

Content of oak moss allergens atranol and chloroatranol in perfumes and similar products

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Chloroatranol and atranol have been identified as the main allergens in the fragrance material of botanical origin, oak moss absolute. A previous study has shown that nearly all individuals sensitized to chloroatranol will elicit to 5 µg/ml in a repeated open application test and that 50% will get a reaction to 0.15 µg/ml under patch test conditions. Thus, chloroatranol is known as a potent allergen. The aim of the current investigation was to quantify exposure to chloroatranol and the chemically related substance atranol in some popular perfumes, eaux de parfum and eaux de toilette available on the European market. In total, 31 products were analysed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS-MS) for their contents of atranol and chloroatranol. The 2 substances were found in 87% ($n=27$) of the products. The median concentration of atranol in perfumes was 0.502 µg/ml and 0.012 µg/ml in eaux de toilette, and 0.235 µg/ml and 0.006 µg/ml for chloroatranol, respectively, in perfumes and eaux de toilette. Chloroatranol was found at a maximum concentration of 53 µg/ml and atranol at one of 190 µg/ml. The wide exposure to oak moss allergens, together with significant amounts of these potent allergens in at least half of perfumes and some eaux de toilettes explains the high frequencies of oak moss absolute allergy. It is suggested that regulations should be introduced aimed directly at these substances, and not just at oak moss absolute.

Key words: atranol; chloroatranol; contact allergy; fragrances; LC-ESI-MS-MS; oak moss absolute; quantitative exposure analysis. © Blackwell Munksgaard, 2004.

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Oak moss absolute is a natural fragrance derived from the lichen *Evernia prunastri* and widely used in fine perfumes, aftershave lotions and other cosmetic products. Oak moss absolute is known for its strong allergenic potential (1). It is a frequent cause of allergic reactions, accounting for about 1/3 of reaction to the ingredients of the diagnostic marker of perfume allergy, the fragrance mix, and showing an increasing frequency over time (2). Oak moss absolute has a very complex chemical composition and several constituents have been suggested as responsible for its strong allergenic properties, including atranorin, chloroatranorin, evernic acid and usnic acid (3, 4).

Oak moss absolute has been a target for research in the EU 5th Framework Programme funded project aiming at preventing fragrance

allergy. By bioassay-guided chemical fractionation (i.e. patch-testing of oak moss-allergic individuals with fractions of oak moss absolute), combined with repeat testing, chemical analysis and chemical structure-activity analysis, it was shown that atranol and chloroatranol – degradation products of atranorin and chloroatranorin, respectively, – were the most potent allergens of oak moss absolute (5). The pathway of degradation of atranorin and chloroatranorin, respectively, to atranol and chloroatranol has been described in a recent publication (5).

This has for the first time made it possible to quantify the exposure to oak moss allergens in consumer products. The aim of the present investigation was to determine the content of atranol and chloroatranol in perfumes and similar products, in order to elucidate the high frequency of

oak moss allergy among patients with fragrance allergy (1). The data obtained may also be used for exposure assessment.

Materials and Methods

Products

The 31 products used in the present investigation were popular perfumes: 12 perfumes, 6 eaux de parfum, 11 eaux de toilette and 2 aftershave lotions, all from major perfume producers and available in Europe. The aim of this investigation was to analyse perfumes, because they contain the maximum amount of a fragrance formula. When perfume of a specific brand was not available, eau de parfum was selected for the analysis. Eau de toilette was considered for the analysis of only those brands for which neither perfume nor eaux de parfum was available. 14 products were purchased in the period 2000–02 and remaining 17 products were bought in 2003.

Analysis

The content of atranol and chloroatranol in the products was determined by a recently developed liquid chromatography-tandem mass spectrometry (LC-MS-MS) with electrospray ionization (ESI) method for the analysis of these compounds in perfumes and similar products (6). Atranol and chloroatranol were synthesized as described before (5).

The analysis was performed using a Perkin Elmer LC system, Series 200 pump and an auto-sampler (Perkin Elmer, Norwalk, CT, USA) coupled to a bench-top triple quadrupole mass spectrometer API 2000 (PE Sciex, Concord, Ontario, Canada). ESI interface operated in negative ionization mode was employed for the introduction of analytes into the MS. The analytes were separated on a Zorbax 5 μ m, SB-C₁₈ column, 150 $\times$ 2.1 mm (Agilent, Palo Alto, CA, USA) with a C₁₈ guard column (Phenomenex, Torrance, CA, USA). The analytical column was kept at a constant temperature of 30°C. The sample injection volume was 10 μ l. A binary mobile phase gradient comprised of water (A) and acetonitrile was used for analyte separation at a flow rate of 0.2 ml/min. The gradient was programmed as follows: linear from 90% A to 5% A in 30 min, maintaining 5% A for 10 min, back to 90% A in 3 min. The system was allowed to equilibrate for 10 min before each injection. Positive identification of the analytes in samples was based on specific parent ion/product ions combination (m/z 151/123 and 151/81 for atranol, and m/z 185/157 and 185/121 for chloroatranol)

and the chromatographic retention time. All samples were analysed in duplicate. Appropriately diluted samples were analysed when the MS signal intensity of the target analytes was beyond the linear range of the respective compounds.

Results

The validity of the LC-ESI-MS-MS used for the determination of atranol and chloroatranol in the present study was checked in a recent study (6). The calibration curves for both compounds were linear ($r^2 > 0.99$) in the range 10–500 ng/ml. The detection limits of atranol and chloroatranol in the products obtained with this method are 5 ng/ml and 2.4 ng/ml, respectively; and the repeatabilities of determination of both atranol and chloroatranol were within 8%. The recoveries of atranol and chloroatranol from the products were $49 \pm 5\%$ and $96 \pm 4\%$, respectively. A rather low recovery of atranol was assigned to the well-known phenomenon of ion suppression, when electrospray technique is used (6). Ion suppression, a common ESI phenomenon, is compound-dependent and matrix-dependent (7). The best way to circumvent the problem is by the use of deuterium-labelled compounds, which were not available in our case. Ion suppression for the atranol signal may be caused by other components present in the perfumes. These compounds may include other polar ionizable molecules present in the perfume products, which may contain over 300 different molecules.

Contents of atranol and chloroatranol in the investigated products are given in Table 1. The results have not been corrected for the recovery. Chloroatranol (0.004 μ g/ml–53 μ g/ml) was found in 87% ($n=27$) of products, and atranol (0.012 μ g/ml–190 μ g/ml) was present in 77% ($n=24$) of products. All products containing atranol, as expected, also contained chloroatranol. However, the absence of atranol in 1 perfume and in 2 eau-de-parfum products, which contained chloroatranol, may be associated with the relatively higher detection limit and lower recovery of atranol, as described before. Thus, all in all, 87% ($n=27$) of the investigated products should be considered to contain the oak moss allergens atranol and chloroatranol. Atranol was generally found at higher concentrations than chloroatranol in the products in the magnitude of a factor 2, which is in agreement with the reported atranol:chloroatranol concentration (2.1:0.9) in oak moss absolute (5). The 4 products not containing atranol and chloroatranol were 1 eau de parfum and 3 eaux de toilette. As eaux de parfum and eaux de toilette contain relatively lower amount

Table 1. Content of atranol and chloroatranol in the investigated products

Product category	Number of products	Content (µg/ml)*	
		Atranol	Chloroatranol
Perfume	12	Range: n.d.†–190.0; median: 0.502; 90% percentile: 74.00 80% percentile: 1.650; 10% percentile: 0.013	Range: 0.004–53.0; median: 0.235; 90% percentile: 10.00 80% percentile: 1.450; 10% percentile: 0.004
Eau de parfum	6	n.d., n.d., n.d., 0.013, 0.016, 0.791	n.d., 0.004, 0.004, 0.013, 0.106, 0.175
Eau de toilette	11	Range: n.d.‡–32.11; median: 0.012	Range: n.d.‡–8.79; median 0.006
Aftershave lotion	2	0.929, 1.878	0.491, 1.016

*Corresponds to p.p.m. when the density of the product is 1 g/ml.

†Atranol was not detected in 1 perfume.

‡Atranol and chloroatranol were not detected in 3 products.

of fragrance compounds compared to those in corresponding perfume products, it cannot be ruled out that the fragrance formulas of these products may contain oak moss absolute.

The median concentrations of both atranol and chloroatranol were higher in perfumes as compared to eaux de toilette. The median concentration of atranol in perfumes was 0.502 µg/ml and 0.012 µg/ml in eaux de toilette; the same values for chloroatranol were 0.235 µg/ml and 0.006 µg/ml, respectively (Table 1).

Discussion

Chloroatranol and atranol were identified as the main allergens in oak moss absolute by a bioguided chemical fractionation procedure (5). Following this, chloroatranol was further subjected to investigation of its elicitation potential in sensitized eczema patients (8). It was shown that 92% of oak moss-sensitized individuals reacted with eczema to the repeated open application of 5 µg/ml chloroatranol in ethanol (8). The same study also showed that the dose eliciting a reaction in 50% of the test subjects under patch test conditions was 0.15 µg/ml (8). These results are put into perspective by the current investigation showing that the range of chloroatranol in the investigated cosmetic products was from 0.004 µg/ml to 53 µg/ml, median 0.2 µg/ml and the 90% percentile 10 µg/ml. There are indications that atranol is a weaker allergen than chloroatranol (5). However, it is also present in higher quantities in the products and that will contribute to the allergenicity of oak moss absolute. The results of the present study showed that 87% of all such products contained oak moss allergens. The wide exposure to oak moss allergens, together with significant amounts of the potent allergens in at least half of the perfumes and some eaux de parfumes, as well as in eaux de toilette may explain the high frequencies of oak moss

absolute allergy and the clinical problems of these patients (2).

According to the EU Cosmetic Directive, the maximum authorized concentration of oak moss absolute in cosmetic products is 0.1% (9). An oak moss absolute sample was found to contain approximately 2.1% atranol and 0.9% chloroatranol (5). Considering this as representative of atranol and chloroatranol content in different oak moss absolute samples, cosmetic products may contain up to 0.0021% (21 p.p.m.) atranol and 0.0009% (9 p.p.m.) chloroatranol. However, the content of atranol and chloroatranol in cosmetic products will depend upon the oak moss absolute used, the degradation of atranorin and chloroatranorin in the product matrix, and finally, upon the matrix effect in the determination of these molecules. Thus, regulation of chloroatranol and atranol in oak moss absolute-containing products appears to be more important than that of oak moss absolute itself. It should be noted that recovery of chloroatranol from perfume/eau de toilette was found to be nearly 100%, while that for atranol was close to 55% (6).

In conclusion, significant and widespread exposure to the potent oak moss allergens, chloroatranol and atranol, was found in perfumes and eaux de toilette. It is suggested that these 2 substances should be direct subjects of regulatory initiatives, the better to prevent contact allergy to fragrances.

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