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Methylisothiazolinone, Chloromethylisothiazolinone and Methyldibromoglutaronitrile in Moist Toilet Papers Analysis and Market Survey

Key words: Methylisothiazolinone, Chloromethylisothiazolinone, Methyldibromoglutaronitrile, Moist toilet papers, Cosmetics

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Introduction

Moist toilet paper is a relatively new product, which has become popular very quickly in the Netherlands over the last few years. These papers are advertised as promoting personal hygiene, which appears to satisfy a consumer need. However, there are distinct disadvantages of the use of moist toilet papers.

Besides the obvious disadvantage that they are more expensive than the usual toilet papers, moist toilet papers can provoke allergic reactions, often apparent from eczema in the anal regions. In fact, this appears a frequent side effect from these products (1). A possible explanation is the sensitivity of the skin area (and mucous membranes) where the product is used, coupled to the fact that this skin area is generally covered. Both conditions increase the risk for allergic reactions. Allergic reactions to moist toilet papers have been described for the preservatives methylisothiazolinone/chloromethylisothiazolinone (2) and for methyldibromoglutaronitrile (3). Besides reactions have been observed for several fragrance ingredients.

To obtain some knowledge about the usage of methylisothiazolinone/chloromethylisothiazolinone and methyldibromoglutaronitrile moist toilet papers 24 different brands available on the Dutch retail market were analyzed for these preservatives.

Methylisothiazolinone and chloromethylisothiazolinone

Solutions of a mixture of methylisothiazolinone and chloromethylisothiazolinone are available under several trade names. The most familiar trade names are

Kathon® CG and Euxyl® K100. Isothiazolinones are effective preservatives in low concentrations. The concentration used for the preservation of cosmetic products is generally lower than 10 mg/kg active ingredients; the EC sets the maximum concentration in cosmetics at a level of 15 mg/kg active ingredients. Until recently, the isothiazolinones were popular preservatives, but the frequency of use is decreasing. The reason for the decline in their popularity is the high sensitizing potential the isothiazolinones showed. Once this knowledge was made public by the popular press in Germany sales of cosmetics containing the isothiazolinones were affected adversely and many manufacturers turned to alternative preservatives.

The risk for allergic reactions is highest in «stay-on» cosmetics (4). For this category of cosmetics CIR (Cosmetic Ingredient Review, an expert panel in the USA that examines the safety of cosmetic ingredients) advises a maximum concentration of 7 mg/kg active ingredients (5). It may be questioned if moist toilet paper should be seen as a stay-on cosmetic. Of course, the paper itself does not stay on the application area, but part of the liquid phase remains behind.

Methyldibromoglutaronitrile

Methyldibromoglutaronitrile is a cosmetic preservative of rapidly increasing significance. It is traded as such under the name Tektamer® and is the main active ingredient of Euxyl® K400. Whereas the frequency of use of the isothiazolinones decreases, it appears that methyldibromoglutaronitrile is used as its substitute. An investigation in 1992 showed that at the time methyldibromoglutaronitrile did not cause many side effects in the Netherlands (3), but with its rapidly increasing use the frequency of allergic reactions also increases (6). In the Netherlands a recent investigation showed that 4.0% of patients visiting dermatological clinics participating in the study and diagnosed to have a contact allergy was allergic to methyldibromoglutaronitrile. With an incidence of 4%, methyldibromoglutaronitrile was the highest scoring preservative in this investigation. In $^1/_3$ of the cases the allergic reaction was caused by moist toilet paper (7). It has also been reported, that methyldibromoglutaronitrile is an important allergen in Italy (8, 9).

The EC limits the maximal concentration of methyldibromoglutaronitrile in cosmetics to 0.1%; in sun protection cosmetics the maximally allowed level is 0.025%.

Experimental

Chemicals

Acetone, methanol, acetonitrile and dichloromethane were all LC-grade. A standardized solution of methylisothiazolinone/chloromethylisothiazolinone (Kathon® CG) was obtained from Rohm and Haas company, whose cooperation

is kindly acknowledged. Methyldibromoglutaronitrile (Tektamer® 38), bronopol and bronidox were all technical quality, and standardized by titration.

Sodium chloride, zinc acetate dihydrate, sodium sulfate, sulfuric acid and acetic acid were all high purity.

Determination of methylisothiazolinone/chloromethylisothiazolinone

Apparatus

LC-system-HP 1090 M HPLC, fitted with a diode array detector (Hewlett Packard, Waldbronn, BRD); Lichrospher® 100 RP 8 analytical column, 5 µm, 250 x 4 mm (Merck, Darmstadt).

Sample preparation

Accurately weigh two tissues in a round bottom flask (W g), add 100.0 ml acetone and shake thoroughly. Transfer 75.0 ml of the solution in acetone obtained into another round bottom flask and vaporize in a rotavapor until a small volume is left. Transfer the extract to a 10-ml volumetric flask using methanol/water (1/1 v/v) and dilute to volume with the same solvent mixture (test solution). Dry and accurately weigh the tissues in order to calculate the amount of liquid phase they contain.

Standard solution Kathon® CG

Accurately weigh 0.2 g Kathon® CG into a 100-ml measuring flask and dilute to volume with water. Dilute 10.00 ml of this solution to a final volume of 100.0 ml with water. This solution should be freshly prepared.

Chromatography

For the determination of the isothiazolinones the chromatograph should be equipped with the diode array detector, detection wavelength set at 274 nm and bandwidth 4 nm using a reference wavelength of 550 nm with a bandwidth of 100 nm. Spectra are collected over the spectral range of 210 to 400 nm for identification purposes whenever one of the isothiazolinone peaks is observed.

Mobile phase

Acetonitrile/water (4/6 (v/v)) during 10 minutes, after which the column is flushed during 10 minutes with acetonitrile/water (9/1 (v/v)) to wash slowly eluting compounds from the column; flow rate: 1 ml/min.

Assay

Inject 10 µl of the test solution and 10 µl of the standard solution and check retention times of the peaks and the spectra obtained. Calculate from the areas obtained the concentrations of methylisothiazolinone and chloromethylisothiazolinone in the sample from:

$$C\ (mg/kg) = \frac{A_S * C_{St} * 13333}{A_{St} * W}$$

where C is the concentration methylisothiazolinone or chloromethylisothiazolinone in the sample in mg/kg, A_S the peak area of the peak obtained for methylisothiazolinone (or chloromethylisothiazolinone) measured in the test solution, A_{St} the peak area for the corresponding analyte in the standard solution, C_{St} the concentration (mg/100 ml) of the corresponding analyte in the standard solution and W the weight of sample taken in grams.

Determination of methyldibromoglutaronitrile

Apparatus

LC-system-HP 1090 M HPLC, HP 1049 A electrochemical detector (Hewlett Packard, Waldbronn, BRD); Lichrospher® 100 RP 8 analytical column, 5 µm, 250 x 4 mm (Merck Darmstadt).

Sample preparation

The same test solution as prepared for the determination of the isothiazolinones is used.

Standard solution

Prepare a stock solution containing 25 mg/100.0 ml methyldibromoglutaronitrile in methanol/water/sulfuric acid 1M (8/2/0.2 (v/v/v)). If desired two additional preservatives, bronopol and bronidox, may be determined in the same run; in that case, dissolve also 25 mg of each of these preservatives in 100.0 ml methanol-water/sulfuric acid 1M (8/2/0.2 (v/v/v)). The standard solutions are prepared by diluting 2.00 ml of the stock solutions to a final volume of 50.0 ml with methanol/water/sulfuric acid 1M (8/2/0.2 (v/v/v)).

Chromatography

Flow rate, 1 ml/min; column oven temperature 40 °C; electrochemical detection, detector configuration: gold working electrode, solid state silver reference electrode; detection mode, reduction pulse mode: +1 V during 10 ms, –1 V during 10 ms, –0.4 V during 100 ms (measuring potential); sensitivity, 500 µA; Response time 2 sec.; thermostatted at 40 °C. Thoroughly degas the mobile phase by flushing with helium. Avoid plastic tubing in the system to reduce detector noise.

Mobile phase

Water – acetone – Sodium sulfate – Sodium chloride = 600 – 400 – 0.02M – 0.002M

Assay

Inject 10 µl of the test solution and 10 µl of the standard solution and check the retention times of the peaks obtained. From the peak heights obtained for the test solution and for the standard solution calculate the concentrations of methyldibromoglutaronitrile (and if desired bronopol and bronidox) in the sample:

$$\% \ (m/m) = \frac{h_{sa} \ x \ C_{st}}{h_{st} \ x \ 750 \ x \ W}$$

where h_{sa} and h_{st} are the heights of the peaks obtained for the test solutions respectively the standard solution, C_{st} the concentration of the standard solution (mg/100 ml), and W the weight of the sample taken.

GC/MS

A few drops of the liquid phase are pressed from the moist toilet paper, to which an equal number of drops of dichloromethane are added. After shaking and separation of the phases 10 µl of the dichloromethane-extract is injected into the GC/MS (in this investigation a Finnigan Matt ITS40 instrument) and analyzed using the following conditions and temperature program: column: DB-5, 30 meters x 0.25 mm, coating 0.25 µm; carrier gas: helium 8 psi; injection temperature 250 °C; transfer line 280 °C. Temperature program: initial temperature 50 °C during 1 minute, increasing with 6 °C/minute to a final temperature of 250 °C. Mass spectrometer: Mass range 35–300 amu, scan rate 1 second, ionization mode: electron impact.

Results and Discussion

Isothiazolinones

The method used for the determination of the isothiazolinones was a slight variation of a method used in a previous investigation done in 1991 of moist toilet papers (10) and is similar to a method presently in discussion for adoption as official EC-method for cosmetic products (11). It was slightly adapted to provide convenient sample preparation and chromatography for moist toilet papers.

Sample preparation involved extraction of the paper with acetone. Subsequent weighing of the dried tissue allows the calculation of the concentration in both the moist tissue and in the liquid phase of the tissues. The method allows the determination of 0.8 mg/kg methylisothiazolinone and 2.10 mg/kg chloromethylisothiazolinone with a relative standard deviation of 4.4%. The recovery from aqueous matrices was 84% ± 1.7% for methylisothiazolinone over the whole concentration range, which ranged from 0.96 mg/kg up to 9.7 mg/kg. For chloromethylisothia-

zolinone, the recovery was 89.2% ± 2.0% at a concentration of 8.4 mg/kg and 85.6% ± 2.0% at 13.6 mg/kg with a range from 2.9 mg/kg up to 29 mg/kg. At the lower level the relative standard deviation was 3.1%, at the higher level 5.2%. A typical chromatogram of a standard solution is shown in figure 1.

Cosmetics contain many ingredients with similar polarities and concentrations as the preservatives, which may easily interfere. Moist toilet papers are no exception. Therefore, diode array detection is highly recommendable, because it allows confirmation of the identity of the peaks of the isothiazolinones.

All samples were analyzed using the gradient elution described. One sample contained methylisothiazolinone and chloromethylisothiazolinone. As a replicate, and for confirmation, this sample was also determined using methanol as the extraction solvent instead of acetone, with a similar result.

Compared with the results of the investigation performed in 1991, in which 9 of 19 samples contained isothiazolinones, the use of the isothiazolinones has clearly decreased.

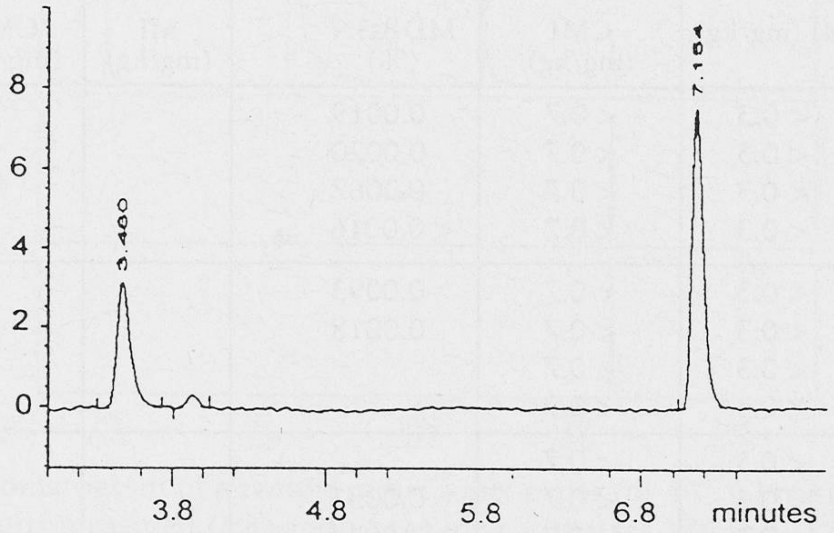

Fig. 1. Chromatogram of an isothiazolinone standard solution. Methylisothiazolinone 0.782 mg/l, retention time 3.48 minutes; chloromethylisothiazolinone 2.345 mg/l, retention time 7.15 minutes. Injected: 10 µl; conditions as described in the text

Methyldibromoglutaronitrile

The method for the determination of methyldibromoglutaronitrile uses HPLC with electrochemical detection and has been described and discussed in detail previously (12). Two additional preservatives, bronopol and bronidox, can be determined concomitantly with the method. For methyldibromoglutaronitrile the method has a recovery from aqueous matrices of 100.4% ± 0.7% at a concentration of 0.075% and of 100.4% ± 0.37% at a concentration of 0.125%. The relative standard deviations at these levels were 0.9% and 0.35% respectively.

The limit of detection, calculated statistically from the calibration curve was 0.0016%, but in actual practice lower concentrations could be detected.

Results of the analyses are shown in table 1. In 21 of 24 brands of moist toilet papers methyldibromoglutaronitrile was identified using the HPLC method. The measured contents varied from 0.0004% (m/m) (0.0007% in the liquid phase) to 0.0372% (m/m) (0.0529% in the liquid phase). Six of these results were lower than the limit of detection (as calculated statistically from the calibration curve), and are therefore not listed quantitatively in the table. For all samples in which methyldibromoglutaronitrile was found the result was verified with GC/MS to exclude the possibility of interference. Confirmation was done based on the retention time in the gas chromatograms obtained (for methyldibromoglutaronitrile approximately

Table 1. Contents of methylisothiazolinone (MI), chloromethylisothiazolinone (CMI) and methyldibromoglutaronitrile (MDBGN) in moist toilet papers and in the liquid phase of these papers

	In tissue			in Liquid phase		
sample	MI (mg/kg)	CMI (mg/kg)	MDBGN (%)	MI (mg/kg)	CMI (mg/kg)	MDBGN (%)
1	< 0.3	< 0.7	0.0019			0.0028
2	< 0.3	< 0.7	0.0020			0.0031
3	< 0.3	< 0.7	0.0067			0.0110
4	< 0.3	< 0.7	< 0.0016 ♣			< 0.0025 ♣
5	< 0.3	< 0.7	0.0093			0.0173
6	< 0.3	< 0.7	0.0018			0.0030
8	< 0.3	< 0.7				
9	< 0.3	< 0.7				
10	< 0.3	< 0.7	+			+
11	< 0.3	< 0.7	0.0053			0.0087
12	< 0.3	< 0.7	0.0044			0.0071
13	< 0.3	< 0.7				
15	< 0.3	< 0.7	0.0080			0.0135
16	< 0.3	< 0.7	0.0368			0.0537
17	< 0.3	< 0.7	0.0372			0.0529
18	3.0	4.0		4.8	6.7	
19	< 0.3	< 0.7	+			+
20	< 0.3	< 0.7	0.0216			0.0363
21	< 0.3	< 0.7	0.0158			0.0259
23	< 0.3	< 0.7	+			+
24	< 0.3	< 0.7	0.0029			0.0047
25	< 0.3	< 0.7	0.0346			0.0484
26	< 0.3	< 0.7				
27	< 0.3	< 0.7	+			+

\+ = sample contains decomposition products from MDBGN
♣ = result below statistical limit of detection, but confirmed by GC/MS

20 minutes) and the mass spectra. Especially the specific masses 66, 106, 185 and 187 are specific for methyldibromoglutaronitrile. Except in the mass spectrum of methyldibromoglutaronitrile, the masses at 66 and 106 are also found in a decomposition product from methyldibromoglutaronitrile with a retention time of approximately 10 minutes. A typical chromatogram of a sample and the mass spectrum of methyldibromoglutaronitrile are shown in figures 2 and 3.

Using GC/MS the results of the HPLC determinations could be confirmed for 15 samples. In the other samples decomposition products could be identified, but the presence of the preservative itself could not be shown. Most probably traces of methyldibromoglutaronitrile were present in these samples, but the sensitivity of the GC/MS confirmation was inadequate to prove these.

Bronidox and bronopol could not be identified in any of the samples.

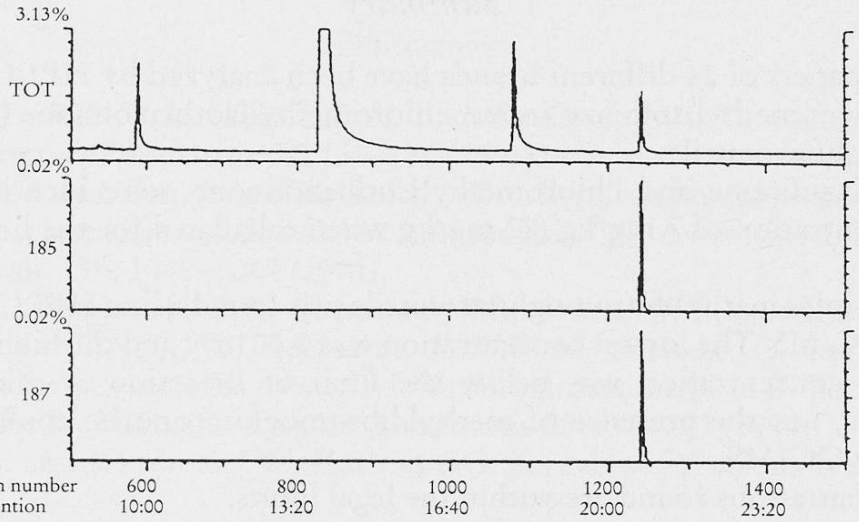

Fig. 2. GC/MS chromatogram of a sample moist toilet paper (nr 16). Chromatograms of the total of all ions measured (tot) and of the ions at masses 185 and 187. Conditions as described in the text

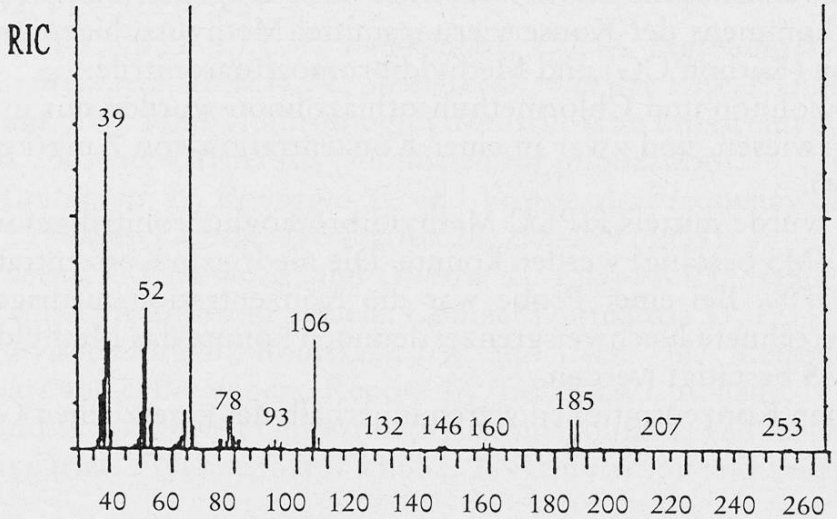

Fig. 3. Mass spectrum of methyldibromoglutaronitrile as found in sample nr 16

The results of this investigation indicate that methyldibromoglutaronitrile is a popular preservative for this product category. Considering its potential for sensitization, coupled to the fact that moist toilet papers are applied at skin areas especially vulnerable (i.e. the mucous membranes of the anal area), the wisdom of such wide application of methyldibromoglutaronitrile in these products may be questioned.

Possibly these doubts regarding the application of methyldibromoglutaronitrile in moist toilet paper should be extended to the product category itself, because a change in preservative would probably only shift the problem to the new compound. It is therefore debatable, whether the benefits of increased hygiene from moist toilet paper, as advertized by the manufacturers, really outweigh the increased risk of contracting an eczema.

Summary

Moist toilet papers of 24 different brands have been analyzed by HPLC for the presence of the preservatives methylisothiazolinone, chloromethylisothiazolinone (Kathon CG) and methyldibromoglutaronitrile.

Methylisothiazolinone and chloromethylisothiazolinone were identified in only one tissue in a concentration of 7 mg/kg (12 mg/kg when calculated for the liquid phase on the tissue).

In fifteen samples methyldibromoglutaronitrile was found using HPLC; its presence was confirmed by GC/MS. The lowest concentration was 0.0018% and the highest 0.0537%. For one sample the concentration was below the limit of detection as calculated from the calibration curve, but the presence of methyldibromoglutaronitrile could nevertheless be confirmed using GC/MS.

All the concentrations found are within the legal limits.

Zusammenfassung

Es wurden 24 verschiedene Marken feuchtes Toilettenpapier mit HPLC analysiert hinsichtlich des Vorkommens der Konservierungsmittel Methylisothiazolinon und Chlormethylisothiazolinon (Kathon CG) und Methyldibromoglutaronitrile.

Methylisothiazolinon und Chlormethylisothiazolinon wurden nur in einem der Toilettenpapiere nachgewiesen, und zwar in einer Konzentration von 7 mg/kg (12 mg/kg in der reinen Flüssigkeit).

In 15 Proben wurde mittels HPLC Methyldibromoglutaronitril gefunden, welches Ergebnis durch GC/MS bestätigt werden konnte. Die niedrigste Konzentration war 0,0018%, die höchste 0,0537%. Bei einer Probe war die Konzentration niedriger als die aus der Kalibrierkurve berechnete Nachweisgrenze; dennoch konnte das Methyldibromoglutaronitril mittels GC/MS bestätigt werden.

Alle gefundenen Konzentrationen waren innerhalb der gesetzlichen Grenzwerte.

Résumé

Les papiers de toilette humides de 24 marques différentes ont été analysés par HPLC pour mettre en évidence les conservateurs méthylisothiazolinone, chlorométhylisothiazolinone (kathon CG) et méthyldibromoglutaronitrile.

Le méthylisothiazolinone et le chlorométhylisothiazolinone ont été identifiés dans un seul tissu à une concentration de 7 mg/kg (12 mg/kg si c'est calculé dans la phase liquide du tissu). Le méthyldibromoglutaronitrile a été trouvé par HPLC dans 15 échantillons; sa présence a été confirmée par GC-MS.

La concentration la plus basse était de 0,0018% et la plus élevée était 0,0537%. Dans un échantillon, la concentration était en-dessous de la limite de détection calculée à partir de la courbe d'étalonnage, néanmoins, la présence de méthyldibromoglutaronitrile a pu être confirmée par GC-MS.

Toutes les concentrations trouvées étaient dans les limites légales.

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