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DETERMINATION OF N-NITROSODIETHANOLAMINE IN COSMETICS

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY AS ALTERNATIVE METHODS TO CHEMILUMINESCENCE DETECTION

ROLF SCHWARZENBACH* and JOACHIM P. SCHMID
Givaudan Research Company Ltd., CH-8600 Dübendorf (Switzerland)
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SUMMARY

For the determination of N-nitrosodiethanolamine (NDELA) in cosmetic products chemiluminescence detection has been widely adopted, but two alternative techniques are now considered. A high-performance liquid chromatographic method using UV detection can be used for the routine determination of underivatized NDELA. A gas chromatographic–mass spectrometric method was also studied. Different derivatives of NDELA and several ionization methods were examined to match the selectivity and sensitivity given by the chemiluminescence detector.

INTRODUCTION

The possible presence of N-nitrosamines and their formation in cosmetic products have created a continuing need for the detection and positive identification of low levels, and numerous methods have been developed[1–6]. The thermal energy analyser, a chemiluminescence detector, is today the most popular detector for nitrosamine analysis. It provides excellent selectivity, combined with detection limits as low as 1–5 ng for most of the compounds. However, the operation of this detector is cumbersome and requires a skilled analyst. As thermal energy analysers are expensive instruments and dedicated to the analysis of nitrosamines, many analysts have attempted to explore other, simpler methods for the detection of these compounds in cosmetic samples[4–6]. These simplified methods can be particularly useful in quality control laboratories in the industry, where a deep knowledge of the sample to be analysed is available.

Based on an extraction and clean-up procedure developed by Sommer and Eisenbrand[7], we have explored the use of a direct high-performance liquid chromatographic (HPLC) method with UV detection, which proved suitable for detecting NDELA at low levels (5 ppb) in known products. As a further alternative to chemiluminescence detection, gas chromatography–mass spectrometry (GC–MS) of NDELA derivatives was examined. This method is highly specific and gives structural information for the positive identification of NDELA.

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EXPERIMENTAL

HPLC system

An M-6000 pump (Millipore Waters, Milford, MA, U.S.A.) was used to force the mobile phase (distilled water) through a 250 × 4.6 mm I.D. stainless-steel column packed with LiChrosorb RP-18, 5 μm (Merck, Darmstadt, F.R.G.) at room temperature. An M-440 UV detector (Millipore Waters) was used to monitor the eluate at 254 nm. To obtain low detection limits, the whole system must be operated under its optimum performance conditions. The water must be degassed carefully in an ultrasonic bath using argon. New columns must be purged with methanol before use to ensure complete wetting of the support surface.

GC system

A Fractovap 2150 gas chromatograph (Carlo Erba, Milan, Italy) was modified for on-column injection, equipped with a glass capillary column (laboratory-made, SE-30, film thickness 0.13 μm, 28 m × 0.3 mm I.D.) and operated at a carrier gas (helium) inlet pressure of 0.8 atm. Injections were made on-column at 100°C. The oven temperature was kept at 100°C for 3 min, then increased at 7°C/min to 240°C.

Mass spectrometer

A Finnigan-MAT (Bremen, F.R.G.) Model 212 mass spectrometer equipped with a combined electron impact–chemical ionization (EI–CI) source was coupled to the GC system by means of an open split interface. The instrument was connected to a Finnigan-Incos data system which controlled all accelerating voltage selected ion monitoring (SIM) scans. For accelerating negative ions a Bertan Model 205A-03R high-voltage power supply was used. The ion source temperture was 250 and 180°C in the EI and CI mode, respectively. Methane (CH4 45) and ammonia (N36) (Carbagas, Rümlang, Switzerland) were used as reactant gases in the CI mode.

Sample preparation

Cosmetic products. The method described by Sommer and Eisenbrand[7] was used for a wide range of cosmetic products. In some instances it was difficult to break the emulsion and the procedure was partly modified. However, clean extracts suitable for HPLC analysis or derivatization followed by GC–MS were obtained in most instances.

A 2-g amount of cosmetic product is mixed with 9 ml of distilled water and 0.5 g of ammonium sulphamate (Fluka, Buchs, Switzerland) is added. To break up the emulsion the mixture is saturated with sodium chloride (Fluka) and 1.5 ml of chloroform (Merck) are added. The mixture is placed on a glass column (20 × 3 cm I.D.) packed with 12 g of Extrelut (Merck). The mixture is allowed to penetrate into the adsorbent for 20 min, then the column is rinsed with 100 ml of cyclohexane–dichloromethane (1:1, v/v) and subsequently connected to a stream of nitrogen to force out all solvents. The NDELA fraction is then eluted with 150 ml of *n*-butanol (Merck). The eluate is evaporated to dryness.

A second glass column (20 × 3 cm I.D.) packed with sodium sulphate–silica gel is prepared as follows: 6 g of sodium sulphate are suspended in methanol–acetone (1:1) and placed at the bottom of the column, then 10 g of silica gel 40 (0.063–0.200 mm; Merck) in methanol–acetone (1:1) are carefully placed on top of the sodium sulphate

layer. The column is washed with *ca.* 20 ml of chloroform–acetone (5:1). The dry residue is dissolved in 20 ml of chloroform–acetone (5:1) and placed on top of the column, which is washed with 60 ml of chloroform–acetone (5:1) and then eluted with 50 ml of acetone. The final eluate is evaporated to dryness and the residue is used for direct analysis by HPLC or derivatized for GC–MS identification.

Alkanolamines. For the determination of NDELA in alkanolamines, a strong cation exchanger was used to bind all the amine according to the procedure proposed by Sommer *et al.*[8].

A 1-g amount of amine (*e.g.*, diethanolamine or triethanolamine) is dissolved in 10 ml of water–methanol (1:1). A 0.2% solution of methyl red (Fluka) in water–methanol (1:1) as indicator is added until the solution is yellow. A cation exchanger in the H^+ form (Amberlite IR-120, 16–45 mesh; Fluka) is added until the colour of the solution changes to red. More cation exchanger is added to adsorb the indicator and to produce an almost colourless solution (20–30 ml are typically used). This suspension is filled in a glass column (20 × 3 cm I.D.) and eluted with 100 ml water–methanol (1:1).

The eluate is evaporated to dryness and the residue is dissolved in 20 ml of chloroform–acetone (5:1) and placed on a glass column (20 × 3 cm I.D.) packed with sodium sulphate–silica gel (this column is prepared in the same way as described above for the determination of NDELA in cosmetic products). The silica gel column is washed with an additional 60 ml of chloroform–acetone (5:1) and finally eluted with 50 ml of acetone. The acetone eluate is evaporated to dryness and the residue can be used for direct separation on a HPLC system or derivatized for GC–MS.

Derivatization. The dry residue is dissolved in 200 μl of MSTFA reagent (N-methyl-N-trimethylsilyltrifluoracetamide; Fluka) and kept at room temperature for 45 min. The reaction mixture is then diluted to 0.5 ml with isooctane (Fluka) and aliquots are analysed by GC–MS for the NDELA bistrimethylsilyl (bis-TMS) ether (see formula II below).

Formation of the NDELA bis-*tert.*-butyldimethylsilyl (bis-tBuDMS) ether (see formula III below) is accomplished by reaction of the dry residue with *tert.*-butyldimethylchlorsilane imidazole reagent (Alltech/Applied Science, Rockwood, Canada) (0.5 ml) at 60°C for 30 min. The reaction mixture is diluted with 1 ml of water and extracted three times with hexane. The hexane extracts are subsequently evaporated to dryness and the residue is dissolved in 0.5 ml of isooctane.

RESULTS AND DISCUSSION

Direct determination by HPLC using UV detection

UV detection of NDELA after HPLC separation is an attractive alternative to chemiluminescence detection because it is simple and allows some of the present limitations of the thermal energy analyser detector to be overcome[9]. Since the early paper of Mitchell and Rhan[10], many analysts have successfully used direct UV detection. Wigfield and Lanouette[11] compared the sensitivity of UV and thermal energy analysis detection and demonstrated clearly that the signal-to-noise ratio in these two techniques is about the same for pure NDELA. In a complicated matrix, however, the determination of small amounts of NDELA is better accomplished by the thermal energy analyser detector owing to its high specificity. Therefore, the better the clean-up, the greater is the possibility of determining NDELA directly by HPLC with

UV detection. As the extraction and clean-up procedure described under Experimental leads to a sample matrix that is sufficiently free from interferences, NDELA can be determined at low ppb levels.

The UV absorption spectrum of NDELA is shown in Fig. 1. The maximum absorbance of this compound is at 234 nm. Using 254 nm in a single-wavelength UV detector, only about 50% of the signal is available. However, at 254 nm fewer interfering compounds absorb and therefore the signal-to-noise ratio may be even better. The transparency of the mobile phase is also higher at longer wavelengths and therefore more energy is available, which leads to a better signal-to-noise ratio. In the examples shown in Fig. 2, 254 nm is used as the detection wavelength. Fig. 2a shows 30 ppb[a] of NDELA in water, worked-up as a sample. The recovery was above 90%. A blank water sample (Fig. 2b) shows no interference in the eluting range of NDELA. Fig. 2c and d show chromatograms of an emulsion without and with 50 ppb of NDELA, respectively. The recovery was in the range 65–80% for this type of product. For shower shampoos, as shown in Fig. 2e and f, the recovery was better than 90%. The shampoo in Fig. 2e was spiked with 50 ppb of NDELA. These chromatograms show the efficiency of the clean-up technique for the HPLC analysis of real samples.

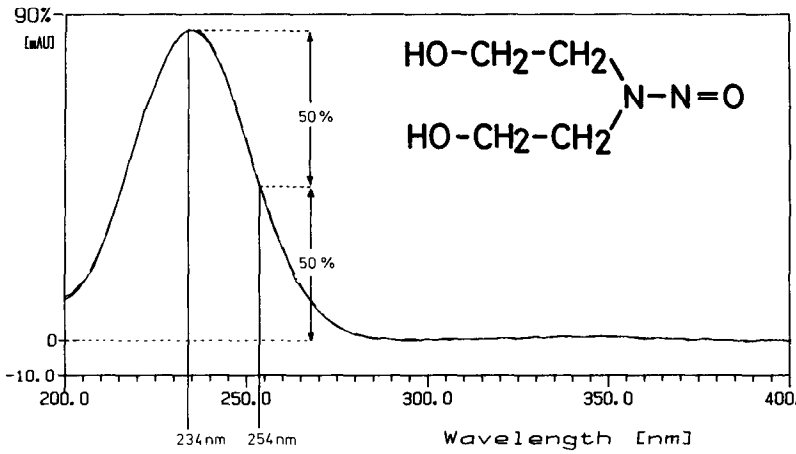


Fig. 1. UV absorption spectrum of N-nitrosodiethanolamine (mol.wt. 134).

The HPLC separation is achieved on a reversed-phase column. Only LiChrosorb RP-18 gives a reasonable retention for NDELA with water as the mobile phase. HPLC with organic mobile phases on silica gel or polar bonded phases is also possible, but such systems are not as easily equilibrated and tend to give lower signal-to-noise ratios.

The NDELA peak is identified by its retention time. Its concentration in the ppb range is too low for identification by a modern photodiode array detector. However, the retention time may be influenced by many factors and the possibility of co-elution with other compounds is high. To overcome this drawback, we used the extreme instability of NDELA to UV light to confirm its identity. The injection solution is

[a] Throughout this article, the American billion (10^9) is meant.

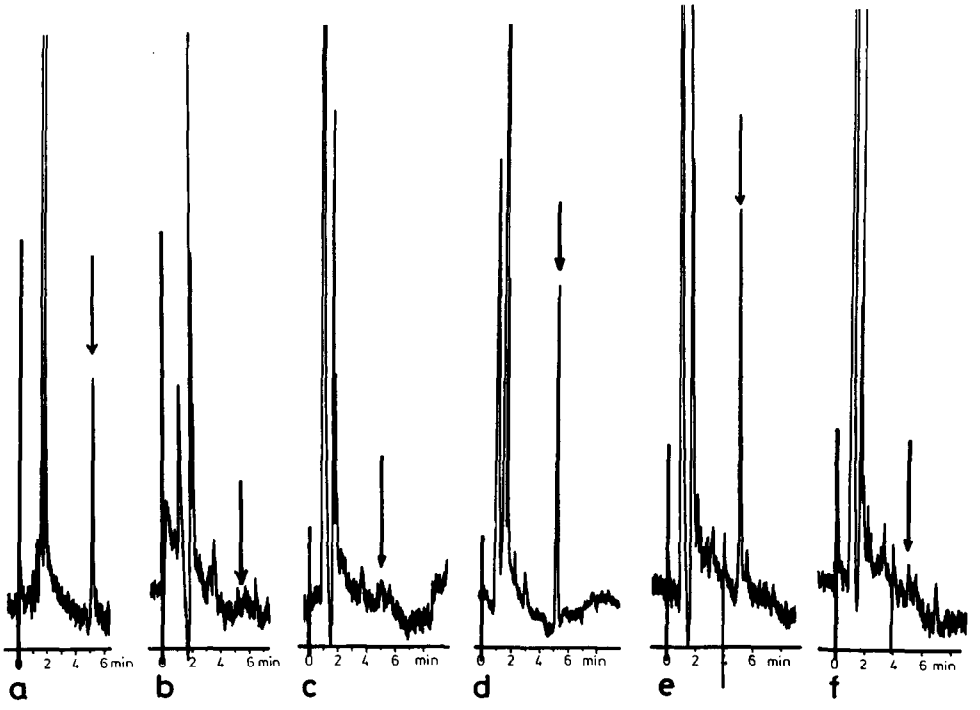
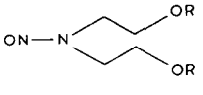


Fig. 2. Examples of HPLC with UV detection at 254 nm: (a) 30 ppb of NDELA in water; (b) blank water; (c) blank emulsion; (d) emulsion containing 50 ppb of NDELA; (e) shampoo containing 50 ppb of NDELA; (f) blank shampoo. Chromatographic conditions: column, LiChrosorb RP-18, 5 μm (250 × 3 mm I.D.); room temperature; mobile phase, water at a flow-rate of 1.0 ml/min.

placed in a quartz cuvette and irradiated for 5 min with a low-power mercury lamp. The disappearance of the peak corresponding to NDELA is taken as good evidence for its identity. This additional test can be used to minimize the possibility of analytical artifacts.

GC–MS of NDELA derivatives

Owing to the chromatographic behaviour and lack of thermal stability of the compound, derivatization of NDELA is essential for GC–MS determinations. NDELA bis-TMS ether (formula II), the most simple silylated derivative of NDELA, is easily formed by reaction with MSHFBA, MSTFA[7] or BSA[11]. For mass spectrometric reasons, the use of alternative silylated derivatives of NDELA such as NDELA bis-tBuDMS ether[12] (formula III) may be more useful.



I R = H(NDELA)
II R = $Si(CH_3)_3$
III R = $Si(CH_3)_2$-*tert.*-C_4H_9

Full scan mass spectra of compounds II and III were recorded under different ionization conditions. The resulting spectra are displayed in Figs. 3 and 4. The EI mass spectra of the two compounds (Figs. 3a and 4a) compare well with the spectra given by

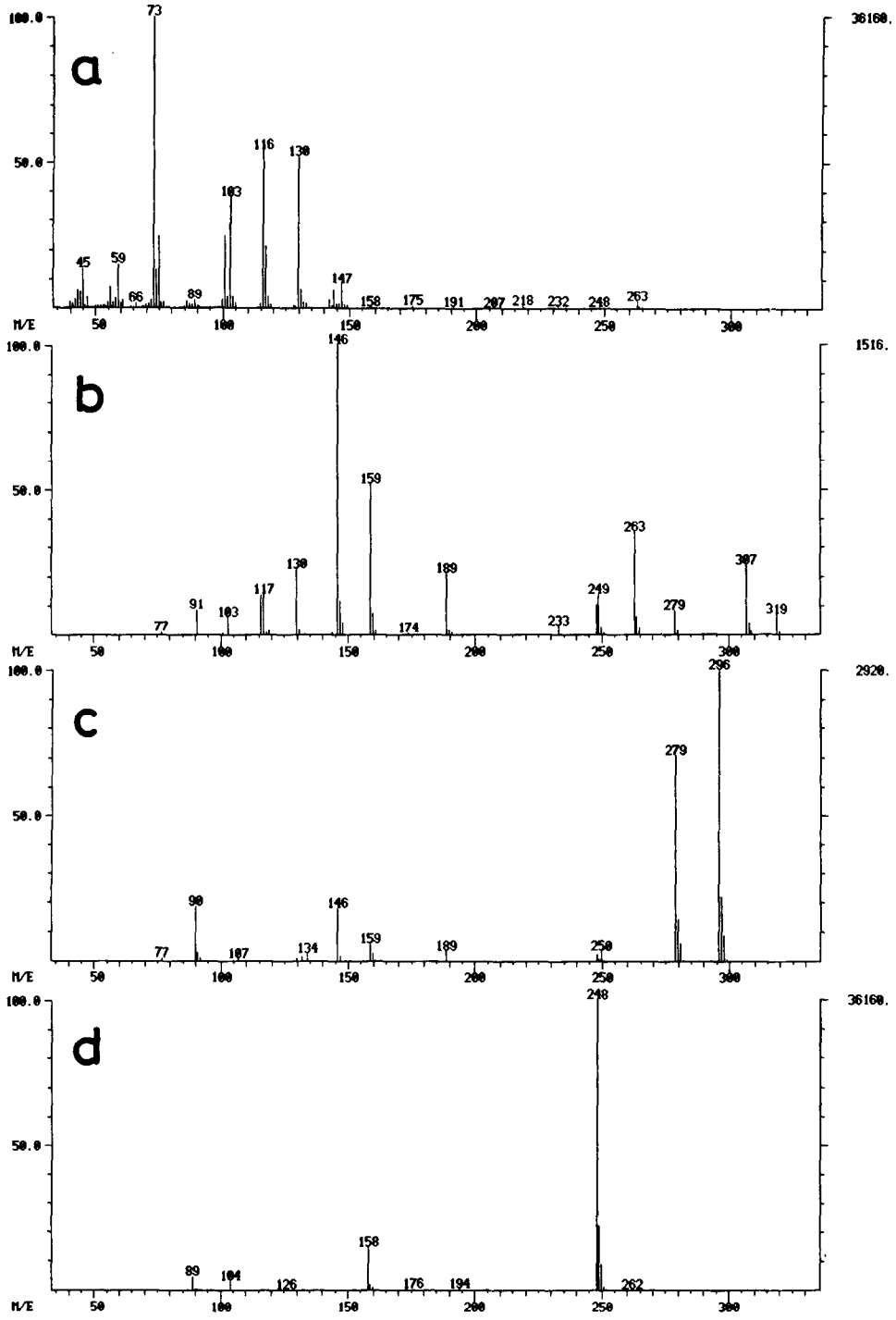


Fig. 3. Mass spectra of II (N-nitrosodiethanolamine bistrimethylsilyl ether, MW 278) using (a) EI, positive ion CI with (b) methane or (c) ammonia and (d) negative ion CI with methane.

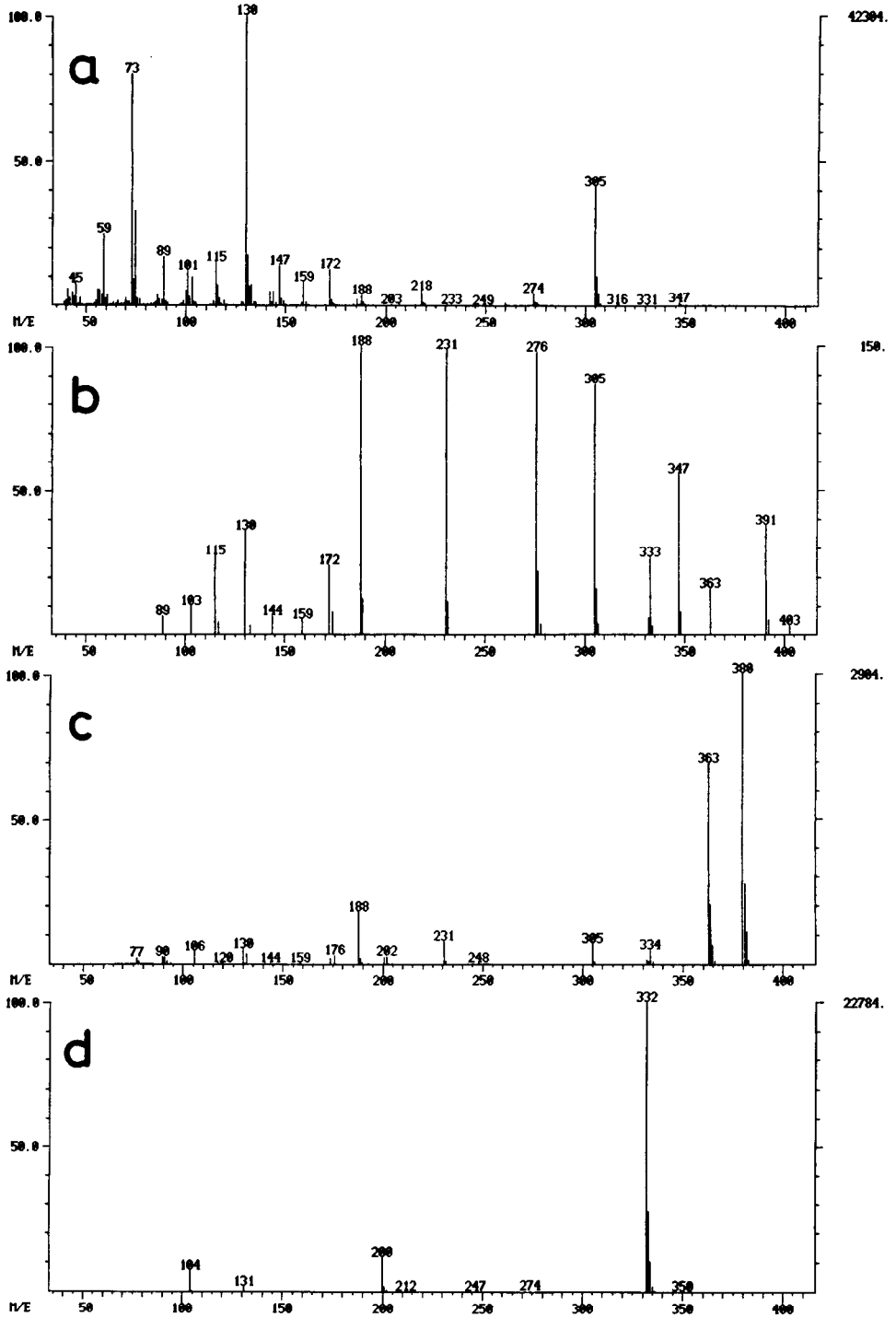


Fig. 4. Mass spectra of III (N-nitrosodiethanolamine bis-*tert.*-butyldimethylsilyl ether, MW 362) using (a) EI, positive ion CI with (b) methane or (c) ammonia and (d) negative ion CI with methane.

Issenberg *et al.*[12]. Although the two derivatives show a similar total ion yield, the distributions of peaks in their EI mass spectra differ considerably. Fig. 3a for compound II shows only a very low abundance at the structurally most characteristic peak of *m/z* 263 (loss of CH_3 from the molecular ion), but includes many strong peaks indicating merely side-chain fragments (*e.g.*, *m/z* 73, C_3H_9Si; *m/z* 103, $C_4H_{11}OSi$; *m/z* 116, $C_5H_{12}OSi$). Fig. 4a for compound III provides a signal of similar structural significance at *m/z* 305 (loss of C_4H_9 from the molecular ion) which, however, is of much higher abundance (*ca.* 45% of the base peak) and therefore much better suited for use in SIM measurements.

Methane positive ion CI mass spectra of the two compounds (Figs. 3b and 4b) are of little interest for the purpose of quantitation or for providing structural information, as the total ion yield is very low and concurrently a wide variety of fragment peaks with little significance are observed, the pseudo-molecular ions (*m/z* 279 and 363 respectively) being of almost no importance. A totally different situation is encountered in the methane negative ion CI mass spectra (Figs. 3d and 4d), where high-intensity peaks are measured for both compounds. The spectra display a base peak that corresponds to a loss of NO from the molecular ion and presumably is formed through a dissociative electron-capture process[13]. Thus the peaks at *m/z* 248 and 332 are excellent possibilities for use in SIM quantifications. For compound II these data are in good agreement with the spectra given by Wigfield and Lanouette[11].

The ammonia positive ion CI mass spectra (Figs. 3c and 4c) were measured in order to complete the information available about the positive ion CI of those compounds. In contrast to methane CI, qualitatively good spectra with high-abundance pseudo-molecular ion peaks of $M+H$ and $M+NH_4$ were observed. The overall sensitivity in this ionization mode, however, turned out to be low in preliminary experiments.

SIM trace determinations of NDELA derivatives

EI SIM measurements on the NDELA bis-TMS-derivative II were tried but turned out to be very unsatisfactory, as can easily be understood from examination of Fig. 3a. The SIM traces on all the mass peaks at *m/z* 103, 116, 130 or 263 included a large number of interfering GC peaks and also showed an elevated baseline due to ion noise, thus leading to an unfavourable signal-to-noise ratio. This situation was encountered with both standard injections and injections of cosmetic emulsion samples. Even increasing the mass spectrometric resolution to $m/\Delta m = 4000$ only slightly improved the results. Switching the ion source to methane negative ion CI provided the selectivity and sensitivity necessary for the analysis of cosmetic emulsions (Fig. 5): injections of an emulsion sample containing 50 ppb of NDELA (Fig. 5b) and of a standard sample with an identical amount of NDELA (Fig. 5a) resulted in very similar SIM mass chromatograms, practically free from interfering peaks, thus indicating the excellent selectivity of the method[14]. Comparison of the analysis of a sample with an unknown NDELA content (*ca.* 7 ppb, Fig. 5c) with the result for a blank injection (Fig. 5d) indicates a good signal-to-noise ratio.

Results of EI SIM analyses of the NDELA bis-tBuDMS derivative III are shown in Fig. 6. The mass chromatograms are not free from interfering peaks from the derivatization step (Fig. 6a) or the emulsion matrix (Fig. 6b–d), but the selectivity obtained by scanning the *m/z* 305 mass peak has drastically increased as compared

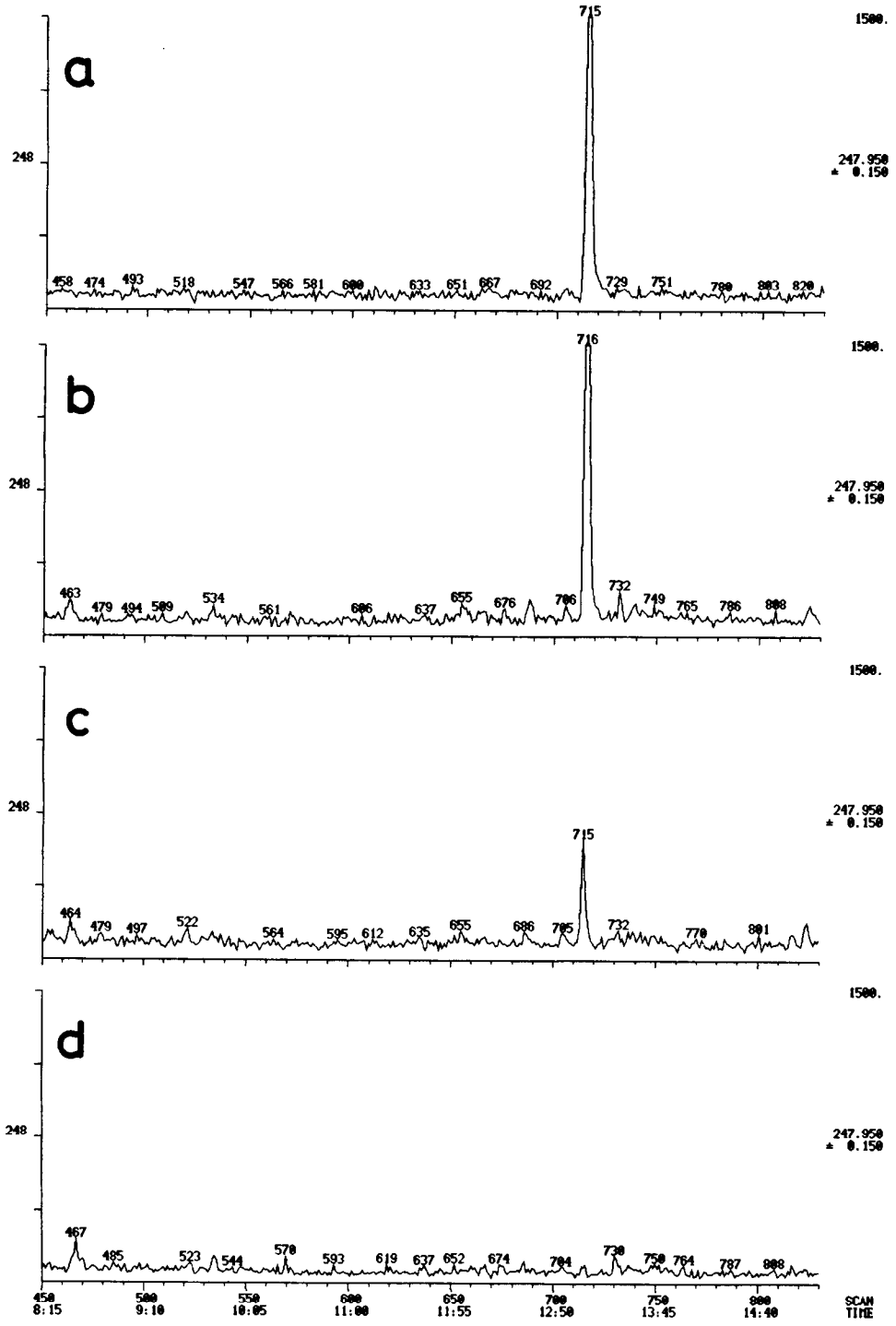


Fig. 5. Negative ion CI (methane) SIM mass chromatograms for m/z 248 ($m/\Delta m$ = 1000). Samples (derivatized with MSTFA): (a) NDELA standard (420 pg of derivative, corresponding to 50 ppb in an injection aliquot); (b) emulsion, after clean-up, containing 50 ppb of NDELA; (c) emulsion, after clean-up, with unknown amount of NDELA; (d) blank (solvent and derivatization reagent). Uniform attenuation in all instances.

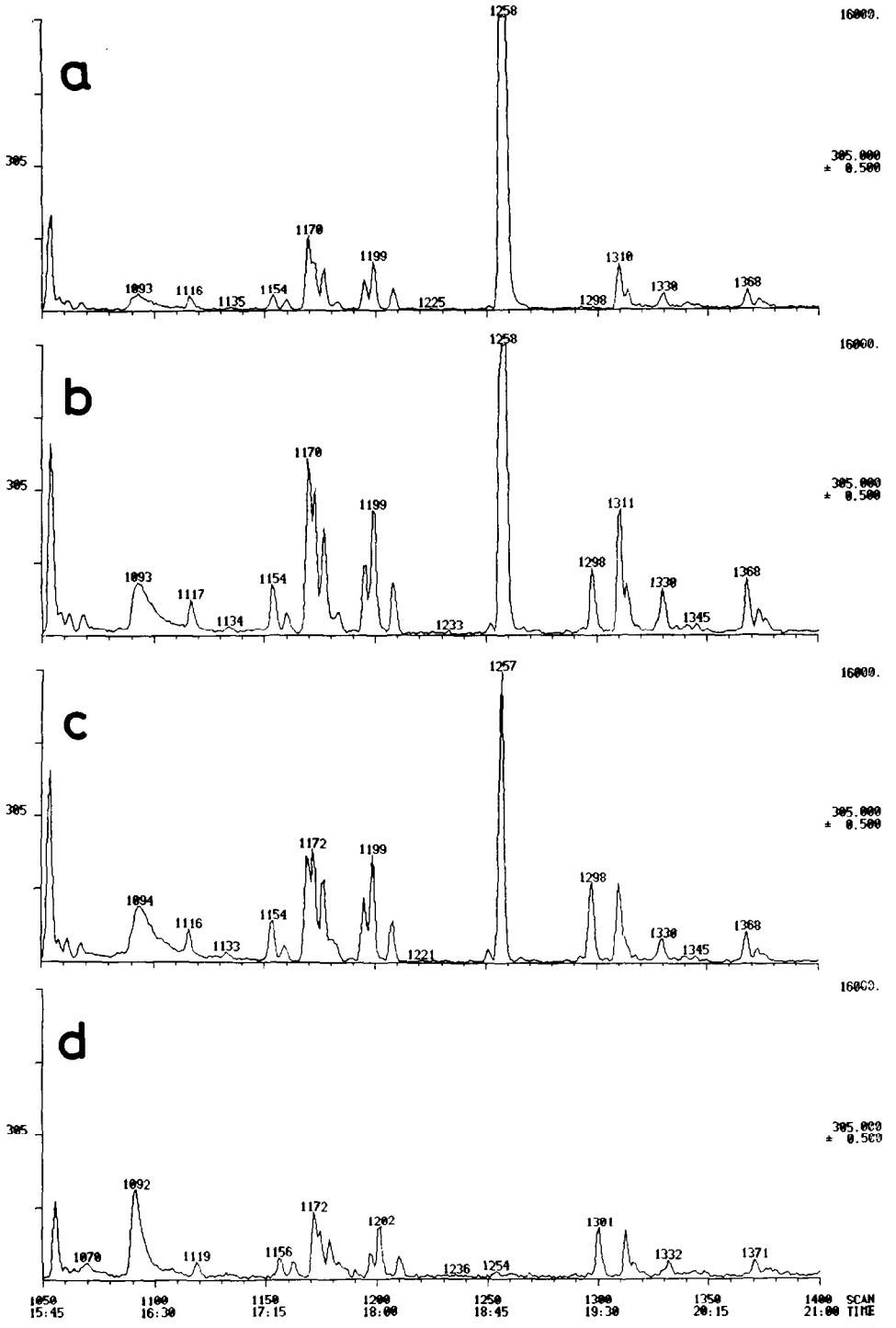


Fig. 6. EI SIM mass chromatograms for m/z 305 ($m/\Delta m$ = 1000). Samples (derivatized with t-BuDMCS–imidazole): (a) NDELA standard (540 pg of derivative, corresponding to 50 ppb in an injection aliquot); (b) emulsion, after clean-up, containing 50 ppb of NDELA; (c) emulsion, after clean-up, with unknown amount of NDELA; (d) sample (c) after irradiation for 30 min with UV light. Uniform attenuation in all instances.

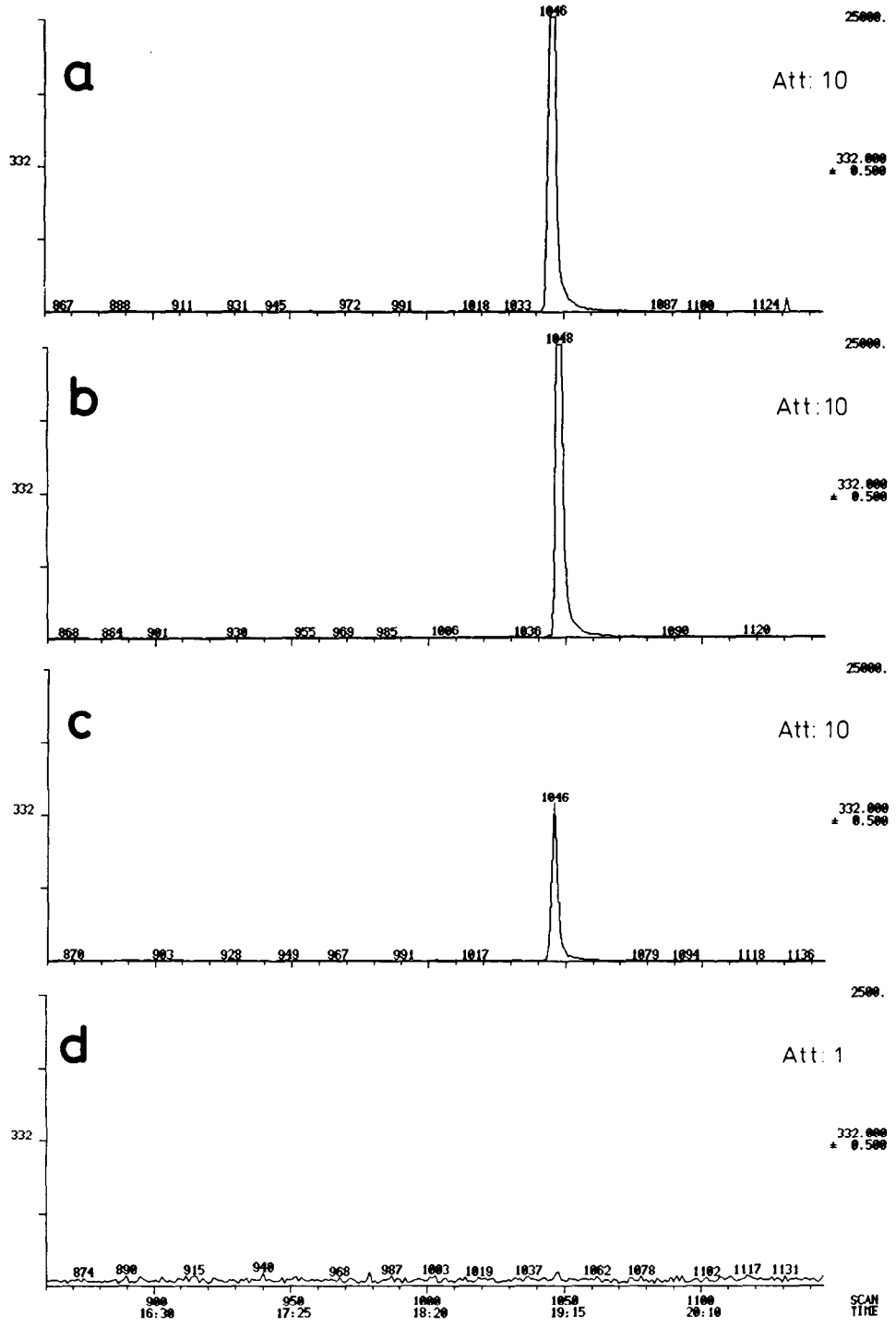


Fig. 7. Negative ion CI (methane) SIM mass chromatograms for m/z 332 ($m/\Delta m$ = 1000). Samples (derivatized with t-Bu DMCS–imidazole): (a) NDELA standard (540 pg of derivative, corresponding to 50 ppb in an injection aliquot); (b) emulsion, after clean-up, containing 50 ppb of NDELA; (c) emulsion, after clean-up, with unknown amount of NDELA; (d) sample (c) after irradiation for 30 min with UV light. Attenuation as indicated.

with EI SIM analyses of derivative II, and it facilitates the determination of an estimated 15 ppb of NDELA with a very good signal-to-noise ratio (Fig. 6c). Fig. 6d was obtained after UV irradiation of an injection aliquot of the sample from Fig. 6c, thus giving a measure of the noise level in the mass chromatograms and further proving the identity of the GC peak with a retention time of 18.75 min.

For the derivative III, a similar sensitivity in methane negative ion CI to that for derivative II could be expected from comparison of the full scan spectra (Figs. 3d and 4d). However, a better signal-to-noise ratio due to the mass shift of 84 units to a mass peak with less background ion noise at m/z 332 could also be anticipated. This indeed occurs, as illustrated very clearly by the SIM mass chromatograms of standard and cosmetic emulsion samples in Fig. 7. Again there are no interfering peaks present, and the chromatogram obtained from the UV-irradiated, and therefore NDELA-free, sample (attenuation factor ten times lower) demonstrates an excellent signal-to-noise ratio. For the sample with an unknown NDELA content (*ca.* 15 ppb, Fig. 7c), this ratio was calculated to be about 200:1.

CONCLUSION

The determination of NDELA still receives attention in spite of the many methods available today. Two alternative means of determining NDELA at the ppb level have been presented here. The mass spectrometer, operated in the negative ionization mode, has been shown to be more sensitive than the thermal energy analyser detector. UV detection after HPLC separation proved to be an easy method for routine determinations of NDELA in known products. The success of these methods was made possible by the use of an efficient clean-up procedure[7,8].

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