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Short communication

Simple and rapid analysis of methyldibromo glutaronitrile in cosmetic products by gas chromatography mass spectrometry

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ABSTRACT

A simple and rapid gas chromatography (GC) method with mass spectrometry (MS) detection has been developed for the determination of methyldibromo glutaronitrile (MDBGN) in cosmetic products. The presence of this preservative in commercial cosmetic samples is prohibited since 2007 because of its allergenic properties. The analyzed products were opportunely diluted in methanol and MDBGN was separated by fused silica capillary column and detected by electron impact (EI)-MS in positive ionization mode with a total run time of 7 min. The assay was validated in the range 0.005–0.100 mg MDBGN per g of examined product with good determination coefficients ($r^2 \geq 0.99$) for the calibration curves. At three concentrations spanning the linear dynamic range of the calibration curves, mean recoveries were always higher than 95% for MDBGN in the tested cosmetics. This method was successfully applied to the analysis of cleansing gels, shampoo and solar waters to disclose the eventual presence of MDBGN illegally added in cosmetic products.

1. Introduction

Methyldibromo glutaronitrile (MDBGN) is a preservative that has been frequently used since the beginning of the 1980s in industrial and cosmetic products. In 1986, the European Union (EU) Scientific Committee on Cosmetology approved its use in cosmetics at a maximum concentration of 0.1% with the exception of sunscreen products where the concentration limit was fixed at 0.025% [1].

Soon after introduction to the market, a first case of contact allergy was reported in a worker exposed to glue preserved with MDBGN [2] and similar cases of contact dermatitis were also described in consumers of different cosmetic products such as soap, cleansing milk, shampoos, moisturizers, bath gel, moistened toilet paper, sunscreen lotion, detergents and eye gel [3]. Finally, in recent years a substantial increase in eczema phenomena cases in people using cosmetic preparations containing methyldibromo glutaronitrile was observed in different European countries [4]. The increase was mainly ascribed to the use of high concentrations (around 0.3%) of this preservative in the cosmetics under suspicion. This especially applied to products designed for prolonged contact with the skin such as creams and lotions (stay-on products) and products used many times a day such as liquid soaps.

In details, the frequency of contact allergy due to presence of MDBGN in cosmetics raised from 0.5% in 1991 to 4% in 1994 in the Netherlands [5]. Similarly, an increased rate of patch test reactivity to MDBGN was reported after the use of some cosmetics in London, UK, for the period 1994–1999 [6]. Finally, a study of the frequency of sensitivity to common preservatives and biocites, that involved 16 centers in 11 countries in Europe, showed a rise in positive reactions to products containing MDBGN from 0.7% in 1991 to 3.5% in 2000 [4]. Exposures mainly occurred not only when using stay-on products but also with rinse-off cosmetics as creams, lotions and liquid soap and moist toilet paper/wipes [7,8].

In light of these evidences and based on the advice from the Scientific Committee on Non-Food Products European Commission in 2002 the use of MDBGN was limited only to rinse-off products at a maximum concentration of 0.1% [9].

However, since the Scientific Committee on Consumer Products (SCCP) affirmed that MDBGN was a skin sensitizer and that no safe use levels in cosmetic leave-on and rinse-off products could be established, in 2006 MDBGN was removed from Annex VI of the EU Cosmetics Directive (List of Preservatives which Cosmetic Products May Contain) and it was no longer allowed in any cosmetic product circulating in the EU [10].

In Italy, the EU Commission Directive 2007/17/EC was implemented by the Ministerial Decree of January 2008, which established the complete banning of cosmetics containing MDBGN starting from June 2008 [11].

Recently, the Italian anti-adulteration and safety bureau (Carabinieri per la Tutela della Salute – NAS) and military police dealing

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with customs, excise and tax crimes (Guardia di Finanza – GdF) seized several cosmetic products sold through legal or illegal circuits due to costumer's alerts on contact allergies occurring after the products use. The reasonable suspicion that MDBGN could be illegally employed in these formulations prompted the bureau to request for specific analysis of seized products.

Literature reports only two methodologies for the determination of MDBGN in cosmetic products. The first method dated back to 1994 and involved a lengthy procedure with the use of a liquid chromatography coupled with electrochemical detector [12]. In the second one, published in 2006, MDBGN was determined together with other bromine preservatives by ultra performance liquid chromatography (UPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS). This latter method was fast and sensitive but not suitable for routine analyses since it implied a high sophisticated and expensive instrument, not normally available in analytical laboratories and a high technical skill for its management [13].

In this study, a simple and rapid gas chromatography (GC) separation method with mass spectrometer (MS) detection was developed to investigate the illegal presence MDBGN in cosmetic products. Indeed, for legal purposes and in order to have a high degree of specificity, the use of mass spectrometry as a detector is the best choice. The developed method has been applied to cleansing gels, shampoo and solar waters sized by anti-adulteration and safety bureau.

2. Experimental

2.1. Chemicals

The pharmaceutical standard (purity > 99%) of MDBGN was purchased from Sigma (Milan, Italy). All reagents were of analytical-reagent grade purchased from Aldrich (Milan, Italy) and were used without further purification.

2.2. Cosmetic products

Six different cosmetic products (four cleaners and two solar waters), seized by NAS and GdF following alerts from consumers were received at the Istituto Superiore di Sanità to be analyzed for the presence of MDBGN.

The products presented a label with generic indications such as:

1. Bubble bath: used for children, 6.7 oz, made in Italy;
2. Cleansing gel: used for cleaning babies, 6.7 oz, made in Italy;
3. Soap cream: used for face and body use, 10 oz, made in Italy;
4. Shampoo: used to clean infant hair, 6.7 oz, made in Italy;
5 and 6 Solar waters: hydrating, ultra fresh and super tanning solar water, without protective factor; in a practical spray bottle by 250 ml.

The first three products' labels reported the list of ingredients and included the presence of MDBGN without the indication of substance percentage.

2.3. Instrumentation and conditions

GC–MS analyses were carried out on a 6890 Series Plus gas chromatograph equipped with an Agilent 7683 autosampler and coupled to a 5973N mass selective detector (Agilent Technologies, Palo Alto, CA, USA). Data acquisition and analysis were performed using standard software supplied by the manufacturer (Agilent Technologies).

Analyte separation was achieved on a fused silica capillary column (HP-5MS. 30 m, 0.25 mm i.d. film thickness 0.25 m) (Agilent Technologies). The oven temperature was programmed at 80 °C for 1 min increased to 190 °C at 5 °C/min and then increased to 290 °C at 5 °C/min and held for 10 min. Split injection mode (15:1) was used. Helium (purity 99%). with a flow rate of 1 ml/min was used as carrier gas. Electron impact (EI)-MS spectrum of the analyte was recorded over the range 40–550*m/z* under the following conditions: the injection port at 250 °C, ion source at 230 °C, quadrupole at 150 °C and interface temperatures at 280 °C, respectively.

2.4. Calibration standards and quality control samples

Standard stock solutions (1 mg/ml) of MDBGN were prepared in methanol and stored at 4 °C. Diluted dispersions of blank bubble bath, shampoo, soap cream and blank solar waters were prepared by transferring 1 g MDBGN-free products (chosen between the ones with composition similar to that of products under investigation, but previously checked for the absence of MDBGN) to a 25 ml volumetric flask and taken to volume with methanol. The dispersions were submitted to ultrasonic bath for 15 min at 37 °C. After centrifugation, the clear supernatant was collected and filtered through a membrane filter.

Calibration standards with different mg amounts (0.005, 0.015, 0.04, 0.06 mg/g) of analyte under investigation were prepared for each analytical batch by adding suitable amounts of standard stock solutions to 1 g blank cosmetic products. Calibration samples were treated and processed as unknown samples. Several aliquots of quality control samples at 0.008, 0.01 and 0.05 mg/g concentration were prepared in blank cosmetic products to be used for the calculation of validation parameters.

2.5. Sample preparation

Aliquots (1 g) of the cosmetic products were accurately weighted into a 25 ml volumetric flasks and 15 ml of methanol were added. Flasks were placed into an ultrasonic bath of 15 min at 37 °C, cooled off at room temperature and taken to volume with methanol. After centrifugation, the clear supernatant was collected and filtered through a membrane filter. A 2 μl filtrate volume was injected into chromatographic column.

2.6. Validation procedures

Prior to application to real samples. the method was tested in a 5-day validation protocol [14,15].

Selectivity, linearity, limit of detection (LOD) and quantification (LOQ), recovery, precision, accuracy and stability were assayed as previously reported for other cosmetic products [16,17].

3. Results and discussion

3.1. GC–EI/MS method development

Fig. 1 reports the EI-MS spectrum of a standard solution of MDBGN in methanol, along with its structural formula explaining the fragmentations of the analyte. As shown in the Fig. 1, the electron ionization at 70 eV completely fragmented the MDBGN molecular ion. Hence, for quali-quantitative analysis of MDBGN. the chosen characteristic mass fragments monitored in the selected-ion monitoring (SIM) mode were: *m/z* 52, 66, 106, 185. The underlined ion was used for the quantification measurement.

3.2. Chromatography and validation results

Representative total ion current and SIM chromatograms of MDBGN obtained following the extraction of a shampoo is shown in Fig. 2. A chromatographic run was completed

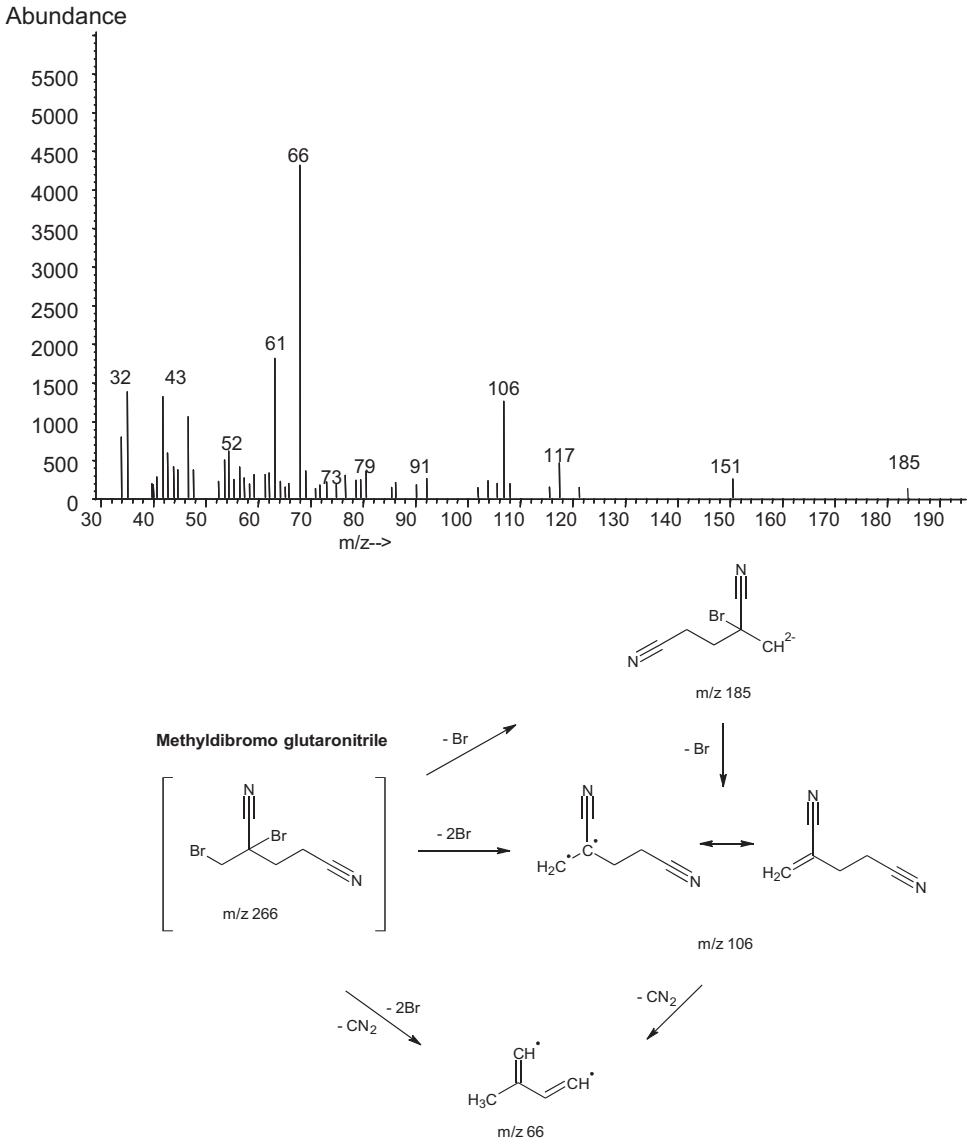

Fig. 1. EI-MS spectrum of a MDBGN standard solution, along with its structural formula explaining the fragmentation of the analyte.

in 8.5 min, and afterwards initial conditions were restored in 2 min.

No additional peaks due to other substances that could have interfered with the detection of compound of interest were observed. Blank samples injected after the highest point of the calibration curve did not present any traces of carryover. Nonetheless, an injection of methanol was introduced between each injection of the batch.

Tables 1 and 2 summarize the method validation data. Linear calibration curves were obtained with a correlation coefficient (r^2) higher than 0.99 for the different cosmetic products. The analytical recoveries (mean ± standard deviation) obtained after liquid–dilution, sonication and filtration at three concentration levels showed that there were no relevant differences between different cosmetic products. Limits of detection and quantification were considered adequate for the purposes of the present study. The results obtained for intra-assay and inter-assay precision and accuracy satisfactorily met the internationally established acceptance criteria [16,17].

3.3. Analysis of products

Results from the analysis of cosmetics samples are listed in Table 3. Presented data are the mean and standard deviation of five different packs of the same batch of each product.

Obtained results were in agreement with what already reported by other authors in 2006 when examining similar cosmetic products [13].

In details, MDBGN was found in only two products: the cleansing gel and the soap cream. In both cases, a percentage less than 0.1%, the maximum allowed concentration of MDBGN until June 2008, was found. However, the products were seized during 2009 and only the manufacture date of the seized batches of each products, held by the anti-adulteration and safety bureau and military police dealing with customs, excise and tax crimes will help in establishing if the products were already prohibited or not.

In conclusion, this paper reports the development of a simple and rapid gas chromatography method with mass spectrometry detection which identifies with a high grade of certainty

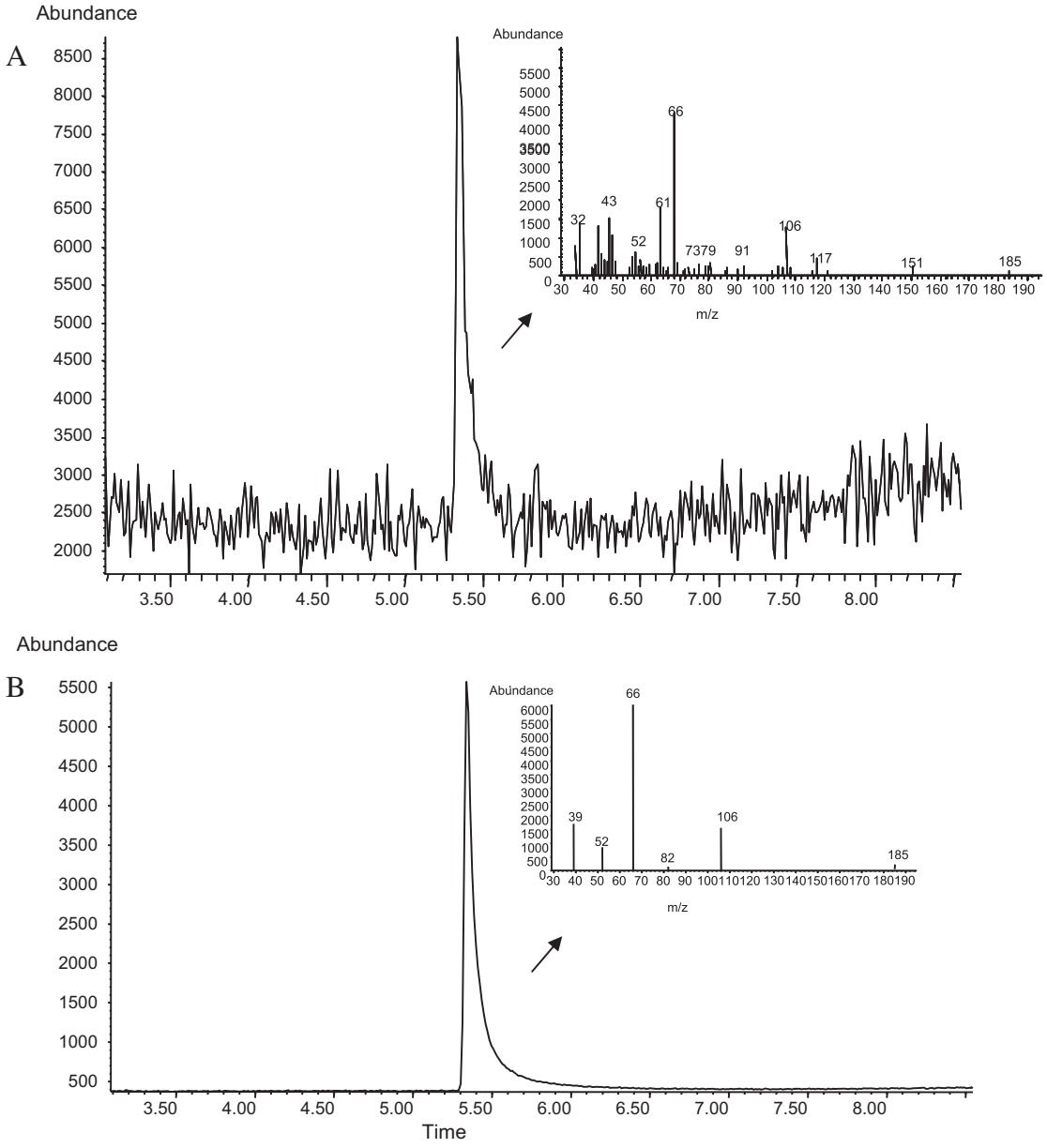

Fig. 2. GC–MS chromatogram of an extract of commercial cosmetic product containing MDBGN. (A) EI total ion current; (B) SIM (selected ion monitoring *m/z* 52,66,106,185).

Table 1
Linearity, analytical recovery and limits of detection and quantification of the assay.

Analyte and cosmetic product MDBGN	Slopes[a]	Intercepts[a]	Correlation Coefficient	Analytical recovery, %[b]			LOD (mg/g)	LOQ (mg/g)
				(0.008 mg/g)	(0.01 mg/g)	(0.05 mg/g)		
Bubble bath	68.031 ± 0.022	0.022 ± 0.002	0.993	96.9	94.7	94.2	0.001	0.005
Cleansing gel	63.044 ± 0.045	0.028 ± 0.005	0.998	97.2	96.2	95.4	0.001	0.005
Shampoo	67.165 ± 0.034	0.033 ± 0.008	0.997	98.3	97.5	95.3	0.001	0.005
Soap cream	69.980 ± 0.022	0.024 ± 0.001	0.998	97.2	96.9	94.5	0.001	0.005
Solar water 1	59.651 ± 0.022	0.022 ± 0.003	0.996	98.2	97.3	94.4	0.001	0.005
Solar water 2	58.432 ± 0.022	0.022 ± 0.004	0.996	97.5	95.3	96.7	0.001	0.005

[a] Mean ± SD of three replicates.
[b] Mean ± SD of four replicates.

Table 2
Intra- ($n = 5$) and inter-assay ($n = 15$) precision and accuracy for the analytes under investigation in quality control samples.

Analyte	Intra-assay precision (%RSD)			Intra-assay accuracy (%Er)			Inter-assay precision (%RSD)			Inter-assay accuracy (%Er)		
MDBGN	0.008 (mg/g)	0.01	0.05	0.008 (mg/g)	0.01	0.05	0.008 (mg/g)	0.01	0.05	0.008 (mg/g)	0.01	0.05
Bubble bath	8.9	7.8	6.9	9.0	10.2	8.9	8.7	7.5	5.5	3.8	5.4	8.8
Cleansing gel	11.8	8.5	9.9	10.8	7.9	11.2	9.2	8.4	6.6	4.2	8.4	3.5
Shampoo	10.4	6.7	7.5	9.5	8.5	4.4	3.8	6.8	5.9	11.5	9.8	5.4
Soap cream	12.5	10.4	9.9	3.5	7.7	9.1	11.3	10.2	4.4	2.2	5.4	3.0
Solar water 1	8.2	4.4	7.8	11.2	5.8	11.5	9.8	5.7	10.3	3.3	9.4	6.7
Solar water 2	7.5	11.3	8.4	6.6	7.8	12.2	11.1	8.8	9.4	3.3	5.4	7.8

Table 3
Concentration and percentage of MDBGN in seized cosmetic products.

Cosmetic product	MDBGN (mg/g)[a]	% MDBGN
Bubble bath	N.D.	N.D.
Cleansing gel	0.010 ± 0.002	0.025
Shampoo	N.D.	N.D.
Soap cream	0.005 ± 0.001	0.012
Solar water 1	N.D	N.D
Solar water 2	N.D	N.D

N.D. = Not detected.

[a] Mean and standard deviation of five different packs of the same batch of each product.

methyldibromo glutaronitrile, a high allergenic preservative which can be illegally added in cosmetic products.

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References

[1] European Commission Opinion of Scientific Committee on Cosmetic Products and non-Food products intended for Consumers. Methyldibromo glutaronitrile. COLIPA No. P77, 2002. http//europa.eu.int/comm./healt/ph_risk/committees/sccp/sccp_opinions_en.htm.
[2] C.G. Mathias, Contact dermatitis to a new biocide (Tektamer38) used in a paste glue formulation, Contact Dermat. 9 (1983) 418.
[3] J.P. Thyssen, J.D. Johansen, T. Mennè, Contact allergy epidemics and their controls, Contact Dermat. 56 (2007) 185–195.
[4] J.D. Wilkinson, S. Shaw, K.E. Andersen, F.M. Brandao, D.P. Bruynzeel, M. Bruze, J.M.G. Camarsa, T. Diepgen, G. Ducombs, P.J. Frosch, A. Goossens, J.M. Lachapelle, A. Lahti, T. Mennè, S. Seidenari, A. Tosti, J.E. Wahlberg, Monitoring levels of biocide sensitivity in Europe. A ten year overview (1999–2000), Contact Dermat. 46 (2002) 207–210.
[5] A.C. De Groot, P.A. De Cock, P.J. Coenraads, Methyldibromo glutaronitrile is an important contact allergen in The Netherlands, Contact Dermat. 34 (1996) 118–120.
[6] J.P. McFadden, J.S. Ross, A.B. Jones, R.J. Rycroft, H.R. Smith, I.R. White, Increased rate of patch test reactivity to methyldibromo glutaronitrile, Contact Dermat. 42 (2000) 54–55.
[7] J.D. Johansen, N.K. Veien, G. Laurberg, Contact allergy to methyldibromo glutaronitrile: data from a 'front line' network, Contact Dermat. 52 (2005) 138–141.
[8] V. Zoli, A. Tosti, S. Silvani, C. Vincenzi, Moist toilet papers as possible sensitizers: review of the literature and evaluation of commercial products in Italy, Contact Dermat. 55 (2006) 252–254.
[9] Scientific Committee on Consumer Products European Commission Opinion on Methyldibromo Glutaronitrile. Available from http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out169_en.pdf (accessed 04.06.02).
[10] Commission Directive 2007/17/EC of 22 March 2007 amending Council Directive 76/768/EEC concerning cosmetic products for the purposes of adapting Annexes III and VI thereto to technical progress – L 82/27, Official Journal of the European Union 23.3.
[11] Updating of the lists annexed to the Law of 11 October 1986, No. 713, on the production and sale of cosmetics, implementing Directive 2007/17/EC of the European Commission. Gazzetta Ufficiale della Repubblica Italiana, January 2008, no. 152.
[12] J.W. Weyland, A. Stern, J. Rooselaar, Determination of bronopol, bronidox, and methyldibromo glutaronitrile in cosmetics by liquid chromatography with electrochemical detection, J. AOAC Int. 77 (1994) 1132–1136.
[13] L. Bendahl, S.H. Hansen, B. Gammelgaard, S. Sturup, C. Nielsen, Hyphenation of ultra performance liquid chromatography (UPLC) with inductively coupled plasma mass spectrometry (ICP-MS) for fast analysis of bromine containing preservatives, J. Pharm. Biomed. Anal. 40 (2006) 648–652.
[14] ICH. Topic Q 2 B Validation of Analytical Procedures: Methodology. The European Agency for the evaluation of Medicinal Products. Available from: http://www.emea.eu.int/htms/human/ich/quality/ichfin.htm. November 1996. London: ICH Technical coordination.
[15] Guidance for Industry Bioanalytical Method validation US Department of Health and Human Services. Food and Drug Administration. May 2001. Available from: http://www.fda.gov/cder/guidance/4252fnl.htm.
[16] D. De Orsi, M. Pellegrini, E. Marchei, P. Nebuloni, B. Gallinella, G. Scaravelli, A. Martufi, L. Gagliardi, S. Pichini, High performance liquid chromatography-diode array and electrospray-mass spectrometry analysis of vardenafil, sildenafil, tadalafil, testosterone and local anesthetics in cosmetic creams sold on the Internet web sites, J. Pharm. Biomed. Anal. 50 (2009) 362–369.
[17] D. De Orsi, M. Pellegrini, S. Pichini, D. Mattioli, E. Marchei, L. Gagliardi, High-performance liquid chromatography-diode array and electrospray-mass spectrometry analysis of non-allowed substances in cosmetic products for preventing hair loss and other hormone-dependent skin diseases, J. Pharm. Biomed. Anal. 48 (2008) 641–648.