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The identification of polyoxyethylene glycols and related compounds by capillary gas chromatography*

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Abstract: A sample work-up method for gas chromatographic profiling of polyoxyethylene glycol (PEG)-related compounds in pharmaceutical matrices is described. After a short sample clean-up, carbon–oxygen linkages are partially cleaved with 0.07 M boron tribromide in dichloromethane at room temperature. The reaction is stopped after 1 min by addition of 0.01 M HCl. The products are trimethylsilylated and injected onto a WCOT 50 m × 0.25 mm CP-SIL 5 CB fused silica column. Eleven model compounds, representing four common types of PEG-derivatives, have been evaluated by this method. The results show that characteristic profiles can be obtained from PEG-derivatives carrying different functional groups. Minimum detectable amounts are in the range of 200 μg.

Keywords: *Polyethylene glycol and related compounds; pharmaceutical adjuvants; capillary gas chromatography; gas chromatography–mass spectrometry; non-ionic surfactants.*

Introduction

Polyoxyethylene glycols (PEG) are condensation products of ethylene oxide and water, having the general formula $HOCH_2—CH_2(O—CH_2—CH_2)_n—OH$. Various physicochemical properties are obtained by the introduction of different functional groups into the basic PEG-chain, giving rise to a wide range of PEG-esters, PEG-ethers or PEG-sorbitan derivatives, which are commonly used in the formulation of cosmetics, foods and detergents. In pharmacy, PEG-related compounds are used as solvents, lubricants, emulsion stabilizers, surfactants or as water miscible excipients for ointments, pessaries and suppositories.

An important aspect of the work of laboratories for quality control of pharmaceuticals is the development of profiling methods for the simultaneous detection and identification of chemically related compounds [1]. Such tests enable the simple screening of adjuvants in pharmaceutical preparations.

Many papers have been published dealing with the analysis of native PEGs. Thin-layer chromatography (TLC) has been successfully applied [2, 3]. Likewise high-performance gel permeation chromatography with refractometric detection can be used for the determination of molecular weight distribution of bulk PEGs [3–6]. The sensitivity of the HPLC-methods can be increased by the use of low wavelength UV-detection [7], UV-absorbing derivatives [4] or by coupling to a mass spectrometer [8]. When differentiation between different types of PEG-related compounds is required, liquid chromatography–mass spectrometry (LC–MS) offers undoubtedly the most powerful tool [9, 10]. However, this technique is not available in most routine laboratories, whilst the more commonly used HPLC- and TLC-methods [11] lack selectivity.

For the analysis of PEGs with molecular weights of 500 or less, gas chromatography has proved to be acceptable [12]. Also, nonylphenolethoxylates with an average number of 3.15 oxyethylene groups have been studied by gas chromatography–mass spectrometry (GC–MS) after isolation from sewage water [13]. Generally it is found that PEG-analogues of higher molecular weight have to be decomposed into more volatile fragments before gas chromatographic analysis can be carried out. Both pyrolysis [14, 15] and ether cleavage methods have been described [16–19]. With

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boron tribromide, PEG is decomposed quantitatively into 2-bromoethanol and 1,2-dibromoethane [16]. The same principle has been applied to some alkylphenolethoxylate surfactants, which were measured as 1,2-dibromoethane in biological samples after cleavage with hydrobromic acid–glacial acetic acid (1:1) [17]. Recently, the degradation products, produced in an excess of acetylchloride of the following PEG-related compounds were studied: PEG-dodecylether, trioxyethylene-decylether [18], PEG 2000, PEG 1500 and two block copolymers of ethylenoxide and α-butyleneoxide [19]. This method is interesting, insofar as it enables decomposition products of both the PEG-chain and the pendant hydrophobic groups to be monitored simultaneously. However, the use of packed column gas chromatography and the high volatility of the halogeno-ethanes, when compared with the side chain products, limits the applicability of the method.

The possibility of identifying PEG-related compounds by means of capillary gas chromatographic profiles, obtained after partial decomposition with boron tribromide has been investigated. Eleven model compounds, representing four common types of PEG-derivatives, have been evaluated in the study which also gave rise to a rapid sample clean-up procedure for pharmaceutical products.

Experimental

Materials

All reagents used were of analytical grade. Dichloromethane, anhydrous sodium sulphate and sodium hydroxide were obtained from Merck (Darmstadt, FRG); heptadecanoic acid from Aldrich Europe (Belgium); boron tribromide, acetonitrile and hexane were purchased from Janssen Chimica (Beerse, Belgium).

The hydrocarbons *n*-decane, *n*-dodecane, *n*-tetradecane, *n*-hexadecane, *n*-octadecane, *n*-eicosane, *n*-docosane and *n*-tetracosane were obtained from Alltech Associates (Deerfield, USA). *N*,*N*-bis-trimethylsilyltrifluoroacetamide (BSTFA) was supplied by Macherey-Nagel (Duren). All reference products, listed below, were of pharmaceutical grade and were supplied by courtesy of several pharmaceutical companies: PEG 6000 (Janssen Chimica, Belgium), PEG 400, PEG 1500, PEG 4000, PEG-400-stearate, Polysorbate 20, Polysorbate 60, Polysorbate 80 (Pharmachemic, Belgium), Cetomacrogol 1000 (Henkel, Dusseldorf, FRG), Tyloxapol (Lab. Thissen, Belgium) and Octoxinol (Ortho Cilag Pharmaceutical Ltd).

Instrumentation

All gas chromatographic experiments were performed on a Varian model 3700 gas chromatograph equipped with a split/splitless capillary injector and a flame ionization detector. The column used was a WCOT 50 m × 0.22 mm i.d. CP-SIL-5 CB fused-silica column with a film thickness of 0.14 μm. Temperature settings were as follows: injector, 250°C; detector, 250°C; oven, 40 to 280°C at 8°C min^{-1}. Integration and calculation of methylene-units (MU) were performed on a Hewlett–Packard model 9816 microcomputer with a modified Nelson model 4416 integration software package.

Electron impact (70 eV) mass spectra (EI) were recorded using a Finnigan TSQ 70 Mass spectrometer. The electron emission current was 200 μA and the ion source temperature was 150°C. Chemical ionization mass spectra (CI) were recorded with isobutane as reagent gas at a pressure of 0.3 torr. The electron emission current was 200 μA and the ion source temperature was 100°C. The mass range was 50–700 mass units at a scan rate of 1 scan s^{-1}.

Sample pretreatment

(a) *Solid and semi-solid dosage forms with a low water content (grounded tablets, contents of capsules, powders, creams, unguents, suppositories etc, . . .).* Ideally, an aliquot containing between 1–10 mg of a PEG-related compound was used for analysis. When the approximate content was unknown, 250 mg of a solid or semi-solid dosage form was used for analysis. The sample was weighed into a test tube, 1 ml of acetonitrile was added and the mixture was shaken for 1 h at room temperature. The tube was then centrifuged at 4000 rpm for 5 min. Subsequently, the supernatant was washed with two 1-ml aliquots of hexane, taken to dryness under a stream of nitrogen.

(b) *Dosage forms with high water content (suspensions, syrups, hydrous solutions and gels).* Ideally, an aliquot containing between 1–10 mg of a PEG-related compound was freeze-dried. To the residue obtained 1 ml of acetonitrile was added and the mixture was

shaken for 1 h at room temperature. After centrifugation at 4000 rpm for 5 min, the supernatant was washed with two 1-ml aliquots of hexane and taken to dryness under a stream of nitrogen.

Chemolysis and derivatization

A 50-μl volume of a 0.002% (w/v) heptadecanoic acid (10 μg) in dichloromethane was added as an internal standard to the dry residue, obtained after sample preparation or to about 5 mg of pure reference compound. The mixture was shaken vigorously for 5 min, 70 μl of a freshly prepared 0.07 M boron tribromide solution in dichloromethane then was added under anhydrous conditions. After exactly 1 min at room temperature, the reaction was stopped by the addition of 0.5 ml of 0.01 M HCl. The organic phase was transferred and the aqueous layer washed with 1 ml of dry dichloromethane. The combined dichloromethane fractions were dried over anhydrous Na_2SO_4 and transferred into a 2-ml vial. A 50-μl volume containing 6.25 μg tetracosane solution in dichloromethane, was added as a second internal standard. The solvent was removed under a stream of nitrogen, carefully avoiding temperatures above 30°C. Trimethylsilyl-derivatives were then prepared by adding 50 μl BSTFA and allowing the reaction to continue for 1 h at 80°C. Finally, 1 μl of the reaction product was injected into the GC or GC–MS system.

Results and Discussion

(a) *Partial chemolysis of native PEGs with boron tribromide*

The complete breakdown of 1 mg PEG 20000 (0.05 μmol) is achieved at 30°C within 15 min with an amount of boron tribromide, exceeding 100 μmol [16]. Other reagents require high temperatures with long reaction times [19] and are therefore less appropriate for pre-column solute modification.

In order to explore the applicability of the above process for solute identification, the possibility of interrupting the decomposition process at an intermediary stage was investigated. PEG 400 and PEG 6000 were studied under varying reaction conditions using 6 mg (±15 μmol) and 6 mg (±0.9 μmol), respectively. The basic procedure was as described in the Experimental section (Chemolysis and derivatization). However, instead of 50 μl dichloromethane and 70 μl of reagent solution, 500 μl and 700 μl, respectively were used. The following boron tribromide solutions were evaluated: 1.73, 0.173, 0.07, 0.035 and 0.0173 M. This corresponds to the addition of 1210, 121, 49, 25 and 12 μmol boron tribromide to about 136 μmol ethyleneoxide-units. The reaction times were 15, 5, 4, 3, 2, 1 and 0.5 min, respectively. All experiments were carried out at room temperature. The results were evaluated by comparing relative peak areas, using heptadecanoic acid as the internal standard. Tetracosane was added before derivatization as a second internal standard, thus providing an internal control of the injected volumes, a recovery of the first internal standard and methylene unit deviations. The highest relative peak areas were obtained after a 1-min reaction with 0.07 M boron tribromide solution. These conditions were not very critical since satisfactory results were still obtained with 0.035 M boron tribromide or with incubation times of 30 s only.

A typical GC-profile from PEG (Fig. 1) shows several pairs of peaks, approaching each other at increasing retention times and finally overlapping at the end of the chromatogram. Both alterations in incubation times or in boron tribromide concentration caused the relative areas of all peaks to decrease simultaneously. On comparing PEG 400, PEG 1500, PEG 4000 and PEG 6000, correlations between the degree of polymerization of the original molecule and the areas or area ratios of certain peaks were not apparent.

Under the above conditions, a detection limit of 1 mg was found for all PEGs studied. However, the detectable amounts decreased down to 200 μg by reducing the volumes of reagent solutions, as described in the Experimental section.

The molecular weights of the cleavage products were derived from the CI-spectra, obtained by GC–MS studies (Fig. 1). Isotope ratios of the $[M+H]^+$-ions in these spectra were in accordance with the presence of 1 and 2 bromine atoms, respectively. In the EI-spectra, several fragments were attributed to consecutive loss of 44 mass units, giving further evidence for the presence of contiguous ethyleneoxide fragments. From these experiments, it was concluded that the decomposition products were identical with homologuous series of monobromo- (MU 11.91, 14.59, 17.25, 19.92, 22.54, 25.23, 27.92) and di-

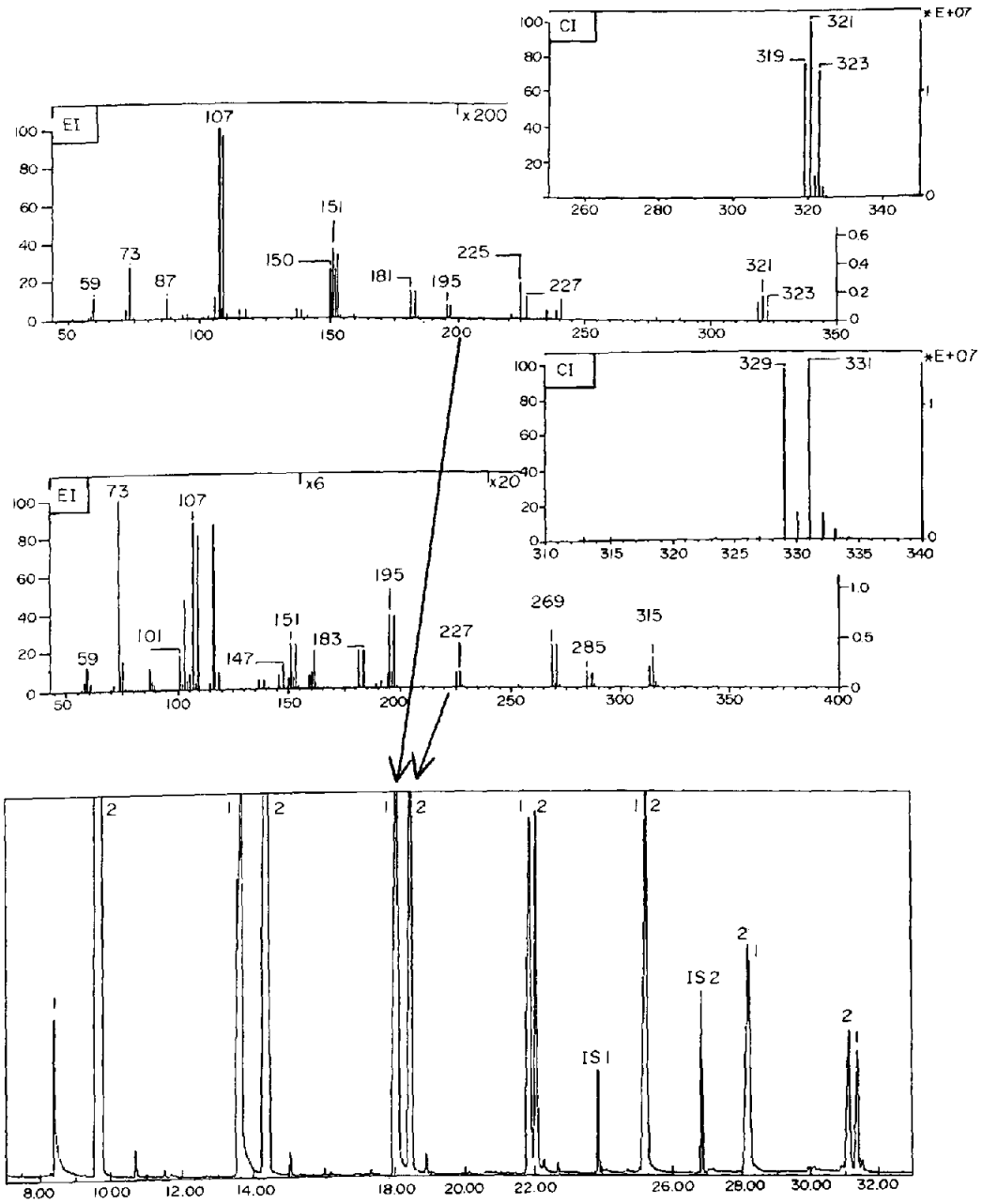


Figure 1
Typical profile and mass spectra of PEG 6000: Key: 1, Br—(CH_2—CH_2—O)$_n$—CH_2—CH_2—Br (dibromopolyethoxylates, DBPE); 2, Br—(CH_2—CH_2—O)$_n$—CH_2—CH_2—OTMS (monobromopolyethoxylates-TMS1, MBPE-TMS); IS1, internal standard 1; IS2, internal standard 2.

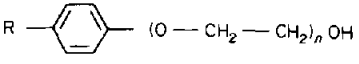
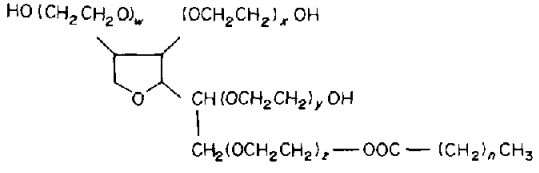
bromopolyethoxylates (MU 11.23, 14.12, 16.97, 19.75, 22.49, 25.29, 28.18), containing between 2 and 8 ethyleneoxide-units.

Oligomeric dihydroxypolyethoxylates were not found. These diol-fragments, if formed by the reaction, are water-soluble and were probably lost during the final extraction.

(b) *Study of commonly used PEG-ethers, esters and sorbitan-esters*

The model compounds used in this study are summarized in Table 1. As shown in Figs 2–4, the same chemical degradation procedure was successfully applied to all types of PEG-related compounds studied. In the first part of the

Table 1
Overview of products evaluated

Group formula	Compound name	Substituent	Number of Et_2O-units	Approx. mol. wt
1. PEG $H-(O-CH_2-CH_2)_n-OH$	PEG 400	—	8–9	380–420
	PEG 1500	—	29–36	500–600
	PEG 4000	—	69–84	3100–3700
	PEG 6000	—	115–160	6000–7500
2. PEG-esters $R-CO-(O-CH_2-CH_2)_n-O-CO-R$ $H-(O-CH_2-CH_2)_n-O-CO-R$	PEG-400-stearate	Stearate	7,5	636
3. PEG-ethers alkoxypolyethoxylates $CH_3(-CH_2)_m-(OCH_2CH_2)_n-OH$	Cetomacrogol 1000	Cetylalcohol	20–24	1122–1326
alkylphenoxypolyethoxylates	Octoxinol	4-(1,1,3,3-Tetra-methylbutyl)phenol	9	646
	Tyloxapol	4-(1,1,3,3-Tetra-methylbutyl)phenol polymer. Formaldehyde	9	2812
4. PEG-sorbitan esters				
	Polysorbate 20	Monolaurate	20	1228
	Polysorbate 60	Monostearate	20	1312
	Polysorbate 80	Monooleate	20	1310

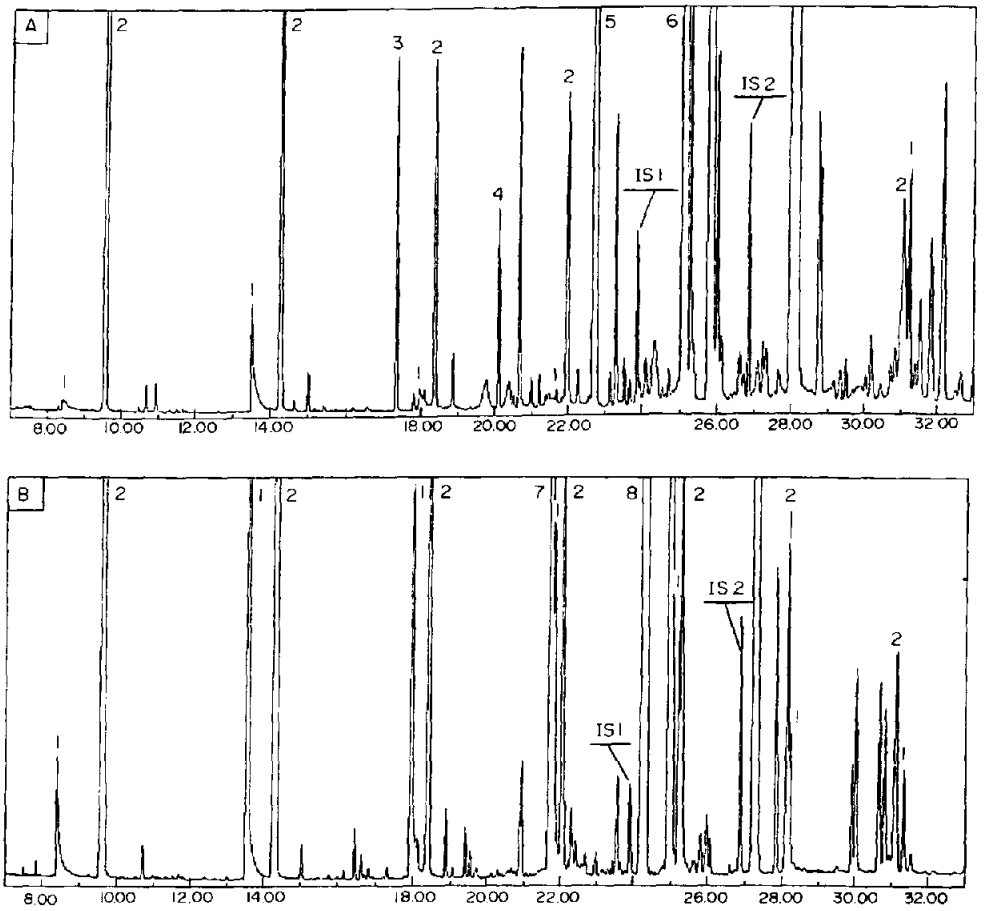


Figure 2
Typical profiles of PEG-400-stearate (A) and Cetomacrogol 1000 (B): Key: 1, DBPE; 2, MBPE–TMS; 3, lauric acid–TMS1; 4, myristic acid–TMS1; 5, palmitic acid–TMS1; 6, stearic acid–TMS1; 7, cetylalcohol–TMS1; 8, stearylalcohol–TMS1; IS1, internal standard 1; IS2, internal standard 2.

chromatogram the typical doublets of peaks from the PEG-chain were clearly distinguished, whilst a typical fingerprint pattern is found in the second part, representing the hydrophobic parts of the molecules. An overview of the retention data of the most prominent peaks in each profile is given in Table 2. No interfering peaks were observed in blank derivatization mixtures.

The profile of Tyloxapol did not differ significantly from PEG, due to the fact that the hydrophobic part of Tyloxapol is a stable formaldehyde polymer resistant to the reagent. In some cases, i.e. PEG-400-stearate and PEG-1000 monocetylether, typical fatty acids or fatty alcohols were identified by retention coincidence. In order to recover acidic substituents, released during the decomposition of PEG-related compounds, acidic conditions were needed in the final extraction step. Therefore, after addition of 10% NaOH as suggested by Drevin and Johansson [16], acidification was necessary. It was found that the same results were obtained when 0.01 M HCl was added immediately to the reaction mixture, omitting the use of 10% NaOH.

As the present study was not focused on the exact identification of the different cleavage products obtained, these have not been evaluated in detail. However, as a confirmation test, a GC–MS run has been carried out for octoxinol. Again, the same monobromo- and dibromopolyethoxylates were found. Additionally, a series of spectra were tentatively identified as a homologuous series of polyethoxylates and monobromopolyethoxylates carrying the hydrophobic substituent (4-(1,1,3,3-tetra-methylbutyl)phenol (Fig. 3).

PEG-related compounds of pharmaceutical grade always consist of mixtures of analogues

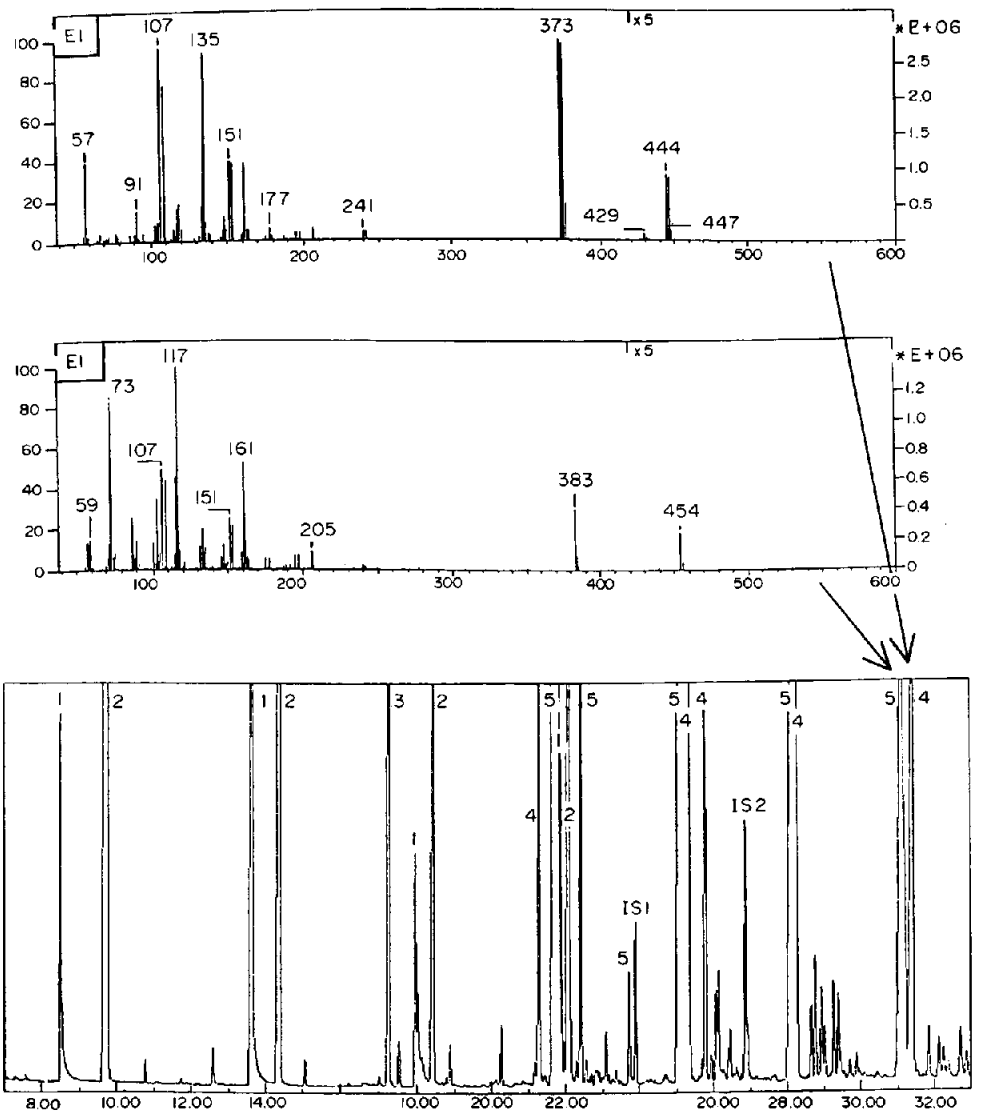


Figure 3
Typical profile and mass spectra of Octoxynol: Key: 1, DBPE; 2, MBPE–TMS; 3, $(CH_3)_3C—CH_2—C(CH_3)_2—C_6H_5—$OTMS; 4, $(CH_3)_3C—CH_2—C(CH_3)_2—C_6H_5—(O—CH_2—CH_2)_n—Br$; 5, $(CH_3)_3C—CH_2—C(CH_3)_2—C_6H_5—(O—CH_2—CH_2)_n—$OTMS; IS1, internal standard 1; IS2, internal standard 2.

with varying PEG-chain lengths, number and position of substituents, carbon number of hydrophobic substituents, etc. Thus, in the polysorbates only the average total number of ethyleneoxide units for each sorbitan molecule is specified [10, 13]. As a consequence, one can expect differences in the profiles between products with the same base name but produced by different manufacturers. Also, it has not been proven by our work that exactly the same profiles are obtained from different brands of the same compound. However, since it was found that all profiles were reproducible within the same batch, the proposed method could be of help for the further differentiation between batches of material and their manufacturers.

(c) *Extraction from pharmaceuticals*

A rapid sample clean-up step allowed extraction of PEG-related compounds from pharmaceutical preparations. The proposed extraction scheme is suitable for detecting PEG-related compounds in drug formulations at the 1% concentration level or higher. Due to interferences from the pharmaceutical matrix, further purification is still required for lower concentrations.

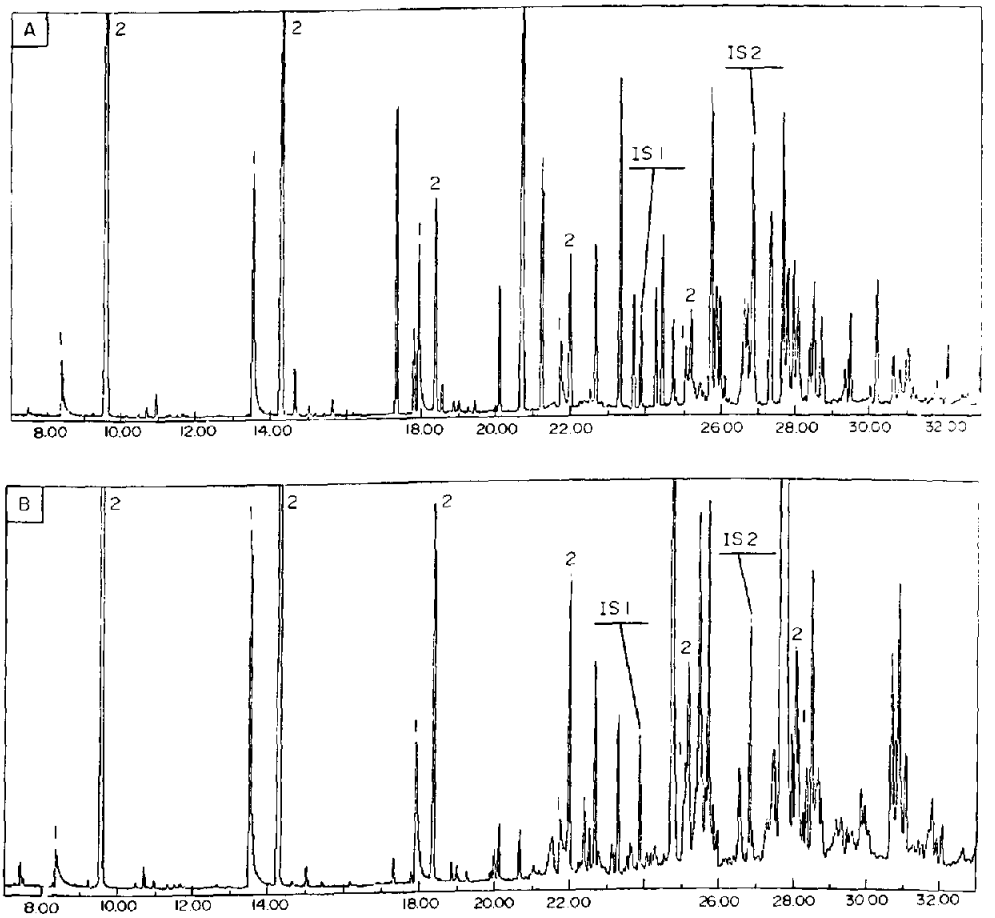


Figure 4
Typical profiles of Polysorbate 20 (A) and Polysorbate 80 (B): Key: 1, DBPE; 2, MBPE–TMS; IS1, internal standard 1; IS2, internal standard 2.

Table 2
Gas chromatographic retention data (methylene-units) of the most prominent cleavage products of PEG-related compounds

PEG (all types)	11.23	11.91	14.12	14.59	16.97	17.25	19.75
	19.90	22.49	22.54	25.23	25.29	27.92	28.18
PEG-400-stearate	11.23	11.91	14.12	14.59	16.52	17.25	18.46
	18.89	19.90	20.50	20.95	22.51	22.61	23.11
	23.25	25.29	25.77	25.85	27.92		
Cetomacrogol 1000	11.23	11.91	14.12	14.59	16.97	17.25	19.72
	19.75	19.90	21.69	21.77	22.30	22.49	22.54
	24.34	24.88	25.23	25.29	27.92	28.18	
Octoxinol	11.23	11.91	14.12	14.59	16.46	16.97	17.25
	19.32	19.63	19.75	19.90	20.19	22.42	22.48
	23.01	25.28	25.32	26.01	26.15		
Tyloxapol	11.23	11.91	14.12	14.59	16.97	17.25	19.75
	19.90	22.49	22.54	23.05	23.22	23.34	23.92
	25.23	25.29	27.92	28.18			
Polysorbate 20	11.23	11.91	14.12	14.59	16.52	16.84	16.97
	17.25	18.46	18.94	19.32	19.75	19.90	20.42
	20.96	21.26	21.74	21.86	22.09	22.49	22.54
	23.01	23.11	23.21	23.80	23.87	24.43	24.76
	24.88	25.03	25.14	25.43	25.54	25.72	

Table 2
Continued

Polysorbate 60	11.23	11.91	14.12	14.59	16.97	17.25	19.75
	19.90	20.42	22.43	22.49	22.54	23.01	23.11
	23.21	24.07	24.16	25.23	25.29	25.43	25.54
	27.92	28.18					
Polysorbate 80	11.23	11.91	14.12	14.59	16.97	17.25	19.75
	19.90	20.42	20.96	22.13	22.49	22.54	22.78
	23.00	23.74	24.58	24.87	25.23	25.29	25.55

Elimination of water before carrying out the ether cleavage is essential, since boron tribromide is very sensitive to moisture. Also, careful temperature control is recommended during the final evaporation step, since a significant decrease of the size of the more rapidly eluted peaks was found to occur at temperatures exceeding 30°C.

Conclusions

Partial cleavage of ether linkages in PEG-related compounds with diluted solutions of boron tribromide is proved to be promising for the identification of such compounds. Typical gas chromatographic patterns have been obtained for 11 model compounds, which demonstrate the validity of the method for the study of bulk materials. For drug formulations, the method has limited sensitivity.

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