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Original Research Article

Rapid Method for the Identification and Quantification of Allantoin in Body Creams and Lotions for regulatory activities

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A B S T R A C T

Allantoin is a white crystalline powder with molecular formular, $C_4H_6N_4O_3$, which is generally produced by the oxidation of uric acid, and by chemical synthesis via purine oxidation. Allantoin is also a natural chemical compound that is produced by many organisms, including animals, bacteria and plants. It is extensively used in the cosmetic industry in body creams, lotions and shower creams formulations as an emollient ingredient, helping to suppress the aggression of exfoliating ingredients. The aim of this study was to develop a rapid but simple spectrophotometric method in the UV/VIS mode for the identification and quantification of allantoin in cosmetic products including body creams and lotions to aid regulatory agencies confirm manufacturer's substantiation claims. Ten (10) body creams and lotion samples purchased randomly from supermarket shops in Calabar, Nigeria, were analysed for allantoin concentration. Allantoin was detected in nine (9) samples at levels 0.51 ± 0.02% to 1.05 ± 0.44%, absorbing at three characteristic and fundamental peaks and shoulders (280nm, with 64sh, 78sh and 84sh), through two of its principal functional groups (O=C and NH_4^+), in the UV/VIS spectrum, and calculated from the product of the reciprocal of wavelength ($^1/\lambda$) and the constant k = 0.129. This study confirmed the concentration of allantoin in the body creams and lotions investigated. The study also confirmed the allantoin type in the creams and lotions to be of different derivatives, mainly Allantoin Polygalacturonic Acid, Allantoin Ascorbate and Allantoin Ethyl p-aminobenzoate.

Introduction

In Nigeria and among the black race, women use skin toning creams and lotions to lighten their skin. Some of these skin lightening products are smuggled into countries like Nigeria because they contain regulated chemicals such as mercuric salts and hydroquinone. Whereas hydroquinone is permitted in cosmetic products up to 2.0%, mercuric salts are out rightly banned in Nigeria, by the National Agency for Food, Drug Administration and Control (NAFDAC). Hydroquinone has been co-

formulated with allantoin in cosmetic products due to the protective effect of allantoin. The aggressive exfoliation of the epidermis by hydroquinone in tropical and sub-tropical regions of the world justifies the inclusion of a skin protecting agent such as allantoin. When its inclusion in a cosmetic product is claimed, it is expected to be substantiated and regulated and confirmed by independent laboratory chemical analysis. Thus this study set out to establish a rapid method for the detection and quantification of allantoin in body creams and lotions using a simple spectrophotometric method to aid regulatory activities.

Most skin lightening creams sold in Nigeria contain hydroquinones and allantoin. While hydroquinone is used as a skin lightener and essentially as an exfoliator of epidermal dead cells of the skin, allantoin functions to reduce the aggression (irritation, burns, sweating) of hydroquinones on the skin and helps to sooth the skin as an emollient agent.

Allantoin is a chemical compound naturally produced by many organisms, including animals, plants, and bacteria. It is also present in plants including, sugar beet, tobacco seed, chamomile, and wheat sprouts (Portugal-Cohen *et.al*. 2009 & 2011). It can be found in botanical extracts of the comfrey plant as well as in urine from cows and other mammals. Chemically synthesize bulk allantoin is reportedly identical to natural allantoin, and is therefore considered safe and non-toxic (Drewes and Staden, 1975, Akema Fine Chemicals, 2006).

A wide range of cosmetic, skin and personal care products contain allantoin as an ingredient. These include sunscreens, lip balm, lipsticks, moisturizing body creams, body lotions, eye creams, toothpaste, mouthwash, acne treatments, skin lighteners and toners, hand creams, shaving creams, bath products, baby powders and hair pomades, hair creams, hair gels and hair dyes. Allantoin is used in oral hygiene products and other toiletries to enhance the activity of active ingredients in the formulation and for synergy. These products usually contain allantoin concentrations between 0.5% to 2.0% percent (Harding *et. al.* 2006).

Biochemically, allantoin speeds up wound healing by stimulating new skin cell growth and is sometimes used to treat radiation skin burns in those who are undergoing radiation treatment for cancer (Harding *et. al.* 2000). However due to its anti-inflammatory properties and its ability to soothe skin irritation, allantoin is a widely used ingredient in skin care products. It is traditionally used to treat wounds, burns, and scars and to reduce inflammation. Allantoin is also well known for its other properties in treating aging and damaged skin. It is a natural anti-irritant, and skin protectant that increases the water content of the extracellular matrix which provides structural support to cells and is an important part of connective tissues. In skin care products, allantoin helps to moisturize and soften skin and acts as a keratinolytic (Loden *et. al.*1999). The epidermis synthesizes a large number of proteins and lipids in the stratum corneum (the outermost layer of the epidermis) to protect the body. When this process, called keratinization, is unbalanced, more keratin than normal is produced and therefore the barrier function is compromised. Allantoin softens the keratin, improves its water holding capacity. This is why consumers generally claim that allantoin leaves the skin feeling smooth and healthy when applied to the face (Drewes and Staden, 1975; Harding *et. al.* 2000)

Allantoin is a natural soothing, anti-irritant,

and skin protectant that increases the water content of the extracellular matrix which provides structural support to cells. It is also an important part of connective tissues. Allantoin increases the smoothness of the skin; promotes cell replication; and promotes the healing of wounds, burns, and scars. It is also called 5-ureidohydantoin or glyoxyldiureide and used as a moisturizing, non-toxic and keratolytic agent (Loden *et. al*. 1999, *Durmus et.al.* 2012) . This property helps the skin to heal more quickly and to bind moisture more effectively, making products containing this substance useful for dry skin and for healing wounds, burns, and scars (Temiz *et.al.* 2009; Durmuz *et.al.* 2012). It is also effective against sunburn, chapped lips, cold sores, diaper rash, and similar skin irritations. Allantoin is a valuable cell-proliferating healing agent which stimulates healthy tissue formation (Loden *et. al.* 1999, Durmus *et.al.* 2012).

The allantois, from which allantoin got its name, is an embryonic excretory organ present in most mammals, except humans and higher apes, in which the compound accumulates. Animals produce allantois as a by-product of purine catabolism, in which the waste product uric acid is oxidized and broken down. Allantoin is odorless, safe, non-toxic, and non-allergenic to humans, in both natural and chemically synthesized form. (Young and Conway, 1942; Borchers, 1977; Linberg and Jansson, 1989).

Several methods have been developed for the identification and quantitative estimation of allantoin in food and cosmetic products (Carlson and Thompson, 2001, Chen et.al. 1996). However, regulatory and enforcement activities require rapid and easy to use methods, and none of the earlier developed methods fits this need. Consequently, this method was developed to meet this need.

Materials and Methods

Materials

Ten (10) different samples of body creams and lotions commonly used as skin lightening products by women were purchased from four (4) supermarket stores in Calabar, Nigeria. Concentrated hydrochloric acid (BDH, England), methanol (Analar grade, BDH, England) and phenolphthalein indicator were purchased from a chemical store in Calabar. Other materials including, a UV/VIS spectrophotometer with a variable wavelength, hot plate with a magnetic stirrer, Whatmann No. 4 filter paper and distil water were available in the research laboratory of the Department of Biochemistry, University of Calabar, Nigeria..

Allantoin undergoes the reactions that are common to the aromatic amino acids. In these reactions, the material in methanol or ethanol absorbs strongly, through two of its principal functional groups (O=C and NH_4^+) in the UV/VIS spectrum, and show three principal characteristic and fundamental peaks and shoulders (280nm, with 64 sh, 78 sh and 84 sh).

The absorbance is inversely proportional to the concentration of allantoin in the given sample. And concentration of Allantoin in sample is calculated as:

% Allantoin = $k.^1/\lambda$

Where, k (constant) = 0.129 and λ is in nm.

Method

Five (5 g) grammes of sample of cream or lotion were weighed into a 250 mL flat-bottomed flask. 50 ml of conc. HCl was added and mixed. A magnetic stirrer was inserted into the flask and heated up to 55^oC for 5 minutes. The thoroughly mixed sample was filtered quickly into a 100 mL beaker

using Whatmann No. 4 filter paper. 50 mL of analar grade methanol was added to the mixture, corked and shaken sufficiently and allowed to stand and cooled to 25^{o}C under refrigeration. The mixture was again filtered between Whatmann No. 4 filter papers.

Using the analar grade methanol as standard, the wavelength of samples was read at 280 nm, to obtain characteristic peaks for degraded allantoin.

Statistical Analysis

All data were expressed as Mean ± SEM of number of replicates (n = 3). Tested products were compared using Analysis of Variance (ANOVA) in the SPSS version 16 software. Values of $p<0.05$ were considered to indicate a significant difference between products.

Results and Discussion

The amphoteric characteristic of Allantoin permits combination with various chemical substances to form salts and complexes. This property allows allantoin to be used in various cosmetic products formulations and productions including moiturizing body creams and lotions, lip balm, lipsticks, eye creams, toothpaste, mouthwash, acne creams, skin lighteners and toners, hand creams, shaving creams, bath oils & soaps, baby powders and dusting powder, hair pomades, hair creams, hair gels and hair dyes. In combination with various therapeutic substances, allantoin synergizes their activity, reducing at the same time their side-effects and toxic properties. The complexes and salts thus formed have been shown to possess useful dermato-therapeutic properties as seen in many creams and lotions used for consumer test studies.

These complexes or products do not interfere with the two main functional groups (O=C and NH_4^+) of allantoin in the UV/VIS spectrum. This makes it possible and easy for the UV/VIS spectrophotometric method to pick the signals of the two chromophores (O=C and NH_4^+) in the UV-spectrum.

The fundamental peak (280 nm) and the three characteristic shoulders (64sh, 78sh and 84sh) are key to this method and these fundamental and characteristic peaks are present in all allantoin containing products. Drewes and Staden (1975) had carried out a rapid estimation of allantoin using the downfield N_3-H proton in the PMR spectrum in plant samples after extraction and concentration of aqueous samples of the plant materials.

Allantoin undergoes the reactions that are common to the aromatic amino acids (phenyl alanine, tyrosine and tryptophan). In these reactions, allantoin either in methanol or ethanol absorbs strongly in the UV/VIS spectrum at 280nm wavelength, and shows three principal characteristic peak shoulders of 64sh, 78sh and 84sh.

Figure.1 Chemical structure of Allantoin

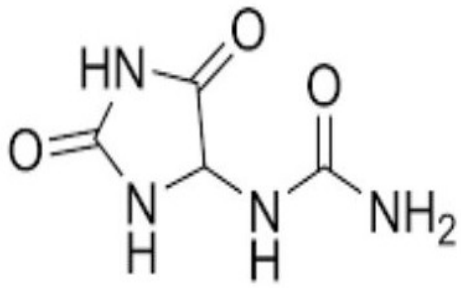

Table.1 Allantoin Concentration in Body Creams & Lotions sold in Calabar Supermarket Stores

S/N	Brand Name of Product	% Allantoin Content	Remarks
1	Vovi skin lightening shower cream	0.69 ± 0.04	Listed & Claimed
2	Jergens Natural Fairness Body Lotion	0.55 ± 0.02	Listed & Claimed
3	Bettina Barty Musk body cream	0.57 ± 0.04	Listed & Claimed
4	Perfect White Cream	0.92 ± 0.04	Listed & Claimed
5	Looking Good skin tone lotion	0.51 ± 0.02	Listed & Claimed
6	Hi White Exclusive Lotion	0.98 ± 0.04	Listed & Claimed
7	Face & Body Maxi-Whitener Lotion	0.79 ± 0.07	Listed & Claimed
8	Smoothly Look Super Whitening Lotion	1.05 ± 0.44	Listed & Claimed
9	Estee Maxitone Skin Beautifying Milk	0.88 ± 0.06	Listed & Claimed
10	Nivea (Control)	ND	Not listed or Claimed

Mean ± S.E.M, n=3. ND= Not detected

Allantoin and allantoin derivatives can be analyzed using a rapid spectrophotometric technique during field inspections of cosmetic production or Customs clearance of imported cosmetic products to confirm claims substantiation of manufacturers and levels of inclusion and allowing large number of samples to be analyzed at the same time.

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