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Chemometric Assisted New Stability Indicating NP-HPLC Method Development and Validation of Doxycycline and Lactobacillus in Combined Dosage Form

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ABSTRACT

A new chemometric assisted by high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection was implemented for the simultaneous determination of Doxycycline and Lactobacillus in capsule dosage form. Two chemometric calibration techniques, principle component analysis (PCA) and partial least squares (PLS) were applied to the peak area at 225 nm of PDA detector responses. The method was carried out on chiral cell OJ-RH (150 X4.6mm, 3.5um) a column with a mobile phase consisting of Hexane and Ethyl acetae in the ratio of (90:10%v/v) and flow rate of 1.0 ml/ min. The detection was carried out at 225nm.The retention time for Doxycycline and Lactobacillus were found to be 3.013and 7.267 min respectively. The method was validated according to the ICH guidelines for specificity, LOD, LOQ, precision, accuracy, linearity and robustness. The method showed good reproducibility and recovery with %RSD less than 2. So the proposed method was found to be simple, specific, precise, accurate and linear. 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 Doxycycline and lactobacillus pharmaceutical capsule dosage form.

KEY WORDS: Doxycycline, Lactobacillus, Method Validation, Chemometrics, ICH Guidelines.

INTRODUCTION:

High Performance Liquid Chromatography is widely applied for separations and purifications in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries 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 NP-HPLC method for the development of 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 that a time.The term chemometrics means biometrics and econometrics which was introduced into the fields of biological science and economics.[2]chemometrics has been developing and is now widely applied to different fields of chemistry, especially analytical chemistry.[3] Two main reasons why chemometrics developedso rapidly at that time:

(1) large piles of data not available before could be acquired from advanced chemical instruments
(2) Advancements in microelectronics technology within that period**.**

The applications of using chemometric techniques in analytical chemistry are numerious including spectroscopy, chromatography spectroscopy, chromatography and other disciplines of analytical chemistry [4]

. Doxycycline acts on the vital functions of the bacteria like preventing the essential protein synthesisthus acts as an antibiotic In bacterial replication, an interaction that is important for translation initiation of proteins occurs at the 3′ end of the 16S rRNA, found on the ribosome on the 30S subunit [1], [2], [3].. doxycycline chemically is (4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aR*)-4-(dimethylamino)-1,5,10,11,12*a*-pentahydroxy-6-methyl-3,12-dioxo-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-2-carboxamide.

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. Lactobacillus is used to restore good bacteria in the intestine which get unbalanced due to the over use of antibiotics or due to various intestinal diseases and infections.

USE OF THIS COMBINATION:

A combination of Doxycycline and Lactobacillus is widely used for Diarrhea, Lyme disease, Chronic prostatitis, Sinusitis, Pelvic inflammatory disease, Acne, Rosacea, Rickettsial infections, Travelers diarrhea, Diarrhea associated with hospitalization and other conditions.The antibiotic property of doxycycline stops the bacterial growth which is essential to carry nvital functions.
. Lactobacillus is used for high cholesterol,lactose intolerance, hives disease and to boost the immune system immune system. Sometimes women used lactobacillus suppositories to treat vaginal infections and urinary tract infections.

2. MATERIALS AND EQUIPMENT:

The developed NP-HPLC method for the simultaneous estimation of Doxycycline and Lactobacillus was carried out on with chiral cell OJ-RH (150 X4.6mm, 3.5um) column. Hexane and Ethyl acetae in the ratio of (90:10%v/v) used as mobile phase and flow rate of 1.0 ml/ min. The detection was carried out at 225nm.

2.1 MATERIALS:

Instruments used- EutechPH meter, Shimadzu Analytical balance, HPLC Water, 2695 separation module, UCA 701 unichrome Ultrasonicator, Empower Software, Version-2.0 Unscrambler, Detector PDA (Photo diode array), Flow rate 1ml/min.

Chemicals and Reagents: Hexane and Ethyl acetate.

Drugs: Doxycycline, Lactobacillus.

3. METHODOLOGY

3.1 METHOD DEVELOPMENT

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

3.2. 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 Of Doxycycline and Lactobacillus. The UV-Spectrum of Doxycycline 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 225 nm was selected for further analysis.

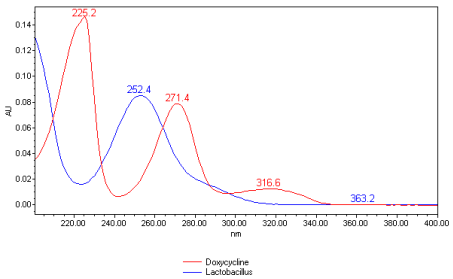

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Figure 01: Overlay spectrum of Doxycycline and Lactobacillus

3.3 OPTIMISED METHOD

3.3.1.Preparation of Buffer solution: HPLC grade methanol is used as diluents and filtered through0.45µm nylon membrane filter.

3.3.2 Mobile Phase: A mixture of Hexane and Ethylacetatae 90:10%v/v was sonicated to degasandfilteredthrough0.45µm nylon membrane filter.

3.3.3. Chromatographic conditions

Preparation of Diluent: Mobile phase is used as diluent.

Column	:	chiral cell OJ-RH (150 X4.6mm, 3.5um)
Mobilephase	:	Hexane and Ethyl acetatae 90:10%v/v
Flow rate	:	1.0mL/min
Detectionwavelength	:	225 nm
Injectionvolume	:	10µl
Temperature	:	Room temperature
Run time	:	10 min

Retentiontime of Doxycycline is about 3.013 min.

Retentiontime of Lactobacillus is about 7.267 min.

3.4. Preparation of standard stock solution: Accurately weighed 5mg of Doxycycline 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 1ml Doxycycline of and 1ml Lactobacillus of solution into 10 ml volumetric flasks and made up to 10ml with methanol and then then transfer this solution into vial using a 1ml syringe.

3.5Preparation of Sample solution: Weighed 10 capsules and weigh and then take 5capsules equivalent of sample into a 100 mL 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 filter.

DRUG	**Area**	**LABELED AMOUNT(mg)**	**AMOUNT PRESENT(mg)**	% **ASSAY**
Doxycycline	3576798	100	50.1	100.4
Lactobacillus	3655443	5billion spores	19.97	99.6

Table:1 Assay Calculations

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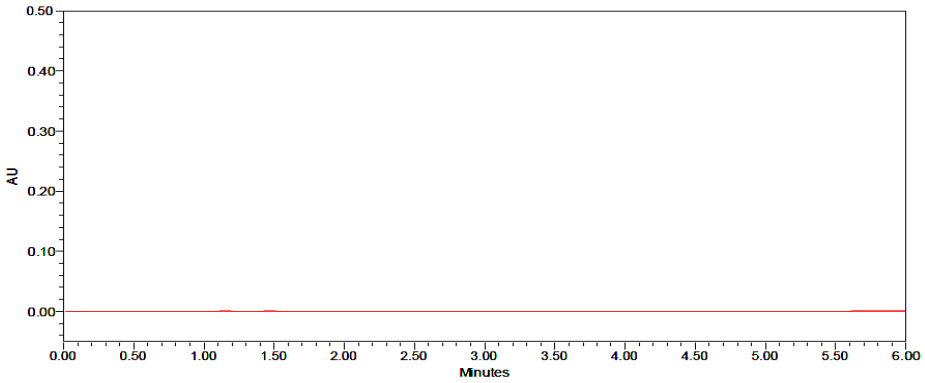

Figure 02: A Representative chromatograph of blank

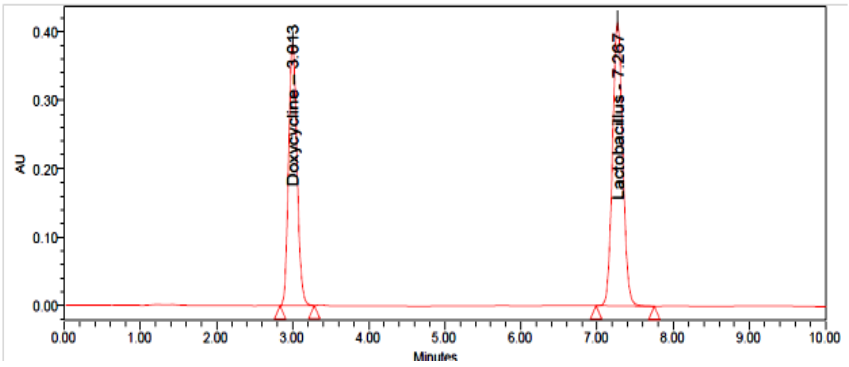

Figure03: A Representative chromatograph of Standard

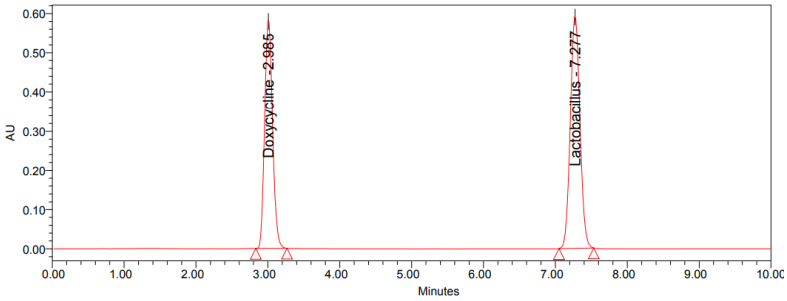

Figure04: A Representative chromatograph of sample

4. 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.

PARAMETER	ACCEPTANCE CRITERIA	DOXYCYCLINE	LACTOBACILLUS

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Linearity Range Correlation Coefficient	Correlation coefficient r 2 > 0.999	r 2 = 0.99947	r 2 = 0.99992
System Precision	RSD < 2%	%RSD = 0.23	%RSD =0.613
Intermediate Precision	RSD < 2%	%RSD = 0.61	%RSD = 1.09
Method precision	RSD < 2%	%RSD = 0.72	%RSD = 0.85
Accuracy	Recovery 98- 102% (individual)	% recovery(50%)= 0.400%	% recovery(50%)= 1.220%
		recovery(100%)=0.610%	recovery(100%)=1.110%
		recovery(150%)=1.000%	recovery(150%)=0.550%
Robustness RSD < 2%	RSD NMT 2% in modified condition	Complies	Complies
	Flow minus	%RSD=0.72	%RSD= 0.56
	Flow plus	%RSD= 0.9	%RSD= 0.81
	Organic plus	%RSD=1.01	%RSD=0.44
	Organic minus	%RSD=0.9	%RSD=1.1
LOD		0.05	0.5
LOQ		0.05	0.5

Table2: Validation parameters for of Doxycycline and Lactobacillus.

4.1 STABILITY STUDIES

S No.	Stability (hrs)	Rt(min)	Peak area	USP Plate count	USP Tailing	% assay
1	INITIAL	3.011	3549487	3514	1.20	100
2	6 HRS	3.016	3545872	3544	1.15	99.9
3	12 HRS	3.012	3540629	3591	1.23	99.7
4	18HRS	3.017	3525414	3588	1.30	99.3
5	24 HRS	3.020	3515356	3565	1.33	99

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Table:03 Results of solution stability for Doxycycline

S No.	Stability (hrs)	Rt(min)	Peak area	USP Plate count	USP Tailing	% assay
1	INITIAL	7.275	3638474	13826	1.35	100
2	6 HRS	7.261	3625781	13870	1.13	99.7
3	12 HRS	7.259	3615692	13720	1.16	99.4
4	18HRS	7.264	3609845	13531	1.19	99.3
5	24 HRS	7.268	3605693	13543	1.24	99.1

Table:04 Results of solution stability for Lactobacillus

		Doxycycline and Lactobacillus				Peak Purity		
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	%Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	29.6	3550486	3550486	100	0	4.112	10.734	Pass
Acid	29.6	3115864	3115864	87.8	12.2	4.136	10.757	Pass
Alkali	29.6	3165584	3165584	89.2	10.8	4.163	10.747	Pass
Peroxide	29.6	3065342	3065342	86.3	13.7	2.584	10.635	Pass
Thermal	29.6	2984654	2984654	84.1	15.9	4.158	10.711	Pass
Hydrolysis	29.6	3539195	3539195	99.7	0.3	4.195	10.711	Pass
Reduction	29.6	3179582	3179582	89.6	10.4	2.531	10.665	Pass
Photolytic	29.6	3146821	3146821	88.6	11.4	4.188	10.715	Pass

Table: 05 Results of forced degradation for doxycycline

	Doxycycline and Lactobacillus				Peak Purity		
Sample	Area Counts	Mean					

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	Weight in mg	Injections	Area Count	% Label Claim	%Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	29.6	3638359	3638359	100	0	0.345	10.844	Pass
Acid	29.6	3248594	3248594	89.3	10.7	0.368	10.832	Pass
Alkali	29.6	3219532	3219532	88.5	11.5	0.347	10.816	Pass
Peroxide	29.6	3086592	3086592	84.9	15.1	1.184	10.336	Pass
Thermal	29.6	3124512	3124512	85.9	14.1	0.368	10.843	Pass
Hydrolysis	29.6	3632487	3632487	99.9	0.1	0.371	10.843	Pass
Reduction	29.6	3202563	3202563	88.1	11.9	1.172	10.306	Pass
Photolytic	29.6	3172547	3172547	87.2	12.8	0.375	10.836	Pass

Table: 06 Results of forced degradation for lactobacillus

5. CHEMOMETRIC ANALYSIS

In this chemometrics assisted HPLC study,PCA,PLS calibrations were used to analyse the drugs of hydrochlorothiazide and triamterene at 276nm 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 using unscrambler (camo software).

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

5.1 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 set.

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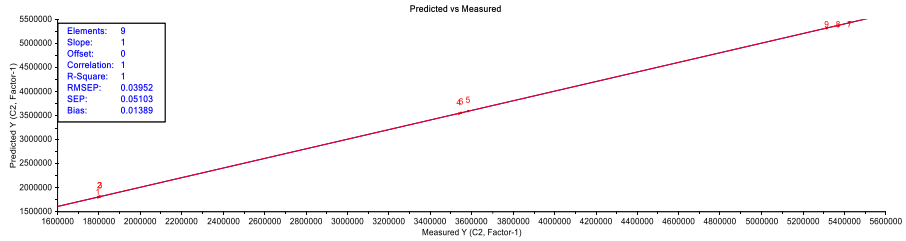

Figure04: PLS of accuracy spectral data of doxycycline

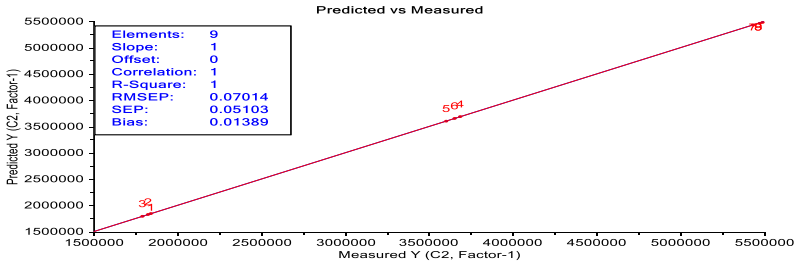

Figure05: PLS of accuracy spectral data of lactobacillus

5.2 PCA approach:

In PCA technique it gives relevant information from data set, and it can be used express the data on the basis of their similarity and differences. It is used to develop correlation structure between variables, and examine the changes. In PCA data transferred to describe the amount of same variability. In these HPLC data analysis the data of drugs of of Doxycycline and Lactobacillus peak area we get the Bio-plot.

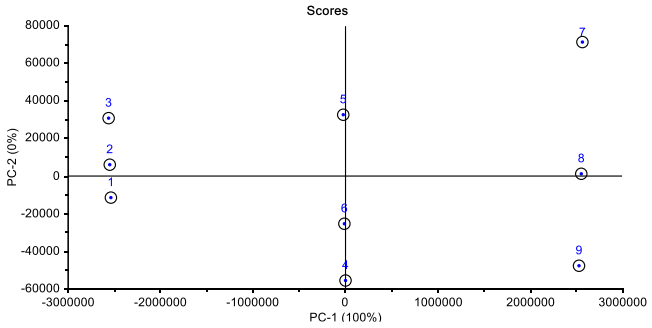

Figure 06: PCA accuracy spectral data of doxycycline and lactobacillus

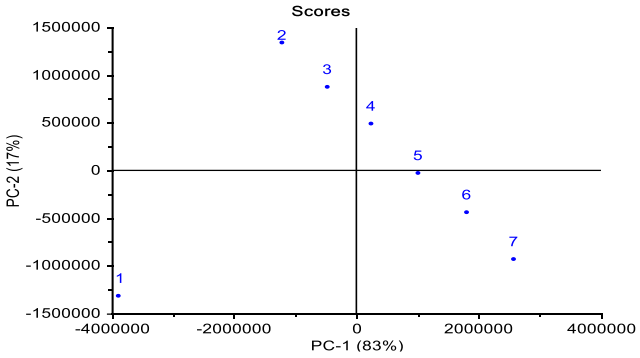

Figure 07: PCA Linearity spectral data of doxycycline and lactobacillus

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6. CONCLUSION

In the present investigation new analytical methods have been developed for the estimation of Doxycycline and Lactobacillus the potent drug. 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 Doxycycline 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 concludes that novel stability indicating method for the determination of drugs in combined dosage form for of Doxycycline and Lactobacillus in according to ICH guidelines and it can be used for meeting the regulatory guidelines for above drugs.

7. CONFLICT OF INTERESTS:

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

8. ACKNOWLEDGEMENT:

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