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Application of Purpald® for determination of 3-cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde

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Synopsis

A spectrophotometric method for determination of 3-cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde was developed. This procedure is based on the reaction of carbonyl compounds with the selective derivatization reagent Purpald® in alkaline solution. The product of reaction is a colored compound with maximum absorption at 538 nm. The linear relationship is in the range of $(2.5–30) \times 10^{-5}$ mol·l^{-1} for 3-cyclohexene-1-carboxaldehyde and $(2.0–20) \times 10^{-5}$ mol·l^{-1} for hydroxyisohexyl 3-cyclohexene carboxaldehyde. The proposed method was successfully used for determination of hydroxyisohexyl 3-cyclohexene carboxaldehyde in a cosmetic product.

INTRODUCTION

3-cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Figure 1) are aldehydes with cyclohexene ring. Hydroxyisohexyl 3-cyclohexene carboxaldehyde is used as a fragrance ingredient in cosmetic products. In the cosmetics industry, the tested compound is known as Lyral®. It may be found in decorative cosmetics, perfumes, shampoos, toilet soaps, and other cosmetics as well as in household detergents and cleaners. The recent dermatological studies have proved that this compound is lipophilic enough to penetrate the skin (1) and it is the reason for induction and elicitation of contact allergy to it (2,3). Further, Scientific Committee on Consumer Products stated that the concentration of up to 0.02% of this fragrance compound in a cosmetic product would induce sensitization or elicit allergic contact reactions in previously sensitized consumers (4). Hydroxyisohexyl 3-cyclohexene carboxaldehyde is listed as suspected allergens in cosmetics by the European Union (5). According to Directive 2003/15/EC, a declaration of the listed 26 "fragrance allergens" on the label of the final product when present over the concentrations 0.001% for "leave-on" and 0.01% for "rinse-off" cosmetics

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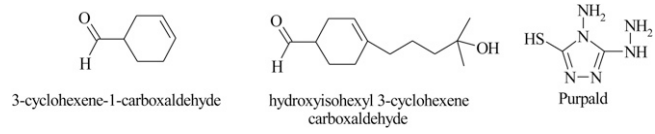

Figure 1. The chemical structure of 3-cyclohexene-1-carboxaldehyde, hydroxyisohexyl 3-cyclohexene carboxaldehyde, and Purpald®.

is required (6). The harmfulness of fragrance ingredients in cosmetic has led to an increased interest in the analyses of these products. Most of the works on determination of volatile allergens used the technique of gas chromatography–mass spectrometry (GC–MS) (3,7) and high-performance liquid chromatography (HPLC) (8).

3-Cyclohexene-1-carboxaldehyde belongs to the same group of compounds as hydroxyisohexyl 3-cyclohexene carboxaldehyde and it is mainly used in organic chemistry (9), organic synthesis (10,11), and production of polymers (12). Moreover, it does irritate the mucous membrane of the respiratory system (13). To our knowledge, a spectrophotometric method has never been used for determination of 3-cyclohexene carboxaldehydes before.

Therefore, the aim of this article was to develop a simple, accurate, and cheap method for the determination of 3-cyclohexene-1-carboxaldehydes. In our study, we used Purpald® (Figure 1) as a specific and sensitive derivatization reagent for the determination of mentioned aldehydes. Purpald® reacts readily with aldehydes and ketones in alkaline solution at room temperature to give a colorless product, but the derivative from an aldehyde can be oxidized to give the purple tetrazine (14). The purple-colored solutions of aldehyde–Purpald® adduct absorb in the visible region at 520–555 nm (15). Several methods and applications have been developed for the detection and determination of aldehydes and other compounds with Purpald®. The reagent has been used in conjunction with aliphatic aldehydes (13,14,16). Purpald® has been used for detecting the presence of aldehydes in disinfectant (17), resin-bound aldehyde groups (18), neutral monosaccharides (19), and lipid aldehydes (20). Several alcohol determination methods use the formation of an aldehyde by means of periodic acid, sodium periodate, or enzyme and its determination using Purpald®, for example, assay of glucosamine, mannitol (21), and glycol (22). Purpald® has also been used in thin layer chromatography as a spray reagent (19). Because of the advantages of this reagent such as selectivity, sensitivity, and ease of reaction we used Purpald® in our studies described later.

EXPERIMENTAL

CHEMICALS AND REAGENTS

Purpald® (4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole), 3-cyclohexene-1-carboxaldehyde, hydroxyisohexyl 3-cyclohexene carboxaldehyde, sodium hydroxide, and all organic solvents (methanol, ethanol, acetonitrile, acetone, dioxane) were obtained from Sigma-Aldrich (Steinheim, Germany), POCH (Gliwice, Poland), or LABSCAN Analytical Science (Dublin, Ireland). All chemicals used were of analytical grade and water was freshly distilled.

Purpald® solution was prepared as 0.1 mol·l^{-1} in 1.0 mol·l^{-1} sodium hydroxide. This solution is not stable and must be used within an hour of preparation.

Stock solutions of 3-cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde were prepared as 0.1 mol·l^{-1} in acetonitrile. One milliliter of these

solutions were diluted to produce working solutions at a concentration of 0.01 $mol \cdot l^{-1}$.

APPARATUS

A double-beam ultraviolet-visible spectrophotometer (Cary 100 Bio-Varian, Palo Alto, CA) with spectral bandwidth 0.2–4.0 nm programmable at 0.1 nm was used in the studies. Spectrophotometric 10-mm quartz cells with Teflon lids were used. In all measurements, the spectral bandwidth was 1.5 nm.

GENERAL PROCEDURE

Suitable volume of working solution of 3-cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde was transferred into a 10-ml in-volume flask and 5-ml in-volume flask, respectively (Table I). Then 1 ml of Purpald® solution and 1 ml of 1 $mol \cdot l^{-1}$ sodium hydroxide solution were added. The mixture was left at room temperature for 45 min and then diluted to the mark with ethanol. The solutions were transferred into a spectroscopic cell and the absorbance of the resulting solution was measured at 538 nm, against blank solution. The blank test was composed of 1 ml Purpald® solution and 1 ml of 1 $mol \cdot l^{-1}$ sodium hydroxide solution.

The absorbance of aldehydes was referred to the standard curves of aldehydes in drug-free samples to determine concentrations. Linearity was established on the basis of the equation $A = aC + b$ where A (AUS) is the absorbance and C ($mol \cdot l^{-1}$) is the concentration of an aldehyde in a sample. The evaluation of the linearity was performed with the use of linear regression analysis.

PRECISION AND ACCURACY

The precision determination was conducted through the analysis of six sample solutions of 3-cyclohexene-1-carboxaldehyde on the same day for intraday precision (repeatability).

Table I
The Results of Compounds Determination; $n = 6$

Compound	Taken ($mol \cdot l^{-1}$)	Found $\bar{x} \pm t_{0,95} \cdot \frac{s}{\sqrt{n}}$ ($mol \cdot l^{-1}$)	RSD (%)	Recovery (%)
3-Cyclohexene carboxaldehyde	2.5×10^{-5}	$(2.8 \pm 0.1) \times 10^{-5}$	2.75	110.8
	5.0×10^{-5}	$(4.8 \pm 0.1) \times 10^{-5}$	2.01	96.0
	1.0×10^{-4}	$(9.6 \pm 0.3) \times 10^{-5}$	3.24	96.0
	2.0×10^{-4}	$(2.02 \pm 0.04) \times 10^{-4}$	2.01	101.0
	3.0×10^{-4}	$(3.0 \pm 0.1) \times 10^{-4}$	3.39	100.0
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	2.0×10^{-5}	$(1.91 \pm 0.03) \times 10^{-5}$	1.56	95.5
	5.0×10^{-5}	$(4.9 \pm 0.1) \times 10^{-5}$	2.28	98.0
	1.0×10^{-4}	$(1.03 \pm 0.02) \times 10^{-4}$	1.47	103.0
	1.5×10^{-4}	$(1.50 \pm 0.03) \times 10^{-4}$	2.13	100.0
	2.0×10^{-4}	$(2.01 \pm 0.06) \times 10^{-4}$	2.73	100.5

For the purpose of accuracy, the known amounts of 3-cyclohexene-1-carboxaldehyde were added to sample solution (recovery test).

The procedure of the test comprised the analysis of three different lotion solutions, in four replicates each, which spiked with 0.5, 1.5, and 2.5 μmol and represented the low, medium, and high concentrations of the linearity concentration range, respectively.

STABILITY OF THE COLOR COMPOUND

The preparation of stability test for 3-cyclohexene-1-carboxaldehyde–Purpald® adduct was conducted. The sample was prepared according to general procedure with spiking of the sample with an appropriate amount of the aldehyde to obtain a solution of 4×10^{-4} mol·l^{-1} concentration. Afterward, the measurement of the absorbance was executed at room temperature for 90 min.

ASSAY HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE IN A DOSAGE FORM

The procedure for determination of hydroxyisohexyl 3-cyclohexene carboxaldehyde in cosmetic is the same as that described for calibration procedure for 3-cyclohexene-1-carboxaldehyde. One milliliter of lotion was transferred in a test tube, then 1 ml of 0.1 mol·l^{-1} Purpald® solution and 1 ml of 1 mol·l^{-1} sodium hydroxide solutions were added. The mixture was left at room temperature for 45 min and then ethanol was added to a volume of 5 ml.

Absorbance of the resulting solution was measured at 538 nm, against blank test. The blank test was composed of 1 ml lotion and 1 ml of 1 mol·l^{-1} sodium hydroxide solution. The mixture was also left at room temperature for 45 min and then ethanol was added to a volume of 5 ml. The amount of hydroxyisohexyl 3-cyclohexene carboxaldehyde in lotion was calculated using the regression equation.

RESULTS AND DISCUSSION

In this work, we used the reaction of aldehydes with Purpald®, which is a specific reagent for aldehydic group as shown in Figure 2. As a result of reaction of aldehydes with described derivatization reagent purple derivatives are formed. This reaction was taken as a base to develop a spectrophotometric method for determination of cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde.

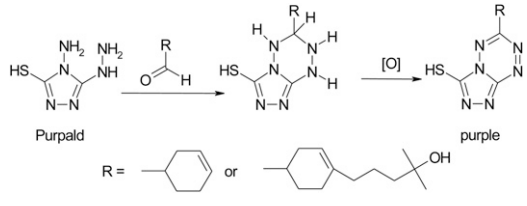

Figure 2. Reaction scheme between Purpald® and carbonyl compounds.

OPTIMIZATION OF ANALYTICAL CONDITIONS

A wide range of parameters leading to the highest absorbance of the corresponding purple product should be taken into consideration to establish optimum conditions for spectrophotometric determination of 3-cyclohexene carboxaldehydes. The optimal conditions for the course of reaction of 3-cyclohexene-1-carboxaldehyde were established in preliminary tests. The following parameters can be enumerated for exemplification: analytical wavelength; optimum reaction time; and concentration of sodium hydroxide, Purpald® solutions, and organic solvent. The selected optimal conditions were used to determine different amounts of both compounds and to establish determination ranges for 3-cyclohexene-1-carboxaldehyde and hydroxyisohexyl 3-cyclohexene carboxaldehyde.

The reaction takes place in an alkaline solution of Purpald® in the presence of oxygen and yields a violet derivative that absorbs most strongly at 538 nm (Figure 3) for both compounds. This is caused by identical structure of chromophores (conjugated system) in both derivatives. According to the literature reports (15,19,26), the purple colored products of this reaction absorb most strongly in the region of 520–550 nm.

The effect of derivatization reaction time on the absorbance value was studied. The maximum intensity was obtained after 45 min of reaction initiation of cyclohexene-1-carboxaldehyde with Purpald® (Figure 4). Several reports have described that in many cases, the time of analysis, which uses Purpald®, ranges from 15 to 25 min (15,21,23,26). The optimal reaction time for the studied aldehydes is 45 min. The structure of aldehyde influences this value. The presence of the ring and the double bond causes a higher steric hindrance, which may slow down the reaction of aldehydes with Purpald®. As a result, the time required to obtain maximum absorbance is longer.

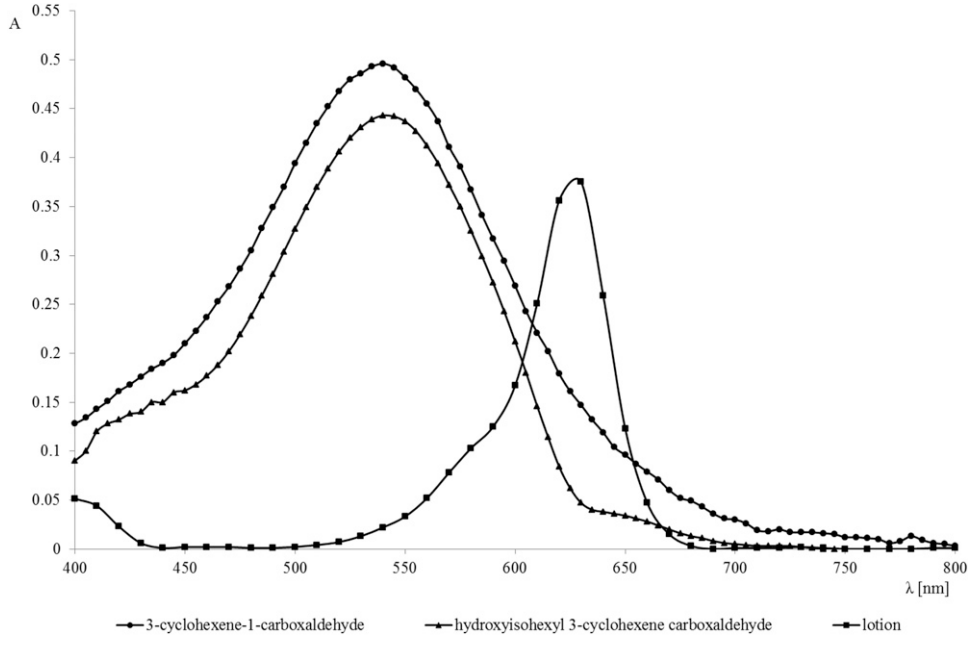

Figure 3. Absorption spectrum of the colored products (produced from the reaction between Purpald® and determined compounds solutions) and absorption spectrum of used lotion; $c_{aldehyde}$ = $2.0 \cdot 10^{-4}$ mol·l^{-1}, $c_{Purpald®}$ = $1.0 \cdot 10^{-2}$ mol·l^{-1}.

The colors of the products are stable for 10 min; therefore, the absorbance decreases with increasing reaction time (Figure 4). Therefore, the measurements of absorbance must be made immediately after stopping the derivatization reaction.

The problem of instability of the solution also applies to Purpald®. The derivatization reagent solution develops slight purple color within approximately half an hour. Therefore, for the determination of compounds with this reagent, the freshly prepared solution must be used.

The concentration of sodium hydroxide solution has a strong influence on the reaction of Purpald® with aldehydes. The best pH for the reaction was that provided by 1 mol·l^{-1} sodium hydroxide solution (Figure 5). Several reports have also described the influence of sodium hydroxide solution on determination of aldehydes (15,17,19). These studies have also chosen 1 mol·l^{-1} sodium hydroxide solution as the optimum concentration for determination of aldehydes. Excess of sodium hydroxide solution causes a great decrease in the intensity of the produced color. The reason is probably too strongly alkaline reaction medium, which negatively influences the stability of the derivative of 3-cyclohexene-1-carboxaldehyde or Purpald® solution.

The next studied effect was the influence of Purpald® solution concentration on absorbance values. The maximum intensity was obtained on using optimal Purpald® solution of 0.1 mol·l^{-1} in 1 mol·l^{-1} sodium hydroxide solution (Figure 6). The similar concentration of Purpald® solution was selected in many reports as the optimal for proposed procedures (15,19,20,22).

During the preliminary studies on the optimization of conditions, the samples were diluted to the mark with methanol. However, this procedure caused the change of color of

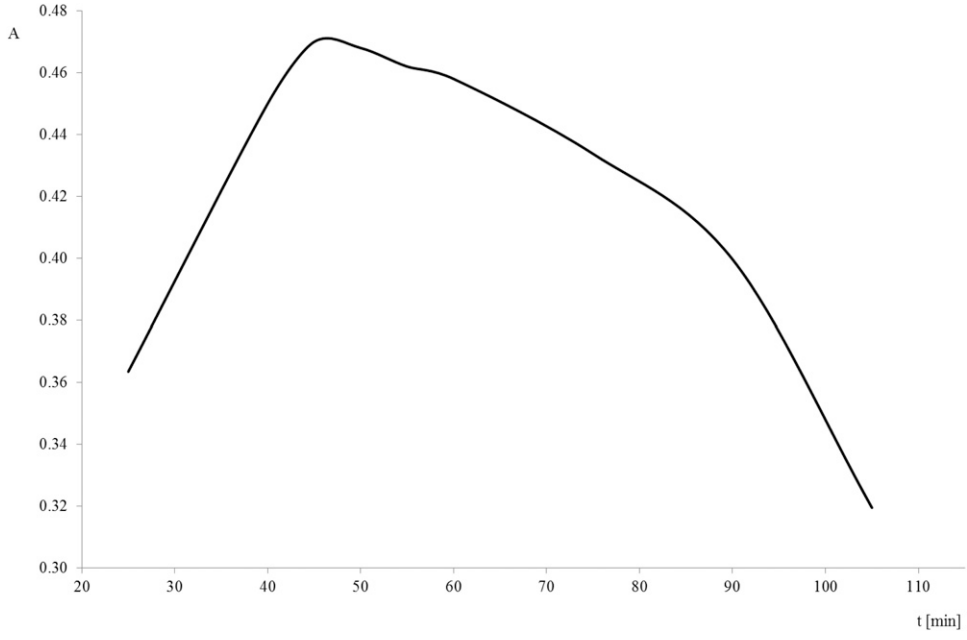

Figure 4. The influence of derivatization reaction time between 3-cyclohexene-1-carboxaldehyde and Purpald® on the absorbance value, $c_{aldehyde} = 4.0 \times 10^{-4}$ mol·l^{-1}, $c_{Purpald®} = 1.0 \times 10^{-2}$ mol·l^{-1}.

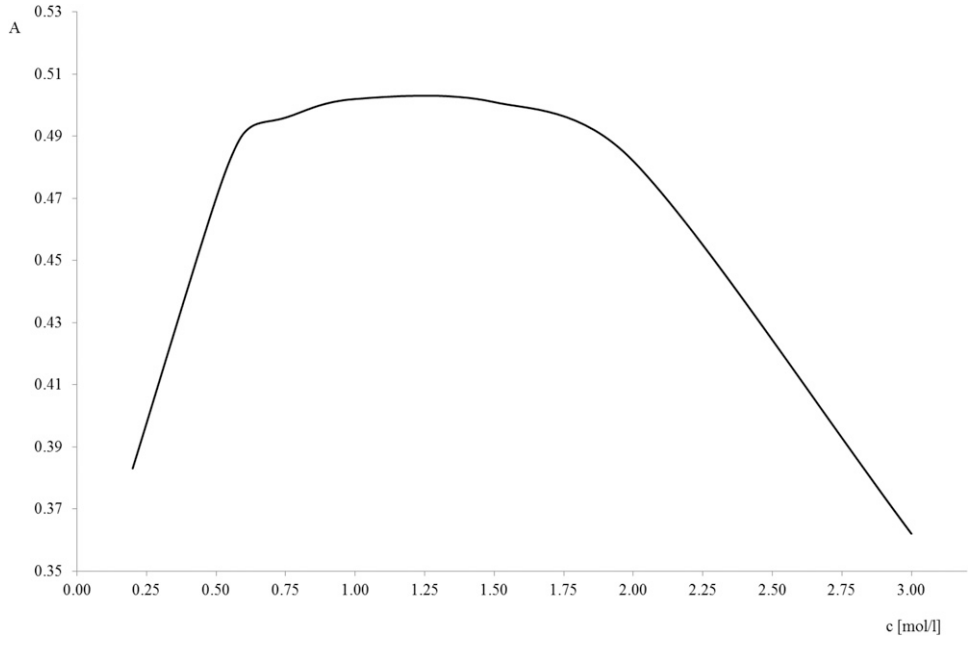

Figure 5. The influence of sodium hydroxide solution concentration on the absorbance value; $c_{aldehyde}$ = 4.0×10^{-4} mol·l^{-1}, $c_{Purpald^{®}} = 1.0 \times 10^{-2}$ mol·l^{-1}.

the blank solution. The influence of different solvents (methanol, ethanol, water, dioxane, and acetone) on Purpald® solution was studied. The aim was to choose one that does not change color of the blank solution, and the absorbance of this solution is low.

The lowest absorbance value was measured for the sample diluted with water, so we used this solvent during the preparation of samples for calibration curve. However, satisfactory results were not achieved. The absorbance–concentration of the aldehyde relationship did not show the linear dependence. The reason could be the fact that water does not stop the derivatization reaction as opposed to organic solvents. Therefore, ethanol was chosen as the suitable solvent. The value of absorbance for the blank solution containing this solvent was lower than that for the sample diluted with methanol. The samples diluted with dioxane and acetone were strongly colored in purple and yellow, respectively.

The method was validated on the basis of the following parameters: linearity, precision, solution stability, and accuracy.

LINEARITY

The working standard of aldehydes was obtained according to experimental part. The reaction mixture was incubated at room temperature for 45 min, the reaction was stopped by adding ethanol and the absorbance was measured at 538 nm. The reference cuvette contained identical concentration of sodium hydroxide and Purpald® solution. The analysis showed that the absorbance obeys Beer's law in the range of 2.5×10^{-5} to 3.0×10^{-4} mol·l^{-1} (0.250–3.00 μmol) for 3-cyclohexene-1-carboxaldehyde and 2.0×10^{-5}–2.0×10^{-4} mol·l^{-1} (0.10–1.00 μmol) for hydroxyisohexyl 3-cyclohexene carboxaldehyde.

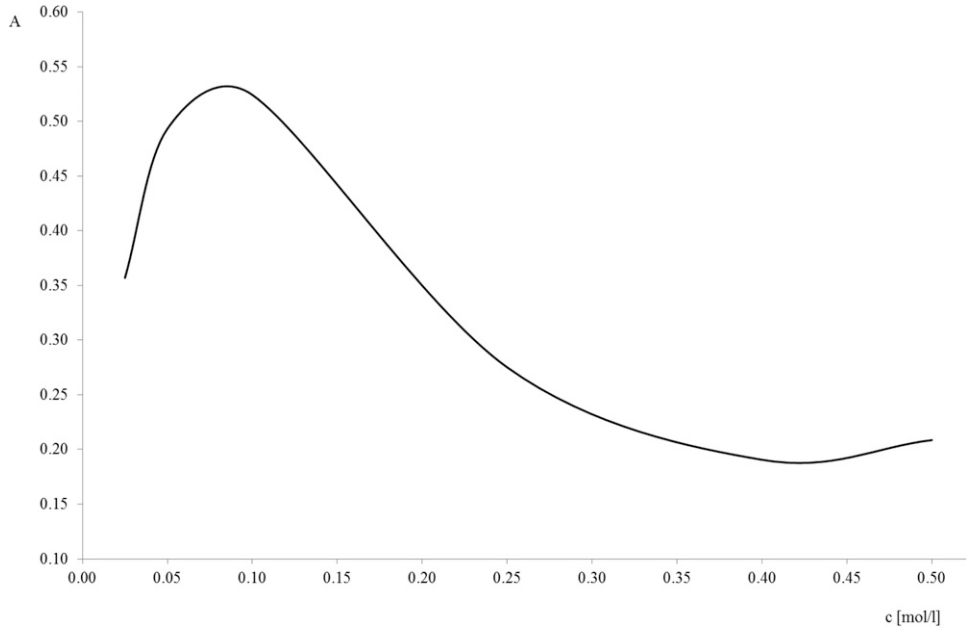

Figure 6. The influence of Purpald® solution concentration on the absorbance value; $c_{aldehyde}=4.0 \times 10^{-4}$ mol·l^{-1}, $c_{NaOH}=1.0$ mol·l^{-1}.

A = 0.1355c − 0.0017 for 3-cyclohexene-1-carboxaldehyde and A = 0.1525c − 0.0489 for hydroxyisohexyl 3-cyclohexene carboxaldehyde were the equations achieved at the least squared regression, with A being the absorbance (AUS) and c the concentration of aldehyde (μmol·l^{-1}).

Standard curves consistently gave r^2 values above 0.999 within a calibration range of the analyte. The molar absorptivity under our measuring conditions was calculated as 1.36×10^3 l·mol^{-1}·cm^{-1} for 3-cyclohexene-1-carboxaldehyde and 1.52×10^3 l·mol^{-1}·cm^{-1} for hydroxyisohexyl 3-cyclohexene carboxaldehyde, which was determined from the linear part of the aldehyde concentration.

PRECISION AND ACCURACY

The method was found to be highly precise (Table I). Intra-batch coefficient of variation at 2.5×10^{-5}, 1.0×10^{-4}, and 3.0×10^{-4} mol·l^{-1} ($n = 6$) was noted to vary between 2.8% and 3.4%. The precision and accuracy results based on absorbance ratios with good recoveries were obtained. There were no significant differences between the added amounts of aldehydes and the amounts found.

INTERFERENCES

Although all the aldehydes are potential interference in the determination of 3-cyclohexene carboxaldehydes by Purpald®, literature has shown that structural differences in the products formed by different aldehydes lead to variations in their spectra (23,24). The important advantage of this reagent is its sensitiveness and specificity for aldehydes. Purpald®

does not react with esters, amides, formic acid, hydroxylamines, quinones, hydrazines, and aminophenols to yield purple products (25). Furthermore, the presented study shows that the developed method can also be applied to the determination of aldehydes in cosmetics because the other ingredients do not react with Purpald®.

The lotion used, which is a mixture of many compounds, also absorbs visible radiation and the maximum absorbance is at a wavelength of 630 nm (Figure 3). The value of absorbance at 538 nm is small, but for low concentrations of the hydroxyisohexyl 3-cyclohexene carboxaldehyde contained in cosmetic it can have a significant effect on the determination. Therefore, to determine the concentration of this aldehyde in cosmetics, we prepared blank solution that contains the lotion, and sodium hydroxide solution without Purpald® solution. This procedure eliminates the influence of other cosmetic ingredients on the determination of hydroxyisohexyl 3-cyclohexene carboxaldehyde.

ASSAY OF HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE FROM COSMETICS PRODUCT

The developed spectrophotometric method for the determination of hydroxyisohexyl 3-cyclohexene carboxaldehyde with Purpald® reaction was successfully applied to cosmetic product lotion. The procedure was based on calibration curve method. The amount of hydroxyisohexyl 3-cyclohexene carboxaldehyde in the tested cosmetic has been determined under optimal conditions and it is $(1.05 \pm 0.06) \times 10^{-4}$ mol·l^{-1} with relative standard deviation at 2.75% (n = 6).

CONCLUSION

In conclusion, the reaction between Purpald® and aldehydes can be carried out quantitatively, which allows the analyses of compounds with carbonyl group. The developed method for determination of aldehydes is simple, cheap, and reproducible. Moreover, to the best of our knowledge, the proposed method is the first analytical procedure for determination of 3-cyclehexene-1-carboxaldehyde and is the first spectrophotometric procedure for the determination of hydroxyisohexyl 3-cyclohexene carboxaldehyde. GC–MS and HPLC can be used for the analysis of volatile chemicals such as perfumes or fragrance mixtures. The linear range in the proposed method was as follows 0.1-2×10^{-2} mol·l^{-1} (5), 5.7×10^{-6}–2.8×10^{-5} mol·l^{-1} (7). However, with the proposed, simple spectrophotometric procedure using selective derivatization reagent, it seems possible to efficiently determine aldehydes also in complex matrices such as cosmetics. The described method of preparation of samples is simple, does not require many reagents, and it is relatively short. Thus, this method could overcome some problems related to sample preparation. The obtained result from the analysis of the commercial products lotion indicates that the developed method can be considered as a tool useful in routine analyses of complex matrices such as cosmetics.

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