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Review Article

In Depth Investigation of Analytical Methods for the Estimation of Antibacterial Drug Mupirocin in Different Matrices: A Review

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Abstract

The top objective of any pharmaceutical industry is to produce products of necessary characteristic and quality in a cost-effective manner. Analytical method development is essential for discovery, development, and evaluation of medicines in the pharmaceutical formulation. It helps in ensuring the safety and efficacy of drugs in different matrices by determining the quantity and quality of Drug. In Research activities literature survey is the vital requirement. Mupirocin belongs to the class of topical antibacterial agent, which is a natural crotonic acid that is extracted from a strain of Pseudomonas fluorescens. It works by inhibiting bacterial protein synthesis by specifically and reversibly binding to bacterial isoleucyl tRNA synthase which is an enzyme that promotes conversion of isoleucine to isoleucyl tRNA. It is used for treatment of primary and secondary skin disorder like impetigo, nasal infection and wound healing. In this article main prominence is given on the various techniques which are used for the estimation of the Mupirocin from various Pharmaceutical dosage forms. Among various methods generally UV and HPLC are the most widely used techniques. Various validation parameters used for method development are being figure out in the article.

Keywords: Mupirocin; Analytical Methods; Antibacterial Agent; Matrices; Estimation

Introduction

Atopic dermatitis (AD) is a multifaceted, chronic relapsing inflammatory skin disease which is also well known to be Atopic Eczema. In Atopic dermatitis, Atopy is defined as an inherited tendency to yield immunoglobulin E (IgE) antibodies in revulsion to minimal amounts of prevailing environmental proteins such as pollen, house dust mites, and food allergens. Dermatitis is being evolved from the Greek "derma", which means skin, and "itis", which means inflammation. The pathogenesis of Atopic dermatitis is thought to be complex interplay between defects in the skin barrier function, immune malfunctioning, and various environmental and infectious factors [1-5].

Mupirocin is a topical antibacterial agent with molecular weight of 500.62g/mol and chemically it is 9-[(E)-4-[(2S, 3R, 4R, 5S)3, 4-dihydroxy-5-[[(2S, 3S)-3-[(2S, 3S) hydroxylbutan-2-yl] oxiran-2-yl] methyl] oxan-2-yl]- 3methylbut-2-enoyl]oxynonanoic acid used in atopic dermatitis for prevention of bacterial infection, it works by interfering RNA and protein synthesis in susceptible bacteria by specifically and reversibly binds to bacterial isoleucyl transfer-RNA (tRNA) synthetase, which is an enzyme that promotes the conversion of isoleucine and tRNA to isoleucyl-tRNA. Inhibition of this enzyme subsequently prevent the incorporation of isoleucyl into bacterial protein. Thus, prevention of the enzyme from functioning properly results in inhibition of bacterial protein and RNA synthesis [6-9].

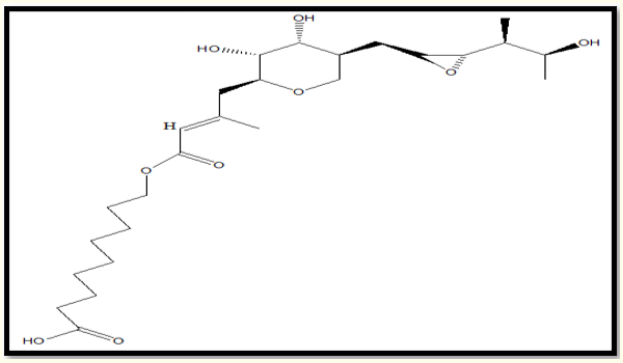

Figure 1: Chemical structure of Mupirocin.

State	
Water solubility	0.0265 mg/ml
Pka	4.83
Log p	2.45
Melting point	77^0C - 78^0C
Storage	Preserve in air tight container

Table 1: Physical properties [10].

Kingdom	**Organic compound**
Super class	Lipids and lipid like molecule
Class	Fatty acid
Subclass	Fatty acid and conjugates
Direct parent	Medium chain fatty acid
Alternative parent	Branched fatty acid/Epoxy fatty acid/ Fatty acid esters/Hydroxy fatty acid/ Dicarboxylic acid and derivatives/ Oxanes/Monosaccharides/Enoate esters/Secondary alcohol/Oxacyclic compounds.
Substituent's	Medium-chain fatty acid/Branched fatty acid/Epoxy fatty acid/Fatty acid ester/Heterocyclic fatty acid/Hydroxy fatty acid/Dicarboxylic acid or derivatives/Oxane/Monosaccharide/Enoate ester.
Molecular framework	Aliphatic heteromonocyclic compounds
External descriptors	Monocarboxylic acid, secondary alcohol, epoxide, triol, alpha,beta-unsaturated carboxylic ester, oxanes

Table 2: Taxonomy [10].

Mupirocin is reported to be active against susceptible aerobic gram-positive cocci, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and other beta-hemolytic streptococci *Streptococcus pyogenes*. It mediates its antibacterial activity by inhibiting the bacterial protein synthesis and formation of bacterial proteins essential for survival. The minimum bactericidal concentration (MBC) against relevant pathogens is generally eight-fold to thirty-fold higher than the minimum inhibitory concentration.

Systemic or percutaneous absorption of mupirocin following dermal application is expected to be minimal in adults and children, but damaged skin may allow enhanced penetration of the drug across the skin barrier, mupirocin undergoes rapid hepatic metabolism to form the principal metabolite monic acid. In various *in vivo* animal and *in vitro* bacterial assays, there was no evidence of genotoxicity caused by mupirocin. In reproduction studies using male and female rats, there were no signs of impaired fertility upon subcutaneous administration of mupirocin [10].

Absorption	**Damage skin enhance penetration**
Metabolized product	Monic acid
Plasma protein binding	95%
Metabolism	Liver
Elimination Half life	20 to 40 min
Excretion	Urinary and renal excretion

Table 3: Pharmacokinetics [10].

Clinical trials

The efficacy and safety of Mupirocin in patients with different types of skin diseases have been tested in numerous clinical trials. The effectiveness of the drug was demonstrated for three 12 week study. In Phase 2 Clinical trial positive nasal culture for staphylococcus aureus were obtained from infants. A 5 day course of mupirocin applied to the nares, umbilicus and perianal (NUP) areas every 8 hours (± 2 hours) vs. no treatment was carried out. In this study from day 1 to day 7 the adverse event regarding Mupirocin were observed. On day 8, primary efficacy of Mupirocin was observed. On day 8, colonization and decolonization were observed through which it was concluded that on applying Mupirocin decolonization of SA in the culture was seen [11].

Generic name	Brand name	Dosage form	Strength	Manufacturer
Mupirocin	T-Bact	Ointment	2% w/w	Glaxo SmithKline Pharmaceuticals Ltd
Mupirocin	I -Bact	Cream	2% w/w	Intas Labs
Mupirocin	Mpower	Ointment	2% w/w	Liva (Zydus Cadila)
Mupirocin	Mucidal	Ointment	2% w/w	Intas
Mupirocin	Mufect	Ointment	2% w/w	Sunpharma
Mupirocin	Mupin	Ointment	2% w/w	Gray Pharma
Mupirocin	Mupinase	Ointment	2% w/w	Cipla
Mupirocin	Mupinova	Cream	2% w/w	Zuventus
Mupirocin	Mupirax	Ointment	2% w/w	Micro Vision
Mupirocin	Mupi	Ointment	2% w/w	Hegde and Hegde Pharmaceuticals
Mupirocin	Supirocin	Ointment	2% w/w	Glenmark Pharmaceutical Ltd
Mupirocin	Enzomac	Ointment	2% w/w	Macleods Pharmaceutical Pvt Ltd
Mupirocin	Mupimet	Ointment	2% w/w	Fourrts India Labs Pvt Ltd.
Mupirocin	Kozibact	Cream	2% w/w	La Med India
Mupirocin	Mupicent	Cream	2% w/w	Synergy Pharmaceuticals
Mupirocin	Mupizer	Cream	2% w/w	Leeford Healthcare Ltd
Mupirocin	Tercin WHP	Powder	2%	Mova Pharmaceutical Pvt Ltd
Mupirocin	Dibact	Powder	2%	Astra Labs
Mupirocin	Mupicol	Ointment	2% w/w	Hi-Cure Biotech
Mupirocin	Mupisar	Ointment	2% w/w	Kyna Pharmaceuticals
Mupirocin	Mupivit	Ointment	2% w/w	Roma Pharmaceutial Pvt Ltd
Mupirocin	Sunbact	Ointment	2% w/w	Sunwin Healthcare
Mupirocin	Aimubact	Ointment	2% w/w	Ai Health Care
Mupirocin	Healiva	Ointment	2% w/w	Bills Biotech Pvt Ltd
Mupirocin	Injayderm	Ointment	2% w/w	Injays Pharmaceuticals
Mupirocin	Dol Bact	Ointment	2% w/w	Dolvis Bio Pharma Pvt Ltd
Mupirocin	**S**tramucin	Ointment	2% w/w	Glenmark Pharmaceuticals
Mupirocin	Brodisym	Ointment	2% w/w	Symbiosis Pharma
Mupirocin and Betamethasone	Mupin B	Ointment	2% w/w, 0.05%w/w	Gray Pharma
Mupirocin and Metronidazole	Co-Mupimet	Ointment	2% w/w, 1% w/w	Fourrts
Mupirocin and Betamethasone	Stemin - MU	Ointment	2% w/w, 0.05%w/w	Ind Swift
Mupirocin and Betamethasone	Supirocin - B plus	Ointment	2% w/w, 0.025%w/w	Glenmark
Mupirocin and Bromelain	Enzoheal	Ointment	2% w/w, 25%w/w	Macleods Pharmaceuticals Ltd.
Mupirocin and Bromelain	Enzomac	Ointment	2% w/w, 5%w/w	Macleods Pharmaceuticals Ltd.
Mupirocin and Fluticasone Propionate	Flutibact	Ointment	2% w/w, 0.005%w/w	Glaxo SmithKline Pharmaceuticals Ltd.
Mupirocin and Mometasone furroate	Sensicort B	Ointment	2% w/w, 0.1%w/w	Zuventus
Mupirocin and Betamethasone	Supirocin - B plus	Ointment	2% w/w, 0.025%w/w	Glaxo SmithKline Pharmaceuticals Ltd.

Table 4: Marketed formulations of mupirocin [12,13].

Analytical methods for estimation of mupirocin in bulk drug, pharmaceutical formulation and biological fluids

There are numerous methods have been reported for estimation of paroxetine in bulk and dosage form as well as in biological fluids.

Spectrometric methods

Bana A., *et al*. specifies analytical method development and validation by UV spectroscopic and RP-HPLC methods for simultaneous estimation of Halobetasol Propionate and Mupirocin in ratio of 1:40. For UV spectroscopic method the wavelength maxima selected were 240nm and 220nm for Halobetasol Propionate and Mupirocin respectively using methanol as a solvent. The Result parameters are tabulated in table 5 [14].

Parameters	Results	
	Mupirocin	Halobetasol Propionate
Linearity Range (µg/ml)	5-30	5.125-5.75
Correlation coefficient	0.9996	0.9995
%Recovery	99.23	100.98
Presicion Interday (%RSD)	0.649	1.355
Precision Intraday (%RSD)	0.356	1.032
Limit of Detection (µg/ml)	0.192	0.819
Limit of Quantification (µg/ml)	0.584	2.48

Table 5: Validation parameters reported by Bana A., *et al*. 2019 [14].

Shende M., *et al*. specifies development and validation of analytical method for simultaneous estimation of Mupirocin and Satranidazole using methanol and water (50:50) as a solvent. The maximum absorbance was found at 220nm for Mupirocin and 213nm for Satranidazole respectively. The results are tabulated in table 6 [15].

Parameters	Results	
	Mupirocin	Satranidazole
Linearity Range (µg/ml)	1-5	0.5-2.5
Correlation coefficient	0.9986	0.9978
%Recovery	101.28	101.52
Presicion Interday (%RSD)	1.42	0.64
Precision Intraday (%RSD)	1.66	1.21
Limit of Detection (µg/ml)	0.13	0.19
Limit of Quantification (µg/ml)	0.46	0.58

Table 6: Validation parameters reported by Shende M., *et al*. 2017 [15].

Attar S., *et al*. specifies stress degradation studies and development of validated UV spectrophotometric method for estimation of Mupirocin using ethanol as a solvent. The wavelength maxima selected was 222nm. The results are tabulated in table 7 [16].

Parameters	Results
Linearity Range (µg/ml)	10-50
Correlation Coefficient	0.9991
%Recovery	98-100
Limit of Detection (µg/ml)	0.06
Limit of Quantification (µg/ml)	0.2
Precision Interday (%RSD)	0.1
Precision Intraday(%RSD)	1.0

Table 7: Validation parameters reported by Attar S., *et al*. 2017 [16].

Parmar A., *et al*. specifies simultaneous estimation of Mupirocin and Mometasone Furoate by Q absorption ratio method using methanol as a solvent, Q absorbance ratio method, involves formation of Q-absorbance equation at 226 nm (isoabsorptive point) and also at 220 nm (λmax of Mupirocin).The results are tabulated in table 8 [17].

Parameters	Results	
	Mupirocin	Mometasone Furroate
	226nm: 220nm	**226nm: 220nm**
Linearity Range (**µg/ml**)	5-25	5-25
Correlation coefficient	0.998: 0.999	0.998: 0.999
%Recovery	99.83-100.68	99.25-101.04
Presicion Interday (%RSD)	0.981-1.139 1.018-1.202	1.051-1.297 1.174-1.423
Precision Intraday (%RSD)	0.851-1.062 0.619-0.915	0.596-0.933 0.993-1.095
Limit of Detection (**µg/ml)**	1.237	0.756
Limit of Quantification (**µg/ml)**	3.75	2.291

Table 8: Validation parameters reported by Parmar A., *et al*. 2015 [17].

Bhageshwar D., *et al*. specifies UV spectrophotometric method for estimation of Mupirocin Calcium using Acetonitrile and Sodium Phosphate buffer pH 6.4 (50:50) as solvent system. Mupirocin Calcium shows maximum absorbance at 220 nm. The other result parameters are tabulated in table 9 [18].

Parameters	Results
Linearity Range (µg/ml)	2-16
Correlation Coefficient	0.9994
%Recovery	99.96
Limit of Detection (µg/ml)	0.45
Limit of Quantification (µg/ml)	2
Ruggedness (%)	99.87

Table 9: Validation parameters reported by Bhageshwar D., *et al.* 2010 [18].

Chromatographic methods

There are numerous high performance liquid chromatography (HPLC) method have been reported for the analysis of Mupirocin in pharmaceutical formulation, bulk as well as in biological fluids.

High performance liquid chromatography (HPLC).

Bana A., *et al.* specifies HPLC method for estimation of Mupirocin and Halobetasol, the chromatographic separation was achieved using CHROMBUDGET C18 (250 × 4.6 mm) 5 µm column as stationary phase, mobile phase consisting of acetonitrile and phosphate buffer (65:35 v/v, pH 3.2), at 1 mL/min flow rate and detection wavelength of 230 nm. The retention time of Halobetasol and Mupirocin was found to be 8.647 ± 0.06 min and 3.357 ± 0.123 min respectively. The other result parameters are tabulated in table 10 [14].

Parameters	Results	
	Mupirocin	Halobetasol Propionate
Linearity Range (µg/ml)	5-30	5.125-5.75
Correlation coefficient	0.9994	0.9994
%Recovery	100.50	100.21
Presicion Interday (%RSD)	0.660	1.021
Precision Intraday (%RSD)	0.788	1.247
Limit of Detection (µg/ml)	0.192	0.819
Limit of Quantification (µg/ml)	0.584	2.48

Table 10: Validation parameters reported by Bana A., *et al.* 2019 [14].

Arapda P., *et al.* specifies stability indicating RP-HPLC method development and validation for simultaneous estimation of Mupirocin and Metronidazole in their combined dosage form. The separation was achieved by using LC – 20 AT C18 (250 mm × 4.6 mm × 2.6 µm), methanol and buffer pH 3.5 (70:30) were used as mobile phase, at flow rate of 1 ml/min. The detection was carried out at 230 nm with retention time of 4.227 and 5.413 min for Metronidazole and Mupirocin respectively. The Result Parameters are tabulated in table 11 [19].

Parameters	Results	
	Mupirocin	Metronidazole
Linearity Range (µg/ml)	10-30	5-15
Correlation coefficient	0.999	0.999
% Recovery	102.5	99.83
Precision Interday (%RSD)	1.544	1.041
Precision Intraday (%RSD)	0.708	0.495
Limit of Detection (µg/ml)	0.735	0.691
Limit of Quantification (µg/ml)	2.227	0.691

Table 11: Validation parameters reported by Arapda P., *et al.* 2018 [19].

Rele R., *et al.* specifies RP-HPLC technique for determination of Mupirocin Lithium in pharmaceutical dosage form using (150 mm × 4.6 mm, 5 µm) column. The mobile phase used for separation was buffer and acetonitrile (74:26 %v/v), with the flow rate of 1ml/min. Study was done at the wavelength of 221 nm. The retention time for Mupirocin Lithium was 5.3min. The result parameters are tabulated in table 12 [20].

Parameters	Results
Linearity Range (µg/ml)	50-150
Correlation Coefficient	0.9998
%Recovery	99.66
Precision	0.441

Table 12: Validation parameters reported by Rele R., *et al.* 2017 [20].

Attar S., *et al.* specifies RP-HPLC method for estimation of Mupirocin using Phenomenex C-18 column (150 x 4.6 mm), methanol and phosphate buffer pH 3 (70:30%v/v) was used as mobile phase, and the flow rate of 1ml/min respectively. The effluent was monitored at wavelength of 220 nm, retention time of Mupirocin was 4.5 min. The result parameters are tabulated in table 13 [21].

Kumari P., *et al.* specifies stability indicating RP-HPLC method development and validation for simultaneous estimation of Mu-

pirocin and Fluticasone using C18 (4.6 × 250 mm, 5 µm) column and mobile phase containing Buffer Orthophosphoric acid and Acetonitrile (55:45). Flow rate was 1 mL/min with the detection wavelength of 230 nm. Retention time of Mupirocin and Fluticasone were found to be 2.146 and 2.770 min. The result parameters are tabulated in table 14 [22].

Parameters	Results
Linearity Range (µg/ml)	10-50
Correlation Coefficient	0.9983
%Recovery	96-98
Precision Intraday (%RSD)	1.29
Limit of Detection (µg/ml)	0.15
Limit of Quantification (µg/ml)	0.47

Table 13: Validation parameters are reported by Attar S., *et al.* 2017 [21].

Parameters	Results	
	Mupirocin	Fluticasone
Linearity Range (µg/ml)	75-450	1.25-7.5
Correlation coefficient	0.999	0.999
%Recovery	98.75	99.42
Presicion Intermediate (%RSD)	1.8	1.6
Limit of Detection (µg/ml)	0.38	1.16
Limit of Quantification (µg/ml)	0.02	0.05

Table 14: Validation parameters reported by Kumari P., *et al.* 2017 [22].

Pradhan P., *et al.* specifies stability indicating method RP-HPLC method for simultaneous estimation of Mupirocin and Beclomethasone dipropionate using LC- 20 AT C18 (250 mm × 2.6 **µm) column**. Mobile phase used for separation was Buffer pH 4.5 and Acetonitrile (60:40 v/v) at the flow rate of 1mL/min. Detection was carried out at 236 nm. Retention time for Mupirocin and Beclomethasone dipropionate was 3.55min and 5.18 min respectively. The result Parameters are tabulated in table 15 [23].

Parameters	Results	
	Mupirocin	Beclomethasone dipropionate
Linearity Range (µg/ml)	20 - 60	0.5 – 1.5
Correlation Coefficient	0.999	0.999
%Recovery	100.22	100.80
Limit of Detection (µg/ml)	0.65	0.032
Limit of Quantification (µg/ml)	1.98	0.098

Table 15: Validation parameters reported by Pradhan P., *et al.* 2017 [23].

Sivannarayana P., *et al.* specifies RP-HPLC method for simultaneous assay of Mupirocin and Metronidazole using C18 column (4.6 ×150 mm, 5µ particle size) with mobile phase containing mixture of phosphate buffer pH 2.5 and acetonitrile (70:30 v/v) at a flow rate of 1 ml/min with UV detection at 220 nm. The retention time for Mupirocin and Metronidazole were found to be 2.153 and 3.157 respectively. The result parameters are tabulated in table 16 [24].

Parameters	Results	
	Mupirocin	Metronidazole
Linearity Range (µg/ml)	20 - 60	10 - 30
Correlation Coefficient	0.997	0.998
%Recovery	99.97	99.99
Precision (% RSD)	0.76	0.58
Limit of Detection (µg/ml)	0.046	0.042
Limit of Quantification (µg/ml)	0.154	0.142

Table 16: Validation parameters reported by Sivannarayana P., *et al.* 2016 [24].

Mahoharan G., *et al.* specifies stability indicating RP-HPLC method for estimation of Mupirocin using C18 column and mobile phase of Methanol and Phosphate buffer (20:80 v/v) at the flow rate of 1 mL/min. Absorption maxima was observed at 270nm. The chromatographic retention time of Mupirocin was found to be 7.3 min. The result Parameters are tabulated in table 17 [25].

Parameters	Results
Linearity Range (µg/ml)	20-100
Correlation Coefficient	0.9987
%Recovery	100.92
Precision Intraday (%RSD)	1.016
Precision Interday (%RSD)	1.007
Limit of Detection (µg/ml)	0.20
Limit of Quantification (**µg**/ml)	0.30

Table 17: Validation parameters reported by Mahoharan G., *et al.* 2016 [25].

Parmar A., *et al.* specifies analytical method for simultaneous estimation of Mupirocin and Mometasone Furoate using Phenomenax-luna C18 (250 x 4.6 mm, 5 µm) column with mobile phase consisting of Acetonitrile: Sodium dihydrogen phosphate buffer (pH 6.8) (70:30 v/v) at a flow rate of 1 mL/min and UV detection at 240 nm. The Result parameters are tabulated in table 18 [26].

High performance thin layer chromatography (HPTLC)

Chhajed S., *et al.* specifies densitometric development and validation of Mupirocin in ointment dosage form using mobile phase

which comprised of dichloromethane: ethyl acetate: methanol (8:1:2, v/v/v). Chromatographic separation of drug was performed on aluminium plates precoated with silica gel 60 F254 as the stationary phase. The densitometric evaluation of separated zones was carried out at 226 nm. A retardation factor of MUP was found to be 0.57 ± 0.02. Linearity of MUP was found to be in the concentration range of 200 - 3000 ng/band. The validation parameters were tabulated in table 19 [27].

Parameters	Results	
	Mupirocin	Mometasone Furroate
Linearity Range (µg/ml)	10-60	1-6
Correlation Coefficient	0.999	0.999
%Recovery	99.8	99.18
Precision Interday (% RSD)	1.069	1.006
Precision Intraday (%RSD)	0.8772	0.715
Limit of Detection (µg/ml)	0.2716	0.0174
Limit of Quantification (µg/ml)	0.8450	0.0517

Table 18: Validation parameters reported by Fawazia Ibrahim., *et al*. 2015 [26].

Parameters	Results
Linearity Range (ng/ml)	200-3000
Correlation Coefficient	0.999
%Recovery	99.172-100.463
Interday Precision (%RSD)	0.563
Intraday Precision (%RSD)	0.601

Table 19: Validation parameters reported by Chhajed S., *et al*. 2013 [27].

LC-MS/MS

Echevarria L., *et al*. specifies development and validation of liquid chromatographic method, LC-MS/MS for *in vitro* Mupirocin quantification in both layer and its penetration studies using C8 (250 mm × 4 mm) LiChrospher column, mobile phase composition was mixture Acetonitrile and Ammonium acetate 0.05M (27.5:72.5 v/v) adjusted to pH 6.3 with acetic acid, with flow rate of 1 ml/min. The analyte was detected at 228nm and the run time was 11 min. Linearity was confirmed in the concentration range 0.2 - 20 µg/mL and the limit of detection was 9.5 µg/mL [28].

Porter R., *et al*. specifies HPLC analysis of Mupirocin in PEG 400 and 3350 using dual UV and evaporative light scattering detection. A gradient LC-MS-compatible method was successfully developed for the separation and identification of impurity and degradates in mupirocin in a complex matrix containing 98% (w/w) of polyethylene glycol 400 and 3350.The method enabled the eluent containing the PEGs to be diverted before it entered and contaminated the mass spectrometer source [29].

Conclusion

The Literature review reveals that numerous methods are available for the estimation of Mupirocin in pharmaceutical formulation. In nutshell, techniques like RP-HPLC, UV, and HPTLC are the most simple, easy and cost efficient methods for estimation of Mupirocin in various formulations. However, techniques like LC-MS/MS can be used for the determination of Mupirocin in the biological samples like plasma. Therefore, this review helps researchers to widen their ideas on different improved aspects for further studies on the evaluation of the drug.

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