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NADIFLOXACIN AND ADAPALENE: A REVIEW ON REPORTED METHODS FOR PURPOSE OF VALIDATION AND QUALITY MONITORING

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ABSTRACT: *Acne vulgaris* is a common chronic inflammatory skin disease found predominantly in adolescents in both sexes. The best acne treatment inhibits bacterial growth, encourages the shedding to the skin cells to unclog pores. Treatment for acne includes topical agents and systemic agents. Its quality monitoring becomes essential for assuring quality products for human care. So, for that purpose a detailed study of review was done including reported and validated methods, official monograph methods for estimation of Nadifloxacin and Adapalene. It was found from a literature survey that among all reported evaluation methods the most widely and predominantly followed method is HPLC. Other methods reported were UV, HPTLC. A conclusion was made that there is lack of evaluation analytical methods for simultaneous estimation of Nadifloxacin and Adapalene in combined dosage form.

INTRODUCTION: *Acne vulgaris* is common chronic inflammatory skin disease found predominantly in adolescents in both sexes. The lesion is formed which are more commonly seen on the face, on upper chest and upper back. The appearance of lesions near puberty is due to physiological hormonal variation. Mild acne is defined as presence of clogged skin follicles which is also known as comedones on to the face with inflammatory lesions. People with mild acne don't get large areas of red, inflamed skin or acne scarring.

Moderate acne occurs when a high number of inflammatory papules and pustules occur on the face when compared to mild cases of acne. They are also found on the trunk of the body. Severe acne occurs when nodules (which is also called as painful bumps) are the characteristic facial lesions and the involvement of trunk is more. Sign and symptoms of acne varies depending upon severity of condition: White heads, Black heads, Small red tender bumps, Pimples, painful lumps (nodules).

Androgen stimulates secretion of the sebaceous gland causing them to enlarge and secrete the natural oil, sebum which rises up to top of the hair follicle and flows out on to the skin surface. In adults who develop acne, androgenic stimulation produces a high response in the sebaceous gland so, the formation of acne occurs when accumulated sebum plugs the pilosebaceous ducts.

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This accumulated material leads to the formation of comedones. Treatment: The best acne treatment inhibits bacterial growth, encourages the shedding to the skin cells to unclog pores. Treatment for acne includes topical agents and systemic agents. Topical agents used in treatment of acne vulgaris: Treatment of acne vulgaris involves retinoids and antimicrobial and some antibacterial drug use. Topical retinoid acts to normalise the maturation of follicular epithelium and reduces inflammation and enhances the penetration of topical medication.

Nadifloxacin is a topical antibiotic that treats bacterial skin infections and acne. It's a second-generation fluoroquinolone that's effective against aerobic and anaerobic bacteria, including Gram-negative bacteria, Gram-positive bacteria, Propionibacterium species, Streptococcus species, and Staphylococcus species. Nadifloxacin works by preventing the synthesis of essential proteins and inhibiting the activity of bacterial enzymes. Nadifloxacin is intended for external use only. Some side effects that may occur during treatment include burning and itching, contact dermatitis, dryness, and skin irritation¹.

Adapalene is a third generation topical retinoid primarily used in the treatment of mild-moderate acne, and is also used off-label to treat keratosis pilaris as well as other skin conditions. Studies have found adapalene is as effective as other retinoids, while causing less irritation. It also has several advantages over other retinoids². The adapalene molecule is more stable compared to tretinoin and tazarotene, which leads to less concern for photodegradation.

Nadifloxacin^{3,4}:

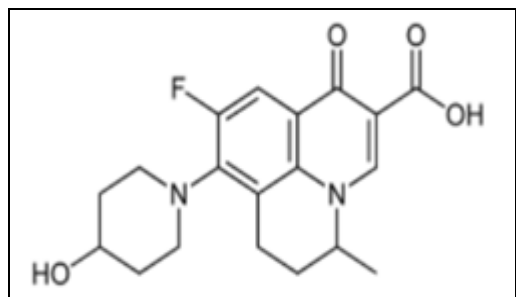


FIG. 1: STRUCTURE OF NADIFLOXACIN

It is also chemically more stable compared to the other two retinoids, allowing it to be used in combination with benzoyl peroxide. Due to its

effects on keratinocyte proliferation and differentiation, adapalene is superior to tretinoin for the treatment of comedonal acne and is often used as a first-line agent.

Chemical Name: 9-Fluoro - 8 - (4 - hydroxyl - 1 - piperidiny) - 5 - methyl - 1 - oxo - 6, 7-dihydro-1H, 5Hpyrido[3, 2,1-ij] quinoline-2-carboxylic acid

Molecular Formula: C₁₉H₂₁FN₂O₄

Molecular weight 360.379 g/mol

Drug Category: Antibacterial

Mechanism of action: Inhibits enzyme DNA gyrase that is involved in bacterial DNA synthesis and replication, thus inhibiting the bacterial multiplication.

Indication: Used in treatment of bacterial skin infection *i.e.* acne vulgaris

Adapalene^{5,6,7}:

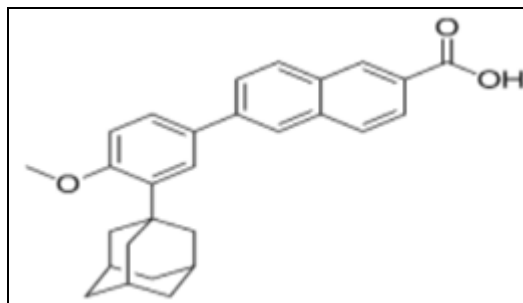


FIG. 2: STRUCTURE OF ADAPALENE

Chemical Name: 6 - [3 - (adamantan - 1 - yl) - 4-methoxyphenyl] naphthalene -2-carboxylic acid.

Molecular Formula: C₂₈H₂₈O₃

Molecular Weight: 412.52 g/mol

Drug Category: Topical retinoid

Mechanism of Action: It acts on retinoid receptor. It is modulator of cell differentiation, keratinization and inflammatory processes which is pathology of acne vulgaris. Indication Used in treatment of acne vulgaris.

Mechanism of Action (in Combination): Nadifloxacin is an antibiotic. It kills bacteria by preventing them from reproducing and repairing themselves.

Adapalene is a form of vitamin A which prevents accumulation of sebum (skin's natural oil), unblocks the pores and allows natural exfoliation of the outer layers of skin. Combination is approved by CDSCO on 17-7-2015. The gel is used in acne vulgaris. The dose is 10 mg of nadifloxacin and 1mg of adapalene.

of pharmaceutical products. So, Analytical Methods are developed and validated as per ICH guideline to assure quality. The methods reported in the literature for evaluation of Nadifloxacin and Adapalene were UV-Visible spectroscopy, HPLC, HPTLC, UPLC. The summary of reported methods is shown in **Fig. 4** and **Fig. 5**.



FIG 3: COMBINATION MARKETED FORMULATION

Literature review shows that many methods has been developed for Nadifloxacin but with other drugs like Mometasone furoate, Terbinafine hydrochloride, Clobetasol Propionate and Miconazole nitrate and also for adapalene in combination with other drugs like Benzoyl peroxide, Clindamycin Phosphate. But no method has been reported for simultaneous estimation of Nadifloxacin and Adapalene. The summary of reported methods is depicted in below **Table 1** and **Table 2**.

Method for Analysis: Quality monitoring is essential to certify the quality, safety, and efficacy

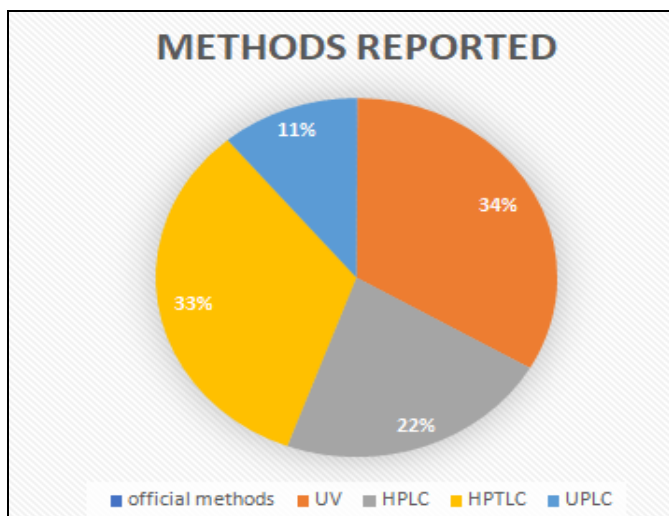


FIG. 4: REPORTED METHODS OF NADIFLOXACIN FROM LITERATURE SURVEY

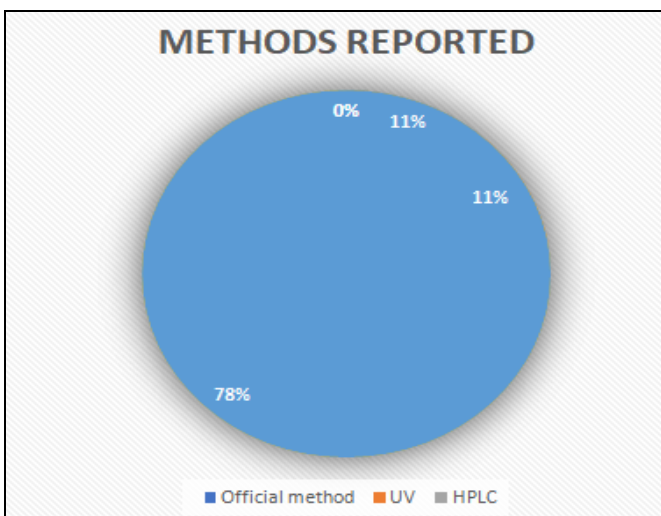


FIG. 5: REPORTED METHODS OF ADAPALENE FROM LITERATURE SURVEY

TABLE 1: REPORTED METHODS FOR NADIFLOXACIN

Sr. no.	Method	Description	Ref.
1	IP 2018	Column: 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm). Mobilephase: A mixture of equal volume of a buffer solution prepared by dissolving 1.927 gm of ammonium acetate in 1000 ml of water, adjusted to pH 3.6 with orthophosphoric acid and acetonitrile. Wavelength: 235 nm Flow Rate: 1 mL/min Injection volume: 10µL.	7
2	Spectrophotometric Estimation of Nadifloxacin in Pharmaceutical Dosage form.	Solvent: Methanol Wavelength: Absorption maxima - 296.5 nm First order derivative spectrophotometry - 278 nm Area under curve (AUC) - 291-301nm	8

3	Development And Validation Of Multiwavelength Method For Simultaneous Estimation Of Nadifloxacin And Ibuprofen In Formulated Hydrogel.	Linearity: 5 – 25 µg/ml Solvent: Methanol Wavelength: NAD- 280nm IBU-222nm Linearity: 2-20µg/ml	9
4	Analytical method development and validation of Nadifloxacin by HPLC	Column: C18 (150 mm x 4.6 mm, 5 µm) Mobile Phase: 0.05% Trifluoroacetic acid : Acetonitrile (65:35 v/v) Wavelength: 237 nm Retention time: 12.3 min Flow rate: 1.2 ml/min Linearity: 0.03 – 5 µg/ml	10
5	A development and validation of RP-HPLC method for simultaneous estimation of Nadifloxacin and Clobetasol Propionate in its dosage form	Column: C18 (250mm X 4.5mm 5µm) Mobile Phase: Acetonitrile: Water (50:50 v/v) Wavelength: 242 nm Retention time: NAD- 2.64min COP- 6.19 min Flow rate: 1 ml/min Linearity: NAD: 20-240µg/ml COP: 1-12µg/ml	11
6	Simultaneous estimation of Nadifloxacin and Mometasone furoate in topical cream by HPTLC Method.	Stationary phase: Silica gel 60 F254 Mobile phase: dichloroethane: diethylether: ammonia: methanol: ethylacetate (6:3:0.2:1.75:3.5 v/v/v/v/v) Wavelength: 254nm Rf value: NAD- 0.12 MOM- 0.85 Linearity: NAD- 1000-3000 ng/band MOM- 100-300 ng/band	12
7	Validated HPTLC method for simultaneous determination of Nadifloxacin, Mometasone furoate and Miconazole nitrate cream using fractional factorial design.	Stationary phase: Silica gel 60 F254 Mobile Phase: Methanol: Ethyl acetate:Toluene:Acetonitrile:3M Ammonium formate in water (1:2.5:6.0:0.3:0.2 v/v/v/vv) Wavelength: 224nm Rf value: NAD -0.23 MOM -0.70 MIN- 0.59 Linearity: NAD- 400-2400 ng/band MOM-100-600 ng/band MIN- 400-2400 ng/band	13
8	Validated stability indicating Thin layer chromatographic (TLC) Determination of Nadifloxacin in Microemulsion and bulk drug formulation.	Stationary Phase: silica gel 60 F254 Mobile Phase: Chloroform : Methanol: Formic acid (7.5 : 2.0 : 0.5 v/v/v) Wavelength: 288nm Rf value: 0.39 Linearity: 50-600µg/ml	14
9	A stability indicating HPTLC method for estimation of Nadifloxacin in topical cream.	Stationary phase: silica gel F-650 Mobile phase: Chloroform: Methanol: Ammonia (4.3:4.3:1.4 v/v/v) Wavelength: 296nm Rf value: 0.62 Linearity: 50-300ng/band	15
10	Stability indicating UPLC method for the estimation of Nadifloxacin, Terbinafine hydrochloride, Mometasone furoate, Methyl paraben and Propyl paraben.	Column: C18 (50mm X 2.1mm ,1.7µm) Mobile phase: A) Buffer (pH 3.5): Acetonitrile mixture (95:5 v/v) B) Buffer (pH 3.5): Acetonitrile mixture (25:75 v/v) Wavelength: 255nm Retention time: NAD-2.6min TER- 6.0 min MOM- 6.9min MP-1.5 min PP-3.4min Flow rate: 0.4ml/min	16

TABLE 2: REPORTED METHODS FOR ADAPALENE

Sr. no.	Method	Description	Ref.
1	UV spectrophotometric method for determination of Adapalene in bulk and pharmaceutical formulation.	Solvent: Methanol Wavelength: 237nm Linearity: 1-25µg/ml	17
2	Determination of Adapalene in gel formulation by conventional and derivative synchronous fluorometric approaches. Application to stability studies and invitro diffusion test.	Solvent: pH 7.0 borate buffer Wavelength: First approach- 389nm Second approach- 1.SDSF -346 nm 2.FDSF- 312.45 nm Linearity: 2-14µg/ml %Diffusion: 65%	18
3	A new HPLC method for development for cleaning validation of Adapalene active pharma ingredient.	Column: C18 (100mm X 4.6mm,3.5µm) Mobile phase: Acetonitrile:0.5% Orthophosphoric acid (35:65 v/v) Wavelength: 230nm Retention time: 4.4min Linearity: 2.5-20µg/ml	19
4	HPLC method development and validation for the estimation of Adapalene in pharmaceutical Formulation.	Column: C18 (250 X 4.6 mm, 5 µm) Mobile phase: Tetrahydrofuran: Acetonitrile: 0.1% Acetic acid in water (20:40:40 v/v/v/v) Wavelength: 270nm Retention time: 10.44min Flowrate: 1.2ml/min Linearity: 10-30µg/ml	20
5	Development of analytical method for simultaneous estimation of Adapalene and Benzoyl peroxide in gel formulation by RP-HPLC.	Column: C8 (250mm X 4.6mm, 5µm) Mobile phase: Acetonitrile: Methanol (90:10 v/v) Wavelength: 245nm Retention time: ADA- 3.7 min BPO- 5.8min Flow rate: 1ml/min Linearity: ADA- 1.9-4.4µg/ml BPO- 48-112µg/ml	21
6	Optimization and validation of HPLC for simultaneous determination of Adapalene and Benzoyl peroxide by surface response methodology.	Column: C18 (250 X 4.6mm, 5µm) Mobile phase: Acetonitrile: Tetrahydrofuran: Trifluoroacetic acid: Water (21: 16: 0.01: 13 v/v/v/v) Wavelength: 270nm Retention time: ADA-13.4 min BPO- 3.82min Flow rate: 1ml/min	22
7	A new RP-HPLC method for estimation of Clindamycin and Adapalene in gel formulation: development and validation consideration.	Column: C18 (250 X 4.6mm, 5µm) Mobile phase: Acetonitrile: Phosphate buffer Ph 3.0(60:40 v/v) Wavelength: 210nm Retention time: CP-3.03 min ADA-4.92 min Flow rate: 1ml/min Linearity: CP -100-500µg/ml ADA- 10-50µg/ml	23
8	A Simple HPLC-DAD	Column: C18 (150 X 4.6mm, 5µm)	24

	Method for Determination of Adapalene in Topical Gel Formulation.	Mobile phase: Acetonitrile: Water (67:33 v/v) (pH adjusted to 2.5 with OPA) Wavelength: 321nm Retention time: 6.8min Flow rate: 1.4ml/min Linearity: 8-16µg/ml Column: C18 (100 X 4.6mm, 5µm)	
9	Estimation of Adapalene through isocratic HPLC method in pharmaceutical gel formulation	Mobile phase: Acetonitrile: Tetrahydrofuran: Phosphate buffer (pH 2.5 0.01M) (30:40:30 v/v/v) Wavelength: 272nm Retention time: 2.4 min Flow rate: 1.5 ml/min Linearity: 14-26 µg/ml Column: C18 (4.6mm×250mm ,5µm)	25
10	Method development of accelerated stability study of Adapalene gel by HPLC in Pharmaceutical Formulations.	Mobile phase: Acetonitrile: Tetrahydrofuran: Trifluoroacetic acid : Water (430:360:210:0.2 v/v/v/v) Wavelength: 235nm Retention time: 7.910min Flow rate: 1ml/min	26
11	Novel Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Clindamycin and Adapalene in Pharmaceutical Dosage Forms.	Mobile phase: Phosphate buffer (pH3.0): Acetonitrile (55:45 v/v) Wavelength: 230nm Retention time: CP- 2.84 min ADA- 3.99 min Flow rate: 1ml/min Linearity: CP- 25-150µg/ml ADA- 2.5-15µg/ml	27
12	Novel Stability-Indicating RP- HPLC Method for the Simultaneous Estimation of Clindamycin Phosphate and Adapalene along with Preservatives in Topical Gel Formulations.	Mobile phase: Ammonium hydrogen Phosphate buffer(pH- 2.50): Acetonitrile (84:16 v/v) Wavelength: 321nm Flow rate: 1ml/min Linearity: CP- 20-1500µg/ml ADA- 0.5-150 µg/ml	28
13	Validated stability indicating analytical method for the determination of clindamycin phosphate and adapalene in topical formulation.	Mobile phase: Acetonitrile: Tetrahydrofuran (65:35 v/v) Wavelength: 210nm Retention time: CP- 4.9min ADA-18.9min Flow rate: 1ml/min Linearity: CP- 100-300 µg/ml ADA- 10-30 µg/ml	29
14	Development and validation of RP-HPLC method for simultaneous	Mobile phase: Water: Acetonitrile: Tetrahydrofuran : Trifluoroacetic acid	30

	determination of Adapalene and Benzoyl peroxide combination Gel.	(29:33:38:0.2 v/v/v/v) Wavelength: 270nm Retention time: ADA- 4.346 min BPO- 10.066 min Flowrate: 1ml/min Column: C18 (150 X 4.6mm, 5µm) Mobile phase: Methanol: orthophosphoric acid: tetrahydrofuran (55:30:15 v/v/v) Wavelength: 260nm Flowrate: 1ml/min Linearity: 20%-200%	31
15	A novel method development and validation for related substances of Adapalene in bulk drug product by HPLC.	Column: C18 (250mm X 4.6mm, 5µm) Mobile Phase: Acetonitrile: Tetrahydrofuran: phosphate buffer (30:40:30 v/v/v) Wavelength: 272nm Retention time: 2.4 min Flow rate: 1ml/min Linearity: 14-26µg/ml	32
16	Qualitative and quantitative estimation by HPLC method in Transdermal formulations:		

CONCLUSION: This review shows detailed study on the reported Spectroscopic and Chromatographic methods developed and validated for the estimation of Nadifloxacin and Adapalene. Literature review suggest that there are various spectroscopic and chromatographic methods available for the estimation of Nadifloxacin and Adapalene alone and in combination with other drugs. HPLC and HPTLC methods were found to be very common.

There is only one HPLC reported method for Nadifloxacin and Adapalene in their combined dosage form. So, there will be a great scope for the method development and validation of same with good precision, accuracy and robust methods for available marketed combined dosage form of Nadifloxacin and Adapalene.

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