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Chromatographic separation of surfactants

XII. Quantification of results in trial high-performance liquid chromatography of ethoxylated surfactants

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ABSTRACT

The high-performance liquid chromatographic (HPLC) analysis of the oligomer distribution of ethoxylated alkylphenols was checked by interlaboratory testing on three samples with low degrees of ethoxylation in ten laboratories. Results for the oligomer composition showed good accuracy, repeatability and reproducibility and indicated that differences in instrumentation, operating conditions and operators' experience did not influence the results. The peaks areas were corrected by different factors depending on whether refractive index or UV detection was used. The mean molecular masses and degrees of ethoxylation calculated from the HPLC data were in very good agreement with indicated or theoretical values, demonstrating the reliability of the HPLC results. Results of thin-layer chromatographic analysis of the same samples with flame ionization detection were very similar to those obtained by HPLC.

INTRODUCTION

The quantitative evalutation of chromatograms obtained by high-performance liquid chromatography (HPLC) of ethoxylated surfactants is a problem. In recent publications mainly separation problems were discussed, only limited attention being devoted to the quantitative evaluation of results[1]. The important requirements for the quantitative evaluation of the composition of oligomer adducts in ethoxylated surfactants are the following: to ensure complete recording of all the oligomers present in the sample; to achieve a sufficient separation of all recorded oligomer peaks; to be certain of their identification; and to have confidence at least in the relative responses of the detection method applied.

To meet these requirements, various ethoxylated surfactants have been analysed by HPLC in our laboratory in recent years applying diol bonded phases and *n*-hexane–2-propanol–water mobile phases[2,3]. The proportions of the components in these mobile phases were adjusted so as to give as complete a separation of oligomers

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as possible in samples of different degrees of ethoxylation with isocratic elution using refractive index (RI) detection. For identification of oligomers in ethoxylated fatty alcohols some pure synthetic standards of lower oligomers were available. Oligomers in other ethoxylates were identified without standards, relying on the sequence continuity of oligomers starting from samples with the lowest degree of ethoxylation.

For the correction of oligomer peaks area with refractive index (RI) detection, relative correction factors were calculated from the differences in n_D values between separate oligomers and the mobile phase applied[3,4]. Appropriate n_D data for ethoxylated surfactants and their oligomers were taken from the literature[5,6] and in some instances were estimated from n_D measurements on samples with different degrees of ethoxylation assuming a simple, smooth dependence of n_D on degree of ethoxylation in order to make inter- and extrapolations of n_D data for individual oligomers.

For UV detection it was found the molar absorptivities of ethoxylated alkylphenols were independent on the polyoxirane chain length[7–10], so that the chromatograms obtained showed the molar distribution of oligomers. Other detection methods for the quantification of ethoxylated surfactants do not appear to have been published.

In spite of numerous applications of HPLC to the separation of ethoxylated surfactants, data on the repeatability and reproducibility are lacking. Only in one paper[11] were coefficients of variation published for nineteen oligomers of ethoxylated alkylphenols. To consider the reliability, accuracy, repeatability and reproducibility of HPLC separations of ethoxylated surfactant oligomers, interlaboratory testing was performed with samples of alkylphenols of low degree of ethoxylation. The choice of samples was limited by the instrumentation and experience available in the participating laboratories and by the effort of analyzing possibly simple samples with a reduced number of oligomers under analogous operating conditions to show the ability of HPLC to differentiate samples with only small variations in their degree of ethoxylation. For the establishment of optimum conditions for interlaboratory testing, preliminary tests in authors laboratory were performed under a wide range of operating conditions with different columns and mobile phase compositions with RI and UV detection. On the basis of this experience, directions and recommendations were despatched to the participating laboratories with three samples. The results of this interlaboratory testing are presented in this paper.

EXPERIMENTAL

HPLC instrumentation

The instrumentation used in the ten participating laboratories is summarized in the Table I. The results were evaluated from the oligomer peaks areas, applying different corrections for RI and UV detection as indicated in the Introduction; correction factors for the evaluation of the mass distribution are given in the Table II. For RI detection they were evaluated from differences in n_D between oligomers[5,6] and the mobile phase; correction factors for UV detection were calculated from comparison of corrected results for RI detection and the peak-area composition with UV detection at 258 nm. Theoretical correction factors for UV detection are also indicated, calculated from the corresponding molecular weights presuming that UV detection gave the molar composition.

TABLE I

HPLC INSTRUMENTATION IN INTERLABORATORY TESTING

Laboratory No.	*Instrument*	*Injector*	*Detector*		*Integrator*
			RI	*UV*	
1	HPP 4001	Rheodyne 7125	RIDK 101	UVD LCD, 254 nm	SP Minigrator
2	SP 8750	SP 8700	–	SP 8440, 258 nm	SP 4270
3	HP 1050	Autosampler	–	UVD, 230 nm	HP 1090 Workstation
4	SP 8100	Valco	–	LCD 2563, 254 nm	SP 4100
5	HPP 4001	LCI 02 septum	RIDK 102	–	CI 100
6	HPP 4001	Stop-flow	RIDK 101	–	–
7	HPP 4001	LCI 02 septum	RIDK 101	–	–
8	PU 4100	Rheodyne 7125	–	PU 4110, 280 nm	PU 4810
9	Knauer FR-30	Rheodyne 7120	Knauer 61.00	Knauer, 258 nm	Chromatopac CR-3-A
10	HPP 5001	Stop-flow	RIDK 101	–	CI 100

Thin-layer chromatography (TLC) with flame ionization detection (FID)

Parallel TLC–FID analyses[12] were performed on an Iatroscan TH-10 Mark III instrument using Chromarod S II for separations with stepwise development using two mobile phases: (a) benzene–ethyl acetate (6:4, v/v) to a distance of 10 cm from the start and (b) ethyl acetate–acetic acid–water (8:1:1, v/v/v) up to a distance of 8 cm. Samples were applied as 2% solutions in chloroform and a 1-μl volume of the solution was applied to one Chromarod at the start. FID was operated with hydrogen at

TABLE II

CORRECTION FACTORS FOR RI AND UV DETECTION AT 258 nm RELATIVE TO OLIGOMER 5

Oligomer No.	*RI detection*	*UV detection*			
		For dodecylphenols		*For nonylphenols*	
		Theory	*Found*	*Theory*	*Found*
1	0.921	0.635	0.721	0.600	0.578
2	0.949	0.726	0.789	0.700	0.712
3	0.969	0.817	0.860	0.800	0.811
4	0.986	0.909	0.933	0.900	0.907
5	1.000	1.000	1.000	1.000	1.000
6	1.013	1.091	1.066	1.100	1.090
7	1.026	1.182	1.136	1.200	1.178
8	1.039	1.274	1.201	1.300	1.266
9	1.051	1.249	1.266	1.400	1.351
10	1.063	1.340	1.331	1.500	1.447
11	1.073	1.548	1.393	1.600	1.558
12	1.083	1.639	1.462	1.700	1.689
13	1.091	1.730	1.526	1.800	1.842
14	1.099	–	–	1.900	2.026
15	1.107	–	–	2.000	2.249
16	1.114	–	–	2.100	2.533

160 ml/min and air at 2200 ml/min. The scanning time was 35 s for each Chromarod. The results were evaluated from the peak areas of the oligomers, applying correction factors considering the relative contributions of different carbon atoms in the molecular structure to the FID response[13], analogous to those applied for the TLC–FID of dodecyl ethoxylates[14].

Conditions for interlaboratory testing

In the directions to the various laboratories, the application of diol bonded phases and RI or UV detection under isocratic conditions or gradient elution with UV detection was recommended, applying *n*-hexane–2-propanol–water mobile phases. The aim was to achieve an acceptable separation of oligomers within the complete distribution by the choice of a suitable mobile phase composition. Provision of at least three complete results for the mass composition of oligomers from subsequent analyses of each of the three samples was requested. The HPLC operating conditions applied in individual laboratories are summarized in the Table III.

Samples

The properties of the samples used are given in Table IV. All other chemicals and solvents used were of analytical-reagent grade.

TABLE III

OPERATING CONDITIONS IN INTERLABORATORY TESTING

Laboratory No.	*Sorbent in 250 × 4 mm I.D. column and grain size*	*n-Hexane–2-propanol–water mobile phase (v/v/v)*	*Flow-rate (ml/min)*	*Sample volume (µl)*	*Sample concentration (%)*
1	Silasorb Diol, 4.7 µm	82:18:0.03	0.8	10	4–5
2	LiChrosorb Diol, 5 µm	75:25:1 or gradient from 90:10: saturated to 70:30:1 in 30 min	0.6	10	2–7
3	Silasorb SPH Amin 10 µm	gradient from 100:0:0 to 0:100:0 in 65 min	1.0	20 or 40	2–5
4	LiChrosorb Diol, 5 µm	82:18:0.03	1.0	10	1
5	LiChrosorb Diol, 5 µm	75:25:1	0.4 or 0.8	3–8	>10
6	LiChrosorb Diol, 5 µm	82:18:0.03	0.5	1–5	20
7	LiChrosorb Diol, 5 µm	70:30:2 for higher and 82:18:0.03 for lower oligomers	0.5	20–25	5–6
8	LiChrosorb Diol, 5 µm	gradient from 90:10:saturated to 75:25:1 in 10 min, then 15 min isocratic	1.0	5	*ca.* 8
9	LiChrosorb Diol, 5 µm	90:10:saturated for lower and 75:25:1 for higher oligomers	0.8	20	2–5
10	LiChrosorb Diol, 5 µm	82:18:0.03	0.8	20	2–5

TABLE IV

SAMPLES ANALYSED IN INTERLABORATORY TESTING

Property	*Dodecylphenol + 6 EO*	*Slovafol 905*	*Emulgator U 6*
Origin	Laboratory sample	Chemical plant, Nováky, Czechoslovakia	Unger, Norway
Designation	DDF	S 905	U 6
Hydrophobe	Dodecylphenol	Nonylphenol	Nonylphenol
Degree of ethoxylation mol EO/mol	6.0	5.0	6.0
Sample composition:			
Free PEG (%)	3.3	2.4	1.2
Free alkylphenol (%)	0.3	0.2	0.1
Oligomer adducts (%)	96.4	97.4	98.7

RESULTS

The data obtained by all the participating laboratories are very numerous and cannot be presented in full. Using isocratic conditions, in six laboratories RI detection and in four laboratories UV detection were applied; gradient elution with UV detection was used in three laboratories. These three groups of mean HPLC results obtained under different conditions are given for the three samples in Tables V–VII together with the TLC–FID results and data for mean molecular mass calculated from these composition data. Some chromatograms produced by the participating laboratories are given in Figs. 1–4.

TABLE V

MEAN COMPOSITION OF DDF AND CORRESPONDING MEAN MOLECULAR MASS DATA

Oligomer No.	*RI detection in 6 labs.*	*UV detection in 4 labs.*	*Gradient elution in 3 labs.*	*TLC–FID in 1 lab.*
1	0.7	0.7	3.6	0.3
2	3.4	3.8		2.2
3	8.9	9.2	8.6	8.9
4	14.5	14.9	14.3	15.2
5	17.1	17.3	18.8	20.0
6	16.1	16.1	15.4	17.7
7	13.6	13.3	13.4	14.8
8	10.4	10.3	10.7	8.7
9	7.0	6.7	6.9	6.1
10	4.4	3.9	4.1	3.1
11	2.3	1.9	2.3	1.8
12	1.2	1.1	1.2	0.9
13	0.4	0.8	0.7	0.3
Mean molecular mass	509.4	506.3	511.0	507.0

TABLE VI

MEAN COMPOSITION OF S 905 AND CORRESPONDING MEAN MOLECULAR MASS DATA

Oligomer No.	*RI detection in 6 labs.*	*UV detection in 4 labs.*	*Gradient elution in 3 labs.*	*TLC–FID in 1 lab.*
1	1.0	1.1	1.0	0.4
2	6.2	6.8	6.2	5.7
3	15.4	15.6	14.6	19.5
4	20.8	20.4	19.1	24.4
5	18.9	18.9	18.6	20.9
6	14.4	14.3	15.4	13.5
7	9.8	9.8	10.4	8.4
8	6.2	6.1	6.7	3.7
9	3.6	3.3	3.7	2.0
10	1.7	1.7	2.2	1.0
11	1.1	1.0	1.2	0.5
12	0.6	0.8	0.6	–
13	0.3	0.3	0.3	–
Mean molecular mass	429.1	427.3	432.8	416.6

The data from repeated HPLC analyses in individual laboratories were checked for standard deviations and corresponding coefficients of variation by appropriate calculations[15]. The calculation of the repeatability of the results (in individual laboratories) and reproducibility (between laboratories) was carried out according to the recommended standard[16]. No outlying values were found. The statistical tests[15] for

TABLE VII

MEAN COMPOSITION OF U 6 AND CORRESPONDING MEAN MOLECULAR MASS DATA

Oligomer No.	*RI detection in 6 labs.*	*UV detection in 4 labs.*	*Gradient elution in 3 labs.*	*TLC–FID in 1 lab.*
1	0.6	0.7	0.6	0.2
2	3.2	3.5	3.2	1.7
3	8.1	8.6	7.6	7.1
4	13.1	13.3	12.3	13.8
5	14.5	14.7	13.9	15.7
6	14.4	14.5	14.3	16.0
7	13.3	13.3	13.4	14.9
8	11.3	11.1	11.9	11.2
9	8.3	7.9	9.2	7.5
10	5.4	5.2	6.0	5.3
11	3.6	3.1	3.6	3.2
12	2.1	2.0	2.1	2.1
13	1.0	1.0	1.4	0.9
14	0.6	0.6	0.3	0.4
15	0.3	0.3	0.2	–
16	0.2	0.2	–	–
Mean molecular mass	480.1	476.0	483.9	483.5

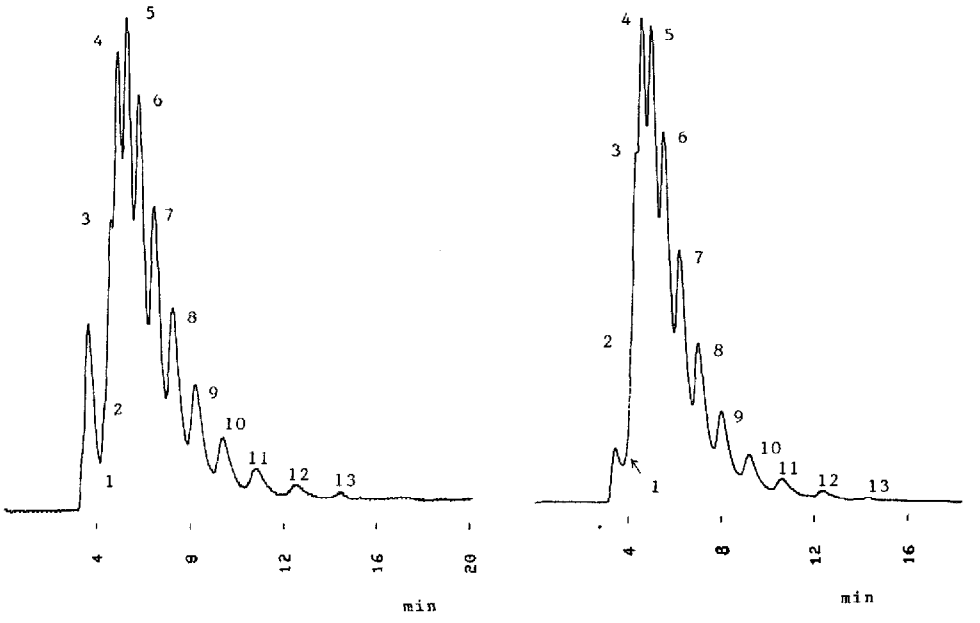

Fig. 1. Separation of DDF with (left) RI and (right) UV detection in laboratory No. 9 using mobile phase with ratio of components 75:25:1; oligomers 1–13.

differences between RI and UV detection with isocratic and gradient elution showed criteria without any significance, *i.e.*, the differences in the results are not caused by differences in the chromatographic variants used.

A survey of the resulting statistical data is presented in Table VIII, grouped according to the oligomers content. The dependence of the relative repeatability and relative reproducibility on the oligomer content is shown graphically in Figs. 5 and 6.

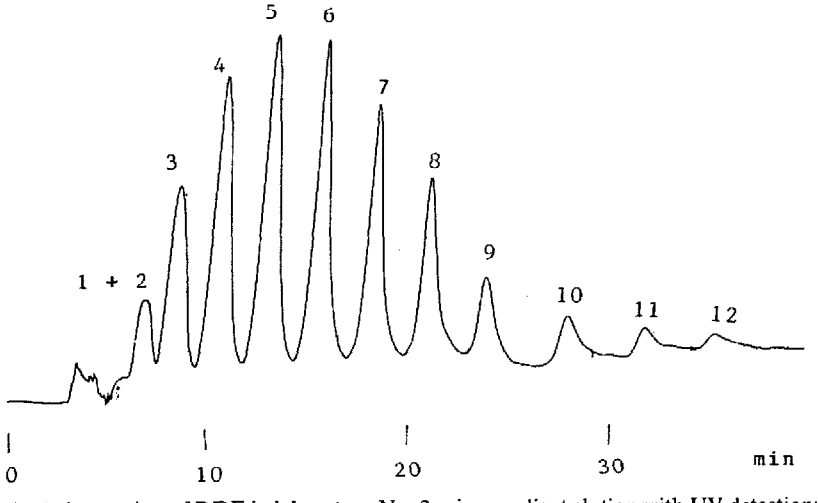

Fig. 2. Separation of DDF in laboratory No. 2 using gradient elution with UV detection; oligomers 1–12.

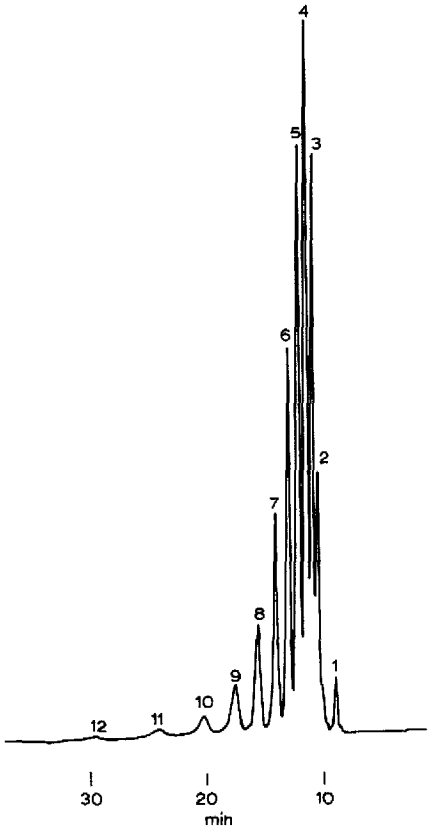

Fig. 3. Separation of S 905 in laboratory No. 5 using mobile phase with ratio of components 75:25:1; oligomers 1–12.

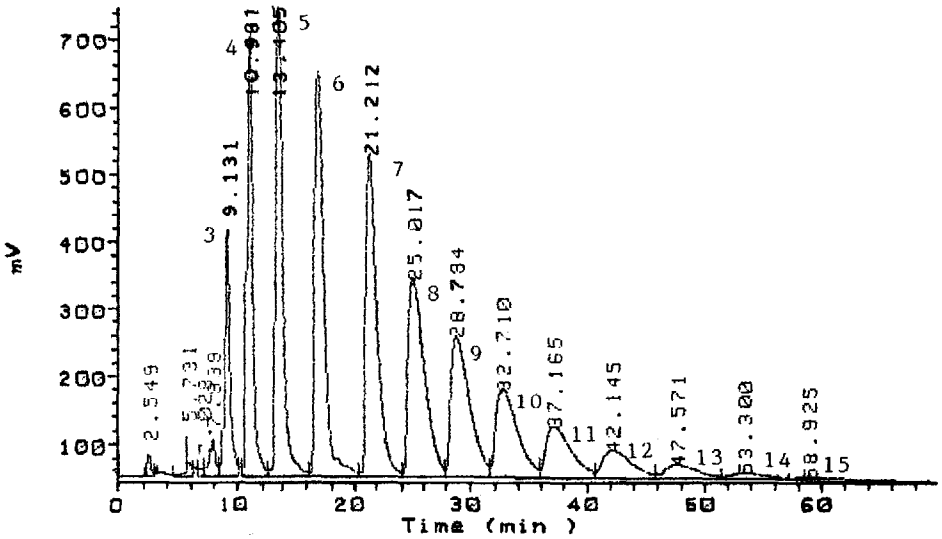

Fig. 4. Separation of U 6 in laboratory No. 3 with linear gradient elution from 100% *n*-hexane to 100% 2-propanol in 65 min with UV detection at 230 nm; up to oligomer 15.

TABLE VIII

STATISTICAL DATA FROM INTERLABORATORY TESTING

Parameter	*Main component oligomers*	*Other substantial oligomers*	*Trace component oligomers*
Contents (%)	13–20	3–12	⩽3
Standard deviation[a]	0.2–0.8	0.4–1.3	>0.3
Coefficient of variation (%)	1–6	6–12	>20
Repeatability	0.2–1.2	0.9–1.3	>0.3
As % of result	1.3–6.5	8–15	>30
Reproducibility	1.1–1.7	1.0–1.7	>0.3
As % of result	9–12	13–20	>40

[a] In separate oligomers content determinations.

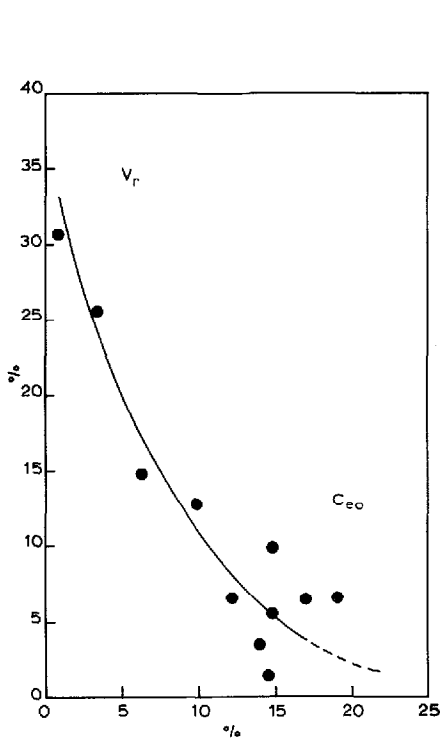

Fig. 5. Dependence of relative repeatability (v_r) in HPLC on relative contents of EO oligomers (c_{eo}).

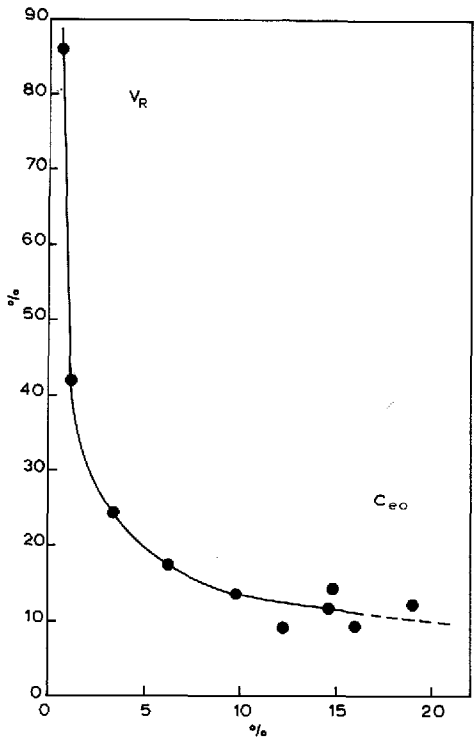

Fig. 6. Dependence of relative reproducibility (v_R) in HPLC on relative contents of EO oligomers (c_{eo}).

DISCUSSION

From the mean results of oligomer mass composition in Tables V–VII it is evident that the three HPLC variants gave nearly identical results with respect to distribution and contents of oligomers. This conformity of results is very valuable for confirming the efficient performance of the analysts in the participating laboratories and an effective arrangement for interlaboratory testing. In spite of the differences in instrumentation, operating conditions and HPLC experience among the laboratories, concordant data were obtained, and the results were not influenced by these differences evidently. In addition, almost identical results were obtained by an independent method, TLC–FID, where the separation of the oligomers was comparable to the HPLC separation with gradient elution, *i.e.*, better than HPLC with isocratic elution[17].

The statistical data (Table VIII) and their dependence on oligomer content (Figs. 5 and 6) showed repeatability values usual in chromatographic practice, *i.e.*, 5% relative or better for the main components, higher for other components and over 20% for trace components; data for relative reproducibility indicate a coincidence between two laboratories of within *ca.* 10% for the results for the main component oligomers, better than 20% for other constituents and worse than 40% for trace components. In this respect the results of interlaboratory testing are satisfactory.

Using the composition data for the calculation of mean molecular mass (MMM) of the corresponding surfactants (Tables V–VII), an even better coincidence is evident (within 1–3% from MMM data), showing the excellent accuracy of the HPLC analyses. Nearly, the same level of coincidence was found between theoretical MMM data calculated from the indicated degree of ethoxylation and MMM data calculated from the mean HPLC data (Table IX) for separate samples. All the HPLC data are lower, however, than those calculated from the degree of ethoxylation; this is evidently caused by the presence of free polyethylene glycol (PEG) in the samples (Table III). When the indicated degree of ethoxylation of the samples is compared with values calculated from the HPLC results considering the free PEG contents, nearly identical values were found (Table X). Thus the reliability of HPLC results is confirmed also by this way.

The capability of HPLC to differentiate between samples with different degrees of ethoxylation is clear; even differences substantially lower than 1 mol EO/mol can be determined owing to the reliability of HPLC.

The results indicate that all the requirements outlined in the introduction for quantitative evaluation in the HPLC analysis of ethoxylates were satisfied and the goals of interlaboratory testing were also met. Complete distributions of oligomers

TABLE IX

MEAN MOLECULAR MASS (MMM) DATA OF ADDUCTS IN ANALYSED SAMPLES

MMM	*DDF*	*S 905*	*U 6*
Theoretical MMM from degree of ethoxylation	526.8	426.6	484.7
Calculated MMM from mean HPLC data	508.3	428.3	478.5

TABLE X

DEGREE OF ETHOXYLATION OF ANALYSED SAMPLES (mol EO/mol)

Degree of ethoxylation	*DDF*	*S 905*	*U 6*
Indicated	6.0	5.0	6.0
Calculated from mean HPLC data, *i.e.*, for adducts	5.64	4.77	5.89
Corrected for free PEG content	5.98	5.00	6.00

were attained and their HPLC peaks were sufficiently separated and identified without difficulty. The applied correction factors for RI and UV detection appeared to be appropriate, giving reliable results; the differentiation between samples with slight variations in their degree of ethoxylation was easy by HPLC.

CONCLUSIONS

The oligomer distribution in ethoxylated surfactants of the alkylphenol type with lower degrees of ethoxylation can be determined by HPLC separation on diol bonded phases using RI or UV detection under isocratic conditions or with UV detection under gradient elution with *n*-hexane–2-propanol–water mobile phases. The results showed high reliability and acceptable accuracy, repeatability and reproducibility. The mean molecular mass data and degree of ethoxylation of the samples analysed can be calculated from the HPLC results, showing even better coincidence with the theoretical values. The coincident results of parallel TLC–FID analyses support the reliability of the HPLC results.

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