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RAPID SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF HEXAMETHYLENETETRAMINE (UROTROPINE) IN FOOT CARE PRODUCTS

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ABSTRACT

Hexamethylenetetramine (urotropine) is a compound that is often used in foot care products. Urotropine (hexamethylenetetramine) has a broad antibacterial effect (due to release of formaldehyde), which ensures the elimination of already occurring microorganisms and prevent the recurrence of infections, so commonly is used in cosmetic products for skin care of the feet (creams, lotions, deodorants, etc.). In cosmetic products are usually dosed at concentrations of 0.05 % to 0.15 %. Formaldehyde is known for its toxic and allergic effect on skin. There is evidence for its possible carcinogenic effects. An easily accessible spectrophotometric method for determination of urotropine in cosmetic products was developed and validated. The method is based on the interaction of urotropine with chromotropic acid in sulfuric acid medium, forming a characteristic colored compound with a maximum light absorption at a wavelength of 570 nm. The LOD is 0.01 %. The LOQ is 0.02 %. Repeatability (SD) at urotropine concentration 0.10 % in basic foot care product with a homogeneous viscous consistency is 0.0022 %. Reproducibility at urotropine concentration 0,10 % in basic skincare product, expressed by RSD, is 1.51 %. The recovery at urotropine concentration 0.10 % in a cosmetic product is 97.17 % (90.00 % - 110.00 %) and at urotropine concentration 0.15 % is 98.33 % (95.00 % - 100.00 %). The developed method was applied for the analysis of five samples of cosmetic products for the hygiene of the feet, containing urotropine, as a preservative. The measured concentrations are within concentration interval permitted by law: from 0.11 % to 0.14 %.The validated spectrophotometric method for urotropine determination in foot care products is rapid, with sufficient sensitivity, accuracy and reproducibility and does not require complicated and expensive equipment. It is easily applied in control of cosmetic products containing the preservative urotropine.

Keywords: urotropine, foot care products, spectrophotometric method.

INTRODUCTION

Hexamethylenetetramine (urotropine) is a compound that is often used in foot care products. The pure substance is a white crystalline powder obtained by reaction of 6 mol of formaldehyde and 4 mol of ammonia. The particle size ranges between 80 and 800 μm. Melting point: 270°C. Urotropine can be absorbed through the skin and it may cause allergic reactions, usually occurring in the form of rashes.

Common complaint is excessive sweating, often accompanied by unpleasant odor, which increases with increased physical activity, in stress and at high temperature during summer months The excessive sweating is often localized in the feet, underarms and palms. Moisture, increased in the skin, creates favourable conditions for the emergence and growth of bacteria and fungi that can give rise to some serious diseases and complications. Odours are resulting from the waste products of vital functions of micro organisms. Most

unpleasant is the excessive sweating of the feet, which is always accompanied by a particularly unpleasant odour. Respect for personal hygiene is one of the most necessary, but unfortunately, not sufficient condition to tackle this unpleasant condition. Therefore, in most cases it is essential to effective antiperspirants. Urotropine (Hexamethylenetetramine) shows a broad-spectrum antibacterial activity (release of formaldehyde), which ensures the elimination of already occurring micro organisms and prevention the recurrence of infections, so commonly used in cosmetic foot care products (creams, lotions, deodorants and others.). In cosmetic products for the hygiene of the feet urotropine is usually dosed at concentrations of 0.05 % to 0.15 %. Formaldehyde is known for its toxic and allergic effect on skin [1]. When uroptopine is used as a preservative at concentrations up to 0.15 % in non-aerosol products, in the literature does not mention its irritant and sensitizer effect on people. This content urotropine release formaldehyde at concentrations less than 0.2 %. But if urotropine is used as the active ingredient in concentrations higher than 0.15 % , in the literature are available data on the sensitizing effect at concentrations equal to or above 0.25 %. There have been studies on the teratogenic and carcinogenic effects of urotropine, but this effects are not proved so far [1-3].

Urotropine and content of formaldehyde in cosmetic products have strict standards in the European and in the harmonized with it Bulgarian legislation. The content of urotropine in preservatives is allowed to 0.15 %, the formaldehyde content in products not intended for oral hygiene – to 0.2 % and in products for oral hygiene -to 0.1 % [4, 5]. In the published methods reference methods for checking composition of cosmetic products (Annex 10 of the Ordinance № 36) [4] no method is mentioned for determination of urotropine. In the Ordinance № 36 and in the literature [4-8] appear sophisticated techniques for formaldehyde determination, when used alone or with other preservatives that are not sources of formaldehyde or in a substance, which is a source of formaldehyde. These methods require complicated and expensive equipment, reagents and materials.

The aim of this study was the development of an easily accessible spectrophotometric method for determination of urotropine in foot care products. The method is based on the interaction of urotropine with chromotropic acid in sulfuric acid medium, forming a characteristic colored compound with a maximum light absorption at a wavelength of 570 nm.

EXPERIMENTAL

Material and methods

As base was used a cosmetic product not containing urotropine, with homogeneous viscous texture and a certified reference substance urotropine. Samples were prepared in the laboratory by mixing of basic cosmetic product with urotropine and subsequent homogenization.

The development of the method is carried out according to BDS EN ISO / IEC 17025:2006 [9] requirements for the parameters: limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility, recovery.

Limit of detection (LOD) and Limit of quantification (LOQ) are defined as follows:

$LOD = C_{bl} \pm 3 \times SD_{bl}$,

where:

C_{bl} is the concentration of urotropine in the blank,

SD_{bl} is standard deviation of the blank samples (n=10).

and

$LOQ = C_{bl} \pm 6 \times SD_{bl}$.

To determine the values of LOD and LOQ were developed n number of samples with basic cosmetic product free of urotropine in it. Demonstration of repeatability and recovery was performed using basic cosmetic product free of urotropine content.

To determine the repeatability, expressed by the standard deviation (SD), samples were tested on different days, at the level of concentrations of urotropine 0.15 %. Reproducibility was expressed by relative standard deviation (RSD).

To determine the recovery were prepared samples contained basic cosmetic product inforced by urotropine as active ingredient at two concentrations - 0.10% and 0.15 %. The concentration is chosen in accordance with the quantities used in cosmetic products.

Equipment, chemicals, standards and reagents

- A UV-VIS Spectrophotometer CARY Varian (Varian Australian Pry.Ltd.) was used with cells of 1 mm thickness

- sulfuric acid, diluted with distilled water (1: 2)

- chromotropic acid (0.1 g of chromotropic acid dissolved in sulfuric acid - 1:2 solution and diluted to

the mark in a volumetric flask of 100.0 cm^3)

- urotropine standard solution in distilled water at a concentration 1.0 mg/cm^3

Sampling and Sample preparation

In an Erlenmeyer flask 200.0 cm^3 were weighed 0.5 g ± 0.0001 grams of basic cosmetic product on an analytical balance. Then 25.0 cm^3 chromotropic acid solution and 50.0 cm^3 of the sulfuric acid solution (1:2) were added. In another Erlenmeyer flask of 200.0 cm^3 were introduced 6.00 cm^3 of urotropine standard solution, 25.0 cm^3 of chromotropic acid solution and 50.0 cm^3 of sulfuric acid solution (1:2). In the third Erlenmeyer flask of 200.0 cm^3 (blank) were introduced 75.0 cm^3 of sulfuric acid solution (1:2).

The three flasks are placed in a boiling water bath for 30 minutes, then immediately cooled to room temperature, transferred into three volumetric flasks of 100.00 cm^3 and completed to the mark with sulfuric acid solution (1:2).

Then the concentration of sample solution at a wavelength (λ = 570 ± 2 nm) was measured against developed the same reference sample of known concentration (6.0 mg, which sets the camera).

RESULTS AND DISCUSSION

The contents of urotropine (X) in cosmetic products, in % is given by:

$$X = \frac{C \times 100}{1000 \times m},$$

where:

C – the concentration of urotropine in mg;

m – mass of sample taken for analysis in g.

The results were processed statistically using statistical software package SPSS 10.0.

To calculate the limit of detection (LOD) and the limit of determination (LOQ) of the method ware prepared using basic cosmetic product containing no urotropine. The results of LOD and LOQ of urotropine are presented in Table 1.

To demonstrate the repeatability of the method are prepared samples of basic foot care product with a homogeneous viscous consistency with the spike at 0.10 % urotropine. Tests were conducted on three consecutive days (Table 2). The good repeatability of the results can be seen from the calculated standard deviation (SD), in terms of repeatability – 0.0022 %.

Table 1. Limit of detection (LOD) and limit of determination (LOQ) of urotropine in basic cosmetic product.

Number of samples n	Concentration (C)		LOD	LOQ
	$\bar{X}$, %	SD	%	%
10	0.0098	0.0010	0.01	0.02

Table 2. Repeatability of the results of determining the concentration (C %) at a rate of 0.10 % urotropine in basic cosmetic product.

Spike level for urotropine' %	Concentration, founded in spike samples (C), %			
	Number of samples n	$\bar{X}$, %	SD, %	RSD, %
0.10	6	0.0952	0.0022	1.51

Table 3. Recovery for urotropine at levels 0.10 % and 0.15 % in basic cosmetic product.

Spike level for urotropine, %	Number of samples n	Concentration, founded in spike samples (C), %			Recovery, %			SD, %	RSD, %
		min	max	$\bar{X}$	min	max	$\bar{X}$		
0.10	6	0.09	0.10	0.0971	90.00	100.00	97.17	7.1671	7.36
0.15	6	0.14	0.15	0.1466	93.33	100.00	98.33	4.0825	4.15

Table 4. Results of determination of urotropine in commercial cosmetic products for foot care.

Code of cosmetic product	Number of measurements (n)	Average results of measurements, %	Standard deviation SD, %	Relative standard deviation RSD, %
1	6	0.11	0.0020	1.38
2	6	0.12	0.0024	1.44
3	6	0.12	0.0038	1.66
4	6	0.11	0.0046	1.77
5	6	0.14	0.0051	2.03

The reproducibility is expressed by the relative standard deviation (RSD) under repeatability conditions: 1.51 %.

The recovery was evaluated at concentrations of urotropine in basic cosmetic 0.10 % and 0.15 % (total for both concentrations 12 samples) and data are presented in Table 3. The resulting recovery was satisfactory and at a concentration of 0.10 % was ranged from 90.00 % to 110.00 % and 0.15 % concentration – was ranged from 95.00 % to 100.00 %.

The developed method was applied to the analysis of five samples of foot care cosmetic products, containing urotropine, as a preservative. The measured concentrations are within permitted by law - from 0.11 % to 0.14 % [4, 5]. The results obtained from tests of commercial cosmetic products are presented in Table 4.

CONCLUSIONS

A spectrophotometric method for determination of urotropine in foot care cosmetic products has been developed and described. The method is based on the interaction of urotropine with chromotropic acid in sulfuric acid medium, forming a colored compound with a maximum light absorption at a wavelength of 570 nm.

The method is validated based on 28 trials and evaluated its parameters. The limit of detection (LOD) is 0.01 %. The limit of determination (LOQ) is 0.02 %. The repeatability (SD) is 0.0022 % at a concentration of urotropine in basic skincare product with a homogeneous viscous consistency 0.10 %. The reproducibility at a concentration of urotropine 0.10 % in basic foot care product with a homogeneous viscous consistency, presented by RSD, in repeatability conditions is 1.51%. The recovery at urotropine concentration of 0.10 % in a cosmetic product is 97.17 % (90.00 % - 110.00 %) and at urotropine concentration of 0.15 % is 98.33 % (95.00% - 100.00 %).

Validated spectrophotometric method for urotropine determination in cosmetic foot care products described here is rapid, with sufficient sensitivity, accuracy and reproducibility and does not require complicated and expensive equipment It is easily applied in control of cosmetic products containing the preservative urotropine.

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