DETERMINATION OF HYDROGEN PEROXIDE

in cosmetic products

"The true method of knowledge is experiment."

William Blake

European Network of Official Cosmetics Control Laboratories (OCCLs)

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High performance liquid chromatography method for the determination of hydrogen peroxide present in or released by tooth whitening or bleaching products

European Directorate for the Quality of Medicines & HealthCare

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High performance liquid chromatography method for the determination of hydrogen peroxide present in or released by tooth whitening or bleaching products

Users should verify the performance of the method in their laboratory for each different matrix.

1. Scope

This procedure describes a method for the quantification of hydrogen peroxide present in or released by tooth whitening products for concentrations ranging from 0.06 % to 10 % (*m/m*) in the finished product.

2. Principle

An HPLC/UV method based on oxidation of triphenylphosphine (TPP) into triphenylphosphine oxide (TPPO) is used for the determination of hydrogen peroxide. In most tooth whitening products, the active ingredient is hydrogen peroxide or a substance able to generate hydrogen peroxide (carbamide peroxide, sodium perborate,[1] calcium peroxide …).

3. Limitation of the method

False positive results due to presence of chlorite or metabisulphite (e.g. as NaOCl and $Na_2O_5S_2$) may be observed. These compounds react with TPP to form TPPO and therefore falsely contribute to the final result.

4. Terms, definitions and abbreviations

- TPP: triphenylphosphine
- TPPO: triphenylphosphine oxide
- H_2O_2: hydrogen peroxide

5. Health, safety and environment

Appropriate safety measures are to be employed when working with chemicals. Generally, these will be defined in each laboratory's safety plan. No extraordinary precautions are necessary here.

1 Attention should be paid to the use of sodium perborate which is classified H360 (reprotoxic) and is regulated by Commission Regulation (EC) No. 790/2009 of 10 August 2009 amending Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures.

6. Apparatus, reagents and solutions

6.1. Apparatus

HPLC system equipped with a Diode Array Detector (DAD)

Column	Nucleosil® C18 Macherey-Nägel® (150 mm × 4.6 mm, 5 µm, 100 Å)
Column temperature	25 °C
Injection volume	10 µL
Detection	225 nm
Flow rate	1.0 mL/min
Runtime	20 min

A gradient elution with water/acetonitrile (see Table 1) is used to separate TPPO from residual TPP.

Table 1 – *Solvent gradient used for the analysis of hydrogen peroxide by HPLC/UV*

Time	H_2O (%) V/V	CH_3CN (%) V/V
0 min	50	50
5.5 min	50	50
6.5 min	0	100
9.0 min	0	100
10.0 min	50	50
20.0 min	50	50

6.2. Accessories

Use standard laboratory glassware, i.e. flasks, pipettes.

6.3. Reagents

Reagents of at least analytical grade are used. Water is to be double distilled or of equal quality.

- Acetonitrile, HPLC grade, CAS [75-05-8]
- Hydrogen peroxide (H_2O_2), 30 % solution in water, CAS [7722-84-1]
- Triphenylphosphine (TPP) 99 %, CAS [603-35-0]
- Urea peroxide 98 %, CAS [124-43-6].

6.4. Solutions

6.4.1. *H_2O_2 standard preparation*

Either a 30 % solution of hydrogen peroxide or urea peroxide may be used for the preparation of H_2O_2 standard solutions.

Prepare a 1 000 µg/mL solution of H_2O_2 by either diluting a 30 % solution of hydrogen peroxide in water or placing 140 mg of urea peroxide (equivalent to 36 % H_2O_2) in a 50 mL volumetric flask with water.

From the previous standard solution (1 000 µg/mL), prepare at least 5, evenly distributed, standard calibration solutions, ranging from 30 to 200 µg/mL of H_2O_2 by dilution in water/acetonitrile (35:65 V/V).

6.4.2. *TPP standard preparation*

Prepare the TPP standard by dissolving 65.5 mg of TPP in acetonitrile in a 25 mL amber volumetric flask. Dilute the solution to volume with the same solvent.

6.4.3. *Blank*

The TPP standard may contain small amounts of TPPO and in order to estimate the contribution of this TPPO to the final result, prepare a blank solution using 1 mL of water instead of the sample solution and applying the entire procedure described under 7.2. If the concentration of TPPO in the blank solution is higher than the limit of quantification (LoQ), perform a blank subtraction.

7. Procedure

7.1. Sample preparations

7.1.1. *Gels*

Weigh 0.1 to 2.0 g of gel[2] in a 50 mL volumetric flask, dissolve in water[2] and make up to the mark with the same solvent.

Determine the content of hydrogen peroxide in the sample solution after H_2O_2 has reacted with TPP to form TPPO (see 7.2).

7.1.2. *Impregnated strips*

The extraction and assay of H_2O_2 is performed in 2 steps:

- The mass of gel present on the strip must first be determined:

1. Introduce a weighed strip into a beaker,
2. Add 200 mL of water,
3. Stir for the period of time indicated as the time of treatment for the product (a minimum of 30 min),
4. Remove the strip without the gel and dry it to constant mass,
5. Determine the exact mass of gel that was present on the strip.

- Assay:

1. Weigh enough strips to obtain a total mass of gel between 0.1 and 2.0 g,[2]
2. Add between 20 mL and 400 mL of water,[2]
3. Stir for the period of time indicated as the time of treatment for the product (a minimum of 30 min),
4. Remove the strip without the gel to determine the exact mass of gel introduced.

Determine the content of hydrogen peroxide in the sample solution after H_2O_2 has reacted with TPP to form TPPO (see 7.2).

7.2. Reaction of H_2O_2 with TPP to form TPPO: samples, standards and blank

Hydrogen peroxide (H_2O_2) oxidises triphenylphosphine (TPP) into triphenylphosphine oxide (TPPO) as shown in the reaction below:

$$\mathbf{TPP + H_2O_2 \rightarrow TPPO + H_2O}$$

- Introduce 5 mL of acetonitrile (6.3), 3 mL of water (6.3) and 1 mL of the TPP solution (6.4.2) into a 10 mL centrifuge tube protected from light,
- Vortex the mixture,
- Add 1 mL of the solution to be examined [either hydrogen peroxide standard solutions (6.4.1), blank (6.4.3) or sample solutions (7.1)],
- Shake for a few seconds using a vortex mixer,
- Wait for at least 2 h before injecting into the HPLC system.

TPP must be present in excess during the reaction step. A peak due to residual TPP (un-reacted) must be present in all HPLC chromatograms.

7.3. Analysis

Each standard calibration solution (6.4.1), sample solution (7.1) or blank (6.4.3) is injected after H_2O_2 has reacted with TPP to form TPPO (see 7.2) into the HPLC system using instrument parameters (6.1).

Note: In order to ensure the absence of interfering compounds in the sample matrix, prepare an additional sample solution without adding the TPP solution: dilute 1 mL of the sample solution (7.1) in acetonitrile/water (1:1) in a 10 mL volumetric flask and inject into the HPLC system.

7.4. Peak identification

The approximate retention times of the analytes are:

2 Sample mass and water volume are adapted to obtain a sample solution with an H_2O_2 concentration within the linearity range (30-200 µg/mL).

Compound	RT (min)	Comment
TPPO	4.04	–
TPP	10.03	A peak due to residual TPP (unreacted) must be present in the HPLC chromatograms

Retention times are given for information and they need to be confirmed in every individual HPLC system. An example of a chromatogram obtained with the 120 µg/mL H_2O_2 standard solution is shown in Annex 1.

8. Calculation

Perform the quantitative determination of H_2O_2 by linear regression based on TPPO peak areas in the chromatograms obtained with the external standard solutions. The calibration curve is linear and the correlation coefficient is equal to or above 0.995. The calibration curve should not be forced through the origin.

The mass fraction of the compound in g for 100 g in the sample is calculated using the following expression:

$$R[\%] = \frac{C \times V \times 100 \times F}{1000 \times SW}$$

Where:

R is the concentration in %

C is the measured concentration of hydrogen peroxide in the sample solution in g/L, interpolated from the calibration curve

V is the volume of dilution for the sample solution in mL

SW is the mass of the sample in g

F is the possible factor for additional dilution.

The amount is expressed in % or in g/100 g and is rounded to the nearest tenth of a percent.

9. Reporting

The test report should contain the following data:

1. Information necessary for the identification of the sample (type, origin and designation of the sample),
2. The date of receipt and date of analysis,
3. The test results (including the measurement uncertainty) and the units in which they have been expressed,
4. Justification of any deviation from the method,
5. Operations not specified in the method or regarded as optional, which might have affected the results.

10. Validation

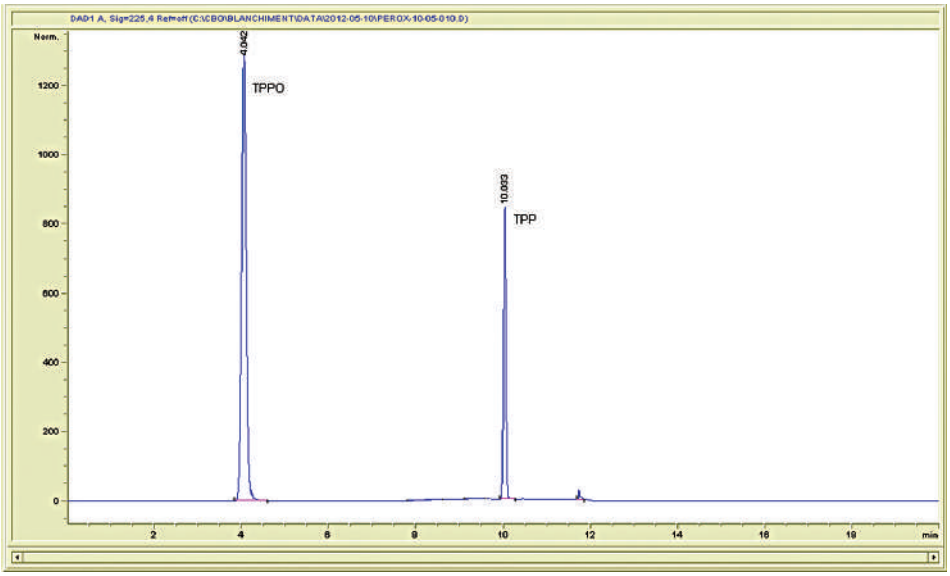
The method was subjected to peer review. Interlaboratory validation data are reported in Annex 2.

11. References

P. Gimeno, C. Bousquet, N. Lassu, *et al.*, High-performance liquid chromatography method for the determination of hydrogen peroxide present or released in teeth bleaching kits and hair cosmetic products, *Journal of Pharmaceutical and Biomedical Analysis* 107 (2015) 386–393.

Annex 1. Chromatogram obtained for a 120 µg/mL H_2O_2 standard solution

For information, an example of the chromatogram corresponding to a 120 µg/mL H_2O_2 standard solution is shown on the right.



Annex 2. Interlaboratory validation data (peer review)

The following table summarises validation data obtained for two different tooth whitening gels by four independent laboratories during a peer-review process. For each product, four sample preparations were performed per day, during two non-consecutive days.

Validation criteria	Validation data	
Linearity range	30–200 µg/mL	$R^2 > 0.9990$ Biases < 5 %
Limit of detection (LoD)		0.02 %
Limit of quantification (LoQ)		0.05 %
Intra-day repeatability** (RSDr%)		2.4 %*
Intermediate precision*** (RSDR%)		3.1 %*
Interlaboratory reproducibility (RSD%)		4.0 %*

* Mean value obtained for the assay of two different tooth whitening gels with hydrogen peroxide contents of 3.4 % and 5.9 %.

** RSDr% = mean intra-day relative standard deviation (RSD).

*** RSDR% = relative standard deviation (RSD) on all individual values.

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