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Development and validation of analytical method for estimation of Antitussive drugs or NSAIDS in multi drug dosage form by HPLC

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Abstract : A Simple, accurate and precise RP-High performance liquid chromatography (HPLC) Method has been development and validation of analytical method for the estimation of Antitussive drugs or NSAIDS in multi drug dosage form. The method has been validated as per the guidelines of ICH. The separation is achieved on Kinetics C18,250×4.6mm,5 micron. Column with flow rate 1.20ml per minutes in gradient modes using Buffer pH-2.5(water modified with OPA) as a mobile base. column oven temperature is maintained at 25^0c. the method is simple, accurate, reproducible and less chemical using and can be used for simultaneous analysis of NSAID'S and Antitussive drugs.
Keyward : Paracetamol,Dextromethorphan,Iupfofen,Buffer-2.50(OPA).

Introduction:-

Ibuprofen is chemically 2[4-(2-methyl propyl) phenyl]propanoic acid. The structural formula is C13H18O2, andmolecular weight is 206. It is non-steroidal antiinflammatory drug (NSAID). It is used for relief ofsymptoms of arthritis, primary dysmenorrheal, and feverand as an analgesic. Ibuprofen is known to have an antiplatelet .Paracetamol is chemically N-(4-hydroxyphenyl) acetamide. It is a centrally and peripherally acting non-opioid analgesic and antipyretic.Dextromethorphan is chemically (morphinan, 3-methoxy-17-methyl(9α-14α Hydrobromide).the structural formula is $C_{18}H_{28}NO_2$and molecular weight is 352.329g/mol. Dextromethorphan hydrobromide (DXM) was an antitussive drug that is found in many over-the-counter cold remedies and cough syrups. Literature survey reveals that there are several HPLC 10-19 methodsreported for the estimation of above mentioned drugs alone or incombination with other drugs in their pharmaceutical dosage formsbut none of the method available for the estimation of these drugs inthe selected combinations. In the analysis of formulation containingtwo or more drugs, one drug can interfere in the estimation ofanother drug. To avoid this, separation of components mixture is usually carried out which make the procedure timeconsuming and complicated and often lacks accuracy. Therefore, itwas thought worthwhile to develop such a method of analysis, which can estimate three drugs in combination without priorseparation.

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Hence on the basis of literature survey, it was thought to develop aprecise, accurate, simple and reliable method for estimation of drug. in tablets using following technique of RP-HPLC method for Paracetamol, dextromethorphan and Ibuprofen.[1-9]

Materials and Method

Methanol HPLC Grade, Paracetamol Working Standard, Dextromethorphan Working Standard, Ibuprofen working standard, ortho phosphoric acid analytical reagent grade (Merck) and water.HPLC instrument, Waters with PDA detector with software empower, Kinetics C-18 column (250 x 4.6 mm, 5 µ)

Method Development[10]

According to literature review, Paracetamol is freely soluble inalcohol and sparingly soluble in water and Dextromethorphan and Ibuprofen is freelysoluble in water and methanol, finally the combination is selected in Gradient mode. Mobile phase composed of water(modified with ortho phosphoric acid (ph-2.50) and methanol. the three step gradient elution program with flow rate of 1.20ml/min,step1 started initially with 46% methanol and 54% buffer for 5min,methanol concentration changed linearity to 70% and buffer 30% buffer in next 15min followed by final step3 reverting methanol concentration back to 46% and buffer 54% in last 15 min thus concluding the method in total run time 30min.

First waters C-18 column with sodium phosphate buffer and methanol in differentproportions tried, dextromethorphan was not eluted then column is changed to Kinetics C-18 different proportions of water andmethanol were tried peak symmetries were good. The mobile phase of Water: Methanol in gradient mode. at flow rate of 1.2 ml/min has separated paracetamol, dextromethorphan and ibuprofen with good peak symmetries andresolution. So that 224nm was selected as the detection wavelength.

Preparation of Standard Solution

Paracetamol standard stock solution :- An accurately weighed quantity of paracetamol 500mg was transferred to the 100ml volumetric flask and dissolved in methanol. The volume was made up to the mark with the same to make (5000ppm)

Dextromethorphan standard stock solution:-

An accurately weight quantity of dextromethorphan 32mg was transferred to 100ml volumetric flask and dissolved in methanol. The volume was made up to the mark with the same to make(320ppm).

Ibuprofen standard stock solution:-

An accurately weighted quantity of ibuprofen 400mg was transferred to the 100ml volumetric flask and dissolved in methanol the volume was made up to the mark with the same to make (4000ppm)

Mix working standard stock solution:-

1ml of paracetamol, 1ml of Dextromethorphan, 1ml of ibuprofen was pipette out from standard stock solution respectively in to a 10ml volumetric flask, and diluted up to mark by methanol and to obtained resultant concentration of 5000ppm,320ppm,4000ppm of paracetamol, dextromethorphan and ibuprofen

Preparation of Sample Solution

For the test solution 20 tablets (marketed preparation contains (PAR) Paracetamol 500 mg, (DMP) Dextromethorphan 32mg, and (IBF) Ibuprofen 400mg) were weighed and the average weight was determined. 20 tablets were triturated and powder. Equivalent to 500 mg of PAR ,32mg of DMP, & 400 mg of IBF was added into a 100 ml volumetric flask the content were mixed with diuent & sonicated for 15 min & same content were filtered through 0.45 µ membrane filter. 1 ml of resultant was taken in a 10 ml of volumetric flask & volume was made up to the mark with diluent.

Optimized chromatographic condition:-

Column:- Kinetics C18,250×4.6mm,5µm
Flow rate:- 1.20ml/min
Wavelength:- 224nm
Injection volume:- 20µl
Column tempecture:- 25^0c
Run time:- 30minutes
Mobile phase:- mixture of methanol, warer(ph-2.50 modified with OPA)
Mobile phasemode:- Gradient mode

Method Validation[11]

System suitability

It is the integral part of many analytical method. the test are based on the concept that the equipment, electronic, analytical operation and sample to be analysed, constitute an integral system that can evaluated as such system sutability for the system shall be determined by using the chromatogram of the standard injection or sample for capacity factor,tailing factor,theoretical plates,resolution factor.

Specificity

Standard, sample solutions and placebo solutions were injectedsimultaneously into the system and results were computed.

Linearity

Standard solutions ranging from 50% to 150% (50%, 75%, 100%,120% and 150%) of the target concentration injected. Peak area Vs Concentration was plotted from this correlation coefficient wasevaluated. Lower level 50% and higher level 150%

Precision

The precision of analytical method is the degree of agreement individual test result when the method is applied repeatedly to multipale of homogenous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation of a series of measurement.

Accuracy

The accuracy of an analytical method is the closeness of test results, obtained by that method to the true value. The accuracy of an analytical method should be established across its range. In the case of the assay of a drug in the formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of drug product components to which known amount of analyte have been added within the range of the method. Average recovery should be 99 to 101 % of drug at each level.

Limit of Detection:

The lowest conc. of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental conditions simply indicates that the sample is below or above certain level. Limit test prescribed as percentage or as parts per million. The limit of detection will not only depend on the procedure of analysis but also on type of instrument.

Limit of Quantitation:

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample. The S/N ratio should not less than 10 and RSD $\leq$ 3% .

Intermediate Precision (Ruggedness)

The ruggeness of analytical method is the degree of reproducibility of test result obtained by the analysis of same sample under a verity of condition such as different laboratories, different analyst, different instrument, different reagent, different tempecture, different days etc. it is normally expressed as the lack of influence on test result of proportional and environmental variables of analytical method.

Robustness

Robustness of the method was evaluated by doing the small changesmobile phase composition and flow rate

Results and Discussion

To develop a simple, precise, accurate, and reliable Reverse PhaseHigh Performance Liquid Chromatographic method for simultaneous estimation of Paracetamol, Dextromethorphan and Ibuprofen different chromatographic conditions were tried. The Kincetics C18 250×4.6mm column, mobile phase containing mixture of methanol: water (ph-2.50 modified with OPA) and the flow rate of 1.2 mL/min found to resolvecomponents with good peak symmetry and theoretical plates. The retention times for Paracetamol, Dextromethorphan and Ibuprofen were found tobe 2.652 min,6.523min and 25.456 min respectively.

The linearity can be expressed as correlation coefficient, i.e 0.991, 0.981 and 0.985 for PAR,DMP and IBF respectively. Correlation coefficient, y- intercept, slope of regression line is shown in fig no.6,7,8. Precision was determined as intermediate precision as per ICH guidelines. It was assessed at 3 concentration levels %RSD obtained was not more than 2% for all the three drugs. The results of precision are shown in table 2. System suitability parameters for proposed method are shown in table 1. Assay of bulk and tablets PAR, DMP and IBF was evaluated. Three replicate determinations were carried out on tablets. Percentage purity was found to be 99.90%, 98.50% and 99.65%. Robustness studies were carried out after deliberate alterations of flow rate, mobile phase compositions and mobile phase pH. It was observed that did not lead to changes of retention times of peak of interest. Percentage of recovery shows that method is free from interference of the excipients used in the formulation shown in **Table 3,5**. And LOD and LOQ determine in table no.4

Table 1:- System stability test results

Sr.no.	Parameter	Paracetamol	Dextromethorphan	Ibuprofen
1	Peak area	12092739	25456667	2462847.3
2	No of theoretical plates	1531592	315331	102295
3	Retention time	2.65min	6.52min	25.45min
4	asymmetry	1.6	0.92	1.02

Table no.2:- Intermediate precision(Ruggedness) evaluation of data

% Assay of LC						
	Paraceatmol		**Dextromethorphan**		**Ibuprofen**	
SR.NO.	SET-1	SET-2	SET-1	SET-2	SET-1	SET-2
1	100.86	102.06	103.46	101.38	101.47	102.20
2	98.16	101.71	103.82	101.23	100.44	100.04
3	100.29	101.94	103.68	102.95	100.41	100.22
Average	100.84		103.65		100.08	
SD	1.48		0.1824		0.6004	
%RSD	1.46		0.1759		0.595	

Table no.3:-Summary of result of Ruggedness study

Parameter	Stastical data	SUMMARY OF MATRIX METHOD		
		Paracetamol	Dextromethorphan	Ibuprofen
Interday	Mean	12559406	234471	2312847.3
	Sd	180791	3128.171	7503.044
Intraday	Mean	12594722.7	265271	2259954
	Sd	153153.5	1854.36	9950.546
Different analyst	Mean	1001.46	66.0340	808.163
	Sd	3.17	0.42865	2.8500

Table no.4:-LOD and LOQ

Sr.no.	API	LOD(µ/ml)	LOQ(µ/ml)
1.	PARACETAMOL	18.807mg	56.993mg
2.	DEXTROMETHORPHAN	1.3864mg	4.201mg
3.	IBUPROFEN	18.639mg	56.482mg

Table no. 5 System suitability of change in Flow Rate

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	1.1ml	1.3 ml	
1	The % RSD of peak area response for five replicate injections	PAR	1.19638072	0.6168374	0.3027782	NMT 2.0
		DMP	1.46200813	1.251964	0.358593	
		IBF	1.74942074	0.1541528	0.8961339	
2	Theoretical plates	PAR	1531592	1173880	1976612	NLT 2000
		DMP	315331	281795	410790	
		IBF	102295	92604	12435	
3	Tailing factor	PAR	1.6	1.92	1.4	NMT 2.0
		DMP	1.7	1.80	1.90	
		IBF	1.95	1.85	1.90	
4	Retention Time (Min)	PAR	2.652	2.651	2.292	
		DMP	6.523	6.524	5.775	
		IBF	25.456	25.454	23.789	

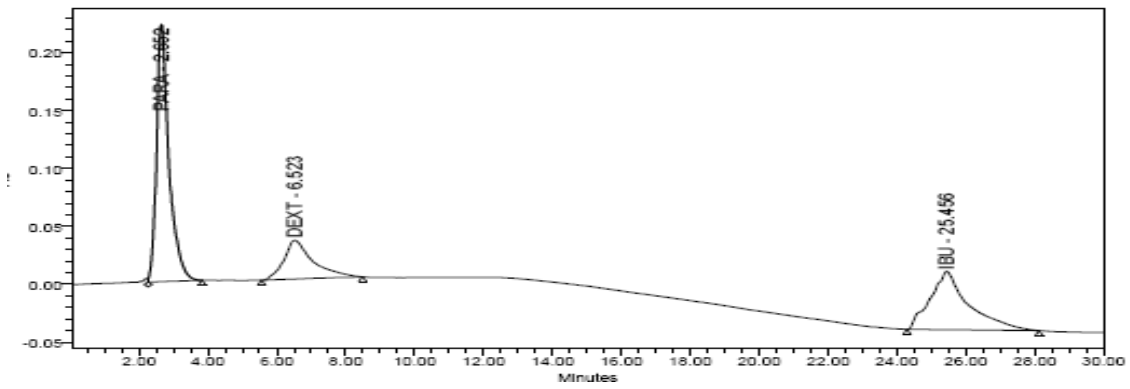


Figure no.1 Chromatogram obtained by laboratory mixture of Paracetamol 2.66, Dextromethorphan 6.52,Ibuprofen 25.45, respectively

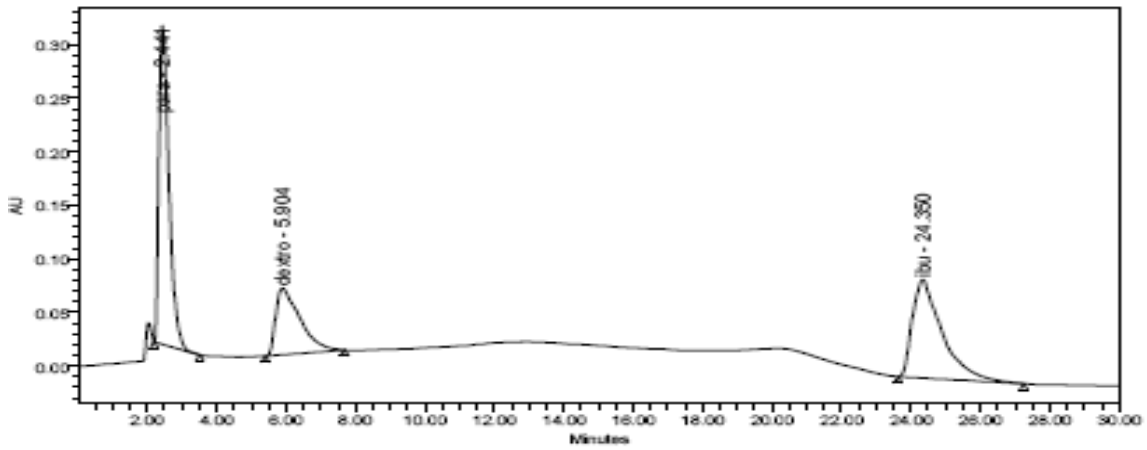


Fig no.2:- Chromatograms of mix. Standard change in pH Buffer pH 2.45

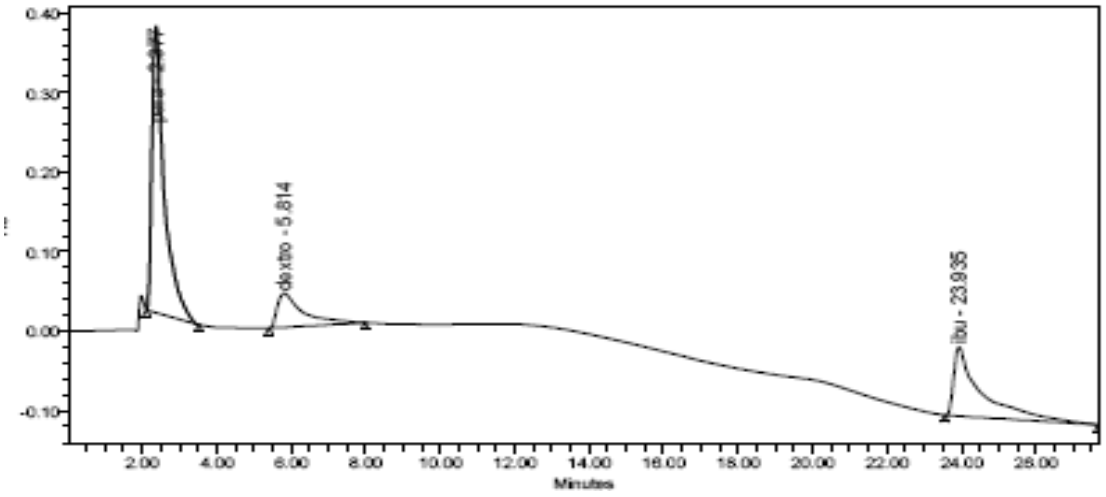


Fig no.3:- Chromatograms of mix. Standard change in pH Buffer pH 2.55

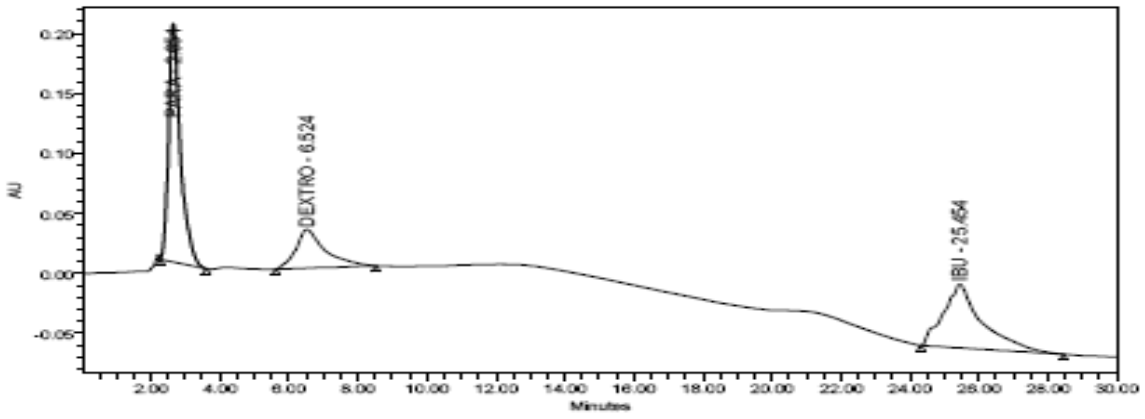
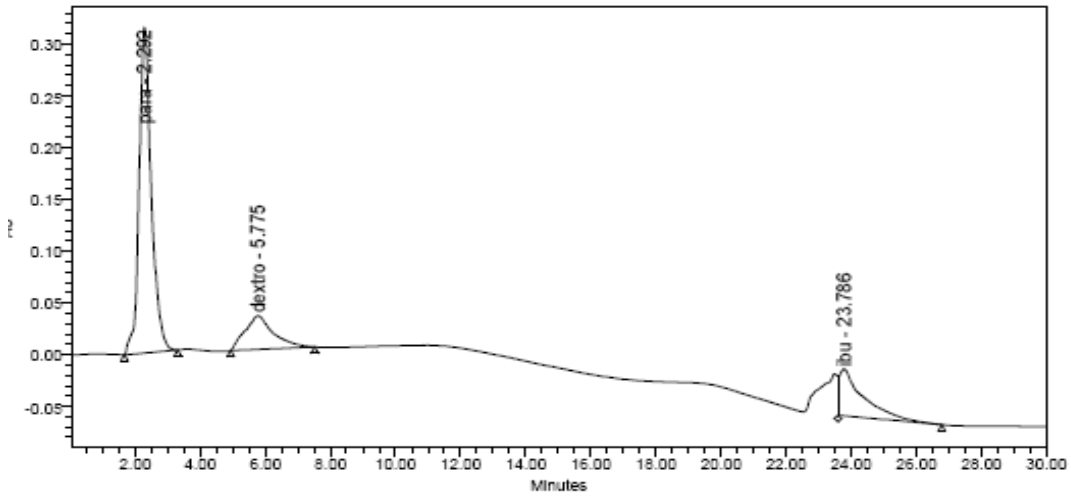


Fig No.4:-Chromatogram of change in flow 1.1 ml/min



Figno.5:- Chromatograms of Change in Flow Rate 1.3 ml/min

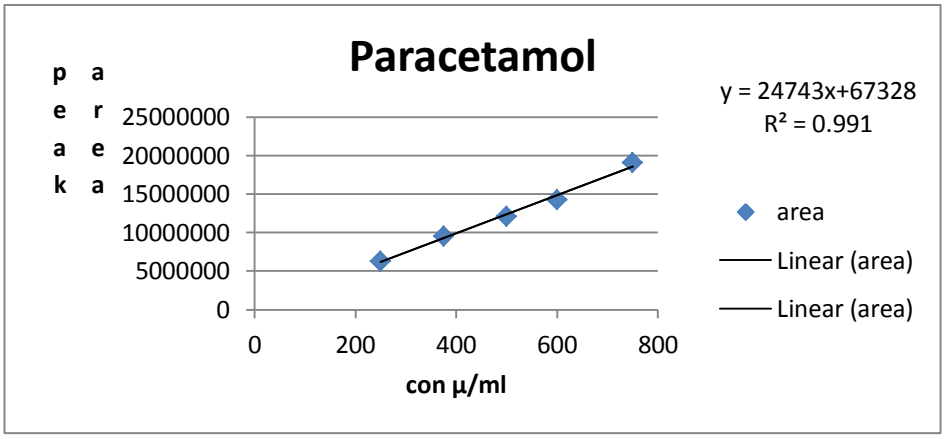


Fig no.6:- Graph of linerarity of paracetamol

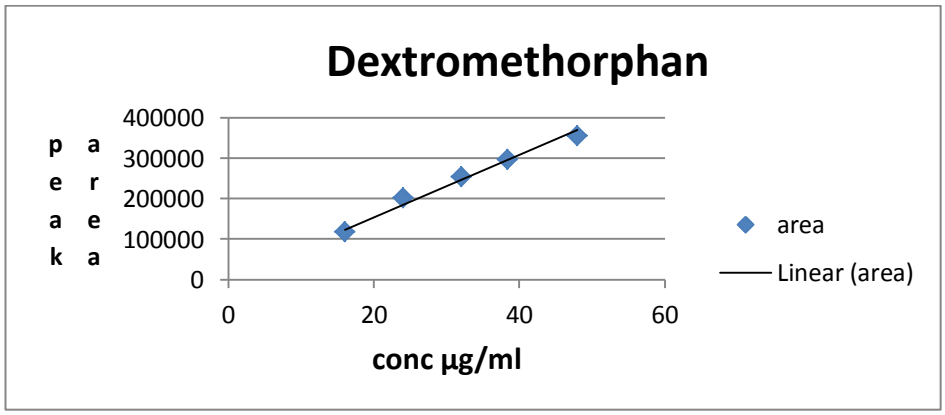


Fig no.7:-Graph of linearity of Dextromethorphan

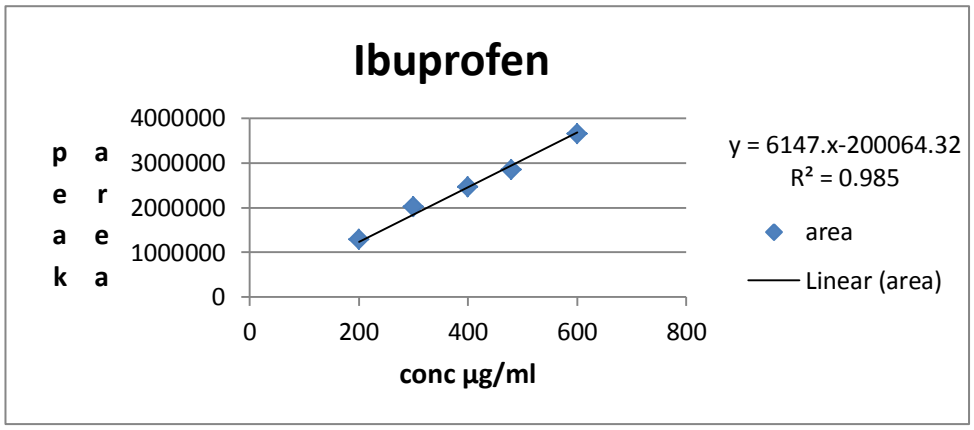


Fig no.8:- Graph of linearity of Ibuprofen

Summary and Conclusion

Combined dose tablet formulation containing Paracetamol, Dextromethorphen and Ibuprofen is available in market for the treatment of Antitissive. Multicomponent formulations are gaining precedence over single component formulations owing to the following reasons:

- ✓ Synergism of effects.
- ✓ Reduction of cost of treatment
- ✓ Increased patient compliance

Due to this rise in the multicomponent formulations, the challenges faced by the analytical chemist are on the rise. Estimation of drugs from a multi-component formulation requires a method capable of discriminating the more than two components. Approaches to multi component analysis can be broadly categorized into those which rely on physical separation of components prior to analysis (e.g. chromatographic methods). The present work involved the development of accurate, precise, and simple suitable RP-HPLC method for estimation of the drugs in multicomponent tablet formulations.

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