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Short communication

# Determination of methyldibromoglutaronitrile in cosmetic products by high-performance liquid chromatography with electrochemical detection Method validation

Suresh Chandra Rastogi [a,*], Claus Zachariae [b], Jeanne D. Johansen [b], Charlotte Devantier [c], Torkil Menné [b]

[a] *National Environmental Research Institute, Frederiksborgvej 399, P.O. Box 358, DK-4000 Roskilde, Denmark*
[b] *National Allergy Research Centre, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark*
[c] *Department of Dermatology, Odense University Hospital, Odense, Denmark*

**Abstract**

An increased frequency of contact allergy to methyldibromoglutaronitrile (MDBGN), a commonly used preservative in cosmetics and other consumer products, has been reported in recent years. A high-performance liquid chromatography (HPLC) method for the determination of MDBGN in cosmetic products has been validated in the present study. The identification is performed by reductive electrochemical detection of the bromine present in the molecule. The method is suitable for compliance testing of cosmetic products as well as for the research to support clinical and epidemiological studies.


*Keywords:* Cosmetics; Methyldibromoglutaronitrile

## 1. Introduction

Methyldibromoglutaronitrile (MDBGN) is used as a preservative in cosmetics and other consumer products. Accumulating evidence from animal and human clinical studies has demonstrated that MDBGN is a clinically significant allergen [1–4]. According to the EU Cosmetic Directive, maximum authorised concentration of this preservatives in cosmetic products is 0.1% [5]. A significant increase in the number of clinical cases of MDBGN contact allergy in recent years required exposure assessment of the population by this compound as well as the knowledge of threshold concentration of the compound that may provoke allergic reaction in the MDBGN sensitised persons. The patients referred mainly to cosmetic products as a cause of allergic reactions. A validated method for the determination of MDBGN in cosmetic products was, therefore, required. Compliance testing of cosmetic products with EU Cosmetic Directive also requires a validated method for the determination of MDBGN. MDBGN in cosmetics has earlier been determined by reversed-phase high-performance liquid chromatography (HPLC) with UV detection [6], normal-phase HPLC with UV detection [7] and reversed-phase HPLC with electrochemical detection [8]. In an earlier study, the HPLC method with electrochemical detection [8] was found to be suitable for the analysis of MDBGN content in cosmetic products [9]. In the present study, we have validated this method for the determination of MDBGN in creams and shampoos. The method has been applied for the analysis of cosmetic products provided by the patients allergic to MDGBN.

## 2. Experimental

### 2.1. Chemicals and other materials

Methyldibromoglutaronitrile was obtained through Lancaster Synthesis (Germany) and HPLC-grade acetone was purchased from Fluka (Buchs, Switzerland). Gradient-grade methanol (LiCHrosolv) and analytical-grade anhydrous sodium sulphate and sodium chloride were from Merck (Darmstadt, Germany). Water of Milli-Q (Millipore, Bedford, MA, USA) quality was used.

* Corresponding author. Tel.: +45-46-30-12-00; fax: +45-46-30-11-14.
*E-mail address:* scr@dmu.dk (S.C. Rastogi).

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Cosmetic products provided by the patients allergic to MDBGN were analysed in the present study.

### 2.2. Calibration standard solutions of MDBGN

A 0.2% (w/v) stock solution of MDBGN was prepared in 80% (w/v) aqueous methanol. The stock solution was appropriately diluted to 1, 2, 5, 10, 20 and 40 μg/ml in 80% methanol.

### 2.3. Sample preparation

Approximately 2–3 g sample was accurately weighed in a 50 ml dark bottle with screw cap. 20 ml of 80% aqueous methanol was added to the sample, mixed and heated at 60 ± 1 °C for 10 min to dissolve the matrix. The contents of the bottle were quantitatively transferred into a 25 ml volumetric flask and that was filled up to mark with methanol and mixed. The sample extracts were analysed within 24 h by HPLC.

### 2.4. High-performance liquid chromatography

An Agilent series 1100 LC comprised of a quaternary pump, a thermosttated column compartment, an autosampler, and an electrochemical detector 1049A, was used. The data collection and treatment were performed using Agilent Chemstation.

HPLC was performed using a Zorbax $C_8$ analytical column (250 mm × 4.6 mm, particle size 5 μm) and a $C_8$ precolumn. Column temperature was set at 40 °C. 10 μl sample extract/calibration standard solutions were analysed by isocratic elution with the use of following mobile phase: 400 ml acetone, 40 ml 0.5 M aqueous sodium sulphate and 10 ml 0.2 M sodium chloride were mixed in a 1000 ml volumetric flask and filled up to the mark with water. The flow of mobile phase was set at 1.0 ml/min. MDBGN was analysed by reductive electrochemical detection using a gold working electrode (Agilent part No. 01049-28802) and an Ag/AgCl as reference electrode. The detector was set at amperometric mode, reduction polarity, −0.500 V potential, 1.0 s response time and 0.5 ua as instrumental full-scale.

## 3. Results and discussion

The principle of the method is the measurement of reduction of Br under the experimental conditions. The robustness of the method was checked by varying the column temperature (±2 °C), mobile phase flow rate (1.0 ± 0.1 ml/min), injection volume (20 μl) and by using another $C_8$ analytical column (Merck). No significant changes were observed in the analytical results by changing these parameters. The method of analysis was validated for the following parameters: repeatability, calibration and recovery of MDBGN from the spiked cosmetic products. The calibration curve of MDBGN was linear $r^2$ (0.998) in the investigated concentration range (1–40 μg/ml). The relative standard deviation of the analysis by six consecutive injections of 5 and 20 μg/ml MDGBN was <2%. The method for sample preparation was found to be suitable for all types of cosmetic products. Recoveries from a cream spiked to 0.005, 0.015, 0.045%

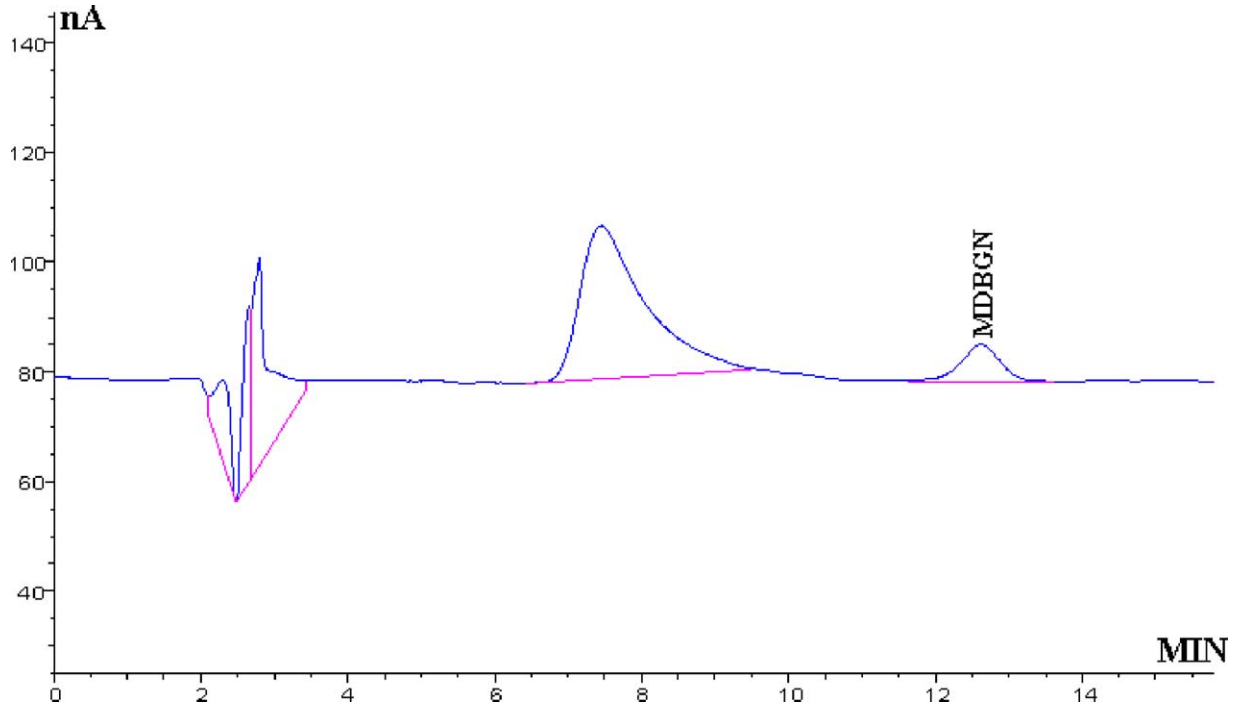


Fig. 1. HPLC chromatogram of a body cream sample containing 0.0134% MDBGN.

MDBGN were 96.8, 99.8 and 100.3%, respectively. The recoveries of a shampoo spiked to 0.005, 0.015 and 0.045% MDBGN were 95.2, 96, and 98%, respectively. The lowest concentration of MDBGN solution that showed a peak without any baseline noise, when 10 μl of this solution was analysed by HPLC, was 0.00005%. Thus, 0.5 ppm was considered as the limit of detection/quantification of MDBGN in the present method.

A typical chromatogram of the analysis of MDBGN in a cosmetic product (a body cream) is shown in Fig. 1. Besides the peak of MDBGN a ghost peak was observed in all chromatograms. The ghost peak was assigned in the previous publication to reduction of oxygen in HPLC system in the previous study [9].

The method was used for the analysis of MDBGN content in 44 cosmetic products provided by the patients allergic to this chemical: 22 creams (face cream, body lotion, hand cream, baby cream, sunscreen lotion, cleansing cream and cream deo), 17 shampoos/liquid soaps, one face water, a hair spray, a mascara, a make-up product and a wipe. Twenty-four of these products were found to contain 0.0011–0.1482% MDBGN. All of these products gave allergic reactions when used by the respective patients. But no allergic reactions were observed when the patients used the cosmetic products without MDBGN. The content of MDBGN was not labelled on three of the products.

## 4. Conclusions

A HPLC method with electrochemical detection has been validated for the determination of MDBGN in cosmetic products. The detection limit of the method is 0.5 ppm. The method is suitable for compliance testing as well as for the research to support clinical and epidemiological studies.

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