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A Review on: Validated Analytical Methods for Determining Preservative Content in Different Dosage Forms

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ABSTRACT

The purpose of this review is to present a thorough summary of the approved analytical techniques for figuring out how much preservative is present in various dosage forms. To maintain product stability and stop microbiological growth, preservatives are frequently used in pharmaceutical and cosmetic products. However, their presence and concentration must be carefully monitored to ensure safety and efficacy. The review discusses various analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and spectrophotometry, which have been widely employed for the determination of preservatives in different dosage forms including oral liquids, ophthalmic solutions, and topical creams. The review also emphasizes the significance of method validation by regulatory guidelines, emphasizing the importance of specificity, linearity, accuracy, precision, and robustness. Also discussed is the importance of sample preparation and extraction methods for intricate matrices. In summary, this review offers significant insights into the present condition of analytical techniques for figuring out the amount of preservatives in various dosage forms. When it comes to the creation and quality assurance of pharmaceutical and cosmetic products, researchers, analysts, and regulatory agencies can all benefit from it.

INTRODUCTION

Preservatives can be manufactured or natural substances that are applied to a variety of items, including paints, meals, medicines, wood, biological samples, and so on, to stop them from decomposing due to microbial growth or undesired chemical changes. Preservatives are necessary for products with high water content to prevent microbial changes and spoilage during storage. Chemicals known as preservatives are regularly added to a variety of foods and medications to prolong their shelf life. Preservatives are used in food to inhibit the growth of potentially harmful microorganisms such as bacteria, yeast, and molds, thereby preventing spoilage and extending the shelf life of the product. Natural foods may lose all of their nutritional content when they are processed, since they may be boiled, mashed, or given chemicals. Then, frequently, the product has additives added to it to restore some nutrients and vitamins lost during food preparation. [1]

Salt and vinegar were the first preservatives to be used, as well as sugar. Nowadays, a large number of preservatives are synthetic compounds. Although most synthetic food and cosmetic additives are generally regarded as safe, some are harmful and cancer-causing, so it is advisable to limit their usage. It is best to stay away from food coloring that is made of artificial chemicals, as they are all potentially dangerous. The effectiveness of antimicrobial preservatives is directly related to their ability to kill living cells, meaning that their toxicity is a crucial factor in determining their usefulness. [1][2][3][4][5]

Ideal Properties of Preservatives: -

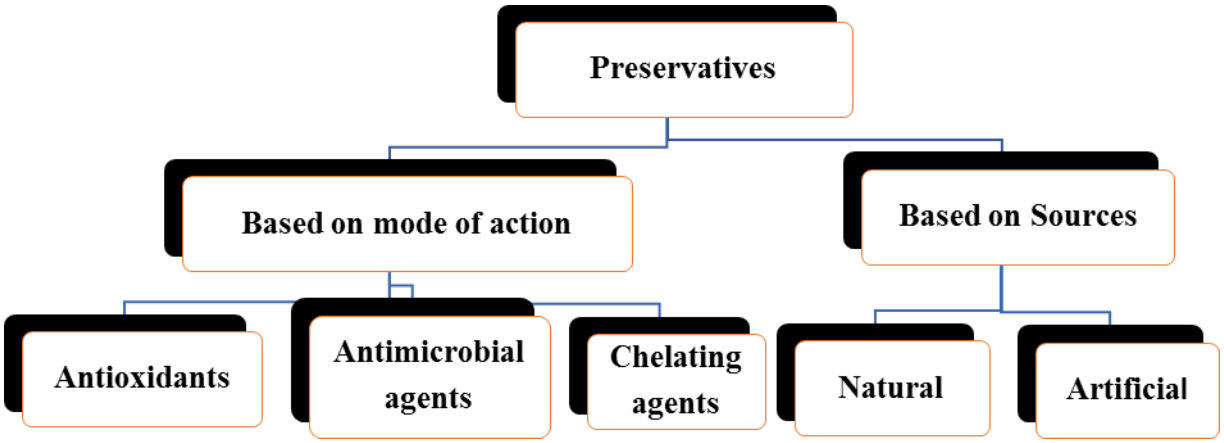
1. It must possess potency to function as a preservative at low concentrations.
2. It should have a broad range of activity and function well as an antimicrobial agent.
3. It should continue to operate during the production, storage, and use of the product.
4. Both chemically and physically, it ought to be stable.
5. It must not irritate.
6. It shouldn't be poisonous.
7. It must be powerful enough to function as a preservative at low concentrations.

8. Compatibility with other ingredients in the formulation is important for the preservative. [3]

Need for Preservatives

1. To enhance the drug's effectiveness and activity.
2. To prevent microbes from attacking our medication.
3. To maintain our product's stability.
4. To improve our product's shelf life.

Classification of Preservatives:-



❖ **Classification according to the mode of action**

A. Antioxidants

➢ Pharmaceutical products contain antioxidants to stop oxidation-related degradation.

➢ For example, substances such as Vitamin E, Vitamin C, Butylated hydroxyanisole (BHA), and Butylated hydroxytoluene (BHT) are effective due to their ability to eliminate living cells. This is because their toxicity plays a significant role in determining their efficacy.

➢ Antioxidants are divided into three categories.

a) Antioxidant synergists

b) True antioxidants

c) Reducing agents

a) Antioxidant synergists

Antioxidant synergists are substances that, while not having strong antioxidant properties on their own, can enhance the antioxidant activity of other compounds by reacting with heavy metal ions that accelerate oxidation. This interaction can boost the overall effectiveness of the primary antioxidants. Antioxidant synergists include citric acid, tartaric acid, lecithin, and editic acid and its salts.

b) True antioxidants

True antioxidants, also known as anti-oxygen, most likely stop oxidation by interacting with free radicals and interrupting the sequence of events. Among those alkygallates are tocopherols, nordihydroguaiaretic acid, butylated hydroxyanisole, and butylated hydroxytoluene.

c) Reducing agents

Reducing agents, which have redox potentials that are lower than the medication or adjuvant, are designed to offer protection, making them more susceptible to oxidation. Free radicals can interact with reducing agents to produce an effect as well. There are several examples, such as ascorbic acid and the sodium and potassium salts of sulfuric acid.

B. Antimicrobial Agents

The preparations contain antimicrobial preservatives to either eliminate or prevent the growth of any microorganisms that may have been unintentionally introduced during production or usage. To maintain sterility during use, they are utilized in sterile preparations like multidose injections and eye drops. They could also be included in aqueous injections, which need to be prepared with aseptic measures because they cannot be sterilized in their final containers. According to British Pharmacopoeia (BP) 5, unless the monograph specifically specifies otherwise, radiopharmaceutical preparations in multidose containers are not required to have antimicrobial preservatives added. E.g. Benzoates Sodium, Benzoate Sorbate.

There are two primary subgroups of antimicrobial preservatives: antibacterial and antifungal. Ascorbic and benzoic acids, along with their salts, and phenolic substances such as parabens (butyl p-hydroxybenzoate, propyl, ethyl, and methyl) are examples of preservatives that have anti-fungal qualities. Biguanides, alcohols, phenols, mercurial, and quaternary ammonium salts are examples of preservatives that have antibacterial qualities.

C. Chelating Agents

Compounds that combine with therapeutic ingredients to create a complex that keeps the formulation from degrading. For example, polyphosphates, citric acid, and sodium ethylenediamine tetraacetic acid (EDTA). [4-6]

❖ **Classification according to the source**

A. Artificial Preservatives

In small quantities, these synthetically created preservatives exhibit antimicrobial activity against a variety of microorganisms. For example, benzoates: nitrites, propionets, and sodium benzoate sorbates.

B. Natural Preservatives

Animals, plants, minerals, and other natural sources are the sources of these medications. Examples include lemon, neem oil, and sodium chloride salt. [7]

Preservatives applied in Different Dosage Forms

Sr no	Formulation	Preservatives
1	Oral Formulation	Sodium Benzoate, Calcium Lactate, Methyl Paraben,Sodium and Potassium ,Propyl Paraben ,Sorbic acid.Ethyl Paraben
2	Dermal Formulation	Phenyl salicylate, Chlorhexidine,Thiomersal,Benzalkonium chloride,Imidurea,Chlorocresol,Cetrimide
3	Dental Formulation	Cetylpyridinium chloride,Methyl parabens,Sodium benzoate Benzoic acid,Ethyl parabens,Potassium sorbate
4	Ophthalmic Formulation	EDTA,Imidurea,Thiomersal,Benzoic acid,Boric acid,Chlorhexidine,Benzalkonium chloride
5	Nasal Formulation	Chlorobutanol,Benzalkonium chloride,EDTA,Potassium sorbate,Chlorocresol Azelastine Hydrochloride
6	Rectal Formulation	Methyl hydroxybenzoate,Benzoic acid,Chlorhexidine gluconate,Sodium benzoate Benzyl alcohol
7	Liquid Formulation	Sorbic Acid,Benzoic Acid,Bronopol,Sodium Benzonate,Propyl Paraben Propylene Glycol,Methyl Paraben
8	Semisolids Formulation	Dichlorobenzyl alcohol,Cetyl trimethyl ammonium bromide,Chlorocresol

Material and Methods

Propyl Paraben and Methyl Paraben

Method 1

Chemicals and reagents

Analytical-grade chemicals were guaranteed for every chemical. Merck Germany provided the HPLC-grade methanol that was accepted. The USP Reference Standard was compared to the Propyl and methylparaben sodium working reference standards were acquired from Fine Chemicals and Pharmaceuticals, Rasula, India. The mobile phase and dilutions were made with highly purified water.

Chromatographic conditions

A 0.45µm membrane filter was used to filter a 35:65 (v/v) combination of water and methanol for the mobile phase. Shimadzu's HPLC C-18 column, named Shim-pack GIST, was used; its dimensions were 4.6 mm x 250 mm,5 µm. The mobile phase flow rate was calibrated at 1.3 mL/min, and the injection volume was 20 mL. The temperature of the

column was kept at 40 °C. Using a UV/visible detector, the analyte concentration was determined at a wavelength of 254 nm.

Preparation of Standard

In order to create the standard stock solution, a 100-mL volumetric flask was filled with 50 mg of propyl paraben sodium and 150 mg of methylparaben sodium. 70 milliliters of purified water were added after that., which is highly purified. Both materials were disintegrated and sonicated for 10 minutes. Well-mixed, ultra-purified water was used to make up the volume. A volumetric flask with a capacity of 50 milliliters was filled with 2 milliliters of the standard stock solution, diluted, and filtered through a 0.45-micron membrane filter to create additional dilutions. The standard concentrations used in the study were 0.02 mg/mL for propylparaben sodium and 0.06 mg/mL for methylparaben sodium.

Preparation of Sample

A 250-milliliter volumetric flask was partially filled with ultrapure water and six milliliters of iron protein succinate syrup to create the sample solution. The 0.45-micron membrane filter was used to filter the volumetric flask. following its placement on a vortex mixer and shaking for about a minute to produce a homogenized solution. [8]

Method 2

Chemicals and reagents

The Indian pharmaceutical company Hetero Drugs provided us with pharmaceutical-grade levetiracetam with a purity of 99.9%. We purchased methyl and propyl parabens from Clariant in Switzerland. 85% orthophosphoric acid and potassium dihydrogen phosphate (Merck, USA), HCl, NaOH, H2O2, and HPLC-grade methanol and acetonitrile were used. The entire trial was conducted using freshly double-distilled water. Egyptian International Pharmaceutical Industry Co., Egypt, is the source of levepsy syrup. from the nearby market, which was labeled to include 100 mg/mL of levetiracetam.

Chromatographic conditions

A Metrohm 744 pH meter was used to adjust the mobile phase PH. Employing an analytical column featuring a reversed-phase C18 Hypersil BDS (Waters, Milford, MA, USA) 150 mm

× 4.6 mm, 3.5 µm, HPLC was used to separate levetiracetam (LTC), propyl paraben (PHB), and methyl paraben (MHB). The following gradient mobile phase system was used: (A) acetonitrile: 0.05 M phosphate buffer (90:10); (B) acetonitrile: 0.05 M phosphate buffer (40:60). The column's temperature was maintained at 40°C. 1.5 ML/min of flow rate adjustment was made. There was 10 µL of injection volume. At 240 nm, detection was accomplished. The mobile phases were filtered and degassed before being used with a 0.45 µm pore size Millipore membrane filter.

Preparation of Standard

Standard stock solution prepared in methanol in a volumetric flask of 100 milliliters. A mixed standard solution of PHB, MHB, and LTC was made in a volumetric flask of 100 milliliters with 1 mL of the preservative stock solution and 100 mg of precisely weighted LTC. A 100-mL methanol fill was added after it was vortexed. For LTC, PHB, and MHB, the final analyte concentrations were 1000, 2, and 20 µg/mL, respectively.

Preparation of samples

Methanol was added to a 100-milliliter volumetric flask after 1 milliliter of the syrup had been moved into it. [9]

Benzalkonium chloride

Method 1

Chemicals and reagents

The German company Merck provided the working standard of 100% pure benzalkonium chloride. glacial acetic acid and acetonitrile (HPLC Grade, SDFCL). Ingredients in filtered water include sodium chloride, sodium acetate, and disodium edetate (Charlo Erba). Examples of betaxolol are 0.5% ophthalmic solution and betaxolol standard (99% purity). obtained from Co. Sudan's Bash Pharma.

Chromatographic conditions

The injection volume is 100 µL, 2.0 mL/min is the flow rate., and 254 nm is the detection wavelength. The temperature inside the column oven is 30 °C, and the mobile phase consists of acetonitrile and 0.1 M sodium acetate (45:55).

Preparation of Standard

Weigh out 100 milligrams of the benzalkonium chloride standard precisely, and then pour it into a 100-milliliter volumetric flask. To prepare a solution containing 1000 µg/ml, fill the flask with purified water and sonicate for one minute.

Preparation of Sample

The placebo solution is made up of sodium edetate, sodium chloride, and betaxolol excipients but does not include benzalkonium chloride. [10]

Method 2

Chemicals and reagents

The working standards for benzalkonium chloride (BKC), phenyl ethanol (PEL), oxymetazoline (OXY), and mometasone (MON) were acquired from Sir Sayyed College in Aurangabad, India. Merck Ltd., Mumbai, India, provided us with sodium hydroxide, hydrochloric acid, hydrogen peroxide, triethylamine, anhydrous potassium dihydrogen orthophosphate, orthophosphoric acid, and HPLC-grade acetonitrile. and oxymetazoline (0.05% W/V) and mometasone furoate (0.05% W/V) nasal spray solution were combined with the preservative's phenyl ethanol (0.25% W/V) and benzalkonium chloride (0.02% W/V).

Chromatographic condition

Empower Software was used to operate an Agilent HPLC system (1100 series) that included a column compartment, auto-injector, quaternary pump, online degasser, and PDA detector. By using a 2.0 ml/min flow rate, a mobile phase comprising 50 mM KH_2PO_4 and acetonitrile in a 60:40 (v/v) ratio was employed. The injection volume was 20 µL; additionally, the

temperature in the column compartment was set to 40°C. All components have a relative absorption maximum in the UV spectrum at 215 nm.

Preparation of Standard

A mobile phase solution was prepared with 250 µg/mL of PEL, 50 µg/mL of MON and OXY, and 20 µg/mL of BKC.

Preparation of Sample

20 mL of mobile phase were added to a 50 mL volumetric flask until it was completely full after 5g of sample or 2.5 mg of MON, had been carefully weighed and transferred. After that, the sample was sonicated for about 20 min to blend the mobile phase and increase the volume to 50 milliliters. [11]

Potassium Sorbate

Method 1

Chemicals and reagents

Suriya Life Science provided pure chlorpheniramine maleate (CTM), Celanese and Germany provided PS, and the USP reference standard provided pure dichloromethorphan-HBr (DTM). In the sample matrix of cough syrup, CTM (2 mg), DTM (5 mg), and potassium sorbate (10 mg) are dissolved to produce the simulation of the cough syrup sample. A combination of HPLC grade and KH_2PO_4 chromatography using ion pairs of reagent sodium 1-heptane sulfonate from TCI was utilized to create aqua DM with high purity using Millipore. Before being used, a sonicator was used to degas the mobile phase and working solutions and filter them through a filter of 0.45 µm.

Chromatographic conditions

The mobile phase used a gradient elution system to extract acetonitrile up to 30% in 15 minutes. It contained acetonitrile (5:95, v/v) and phosphate buffer with a pH of 2.5, which included sodium-heptane sulfonate as an ion pair. At a 1 mL/min flow rate, using a 20 µL injection volume, a 35°C column temperature, and using 230 nm as the detection wavelength.

Preparation of Standard

A volumetric flask with a 50-milliliter capacity should be filled with 20 mg, 50 mg, and 10 mg of the CTM, DMP, and PS, respectively. After adding the methanol and phosphate buffer pH 2.5 (10:90) solution, sonicate for fifteen minutes in a bath Sonicator and make up the volume.

Preparation of Sample

Measure 80 mg CTM and 200 mg DTM in a 50-milliliter volumetric flask, then dissolve them in a methanol-phosphate buffer mixture (100:90) until the specified limit (solution A). Additionally, weigh 400 mg of KS in a separate 50-milliliter volumetric flask, and after that, dissolve them in the same blended solution until the designated limit (solution B). [12]

Method 2

Chemicals and reagents

The following products were obtained by applying the USP Pharmacopeia convention standards: (HCA) hydrocortisone acetate (99.3% approved purity), (PRM) pramoxine hydrochloride (99.8% approved purity), sorbic acid (99.8% approved purity), and potassium sorbate (PS) (100.0% approved purity). The phosphate buffer, orthophosphoric acid, and acetonitrile have been obtained in analytical/HPLC grade from EMD Millipore in Massachusetts, USA. Koptec, Pennsylvania, USA, was the source of the alcohol proof. Symbiotic Pharma Ltd., India, USP, and Indore were the suppliers of HCA contaminants. PRM contaminants were obtained from PA, TCI Chemicals, TRC Standards, Canada, Ontario, and the USA.

Chromatographic conditions

Mobile phase-A, comprising 10 mM potassium dihydrogen phosphate buffer (pH 7.5) and acetonitrile in a 95:5 (v/v) ratio, was used to separate PRM, HCA, and sorbate. Acetonitrile and water were mixed 900:100 (v/v) in phase B of the mobile phase. The gradient elution method was used with a 0.2 ML/min UPLC flow rate. When using the Acuity UPLC, 1.7 µm, 150 mm × 2.1 mm, BEH column, C18, the temperature was 30 °C. Using a PDA detector, at 254 nm and 225 nm, respectively, HCA, PRM, and sorbate were found. Two distinct types of diluents were used to prepare the sample, using a 1 µl injection volume.

Preparation of Standard

To create the composite standard, diluent-2 was used to obtain the following concentrations: PRM at 50 µg/mL, HCA at 125 µg/mL, potassium sorbate at 5 µg/mL, and sorbic acid at 5 µg/ml.

Preparation of Sample

Fill a 50-milliliter volumetric flask with 31.25 mg of HCA. and sonicate for 20 minutes after adding 20 mL of diluent-1. Mix well and dilute with diluent to volume. Pass the filtrate through a 0.45 µm nylon syringe filter, discarding the first 3 millilitres. Using a pipette, transfer 5.0 millilitres of the preparation into a 25-milliliter volumetric flask. Dilute with diluent-2 to volume, then thoroughly mix. [13]

Benzyl alcohol

Method 1

Chemicals and reagents

Triethylamine, phosphoric acid, and HPLC-grade acetonitrile were produced by UK-based Fischer Scientific and obtained from their regional agent. Our labs had access to the working-standard materials that were utilized.

Chromatographic conditions

A Shimadzu Nexera LC-30AD system manufactured by Shimadzu Corporation located in Kyoto, Japan, with an SPD-20A UV detector, DGU-20A5 degasser, and LC-30 AD-LPGE pump was used for the analysis. 1µL of the samples was injected using a Shimadzu SIL-30AC auto sampler onto a 40°C-stored Thermo Scientific, USA; Hypersil BDS C18 column, 5 µm 4.6 mm x 150 mm. At 220 nm, the detection was finished. The mobile phase was supplied at a flow rate of 1.0 mL/min and ultrasonically degassed for five minutes via a membrane filter with a diameter of 0.22 µm. (Chromatech, UK) or an equivalent.

Preparation of Standard

500 µg/ml of benzyl alcohol and 200 µg/ml of tolfenamic acid were contained in the standard solution, made by dissolving standard substances (working standards) in the mobile phase, sonicating for five minutes, and diluting the mixture to the appropriate level with the mobile phase.

Preparation of Sample

Tolfenamic acid (20 mg) and benzyl alcohol (50 mg) were added to a precisely measured volume of the formula in a 50- 50-millilitre volumetric flask. Approximately thirty milliliters of mobile phase were added to this flask. After that, it was ultrasonically sonicated for five minutes to reduce the volume to fifty milliliters. 5-milliliter of the previous solution were diluted to produce 10-milliliter. [14]

Method 2

Chemicals and reagents

Fulvestrant provided the reference standard, according to Manasa Life Sciences. The filler injection was purchased from a store. Acetonitrile, methanol, water, benzyl benzoate, and benzyl alcohol were supplied by Merck.

Chromatographic conditions

Chromatographic separation of the drug and co-solvents was achieved with a Phenomenex Luna column C8, 150 × 4.6 mm, 3 µm. Deionized water in mobile phase (A), and acetonitrile were used in the mobile phase (B). The wavelength detection for benzyl benzoate and benzyl alcohol was found to be 254 nm, and for Fulvestrant, it was found to be 280 nm at a column temperature of 35 °C, 1.5 mL/min as the flow rate, and 10 µL of injection volume.

Preparation of Standard

150 mg of Fulvestrant reference standard, 450 mg of benzyl benzoate, and 300 mg of benzyl alcohol are in a 50-milliliter volumetric flask. Add 35 milliliter of methanol to the flask. Vortex the mixture for 5 minutes. Dilute the solution with methanol to reach the 50-milliliter volumetric mark. Thoroughly mix the solution.

Preparation of Sample

Separate 50-mL volumetric flasks should be used for the measurement of the 150 mg placebo and the Fulvestrant injection. To every flask, add 35 milliliters of methanol. Give every combination a five-minute vortex. To get the volume to 50 milliliters, dilute each of the two solutions separately with methanol. Add both solutions and stir well. [15]

Chlorhexidine gluconate

Method 1

Chemicals and reagents

Drogsan Pharmaceuticals generously provided Roben® (0.15% BH and 0.12% CG (w/v)) gargles, spray, and chlorhexidine gluconate. The sprays, Far-hex® and Orohex®, 0.12% chlorhexidine gluconate and 0.15% benzydamine hydrochloride (w/v), were kindly provided by neighborhood chemists. We bought acetonitrile from Riedel-de-Hain and HPLC-grade methanol from Merck. A Milli-Q water purification system was used to create ultrapure water.

Chromatographic conditions

Utilizing an analytical column of Nucleosyl 100-5 C18 (250 mm x 4.6 mm 5µm), chromatographic separation was carried out. With a 1 mL/min flow rate, the compounds were clustered in a mobile phase containing 40 mM triethylamine (TEA) with phosphate buffer and acetonitrile (10 mM, pH 3.0) at 65:35 v/v. At 230 nm, the UV detector was set. The Agilent 8453, in conjunction with the DAD UV-VIS spectrophotometer, was used to conduct the spectrophotometric analysis.

Preparation of Standard

BH and CG Standard stock solutions were made at 1000 µg/mL in MeOH. Using the mobile phase of the HPLC system, this stock was used to dilute working solutions. Additionally, 100 µg/mL of hydrochlorothiazide was added to these solutions as an internal standard. We sonicated each solution for ten minutes. For the UV derivative spectrophotometric analysis, in a volumetric flask, working solutions were quickly diluted with methanol.

Preparation of Sample

To analyze Sample Solutions preparations for HPLC, 20 µL of the preparation was taken, diluted in a volumetric flask to 1 milliliter, and then 20 µL of HCT (IS) was added to the vial. The preparation was taken down to 130 µL and diluted in a volumetric flask to 10 milliliters for UV analysis. The synthetic drug was made by mixing in 0.15% BH and 0.12% CG to the combination of placebos, which consisted of 20% sorbitol, 1% Lutron, 70% ethanol, and essential oils of mint and lemon. [16]

Method 2

Chemicals and reagents

The Greek military laboratories provided a chlorhexidine gluconate (CHG) 20% water solution and chlorhexidine (CE) powder with 97.7% purity. The CHA Secondary Reference Standard, obtained from the same source, is used for the preparation of the CHA solution. As required by the Military Health System (MHS) and the US Armed Forces, this solution is used for several purposes, including wound care. The preparation involves stirring the CE powder and CHG solution, and the final product is a chlorhexidine acetate solution suitable for medical use.

Chromatographic condition

A combination of MeOH and 50 mM $NaH_2PO_4 \cdot H_2O$ at a ratio of 85:15 v/v made up the mobile phase. Before using orthophosphoric acid to bring the pH of the aqueous solution down to 3.0, 0.2% Et_3N was added during its production. Because the process was conducted in an isocratic environment, the mobile phase's composition did not change. 205 nm was the detecting wavelength, and the flow rate was fixed at 1.0 mL/min. Finally, in a column oven, the temperature was set to 25°C., and a 10 µL injection volume was used.

Preparation of Standard

An 85:15 v/v MeOH-H2O diluent was used to dissolve the precisely weighed portions of CHA (75.0µg/mL) and CE (750µg/mL) that were placed in a 100-milliliter volumetric flask. At the appropriate ratios, standard solutions were obtained by diluting each stock. At 5°C, the solutions were kept in a refrigerator.

Preparation of Sample

With the same diluent, a precise volume of antiseptic solution (1 mL) was diluted in a volumetric flask measuring 100 milliliters to create a stock solution. This resulted in CE and CHG concentrations of 1500 µg/mL and 150 µg/mL, in that order. In a 20-milliliter volumetric flask, 2 milliliters of the stock were diluted to create the sample solution (test solution). Before starting each analytical step, a PVDF 0.45 µm syringe filter was used to filter the sample solution. [17]

Methyl Paraben and Ethyl Paraben

Method Parameters	Method 1	Method 2
Technique	RP-HPLC	RP-HPLC
Mobile Phase	Methanol: water (65:35)	(A) phosphate buffer : acetonitrile (90:10) (B) phosphate buffer : acetonitrile (40:60)
Column	C-18 4.6 mm x 250 mm, and 5 µm	C-18 150 mm × 4.6 mm, 3.5 µm
Column Temperature	40^0C	40^0C
Preparation of Standard	To create the standard stock solution, a 100-mL volumetric flask was filled with 50 mg of propyl paraben sodium and 150 mg of methyl paraben sodium. 70 milliliters of purified water were added after that., which is highly purified. Both materials were disintegrated and sonicated for 10 minutes. Well-mixed, ultra-purified water was used to make up the volume. A volumetric flask with a capacity of 50 milliliters was filled with 2 milliliters of the standard stock solution, diluted, and filtered through a 0.45-micron membrane filter to create additional dilutions. The standard concentrations used in the study were 0.02 mg/mL for propylparaben sodium and 0.06 mg/mL for methyl paraben sodium.	standard stock solution prepared in methanol in a volumetric flask of 100 milliliters. A mixed standard solution of PHB, MHB, and LTC was made in a volumetric flask of 100 milliliters with 1 mL of the preservative stock solution and 100 mg of precisely weighted LTC. A 100-mL methanol fill was added after it was vortexed. For LTC, PHB, and MHB, the final analyte concentrations were 1000, 2, and 20 µg/mL, respectively.
Preparation of Sample	A 250-milliliter volumetric flask was partially filled with ultrapure water and six milliliters of iron protein succinate syrup to create the sample solution. The 0.45-micron membrane filter was used to filter the volumetric flask. following its placement on a vortex mixer and shaking for about a minute to produce a homogenized solution.	Methanol was added to a 100-milliliter volumetric flask after 1 milliliter of the syrup had been moved into it
Flow rate(mL/min)	1.3	1.5
Injection volume	20 µL	10 µL
Detector	UV/Vis detector	UV/Vis detector
Detector Wavelength	254nm	240nm

Benzalkonium chloride

Method Parameters	Method 1	Method 2
Technique	HPLC	HPLC
Mobile Phase	acetonitrile:0.1M sodium acetate (45:55)	Acetonitrile: KH_2PO_4 (40:60)
Column	Column L10, CN 250 mm x 4.6mm ,10 µm	C-18 150 mm x 4.6mm, 5 µm
Column Temperature	30^0C	40^0C
Preparation of Standard	Weigh out 100 milligrams of the benzalkonium chloride standard precisely, and then pour it into a 100-milliliter volumetric flask. To prepare a solution containing 1000 µg/ml, fill the flask with purified water and sonicate for one minute.	A mobile phase solution was prepared with 250 µg/mL of PEL, 50 µg/mL of MON and OXY, and 20 µg/mL of BKC.
Preparation of Sample	The placebo solution is made up of sodium edetate sodium chloride and betaxolol excipients, but does not include benzalkonium chloride.	20 mL of mobile phase were added to a 50 mL volumetric flask until it was completely full after 5g of sample, or 2.5 mg of MON, had been carefully weighed and transferred. After that, the sample was sonicated for about 20 min to blend the mobile phase and increase the volume to 50 milliliters.
Flow rate (mL/min)	2	2
Injection volume	100 µL	20 µL
Detector	UV Visible detector	PDA detector
Detector Wavelength	245nm	215nm

Potassium Sorbate

Method Parameters	Method 1	Method 2
Technique	HPLC	UPLC
Mobile Phase	acetonitrile: phosphate buffer (5:95)	(A) Potassium dihydrogen phosphate buffer:acetonitrile (95:5) (B) acetonitrile:water (900:100)
Column	C-18 15 cm x 4.6 mm x 5 µm	C-18 150 mm x 2.1 mm x1.7 µm
Column Temperature	35^0C	30^0C
Preparation of Standard	A volumetric flask with a 50-milliliter capacity should be filled with 20 mg, 50 mg, and 10 mg of the CTM, DMP, and PS, respectively. After adding the methanol and phosphate buffer pH 2.5 (10:90) solution, sonicate for fifteen minutes in a bath sonicator and make up the volume.	To create the composite standard, diluent-2 was used to obtain the following concentrations: PRM at 50 µg/mL, HCA at 125 µg/mL, potassium sorbate at 5 µg/mL, and sorbic acid at 5 µg/mL.
Preparation of Sample	Measure 80 mg CTM and 200 mg DTM in a 50-milliliter volumetric flask, then dissolve them in a methanol-phosphate buffer mixture (100:90) until the specified limit (solution A). Additionally, weigh 400 mg of KS in a separate 50-milliliter volumetric flask, and after that, dissolve them in the same blended solution until the designated limit (solution B).	Fill a 50-milliliter volumetric flask with 31.25 mg of HCA. and sonicate for 20 minutes after adding 20 mL of diluent-1. Mix well and dilute with diluent to volume. Pass the filtrate through a 0.45 µm nylon syringe filter, discarding the first 3 milliliters. Using a pipette, transfer 5.0 milliliters of the preparation into a 25-milliliter volumetric flask. Dilute with diluent-2 to volume, then thoroughly mix.
Flow rate (mL/min)	1	0.2
Injection volume	20 µL	1 µL
Detector	UV Detector	PDA Detector
Detector Wavelength	230nm	254nm

Benzyl alcohol

Method Parameters	Method 1	Method 2
Technique	RP- HPLC	HPLC
Mobile Phase	Acetonitrile:50 mM triethylamine (70: 30)	0.01M monobasic phosphate with orthophosphoric acid:acetonitrile (1:1)
Column	C-18 4.6 mm x 150 mm, 5 µm	C-18 250 × 4.6 mm , 5 µm
Column Temperature	40^0C	35^0C
Preparation of Standard	500 µg/ml of benzyl alcohol and 200 µg/ml of tolfenamic acid were contained in the standard solution, made by dissolving standard substances (working standards) in the mobile phase, sonicating for five minutes, and diluting the mixture to the appropriate level with the mobile phase.	150 mg of Fulvestrant reference standard, 450 mg of benzyl benzoate, and 300 mg of benzyl alcohol are in a 50-milliliter volumetric flask. Add 35 milliliters of methanol to the flask. Vortex the mixture for 5 minutes. Dilute the solution with methanol to reach the 50-milliliter volumetric mark. Thoroughly mix the solution.
Preparation of Sample	Tolfenamic acid (20 mg) and benzyl alcohol (50 mg) were added to a precisely measured volume of the formula in a 50- 50-milliliter volumetric flask. Approximately thirty milliliters of mobile phase were added to this flask. After that, it was ultrasonically sonicated for five minutes to reduce the volume to fifty milliliters. 5-milliliter of the previous solution were diluted to produce 10-milliliter.	Separate 50-mL volumetric flasks should be used for the measurement of the 150 mg of placebo and the Fulvestrant injection. To every flask, add 35 milliliters of methanol. Give every combination a five-minute vortex. To get the volume to 50 milliliters, dilute each of the two solutions separately with methanol. Add both solutions and stir well.
Flow rate (mL/min)	1	1
Injection volume	10 µL	10 µL
Detector	UV detector	UV detector
Detector Wavelength	220nm	280nm

Chlorhexidine gluconate

Method Parameters	Method 1	Method 2
Technique	RP- HPLC	HPLC
Mobile Phase	40mM triethylamine containing phosphate buffer: acetonitrile (65:35)	A mixture of MeOH :50 mm $NaH_2PO_4.H_2O$ (85:15)
Column	C-18 250 × 4.6 mm,5µm	C-18 4.6 x 150 mm,5 µm
Column Temperature	100^0C	25^0C
Preparation of Standard	BH and CG Standard stock solutions were made at 1000 µg/mL in MeOH. Using the mobile phase of the HPLC system, this stock was used to dilute working solutions. Additionally, 100 µg/mL of hydrochlorothiazide was added to these solutions as an internal standard. We sonicated each solution for ten minutes. For the UV derivative spectrophotometric analysis, in a volumetric flask, working solutions were quickly diluted with methanol.	An 85:15 v/v MeOH-H_2O diluent was used to dissolve the precisely weighed portions of CHA (75.0µg/mL) and CE (750µg/mL) that were placed in a 100-milliliter volumetric flask. At the appropriate ratios, standard solutions were obtained by diluting each stock. At 5°C, the solutions were kept in a refrigerator.
Preparation of Sample	To analyze Sample Solutions preparations for HPLC, 20 µL of the preparation was taken, diluted in a volumetric flask to 1 milliliter, and then 20 µL of HCT (IS) was added to the vial. The preparation was taken down to 130 µL and diluted in a volumetric flask to 10 milliliters for UV analysis. The synthetic drug was made by mixing in 0.15% BH and 0.12% CG to the combination of placebos, which consisted of 20% sorbitol, 1% Lutron, 70% ethanol, and essential oils of mint and lemon.	With the same diluent, a precise volume of antiseptic solution (1 mL) was diluted in a volumetric flask measuring 100 milliliters to create a stock solution. This resulted in CE and CHG concentrations of 1500 µg/mL and 150 µg/mL, in that order. In a 20-milliliter volumetric flask, 2 milliliters of the stock were diluted to create the sample solution (test solution). Before starting each analytical step, a PVDF 0.45 µm syringe filter was used to filter the sample solution.
Flow rate (mL/min)	1	1
Injection volume	1 µL	10 µL
Detector	UV Visible detector	UV Visible detector
Detector Wavelength	230nm	271nm

Conclusion

In conclusion, the creation and verification of analytical techniques to ascertain the amount of preservatives present in various dosage forms is essential to guaranteeing the security and effectiveness of pharmaceutical goods. For this, several methods, including titration, UV-Vis spectroscopy, GC, and HPLC, have been effectively applied. Nevertheless, the kind of preservative, the dosage, and the legal requirements all play a role in choosing the right procedure. Analytical methods must be validated to show their robustness, accuracy, precision, specificity, and reliability. It is essential to validate methods in compliance with regulatory standards, such as those outlined by the FDA, USP, and ICH, to ensure that the results are accurate and repeatable.

In general, the creation and approval of analytical techniques for figuring out the amount of preservatives in various dosage forms need to give careful thought to several variables, including the preservative's physicochemical characteristics, the dosage form's suitability, and legal requirements. Pharmaceutical businesses can guarantee the quality and safety of their goods by utilizing appropriate methodologies and adhering to validation requirements.

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