Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i04.3978

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SOME TOPICAL ANTIMICROBIAL FORMULATIONS ACCORDING TO ICH GUIDELINES

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Abstract
The development of methods is essential for stability studies, drug testing, manufacturing processes, and long-term drug performance. The International Council for Harmonization offers standards for analytical method validation. Scientists all over the world are creating new techniques for the same with the least amount of time and money spent. Numerous investigations have been conducted in the past to develop methods for applying these topical antimicrobials; however, certain antimicrobials have not been addressed. The focus of this review article is on specific information about the techniques created for different antimicrobials.

Keywords: Method development, Validation, Antimicrobial formulations, ICH guidelines

Introduction

Skin that is in good health provides a strong natural defense against microbial invasion. If this barrier is compromised, a person may be more prone to infection. Therefore, cutaneous bacterial infections can result from a variety of physical traumas such as abrasions, penetrations, cuts, and burns; pre-existing dermatoses with impaired barrier states; undernutrition; diabetes mellitus; and various congenital and acquired immunodeficiency syndromes.

Community pharmacists frequently prescribe topical antimicrobial medications, especially for bacterial infections on the skin. It can be used for both prophylaxis and the treatment of superficial infections. Its function is to either kill or inhibit the growth of microorganisms. Nowadays, topical antibacterial medications come in a variety of forms, including liquid, gels, creams, ointments, and sprays. They can be combined with corticosteroids or other antibiotics, or they can contain just one antibiotic ingredient. A range of topical antibacterial agents are available from community pharmacies that may be used to treat acne vulgaris, pyoderma, including impetigo, folliculitis, rosacea, infected eczema, secondary infection from traumatic skin lesions, minor cuts and burns, and eye and ear infections. (Amirthalingam *et al*., 2015; Williamson *et al*., 2017).

Various infections and related antimicrobials

Skin infection

Tetracyclines have been a widely used systemic treatment for acne. Nevertheless, they are not very appropriate for topical application due to their chemical instability and propensity for oxidative degradation. Certain medications, like erythromycin and clindamycin, prevent the synthesis of both proteins and lipase, which makes them perfect for infections of this kind. Lipase is necessary for the

hydrolysis of serum triglycerides into glycerol, which is a substrate that Propionibacterium acnes, a common acne-causing agent, uses. Benzoyl peroxide and retinoids have a desquamating effect that increases the penetration of antibiotics, which justifies their combined use (Gamble *et al*., 2012).

Ear infections

When it comes to treating ear infections, topical antibiotics have been proven to be just as successful as oral ones. Topical and combination (intravenous and topical) ciprofloxacin were found to be equally effective in treating patients with chronic suppurative otitis media. Clinical cure rates of 65%–80% were noted within 10 days of treatment in a systematic review by Rosenfeld et al. assessing the use of topical antimicrobials for otitis externa, indicating a high efficacy. On the other hand, data from randomized controlled trials involving children who have grommets (a middle ear ventilation tube and ear discharge) clearly show that oral administration is not as effective as it is (Rodriguez *et al*., 1993).

Vulvo-vaginal infections

To treat bacterial vaginosis (BV), topical and oral formulations of metronidazole and clindamycin are utilized. Topical treatments have better compliance, less correlation with a subsequent Candida infection, and fewer gastrointestinal (GI) side effects (Vanić *et al*., 2021).

Dental infection

Topical antibacterial agents, such as povidone-iodine and chlorhexidine, reduce the development of early childhood caries in children who are at high risk. Gum infections are frequently treated with minocycline, metronidazole, slow-release doxycycline gels, and thin tetracycline strips positioned between the infected gum and tooth. Topical treatments provide relief to the affected gum tissue directly, in contrast to oral antibiotics. Topical amoxicillin is used to prevent endocarditis and lowers the post-procedural incidence of bacteraemia in patients undergoing dental procedures (Lopez *et al*., 1999; Mombelli and Samaranayake, 2004).

Forms of topical drug delivery

Paste

A concentrated mixture of powder, water, and oil.

Solution

Lotion or water with alcohol that has dissolved powder in it.

Lotion

usually regarded as thicker than a solution and more likely to include oil in addition to alcohol or water. Shake lotion must be shaken into suspension before use because it breaks down over time.

Cream

thicker than a lotion but still holding its form, like a 50/50 oil and water emulsion. needs a preservative to increase its shelf life. frequently hydrating.

Powder

Solid, like corn starch (a vegetable) or talc (a mineral).

Transdermal patch

The adhesive in the drug delivery system makes precise dosing possible.

Gel

A semisolid aqueous or alcoholic monophasic emulsion that liquefies when it comes into contact with skin; it is frequently based on cellulose. frequently contains fragrances and preservative (Ray *et al*., 2019; Paulson, 1999).

Factors affecting topical infection

Thin skin absorbs more than thick skin; skin thickness varies according to age, body location, and type of skin condition.

The function of the skin barrier is compromised by dermatitis, ichthyosis, and keratolytic agents (like salicylic acid), which means that medication is absorbed more readily by damaged skin than by healthy skin. • Where there is occlusion, like in skin folds, under dressings, or in greasy ointment formulations, the active ingredient is absorbed more readily.Lipophilic compounds are better absorbed than hydrophilic ones; higher concentrations of the active ingredient may penetrate more than lower concentrations; small molecules are more easily absorbed through the skin than large molecules;The formulation's other ingredients may work together to change the potency or rate of absorption. (Subedi *et al*., 2010; Kesarwani *et al*., 2013).

Classification of antimicrobial agents

Antifungal agent

Antifungal medications are used to treat fungal infections. Examples include ringworm, yeast infections, and infections of the skin and nails. Inhaling fungus spores can cause respiratory illnesses. People with compromised immune systems are more susceptible to fungal infections, which require the use of antifungal medications. Antifungals are medications that kill or stop the growth of the fungi that cause infections. Another name for them is antimycotic agents (Veterinary and Oltean, 2021).

Antibacterial

An antibacterial agent stops the growth and multiplication of bacteria. Right now, antibacterial agents are the most common. Described as materials that disinfect surfaces, get rid of potentially dangerous bacteria, and balance out household cleansers. Only in cases where clinical or laboratory data point to a bacterial infection should they be used (Aziz *et al*., 2019).

Antibacterial agents fall into two groups based on how well they work and how much residue they leave behind: First-class bacteria quickly destroy other bacteria, but they also disappear (evaporate or disintegrate) and leave no active residue in their wake (they are called non-residue-producing). Examples of this category include peroxides, alcohols, chlorine, and aldehydes (Esmatabadi *et al*., 2017).

Antiviral agents

Viral infections are treated with drugs in the antiviral medication class. Certain viruses infiltrate our bodies and give rise to a host of illnesses. Meanwhile, while most antivirals target particular viruses, broad spectrum antivirals combat a variety of viruses. Antiviral medications are mainly used to treat HIV, herpes, influenza A and B, and hepatitis B and C (Ianevski *et al*., 2022).

Anti-parasitic agent

Antiparasitic medications are used to treat and prevent infections brought on by helminths, ectoparasites, and protozoa. Numerous diseases caused by parasites are treated by different classes of anti-parasitic drugs. This activity explains how to treat diseases like scabies, pneumocystis, trypanosomiasis, and malaria with major anti-parasitic drug classes, including their uses, modes of action, side effects, and contraindications (Kandeel *et al*., 2022).

Analytical method development and validation for antimicrobial drugs

Need of method development and validation

- The current approach may be excessively expensive, require a lot of time or energy, or not be fully computerized.
- The current method may contain too many mistakes, be prone to infection, or be untrustworthy.
- There might be a desire for an opportunity technique to verify analytical records initially obtained through current strategies, for criminal or scientific purposes. The unique pattern matrix won't contain a method that works for a given analyte. The current strategy might not provide enough sensitivity.
- For requirements related to regulations, it's necessary.
- Main drug selection criteria for improving a brand-new analytical technique
- The medication or medication combination might not be consistent across pharmacopoeias.
- Because of patent regulations, it may not be possible to find the appropriate analytical method for the medication in the literature.
- The interference caused by the formula excipients may prevent analytical techniques for the drug from being available in the form of a formula. (Ravishankar *et al*., 2015).

Reported methods developed for estimation of various antimicrobial drugs

For anti -fungal drugs

Name of drug	Uses	Reported methods	References
Hydrocortisone and clotrimazole	Utilized to treat candidiasis and a range of dermatophyte infections.	The USP L1 (250 × 4.6) mm column has 5 µm-sized particles. For this investigation, acetonitrile and buffer are used as the mobile phase in a 75:25 ratio, respectively. One milliliter per minute was the constant flow rate. A UV detector was utilized to detect the drug at a wavelength of 254 nm.	(Iqbal *et al*.,2020)
Micafungin	Used to prevent Aspergillus and Candida infections in patients receiving hematopoietic stem cell transplantation, as well as to treat esophageal candidiasis.	Phenomenax Aeris peptide XB C18 (150×2.1 mm i.d., 1.7 µ particle size) column maintained at 45 °C temperature with mobile phase consisting of 0.01 M phosphate buffer pH 2.9 and acetonitrile in a gradient program was used to successfully separate Micafungin sodium and its synthetic impurities. The detection wavelength was 279 nm, and the mobile phase flow rate was 0.3 ml/min.	(Joshi *et al*., 2016)
Prednicarbate (PC),	Used for fungal	A Hypersil GOLD C18, 5 µm, 250 mm × 4.6 mm i.d.	(Amrutiya *et al*., 2010)

mupirocin (MP) and ketoconazole (KT)	infection	column is used in the proposed RP-HPLC method. The mobile phase is made up of methanol-water (80:20, v/v) adjusted to pH 5.0 with orthophosphoric acid in isocratic mode at a flow rate of 0.5 mL/min and UV detection at 243 nm.	
Luliconazole	Used to treat infections brought on by yeast or fungi.	Used ACN (60:40) ammonium acetate buffer. The inertsil ODS 3V (4.6*150 mm, 5µm) column was utilized, and its flow rate was 1.0 ml/min. The wavelength of detection for methyl paraben was 254 nm and for luliconazole was 294 nm.	(Majumder *et al*., 2019)
Clotrimazole (CT) and beclomethasone dipropionate (BD)	Used to treat infections causing jock itch, athlete's foot, and ringworm	Using a Kromasil C18 analytical column (150 mm x 4.6 mm, 5 [micro]m), HPLC was performed. The mobile phase was a 70:30, v/v mixture of acetonitrile and water, with a flow rate of 1 ml/min and a detector wavelength of 254 nm.	(Dhudashia *et al*., 2013)
Econazole nitrate	Used to treat Athlete's foot, jock itch, and ringworm	Used pH 2.5 phosphate buffer at 20 mmol L^{-1} concentration, +30 kV applied voltage in a 31.5 cm × 50 µm I.D. capillary. Direct UV detection at 200 nm	(Gaona-Galdos *et al*., 2008)
Halometasone, fusidic acid	Used for treating skin infection	Method development and validation were carried out using gradient elution at 240 nm detector wavelength on an Agilent Zorbax CN (Cyano) column measuring 5 µm (250 x 4.6 mm).	(Goswami *et al*., 2013)
Bifonazole	Used in treatment of superficial skin infection	Utilized a hemochrom C18 (150 x 4.6 mm x 5µ) and acetonitrile and 0.05% TFA (20:80) at a flow rate of 1 milliliter per minute for the mobile phase. At	(Prajapati *et al*., 2023)

		256 nm wavelength, the retention time was determined to be 6.871 minutes.	
Sertaconazole Nitrate	Prevent fungus and yeast growth	Used a C18 column (250 × 4.6 mm, 5 µm) with an isocratic mode flow rate of 1.2 mL/min and a blend of acetonitrile, methanol, and 30 mM potassium phosphate buffer (pH 3.0) (60: 20: 20%, v/v). Using a multi-channel wavelength detector set at 260 nm, all eluents were found.	(Sonawane *et al*., 2019)

For anti -bacterial drug

Name of drug	Uses	Reported method	References
Pretomanid	Used to treat MDR TB	Employed Xtimate C18 column with methanol (250mm × 4.6mm; 5µm): Acetonitrile: Ammonium phosphate buffer at a ratio of 55:40:05 (v/v/v) used as the mobile phase, with a 262 nm wavelength for detection at a flow rate of 1.0 mL/min.	**(**Rao *et al*., 2023)
Ceftolozane and Tazobactam	Used to treat pneumonia	Used XTerra C18 (molecule size: 5 µm, 4.6 x 150 mm). 260 nm was used to estimate pH 4.6 on a portable stage that contained a phosphate cradle and acetonitril at a stream pace of 1 ml/min.	**(**Krantikumar *et al*., 2022)
Ciprofloxicin	Used to treat bone and joint infections, intra-abdominal infections, certain types of infection	The developed UV-spectrophotometric method made use of a solvent system consisting of methanol and water (50:50 v/v). The maximum absorbance wavelengths for ciprofloxacin and curcumin were found to be 275 nm and 430 nm, respectively.	**(**Sanjay *et al*., 2020)
Cefdinir and Cefixime	Used for treating Middle ear infections. Tonsillitis. Strep Throat.	Used guard cartridge (Perkin Elmer C18; 30 mm × 4.6 mm, 10 µm) to shield the Supelco Discovery HS C18 (150 mm × 4.6 mm, 5 µm) analytical column. The mobile phase was pumped at a flow rate of 2.0	**(**Khan *et al*., 2011)

		mL min−1 and the column eluents were observed at a wavelength of 285 nm. The mobile phase was methanol/acetonitrile (50/50, v/v):0.05% trifluoroacetic acid (19:81, v/v), operated at 50 °C column oven temperature..	
Ambroxol hydrochloride and azithromycin	Used to treat respiratory tract infection	Utilized an analytical column, Xterra RP18 (250 mm × 4.6 mm, 5 μm). The mobile phase was a mixture of acetonitrile and dipotassium phosphate (30 mM) (50:50, v/v) (pH 9.0), with a flow rate of 1.7 ml/min and a detector wavelength of 215 nm.	**(**Shaikh et al., 2008)
Besifloxacin Hydrochloride and Phenoxyethanol	Used to treat bacterial conjunctivitis	The percentage of recovery ranges from 98% to 101%, and the RSD for all recovery values is within acceptable bounds at 1.41%. The conditions were optimized by the HPLC method to produce a suitable separation of the eluted compound.	(Kundu *et al*., 2023)

For antiviral drugs

Name of drug	Uses	Reported method	Reference
Valacyclovir	Used to treat herpes virus infections	The n-hexane, ethanol, and diethylamine (30:70:0.1, v/v/v) were the components of the mobile phase system used with a Chiralpak AD (250 mm × 4.6 mm, 10 μm) column.	**(**Jadhav *et al*., 2007)
Baloxavir marboxil (BXM)	Used for treatment of influenza A and influenza B flu.	Utilized C18 (100 × 4.6 mm, 5 μm) in conjunction with a binary solvent delivery system (A:0.1% trifluoroacetic acid in water; B:0.1% trifluoroacetic acid in acetonitrile) with a 260 nm detection wavelength, 57°C column temperature, 1.2 mL/min flow, and 10 μL injection volume.	**(**Nagulancha *et al*., 2023)
Famciclovir	Used to treat herpes zoster	Utilized an RP-C18 column with a mobile phase that was pH 3.05-adjusted using orthophosphoric acid and methanol (75:25 v/v). At 221 nm, the mobile phase was detected after being pumped at a flow rate of 1 mL/min.	**(**Velivela *et al*., 2016)

Daclatasvir	Used to treat a certain type of chronic hepatitis C	Employed a Waters XTerra RP18 (150 mm × 4.6 mm, 3.5 µm) column with a mobile phase consisting of acetonitrile (56:44, v/v) and ammonium acetate buffer (pH 5.0, 10 mM), with UV detection set at 318 nm.	**(**Nannetti *et al*., 2017)
Penciclovir	Used to treat herpes simplex virus	Utilized a mobile phase of 0.01 M and a stationary phase consisting of a Kromosil C18 column (250 x 4.6 mm, 5 µm). UV detection at 286 nm, and a ratio of 40:40:20 (v/v) sodium di hydrogen phosphate, acetonitrile, and water at a flow rate of 1.0 ml/min	**(Ch *et al*., 2011)**
lamivudine, tenofovir & efavirenz	Used to treat human immunodeficiency virus (HIV) infection.	A mobile phase consisting of 10 mM ammonium formate, methanol, and acetonitrile in a 50:25:25 (v/v/v) ratio was used in an Xterra, C18 (2), 150 X 4.6 mm, 5 µm column, flowing at a rate of 1.0 mL/min. For the mass spectrometric detection, a turbo ion-spray interface (TIS) operating in positive ionization mode was employed. Mamigudine (m/z 230.1/112.1), tenofovir (m/z 288.0/176.2), efavirenz (m/z 316.2/168.1), emtricitabine (m/z 248.1/130.1), and abacavir (m/z 287.2/191.2) were the MRM transitions that were observed.	**(**Akiladevi and Mounika, 2021)
Entecavir	Used to treat liver infection caused by hepatitis B virus.	An Agilent HC-C18 (250 x 4.6 mm i.d., particle size 5 µm) column was utilized, and the mobile phase consisted of a 95:5 buffer:acetonitrile ratio. At 253 nm, the effluents were detected using a flow rate of 1 mL/min. The 6.98-mile retention time was discovered.	**(**Mounika *et al*., 2017)
Imiquimod	Used to treat external genital or anal warts.	The UV detection at 242 nm and a C8 column were used. Acetonitrile:acetate buffer (pH 4.0, 100 mm):diethylamine (30:69.85:0.15, v/v) was the mobile phase used, and the flow rate was 1 mL/min. The running time was restricted to 6.0 minutes, and imiquimod eluted at 4.1 minutes.	**(**Paula *et al*., 2008)

Trifluridine	Used to treat eye infections	In ophthalmic preparations, a C18 Shim-pack GWS HPLC packed column (250 mm × 4.60 mm, 5 µm) was utilized. Acetonitrile-containing mobile phase: With diluted trifluro acetic acid (70:30 v/v) in isocratic mode and a flow rate of 1.0 mL/min, a pH of 3.5 was achieved using a 10 mM potassium dihydrogen phosphate buffer (detection wavelength: 272 nm).	**(**Bandaru and Annapurna, 2022)

ICH Guidelines

- Harmonized scientific and technical principles are established for analytical procedures throughout the whole analytical procedure lifecycle by the ICH Q14 and ICH Q2(R2) guidelines.
- By implementing the ICH Q14 principles, industry and regulators can communicate more effectively about regulations and enable more effective, sound, scientific, and risk-based approval processes, as well as post-approval change management for analytical procedures.
- ICH Q2(R2) has been updated to incorporate newer technologies and will still serve as a general framework for the principles of analytical procedure validation (e.g., for biological products or multivariate analytical procedures) (Ermer and Miller, 2006).

Harmonization under ICH leads to

- Regulatory review processes that are more effective
- Information sharing amongst regulatory authorities that is more effective; product launch times that are shorter
- Patient burdens that are lessened by avoiding needless repetition of clinical trials and post-market clinical evaluations
- And the elimination of needless animal testing without sacrificing efficacy or safety (Breaux *et al*., 2003)

Validation parameters according to ICH guidelines

Specificity

The term "specificity" describes an analytical method's capacity to identify and measure analytes in complicated mixtures. When identifying contaminants and validating identification tests, a specificity investigation must be carried out. The ability of HPLC to produce interference-free signals is one of its key features.

According to the ICH guideline, specificity is the capacity to definitively evaluate the analyte in the presence of potentially present other compounds. These are typically things like matrices, degradants, and impurities. The following implications flow from the definition.

Identification examination To ensure the identity of an analyte, identification tests should be able to distinguish between compounds with closely related structures that are predicted to be present.

Purity test

To guarantee that the analytical method used enables an accurate statement of the impurity content of an analyte, such as the presence of heavy metals, related substances, and residual solvents, etc. (Kumar V, Bharadwaj R, GG SK. An Overview on HPLC Method Development. Optimi *et al*., 2015).

Assay

This enables an accurate report on the potency or content of the analyte in a sample in order to arrive at an accurate result (Prathap *et al*., 2012).

Linearity and Range

A method's linearity is determined by how closely a response vs. concentration calibration plot resembles a straight line (Sahu et al., 2018). One way to evaluate linearity is to run single measurements at various analyte concentrations. Regression using linear least-squares is then used to process the data. The required linearity information is provided by the plot slope, intercept, and correlation coefficient that result.

Precision

An analytical procedure's precision, which is broken down into three categories, is the degree of agreement between several measurements obtained from multiple samplings of the same homogenous sample under comparable analytical conditions.

Repeatability

Accuracy in the same operating environment, with the same analyst for a brief duration.

Intermediate precision

Method is tested on multiple days, instruments, analysts, etc.

Reproducibility

Studies conducted between laboratories According to the ICH guidelines, repeatability must be properly conformed using a minimum of six determinations at 100% of the test concentration or at least nine determinations with a specified range for the procedure (e.g., three concentrations / three replicates each) (Swartz and Krull, 2018).

Accuracy

The degree to which the measured value agrees with the true value is known as measurement accuracy. A sample whose "true value" is known is analyzed using a high accuracy method, and the measured value matches the true value exactly. Recovery studies are usually used to represent and determine accuracy. Three methods exist for assessing accuracy.

Evaluation in relation to a benchmark.

Analyte recovery that was spiked into an empty matrix.

Adding the analyte as usual.

Limit of detection

The minimal level at which the analyte can reliably detect—but not necessarily quantify as precise value—under the specified experimental conditions is known as the limit of detection, or LOD, and is established by analyzing samples with known analyte concentrations. It is typically expressed as an analyte's concentration (ppm).

- Visual assessment
- Signal-to-noise ratio
- Response standard deviation
- Standard deviation of the linearity plot's slope

The formula for calculating LOD is LOD = 3.3 δ/S Where δ = standard deviation of intercepts of calibration curves. S = the slope of the linearity plot.

Limit of quantitation

The lowest amount of medication in a sample that can be accurately and precisely estimated under approved experimental conditions is known as the lower limit of quantification, or LOQ. The ICH

suggests the following four techniques for LOQ estimation, which are similar to LOD. The methods that are appropriate are:
Visual assessment
Signal-to-noise ratio
Response standard deviation
Slope of linearity plot standard deviation
The formula for calculating LOQ is LOQ = 10 δ/S
Where δ = standard deviation of response. S = Mean of slopes of the calibration curves

Robustness
An analytical method's robustness is determined by how well it can withstand small, intentional changes to its parameters. The pH, sample temperature, flow rate, column temperature, and mobile phase composition are examples of the variable method parameters in the HPLC technique.

System Suitability Test
The pharmaceutical industry initially thought that suitability testing could determine whether a chromatographic system was suitable for a specific analysis and could be used on a daily basis in pharmaceutical laboratories where the quality of the results was the most crucial factor.
Arguably, the most significant analytical method in pharmaceutical analysis is HPLC. HPLC analysis requires a proficient operator. Any information package that is sent to international regulatory bodies in support of applications for clinical trials or the marketing of novel products must include method validation. Analytical procedures, such as those listed in the pertinent pharmacopoeia or other accepted standard references, ought to be validated. Every test method's suitability should be thoroughly documented and confirmed under actual usage circumstances. The International Conference on Harmonization (ICH) guidelines pertaining to the validation of analytical methods should be taken into consideration when validating methods. In general, bioanalytical method validation is crucial for assessing and interpreting bioequivalence, bioavailability, toxicokinetic studies, and pharmacokinetic data. In fact, this allows for the quantitative analysis of the drug and its metabolites in the biological fluid.

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