Online Extraction and Determination of Octylglucoside by Reversed-Phase High-Performance Liquid Chromatography with Evaporative Light-Scattering Detection

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Abstract

A reversed-phase high-performance liquid chromatography method using evaporative light-scattering detection is developed for the determination of residual octylglucoside (OG) levels after a detergent exchange step for in-process samples of a vaccine antigen. The reversed-phase column not only provides separation of the OG but also functions as an extraction column to remove the vaccine antigen from the sample, thereby eliminating off-line sample manipulations. In addition to column selection, the mobile phase is optimized to enhance extraction and separation. The vaccine antigen is irreversibly bound to the column, allowing nonprotein components to interact with the column for separation and elution. The assay is linear over the range of 0.00050–0.050% OG. Precision tested at 0.0010% and 0.0050% OG is 2.9% and 7.2% relative standard deviation, respectively. The limits of quantitation and detection are determined to be 0.00050 and 0.000125% OG, respectively. Accuracy is determined to be 103 and 98%, based on spike recoveries of 0.0010% and 0.0050% OG, respectively.

Introduction

Octylglucoside (OG) is a nonionic surfactant belonging to the general class of alkyl-polyglycosides, which are composed of one or more carbohydrates and a fatty alcohol alkyl chain typically ranging from 7 to 15 methylene groups. Industrial processes usually produce a mixture of alkyl-polyglycosides consisting of multiple glucose units and varying alkyl chain lengths; therefore, these preparations may not be discrete molecules. The alkyl-polyglycoside (OG) used in this study is composed of a discrete species that includes a monoglycoside with an eight-carbon fatty alcohol chain.

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Alkyl-polyglycosides not only have good toxicological, dermatological, and ecological properties (uses include cosmetic formulations and cleaning agents), but are also used as solubilizers of membrane proteins (1). The solubilization properties are useful in the production of biopharmaceuticals. Thus, the determination of OG concentration, as a potential process-related impurity, is important in pharmaceutical-grade protein preparations.

A number of analytical methods have been described for the analysis of alkyl-polyglycosides. These assays can be subdivided into two categories: bulk-type analysis (measurement of total amount) and discrete molecule analysis requiring a separation step prior to detection. Enzyme-based assays have been developed to quantitate alkyl-polyglycosides (2–3). The carbohydrate and fatty alcohol chain are conducive to enzymatic cleavage or reaction (or both). Incubation with a carbohydrolase releases the glucose moiety, which is then exposed to an enzyme electrode coated with glucose oxidase, thereby producing hydrogen peroxide. The amount of hydrogen peroxide formed is correlated to the concentration of the alkyl-polyglycoside(s). Following the quantitation of glucose after cleavage of the glucose moiety, an alcohol dehydrogenase is added, forming the reduced form of nicotinamide adenine dinucleotide, which is then measured spectrophometrically and correlated to the amount of the fatty alcohol chain. Photometric determination of alkyl-polyglycosides in various industrial preparations was described using anthrone to form a color derivative, measured at 620 nm (4).

The inherent structural characteristics of alkyl-polyglycosides preclude the use of more desirable methods of analysis, particularly detection after chromatographic separation. Because of the absence of strong chromophores, UV detection requires low (190–200 nm) wavelengths. A high-performance liquid chromatography (HPLC) method using UV and refractive index (RI) detection has been demonstrated (5). This low wavelength precludes the use of a majority of HPLC solvents that are not transparent. Even though RI detection is compatible with any solvent,

provided that the RI of the solvent and analyte differ, the required level of sensitivity may not be achieved, and gradient elution is not practical. To circumvent these detection issues, a high-performance thin layer chromatography (HP-TLC) method was described using a tymol/sulfuric acid and sulfuric acid/heat for visualization (5–6). Although HP-TLC can analyze multiple samples within a single plate, the required sensitivity might not be obtained. Mass spectrometry (MS) provides a very sensitive detection platform, especially coupled to a separation system. Gas chromatography (GC) and HPLC have been coupled to a mass spectrometer for the determination of alkyl-polyglycosides (7–8). The low volatility of alkyl-polyglycosides requires derivitization to trimethylsilylethers for GC separation. Derivitization increases the number of sample manipulation steps and thus increases analyte losses. The HPLC–MS method was developed for the analysis of a mixture of alkyl-polyglycosides and metabolic products in municipal wastewater. High sensitivity was achieved in spite of analyte recoveries in the range of 66–98% using solid-phase extraction (SPE).

The lack of significant UV chromophores and low volatility make alkyl-polyglycosides ideal analytes for evaporative light-scattering detection (ELSD). A primary criterion for ELSD is that all components comprising the mobile phase must be volatile. An extensive study of the behavior of alkyl-glycoside homologues on several reversed-phase (RP) columns with ELSD showed that retention not only was affected by the length of the fatty alcohol chain but also was influenced by the nature of the glycoside S- or O-bonding (9). SPE was used to identify an alkyl-diglycoside from a technical-grade preparation of alkyl-polyglycoside containing greater than 50% alkyl-monoglycosides (10). The extraction on a styrene–divinylbenzene SPE provided separation and enrichment for RP-HPLC–MS and NMR structural characterization.

RP-HPLC of low-molecular-weight analytes in complex biological samples containing excessive amounts of proteins usually requires removal of the protein species to minimize chromatographic degradation. To eliminate and minimize protein interactions with the stationary phase, restricted-access materials (RAMs) have been developed (11–14). The RAMs are applied primarily to a biological-type sample (such as serum) in which the analyte is typically a small-molecule drug. The RAM does not allow the contaminating proteins to interact with the hydrophobic stationary phase and are eluted in the void volume; on the other hand, the small molecules are able to interact with the hydrophobic phase. An alternative procedure was investigated: instead of eliminating the interaction of the protein with the stationary phase, conditions were optimized to irreversibly bind the protein species onto the stationary phase and allowing the low-molecular-weight components to interact and separate.

This paper describes the analysis of a single discrete alkyl-monoglycoside. High-purity OG is used in the purification of a vaccine antigen and is considered to be a potential process-related impurity above a minimum level, thereby requiring a sensitive method for determination by HPLC with ELSD. This analytical method is quality control "friendly" and is capable of high throughput.

Table I. Gradient and Flow-Rate Program

Time	Flow rate (mL/min)	%A	%B
0.0	0.75	90	10
4.9	0.75	90	10
5.0	0.35	90	10
5.05	0.35	65	35
8.05	0.35	15	85
10.0	0.35	15	85
11.0	0.35	90	10
11.1	0.75	90	10
14.9	0.75	90	10
15.0	0.35	90	10

Experimental

Reagents, solutions, and OG standards

HPLC-grade acetonitrile, sodium phosphate dibasic (heptahydrate), and sodium chloride were obtained from J.T. Baker (Muskegon, MI). Sodium phosphate monobasic (monohydrate) was obtained from EM Science (Gibbstown, NJ). HPLC-grade water was prepared from a Millipore Milli-Q system (Bedford, MA). Analytical-grade (≥ 99%) *n*-octyl-β-D-glucopyranoside (OG) was from Alexis Biochemicals (San Diego, CA) and protein-grade Triton X-100 was from Calbiochem (San Diego, CA). The final vaccine antigen (formulation) buffer composition consisted of 27.5mM sodium phosphate–165mM sodium chloride–0.050% Triton X-100 (pH 7.2). A 1.0% OG stock solution was prepared by dissolving 0.10039 g in 10 mL of the vaccine antigen buffer. The

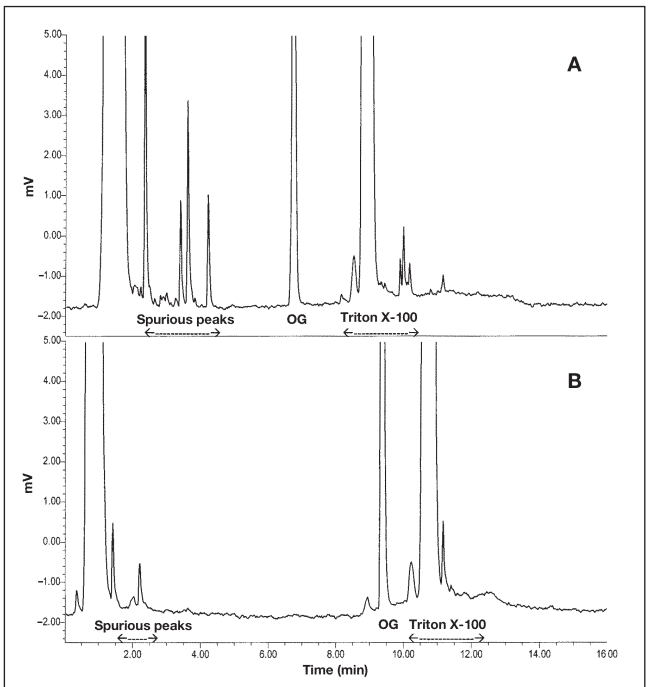

Figure 1. (A) 0.0050% OG standard (500 ng injected), initial gradient conditions: 0.0–4.0 min (20–35% B at 0.35 mL/min), 4.0–6.0 min (35–95% B) at 0.35mL/min), 6.0–8.0 min (95% B at 0.35 mL/min); (B) 0.0050% OG standard, optimized gradient conditions are described in the text.

following OG concentrations (v/v) were prepared by serial dilutions of the 1.0% solution for generation of a calibration curve: 0.10, 0.050, 0.010, 0.0050, 0.0025, 0.0010, and 0.00050%. All dilutions were performed with the vaccine antigen buffer. Reference material of the vaccine antigen was used for method development studies, and samples (peak pools) for analysis were obtained from a cation exchange column (Shire Biologics, Northborough, MA).

HPLC system and method parameters

The HPLC system consisted of a Waters Alliance 2690 (Milford, MA) and a Sedex 75 ELSD (Cedex, France). A Vydac Protein C4 column (150 × 2.1 mm, 300 Å, 5 µm) (Southborough, MA) was used at ambient temperature. Mobile phases A and B were HPLC water and acetonitrile, respectively. The optimized gradient and flow rates are listed in Table I. The ELSD parameters were: temperature at 40°C, pressure of 3.9–4.2 bar, and gain (sensitivity) of 7. The injection volume was constant at 10 µL.

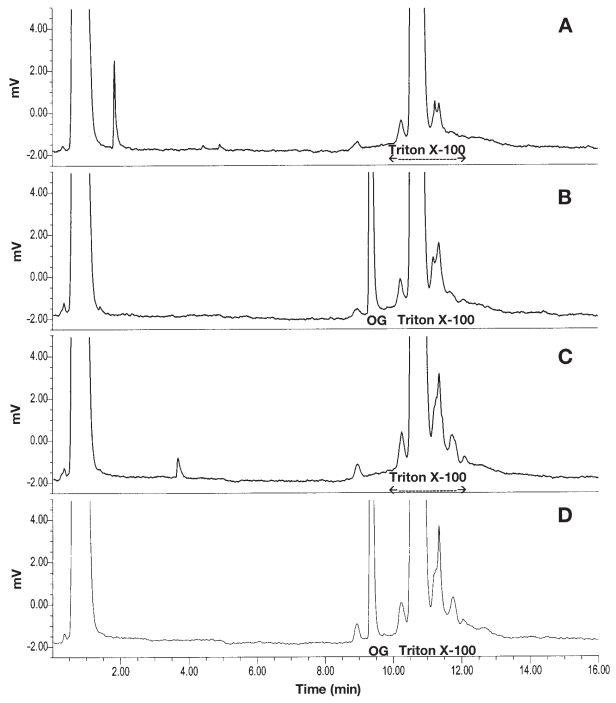

Figure 2. Representative chromatograms of (A) vaccine antigen buffer, (B) 0.0050% OG standard (500 ng injected), (C) vaccine antigen, and (D) 0.0050% OG in vaccine antigen (2.5 µL of 1.0% OG diluted to 500 µL with antigen). The conditions are described in the text.

Table II. Effect of ELSD Temperature on OG Peak Area and Height

	OG peak area		OG peak height	
Injection no.	40°	60°	40°	60°
1 (vial 1)	254120	203631	51054	41526
2 (vial 1)	255222	213656	49013	42678
1 (vial 2)	–	208305	–	41060
2 (vial 2)	–	217493	–	43737

Results and Discussion

Chromatographic optimization studies

The methodology was designed to perform an online extraction of the vaccine (protein) antigen. The online extraction would be advantageous by eliminating sample preparation step(s) in which analyte losses can occur. Removal of the antigen was necessary because of the possible interferences caused by the greater amount of antigen relative to the potential trace levels of OG. Online extraction and irreversible column retention of the vaccine antigen were successful, as a result of the mobile phase solvents and column selected.

During late-stage purification, a detergent exchange step (OG to Triton X-100) was used through cation-exchange chromatography. The analyte mixture not only consisted of the protein antigen, but also Triton X-100 and nonvolatile buffer components (phosphate and sodium chloride). The HPLC conditions were designed to allow Triton X-100 and OG to interact with the stationary phase and were resolved by gradient elution. The nonvolatile components, especially the sodium chloride, were problematic. Injections of the antigen buffer produced spurious peaks that were random not only in number, but also in retention time and intensity, regardless of injection, and were observed generally within the first 10 min of the chromatographic separation. At times these spurious peaks would coelute with OG prepared in this buffer. The primary source of these peaks was sodium chloride, although nonvolatile phosphate was also present. The phosphate did not produce significant spurious peaks because of its considerably lower concentration with respect to the sodium chloride.

A number of method optimization studies were conducted to

Table III. OG Linearity and Precision Results

OG standard (%)	Injection no. peak area	Mean peak area (%RSD peak area)	Peak area log	Mean log area (%RSD log area)
0.00050	1) 4385	4385	1) 3.64	3.64
	2) 4017	(6.4)	2) 3.60	(0.78)
	3) 4568		3) 3.66	
0.0010	1) 13580	13580	1) 4.13	4.11
	2) 12827	(3.8)	2) 4.11	(0.41)
	3) 12597		3) 4.10	
0.0025	1) 70649	70649	1) 4.85	4.81
	2) 63270	(8.1)	2) 4.80	(0.79)
	3) 59380		3) 4.77	
0.0050	1) 191016	191016	1) 5.28	5.27
	2) 181177	(2.8)	2) 5.26	(0.24)
	3) 182445		3) 5.26	
0.010	1) 544653	544653	1) 5.74	5.73
	2) 533024	(2.0)	2) 5.73	(0.15)
	3) 523075		3) 5.72	
0.050	1) 5287152	5287152	1) 6.72	6.72
	2) 5200629	(0.83)	2) 6.72	(0.05)
	3) 5231605		3) 6.72	
0.10	1) 9328645	9823645	1) 6.97	6.97
	2) 9271286	(1.8)	2) 6.97	(0.11)
	3) 9588271		3) 6.98	

eliminate or minimize the number and randomness (i.e., elution time) of the spurious peaks. Total elimination of the spurious peaks was not possible; however the randomness was controllable. The mobile phase and gradient conditions were adjusted to elute the spurious peaks to occur within a specified elution time (Table I, lines 1 and 2). By an increase of flow rate during isocratic conditions, the spurious peaks eluted within the first 3–4 min. Representative chromatograms illustrating spurious peaks before and after method optimization are shown in Figure 1. In Figure 1A, spurious peaks are observed between the nonretained peak and OG (500 ng injected); although a single chromatogram is illustrated, these peaks varied from injection to injection without regard to sample composition (i.e., OG concentration). Figure 1B is also a 500-ng OG injection with the optimized chromatographic conditions in which spurious peaks are clearly not an issue.

Studies were conducted to optimize the separation of OG and Triton X-100. A two-step gradient was required because of the mobile phase constraint imposed by the conditions for elution of the spurious peaks. The flow rate was reduced at 5.0 min, and a step gradient (0.05 min) was used to quickly increase the acetonitrile concentration (see Table I, line 4), because no components eluted within this change in acetonitrile. To resolve the OG and Triton X-100, a 3.0-min gradient (Table I, line 5) was used, as shown in Figure 1B.

To demonstrate the irreversible column binding of the vaccine antigen, injections of the antigen with and without OG were evaluated (Figure 2A–D). The antigen buffer is shown in Figure 2A, in which the only component detected as a series of peaks is the Triton X-100. A standard solution of 0.0050% OG in the buffer is shown in Figure 2B; very good resolution was observed between the OG and Triton X-100 peaks. The vaccine antigen is shown in Figure 2C, in which there is no detection of either the vaccine antigen or OG. The vaccine antigen spiked with 0.0050% OG is shown in Figure 2D. The presence of the vaccine antigen did not interfere with the recovery of OG as demonstrated by comparison of OG peak areas. The mean peak areas (n = 4) of the standard OG solution and the OG spike in the vaccine antigen were 195866 and 201234, respectively, demonstrating greater than 97% recovery.

Table IV. OG Accuracy Results

OG standard (%)	Determined concentration (%)	Mean %OG (%RSD)	%Normalized residuals*
0.00050	1) 0.00048	0.00047	1) –4.6
	2) 0.00045	(4.2)	2) –9.8
	3) 0.00049		3) –2.0
0.0010	1) 0.0099	0.0096	1) –1.0
	2) 0.0095	(2.5)	2) –4.6
	3) 0.0094		3) –5.7
0.0025	1) 0.0029	0.0027	1) 14.7
	2) 0.0027	(5.7)	2) 6.8
	3) 0.0026		3) 2.5
0.0050	1) 0.0054	0.0053	1) 8.9
	2) 0.0053	(1.9)	2) 5.3
	3) 0.0053		3) 5.8
0.010	1) 0.011	0.011	1) 7.1
	2) 0.011	(1.3)	2) 5.6
	3) 0.010		3) 4.3
0.050	1) 0.046	0.046	1) –7.2
	2) 0.046	(0.54)	2) –8.2
	3) 0.046		3) –7.8
0.10	1) 0.080	0.081	1) –19.7
	2) 0.080	(1.2)	2) –20.1
	3) 0.082		3) –18.2

* Normalized residuals = $100 \times [(\text{Amount}_{measured} - \text{Amount}_{expected})/\text{Amount}_{expected}]$. The $\text{Amount}_{measured}$ is based on converting the peak area of each injection to concentration based on the calibration curve. The $\text{Amount}_{measured}$ is the concentration prepared.

ELSD optimization studies

The primary ELSD parameter evaluated was temperature (40°C and 60ºC). Injections of 0.0050% OG were performed at these two temperatures; peak areas and heights are listed in Table II. Greater response was observed at 40°C with respect to peak area and height.

Sample treatment

Samples (peak pools) for analysis were obtained from the cation-exchange chromatography step. Neat injections were performed; however, atypical profiles were observed after several

Table V. OG Limit-of-Detection Study

%OG	Peak area	Mean peak area (%RSD)	Log peak area	Calculated log (%OG)	Normalized residuals (%)
0.000050	1) 484	606	2.68	–4.01	95
	2) 566	(23)	2.75	–3.96	117
	3) 807		2.91	–3.86	175
	4) ND*		–	–	–
	5) 565		2.75	–3.97	117
	6) ND*		–	–	–
0.000125	1) 1476	1047	3.17	–3.69	64
	2) 1148	(24)	3.06	–3.76	39
	3) 946		2.98	–3.82	22
	4) 716		2.85	–3.90	1
	5) 1071		3.03	–3.78	33
	6) 927		2.97	–3.82	21
0.00025	1) 2014	2204	3.30	–3.60	1
	2) 1761	(20)	3.25	–3.64	–8
	3) 2986		3.48	–3.48	31
	4) 2455		3.39	–3.54	15
	5) 1887		3.28	–3.62	–3
	6) 2123		3.33	–3.58	5
0.00050	1) 6237	5644	3.79	–3.27	7
	2) 5839	(7.1)	3.77	–3.29	3
	3) 5711		3.76	–3.30	1
	4) 5688		3.75	–3.30	1
	5) 5250		3.72	–3.32	–4
	6) 5138		3.71	–3.33	–6
0.0010	1) 14715	14762	4.17	–3.02	–5
	2) 14208	(4.4)	4.15	–3.03	–7
	3) 15594		4.19	–3.00	–1
	4) 13866		4.14	–3.04	–9
	5) 15282		4.18	–3.01	–2
	6) 14906		4.17	–3.02	–4

* Not detected.

samples. The atypical profiles were a result of increased levels of the vaccine antigen and Triton X-100, relative to the reference material (0.55 mg/mL antigen and 0.050% Triton X-100). Method development was based primarily on the reference material at a specific level of antigen and Triton X-100. Therefore, to analyze samples, the vaccine antigen concentration was diluted to the level of the reference material, and as a consequence the level of Triton X-100 was also reduced. The dilution buffer did not contain Triton X-100, only phosphate and sodium chloride.

Evaluation of assay performance

Linearity and precision were established by using an external standard method, with triplicate injections for each standard. The response of the ELSD was nonlinear, and a logarithmic function best fits the data (15), thus raw peak areas and concentrations of each OG standard were transformed to logarithms, and a log–log plot was generated. To obtain the OG concentration in samples, the OG peak area was converted to a log value. The resulting log peak area was then converted to log concentration (based upon the log–log regression equation). To report OG concentration, the antilog of the log concentration was determined. The log–log OG (ranging from 0.00050% to 0.050%) calibration curve was described with the following regression equation:

$$y = 1.55x + 8.79,\ R2 = 0.9979 \qquad \text{Eq. 1}$$

Two measurements of precision were based upon raw and log peak areas. Raw peak area, rather than log peak area, better represents precision because log values exhibited insignificant variability. The precision criterion was set at 10% relative standard deviation (RSD). Linearity and precision data are listed in Table III. Precision of raw peak areas ranged from 1.8% to 8.1% RSD for the linearity range of 0.00050–0.10% OG (Note: variability appears to be underestimated based on log peak area).

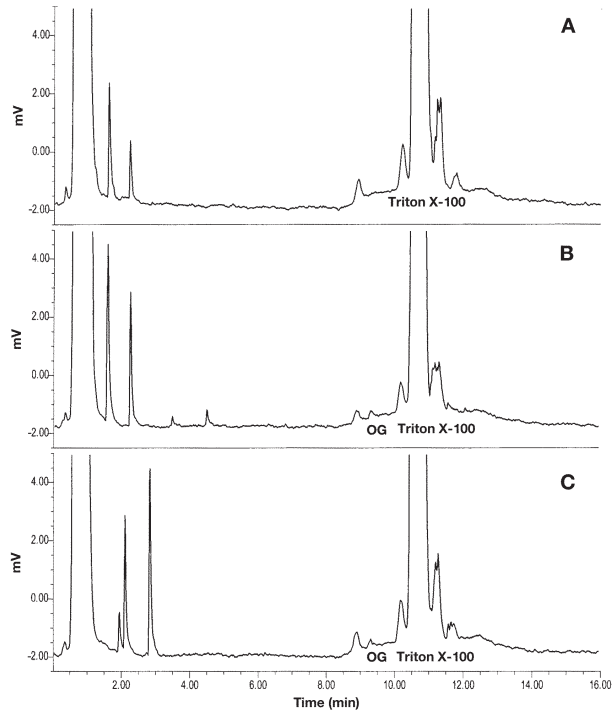

Figure 3. Representative limit-of-detection chromatograms of (A) vaccine antigen, (B) 0.000125% OG in vaccine antigen (12.5 ng injected), and (C) 0.000125% OG standard. The conditions are described in the text.

Accuracy (recovery) was determined for each standard, based on percent-normalized residuals of the linearity curve. An accuracy criterion of ± 15.0% was established. Table IV lists the accuracy data for each OG standard. The 0.10% OG standard exhibited accuracy values greater than the established criteria and thus was

Table VI. OG Spike Accuracy Study

Sample	Peak area (OG)	Log peak area	Determined log (%OG)
Vaccine Antigen	Not Applicable	—	—
Antigen + 0.0010% OG	1) 15729	4.20	–2.96
	2) 13709	4.14	–3.00
	3) 15608	4.19	–2.97
	4) 15224	4.18	–2.97
	5) 14589	4.16	–2.98
	6) 12833	4.11	–3.02
Antigen + 0.0050% OG	1) 161144	5.21	–2.31
	2) 149591	5.17	–2.33
	3) 173744	5.24	–2.29
	4) 170578	5.23	–2.30
	5) 168571	5.23	–2.30
	6) 146955	5.17	–2.34
0.0010% OG	1) 13580	4.13	–3.00
	2) 12827	4.11	–3.02
	3) 12597	4.10	–3.03
0.0050% OG	1) 191016	5.28	–2.26
	2) 181177	5.26	–2.28
	3) 182445	5.26	–2.28

$$\%\text{Recovery} = 100 \frac{(\text{Spiked sample concentration} - \text{nonspiked sample concentration})}{\text{Spiked of level concentration}}$$

Nonspiked sample concentration = 0, because there was no measurable level of OG in the antigen.

Table VII. OG Precision Study

Sample (%OG)	Peak area (OG)	Mean peak area (%RSD)	Log peak area	Mean log peak area (%RSD)
0.0010	1) 11899	12593	1) 4.08	4.10
	2) 12773	(2.9)	2) 4.11	(0.32)
	3) 12819		3) 4.11	
	4) 12798		4) 4.11	
	5) 12828		5) 4.11	
	6) 12438		6) 4.09	
0.0050	1) 157622	168118	1) 5.20	5.22
	2) 159015	(7.2)	2) 5.20	(0.58)
	3) 165724		3) 5.22	
	4) 189368		4) 5.28	
	5) 162007		5) 5.21	
	6) 174969		6) 5.24	

not included in the linearity curve. Percent-normalized residuals ranged from –9.8 to +14.7. The limit of quantitation (LOQ), with suitable accuracy and precision, was defined by the lowest OG standard (0.00050%).

The limit of detection (LOD) was experimentally determined, because a theoretical determination was not practicable (data not shown), with the following precision and accuracy criteria of ≤ 50% RSD and ≤ 100% normalized residuals, respectively. Further OG dilutions (Table V) below the LOQ were prepared. The LOD is primarily based on detection and not quantitation. Percent normalized residuals support the LOD level because dilutions of less than 0.0050% OG cannot be accurately measured. The LOD was experimentally determined to be 0.000125% OG (1.25 ng/µL corresponding to 12.5 ng injected). To confirm the LOD, a vaccine antigen solution containing 0.000125% OG was prepared and injected (Figure 3A–C). There was no detectable level of OG in the vaccine antigen (Figure 3A). An OG peak was detected in the 0.000125% standard (mean peak area = 1434, 30% RSD) as well as in the spiked (mean peak area = 1564, 32% RSD) antigen solution. Excellent agreement between the antigen spiked with OG and standard OG solution was exhibited.

OG spike studies (at 0.0010% and 0.0050%) were conducted for the determination of accuracy in the vaccine antigen. Six individual samples at each spike level were prepared and each injected once. Table VI lists the accuracy study data in addition to the spiked sample; the spike concentration (in buffer only) was also determined in a single preparation with triplicate injections. Very high recoveries of 103.7% and 97.8% were observed for 0.0010% and 0.0050% OG, respectively. The high recovery indicates that the OG did not complex to the antigen and, even if so, the chromatographic conditions appeared capable of disrupting any potential complexes.

Precision (%RSD) was not only evaluated by data from the linearity study (each standard in triplicate), but also by single injections of six individual preparations at 0.0010% and 0.0050% OG. Table VII lists the precision data, comparing the %RSD of the log peak area and raw peak area. Precision is 2.9% and 7.2% for the 0.0010% and 0.0050% OG, respectively.

Conclusion

An RP-HPLC assay, using ELSD, has been developed and qualified for the analysis of levels of OG during purification of a vaccine antigen. The methodology involved the incorporation of an online extraction for the removal of the vaccine antigen from the sample during chromatographic separation of OG and Triton X-100. Mobile and stationary phases were optimized to irreversibly bind the antigen and resolve the OG and Triton X-100. The flow rate and gradient program was also optimized to control the elution of spurious peaks generated from the antigen buffer containing nonvolatile sodium chloride and phosphate.

The assay exhibited very good precision, accuracy, and robustness. Precision and accuracy criteria of the OG standards defined the linearity encompassing the range of 0.00050–0.050% OG. In addition, precision and accuracy were evaluated for individually prepared OG standards at two levels. The LOD was experimentally determined to be 0.000125% OG.

Atypical profiles were observed during sample analysis and were determined to be a function of vaccine antigen and Triton X-100 concentrations, each greater than the reference material used for assay development. A normalization of vaccine antigen concentration to the reference material produced typical profiles. The column exhibited very good chromatographic performance even with multiple antigen injections (5.5 µg/injection). A single column was qualified and used for sample analysis. Approximately 48 and 64 injections of the vaccine antigen and OG standards were injected; at no point was chromatographic performance degraded. No detectable levels of OG (< 0.000125%) were observed in vaccine antigen cation-exchange peak pools.

This online extraction methodology may be useful for the analysis of other low-molecular-weight process reagents during the manufacture of biopharmaceuticals.

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References

1. L.M. Hjelmeland. *Solubilization of Native Membrane Proteins. Methods in Enzymology*, vol. 182, M.P. Deutscher, Ed. Academic Press, Boston, MA, 1990, pp. 253–64.
2. L.W. Kroh, T. Neubert, and E. Raake. Enzymatic analysis of alkyl polyglycosides. *Tenside Surf. Det.* **36:** 19–21 (1999).
3. R. Bastl-Borrmann and L.W. Kroh. Novel enzymatic assay for determination of alkyl polyglycosides with short chain fatty alcohols. *Fresenius J. Anal. Chem.* **371:** 939–43 (2001).
4. N. Bushmann and S. Wodarczak. Analytical methods for alkylpolyglucosides. *Tenside Surf. Det.* **32:** 336–39 (1995).
5. H.S. Klaffke, T. Neubert, and L.W. Kroh. Analysis of alkyl polyglucosides using liquid chromatographic methods. *Tenside Surf. Det.* **35:** 108–111 (1998).
6. N. Bushmann, L. Merschel, and S. Wodarczak. Analytical methods for alkyl polyglucosides. *Tenside Surf. Det.* **33:** 16–20 (1996).
7. P. Billian and H.-J. Stan. Gas chromatography/mass spectrometry of alkyl polygulcosides as their trimethylsilylesters. *Tenside Surf. Det.* **35:** 181–84 (1998).
8. P. Eichhorn and T.P. Knepper. Investigations on the metabolism of alkyl polyglucosides and their determination in waste water by means of liquid chromatography–electrospray mass spectrometry. *J. Chromatogr.* **854:** 221–32 (1999).
9. M. Lafosse, P. Marinier, B. Joseph, and M. Dreux. Study of amphiphilic behavior of alkylglycoside surfactants using reversed-phase liquid chromatography. *J. Chromatogr.* **623:** 277–87 (1992).
10. P. Billian, W. Hock, R. Doetzer, H-J. Stan, and W. Dreher. Isolation of n-decyl-α(1→6) Isomaltoside from a technical APG mixture and its identification by the parallel use of LC–MS and NMR spectroscopy. *Anal. Chem.* **72:** 4973–78 (2000).
11. K. Kimata, K. Hosoya, N. Tanaka, T. Araki, R. Tsuboi, and J. Haginaka. Effect of stationary phase structure on retention and selectivity of restricted-access reversed-phase packing materials. *J. Chromatogr.* **558:** 19–30 (1991).
12. L.J. Glunz, J.A. Perry, B. Invergo, H. Wagner, T.J. Szczerba,

J.D. Rateike, and P.W. Glunz. The semipermeable surface, a new restricted access medium. *J. Liq. Chromatgr.* **15(8):** 1361–79 (1992).
13. Z. Yu, D. Westerlund, and K.-S. Boos. Evaluation of liquid chromatographic behavior of restricted-access media precolumns in the course of direct injections of large volumes of plasma samples in column-switching systems. *J. Chromatogr.* **704:** 53–62 (1997).
14. C.T. Fleischer and K.-S. Boos. Highly selective solid-phase extraction of biofluids using restricted-access materials in combination with molecular imprinted polymer. *American Lab.* **33(10):** 20–25 (2001).
15. J.V. Amari, P.R. Brown, and J.G. Turcotte. Optimization of detection for HPLC separations of glycerophospholipids: Part 2. *American Lab.* **24(5):** 26–36 (1992).

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