Validation of analytical method for the quantitative determination of preservative acids allowed in Eco label cosmetic products

**Keywords:** preservative acids, natural products, quantitative LC-UV method

**Introduction**

Preservative is a natural or synthetic substance incorporated into cosmetics in order to avoid chemistry (oxidation) or microbiologically alterations. It prevents microorganisms’ proliferation such as fungi that may create skin allergies in contact with the skin. These preservatives are essential for any cosmetic product, organic or not.

The origin of preservatives may be synthetic or natural. To be used in a cosmetic product in Europe, they must be listed in Annex V of EC Regulation N°1223/2009. There are fifty original synthetic antimicrobial preservatives authorized in Europe.

Preservation of a natural cosmetic product should logical be ensured by a natural way. Because of the difficulty of defined properly the efficiency of natural preservatives (absence of minimum inhibitory concentration (MIC), absence of toxicological data) some synthetic preservatives are authorized by labels for their occurrence in nature, their low toxicity and their limited irritant or allergenic reactions. The main synthetic preservatives allowed in natural products are: benzyl alcohol, benzoic acid and its salts, dehydroacetic acid and its salts, salicylic acid and its salts, sorbic acid and its salts (Table 1).

**Table 1 Synthetic preservatives authorized by European Eco labels.**

| Country | Label            | Benzoic acid | Sorbic acid | Salicylic acid | Dehydroacetic acid | Benzy alcohol | Propionic acid | Formic acid |
|---------|-----------------|--------------|-------------|---------------|-------------------|---------------|----------------|-------------|
| Germany | BDIH            | X            | X           | X             | X                 | X             |                |             |
| France  | Ecocert/Cosmebio | X            | X           | X             | X                 | X             |                |             |
| France  | Nature & Progrès | X            | X           | X             | X                 | X             |                |             |
| UK      | Soil association | X            | X           | X             | X                 | X             |                |             |
| France  | Natrue          | X            | X           | X             | X                 | X             |                |             |
| France  | Cosmos          | X            | X           | X             | X                 | X             |                |             |

**Table 2 Restriction limits in finished products**

| Substance                     | Product, body part          | Maximum authorized concentration | Limitations and requirements                                                                 |
|-------------------------------|-----------------------------|----------------------------------|---------------------------------------------------------------------------------------------|
| Benzoic acid and its sodium salt | Rinse-off products, except oral care products | 2.5% (acid)                      | Not to be used in preparations for children under three years old, except for shampoo        |
|                               | Oral care products          | 1.7% (acid)                      | Not to be used for children under three years old (1)                                        |
|                               | Leave-on products           | 0.5% (acid)                      | Prohibited in aerosol dispensers (sprays)                                                    |
| Salicylic acid and its salts  |                             | 0.5% (acid)                      |                                                                                             |
| Sorbic acid and its salts     |                             | 0.6% (acid)                      |                                                                                             |
| Dehydroacetic acid and its salts |                             | 0.6% (acid)                      |                                                                                             |
| Benzyl alcohol                |                             | 1%                               |                                                                                             |
Material and method

Standards, reagents and solvents

All solvents were analytical grade or HPLC grade. The selected compounds used in the present work are: Benzoic acid (BA), Sorbic acid (SA), Salicylic acid (SAA), Dehydroacetic acid (DHA), Benzyl alcohol (BA). They were acquired from Sigma-Aldrich (Saint-Quentin Fallavier, France). Methanol was supplied by Sigma-Aldrich (Saint-Quentin Fallavier, France). Citric acid and Acetate ammonium were supplied by Fisher Chemical (Illkirch, France) and VWR (Fontenay-sous-Bois, France). Purified water from Milli-Q was used (Millipore).

LC/UV conditions

The liquid chromatography was performed by an Agilent 1220 Infinity LC system including a degasser and a variable wavelength UV/Vis detector, connected to Open Lab Station. The LC columns were a ZORBAC Eclipse Plus C18 (4.6x150nm, 3.5µm). The optimal chromatographic separation of the acid preservatives was obtained under the following conditions: the mobile phase in isocratic composed of a buffer mixture methanol-acetate (pH 4.4) (35:65v/v) at a flow rate of 1 mL/min, 5µL of the sample was injected, and the wavelength was set at 230nm.

Calibration standard solutions

Preparation of stock standard solutions: For the preparation of preservatives stock standard solutions, separate stock solutions of 100µg/mL were prepared by dissolving an appropriate amount of each compound in methanol. Mixed stock standard solutions of 10µg/mL (BA, SA, SAA, and DHA) were prepared by mixing 1 mL of each stock solution and fill up to 10mL.

Calibration curve of standard solutions: The calibration curves are obtained by diluting mixed stock standard solutions to six points of concentrations between 10 and 1000µg/mL.

Assay validation

Validation of the chromatographic method is based on ISO 1278:2011 concerning the validation of analytical results using chromatographic techniques in cosmetic. Validation criteria’s are: range linearity, accuracy, precision, repeatability and quantification/detection limits.3,4

Extraction of preservatives from cosmetic samples

About 1g of cosmetic product was weighed into a plastic falcon tube, 10mL of methanol was added, after vortex the tube was immersed for 30minutes in an ultrasonic thermo stated bath at 60°C to melt any lipid phase and to facilitate the extraction of active compounds into the methanol phase. Then, the tube was centrifuged for 20 minutes at 3000rpm. The supernatant was filtered with 4µm filter and 1mL volume of the supernatant was introduced in sample vial. Some matrixes have to be diluted to fit with the validated linear regression curve.

Results and discussion

Chromatography

As shown in Figure 1, good chromatographic separation was achieved for all the analytes. SAA, BA, SA, and DHA were separated in 10minutes.
Table 3 Precision, recovery and repeatability results

| Analytes | Expected concentration (µg/mL) | Observed concentration (µg/mL) | Precision (%) | Recovery (%) | Repeatability (%) |
|----------|--------------------------------|--------------------------------|---------------|--------------|-------------------|
| BA       | 100                            | 94.26                          | 1.63          | 5.74         | 2.15              |
|          | 500                            | 510.47                         | 0.26          | 2.10         | 0.88              |
|          | 1000                           | 994.35                         | 0.42          | 0.56         | 0.51              |
|          | 100                            | 85.91                          | 0.13          | 19.52        | 2.97              |
| SAA      | 500                            | 518.64                         | 1.14          | 1.46         | 0.82              |
|          | 1000                           | 990.12                         | 0.27          | 0.58         | 0.79              |
|          | 100                            | 80.48                          | 2.86          | 19.52        | 2.34              |
| SA       | 500                            | 907.31                         | 1.16          | 1.46         | 0.51              |
|          | 1000                           | 994.20                         | 0.75          | 0.58         | 0.54              |
|          | 100                            | 104.27                         | 4.66          | 4.27         | 4.33              |
| DHA      | 500                            | 504.36                         | 2.50          | 0.87         | 1.62              |
|          | 1000                           | 999.01                         | 0.27          | 0.10         | 0.35              |

Application

This validated method applied by our laboratory in order to monitor the preservative concentrations in eco label cosmetic products in compliance with the regulation 1223/2009. Quantitative determination by LC/UV of the main synthetic preservatives in a commercial cream marketed in Europe is presented in Figure 3. Result has shown that dehydroacetic acid was found at a concentration of 647ppm. No other preservatives have been detected with this method. DHA concentration is in compliance with the regulation 1223/2009 (<0.6%).

Figure 3 Quantitative determination of DHA concentration in emulsion.

Conclusion

To ensure the preservation of cosmetics and consumer safety in compliance with the authorized concentrations as set up by the Regulation 1223/2009, EXPERTOX laboratory has developed and validated a quantitative determination and extraction methods of 4 main synthetic preservatives authorized by the organic labels: benzoic acid, sorbic acid, salicylic acid and dehydroacetic acid.

LOQ is below the regulatory limit concentrations of the 4 preservatives acids, between 0.5% and 2.5%, as part of the quality control in compliance with 1223/2009 Regulation. The analytical results had permitted the validation of all criteria’s which ensure the performance, reliability and quality of the final results. Chromatographic and extraction methods were validated.

Even if the labels focus on the origin, on the green process and on the good toxicological profiles of raw materials, certified organic products do not imply a perfect tolerance as the consumer could think. Preservatives can induce allergic reactions despite their limited toxicological profiles. The preservation of biological or natural cosmetic products requires the development of new approaches to tend to low-risk microbiological products. The researches on packaging in order to limit the risk of contamination (one-use, airless ...) is a delicate way since it must remain compatible with the ecological requirements (limitation of packaging). The self-protection concept is increasing, it needs the presence of substances which get bactericidal-fungicidal properties without preservatives such as alcohols (ethyl, benzyl), aliphatic glycols (propylene glycol, glycerine), soaps, some fatty acids (monolaurin lipoaminoacids), antioxidants that work synergistically with traditional preservatives (BHT, EDTA...) and finally, the substances from plants or rich of substances with microbiological properties.

Acknowledgements

None.

Conflicts of interest

Author declares that there is none of the conflicts.

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