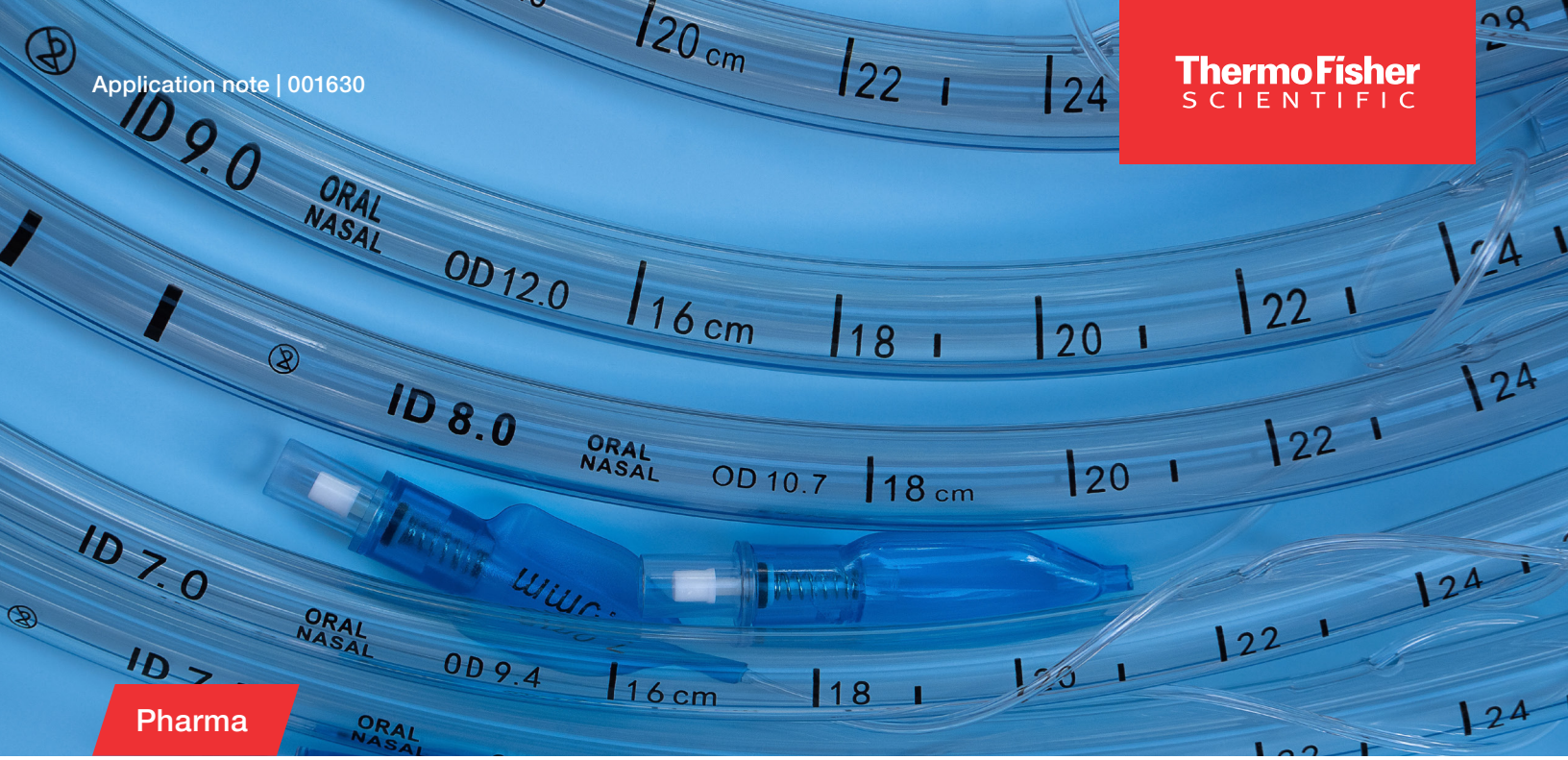

A reliable UHPLC/UV/CAD/MS multidetector method for routine quantification and library matching of extractables and leachables in pharmaceutical-grade plastics

Authors

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Keywords

Vanquish Inverse Gradient LC system, charged aerosol detector, CAD, ISQ EC MS, inverse gradient, extractables and leachables (E&L), compound identification, single quadrupole mass spectrometry, epoxidized soybean oil, process impurities

Application benefits

- Introduce a routine method for extractables and leachables (E&L) quantification and single-quadrupole MS library data generation
- Determine which analytes behave as non-volatiles with CAD and can therefore be accurately quantified using surrogate standards
- Apply a fit-for-purpose, easy-to-use single quadrupole mass spectrometer (MS) for matching compounds using relative retention time and molecular ion masses
- Improve quantification accuracy and reduce response factor variation using an inverse gradient
- Employ the inverse gradient without adversely affecting MS LOQs or introducing ammonium or other adducts
- Use an in-line mixer to combine the analytical and inverse gradients and improve response uniformity

Goal

An in-house method will be used for the study of E&L substances in common medical and pharmaceutical-grade plastics. The method uses multiple orthogonal detectors to facilitate analysis of a chemically diverse range of analytes. The method uses an inverse gradient technique that has been further refined by implementing an in-line mixer after the column and by optimizing gradient delay times. A previously published calculation

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was applied for determining whether analytes behave with CAD as non-volatiles or semi-volatiles. The described approach was explored for its ability to minimize inter-analyte response factor variation and to increase quantitative accuracy, thus minimizing the uncertainty factor used in setting analytical evaluation thresholds. The overall goal of the described method is to address key requirements in E&L studies to protect against the occurrence of potentially harmful substances in a highly efficient and cost-effective manner.

Introduction

Studies designed to ensure that no potentially harmful substances are released into the pharmaceuticals we use, the food we eat, and the water we drink involve complex samples. Detection of unknown or unexpected peaks in such complex samples requires multiple orthogonal detectors. Beyond mere detection, laboratories also frequently require identity confirmation and quantitation of these compounds to determine their nature and whether they are below acceptable concentration limits. A reliable method based on years of experience[1] and used regularly in our labs combines UV, MS, and charged aerosol detectors to characterize these important samples. The UV detector offers accurate quantification if substances contain chromophores and if standards are available. The CAD delivers universal detection of non- and semi-volatile compounds. Additionally, its near uniform response allows straightforward quantification without individual reference standards. MS in combination with a compound library offers identity confirmation of the detected compounds. An established method on a multi-detector platform was used to efficiently and cost-effectively analyze potentially harmful substances extracted from medical-grade sterile PVC and polyurethane (PU) -based wound dressings.

Experimental

Chemicals

Chemical name	Part number	CAS Nr.
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A	7732-18-5
Acetonitrile, Optima™ LC/MS grade, Fisher Chemical™	A955	75-05-8
Methanol, Optima™ LC/MS grade, Fisher Chemical™	A456-212	67-56-1
Ammonium acetate, CHROMASOLV™ LC-MS Ultra, Honeywell	14267	631-61-8
Plastic additive 04 CRS Epoxidized Soybean Oil (ESBO), LGC Standards	EPE0800000	8013-07-8
Irganox™ 245, BLD Pharmatech Ltd. via Merck	BD20458-5G	36443-68-2
Diisobutyl phthalate, Supelco via Merck	43540-100MG	84-69-5
Sodium 1-octanesulfonate hydrate, HPLC grade, 99+%, Thermo Scientific™	042636.06	5324-84-5
1-Hydroxycyclohexyl phenylketone, 98%, Thermo Scientific™	L11377	947-19-3
5-Amino-1-pentanol, 50% wt.% aqueous solution, Thermo Scientific™	387910250	2508-29-4
Bis(4-chlorophenyl) sulfone, 99%, Thermo Scientific™	A18938	80-07-9
Diphenyl phthalate, 98%, Thermo Scientific™	A14449	84-62-8
Oleamide, Thermo Scientific™	467650050	301-02-0

Sample handling

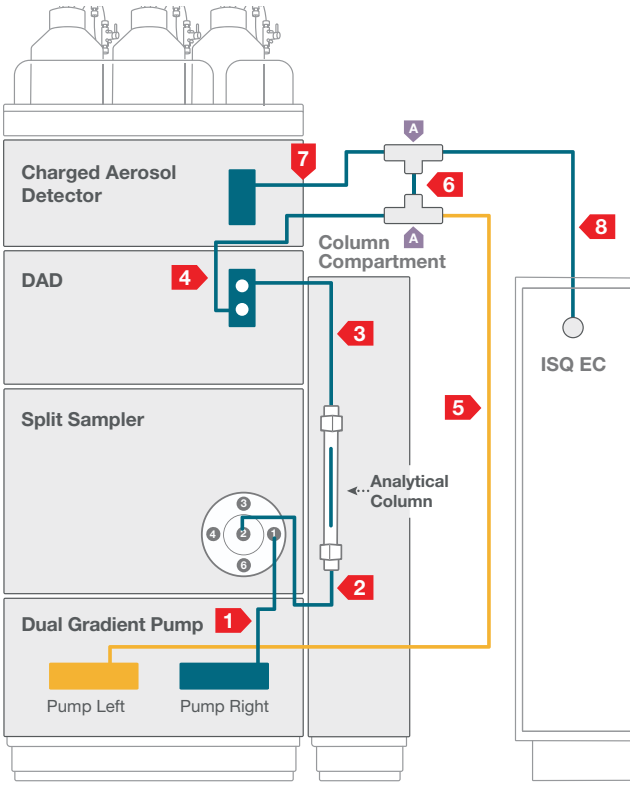
Item name	Part number
Fisherbrand™ Isotemp™ Stirring Hotplate	S14365
Fisherbrand™ Mini Centrifuge	12-006-901
Fisherbrand™ Mini Vortex Mixer	14-955-152
Vials (amber, 2 mL), Fisher Scientific™	03-391-6
Cap with Septum (Silicone/PTFE), Fisher Scientific™	13-622-292
Fisherbrand™ microcentrifuge tubes (2 mL, DNase/RNase Free)	14-666-315
Thermo Scientific™ Regenerated Cellulose Syringe Filter	12316958

Instrument setup for inverse gradient

A Thermo Scientific™ Vanquish™ Flex Inverse Gradient LC system for Inverse Gradient was used consisting of:

- Thermo Scientific™ Vanquish™ System Base Horizon/Flex (P/N VF-S01-A-02)
- Thermo Scientific™ Vanquish™ Dual Pump F (P/N VF-P32-A-01)
- Thermo Scientific™ Vanquish™ Split Sampler FT (P/N VF-A10-A-02)
- Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A-03)
- Thermo Scientific™ Vanquish™ Diode Array Detector FG (P/N VF-D11-A) with a standard Vanquish flow cell with a 13 µL illuminated volume and a 10 mm flow path (P/N 6083.0510)
- Thermo Scientific™ Vanquish™ Charged Aerosol Detector F (P/N VF-D20-A)
- Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer (P/N ISQEC-LC)

The flow paths from the left and right pumps are detailed in Figure 1. A similar configuration that incorporates a switching valve for increased robustness is detailed in Application Note 72869.[2] Thermo Scientific™ Viper™ Fingertight Fitting systems with 100 µm inner diameter were used. The capillaries from T-piece to the CAD and MS were of the same length. A capillary mixer was added directly before the CAD/MS split to ensure proper mixing of the analytical and inverse gradients.



No.	Additional part	Description
A	Two T-pieces	Standard 500 µm ID P/N 6263.0035

No.	Connection between	Description
1	Pump right outlet – Injection valve port 1 (= “From pump”)	Viper capillary, ID × L 0.10 × 350 mm, MP35N, P/N 6042.2340
2	Injection valve left port 2 (= “To column”) – Column inlet	Active pre-heater, 0.10 × 380 mm, MP35N, P/N 6732.0110
3	Column outlet – DAD inlet	Post-column cooler, ID × L 0.10 × 240 mm, MP35N, P/N 6732.0510
4	DAD outlet – T-piece	Viper capillary, ID x L 0.10 × 550 mm, MP35N, P/N 6040.2360
5	Pump left outlet – T-piece	Viper capillary, ID x L 0.10 × 950 mm, MP35N, P/N 6042.2395
6	T-piece – T-piece	Viper capillary mixer, 25 µL, MP35N, P/N 6042.3020
7	T-piece – Charged Aerosol Detector inlet	Viper capillary, ID x L 0.10 × 650 mm, MP35N, P/N 6042.2370
8	T-piece – MS inlet	Viper capillary, ID x L 0.10 × 650 mm, MP35N, P/N 6042.2370

Figure 1. Schematic diagram of inverse gradient setup

Sample preparation

The 500 µg/mL stock solutions were prepared by adding 50 mg of each compound in the standard mixture (see Table 4) to a 100 mL volumetric flask and filling to the line with methanol. A 10 µg/mL working solution was prepared by adding 2 mL of each stock solution to a 100 mL flask and filling to the line with a diluent consisting of 30/70 water/methanol (v/v). The pharmaceutical grade PVC IV tubing and wound dressings were cut into 100 mg samples with scissors and incubated in 2 mL microcentrifuge tubes for 72 h at 50 °C in 1 mL of 50/50 water/isopropanol (v/v). The samples were filtered through a 0.2 µm regenerated cellulose syringe filter and analyzed directly.

Mobile phase preparation

For aqueous mobile phase A, 0.19 g ammonium acetate was added to a clean mobile phase bottle. Using a graduated cylinder, 1,000 mL water was added to yield an approximately 2.5 mM solution. For solvent B, the same procedure was followed with 1,000 mL methanol instead of water. Best practices for mobile phase preparation were implemented according to Technical Guide 73914.[3] This included preparation of the aqueous solvent daily, preparation of the organic solvent every two days, avoiding unnecessary solvent filtration, and avoiding exposure of mobile phases to pH electrodes. These practices helped to minimize baseline noise and drift.

Injection sequence

A specific injection sequence, shown in Table 2 and programmed into the eWorkflow™ for this publication, was used to confirm system suitability, perform periodic checks on the system performance, and minimize carryover. The eWorkflow is available on Thermo Scientific™ AppsLab Library of Analytical Applications. Although the needle is in the pressurized flow path during the run, holding a large volume at system pressure prior to injection facilitates sample desorption from the inner needle walls.

Chromatographic conditions

Table 1. Chromatographic conditions

Column	Thermo Scientific™ Hypersil GOLD™ C18 3.0 × 100 mm, 1.9 µm particle size, P/N 25002-103030		
Mobile phase	A: 2.5 mM ammonium acetate in water B: 2.5 mM ammonium acetate in methanol		
Gradient	**Time (min) **For inverse gradient timing, add 1.358 min**	**Standard gradient, right pump, mobile phase B (%)**	**Inverse gradient, left pump, mobile phase B (%)**
	-1.25	20	100
	0	20	100
	5	60	60
	20	100	20
	30	100	20
	30.1	20	100
	35	20	100
Flow rate	0.45 mL/min, both left and right pump		
Column compartment and active preheater temperature	60 °C, forced air mode		
Autosampler temperature	10 °C		
Autosampler wash solvent	75:25 methanol:water (v/v), needle wash type “both”		
Injection volume	10 µL		
Sample loop capacity	25 µL		
UV detector settings	λ = 220, 230, 250, 270 nm 20 Hz data collection rate 0.2 s response time		
CAD settings	Evaporation temperature (EvapT) 35 °C 10 Hz data collection rate 5.0 s filter Power function value 1.10		

Table 2. Injection sequence

Solution	Number of injections
Method blank (30:70, Water:MeOH)	At least 2
Irganox 245 working standard	6
Sample mixture working solution	1 injection per sample
Irganox 245 working standard	1 injection after every 6 sample injections and at the end of the sequence.
Method blank (30:70, Water:MeOH)	Large volume injections (up to the 25 µL injector loop capacity) as needed to minimize carryover

MS settings

Instrument settings for the ISQ EC single quadrupole mass spectrometer are shown in Table 3. Two injections for each sample were run: one full scan injection with three full scans (negative scan from 90 to 1,250 *m/z*, positive scan from 90 to 400 *m/z* and positive scan from 400 to 1250 *m/z*) and one SIM scan injection with pre-programmed SIMs based on typical extractables of concern for polyurethane or epoxidized soybean oil.[5] Mass lists for unknowns were taken from Reference 6 (ESBO) and Reference 7 (PU). The SIM scan method would have accommodated one full scan. Instead, an additional "full scan" injection was made, which had one negative mode scan plus two positive mode scans, implemented to promote collection of more ions in the higher mass range.

Instrument methods with the SIM scan timing and mass settings for the 13 components in the standard mixture and for the putative unknowns in the polyurethane and ESBO (PVC) samples are also available in the AppsLab Library.

The ISQ EC mass spectrometer is designed with robust default settings that need minimal optimization. Sliders for sensitivity, mobile phase volatility, and thermal stability are present in the user interface. The sweep gas "sensitivity" slider was moved down one position below the default to increase the sensitivity. Using the "sensitivity" slider in the default position is needed only if too much low molecular weight matrix drowns the signal. The "stability" slider for the ion transfer tube temperature was used at one position above the default because all analytes were expected to be relatively thermally stable. The SIM scan width was set to 0.1 amu, which is the proper default value for SIM scan width for the ISQ EC, based on empirical results.

A source CID of 25 V for oleamide was employed to reduce the presence of its dimer. All other SIM scans used a source CID of 10 V. Although some analytes were only found as the ammonium adducts, increased CID voltage did not produce protonated species. This could be expected because ammonia is part of the eluent additive. The Genesis detection algorithm, a MS peak detection algorithm also used in Thermo Scientific™ Xcalibur™ software and Thermo Scientific™ TraceFinder™ software, was used. This processing method is available as part of the eWorkflow in the AppsLab Library.

Table 3. Instrument and scan settings for the mass spectrometer. Methods detailing the SIM scan timing and masses for polyurethane and epoxidized soybean oil are available in the eWorkflow in the AppsLab Library.

Instrument settings	
Vaporizer temperature	255 °C
Ion transfer tube temperature	350 °C
ESI source voltage	+ 3,000 V - 2,000 V
Sheath gas pressure	46.4 psi
Aux gas pressure	5.3 psi
Sweep gas pressure	0.1 psi
Total pump flow	0.45 mL/min (approximate value. Note that, despite the equal split with identical capillaries, internal MS capillaries lead to slightly lower flow rate on the MS side relative to the CAD side)
SIM scan settings	
Method type	Component mode
Acquisition rate: Minimum baseline peak width Desired scans per peak	 10 s 6
SIM scan width	0.1 amu
Full scan settings	
Method type	Scan mode with 3 scans: 1) 90–400 amu, + 2) 400–1250 amu, + 3) 90–1250 amu, -
Source CID voltage	10

Chromatography Data System

The Thermo Scientific™ Chromeleon™ 7.3.1 CDS was used for data acquisition and analysis.

Table 4. Table of SIM Windows for three different scans (standard mixture (SM), polyurethane (PU), and (PVC))

Class	Compound name	Start time [min]	End time [min]	Mass [*m/z*]	Ion polarity
SM	Irganox 245	14.3	15.8	604.39	+
SM	Irganox 245	14.3	15.8	585.25	-
SM	Diisobutyl phthalate	12.3	13.8	279.22	+
SM	1-Octanesulfonate	7.3	8.2	193.09	-
SM	Oleamide	17.5	18.5	282.35	+
SM	Diphenylphthalate	10.5	12.5	319.14	+
SM	bis(4-chlorophenyl) sulfone	9.8	10.8	304.05	+
SM	1-Hydroxycyclohexylphenyl ketone	8.5	9.5	205.23	+
SM	5-Amino-1-pentanol	1	1.8	104.27	+
SM	Erucamide	20	20.8	338.3	+
SM	Stearic acid	18.3	19.3	283.3	-
SM	Palmitic acid	16.8	17.6	255.2	-
SM	Bisphenol A	7.8	8.8	227.1	-
PVC	DG(36:4-eO)	1	30	698.52	+
PVC	TG(54:8-eO)	1	30	1020.7	+
PVC	Isooctyl phthalate or isomer	1	30	391.28	+
PVC	Bis(2-ethylhexyl)adipate	1	30	371.32	+
PVC	TG(54:6-eO)+H_2O*	1	30	1010.75	+
PVC	TG(54:7-eO)	1	30	1006.72	+
PVC	DG(36:3-eO)	1	30	684.54	+
PVC	Dioctyl phthalate or isomer	1	30	408.31	+
PVC	TG(54:6-eO)	1	30	992.74	+
PVC	DG(34:2-eO)	1	30	642.53	+
PVC	TG(54:5-eO)	1	30	978.76	+
PVC	Trioctyl trimelitate (TOTM)	1	30	547.4	+
PU	Methyl stearate	1	30	299.3	+
PU	Butylated hydroxytoluene	1	30	219.2	-
PU	4-(ethylphenylamino)-benzaldehyde	1	30	226.1	+
PU	2-[2-Methoxy-5-(1,1,3,3-tetramethylbutyl)phenyl]-2H-benzotriazole	1	30	338.22	+
PU	Methyltris(trimethylsiloxy)silane	1	30	311.13	+
PU	3,5-bis(1,1-dimethylethyl)-4-hydroxy-benzenepropanoic acid, methyl ester	1	30	293.2	+
PU	Triethyl phosphate	1	30	183.1	+
PU	1-phenyl-ethanone-(1-phenylethylidene)hydrazone	1	30	237.1	+
PU	(+,-)-Epi-perhydrohistrionicotoxin	1	30	296.3	+
PU	9-Octadecenamide	1	30	282.3	+
PU	Triamcinolone acetonide	1	30	435.22	+

Results and discussion

Determining analyte suitability to universal quantification by CAD

CAD has been well described for its ability to accurately quantify analytes using surrogate standards.[11] A key requirement is that analytes behave as non-volatiles under the method conditions. This non-volatile behavior was defined as $Q_{50/35} > 85$,[4] which is the ratio of the peak area detected in the CAD at EvapT 50 °C to the peak area at EvapT 35 °C. Of twelve standard substances tested, six met this threshold. Of eight unknowns in the PVC sample, three met this threshold. Of six unknowns in the polyurethane sample, three met this threshold. This simple calculation is a valuable and unique tool for determining whether unknowns can reliably be quantified in CAD by a universal standard. There is no comparable simple determination that can be made to improve quantification reliability of unknowns by MS or UV detection.

Determination of power function value and calibration functions

For one representative non-volatile component, Irganox 245, an optimal power function value of 1.1 was determined according to the guidance in TN 73299.[7] For all non-volatiles, relative amount deviations of less than ±15% for all calibration data points were found for linear least squares regression calibration curves when weighting the calibration data by 1/amount. For semi-volatile substances, log-log calibration curves (Chromeleon "Power" Curve Fit Type) with no weighting that passed through the origin were generated. The residual values were also less than ±15% for all calibration data points. Quantification was not attempted for unknown semi-volatile substances.

Implementation of the inverse gradient

Because CAD response is greater with higher organic content in the mobile phase, a second pump was used to produce a gradient from high organic to low organic at the same flow rate as the standard gradient. This "inverse gradient" had a shorter path to the detector because it did not flow through the column and the autosampler. A delay was programmed to ensure that the CAD and mass spectrometer worked under effectively isocratic eluent conditions. The inverse gradient was calculated to have a 610 µL shorter flow path using Chromeleon CDS's fluidic configuration manager and the inverse gradient wizard with the economical "keep solvent composition" option.[8,9] Alternatively, the "maximize %B" option could have been chosen for greater sensitivity.

Advantage of the in-line mixer

The capillary mixer, shown in Figure 1 as capillary 6, was used to improve the uniformity of response for non-volatile compounds when the inverse and analytical flows are combined and then immediately split again into the CAD and MS. This finding has been explored in detail and will be presented in another publication.[10]

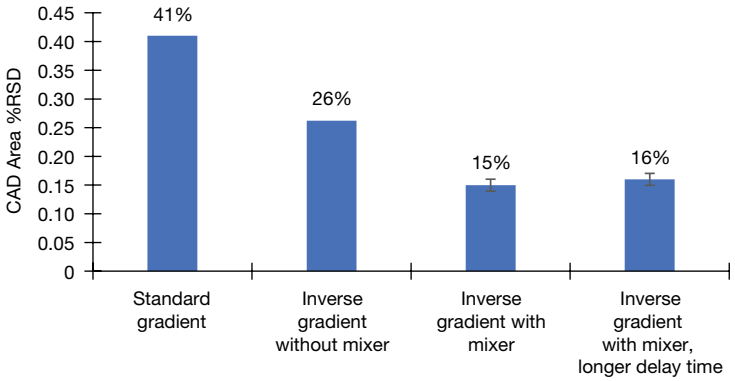


Figure 2. The CAD inter-analyte area %RSD for six non-volatile substances using the 10 µg/mL standard mixture working solution improved upon implementation of the inverse gradient (41% RSD to 26% RSD) and again upon insertion of the capillary mixer (26% to 15%). Implementation of the actual, longer delay time did not improve the %RSD in this case.

Gradient delay

No difference between the wizard-calculated and the empirically determined delay was observed (Figure 2). The inverse gradient was calculated to have a 610 µL or 1.35 min shorter flow path using Chromeleon CDS's fluidic configuration manager. This delay was used in the inverse gradient method wizard to generate a method with a delayed start of the gradient for the left pump. Because a few capillaries that differed from the default inverse gradient setup were used to accommodate the CAD-to-MS distance, an empirically determined delay of 707 µL or 1.57 min was found by sending a pulse of acetonitrile through the left and then the right pumps and determining the difference in arrival time at the CAD. Both the wizard-calculated and the empirically determined delay led to similar responses (Figure 2) although this will not always be true for all system setups.

Advantage of the inverse gradient

The inverse gradient normalized the CAD peak areas for the non-volatile components and brought the individual calibration curves closer together (Figure 3). The inter-analyte %RSD in relative response was 15% with the inverse gradient and 41% without the inverse gradient (Figure 3). The working solution, in which every standard was present at 10 µg/mL, yielded results from 10.32 (Irganox 245) to 14.82 µg/mL (1-octanesulfonate), when evaluated using the calibration curve for Irganox 245 in the inverse gradient. Without the inverse gradient and choosing Irganox 245 as the universal calibrant, the components in the working solution were evaluated with much worse accuracy from 4.5 (5-amino-1-pentanol) to 20.6 µg/mL (erucamide).

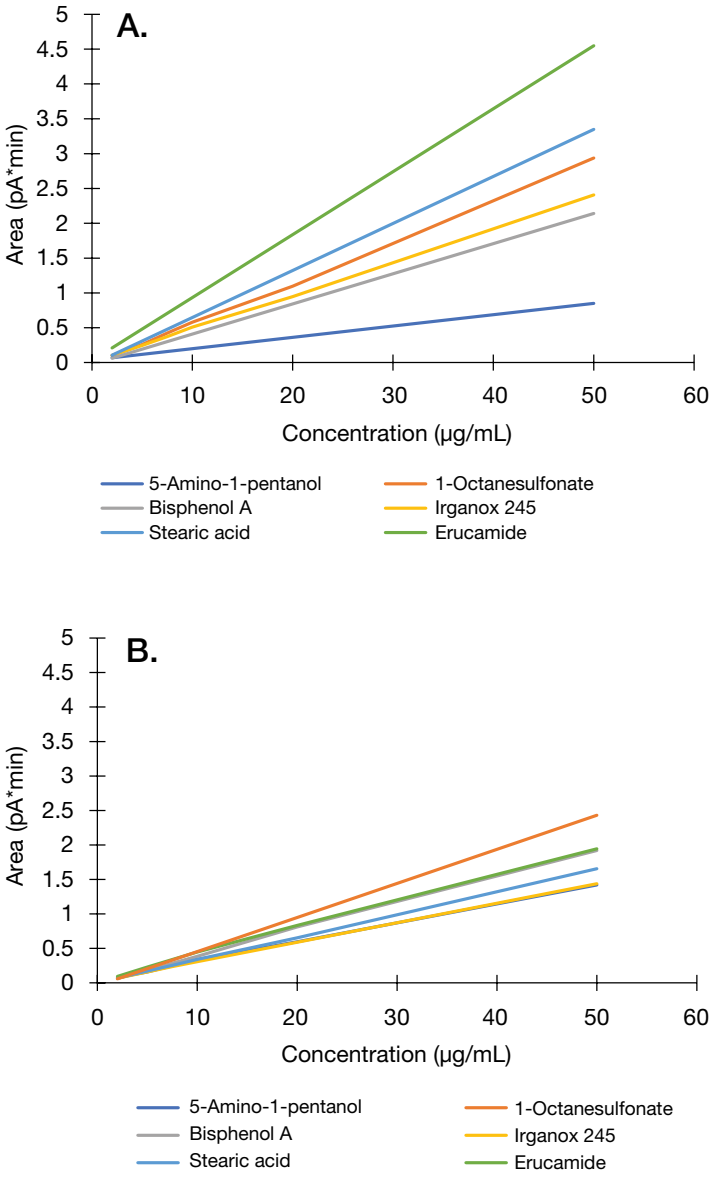


Figure 3. Calibration curves for standards with standard gradient (A) and non-volatiles with the inverse gradient (B). The %RSD in relative response was 15% with the inverse gradient and 41% without the inverse gradient.

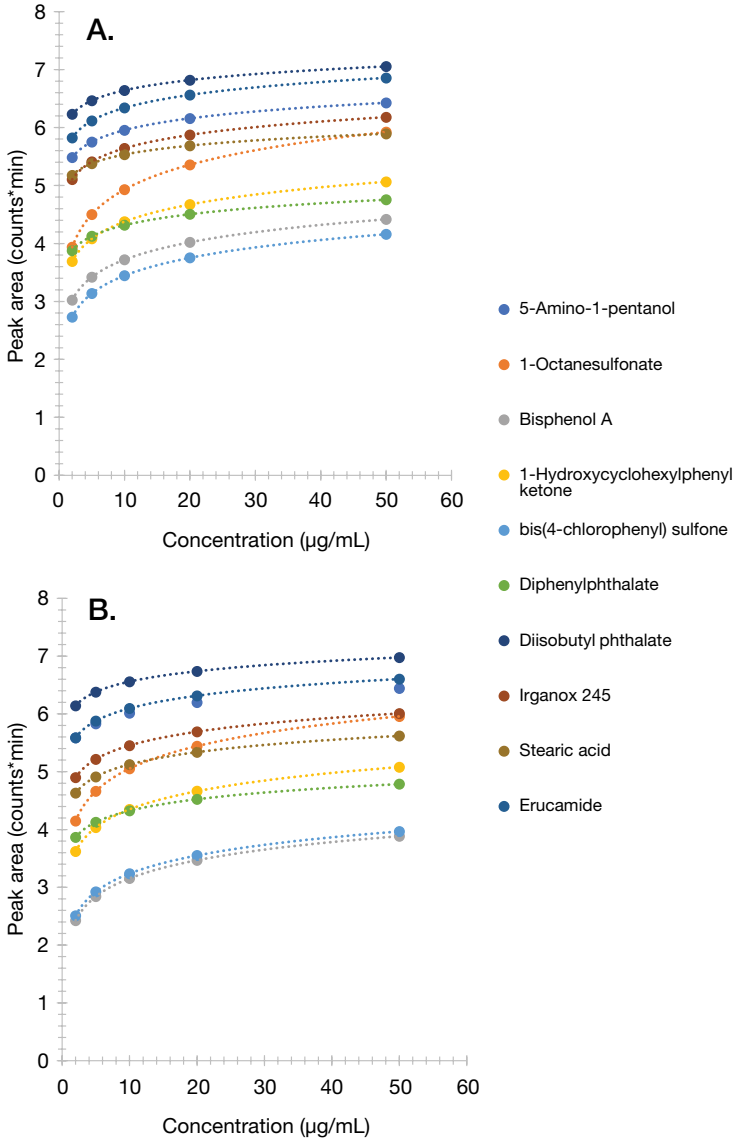


Figure 4. Calibration curves for the ISQ EC single quadrupole mass spectrometer with the standard gradient (A) and with the inverse gradient (B)

As shown in Figure 5, there was no baseline drift over the gradient in the CAD standard gradient as long as the aqueous mobile phase was exchanged daily, the organic mobile phase was exchanged every two days, and both phases were prepared with MS-grade solvents and reagents without filtering or use of a pH meter.[3] With the inverse gradient, the earliest peaks are larger (area of 5-amino-1-pentanol is 142% of the standard gradient area) and the latest peaks are smaller (area of erucamide is 47% of the standard gradient area). The earlier peaks are larger with the inverse gradient because these analytes pass through the detector in a more methanol-rich eluent. Higher methanol content means lower surface tension and lower viscosity (after 40% MeOH) and these analyte-independent effects lead to improved mass transport efficiency to the CAD and higher signal.[11] The opposite is true for later peaks.

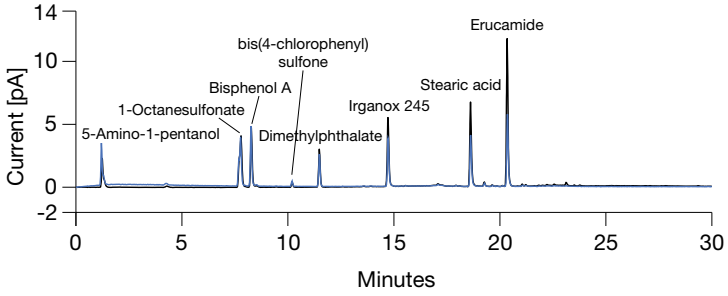


Figure 5. Chromatogram of working solution with standard gradient (black) and inverse gradient (light blue)

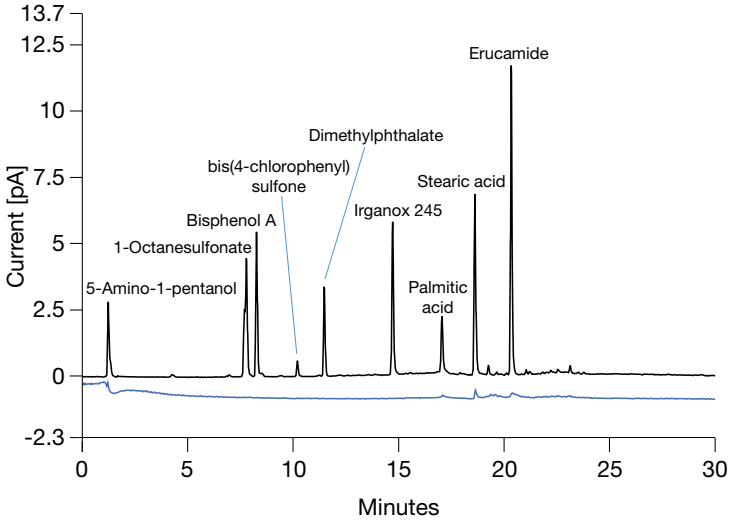


Figure 6. Evaluation of chromatographic interference in the CAD shows small peaks due to carryover from erucamide and stearic acid (blue is matrix injection and black is working solution).

The inverse gradient was designed such that it passed through the MS and the CAD. This decision ensured that the split between the CAD and the MS was not affected by backpressure changes over the gradient and did not adversely affect the LOQs in the MS (Table 7). The inverse gradient did not lead to increased adduct formation (i.e., ammonium adduct formation) in the MS.

Utility of ISQ EC single quadrupole MS for E&L substances

A single quadrupole in the extractables and leachables laboratory offers a less complex workflow relative to other MS systems, thereby saving analyst training and familiarization time. Single quadrupole systems find good use in routine testing to identify and quantify targeted compounds or perform straightforward unknowns testing with in-house compound databases. In addition to quantification of toxic substances in leachables* samples, for which the peaks have already been identified by high-resolution MS, the monitoring of leaching over time is another common workflow. Conclusive analyte identification by molecular formula and fragmentation patterns is not possible with a single quadrupole MS because of limited instrument capabilities.

System suitability: Chromatographic non-interference and precision

Chromatographic non-interference was evaluated daily using the CAD channel of the last injection of the method blank (30:70 water:MeOH) prior to the working standard. Small but persistent peaks in the CAD at the retention time of stearic acid (5% CAD peak area of 10 µg/mL standard mixture), palmitic acid, and erucamide (both 1% CAD peak area of the standard mixture) were present after repeated injections of the 10 µg/mL standard mixture (Figure 6). In the MS, small peaks (<1%) in the negative mode were present only for stearic and palmitic acid. In the $UV_{230\ nm}$, small peaks at <0.1% persisted for Irganox 245, bisphenol A, and bis(4-chlorophenyl)sulfone. These persisted after washing the column with 100% acetonitrile and the system with 100% acetonitrile and then 100% isopropanol. These peaks did not affect quantitation of the samples or the calibration curve, although a method blank chromatogram could be subtracted from each sample chromatogram if needed for quantification of unknowns.

Instrument precision was evaluated using six replicate injections of the Irganox 245 10 µg/mL working standard and all working standard injections made over the course of the sequence (one every six sample injections and at the end of the sequence). The peak area %RSD in the 230 nm UV channel for Irganox 245 was 0.17% over the six replicate injections and 1.13% over 12 injections.

*A leachables study involves exposure of samples to physiologically relevant buffers over a shelf-life relevant time span, whereas an extractables study generally involves extracting a sample with organic, non-physiologically relevant solvents to pull out and identify as many potentially toxic substances as possible.

Limit of detection, analytical evaluation thresholds, uncertainty factors, and range of calibration

Toxicity of a substance depends on how much is present in a sample. An E&L analysis should accurately quantify concentrations of potentially hazardous substances. If accurate quantification is not possible, the concentration must be estimated, erring on the side of overestimation and never underestimation.

For accurate quantification of known standards, a calibration concentration range of 2–50 µg/mL was chosen based on the signal-to-noise ratios of actual standards injected at the given concentrations. The extractables and leachables scientific literature also shows use of standards at these concentrations.[4,12–14] This concentration range is relevant for evaluating the toxicity of contaminants leaching from food contact materials as well as for pharmaceutical packaging and medical devices.[15] Specifically, the guidelines stipulate a threshold of 120 µg/day for devices that have contact with patients for 30 days or less, such as wound dressings or i.v. tubing, and a threshold of 1.5 µg/day for lifetime exposure. The multidetector system is not sensitive enough for analytical evaluation thresholds in the low ppb (or ng/mL) range, such as those needed for assessing risk over lifelong exposure times, and strategies for quantification with a high-resolution mass spectrometer must be employed.

For estimation of unknown substances, accurate estimation or overestimation is desired. Underestimation could lead to accidental disregard of toxic levels of a substance. Relative response factors (Table 6) assist in estimation. If an unknown substance has a relative response factor of less than one, the estimated concentration will be underestimated and toxic levels of the substances may be present, but not reported. The use of internal standard Irganox 245 yielded relative response factors in the CAD of greater or equal to one for all non-volatiles. For the MS, normalization to Irganox 245 yielded response factors over more than three orders of magnitude from 0.004 to 11.1. For the UV at 230 nm, normalization also yielded response factors over three orders of magnitude, from 0.003 to 10.8.

Quantification and relative response factor

The expedient choice of Irganox 245, the substance with the lowest relative response factor of all the non-volatiles, as a universal calibrant in the CAD led to a slight overestimation of the concentrations. Avoiding underestimation is important when evaluating whether a certain substance might be present in amounts that exceed safe limits for toxicity. The working solution, in which every standard was present at 10 µg/mL, yielded results from 10.32 (Irganox 245) to 14.82 µg/mL (1-octanesulfonate).

Bisphenol A failed the $Q_{50/35}$ test but also had a response factor close to 1 at an EvapT 35 °C. If it were an unknown, it could not be quantified by CAD. Bisphenol A would pass a $Q_{40/35}$ test, where the higher temperature would be 40 instead of 50 °C. Implementation of this change was not pursued beyond preliminary investigations because of the potential for misidentification of semi-volatiles as non-volatiles. Because it is a known substance for which the calibration curve lines up with the other non-volatiles at 35 °C, it was quantified by the Irganox 245 curve. The uncertainty for the estimated amount of the non-volatile unknowns is given by the uncertainty factor (UF), calculated by the expression:

$$UF = 1/(1\text{-}RSD) = 1/(1\text{-}0.15) = 1.18$$

Where RSD is the peak area %RSD for non-volatiles.[13] This uncertainty factor equation is not useable for the MS and the UV in this case, because both detectors had response factors over three orders of magnitude and RSDs greater than 1.

Table 5. Relative retention times (RRT) for entry into single quadrupole MS compound database. Numbers in parentheses refer to peak numbers for Figure 7 or Figure 8. The table is ordered by CAD non-volatility factor for the compounds in the standard mixture, then by relative retention time to Irganox 245 (RRT) for the PU compounds and RRT for the ESBO compounds.

	RRT	RT (min)	*m/z* found	CAD non-volatility factor $Q_{50/35}$	Estimated amount
5-Amino-1-pentanol	0.08	1.24	104.27 $(M+H)^+$	0.86	*11.27
1-Octanesulfonate	0.53	7.79	193.09 $(M-H)^-$	0.85	*15.22
Bisphenol A	0.56	8.27	227.1 $(M-H)^-$	0.47	*#12.89
Irganox 245	1.00	14.71	604.39 $(M+NH_4)^+$ 585.25 $(M-H)^-$	1.13	*10.21
Palmitic acid	1.16	17.05	255.2 $(M-H)^-$	0.89	n.d.d.
Stearic acid	1.26	18.61	283.3 $(M-H)^-$	0.86	*11.37
Erucamide	1.61	20.34	338.3 $(M+H)^+$	0.96	*15.12
Oleamide	1.21	17.83	282.4 $(M+H)^+$	0.54	*11.50
1-Hydroxycyclohexylphenyl ketone	0.58	8.54	205.2 $(M+H)^+$	—	*†9.97
bis(4-chlorophenyl) sulfone	0.69	10.20	304.1 $(M+H)^+$	0.46	*#10.11
Diphenylphthalate	0.78	11.47	319.1 $(M+H)^+$	0.45	*#10.17
Diisobutyl phthalate	0.83	12.55	279.2 $(M+H)^+$	—	*†9.89
(PU 1) Unknown PU Peak, UV-silent	1.14	16.78	280.3 (+)	0.42	n.d.s.v.
(PU 2) Putative 9-octadecenamide in PU wound dressing, UV-silent	1.21	17.83	282.4 $(M+H)^+$	0.58	n.d.s.v.
(PU 3) Unknown PU Peak, UV-silent	1.26	18.53	148.3 (+)	0.75	n.d.s.v.
(PU 4) Unknown PU Peak, UV-silent	1.28	18.83	338.4 (+)	0.92	§0.8 (0.7–1.0)
(PU 5) Unknown PU Peak, visible at 230 nm	1.51	22.14	—	1.2	§0.4 (0.4–0.5)
(PU 6) Unknown PU Peak, visible at 230 nm	1.57	23.03	—	0.875	§0.25 (0.2–0.3)
(ESBO 1) Unknown ESBO Peak	0.08	1.2	371.3, 713.0, 753.0 (+)	0.89	§6.3 (5.3–7.4)
(ESBO 2) Unknown ESBO Peak	1.16	17.05	255.2 (-)	0.34	n.d.s.v.
(ESBO 3) Unknown ESBO Peak, DG(36:4-eO)	1.21	17.78	698.0, 739.0 $(M+H)^+$	1.06	§18.4 (15.6–21.7)
(ESBO 4) Unknown ESBO Peak	1.22	17.96	282.4 (+)	0.58	n.d.s.v.
(ESBO 5) Unknown ESBO Peak, TG(54:8-eO)	1.26	18.47	1021 $(M+H)^+$	1.12	§3.6 (3.0–4.2)
(ESBO 6) Unknown ESBO Peak	1.27	18.64	283.2 (+)	0.52	n.d.s.v.
(ESBO 7) Unknown ESBO Peak, TG(54:7-eO)	1.35	19.79	1007 $(M+H)^+$	1.03	§31.7 (26.9–37.4)
(ESBO 8) Unknown ESBO Peak, TG(54:6-eO)	1.42	20.96	993 $(M+H)^+$	0.71	n.d.s.v.

*For 10 µg/mL sample

§Based on Irganox 245 calibration curve

#Based on own log-log calibration curve (semi-volatile)

†Based on own linear $UV_{230\ nm}$ calibration curve

n.d.s.v. not determined because of semi-volatile unknown

n.d.d. not determined because of degraded sample

Table 6. Comparison of response factors relative to the standard, Irganox 245, and response at EvapT 50 °C and Evap T 35 °C. Analytes shaded in green are semi-volatiles. Uncertainty is RSD from n = 3.

	Relative response, CAD	Relative response, MS	Relative response, $UV_{230\ nm}$	CAD nonvolatility factor $Q_{50/35}$
5-Amino-1-pentanol	1.14 ± 0.03	2.6 ± 0.2	1.1 ± 0.1	0.86
1-Octanesulfonate	1.54 ± 0.02	0.35 ± 0.02	—	0.85
Bisphenol A	1.39 ± 0.02	0.005 ± 0.001	5.2 ± 0.2	0.47
Irganox 245	1	1	1	1.13
Palmitic acid	1.0*	0.22*	—	0.89
Stearic acid	1.25 ± 0.06	0.49 ± 0.09	—	0.86
Erucamide	1.37 ± 0.04	3.14 ±0.39	0.02 ± 0.01	0.96
Oleamide	1.19 ± 0.01	2.7	0.006 ± 0.0002	0.54
1-Hydroxycyclohexylphenyl ketone	—	0.07 ± 0.01	2.4 ± 0.1	—
bis(4-chlorophenyl) sulfone	0.11 ± 0.02	0.004 ± 0.004	3.8 ± 0.1	0.46
Diphenylphthalate	0.66 ± 0.01	0.07 ± 0.01	2.7 ± 0.1	0.45
Diisobutyl phthalate	—	12.1 ± 0.9	10.6 ± 0.4	—

*Indicates that result was taken from previous multi-detector experiments due to a degraded palmitic acid standard[2]

Table 7. Comparison of sensitivity on HPLC-CAD, HPLC-UV, and HPLC-Single Quadrupole MS in µg/mL. Method LOQ was determined by analysis of a standard at the actual concentration of the LOQ (S/N > 10), not by extrapolation. Noise was calculated by the root of mean squares method, and the time range for the noise calculation was twenty times the peak width at 50% max height.

Compound	Method LOQ, CAD (µg/mL)	Method LOQ, CAD, standard gradient (µg/mL)	Method LOQ, UV_{230} (µg/mL)	Method LOQ, MS (µg/mL)	Method LOQ, MS, standard gradient (µg/mL)
5-Amino-1-pentanol	2	2	2	2	2
1-Octanesulfonate	2	2	>50	2	2
Bisphenol A	2	2	2	5	5
Irganox 245	2	2	2	2	2
Palmitic acid	2	2	>50	2	2
Stearic acid	2	2	>50	2	2
Erucamide	2	2	>50	2	2
Oleamide	2	2	50	<10	<10
1-Hydroxycyclohexylphenyl ketone	>50	>50	2	2	2
bis(4-chlorophenyl) sulfone	5	5	2	2	2
Diphenylphthalate	2	2	2	2	2
Diisobutyl phthalate	>50	>50	2	2	2

LOQ (S/N > 10) was set based on measurement of a standard and the most dilute standard was 2 µg/mL. The LOQs in the CAD and the MS were the same with and without the inverse gradient (Figure 4 and Table 7). LOQ was determined by analysis of a standard at the actual concentration of the LOQ, not by extrapolation. Noise was calculated by the root of mean squares method and the time range for the noise calculation was twenty times the peak width at 50% max height. The inverse gradient did not pass through the UV.

Analysis of unknowns

The CAD is highly suited to analysis of complicated, chromophore-deficient, potentially toxic oligomers that can be extracted from plastic materials.[4] This analysis does not rely on ionization efficiency or the presence of a chromophore.

To demonstrate the utility for unknown extractable analysis, a wound dressing containing polyurethane was analyzed on the multidetector system (Figure 7) after extraction with 50/50 isopropanol/water for 72 hours. Using CAD and MS, six unknown peaks were observed and three of the six were non-volatiles that could be quantified by CAD. When compared with a mass list of potentially hazardous PU extractables,[6] one peak was tentatively identified as 9-octadecenamide.

Samples of ESBO and sterile medical PVC tubing made with ESBO were also analyzed on the multidetector system (Figure 8) after extraction in isopropanol and water. Eight unknown peaks were observed in the tubing and four were non-volatiles that could be quantified by CAD. The four assignable peaks matched the masses of di- and tri-glycerides (DG, TG), all of which are non-volatile substances. The abbreviation TG(54:6-eO) stands for a triglyceride with 54 carbons in the side chains and six epoxide groups. The peaks assigned to masses of substances with the most epoxide groups eluted first, as carbon chains containing epoxide groups are more hydrophilic than carbon chains. The peak assignment is based only on matching of the observed mass and RRT and should be confirmed with a high-resolution mass spectrometer.

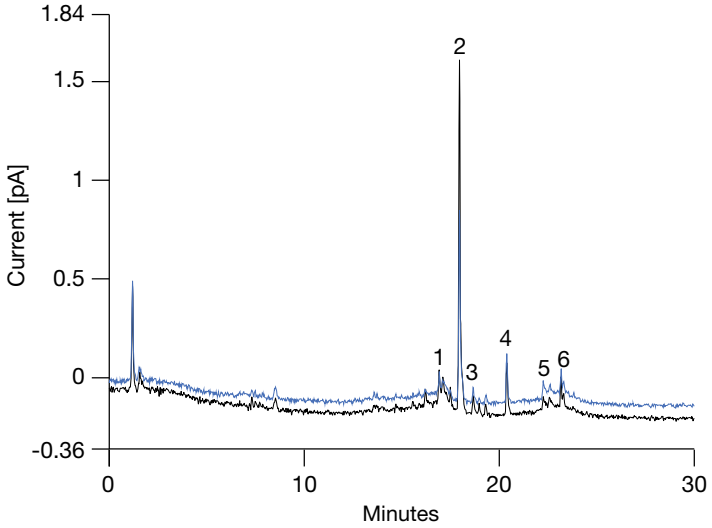


Figure 7. CAD chromatograms of a sample of polyurethane wound dressing extracted with 50:50 isopropanol:water for 72 h at 50 °C. The black trace shows analysis at EvapT = 35 °C and the blue trace shows analysis at EvapT = 50 °C. Peak numbers match those in Table 5.

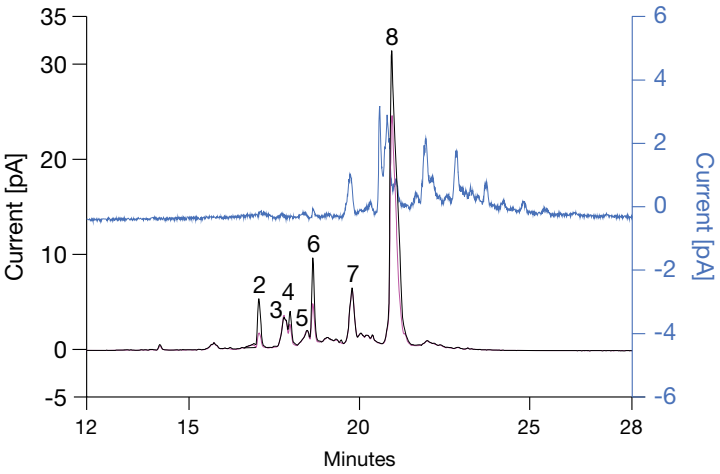


Figure 8. CAD chromatograms of a sample of pharmaceutical grade PVC tubing extracted with 50:50 isopropanol:water for 72 h at 50 °C (black trace) and an epoxidized soybean oil standard (blue trace). The black trace shows analysis at EvapT = 35 °C and the blue trace shows analysis at EvapT = 50 °C. Peak numbers match those in Table 5.

Conclusion

- A well-established method for extractables and leachables quantification and for producing data for a single-quadrupole MS library was implemented along with laboratory best practices and streamlined data analysis to save time and money and improve detector signal and quantification accuracy.
- The uncertainty factor was minimized and quantification was improved through three improvements: 1) classifying unknowns as non-volatile or semi-volatile, 2) using an inverse gradient, and 3) mixing with an inline capillary mixer after the merge point of the analytical and inverse gradients and before the flow split.
- The inverse gradient did not adversely affect MS LOQs and improved the uncertainty factor.
- Optimization of detector settings was simplified by using a single quadrupole MS.
- An in-house compound database was strengthened with analyte RRT and mass from the single quadrupole MS.
- The baseline drift in CAD and MS was avoided by remaking the mobile phases every day or every second day.

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