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Preformulation Studies of Methotrexate and Aceclofenac for Formulation and Development of Solid Lipid Nano-Particles: Novel Drug Delivery

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ABSTRACT: The primary objective of pre-formulation testing is to generate information that formulators can utilize to design stable, bioavailable, secure and efficient dosage forms.Pre-formulation research considers the early optimization of drug delivery by identifying the physiochemical characteristics of the substance that may impact medication performance.By using capillary fusion, the melting point was found.The drugs MTX and ACE, selected for the present study, were identified using various literature-reported methods, including melting point determination, partition coefficient analysis, and absorption maxima (λmax) measurement FTIR spectroscopy and drug excipient interaction studies. The obtained data from these studies were matched with the data given in standard monographs.The melting point for MTXandACE was recorded at 180-191˚C and 147-153ºCrespectively. The partition coefficient value log P was found to be -1.9 ± 0.25 and 1.86 ± 0.75 respectively. Data of solubility study indicates that Methotrexate was freely soluble in acetone and pH 6.8 Phosphate buffer, insoluble in water, chloroform and methanol and slightly soluble in 0.1 N HCl.Aceclofenacwas soluble in acetone, methanol & chloroform, sparingly soluble in methanol &alchohol.The FTIR peak of test sample Methotrexate &Aceclofenacwere matches with that of standard spectra especially in fingerprint region thereby confirming the identity of both drug.

Keywords: Aceclofenac, Methotrexate, Pre-formulation, FT-IR,DSC

I. INTRODUCTION:

Developing a dosage form is frequently required to improve patient compliance by achieving the intended release pattern and an efficient therapeutic response. The aforementioned medical and economic motivators have led to a rise in improved drug delivery research and development effort during the past 20 years.[1-3]One promising instrument for the twenty-first century is nanotechnology. In the current pharmaceutical environment, the idea of a novel drug delivery method utilizing nanomedicine is now well established. Using a variety of nano-medicines, enabled precise molecular-level illness diagnosis and therapy.[4, 5]The goal of the preformulation study is to create a dosage form that is safe, efficacious, stable, and elegant by determining its kinetic rate profile and compatibility with othercomponents and determine the new medicinal compounds' physico-chemical parameters. The synthetic chemist, either by themselves or in collaboration with experts in other fields, such as preformulation, may document some information throughout the early stages of a novel therapeutic substance's development that is suitably categorized as preformulation data.[6-8]

Prior to beginning preformulation studies, we should be aware of the drug's characteristics, potency in comparison to competing products, dosage form, stability and decay data from literature searches, the suggested drug administration route, formulation approaches, bioavailability, and pharmacokinetics of chemically related drugs.[8-9] Additionally, it involves initial research and molecular analysis to assess the extent of each suspected problem area (Step I); if a deficiency is found, a molecular alteration ought to be carried out (Step II). Molecular modifications such as salts, prodrugs, solvates, polymorphs, or even novel analogues are used to address this weakness.[10]

The first stage in the methodical creation of a pharmacological substance's dosage forms is pre-formulation testing. It is the study of a drug substance's both individually and in conjunction with excipients, in form of physical & chemical characteristic. The primary objective of pre-formulation testing is to generate information that formulators can utilize to design stable,

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bioavailable, secure and efficient dosage forms. [11-12]

Pre-formulation research considers the early optimization of drug delivery by identifying the physiochemical characteristics of the substance that may impact medication performance. This aids in the creation of a safe, effective, & stable dosage form with high bioavailability.

II. MATERIALS AND METHODS

2.1 Materials

Aceclofenac and methotrexatewas purchased from Healthy Life Pharma Pvt. Ltd. Mumbai, Stearic acid, Tween 80 was received as gift sample from Molychem, Mumbai, India.Hydrochloric acid, Sodium hydroxide and Methanol were purchased from SD finechem. Limited, (Mumbai, India).

2.2 Methods

Selection of suitable lipids and surfactants: Based on drug solubility and compatibility, lipids (stearic acid) were chosen. On the basis of literature analysis and their safety profile, surfactants were chosen. The surfactants had chosen included sodium taurocholate and Tween 80.

2.1 Physiochemical Characterization of Drugs

Drugs FTIR spectroscopy data, melting point determination, partition coefficient determination, calculation of absorption maxima (λ_{max}), and medication excipient interaction investigations were all used to identify ACE.

2.2Solubility Study of drugs in different lipids

The drug's solubility in a range of lipids was assessed in order to identify the lipid with the greatest potential for both drugs. A fixed weight (100 mg) of fat was melted in a glass vial. The vial's medication content was progressively increased. The mixture stated before was heated over the lipid's melting point. A translucent solution, which acts as the experiment's endpoint, indicates that the medication has been dissolved into the melting lipid.[13-14]

2.3FTIR for Compatibility study

To verify any possible chemical interactions between the medicationMTX, ACE and polymers such chitosan and stearic acid, FTIR research was performed. The drug was investigated using FTIR by first mixing it with dried KBr and then running spectra between 4000 cm^{-1} and 400 cm^{-1} with an FT/IR 4100-TypeA.[14] Subsequent samples of the same drug were compared to the original running spectra when significant peaks associated with the key functional groups were identified.[15-16]

2.4Standard calibration curves for MTX and ACEin PBS pH 7.4

The maximum absolute wavelength for aceclofenacwas discovered to be 303 &276 nm. Drug sample dilutions of 10 to 50 µg/mL were made in triplicate. Absorbance (y) and concentration (x) are used in a regression analysis to derive the calibration equation and correlation coefficient.

III. RESULTS AND DISCUSSION

3.1 Physiochemical Characterization of Drugs:

Organoleptic properties indicated that the drugswere crystalline in nature, MTX was orangeand ACE waswhitein color and have no odor. The melting point for MTXandACE was recorded at 180-191˚C and 147-153ºCrespectively. The partition coefficient value log P was found to be -1.9 ± 0.25 and 1.86 ± 0.75 respectively for MTX and ACE. All the physiochemical properties were found to be in range according to specification.Melting point and partition coefficient value of both drugs was matched with standard value given in standard monograph table 1.

Table 1 Result of Physiochemical Properties of the Methotrexate and Aceclofenac

Characters	Inference	
	Methotrexate	**Aceclofenac**
Color	Orange powder	White powder
Odor	odorless	odorless
Nature	crystalline powder	crystalline powder
Melting Point	185-190˚C	147-153ºC
Partition Coefficient	-1.6 ± 0.2	1.86 ± 0.75

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3. 2 Solubility Study:

The solubility of MTX and ACEin different solvents likewater, 0.1N HCl, PBS pH 6.8 (PBS)and indifferent lipid such asCholesterol, Stearic acid was determined by using standard procedure.Table2A, B.

Table 2A Solubility Profile ofdrugs in different solvent

Sr. No.	Medium	Solubility (mg/ml)	
		Methotrexate	**Aceclofenac**
1	Water	Insoluble	Poorly soluble
2	0.1 N HCl	Slightly soluble	Slightly soluble
3	pH 6.8 PBS	Freely soluble	Freely soluble
4	Ethanol	Insoluble	Sparingly soluble
5	Stearic acid	Freely soluble	Freely soluble
6	Cholesterol	Sparingly soluble	Slightly soluble
7	Witepsol H 32	Sparingly soluble	Sparingly soluble

Data of solubility study indicates that Methotrexate was freely soluble in acetone and pH 6.8 Phosphate buffer, insoluble in water, chloroform and methanol &Slightly soluble in 0.1 N HCl.

In case of aceclofenac solubility study, it was soluble in acetone, methanol & chloroform, sparingly soluble in methanol &alchohol. Aceclofenac is insoluble in the water. The solubility study concludes that aceclofenac was hydrophobic drug.

Table 2B: Solubility of MTX and ACE in different solvents & lipids

S.No	Solvents	Amount of drug dissolved	
		Methotrexate	**Aceclofenac**
1	Water	Insoluble	Insoluble
2	0.1N Hcl pH1.2	95µg /ml	230µg/ml
3	PBS pH6.8	390µg/ml	720µg/ml
4	Stearic acid	810µg/ml	770µg/ml
5	Witepsol H 32	225µg/ml	345µg/ml
6	Tween 80	310µg/ml	290µg/ml

3.3 Compatibility Studies:

For the determination of compatibility of drugs and excipients to each other, FTIR spectra and DSC plot were carried out.

3.3.1 FT-IR spectral analysisof Drug and Polymers:

To determine the main functional groups in each medication, FTIR analysis was performed. The main application of infrared spectroscopy is to identify and determine the purity of a substance. The drug and polymer must be compatible for a successful formulation. The potential information regarding the drug-polymer interaction is provided by FT-IR spectroscopy. The drug's legitimacy was demonstrated by the absence of differences in the absorption peak pattern between the official spectra listed in the Indian Pharmacopoeia and the measured FTIR spectra.Only after a thorough analysis of the drug's and the polymers' physicochemical characteristics was the formula optimized. For a formulation to be developed, the medication and polymer must get along. The potential interaction between the medication and polymer is described by the FTIR. FTIR was applied to samples of Drug MTX, ACE coating agent CS, and physical drug mixture with polymers.

3.3.2 FT-IR studies for drug Methotrexate:FT-IR tests were conducted for bulk medication and SLNs loaded with MTX and ACE.

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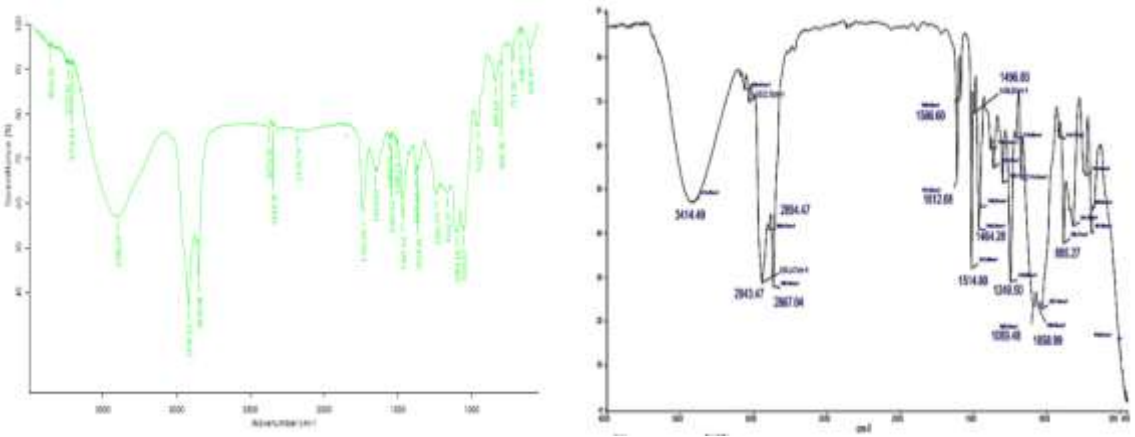

Fig. 1: FTIR of Standard &SampleMethotrexate

3.3.3 FTIR Interpretation of Methotrexate and Physiological Mixture:-

Table 3A: Interpretation of FTIR spectra of Methotrexate

Stretching type	Spectra Range Standard cm^{-1}	Drug sample peak
N-H stretch	3550-3200	3340.8
O-H stretch	3500-3300	3435.5
C-H Stretching (aromatic)	3150-2900	3050.5
C-H Stretching (aliphatic)	3000-2800	2965.4
C=O Stretching	1850-1600	1680
C-C stretch(in ring)	1600-1585	1610.5
C-C stretch	1610-1450	1585.1

The FTIR spectrum of MTX (Figure 1) showed characteristic absorption bands that matched its structure (Figure 1). MTX showed an absorption band at 3340 cm^{-1} as a broad peak corresponding to N-H stretching from amine groups, while the band at 3435.5 cm^{-1} was due to the presence of hydroxylgroup. The small peak at 1610.5 cm^{-1} attributes to C-C stretch, while C=H aromatic ring stretching was observed at 3050.5 cm-1 as well between 1585.1 cm^{-1} C–C stretching.

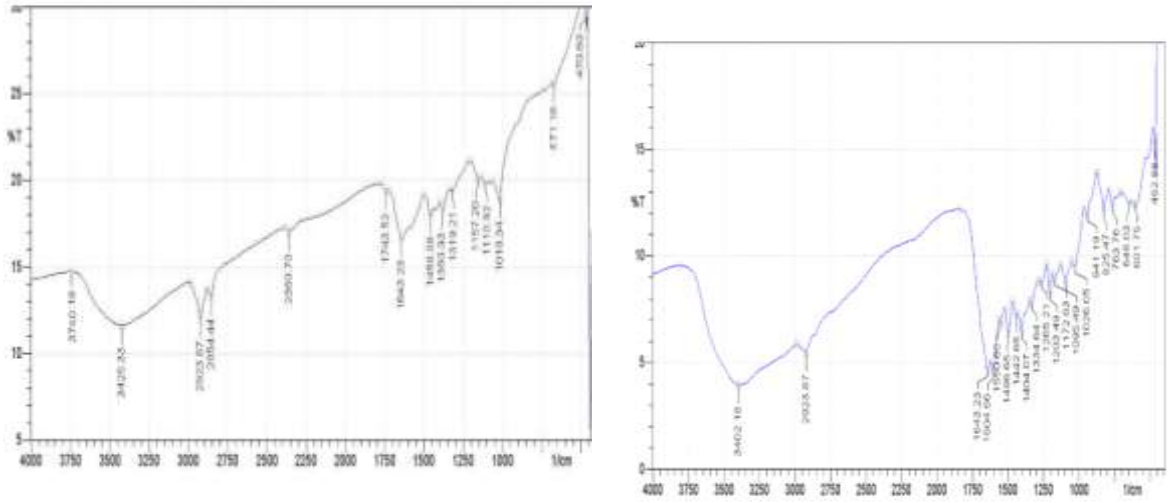

Fig. 2,3: FTIR Spectra of Chitosan&mixture of MTXwith polymers

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Table 3B: Interpretation of FT-IR Spectrum of Chitosan

Stretching type	Spectra Range Standard cm^{-1}	Observed peak cm^{-1} Drug sample
O-H Stretching	3500-3300	3435.5
N-H Stretching	3550-3200	3340.2
C-H bending	3000-2850	2890.2
N-H bending	1780-1570	1625
C-O-C stretching	1210-1040	1222

Table 3C: Analysis of the FT-IR spectra of a methotrexate and polymer mixture

Stretching type	Spectra Range Standard cm^{-1}	Observed peak cm^{-1} Drug sample
O-H stretch	3500-3300	3408
N-H stretch	3550-3200	3390
C-H stretch (aliphatic)	3000-2800	2915
C-N Stretching	1370-1160	1203
C-O-C stretching	1210-1040	1090

When MTX was physically mixed with CS and other polymers, the FT-IR spectra showed these distinctive bands of MTX appearing intact. Consequently, the FT-IR study verified that the medication and polymers did not interact.

3.3.4 FT-IR studies for drug Aceclofenac:

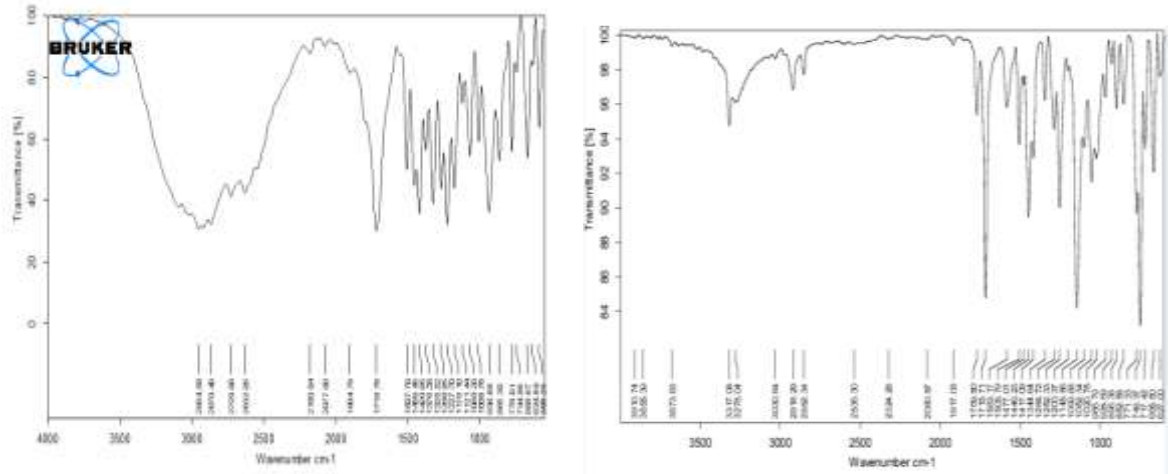

Fig: 4, 5 Standard FTIR spectra of ACE &mixture of ACEwith polymers

3.3.5 FTIR Interpretation of Aceclofenac and Physiological Mixture:-

Table 4A: Interpretation of FTIR spectra of Aceclofenac

Stretching type	Spectra Range Standard cm-1	Observed peak cm-1 Drug sample
N-H stretching	3500-3300	3359
O-H stretching	3100-2550	2875.2
C=O stretching	1850–1550	1710.5
C-C stretching for NH	3000-2800	2910.5
O-H in plane bending	1450-1350	1314.8
C-Cl	850–550	715.5

Table 4B: Interpretation of FT-IR of mixture of Aceclofenacwith Polymers

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Stretching type	Spectra Range Standard cm^{-1}	Observed peak cm^{-1} Drug sample
O-H stretch	2970-2535	2870
N-H stretch	3450-3210	3340
C-H stretch (aliphatic)	3000-2850	2980
C-C and C-N stretch	1370-1160	1203
C=O stretch	1540–1870	1710

Interpretation:-The FTIR peak of test sample Aceclofenacwere matches with that of standard spectra especially in fingerprint region thereby confirming the identity of aceclofenac.

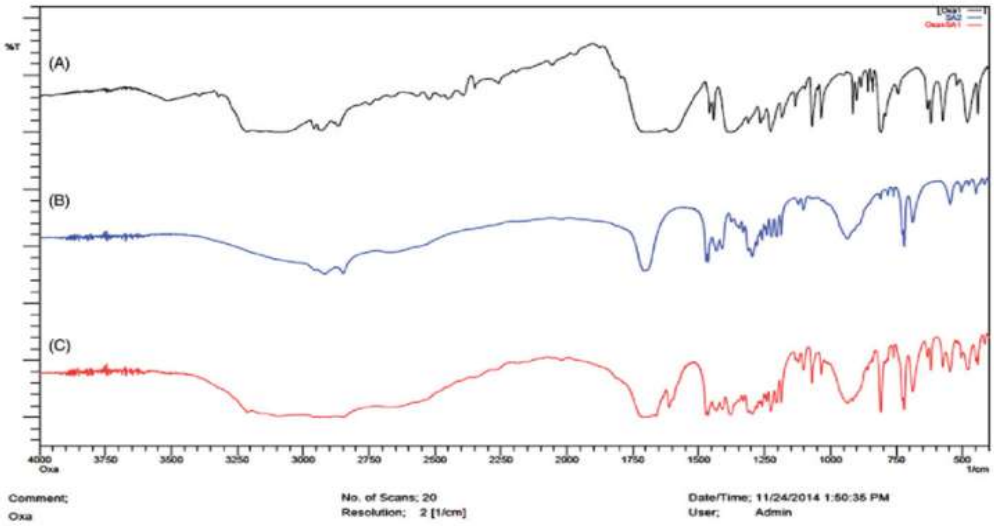

Fig. 6: FTIR spectra of (a) Aceclofenac, (b) Chitosan (CS) and Stearic Acid

3.4Differential Scanning Calorimetry:

DSC investigations were conducted across a range of temperatures of 100 º C to 300 º C at 20 º C increments of temperature. Melting point was recorded as 187.2ºC and 153.9 ºC respectively which was almost similar to the value obtained through capillary fusion method, thus confirming the identity and purity of methotrexate &aceclofenac. The thermograph is shown in fig 7

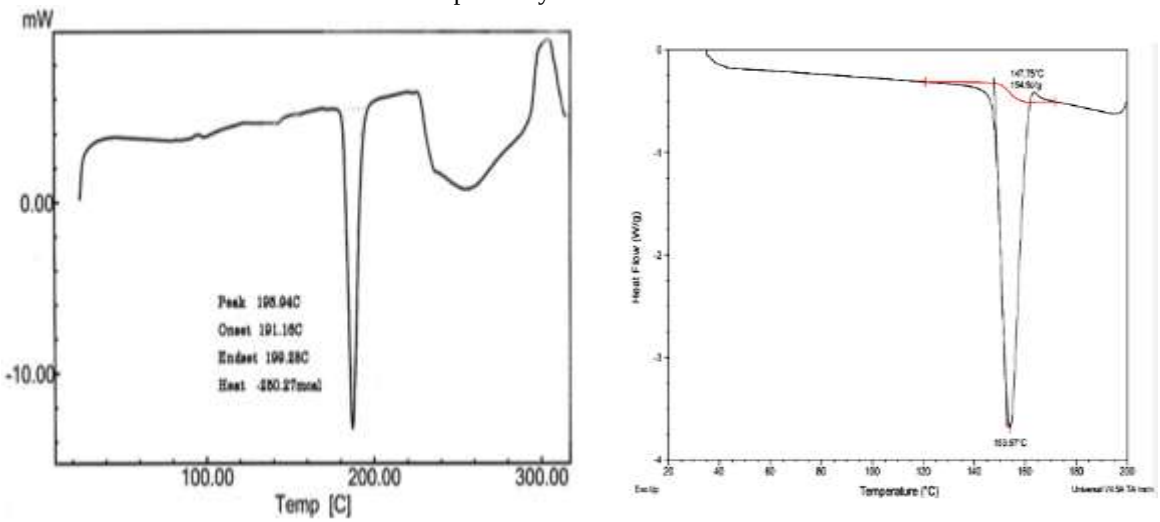

Fig 7:Differential scanning calorimetry curves for Pure MTX and ACE

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3.5 Spectrophotometric Analysis of MTX and ACE:

3.5.1 Preparation of Standard calibration curves for Methotrexate:

Absolute wavelength (λ_{max}) for MTX was found to be 303nm Different dilutions (10, 10, 20, 30, 40& 50µg/mL) of sample were prepared in triplicate. Regression analysis is utilized to study the calibration equation & correlation coefficient based on absorbance (y) and concentration (x). The regression equation in PBSpH 6.8 was found to be y = 0.0157x-0.151. Over the range of 10to 50µg/mL, the MTX calibration curve was linear. The coefficient of determination (R^2) was 0.996. The values are displayed in the table below, which demonstrates their compliance with Beer's law.

Table 5A: Calibration curve data of MTX in PBSand 0.1N HCl

S.No	Concentration (µg/ml)	Avg. AbsorbancePBS pH 6.8	Avg. Absorbance 0.1 N HCl pH 1.2
1	0	0	0
2	10	0.158	0.196
3	20	0.330	0.380
4	30	0.498	0.625
5	40	0.605	0.790
6	50	0.795	1.02

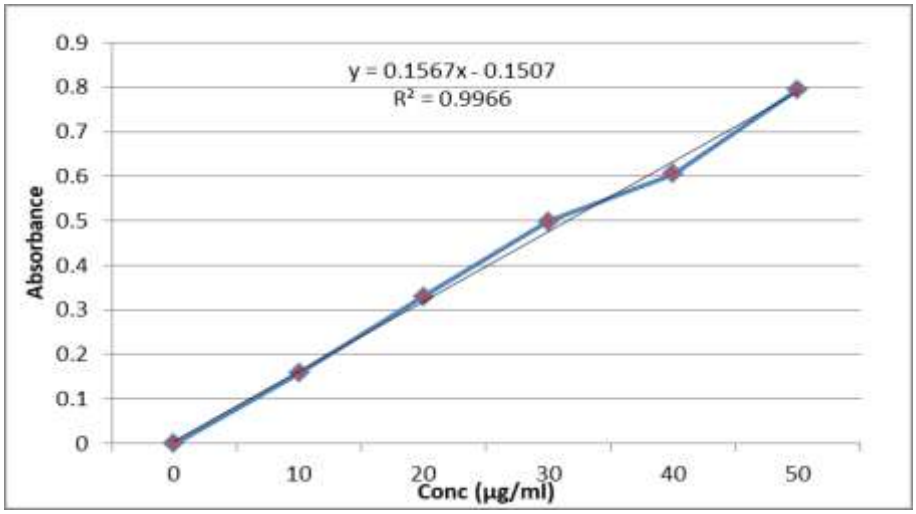

Fig: 8 Standard curve of Methotrexate in PBS at pH 6.8 (303nm)

3.5.2 Preparation of Standard calibration curves for Aceclofenac in Phosphate buffer pH 6.8:

The standard plot of **Aceclofenac** was prepared in triplicate using a concentration range 10 to 50µg /ml.Absorbance was measured for each solution at λ_{max} of 276 nm using UV-Visible spectrophotometer, and the curve was plotted between absorbance and concentration of aceclofenac.

Table 5B: Calibration curve data of Aceclofenac in PBS pH 6.8 and Ethanol

S.No	Concentration (µg/ml)	Avg. AbsorbanceEthanol (276nm)	Avg. AbsorbancePBS pH 6.8 (276nm)
1	0	0	0
2	10	0.234	0.098
3	20	0.454	0.225
4	30	0.677	0.31
5	40	0.864	0.405
6	50	1.08	0.49

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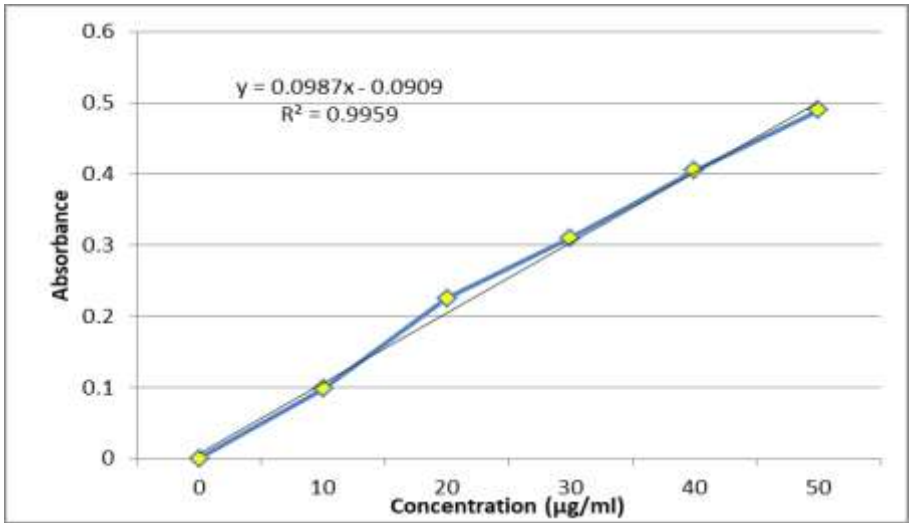

Fig: 9 Calibration curve of Aceclofenacin PBS pH 6.8 at 276nm

IV. CONCLUSION:

- Organoleptic properties of both drug indicated that they was crystalline,almost orange in color and odorless. A prominent endothermic peak was visible on the DSC thermo gram of MTX at 187.2°C and ACE at 153.9 ºC. The partition coefficient was found to be -1.6 ± 0.2 and 1.86 ± 0.75.
- The solubility of both drugs in different media stearic acid (810µg/ml and 770 µg/ml) and PBS pH 6.8 (390 µg/ml and 720mg/ml) indicates free solubility of drug.
- Calibration curves were prepared in 0.1N HCl& PBS pH 6.8. Calibration curve data were subjected to linear regression analysis. R^2 values in both solvents were found to be near 0.99, indicating good linearity.
- FTIR spectra of MTX showed characteristic IR band at 3408.2cm-^{1} which can be assigned to O-H stretch, 1648 assigned to stretching of C=0 group, 2915 assigned to stretching of aliphatic C-H group, which confirmed its identity and purity.
- FTIR spectra of ACE showed characteristic IR band at N-H stretching 3359cm^{-1} , 2875.2 assigned to O-H in stretching bending, 2910 cm-^{1} for C-C stretching for NH which confirmed its identity and purity.Therefore, it was noted that the chosen excipients worked well with both medications.

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