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Determination of bisphenol diglycidyl ethers in topical dosage forms

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Abstract

A method involving extraction and LC–ESI–MS-MS detection of BADGE, BFDGE, BADGE·H_2O, BADGE·$2H_2O$, BADGE·HCl, BADGE·H_2O·HCl, BADGE·2HCl and BFDGE·2HCl in aqueous cream was developed and validated. Initially, empty internally lacquered aluminum container closure systems were extracted with isopropanol as an attempt to estimate the upper limit of extractable bisphenol diglycidyl ethers present in lacquer. Six of the eight potential bisphenol diglycidyl ethers were quantified. In an accelerated experiment, on aqueous cream stored in lacquered aluminum tubes at 70 °C, all derivatives except BADGE·2HCl and BFDGE·2HCl were extracted from cream samples and quantified as an attempt to estimate the upper limit of compounds leaching to the cream. Detection limits were from 0.3 ± 0.2 to 3.4 ± 0.7 μg l^{-1}. Recoveries were determined for all compounds at three concentration levels (mean $63 \pm 6\%$). Mean inter-day and mean intra-day precision was 7 ± 2 and $13 \pm 6\%$, respectively. Three commercially available creams were obtained from a local community pharmacy and analysed for bisphenol diglycidyl ethers. BADGE, BADGE·H_2O, BADGE·$2H_2O$ and BADGE·H_2O·HCl were detected and quantified. In conclusion, the developed method allows for the extraction and detection of bisphenol diglycidyl ethers originating from the epoxy phenol lacquer used in aluminum tubes. This study does not indicate that they leach into aqueous cream in significant amounts under normal storage conditions.

Keywords: Container closure system; Aqueous cream; Anti-androgen; Epoxy phenol lacquer; Mass spectrometry

1. Introduction

The presence of diglycidyl ethers of bisphenol A (BADGE) and bisphenol F (BFDGE) and their hydrolysed and chlorinated derivatives (Fig. 1) as leachables in canned food and drinks has received much attention lately due to the suspected mutagenic, genotoxic and anti-androgenic effects of the compounds [1–8].

BADGE and BFDGE are used as precursors for epoxy phenol lacquers coating the inside of the majority of cans used in food industry. The compounds can leach into canned products from the lacquer, which is used to inhibit interactions between the can itself and the product [9]. A specific migration limit with regards to foodstuffs and food simulants for the sum of BADGE and derivatives (BADGE·H_2O, BADGE·HCl, BADGE·2HCl and BADGE·H_2O·HCl) has been set by the European Commission at 1 mg kg^{-1} [10]. Although similar types of lacquers are being used in container closure systems intended for pharmaceutical semi-liquid topical dosage forms (creams, gels and ointments), no study that the authors are aware of has yet been conducted to show the possible contamination of these products by BADGE, BFDGE and their derivatives.

Previously, the use of HPLC, either combined with fluorescence and/or MS detection for the determination of a variety of BADGE, BFDGE and their derivatives in canned food, food simulants and human plasma has been described [11–18]. Furthermore, GC–MS has been applied for the determination of BADGE and BFDGE in food simulants and wastewater [19,20]. Schaefer and Simat [21] used HPLC with UV, fluorescence and MS detection to analyse 42 migrants ($M_W < 1000$ Da) with a bisphenol backbone from epoxy-based can coatings and with a limit of quantitation of 5 μg BADGE kg^{-1}.

The importance of detecting and quantifying any BADGE and BFDGE derivative in pharmaceutical semi-liquid topical dosage forms can be realised when considering the exposure route. In contrast to food and drinking products intended for oral intake, creams, gels and ointments are intended for dermal application. By dermal exposure, the compounds may cross the skin in order to reach the blood stream, whereas if exposure occurs orally, the compounds have to pass the harsh environment of the gastro-intestinal tract and potentially undergo first-

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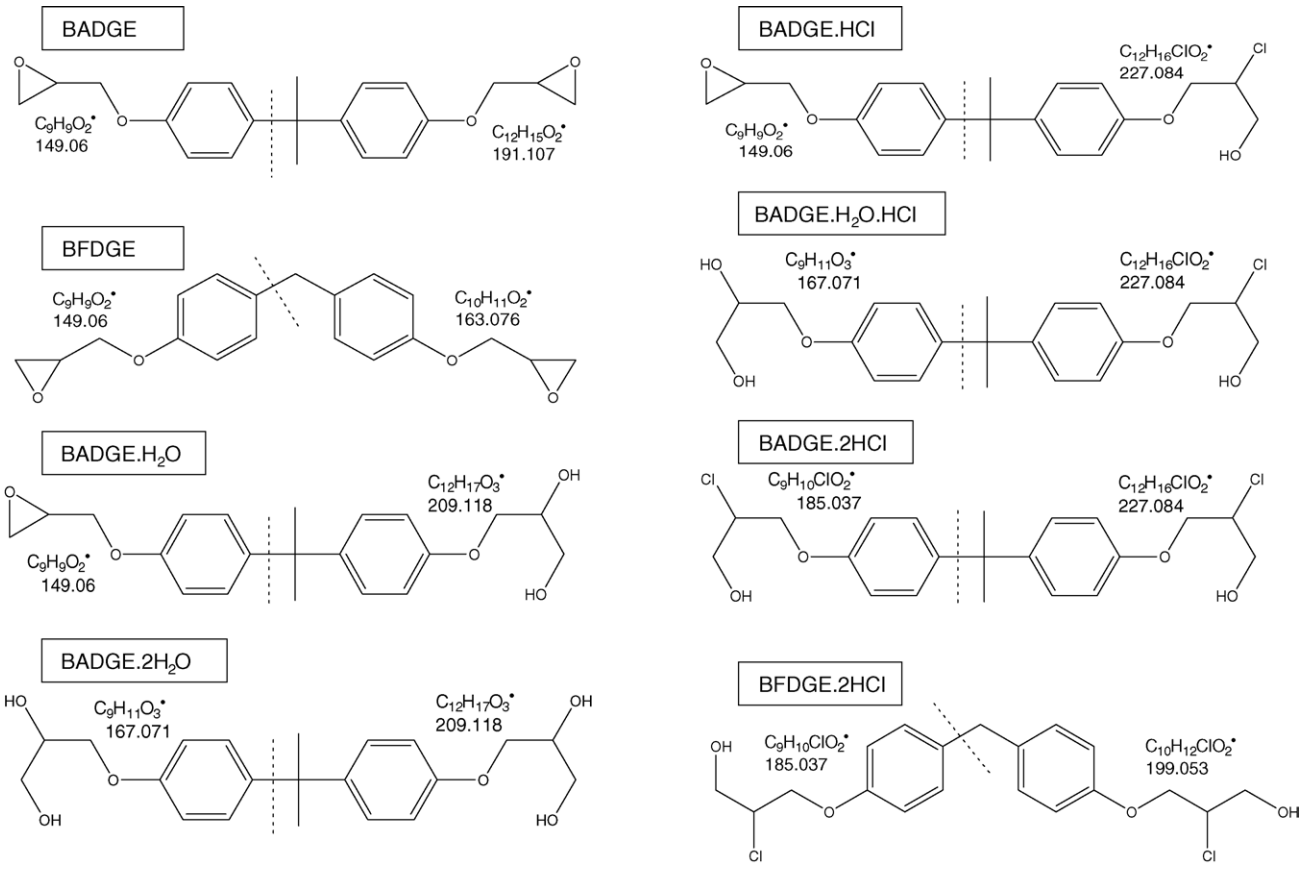

Fig. 1. Structures of the investigated compounds. Fragments used for the MS-MS analyses are indicated. *Abbreviations*: BADGE: bisphenol A diglycidyl ether; BFDGE: bisphenol F pdiglycidyl ether. H_2O or HCl indicates the addition of water (hydrolysis) or of hydrochloric acid to BADGE or BFDGE.

pass metabolism in the liver. First-pass metabolism most often results in detoxification and faster excretion of the xenobiotic. As reviewed by Santerre et al. [22], BADGE·$2H_2O$ has previously been identified as a hydrolysis product of bis-GMA in the presence of enzymatic activity. In another study, BADGE·$2H_2O$ was quantified in urine from children receiving dental composites and/or sealants [23]. BADGE has also been found to be rapidly hydrolysed by microsomal and cytosolic fractions of mouse liver and skin [24]. BFDGE·$2H_2O$ has been shown to form rapidly from BFDGE in vivo in rat and human hepatic S9 fraction [25]. In the same study, the cytotoxicity, the mutagenicity and the estrogenicity of the parent compound and the identified metabolites were evaluated in vitro. In all cases, BFDGE was found to be more toxic than BFDGE·$2H_2O$ indicating a detoxification of BFDGE by the hepatic S9 fraction. Neither of the compounds was found to stimulate the proliferation of the estrogens-dependent MCF-7 human breast cancer cells. Hanaoka et al. [26] found the concentration of BPA in urine from workers spraying BADGE with mixed organic solvents as an epoxy resin-hardening agent to be significantly higher than the concentration of BPA in urine from a control group. In this work, no other metabolites of BADGE were analysed.

Although enzymatic activity is present in skin, the activity is of minor importance with respect to the degradation and detoxification of BADGE, BFDGE and their derivatives when compared to the activity in the liver. After dermal exposure of ^{14}C-BADGE in mice, Climie et al. [27] were able to detect BADGE and metabolites in both urine and faeces for 8 days. Furthermore, local treatment of skin conditions associated with a reduced barrier function (damaged skin, wounds, psoriasis, etc.), may lead to higher concentrations of leachable xenobiotics in the blood stream, when compared to application of cream or ointment to normal skin with an intact barrier function.

The primary aim of the present study was to develop an analytical method for the analysis of BADGE, BFDGE and their derivatives in internally epoxy phenol lacquered aluminum container closure systems intended for semi-liquid pharmaceutical dosage forms. Secondly, the aim was to prepare and analyse aqueous cream samples from the same type of container closure systems and estimate the leaching potential of the compounds from the lacquer into the cream.

Initially, the method was applied to new internally lacquered aluminum tubes, which were extracted using isopropanol at 70 °C for 48 h. This approach was taken in order to obtain a theoretical upper limit for the amount of extractable compounds in the lacquer.

Secondly, an accelerated study (70 °C, 120 h) of a model cream stored in internally lacquered aluminum tubes was performed. This experiment was intended to mimic a worst-case scenario with regards to the amount of compounds capable of leaching into the cream.

The use of elevated temperature was chosen to simulate long-term storage at room temperature [28]. This approach is generally accepted in order to obtain knowledge of the stability of a drug or a drug product (see e.g. ICH Q1A (R2) guideline [29]).

Finally, to show the general applicability of the method, three commercially available aqueous creams from internally lacquered aluminum tubes marketed in Denmark were analysed.

2. Materials and methods

2.1. Chemicals

Bisphenol A diglycidyl ether (BADGE) ≥97.0% (Cas number: 1675-54-3), bisphenol F diglycidyl ether (BFDGE) ≥95.0% (Cas number: 2095-03-6), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O) ≥95.0% (Cas number: 76002-91-0), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$) ≥97.0% (Cas number: 5581-32-8), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl) ≥90% (Cas number: 13836-48-1), bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·H_2O·HCl) ≥95.0% (Cas number: 227947-06-0), bisphenol A bis(3-chloro-2-hydroxy-propyl) ether (BADGE·2HCl) ≥97.0% (Cas number: 4809-35-2) and bisphenol F bis(3-chloro-2-hydroxypropyl) ether ∼95% (BFDGE·2HCl) (no Cas number) were all obtained from Sigma–Aldrich Chemie GmbH (Schnelldorf, Germany).

Stock solutions of all compounds (1000 mg l^{-1}) were made in methanol and stored at −18 °C. No breakdown of the parent compounds was observed under these conditions for up to 3 months (data not shown).

White soft paraffin, liquid paraffin, cetostearyl alcohol, macrogol cetostearyl ether and phenoxyethanol were kindly supplied by LEO Pharma A/S (Ballerup, Denmark).

All other chemicals used were of analytical reagent grade and were obtained from Merck (Ballerup, Denmark).

2.2. Model cream

One thousand gram of a custom-made model cream was used for the experiments. The cream was a slightly modified *Aqueous Cream* as described in the *British Pharmacopoeia 2003* [30]. *Cetomacrogol Emulsifying Ointment* was used instead of *Emulsifying Ointment*. The purpose of the modification was to make the cream resemble a typical aqueous cream on the Danish market. In brief, cetostearyl alocohol was melted together with macrogol cetostearyl ether. This mixture was subsequently melted together with white soft paraffin and liquid paraffin. Finally, water was added while stirring to reach a final mass of 1000 g. The cream was kept at room temperature in sealed glass containers until use.

2.3. Aluminum tubes

New internally lacquered aluminum tubes (volume 5 ml) were kindly provided by LEO Pharma A/S.

2.4. LC–ESI–MS-MS

The LC–ESI–MS-MS analyses were performed on an Agilent 1100 series HPLC (Agilent Technologies, Inc., Palo Alto, CA, USA) coupled to a PE Sciex 3000 triple quadropole mass spectrometer equipped with a turbo ionspray. Data were collected using the Analyst® 1.4 software (MDS Sciex, Concord, Ont., Canada).

The column used was a Waters Xterra® MS C_{18}, 3.5 μm, 2.1 mm × 100 mm (Waters Corporation, Milford, MA, USA). The flow rate was 0.2 ml min^{-1} and column temperature controlled at 45 °C. A methanol gradient was used for elution of the eight compounds within 27 min. Initially, 54% methanol was held for the first 5 min. Hereafter, the methanol was linearly increased to 66% in 10 min, further increased to 78% in 1 min, and then brought back to 54% in 2 min and kept for the remaining 9 min. The eluents were buffered with 0.1% formic acid and contained 5 mM ammonium formiate. The MS-MS detection was performed in the positive ion mode using multiple reaction monitoring (MRM). MRM transition (see Fig. 1) and compound dependent parameters (declustering potential (DP), focusing potential (FP), collision energy (CE) and cell exit potential (CXP)) were optimised using the automated infusion analysis program (see Table 1).

Gas parameters (curtain gas (CUR), nebuliser gas (NEB), collision gas (CAD), temperature (TEM) and needle voltage

Table 1
MS parameters for the investigated compounds

MS parameters	Compound							
	BADGE	BFDGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·HCl	BADGE·H_2O·HCl	BADGE·2HCL	BFDGE·2HCl
Mass	340.17	312.14	358.18	376.19	376.14	394.16	412.12	384.09
Q1 (*m*/*z*)	358.3 $[M+NH_4]^+$	330.4 $[M+NH_4]^+$	376.4 $[M+NH_4]^+$	394.4 $[M+NH_4]^+$	394.3 $[M+NH_4]^+$	412.3 $[M+NH_4]^+$	430.3 $[M+NH_4]^+$	402.2 $[M+NH_4]^+$
Q3 (*m*/*z*)	191.1	163.2	209.1	209.1	227.1	227.5	227.1	199.0
DP (V)	30	28	28	40	30	20	28	35
FP (V)	100	100	80	110	80	100	100	100
EP (V)	8	6	8	7	8	8	6	5
CE (V)	21	19	19	22	19	24	24	18
CXP (V)	12	10	14	14	14	14	14	12
Period	3	2	2	1	3	2	3	3

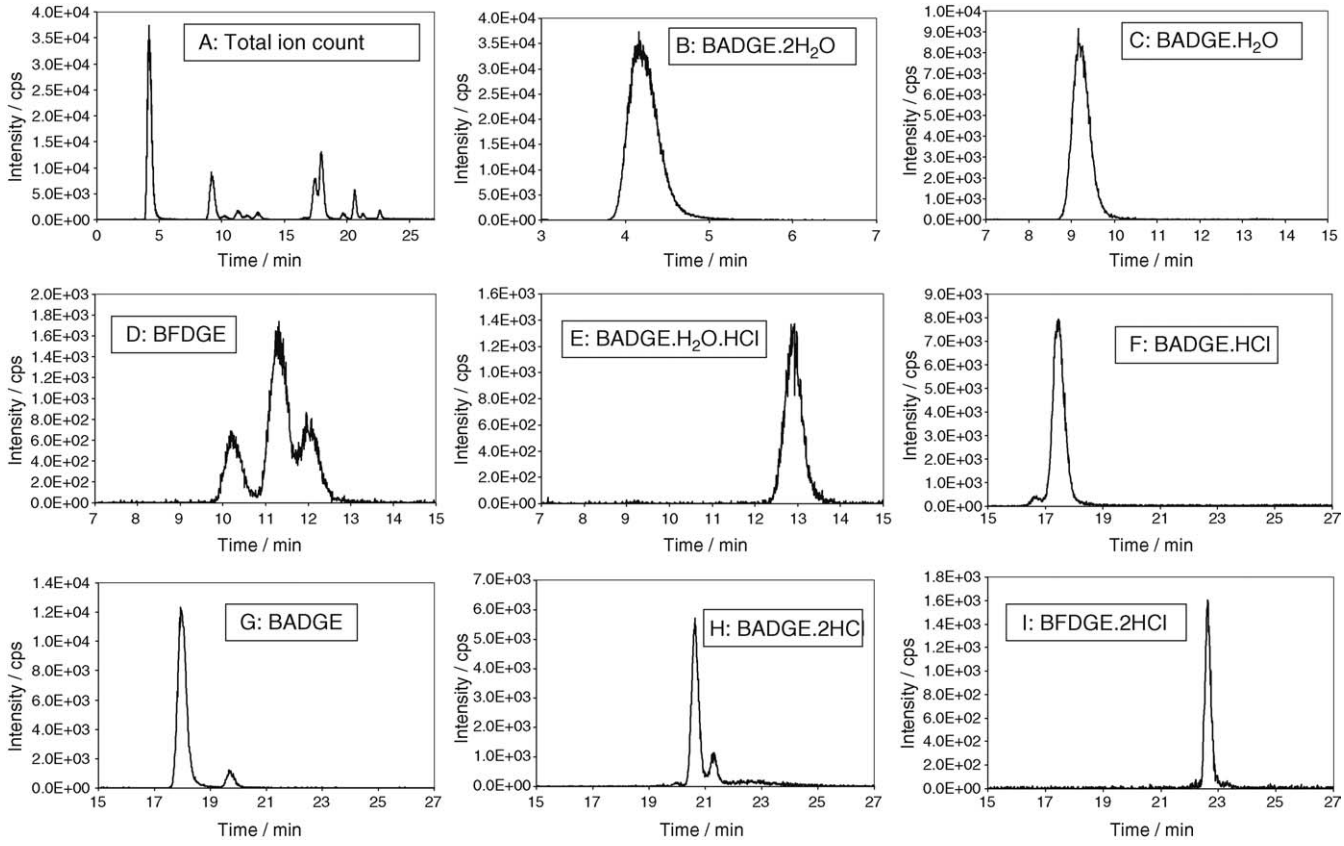

Fig. 2. MRM spectra of all standards (10 mg l^{-1}): (A) total ion count and (B–I) extracted ion counts corresponding to all eight analytes.

(IS)) were optimised using the automated flow injection analysis program. Nitrogen was used for all gas purposes. The analysis time was split into three periods denoted periods 1, 2 and 3, respectively. This was done in order to increase the sensitivity. The duration of each period was 7, 8 and 12 min, respectively. Gas parameters for each period were: CUR (l min^{-1}): 9, 8 and 7, respectively; NEB (l min^{-1}): 12, 8 and 8, respectively; CAD (l min^{-1}): 10, 8 and 8, respectively; TEM (°C): 450, 475 and 400, respectively; IS (V): 5500, 4000 and 3500, respectively. Representative MRM spectra of all eight analytes are given in Fig. 2.

2.5. Sample preparation

An aliquot of 250 mg cream was suspended in 1 ml dimethyl sulfoxide (DMSO) and 200 μl *n*-heptane in a 1.5 ml Eppendorff tube. The suspension was vortexed for 2 min at 2800 rpm and centrifuged for 5 min at 8000 × *g* in a Sigma 1–13 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Seven hundred and fifty microliter of the lower clear solution was carefully aspirated using a 23-gauge needle and diluted to approximately 20 ml with milliQ water. The diluted solution was transferred to a 3cc Varian Bond Elut C_{18} solid phase extraction (SPE) cartridge (Varian, Inc., Palo Alto, CA, USA), which had previously been activated with 2 ml methanol and equilibrated with 2 ml milliQ water. Before elution with 1 ml methanol, the SPE cartridge was washed with 2 ml 5% (volume) methanol. After the final wash with 5% methanol and after elution of the analytes, the solvents were carefully removed from the SPE cartridge in order to obtain a well-defined elution volume. The eluate was diluted by adding 500 μl of milliQ water. An aliquot of 10 μl was injected into the LC–MS-MS system.

2.6. Extraction of aluminum tubes

An aliquot of 2 ml isopropanol was used for the extraction of the aluminum tubes, which occurred at 70 °C for 48 h. This approach is in accordance with USP ⟨661⟩ on containers [31]. The isopropanol was filled into the tubes, which were subsequently closed by folding. At times 1, 2, 4, 7, 25 and 48 h, samples were taken by carefully unfolding the tubes and aspirating the isopropanol. The experiments were performed in duplicate and samples were analysed in triplicate. The extracts were diluted to a final volume of 40 ml with milliQ water. The diluted extracts were transferred to 3cc Varian Bond Elut C_{18} SPE cartridges as described in Section 2.5 and subsequently analysed.

2.7. Accelerated cream extraction study

Fifteen new 5 ml internally lacquered aluminium tubes were filled with 5 g of model cream. The filled tubes were placed in an oven at 70 °C. At times 24, 48, 72, 96 and 120 h, the tubes were allowed to cool for 15 min before 3× 250 mg cream from

Table 2
Validation parameters

Analytical parameters	Compound							
	BADGE	BFDGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·HCl	BADGE·H_2O·HCl	BADGE·2HCL	BFDGE·2HCl
R_t (min)	18.0	10.0, 11.1, 11.8	8.9	3.9	20.7	12.6	22.7	17.3
LOD	0.3 ± 0.2	0.6 ± 0.4	0.4 ± 0.3	0.6 ± 0.1	1.5 ± 0.2	1.0 ± 0.2	3.4 ± 0.7	1.6 ± 0.4
LOQ	1.0 ± 0.6	1.9 ± 1.2	1.4 ± 0.8	2.0 ± 0.4	4.8 ± 0.7	3.4 ± 0.5	11.3 ± 2.2	5.3 ± 1.3
Slope	9421	4505	2806	2380	1255	1200	271	1039
ITC	−13074	68849	−45428	−5259	−14017	−19419	−2317	−8537
R^2	0.997	0.995	0.993	0.997	0.991	0.995	0.989	0.995
Range	LOD–1000	LOD–2000	LOD–2000	LOD–6000	LOD–2000	LOD–2000	10–1000	LOD–1000
Inter-day, $n = 60$	7.9%	7.0%	7.9%	4.5%	7.5%	5.4%	9.8%	7.4%
Intra-day, $n = 3$	9.7%	3.4%	7.5%	13.2%	15.5%	12.1%	22.4%	17.0%

BFDGE was detected and quantified as three isomers, hence the three values of R_t. Limits of detection (LOD) and limits of quantification (LOQ) (given in $\mu g\,l^{-1}$) were defined as three and 10 times the signal-to-noise ratio, respectively, and calculated using the Analyst® 1.4 software. Slope and ITC demotes the slope and the intercept of the linear calibration curves. Corresponding R^2 values are given. Range is the linear range of the calibration curve and is given in $\mu g\,l^{-1}$.

each tube were prepared and analysed in triplicate as described in Section 2.5.

2.8. Analysis of commercially available creams

Three different commercially available creams in internally lacquered aluminium tubes were obtained from a local community pharmacy. To obtain realistic worst-case conditions, the creams were all obtained among the used drugs delivered back to the pharmacy by customers with the purpose of safe destruction. The three creams were in tubes of 15, 15 and 10 g, respectively. They were all over due and should have been used before 09, 2004; 01, 2003; and 10, 2003, respectively, as indicated on the tube. The creams were prepared and analysed in triplicate as described in Section 2.5.

2.9. Statistics

For the statistic calculations, single factor ANOVA analyses were performed in Microsoft® Excel 2002 (Microsoft Corporation, Redmond, WA, USA).

3. Results

The extraction study of the new internally lacquered aluminum tubes showed that all compounds except BFDGE and BFDGE·2HCl could be measured above their limits of quantification (LOQs) (see Table 2).

The results of the extraction study are given in Fig. 3. BADGE·H_2O was present at highest concentration (1.2 ± 0.1 mg l^{-1} after 48 h).

In the accelerated cream extraction study BADGE, BADGE·H_2O, BADGE·$2H_2O$ and BADGE·H_2O·HCl were all measured above their LOQs in all samples (Fig. 4). BADGE·2HCl BFDGE and BFDGE·2HCl could not be detected above their LODs. The concentrations of BADGE, BADGE·HCL and BADGE·H_2O·HCl did not differ significantly ($P < 0.05$) from 24 to 120 h, whereas the concentration of BADGE·H_2O decreased significantly ($P < 0.05$) from $43.7 \pm 18.0\,\mu g\,l^{-1}$ at 24 h to $30.6 \pm 22.7\,\mu g\,l^{-1}$ at 120 h. The concentration of BADGE·$2H_2O$ increased significantly ($P < 0.05$) from $84.5 \pm 32.5\,\mu g\,l^{-1}$ at 24 h to $163.1 \pm 25.7\,\mu g\,l^{-1}$ at 120 h.

Mass spectra corresponding to the analysis of the extracted and cleaned-up cream after 120 h at 70 °C is shown in Fig. 5.

For all three commercially available creams the analyses showed quantifiable concentrations of BADGE·H_2O (25.7 ± 5.9, 24.4 ± 9.4 and $26.2 \pm 15.2\,\mu g\,l^{-1}$ for creams 1, 2 and 3, respectively), BADGE·$2H_2O$ (13.3 ± 0.9, 6.4 ± 0.6 and $12.0 \pm 4.9\,\mu g\,l^{-1}$ for creams 1, 2 and 3, respectively) and BADGE·H_2O·HCl (26.2 ± 7.7, 24.9 ± 8.7 and $28.1 \pm 10.7\,\mu g\,l^{-1}$ for creams 1, 2 and 3, respectively). BADGE

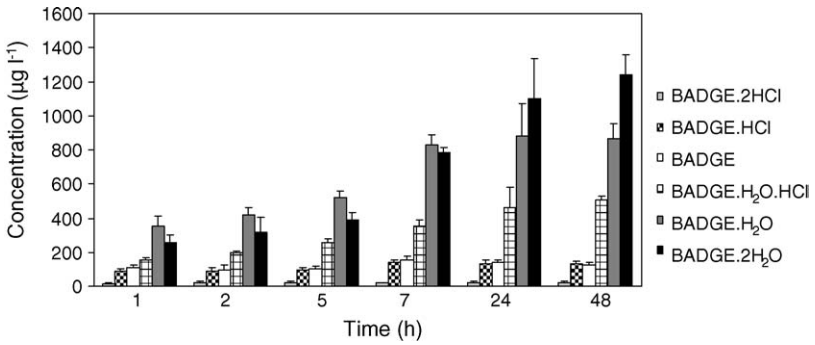

Fig. 3. Concentrations of BADGE·2HCl, BADGE·HCl, BADGE, BADGE·H_2O·HCl, BADGE·H_2O and BADGE·$2H_2O$ extracted from 5 ml lacquered aluminium tubes with 2 ml isopropanol for 48 h at 70 °C.

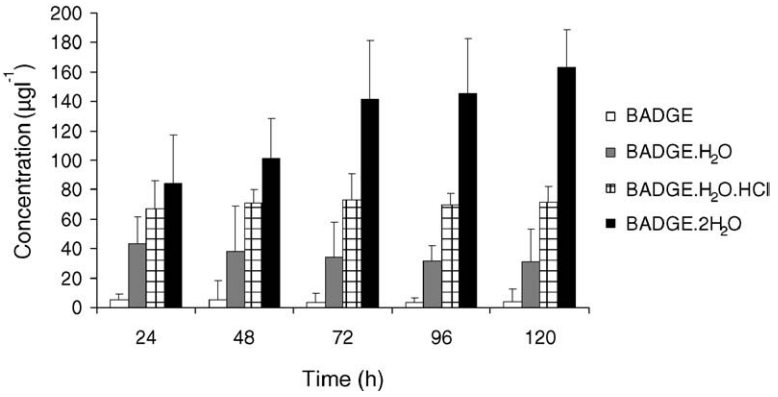

Fig. 4. Concentrations of BADGE, BADGE·H_2O, BADGE·H_2O·HCl and BADGE·$2H_2O$ extracted from aqueous cream from 5 ml lacquered aluminium tubes for 120 h at 70 °C.

Table 3
Recovery (%)

Compound	10 μg l^{-1}	100 μg l^{-1}	1000 μg l^{-1}
BADGE	76 ± 6	71 ± 3	63 ± 5
BFDGE	63 ± 3	55 ± 5	57 ± 3
BADGE·H_2O	48 ± 9	54 ± 12	40 ± 9
BADGE·$2H_2O$	NA	NA	58 ± 3
BADGE·HCl	91 ± 10	73 ± 2	56 ± 3
BADGE·H_2O·HCl	86 ± 6	70 ± 5	58 ± 4
BADGE·2HCl	65 ± 13	69 ± 4	58 ± 3
BFDGE·2HCl	62 ± 5	65 ± 3	49 ± 5

Recovery of the compounds from aqueous cream was established at three levels (three samples at each level analysed in triplicate).

was quantified in one cream only at a concentration of 2.4 ± 0.8 μg l^{-1}.

3.1. Validation

For the calibration curves, working solution A (10,000 μg l^{-1}) was prepared by adding an aliquot of 100 μl of a standard stock solution (1000 mg l^{-1} in methanol) of each of the eight standard substances to a 10 ml measuring flask and diluting to volume with DMSO. Working solution B (1000 μg l^{-1}) was prepared by transferring an aliquot of 1 ml of working solution A to a 10 ml measuring flask and diluting to volume with DMSO. Calibration curves were established (as described in Section 2.5) at 0.2, 0.5, 1, 10, 50, 100, 500, 1000, 2000, 3000, 4000, 5000 and 6000 μg l^{-1} corresponding to 1.6–48,000 μg analyte per kg cream. Validation parameters are given in Table 2.

The recovery was established at three levels (10, 100 and 1000 μg l^{-1}, respectively). At each level, three samples (prepared as described in Section 2.5) and three standards prepared in methanol:milliQ water (1:1, v/v), respectively, were analysed in replicates of three. The results are given in Table 3.

4. Discussion

A custom-made model cream was used in this study. This approach was taken in order to minimise the risk of contamination from ingredients, containers and equipment used in a commercial production. At 70 °C, the cream became much less viscous when compared to room temperature. This was believed to facilitate a more homogeneous mixing of the extractables into the cream, thereby avoiding any biased sampling with regards to concentration gradients over layers of cream in the tube. After heating to 70 °C for up to 72 h and subsequent cooling to room temperature, the cream still appeared to possess the same physical properties as unheated cream (appearance, viscosity and structure).

A mixture of DMSO and *n*-heptane were used to suspend the cream. This composition of solvents was chosen among others (data not shown) because it easily facilitated a homogeneous suspension of the cream and a clean separation of the layers after centrifugation. Furthermore, the composition of the solvents made it possible to extract the analytes from the cream into the clear layer, which appeared after centrifugation of the cream suspension.

The analytical method enabled low μg l^{-1} quantification of eight BADGE and BFDGE derivatives including BADGE and BFDGE from aqueous cream. Aqueous cream is an emulsion of oil-in-water. The nature of the cream makes it a challenge to extract neutral compounds like BADGE, BFDGE and their derivatives without extracting the fatty compounds from the cream, which may interfere with the LC–ESI–MS-MS analyses and apparatus. In the present study, this may explain the relatively low recovery values around 65%. For the analyses of the model cream and the commercially available creams 1 ml of methanol was used to elute the analytes from the SPE column. Smaller elution volumes were tried unsuccessfully (data not shown). To obtain useful chromatographic conditions, the eluate was diluted with 500 μl of milliQ water reaching a final volume of 1.5 ml. Only 10 μl was used for the injection into the HPLC. In order to lower the LODs and LOQs, evaporation of the solvent and reconstitution in a smaller volume after the elution from the SPE column was investigated. This approach was, however, not possible due to insoluble residues in the vial after evaporation of the solvent. Although we did not succeed in concentrating the sample in the present study, we consider this to be an important step to

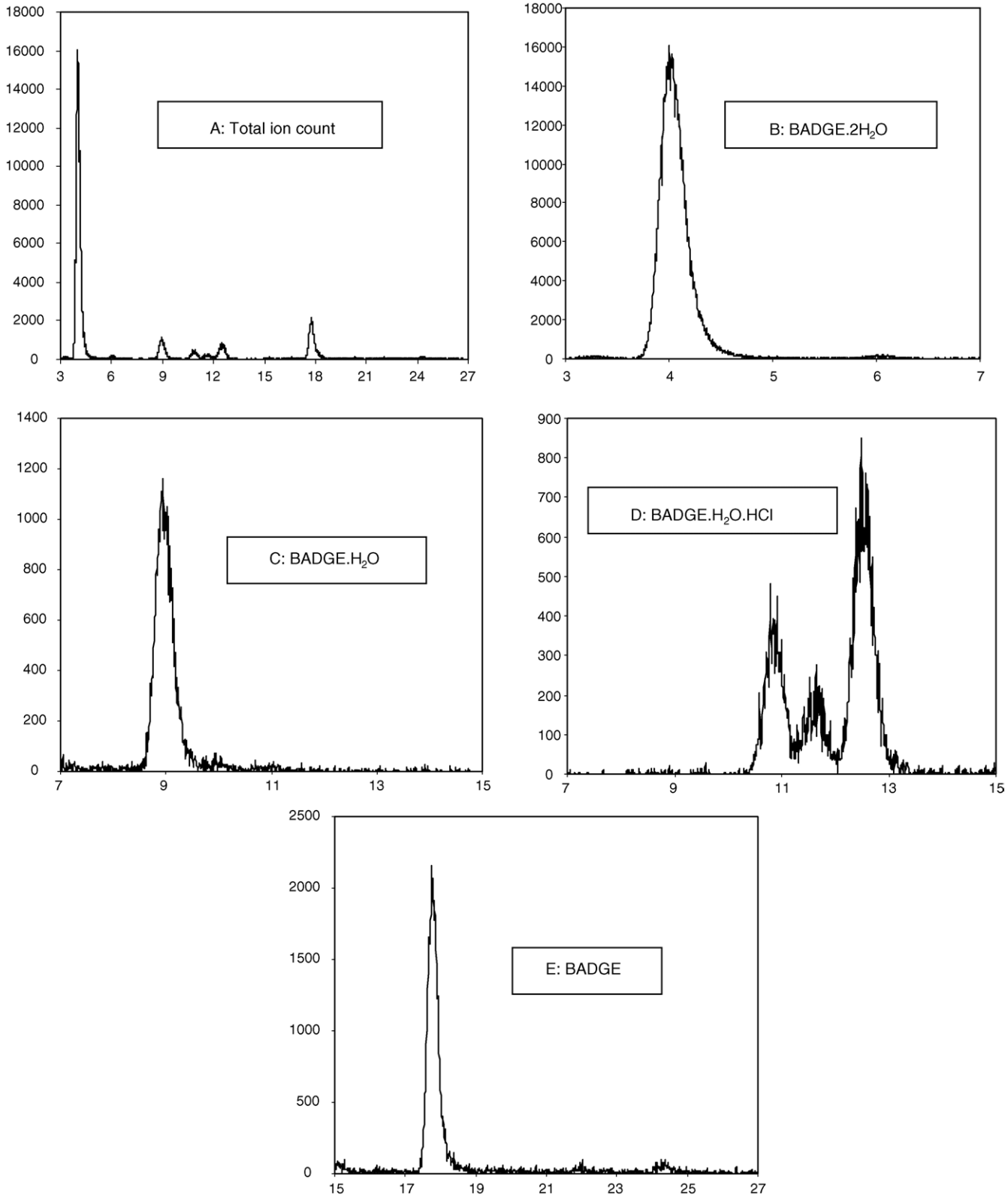

Fig. 5. MRM spectra corresponding to the extracted and cleaned-up cream after 120 h at 70 °C. (A) Total ion count, (B–E) extracted ion counts corresponding to BADGE·2H_2O, BADGE·H_2O, BADGE·H_2O·HCl and BADGE, respectively. X-axes are time (min) and Y-axes are intensity (cps).

focus on if lower LODs and LOQs should be needed for future applications.

Ammonium adducts of the BADGE and BFDGE derivatives were used quantitatively in the analyses. Sodium adducts were also frequently observed during the method development. At several occasions the sodium adducts resulted in higher abundances than the ammonium adducts in single MS scans (Q1 scans). The ammonium adducts were chosen over the sodium adducts because they gave rise to considerably better fragmentation patterns and thus lower LODs and LOQs (data not shown).

The BADGE and BFDGE derivatives studied in this paper exhibit masses in the range 312–412 Da (BFDGE and BADGE·2HCl, respectively) and have estimated $\log K_{ow}$ values varying from 1.93 to 4.57 [32] (BADGE·$2H_2O$ and BADGE·2HCl, respectively). From these physicochemical properties, the resulting $\log K_p$ values can be estimated using the equation: $\log K_p = 0.7811 \log K_{ow} - 0.0115 M_W - 2.19$ [33] to be −5.01, −4.18, −3.89, −3.51, −3.46, −3.39, −3.37 and −3.10 for BADGE·$2H_2O$, BADGE·H_2O·HCl, BADGE·H_2O, BFDGE·2HCl, BFDGE, BADGE·HCl, BADGE·2HCl and BADGE, respectively. $\log K_p$ values are used to characterise the rate at which a given compound crosses the stratum corneum.

When considering that BADGE·H_2O, BADGE·$2H_2O$ and BADGE·H_2O·HCl were found in quantifiable concentrations in all three commercially available creams, and that the same three compounds along with BADGE were quantifiable in the accelerated cream study, the greatest concern regarding permeation of skin should be with regards to BADGE. This is evident from the estimated $\log K_p$ values that predict BADGE to possess the highest skin permeation rate of the compounds studied. Furthermore, BADGE has previously been found to be approximately 10 times more potent than BADGE·H_2O in in vitro mutagenicity assays, where BADGE·$2H_2O$ and BADGE·2HCl did not have any effect [4]. In in vitro mutagenicity and genotoxicity assays, BADGE has also been found to be more potent than BFDGE [6].

The concentrations of BADGE ($\sim 4\,\mu g\,l^{-1}$) found in the cream from the accelerated study, which should resemble an extreme worst-case situation is, however, orders of magnitude lower than the concentrations considered to cause any effect with regards to mutagenic, genotoxic and anti-androgen effects in vitro [3,6,8]. The specific migration limit set by the European Commission at $1\,mg\,kg^{-1}$ for the sum of BADGE, BADGE·H_2O, BADGE·HCl, BADGE·2HCl and BADGE·H_2O·HCl [10] is also much higher than the concentrations found in the cream from the accelerated study. Finally, the aqueous creams analysed in the accelerated study contained only 5–15% of the amounts of BADGE and BFDGE derivatives extracted by isopropanol, whereas the commercially available cream only contained 1–6% of the amount found in the isopropanol extract.

5. Conclusions

Although aqueous cream does not seem to be very effective in extracting BADGE, BFDGE and their derivatives from tube lacquers, and although the levels of the compounds in semi-liquid topical dosage forms found in this study seem to be safe, more data is needed with regards to in vivo effects of these chemicals both alone and in mixture. Furthermore, the permeation of the chemicals present in dosage forms used in chronic treatment of skin disorders associated with damaged skin and reduced barrier function needs to be evaluated.

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