Research Article

Qualitative analysis of cetomacrogol creams by thin-layer chromatography–flame ionization detection (TLC–FID)

Lien Secretin[1] · Felix Anyakudo[1,2] · Liesbeth Gilissen[3,4] · An Goossens[3,4] · Ann Van Schepdael[1]

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Abstract
Buffered cetomacrogol cream has been described as the cause of iatrogenic allergic contact dermatitis, while patch testing with all ingredients was, in most cases, unable to identify the sensitizing culprit. Several hypotheses had been put forward, among which the formation of a new allergen by interaction of some of the ingredients, so-called 'compound allergy'. In order to investigate this hypothesis, a method for the qualitative analysis of cetomacrogol creams, using thin-layer chromatography with flame ionization detection (TLC–FID), is presented. All cetomacrogol cream components, i.e., a preservative and excipients were completely separated. A two-step elution system was used to separate the analytes on the Chromarods: in the first step we separated and focussed the paraffins and cetostearyl alcohol with the use of hexane-methanol–methyl *tert*-butyl ether (100:3:6, v/v). After drying, the same rod was then redeveloped using methanol to resolve sorbic acid from cetomacrogol 1000, whereupon detection of the cream components could be performed by direct flame ionization detection on the Chromarods. The developed method was then applied for the analysis of commercial non-buffered and buffered cetomacrogol cream samples. No newly formed allergen could be detected, thus excluding 'compound allergy'. This method proved to be simple, cheap, and fast, enabling the separation of the auxiliary substances present in cetomacrogol cream.

Keywords Allergic contact dermatitis · TLC–FID · Cetomacrogol cream · Excipient

1 Introduction

A retrospective study, published in 2019 [1], described several cases of allergic contact dermatitis from buffered cetomacrogol cream, used to treat various skin diseases, and confirmed by positive patch-test reactions to it. However, positive reactions during patch-testing with the non-buffered cetomacrogol cream, and particularly with the individual cream ingredients were seldom observed, hence, the culprit allergen was not identified.

Several hypotheses had been put forward by the authors, among which the formation of a new allergen by interaction of some of the ingredients, so-called 'compound allergy'. In order to investigate this hypothesis, qualitative analysis of the buffered cetomacrogol creams was performed.

Cetomacrogol cream is a hydrophilic cream base, thus an oil-in-water emulsion that consists of several excipients with different polarities [2], as well as a preservative.

Several chromatographic methods for the identification of the active ingredients present in pharmaceutical creams exist, such as TLC described in pharmacopoeias, or high performance liquid chromatography (HPLC) [3, 4] and gas chromatography [5]. In the existing methodologies a

✉ Ann Van Schepdael, Ann.VanSchepdael@kuleuven.be | [1]Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, KU Leuven – University of Leuven, O&N2, Herestraat 49/923, 3000 Leuven, Belgium. [2]Bureau Veritas-Antwerpen, Belgium, Romeynsweel 14, 2030 Antwerp, Belgium. [3]Department of Microbiology, Immunology and Transplantation, Allergy and Clinical Immunology Research Group, KU Leuven – University of Leuven, Herestraat 49, 3000 Leuven, Belgium. [4]Department of Dermatology, Contact Allergy Unit, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium.

(rather complicated) clean-up procedure is usually applied to the creams, because the excipients themselves may interfere in the chromatographic process. However, the complete recovery of the target compound is not always guaranteed. Here we will only focus on the separation and identification of the cetomacrogol cream components, i.e., the preservative and excipients.

TLC separation of excipients present in cream bases has been reported in the literature: Van de Vaart et al. [6] described two TLC systems for the separation of common cream excipients, i.e., three cream bases of the Formularium Nederlandse Apothekers (FNA) and four commercial cream bases. Whitmore et al. [7] discussed a simple method for the separation of non-ionic detergents in the presence of the common natural lipids: Lubrol WX®, Triton X-100®, and Brij 58®.

Detection with UV light and spray reagents is used in classical TLC measurement for determination of analytes, in general, but the use of flame ionization detection (FID) as detector could avoid this, the latter having often been described in combination with TLC. The book entitled 'Thin-Layer Chromatography with Flame Ionization Detection' from Mojmir Ranny provided an extensive explanation on the use of TLC–FID, along with some specific applications and new perspectives [8]. Various of its applications have been reported by several authors, such as the separation of marine lipids into seven classes [9], the analysis of neutral lipids and phospholipids in marine animals [10], as well as of petroleum fractions [11]. Moreover, Anyakudo et al. [12] recently presented an extensive review on the subject.

Here we present TLC–FID as a fast and reliable technique, able to separate and simultaneously analyze all the excipients present in cetomacrogol cream, without any need for an additional clean up procedure.

2 Experimental

2.1 Apparatus and experimental conditions

A 3202/IS-02 semi-automatic sample spotter (SES, Schaidt, Germany) was used for sample application. Chromatographic separation was performed on Chromarods S-V (silica gel, 5 µm particle size, 60 Å pore diameter), and peak detection using an FID, Iatroscan MK6 (Iatron Labs, Tokyo, Japan). Hydrogen—and air flow were set at 160 mL/min and 2 L/min, respectively. A scan speed of 25 s was used for sample analysis.

2.2 Chemicals

Analytical reagent grade dichloromethane (CH_2Cl_2) ≧ 99.8% was purchased from Fisher Chemical (Hampton, New Hampshire, USA). Methanol (MeOH) 99.8% for HPLC and ammonium hydroxide 25% solution in water for analysis (NH_4OH) were both from Acros Organics (Geel, Belgium). *n*-Hexane, methyl *tert*-butyl ether (MTBE) and acetone were all purchased from VWR (Haasrode, Belgium).

The reference substances (all Ph. Eur. approved) cetostearyl alcohol and white petrolatum were obtained from Fagron (Nazareth, Belgium), Cetomacrogol 1000® from FSA chemicals (Sint-Lenaarts, Belgium), liquid paraffin from Fraver (Kontich, Belgium), and sorbic acid from Fluka Chemika (Bucharest, Romania).

The four cetomacrogol cream bases were: a non-buffered cream base from company X and three buffered cream bases from companies X, Y, and Z. The typical composition of buffered cetomacrogol cream is shown in Table 1 [2].

2.3 Sample preparation

5 mg/mL reference solutions of cetostearyl alcohol, cetomacrogol 1000, liquid paraffin, and white petrolatum were prepared in CH_2Cl_2. A 0.5 mg/mL reference solution of sorbic acid was prepared in acetone.

A cream sample solution of 45.5 mg/mL was used. For this solution, 500 mg of the cream was diluted in 7 mL CH_2Cl_2 and vortexed until the cream was completely dispersed (appr. 1 minute). Then, 4 mL of EtOH was added and the sample was vortexed again until a clear solution was obtained. It was necessary to vortex the sample

Table 1 Composition of buffered cetomacrogol cream according to Therapeutisch Magistraal Formularium (TMF) [2]

Buffered cetomacrogol cream	
Cream excipient	Amount (g)
Cetostearyl alcohol	7.2
Cetomacrogol 1000	1.8
White petrolatum	15
Liquid paraffin	6
Potassium sorbate[a]	0.27
Sodium dihydrogen phosphate dihydrate	0.30
Diluted phosphoric acid (min. 85%) or NaOH (1 M)	Until pH 5
Purified water q.s. ad	100

[a]Creams from the companies X, Y, and Z were prepared with sorbic acid instead of potassium sorbate

solution until a(n) (almost) clear solution was reached, prior to spotting. Spotting volumes of 1µL for both the reference solutions and cream sample solutions were used.

2.4 Elution system

Two developing tanks were used for this work. The first tank contained *n*-hexane-MeOH-MTBE (100:3:6, v/v) while the second tank only contained MeOH. Precut filter paper was inserted to the back and sides of both tanks before addition of the solvent to a height of about 1.5 cm.

2.5 Analysis procedure

The method for TLC–FID analysis of the cream excipients consisted of the following steps:

1. Sample preparation as described in Sect. 2.3.
2. Application of 1 µL solution at the origin of the Chromarods using a semi-automatic spotter. The rod holder contained 10 Chromarods.
3. Development of the Chromarods in the first tank until a height of 10 cm.
4. Air drying of the developed Chromarods.
5. Re-development of the Chromarods in the second tank until a height of 3 cm.
6. Drying of the Chromarods in an oven at 50 °C for about 2 min.
7. Detection of the separated cream excipients on the Chromarods by the Iatroscan MK6.

Sample analysis was carried out using a scan speed of 25 s, being the time needed for the FID to traverse the Chromarod from the top (10 cm) to the origin (0 cm), resulting in ionization and subsequent detection of a compound. Here, the position of the cream excipients on the rod is expressed as scan time, shown as the retention time on the chromatogram, in contrast to Rf values on a classical TLC plate.

3 Results and discussion

3.1 Method optimization with the reference solutions

As a starting point, the separation of cream excipients was initially performed on TLC plates. The mobile phase of system A (Table 2), and a TLC plate with silica gel bound to a plastic carrier (Silica Gel 60F_{254} plastic sheets, 20 × 20 cm; Merck) gave a good separation of all the cream excipients, as shown in Fig. 1 [7]. Sorbic acid was detected with the use of short UV light (254 nm) and resulted in an Rf value of 0.05, while the other cream components (cetostearyl alcohol, cetomacrogol 1000 and the paraffins, i.e., liquid paraffin and white petrolatum) were viewed with long UV light (365 nm) after spraying with the reagent 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANS) [6], giving Rf values of 0.75, 0.63, 0.86 and 0.86, respectively.

To be sure that all the separated cream components could be visualized, a universal detection method, i.e., flame ionization detection was proposed.

However, when system A was used on the Chromarods, the paraffins (liquid paraffin and white petrolatum) and cetostearyl alcohol co-eluted while sorbic acid and cetomacrogol 1000 could not be resolved.

Table 2 Method optimization performed with the reference solutions

System	Mobile phase	Scan speed (s/scan)	Amount spotted (µL)
A	CH_2Cl_2 100 mL MeOH 18 mL NH_4OH 25% 1 mL	30	0.4
B	CH_2Cl_2 100 mL MeOH 18 mL	30	0.4
C	CH_2Cl_2 100 mL MeOH 18 mL	60	0.4
D	CH_2Cl_2 65 mL MeOH 45 mL	25	1
E	CH_2Cl_2 100 mL MeOH 5 mL NH_4OH 25% 2 mL	25	1
F	*n*-Hexane 100 mL MeOH 5 mL NH_4OH 25% 2 mL	25	1
G	1. *n*-Hexane 100 mL → 10 cm MeOH 6 mL 2. *n*-Hex 100 mL → 4 cm MeOH 20 mL	25	1
H	1. *n*-Hexane 100 mL → 10 cm MeOH 6 mL 2. CH_2Cl_2 100 mL → 4 cm MeOH 5 mL	25	1
I	1. *n*-Hexane 100 mL → 10 cm MeOH 6 mL 2. MeOH → 3 cm	25	1
J	1. *n*-Hexane 100 mL → 10 cm MTBE 6 mL 2. MeOH → 3 cm	25	1
K	1. *n*-Hexane 100 mL → 10 cm MeOH 3 mL MTBE 6 mL 2. MeOH → 3 cm	25	1

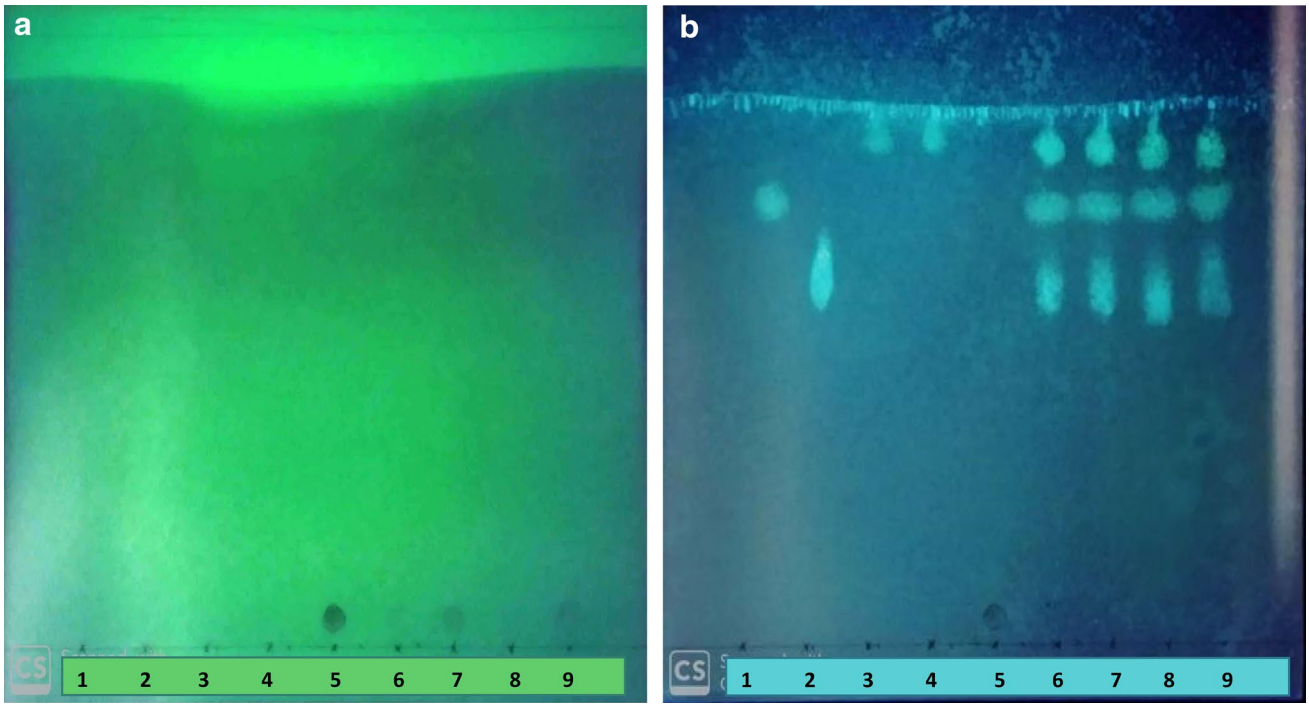

Fig. 1 Identification and separation of all cream excipients with mobile phase A on a TLC plate. **a** Detection with short UV light (254 nm) **b** followed by 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANS) spray reagent in combination with long UV light (365 nm). Spotted samples (left to right): 5µL cetostearyl alcohol (5 mg/mL) 1, 5µL cetomacrogol 1000 (5 mg/mL) 2, 5µL liquid paraffin (5 mg/mL) 3, 5µL white petrolatum (5 mg/mL) 4, 5µL sorbic acid (0.5 mg/mL) 5, 10µL non-buffered cream from company X (45.5 mg/mL) 6, 10µL buffered cream from the companies X, Y, and Z (45.5 mg/mL) 7, 8, and 9

Several mobile phases with varying degrees of polarities (Table 2) were then investigated with the aim of achieving a good separation of all the cream excipients.

Due to significant differences in polarity of the components used in cetomacrogol cream, it was difficult to find a single solvent that was able to separate all target components into distinct peaks. We resorted to a two-step elution procedure, in which a relatively less polar mobile phase was used to develop the rod to a distance of 10 cm, resulting in the separation of the paraffins and cetostearyl alcohol migrating towards the top of the Chromarod into two distinct peaks, while sorbic acid and cetomacrogol 1000 remained at the origin. After drying, the Chromarod was further redeveloped up to a distance of 3 cm in a second tank containing methanol, which, being polar, was able to resolve sorbic acid from cetomacrogol 1000.

The results shown in Table 2 allow to conclude that none of the investigated mobile phases were able to provide a satisfactory peak shape and separation, except for system K.

The scan times, expressed as retention times on the chromatogram, found by using the different elution systems (Table 2) are reported in Table 3.

A chromatogram of the reference solution, obtained with system K, is illustrated in Fig. 2.

A good separation of all the cream components was obtained, even though broad peaks were still present. This could be attributed to (1) the high concentration of the spotted creams (45.5 mg/mL), necessary in order to detect all the components, (2) the limited separation power of the sintered silica of the Chromarods, due to a low number of theoretical plates, causing a bad efficiency; unfortunately, the number of plates would never get better because no other stationary phases than silica can be used, and/or (3) the fact that liquid paraffin, white petrolatum, and cetostearyl alcohol are mixtures.

A broad smeared out peak of cetomacrogol 1000 is seen on the TLC-plates and on the rods, which could be due to its chemical structure, having a molecular weight distribution of polyethylene glycol hexadecyl ethers with different levels of ethoxylation. Cetomacrogol 1000 is found near the origin of the Chromarod, the site where the sample solutions are spotted, where also dust particles and impurities may be present. To minimize these at the origin, a severe and proper cleaning procedure of the Chromarods prior to spotting is recommended. Cleaning the rod is done by running several blank scans from the instrument menu on the unspotted Chromarods at a scan speed of 30–60 s, making sure all the previously spotted samples are ionized and removed from the rod

Table 3 Scan time of each cream excipient using the respective systems mentioned in Table 2

System	Scan time (min)			
	Paraffins	Cetostearyl alcohol	Sorbic acid	Cetomacrogol 1000
A	0.097	0.110	/[a]	/
B	0.100	0.127	/	/
C[b]	/	/	/	/
D	0.104	0.104	0.104	0.347
E	0.047	0.096	/	0.363
F[c]	*0.033*	*0.153*	0.363	0.363
G	*0.053*	*0.204*	*0.303*	*0.370*
H	*0.048*	*0.175*	*0.292*	*0.357*
I	*0.054* *0.038*	*0.166* *0.144*	*0.274* *0.257*	*0.329* *0.336*
J	*0.115* *0.111*	0.270 0.267	0.270 0.267	*0.342* *0.336*
K	*0.054* *0.050* *0.055* *0.067*	*0.119* *0.179* *0.178* *0.167*	*0.282* *0.265* *0.268* *0.268*	*0.352* *0.340* *0.343* *0.345*

[a]/means that the result is not clear because no peak was identified on the chromatogram. This can either be explained by an error in the spotting procedure or by using a wrong/too high scan speed

[b]A scan speed of 60 s was used whereby no peaks were detected. This is consistent with a previous study, in which the FID responses of the analytes initially increased up to a scan speed of 30 s and then rapidly decreased with further increases of scan speed [13]

[c]Italics numbers represent a good separation between the components, expressed as one single peak distinguished from other ones

[13]. Another option could be soaking the Chromarods in concentrated nitric acid followed by rinsing with water.

3.2 Analysis of a buffered cetomacrogol cream

Following a good separation of all the cream components, the analysis of the non-buffered (Fig. 3a) and the buffered cetomacrogol cream (Fig. 3b) was performed.

The buffered cetomacrogol cream did not show an extra peak in comparison to the non-buffered cream, hence, no newly formed allergen could be detected and the hypothesis regarding 'compound allergy' could be excluded. This still does not exclude the possibility of a newly formed small peak appearing beneath another broad one, but this would be extremely exceptional since a significant increase in area under the curve for any cream component could not be observed.

We have to admit though that the TLC–FID technique has its limitations: the compounds are only identified on the basis of retention times in comparison to reference standards, and the sample is being destroyed during FID detection.

However, also when pharmaceutical creams containing active pharmaceutical ingredients (APIs) need to be analyzed, this method could offer an elegant alternative for avoiding sample preparation procedures. If a proper separation can be achieved between the cream excipients and the API, much time could be spared since a clean-up prior to UV [14] or HPLC analysis would not be needed.

In addition, all cream components could be revealed without the need to use two separate detection systems, i.e., UV and a spray reagent.

4 Conclusion

Thin layer chromatography–flame ionization detection showed to be a quick and inexpensive technique that can be used to perform qualitative analysis of

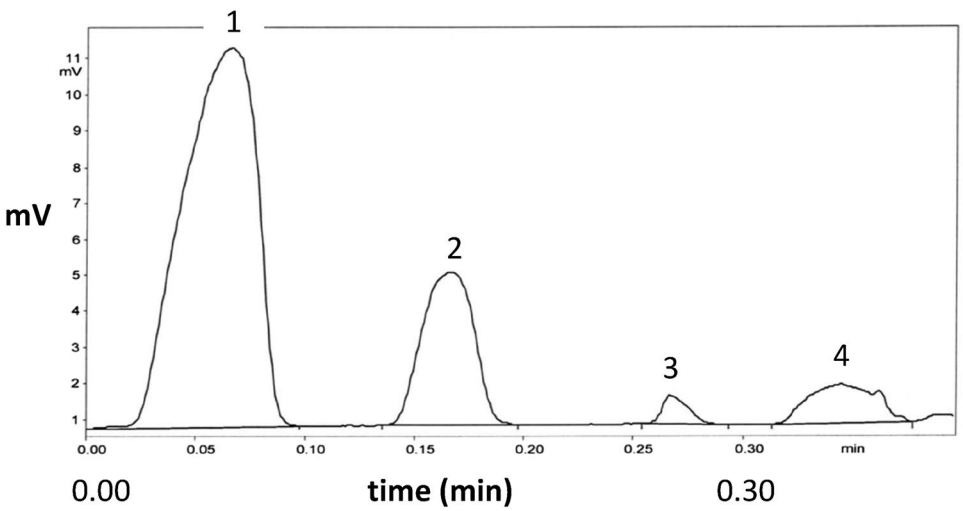

Fig. 2 Chromatogram of the reference solutions obtained with the optimized method using *n*-hexane-MeOH-MTBE (100:3:6, v/v) over a 10-cm distance in the first tank, and MeOH over a 3-cm distance in the second tank. The peaks from left to right, with their respective scan times between brackets are: the paraffins 1 (0.067 min), cetostearyl alcohol 2 (0.167 min), sorbic acid 3 (0.268 min) and cetomacrogol 1000 4 (0.345 min)

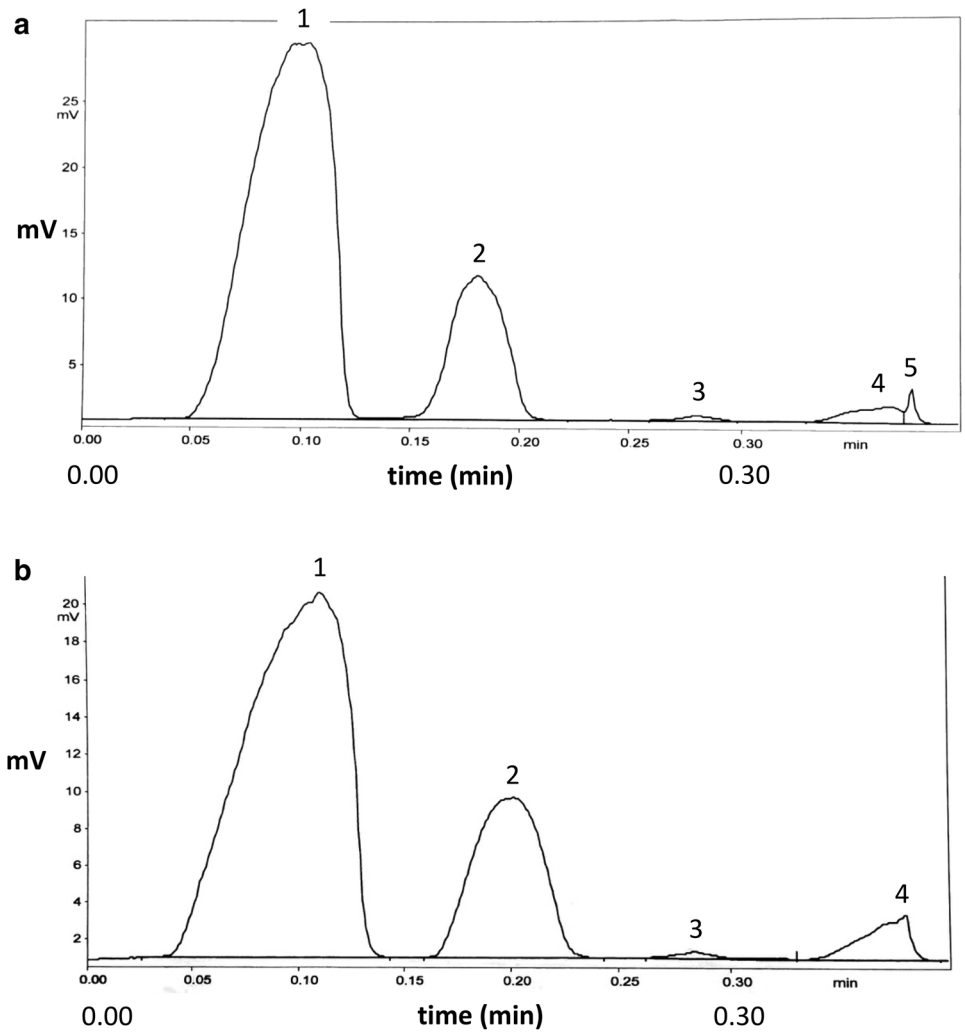

Fig. 3 Typical chromatograms obtained with an elution system using *n*-hexane-MeOH-MTBE (100:3:6, v/v) over a 10-cm distance in the first tank, and MeOH over a 3-cm distance in the second tank. The peaks from left to right, with their respective scan times between brackets are: **a** for the non-buffered cetomacrogol cream: the paraffins 1 (0.102 min), cetostearyl alcohol 2 (0.181 min), sorbic acid 3 (0.281 min) and cetomacrogol 1000 4 (0.365 min); **b** for the buffered cetomacrogol cream: the paraffins 1 (0.09 min), cetostearyl alcohol 2 (0.176 min), sorbic acid 3 (0.268 min) and cetomacrogol 1000 4 (0.363 min)

cetomacrogol creams without the need for a complicated clean-up procedure. We could demonstrate that the buffered version of cetomacrogol cream, held responsible for several cases of ACD, did not reveal the presence of an additional component compared to the non-buffered version. Thus, a new allergen formed by interaction of certain ingredients present in the formulation responsible for ACD by a so-called compound allergy, could be excluded.

The method presented here proved to be simple, cheap, and fast. It was able to separate and simultaneously analyze, in a qualitative fashion, the auxiliary substances present in cetomacrogol cream.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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