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Rapid quantification of iodopropynyl butylcarbamate as the preservative in cosmetic formulations using high-performance liquid chromatography–electrospray mass spectrometry

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Abstract

A simple, rapid and reproducible method for identification and quantification of iodopropynyl butylcarbamate (IPBC) in different cosmetic formulations is presented. The determination was carried out using a high-performance liquid chromatography (HPLC) procedure on a reversed phase column coupled to a single quadrupole mass spectrometer (MS) via an electrospray ionization (ESI) interface. Detection was performed in the positive selected ion-monitoring mode. In methanol/water extracts from different cosmetic formulations a detection limit between 50 and 100 ng/g could be achieved. A routine analytical procedure could be set up with good quantification reliability (relative standard deviation between 0.9 and 2.9%). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Iodopropynyl butylcarbamate; Cosmetics; Preservative; Liquid chromatography; Electrospray ionization mass spectrometry

1. Introduction

Fungi and yeasts cause considerable problems in the preservation of cosmetic products [1]. Approx. 10% of the microbial contamination of cosmetics and toiletries are caused by fungi and yeasts. Due to its good antimicrobial, technical and ecological properties, the preservative IPBC today finds increasing use in rinse-off and leave-on applications. Its antimicrobial properties include a very good fungicidal effect in low application concentrations as well as considerable bactericidal efficacy [1,2]. Its technical features make it particularly suitable for use in cosmetic formulations. IPBC has a good solubility in ionic and non-ionic surfactants, emulsifying agents and polar solvents. Additionally, IPBC has very good

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dermatological characteristics. It has been classified in the category 'allergic reactions unknown or extremely rare' [3]. Technical grade IPBC has an acute oral LD_{50} of 1580 mg/kg in rats [4]. At present the maximum permissible concentration of IPBC in cosmetic products is 0.05% [5]. Apart from the preservation of a wide range of cosmetic formulations, IPBC is used also as a wood preservative. Other fields of application are the preservation of paints, coatings, textiles, paper, metal working fluids, and adhesives [6].

IPBC has the chemical formula shown in Fig. 1 [7]. It decomposes at 100°C, but it is stable to hydrolysis and degradation processes in pH 5 buffered solution [8].

Although it is well known that the instability of IPBC at high temperatures limits its use in gas chromatography (GC) techniques, several studies have discussed separation and determination of IPBC by GC [9], electron capture GC [10] and GC/mass spectrometry [11,12]. The results reveal why LC often is the method of choice for trace determination of polar, non-volatile and/or thermally unstable carbamates [13]. An overview of the analysis of carbamate insecticides and pesticides using liquid chromatography (LC) with different detection techniques has been provided by Nunes and Barcelo and Pleasance et al. [13,14]. Di Corcia et al. has described the feasibility of using reversed-phase high-performance liquid chromatography/mass spectrometry (HPLC/MS) with an electrospray ionization (ESI) interface for measuring carbamate insecticides in fruits and vegetables [15].

The aim of the present work was to develop an efficient, sensitive and reproducible analytical method for the determination of IPBC as the preservative in different cosmetic products. The method was carried out using a HPLC procedure on a reversed phase column coupled to a single quadrupole mass spectrometer via an ESI interface.

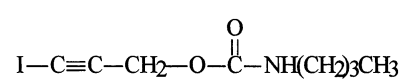

Fig. 1. Chemical formula for IPBC [7].

2. Experimental

2.1. Instrumentation

HPLC operations were carried out with an HP Series 1100 system (Hewlett Packard, Palo Alto, CA) with degasser, binary pump and column oven. Chromatographic separations were performed on a GROM Sil 120 ODS 5-ST (end capped, 5 μm particle size, 60×2 mm i.d.) column with a precolumn (GROM Sil 120 ODS 5-ST, end capped, 5 μm particle size, 10×2 mm i.d.; GROM Analytik + HPLC GmbH, Herrenberg-Kayh, Germany). Atmospheric pressure ESI MS was performed on a HP Series 1100 MSD quadrupole mass spectrometer (Hewlett Packard, Palo Alto, CA).

2.2. Reagents

Crystalline IPBC standard was obtained from Lonza (Annadale, USA). Methanol (analytical grade) and tetrahydrofuran (HPLC grade) were purchased from Merck (Darmstadt, Germany). Deionized water from a Seralpur-Delta system (Alhaeuser, Ransbach-Baumbach, Germany) was used for sample extraction and as a component of the mobile phase.

Four different IPBC-containing cosmetic products and the corresponding placebos were provided by Beiersdorf AG (Hamburg, Germany). The following cosmetic formulations were analyzed: water-in-oil emulsion (W/O emulsion, 58% water), oil-in-water emulsion (O/W emulsion, 69% water), phase-inversion-temperature emulsion (PIT emulsion, 66% water), aqueous solution (94% water).

2.3. Sample preparation

A 0.5 g sample of a cosmetic product (0.01% IPBC) was mixed with 1 ml of tetrahydrofuran and 5 ml of methanol by ultrasonication at room temperature for 5 min. This mixture was diluted to 10 ml with water and the ultrasonication procedure repeated. Then the mixture was centrifuged at 5500 rpm for 10 min. Approx. 4 ml of the supernatant were passed through a 0.2 μm syringe

filter; 5 μl of the filtrate were injected by autosampler into the HPLC/MS system without any further preparation.

2.4. Chromatography

The mobile phase was a binary gradient formed from water (mixture A) and methanol (mixture B). The steps of the gradient program were: 0 min 45% A to 55% B; 5 min 45% A to 55% B; 6 min 0% A to 100% B; 20 min 0% A to 100% B. The flow rate was 200 μl/min. The column was equilibrated for 10 min under the initial conditions before each analysis. Chromatographic separations were performed at a column temperature of 25°C.

2.5. Mass spectrometry

The total HPLC effluent was delivered into the ESI ion source and nebulized by a nitrogen gas stream of 50 psi. The mobile phase was evaporated by a drying gas flow of 12 l/min at a temperature of 105°C. Detection was performed in the positive ion mode. The capillary voltage was set to −4500 V. For collision induced dissociation a fragmentor voltage of 60 V was applied.

2.6. Method validation

To determine the quantitative range, calibration graphs were prepared by plotting the peak area against the known concentration. Interference from other cosmetic constituents with the ionization process of IPBC was evaluated by treating standards with extracts of the corresponding placebos. Portions of placebos (1 g) were extracted according to the above procedure and 500 μl aliquots of the filtered extracts spiked by adding 500 μl of an IPBC solution in methanol. Six concentrations of IPBC, which ranged from 0.1 to 10 μg/ml were analyzed in triplicate.

To evaluate the recovery, placebos were spiked with 50 μg/g by addition of 500 μl of an IPBC solution in methanol. Samples were allowed to equilibrate for 10 min prior to extraction and then processed according to the above procedure. The recovery assays were performed six times in replicate.

To estimate the repeatability and the intermediate precision (inter-day precision) of the developed method, commercial cosmetic products with 100 μg/g IPBC as preservative were analyzed. Each cosmetic product was measured six times in replicate (intra-day repeatability) and twice on three days (intermediate precision), respectively.

3. Results and discussions

3.1. Chromatographic separation

The mobile phase was selected on the basis of the chromatographic separation and MS detection. To separate IPBC from the remaining constituents of the sample a gradient of methanol/water is used. The analyte is eluted after approx. 4 min. Apart from short analysis times, the separation of IPBC from the polar surfactants and emulsifying agents was particularly important for development of the chromatographic method. Furthermore the gradient enables elution of nonpolar cosmetic ingredients from the reversed-phase column after 20 min.

To optimize the sensitivity of MS detection, the dependence of the ion signal sensitivity on the composition of the mobile phase was evaluated. This was done by changing the organic solvent. When methanol was replaced with acetonitrile, the ion signal sensitivity was reduced by 35%. Di Corcia et al. obtained similar results for carbamate insecticides. They described this effect by the basic nature of acetonitrile, which probably can compete with analyte molecules for formation of adducts with inorganic cations [15].

To prevent overloading of the ion source, a 2 mm i.d. microbore column was used with a flow rate of 200 μl/min, making it unnecessary to split the flow.

Fig. 2 shows a HPLC/MS chromatogram obtained for a PIT emulsion with an IPBC content of 50 μg/g. Apart from the total ion current, the extracted mass traces of IPBC are shown in this chromatogram.

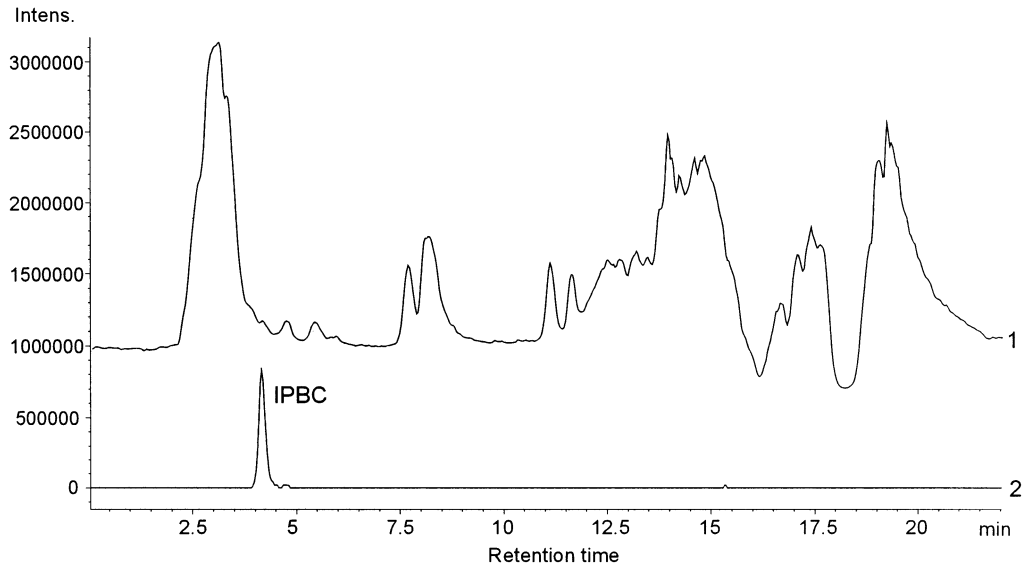

Fig. 2. HPLC–ESI–MS chromatogram of a sample (PIT emulsion; 50 μg/g IPBC) prepared by the described method: 1, total ion current (m/z 100–1000); 2, extracted mass traces of IPBC (m/z 282 $[M + H]^+ + m/z$ 304 $[M + Na]^+$).

3.2. Mass spectrometric detection

Both mass-selective detection and UV detection at 235 nm can be used for the determination of IPBC. However, its selectivity and sensitivity limits the use of UV detection mainly to samples with little matrix interference. In contrast, a major advantage of mass-selective detection is that it can detect small amounts of analytes in highly matrix-loaded samples. Since the chromatograms of the samples are shown as extracted ion traces by means of mass-selective detection, no baseline separation of IPBC from the sample matrix is necessary for quantification. Quantification without previous chromatographic separation (flow injection) is not recommended, since the ion source could be overloaded due to matrix effects. This leads to a strongly reduced sensitivity.

The most sensitive detection of IPBC is achieved with electrospray MS in the positive-ion mode. Fig. 3 shows a mass spectrum displaying two peaks, one each for the $[M + H]^+$ and $[M + Na]^+$ ions. The Na adduct is usually the more abundant of the two.

To increase signal intensity the ion current profiles for the two signals were summed. This also improved the repeatability of detection, since the ratio of sodium adduct ion to hydrogen adduct ion is influenced by the sample matrix. In this way the repeatability (e.g. O/W emulsion) of the determination could be improved from 7.99% $[M + H]^+$ and 3.70% $[M + Na]^+$ to 2.86%, respectively.

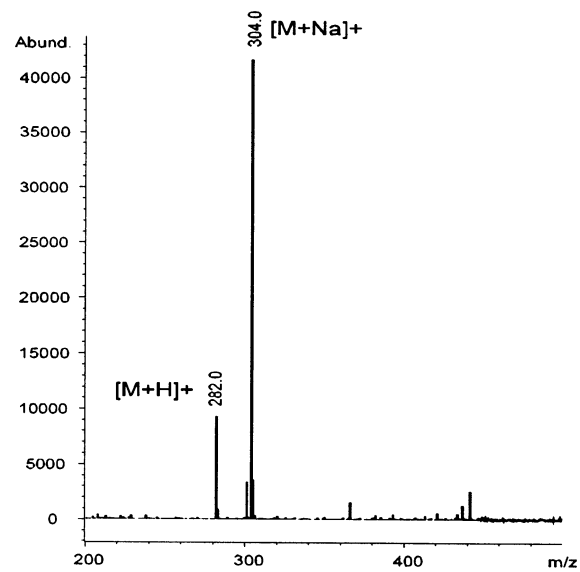

Fig. 3. Positive electrospray mass spectrum of IPBC.

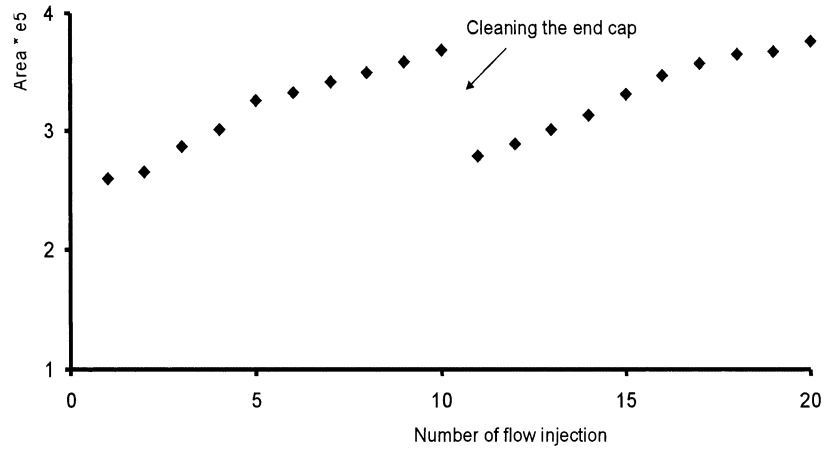

Fig. 4. Effect of cleaning the end cap of the transfer capillary on the signal intensity (m/z 282 + 304) during 20 injections of IPBC standard solutions.

The SIM (selected ion monitoring) mode was chosen for quantification because of its higher sensitivity. In the SIM mode the detector scans only two (m/z 282 and 304) programmed mass ranges. Compared to the full scan mode the signal to noise ratio is much lower so that up to 50 times (O/W emulsion) better detection limits are achieved.

The optimum ESI/MS conditions were developed using the flow injection technique and an IPBC solution in methanol. At a drying gas temperature of 200°C, peak areas increased with an increasing number of injections. After cleaning the transfer capillary (ion transfer from the ion source to the quadrupole) end cap the peak areas again decreased to the starting level. The increase in peak areas after cleaning the transfer capillary end cap is shown in Fig. 4.

It is assumed that the thermally unstable IPBC is pyrolyzed at a temperature of 200°C at the metal end cap. After several injections, deposition and film formation increasingly reduce the contact area of the end cap, so that pyrolysis decreases from injection to injection. After approx. 50 injections the peak areas are constant.

A reproducible result could be obtained only by reducing the drying gas temperature to 105°C. In order to still achieve a sufficient sensitivity with electrospray ionization of the IPBC, the gas flow and nebulizer pressure were increased. The drying gas temperature was not reduced below 105°C as it was assumed that separation of the mobile phase from the analytes in the ion source would be insufficient at lower temperatures.

3.3. Method validation

The calibration graphs (external standard mode) obtained under the chromatographic conditions described were constructed by plotting peak areas against concentrations. A good linearity was achieved in the range of 0.1–10 μg/ml. The regression equations of the curves and the correlation coefficients for all four types of cosmetic formulations were calculated as follows:

O/W emulsion $y = 0.4005x + 0.0197$ ($r = 0.9998$)

W/O emulsion $y = 0.3589x + 0.0265$ ($r = 0.9995$)

PIT emulsion $y = 0.2971x + 0.0283$ ($r = 0.9990$)

Aqueous solution $y = 0.6225x + 0.0241$ ($r = 0.9999$)

To optimize the extraction conditions different solvent mixtures were examined. The extraction method described above gave best results for recovery, peak shape and sensitivity. The recoveries

Table 1
Analytical parameters of the described method for the determination of IPBC in the different cosmetic products

Formulation	LOD[a] (ng/g)	LOQ[b] (ng/g)	Recovery[c] (%)	RSD[d] (%)	RSD[e] (%)
O/W emulsion	100	300	104.0	2.86	4.59
W/O emulsion	100	300	104.0	2.21	3.87
PIT emulsion	100	300	102.0	0.85	1.98
Aqueous solution	50	150	99.4	1.23	2.89

[a] Limit of detection (S/N 3:1).
[b] Limit of quantitation (S/N 10:1).
[c] Six repeated measurements of spiked placebos at a concentration of 50 μg/g.
[d] Relative standard deviation (repeatability), six repeated measurements of commercial products at a concentration of 100 μg/g.
[e] Relative standard deviation (intermediate precision), two repeated measurements on three days of commercial products at a concentration of 100 μg/g.

were determined using spiked placebos. The repeatability of the described method was investigated using commercially available cosmetic products.

The limit of detection and quantitation for IPBC in cosmetic formulations was also determined by means of spiked placebos. The analytical parameters of the described method for the investigated cosmetic formulations are shown in Table 1.

4. Conclusions

The described method is suitable for measurement of IPBC in a wide variety of cosmetic formulations. A simple sample preparation and short retention times permit rapid quantification of IPBC in complex samples.

Since IPBC is thermally labile and not amenable to GC techniques the use of HPLC is preferrable. In terms of sensitivity and selectivity mass-selective detection is a significant improvement over UV detection, especially for analysis of highly matrix-loaded samples.

References

[1] C.S. Koch, R. Grüning, SÖFW-J. 122 (1996) 973–979.
[2] C.S. Koch, R Grüning, Parfüm. Kosmet. 78 (1997) 26.
[3] H.P. Fiedler, Blaue Liste — Cosmetic Ingredients, 2nd Edition, Editio Cantor Verlag, Aulendorf (Germany), 1993.
[4] J. Hansen, Mod. Paint Coat. 74 (1984) 50–56.
[5] EC Directive, Paragraph 3a (98/62/EEC), (23th Amendment of the Directive to the EC Cosmetics Directive 76/768/EEC).
[6] R. Grüning, Cosmet. Toilet. 112 (1997) 59–65.
[7] J.A. Wenninger, G.N. McEwen, International Cosmetic Ingredient Dictionary, 6th Edition, The Cosmetic, Toiletry and Fragrance Association, Washington, DC, 1995.
[8] N.D. Henderson, BC Enviroment, BC Environment, Victoria, BC, 1992 NTIS No. MIC-92-06743.
[9] D. Lee, K. Tsunoda, M. Takahashi, Mokuzai Gakkaishi 37 (1) (1991) 76–81.
[10] P.D. Gabriele, R.M. Ianunucci, J. Caot. Technol. 56 (1984) 33–48.
[11] D. Lee, K Tsunoda, M. Takahashi, Mokuzai Gakkaishi 37 (3) (1991) 261–265.
[12] W. Horn, R. Marutzky, Fresenius J. Anal. Chem. 348 (1994) 832–835.
[13] G.S. Nunes, D. Barceló, Trends Anal. Chem. 18 (1999) 99–107.
[14] S. Pleasance, J.F. Anacleto, M.R. Bailey, D.H. North, J. Am. Soc. Mass Spectrom. 3 (1992) 387–397.
[15] A. Di Corcia, C. Crescenzi, A. Lagana, E. Sebastiani, J. Agric. Food Chem. 44 (1930–1938) 1996.