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Development and Validation of High Performance Liquid Chromatographic Method for Estimation of Clindamycin Phosphate

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Rahul Srivastava*[1], Urmila Nishad[2], Shailendra Kumar Yadav[3]

1.Aryakul College Of Pharmacy And Research, India. 2. Gaya Prasad Institute For Human Excellence Of Pharmacy, India. 3.Hygia Institute of Pharmaceutical Education And Research, India.

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ABSTRACT

Clindamycin phosphate is used in treatment of different kinds of infections and some of their combinations are given for the treatment of malaria and Clindamycin is also used for the treatment of Acne. In this project, we worked on the Clindamycin phosphate microsphere for topical delivery and the microsphere was prepared by the method of Double emulsion solvent diffusion method. For the routine analysis of Clindamycin like sensitivity and reproducible quantitative analysis are described by HPLC. All the required data are analysed and summarized in this project work *i.e.* Linear response, Co-efficient of Correlation, Retention time etc. where these methods are very simple and easy for analysing sample in a short period of time.

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INTRODUCTION

It is an ideal controlled drug delivery system which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. The concept of microencapsulation was initially utilized in carbonless copy papers. More recently it has received increasing attention in pharmaceutical and biomedical applications. The first research leading to the development of microencapsulation procedures for pharmaceuticals was published by Bungenburg de Jong and Kass in 1931 and dealt with the preparation of gelatin spheres and the use of gelatine coacervatio process for coating. In the late 1930s, Green and co-workers of National cash register co. Dayton, Ohio, developed the gelatine coacervation process. Since then may other coating materials and processes of application have been developed by the pharmaceutical industry for the microsphere of Medicines. Over the last 25 years, numerous patents have been taken out by pharmaceutical companies for microencapsulated drugs. Microsphere is a rapidly expanding technology. As a process, it is a means of applying relatively thin coating to small particles of solids or droplets of liquids and dispersions.

Microspheres are defined as "solid spherical particles containing dispersed drug in either solution or microcrystalline form." They are ranging in size from 1 to 1000 micrometer. Microcapsules are small particles that contains an active agent as a core material and coating agent as shell, at present, there is no universally accepted size range that particle must have in order to be classified as microcapsules. Microsphere provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials.

However, the terms microcapsule and microspheres are often used synonymously. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer.

Solid biodegradable microcapsules incorporating a drug dispersed or dissolved throughout the particle matrix have the potential for the controlled release of drug.

A wide range of core materials have been encapsulated including adhesives, agrochemicals, live cells, active enzymes, flavour fragrances, pharmaceuticals, and inks.

Most capsule shell materials are organics, polymers, but fats and waxes are also used. Microcapsules can have a variety of structures some have a spherical geometry with a continuous core region surrounded by a continuous shell as shown in fig. 1 (A), other have an irregular geometry and contain a number of small droplets as particles of core material as shown in fig.1 (B). Microcapsules are used in a wide range of oral and injected drug formulation. Encapsulated adhesive resins coated on automotive fasteners are routinely used to assure that such fasteners are firmly set when installed. Microcapsules are also the basis for a number of long acting commercial pesticides and herbicides. Improvement of these products and development of new ones is an ongoing process that involves a large number of development groups globally.

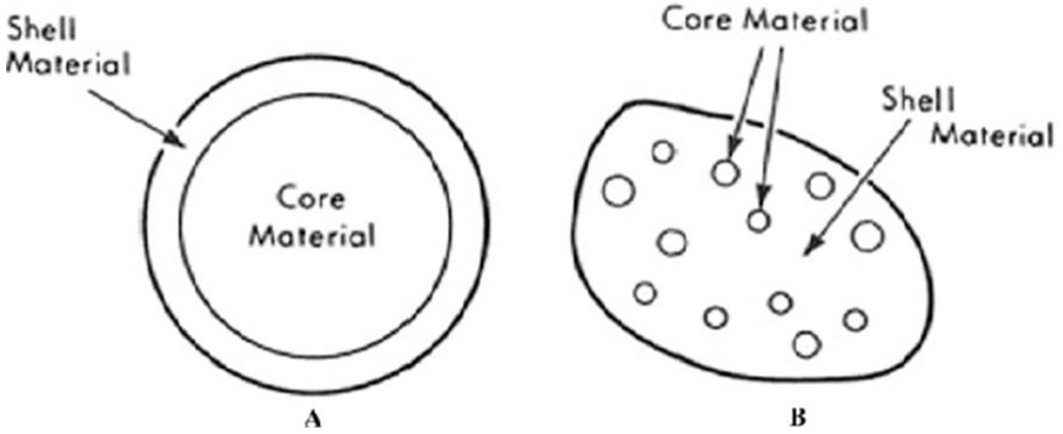


Figure No. 1: (A) Continuous core/shell microcapsules. (B) Multinuclear microcapsule.

Merits of Microspheres-

1. Microsphere change liquid to solid.

2. Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug.

3. Microspheres also used as taste masking agent.

4. Microsphere protects materials from environment for examples by preventing oxidization. E.g. Vitamin A, Palmitate

5. Microspheres are normally used to enhance material stability, reduce adverse or toxic effects as extend material release for different applications in various fields of manufacturing.

6. Microspheres solidify tacky materials and increase its fluidity. E.g. Thiamine HCL.

Demerits of Microspheres-

1. Drug entrapment is low because some portion of drug is lost in the dispersion vehicle.

2. The industrial scale of microspheres formulation is difficult because to maintain size of microspheres at industry level is difficult.

3. The manufacturing of microspheres involves use of solvents which make the process costly.

4. As compared to the extended release tablets and capsules, the manufacturing of microspheres is much more complicated.

5. Time consuming process as much time period for required for emulsification, vaporization of solvent and rigidization of microspheres.

INTRODUCTION TO DRUG CLINDAMYCIN PHOSPHATE-

A. Generic name:

(Clindamycin Phosphate)

B. Chemical name:

Methyl7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2 pyrrolidinecarboxamido)-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-(dihydrogen phosphate)

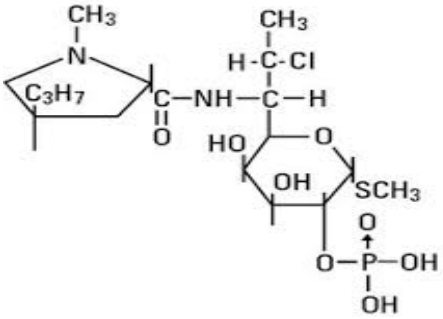
C. Empirical formula:

C18H34ClN2O8PS

D. Molecular weight:

505.0 gm/mol

E. Structural formula:



F. Description:

Clindamycin Phosphate is a white or almost white, crystalline powder.

G. Solubility:

Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS and acetone R.

H. Category:

Antibacterial drug

I. Dose:

Oral150–300 mg q 6 hr., up to 300–450 mg q 6 hr. in more severe infections.

J. Clinical pharmacology:

Although Clindamycin phosphate is inactive *in vitro*, rapid *in vivo* hydrolysis converts this compound to the antibacterial active Clindamycin. Cross resistance has been demonstrated between Clindamycin and Lincomycin. Used for the treatment of serious infections caused by susceptible anaerobic bacteria, including Bacteroides spp., Peptostreptococcus, anaerobic Streptococci, Clostridium spp., and microaerophilic Streptococci.

Systemic/vaginal clindamycin inhibits protein synthesis of bacteria by binding to the 50S ribosomal subunits of the bacteria. Specifically, it binds primarily to the 23s RNA subunit. Topical Clindamycin reduces free fatty acid concentrations on the skin and suppresses the growth of Propionibacterium acnes (Corynebacterium acnes*)*, an anaerobe found in sebaceous glands and follicles.

K. Pharmacokinetic parameters of Clindamycin Phosphate –

Pharmacokinetic parameters of Clindamycin Phosphate are shown below-

TABLE No. 1: PHARMACOKINETIC PARAMETERS OF CLINDAMYCIN PHOSPHATE

Pharmacokinetic Parameters	Values
Availability (%)	90 % (oral), 4-5 % (topical)
Urinary Excretion	Around 20 %
Bound in Plasma (%)	92-94 %
Half-Life (hours)	2-3 hr.

TABLE No. 2: APPLICATIONS OF EC IN PHARMACEUTICAL FORMULATION

Use	Concentration (%)
Microencapsulation	10.0–20.0
Sustained-release tablet coating	3.0–20.0
Tablet coating	1.0–3.0
Tablet granulation	1.0–3.0

TABLE No. 3: PHARMACOPEIAL SPECIFICATIONS OF ETHYLCELLULOSE

Test	PhEur 2005	USP NF 23
Loss on drying	≤3.0%	≤3.0%
Residue on ignition	—	≤0.4%
Sulfated ash	≤0.5%	—
Lead	—	≤10 ppm
Heavy metals	≤20 ppm	≤20 µg/g
Acetaldehyde	≤100 ppm	—
Chlorides	≤0.1%	—
Organic volatile	—	+
impurities		
Assay (of ethyl groups)	44.0–51.0%	44.0–51.0%

TABLE No. 4: USES OF CARBOMERS

Uses	Concentration (%)
Emulsifying agent	0.1–0.5
Gelling agent	0.5–2.0
Suspending agent	0.5–1.0
Tablet binder	5.0–10.0

TABLE No. 5: PHARMACOPEIAL SPECIFICATIONS OF CARBOMER

Tests	PhEur 2005	USP NF 23
Identification	+	+
Characters	+	—
Aqueous viscosity (mPas)	300–115 000	—
Carbomer 934P (0.5% w/v)	—	29 400–39 400
Loss on drying	≤3.0%	≤2.0%
Sulfated ash	≤4.0%	–
Heavy metals	≤20ppm	≤ 0.002 %
Benzene	≤ 2ppm	—
Carbomer 934P	—	≤ 0.01 %
Free acrylic acid	≤ 0.25 %	—
Organic volatile impurities	—	+
Assay	56.0–68.0%	56.0–68.0%

MATERIALS AND METHOD

Apparatus- Shimadzu- RPHLC with UV visible detector

Photodiode array detector, manual injector with a 20µ loop C18 column (250mm x 4.6mm, 5µm particle size) Analytical Balance, Ultrasonic, Micropippetes.

Reagents- Clindamycin, Methanaol, Acetonitrile, Water membrane filter (0.45µm-47mm).

Chromatographic condition-

Phenomenex C18 column (250mm x 4.6mm)

Acetonitrile: Methanol- (95:5 v/v)

Flow rate- 1.0ml/min

Injection volume- 10µL

Detection- Monitored at 210mm using U V detector

Preparation of mobile phase-

The ratio of mobile phase was prepared from Acetonitrile and Methanol (95:5v/v) and separated into clean bottle by the help of nylon 0.45µm-47mm membrane filter.

Preparation of Clindamycin phosphate standard stock solution-

Weigh accurately 22mg clindamycin phosphate and transfer into 100 ml volumetric flask and makeup volume up to the mark to obtain standard solution and that's having concentration of 220µ/ml of clindamycin phosphate.

Determination of Analytical wavelength-

Method of Validation (Calibration Curve – Linearity)-

The calibration curve plotted over the concentration range 22-220µ/ml for clindamycin phosphate. Transferring the measured standard solution of Clindamycin Phosphate (1, 2, 4, 6, 8, 10 ml) into 10 ml volumetric flask and make volume up to the mark with distill water and 10µl of each solution were injected into HPLC.

Accuracy-(Precision)-

There are the known amount of standard solution of Clindamycin Phosphate were added to 50, 100, 150% of pre quantified sample solution of Clindamycin Phosphate and that was determined by calculating recoveries by standard additional method.

Method Precision-(%Repeatability)-

For the accuracy, the precision was checked by injecting 88µg/ml of Clindamycin Phosphate under the same chromatographic condition and retention time and % relative standard deviation should not be exceeded by 2%.

Intermediate Precision (Reproducibility) Relative Standard Deviation (RSD)-

There is a method for Interday and Intraday precision where the sample analyzed by corresponding response by 3 times on the same day and on different 3 days over 1 week period for 3 different concentration of standard Clindamycin Phosphate (88µ/ml) and that was shown in terms of RSD.

Limit of Detection (LOD) and Limit of Quantification (LPQ)-

LOD and LOQ were determined by signals to noise ratio (i.e.) 3.5 LOD and 11 LOQ) and put it into equation as per International Conference on Harmonization (ICH).

$$LOD = 3.5x6/s$$

$$LOQ = 11x6/s$$

Where,

6= Standard deviation of response

S= Slope of calibration curve

RESULT AND DISCSSION

Method development-

Good peak sematary for Clindamycin Phosphate was obtained with mobile phase (95:5) v/v at the flow rate of 1.0ml/min. and that are done for optimize the RP-HPLC. The peak was obtained at 22µg/ml and 220µg/ml and that was compared with different spectra of standard solution for confirmed peak purity. Then values of UV-visible spectrum of standard solution shown good correction in system suitability for Clindamycin Phosphate.

TABLE NO. 6: SYSTEM SUITABILITY PARAMETERS OF CHROMATOGRAM FOR CLINDAMYCIN PHOSPHATE

Parameter	22µg/mlRSDa	220µg/mlRSDa
Retention Time	2.53+-0.04	2.52+-0.04
Tailing Factor	1.12+-0.24	1.15+-0.27
Theoretical Plates	6240.50+-0.32	6457.89+-0.32
Resolution	3.46+-0.21	3.59+-0.13

RSDa= Relative Standard Deviation

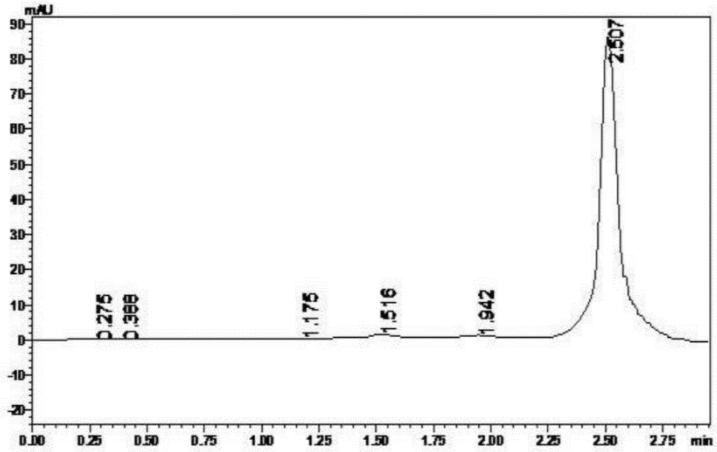


Figure No. 2: 22µg/ml chromatogram of Clindamycin Phosphate

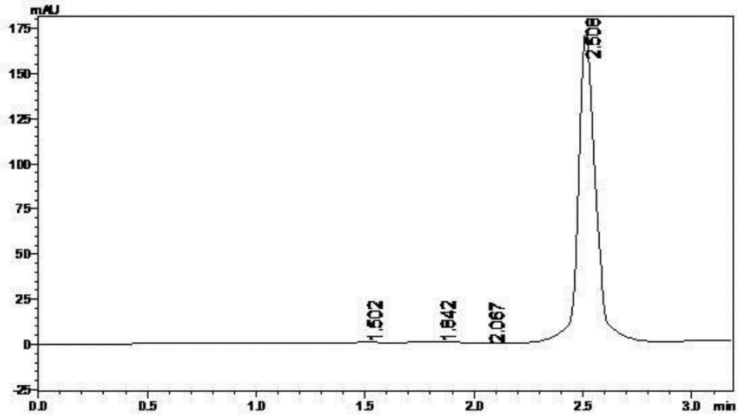


Figure No. 3: 220µg/ml chromatogram of Clindamycin Phosphate

Linearity-

The calibration curve was validated by the high values of correlation coefficient of regression. Where Linearity Correlation was obtained between peak area v/s concentration of Clindamycin Phosphate in ranges of 22-220µg/ml.

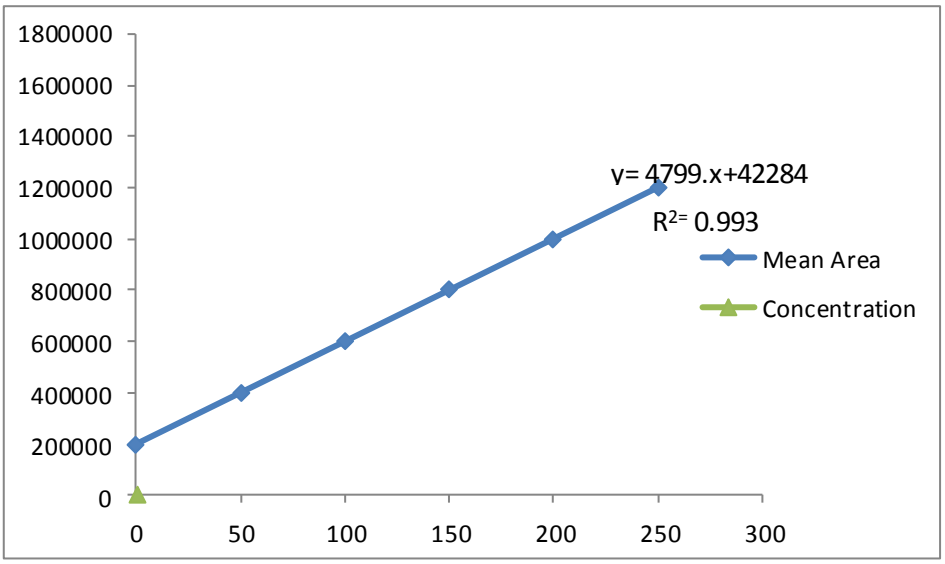


Figure No. 4: Calibration curve of Clindamycin Phosphate by using HPLC

TABLE NO. 7: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER

Parameters	**HPLC method for CP**
Detection wavelength (nm)	210nm
Concentration range	22-220 (µg/ml)
Slope	4801
Intercept	42286
Correlation Coefficient	0.995
LOD µg/ml)	2.10µg/ml
LOQ µg/ml)	6.33µg/ml
Accuracy	
S1	100.8%+-0.28%
S2	100.7%+-0.39%
S3	100.4%+-0.35%
Repeatability (%RSD)	0.37
Precision (%RSD)	
Intraday	0.28%-0.33%
Intraday	0.33%-0.38%

LOD- Limit of Detection

LOQ- Limit of Quantification

RSD- Relative Standard Deviation

Accuracy-

The recoveries obtained 100.63+-0.34 for Clindamycin Phosphate and the standard deviation for low value indicates the proposed method is accurate where standard method used for recovery experiment and result of recovery are shown in table given below.

TABLE No. 8: RECOVERY DATA FOR PROPOSED METHOD

Drug	Level	Amount of Sample	Amount of Standard Spiakal (%)	Mean % Recovery +-RSD
Clindamycin Phosphate	1	72.0	82	100.8+-0.28
	2	90	102	100.7+-0.39
	3	107.0	122	100.4+-0.35

Method Precision (% Repeatability)-

0.37% was found to be RSD value for Clindamycin Phosphate RSD values were found to be < 1% which indicates the proposed method is precise.

TABLE No. 9: PRECISION DATA FOR Clindamycin Phosphate

Clindamycin Phosphate	Retention Time	Peak Value
1	2.701	856293
2	2.703	586190
3	2.693	861104
4	2.704	863412
5	2.503	856994
6	2.496	856899
Mean	2.633333333	858482
SD	0.00626874	3033.33
%CV	0.190795525	0.373104

Intermediate Precision-

Variation in RSD values for Clindamycin Phosphate is 0.28%-0.33% Interday and 0.33%-0.38% Intraday.

LOD and LOQ-

Values of LOD and LOQ for Clindamycin Phosphate was found to be 2.10µg/ml and 6.33µg/ml respectively.

CONCLUSION

The methods used for analysis of linear response in range of 22-46µg/ml with coefficient correlation (r^2) 0.996 for Clindamycin Phosphate and their retention time less than 10 minutes.

Reproducibility, sensitivity, precise quantitative method are provided by HPLC method used for routine analysis of Clindamycin Phosphate based on different results obtained during analytical work.

REFERENCES

1. Patel A, Ray S, Thakur RS. in-vitro *e*valuation and optimization of controlled release floating drug delivery system of metformin hydrochloride. Drug del. 2006; 14 (2): 57-64.
2. Vyas S P., Khar R K., Targeted and Controlled Drug Delivery Novel Carrier System. Ist Ed., CBS Publishers and Distributors, New Delhi, 2002; 417-454.
3. Bajaj A. and Wokade R., Gelatin microspheres for topical delivery of Vitamin A palmitate, *NSHM Journal of Pharmacy and Healthcare Management*, 02; 2011;61-65.
4. Das M.K. and Rao K., Microencapsulation of zidovudine by double emulsion solvent diffusion technique using ethylcellulose, Indian journal of pharmaceutical science, 69(2); 2007; 244-250.
5. Garud N. and Garud A., Preparation and in-vitro *e*valuation of metformin microspheres using non-aqueous solvent evaporation technique, Tropical journal of pharmaceutical research, 2012; 11(4); 577-583.
6. Golmohammadzadeh S., Malaekeh-Nikouei B., Hosseini M. and Nassirli H., Preparation and characterization of liposomes encapsulated with clindamycin and tretinoin, Pharmacy globale international journal of comprehensive pharmacy, 2(6); 2011; 1-4.
7. Lachman L, Lieberman H A., Pharmaceutical Dosage Forms, microspheres, Vol. 12, Marcel Dekker, Inc. New York, 2000.
8. Jain N K. Controlled Novel Drug Delivery. Its Ed. CBS Publishers and Distributors, New Delhi, 2002, 236-255.
9. Jain N K. Progress in Controlled and Novel Drug Delivery Systems. 1st Ed. CBS Publishers and Distributors, New Delhi, Bangalore, 2004; 84-85.
10. Gupta R., Varma H., Anti-ulcer potential of ethylcellulose floating microspheres containing ranitidine hydrochloride in experimental rodents, Asian Journal of Pharmaceutical and Clinical Research, 5(2); 2012; 205-209.

	***Author Name – Rahul Srivastava*** *Aryakul College of Pharmacy And Research* *Gauri bijnaur road adjacent of CRPF camp*
	***Urmila Nishad*** *Gaya Prasad Institute of Human Excellence for Pharmacy* *Near malihabad station*
	***Shailendra Kumar Yadav*** Hygia Institute of Pharmaceutical Education And Research