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CHEMOMETRIC ASSISTED NP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF NORFLOXACIN AND LACTOBACILLUS

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ABSTRACT:
A new chemometric carried out by High Performance Liquid Chromatography (HPLC) through photodiode array (PDA) detection was executed for the simultaneous estimation of tablet dosage form. Two chemometric calibration techniques, principle component analysis (PCA) and partial least squares (PLS) were applied to the peak area at 242 nm of PDA detector responses. Chromatographic separation of Norfloxacin and Lactobacillus was achieved on Waters Alliance-e2695, by using Chiral Cell ODH 150 × 4.6 mm, 5µ column and the mobile phase containing Hexane : IPA : Ethanol in the ratio of 20:40:40% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 242 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Norfloxacin and Lactobacillus were NLT 2000 and should not more than 2 respectively. The % Relative standard deviation of peak areas of all measurements always less than 2.0**.** The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Norfloxacin and Lactobacillus and study of its stability. The 'UNSCRAMBLER (camo)'software was used for the numerical calculations. All of the two-chemometric analysis methods in this study can be satisfactorily applied for the quantitative analysis of Norfloxacin and Lactobacillus in pharmaceutical tablet dosage form

KEYWORDS: NP-HPLC, Norfloxacin, Lactobacillus, PCA, PLS, Unscrambler.

INTRODUCTION

In the assurance of the quality of the bulk drugs and Pharmaceutical preparations the role of data analysis is vital. The pharmacopoeias may not provide the standard analytical procedure for the determination of the newer drugs and formulations. Thus, it is essential to develop chemometric assisted RP-HPLC method to develop a rapid qualitative analysis Pharmaceutical properties of intermediate and finished dosage forms[1].

The chemometric methods are one type of multivariate analysis that is considering more than one variable at the at a time[2]. Thus, it does not exist in one dimensional data[3]. The science of chemometrics can be briefly described as the interaction of certain mathematical and statistical methods to chemical problems. It has developed as a consequence of a change of in the data obtained with the chemistry with the emergence of the new analytical techniques as well as microprocessors [4].The applications of using chemometric techniques in analytical chemistry are now numerous and applications have been revealed in spectroscopy, chromatography and other disciplines of analytical chemistry[5] Least square approach involves mathematical modelling by which the square of residual (difference between actual and predicted concentration) is minimized to lowest level[6]

These methods first calibrate the mathematical model by using absorbance data of calibration standards with known concentration and then predict the concentration of un-known samples from their absorbance data. If there are m number of calibration standards and l chemical components (drugs) and is the number of wavelengths considered, all methods involve presentation of absorbance data as a matrix with m rows and n columns, concentration data as a matrix with m row and l columns. Stability is defined as "An ability of pharmaceutical product to retain its physical, chemical, microbiological properties within specifications throughout its shelf life" according to ICH guidelines[7]. The solution stability of Norflaxallin and Lactobacillus in diluents can be determined by storing sample solution and tightly cab volumetric flask at room temperature for 24 hr.Method validation can be defined as per ICH as

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"establishing evidence, which provide a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.[8]

NORFLOXACIN AND LACTOBACILLUS COMBINATION

Norfloxacin + Lactobacillus is a combination of two medicines. Norfloxacin is an antibiotic. It works by stopping the action of a bacterial enzyme called DNA-gyrase. This prevents bacterial cells from dividing and repairing, thereby killing them. Lactobacillus is a probiotic. It works by restoring the balance of good bacteria in the intestine that may get upset after antibiotic use or due to intestinal infections. This is how it works to treat bacterial infections.

MATERIALS AND EQUIPMENT

The developed NP-HPLC method for the estimation of Norfloxacin and Lactobacillus was carried out on Chiral Cell ODH 150x4.6mm, 5µ column using mobile phase composition of a mixture Hexane: IPA Ethanol in the ratio of 20:40:40% **v/v**. with flow rate of 1.0 ml /min at 242 nm.

MATERIALS

Instruments used-HPLC, Empower version 2.0 software, UV-Visible detector, Shimadzu Analytical balance.

Chemicals and Reagents: HPLC grade Water,Isopropyl alcohol ,Ethanol and hexane

Drugs- Norfloxacin and Lactobacillus

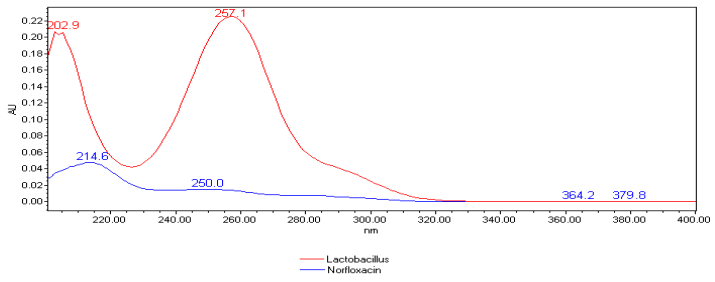
METHODOLOGY

METHOD DEVELOPMENT

In the present investigation, we have developed a simple and sensitive NP-HPLC method for quantitative estimation of Norfloxacin and Lactobacillus in bulk drug and Pharmaceutical dosage forms. These are trails performed for HPLC method development of Lactobacillus and Lactobacillus.

Selection of wave length (For Detection) In setting up the conditions for development of assay method, the choice of detection wavelength was based on the scanned absorption spectrum for Norfloxacin and Lactobacillus. The UV-Spectrum of Norfloxacin and Lactobacillus was obtained separately by scanning the sample over the wave length range 200-400nm against blank as methanol. After thorough examination of the spectra, the wave length 242 nm was selected for further analysis. Shown in **Figure I**

Figure I : Overlay spectrum of Norfloxacin and Lactobacillus



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OPTIMIZED METHOD:

Preparation of Buffer solution: Mix Ethanol in 1litre water, filtered through 0.45µm nylon membrane filter.

Mobile Phase A mixture of Hexane and IPA: with Ethanol in the ratio of (20:40:40)%v/v was sonicated to degas andfiltered through 0.45µm nylon membrane filter.

Chromatographic conditions

Column : Chiral Cell ODH 150x4.6mm, 5µ

Mobile phase ratio : Hexane, THF and Acetic acid 96.5+3+0.5

Detection wavelength : 242 nm

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 10min

Retention time of Norfloxacin is about 3.003 min.

Retention time of Lactobacillus is about 7.267 min.

Preparation of standard stock solution: Accurately weighed 5mg of Norfloxacin and 5mg of Lactobacillus were transferred into two different 10ml volumetric flasks, make up the flasks with methanol and sonicate for 5 minutes then take 0.8ml of Norfloxacin and 0.24ml of Lactobacillus solution into a 10 ml volumetric flasks and made upto 10ml with methanol and then then transfer this solution into vial using a 1ml syringe.

Preparation of Sample solution: Weighed 1 tablet and crush in to powder and take equivalent weight of sample in to a 100ml volumetric flask. Added 70 mL of diluent, sonicate to dissolve and diluted to volume diluent. Further diluted 5 mL to 50 mL with the diluent. Filter through 0.45µ Nylon syringe filte

Table: I Assay Calculations

Drug	**sample area**	**Std. wt**	**Sample wt.**	**Label amount (mg)**	**% assay**
Norfloxacin	3245936	25	39.1	400	100.7
Lactobacillus	1139893	6	39.1	96	101.1

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Figure II Representative chromatogram of Blank

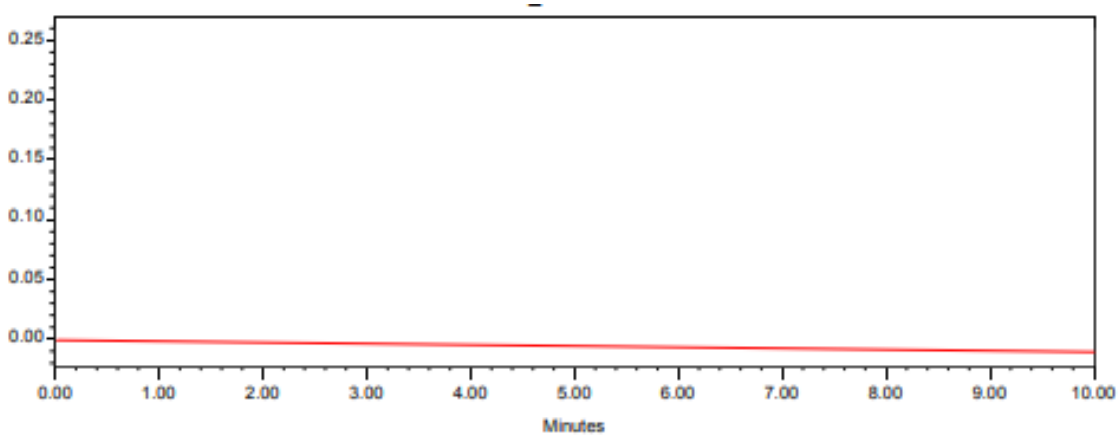


Figure III A Representative chromatogram of Standard

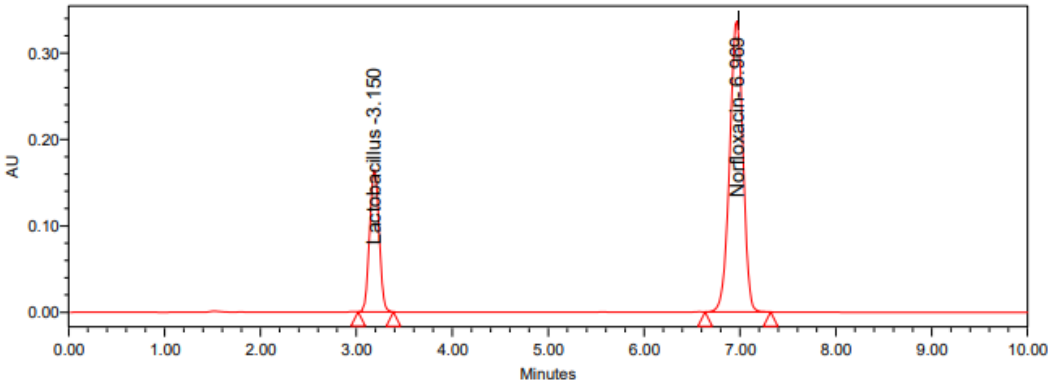
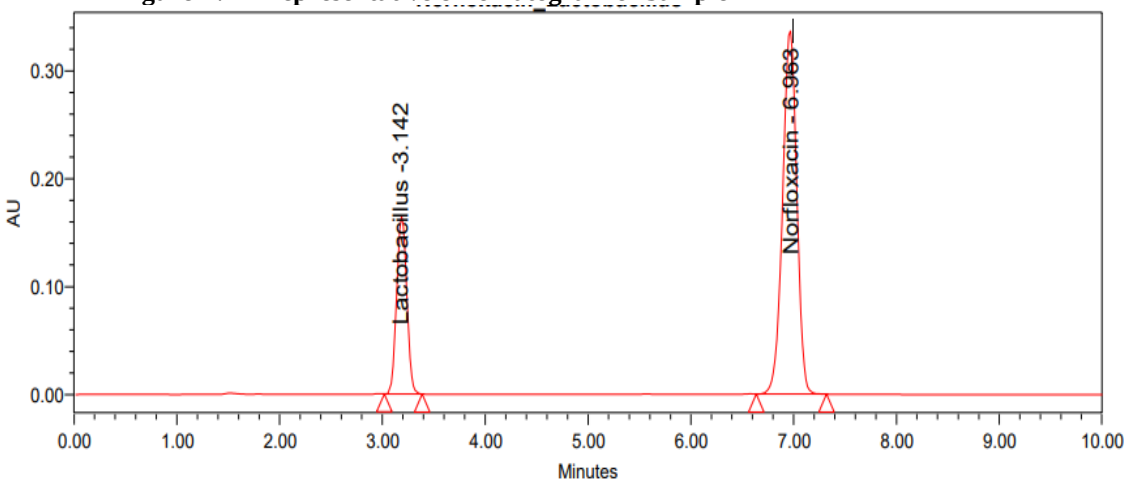


Figure IV A Representative chromatogram of Sample



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METHOD VALIDATION

Analytical method validation is a process of performing several tests designed to verify that an analytical test method is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely. There are several parameters that are considered in the method validation process as per International Conference of Harmonization (ICH) guidelines and the values for these parameters are as follows.

Table: II Validation parameters for Norfloxacinand Lactobacillus

PARAMETER	ACCEPTANCE CRITERIA	NORFLOXACIN	LACTOBACILLUS
Correlation Coefficient Linearity Range	Correlation coefficient $r^2 > 0.999$	$r^2 = 0.9998$	$r^2 = 0.9999$
System Precision	RSD < 2%	%RSD =0.303	%RSD =0.297
Intermediate Precision	RSD < 2%	%RSD = 0.7 .	%RSD = 0.9
Method precision	RSD < 2%	%RSD = 0.69	%RSD = 1.06
Accuracy	Recovery 98 - 102% (individual)	% recovery=100.2	% recovery=99.5
Solution Stability	> 12 hour	Stable up to 24 hour	Stable upto 24 hour %RSD =0.868
Robustness	RSD NMT 2% in modified condition Flow minus Flow plus Organic plus Organic minus	Complies %RSD=0.51 %RSD= 0.15 %RSD=0.66 %RSD=0.72	Complies %RSD= 1.25 %RSD= 1.45 %RSD=1.51 %RSD=1.15
LOD		0.25	0.06
LOQ		0.5	0.6

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Table no: III Stability studies of Norfloxacin

S.No.	Stability (hrs.)	Rt (min)	Peak Area	USP Plate count	USP Tailing	%Assay	% Deviation
1	INITIAL	6.952	3220874	12264	0.96	100	0.00
2	6 HRS	6.948	3215781	12654	0.91	99.8	-0.20
3	12 HRS	6.953	3212367	12239	0.95	99.7	-0.30
4	18HRS	6.958	3201481	12580	0.97	99.4	-0.60
5	24 HRS	6.956	3195743	12447	1.00	99.2	-0.80

Table no: IV Stability studies of Lactobacillus

S.No.	Stability (hrs.)	Rt (min)	Peak Area	USP Plate count	USP Tailing	%Assay	% Deviation
1	INITIAL	3.156	1127154	4674	1.11	100	0.00
2	6 HRS	3.150	1123587	4612	1.04	99.7	-0.30
3	12 HRS	3.155	1120046	4654	1.10	99.4	-0.60
4	18HRS	3.161	1119533	4698	1.11	99.3	-0.70
5	24 HRS	3.160	1117124	4702	1.08	99.1	-0.90

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Table no V Disintegration results of Norfloxacin

		Norfloxacin				Peak Purity		
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	%Degra dation	Purity Angle	Purity Thresold	Pass/Fail
Control	39.1	3220769	3220769	100	0	4.162	10.823	Pass
Acid	39.1	2796451	2796451	86.8	13.2	4.182	10.711	Pass
Alkali	39.1	2835347	2835347	88	12	4.256	10.777	Pass
Peroxide	39.1	2848347	2848347	88.4	11.6	2.552	10.714	Pass
Thermal	39.1	3201604	3201604	99.4	0.6	4.148	10.685	Pass
Hydrolysis	39.1	3214604	3214604	99.8	0.2	4.196	10.658	Pass
Reduction	39.1	2870457	2870457	89.1	10.9	2.525	10.539	Pass
Photolytic	39.1	2838904	2838904	88.1	11.9	4.132	10.225	Pass

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Table no V1 Disintegration results of Lactobacillus

		Lactobacillus				Peak Purity		
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	%Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	39.1	1127672	1127672	100	0	4.185	10.719	Pass
Acid	39.1	983597	983597	87.3	12.7	4.191	10.726	Pass
Alkali	39.1	949069	949069	84.2	15.8	4.195	10.715	Pass
Peroxide	39.1	1009412	1009412	89.5	10.5	2.533	10.605	Pass
Thermal	39.1	1123412	1123412	99.7	0.3	4.182	10.732	Pass
Hydrolysis	39.1	1121412	1121412	99.5	0.5	4.188	10.715	Pass
Reduction	39.1	1001741	1001741	88.9	11.1	2.533	10.613	Pass
Photolytic	39.1	984577	984577	87.3	12.7	4.182	10.721	Pass

Chemometric Analysis

In this chemometrics assisted HPLC study , PCA, PLS calibrations were used to analyse the drugs of Norfloxacin and Lactobacillus at 246 nm by using PDA detector. The data obtained from analysed drugs were stored in computer having required software to perform chemometric analysis.

Acquisition software: In present study we are using following chemometric techniques.

- Principal component analysis(PCA)
- Partial least squares technique(PLS)

We are download the unscrambler (camo software), it facilitates the PCA,PLS analysis more robust, accessible.

3.1 PLS Approach:

PLS calibration using the orthogonalized PLS algorithm involves, simultaneously, independent and dependent variables on the data compression and decomposition operations. In the HPLC data analysis, HPLC-PLS calibration was obtained by decomposition of both the drugs of concentration , peak area matrix into latent variables. PLS calibration was obtained using the relationship between the decomposed peak area data and concentration

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Figure V PLS of accuracy spectral data of Norfloxacin

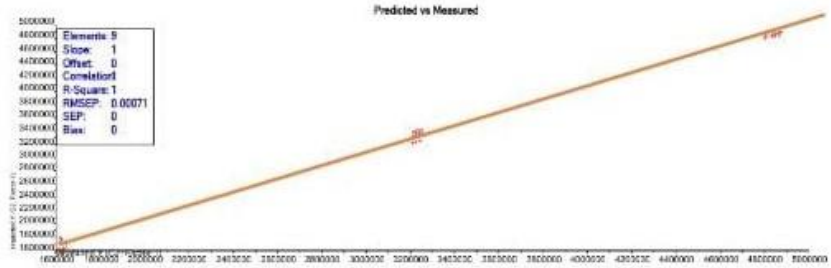

Figure VI PLS of accuracy spectral data of Lactobacillus

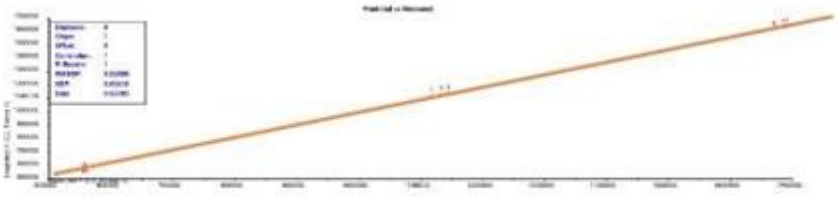

Figure VII PLS of accuracy spectral data of Norfloxacin & Lactobacillus

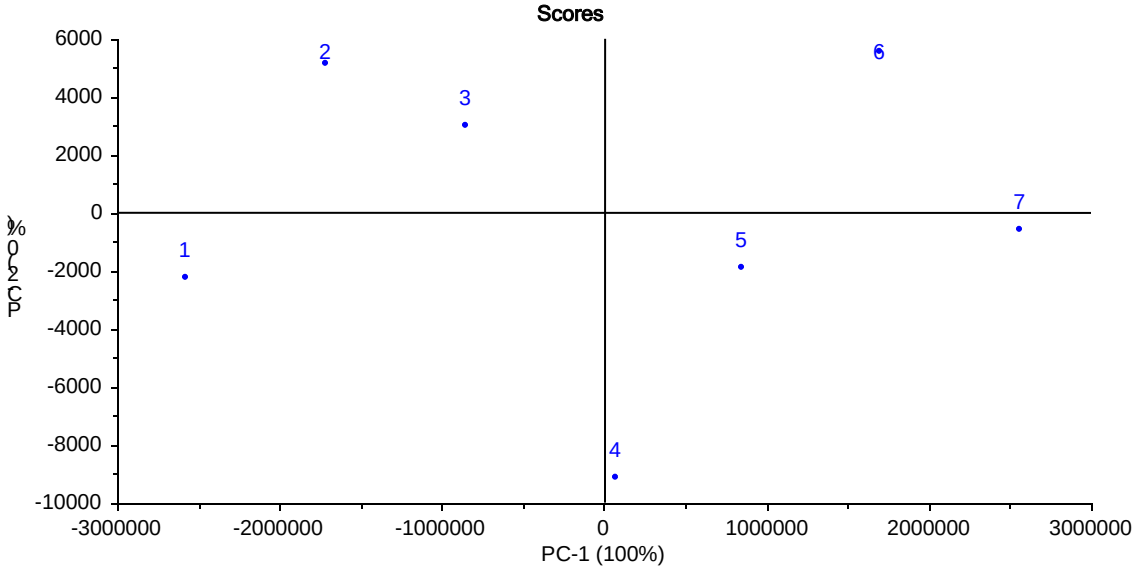

Figure VIII PCA Linearity Spectral Data of Norfloxacin and Lactobacillus

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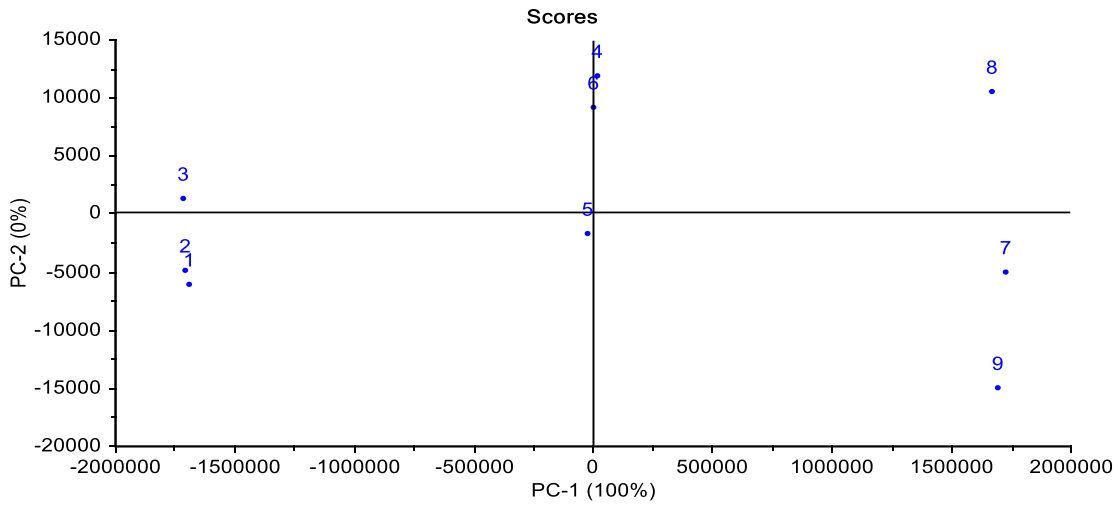

CONCLUSION

In this investigation new analytical methods have been developed for the estimation of the potent drug Norfloxacin and Lactobacillus. This study contains evaluation of HPLC data for the chemometric techniques of PCA and PLS. These chemometric methods could be applied with great success for the simultaneous determination of Norfloxacin and Lactobacillus in the pharmaceutical formulation without the interference of each other.

The two chemometric method that i.e. PCA and PLS are found to be simple, precise, accurate, rapid and economical method for their simultaneous determination. The methods were successfully validated and found suitable for quality control laboratories.

It conclude that novel stability indicating method for the determination of drugs in combined dosage form for Norfloxacin and Lactobacillus in according to ICH guidelines and it can be used for meeting the regulatory guidelines for above drugs

CONFLICT OF INTERESTS:

The authors declare that they have no conflict of interests regarding this research work.

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