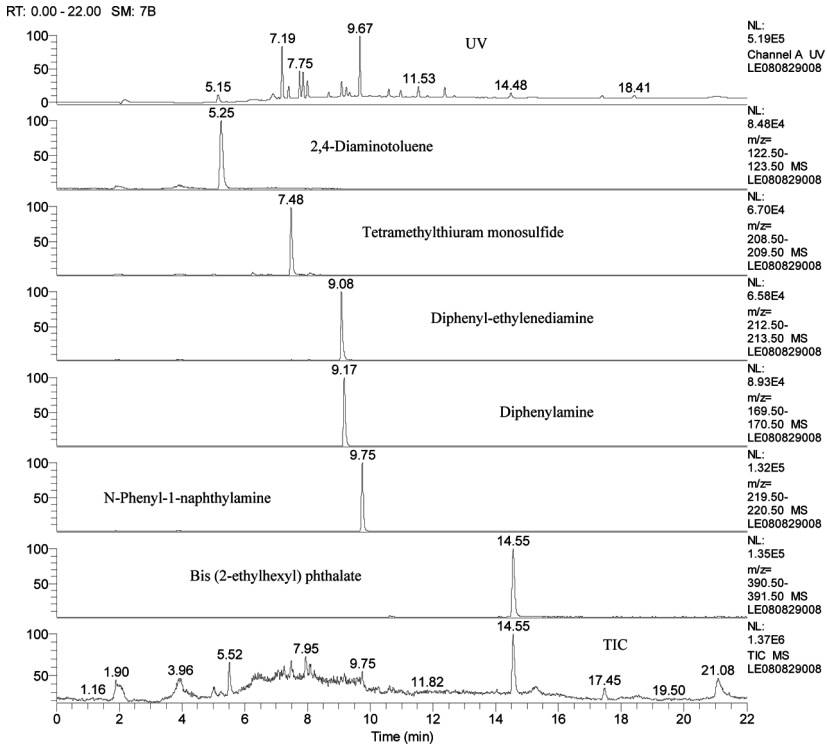


Figure 18. Positive ion Electrospray (ESI) LC/MS analysis of a test mixture of extractables/leachables (see Figure 3 for the HPLC platform and analytes). Note that certain analytes are highlighted with mass chromatograms of their molecular ions. The on-line UV chromatogram is on the top and the Total Ion Chromatogram (TIC) is on the bottom.

document the presence of PBT oligomers (dimer through tetramer) in the PBT extract and note that 2,2′-methylenebis(6-*tert*-butyl-4-methyl phenol), a commonly employed antioxidant (see above), was the major extractable in the acetal extract. Other extractables from the acetal included ricinoleic acid, from calcium ricinoleic acid, a formaldehyde scavenger, and N,N′-ethylene-bis-stearamide, a process lubricant. Once the identification of the extractables was completed (via both LC/MS and GC/MS), LC/MS methods were developed for the quantitation of specific extractables, including the PBT oligomers and the bisstearamide.

Mortensen et al.[83] reported the development and validation of an LC/ESI-MS/MS method for the direct analysis of water and 3% acetic acid extracts of polyurethane products for twenty primary aromatic amines. The detection limits of the method ranged from 0.3 to 3 μg/L and the within laboratory reproducibility of the method was from 4%

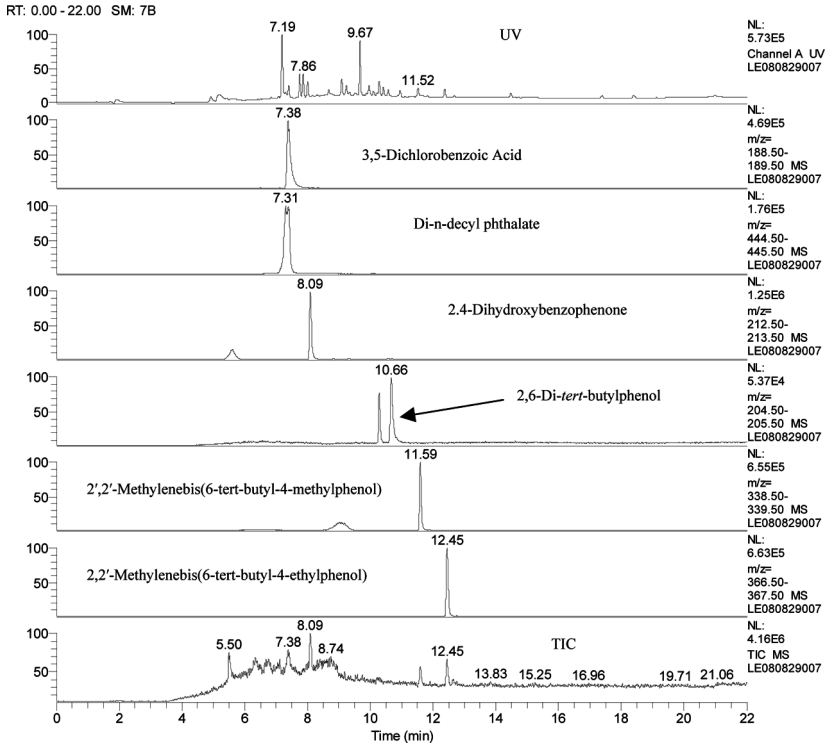


Figure 19. Negative ion Electrospray (ESI) LC/MS analysis of a test mixture of extractables/leachables (see Figure 3 for the HPLC platform and analytes). Note that certain analytes are highlighted with mass chromatograms of their molecular ions. The on-line UV chromatogram is on the top and the Total Ion Chromatogram (TIC) is on the bottom.

to 19% at the 2 μg/L level. The authors report that "high" levels of 4,4′-methylenedianiline and aniline were extracted from about half of the cooking utensils that were tested. Rogalewicz et al.[84] utilized LC/MS to identify the organic extractables associated with resin-modified glass-ionomer cements used as dental fillings. These authors found almost thirty compounds in the extracts of the tested materials, with the main identified extractables including monomers such as bisphenol A glycidyl dimethacrylate, ethoxylated bisphenol A dimethacrylate, urethane dimethacrylate, triethylene glycol dimethacrylate, 2-hydroxyethyl methacrylate, and additives such as diphenyliodonium chloride, camphorquinone, BHA and 4-(dimethylamino) ethyl benzoate.

Jenke et al. have used LC/MS methodologies to identify extractables or leachables from several pharmaceutically relevant systems. In one such application, extractables from a polyolefin plastic container were

identified and quantified in eight solvent systems representing the types of buffers and media used in biopharmaceutical manufacturing.[85] Extractables that were examined in this study included various cyclic esters, decomposition products of hindered phenol antioxidants, organic acids (e.g., C_6, C_8), caprolactam, erucamide and toluenesulfonamide (associated with the container's printing). In a similar manner, these researchers used LC/MS methods (among others) to identify extractables from four different bags used in pharmaceutical processing, two of which were polyolefins and two of which were EVA-based.[86] Several of the identified extractables, including myristic acid, palmitic acid, stearic acid, hexadecanamide, octadecanamide and erucamide, were chosen as targets whose levels were measured in a number of extracting media by LC/MS. Lastly, these investigators identified and quantified the extractables from silicone- and Santoprene-based plastic tubing that might be utilized in a production suite using headspace GC/MS, GC/MS and LC/MS.[87] Static extracts of the tubing materials, using water and ethanol as the extracting solvents, were characterized to ascertain the extractables profiles of the materials of interest. The major extractables from the silicone tubing were a homologous series of silicone oligomers (linear and cyclic polydimethylsilicones), dioctyl phthalate, dioctyl adipate, and a homologous series of propylene glycol oligomers. The extractables profiles of different silicon tubing from different vendors were quite variable. An LC/MS method was developed and qualified for the purpose of measuring the concentration of target extractables in dynamic (flow) extractions of the tubing materials. These authors noted that while the extractables profiles obtained under ''aggressive'' static extraction conditions (with ''aggressive'' solvents) were extensive (many compounds at high concentrations) for both types of tubing, the leachables profile, obtained under more or less ''real world'' dynamic conditions with more or less ''real world'' solutions were trivial (few compounds at low concentrations).

Pan et al.[28] used LC/ESI-MS along with GC/MS to identify an unknown leachable present at 0.19% in an ophthalmic drug product (see previous discussion under LC/UV applications). Identification of the leachable as a monomethyl derivative of mephenesin required the use of GC/MS as LC/MS alone proved incapable of accomplishing the complete structural analysis. Systematic extractions of various components and component combinations of the packaging were employed to establish the source of the leachable as the varnish applied to the drug product label. Soeborg et al.[88] used LC/ESI-MS to detect and quantify various bisphenol diglycidyl ethers in extracts of internally lacquered aluminum container closure systems and a topical cream stored in this container closure system. Three commercially available topical creams were also analyzed for these potential leachables and four were detected and quantified. Spahl et al.[89] used LC/MS and GC/MS to identify and

quantify potential leachables from polymerized specimens of four universal hybrid-type dental composite resins. Note that these workers employed particle beam LC/MS, which requires that analytes be amenable to gas phase ionization by either Electron Ionization (EI) or Chemical Ionization (CI).[58–60,66]

Additional works of interest include the identification of some plastic and latex additives in fruits by Pico et al.[90] using UPLC with a quadrupole time-of-flight mass spectrometer (QTOF). The QTOF is capable of acquiring MS/MS and accurate mass information on trace level chemical entities. The main purpose of the work was to identify unknown pesticides in the fruit. Guo et al.[91] characterized typical background interferences in API LC/MS as plastic additives, including phthalates, phosphates, sebacates, adipates and silicones. Kaerrman et al.[92] used solid-phase extraction with LC/ESI-MS and LC/MS/MS to detect and quantify various perfluorochemicals in human whole blood. These compounds have various applications, including uses as surfactants and plastic additives.

Other hyphenated techniques besides LC/MS have also been applied and are worthy of mention. A novel approach for the analysis of complex polymers, two-dimensional (2D) chromatography (HPLC and Size Exclusion Chromatography (SEC)), is described by Heinz et al.[93] Blends of styrene-butadiene rubber and butyl rubber were separated by gradient HPLC using a polar stationary phase and chloroform-cyclohexane as the eluent, and the chemical structures of the blend components were determined by HPLC-FTIR (Fourier Transform Infrared Spectroscopy) which revealed information on styrene and butadiene content and the conformation of butadiene units. The 2D combination was able to achieve complete separation of the blends with respect to chemical composition and molar masses. In a separate report Heinz et al.[94] used temperatures above 130°C for the analysis of ethylene - methyl methacrylate block copolymers that dissolve in organic solvents only at high temperatures. The study used high-temperature SEC, hyphenated SEC-FTIR, and Crystallization Analysis Fractionation for the analysis of block copolymers of different compositions. Even though these particular applications stray somewhat from the extractables/leachables theme of this review, they are worthy of mention as novel chromatographic applications to rubber and plastic potential extractables/leachables.

"Special Case" Leachables

The USFDA has recognized that certain compounds and compound classes are worthy of special consideration as leachables and potential leachables due to possible special safety concerns and for various historical reasons.[10] For inhalation drug products, these include Polyaromatic

Hydrocarbons (PAHs; also referred to as Polynuclear Aromatic Hydrocarbons, PNAs), volatile N-nitrosoamines, and 2-mercaptobenzothiazole.[7,8,10] HPLC systems with various detection techniques, including LC/UV and LC/MS, have been employed for these analytes. HPLC with either UV or fluorescence detection has been used to analyze PAHs in various matrices.[95–106] LC/MS[107–110] and HPLC with fluorescence detection[111] have been applied to volatile N-nitrosamines in various matrices. Table 3 lists several references to 2-mercaptobenzothiazole analysis by LC/UV, and LC/MS and LC/MS/MS methods have been developed for 2-mercaptobenzothiazole determination in wastewaters.[112–115]

At the time of this writing, Bisphenol A is receiving significant media attention as well as attention from the regulatory authorities suggesting that it has the potential for "Special Case" status in the future. A search of the available scientific literature for the past 20 years revealed many references connecting Bisphenol A and HPLC and LC/MS, but that is the potential subject of another review.

CONCLUSIONS

The issue of extractables and leachables in pharmaceutical container closure systems and drug products is a significant one. The Product Quality Research Institute's recent publication of safety and analytical thresholds for leachables in inhalation drug products,[10–12] along with "Best Practice" recommendations for extractables/leachables pharmaceutical development programs for these drug products, also includes an HPLC platform for extractables/leachables "profiling" which can be the basis for both LC/UV and LC/MS methods. This is an attempt to create an HPLC platform which can be applied to the diversity of organic chemical types which can appear as extractables/leachables. It is envisioned that in the near future significant work, including real world pharmaceutical applications, will be published on extractables/leachables *qualitative, quantitative*, and *structural analysis*. It is anticipated that these published works will include detailed LC/MS investigations, specific and highly sensitive LC/MS quantitative methods, novel HPLC platforms for both target compound analysis and broad spectrum profiling, as well as application of new technologies such as UPLC and novel LC/MS technologies.[60,65,66]

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