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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF METHYLISOTHIAZOLINONE IN COSMETIC PRODUCTS

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ABSTRACT

Cosmetic products commonly contain preservatives to prevent microbial growth and ensure product safety. Methylisothiazolinone (MIT) is a widely used preservative in various personal care products. The accurate and reliable estimation of MIT is essential for quality control and regulatory compliance. This study aimed to develop and validate a UV spectroscopic method for the quantitative determination of MIT in cosmetic products. The UV spectroscopic method was developed based on the absorption characteristics of MIT in a suitable solvent. The method involved the preparation of standard solutions of MIT and the measurement of their absorbance at wavelength 270nm using a UV-visible spectrophotometer. The calibration curve was constructed by plotting the concentration of MIT against the corresponding absorbance values. The method was optimized by evaluating various parameters such as solvent selection, wavelength, and linearity range. The developed UV spectroscopic method was validated according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was found to be specific, as no interference from excipients or other common preservatives was observed. The linearity of the method was established over a concentration range of 20 to 100 µg/mL, with a correlation coefficient (R^2) of 0.96. The accuracy and precision of the method were evaluated through recovery studies and intra-day and inter-day variability studies, respectively. The validated UV spectroscopic method was successfully applied for the estimation of MIT in a range of cosmetic products, including facewash and shampoos. The proposed UV spectroscopic method provides a cost-effective, rapid, and reliable alternative for the routine analysis of

MIT in cosmetic products. In conclusion, this research presents the development and validation of a UV spectroscopic method for the estimation of MIT in cosmetic products. The method demonstrated good linearity, specificity, accuracy, and precision. It can serve as a valuable tool for routine quality control analysis and regulatory compliance in the cosmetic industry, contributing to the safety and efficacy of cosmetic products.

KEYWORD: Methylisothiazolinone, UV spectroscopy, Method development, Validation, Preservatives, etc.

INTRODUCTION

Cosmetics: As per Section 3 (a) of the Drugs and, Cosmetics act 1940 and Rules 1945Cosmetics means any articles intended to be rubbed, poured, sprinkled, or sprayed on, or introduce, otherwise applied to, the human body or any part there of for cleansing, beautifying, promoting attractiveness, or altering the appearance, and include any articles intended for use as a component of cosmetics.[1]

Cosmetics are constituted mixtures of chemical compounds derived from either natural sources, or synthetically created ones. Cosmetics have various purposes. Those designed for personal care and skin care can be used to cleanse or protect the body or skin. Cosmetics designed to enhance or alter one's appearance (makeup) can be used to conceal blemishes, enhance one's natural features (such as the eyebrows and eyelashes), add color to a person's face, or change the appearance of the face entirely to resemble a different person, creature or object. Cosmetics can also be designed to add fragrance to the body.[2]

COSMACEUTICALS

Cosmeceuticals are cosmetic products with bioactive ingredients purported to have medical benefits. In America, there are no legal requirements to prove that these products live up to their claims. The name is a portmanteau of "cosmetics" and "pharmaceuticals". **Nutricosmetics** are related dietary supplement or food or beverage products with additives that are marketed as having medical benefits that affect appearance.[1]

Types of Cosmeceuticals Product[1]

1) Shampoo
2) Rinse
3) Beauty lotion

4) Cream, Milky lotion, Hand cream, Cosmetic oil
5) Shaving preparation
6) Sunscreens
7) Face packs
8) Medicated Soap

Cosmeceuticals Product[2]

Figure 1: Cosmetics.

Figure 2: Cosmetic application

PRESERVATIVES

A preservative is a substance or a chemical that is added to products such as food products, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, and many other products to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical. Chemical preservation entails adding chemical compounds to the product. Physical preservation entails processes such as refrigeration or drying. Preservative food additives reduce the risk of foodborne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality. Some physical techniques for food preservation include dehydration, UV-C radiation, freeze-drying, and refrigeration. Chemical preservation and physical preservation techniques are sometimes combined.[3]

Cosmetics Preservation – An Ultimate Guide to Select the Right Preservative

Cosmetic products require preservation against microbial contamination to guarantee consumer's safety and to increase their shelf-life. Preservatives are essential ingredients that help preserve the integrity and stability of cosmetic products that can be damaged by microorganisms. While there is a wide variety of preservatives available, either synthetic or natural, choosing the right one for your cosmetic formulation could be a task.[4]

Why is preservation important? Several products in the market contain ingredients which make cosmetic systems very difficult to preserve. For example, products often contain significant amounts of water and other organic or inorganic ingredients that may be susceptible to contamination by microorganisms like bacteria, fungi, and molds.[4]

Most cosmetic care products must be protected against microbial growth – for both consumer protection and formulation stability reasons. Growth of microorganisms might result in degradation of ingredients and consequently in deterioration of physical and chemical stability.

In order to prevent such happenings, formulators include preservatives in their formulations. Depending on the type of preservative selected, they can:

- Kill or inhibit the growth of micro-organisms, and
- Protect a formula from microbial contamination

Moreover, microbial growth can also be prevented by using sterile or aseptic production and filling into packaging material, preventing microbial contamination during storage and usage. Also, while applying a cosmetic for personal care use it should be assured that its free from any contamination. The product must be preserved adequately to kill the microorganisms that are introduced by the consumers themselves.[4]

Cosmetics Preservatives – Types of Chemistries Available[4]

Different preservatives have different ways of working based on their chemistry. Tables below lists some common types of preservatives used in cosmetic formulations and their key features, activity spectrum and functioning range as well as suggested use levels.

Preservative Types	Features	Examples
Organic Acids	○ Effective with fungi but offer least protection against bacteria. ○ Effective only in acidic conditions (pH 5.0) ○ Need an aqueous base, in order to be used ○ Considered natural alternatives while developed synthetically ○ Need to be combined with other preservatives to	○ Benzoic acids and salts ○ Sorbic acid and derivatives ○ Salicylic Acid and derivatives ○ Formic acid and derivatives ○ Dehydroacetic acid, formic acid, undecylenic acid, citric acid, etc

	provide broad-spectrum action.	
Alcohols and Phenols	○ Effective against a wide range of microbes ○ Combination of phenethyl alcohol with caprylyl glycol shows a synergistic antimicrobial effect.	○ Triclosan ○ Phenoxyethanol ○ Benzyl alcohol ○ Dichlorobenzyl alcohol ○ Phenethyl alcohol
Isothiazolinone	○ Activity is related with the thiol and amine groups of their structures ○ Often masked under the chemical names of their mixtures ○ Can cause skin irritation / allergic reactions	○ Methylisothiazolinone ○ Benzisothiazolinone ○ **Methylchloroisothiazolinone**
Urea Compounds / Formaldehyde releasing preservatives	○ Effective in fighting bacteria but weak fungal efficacy ○ Low levels required for use as these release formaldehyde needed for preservation ○ Can cause skin irritation / allergic reactions ○ Used in skin care and toiletries	○ DMDM Hydantoin ○ **Imidazolidinyl Urea** ○ Diazolidinyl Urea ○ Bronopol

Selected Preservatives: *Methylisothiazolinone*

Category: Isothiazolinone

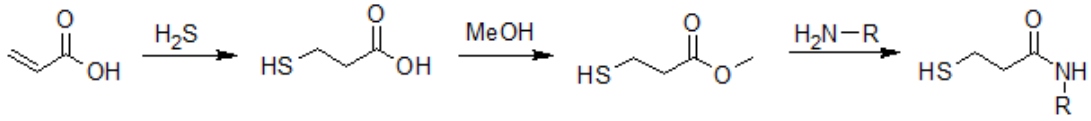
ISOTHIAZOLINONE

Isothiazolinone (sometimes **isothiazolone**) is an organic compound with the formula $(CH)_2$ SN(H)CO. A white solid, it is structurally related to isothiazole. Isothiazolone itself is of limited interest, but several of its derivatives are widely used preservatives and antimicrobials.[5]

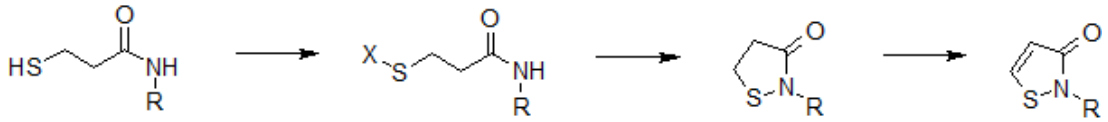
Synthesis

Compared to many other simple heterocycles, the discovery of isothiazolinone is fairly recent, with reports first appearing in the 1960s.

Isothiazolinones can be prepared on an industrial scale by the ring-closure of 3-mercaptopropanamides. These in turn are produced from acrylic acid via the 3-mercaptopropionic acid:



Ring-closure of the thiol-amide is typically effected by chlorination or oxidation of the 3-sulfanylpropanamide to the corresponding disulfide.



Many other routes have been developed, including addition of thiocyanate to propargyl amides.[5]

Mechanism of action

The antimicrobial activity of isothiazolinones is attributed to their ability to inhibit life-sustaining enzymes, specifically those enzymes with thiols at their active sites. It is established that isothiazolinones form mixed disulfides upon treatment with such species.[15]

Application

The principal isothiazolones are:

- Methylisothiazolinone (MIT, MI)
- Chloromethylisothiazolinone (CMIT, CMI, MCI)
- Benzisothiazolinone (BIT)
- Octylisothiazolinone (OIT, OI)
- Dichlorooctylisothiazolinone (DCOIT, DCOI)
- Butylbenzisothiazolinone (BBIT)

These compounds all exhibit antimicrobial properties. They are used to control bacteria, fungi, and algae in cooling water systems, fuel storage tanks, pulp and paper mill water systems, oil extraction systems, wood preservation, and some paints. They are antifouling agents. They are frequently used in shampoos and other hair care products.[5]

Selected Preservative

METHYLISOTHIAZOLINONE

Definition

Methylisothiazolinone, **MIT** or **MI**, is the organic compound with the formula $S(CH)_2C(O)NCH_3$. It is a white solid. Isothiazolinones, a class of heterocycles, are used as

biocides in numerous personal care products and other industrial applications. MIT and related compounds have attracted much attention for their allergenic properties, e.g. contact dermatitis.

In 2010, the Panel published a final report of the safety assessment of methylisothiazolinone (MI) with the conclusion that "MI is safe for use in cosmetic formulations at concentrations up to 100 ppm (0.01%)." 1 At the March 2013 CIR Expert Panel meeting, the Panel reviewed newly provided clinical data indicating a higher than expected frequency of individuals who have allergic reactions to the preservative MI. In some cases, comparative data were available indicating a higher frequency of positive reactions than currently seen with the combination preservative, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI). The Panel reopened this safety assessment to gather and evaluate additional data.[6]

Chemical Structure

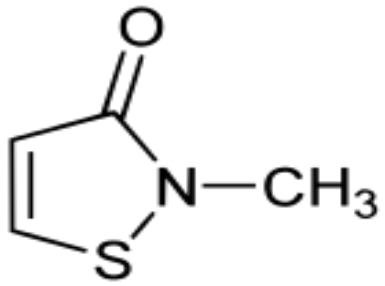


Figure 3: Methylisothiazolinone.[10]

Preparation: It is prepared by cyclization of *cis*-N-methyl-3-thiocyanoacrylamide:[6]

$NCSCH = CHC(O)NHCH_3 \rightarrow SCH = CHC(O)NCH_3 + HCN$

Preferred IUPAC name

2-Methyl-1,2-thiazol-3(2*H*)-one

Application

Methylisothiazolinone is used for controlling microbial growth in water-containing solutions. It is typically used in a formulation with 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), in a 3:1 mixture (CMIT:MIT) sold commercially as *Kathon*. Kathon is supplied to manufacturers as a concentrated stock solution containing from 1.5–15% of CMIT/MIT. Kathon also has been used to control slime in the manufacture of paper products that contact food. In addition, this product serves as an antimicrobial agent in latex adhesives and in paper coatings that also contact food.[7]

TOXICOLOGICAL STUDIES

Acute Toxicity: In acute oral toxicity studies, MI was slightly toxic in rats in concentrations ranging from 9.69% to 99.7%. At 9.69%, the LD50 for male and female rats was 274.6 and 105.7 mg/kg body weight, respectively. Rats that died during these studies had reddened intestines and/or stomach mucosa, clear or red/yellow fluid in the intestines and/or stomach; blackened intestines and distended stomachs. Studies on body lotion, shampoo, and sunscreen formulations in rats containing 100 ppm MI found no treatment related effects and an LD50 greater than 2000 mg formulation/kg body weight. Slight toxicity, including gastrointestinal changes, was observed in mice that orally received 97.5% MI. The LD50 was 167 mg/kg body weight. An acute oral toxicity study of the metabolite NMMA found the substance slightly toxic.

The calculated oral LD50 for NMMA in males and females was 3550 and 4100 mg/kg body weight, respectively. MI at 97.5% was slightly toxic in rats in an acute dermal toxicity study. The substance was corrosive to the skin. The LD50 was calculated to be 242 mg/kg body weight. In another acute dermal toxicity study, 9.69% MI was corrosive to rat skin, but no deaths occurred during the study. The LD50 was greater than 484.5 mg/kg body weight. Acute inhalation toxicity studies in rats found that 53.52% and 97.8% MI were slightly toxic after 4 h exposures. The LC50 were 0.35 and 0.11 mg/L. Rats that died during these studies had reddened lungs and distended gastrointestinal tracts. Mice exposed to 10 minutes of atomized 98.6% MI had up to 47% decrease in respiratory rates that equated to moderate responses for sensory irritation.[7]

Repeated Dose Toxicity: No toxic effects were observed when 97.5% MI was administered to rats in drinking water for 13 weeks at concentrations of 0, 75, 250, or 1000 ppm. Dogs that were fed diets prepared with 51.4% MI for 3 months had a NOAEL of 1500 ppm. In a subchronic study, rats fed the metabolites NMMA [and malonic acid (MA), up to 220 ppm and 44 ppm in the diet, respectively]* for 3 months had no effects observed in body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, or gross pathologic changes. Beagle dogs that received these metabolites [up to 500 ppm NMMA and 100 ppm MA]* in their diets for 3 months had no systemic toxicity. Bracketed text presents corrections to the original report.[7]

Reproductive and Development Toxicity: In a teratogenicity study, MI was administered by daily single oral doses to pregnant rats at doses of 5, 20, or 60 (reduced to 40) mg/kg body

weight/day on gestation days 6-19. Females in the high dose group had clinical signs of rales, gasping, and labored breathing and at necropsy had red areas in the glandular portion of the stomach and lungs. No treatment-related effects were observed in the fetuses. The maternal and developmental NOAEL were 20 mg/kg/day and 40 mg/kg/day, respectively. In a teratogenicity study of MI in rabbits, pregnant females received daily single oral doses of 3, 10, or 30 mg/kg/day MI on gestation days 6-28. Maternal effects in the 30 mg/kg/day group included decreased defecation and dark red areas in the stomach. The maternal NOAEL was 10 mg/kg/day. No treatment-related effects were observed in the fetuses and the developmental NOAEL was determined to be 30 mg/kg/day. A two-generation reproduction toxicity test found that MI in drinking water at concentrations up to 1000 ppm was not a reproductive toxicant.[7]

CARCINOGENICITY: Studies of the carcinogenicity of the sole ingredient MI were not available; however, a 2 year drinking water study in rats concluded that the mixture MCI/MI tested up to 300 ppm was not a carcinogen.[7]

GENOTOXICITY: MI (up to 1000 µg/plate) and the metabolite NMMA (up to 5000 µg/plate) were not mutagenic in the Ames test when tested with and without metabolic activation. In a Chinese hamster ovary cell assay, 97.5% pure MI was non-mutagenic when tested with and without metabolic activation (0.5 - 40.0 µg/ml). However, another CHO assay that studied MI at 97.5% a.i. (0.0785 - 5000 µg/ml) found significant increases in cells with chromosome aberrations, with and without metabolic activation. The aberrations were accompanied by significant cytotoxicity, which may have caused a false positive in this assay. MI was non-mutagenic in an unscheduled DNA synthesis assay and in a micronucleus test.[7]

NEUROTOXICITY: An acute in vitro neurotoxicity study of MI (up to 300 µM) in embryonic rat cortical neurons and glia observed widespread neuronal cell death within 24 h in the cortical cultures. Gliotoxicity was low. A 14-hour in vitro neurotoxicity study of MI (up to 3.0 µM) from the same laboratory concluded that prolonged exposure to MI and related isothiazolones may damage developing nervous systems. However, no evidence of neurotoxicity has been observed in vivo.[7]

IRRITATION AND SENSITIZATION

Irritation

Non-Human- A bovine cornea study classified MI [neat] as mildly irritating. Ocular irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm MI found the formulations non-irritating in rabbit eyes. Undiluted 97.8% MI was corrosive to intact rabbit skin after an exposure period of 1 h. Rabbit dermal irritation studies of MI at 9.69% and 10% concluded the chemical was non-irritating. In EpiDerm skin constructs, 1.7% MI applied for 3 or 60 minutes were non-corrosive. In the same study, 51.5% MI was non-corrosive in the 3 minute exposure but corrosive at the 60 minute exposure.

Human - A single 24-hour application of 100 ppm MI in 40 volunteer subjects did not produce skin irritation. Respective skin irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm MI also found MI to be nonirritating.[7]

ANALYTICAL METHODS USED TO DETERMINE PRESERVATIVES

As mentioned before, in order to protect consumer health and ensure compliance to existing government regulations, there is a practical demand for the development of analytical methods to identify and determine preservatives in cosmetics both accurately and sensitively. In spite of the relatively high number of preservatives used in cosmetics, and their restrictions, there are not many official analytical methods to control all these substances in these products. As mentioned in Section 2.1, within the EU framework there are several official analytical methods proposed to determine some preservatives in cosmetic products, which are described in different EU Directives and grouped in a book edited by the European Commission (1999) As was also mentioned in Section 2.1, there are no official methods for preservative determination published by FDA and Japanese authorities.

On the other hand, most papers on preservative analyses can be found in the analytical chemistry databases, most of which are focused on food products, given the major implications of these products have on health. However, a relatively high number of articles have reported the analysis of preservatives in cosmetic products for external use. For easy consultation, for each paper it shows the preservative studied, the cosmetic matrix, the employed analytical technique, a brief description of sample pre-treatment and, when present, the results of the analysis in terms of limit of detection (LOD) and limit of quantification (LOQ). In order to use or adapt a method cited in literature or develop a new one, it is necessary to know in which concentration preservatives are generally added and, if available,

the threshold concentration. As mentioned before, concerning the maximum permissible concentrations, some discrepancies exist between different legislations as well as the kind of cosmetic to which the preservative is added. So for example, the cosmetic ingredient review (CIR) expert panel from CTFA concluded that iodo-propynyl butyl-carbamate is safe as a cosmetic ingredient in concentrations lower than or equal to 0.1% (non-aerosolized products).

However, the Blue List of Germany's regulatory agency lists all this compound can give rise to unusual allergic reactions. Most cosmetic formulations require concentrations below 0.0125% of this preservative for proper preservation. In the EU the concentration approved when used as a preservative is up to 0.05% but it is not allowed in oral hygiene or lip-care products. Another point that should also be taken into account concerns the restrictions for a mixture of preservatives. For example, according to EU Cosmetics Directive, the maximum admitted concentration for parabens, expressed as p-hydroxybenzoic acid content, is 0.4% if only one paraben is present and 0.8% if a mixture of them is used.[8]

TREATMENT OF THE SAMPLE AND EXTRACTION

Preservative determination in cosmetics is often difficult due to matrix complexity, therefore great care must be devoted to developing suitable extraction procedures and reliable evaluation of the mean recovery values. The procedure used to extract preservatives from cosmetics depends on the nature of the products (emulsion, cream, shampoos, etc.) and also the characteristics of the analytical techniques to be employed to determine the active substances. For chromatographic analyses, based on liquid, gas or thin layer chromatographic techniques, it is generally possible to dilute aqueous samples in a suitable solvent or extract target compounds with organic or hydro-organic mixtures. For example, Hashim et al. (2005) determined different parabens contained in different products (gel, creams, lotions, etc.) by simple vortex extraction with a water/acetonitrile mixture (under heating) and analysed by liquid chromatography (LC).[21,24]

However, sometimes, the extraction process also needs a clean-up step using a suitable solid-phase extraction (SPE) sorbent, as done by Matissek (1986) or Hild (1993), for example, who employed silica gel or octadecyl silica cartridges to clean-up thiazolone type preservatives, respectively, or Baltes and Hirsemann (1986), who used a cation exchange sorbent to determine basic preservatives. By choosing the correct volume of eluting solvent, this step can also represent a pre-concentration process. The clean-up is necessary to determine preservatives in complex matrices or heterogeneous samples, in which interference problems

are likely. To a lesser extent, supercritical fluid extraction (SFE) with carbon dioxide as extractant (e.g. Wang and Chang, 1998) or solid-phase micro-extraction (SPME) (e.g. Rivero and Topiwala, 2004), which do not require great amounts of solvent, have been also used for preservative determination. The organic solvents most commonly used in the extraction–preconcentration step and sample dilution are hexane, methanol, ethyl ether, ethyl acetate and acetone.

When determination involves the separation of compounds with different acid–base properties, i.e. acidic, basic and neutral analytes, separation occurs as a function of Ph: acidic and basic molecules are extracted making use of hydro-organic mixture also containing acids or bases while neutral species are dissolved in organic phases. The first pre-treatment procedure for non-aqueous formulations is represented by the homogenisation of the sample that is obtained by stirring, ultrasonication or centrifugation and filtration. Sometimes, heating or cooling processes are employed, to favour substance transport between phases or to induce precipitation.[8,19,22]

Mostly Uses Analytical Techniques

The most commonly used methods to determine congeners or classes of preservatives. However, one must bear in mind that cosmetic formulations very often contain mixtures of preservatives belonging to different chemical classes and characterized by different functional groups. Therefore, multicomponent analysis methods are required. In this sense, chromatographic techniques are those most commonly used to determine preservatives in cosmetic products. Within this group of techniques, LC is the most commonly used technique to separate and determine preservatives, in particular both ion-pair and reversed-phase LC with UV/V is detection.[22,23]

Thin layer chromatography (TLC), as well as capillary electrophoresis (CE) and capillary zone electrophoresis (CZE) have been widely used. Recently, CE has become a popular separation technique in preservative analysis, used to determine both charged and hydrophobic compounds after addition to the running buffer of a surfactant or a modifier. Also, papers have been published related to gas chromatography (GC) with flame ionization detector (FID), electron capture detector (ECD) or mass spectrometry (MS) detector used for preservative determination. In summary, according to the analysis to be done, the kind and concentration level of the preservatives of interest, the matrix in which they are contained, the degree of accuracy required and the instrumentation available, readers can adapt the most

suitable methodology to their individual needs, including the extraction step and the analysis itself.[8,25]

Analytical techniques[25,26]

1. Titrimetric techniques
2. Chromatographic techniques

a) Thin layer chromatography
b) High performance thin layer chromatography
c) High-performance liquid chromatography (HPLC)
d) Gas chromatography

3. Spectroscopic techniques

Spectrophotometry[18]

Another important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions (Gorog, 1995). Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years (Tella et al., 2010; Venugopal and Sahi, 2005; Sharma et al., 2008; Ieggli et al., 2005). The colorimetric methods are usually based on the following aspects: Complex-formation reaction. Oxidation-reduction process. A catalytic effect. It is important to mention that colorimetric methods are regularly used for the assay of bulk materials. For example, the blue tetrazolium assay is used for the determination of corticosteroid drug formulations (Gorog and Szasz, 1978; Gorog, 1983).

The colorimetric method is also exploited for the determination of cardiac glycosides and is presented in European Pharmacopoeia. Several approaches using spectrophotometry for determination of active pharmaceutical ingredients in bulk drug and formulations have been reported and details of these methods are recorded. Derivative spectroscopy uses first or upper derivatives of absorbance with respect to wavelength for qualitative investigation and estimation. The concept of derivatizing spectral data was first offered in the 1950s, when it was shown to have many advantages. However, the technique received little consideration primarily due to the complexity of generating derivative spectra using early UV–Visible spectrophotometers. The introduction of microcomputers in the late 1970s made it generally

convincing to use mathematical methods to generate derivative spectra quickly, easily and reproducibly. This significantly increased the use of the derivative technique.

The derivative method has found its applications not only in UV spectrophotometry but also in infrared (McWilliams, 1969), atomic absorption, fluorescence spectrometry (Snelleman et al., 1970; Konstantianos et al., 1994), and fluorimetry (O'Haver, 1976; John and Soutar, 1976). The use of derivative spectrometry is not restricted to special cases, but may be of advantage whenever quantitative study of normal spectra is problematic. Disadvantage is also associated with derivative methods; the differential degrades the signal-to-noise ratio, so that some form of smoothing is required in conjunction with differentiation (O'Haver and Begley, 1961).

A. Near infrared spectroscopy (NIRS)
B. Nuclear magnetic resonance spectroscopy (NMR)
C. Fluorimetry and phosphorimetry
D. Electrochemical methods
E. Kinetic method of analysis
F. Electrophoretic methods
G. Flow injection and sequential injection analysis
H. Hyphenated technique

VALIDATION TECHNIQUE[25,26,27,28]

Validation is a fundamental requirement under Good Laboratory Practices and it is equally important to maintain documentary records of all such activities. It is mandatory to validate the performance parameters as suggested in the article. Besides these some additional performance tests will ensure results that would be acceptable in any other laboratory.[9]

Wavelength Accuracy: Wavelength accuracy establishes the closeness of the recorded wavelength to the true value. It can be established by using either absorption or emission standards.[9]

Accuracy

The ability of an instrument to measure the accurate value is known as accuracy. In other words, it is the closeness of the measured value to a standard or true value. Accuracy is obtained by taking small readings.[16]

Precision

The closeness of two or more measurements to each other is known as the precision of a substance.[16]

Repeatability

The variation arising when the conditions are kept identical and repeated measurements are taken during a short time period.[16]

Reproducibility

The variation arises using the same measurement process among different instruments and operators, and over longer time periods.[16]

Linearity

Linearity is an indicator of the consistency of measurements over the entire range of measurements. In general, it is a good indicator of performance quality of a sensor, but on its own, it can be a misleading indicator. In simple terms, linearity tells us how well the instrument measurement corresponds to reality.[16]

MATERIALS AND METHOD

Materials

Methylisothiazolinone containing products were procured from the local pharmacy. Methanol & Sodium phosphate buffer were used throughout the process.

Instruments

Shimadzu double beam UV – visible spectrophotometer 1900i Ultra with matched pair Quartz cells corresponding to 1cm path length & spectral bandwidth of 1mm. Bath sonicator & Electronic weighing balance. Magnetic stirrer machine, Beaker, filtration apparatus was used.

Selection of solvent

- **Trial-1**: The sample was treated with water at first but there were lots of impurities were found and can't get the spectra.
- **Trial-2**: Then the sample was treated with ethanol & still the spectra was not found due to presence of other impurities.
- **Trial-3**: Then it was treated with Methanol but the spectra was incorrect.

- **Trial-4**: After that a solution of Methanol & Sodium phosphate(30:70) buffer was used and the sharp peak was found at 270nm.

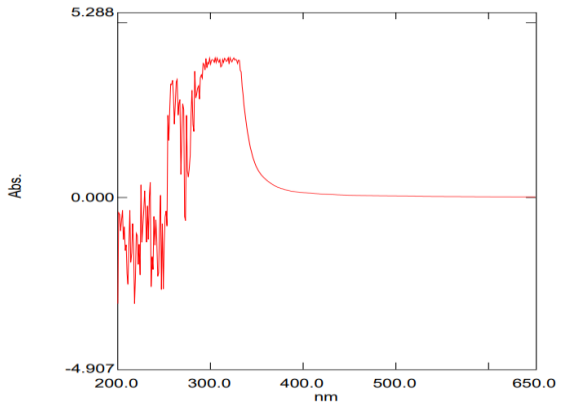


Figure 4: Spectrum of MIT with water

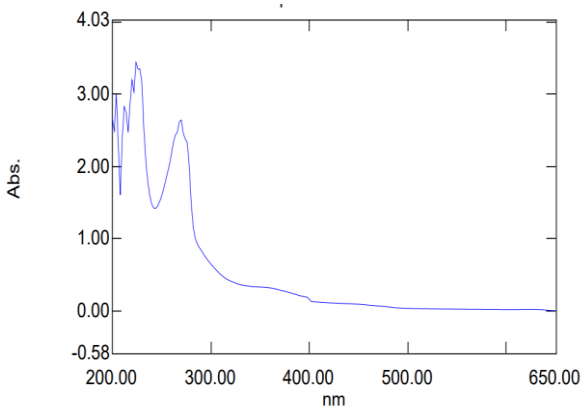


Figure 5: Figure: Spectrum of MIT with Methanol: Phosphate buffer.

Preparation of standard & sample solution

Stock solution

1gm Methylisothiazolinone Accurately weighed & transferred into a 1000ml volumetric flask and diluted up to the mark with the mixture of methanol and sodium phosphate buffer. Then 10ml Methylisothiazolinone stock solution was transferred to the 100ml volumetric flask and diluted up to the mark & was sonicated for 25-30 min., then a series of solution were prepared by 2, 4,6,8, 10 ml of diluted solution into 10ml volumetric flask and diluted with the methanol and sodium phosphate buffer solution.

Method of Validation- The Proposed method was developed by using linearity, accuracy, precision & ruggedness as per ICH guidelines, 1996.

Linearity: The linearity of the proposed assay was studied in the concentration of 2-8ppm at 270nm. The calibration data showed a linear relationship between concentrations.

Table 1: Linearity.

Sr.no	Sample concentration	Absorbance
1	20µg/ml	0.642
2	40µg/ml	1.300
3	60µg/ml	2.094
4	80µg/ml	2.860
5	100µg/ml	2.970
6	R^2	0.9612

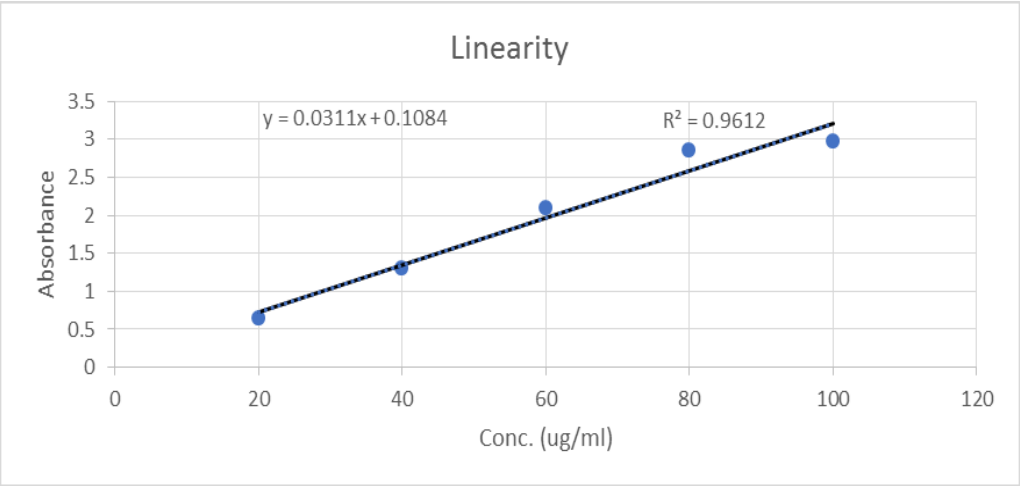


Figure 6: Graph of Linearity.

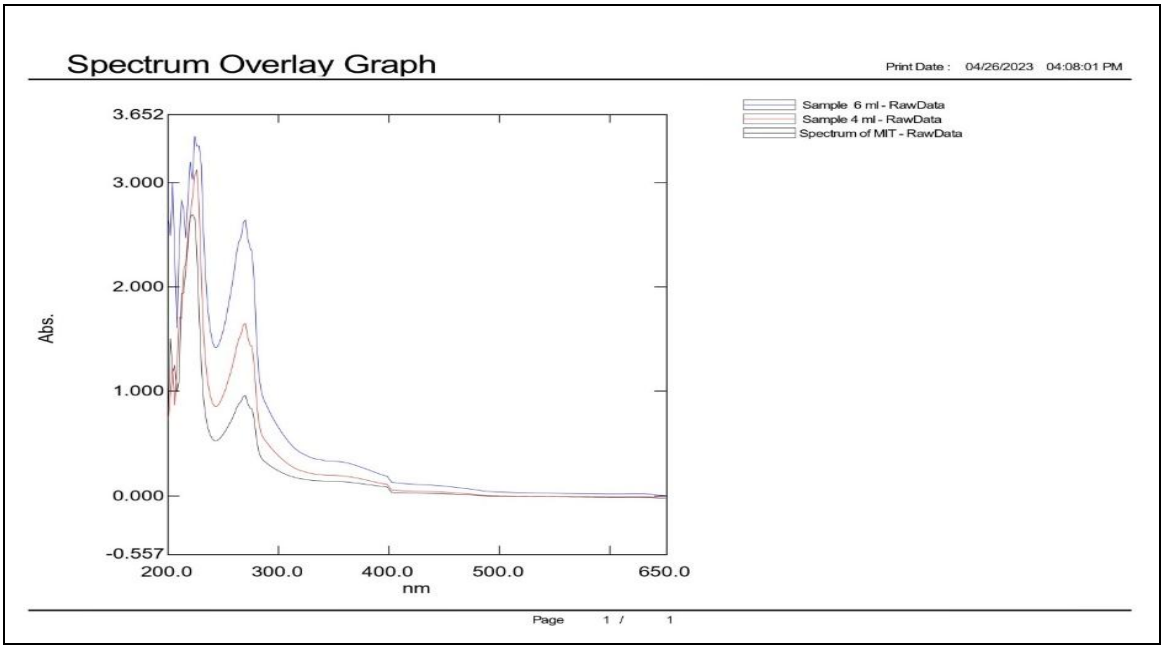


Figure 7: Overlay Spectrum of linearity range.

Precision

The precision of the method was demonstrated by inter- day and intra – day. Ten sample Solution were made and the % RSD was calculated.

Table 2: Intraday Precision and Inter day Precision data.

	Intraday Precision		Inter day Precision	
Sr.no	Sample	Absorbance	Sample	Absorbance
1	40ug/ml	1.603	40ug/ml	1.623
2	40ug/ml	1.641	40ug/ml	1.511
3	40ug/ml	1.645	40ug/ml	1.595
4	40ug/ml	1.640	40ug/ml	1.592

5	40ug/ml	1.650	40ug/ml	1.625
6	40ug/ml	1.652	40ug/ml	1.597
7	40ug/ml	1.656	40ug/ml	1.598
8	40ug/ml	1.655	40ug/ml	1.613
9	40ug/ml	1.650	40ug/ml	1.589
10	Sample 10	1.665	Sample 10	1.578
	MEAN	1.6457	MEAN	1.5921
	%RSD	0.015875	%RSD	0.01968

Repeatability

Table 3: Repeatability Data.

Sr.no	Sample	Absorbance
1	40ug/ml	1.603
2	40ug/ml	1.641
3	40ug/ml	1.645
4	40ug/ml	1.640
5	40ug/ml	1.650
6	40ug/ml	1.652
7	40ug/ml	1.656
8	40ug/ml	1.655
9	40ug/ml	1.650
10	40ug/ml	1.665
	MEAN	1.6457
	%RSD	0.015875

Recovery Study

Table 4: Recovery Study Data.

Sr.no	Sample name	Recovery level	Label claim	Amount of drug added	Absorbance	Recovery (%)
1	Dove Shampoo	· 80%	· 10mg	· 8mg	1.297	99.23
		· 100%	· 10mg	· 10mg	1.562	100.16
		· 120%	· 10mg	· 12mg	1.785	99.76
2	Pantene Shampoo	· 80%	· 10mg	· 8mg	1.431	99.14
		· 100%	· 10mg	· 10mg	1.723	99.93
		· 120%	· 10mg	· 12mg	1.979	100.32
3	Tresemme Shampoo	· 80%	· 10mg	· 8mg	2.153	98.76
		· 100%	· 10mg	· 10mg	2.382	99.58
		· 120%	· 10mg	· 12mg	2.517	101.38

Application of developed method on Marketed preparations containing methylisothiazolinone

Sample solution

Marketed product containing Methylisothiazolinone like Shampoo, Facewash were procured from the local pharmacy shop. 5ml sample was diluted with the methanol and sodium

phosphate buffer solution (15:35 ml) to make the solution of 50ml. Then it was placed on magnetic stirrer for 1 min after that the solution was sonicated for 25-30min. After sonication the beaker was cool down and was filter by Whattman filter paper. Then a 4 ml stock sample was prepared in 10ml volumetric flask and volume makeup to get concentration of 40ug/ml for each marketed preparation.

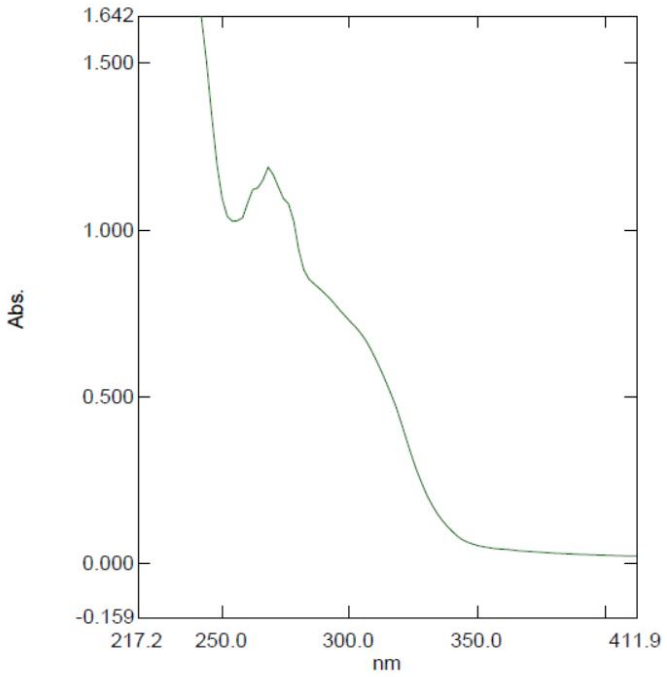


Figure 1: Spectrum of Sample (Shampoo).

Assay of Marketed Product

Table 5: Assay of Marketed Product.

Sr. No.	Sample (40 µg/ml)	Absorbance at 274nm	% Assay
1	Dove Shampoo	1.450	88.14 %
2	Pantene Shampoo	1.245	75.68 %
3	Tressemee Shampoo	2.374	83.52 %

RESULT AND DISCUSSION

Solubility of Methylisothiazoline

Methylisothiazoline was found soluble in Methanol, Acetonitrile, Chloroform.

Melting point of Methylisothiazoline

The melting point of methylisothiazoline was found to be 254-256^{o}C.

Linearity: The regression equation for the calibration plot was y = 0.0311x + 0.1084 and Regression Coefficient R^2 = 0.9612. These results showed there was a good linear relationship between absorbance and the amount of analyte in the range studied.

Accuracy: The calculated recovery and percentage recovery values were (99.96 % - 100.7 %). Percent Recovery was within range, indicates that the method is accurate. Precision:

Intra-day precision and Inter-day precision were within the acceptable range indicative of good method precision.

CONCLUSION

The proposed UV Spectrophotometric method can considered simple, fast and economical which is also applied in many studies such as determination of Methylisothiazolinone in cosmetic formulations such as face wash, shampoo etc. The method is valid compliance with ICH guidelines and appropriate for estimation of methylisothiazolinone with excellent linearity, Intra da precision, inter-day precision and repeatability.

The proposed methods are used for the routine analysis of the drugs in the quality control. In view of these facts, the found method could be a subject of further investigations for developing a selective and accurate method for quality control.

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