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CHEMOMETRIC ASSISTED NEW STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF BOSWELLIA SERRATA (AFLAPIN) AND COLLAGEN TYPE II IN COMBINED DOSAGE FORM

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ABSTRACT:

Anewchemometricassistedbyhigh-performanceliquidchromatography (HPLC)withphotodiodearray(PDA)detectionwasimplementedforthesimultaneousdeterminationofBoswelliaserrata (AFLAPIN)andCollagen type IItabletdosageform.Twochemometric calibration techniquesPrincipal component analysis (PCA) and partialleastsquares(PLS)wereappliedtothepeakareaat221 nmofPDAdetectorresponses. The method was carried out on a Luna Phenyl Hexyl(150X4.6mm, 3.5µ),columnwithamobilephaseconsistingofHexane and IPA (20:80v/v) with 0.1% Acetic acid and flow rate of 1.0 ml/ min. The detection was carried out at 221nm.TheretentiontimeforBoswelliaserrata (AFLAPIN)andCollagen type IIwerefoundtobe4.1and6.2min respectively. The method was validated according to the ICH guidelines forspecificity, LOD, LOQ, precision, accuracy, linearity and robustness. The methodshowed good reproducibility and recovery with %RSD less than 2. Sothe proposedmethodwasfoundtobesimple,specific,precise,accurateandlinear.The'UNSCRAMBLER (camo)'software was used for the numerical calculations. All ofthe two-chemometric analysis methods in this study can be satisfactorily applied forthe quantitative analysis of Boswelliaserrata (AFLAPIN) and Collagen type II in pharmaceutical capsule dosageform

KEYWORDS: Boswelliaserrata (AFLAPIN)**,**Collagen type II, RP-HPLC, unscramble software, PLS, PCA.

INTRODUCTION:

In data analysis the qua;ity assurance of the bulk drugs and pharmaceutical preparations plays a vital role. 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 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. [2]Thus, it does not exist in one dimensional data.[3] the science of chemometric 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 [3].

The resinous part of Boswelliaserrata contains,monoterpenes, diterpenespentacyclictriterpenic acids (boswellic acids); tetracyclic triterpenic acids. The 4 major components are β-Boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, acetyl-11-keto-β-boswellic The 20% concentration of acetyl-11-keto-β-boswellic acid is known as aflapin.It is used as Antiseptic, Antiarthritic And Antiinflammatory[5-6]It is believed that type II collagen will be transported across the gut epithelial cells to the underlying immune cells in the Peyer's patches where it activates naive T cells to become T regulatory (Treg). The activated Treg cells then migrate from the GALT through the lymphatic system and enter circulation. When they recognize a compound similar to what

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was ingested (e.g., type II collagen in joint cartilage), the Treg cells secrete anti-inflammatory cytokines such as TGFbeta, interleukin (IL)-4 and IL-10. This action suppresses the action of cells involved in the normal breakdown of collagen and other extracellular matrix proteins[8]

USE OF THIS COMBINATION

This combination is used in osteoarthritis and various other joint diseases treatment. This combination is agood painkiller and also prevents cartilage loss. It maintains overall joint health by providing complete protein supplement for cartilages and joints.

Collagen type II is present as protein in various body parts of humans and animals like cartilage,bone and other tissues. Collagen type II helps by directly acting on the body and producing substances that reduce swelling and also to fight pain. Aflapin directly acts on leukotrienes formation, thus reducing inflammation.alflapin is used in the treatment of osteoarthritis, rheumatoid arthritis, asthma and can be an effective painkiller and may prevent the loss of cartilage

MATERIALS AND EQUIPMENT:

The developed RP-HPLC method for the simultaneous estimation of Boswelliaserrata (AFLAPIN) and collagen type II on with Luna Phenyl Hexyl(150X4.6mm, 3.5µ). Hexane and IPA 20:80 with 0.1% Acetic acid was used as mobile phase and flow rate of 1.0 ml/min. The detection was carried out at 221 nm and ambient column temperature was maintained

MATERIALS:

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

Chemicals and Reagents: HPLC grade Water, Hexane,Iso propyl alcohol, acetic acid.

Drugs-Boswelliaserrata (AFLAPIN) and collagen type II

METHODOLOGY:

METHOD DEVELOPMENT

In the present investigation, we have developed a simple and sensitive RP-HPLC method for quantitative estimation ofOfBoswelliaserrata (AFLAPIN) and collagen type II in bulk drug and pharmaceutical dosage forms. These are trails performed for HPLC method development of Boswelliaserrata (AFLAPIN) and collagen type II.

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 Boswelliaserrata (AFLAPIN) and collagen type II . The UV-Spectrum of Boswelliaserrata (AFLAPIN) and collagen type II 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 221 nm was selected for further analysis as shown in **Figure I**

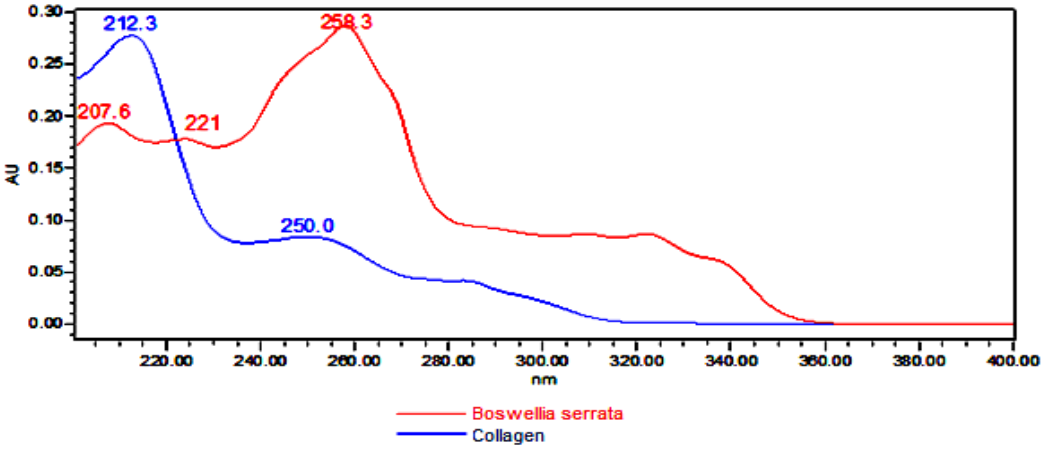

Figure I: Overlay spectrum of Boswelliaserrata (AFLAPIN) and collagen type II

3.3. OPTIMIZED METHOD:

3.3.1.Preparation of Buffer solution: Mix 0.1ml Acetic acidin1litrewater,filteredthrough0.45µmnylonmembranefilter.

3.3.2 Mobile Phase: A mixture of Hexane and IPA in the ratio of 20:80%v/v with 0.1% Acetic acid was sonicated to degasandfilteredthrough0.45µmnylonmembranefilter.

3.3.3. Chromatographic conditions

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DRUG	Area	LABELED AMOUNT (mg)	AMOUNT PRESENT (mg)	%ASSAY
Boswelliaserrata (AFLAPIN)	2531569	50	50.1	100.4
Collagen Type-II	1193634	20	19.97	99.6

PreparationofDiluent: Acetonitrile: Buffer (25:75v/v)

Column	Luna Phenyl Hexyl(150X4.6mm, 3.5µ)
Mobilephase	Hexane And IPA (20:80v/v) with 0.1% Acetic acid
Flow rate	1.0mL/min
Detectionwavelength	221 nm
Injectionvolume	10µl
Temperature	Roomtemperature
Run time	8 min

Table-I: Chromatographic conditions

RetentiontimeofBoswelliaserrata (AFLAPIN) isabout2.791mi
Retentiontime ofCollagen Type-II isabout5.136min.

Preparation of standard stock solution: Accurately weighed 5mg of Boswelliaserrata and 5mg of collagen type II were transferred into two different 10ml volumetric flasks, make up the flasks with methanol and sonicate for 5 minutes then take 1ml of Boswelliaserrata and 0.4ml of collagen type II solution into 10 ml volumetric flasks and made up to10ml with methanol and then transfer this solution into vial using a 1ml syringe.

Preparation of Sample solution: Weighed 10 capsules and weigh and then take 5capsules equivalent of sample into a 100 mL volumetric flask. Added 70 mL ofdiluent sonicate to dissolveand dilutedto volume diluent. Further diluted 5 mL to 50 mLwiththediluent.Filterthrough0.45µNylonsyringefilter.

Table: IIAssay Calculations

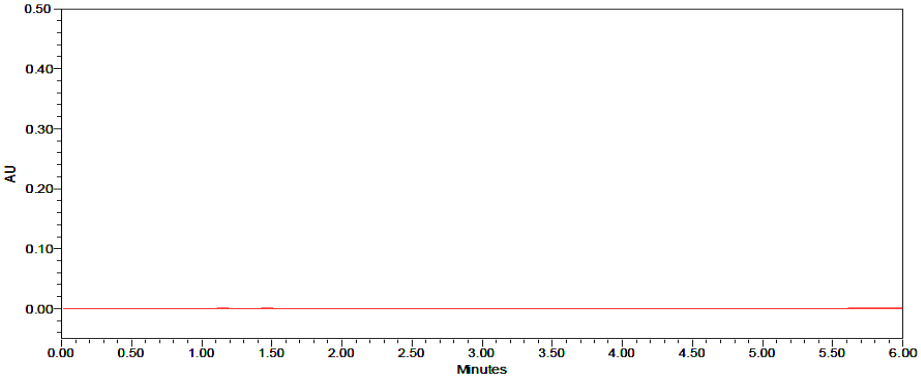

Figure II: A Representative chromatograph of blank

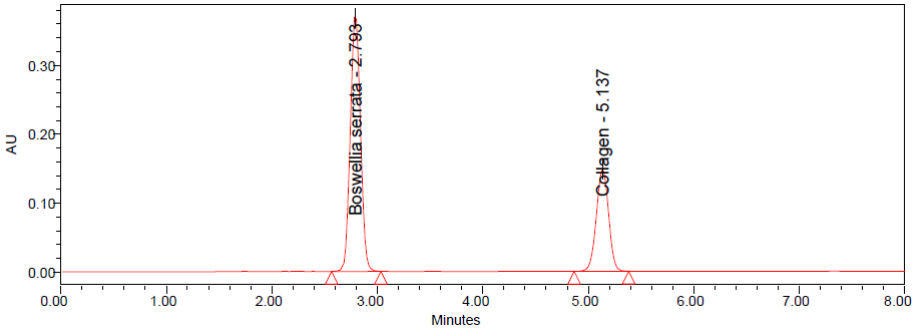

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Figure III: A Representative chromatograph of Standard

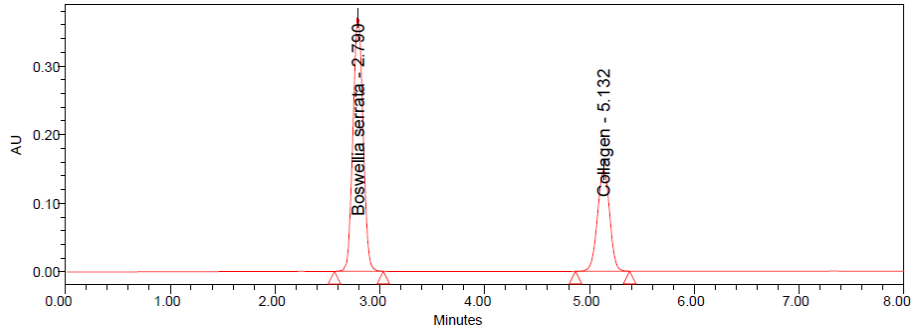

Figure IV: A Representative chromatograph of sample

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 asfollows.

PARAMETER	**ACCEPTANCE CRITERIA**	**BOSWELLIA SERRATA (AFLAPIN)**	**COLLAGEN TYPE II**
Linearity Range Correlation Coefficient	Correlation coefficient r 2 > 0.999	r 2 = 0.99947	r 2 = 0.99992
System Precision	RSD < 2%	%RSD = 0.144	%RSD =0.41
Intermediate Precision	RSD < 2%	%RSD = 0.092	%RSD = 1.23
Method precision	RSD < 2%	%RSD = 0.94	%RSD = 1.12
Accuracy	Recovery 98- 102% (individual)	% Recovery (50%) = 99.6 %	% Recovery (50%) = 99.05 %
		recovery (100%) =101.4	recovery (100%) =100.3 %
		recovery (150%) =99.8	recovery (150%) =100.3
Robustness RSD < 2%	RSD NMT 2% in modified condition	Complies	Complies
	Flow minus	%RSD= 0.61	%RSD= 1.01
	Flow plus	%RSD= 0.8	%RSD= 1.12
	Organic plus	%RSD=1.15	%RSD=1.46
	Organic minus	%RSD=0.78	%RSD=0.78
LOD		0.005	0.02
LOQ		0.05	0.2

Table: III- Validation parameters for of Boswelliaserrata (AFLAPIN) and collagen type II

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Table: IV - Results of forced degradation for Boswelliaserrata (AFLAPIN)

S.No.	Stability(hrs.)	Rt(min)	Peak Area	USP Platecount	USP Tailing	%Assay	% Deviation
1	INITIAL	2.777	2520286	3792	1.16	100	0.00
2	6 HRS	2.766	2513277	9166	0.95	99.7	-0.30
3	12 HRS	2.759	2520624	3761	1.20	99.4	-0.60
4	18HRS	2.761	2518056	3776	1.17	99.2	-0.80
5	24 HRS	2.768	2511947	3781	1.13	99	-1.00

3.4. STABILITY STUDIES

S.No.	Stability(hrs.)	Rt(min)	Peakarea	USP Platecount	USPTailing	% assay	% Deviation
1	INITIAL	5.123	1195540	9201	0.94	100	0.00
2	6 HRS	5.121	1192520	3324	1.61	99.7	-0.30
3	12 HRS	5.123	1203847	9158	0.93	99.3	-0.70
4	18HRS	5.125	1196328	9171	0.99	99.1	-0.90
5	24 HRS	5.130	1203809	9176	1.01	98.7	-1.30

Table: V- Results of forced degradation for collagen type II

		Boswelliaserrata (AFLAPIN)				Peak Purity		
	Sample Weight in mg	Area Count Injections	Mean Area Count	% Label Claim	%Degradation	Purity Angle	Purity Threshold	Pass/Fail
Control	14	2516196	2516196	100	0	0.338	10.826	Pass
Acid	14	2185241	2185241	86.8	13.2	0.114	1.236	Pass
Alkali	14	2202147	2202147	87.5	12.5	0.125	1.241	Pass
Peroxide	14	2192478	2192478	87.1	12.9	0.15	1.186	Pass
Thermal	14	2251478	2251478	89.5	10.5	0.143	1.247	Pass
Hydrolysis	14	2506204	2506204	99.6	0.4	0.138	1.256	Pass
Reduction	14	2225530	2225530	88.4	11.6	0.148	1.242	Pass
Photolytic	14	2501619	2501619	99.4	0.6	0.148	1.242	Pass

Table:VI- Results of forced degradation for of Boswelliaserrata (AFLAPIN)

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		collagen type II				Peak Purity		
	Sample Weight in mg	**Area Counts** Injections	**Mean Area Count**	**% Label Claim**	**%Degra-dation**	**Purity Angle**	**Purity Threshold**	**Pass/Fail**
Control	14	2516196	2516196	100	0	0.338	10.826	Pass
Acid	14	2185241	2185241	86.8	13.2	0.114	1.236	Pass
Alkali	14	2202147	2202147	87.5	12.5	0.125	1.241	Pass
Peroxide	14	2192478	2192478	87.1	12.9	0.15	1.186	Pass
Thermal	14	2251478	2251478	89.5	10.5	0.143	1.247	Pass
Hydrolysis	14	2506204	2506204	99.6	0.4	0.138	1.256	Pass
Reduction	14	2225530	2225530	88.4	11.6	0.148	1.242	Pass
Photolytic	14	2501619	2501619	99.4	0.6	0.148	1.242	Pass

Table: VII- Results of forced degradation for collagen type II

STASTICAL ANALYSIS-CHEMOMETRIC ANALYSIS

In this chemometrics assisted HPLC study PCA,PLS calibrations were used to analyse the drugs of **Boswelliaserrata (AFLAPIN) and collagen type II**

at 221 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 followingchemometric techniques using unscrambler (camo software).

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

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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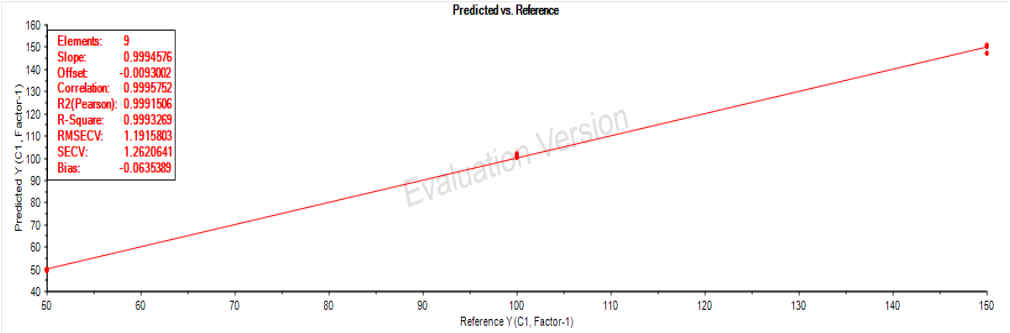

Figure:V-PLSofaccuracyspectraldataofBoswelliaserrata (AFLAPIN)

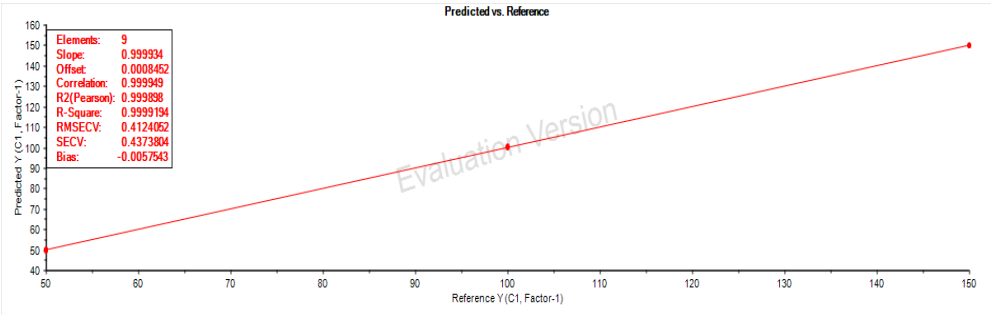

Figure:VI-PLSofaccuracyspectraldataofcollagen type II

1.1. 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 developcorrelation 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 Boswelliaserrata (AFLAPIN) and collagen type II peak area we get the Bio-plot.

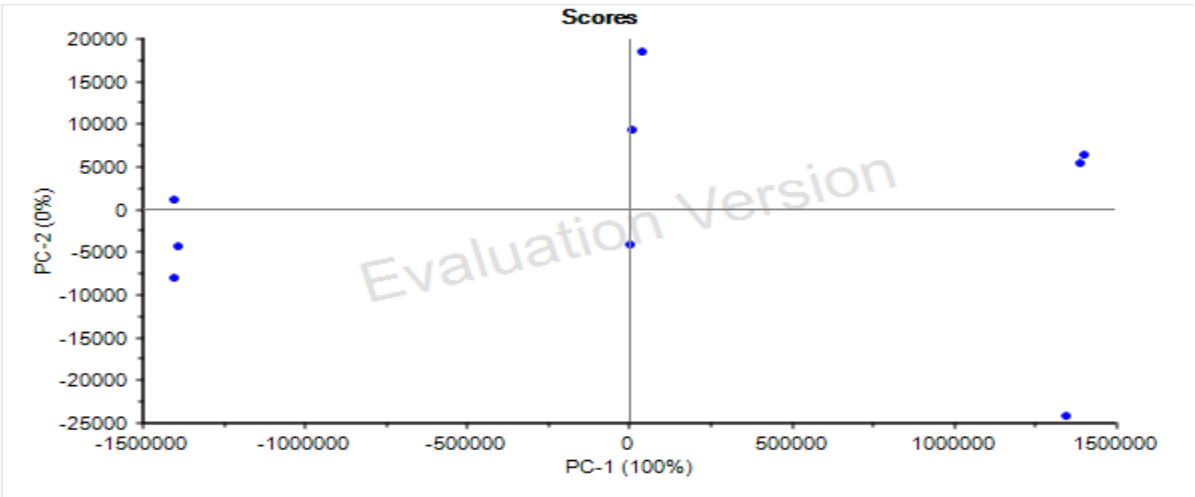

Figure:VII- PCA accuracy spectral data of Boswelliaserrata (AFLAPIN) and collagen type II

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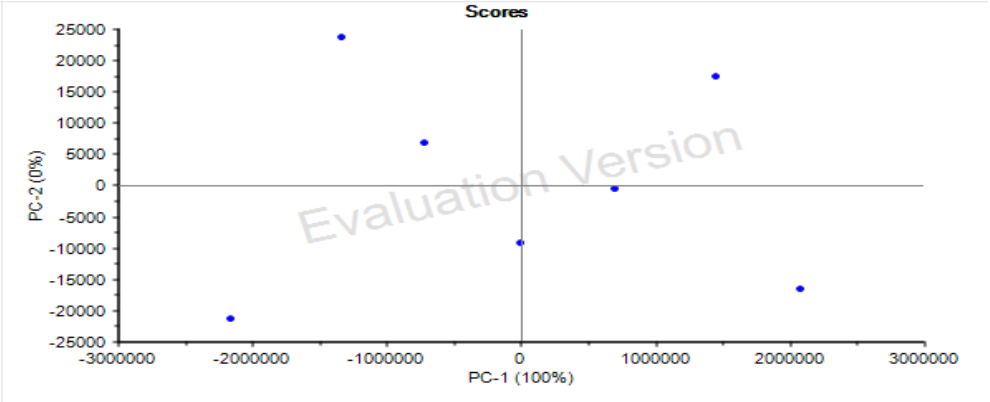

Figure VIII: PCA Linearity spectral data of Boswelliaserrata (AFLAPIN) and collagen type II

2. CONCLUSION

In the present investigation new analytical methods have been developed for the estimation of the potent drug Boswelliaserrata (AFLAPIN) and collagen type II. 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 Boswelliaserrata (AFLAPIN) and collagen type II 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 Boswelliaserrata (AFLAPIN) and collagen type IIin according to ICH guidelines and it can be used for meeting the regulatory guidelines for above drugs.

3. CONFLICT OF INTERESTS:

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

4. ACKNOWLEDGEMENT:

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5. REFERENCES

1. Shanta k et al/Chemometric assisted RP-HPLC quantitative estimation and validation of hydrochlorothiazide and triamterene in tablet dosage formInt.J of pharmacy and Analytical research vol-6(3),2017[442-4=8].
2. Riddhi Patel and RajashreeMashru. Development and Validation of Chemometric Assisted Methods and Stability Indicating RP-HPLC Method for Simultaneous Estimation of RasagilineMesylate and Pramipexole in Synthetic MixtureActa Scientific Pharmaceutical Sciences 3.8 (2019): 154-168.
3. K. Smilde, Y. Wang, B. KowalskiTheory of medium-rank second order calibration with restricted-Tucker modelsJournal of Chemometrics 8 (1994) 21–36.
4. Jing D and Linfang H. Application of chemometrics in quality evaluation of medicinal plants. J Medicinal Plants Res. 2006; 5: 4001-4008
5. M.K. Menon and A. Kar.1970. Analgesic and psychopharmacological activity of gum resin of Boswelliaserrata. Planta Med. 19: 51-4
6. Vernon, R., 1969, Lymphocyte to macrophages transformations in the peritoneal cavity proceeding the mobilization of peritoneal macrophages to inflamed areas, Nature, 222, 1268- 1288.
7. Singh, G. B and Atal, C. K.,1984, Pharmacology of an extract of salaiguggulexBoswelliaserrata, Indian Journal of Pharmacology, 16, 51.
8. x. Gupta RC, Canerdy TD, Lindley J, et al. Comparative therapeutic efficacy and safety of type-II collagen (UC-II), glucosamine and chondroitin in arthritic dogs: pain evaluation by ground force plate. J AnimPhysiolAnimNutr 2012;96(5):770-7. PMID: 21623931.