

# Determination of polyethylene glycols of different molecular weight in the stratum corneum

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## Abstract

We developed a sensitive method for determination of polyethylene glycols (PEGs) of different molecular weight (MW) in the human stratum corneum (SC) obtained by tape stripping. The analysis is based on derivatization with pentafluoropropionic anhydride (PFPA) and gas chromatography–electron capture detection (GC–ECD). The identification and quantification of PEGs was done using individual oligomers. The method showed to be suitable for studying permeability in normal and impaired skin with respect to MW in the range of 150–600 Da. © 2004 Elsevier B.V. All rights reserved.

**Keywords:** Polyethylene glycol (PEG); Tape stripping; Percutaneous penetration; Stratum corneum

## 1. Introduction

Penetration of chemicals into the body is primarily prevented by the outermost layer of the epidermis, the stratum corneum (SC). The molecular weight (MW) plays a significant role in the percutaneous penetration of chemicals. The MW cut-off for normal skin has been reported to be approximately 500 Da [1]. However, it has been shown recently that in the compromised skin, this cut-off is shifted to higher MW [2,3]. As a result, the impaired skin barrier enables the entering of macromolecules, e.g. protein allergens leading to physiological effects. Since such compromised skin is common due to chemical or physical damage as well as in diseased skin, an evaluation of increased permeability is important. For studying the permeability of the healthy and affected skin, polyethylene glycols (PEGs) are suitable model compounds. PEGs have been extensively used in research of intestinal permeability since their introduction by Chadwick et al. [4–7]. They are available in a wide range of MW and their water/octanol partitioning ( $P_{o/w}$ ) coefficient does not change greatly with molecular mass [8]. Due to low toxic-

ity, they are used in many fields of cosmetic, chemical and pharmaceutical industries.

For the determination of PEGs in biological material, several high-performance liquid chromatographic (HPLC) and gas chromatographic (GC) methods have been described [4,9–16]. The majority of the HPLC methods are based on refractive index detection. However, the HPLC methods were primarily developed for the analysis of PEGs in urine or blood and could not be applied in the present study due to their low sensitivity. Higher sensitivity was reported in the GC methods which were based on derivatization of hydroxy groups followed by gas chromatography–electron capture detection (GC–ECD) [9]. Derivatization with different reagents such as acetic acid anhydride, trifluoroacetic acid (TFAA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) was investigated. However, the major problem in these procedures was a poor stability of the derivatives [4,5,9,16].

In all reported methods, polydisperse mixtures containing PEGs of different MW were used and the identification and quantification of the PEG was performed only indirectly. Recently, PEGs are also available as individual monodisperse oligomers, which enabled us to validate the method. We developed a sensitive GC method for quantification of

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PEG oligomers of different MW in human SC. The method was applied for studying the differences in percutaneous penetration between normal and impaired skin.

## 2. Experimental

### 2.1. Chemicals and materials

PEG 150.17 Da (PEG150) was purchased from Sigma, The Netherlands and PEG 282.34 Da (PEG282) was purchased from Acros Organics, NY, USA. PEGs 326.4 Da (PEG326), 370.4 Da (PEG370), 502.6 Da (PEG502), 546.7 Da (PEG546) and 590.7 Da (PEG590) (all oligomers have chemical purity >99%, and oligomer purity >95%) were purchased from PolyPure, Norway. Polydisperse PEG600 (average MW) was purchased from Sigma, The Netherlands. Pentafluoropropionic anhydride and sodium lauryl sulfate (SLS) (>99% GC) were purchased from Fluka, The Netherlands and (1*S*,2*S*)-(–)-phenylpropylene oxide (PPO) (98%, GLC) was purchased from Aldrich, USA. Dichloromethane (p.a.), ethyl acetate (p.a.), *n*-hexane (Unisolv), hydrochloric acid (37%, p.a.), methanol (Lichrosolv), sodium hydroxide (p.a.) and sulphuric acid were purchased from Merck (The Netherlands). Pyridine was purchased from Alltech, The Netherlands. Bio-Rad protein assay (Cat. No. 500-0112) was purchased from Bio-Rad Laboratories (Germany). Diamond Ultra Clear tape (19 mm × 33 m) was purchased from Sellotape® Company, The Netherlands and Finn chambers® (18 mm in diameter) were purchased from Epitest Ltd., Finland.

### 2.2. Percutaneous penetration study

The volunteers were Caucasians of both genders aged 18–55 years ( $n = 20$ ) and had no visible skin damage and no history of dermatological diseases. The subjects participating in the study were asked not to use lotion, cream or soap on the lower arm 3 days prior to and during the experiment. Written informed consent was obtained from all subjects prior to the experiment. The Ethical Committee of the Academic Medical Center approved the experiment protocol.

An application mixture of PEG was made by dissolving 47.5 mg PEG150, 50.1 mg PEG282, 102.9 mg PEG326, 199.1 mg PEG370, and 10 g of polydisperse PEG600 in 2 ml of water. One hundred and eighty microliters of the mixture was spread onto the filter paper in the Finn Patch test chamber®. After the whole amount of the mixture was absorbed, two chambers were applied for 6 h onto the lower volar forearm of the volunteers. Two other chambers containing the application mixture were applied onto the skin site pre-treated with SLS. The treatment with SLS was performed 24 h before the application of PEG. This was done by applying 200  $\mu$ l of 5% (w/w) water solution of SLS for 4 h. Immediately after the removal of the chambers, the residue of the PEGs on the skin was gently wiped off with wet and,

subsequently, dry paper tissue. Medical adhesive tape with a round marked area of 18 mm in diameter was fixed around the application site to ensure that the tapes were consistently applied to the same site. Fifteen minutes after the end of exposure, the SC was repeatedly stripped with pre-cut Diamond tape pieces, i.e. strips. Prior to the experiment, the tape was mounted on a glossy side of a commercially available paper sheet and cut to the size of 1.9 cm × 2.0 cm. The tape was applied with tweezers to the test site and rolled over 20 times with a 1 kg stainless steel roller. The tapes were peeled off with one quick movement multidirectionally. Each skin site was striped 15–25 times until the SC was totally removed as observed by the shiny appearance of the skin and by measuring transepidermal water loss (TEWL) using portable VapoMeter (Delfin Technologies Ltd., Finland). Each subsequent tape was placed into a 20-ml glass vial and stored at –18 °C until analysis. For the calibration line and quality control (QC) samples, tape strips containing non-exposed SC of a volunteer were used (blank samples).

### 2.3. Analysis of PEGs

#### 2.3.1. Calibration samples

The stock standard solution was prepared by dissolving 9.5 mg PEG150, 32.9 mg PEG282, 49.5 mg PEG326, 63.2 mg PEG370, 289.0 mg PEG502, 455.1 mg PEG546 and 871.6 mg PEG590 in 10 ml of distilled water. A working standard solution of PEGs was prepared by diluting 40  $\mu$ l of stock solution with 20 ml of methanol. The calibration standards were prepared by adding 10–160  $\mu$ l of the working standard to the 500  $\mu$ l methanol solution of the blank samples. For internal standardization, 10  $\mu$ l of the working standard of (1*S*,2*S*)-(–)-phenylpropylene glycol (PPG) in ethyl acetate was added. The concentration of PEGs in the SC was calculated by internal standardization using peak height measurements. All samples were analyzed in duplicates. Since PPG was not commercially available, it was synthesized by acid-catalyzed hydrolysis of PPO as described previously [17].

#### 2.3.2. Extraction and derivatization

Two milliliters of methanol was added to the glass vials containing tape strips and they were mechanically shaken for 1 h. Five hundred microliters of aliquot of the methanol phase was transferred to 4 ml vials. Ten microliters of PPG as internal standard (IS) was added. The methanol aliquots were evaporated to dryness. To each sample, 500  $\mu$ l of dichloromethane, 20  $\mu$ l of pyridine and 20  $\mu$ l of PFPA were added. The reaction mixtures were heated at  $70 \pm 5$  °C for 30 min. After cooling down to room temperature the samples were evaporated and the residue was dissolved in 1 ml of *n*-hexane to which 15  $\mu$ l of pyridine was added. Samples were vortexed for 1 min and transferred to safe-lock tubes, which were centrifuged for 15–30 s ( $11\,860 \times g$ ) prior to the GC–ECD analysis. The samples were analyzed during a time period of 14 h.

### 2.3.3. GC–ECD analysis

The GC–ECD analysis was carried out with a Hewlett-Packard 5890 GC (Hewlett-Packard, USA) equipped with a  $^{63}\text{Ni}$  ECD. The column was HP-1 (25 m  $\times$  0.32 mm, 0.17  $\mu\text{m}$  film thickness, Agilent technologies, USA). The initial column temperature was 60  $^{\circ}\text{C}$  and the temperature was increased after 6 min to 150  $^{\circ}\text{C}$  at 15  $^{\circ}\text{C min}^{-1}$  and then programmed at 30  $^{\circ}\text{C min}^{-1}$  to 340  $^{\circ}\text{C}$  where it was held for 4 min. The injector and detector temperature was 350  $^{\circ}\text{C}$  and the column head pressure was 110 kPa. The sample (1  $\mu\text{l}$ ) was injected by the split injection technique (split ratio 1:40). The deactivated cup inlet liner suitable for split injection of high MW compounds (Cat. No. 20510) was purchased from Supelco, USA.

### 2.3.4. Determination of the stability of PFPA derivatives

To determine the stability of PEG derivatives, a standard SC sample was prepared by adding 80  $\mu\text{l}$  of working standard (see Section 2.3.1) to 500  $\mu\text{l}$  methanol solution of the blank samples. After extraction and derivatization (see Section 2.3.2), the hexane layer was distributed into the auto sampler vials and repeatedly injected into the GC–ECD during 14 h.

### 2.3.5. Recovery and precision

To determine the recovery, seven blank tape strips containing SC were placed into 20-ml glass vials and spiked with a working standard solution (see Section 2.3.1). The SC tape strips were dry within 1 h and thereafter analyzed according to the procedure (see Section 2.3.2). The same amount of the working standard solution was directly added to seven glass vials containing 2 ml methanol (100% recovery). The samples were then analyzed according to the standard procedure (see Section 2.3.2). The extraction recovery from the tape strips was determined by comparing the peak height ratios of standards not extracted with those of standards extracted from the tape strips.

The repeatability (within-run precision) and reproducibility (between-run precision) of the method were determined by analysis of seven SC strips spiked at two and one concentration levels, respectively.

### 2.4. Protein analysis in the tape strip

The protein analysis was used to measure the amount of the SC removed by each tape strip, and to assess the depth of the consecutive SC strip [18,19].

The methanol residue containing tape strip with precipitated proteins on it after PEG analysis was evaporated. One milliliter of 1 M NaOH was added to the strip and the vials were shaken for 2 h. The samples were left at room temperature overnight and the next day they were shaken for 2 h one more time. One milliliter of 1 M HCl was added to the vials to neutralize the basic solution. The protein assay was based on the modified method of Dreher et al. and performed according to Bio-Rad DC protein microassay using commercially

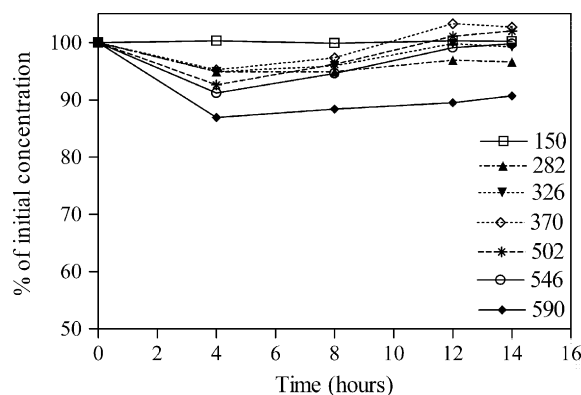


Fig. 1. Stability of PEG derivatives expressed as percentage of initial concentration.

available bovine serum albumin (BSA) for standardization [20,21]. Absorbance at 655 nm was measured using the Bio-Rad 680 microplate reader (Bio-Rad, USA).

## 3. Results and discussion

### 3.1. Derivatization of the hydroxy groups with PFPA

To enable sensitive ECD detection and improve chromatographic performance, hydroxy groups were acylated with PFPA. In a pilot study, we investigated the optimal temperature and time duration for the derivatization. As concluded from the peak heights of formed derivatives, the derivatization performed at 70  $^{\circ}\text{C}$  was completed within 30 min. The addition of pyridine as an acid scavenger improved the derivatization; the absolute peak heights of the derivatives were higher for a factor of 10 when pyridine was added. Higher amounts of pyridine did not lead to a further increase in peak heights. Also, the stability of the derivatives was improved by addition of pyridine. The deterioration of the derivatives for all oligomers during a period of 14 h was limited and within the error of the method (Fig. 1), except for oligomer with MW of 590 Da, for which the loss was somewhat higher (up to 15% within 14 h). The stability of the derivatives was better than that reported in the study of Fakt and Ervik [9]. They also used PFPA for acylation of polydisperse PEG400 reporting gradual deterioration of derivatives after only 1 h. After 24 h, the percentage of initial concentration in that study was about 60%. It was inconclusive from the article if all oligomers in PEG400 were investigated or just the highest peak in the mixture, which corresponded to MW of 326.4 Da. In their procedure, they used toluene as a solvent with no addition of pyridine.

### 3.2. Chromatography

Fig. 2 shows chromatograms of blank SC sample, blank SC sample spiked with mixture of individual oligomers and SC sample of a volunteer exposed to PEGs (fifth strip). As can

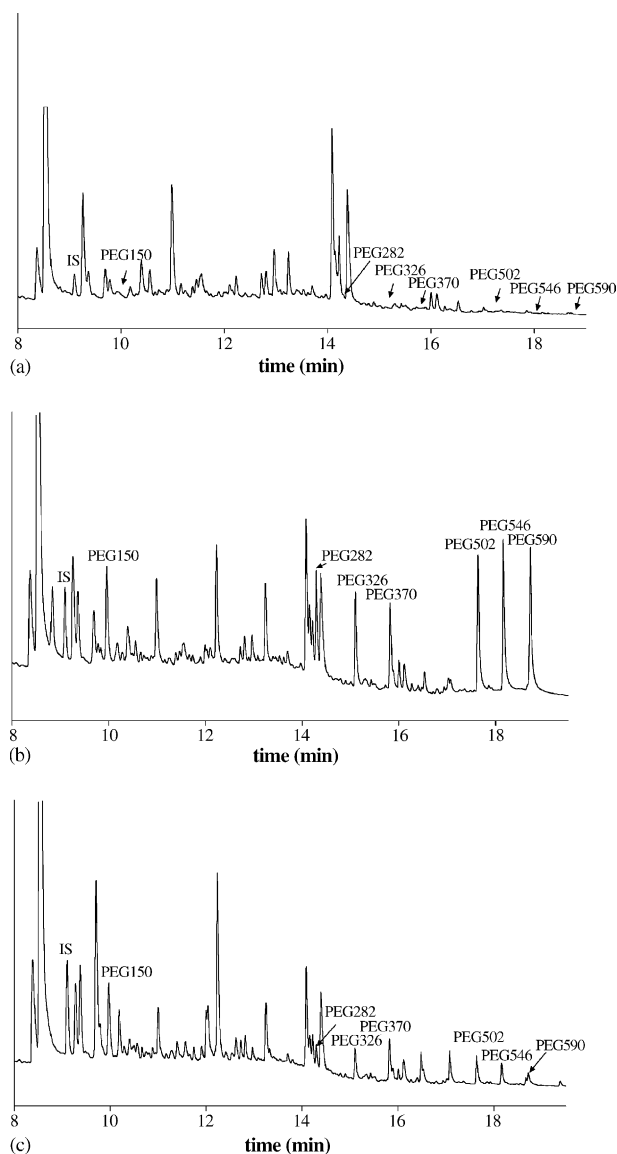


Fig. 2. Representative GC–ECD chromatograms of: (a) blank SC sample; (b) blank SC sample spiked with standard solution of individual PEG oligomers; and (c) SC sample obtained from a volunteer exposed to PEGs for 4 h at a skin site pre-treated with SLS (fifth strip).

be seen, good separation of all oligomers was achieved. In a pilot study, we investigated chromatographic performance using an identical column with a thicker stationary phase film (0.52  $\mu\text{m}$ ). However, the peaks of PEGs, especially those of higher MW, showed more peak tailing on that column. As concluded from the peak heights of the oligomers, we did not observe any deterioration of the polymers at high injector and column temperature (350 and 340  $^{\circ}\text{C}$ , respectively). This is consistent with the findings of Onigbinde et al., who also reported stability of the PEGs at the temperature of 330  $^{\circ}\text{C}$  [22]. The blank sample showed no major interfering peaks except for PEG282 and PEG590, although for these oligomers reproducible results were obtained (Table 1).

### 3.3. Identification and quantification of oligomers

Identification of oligomers in the application mixture and in the SC samples was achieved by direct comparison with individual oligomer standards which were analyzed under the same chromatographic conditions. This is in contrast with the previous studies, where the identification of the oligomers in the polydisperse mixtures was performed only indirectly [2–5,9,11,14,15]. In most of these studies, the identification of the PEG oligomers in the chromatogram was done by the comparison of the retention time with that of a smaller individual oligomer (di-, tri- or tetraethylene glycol) which was available as a pure chemical [11,14,15]. From this, the other oligomers were identified by sequential counting of the peaks. Another approach was to assign a MW closest to the average MW of the polymer to the highest peak in the chromatogram [2,3]. The standardization of these methods was also performed indirectly. The amount of each oligomer in the mixture was assessed from the relative peak areas, assuming equal detector response for oligomers of different MW. Under the assumption that the polydisperse mixture contained no impurities and that all oligomers in the mixture could be quantified from the chromatogram, this approach is less critical by HPLC methods. However, by GC methods, due to possible discrimination in the injector that might occur for higher MW compounds, the peak area might not be proportional to the mass fraction in the polydisperse mixture. In the study of Fakt and Ervik, the highest peak in a GC chromatogram of polydisperse PEG400 was reported to correspond to MW of 326 Da [9]. However, as confirmed by gel permeation chromatography, mass spectrometry and HPLC, in the polydisperse PEG400, PEG370 and PEG414 have the highest mass fraction [11,13]. This clearly illustrates the advantage of using individual oligomers for the standardization of the method.

### 3.4. Extraction recovery

The results of extraction recovery of PEG oligomers from the tape are summarized in Table 1. Recoveries ranged from 88 to 97% and seemed not to be dependent on MW of oligomers. This is in contrast with the study of Ruddy and Hadzija, who reported recovery dependence on MW [11]. The recoveries in that study were found to increase with increasing MW ranging from 18 to 86% for PEG282 and PEG854, respectively. In that study, PEGs were isolated and purified by solid-phase extraction using large pore kieselguhr (Extrelut QE) cartridges.

### 3.5. Linearity

To determine the linearity of the assay the standard samples prepared as described in Section 2.3.1 (concentration ranges are reported in Table 1) were analyzed on seven different days. The regression analysis was performed using the least square method. The ratios of the peak heights of individ-

Table 1  
Analytical parameters of GC–ECD assay of PEG oligomers in human SC

	N	PEG150	PEG282	PEG326	PEG370	PEG502	PEG546	PEG590
Linear range ( $\mu\text{g cm}^{-2}$ )	7	0.026–0.42	0.09–1.5	0.14–2.2	0.2–2.8	0.8–13	1.3–20	2.4–38
LOD ( $\mu\text{g cm}^{-2}$ )	7	0.007	0.013	0.014	0.003	0.051	0.031	0.082
Repeatability (CV, %)	7	6.8	6.9	7.3	8.7	6.5	7.2	9.6
Concentration level ( $\mu\text{g cm}^{-2}$ )		0.21	0.72	1.2	1.6	6.4	10.4	19.2
Repeatability (CV, %)	7	5.2	9.9	9.3	7.8	6.2	7.0	8.5
Concentration level ( $\mu\text{g cm}^{-2}$ )		0.05	0.18	0.3	0.4	1.6	2.6	4.8
Reproducibility (CV, %)	5	3.2	9.3	10.7	10.8	9.5	10.1	12.8
Concentration level ( $\mu\text{g cm}^{-2}$ )		0.21	0.72	1.09	1.39	6.35	10.01	19.16
Recovery (%)	7	94	88	93	97	96	96	93
Concentration level ( $\mu\text{g cm}^{-2}$ )		0.21	0.72	1.2	1.6	6.4	10.4	19.2

CV = coefficient of variation; LOD = limit of detection.

ual oligomers of PEGs versus IS were linearly related to the oligomer concentrations within the range of concentrations studied. The correlation coefficients ( $R$ ) of the calibration curves ranged from 0.9909 to 0.9942.

### 3.6. Precision and limit of detection and quantitation

The limit of detection (LOD) was defined as three times the standard deviation (S.D.) obtained by repeated analysis of seven blank samples. The lower limit of quantitation (LLOQ) defined as the lowest standard on the calibration curve ranged from  $0.026 \mu\text{g cm}^{-2}$  for PEG150 to  $2.4 \mu\text{g cm}^{-2}$  for PEG590 (Table 1). As estimated from the injected amount of PEG

that corresponds to the LLOQ, our method was much more sensitive than the existing GC method of Fakt and Ervik (0.4 and 16 ng, respectively) [9].

The coefficient of variation (CV) for all oligomers was less than 10% for repeatability and 13% for reproducibility. The results are summarized in Table 1.

### 3.7. Skin penetration study

The concentration of PEG oligomers was determined in the SC tape strips of the normal skin and in the skin after acute disruption by SLS. The amount of PEG oligomers in a subsequent strip normalized for the applied concentration was

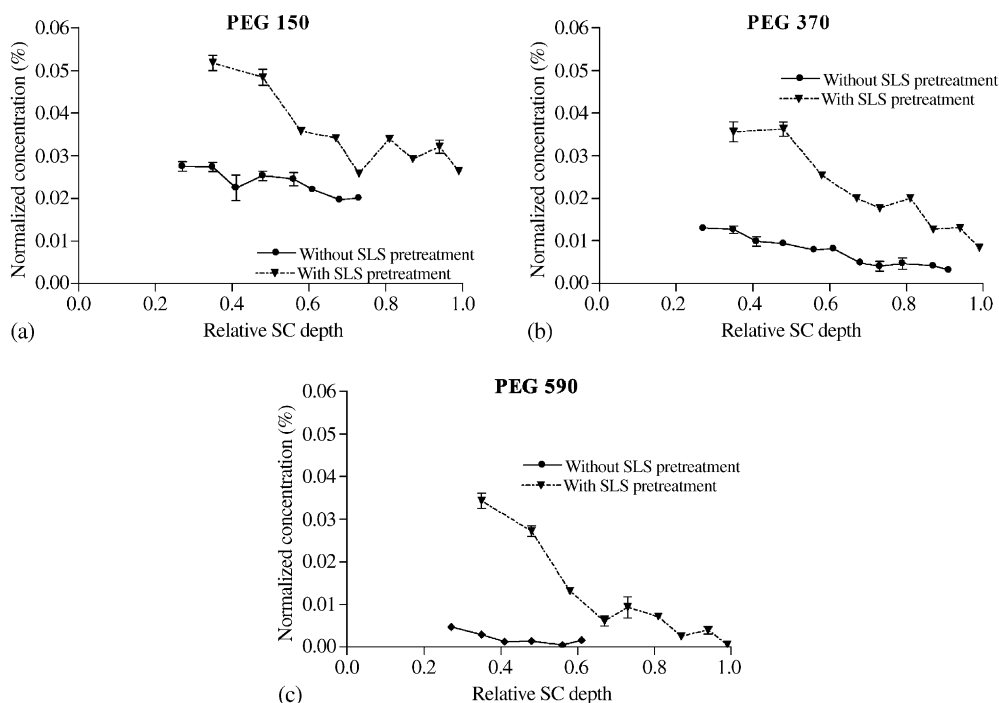


Fig. 3. The concentration of PEG150 (a), PEG370 (b) and PEG590 (c) in the SC normalized for the applied concentration (%) in a volunteer exposed to PEGs for 6 h. The relative SC depth was calculated from the ratio of the SC mass removed till  $i$ -th tape strip and the total SC mass removed by all tape strips.

plotted as a function of the SC depth. This relationship was used for the estimation of the penetration parameters using the approach based on Fick's second law of diffusion described in details elsewhere [19]. Fig. 3a–c illustrates the effect of SLS on the penetration of PEG150, PEG370 and PEG590 in a volunteer. The penetration of all PEG oligomers into the SC was enhanced in the skin impaired by SLS. Furthermore, the penetration enhancement was more prominent for PEGs with higher MW, which is in agreement with in vitro study in murine skin of Tsai et al. [3]. The results of this study will be published elsewhere.

#### 4. Conclusions

Our method, using individual oligomers, has proper identification, quantification and high sensitivity. The described method of extraction of the PEG oligomers with different MW from tape strips was fast and efficient showing no recovery dependence on MW. The derivatization procedure produced sufficiently stable derivatives for the analytical purpose. The method proved to be suitable for studying percutaneous penetration of PEGs with MW in the range from 150 to 600 Da in normal and impaired skin.

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