DETERMINATION OF 2-PHENOXYETHANOL IN HAND CREAM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING THE INTERNAL STANDARD METHOD

Alcohols
The properties of microbial destruction are predominantly observed for monohydric alcohols. The mechanism of the biological action of alcohols is related to the dehydration process of proteins, which leads to the change of the second-order structure of proteins and hence impairing their function. It should be emphasized that alcohols have the ability to destroy vegetative forms of microorganisms as well as the significant types of viruses, but they do not act on their spore form. In case of injury, the precipitation of proteins that form the protective layer against microorganisms is observed within the wound area, which further increases the effectiveness of the antimicrobial action of the alcohols. For these reasons, many different types of monohydric alcohols are commonly used in cosmetics. The most commonly found compounds in cosmetic products include ethanol and 2-phenoxyethanol. 2-Phenoxyethanol is mainly known as a preservative commonly used in cosmetic products, most commonly used in combination with other anti-microbial compounds (eye make-up products, fragrances, roses, sleepers and makeup bases, lipsticks, soaps, baby products, tanning products, as well as facial, body and feet powders). It shows antiseptic and antimicrobial effect, which prolongs shelf life of cosmetics. In addition, it acts as an active ingredient in deodorants and repellents in insect repellents. Due to the possibility of an allergic reaction, the amount of 2-phenoxyethanol used in cosmetics is strictly controlled and the permissible concentration of the compound in question in such products is 1% [1,2]. The purpose of this exercise is to determine 2-phenoxyethanol in the selected hand care cream.

References:

INTERNAL STANDARD METHOD
This is a method of adding to a sample a known amount of a component, so called an internal standard (IST), which is however different from the investigated compound and cannot be present in the analysed samples before it is added. In order to obtain a calibration curve, a constant and known amount of internal standard is added to a number of standard solutions with different concentrations of the substance to be measured. Based on the obtained chromatograms of the standard solutions, the concentration of the analyte is calculated as a function of the ratio of the analyte peak and the internal standard peak areas.

Figure 1 shows the calibration curve for the substance determined by the internal standard method. In order to determine the content of the substance by internal standard method in the investigated sample (known mass or volume), a known amount of internal standard is added to it. Then the calculation of the ratio of the analyte peak and the internal standard peak areas is done on the basis of the recorded chromatogram.

Fig. 1. The calibration curve for the substance determined by the internal standard method.
The content of the substance is determined by graphical interpolation or is calculated from the equation of calibration curve or using (1) and (2) relationships where the calibration curve is a linear relation of \( f(x) = ax \) type:

\[
C = \frac{A_i / A_{IST}}{R_f} \quad (1)
\]

\[
R_f = \frac{A_i / A_{IST}}{C_i} \quad (2)
\]

Where: \( C \) – denote the concentration of the determined substance, 
\( A_i \) – the peak area of the analyte, 
\( A_{IST} \) – the peak area of the internal standard, 
\( R_f \) – response coefficient, 
\( A_{i, A_{IST}} \) – the peak areas for the calibration solution for analyte and internal standard respectively.

Figure 2 shows the chromatogram of the reference mixture of the investigated compounds (A,B,C and D) and internal standard (IST).

![Chromatogram](image)

Fig.2. A chromatogram of mixture of investigated substances A, B, C, D and internal standard (IST)

The internal standard should meet the following requirements:
- must be well separated from other compounds in the sample
- retention time should be similar to the retention time of the analyte (s)
- is not present in investigated samples
- its physico-chemical properties are similar to analyte, this is particularly important at the stage of sample preparation (purification, enrichment or derivatisation)
- should be as clean as possible
- should be chemically stable
- the detector response for the internal standard should be similar to the response of the determined substances

This method is particularly applicable to analytical methods requiring a complex, multi-stage sample preparation process (isolation, enrichment, derivatisation, etc.), which can cause losses in amount of analytes. Adding an internal standard to the sample before the sample preparation for chromatographic analysis makes it possible to correct these losses.

The use of the internal standard method also allows the results to be independent of fluctuations in the quantity of the dose of the injected sample. The limitation in using this method can be the case of the very rich matrix in which the analytes are determined. Sometimes it may be difficult to choose the right substance as an internal standard. In practice, this method is also more laborious than the calibration curve method (external standard).
Equipment:

Hewlett Packard Liquid Chromatograph consisting of the following modules:

1. Solvent shelf – the area to store the mobile phase solvents, furthermore it allows degassing of individual component of the mobile phase with helium.

2. Chromatography Controller (HP 1050 Controller) - allows you to set the desired phase composition and flow velocity, as well as program the tables responsible for analysis (e.g. gradient analysis).

3. HP 1050 Series Pump - is responsible for the automatic mixing of the mobile phase components in predetermined proportions and providing the elution mixture to further modules of the HPLC system. It is responsible for monitoring the system pressure. The maximum operating pressure of the pump is 4300 psi / 300 bars.

4. UV-Vis Single Detector (HP 1050 series λ Absorbance Detector) - allows you to work at a selected wavelength of 190-600 nm. It is equipped with a deuterium lamp and measuring cell with a volume of 14 μL and an optical path length of 8 mm.

5. Injector valve (HP 1050 Series) - is used to inject samples onto a chromatography column. It is equipped with an injection loop of 20 μL volume.

6. Computer with installed communication card and Clarity Software - used to collect and process the recorded data.

7. Chromatographic conditions:

<table>
<thead>
<tr>
<th></th>
<th>Column</th>
<th>HP ODS C18 (250 mm x 4.0 mm; 5 μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile (AcN) content</td>
<td>30 % (v/v)</td>
<td></td>
</tr>
<tr>
<td>Water (H₂O) content</td>
<td>70 % (v/v)</td>
<td></td>
</tr>
<tr>
<td>The flow velocity of mobile phase</td>
<td>0.8 mL/min</td>
<td></td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 μL</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>223 nm</td>
<td></td>
</tr>
</tbody>
</table>

Experimental Part:

1. Preparation of solutions for injection. Add the standard solutions of benzoic acid and 2-phenoxyethanol (both with the concentration 10⁻³ mol/dm³) and acetonitrile to the labelled vials according to the following table. Cover vials with stoppers and thoroughly mix the solutions.

<table>
<thead>
<tr>
<th>Vial number</th>
<th>Volume of benzoic acid μL</th>
<th>Volume of 2-phenoxyethanol μL</th>
<th>Volume of acetonitrile μL</th>
<th>The peak area for benzoic acid</th>
<th>The peak area for 2-phenoxy ethanol</th>
<th>The peak areas ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>300</td>
<td>900</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>600</td>
<td>600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>900</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>1200</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inv.Samp.</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Add about 0.1g dose (write down the exact mass) of the investigated hand cream to a small covered vial, and then add 4mL of acetonitrile and 1 mL of standard solution of benzoic acid with the concentration 10⁻³mol/dm³. Close tightly the vial and shake vigorously for 1 minute. Then place the solution in an ultrasonic bath and turn on the equipment setting time for 10 minutes. After a set time, take about 2 mL of solution and filter through the filter system according to the instruction pass by the supervisor.

3. Injecting samples onto the column: After preparing the solutions, inject samples onto the column. After performing the chromatogram write down the area under the peaks of the investigated compounds and place them in the table. Calculate the ratio of the area of the studied peaks - the results put in the table
4. Elaboration of results:
Make a plot of the relationship between the ratio of the peaks areas and the concentration of the internal standard. Using the graph, calculate the quantitative composition of the tested compound in prepared solution. Finally, calculate the percentage of 2-phenoxyethanol in the investigated hand cream and compare it with the maximum allowed value of its concentration.

Place for calculations: