

A multi-detector platform comprising UV/Vis, charged aerosol, and single quadrupole mass spectrometric detection for comprehensive sample analysis

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Keywords

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Goal

Reliable verification and quantification of the presence of extraneous compounds present in a sample, such as impurities, degradation products, or extractables and leachables.

Application benefits

- Multiple complementary detectors discover peaks that would be overlooked in standard UV-MS setups.
- Unknowns are quantified by CAD and identified by MS. Known substances are quantified by CAD or UV.
- Atmospheric-pressure chemical ionization (APCI) offers an alternative ionization mode to heated electrospray ionization (HESI) for poorly ionizable compounds, like some extractables and leachables.

Introduction

Chromatographers can only analyze the contents of a sample when detectors produce a response. If the contents of a sample are well-defined, detector choice may be easy. For example, if all components are known to have a chromophore, a UV detector is well-suited. For poorly defined samples, such as samples with impurities, degradation products, or contamination by

extractables and leachables, a single detector will miss some components. Multiple detectors are needed to fully evaluate the sample.

Comprehensive sample analysis with multiple complementary detectors is essential for determining the presence of unknown or unexpected compounds. 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. The UV detector offers accurate quantification of chromophore-containing substances. The charged aerosol detector (CAD) delivers universal detection of non- and semi-volatile compounds, making it an ideal second detector. Additionally, its near uniform response enables quantification without reference standards. Mass spectrometry (MS) offers identity confirmation of the detected compounds. These three detection techniques

provide a comprehensive sample analysis platform, which was expanded further by applying two different ionization modes—HESI and APCI—for MS detection. This platform was used to analyze impurities extracted from cell culture bags.

Experimental

Chemicals and reagents

Eighteen chemicals, selected from literature reports of extractables present in cell culture bags,¹⁻³ were used as reference standards. Grades and purities are listed in Table 1. Fisher Scientific™ Optima™ LC/MS-grade acetonitrile (P/N A955-212), formic acid (P/N A117-50), isopropanol (A461-212), acetone (P/N A949), *n*-hexane (P/N H306), and methanol (P/N A456-212) were used. Ultrapure water was produced by a Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System.

Table 1. Chemicals in calibration standards

#	Analyte	CAS	Purity	Amount in 10 mL (mg)	Solvent
1	Phthalide	87-41-2	0.98	10.20	methanol
2	Phthaldialdehyde	643-79-8	0.99	10.10	methanol
3	Bis(2-hydroxyethyl) terephthalate, (BHET)	959-26-2	0.945	10.58	methanol
4	Dimethyl phthalate	131-11-3	0.99	10.10	methanol
5	Bisphenol A	80-05-7	0.99	10.10	methanol
6	Butylparaben	94-26-8	0.99	101.0	methanol
7	Tinuvin™ P	2440-22-4	0.97	10.31	isopropanol
8	Azobenzene	103-33-3	0.98	10.20	methanol
9	2,4-di- <i>t</i> -Butylphenol	128-39-2	0.99	10.10	methanol
10	Butylhydroxytoluene, (BHT)	128-37-0	0.99	10.10	methanol
11	Palmitic acid	57-10-3	0.99	10.10	acetone
12	Erucamide	112-84-5	0.85	11.76	methanol
13	Stearic acid	57-11-4	0.985	10.15	acetone
14	Tinuvin™ 234	70321-86-7	unknown	10.31	methanol
15	Irganox™ 1010	6683-19-8	0.98	10.20	methanol
16	Irgafos™ 168	31570-04-4	0.98	10.20	acetone
17	Eicosane	112-95-8	0.99	101.0	<i>n</i> -hexane
18	Tetracosane	646-31-1	0.99	101.0	<i>n</i> -hexane

System

A Thermo Scientific™ Vanquish™ Flex UHPLC system was used in two different configurations for chromatographic analysis (Figure 1 and Figure 2). In the standard setup (Figure 1), a quaternary low-pressure mixing pump delivered the analytical gradient. In the inverse setup (Figure 2), a pump module that contained two ternary low-pressure pumps delivered both the analytical gradient and the inverse gradient. The inverse gradient resulted in a constant solvent composition during the CAD and MS detection, which improved detector response uniformity. Response uniformity of CAD is fully explained in Technical Note 72806.⁴ The complementary ionization methods, heated electrospray ionization (HESI) and atmospheric pressure chemical ionization (APCI), increased detection scope.

For the inverse gradient setup, capillaries with the Vanquish standard 100 µm inner diameter (ID) delivered the analytical gradient to the analytical column and the flow cell (see Figure 2). The high viscosity of isopropanol required 130 µm ID capillaries downstream of the flow cell to keep the backpressure on the flow cell below 120 bar. Although capillaries with the smallest possible inner diameter are desirable to reduce peak broadening, the larger capillaries were needed in this case to allow a reasonable flow rate. The inverse gradient was delivered using 130 µm ID capillaries. Passive flow splitting with a ratio of 2:1 (CAD:MS) was accomplished using a T-piece. The split ratio between CAD and MS was achieved by generating double the backpressure on the branch leading to the MS relative to the one leading to the CAD. To ensure this required backpressure ratio, a 100 µm ID capillary (8) was used between the flow splitter and the CAD vaporizer needle.

The 2-position, 6-port valve in the column compartment was used to divert the flow to waste when washing the column to minimize fouling of the mass spectrometer and charged aerosol detector.

As recommended for MS applications, 200 µL mixers were used instead of standard 350 µL mixers. The smaller mixers reduced the gradient delay volume, allowing for a faster method.

Required modules

- For standard setup:
 - Thermo Scientific™ Vanquish™ Quaternary Pump F (P/N VF-P20-A)
 - 200 µL static mixer (P/N 6044.5110)
- For inverse gradient setup:
 - Thermo Scientific™ Vanquish™ Dual Pump F to replace Quaternary Pump (P/N VF-P32-A)
 - Two 200 µL static mixers (P/N 6044.5110)
- Thermo Scientific™ Vanquish™ Split Sampler FT (P/N VF-A10-A)
- Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A)
 - 2-position/6-port valve (P/N 6036.1560)
- Thermo Scientific™ Vanquish™ Diode Array Detector FG (P/N VF-D11-A)
 - 2.5 µL titanium flow cell (P/N 6083.0550)
- Thermo Scientific™ Vanquish™ Charged Aerosol Detector F (P/N VF-D20-A)
- Thermo Scientific™ Vanquish™ ISQ EC/EM Single Quadrupole Mass Spectrometer (P/N ISQEC-LC/ISQEM-ESI-APCI)
- Thermo Scientific™ Vanquish™ System Base F (P/N VF-S01-A)

Chromatography data system

The ISQ EC/ISQ EM mass spectrometer is fully integrated into Chromeleon 7.2.9 CDS, which was used for system operation and subsequent data analysis.

Standard setup

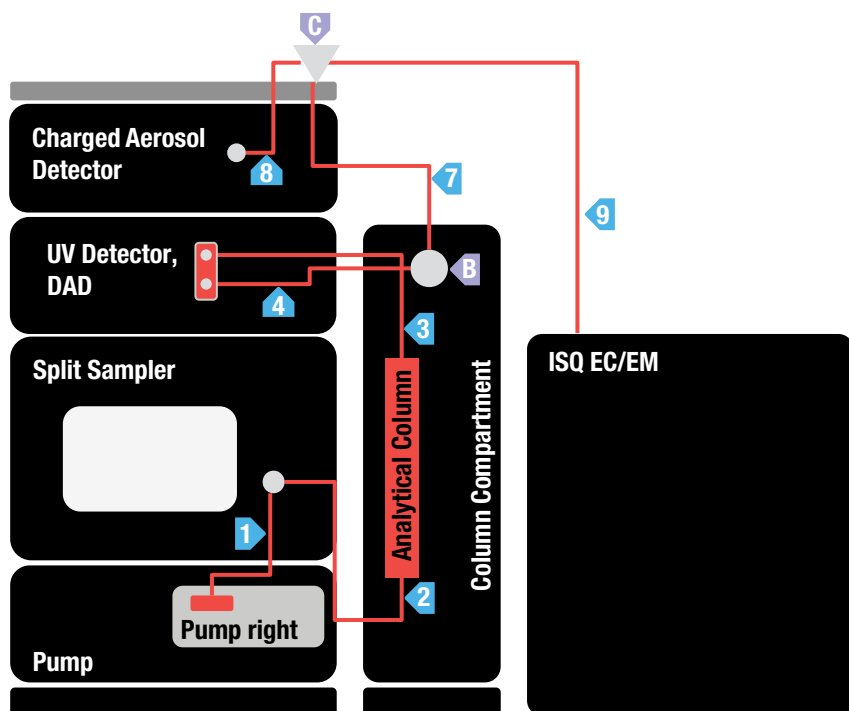


Figure 1. Schematic display of standard setup. It uses solely 100 µm ID (inner diameter) capillaries of a biocompatible nickel-cobalt-chromium alloy, MP35N.

Flow splitters and valves

No.	Additional part	Description
B	Diverter Valve	Vanquish 2-position, 6-port valve, P/N 6036.1560
C	T-piece	150 µm I.D., Vici ZT1XCS6-M

Capillaries

No.	Connection between	Description
1	Pump right outlet – Injection valve port 1	Thermo Scientific™ Viper™ capillary, ID × L 0.10 × 350 mm, P/N 6042.2340 (all capillaries in Figure 1 are MP35N)
2	Injection valve left port 2 – Column inlet	Active pre-heater, 0.1 × 380 mm, P/N 6732.0110 (optional passive pre-heater, P/N 6732.0174)
3	Column outlet – DAD	ID × L 0.10 × 300 mm, insulated, P/N 6083.2405
4	DAD – Diverter Valve	ID × L 0.10 × 250 mm, P/N 6042.2330 For VWD: ID × L 0.10 × 350 mm, P/N 6042.2340
7	Diverter Valve – Flow Splitter	ID × L 0.10 × 650 mm, P/N 6042.2370
8	Flow Splitter – Charged Aerosol Detector inlet	ID × L 0.10 × 450 mm, P/N 6042.2350
9	Flow Splitter – MS inlet	ID × L 0.10 × 750 mm, P/N 6042.2390

Inverse gradient setup

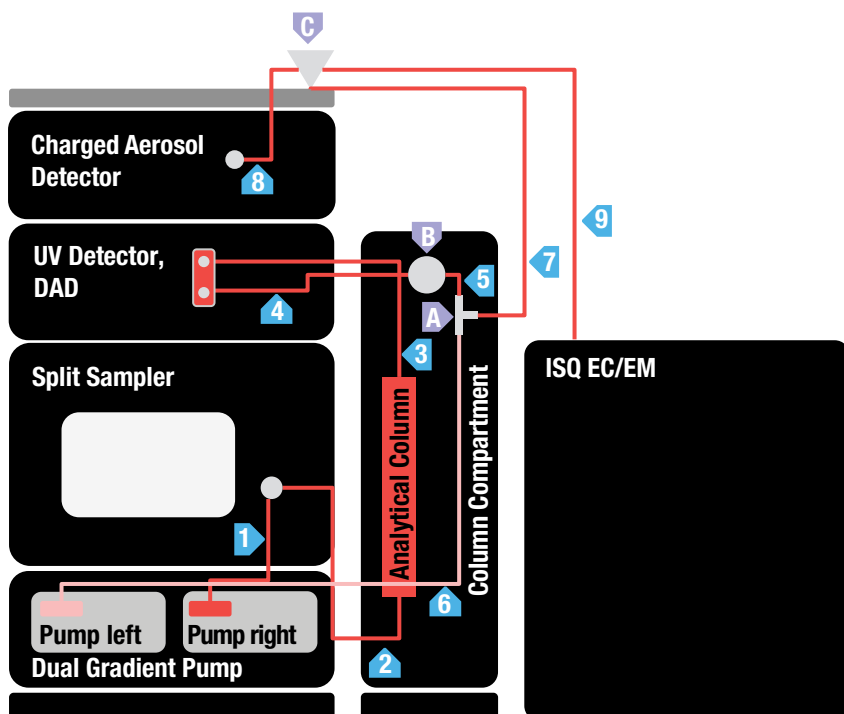


Figure 2. Schematic display of inverse gradient setup. The 100 μm ID capillaries were used for the analytical gradient delivery to the analytical column and the flow cell. Downstream of the flow cell, 130 μm ID capillaries were used. One 100 μm ID capillary (8) was used downstream of the flow cell to ensure proper flow splitting. The inverse gradient was delivered using 130 μm ID capillaries.

Flow splitters and valves

No.	Additional part	Description
A	T-piece	500 μm ID, P/N 6263.0035
B	Diverter Valve	Vanquish 2-position, 6-port valve, P/N 6036.1560
C	T-piece	150 μm I.D., Vici ZT1XCS6-M

Capillaries

No.	Connection between	Description
1	Pump right outlet – Injection valve port 1	Viper capillary, ID \times L 0.10 \times 350 mm, MP35N, P/N 6042.2340
2	Injection valve left port 2 – Column inlet	Active pre-heater, 0.1 \times 380 mm, MP35N, P/N 6732.0110
3	Column outlet – DAD	ID \times L 0.10 \times 300 mm, MP35N, P/N 6083.7950
4	DAD – Diverter Valve	ID \times L 0.13 \times 250 mm, SST, P/N 6040.2325
5	Diverter Valve – T-piece	ID \times L 0.13 \times 65 mm, SST, P/N 6040.2307
6	Pump left outlet – T-piece	ID \times L 0.13 \times 950 mm, SST, P/N 6040.2340
7	T-piece – Flow Splitter	ID \times L 0.13 \times 350 mm, SST, P/N 6040.2335
8	Flow Splitter – Charged Aerosol Detector inlet	ID \times L 0.10 \times 350 mm, MP35N, P/N 6042.2340
9	Flow Splitter – MS inlet	ID \times L 0.13 \times 750 mm, SST, P/N 6040.2320

Chromatographic procedure and detection parameters

Reversed-phase chromatography was performed using a solid core silica column and a water-to-isopropanol gradient (Table 2). The inverse gradient was programmed for a delay equivalent to 364 μL so that both the inverse and analytical gradients would arrive at the T-piece simultaneously. The delay for the inverse gradient was calculated automatically by a wizard in the Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) based on the column volume and fluidic setup.

Table 2A. Chromatographic conditions

Parameter	Setting
Column:	Thermo Scientific™ Accucore™ C18, 100 x 2.1 mm, 2.6 μm (P/N 17126-102130)
Eluents:	A: 4 mM Formic acid in water, pH 3.1 B: Isopropanol
Injection Volume:	2 μL
Analytical Gradient:	0.5 mL/min flow rate
	<i>Time [min]</i> %B
	0 5
	10.5 100
	12 100
	12.1 5
	16 5
Inverse Gradient:	0.5 mL/min flow rate
	<i>Time [min]</i> %B
	0 100
	0.728 100
	11.228 5
	12.728 5
	12.828 100
	16 100
Column Temp.:	45 °C forced air mode, 45 °C active preheater
Sampler Temp.:	4 °C

Table 2B. Detector settings

Parameter	Setting
UV Settings:	10 Hz data collection rate, 0.5 s response time, 4 nm bandwidth, 210, 220, 254, 280, 300, 320 nm and 190–345 nm (3D field)
CAD Settings:	35 °C evaporator temperature, 3.6 filter, 1.0 power function value, 10 Hz data collection rate
MS Settings:	Default HESI and APCI source settings for 0.167 mL/min (standard setup) or 0.333 mL/min flow rate (inverse gradient setup)
MS Full Scan, +: Time	0–16 min
Mass range	130–1250 <i>m/z</i>
Dwell Time	0.1 s
Polarity	Positive
Source CID voltage	10 V
MS Full Scan, -: Time	0–16 min
Mass range	130–700 <i>m/z</i>
Dwell Time	0.1 s
Polarity	Negative
Source CID voltage	10 V

Preparation of eluent

Eluent A was prepared by adding 150 μL formic acid to 1000 mL water.

Preparation of standard solution

The standard solution of 18 reference standards was prepared by first preparing each standard (except for butylparaben, eicosane, and tetracosane) as a 1 mg/mL solution in a 10 mL volumetric flask using the mass given in Table 1 and adding the solvent specified in Table 1 to the fill line. Butylparaben, eicosane, and tetracosane were prepared at 10 mg/mL. The mass in Table 1 was calculated based on purity and, for likely cations, salt formation with formic acid in the CAD.

Salt formation in the CAD and its use to stabilize semi-volatiles is addressed in Technical Note 72806.⁴ From the individual solutions, calibration standards containing all 18 standards were prepared in methanol. The calibration concentrations were 1, 2, 5, 10, 20, and 50 µg/mL, except for the semi-volatiles butylparaben, eicosane, and tetracosane, which were present in the calibration solutions at 10, 20, 50, 100, 200, and 500 µg/mL because of the partial loss of these substances in the evaporation tube.

Preparation of samples

Four different types of single-use cell culture bags, the inner layer of which was made of ethylenevinyl acetate and different density grades of polyethylene, were investigated. Extracts were prepared by rinsing with 50:50 isopropanol/water (v/v) and reducing the extracts to dryness using a vacuum concentrator. Samples were reconstituted in 70 µL 50:50 isopropanol/water (v/v).

Note on Workflow: HESI and APCI

Although HESI and APCI data are reported side-by-side in this text and although the ISQ EM can be provisioned with a single dual HESI/APCI source, the data are not measured on the same injection. Collection of both HESI and APCI data for a particular sample requires two

separate injections and instrument methods. Physical changes to the source are also required to switch modes. Collection of HESI data requires a HESI spray capillary probe. Collection of APCI data requires an APCI probe and the APCI corona needle. A physical switch on the source moves the APCI corona needle in and out of position.

Results and discussion

The 18 reference standards were analyzed with both setups. The CAD and the diode array UV/Vis detector were used to determine peak retention times. The CAD and UV detector were complementary; some peaks were only detected by the CAD, while some were only detected by the UV/Vis detector. By combining detection techniques, all 18 standards could be measured with the standard and the inverse gradient setups. Thirteen standards were detected by the UV/Vis detector, 11 by CAD and six by both detectors (Table 3). Representative UV/Vis and CAD chromatograms are shown in Figure 3. Seven analytes could not be detected by the CAD as they were too volatile.⁵ However, they possessed a sufficiently active chromophore to allow their measurement by UV/Vis detection. Conversely, five analytes with poor chromophores were not detected by UV/Vis detection but were detected by the CAD. Two analytes did not show a strong MS signal, most likely due to poor ionizability.

Table 3. Summary of results for each reference standard. Detectability with UV, CAD, MS with HESI, and MS with APCI is indicated with check marks. Cells in dark gray indicate substances for which the relative MS peak area using one ionization mode was at least 20-fold greater than the relative peak area using the other source. [M] refers to the monoisotopic mass. LOQ refers to the CAD limit of quantification (except where noted as UV) defined as a signal-to-noise (S/N) ratio of larger than 6:1 for the standard at a given concentration, relative to the noise measured in a blank sample.

#	Analyte	UV	CAD	MS (HESI)	MS (APCI)	[M]	Detected m/z, HESI / APCI	Detected Ion HESI / APCI	LOQ (µg/mL)
1	Phthalide	✓		✓	✓	134.0	135.1	[M+H] ⁺	5 (UV)
2	Phthaldialdehyde	✓		✓	✓	134.0	135.1	[M+H] ⁺	5 (UV)
3	BHET	✓	✓	✓	✓	254.1	255.1	[M+H] ⁺	1
4	Dimethyl phthalate	✓		✓	✓	194.1	195.1	[M+H] ⁺	1 (UV)
5	Bisphenol A	✓	✓	✓	✓	228.1	227.1 / 228.1	[M-H] ⁻ / [M] ⁺	1
6	Butylparaben	✓	✓	✓	✓	194.1	195.1	[M+H] ⁺	50
7	Tinuvin P	✓		✓	✓	225.1	226.1	[M+H] ⁺	1 (UV)
8	Azobenzene	✓		✓	✓	182.1	183.1	[M+H] ⁺	1 (UV)
9	2,4-di-t-Butylphenol	✓		✓	✓	206.2	205.2 / 206.2	[M-H] ⁻ / [M] ⁺	1 (UV)
10	BHT	✓		✓	✓	220.2	219.2 / 220.2	[M-H] ⁻ / [M] ⁺	1 (UV)
11	Palmitic acid		✓	✓	✓	256.2	255.2	[M-H] ⁻	1
12	Erucamide		✓	✓	✓	337.3	338.3	[M+H] ⁺	1
13	Stearic acid		✓	✓	✓	284.3	283.3	[M-H] ⁻	1
14	Tinuvin 234	✓	✓	✓	✓	447.2	448.2	[M+H] ⁺	1
15	Irganox 1010	✓	✓	✓	✓	1176.8	1193.8 / 1176.8	[M+NH ₄] ⁺ / [M] ⁺	1
16	Irgafos 168	✓	✓	✓	✓	646.5	647.5	[M+H] ⁺	1
17	Eicosane		✓			282.3			50
18	Tetracosane		✓			338.4			50

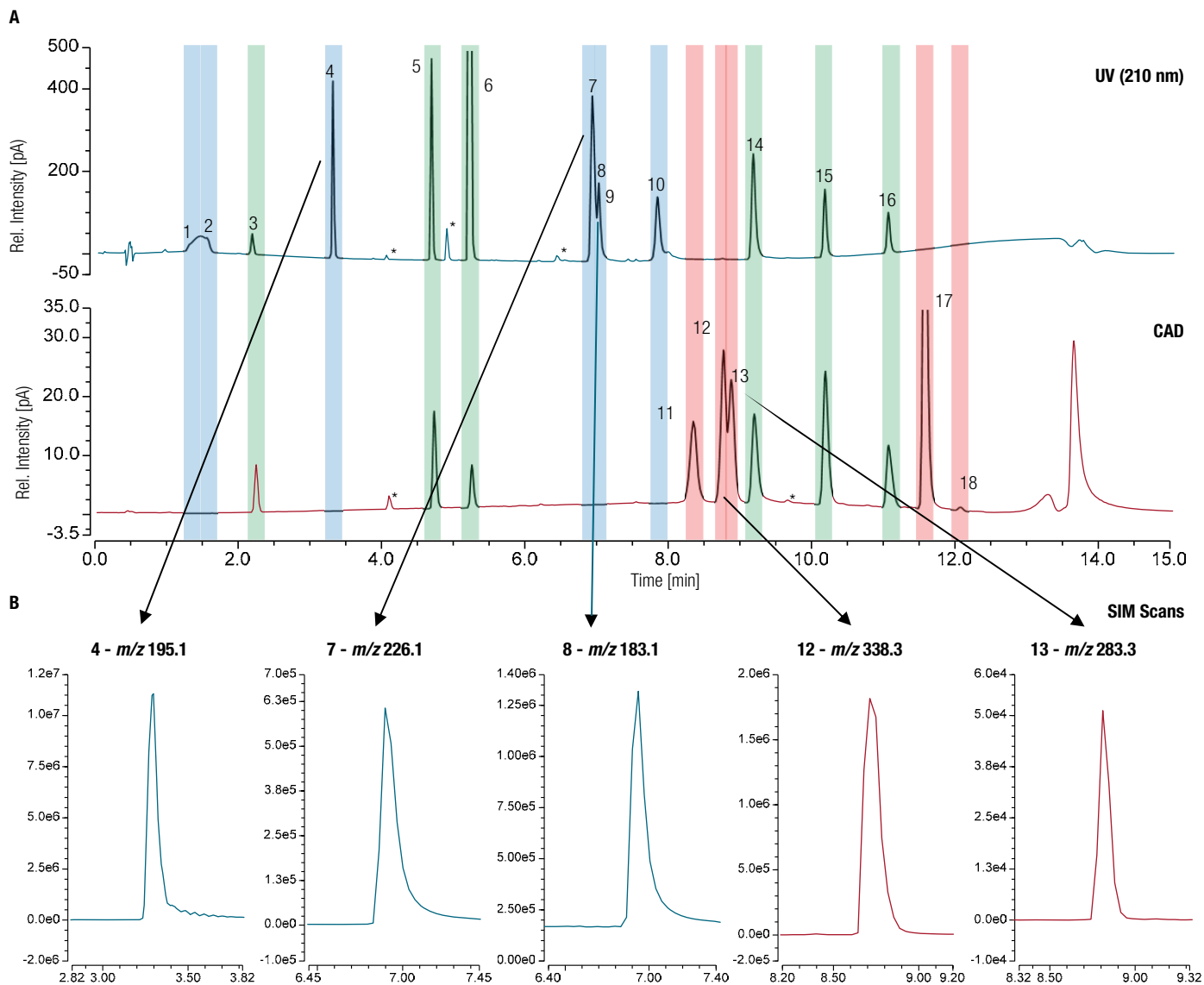


Figure 3. (A). Representative UV/Vis and CAD chromatograms of an analysis of a reference standard mix using the standard setup. Blue indicates analytes detected only by UV/Vis, red highlights analytes detected only by CAD, and green indicates analytes detected with both UV/Vis and CAD. Asterisks indicate impurities present in analytical standards. Sample concentration was 50 $\mu\text{g/mL}$, except for butylparaben, eicosane, and tetracosane. These substances were prepared at 500 $\mu\text{g/mL}$ because they are semi-volatiles with higher CAD LODs. The identification numbers refer to the standard names listed in Table 3. **(B).** Representative extracted ion chromatograms (XICs) of 5 analytes using the HESI source. The identification numbers in the heading refer to the names in Table 3. The shown m/z are the values used for the single ion monitoring (SIM) scans.

Sixteen compounds could be clearly detected with the single quadrupole mass spectrometer (Table 3) by HESI and APCI. Mass confirmation was based on detection of the respective m/z species in positive or negative mode in full scan and SIM scan at the same elution time as observed by UV/Vis detection or charged aerosol detection. Five representative extracted SIM scans are

shown in Figure 3. Substances that showed better relative peak areas with APCI were azobenzene and BHT. Samples with better relative peak areas with HESI were BHET, palmitic acid, and stearic acid. Extracted SIM scans comparing detectability of BHT and palmitic acid with APCI and HESI are shown in Figure 4.

uniformity of the CAD with inverse gradient is readily apparent. The response curves in Figure 5B show greater similarity than the ones in Figure 5A, as reflected by the greater similarity between the response curves. The inverse gradient results in shallower response curves for substances that elute in predominantly aqueous conditions and steeper response curves for substances that elute in mostly organic conditions.⁴ This equalization facilitates the use of a single calibrant for quantification of analytes where standards may not be available. This capability is demonstrated in Figure 6, which shows quantification of different analytes using a single calibrant

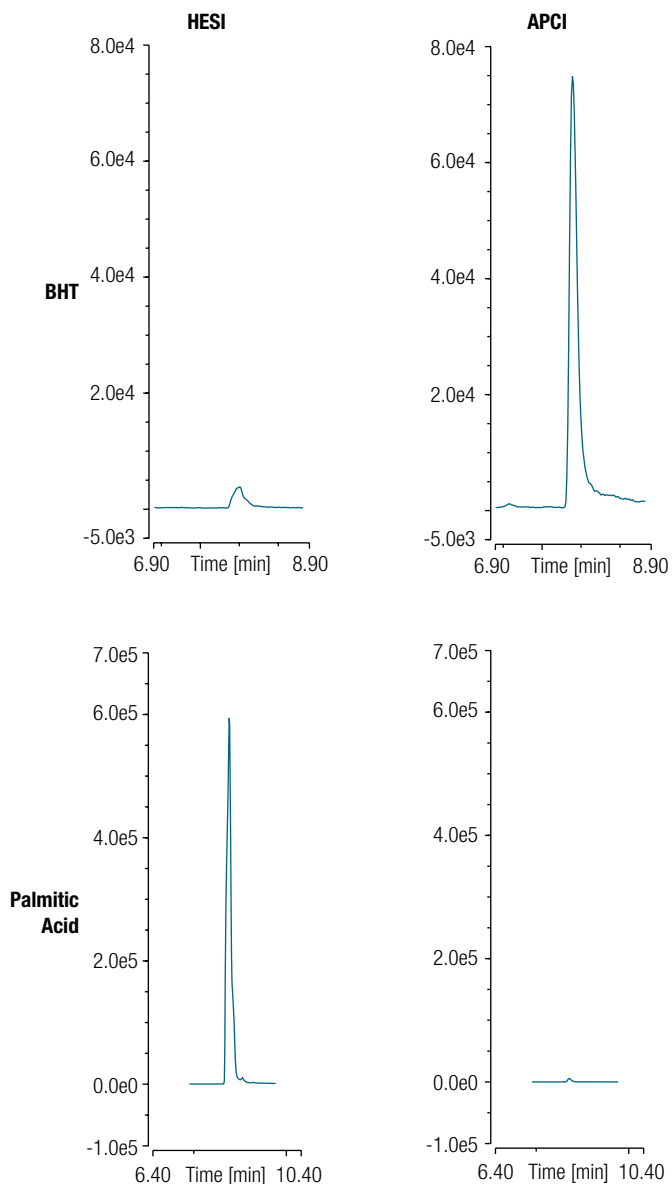


Figure 4. Examples of substances that showed differences in detection sensitivity between APCI and HESI. BHT was better detected with APCI, palmitic acid with HESI. (Top row, BHT, HESI shows SIM scan with negative polarity for m/z 219.2. APCI shows SIM scan with positive polarity for m/z 220.2, bottom row, palmitic acid, SIM scans with negative polarity for m/z 255.2).

Calibration curves for quantification by CAD in the presence and absence of the inverse gradient were compared (Figure 5). With the inverse gradient, the overlap of the calibration curves, and consequently the uniformity of response, was better than without.

The overall benefit of the inverse gradient is that response is more uniform and quantification with a universal calibrant is possible. Improved response

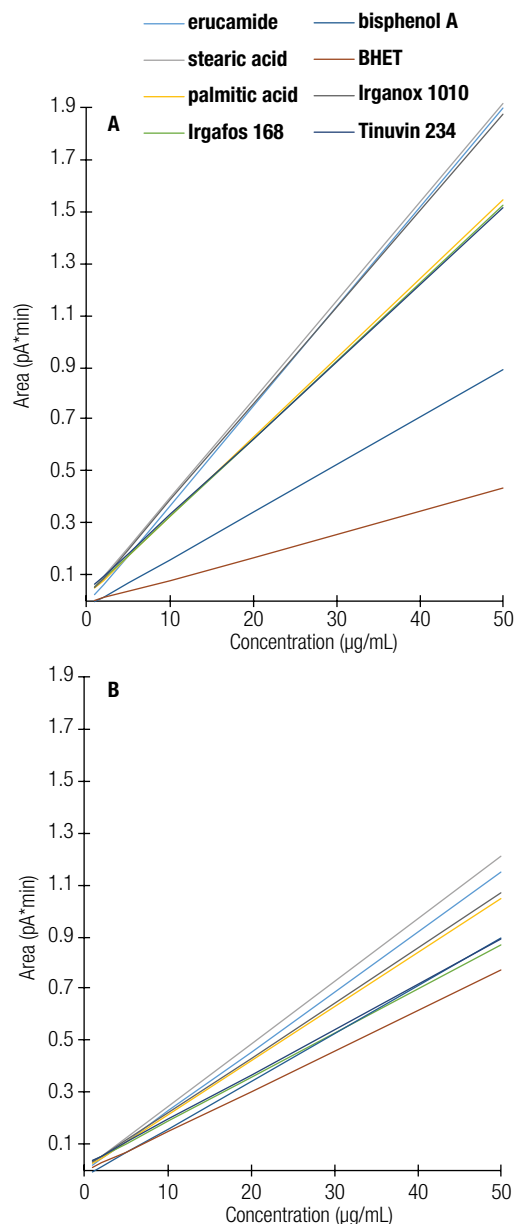


Figure 5. Calibration curves for quantitation by CAD without (A, top) and with (B, bottom) the inverse gradient. Curves for butylparaben, tetracosane, and eicosane are not shown because their volatility results in a limit of quantification of 50 $\mu\text{g/mL}$.

(bisphenol A). Bisphenol A was chosen because it elutes at roughly the midpoint of the gradient. When the inverse gradient was applied, estimated amounts for eight analytes were much closer to the target of 20 $\mu\text{g/mL}$ than in the absence of the inverse gradient. The three semi-volatile analytes, butylparaben, eicosane, and tetracosane, had a lower than expected response and could not be accurately quantified by a single universal calibrant (see also Figure 6). They are not shown in Figure 5 because they could not be quantified below a concentration of 50 $\mu\text{g/mL}$. Apart from analyte volatility, salt formation can also affect response; these factors and methods for identifying semi-volatiles are explained in detail in Technical Note 72806.⁴

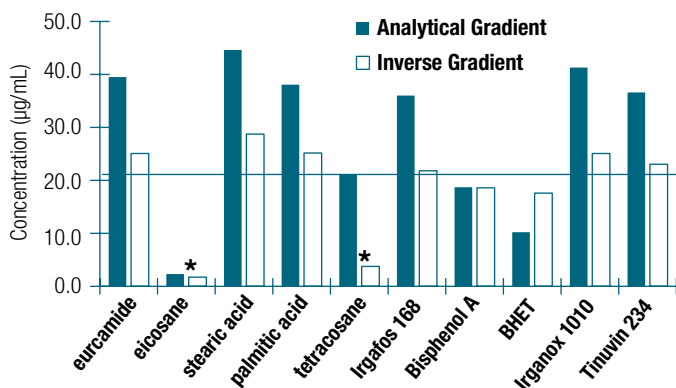


Figure 6. Calibration using a universal calibrant (bisphenol A) with and without the inverse gradient. Using an inverse gradient setup results in a more uniform signal response for the CAD and hence more accurate quantitation with a universal standard. The asterisks indicate semivolatiles, which have a lower response and a limit of quantification of only 50 $\mu\text{g/mL}$.

Comparison of the response uniformity of UV and CAD (Figure 7) confirms that the CAD is more accurate for the quantitation of unknowns. The response of the UV detector to a small molecule depends on the molar

extinction coefficient of the molecule, which can range from 0 to over 10,000 $\text{M}^{-1}\text{cm}^{-1}$. Thus, analytes can show a strong or a weak response at a given wavelength depending upon the chromophore present. CAD response is largely independent of an analyte's chemical and physical properties.⁵

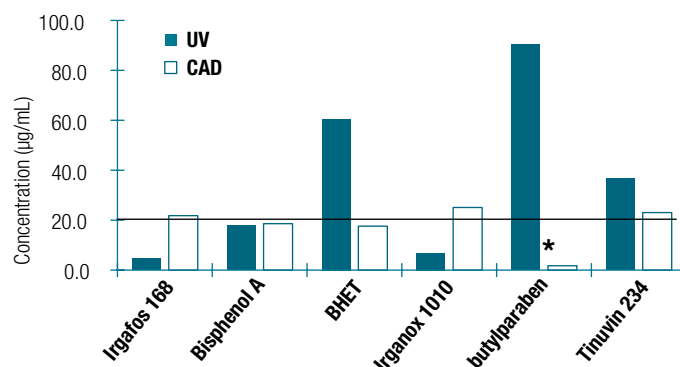


Figure 7. Comparison of quantification of a reinjected 20 $\mu\text{g/mL}$ standard by UV and CAD using a universal calibrant (bisphenol A). The CAD provides uniform response and thus more accurate quantitation than UV if a universal calibrant is employed. Butylparaben was identified as a semi-volatile in the CAD and could not be quantified by the universal calibrant because of its limit of detection of 50 $\mu\text{g/mL}$.

Sample analysis

Analysis of contaminants that leach from cell culture bags used in biopharmaceutical production is critical to prevent toxic extractables and leachables from ending up in the final product and potentially harming the patient. The inner layers of four different brands of single-use cell culture bags were extracted with isopropanol and water. The polymer composition of the inner layer of these bags was ethylenevinyl acetate and different density grades of polyethylene. Extract analysis revealed high levels of the UV-invisible slip agent, erucamide, in three out of four extracts (Table 4 and Figure 8).

Table 4. Known and unknown extractables from cell culture bags (Samples A, B, C, and D) and from the microcentrifuge tubes used to prepare the samples (labeled with a dash, —). Eleven additional smaller peaks (data not shown), were also detected and quantified by CAD. Abbreviations: bDtBPP = bis(2,4-di-*tert*-butylphenyl)phosphate; TBPP-ox = oxidized Irgafos 168; IPA = isopropanol; RT = retention time.

RT (min)	UV	CAD	HESI - MS	Amount (µg/mL)					Detected Mass	Possible Identity
				—	A	B	C	D		
7.14		✓	✓	5.5	5.2	5.2	5.6	5.2	325.3 (+)	unknown
7.29	✓	✓	✓	x	1.9	1.7	3.1	x	374.3 (+)	unknown
7.66		✓	✓	5.9	4.6	8.9	3.8	6.2	375.4 (-)	unknown
7.73	✓	✓	✓	149	144	111	144	136	473.4 (-)	bDtBPP, [M-H] ⁻
8.28	✓	✓	✓	124	131	95.6	132	115	403.4 (-)	unknown
8.72	✓	✓	✓	x	31.8	31.9	36.8	2.1	338.3 (+)	erucamide, [M+H] ⁺
8.83		✓	✓	3.0	3.7	3.5	32.1	4.0	283.3 (-)	stearic acid, [M+H] ⁺
10.17	✓	✓	✓	1.9	15.8	16.2	15.2	8.2	663.5, 685.5, 723.5 (+)	TBPP-ox, [M+H] ⁺ , [M+Na] ⁺ , [M+H+IPA] ⁺⁺
10.88	✓	✓	✓	1.4	1.9	2.0	1.7	3.1	279.2, 366.2 (+)	unknown

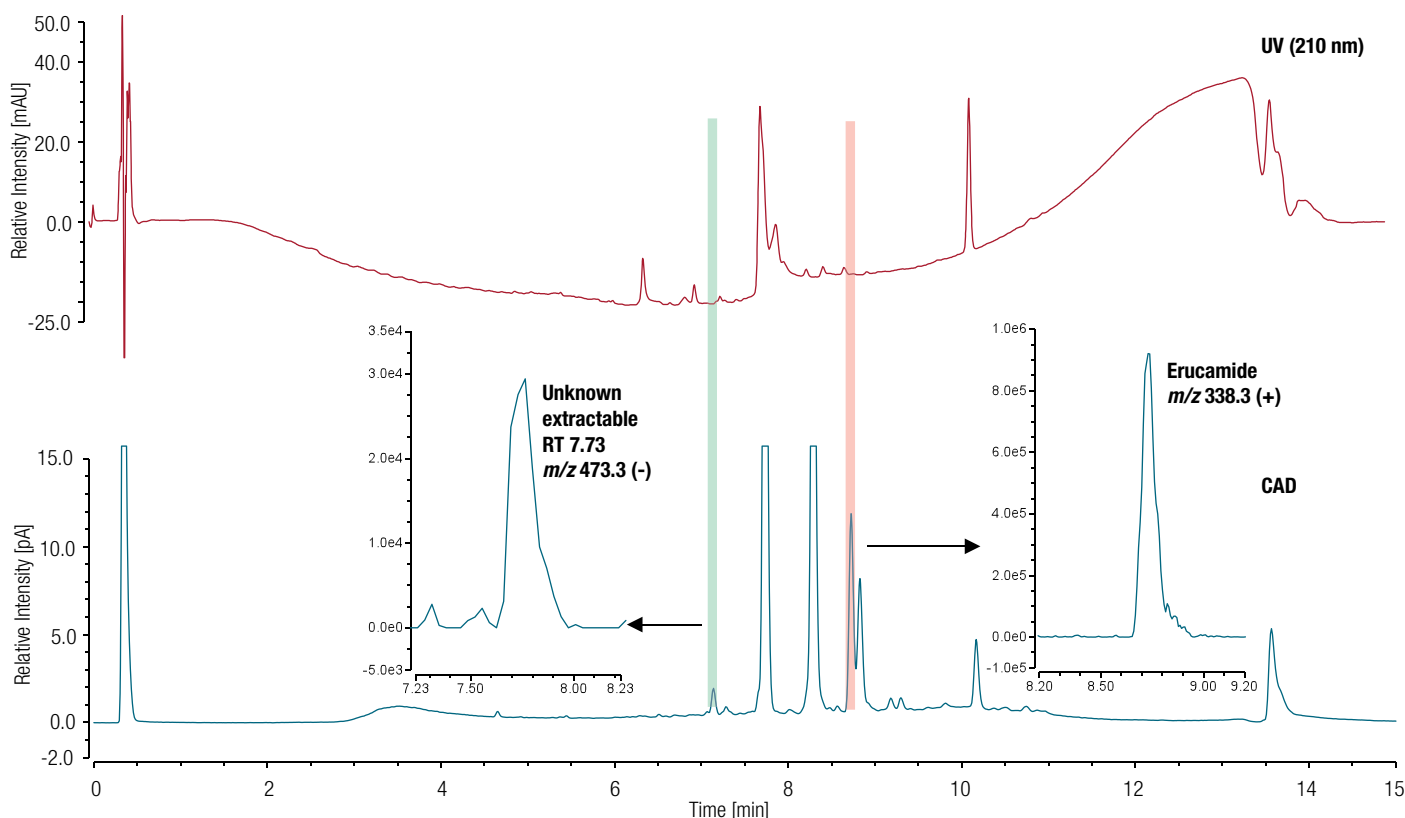


Figure 8. Analysis of cell culture bag lining extracts by UV/Vis detector, the CAD, and the MS. UV (210 nm) and CAD chromatograms of Sample C. Several extractables were detected with both detection modes. Insets: XICs of full scans using HESI of two extractables found in several samples. One is an unknown extractable with a retention time of 7.73 minutes and an *m/z* of 473.3 in negative mode. The other is erucamide.

Two derivatives of Irgafos™ 168 were also present in many of the samples (Table 4), as previously described.¹ In total, all 19 unknowns and two known substances (stearic acid and erucamide) found in the bags were quantified by the universal calibration curve, that for

bisphenol A (Figure 9 and Table 4). The MS allowed mass assignments for all extracted substances. Two unknowns, bis(2,4-di-*tert*-butylphenyl)phosphate and an oxidized form of Irgafos 168, could be identified based on their detected mass and previous reports.¹

Ionization mode choice

Either HESI or APCI proved suitable for this analysis. Sixteen of eighteen substances in the calibration standard were detected with both sources, however sensitivities varied between the ionization modes. Ionization mode choice is based on analyte type. APCI is a gas phase ionization process that is well-suited to analytes that do not protonate or deprotonate in the liquid phase, in contrast to HESI, which is a process that relies on ions present in the liquid phase. Conversely, HESI is more suitable for thermolabile compounds that suffer under the harsher conditions of APCI. If in doubt, analyzing the same sample by the two different ionization modes minimizes the risk of missing peaks due to poor compound ionization.

Platform choice

When the sample contains unknown substances for which standards do not exist, the inverse gradient multidetector setup should be used to quantify these substances by CAD. Peak identification should be performed by MS and supported by UV 3D/contour plots. If standards exist for all peaks in a sample, a multi-detector setup with only an analytical gradient can be used. Quantification is performed by the complementary CAD and UV detectors, and MS should be used for peak confirmation.

Conclusion

- The UV/Vis detector and the CAD complement each other, resulting in comprehensive sample analysis.
- The inverse gradient multi-detector setup enables the use of a single calibrant that allows quantification of compounds that are not available as reference standards or whose identity is unknown.
- Mass spectrometric detection provides additional information on the detected analytes enabling confirmation of known compound identities or tentative identification of unknown compounds. For more complete sample characterization two ionization modes, APCI and HESI, can be applied.

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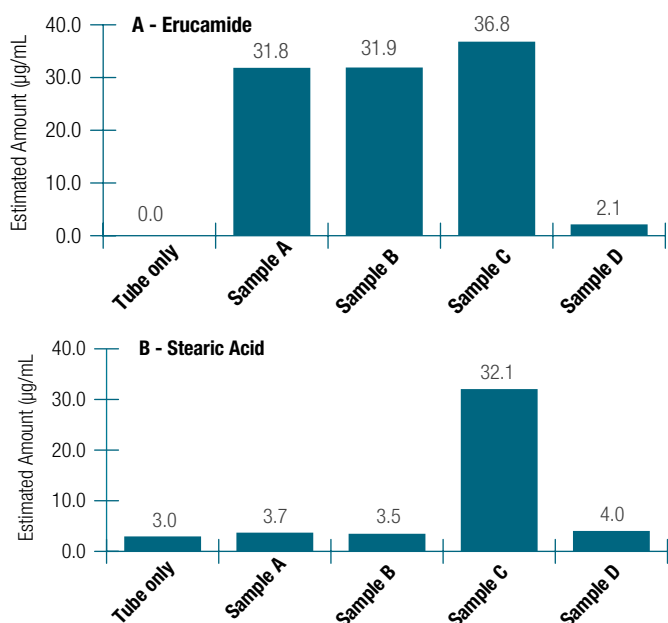


Figure 9. Analysis of cell culture bag lining extracts by UV, CAD, and MS. (A). Estimated amounts of the slip agent, erucamide. **(B).** Estimated amounts of the coating agent, stearic acid.

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