Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 964–970
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A reversed phase HPLC assay for the simultaneous quantitation of non-ionic and ionic surfactants in bioprocess intermediates

Lorenzo H. Chen *, Colleen E. Price, Aaron Goerke, Ann L. Lee, Pete A. DePhillips

Department of Biologics Development and Engineering, Merck Research Laboratories, West Point, P.O. Box 4, WP42A-20, PA 19486, USA

Received 12 July 2005; received in revised form 23 August 2005; accepted 24 August 2005
Available online 19 October 2005

Abstract

This report describes a rapid and accurate reversed phase HPLC method for the simultaneous quantitation of multiple surfactants in various bioprocess solution matrices including cell lysates. Separation and quantitation of a mixture of the cationic detergent domiphen bromide from the non-ionic detergent Triton X-100 in crude cell mixtures can be achieved within 15 min using a TSK-gel C18-NPR reversed phase column and an aqueous mobile phase gradient of acetonitrile:water with the reagent PIC-B8 as ion-pairing modifier. The linear dynamic range for quantitation of domiphen bromide (DB) and Triton in this assay extends from 20 to 2000 μM. Linear regression analyses from the standard curve determinations showed an R^2 of ≥0.990. The assay does not show any interferences from proteins or other cellular contaminants such as nucleic acids. The assay has been used to evaluate clearance of these compounds throughout the purification process of an adenovirus-based vaccine candidate, as well as to determine the effects of process changes on detergent clearance.
© 2005 Elsevier B.V. All rights reserved.

Keywords: RP-HPLC; Triton; Adenovirus; Process monitoring

1. Introduction

Detergents are employed in a myriad of uses ranging from the biochemical solubilization of membranes during cell lysis to viral inactivation [1] to bactericides in the formulation of creams or tablets [2]. With these widespread uses, a number of sensitive and high resolution techniques have been developed for the determination of low concentrations of these analytes in different matrices. Many of these techniques and some applications have been reviewed by Vogt and Heinig [3], in which a substantial number of these assays were directed at evaluating trace detergents and their degradation products in environmental analyses. Morelli and Szajer [4,5] also provided additional and rather comprehensive reviews on surfactant analyses and methodology. Other examples of assays to determine the presence of detergents in wastewater streams include that of Barco et al. [6]. This mass spectrometric assay provides high sensitivity and quantitation with the application of some internal standards. Other assays for the evaluation of surfactants in cosmetics and commercial rinses utilize ion-associated titrimetry [7] and GC/MS [2]. The applicability of capillary electrophoresis to detergent analyses is reviewed by Heinig and Vogt [8].

In process development and production of biologics, the quantitation of detergents is more complex than in trace analyses from aqueous matrices. In some cases, in-process decisions are dependent on these evaluations, and a fast and simple assay using routine laboratory equipment can be of significant value. In many circumstances, more than one type of surfactant is used in various steps of the purification process. For example, cell or membrane lysis can be achieved by treatment with the non-ionic Triton X-100. This application of the surfactant also aids in the inactivation of some types of enveloped viruses such as HIV and hepatitis B and C viruses. This is the rationale employed for the production of human plasma where Triton, PS-80 and other non-ionic surfactants are added to the plasma and incubated [1]. After the incubation period, the biologically active plasma proteins remain intact and are then purified chromatographically. In other processes, such as the large scale purification of plasmid DNA, the cationic detergent cetrimonium bromide (CTAB) facilitates plasmid DNA precipitation [9]. As the prod-

Abbreviations: bql, below quantifiable level; CH_3CN, acetonitrile; CTAB, cetrimonium bromide; DB, domiphen bromide; RP-HPLC, reversed phase high performance liquid chromatography

* Corresponding author. Tel.: +1 215 652 2984; fax: +1 215 993 3348.
E-mail address: Lorenzo_chen@merck.com (L.H. Chen).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.08.033

uct is purified, other surfactants such as polysorbates can be introduced as stabilizers to prevent aggregation by decreasing surface adsorption. As a result, purification steps are introduced that are directed at the removal of detergents, ranging from physical methods (such as diafiltration, resin adsorption, gel chromatography and dilution) to chemical methods that include co-precipitation. In the midst of these myriad applications, it is therefore essential to demonstrate process consistency and control by monitoring clearance of detergents such as the non-ionic Triton and/or cationic detergents that have been introduced throughout the purification process. The residual concentrations of each of these surfactants may vary widely depending on the step of the process and the stage or extent of purification. Thus, any assay to determine the clearance of these detergents would necessarily need to employ a wide linear dynamic range. In addition, there are potential matrix effects because of the presence of the product (primarily proteinaceous in nature) as well as residual host cell nucleic acids, carbohydrates and proteins. Furthermore, in a process where ionic, non-ionic and zwitterionic surfactants are used, mixed micelles of these surfactants can develop, complicating their separation prior to quantitation of the individual detergents. To enable in-process decisions to be made in a timely fashion, it is essential to evaluate large numbers of samples quickly with conventional equipment.

We present a reversed phase assay for the simultaneous separation and determination of surfactants commonly used in bioprocesses, and without adverse effects from the solution matrices containing mixed micelles, residual proteins and nucleic acids. The assay is robust, accurate and fast and requires only routine laboratory equipment such as an HPLC with at least dual pumps and a diode array detector.

2. Materials and methods

2.1. Apparatus and chemicals

All studies were carried out on an Agilent 1100 HPLC system equipped with an inline vacuum solvent degasser, thermostatted autosampler and column chambers, quaternary pumps and a photodiode array detector. Instrument control and data analyses were carried out with the Chemstation software. Triton® X-100 standards were purchased from Pierce Chemical and domiphen bromide (DB) from Sigma–Aldrich. HPLC grade water was provided from an in-house source and acetonitrile (CH_3CN) was obtained from EM Sciences. Octane sulfonic acid (PIC B8) and other PIC reagents were purchased from Waters Corp. All other reagents were of analytical grade. For Triton X-100, a formula weight of 628 g/mol was used based on manufacturer. Thus, a 1% (v/v) solution is equivalent to 16 mM. Likewise, a 1% (v/v) solution of DB is equivalent to 24 mM based on a formula weight of 414 g/mol.

2.2. Chromatographic conditions

The mobile phase A consisted of 10 mM PIC B8 in H_2O and mobile phase B consisted of 10 mM PIC B8 in 90% CH_3CN:H_2O (v/v). Prior to the first injection, the column was equilibrated in 5% B at a flow rate of 1.0 mL/min. Upon injection of 50 μL of sample, the gradient was increased from 5 to 100% B over 10 min. After a 2 min hold at the end of the gradient (100% B), the column was re-equilibrated at 5% B. The autosampler and column were maintained at ambient temperature (20–25 °C) and the autosampler needle utilized CH_3CN as the needle wash solvent. The absorbance was monitored at 276 nm.

2.3. Columns, standard solutions and sample preparations

A TSK-gel C18-NPR (4.6 mm × 35 mm, TosoHaas) column was used in all experiments indicated. New HPLC columns were conditioned prior to analyses by the application of at least six consecutive injections of a mixture of 250 μM Triton X-100 (0.0162%) and 250 μM DB solutions (0.0104%). Column conditioning was considered successful when the retention times and detergent peak areas of the three most recent injections were within 5%. Columns were replaced when the retention times and peak areas of the control samples drifted outside of the above specifications. Positive control samples containing 0.1% Triton X-100 (v/v) (1600 μM) and 0.1% DB (v/v) (2400 μM) were interspersed at intervals of every 10 sample injections after the standard curve. All of the control solutions and samples were prepared fresh on the day of use.

Each sample was subjected to two dilutions prior to the sample preparation, unless otherwise stated. Sample pretreatment consisted of the addition of an equal volume of CH_3CN to the diluted sample. This mixture was mixed (by vortexing) and centrifuged for 20 min at 5100 × *g*. Although protein and detergent co-precipitation was never observed, an aliquot of the supernatant was then carefully aspirated into a vial for injection on to the column.

2.4. Calculations

Although the linear dynamic range for the analyses extended from 20 to 2000 μM, there was a better fit to the data if the ranges were divided into two curves, designated a high and a low curve, with ranges of 20–200 and 200–2000 μM, respectively (see Section 3). The concentrations of each of the samples were determined by calculated interpolation of the observed peak areas to the respective standard curves generated with SigmaPlot software (SPCC, Inc.).

3. Results and discussion

3.1. Chromatography

Several HPLC assays for the detection and quantitation of Triton X-100 have been developed. Pardue and Williams demonstrated adequate recovery and yield using ion exchange resins in the absence of ion-pairing agents [10]. A reversed phase quantitation of Triton, using an CH_3CN and water gradient, has been described by Varughese et al. using an octadecyl silane column with a refractive index detector to evaluate the effect of increasing methylene groups on the retention times of various non-ionic surfactants [11]. Using a silica column and aqueous solvents,

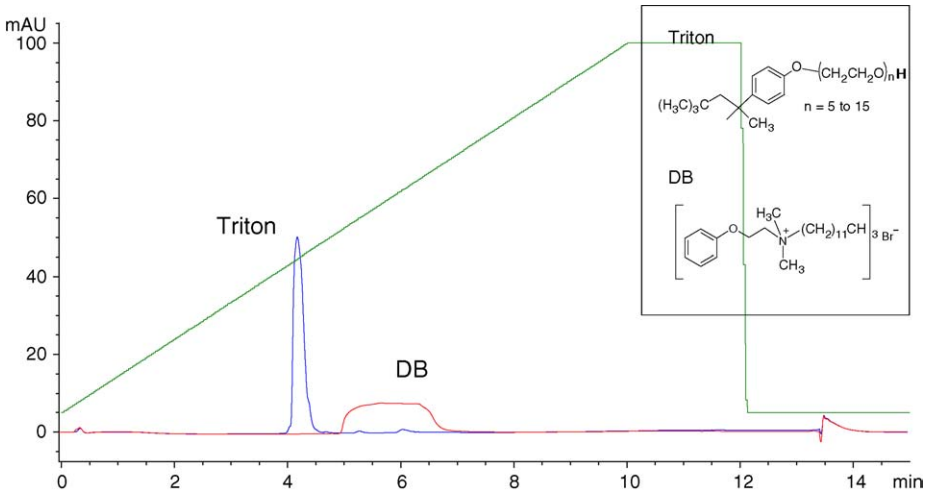

Fig. 1. Reversed phase HPLC chromatogram of 0.02% Triton X-100 (v/v, blue trace) and 0.02% domiphen bromide (v/v, red trace) without organic modifiers. Elution is achieved with a linear gradient from 5% CH_3CN to 95% CH_3CN over 12 min. The attached inset shows the chemical structures of the two analytes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Chen et al. were able to quantitate levels of cationic surfactants present in household cosmetics as well as in some pharmaceutical formulations [12]. The mass spectrometric method of Barco et al., applied to wastewater treatment samples, offered high sensitivity and quantitation using radiolabeled triethoxylated nonylphenol and sodium dibutylnaphthalenesulfonate as internal standards [6].

These assays measured similar types of surfactants in solution and did not address a mixture of surfactants as would be observed in a biological purification process. In a solution containing a mixture of non-ionic Triton X-100 and cationic DB, the separation and quantitation under any of the above liquid chromatographic conditions was not satisfactory. In Fig. 1, the separation and quantitation of Triton and DB by reversed phase high performance liquid chromatography (RP-HPLC) was attempted using a water–CH_3CN gradient without ion-pairing agents. The results demonstrate that, while a well-resolved and symmetrical peak corresponding to Triton was readily obtained, the DB eluted in a broad peak and with a slightly later retention time than Triton. The poor resolution of DB made it difficult to quantify this species. Different chromatographic conditions, such as variations in column temperature and column polarity, were also investigated but with no enhancement of the DB signal. The best results were obtained using a C18-NPR column, but required use with various paired-ion chromatography reagents available from Waters Inc. These commercially available reagents are composed of compounds of variable alkyl chain lengths tethered to a sulfonic acid moiety. While the effects of these reagents on the column resin are not completely understood, it is apparent that they mitigate the effect of the positively charged DB on the column. Other sulfonic acid derivatives with variable alkyl chain lengths as well as other positively charged ion-pairing agents were evaluated, but the best separation and resolution was observed with the addition of octylsulfonic acid to the mobile phase. The Triton and DB analyte peaks are well resolved and sufficiently separated from each other, facilitating quantification of these detergents (Fig. 2).

3.2. Recoveries and ruggedness

Once a baseline separation of Triton and DB was accomplished and a well-resolved Gaussian peak was obtained for DB, the effects of sample matrices were investigated using various cellular components of cells lysed by Triton. In order to demonstrate a lack of interference from the most crude sample matrix (clarified lysates), samples without Triton and DB were injected, analyzed and the chromatograms were compared to those in a similar matrix with detergents. These results, also shown in Fig. 2, demonstrate no overlap of the relevant UV signals from Triton or DB. Although there is a small peak eluting about 1 min after the later-eluting analyte (DB), it is well separated from DB, such that it does not interfere with the integration of the DB peak area. Additional experiments have indicated that this small peak is a cell-based impurity and its presence is dependent on the initial cell lysis conditions. The purpose of the sample pretreatment is to further clarify the matrix by denaturing residual proteins and removing them from solution by centrifugation. The results from the injection of the clarified lysate alone indicate that this pretreatment was sufficient to remove host cell residuals that could interfere with the detergent signal. We then evaluated the effect of this pretreatment on the detergent recoveries. Sample recoveries were evaluated from the entire sample pretreatment, as well as for the individual steps in the pretreatment, to determine if there is any significant detergent loss. Table 1 demonstrates that there is no loss of either Triton or DB for any of the individual permutations of the sample pretreatment step.

As a measure of assay ruggedness, spike recovery experiments were conducted by adding known concentrations of DB and Triton to each process intermediate. The results are shown in Table 2 and indicate good recovery in all process matrices except for Step 4. This matrix is the result of a concentration step in the process and contains significantly elevated levels of surfactants, NaCl and cellular components. Recovery is vastly improved after additional dilution of this solution. Thus, the entire pretreatment enables this assay to be used throughout the

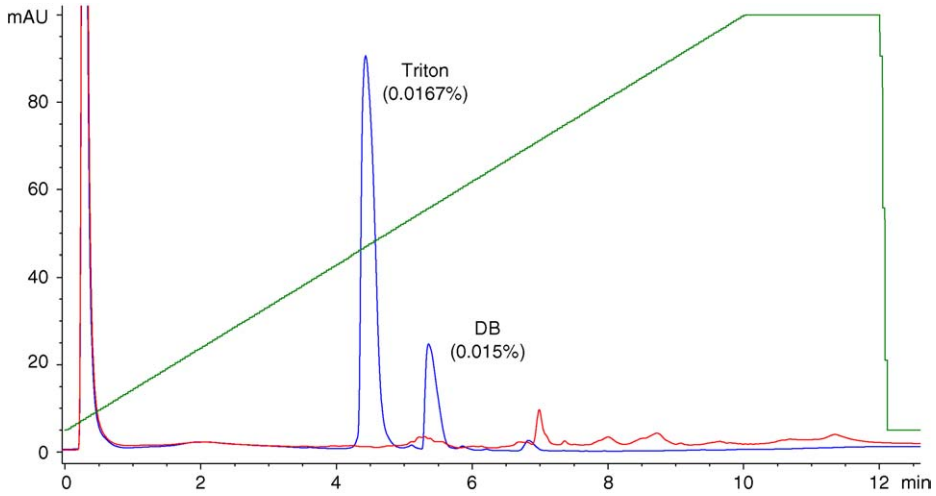

Fig. 2. Comparison of the effect of sample matrix on the elution of Triton and DB. The chromatograms of the Triton and DB peaks were obtained upon addition of 10 mM PIC-B8 ion-pairing reagent to the CH_3CN mobile phases, and injection of a process sample (blue trace). The chromatogram of a process sample containing no detergents is superimposed (red trace) and demonstrates that the Triton and DB peaks are well resolved from possible contaminant peaks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

entire process to determine the concentrations of these surfactants.

3.3. Accuracy, precision and limits of detection

Calibration curves demonstrated assay linearity over the entire concentration range of 20–2000 μM for both Triton X-100 and DB (Table 3). The accuracy of the assay was determined by examination of the back-calculated values of the predicted concentrations, based on the observed slopes and intercepts (Table 3, column 2). As shown in Fig. 3A (in the case of DB), the observed accuracy of the assay, if interpolated over the entire dynamic range, is somewhat poor, especially at lower concentrations, where the curvature in the data points is obvious, and the backfits to the data demonstrate the lack of fit (Table 3, column 3). To obtain the best accuracy for the data for either detergents, the ranges were deconstructed into two curves covering 20–200 μM (designated "low curve") and 200–2000 μM ("high curve") (Fig. 3B and C). The back calculations of the same data using these two curves provided much better accuracy (Table 3, columns 4 and 6). With the application of the separate curves for both detergents, the linear dynamic range of the assay was maintained and the accuracy of the results was enhanced to within 10% of the expected values.

The limit of quantitation (LOQ), defined as the lowest concentration of the analyte(s) in a sample that can be determined with acceptable precision and accuracy in the various sample matrices, was estimated at 10 μM; the accuracy at this concentration is less than 20%. The limit of detection, defined as the lowest concentration of the analyte(s) that can be detected, was 5 μM for each of these surfactants (data not shown). At this concentration, the accuracy of the assay was estimated at greater than 20%.

In further validation of the assay, the day-to-day and within-day precision of the assay were also examined with respect to both surfactants, and the results are tabulated in Table 4. For both Triton X-100 and DB, the within-day precision was determined from two injections of the sample. The day-to-day variability was obtained from the results of the assay carried out on five non-consecutive days. Furthermore, these experiments were all carried out in the presence of the various sample matrices from the purification process. The results for Triton X-100 indicate that the intra-day variability is less than 7%, whereas the

Table 1
Recoveries using various sample pretreatment steps

Sample	Triton recovery (%)	DB recovery (%)
Control	–	–
CH_3CN, no centrifugation	108.3	111
No CH_3CN, centrifugation	101.9	101.6
CH_3CN, centrifugation	109	111.7

The control sample contained DB and Triton, both at 30 μM, dissolved in H_2O. The usual pretreatment of adding equal volumes of H_2O was not applied here. In the second experiment, an equal volume of CH_3CN was added to the sample with no subsequent centrifugation. The third permutation of the pretreatment involved the centrifugation without addition of CH_3CN. Finally, the entire sample pretreatment was applied. The results indicated that none of the pretreatment steps, taken together or individually, contributed to significant loss of the detergent being evaluated.

Table 2
Determination of assay interferences by sample matrices

Sample type	Triton recovery (%)	DB recovery (%)
Step 2	84.4	99.4
Step 3	82.9	99.4
Step 4	11.6	6.1
Step 5	100.3	100.9
Step 6	101.2	102.4
Step 7	98.6	100.6

Process samples were analyzed undiluted (prior to pretreatment). A mixture containing 300 μM each of Triton and DB was then added to each sample and the samples were re-analyzed. The percent recovery is defined as the (([detergent] in the spiked adenovirus sample − [detergent] in the unspiked adenovirus sample)/([detergent] of the spike)) × 100.

Table 3
Accuracy of Triton X-100 and domiphen bromide measurements

[Triton] (μM)	Full range calculated [Triton] (μM)	Full range (% difference)	Lower range calculated [Triton] (μM)	Lower range (% difference)	Upper range calculated [Triton] (μM)	Upper range (% difference)
10	37	−270.53	12	−19.61	–	–
25	45	−80.32	21	14.49	–	–
50	70	−39.41	50	−0.53	–	–
100	114	−13.99	102	−2.21	–	–
200	197	1.68	199	0.41	217	−8.58
250	239	4.40	–	–	259	−3.49
300	284	5.18	–	–	303	−1.11
400	383	4.33	–	–	400	0.06
500	460	8.04	–	–	475	4.91
1000	978	2.20	–	–	984	1.60
1500	1507	−0.44	–	–	1503	−0.19
2000	2022	−1.10	–	–	2009	−0.44
[DB] (μM)	Full range calculated [DB] (μM)		Lower range calculated [DB] (μM)		Upper range calculated [DB] (μM)	
10	48	−384.32	11	−6.93	–	–
25	57	−127.81	21	14.01	–	–
50	81	−62.14	52	−4.17	–	–
100	120	−20.19	102	−1.69	–	–
200	197	1.52	199	0.48	228	−13.77
250	233	6.62	–	–	263	−5.20
300	273	8.88	–	–	302	−0.61
400	375	6.19	–	–	401	−0.22
500	451	9.75	–	–	475	5.04
1000	944	5.61	–	–	954	−4.62
1500	1520	−1.35	–	–	1514	−0.95
2000	2034	−1.70	–	–	2014	−0.69

The full range calculations are based on the data curve spanning 10–2000 μM. The recalculated values for the lower range span 10–200 μM; the upper range spans 200–2000 μM.

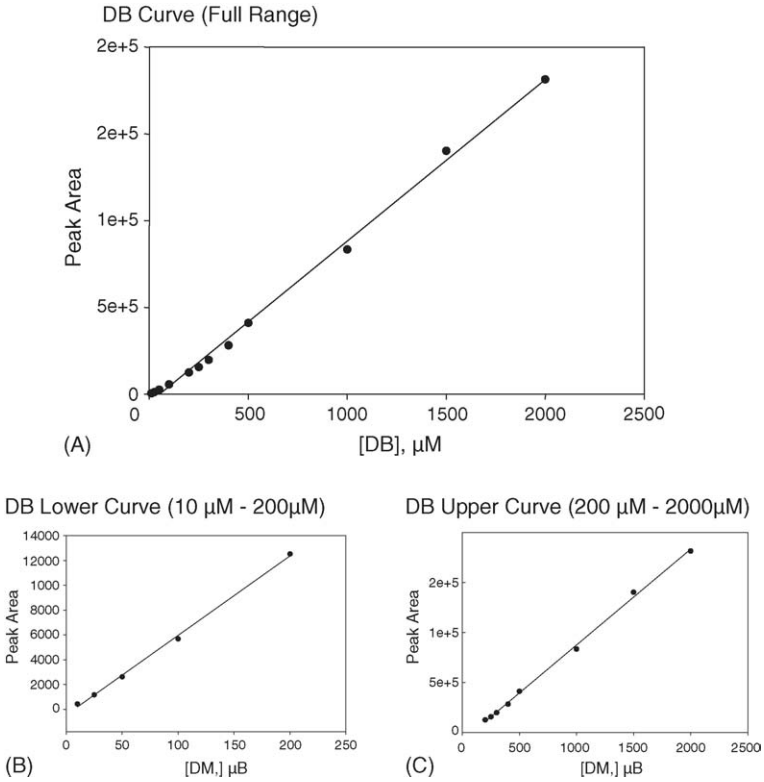

Fig. 3. DB standard curves. (A) Full range curve from 10 to 2500 μM, where the curvature and lack of accuracy at the lower concentrations is observed. In the evaluation of the full curve, $R^2 = 0.996$. (B) "Low curve" (10–200 μm) and (C) "high curve" (200–2000 μm), respectively. Linearity (R^2 value) of "low curve" = 0.998 and of "high curve" = 0.998.

Table 4
Intra-day and inter-day precision for: (A) Triton X-100 and (B) domiphen bromide

Sample type	*n* (for each day)	Intra assay					Inter assay, *n* = 5 (%)
		Day1 (%CV)	Day 2 (%CV)	Day 3 (%CV)	Day 4 (%CV)	Day5 (%CV)	
(A) Triton							
Cell lysis	2	3.73	1.25	3.36	–	3.74	5.31
Step 2	2	1.00	1.80	1.52	1.14	4.68	4.71
Step 3	2	2.73	1.61	224	0.64	2.78	3.93
Step 4	2	1.09	6.17	3.51	2.31	5.48	2.14
(B) DB							
Cell lysis	2	bql	bql	bql	bql	bql	bql
Step 2	2	3.66	1.20	7.10	9.29	2.08	3.55
Step 3	2	1.33	2.48	4.06	5.84	2.11	3.58
Step 4	2	6.38	10.73	9.28	6.64	10.26	6.03

variability is only slightly higher (less than 11%) for DB. Not surprisingly, the largest variability for both surfactants occurs in the sample matrix in which a concentration step occurs. By implementing the proper dilution scheme for this step, however, this issue was resolved. The day-to-day precision of the assay with respect to both surfactants is excellent, with a variability of less than 6% for Triton X-100 and less than 7% for DB.

3.4. Process development applications

Having established the accuracy and precision of the assay (Tables 2 and 4), its utility was demonstrated in two experiments. It was first used in determining the clearance of these surfactants by monitoring of in-process samples in the purification of an adenovirus vaccine candidate under development [13]. In this process, cell lysis is facilitated by the addition of 0.1% Triton X-100 (w/v). In Table 5, this step is designated LYS. DB is added to 0.04% (w/v) to selectively precipitate host cell nucleic acids and cell debris, which are removed by depth filtration, and the resulting clarified lysate (CL) is concentrated 20-fold by ultrafiltration with a buffer exchange. The ultrafiltration product (UF1P) is diluted for application to an anion exchange column. The eluted product from this column (AEP) is subjected to another buffer exchange by ultrafiltration (product designation UF2P). The DB and Triton X-100 levels from two purification lots are shown in Table 5. Significant clearance of both Triton and DB is obtained after the AEP step and following the next ultrafiltration step, these detergents are cleared below the LOQ.

In a second example, the assay was used in monitoring the kinetics of adsorption of a Triton X-100 and DB mixture containing an adenovirus product on a series of Amberlite XAD resins in order to determine the duration of the adsorption cycle time. The resins tested and the analytical results are listed in Table 6. In all samples, the loading was defined as the maximum quantity of detergent that may be bound such that the bulk concentrations remain below the assay limit of detection (0.001%). It can be predicted that the size of the resin pores (<600 Å) and the resin neutrality are responsible for the high product (adenovirus) recoveries. A typical observation is that, the larger the surface area (800 m^2/g) of the resins, the greater the adsorption capacity, and this seems to be the case for the commercial resins used. Under these experimental conditions, the results demonstrated that XAD-16 and XAD-1600 adsorbed more residual detergents, yet ensured maximum product recovery (Table 6). The only resin that displayed loss was XAD-1180. In this case, it may be that the relatively large pores (450 Å) coupled with the chemical nature of the resin surface could have resulted in the adsorption/aggregation of the adenovirus product. Following

Table 5
Process monitoring of the clearance of: (A) Triton and (B) domiphen bromide across the purification process of an adenovirus vaccine candidate

	Lot 1	Lot 2
Triton (w/v, %)		
LYS	0.1095	0.134
CL	0.091	0.082
UF1P	0.136	0.113
AEP	0.007	0.003
UF2P	0.0012	<0.0012
DB (w/v, %)		
LYS		
CL	0.023	0.021
UF1P	0.44	0.031
AEP	0.006	0.003
UF2P	0.0008	<0.0008

Abbreviations: LYS, cell lysate; CL, clarified lysate; UF1P, ultrafiltration product 1; AEP anion exchange product; UF2P, ultrafiltration product 2.

Table 6
Evaluation of the adsorption capacities of various resins for Triton and domiphen bromide

Resin	Product recovery (%)	Triton X-100 loading (g detergent/(g resin))	Domiphen bromide loading (g detergent/(g resin))
XAD-4	98	0.045	0.028
XAD-16	100	0.150	0.044
XAD-1600	99	0.190	0.066
XAD-1180	90	0.083	0.046
XAD-7	102	0.037	0.017
XAD-761	101	0.022	0.018

Product (adenovirus) recovery is determined from the results of an anion exchange assay.

Table 7
Adsorption of Triton, domiphen bromide and product as a function of column residence times

Residence time (min)	Triton X-100 capacity (g detergent/(g resin))	Domiphen bromide capacity (g detergent/(g resin))	Product recovery (%)
2.5	0.05	0.03	101
4.7	0.15	0.04	100
7.1	0.18	0.05	100

Product recovery is determined from the results of an anion exchange assay.

these batch experiments, a pilot-scale chromatographic experiment using XAD-1600 was performed to confirm the observed results. The solution of 0.1% Triton X-100 and 0.02% DB was pumped through a column packed with XAD-1600. For product prepared in this way, residual DB and Triton X-100, protein, DNA and adenovirus content were determined across different residence times and compared to their initial values. These results are shown in Table 7. During this manufacturing procedure, no product, DNA or proteins were adsorbed. The concentration of DB and Triton X-100 was below the detection limits. In-process monitoring of these residual detergent impurities shows that increased residence time will result in the higher capacities obtained in batch operation. The application of this simple assay provided an efficient monitoring tool for removal and quantification of detergent impurities in process development and for scale-up to commercial production.

In conclusion, a fast and simple RP-HPLC assay has been developed for the simultaneous determination of non-ionic and cationic detergents in biological purification processes. The ruggedness and accuracy have been demonstrated in the determination of the clearance of DB and Triton. The adsorption capacities of various resins were determined. Although two surfactants were tested here, this method has also been shown to be applicable to the testing of other cationic surfactants such as cetylpyridinium chloride and CTAB in the presence of Triton.

References

[1] A. Strancar, P. Raspor, H. Schwinn, R. Schütz, D. Josic, J. Chromatogr. A 658 (1994) 475–481.
[2] P.-C. Tsai, W.-H. Ding, J. Chromatogr. A 1027 (2004) 103–108.
[3] C. Vogt, K. Heinig, Fresenius J. Anal. Chem. 363 (1999) 612–618.
[4] J.J. Morelli, G. Szajer, J. Surfactants Detergents 3 (2000) 539–552.
[5] J.J. Morelli, G. Szajer, J. Detergents Surfactants 4 (2001) 75–83.
[6] M. Barco, C. Planas, O. Palacios, F. Ventura, J. Rivera, J. Caixach, Anal. Chem. 75 (2003) 5129–5136.
[7] T. Sakai, N. Teshima, Y. Takatori, Anal. Sci. 19 (2003) 1323–1325.
[8] K. Heinig, C. Vogt, Electrophoresis 20 (1999) 3311–3328.
[9] R. Lander, M. Winters, F. Meacle, B. Buckland, A. Lee, Biotechnol. Bioeng. 79 (2002) 776–784.
[10] K. Pardue, D. Williams, BioTechniques 14 (1993) 580–583.
[11] P. Varughese, M.E. Gangoda, R.K. Gilpin, J. Chromatogr. 499 (1990) 469–475.
[12] F.-A. Chen, K.-S. Wu, M.-C. Huang, C.-Y. Chen, A.-B. Wu, J. Food Drug Anal. 9 (2001) 191–198.
[13] A.R. Goerke, B.C.S. To, A.L. Lee, S.L. Sagar, J.O. Konz, Biotechnol. Bioeng. 91 (2005) 12–21.