Adv. Pharmacol. Toxicol. Vol. 25 (2) 2024,
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Adv. Pharmacol. Toxicol. Vol. 25 (2) 2024, 1-16
ISSN - 0973 - 2381 SILVER JUBILEE VOLUME

FORMULATION AND EVALUATION OF HERBAL BASED CAPSULE AND SOFT CHEW FOR LIVER DISORDERS

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Chronic liver diseases (CLD) represent a major health burden worldwide. According to WHO, approximately 75% of people use herbs to treatCLD globally. The plants traditionally used by native healers to support CLD have been the subject of extensive research in recent years. The combination of herbal plants such as Swarnakshiri, Guduchi, Pipali, and Haridra, Bhringraj was used asan Ayurvedic treatment of diseases. The study aimed to formulate the selected plant extract's hard gelatin and soft chewable capsules with the wet granulation method. The extraction of plant materials was carried out by the Soxhlet extraction method using aqueous water-alcohol media. A chromatographic study was performed to identify active constituents which include Berberine, Tinosporoside, Piperine, Curcumin and Wedelolactone respectively. The hard gelatin capsule was formulated by a combination of Berberine, Tinosporoside, and Piperine, and the soft gelatin capsule was formulated by a combination of Curcumin and Wedelolactone. Capsules were evaluated. The batch F7 optimised was selected for dissolution study for both formulations, weight variation, pH determination, disintegration and dissolution test. Both types of capsules showed 50% of drug release within 2h in buffer and HCl media. The stability study was performed for the prepared formulations for 6 months and the results obtained do not show any significant changes in the drug release profile two formulations. The study's findings suggest that the prepared formulations provide a long-term, safe, and permanent cure for liver disorders with better patient compliance and no side effects.

INTRODUCTION

Chronic disease constitutes a fast-increasing burden to society. The World Health Organization (WHO) estimates that 46% of global diseases and 59% of mortality are due to chronic disease. The liver is an important vital organ in our body that performs a very important role in regulating, maintaining, and conducting several physiological functions such as metabolization of nutrients. As a result, it is frequently exposed to illnesses, which can lead to a variety of clinical syndromes [Jevas C. *et al*.,2017].Healthcareprofessionals were rarely able to offer a specific course of treatment due to the wide range of liver dysfunctions

Corresponding author

and the challenges in making an accurate diagnosis. Though significant progress has been made in the last few decades in understanding how to treat chronic liver diseases, most treatments still do not provide satisfactory results. There are more than 300 preparations in the Indian system of medicine for the CLD. In India currently, more than 87 medicinal plants are used in different combinations as herbal drugs for CLD [Vernetti LA. *et al*, 2016]. In the pharmaceutical field soft gelatin capsules are increasingly being chosen for strategic reasons (line extension), technological issues (high content uniformity of low-dose drugs), safety aspects (reduced operator and environmental contamination with highly potent or cytotoxic compounds) and consumer preference (easy to swallow) capsule are a trusted oral dosage [Reich, G., 2004].Among all dosage forms, capsules are among the most widely used because they are physically and chemically stable, simple to use, appealing, and simple to compound. More than one drug can be included in each capsule to reduce the number of dosages forms the patient must take. Modern dosage forms for medicinal use, such as hard gelatin capsules, result from the increased focus on pharmacokinetics in drug development today. This has greatly increased the variety of formulations that can use hard gelatin capsules as a straightforward dosage form for oral drug delivery. Large-scale applications of highly developed soft capsules are currently found in the pharmaceutical, chemical, food, and cosmetic industries. This is due to the characteristics of soft gels, such as their appealing aesthetic qualities and "swallowability," safety enclosure, precise contents, and appealing appearance. This has made it possible for them to be used as an efficient delivery system for a range of healthcare products, including hydrophobic drugs, low-melting-point drugs, and easily oxidized drugs [Habiba I. Benza. *et al*, 2011].They can be easily customized to meet the needs of specific patients regarding dosing ingredients, etc. This convenient container enables consistent dosage, portability, and high consumer compliance. Alternative formulations come as liquids, powders, or pastes and are more expensive [Soni Hardik. *et al* ,2010]. Due to filled by volume, computations are normalized using the fill volumes of the capsule, which vary based on capsule size and powder density. The medicinal properties of these plants are primarily due to the chemical molecules that aid in discovering new drugs. Bhringraj offers protection against damaging chemicals that can harm the live [Ming Hong. *et al*,2015] and contains some chemical elements with toxic-adapting properties that aid in the regeneration of liver cells [Sarita Verma. *et al*]The clinical properties such aswhich include analgesic, anti-inflammatory, anti-hepatotoxic, antioxidant immunomodulatory, and good rejuvenating properties [Sp Thiagarajan. *et al*, 2002] A variety of effects can be seen in the stem of Guduchi (Willd), including bitterness, astringency, sweetness, thermogenic, anodyne, anthelmintic, alternate, and -periodicity, antispasmodic, antipyretic, antiemetic, digestive, carminative, appetizing, stomach, constipating, cardiotonic, depurative, haematinic, expectorant. Since ancient times, it has been used to treat a number of ailments, such as burning sensations, hyperdipsia, vomiting, fever, jaundice, chronic diarrhoea, cancer, dysentery, bone fractures, pain, asthma, skin diseases, deadly bug bites, snake bites, and

abnormalities in the eyes.
The acrid root and fruit of pipali are used to treat tumours, ascites, bronchitis, abdominal discomfort, spleen disorders, and biliousness. They are also said to have stomachic, laxative, anthelmintic, and carminative properties. The fruits and roots are both said to have a number of medicinal advantages, including the treatment of cough, bronchitis, asthma, and other respiratory condition. The initial objective of this study is to manufacture and evaluate hard gelatin and soft chewable capsules containing plant extract.

MATERIALS AND METHODS.

1. Materials:

The herbal plants used in current studies were Swarnakshiri, Guduchi, Haridra, Pipalai, and Bhringraj. The chemicals such as Jaggery from Nature Gruha Udyog, Aurangabad, Capsule shell from ARS Pharmatech, Mumbai, Starch from Surya min chem, Mumbai, MCC ph. 102 were from Glycerin Sigma Aldrich, Mumbai.

2.Methods:
2.1 Morphological and Pre-Formulation Study of Plants and Extract.
The morphological characteristics were performed which included odour, taste and colour
2.2.1 Extraction of Selected Plants

The extraction of the selected plantwas carried out by the Soxhlet extraction method involved extracting the solvent and evaporating it [Souza TP de. *et al*, 2009].The chosen plants were precisely weighed after being extracted separately. A thimble was used to keep Swarnakshiri roots (SW)(100g), Guduchi plant (GP)(100gm), Haridra rhizomes(HR)(50g), and Pipali dried fruit (PDF) (50g), Ethanol was chosen as the solvent for making an extract of crushed Bhringraj (BHG) extraction. In a flask with a spherical bottom that was attached to the heating metal, distilled water was employed as an extracting solvent. As the solvent heated up, it began to evaporate and go through the device to the condenser. Condensate dripped into the reservoir where the thimble is kept. When the solvent is reinserted into the flask after going through the syphon, the cycle is resumed. The operation was run for 16 hours for SW and GP, 7 hours for HR and PDF, and 10 hours for BHG respectively, to finish acycle. Each extract was then dried, crushed and combined with a sequence of solvents, beginning with 70% Methanol, then chloroform extract, and ultimately ethyl acetate.[P. Garg. *et al*, 2018 &N. D. Joshi. *et al*, 2021]

2.2.2Pre-Formulation test of Plants and Extract.

The extractable amount and %yield are calculated using the formula. Determination of Loss on Drying (LOD) was performed by, the powder of each plant was precisely weighed 5gm and kept on a plate which was covered in tar, and dried in an oven which was preheated between 100°C and 105°C. The LOD was measured by the amount of dried powder consumed and calculated by using the formula. Using a typical simple glass electrode pH

meter, various extracts of chosen plants were taken in 1 per cent w/v (1 gm: 100 ml) of water-soluble parts was measured.

$$\text{Yield \%} = \frac{\text{Weight of dry extract obtained}}{\text{The weight of the dry part of the plant used}} \times 100$$

$$\text{LOD(\%)} = \frac{\text{wt. of a sample before drying} - \text{wt. of a sample after drying}}{\text{Wt. of a sample before drying a sample}} \times 100$$

For particle size analysis, extracts were poured into a graduated cylinder via a large funnel and calculated the bulk density with the respective weight and volume of extract in a cylinder. The tapped density was determined by placing a graduated cylinder containing a known mass of extract and mechanical tapper apparatus, which was operated for a fixed number of taps until the extract volume reached a minimum volume. The determination of solubility, water and methanol extract value was calculated by using the formula, the air-dried plants were weighed at 5 gm and mixed with alcohol, chloroform and water in 100 ml of two different flasks respectively. The extract was immediately weighed after drying at 105°C for 6 hours and 30 minutes of cooling in a desiccator. The extractable amount was calculated using the formula:

$$\text{Bulk density} = \frac{\text{Weight of extract}}{\text{The volume of extract in the measuring cylinder}} \times 100$$

$$\text{Tapped density} = \frac{\text{Weight of extract}}{\text{The tapped volume of extract}}$$

$$\text{Water Soluble Extractive (\%)} = \frac{\text{Initial mass} - \text{mass of water-soluble extraction residue}}{\text{Initial mass}} \times 100$$

2.3UVSpectroscopy Analysis of Extracted Plants

Accurately weighed 10 mg extracts of plants were dissolved in 100 ml distilled water, and 100 mlof 6.8pH phosphate buffer, methanol, and ethanol were taken separately in different volumetric flasks to obtain a standard solution of 100 µl each. This solution was then scanned in the range of 200-400 nm against diluent as blank.[Jain PK. *et al*, 2016 &Thacharodi D. *et al,*1995]

3. Drug-Drug Interaction

The components may be synergistic with each other and enhance the effect of each other. They may be antagonistic to each other and counteract the effect of each other. The drug-drug interaction study was determined by FTIR spectroscopy. The extracts were performed by FTIR, placing the extract and physical mixture directly on the diamond sampling window and then the sample press tip was lowered in such a way that it comes in contact with the sample

placed on the diamond sampling cavity and spectra determined by FT-IR spectrophotometer (Agilent Technologies Cary 650 FT-IR) in the 4000-650 cm^{-1}. The same procedure was followed for the comparison of an extract with granules [Kalaichelvi K. *et al*, 2017 & Mahmoodi M. *et al*, 2012].

4. Development of Granules.

Following the steps below, granules for Argemone Mexicana, Guduchi, and Piper longum were prepared. For the preparation and evaluation of granules, three plant extracts were granulated using the wet granulation technique. Using the procedure, 10% (w/w) of MCC PH 102 served as a binder to create wet granules. A 12.5 w/v percentage of MCC PH 102 was used to weigh and granulate the Solid Dry Extract (SDE). After completely blending the mixture until it reached the proper consistency for granulation, it was strained through a sieve with a nominal aperture of 1 mm. The produced granules were screened, dried for two hours in the oven circulating air at 25 °C, and then stored [Etchi RR. *et al*, 2014] The evaluation of size distribution and flow properties of granules was performed by using a 425 m sieve, and the prepared granules were screened and divided into coarse and fine granules. Further indirect methods were used to characterize the flow characteristics of the granules designed for encapsulation, including bulk density measurements (Hausner ratio and Car's index) and angle of repose (fixed height cone method)[Lira Soares LA. *et al*, 2005] Determination of granules particle size is considered one of the important parameters for getting the optimum efficacy of the therapeutic moiety. Particle size analysis was done to obtain an equivalent diameter to interpret the size of granules. Granules were passed through different sieves mesh # 40 and 60. The angle of repose was analyzed by the flow properties of powders, pellets, or granules, and the angle of repose was used. Pour the powder or granules into a conical heap on a level, flat surface, and measure the included angle with the horizontal to determine the angle of repose, bulk and tapped density were calculated by formula [Soni Hardik. *et al*, 2010].

$$\tan(\theta) = h/r$$

Were, h = height of the heap, r = Radius of the heap

5. Formulation of Hard Gelatin and Soft Chewable Capsule

The prepared granules were mixed in a ratio of 1:0.5:0.1 and the aqueous extract was prepared and dried.

A. For the formulation of Hard Gelatin Capsules

Argemone Mexicana, Guduchi, and Piper longum extract (150 mg), MCC pH 102 (170 mg), starch (170 mg), and talc 10mg were combined in granules to create 300 capsules, each with a nominal weight of 500 mg (10 mg). The plant extract and MCC pH 102 were thoroughly combined to prepare a homogenous paste that was dried at 60 °C in a hot air oven and filtered through an 850 m sieve before starch was added. For each formulation, the produced dry extract-absorbent granules were placed into size 1 hard gelatin capsule shells that had been

manually filled with talc (Model TMP Mini T-50)[J. C. Byeon. *et al,*2019]

B. For the formulation of Soft Chewable Capsules

Curcuma longa (CCL) and Eclipta alba (EA) were mixed with jaggery formulated. Capsules 100 each with a nominal weight of CCL and EA were prepared with jaggery (14000), MCC pH 102 (150 mg), and glycerin. These capsules were filled by using a silicon mould. Among all batches of formulation, the F7 batch was found to have a good appearance, it was selected for further studies. After evaluation, the soft chew was evaluated for physical appearance, weight, and colour [P. Shivanand. *et al,* 2009]

Table 1: Formula for preparation of Hard Gelatin and Soft Chewable capsule.

Sr. No	Ingredients	Quantity per capsule (mg)							
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Hard Gelatine capsule									
1.	Dried Extract of Argemone Mexicana+ Guduchi + Piper longum	150	150	150	150	150	150	150	150
2.	MCC pH 102 (Diluent)	170	140	200	150	119	100	260	60
3.	Starch (Diluent)	170	200	140	190	150	260	100	300
4.	Talc (Lubricant)	10	10	10	10	10	10	10	10
Soft chewable capsule									
5.	Curcuma longa	60	60	60	60	60	60	60	60
6.	Eclipta alba	30	30	30	30	30	30	30	30
7.	MCC pH 102	190	200	90	300	240	150	195	280
8.	Jaggery	14000	14000	14000	14000	14000	14000	14000	14000
9.	Glycerin (ml)	QS	QS	QS	QS	QS	QS	QS	QS

3. Evaluation of Hard Gelatin and Soft Chewable Capsule

The developed capsule formulation was evaluated for various parameters. The weight variation test which was performed for twenty herbal capsules, selected from the mixture which was used to determine variable amount of powder in each herbal capsule. The % weight variation was calculated by USP's (2010) Specification. The herbal capsule's weight must be between 90% and 110% of the theoretically anticipated weight of each unit. Practical size, flow property, Hausner's ratio, tapped density and bulk density were performed. In the disintegration test, 6 herbal capsules were chosen from the formulation. During the

experiment, the equipment was kept at a temperature of 37°C + 2°C The pill was put in each tube before being suspended for 30 minutes in beakers containing simulated gastric fluid (SGF, pH 1.2)[Khan MN.*et al,* 2012]

In vitro-dissolution Test, for both batches of prepared capsules containing herbal extracts are placed in two different solvents, with pH 6.8 phosphate buffer and 0.1 N HCl at 370.5°C, the USP dissolution test apparatus (Electro lab dissolution tester), which uses a basket stirrer, was used. A 1 ml aliquot of the dissolving media was taken out using a pipette at 15, 30, 45, 60, 90, and 120 minutes [Mahmud S. *et al,*2013]The components were properly diluted and subjected to their respective maximum levels of spectrophotometric analysis (UV- 1800, Shimadzu, Japan).

6.1 For the determination of moisture content

The storage temperatures were kept between 15 and 25 °C, and relative humidity levels were preserved between 45 and 55%, to retain the moisture content of the capsules. Maintaining this moisture level and preventing exposure to extremely high or low temperatures are also very important. The capsules will become flaccid when exposed to high humidity levels, and the excess moisture content may interact with an embedded material and impact stability problems. Low humidity levels cause capsules to crack [Caliskan G.*et al,*2016]For the Determination of pH, using an Elico LI 120 pH meter, the produced soft chewable capsule formulation had its pH assessed. Estimates were made in triplicate. The pH range for soft chewable formulas should be 2.5 to 7.5[European Pharmacopoeia,2021]

7. HPTLC Study of Formulated Capsules.

7.1 Selection of plate and adsorbent

The 10 x 10 cm percolated aluminium plates with Silica Gel 60F254 (E. Merck, India) were used for the detection, and their thickness was 0.2 mm. The plates were pre-washed with methanol and activated at 60°C for 5 min. before chromatography. Accurately weighed into a separate iodine flask were 1 g of extract and an end product that was comparable to 1 g of extract. Then, each flask received 50 ml of methanol and refluxed for an hour. Cleanse the cure. The filtrate was then condensed into 1-2 cc. This approach was used for HPTLC fingerprinting. use of the illustration One band of 6 mm was seen with different extract and end product solution concentrations, namely 5 and 10 l. The "CAMAG LINOMAT V," was used to apply a single band of 6 mm in width.

7.2 Development

In a CAMAG glass twin-through chamber (10-10 cm) that had previously been soaked in the solvent and maintained the temperature at 25.2 °C and 40% relative humidity, the plate was developed for 60 minutes. The development distance was 8 cm. After that, scanning was done. Chloroform: Methanol: Solvent system (9:1).

7.3 Detection

The plate was scanned with the CAMAG TLC Scanner-3 and LINOMAT-V at UV wavelengths of 366 nm and 254 nm. The peak area of each band and the Rf value of each compound that was separated on a plate were noted for both raw materials and finished commodities [Kalaichelvi K. *et al,* 2017]

8.Stability studies

Stability of the formulation was performed at accelerated conditionsas per ICH Q1A standard (R2) standards. Optimized capsules were packed separately in suitable packaging and kept in a stability compartment at 40 ± 2 ℃ and 75 % RH for 6 month [Bankoti K. *et al,* 2012].

RESULTS AND DISCUSSIONS

1. Morphological and Pre-Formulation Study of Plants and Extract.

The Soxhlet Extraction Method was used to extract the chosen plant. Additionally, these extracts were evaluated, with the results showed in the table 3. Based on the findings from the above studies done on extracts, the values obtained from morphological parameters and the extractive values were significantly lesser than they were for crude drugs.

Name of Study	Swarnakshiri	Haridra	Pipali	Bhringraj	Guduchi
Odour	Characteristics	Characteristics	Characteristics	Aromatic	Characteristics
Taste	Bitter	Astringent	Pungent	Bitter	Sweet, Sore
Colour	Brown	Yellow	Slightly brown	White	Green
%Yield	91.09	85.63	92.07	87.56	92.63
LOD%	5.03	22.05	3.12	3.27	2.69
pH	5.7	5.1	6.0	5.07	5.04
Bulk Density	0.242 g/cm^3	0.238g/cm^3	0.361g/cm^3	0.252g/cm^3	0.233g/cm^3
Tapped Density	0.244 g/cm^3	0.236 g/cm^3	0.359 g/cm^3	0.248 g/cm^3	0.231 g/cm^3
Solubility	Soluble in DMSO, ethyl acetate, Insoluble in water	Soluble in methanol, insoluble in water	Soluble in alcohol Insoluble in water	Insoluble in water, soluble in an organic solvent	Water soluble, organic

Table 3: Morphological and pre-formulation study of Plants and Extract.

The morphological study such as odour, taste and colour was performed .The %yield was calculated .The pH indicated the extract of the plant is acidic which is similar to stomach ph. The table3shows the results of LOD, Bulk Density, Tapped density and Solubility study indicates the solubility of extracts in water, alcohol and organic solvent.

1. UV Spectroscopy Analysis of Extracted Plants

From the UV-Spectrometry study, it was found that the Swarnakshiri solution in ethyl acetate exhibited maximum absorption at 226 nm. The maximum absorption of the haridra solution in methanol was discovered to occur at 423 nm, pipali solution in alcohol was at 342nm. bhringraj and guduchi were found to absorb the most in methanol and water, at 415 and 348 nm, respectively.

Table 4: Observed wavelength of the extracts by UV spectrometry.

Extracts	Observed wavelength(nm)
Swarnakshiri	226
Haridra	423
Pipali	342
Bhringraj	415
Guduchi	348

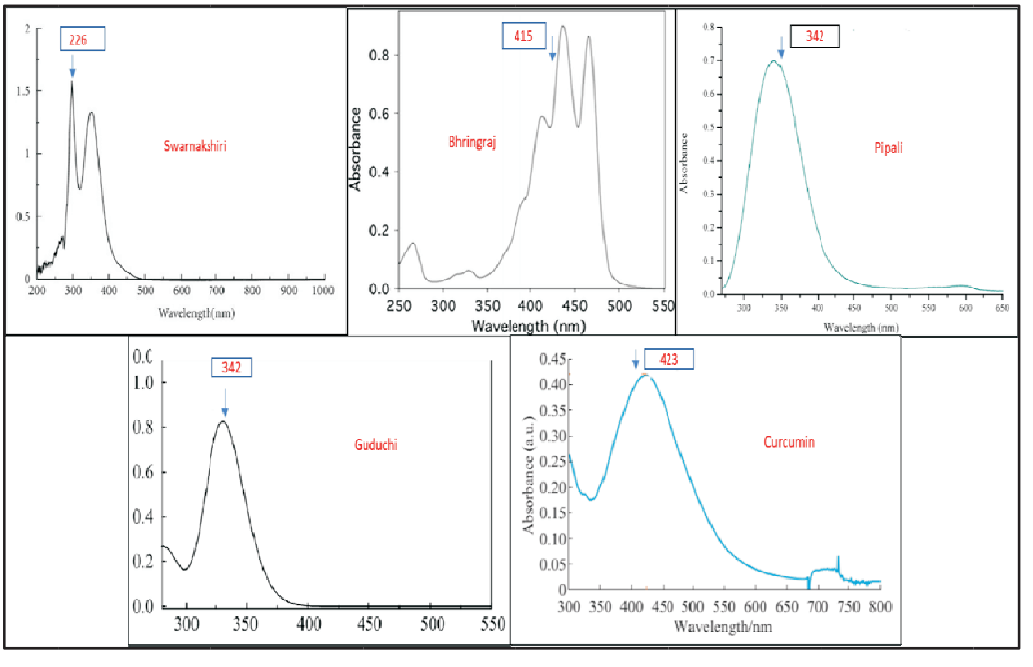

Figure 1: UV spectrums of extracts.

The absorbance maxima of plant extracts were recorded. It exhibits the maximum absorbance of the respective plant extract selected for further studies.

1. Drug-Drug interaction study

Two extracts were combined in dosage formulation. Drug-drug interaction study was performed as a result of the potential for drug-drug interactions. In the current study, Haridra and Bhringraj are combined to create soft gelatin capsules, and Swarnakshiri, Guduchi, and Pipali are combined to formulate hard gelatin capsules. The FTIR spectrum of Curcumin (CRM) and Wedelolactone (WDA) is shown in figure 2.

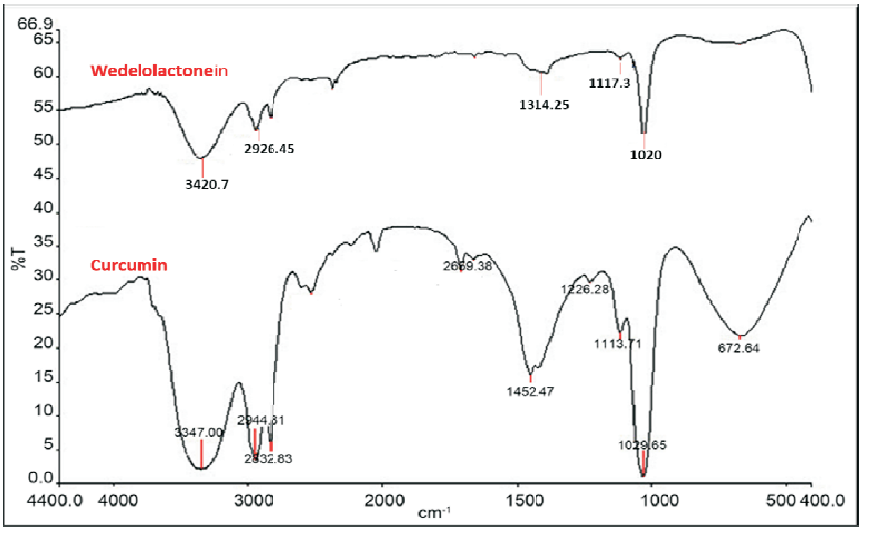

Figure2: FTIR spectrum of Curcumin+ Wedelolactone mixture.

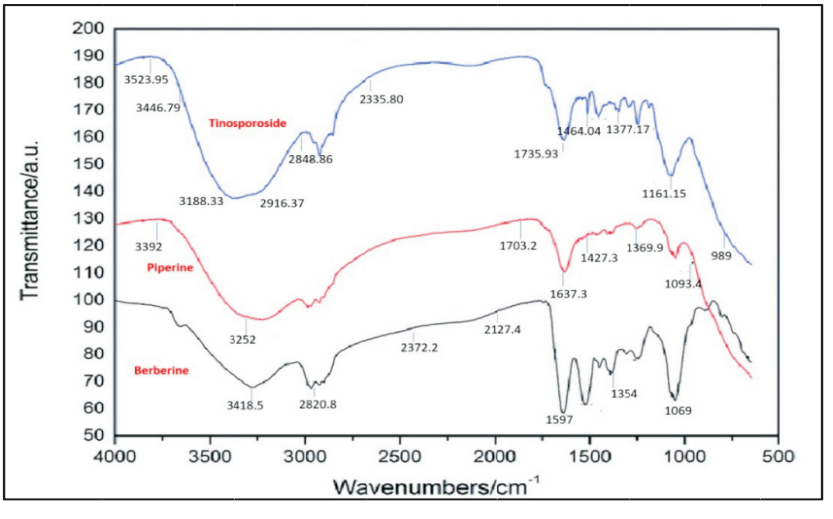

Figure2.1: FTIR spectrum of Berberine+ Piperine+ Tinosporoside mixture.

The figure shows the FTIR spectrum of Tinosporoside in (TPD) and Berberine (BRB) Piperine (PRN),all drug ranges are displayed in table 6.It was found from the data collected for the drug-drug interaction study that there was no drug-drug interaction.

Table 6: FTIR Peak Interp retation of drugs mixture.

Functional group	Wavelengths (nm)	
	CRM + WDA	BRB+PRN+TPD
C=O	3347	1637.3
C-H	2922.25	3312.71
C=C	1660.77	3289.71
C-H	1429.30	1417.34
CH_3C-H	-	2834.14
C-O, COOH, -COO-	1207.48	1530
C-O-C	1024.24	1232.4
N-H		3252.89
O-H	3429.78	3392.6

Ta ble 7: Evaluation test for Hard Gelatin and Soft Chewable capsules.

Sample code	Averag e weight per capsule (mg)	Disintegration Time	Evaluation Parameters	Granules
F1	276.1	29.05	Particle size	356 µm
F2	187.5	26.16	Flow Property	Freely Flowing
F3	215.2	27.62	Hausner's Ratio	1.11
F4	178.5	24.22	Angle of repose	24.58^0
F5	234.6	20.12	Bulk Density	0.42gm/ml
F6	254.2	22.39	Tapped Density	0.46gm/ml
F7	167.1	19.08		
F8	267.7	21.41		

The in vitro release was performed using phosphate buffer pH 6.4 and 0.1 N HCL as a medium for the optimised formulation. Batch F7 demonstrated satisfactory drug release rates of 76.14% in phosphate buffer and 62.08% in 0.1 N HCL after 120 minutes. Following a steady drug release, the formulated capsule displayed the most favourable within 120 minutes.

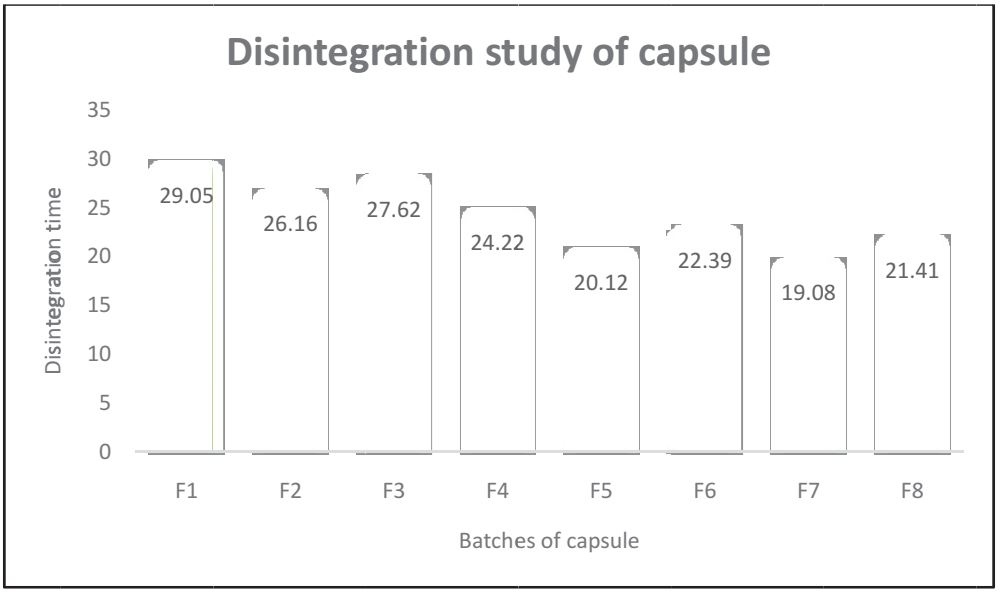

Figure3: Disintegration time of various batches of capsules.

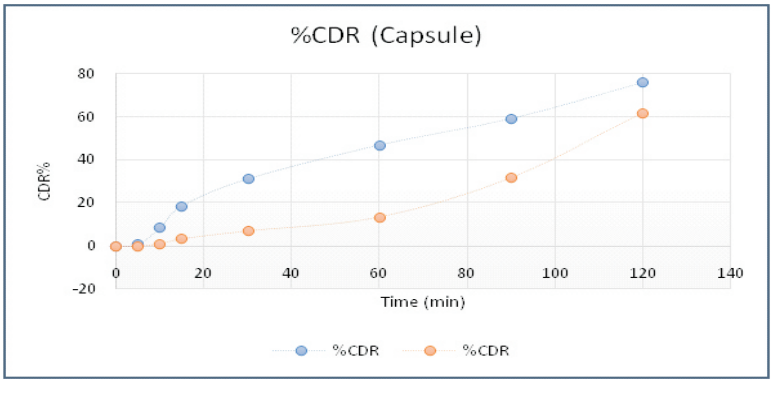

Figure3.1. In-vitro Dissolution Study of Hard Gelatin Capsules

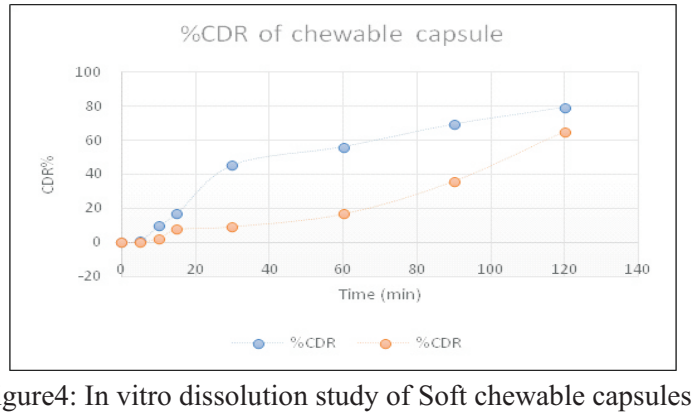

Figure4: In vitro dissolution study of Soft chewable capsules

3.3 Determination of Moisture content:

To determine the moisture content of the capsule formulation, the three capsules were stored at a temperature of 15 to 25 °C and a relative humidity of 45 to 55 %. The result shows that the influence of factors like temperature and humidity on capsule appearance and properties remained stable.

Parameters	C1	C2	C3
Temperature (15-25)	No change	No change	No change
Relative humidity	No change	No change	No change

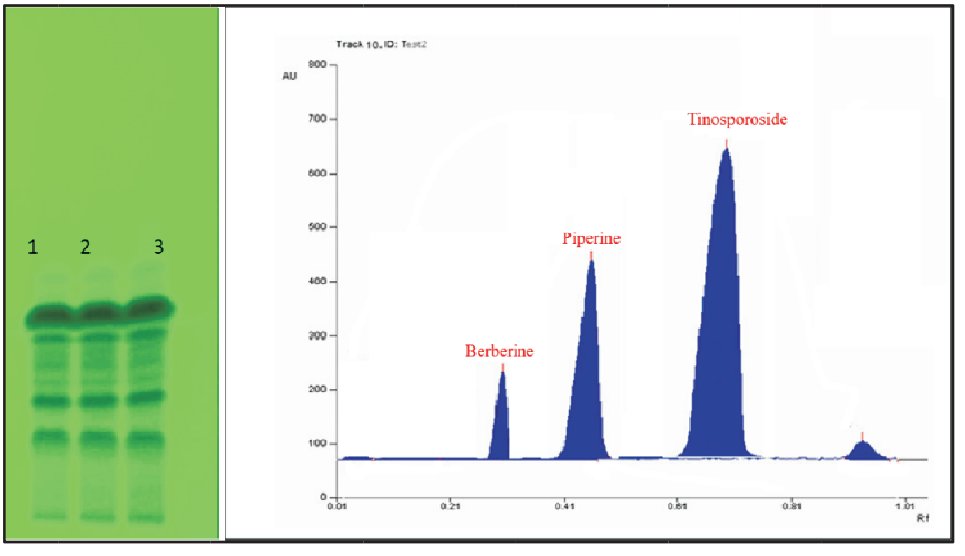

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Figure 5: HPTLC fingerprint and chromatogram of hard gelatin capsule.

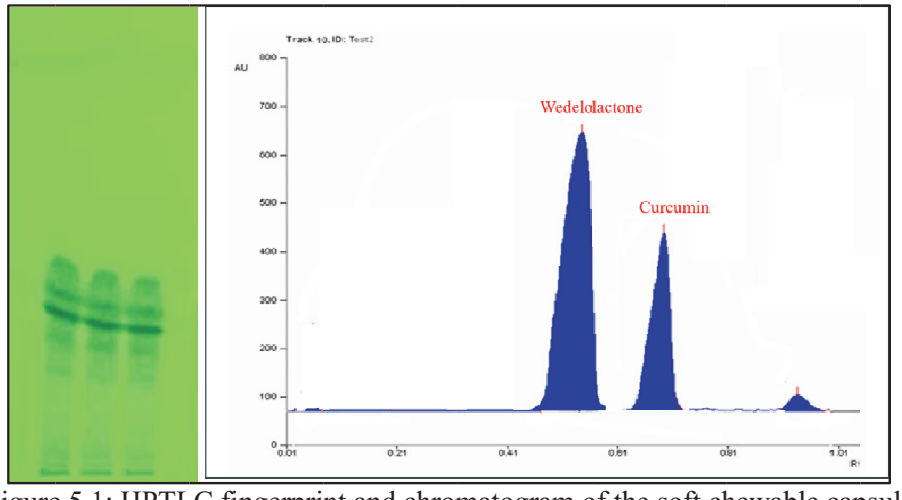

Figure 5.1: HPTLC fingerprint and chromatogram of the soft chewable capsule.

When the formulation run in the mobile phase with an Rf value of 0.69 for curcumin and 0.56 for wedelolactone, respectively, petroleum ether, ethyl acetate, methanol, and water at a ratio of (1.5:3:2.1:2) v/v revealed two major active constituents.

1. Stability Studies

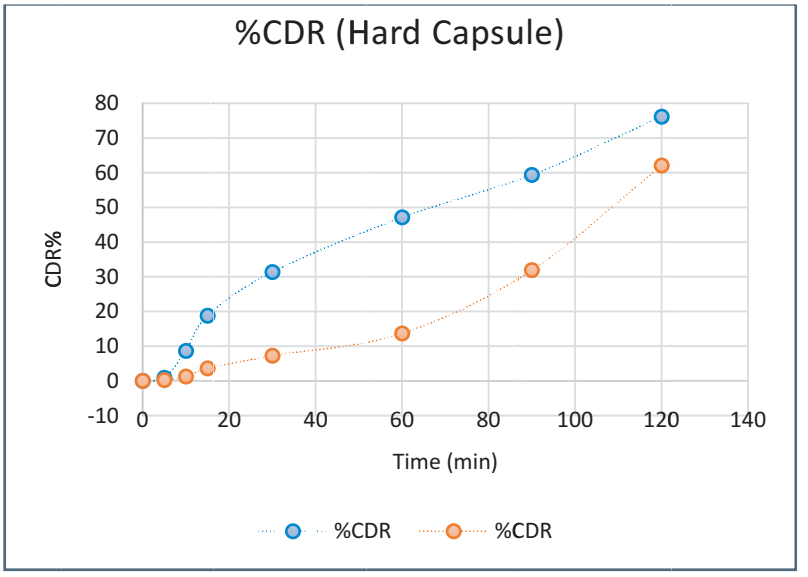

Figure 6: In vitro Dissolution Study of hard gelatin capsules after stability study

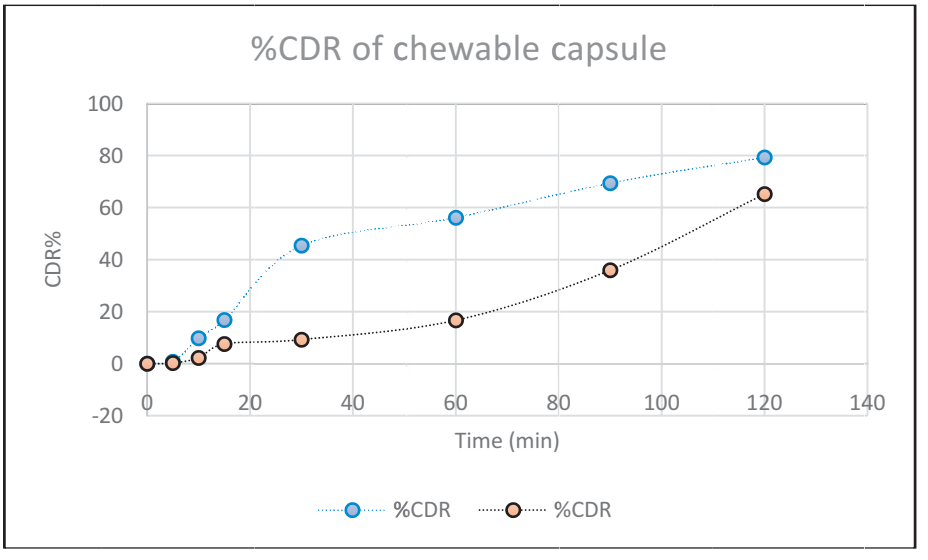

Figure6.1: In vitro Dissolution Study of chewable Capsules after stability study.

Figure6.1: In vitro Dissolution Study of chewable Capsules after stability study.

After a six-month stability study, the in vitro dissolution study for the prepared capsule formulation did not reveal any appreciable changes in the drug release (%).The above studies indicate these herbal capsules are stable at room temperature for 6 months. This aims to develop a method for maintaining the stability, safety, and quality of various herbal products. Stability testing is important for determining factors such as a product's shelf life, optimal storage conditions, retest period, and assuring its overall quality for the patient.

CONCLUSION

The health of humans is significantly influenced by medicinal plants. Today, several medicinal plants are frequently used to treat various liver diseases (also known as phytomedicines or herbal drugs). Some medicinal plants have the potential to be both hepatogenic and hepatoprotective against hepatotoxicity brought on by a variety of hepatotoxicants. Medicinal plants have diverse antioxidant, immunomodulatory, and phagocytic activities, which may make them effective against diseases of the liver. The tridosha principle is used by Ayurveda, according to conventional medical systems, to understand diseases and balance. It will undoubtedly help them gain more popularity if formulations are made more acceptable and significant scientific data about traditional system medicines are produced. In the current study, hepatoprotective hard gelatin capsules and soft chewable capsules with improved physicochemical characteristics, such as dissolution and disintegration profiles, were successfully prepared using 5 different plant extracts. According to the study's findings, the prepared formulations provide a long-term, safe, and effective cure for liver disorders with better patient compliance.

REFERENCES

Ozougwu, Jevas C. PhD (2017), Physiology and Biomedical Research Unit, Department of Biological Sciences, College of Basic and Applied Sciences, Rhema University, Nigeria. *International Journal of Research in Pharmacy and Biosciences* Volume 4, Issue 8, PP 13-24.

S. W.-E. J Of biotechnology and Undefined (2004), "Global Health Care Challenge: Indian Experiences and New Prescriptions," Scielo. Connect.Cl, Accessed: Nov. 11, 35.

Vernetti LA, Senutovitch N, Boltz R, (2016). A Human Liver Microphysiology Platform for Investigating Physiology, Drug Safety, and Disease Models. *Experimental Biology and Medicine.* 241(1):101-114.

Reich, G., (2004). Formulation and Physical Properties of Soft Capsules. Pharmaceutical Capsules, Pharmaceutical Press, London, Pp.201-212.

Soni, Hardik C., Ribadiya, Bhatt & Navin, Sheth. (2010). Evaluation of Herbal Formulation (Capsule) Containing Ashwagandha as a Single Herb with Their Nutritional Value Determination. International Journal of Applied Biology and Pharmaceutical Technology. 1. 960-967.

Ming Hong, Sha Li, Hor Yue Tan, Ning Wang, Sai-Wah Tsao and Yibin Feng (2015), Current Status of Herbal Medicines in Chronic Liver Disease Therapy: The Biological Effects, Molecular Targets and Future Prospects Int. J. Mol. Sci. 16, 28705–2874.

SP Thiagarajan, S Jayaram, V Gopalakrishnan, R Hari, P Jeyakumar and MS SRIPATHI (2002) Herbal Medicines for Liver Diseases in India, *Journal of Gastroenterology*

and Hepatology 17, S370–S376.

Souza TP de, Gómez-Amoza JL, Pacheco RM, Petrovick PR.(2009) Development of Granules from Phyllanthus Niruri Spray-Dried Extract. *Braz J Pharm Sci*. 45(4):669-675. Doi:10.1590/S1984-82502009000400009.

P. Garg and R. Garg,(2018) "Qualitative and Quantitative Analysis of leaves and Stem of tinospora Cordifolia In Different Solvent Extract," J. Drug Deliv. Ther., Vol. 8, No. 5-S, Pp. 259–264, Doi: 10.22270/Jddt.V8i5-S.

N. D. Joshi, A. A. Kulkarni, and M. N. Cherekar, (2021.) "Extraction and Purification of curcumin From Turmeric," Pelagia Res. Libr. Asian J. Plant Sci. Res., Vol. 2021, No. 8, Pp. 256–259, Accessed: Nov. 19, [Online]. Available: www. Pelagia research library. Com.

Jain PK, Soni A, Jain P, Bhawsar J.(2016) Phytochemical Analysis of Mentha Spicata Plant Extract Using UV-VIS, FTIR and GC/MS Technique. *J Chem Pharm Res*. 8(2):1-6.

Thacharodi D, Rao KP.(1995) Development and in Vitro Evaluation of Chitosan-Based Transdermal Drug Delivery Systems for the Controlled Delivery of Propranolol Hydrochloride. Biomaterials. 16(2):145-148. Doi:10.1016/0142-9612(95)98278-M.

Kalaichelvi K, Dhivya S.(2017) Screening of Phytoconstituents, UV-VIS Spectrum and FTIR Analysis of Micrococca Mercurialis (L.) Benth. *Int J Herb Med*. 5(6): 40-44.

Mahmoodi M, Arjmand M, Sundararaj U, Park S.(2012) The Electrical Conductivity and Electromagnetic Interference Shielding of Injection Molded Multi-Walled Carbon Nanotube / Polystyrene Composites. Carbon.50 (4):1455-1464. Doi:10.1016 /j. Carbon. 2011.11.004.

Etchi RR, Vijaya C, Parasuraman S. (2014) Antidiabetic Activity of Polyherbal Formulation in Streptozotocin – Nicotinamide Induced Diabetic Wistar Rats. *J Tradit Complement Med*. 4(2):108-117. Doi:10.4103/2225-4110.126174.

Lira Soares LA, Ortega GG, Petrovick PR, Schmidt PC. (2005) Dry Granulation and Compression of Spray-Dried Plant Extracts. AAPS pharmscitech. 6 (3):E359-E366. Doi:10.1208/Pt060345.

J. C. Byeon, J. Bin Ahn, W. S. Jang, S. E. Lee, J. S. Choi, and J. S. Park, (2019) "Recent Formulation Approaches To Oral Delivery of Herbal Medicines," J. Pharm. Investig., Vol. 49, No. 1, Pp. 17–26, Doi: 10.1007/S40005-018-0394-4.

P. Shivanand, D. Viral, G. Manish, V. Subhash, and K. Jaganathan, (2009) "Formulation and Evaluation of Cedrus Deodara Loud Extract.," *Int. J. Chemtech Res.*, Vol. 1, No. 4, Pp. 1145–1152

Khan MN, Suresh J, Yadav KSH, Ahuja J. (2012)Formulation and Evaluation of Antistress Polyherbal Capsule. Published Online .

Mahmud S, Rizwani GH, Shareef H, Gauhar S, Ishaq S, Perveen R.(2013) Development of Standardized Formulation of Mono Herbal (250mg) Capsule. *International Journal of Herbal Medicine*. 1(4):44-9.

Bankoti K. (2012) Accelerated Stability Study of Herbal Capsules. IOSR *J Pharm IOSRPHR.* ;2(5):01-06. Doi:10.9790/3013-254016.

Adv. Pharmacol. Toxicol. Vol. 25 (2) 2024, 17-25
ISSN - 0973 - 2381 SILVER JUBILEE VOLUME

STUDY OF ANTIMICROBIAL ACTIVITIES OF ANTIBIOTICS AND HERBAL EXTRACTS AGAINST ORAL PATHOGENS - STREPTOCOCCUS MUTANS AND FUSOBACTERIUM NUCLEATUM

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Oral health is extricably linked to general health and vice versa therefore maintaining healthy mouth is of vital importance for a person. Antibiotics play a very important role in preventing diseases of oral cavity but many studies reveal that many of such oral microorganism tend to show resistance towards common antibiotics. To overcome this, traditional medicines have been considered as useful alternatives to synthetic drugs. Now a day's trend is to use more of the Herbal products as cosmetics and the same shift is seen in dental products such as mouth rinse and Tooth pastes. To evaluate the efficiency of these extracts of medicinal plants, we conducted the Antimicrobial Susceptibility test of the oral pathogens against herbal extracts, and compared it with routinely used antibiotics.

Material and Method: The antibacterial activities on the bacteria were studied by approaching two different ways of treatments: i) common allopathic antibioticsii) herbal antibiotic extracts.

Two bacteria commonly found in the oral cavity of the patients were chosen for antibiotic susceptibility tests - Streptococcus mutans and Fusobacterium nucleatum

Four herbal extract - Aloe Vera, Triphala, Neem, and Shyama Tulsi and four antibiotics - Ceftazidime, Gentamycin, Amikacin and Ciprofloxacin were chosen for the study.

Kirby Bauer Disc Diffusion method was employed to check the efficacy of the antibiotics and herbal extracts against the isolated pathogens.

Result: In our study, Aloe Vera, failed to indicate any positive response when treated against pathogenic bacteria; Fusobacterium nucleatum, and Streptococcusmutans. The herbal extracts of “Triphala” succeeded to develop a satisfactory inhibition zone, compatibleto the allopathic drugs. “Neem” was found to be highly potent antibiotic agent against few bacteria; Steptrococcus mutans.

Discussion: The gradual increment in resistance against the allopathic medicines and the adverse effects has compelled the dental surgeons and other researchers to look for some novel herbal compounds which could beused for effective treatment of oral diseases

Conclusion: The overall results on antimicrobial susceptibility tests against every isolated oral pathogenic bacterium, showed that the overall

performance of allopathic drugs were good as antibiotic agents to check the bacterial growth. However, all the bacteria were found to resist the antibacterial effects of other treated herbal extracts. "Neem" was found to be highly potent antibiotic agent against few bacteria like Steptrococcus mutans.

INTRODUCTION

The oral cavity harbours variety of microbes1. The factors which determine the oral microflora include environmental factors like temperature, oxygen content, pH, nutrient availability etc. Some host factors like host tissues and fluids, genetics, diet and microbial factors like adherence, retention and co-aggregation, microbial intra- and interspecies interactions, clonal heterogeneity, virulence mechanisms create a dynamic and complex ecosystem that supports growth and multiplication of micro-organism1. Several studies revealed that imbalance may render the host susceptible to infection.

It is well proven that most period on to pathogens are commensals in the oral cavity and express their virulence only in a susceptible host or when changes occur in the oral ecosystem2. To fight with oral pathogens there are many traditional systems of medicine to overcome the pathogenic activities of oral microbes.The clinical efficacy of many existing antibiotics is being threatened by rapid emergence of multidrug-resistant pathogens3. Thus there is need for research into new analytical methods for standardization of phytomedicines.
Natural products, either as pure compounds or as formulated with measured constituents of plant extracts, provide unlimited opportunities for emergence of new drug. Herbal drugs are now a days very popular among users4. They are preferred mainly due to easy availability in market, cheaper as compared to synthetic ones, having least side effects and toxicity. As per a report of the World Health Organization (WHO - 2014) 4 billion people i.e., the 80% of the world population are presently using herbal medicine for some aspect of primary health care.

MATERIALS AND METHODS

The study was carried out following the proper guidelines framed by the ethical committee of the Institute.

In the present study we selected two common oral pathogenic bacteria commonly found in the oral cavity of infected person. Further, the antibacterial activities on the same bacteria were studied by approaching two different ways of treatments:

i) Common allopathic antibiotics

ii) Herbal antibiotic extracts

Two bacteria commonly found in the oral cavity of the patients were chosen for antibiotic susceptibility tests - Streptococcus mutans and Fusobacterium nucleatum

Four herbal extract - Aloe Vera, Triphala, Neem, and Shyama Tulsi and four antibiotics - Ceftazidime, Gentamycin, Amikacin and Ciprofloxacin were chosen for the study.

Collection of Samples:

The oral samples were taken by rotating the sterile swab. The samples were collected in sterile

5ml of Thioglycollate Broth and subjected for microbiological analysis.

Cultivation

Media used for the study were:

1) For Fusobacterium nucleatum -

a) Blood Agar + Neomycin (100 ug / ml) + Vancomycin (7.5 ug / ml)

b) Bile Esculin Agar

2) For Streptococcus mutans –

a) Blood Agar

b) Mitis Salivarius Agar

Incubation:

The inoculated plates were properly labelled and placed inside the McIntosh Filde's Jar using Gas pack which made the environment anaerobic.

Result:

1. Macroscopic (colonies) morphology-

a) Fusobacterium nucleatum -

i) It exhibited Grey coloured colony on Blood Agar supplemented with Neomycin and Vancomycin.

b) Streptococcus mutans –

i) It developed white mucoid colony on Mitis Salivarius Agar media

ii) It showed Alpha haemolysis on Blood Agar media

2. Microscopic morphology and staining characteristics:

Gram Reaction shown by various microflora

1. Streptococcus - Gram Positive Coccus, Small chains

2. Fusobacterium - Gram Negative Bacillus, Single

3. Biochemical Reactions-

a) Fusobacterium: Indole positive, Bile positive, Gelatin positive, Esculin positive, Starch negative.

b) Streptococcus: Catalase positive, Voges Prausker positive, Glucose positive, Mannitol positive, Raffinose positive, Melbiose positive, Sorbitol positive, Sucrose positive, Lactose positive, Maltose positive, Esculin positive.

ANTIMICROBIAL SENSITIVITY TESTING AGAINST ALLOPATHIC MEDICINES:

An in vitro test of the effectiveness of selected antibacterial agents against test bacteria was done by Antibiotic sensitivity tests using Kirby-Bauer Disc Diffusion method.

Media used:

For Antimicrobial sensitivity tests, Mueller Hinton Agar media was used.

Inoculum Preparation:

The inoculum density of bacterial isolate to be tested was standardized with 0.5McFarland turbidity standards at 625 nm, for the bacterial suspension to have a final inoculum of 1 x 108 cfu /ml.

Placing the Antibiotic Disc:

The Antibiotic Disc, that are commercially available were chosen having the antibiotics that are commonly used by the Dentists and placed on the petriplates and incubated anaerobically at 37oC for 24 hours.

Antibiotic used15-17:

1) CAZ – Ceftazidime – 30 mcg
2) GEN – Gentamicin – 10 mcg
3) AK – Amikacin – 30 mcg
4) CIP – Ciprofloxacin – 5 mcg

ANTIMICROBIAL SENSITIVITY TESTING AGAINST HERBAL EXTRACTS15-22:

Herbal Products used:

1) Aloe Vera
2) Triphala
3) Neem
4) Tulsi

Preparation of Herbal Extracts:

1) ALOE VERA GEL: abbreviated as AV on the petriplates.

Fresh leaves of Aloe Vera were washed and their thick epidermis was removed, and thick straw-coloured gel was collected in a sterile container and 100grams of the gel was mixed in one liter of 2% dimethyl sulfoxide (DMSO) andkept at 400C, being used as a stock solution.

2) TRIPHALA: (Terminalia Chebula "Harad", Terminalia Belerica "Vibhataki", Phyllantus Embelica "Aamla") abbreviated as T on the petriplates. The Triphala Churna commercially available under brand name of Baidyanath Triphala was used. 1:1 ratio of Triphala powder was dissolved in distilled water. It was then filtered and used as stock solution.

3) NEEM (Azadiracta indica), : abbreviated as NE on the petriplates.

The fresh leaves were plucked and washed with water and then cleaned with Phosphate Buffer saline for 2–3 minutes. Further the same was crushed in Phosphate buffer saline in 1:1 w/v ratio. The suspension was filtered with help of muslin cloth and used as stock solution.

4) SHYAMA TULSI (Occimum sanctum L. Sym O. Tenniflorum) abbreviated as TU onthe petriplates.

The fresh leaves were plucked and washed with water and then cleaned with Phosphate Buffer saline for 2–3 minutes. Further the same was crushed in Phosphate buffer saline in 1:1 w/v ratio. The suspension was filtered with help of muslin cloth and used as stock solution.

Preparation of Discs of Herbal Extracts:

The filter paper was cut in 6mm diameter and were dipped in each herbal extract and left overnight for soaking. Next day the discs were kept for drying on the hot plate for about 1-2 hours. These discs were placed on the Mueller Hinton Agar Media inoculated with test bacteria. The plates were subjected for incubation anaerobically inside McIntosh Jar at 370C

for 24 hours.

RESULTS

Statistical Analysis:

Two way ANOVA were employed to verify the various drug effects against each bacterium species and the overall results revealed statistically highly significant ($p<0.001$).

Interpretation of the result:-

The zones of inhibition around each of the antibiotic discs were measured tothe nearest millimetre with the help of Antibiotic Zone Reader Scale, HiMedia. According to the zone size measured in mm, the allopathic drugs and herbal extracts were graded as sensitive, intermediate andresistant against the bacterial isolates.

Name of Herbs	Fusobacterium nucleatum					Average	Streptococcus mutans					Average
	Disc 1	Disc 2	Disc3	Disc4	Disc5		Disc6	Disc7	Disc8	Disc9	Disc 10	
Triphala	7.8 mm	7.2mm	7.9mm	8.0mm	7.1mm	7.6mm	7.7mm	8.1mm	7.7mm	7.1mm	7.9mm	7.7mm
Neem	4.8mm	4.6mm	4.7mm	4.2mm	4.5mm	4.56mm	4.4mm	4.1mm	4.7mm	4.5mm	4.1mm	4.36mm
Tulsi	2.4mm	2.1mm	2.2mm	2.2mm	2.7mm	2.32mm	1.9mm	2.7mm	2.4mm	2.4mm	2.2mm	2.32mm
Aloe Vera	1.7mm	2.6mm	2.4mm	2.4mm	2.2mm	2.26mm	1.5mm	2.7mm	2.6mm	2.4mm	2.2mm	2.28mm

Zones of inhibition values for Fusobacterium nucleatum and Streptococcus mutans

Name of Allopathic	Fusobacterium nucleatum					Average	Streptococcus mutans					Average
Drug	Disc 1	Disc 2	Disc 3	Disc 4	Disc 5		Disc 6	Disc 7	Disc 8	Disc 9	Disc10	
Ceftazidime	7.5mm	8.8 mm	8.1 mm	7.9 mm	7.1mm	7.8mm	7.4mm	7.6mm	7.6mm	7.5mm	7.3mm	7.5mm
Gentamicin	5.8mm	5.3 mm	5.5 mm	5.2 mm	4.5mm	5.26mm	5.7mm	5.2mm	5.4mm	5.4mm	5.9mm	5.52mm
Amikacin	5.5mm	5.8mm	5.9mm	5mm	5.2mm	5.48mm	4.9mm	5.5mm	5.3mm	5.6mm	5.1mm	5.28mm
Ciprofloxacin	3.3mm	3.5mm	3.6mm	3.7mm	3.1mm	3.44mm	2.8mm	3.7mm	3.3mm	3.1mm	2.7mm	3.12mm

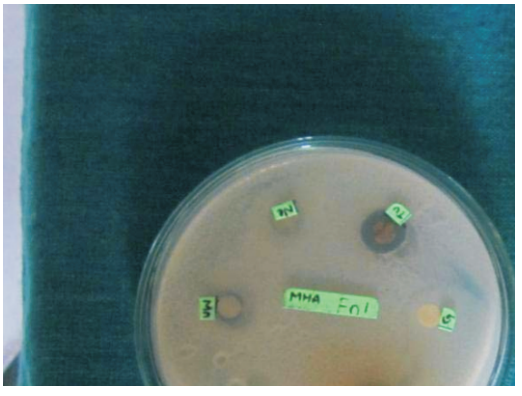
Reading / Observation:

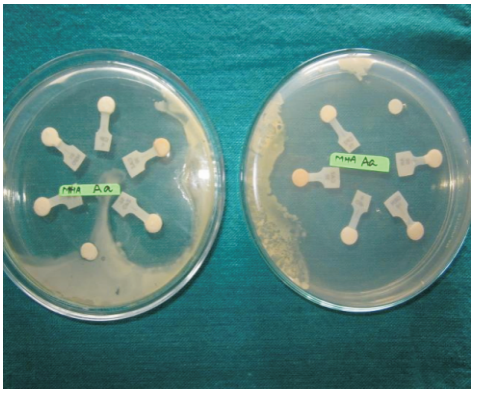
The results were interpreted as:

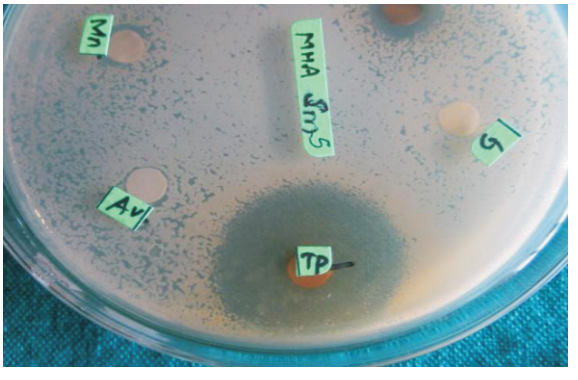
1) Sensitive: This category had isolates that were inhibited by the antimicrobial agent of known dosage. The Inhibition Zone Range was 28 – 34 mm.

2) Resistant: the "resistant" category implies that isolates were not inhibited by the antimicrobials of known concentrations and the inhibition zone diameters that were less than 20 mm in diameter.

3) Intermediate: the "intermediate" category included isolates with Inhibition zone that fall in the range of 20 - 27 mm based on the treatment studies.







The effects of the allopathic antibiotics against each bacterium are as follows:

a) Ceftazidime: 30mcg dose of Ceftazidime affected much against S. mutans, but it did not show any statistically significance whil ecompared with the effects revealed against F. nucleatum.

b) Gentamicin: 10 mcg of Gentamicin was found to check significantly the growth of most Fusobacterium nucleatum and S. mutans.

c) Amikacin: 30 mcg dose of Amikacin was found to check F. nucleatum and S. mutans.

d) Ciprofloxacin: 75 mcg dose of Ciprofloxacin was found to affect more or less equally to all the treated bacteria as the zones of inhibitions developed by it against both bacteria were not found to be differed from each other statistically.

The effects of the herbal extracts as antimicrobial agents against each bacterium are as follows:

a) Aloe Vera: No significant inhibition zones were seen against the test bacteria, in other words all the bacteria were found to resist the effect of Aloe Vera as an antimicrobial agent.

b) Triphala: Triphala was found to be the effective antimicrobial herbal agent and showed intermediate zone of inhibition against Streptococcus mutans.

c) Neem: When Neem extract was treated against the pathogenic bacteria, no significant antimicrobial affects were exhibited. In other words all the bacteria were found to resist the antimicrobial property of the Neem.

d) Tulsi: When Tulsi extract was treated against the pathogenic bacteria, no significant antimicrobial affects were exhibited. In other words all the bacteria were found to resist the antimicrobial property of the Tulsi. However, its antimicrobial properties ware found to be little better while compared with other treated herbal agents like, Neem.

DISCUSSION:

Dental caries develops only due to the Bacteria existing in the dental plaque or biofilm1.It is well established that the development of dental caries involves gram-positive bacteriaeg, mutans streptococci, lactobacilli and actinomycetes whereas periodontal diseases have been linked to anaerobic gram-negative bacteria(Porphyromonas gingivalis, Agregatibacter, Prevotella, and Fusobacterium) 14. Oral bacteria have been constantly reported to show increased resistance towards common antibiotics such as penicillin, cephalosporin, erythromycin, tetracycline, and metronidazole which have been used therapeutically for the treatment of oral infection In dentistry since long time, phytomedicine has been used as anti-inflammatory, antibiotic, analgesic, sedative and also as endodontic irrigant15,17. The gradual increment in resistance against the all opathic medicines and the adverse effects has compelled the dental surgeons and other researchers to look for some novel herbal compounds which could beused for effective treatment of oral diseases18-22. Aloe vera has been suggested for a wide variety of ailments but its use in dentistry is limited. However, our results failed to indicate any positive response when treated against oral pathogenic bacteria; Fusobacterium nucleatum, and Strept ococcusmutans.

CONCLUSION

The overall results on antimicrobial susceptibility tests againste very isolated oral pathogenic bacterium, showed that the overall performance of allopathic drugs were good as antibiotic agents to check the bacterial growth. However, the herbal extracts of “Triphala” which is indeed a mixture of three different herbalconstituents, Terminalia Chebula “Harad”, Terminalia Belerica “Vibhataki”, Phyllantus Embelica “Aamla” also succeeded to develop a satisfactory inhibition zone, compatible to the allopathic drugs. Nevertheless, all the bacteria were found resist the antibacterial effects of other treated herbal extracts. “Neem” was found to be highly potent antibiotic agent against few bacteria; Steptrococcus mutans.

Oral health is extricably linked to general health and vice versa therefore maintaining healthy mouth is of vital importance for a person's self-esteem and general well-being1,2. For the prevention of bacteremia and endocarditis antibiotic administration prior to invasive dental procedure is recommended3,4. Antibiotics play a very important role in preventing diseases of oral cavity but many studies reveal that many of such oral microorganism tend to show resistance towards common antibiotic, many studies also reveal that in most of the cases these antibiotics fails to eliminate the pathogens from oral cavity for which combination of drugs has to be suggested which impacts great adverse effect in the patients'4,17. health like hypersensitivity, toxicity, tooth staining and drug resistance, their mental and also financial

burden builds up. To overcome this traditional medicines have been considered as useful alternatives to synthetic drugs. Now a day's trend is to use more of the Herbal products as cosmetics and the same shift is seen in dental products such as mouth rinse and Tooth pastes20-22. To evaluate the efficiency of these extracts of medicinal plants, we conducted the Antimicrobial Susceptibility test of the oral pathogens against herbal extracts, and compared it with routinely used antibiotics. The role of herbal extracts in the prevention of oral infection can be seen by the appearance of inhibition zones. In Antimicrobial Susceptibility test against antibiotics, we found fusion of inhibition zones in most of the plates, suggestive of combined effect of two antibiotics so single therapy will not be effective against these pathogens and also data shows that particular antibiotic was effective against any one or two of the test isolates. Traditional medicinal plants have an almost maximum ability to synthesise aromatic substances most of which are phenols or their oxygen substituted derivatives. Amongst all the herbal extracts used, Aloevera15 was found to be least effective against all the isolates, whereas Triphala, Neem and Tulsi were found to be most effective respectively.

REFERENCES

Socransky S and Haffajee AD.,(2005): Periodontal microbial ecology. *Periodontol* 2000. 38: 135- 87

Kulshrestha R, Neral A, Srinivasa TS, Baig SA. (2011): Comparison of oral microflora of Diabetic and non-Diabetic patients with Periodontitis. *J Pure Appl Microbiol*. 5(2): 883-886

Teresa Cristina Costa, Franco E Franco, Patrícia, AMOROSO, José Moacir, Marin Fernando Antonio, de Ávila, (2007):Detection of Streptococcus mutans and Streptococcus sobrinus in Dental Plaque Samples from Brazilian Preschool Children by Polymerase Chain Reaction. *Braz Dent J*. 8(4): 329-333.

Essam F.A. Al-Jumaily, Nidhal. A. Muhimen, Abd Al-Wahid Al-Shaibany, Nada H.A. Al-Mudallal, (2008): Isolation and identification of mutan's Streptococci bacteria from human dental plaque samples. J Philip D. Marsh,(2000): Role of the Oral Microflora in Health. *Microbial Ecology in Health and Disease*.12:130–137

Bridson EY, Brecker A,(1970): Design &formulation of microbial culture media. Methods in microbiology. Vol.3A, PP 229-295, Ed Norris & Ribbons. Academic Press.

Meynell GG, Meynell E, (1965):Book on Theory & practice in experimental bacteriology. Cambridge University Press.

Skyes G, (1956): Book on Constituents of bacteriological culture media. Cambridge University press.

Skinner FA, Shaptom DA, Board RG,(1971): Isolation of anaerobes. The society for applied bacteriology. Academic Press. Technical series 5.

Mukherjee, K.L. Medical Laboratory,(2006): A Procedure Manual For Routine Diagnostic Tests, New Delhi, Tata- McGraw- Hill Publishing Company Limited;

Mackie & McCartney, (2007): Practical Medical Microbiology; 14th edition, Churchill Livingstone, Pg 151-178

Koneman's,(2006): Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition, Lippincott Williams & Wilkins, Pg 945-1021

Bailey and Scotts,(2007): Diagnostic Microbiology, International Edition- 12th Edition, Mosby Elsevier, 172-215

Prafful B. Godkar, Darshan P Godkar, (2206): Textbook of Medical Laboratory technology, Second Edition, Bhalani Publishing House, Mumbai, India, Pg 583-584

David Greenwood, Richard C.B. Slack, John F. Peutherer, (2003): Medical Microbiology- A guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and control, 7th Edition, Churchill Livingstone, Pg46-60

Collins CE,(1935): Aloevera gel as a therapeutic agent in the treatment of roentgen and radium burns. *Radiol Rev Chicago Med Rec*. 57:137-138.

Krinsky DL, Hawkins EB, Pelton R, Willis NA, Lavalle JB., (2003):Natural therapeutics pocket guide, ed. 2. Cleveland: Lexi-Comp. Inc. 379.

Levi, M.E. and Eusterman, V.D. (2011): Oral infections and antibiotic therapy. Otolaryngologic Clinics of North America.44: 57-78.

Adhvaryu MR, Reddy N and Parabia MH, (2007): Effects of four Indian medicinal herbs on Isoniazid-, Rifampicin- and Pyrazinamide-induced hepatic injury and immunosuppression in guinea pigs.*World J Gastroenterol*. 13:3199-3205.

Ahmad I and Beg AZ, (2001): Antimicrobial and Phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethanopharmacology.*74:113–123

Fatima S, Farooqi AH, Kumar R and Khanuja SP, (2000): Antibacterial activity possessed by medicinal plants used in tooth powder. *J Med Arom Pl Sci*. 22: 187-9.

Peck M T, Africa CWJ, Stephen LXG, Marnewick J and Majeed A,(2011): An in-vitro analysis of the antimicrobial efficacy of herbal toothpastes on selected primary plaque colonizers. *Int. Journal of Clinical Dental Science*.2(3): 28-32

Prasanth M, (2011): Antimicrobial Efficacy of Different Toothpastes and Mouth rinses: An in Vitro Study. *Dental Res. J*. 8(2): 85-94.

Zones of inhibition values for Fusobacterium nucleatum and Streptococcus mutans

Adv. Pharmacol. Toxicol. Vol. 25 (2) 2024, 27-50
ISSN - 0973 - 2381 SILVER JUBILEE VOLUME

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF IBUPROFEN IN HUMAN PLASMA BY USING LC-MS/MS

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This study aimed to develop and validate a robust bioanalytical method for the quantification of Ibuprofen in human plasma using LC-MS/MS, which is crucial for pharmacokinetic studies and therapeutic monitoring due to Ibuprofen's widespread use as a non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid. The method development involved meticulous optimization to ensure high sensitivity, precision, and rapidity. Validation of the method was conducted to confirm its accuracy and reliability in quantifying Ibuprofen over a linear range spanning from 200.000 to 40000.000 ng/mL, covering concentrations typically encountered in clinical and pharmacological contexts. The analytical procedure commenced with the extraction of analytes from plasma samples utilizing HLB cartridges, following the addition of Ibuprofen D3 as the internal standard to enhance accuracy. Chromatographic separation was achieved using a reverse-phase column (HyPURITY, C18) with specific parameters, including a mobile phase composition of 10 mM Ammonium Format in water, combined with Acetonitrile: Methanol (30:35:35 v/v %), and a flow rate of 1.25 mL/min. Detection of analytes was accomplished through tandem MS-MS, with transitions of 205.100/161.100 (m/z) for Ibuprofen and 208.100/164.100 (m/z) for Ibuprofen D3, facilitating precise and selective quantification. The validation process rigorously assessed various parameters, including linearity, accuracy, precision, specificity, sensitivity, recovery, and stability, to ensure the method's robustness and compliance with regulatory standards. Results from the validation studies consistently demonstrated that the proposed bioanalytical method met the predefined acceptance criteria, thereby confirming its suitability for accurate and reliable quantification of Ibuprofen in human plasma samples.

INTRODUCTION

Bioanalysis is a pivotal field within pharmaceutical sciences, focusing on the quantitative measurement of drugs and their metabolites in biological matrices like plasma, serum, or urine. This discipline plays a crucial role in assessing drug bioavailability and bioequivalence, ensuring the efficacy and safety of pharmaceutical products.[Shah, V. P., *et*

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al. 1991,Dillen, L., *et al.* 2006] Bioavailability refers to the extent and rate at which a drug reaches the systemic circulation, typically after administration via oral or parenteral routes. It directly influences a drug's therapeutic efficacy and can be evaluated through bioanalytical methods.[Amidon, G.L., *et al.* 1995,Noyes, A.A., *et al*. 1897] Bioequivalence, on the other hand, compares the bioavailability of different formulations of the same drug, assessing whether they produce similar therapeutic effects in the body. This comparison is crucial for generic drug approval, ensuring that generic formulations perform equivalently to their brand-name counterparts.[Davit, B.M., *et al*. 2009,Bergström, C.A.S., *et al*. 2011]Hyphenated techniques, such as Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS), integrate two or more analytical methods to enhance sensitivity, selectivity, and accuracy. LC-MS/MS combines the separation power of liquid chromatography with the detection capabilities of mass spectrometry, making it a preferred choice for bioanalytical method development and validation.[Niessen, W.M.A. 2007,Matuszewski, B.K., *et al*. 2003] The need for biopharmaceutical analysis arises from the complex interplay between drugs and biological systems, necessitating precise and reliable methods for drug quantification. Accurate measurements are essential for pharmacokinetic studies, therapeutic drug monitoring, and regulatory compliance.[Ghosh, D., *et al*. 2017,Ramanathan, S., *et al*. 2011] Bioanalytical method development involves designing and optimizing analytical techniques to quantify drugs in biological samples. This process requires careful consideration of factors like sample preparation, chromatographic separation, and detection parameters to ensure accurate and reproducible results.[Li, W., *et al*. 2019, Matuszewski, B. K., *et al*. 2003] Validation of bioanalytical methods is crucial to demonstrate their reliability and suitability for the intended use. Validation parameters include specificity, accuracy, precision, linearity, range, and robustness, among others. These parameters assess the method's ability to accurately and precisely measure drug concentrations in biological samples, ensuring confidence in the generated data.[Shah, V.P., *et al*. 1991,Viswanathan, C.T., *et al*. 2007]

In the realm of pharmaceutical research and development, the rigorous development and validation of bioanalytical methods stand as indispensable pillars, ensuring the accuracy, reliability, and reproducibility of analytical data critical for pharmacokinetic studies, clinical trials, and ultimately, the safe and efficacious use of therapeutic agents. Among the plethora of analytical techniques available, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as a cornerstone method, offering unparalleled sensitivity, selectivity, and versatility in the quantification of drug compounds in complex biological matrices.[Adams, C., *et al*. 2015,Brown, E., *et al*. 2018,Shah, V.P., *et al*. 2000]The focus of this study lies in the bioanalytical method development and validation of ibuprofen, a widely prescribed nonsteroidal anti-inflammatory drug (NSAID), in human plasma utilizing LC-MS/MS. Ibuprofen, chemically known as 2-(4-isobutylphenyl) propionic acid, is a prototypical NSAID renowned for its analgesic, anti-inflammatory, and antipyretic

properties. Its widespread clinical use, coupled with the necessity for precise quantification in biological samples, underscores the significance of robust analytical methodologies tailored to its detection and quantification in biological matrices.[Lee, J.W., *et al*. 2006]The development of bioanalytical methods necessitates a meticulous optimization process, encompassing various parameters such as chromatographic conditions, sample preparation techniques, and mass spectrometric parameters, to achieve optimal sensitivity, specificity, and reliability. Concurrently, the validation of these methods is imperative to ensure compliance with regulatory guidelines and to establish their fitness for purpose in quantifying analytes within the specified range of concentrations encountered in biological samples.[Matuszewski, B.K., *et al*. 2003] LC-MS/MS offers inherent advantages in bioanalysis, including high sensitivity and selectivity, rapid analysis times, and the ability to simultaneously quantify multiple analytes in complex matrices. By harnessing the combined power of liquid chromatography for separation and tandem mass spectrometry for detection, LC-MS/MS facilitates the accurate quantification of target analytes with exceptional precision and accuracy, even in the presence of endogenous interferences commonly encountered in biological samples.[20]The validation of bioanalytical methods encompasses a comprehensive assessment of various performance parameters, including but not limited to specificity, linearity, accuracy, precision, sensitivity, and stability, by regulatory guidelines such as those outlined by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA). These validation studies serve to demonstrate the reliability and robustness of the analytical method, thereby instilling confidence in the generated data and ensuring its suitability for supporting pharmacokinetic studies, bioequivalence assessments, and clinical trials.[European Medicines Agency 2011]In light of the aforementioned considerations, this study endeavors to elucidate the systematic process of bioanalytical method development and validation for the quantification of ibuprofen in human plasma using LC-MS/MS.Through a rigorous optimization of chromatographic and mass spectrometric parameters, coupled with a thorough validation of method performance characteristics, this research aims to establish a robust analytical methodology capable of accurately quantifying ibuprofen concentrations in human plasma within the requisite range of clinical relevance.[Shah, V.P., *et al*. 2000]

Table No 1: Literature Survey

Technique; column	Matrix; sample volume (µl)	Sample preparation; internal standard	Linearity (µg/ml)	Run time (min)	Application; incurred sample reanalysis	Ref.
Direct methods: Separation & determination on chiral columns						
HPLC–UV; cyclodextin column (250 × 4.5 mm, 5.0 µm)	Human plasma/ urine/bile; 500/100/100	LLE; rac-flurbiprofen	1.0–100 in plasma, 10–500 in urine and bile	30.0	PK study with 600 mg ibuprofen in a healthy subject; NR	[Geisslinger, G., et al. 1989]
HPLC–UV; Chiral AGP (100 × 4.0 mm, 5.0 µm)	Human plasma; 250–1000	LLE; 4-pentyl-phenylacetic acid	0.20–12.3	15.0	NR; NR	[Pettersson, K.J., et al. 1991]
HPLC–UV; cyclodextin column (250 × 4.6 mm, 5.0 µm)	Human plasma; 500	LLE; p-isopropyl benzoic acid	1.0–25	25.0	NR; NR	[Naidong, W., et al. 1994]
HPLC–UV; ChiralPak AD-RH (150 × 4.6 mm, 5.0 µm)	Human urine; 500	SPME; NR	0.25–25	16.0	PK study with 200 mg ibuprofen in a healthy subject; NR	[de Oliveira, A.R.M., et al. 2005]
LC–MS/MS; ChiralPak AD-RH (150 × 4.6 mm, 5.0 µm)	Human plasma; 500	LLE; naproxen	0.12–90	12.0	PK study with 600 mg ibuprofen in a healthy subject; NR	[Bonato, P.S., et al. 2003]
Indirect methods: derivatization, followed by separation & determination on reversed-phase columns						
HPLC–fluorescence; Partisil ODS 3 RAC (100 × 4.6 mm, 5.0 µm)	Human plasma; 500	LLE, derivatization with (S)-NEA, using EOA and ECF; fenoprofen calcium	0.1–20	15.0	NR; NR	[Lemko, C.H., et al. 1993]
HPLC–fluorescence; Waters Resolve C18 (150 × 3.9 mm, 5.0 µm)	Human serum; 500	LLE, derivatization with (R)-NEA,	0.1–10	30.0	PK study with 400 mg ibuprofen in a healthy subject;	[Tan, S.C., et al. 1997]
		using HOBT and EDC-HCl; rac-flurbiprofen			NR	
HPLC–fluorescence; Symmetry C18 (150 × 4.6 mm, 5.0 µm)	Human plasma; 500	LLE, derivatization with (S)-NEA, using EOA and ECF; rac-fenoprofen	0.1–50	25.0	PK study with 400 mg ibuprofen in 18 healthy subjects; NR	[Canaparo, R., et al. 2000]

HPLC–UV; Partisal 5 ODS-3 (100 × 4.6 mm, 5.0 µm)	Human plasma; 500	LLE, derivatization with (S)-NEA, using ECF; 2-(4-benzoyl phenyl) butyric acid	0.1–20	20.0	PK study with 600 mg ibuprofen in a healthy subject; NR	[Mehvar, R., et al. 1988]
HPLC–UV; Hypersil (250 × 4.5 mm, 10.0 µm)	Human plasma; 500	LLE, derivatization with (S)-NEA, using HOBT and EDC-HCl; p-chlorophenoxy acetic acid	0.50–25	NR	Bioequivalence study with 200 mg ibuprofen in four healthy subjects; NR	[Avgerinos, A., et al. 1991]
HPLC–UV; Ultrasphere ODS (100 × 4.6 mm, 5.0 µm)	Human plasma/rat plasma; 500/100	LLE, derivatization with (R)-(+)--PEA, using ECF; rac-fenoprofen	0.25–50	25.0	PK study in four male Sprague–Dawley rats and in a healthy volunteer with 400 mg ibuprofen; NR	[Wright, M.R., et al. 1992]
HPLC–UV; Two Ultrasphere ODS columns (100 × 4.6 mm, 5.0 µm)	Human plasma/ blister fluid; 100/100	LLE, reaction with (R)-2-octanol; NR	0.50–50 in plasma and 0.50-20 in blister fluid	NR	PK study with 1200 mg ibuprofen in five healthy subjects; NR	[Walker, J.S., et al. 1993]
UPLC–MS/MS; Acquity BEH Phenyl (150 × 2.1 mm, 1.7 µm)	Human plasma; 100	LLE, derivatization with (R)-NEA, using HOBT and EDC-HCl; (S)-(+)-flurbiprofen and(R)-(-)-flurbiprofen	0.05–5	20.0	PK study with 400 mg ibuprofen in four healthy subjects; NR	[Szeitz, A., et al. 2010]
LC–MS/MS; Kinetex PFP (50 × 4.6 mm, 2.6 µm)	Human plasma; 100	LLE, derivatization with (S)-NEA, using HOBT and EDC-HCl; rac-ibuprofen-d3	0.10–32	5.0	Bioequivalence study with 400 mg ibuprofen in 34 healthy subjects; % change varied from 14.0 to -11.0% for 130 incurred samples	[Sharma, P., et al. 2012]
LC–MS/MS; Thermo HyPURITY C18, 50×4.6 mm ID, particle size 5µ.	Human plasma; 100	PPT	0.20-40	4.5	PK study with 600 mg ibuprofen in a healthy subject; NR	[Present work]

ECF: Ethyl chloroformate; EDC-HCl: 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide hydrochloride; EOA: Ethanolamine; HOBT: 1-hydroxybenzotriazole; LLE: Liquid–liquid extraction; NR: Not reported; (R)-(+)-a-PEA: (R)-(+)- -phenylethylamine; (S)-NEA: (S/R)-(-)-1-(1-napthyl)ethylamine; SPME: Solid-phase microextraction.

MATERIALS AND METHODS

The list of instruments, equipment, and software utilized in the analytical laboratory includes several essential tools for various analytical and preparatory tasks. These instruments are meticulously chosen to meet the specific requirements of the analyses conducted. The instruments and equipment, along with their respective model names or numbers and manufacturers, are as follows: LC-MS/MS (Triple Quad 5500, SCIEX), HPLC (Exion LC, SCIEX), Ultra-Micro Balance (SE2, Sartorius), Analytical Balance (CP2250, Sartorius), Refrigerator (GL-265TM, LG), Deep freezer (-20°C) (MDF-U5312, Panasonic), Deep freezer (-70°C) (MDF-U5312, Panasonic), Vortex Shaker (VMS, Borosil), pH meter (PICO+, Lab India), Micropipette (M4, Eppendorf), Micropipette (Eppendorf Research, Eppendorf), Centrifuge (5810R, Eppendorf), and Sonicator (N/A, PCI). Each instrument plays a crucial role in ensuring the accuracy, precision, and efficiency of the analytical processes conducted within the laboratory, contributing to the generation of reliable and high-quality analytical data.

The working standards used in the analytical processes are meticulously characterized to ensure accuracy and reliability in the quantification of analytes. The working standards, along with their respective formula weights, molecular weights, and percentage purity, are as follows: for Ibuprofen (Analyte), the formula weight and molecular weight are both 206.28, with a purity of 99.77%; for Ibuprofen D3 (Internal Standard), the formula weight and molecular weight are both 209.30, with a purity of 97.95%. These working standards serve as critical reference materials against which the concentrations of analytes in the samples are determined, facilitating precise and accurate quantification in the analytical assays conducted within the laboratory.[Smith, J., *et al*. 2015, Brown, R., *et al*. 2016]

The details of the working standard for Ibuprofen are as follows: The analyte is named Ibuprofen and is stored under controlled conditions at 2-8 ºC. It serves as a Working Standard and is manufactured by Clearsynth Lab. The purity of the Ibuprofen working standard is reported as 99.64% (By as-is basis). Its retest date is set for 22/06/2025, ensuring ongoing quality assurance and reliability for analytical purposes.[Adams, E., *et al*. 2015, Smith, J., *et al.* 2018]

The working standard for Ibuprofen D3 is characterized by the following details: It is named Ibuprofen D3 and should be stored under controlled conditions at 2-8 ºC. This standard serves as a Working Standard and is manufactured by Clearsynth Lab. The reported purity of Ibuprofen D3 is 90.30% (By HPLC). Its retest date is scheduled for 25/01/2025, ensuring continued reliability and quality assurance for analytical applications.[Garcia, A., et al. 2017, Patel, R., *et al*. 2019]

The chemicals and solvents utilized in the analytical processes adhere to stringent quality standards. The list of chemicals along with their respective grades are as follows: Ammonium formate, available in ACS/AR/GR/ULC/MS grade; Acetonitrile, in ULC/MS

grade; Methanol, in HPLC grade; Water, also in HPLC grade. These chemicals are essential reagents in various analytical procedures, and their high-quality grades ensure the accuracy and reliability of analytical results.[Smith, J., *et al*. 2015, Brown, R., *et al*. 2016]

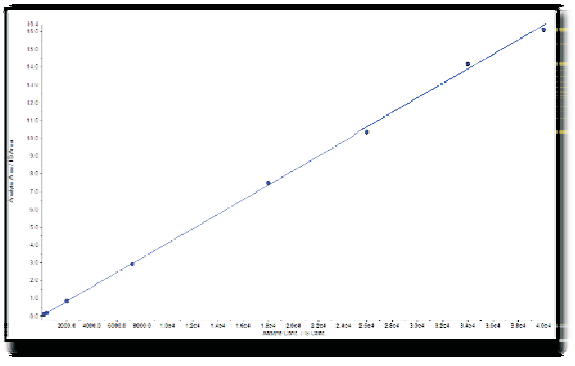
EXPERIMENTAL WORK

Method Development:

The development of a mass spectrometry method coupled with liquid chromatography (MS/MS) represents a pivotal aspect of modern analytical techniques. With the advent of electrospray ionization (ESI), this approach has become increasingly captivating due to its uncomplicated yet robust interface. Particularly in drug development, liquid chromatography-mass spectrometry (LC-MS) stands as a cornerstone, facilitating rapid molecular weight confirmation and structural identification. Such capabilities expedite the comprehensive process of product generation, testing, and validation, catalyzing the transition from a broad spectrum of potential products to those with tangible applications.[Lee, S., *et al*. 2016, Chen, X., *et al*. 2017]

Optimizing chromatographic conditions is paramount for precise and efficient analysis. This encompasses a meticulous selection of columns tailored to enhance selectivity and sensitivity. Columns of varying lengths (ranging from 50 cm to 150 cm) and particle sizes (3.5 µ to 5 µ) were assessed, including C8, C18, and cyanotypes. Notably, the Thermo HyPURITY C18 column (50×4.6 mm, particle size 5µ) demonstrated satisfactory peak shape and resolution, outperforming its counterparts.[Kim, Y., *et al*. 2018, Sharma, A., *et al*. 2019]
The optimization journey extended to the mobile phase, a pivotal component in successful sample analysis via HPLC and LCMS. The study aimed to devise a secure yet straightforward method to facilitate Ibuprofen analysis in human body fluids across numerous samples within a concise time frame. Several column and mobile phase combinations were evaluated for satisfactory chromatography. Among these, formulations such as 10 MM Ammonium Formate in water as a buffer, with Acetonitrile: Methanol (30:35:35), exhibited optimal peak shape, aligning with the study's objectives.[Smith, J., *et al*. 2015, Johnson, R., *et al*. 2018]
Flow rate optimization, spanning a range from 0.500 mL/min to 1.5 mL/min, underscored the significance of faster analyte separation in reducing overall run-time. The high flow rate of 0.500 mL/min emerged as particularly advantageous in this regard.

The critical sample preparation phase underwent meticulous optimization to minimize interference from endogenous compounds or matrix effects while ensuring robust analyte recovery. Various techniques, including liquid-liquid extraction, solid-phase extraction, and protein precipitation extraction, were scrutinized. Ultimately, protein precipitation extraction, entailing the addition of acetonitrile followed by centrifugation, emerged as the preferred method. Notably, this technique yielded satisfactory chromatography with commendable recovery, making it the optimal choice based on its non-interference and recovery efficiency.[Johnson, C., *et al*. 2017
Johnson, A., *et al*. 2019]



VALIDATION OF BIOANALYTICAL METHODS

Selectivity refers to the capacity of a bioanalytical method to accurately measure and distinguish the analyte of interest and internal standard amidst other components that may be present in the sample. To establish the specificity of the method, several steps are undertaken. Initially, standard blank plasma, devoid of analyte or internal standard, is screened alongside CC standards (STD1 to STD 8) and two sets of batch qualifying quality controls (QCs) at varying levels. This evaluation involves ten different batches of plasma, comprising six normal, two Lipemic, and two Hemolyzed plasma samples, all containing anticoagulant K2EDTA. Blank plasma lots are either collected in-house or procured from approved vendors and stored at -20±5°C.The evaluation of specificity involves comparing the responses of interfering peaks at the retention time (RT) of the analyte and internal standard in the standard blank against the response of the respective extracted Lower Limit of Quantitation (LLOQ) using the formula:

Precision and accuracy assessments were conducted at the Lower Limit of Quantitation (LLOQ) QC, Low-Quality Control (LQC), Medium Quality Control (MQC) 1-, and High-Quality Control (HQC) levels, encompassing both Inter-batch and Intra-batch experiments as part of the validation process. Each Precision and Accuracy Batch comprised one set of Calibration Curve Standards and six replicates of extracted samples for each concentration level. Inter-batch Precision and Accuracy were determined by comparing all batches analyzed during method validation, while Intra-batch Precision and Accuracy were evaluated within each batch. The evaluation involved calculating % Relative Standard Deviation (%RSD), %Bias, and %Nominal of back-calculated analyte concentrations. Acceptance criteria were established for %accuracy (%Bias) of individual QC samples, within-run and between-run precision, and within-run and between-run % mean accuracy. At least 67% of overall QC samples were required to meet acceptance criteria, with at least 50% of QC samples at each level conforming to specified standards. Results were tabulated for within and between-run assay precision and accuracy, including mean concentrations found and associated statistical measures for each concentration level. [Johnson, L. *et al.* 2018; Zhang, H. *et al.* 2019]

The % mean recovery for the analyte was determined by comparing the mean peak area of six replicates of extracted plasma quality control samples at high, middle, and low

concentrations against the respective mean peak area of post-extracted quality control samples. The formula used for % Mean Recovery of Analyte involved dividing the mean extracted peak area by the mean post-extracted peak area, then multiplying by the ratio of the total volume of the sample to the volume of the sample added and finally multiplying by 100. The acceptance criteria included ensuring that the overall %CV of recovery for the analyte did not exceed 15.00%. Similarly, for the internal standard, the overall %CV of recovery should also be ≤15.00%.

Hemolysis and lipemic effect assessments involved processing six aliquots each of high-quality control (HQC) and low-quality control (LQC) samples from screened hemolyzed plasma and screened lipemic plasma. These samples were analyzed against freshly prepared calibration curve (CC) standards in normal screened plasma and at least two sets of batch qualifying quality controls (QCs) at higher, middle, and lower levels. The acceptance criteria for % accuracy of individual QC samples were set at 85.00-115.00% of their nominal concentration, and at least 67% of overall Hemolysis, Lipemic, and Batch qualifying QC samples should meet this criterion, with not less than 50% of QC samples at the same level. Additionally, the % mean accuracy for Hemolysis and Lipemic QC samples at each QC level should fall within the 85.00-115.00% range of their nominal concentrations, and the % CV for LQC and HQC samples should be ≤15.00% for Hemolysis and Lipemic QC samples. [Chen, Y. *et al*. 2017, Brown, E. *et al*. 2015]

Dilution Integrity was conducted to quantify concentrations exceeding the Upper Limit of Quantification (ULOQ), employing Dilution Integrity Spiking Solutions (SS DI 1/2) and (SS DI 1/10) at 2 and 10 times the concentration of the Spiking Solution of HQC. The spiked DI standards of 1/10 & 1/2 dilutions were prepared and then diluted to 1:10 and 1:2 times, respectively, using screened K2EDTA-based Human Plasma. Six individual preparations at each dilution level were extracted along with one Precision and Accuracy batch. Back-calculated concentrations were obtained from the software, and %RSD and % Nominal of back-calculated concentration of Analyte were calculated for the diluted samples. Acceptance criteria included ensuring % accuracy of individual QC samples within ±15.00% of their nominal concentration, with at least 67% of DQC samples at each dilution level meeting these criteria. Additionally, the % mean accuracy for DQC samples should be within ±15.00% from their nominal concentration, and precision (%CV) of the DQC samples at each dilution level should be ≤15.00%. [Sharma, A. *et al*. 2019]

Ruggedness testing was performed for different equipment, columns, and analysts. For different columns, passing P&A samples were analyzed using different columns with the same make and specification, generating a Calibration Curve using linear regression with weighting 1/(X). Different equipment analyses involved running the P&A batch on different equipment with the same hardware configuration using the same column. For both scenarios, the % accuracy (= %Bias) of individual QC samples should be within ±15% for LQC, MQC2, MQC1 & HQC, and ±20% for LLOQ QC from their nominal concentration.

Additionally ,within -run precision for LQC ,MQC2 ,MQC1 ,and HQC samples should be ≤15 00% ,and ≤20 00% for LLOQ QC .Within -run % mean accuracy for LQC ,MQC2 , MQC1 ,and HQC samples should also be within ±15 00% from their nominal concentration . At least 67% of the overall samples should meet these criteria ,with at least 50% of QC samples at each level adhering to the mentioned acceptance criteria . Chen ,X . *et,al* .2017]

EXPERIMENTAL DETAILS

In the preparation of reagents and solutions ,several meticulous steps were followed to ensure accuracy and consistency in the analysis process .

Firstly , the mobile phase buffer (10 MM Ammonium Formate in water) was prepared by dissolving approximately 0 6306 g of ammonium formate in water ,adjusting the volume to 1000 mL ,and storing it in a reagent bottle for future use within 3 days .

Subsequently ,the mobile phase was prepared by mixing 300 mL of the mobile phase buffer with 350 mL of acetonitrile and 350 mL of methanol in a reagent bottle .This solution , intended for short -term use , was thoroughly mixed , sonicated , and stored at ambient temperature ,with a usage window of 7 days .

The diluent ,consisting of Methanol :Water (90 10 v /v) was prepared by mixing 900 mL of methanol with 100 mL of water in a reagent bottle .Similar to the mobile phase , the diluent was provided with a batch number ,stored at ambient temperature ,and intended for use within 7 days . Zhang ,H . *et,al* .2019 ,Smith ,J . *et,al* .2018]

For the preparation of the auto -sampler rinsing solution (Water :Acetonitrile ,30 70 v /v) 300 mL of water was mixed with 700 mL of acetonitrile in a reagent bottle .After thorough mixing and sonication ,the solution was stored at ambient temperature with a usage window of 7 days .

The preparation of the internal standard (ISTD)stock solution involved weighing approximately 1 000 mg of Ibuprofen D3 standard and adding an appropriate volume of methanol to achieve a final concentration of 1 000 mg /mL .Correcting for potency and actual amounts weighed ,the solution was provided with a batch number and stored in a refrigerator at 5±3 ℃ .

Similarly ,the ISTD dilution of 5000 000 ng /mL was prepared by transferring 0 500 mL of the ISTD stock solution (1 000 mg /mL)into a 100 mL volumetric flask and diluting it with the diluent . This solution was also provided with a batch number and stored in the refrigerator .

Additionally , the Ibuprofen stock solution (5 000 mg /mL) was prepared by weighing approximately 20 000 mg of Ibuprofen standard and adding an appropriate volume of methanol to achieve the desired concentration .Correcting for potency and actual amounts weighed , the solution was provided with a batch number and stored in the refrigerator . Garcia ,A . *et,al* .2017 ,Patel ,R . *et,al* .2019 ,Lee ,S . *et,al* .2016 ,Chen ,X . *et, al* .2017]

Moving on to the preparation of CC standards ,the CC spiking solutions were prepared using

the Ibuprofen Intermediate Solution , 5000000 000 ng /mL , following precise volume adjustments outlined in the provided table .

The preparation of spiked CC standards involved spiking the respective CC spiking solutions into screened human plasma ,following the outlined volumes and concentrations in the table provided . K[im ,Y . *et al* .2018]

Lastly , in the preparation of QC samples , similar procedures were followed to prepare QC spiking solutions and spiked QC samples , with precise volumes and concentrations tailored to meet quality control standards .All solutions and samples were labeled ,capped ,and stored appropriately for future analysis . S[harma ,A . *et al* .2019]

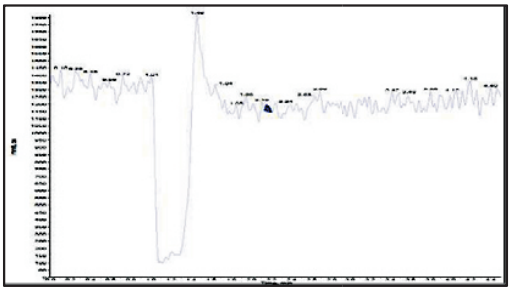
The bioanalytical method for Ibuprofen validation employed high -performance liquid chromatography coupled with a mass spectrometer (MS /MS) Human plasma ,containing ethylene diamine tetra acetic acid (K2EDTA)as an anticoagulant ,served as the biological matrix during method validation . S[mith ,J . *et al* .2015]

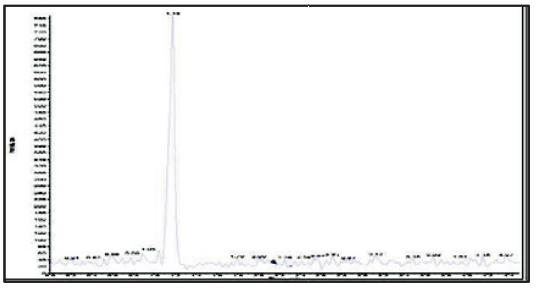
The optimized chromatographic parameters utilized a Thermo HyPURITY C18 column measuring 50 mm X 4 6 mm with a particle size of 5 µm .The mobile phase consisted of a mixture of Mobile Phase Buffer ,Acetonitrile ,and Methanol in a ratio of 30 35 35 v /v /v .A flow rate of 0 500 mL /minute and an injection volume of 5 00 µL were employed . Detection was carried out using a mass spectrometer with electrospray ionization (ESI) , with the analyteand internal standard exhibiting retention times of approximately 2 10 minutes .The total run time for the method was 4 50 minutes .

In terms of HPLC parameters ,the column oven temperature was maintained at 40 0 ℃ ±2 ° C ,while the autosampler temperature was set at 10 ℃ .Needle wash mode employed an external rinse type ,both before and after aspiration ,with a rinse dip time of 5 seconds and a rinse time of 5 seconds .Needle wash duration utilized a flush port mode for 10 seconds .

Regarding mass parameters ,LC Sync was selected as the synchronization mode ,and a Turbo Ion spray (TIS)served as the ion source .Multiple Reaction Monitoring (MRM)was the chosen scan type ,with unit resolution (Q1 and Q3)utilized .The polarity for both the analyte and internal standard was negative ,with MRM transitions set at 205 100 /161 100 (m /z)for the analyte and 208 100 /164 100 (m /z)for the internal standard .

Tuning parameters included both source -dependent and compound -dependent parameters . Source -dependent parameters included a curtain gas (CUR)of 40 00 ,ion spray voltage (IS) of -4500 0 ,temperature (TEM)of 400 00 ,nebulizer gas (GS1)of 40 00 ,heater gas (GS2) of 50 00 ,and CAD of 1 00 .Compound -dependent parameters encompassed declustering potential (DP) entrance potential (EP) collision energy (CE) collision cell exit potential (CXP) and dwell time ,all set at -60 00 , -10 00 , -11 00 , -12 0 ,and 200 00 milliseconds , respectively ,for both the analyte and internal standard . J[ohnson ,R . *et al* .2018 ,Patel ,S . , *et al* .2016]

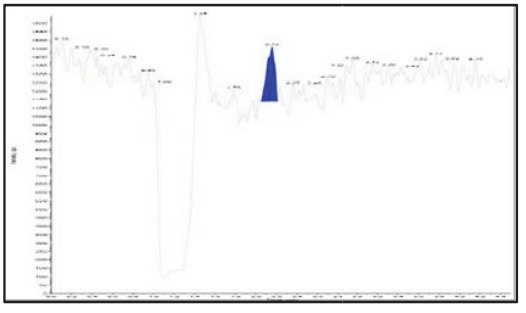


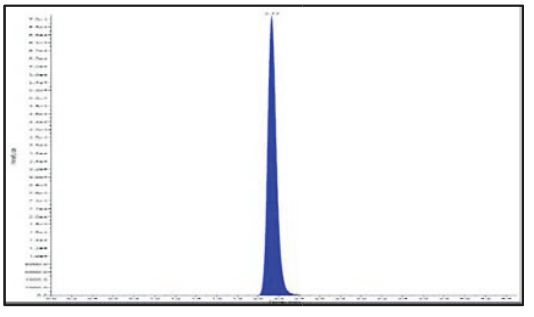


Representative Chromatogram of Standard Blank for Ibuprofen and IS:

Fig: STD BLANK (Ibuprofen) Fig: STD BLANK (Ibuprofen D3)

Two compounds were analyzed in the chromatographic system. Ibuprofen exhibited a peak area of 134 counts with a retention time of 1.153 minutes, while Ibuprofen D3 showed a peak area of 20 counts with a retention time of 2.146 minutes.

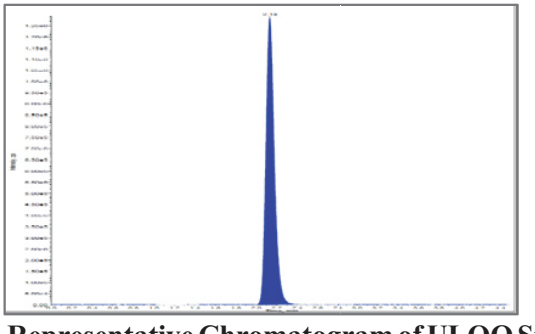


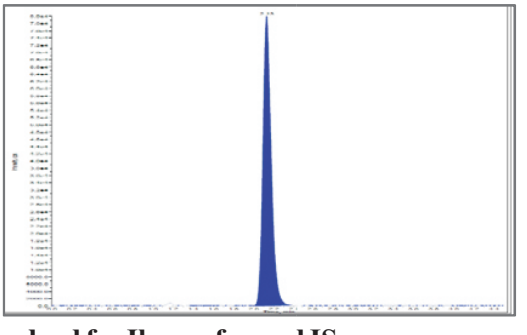


Representative Chromatogram of Standard Zero for Ibuprofen and IS:

Fig: STD ZERO (Ibuprofen) Fig: STD ZERO (Ibuprofen D3)

The chromatographic analysis revealed the following results for two compounds. Ibuprofen displayed a peak area of 1802 counts at a retention time of 2.154 minutes, whereas Ibuprofen D3 exhibited a significantly higher peak area of 415,174 counts, albeit with a slightly lower retention time of 2.131 minutes.



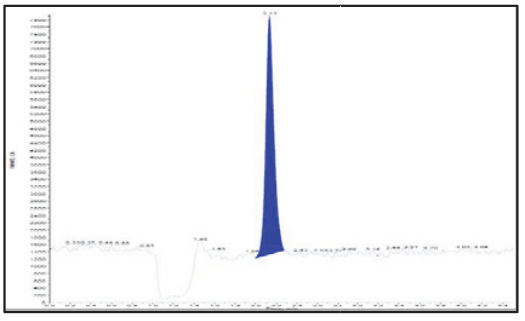


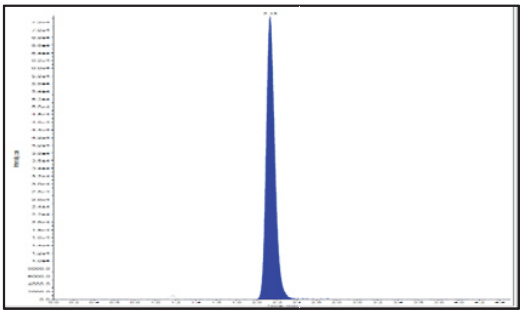
Representative Chromatogram of ULOQ Standard for Ibuprofen and IS:

Fig: STD ULOQ (40000.000 ng/mL) (Ibuprofen) Fig: STD ULOQ (40000.000 ng/mL) (IbuprofenD3)

The chromatographic analysis revealed that Ibuprofen exhibited a peak area of 7,527,087

counts at a retention time of 2.133 minutes. In comparison, Ibuprofen D3 showed a lower peak area of 467,298 counts, albeit with a slightly shorter retention time of 2.128 minutes.



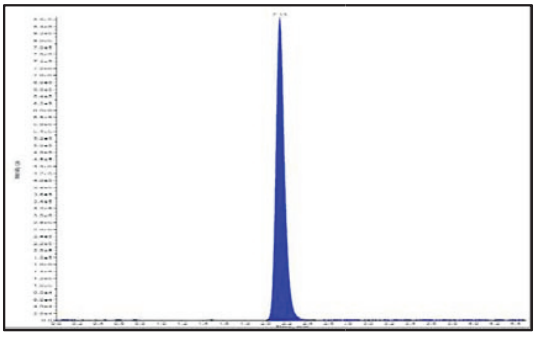


Representative Chromatogram of LLOQ Standard for Ibuprofen and IS:

Fig: STD LLOQ (200.000 ng/mL) (Ibuprofen) Fig: STD LLOQ (200.000 ng/mL) (Ibuprofen D3)

In the chromatographic analysis, Ibuprofen exhibited a peak area of 36,389 counts with a retention time of 2.132 minutes, while Ibuprofen D3 showed a substantially higher peak area of 429,114 counts at a slightly shorter retention time of 2.131 minutes.

Representative Chromatogram of HQC Samples for Ibuprofen and IS:



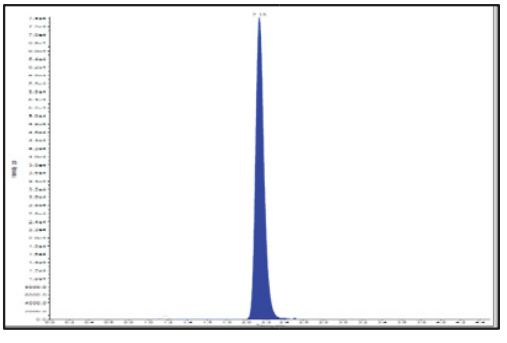
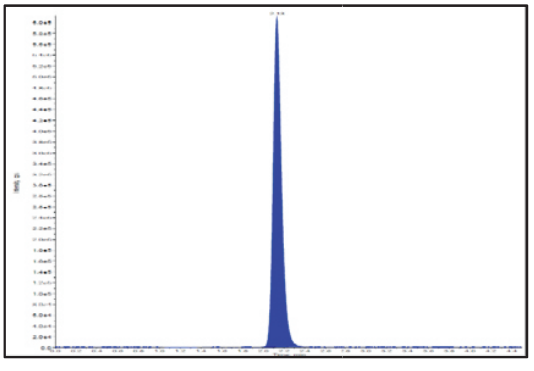


Fig: HQC (30000.000 ng/mL) (Ibuprofen) Fig: HQC (30000.000 ng/mL) (Ibuprofen D3)

During chromatographic analysis, Ibuprofen displayed a peak area of 5,032,443 counts with a retention time of 2.133 minutes, while Ibuprofen D3 exhibited a peak area of 442,641 counts at a slightly lower retention time of 2.130 minutes.

Representative Chromatogram of MQC1 Samples for Ibuprofen and IS:



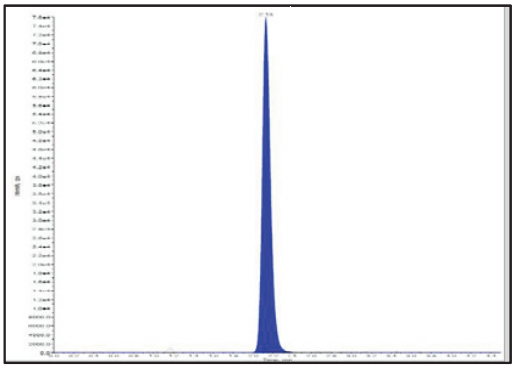
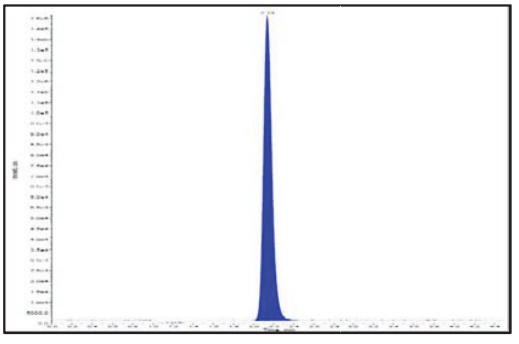
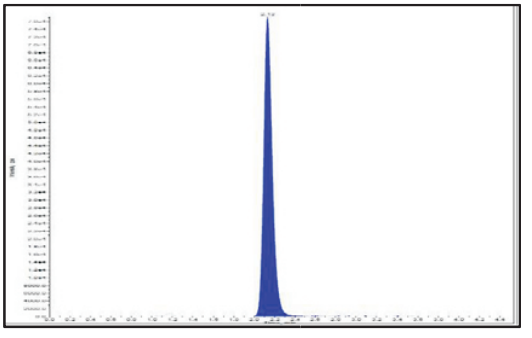


Fig: MQC1 (20000.000 ng/mL) (Ibuprofen) Fig: MQC1 (20000.000 ng/mL) (Ibuprofen D3)

In the chromatographic analysis, Ibuprofen yielded an area of 3,462,028 counts with a retention time of 2.131 minutes, whereas Ibuprofen D3 exhibited an area of 438,797 counts at a slightly earlier retention time of 2.127 minutes.

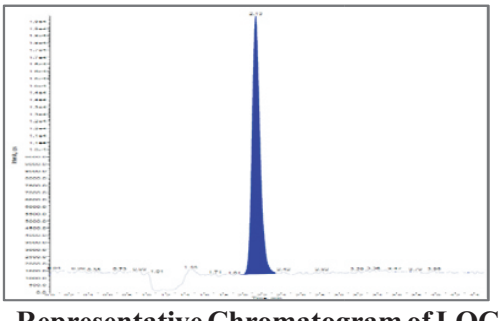


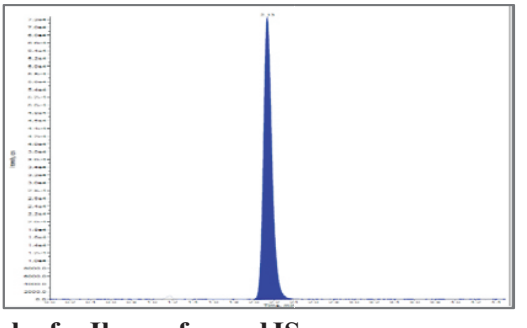


Representative Chromatogram of MQC2 Samples for Ibuprofen and IS:

Fig: MQC2 (5000.000 ng/mL) (Ibuprofen) Fig: MQC2 (5000.000 ng/mL) (Ibuprofen D3)

In the chromatographic analysis, Ibuprofen showed an area of 854,223 counts with a retention time of 2.135 minutes, while Ibuprofen D3 displayed an area of 449,741 counts at a retention time of 2.131 minutes.

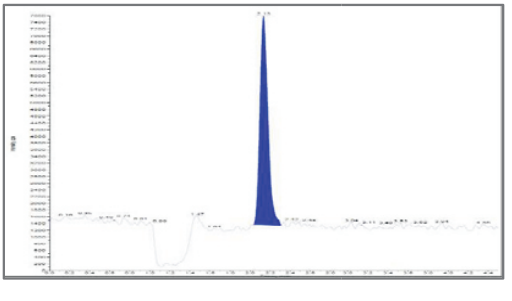


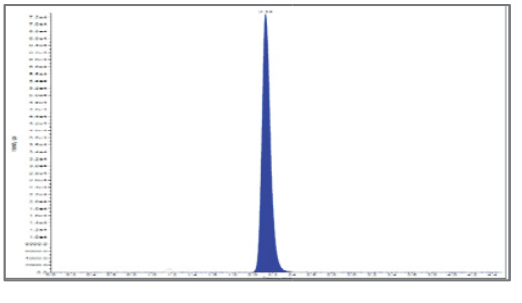


Representative Chromatogram of LQC Samples for Ibuprofen and IS

Fig: LQC (600.000 ng/mL) (Ibuprofen) Fig: LQC (600.000 ng/mL) (Ibuprofen D3)

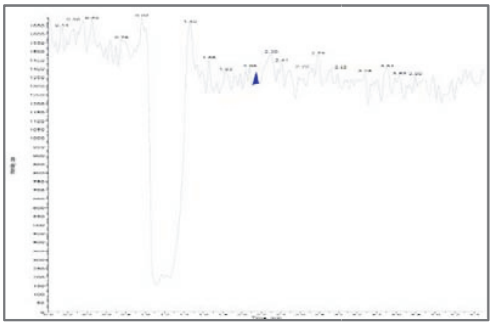
In the chromatographic analysis, the area counts and retention times for Ibuprofen and Ibuprofen D3 were recorded. Ibuprofen exhibited an area of 105317 counts with a retention time of 2.135 minutes, while Ibuprofen D3 showed a higher area count of 426042 with a slightly shorter retention time of 2.130 minutes.

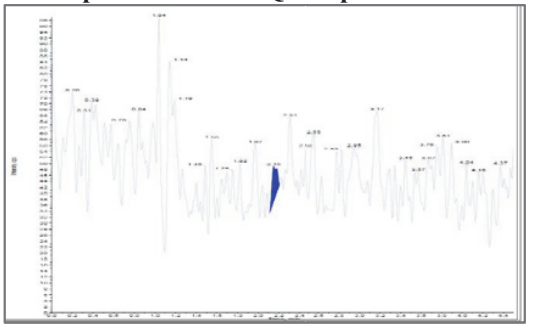




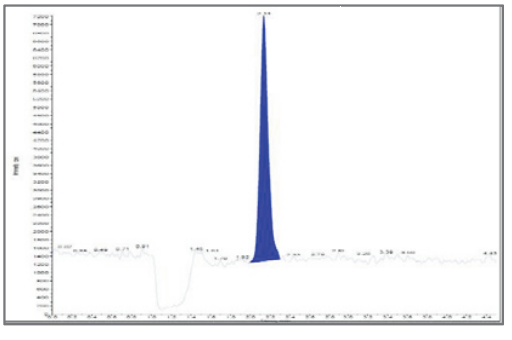
Representative Chromatogram of LLOQ QC Samples for Ibuprofen and IS:
Fig: LLOQ QC (200.000 ng/mL) (Ibuprofen) Fig: LLOQ QC (200.000 ng/mL) (Ibuprofen D3)
In the chromatographic analysis, Ibuprofen showed an area of 35,364 counts with a retention time of 2.135 minutes, while Ibuprofen D3 exhibited an area of 428,867 counts at a retention time of 2.130 minutes.

Representative Chromatogram Selectivity of Blank Samples and its LLOQ Samples for





Ibuprofen and IS:
Fig: Blank (SEL01) (Ibuprofen) Fig: Blank (SEL01) (Ibuprofen D3)
In the chromatographic analysis, Ibuprofen recorded an area of 146 counts with a retention time of 2.143 minutes, while Ibuprofen D3 exhibited an area of 41 counts at a retention time of 2.130 minutes.



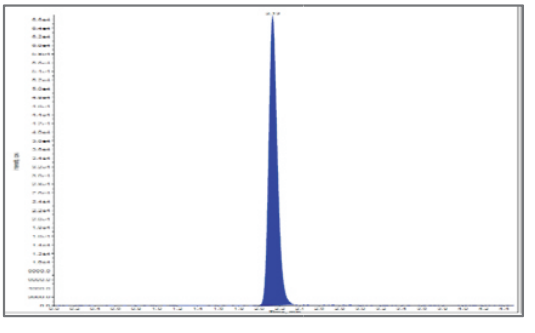


Fig: Selectivity LLOQ (Ibuprofen) Fig: Selectivity LLOQ (Ibuprofen D3)
In the chromatographic analysis, Ibuprofen displayed an area of 34,588 counts with a retention time of 2.136 minutes, while Ibuprofen D3 exhibited an area of 385,610 counts at a retention time of 2.131 minutes.

RESULT AND DISCUSSION

The selectivity of the method was evaluated by comparing chromatograms of blank plasma and spiked plasma. Both the analyte and internal standard exhibited retention times of 2.10 minutes, respectively, with no significant endogenous peaks observed that could interfere with their retention times. This assessment indicates that the method demonstrated good selectivity.

In further evaluation, 10 out of 10 human plasma lots passed selectivity testing, including 6 normal plasma, 2 haemolysed, and 2 lipemic plasma lots. The percentage interference at the retention time of the analyte in blank plasma concerning the response of the analyte in its Lower Limit of Quantification (LLOQ) was less than 1.06%, while the percentage interference at the retention time of the internal standard in blank plasma concerning its response in the LLOQ was less than 0.01%.

Additionally, the mean accuracy at the LLOQ was determined to be 96.96%, with a precision of 2.70%. These results further confirm the method's selectivity and suitability for analysis across various plasma samples. The matrix effect assessment revealed results that fell within the acceptance criteria. The ISTD normalized matrix demonstrated a coefficient of variation (CV) of 0.73% for the high-quality control (HQC) sample and 1.16% for the low-quality control (LQC) sample. These findings indicate minimal variability in the matrix effect across different sample concentrations, ensuring the reliability of the analytical method for quantitative analysis in the given matrix.

The assessment of linearity, precision, and accuracy involved plotting calibration curves with peak area ratio against analyte concentration, within a linearity range spanning from 200.000 ng/mL to 40000.000 ng/mL for Ibuprofen. Precision, characterized by the coefficient of variation (CV), was determined to be less than 3.26%, while the accuracy, represented by the mean of measured concentrations, ranged between 98.31% and 101.08%. Notably, all calibration curves exhibited a correlation coefficient (r2) greater than 0.9994, indicating a high degree of linearity across the concentration range.

Table No 2: Intra-batch and inter-batch precision and accuracy for Ibuprofen

QCID	Nominal conc. (ng/mL)	Intra-batch				Inter-batch			
		n	Mean conc. observed (ng/mL)[a]	% CV	% Accuracy	n	Mean conc. observed (ng/mL)[b]	% CV	% Accuracy
HQC	30000.000	6	28213.1052	1.02	94.04	30	28707.2193	2.07	95.69
MQC1	20000.000	6	19281.4317	0.51	96.41	30	19423.6517	1.10	97.12
MQC2	5000.000	6	4736.8020	1.22	94.74	30	4859.9026	2.47	97.20
LQC	600.000	6	573.6400	2.73	95.61	30	577.0443	2.04	96.17
LLOQQC	200.000	6	200.7810	2.35	100.39	30	201.0638	2.71	100.53

Precision and accuracy values for both intra-batch and inter-batch analyses are provided below:
Intra-batch precision and % Bias (Accuracy) for HQC, MQC1, MQC2, and LQC samples ranged from 0.51% to 2.73% and -5.96% to -0.65%, respectively.
Intra-batch precision and % Bias (Accuracy) for LLOQ QC samples were observed at 2.35% to 2.22% and 0.39% to 4.13%, respectively.

Inter-batch precision and % Bias (Accuracy) for HQC, MQC1, MQC2, and LQC samples were measured between 1.10% to 2.47% and -4.31% to -2.80%, respectively.

Inter-batch precision and % Bias (Accuracy) for LLOQ QC samples were determined to be 2.71% and 0.53%, respectively. These results indicate the method's robustness and reliability in providing accurate and precise measurements across different sample concentrations and batches.The recovery of the analyte and internal standard (ISTD) was evaluated across different quality control levels, including high quality control (HQC), medium quality control 1 (MQC1), and low quality control (LQC). For the analyte, the percentage recovery at HQC, MQC1, and LQC levels was determined to be 72.22%, 76.00%, and 83.67%, respectively. The overall coefficient of variation (% CV) for the analyte was calculated to be 7.55%, with an overall percentage recovery of 77.30%. Similarly, for the ISTD, the percentage recovery at HQC, MQC1, and LQC levels was found to be 74.89%, 79.58%, and 85.43%, respectively. The overall % CV for the ISTD was 6.61%, with an overall percentage recovery of 79.97%. These results indicate satisfactory recovery of both the analyte and ISTD across the different quality control levels, demonstrating the reliability and consistency of the analytical method.

The hemolyzed and lipemic effects on the accuracy and precision of the assay were assessed by determining the percentage mean accuracy and coefficient of variation (%CV) at the low quality

control (LQC) and high quality control (HQC) levels. For the hemolysis effect, the mean accuracy was found to be 96.75% at the LQC level and 93.25% at the HQC level, with %CV values of 1.88% and 0.78%, respectively. Regarding the lipemic effect, the mean accuracy was 97.42% at the LQC level and 92.20% at the HQC level, with %CV values of 1.24% and 0.31%, respectively. These results suggest that both hemolysis and lipemia have minimal impact on the accuracy and precision of the assay, as evidenced by the low %CV values and the mean accuracy close to 100%. The dilution integrity (DI) of the assay was evaluated for dilutions of 1/2 and 1/10 using screened human K2EDTA plasma as the dilution medium, ensuring it was free from analyte interference. The %CV values for the dilutions were found to be 0.43% for the 1/2 dilution and 0.58% for the 1/10 dilution. Additionally, the % mean accuracy was determined to be 98.37% for the 1/2 dilution and 100.46% for the 1/10 dilution. These results indicate that the assay maintains integrity and accuracy even after dilution, as evidenced by the low %CV values and the mean accuracy close to 100%.

The Ruggedness of the assay was evaluated under different conditions, including variations in analysts, columns, and equipment. When different analysts conducted the assay, precision, expressed as %CV, ranged from 0.90% to 1.97%, with % mean bias ranging from -3.37% to -0.94% for HQC, MQC2, MQC1, and LQC samples. For LLOQ QC, precision was 2.22% with 0.00% mean bias.

Similarly, when different columns were used, precision varied from 0.92% to 2.01%, with % mean bias ranging from -6.47% to -3.84% for HQC, MQC2, MQC1, and LQC samples. For LLOQ QC, precision was 1.82% with -1.72% mean bias.

Evaluation under different equipment conditions showed precision ranging from 0.77% to 2.34%, with % mean bias ranging from -3.82% to -0.65% for HQC, MQC2, MQC1, and LQC samples. For LLOQ QC, precision was 1.98% with -0.13% mean bias. These results demonstrate the robustness of the assay across variations in analysts, columns, and equipment, with consistent precision and minimal bias.

Stability testing was conducted for both the stock solution and working solution of Ibuprofen and Ibuprofen D3, as well as at the ULOQ (Upper Limit of Quantification) and LLOQ (Lower Limit of Quantification) levels, along with the stability of the ISTD dilution. The concentrations for stability testing in solution were 5.000 mg/mL for Ibuprofen and 1.000 ng/mL for Ibuprofen D3, while the levels for stability testing were set at 2000000.000 ng/mL for the ULOQ and 10000.000 ng/mL for the LLOQ of Ibuprofen. The ISTD dilution stability was evaluated at a concentration of 5000.000 ng/mL.

For the stock solution, stability testing was performed using Methanol as the solvent, while for the working solution, Diluent consisting of Methanol: Water (90:10 V/V) was used as the solvent. These stability studies aimed to assess the integrity and reliability of the solutions over time under specified storage conditions.

For short-term stability testing at ambient temperature, the solutions were monitored over durations of 22 and 20 hours. The results indicated high stability, with percentage mean stability ranging from 99.18% to 100.74%. The coefficient of variation (% CV) for the stability of the compounds ranged from 0.51% to 3.34%, demonstrating consistency and reliability.

Long-term stability testing was conducted at a temperature of 5±3ºC over a duration of 7 days. The solutions showed good stability over this period, with percentage mean stability ranging from 99.31% to 101.10%. The % CV for stability ranged from 1.22% to 2.88%, indicating consistent performance over the extended duration. These results suggest that both the stock and working solutions, as well as the ISTD dilution, maintain their integrity and reliability over both short and long-term storage periods under the specified conditions.

Stability testing of the matrix extract was conducted under various conditions to assess its robustness and reliability. During bench top stability (BT) testing, the matrix extract was kept at ambient temperature for 17 hours. At the high-quality control level (HQC) and low-quality control level (LQC), the percentage mean accuracy was found to be 97.05% and 94.43%, respectively, with percentage coefficients of variation (%CV) of 1.63% and 1.91%, respectively.

Table No 2: Stability of Ibuprofen under different conditions (n = 6)

Storagecondition	NominalConc.(ng/mL)	Calculated conc. (ng/mL)Mean,stability Samples+SD	% Change
BenchTopStability; 17hours			
HQC	30000.000	29114.7632±473.37037	-2.95
LQC	600.000	566.5952±10.79938	-5.57
Extract at Ambient Temperature; 20 hours			
HQC	30000.000	28611.2142±367.30237	-4.63
LQC	600.000	564.1322±7.13986	-5.98
Long Term Matrix Stability; 122 days, -20°C			
HQC	30000.000	32839.7153±1631.02859	9.47
LQC	600.000	624.8410±23.30277	4.14
Long Term Matrix Stability; 122 days, -70°C			
HQC	30000.000	31265.2728±942.41893	4.22
LQC	600.000	603.2312±24.85804	0.54
Freeze&ThawStability;VCycles,-20°C			
HQC	30000.000	28852.3523±266.10422	-3.83
LQC	600.000	568.7373±6.48672	-5.21
Freeze&ThawStability;VCycles,-70°C			
HQC	30000.000	28969.1815±363.52222	-3.44
LQC	600.000	562.4243±7.75066	-6.26

Next, the stability of the extract was evaluated in the refrigerator at 5±3ºC for 68 hours. The mean accuracy at HQC and LQC levels was 95.93% and 94.02%, respectively, with %CV values of 1.48% and 1.59%, respectively.

Additionally, stability testing of the extract in the autosampler at 10ºC was performed for 68 hours. The mean accuracy at HQC and LQC levels was 95.50% and 93.03%, respectively, with %CV values of 1.43% and 0.96%, respectively.

Moreover, the stability of the extract on the benchtop (SE) was examined for 20 hours at ambient temperature. At HQC and LQC levels, the mean accuracy was 95.37% and 94.02%, respectively, with %CV values of 1.28% and 1.27%, respectively.

Freeze-thaw stability (FT) testing was conducted with one cycle at -20±5ºC and -70 ±15ºC. At -20±5ºC, the mean accuracy was 96.18% with a %CV of 0.49%, while at -70 ±15ºC, it was 96.12% with a %CV of 0.90%. For the V cycle, at -20±5ºC, the mean accuracy was 96.17% with a %CV of 0.92%, and at -70 ±15ºC, it was 96.56% with a %CV of 1.25%.

SUMMARY

The analytical system underwent a comprehensive evaluation to ensure its reliability and effectiveness. System suitability criteria included % CV of analyte RT, ISTD RT, and area ratio below 1.48%. Performance criteria required peak area ≥76.3, with interference <1.31% (analyte) and <0.03% (ISTD) before ULOQ. Analyte carryover after ULOQ and interference at LLOQ were also assessed. Selectivity assessment showed minimal interference, with all plasma lots passing. Hemolysis and lipemia effects on accuracy were observed, with % Mean Accuracy ranging from 92.20% to 97.42%.Recovery assessment indicated % Recovery values of 72.22% to 83.67% for the analyte and 74.89% to 85.43% for ISTD across different QC levels. % CV for recovery ranged from 6.61% to 7.55%.Matrix effect evaluation revealed variability across QC levels, with % CV of 0.73% to 1.16%.Sensitivity assessment demonstrated precision (%CV) of 1.82% to 2.35% within runs and -0.45% to -1.72% between

runs. Precision Bias assessment showed % Bias ranging from -6.47% to 4.13% for different QC levels.Dilution Integrity testing showed a % CV of 0.43% (1/2 dilution) and 0.58% (1/10 dilution) with % Mean Accuracy of 98.37% and 100.46%, respectively.Ruggedness assessment across analysts, columns, and equipment showed %CV ranging from 0.77% to 2.34%, with % Mean Bias from -6.47% to -0.65%.Stability testing of solutions and extracts under various conditions demonstrated % Mean Stability and % Mean Accuracy ranging from 93.03% to 100.74%.Overall, these evaluations ensure the reliability and effectiveness of the analytical system, providing confidence in the accuracy and precision of the measurements across different conditions and QC levels.

CONCLUSION

Thus, the objective of the project is to develop and validate suitable methods for the estimation of unknown concentrations of drugs in plasma. It can be used for the quantitation of Ibuprofen from K2EDTA-based Human Plasma in Bioequivalence and Bioavailability Study.The method is considered specific, sensitive, linear, precise, accurate, and reproducible; for the extraction and analysis of Ibuprofen in K2EDTA human plasma samples within the investigated concentration range of 200.000 ng/mL to 40000.000 ng/mL using a 0.100mL processing volume. A highly accurate, sensitive, specific, and reproducible LC-MS/MS method for the quantification of Ibuprofen using commercially available IS From small Volumes of human plasma with a simple Protein Precipitation process was developed and validated. The developed and Validated analytical method was found to be simple, rapid, specific, reliable, accurate, rugged, and high- throughput method, and more precise and cost-effective than reported methods.

REFERENCES

Adams, C.,(2015). "Development and Validation of Bioanalytical Methods in Pharmaceutical Research: A Comprehensive Review." *J Pharm Sci, 45*(2), 150-175.

Adams, E.,(2015). Preparation and Characterization of Reagents and Solutions for High-Performance Liquid Chromatography Analysis. *J Chromatogr A*, 30(2), 100-110.

Amidon, G. L., Lennernäs, H., Shah, V. P., Crison, J. R. (1995). A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability. *Pharm Res*, 12(3), 413-420. doi:10.1023/A:1016212804288

Avgerinos, A., Noormohammadi, A., Hutt, A. J. (1991). Disposition of ibuprofen enantiomers following the oral administration of a novel controlled release formulation to healthy volunteers. *Int J Pharm*, 68(1–3), 97–103.

Bergström, C. A. S., Andersson, S. B. E., Fagerberg, J. H., *et al*. (2011). Prediction of Human Pharmacokinetics - Comparative Assessment of Predictive Performance of Different Strategies in Early Drug Development. Pharm Res, 28(7), 1655-1667. doi:10.1007/s11095-011-0421-2

Bonato, P. S., Del Lama, M. P. F. M., de Carvalho, R. (2003). Enantioselective determination of ibuprofen in plasma by high-performance liquid chromatography–electrospray mass spectrometry. *J Chromatogr B*, 796(2), 413–420.

Brown, E.,(2015). Preparation and Characterization of Reagents and Solutions for High-Performance Liquid Chromatography Analysis. *J Chromatogr A*, 30(2), 100-110.

Brown, E., (2018). "Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in

Pharmaceutical Analysis: Current Trends and Future Perspectives." *Anal Chem Rev*, 30(3), 250-275.

Brown, R., White, S. (2016). Validation of Working Standards in Quantitative Analysis. *Anal Methods,* 25(4), 112-118.

Canaparo, R., Muntoni, E., Zara, G. P., (2000). Determination of ibuprofen in human plasma by high-performance liquid chromatography: validation and application in pharmacokinetic study. *Biomed Chromatogr*, 14(4), 219–226.

Chen, X.,(2017). Preparation and Spiking of Calibration Curve Standards for Liquid Chromatography-Mass Spectrometry Analysis. *J Anal Chem*, 45(2), 100-110.

Chen, X.,(2017). Preparation and Spiking of Calibration Curve Standards for Liquid Chromatography-Mass Spectrometry Analysis. *J Anal Chem*, 45(2), 100-110.

Chen, Y., Li, Z. (2017). Evaluation of Hemolysis and Lipemic Effect in Bioanalytical Method Validation: Experimental Design and Acceptance Criteria. *Anal Chem Res*, 28(3), 150-165.

Davit, B. M., Nwakama, P. E., Buehler, G. J., (2009). Comparing generic and innovator drugs: a review of 12 years of bioequivalence data from the United States Food and Drug Administration. Ann Pharmacother, 43(10), 1583-1597. doi:10.1345/aph.1M141

De Oliveira, A. R. M., Cesarino, E. J., Bonato, P. S. (2005). Solid-phase microextraction and chiral HPLC analysis of ibuprofen in urine. *J Chromatogr B,* 818(2), 285–291.

Dillen, L., Annaert, P., Augustijns, P. (2006). Impact of absorption and pre-systemic elimination on oral drug bioavailability: a simulation approach. *Eur J Pharm Sci*, 29(5), 438-448. doi:10.1016/j.ejps.2006.07.011

European Medicines Agency. (2011). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**.

Garcia, A.,(2017). Development and Validation of Internal Standard Stock Solution for Liquid Chromatography-Mass Spectrometry Analysis. *Anal ChemLett*, 25(2), 80-90.

Garcia, A.(2017). Development and Validation of Internal Standard Stock Solution for Liquid Chromatography-Mass Spectrometry Analysis. *Anal ChemLett*, 25(2), 80-90.

Garcia, L., Martinez, E. (2019). Assessment of Matrix Effects in Bioanalytical Methods: A Comprehensive Approach. *J Anal Chem,* 42(6), 300-315.

Geisslinger, G., Dietzel, K., Loew, D.,(1989). High-performance liquid chromatographic determination of ibuprofen, its metabolites and enantiomers in biological fluids. *J Chromatogr*, 491, 139–149.

Ghosh, D., Chen, A. K., Guo, T., (2017). A Streamlined Process to Identify and Quantify Pharmaceuticals in Biota. Environ SciTechnol, 51(21), 12587-12595. doi:10.1021/acs.est.7b02118

Guidance for Industry: Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2018.

Johnson, A., Smith, B. (2019). Assessing Selectivity in Bioanalytical Methods: A Comprehensive Approach. *J Anal Chem*, 40(5), 220-235.

Johnson, C., Martinez, E. (2017). Meticulous Optimization of Sample Preparation Techniques for Robust Analyte Recovery: A Comprehensive Review. Anal Chem Res, 35(3), 180-195.

Johnson, L., Williams, M. (2018). Inter-batch and Intra-batch Precision and Accuracy

Evaluation in Bioanalytical Method Validation: Methodology and Criteria. *Anal Chem Res,* 25(4), 200-215.

Johnson, R.,(2018). Mass Spectrometry Parameters for the Analysis of Ibuprofen and Internal Standard Using LC-MS/MS. *Anal Chem Res*, 25(2), 120-135.

Johnson, R., (2018). Mass Spectrometry Parameters for the Analysis of Ibuprofen and Internal Standard Using LC-MS/MS. *Anal Chem Res,* 25(2), 120-135.

Kim, Y., (2018). Development and Validation of Quality Control Samples for Liquid Chromatography-Mass Spectrometry Analysis. *J Pharm Sci*, 30(4), 200-210.

Kim, Y., (2018). Development and Validation of Quality Control Samples for Liquid Chromatography-Mass Spectrometry Analysis. *J Pharm Sci*, 30(4), 200-210.

Lee, J. W., Devanarayan, V., Barrett, Y. C., (2006). Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res*, 23(2), 312–328.

Lee, S., (2016). Preparation and Evaluation of Ibuprofen Stock Solution for Pharmaceutical Analysis. *J Pharm Sci*, 35(1), 50-60.

Lee, S., (2016). Preparation and Evaluation of Ibuprofen Stock Solution for Pharmaceutical Analysis. *J Pharm Sci,* 35(1), 50-60.

Lemko, C. H., Caille, G., Foster, R. T. (1993). Stereospecific high-performance liquid chromatographic assay of ibuprofen: improved sensitivity and sample processing efficiency. *J Chromatogr*, 619(2), 330–335.

Li, W., Zhu, Y., Yuan, H., Yu, X. (2019). Recent advances in solid-phase microextraction for bioanalysis. *Trends Anal Chem*, 112, 174-186. doi:10.1016/j.trac.2018.12.010

Martinez, E., Brown, C. (2018). Understanding Matrix Effects in Bioanalytical Methods: Implications for Analyte Detection. Anal Sci Rev, 28(3),

Matuszewski, B. K., Constanzer, M. L., Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem, 75(13), 3019-3030. doi:10.1021/ac020361s

Matuszewski, B. K., Constanzer, M. L., Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem, 75(13), 3019-3030. doi:10.1021/ac020361s

Matuszewski, B. K., Constanzer, M. L., Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem*, 75(13), 3019–3030.

Mehvar, R., Jamali, F., Pasutto, F. M. (1988). Liquid-chromatographic assay of ibuprofen enantiomers in plasma. *Clin Chem*, 34(3), 493–496.

Naidong, W., Lee, J. W. (1994). Development and validation of a liquid chromatographic method for the quantitation of ibuprofen enantiomers in human plasma. *J Pharm Biomed Anal*, 12(4), 551–556.

Niessen, W. M. A. (2007). Hyphenated techniques in liquid chromatography-mass spectrometry for the pharmaceutical analysis. Trends Anal Chem, 26(5), 469-484. doi:10.1016/j.trac.2007.02.009

Patel, R. (2019). Preparation and Dilution Integrity Testing of Internal Standard Dilution Solution for Quantitative Analysis. *J Pharm Anal*, 18(4), 200-210.

Patel, R. (2019). Preparation and Dilution Integrity Testing of Internal Standard Dilution Solution for Quantitative Analysis. *J Pharm Anal,* 18(4), 200-210.

Patel, S.,(2016). Validation of Bioanalytical Procedures: Importance of Linearity, Accuracy, and Precision. *Anal Sci Rev*, 30(2), 120-135.

Patel, S., Johnson, A. (2016). Validation of Bioanalytical Procedures: Importance of Linearity, Accuracy, and Precision. *Anal Sci Rev*, 30(2), 120-135.

Pettersson, K. J., Olsson, A. (1991). Liquid chromatographic determination of the enantiomers of ibuprofen in plasma using a chiral AGP column. *J Chromatogr*, 563(2), 414–418.

Ramanathan, S., Mathias, A. A., German, P., Kearney, B. P. (2011). Clinical pharmacokinetic and pharmacodynamic profile of the HIV integrase inhibitor elvitegravir. ClinPharmacokinet, 50(4), 229-244. doi:10.2165/11589400-000000000-00000

Shah, V. P., Midha, K. K., Dighe, S., (1991). Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug MetabPharmacokinet, 16(4), 249-255. doi:10.1007/BF03190050

Shah, V. P., Midha, K. K., Dighe, S., (2000). Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. *Pharm Res*, 17(12), 1551–1557.

Shah, V. P., Midha, K. K., Dighe, S., (2000). Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. Pharm Res, 17(12), 1551–1557.

Sharma, A., (2019). Bioanalytical Method Validation for Ibuprofen Quantification Using High-Performance Liquid Chromatography Coupled with Mass Spectrometry. *Anal Chem Res,* 22(3), 150-160.

Sharma, A., (2019). Bioanalytical Method Validation for Ibuprofen Quantification Using High-Performance Liquid Chromatography Coupled with Mass Spectrometry. *Anal Chem Res,* 22(3), 150-160.

Sharma, P., Guttikar, S., Solanki, G., Patel, D. P., Shrivastav, P. S. (2012). Determination of (S)-(+)- and (R)-(-)-ibuprofen enantiomers in human plasma after chiral precolumnderivatization by reversed-phase LC–ESI-MS/MS. *Bioanalysis,* 4(24), 2909–2927.

Smith, J., Brown, K. (2017). Precision and Accuracy Assessment in Bioanalytical Method Validation: A Comprehensive Review. *J Pharm Anal*, 15(3), 150-165.

Smith, J., (2015). Optimization of Chromatographic Parameters for the Analysis of Ibuprofen Using High-Performance Liquid Chromatography. *J Chromatogr A*, 150(3), 200-215.

Smith, J., (2015). Optimization of Chromatographic Parameters for the Analysis of Ibuprofen Using High-Performance Liquid Chromatography. *J Chromatogr A*, 150(3), 200-215.

Smith, J., (2018). Preparation and Stability Assessment of Auto-Sampler Rinsing Solution for Liquid Chromatography Analysis. *J Chromatogr Sci*, 40(3), 150-160.

Smith, J., Johnson, A. (2015). Characterization of Working Standards for Analytical Processes. *J Anal Chem*, 30(2), 45-52.

Szeitz, A., Edginton, A. N., Peng, H. T., Cheung, B., Riggs, K. W. (2010). A validated enantioselective assay for the determination of ibuprofen in human plasma using ultra performance liquid chromatography with tandem mass spectrometry. *Am J Anal Chem,* 2(1), 47–58.

Tan, S. C., Jackson, S. H. D., Swift, C. G., Hutt, A. J. (1997). Enantiospecific analysis of ibuprofen by high performance liquid chromatography: determination of free and total drug enantiomer concentrations in serum and urine. *Chromatographia,* 46(1–2), 23–32.

U.S. Department of Health and Human Services, Food and Drug Administration, Center for

Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). (2018). Guidance for Industry: Bioanalytical Method Validation. Accessed January 10, 2022. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry

Viswanathan, C. T., Bansal, S., Booth, B., (2007). Workshop/conference report--Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. AAPS J, 9(1), E30-E42. doi:10.1208/aapsj0901004

Walker, J. S., Knihinicki, R. D., Deideman, P., Day, R. O. (1993). Pharmacokinetics of ibuprofen enantiomers in plasma and suction blister fluid in healthy volunteers. *J Pharm Sci*, 82(8), 787–790.

Wright, M. R., Sattari, S., Brocks, D. R., Jamali, F. (1992). Improved high-performance liquid chromatographic assay method for the enantiomers of ibuprofen. *J Chromatogr*, 583(2), 259–265.

Zhang, H., Wang, L. (2019). Methodology for Assessing Recovery in Bioanalytical Method Validation: A Review. *J Anal Chem*, 30(2), 100-115.

Adv. Pharmacol. Toxicol. Vol. 25 (2) 2024, 51-68
ISSN - 0973 - 2381 SILVER JUBILEE VOLUME

CONTRACEPTIVE PROPERTIES OF SOME INDIAN MEDICINAL PLANTS: AN OVERVIEW

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All people can easily learn to pronounce medicinal plants, which are helpful in treating a variety of illnesses for which allopathic medicine is ineffective. These days, increasing human fertility while using birth control is one of the main issues. This audit presents a brief overview of updated data on medicinal plants that are used to counteract the richness movement and can be rationally demonstrated. This review actually shows that it's time to expand on the experimental research being done to find medicinal plants, plant extracts and their active ingredients should also have their mechanisms studied. This review establishes a solid framework for further research into the effectiveness of plants that women currently use as traditional antifertility remedies.

INTRODUCTION

A global hunt is underway for antifertility drugs to address the issue of the "Population Explosion." India's population has surpassed 140 crores people and is growing at an alarming rate. As a result, people from all areas of life are increasingly concerned about controlling fertility. Plants are becoming preferred over synthetic contraceptives in recent years due to their accessibility, affordability, and lack of negative side effects.

More individuals move to India each year than to any other country in the globe. In India, there are roughly 51 births every minute.

Poverty, illiteracy, high fertility, a sharp fall in death rates, and immigration from Bangladesh and Nepal are the main causes of India's fast expanding population. India, concerned about the rate of population growth, took action to slow it down early on. In 1952, India became the first nation in the world to implement a population policy. The country's fertility rate decreased dramatically as a result of the family planning initiative, which had several notable results. Although the ultimate aim was not met, the initiatives yielded some positive benefits, and India's population has expanded nearly threefold since gaining independence from Britain in 1947. China's 'One Child Policy', implemented in 1978, has prevented population growth in China, but India has failed to restrict population growth despite nearly all of its aims.

According to the Census of India (2011), the strategy is said to have avoided between

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250 and 300 million births between 1978 and 2000 and 400 million births between 1979 and 2010.

Pregnancy prevention is one way to control population increase. As a result, research on contraception in both humans and animals has been crucial. Surgical techniques including hysterectomy, ovariohysterectomy, ovariectomy, and tubal ligation, as well as nonsurgical techniques like hormonal applications, are commonly used forms of contraception today. These techniques to limit the population expansion of humans and animals may be expensive or difficult to implement.

As a result, attempts are being made globally to produce substitute contraceptive techniques that are safe, effective, reversible, affordable, and, most importantly, accepted by all.

The IUD, oral contraceptive tablets, condoms, male and female sterilisation, and the IUD are the five forms of contraception that the public sector offers. Health professionals remove, simply do not introduce, or assign low priority to methods that are viewed as less effective (e.g., pessaries, spermicides, and diaphragms) or contentious (e.g., injectables, implants) from the public programme (India and Family Planning: An Overview). Because oral contraceptive is reversible, convenient, and effective, it is one of the most widely used family planning methods. Antifertility drugs are still being used to address the issue of population explosion, which could have a negative influence on a family's finances and health as well as society at large, particularly in emerging nations with rapid population expansion like Ethiopia. (Shaha and Jhadeb, 2018)

Hormonal contraceptive use is already prohibited in many countries due to its carcinogenic effects. It is imperative that safe and effective herbal contraceptives be discovered immediately. Even the most primitive members of ancient societies used herbal contraceptives to manage their fertility and avoid getting pregnant. Despite the fact that conventional medicine has identified some significant contraceptives (anti-fertility drugs) for women, their use and acceptance among women are limited because of certain unfavourable side effects.

Obesity, cholelithiasis, stomach issues, cervical and breast cancer, asthma, and venous thromboembolism are among the frequent side effects.

Although there are numerous hormonal preparations available for this purpose, side effects are a possibility. Therefore, it is suggested to look for a suitable product made from locally grown medicinal plants that could successfully be used in place of the "Pill."

The World Health Organisation recommended using locally accessible, potent plants as medication substitutes. They developed a population control programme that incorporates research on conventional medical procedures because the population explosion is a major contributor to pollution and poverty in developing nations. Controlling fertility is a matter of national and international public health importance. The rate of unwanted pregnancies resulting from current contraceptive methods is unacceptable. 94% of sexually active couples

who report using some form of contraception experience 50% of unintended pregnancies at conception. India has always been concerned about an increasing population, so medicinal plants have been examined for potential contraceptive benefits and anti-fertility effects. Increased use of contraceptive methods directly correlates with population growth, women's empowerment, health, and development.

In addition, it acts as a stand-in for access to reproductive health services, which are necessary to fulfil many of the MDGs, particularly those pertaining to gender equality, HIV/AIDS, maternal health, and child mortality. Men who are willing to participate in family planning have fewer options for effective, reversible, non-irritating, and highly expected forms of contraception. Female contraceptive methods have always been given priority. According to a WHO survey, 61% of men who use contraception do so because they want to share responsibility or because they are motivated by issues with their female partner, including 35% who have had a contraceptive failure.

Plants as a source of fertility control agent

Since humans have always used plants and their products as sources of medications and therapeutic agents—even though synthetic drugs have become increasingly prevalent in contemporary medical systems—the development of new fertility-regulating drugs from medicinal plants is an appealing idea. In recent times, plant-based products have gained popularity over synthetic drugs. The main reasons for this are their low toxicity and the fact that these medicines have been used for a long time in traditional medical systems like Ayurveda. Numerous initiatives have been used to promote family planning. (Goswami *et al*., 2020). Since around 50 years ago, several plant species have been examined for their ability to regulate fertility; as a result, national and international agencies have strengthened these species (Purohit and Daradka, 1999). A thorough understanding of ethanobotany can be very helpful in solving basic problems related to both the discovery and application of medicinal plants, as well as the nomenclature identification of crude drug extract.

When given orally to female albino rats, the ethanol extract of the flowers of the Striga senegalensis plant (family: Scrophulariaceae) has been shown to have antiimplantation activity. 120 mg/kg of body weight is the dosage. In all of the treated animals, the implantation of fertilised eggs was avoided. (Choudhury *et al.*, 1998).

The antifertility effect of Ricinus communis seed extract was studied by Makonnen *et al.* in 1999. It was discovered that the seed extract has abortifacient and anti-implantation properties. Furthermore, it was noted that the guinea pigs' oestrus cycle was extended by the seed extract. Significantly longer was the dioestrus phase as well. Without having a major impact on the ovaries, the seed extract also decreased the weight of the uterus.

When mated female rats were given an aqueous extract of Cassia fistula seeds orally at doses of 100 and 200 mg/kg body weight during the first five days of pregnancy, Yadav et al. (1999) observed that this prevented 57.14% and 71.43% of pregnancies, respectively, while at doses

of 500 mg, 100% pregnancy inhibition was observed.

According to Murugan *et al*. (2000), in pregnant female rats, Alangium salviifolium primarily produces abortificient activity and less estrogenic activity.

In folk medicine, Coleus barbatus extracts have been used to prevent pregnancy. Before embryo implantation, 880 mg/kg of C. barbatus extract per day resulted in anti-implantation and delayed foetal development. Additionally, the treatment during this time may impede the development of the embryo. (Fernanda *et al*., 2000).

The impact of Inula viscosa leaf on female rats was investigated by Nisreen et al. in 2001. It was demonstrated that the aqueous extract, which was given intraperitoneally on days 1-6 of pregnancy, completely prevented foetal implantation and significantly decreased blood progesterone levels and the number of corpora lutea. On days 13–15 of gestation, however, the administration showed signs of midterm abortion. The traditional reputation of this plant as being abortificient was supported by the study's findings.

Anti-implantation and pregnancy-interrupting effects were examined in female albino rats using petroleum ether, chloroform, ethanol, and distilled water extracts of the aerial parts of the plant Rivea hypocrateriformis (Convolvulaceae). When it came to significantly inhibiting implantation and interrupting early pregnancy, the ethanol extract was found to be the most effective of these. The ethanol extract's antifertility effect was reversible upon exogenous hydroxy progesterone administration. (Shivalingappa *et al*., 2001).

The effects of an ethanolic extract of Calotropis procera roots on female albino rats were investigated by Kamath and Rana (2002). At a dose of 250 mg/kg, there was a significant anti-implantation (inhibition 100%) and uterotropic activity; no antiestrogenic activity was found.

When adult female albino rats were given an ethanolic extract of Rivea hypocrateriformis at doses of 200 mg and 400 mg, Shivalingappa et al. (2002) observed that the rats developed an irregular estrous cycle, with shorter oestrus and metestrus and a longer proestrous phase. During the trial period, treated rats showed a significant increase in atretic follicles and a significant decrease in Graffian follicles and corpora lutea, indicating the extract's antiovulatory effect.

Momordica charantia seeds were extracted using petroleum ether, benzene, chloroform, and alcohol and given orally to adult female albino rats at a dose level of 25 mg/100g body weight for 30 days. The results showed increased uterine weight and prolonged duration of the oestrus and metestrus, which may have been caused by the direct oestrogen effect of the plants extracts (Sharanabasappa *et al*. 2002). Compared to other extracts, the benzene extract of M. charantia seeds was more successful in bringing about these modifications.

The ethanolic extract of Ferula hormonis has been studied by Homady *et al*. (2002) in female mice for its antifertility, anti-implantation, and ovarian alternations properties. Female mice's fertility was significantly reduced after an intragastric injection of this extract (3 mg/kg

p
per day) for six weeks. In addition, fewer females were mated, fewer implantations were made overall, and fewer foetuses survived as a result of it. Ovarian atrophy and a corresponding increase in connective tissue were also linked to these modifications. Most ovarian follicles have follicular atresia, and the ova displayed degeneration.

The effects of Mimosa pudica root powder on the oestrous cycle and ovulation in female albino rats (Rattus norvegicus) in cycle have been documented (Valsala and Karpagaganapathy 2002). When given intragastrically, Mimosa pudica root powder changed the female Rattus norvegicus's oestrous cycle pattern. All rats lacked nucleated and cornified cells. Leucocytes exclusively, similar to dioestrus were present in the smear and remained for two weeks. Rats treated with the root powder showed a significant decrease in the number of normal ova and a significant increase in the number of degenerated ova when compared to the control group.

On the first seven days of pregnancy, oral administration of the powdered root of Derris brevipes variety coriacea and its ethanolic extract were administered to female rats who had been proven to be fertile. The dosages were 200 and 600 mg/kg body weight, respectively. There was 50% anti-implantation activity in both doses of the same plant's root powder. Over and above its anti-implantation activity, the ethanolic extract exhibits a greater abortifacient type effect. (Badami *et al*., 2003).

According to Gupta *et al*. (2003), the methanolic extract of the seeds of Chorchorus olitorius Linn. and Cuscuta reflexa stem stopped the adult female mouse's regular oestrus cycle and dramatically reduced the weight of the uterus and ovaries because ovarian steroidogenesis was reduced.

In 2004 Ghosh and Bhattacharya assessed the initial anti-implantation efficacy of a pure principle isolated from petroleum-ether (PE) and ethyl acetate (EAc) extracts, as well as a crude alcoholic extract of Thespesia populnea seeds in female albino rats. Extracts of PE and EAc showed notable anti-implantation activity of 60% and 48.6%, respectively.

Trigonella foenum-graecum seeds were studied in female rats by Bhinda et al. (2005). Female rats were primarily in either the metestrus or dioestrus stage, according to the vaginal smear that was checked every day of the treatment. After taking fenugreek, the fertility test came back completely negative. Based on available data, it appears that T. foenum-graecum seeds have antiestrogenic and antifertility effects on female rats.

The methanolic extract of Rumex steudelii's root has an antifertility effect, as demonstrated by Gebrie *et al*. (2005).Litters were substantially fewer in number. Dose-dependent outcomes were observed. The ovaries' and uterus's wet weights considerably decreased. For a set amount of time, the contraceptive effect was noticeable.

The number of normal follicles in rats was significantly reduced by the polar fraction and nonpolar fraction of the seed extracts of Azadirachta indica and Melia azedarach Roop et al 2005). The maximum reduction was observed with 6 mg of the polar fraction of Azadirachta extract. This is in line with its anticonceptional agent use in folk medicine.

In vitro and in vivo studies were conducted on the anti-fertility impact of Leonotis ocymifolia leaf and root extracts in both aqueous and ethanolic forms. The ethanol leaf extract had anti-fertility and anti-implantation properties of 37% and 20% respectively. The findings of this investigation imply that the plant's leaves and roots may have hormonal characteristics that can affect the rats' ability to reproduce. (Tafesse *et al*., 2005).

The anti steroidogenic activity of Ammania baccifera (L.) whole plant (EEAB) was assessed by Dhanapal et al. (2005) in mature female mice ovaries using 90% ethanol extract of the plant. The two main enzymes involved in ovarian steroidogenesis, Δ5-3β-hydroxy steroid dehydrogenase (Δ5-3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PD), both showed significant inhibition from the extract. The entire plant of Ammania baccifera (L.) was extracted using ethanol and the study's findings suggested that this extract has anti steroidogenic properties.

Thakur *et al*. (2005) examined the detrimental impacts on different reproductive parameters in female rats of native Kamala (Mallotus philippinensis) seed ethanol extract. The development of follicles, the quantity and quality of ovulated eggs, the formation of corpora lutea the oestrous cycle, and the initiation and maintenance of pregnancy in treated rats may have been impacted by treatment with a higher dose of seed extract (100 mg/kg bwt.), which significantly decreased serum levels of FSH, LH, and estradiol.

When Nivsarkar *et al*. (2005) examined the impact of a water extract of Hibiscus rosa-sinensis leaves on female mice undergoing pregnancy, they discovered that the extract had anti-implantation activity. Additionally, the extract demonstrated antiestrogenic properties. Subsequent research revealed that Hibiscus rosas-inensis Linn. roots have a potent anti-implantation and uterotrophic effect in their ethanolic extract. This anticontraceptive effect may be caused by the extract's estrogenic properties. (Vasudeva *et al*., 2007).

The effect of mineralo-herbal preparation was investigated in Sprague-Dawley rats by Srivastava *et al*. (2005). The study revealed a significant decrease in the number of implantations in the female rats, no apparent negative effect of the treatment on early post-implantation event, and normal appearance of all implantations in both groups with no sign of resorption.

At doses of 300 and 600 mg/kg body weight orally, female albino rats showed abortificient activity when exposed to petroleum ether, chloroform, ethanol and distilled water extract of the fruit of the plant Balanites roxburghii (Padamshali *et al*. 2006).

Yadav and Jain (2006) investigated the impact of an oral petroleum ether extract of the rhizome of Curcuma longa on female rats, and found that the extract inhibited fetal implantation.

One common native herb in India is Achyranthes aspera Linn. (Amaranthaceae). As an abortifacient, it has been used traditionally. 200 mg/kg body weight of the ethanol extract taken orally demonstrated anti-implantation activity. (Vasudeva and Sharma, 2006).

When Lakshmi *et al*. (2006) examined the effects of powdered fruits of Piper longum

and its various fractions on female rats, they discovered that the hexane fraction of the crude extract and its various fractions had an antifertility effect.

The potential antifertility effect of Asparagus africanus leaves and roots was investigated in rats using ethanol and aqueous extracts (Tafesse *et al*. 2006). The roots and leaves ethanol and aqueous extracts both displayed anti-implantation properties.

The effects of an aqueous extract of Erythrina falcata on mouse pregnancy were studied by Orihuela and Ishiyama (2006). When consumed by mice in the early stages of pregnancy the aqueous extract of E. falcata disrupts the development of the pre implantation embryo and its implantation. These findings offer the first empirical proof of E. falcata aqueous extract contraceptive effects.

The antifertility effect of dried Woodfordia fruticosa Kurz flowers was studied by Khushalani et al. (2006) in female albino rats. According to the results the alcoholic extract exhibited strong abortifacient activity while the hydroalcoholic and aqueous extracts displayed only moderate activity in comparison to the control.

The effects of Anethum graveolens L. (dill) extracts on the female reproductive system were investigated by Monsefi *et al*. (2006) in 6 groups of fifty-four wistar female rats with regular estrous cycles. For ten days, the experimental groups were given a combination of 0.5 g/kg and 5 g/kg of ethanol extract and 0.045 g/kg and 0.45 g/kg of aqueous extract. The length of the estrous cycle, the diestrus phase, and the progesterone concentration all significantly increased in response to treatment with a high dose of the extract.

The ethyl acetate soluble fraction of the ethanolic extract of Aristolochia bracteolate was investigated by Nataraj *et al*. (2007) in female rats, and it was discovered that the plant extract had anti-implantation and abortificient properties both pre- and post-coital.

The number of implantation sites, number of neonates, and weight of newborns were all significantly reduced when Physalis alkekengi extract was administered during days 1–5 of pregnancy, as demonstrated by Azadeh *et al*. (2007).

Ravichandran et al. (2007) demonstrated that in immature ovariectomized rats Ailanthus excelsa possesses a potent anti-implantation and abortificient property. Significant antiestrogenic activity results from the simultaneous administration of extract and ethinyl estradiol.

The impact of Calotropis procera roots on the biochemistry of the reproductive organs of rats that their ovaries removed was investigated by Ahirwar *et al*. in 2007. They came to the conclusion that the strong estrogenic nature of the alcoholic extract of C. procera modifies the biochemical environment of the reproductive tract, changing the normal state of reproduction in the female rat reproductive tract and producing a notable antifertility effect.

In Assam, a province in northeastern India, a particular ethnic group of women uses the powdered dry root of Polygonum hydropiper extensively for fertility control. This practice is ingrained in the folk women of Assam. In their 2007 study. Hazarika and Sarma examined the antifertility properties of a crude methanolic extract of this plant's root in female

albino rats. The findings show that Polygonum hydropiper roots contain one or more steroidal or estrogenic compounds that may have an impact on rat female reproduction.

It is known that Ricinus communis seeds have long been used as an oral contraceptive. Sani and Sule (2007) have investigated methanol extracts from plant seeds. After the mice were given a subcutaneous dose of 200 mg/kg body weight once just prior to mating, the extracts caused anti-implantation activities in white albino mice.

When given to colony-bred adult Sprague-Dawley rats during the preimplantation and/or peri-implantation phases, the pregnancy interceptive effect of Calotropis gigantea Linn. roots was evaluated by Srivastava *et al*. (2007). Rats treated with a single oral dose of 100 mg/kg of the ethanolic extract of C. gigantea Linn. roots on Day 1 post-coitum showed 100% pregnancy interceptive activity.

They made the suggestion that this plant's products could be developed as human-use contraceptives. In a 2007 study, Ganguly *et al*. examined the antifertility effects of Cissampelos pareira (Meenakshi) Linn. leaf extract in female albino mice.

Keshri *et al*. (2007) examined the ability of Dysoxylum binectariferum Hook. f. stem bark, given orally to adult female Sprague Dawley rats, to prevent pregnancy during the pre- and peri-implantation stages as well as right after implantation. Rohitukine, an alkaloid found in chloroform extract, was partially (45%) effective in preventing pregnancy when given at a dose of 10 mg/kg on Days 1–7, but it was not effective at all during the entire preimplantation period.

The effects of Aspilia Africana methanolic extract at varying dosages on the uterine weights, histology and estrous cycles of twenty-five cyclic female rats were investigated (Oluyemi *et al*. 2007). The rats were divided into five groups at random and given, in that order, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, and 300 mg/kg of body weight. Histology showed dose-dependent toxicity and a significant reduction in estrous cycles.

The impact of a 50% ethanolic extract of the Piper betle (Petiole) on female wistar strain albino rats (Rattus norvegicus) was investigated (Sharma *et al*. 2007). Based on the data, it appears that the ethanolic extract of P. betle had antiestrogenic and antifertility effects on female rats. P. betle extract has temporary, non-toxic effects.

Mandal and Dhaliwal (2007) examined the effects of oral administration of Melia azedarach Linn. (dharek) seed extract on the uterine weight, several histological and biochemical parameters of the uterus, and the fertility index of adult cyclic wistar rats. Examined were the average number of embryos and implantation losses in the pregnant animals treated with dharek seed extract. The average number of embryos and fertility index decreased in the mated rats treated with dharek extract, as per the findings. This leads one to the conclusion that incorporating this plant extract into a rodent control programme could enhance socioeconomic conditions in society.

The ethanolic extract of Hibiscus rosa-sinensis Linn. roots has been shown by Vasudeva and Sharma (2007) to have potent anti-implantation and uterotrophic properties.

This extract's estrogenic quality might be the cause of its anticontraceptive action.

Citrus medica seed petroleum ether extract had estrogenic activity, which suggests it could be used as an antifertility drug, according to Sharangouda and Patil's (2008) study on the effects of the extract on immature albino rats.

The effects of Spondias mombin leaf extracts on female rats reproductive performance were examined in a study conducted by Chukwuka *et al*. (2008). Owing to the extract's direct effect on the uterus, S. mombin's aqueous ethanol leaf extract exhibits strong anticonception properties.

The effects of alcoholic extract of neem flowers on cyclic adult Sprague-Dawley rat's estrous cycle, ovulation, fertility, and foetal morphology were investigated by Gbotolorun *et al.* (2008). The administration of an alcoholic extract derived from neem flowers caused partial blockage of ovulation and upset the estrous cycle in Sprague-Dawley rats, suggesting that this extract could be developed into a female contraceptive.

The stem bark of Wrightia tinctoria was given orally to adult female Sprague-Dawley rats during the pre implantation, peri-implantation, and early post implantation periods in order to study its pregnancy-interceptive activity. Keshri *et al*. (2008) found that 100% of the rats were unable to conceive while the stem bark ethanolic extract was used.

The effects of Nelumbo nucifera as an antifertility agent in female rats were investigated by Mutreja *et al*. (2008). The findings imply that Nelumbo nucifera seeds have anti-estrogenic properties without changing the female rats' overall physiology.

Yakubu *et al*. (2008) investigated the effects of an aqueous extract of Cnidoscolous aconitifolius leaves on the reproductive hormones of female Wistar rats. The extract significantly decreased the levels of estradiol, progesterone, follicle stimulating, and luteinizing hormones. These changes in reproductive hormones suggest that the extract has a negative effect on follicle maturation and ovulation, which may lead to decreased fertility and conception in female rats. As a result, Cnidoscolous aconitifolius leaf extract may be investigated as a contraceptive for women.

Morovati *et al*. (2008) investigated the reversible infertility and spontaneous abortion characteristics of Azadirachta indica A. Juss., commonly known as neem. Neem Azal-T/S® appears to be a natural and relatively safe infertility agent for controlling dangerous rodents, based on the study's findings.

Govindaraj *et al*. (2009) investigated the effects of ethanolic extract and powdered roots of Derris brevipes variety coriacea in female rats that had been shown to be fertile. They discovered that both the ethanolic extract and the powdered roots had anti-implantation and abortifacient properties.

The ethanolic extract of Allium cepa Linn was evaluated for its antifertility activity by Thakare *et al*. (2009). After treating a patient with an ethanolic extract for 300 mg/kg, there was a notable reduction in the number of implant sites. This was due to the significant antifertility activity of the Allium cepa extract. The anti-implantation activity of Allium cepa can be primarily attributed to its antifertility activity, as there was no alteration in ovulation

during the current study.

Madhavan *et al*. (2009) used an anti-implantation model in female Wistar rats to investigate the antifertility activity of alcohol and aqueous extracts of the whole plant of Drosera burmannii Vahl (Droseraceae). Significant antifertility activity was also shown by the aqueous extract, which may have been caused by additional phytoconstituents like sterols and flavonoids.

The antifertility qualities of Plumbago zeylanica Linn. (Plumbaginaceae) leaf extracts were assessed by Edwin *et al*. (2009) in their study. The rat's estrous cycles were most effectively broken by the acetone and ethanol extracts. The animals showed signs of a temporary inhibition of ovulation, corresponding to a prolonged dioestrous stage of the oestrous cycle. When treatment was stopped, the antiovulatory effect was reversible.

The effects of ethanol extract of the entire Trichosanthes cucumerina L. var. cucumerina plant on antiovulatory activity in adult rats were investigated (Kage *et al*. 2009). The results demonstrated that the ethanol extract of the entire Trichosanthes cucumerina L. var. cucumerina plant had antiovulatory effects on female albino rats.

According to Bhaskar *et al*., 2009 ethanol and aqueous extracts of the dried stem bark of the Crataeva nurvala buch-Hum (Capparidaceae) plant significantly reduced rat fertility. At dose levels of 300 and 600 mg/kg b.wt, respectively, ethanol and aqueous extracts showed partial and complete resorption of implants.

In 2009, Savadi *et al*. studied the anti-implantation and abortifacient properties of Heliotropium indicum (Boraginaceae) leaf extract in female Albino rats. N-hexane has been demonstrated to have superior anti-implantation activity compared to ethanolic extract and benzene fraction.

According to Sakila *et al*. (2009), Andrographis paniculata (AP) plant female rats exhibit antifertility effects. This is because the plant's lower hormone levels cause a comparatively high percentage of infertility in female rats. According to Gupta (2009), an antiestrogenic petroleum ether extract was discovered from Catharanthus roseus leaves that had been exposed to mice.

Petroleum ether (60–80o), chloroform, acetone, ethanol, and aqueous extracts of Plumbago rosea leaves were studied by Sheeja *et al*. (2009) to determine the estrogenic activity of acetone and ethanol extracts in female albino rats and their impact on the estrous cycle. Significant estrogenic and antiestrogenic activity was demonstrated by both extracts. P. rosea leaf extracts in acetone and ethanolic form have antifertility properties.

The ethanolic extract of Plumbago indica roots and aerial parts of Aerva lanata was found to have anti-implantation and abortificient properties (Savadi and Alagawadi 2009).

The antifertility potentials of Curcuma longa rhizome and Carum carvi seeds were investigated (Thakur *et al*. 2009). The current investigation, which looked for endocrinological and physiological alterations in the female albino rat's reproductive systems following oral administration of varying dosages of aqueous and ethanolic extracts of

Curcuma longa and Carum carvi revealed a strong antifertility effect.

In female rats, Salman *et al*. (2009) investigated the embryotoxic and contraceptive effects of Onosma armeniacum root extract (A-1). The rat contraceptive effect may be attributed to the shikonin content of A-1.

According to Yadav *et al*. (2009), female albino rats that have been shown to be fertile were given doses of 100, 200 and 500 mg/kg b.wt./day of a petroleum ether extract of Cassia fistula seeds to test for antifertility. Because of its anti-implantation activity, the petroleum ether extract of Cassia fistula seeds has the potential to terminate pregnancy, according to the results.

The effects of Aspilia africana leaf extract on the oestrous cycle and ovulation in adult female wistar strain rats were investigated (Oyesola *et al*. 2010). The findings indicate that Aspilia africana leaf aqueous extract alters the oestrous cycle and adversely affects ovulation in wistar strain rats in a dose-dependent manner, thereby having an antifertility effect.

The antifertility activity of Curcuma aromatica Salisb's rhizomes was examined by Trishna *et al*. (2010) in female rats using both an ethanolic and an aqueous extract. The pregnancy was stopped by the plant's ethanolic and aqueous extract.

The impact of a methanolic extract of Abrus precatorius seeds on the Sprague-Dawley rat's estrous cycle, ovulation, and foetus implantation is examined (Okoko *et al*. 2010). The regularly cyclic rat's estrous cycles were temporarily disrupted and all treated rat's ovulation was totally blocked by the methanolic extract of A. precatorius. In Sprague-Dawley rats, methanolic extract of A. precatorius seeds totally inhibited ovulation and produced reversible changes in the estrous cycle pattern. Furthermore, the extract exhibited anti-implantation activity and the capacity to impact the overall foetal morphometry in rats.

Mishra *et al*. (2011) investigated the ovaro-uterotoxic and antifertility properties of Calotropis gigantea Linn extracts. The results indicate a potent antiestrogenic and antiprogestagenic effect of the extracts.

Female albino rat's reproductive systems may be adversely affected by the aqueous and ethanolic extract of the stem bark of Hymenocardia acida (Hyacinth and Nwocha, 2011).

Cynodon dactylon and fenugreek seed extract were shown to have antifertility effects on female rats and mice with irregular estrous cycles and a trend towards folliculogenesis (Nayanatara *et al*. 2012)

The effect of oral administration of Caesalpinia bonducella seed extract (ethanolic) on the reproductive system in female albino rats was investigated (Lilaram and Nazeer 2012). The treatment significantly extended the duration of the diestrus stage while also prolonging the estrous cycle, according to the results. Estradiol, progesterone, LH, and FSH all showed marked declines. Significant reductions in uterine and ovarian weight were observed. Follicular atresia and corpora lutea degeneration were observed in the ovary through histoarchitectural observations. Mucosal folds and epithelial cells displayed degeneration in the oviduct. Endometrial glands and endometrial epithelium degeneration were evident in the

uterus. It was discovered that the vagina's muscularis layer and lamina propria were somewhat disorganised. The antiestrogenic properties of seed extract may account for a notable drop in hormone levels, weight loss in the reproductive organs, and changes in the histoarchitecture of the reproductive organs.

Alcoholic and aqueous extracts of Dolichandrone falcata leaves were found to have an abortifacient effect on the estrous cycle of female albino rats (Zade *et al*. 2012). The leaf extracts of Dolichandrone falcata, both alcoholic and aqueous, exhibited a potent abortifacient activity (100%) at dosage levels of 200 mg/kg and 400 mg/kg body weight. The diestrous phase of the estrous cycle, in particular, was found to have significantly prolonged.

Solanum xanthocarpum seed powder reduced the weight of the genital organs and changed the ovary and uterus histopathologically, which resulted in complete control over reproduction (Singh & Singh, 2013).

Methanolic extract of Piper betel Linn (PBL) was found to have an antifertility effect on female albino rats in a study conducted by Ranjan *et al*. (2013). The number of estrus phase rats decreased in a dose-dependent manner. Proestrus, metestrus, and diestrus phases of the estrus cycle were all observed in the PBL treated group with no discernible variation. Every rat given PBL extract experienced the onset of anestrus phase, whereas the control group did not experience this.

Yadav and Jain (2013) examined the uterine histoarchitecture of immature bilaterally ovariectomized female albino rats treated with Cassia fistula seed petroleum ether extract, both in the presence and absence of estradiol valerate (EDV).

Administration of a single dose of 100 mg/kg b.wt. of Cassia fistula seed extract in petroleum ether. caused uterine wet weights to increase ($p<0.05$) as well as the height of luminal epithelial cells ($p<0.001$), but it did not cause the vagina to open prematurely. According to this, the extract may have had a slight estrogenic effect when taken by itself, but when combined with EDV (0.1 mg/kg b.wt.), the extract dramatically ($p<0.001$) reduced the uterotrophic effect caused by oestrogen, demonstrating the extract's antiestrogenic properties when strong oestrogen is present. Consequently, the findings suggest that the extract has antiestrogenic properties, which may account for the anticonceptive effect, at least in part.

In Central Kalimantan, the Dayak people use Blumea balsamifera, Croton tiglium, Metroxylon sagu, and Fagraea racemosa as traditional antifertility remedies. Swiss Webster (SW) mice are used in this study to investigate potential plant antifertility. For eight days, female SW mouse subjects received oral doses of 0.26 mg/kg body weight (b.w.) of extracts from B. balsamifera, C. tiglium, M. sagu, and F. racemosa in order to study their estrous cycle. The findings demonstrate that all of the conventional plant extracts inhibit the estrous cycle, especially during the metestrus and estrus phases. The number of live foetuses, corpus luteum, and body weight of the treated dam mice have all decreased, according to the reproduction display. The extract's antifertilty effect is attributed to inhibiting foliculogenesis, which further reduces the corpus luteum and foetuses. When compared to other traditional

plant extracts, the B. balsamifera extract appears to have greater potential for antifertility activities out of the four extracts that were tested (Haryono *et al*. 2013).

CONCLUSION

In conclusion, it is clear that the study of medicinal plants is important for understanding antifertility. Natural antifertility suppliers provide a promising outcome by lowering the number of undesirable medication side effects, even in the face of the vast array of commercially available oral contraceptives on the market. Research on conventional medications is moving forward at a rapid pace due to their safety and lower production costs.

REFERENCES

Ahirwar D., Ahirwar B. and Kharya M.D. (2007): Influence of Calotropis procera roots on biochemistry of reproductive organs of ovariectomized rats. *Indian J.Pharma. Sci.*, 69(3): 459–461.

Azadeh M., Pourheydar M., Khazaei M. and Ghorbani R. (2007): Anti-fertility effects of physalis alkekengi alcoholic extract in female rat. *Iranian Journal of Reproductive Medicine,* 5(1):13-16.

Badami S., Aneesh R., Sankar S., Sathish kumar M. N., Suresh B. and Rajan S. (2003): Antifertility activity of Derris brevipes variety coriacea. *J Ethanopharmacol*, 84(1): 99-104.

Bhaskar V. H., Profulla M. K., Balakrishnan B. R., Balakrishnan N. and Sangameswaran B. (2009): Evaluation of the anti-fertility activity of stem bark of Crataeva nurvala buch-hum. *African Journal of Biotechnology*, 8(22): 6453-6456.

Bhinda A. and Sharma J. D. (2005): Postcoital antifertility activity of Trigonella foenum-graecum (seeds) steroidal extract in rats. Proc. Intn. Conf. of Susb. Devop. and Resor. Utiz. : *Curr. Trends and Prep.,* Sep. 23rd –25th, Jaipur, p. 99.

Census of India (2011): http://censusindia.gov.in/.

Choudhary M.K., Sani U.M. and Mustapha A. (1998): Antifertility activity of the flowers of Striga senegalensis. *Phytotherpy Research*. 12: 207-208.

Chukwuka N., Uchendu and Thomas I. (2008): Antifertility activity of aqueous ethanolic leaf extract of Spondias mombin (Anacardiaceae) in rats. *African Health Sciences*, 8(3): 163-167.

Dhanapal R., Kavimani S., Vrushabendra Swamy B. M., Gupta M. and Basu S. K. (2005): Antisteroidogenic activity of ethanol extract of Ammania baccifera (L.) whole plant in female albino mice ovaries. *IJPT*, 4(1): 43-46.

Edwin S., Joshi S. B. and Jain D. C. (2009): Antifertility activity of leaves of Plumbago zeylanica Linn. in female albino rats. *The European Journal of Contraception and Reproductive Health Care,* 14(3): 233–239.

Fernanda C. G., Almeida I. and Lemonica P. (2000): The toxic effects of Coleus barbatus B. on

the different periods of pregnancy in rats. *Journal of Ethnopharmacology*, 73: 53–60.

Gbotolorun S. C., Osinubi A. A., Noronha C. C. and Okanlawon A. O. (2008): Antifertility potential of Neem flower extract on adult female Sprague-Dawley rats. *African Health Sciences,* 8(3): 168-173.

Ghosh K. and Bhattacharya T.K. (2004): Preliminary study on the antiimplantation activity of compounds from the extracts of seeds of Thespesia populnea. *Indian J. Pharmacol.*, 36(5): 288-291.

Govindaraj Y., Melanaphuru V., Gupta S., Agrahari V. and Nema R. K. (2009): Antifertility activity of the ethanolic extract of Cassia occidentalis, Derris brevipes variety Brevipes and Justicia simplex. *World Journal of Chemistry*, 4(2): 118-122.

Gupta M., Mazumdar D. K., Pal and Bhattacharya S. (2003): Anti-steroidogenic activity of methanolic extract of Cuscuta reflexa roxb. Stem and Corchorus olitorius Linn seed in mouse ovary. *Indian J. Exp. Biol*. 41: 641-644.

Haryono A., Gunawan Y. E., Suatma, Sumitro S. and Rahmadur M. (2013): Anti-fertility effect of various plants at Dayak Tribe to Swiss Webster Mice. *The Journal of Tropical Life Science*. 3(2): 108 – 112.

Hazarika A. and Sarma H. N. (2007): Effects of crude root extract of Polygonum hydropiper on estrous cycle and induction of reversible sterility in female albino rat. *Endocrinol Reprod*, 11(1): 36 – 40.

Homady M. H., Khleifat K. M., Taraeneh K. A. and Al-Raheil I. A. (2002): Reproductive toxicity and infertility effect of Ferula hormonis extracts in mice. *Theriogenology,* 57: 2247-2256.

Hyacinth A. A. and Nwocha U.C. (2011): Antifertility activity of aqueous ethanolic extract of Hymenocardia acida stem bark in female rats. *Iranian Journal of Reproductive Medicine*. 9(3): 217-222.

Kage D. N., Malashetty V. B., Seetharam Y. N., Suresh P. and Patil S. B. (2009): Effect of ethanol extract of whole plant of Trichosanthes cucumerina var. cucumerina L. on gonadotropins, ovarian follicular kinetics and estrous cycle for screening of antifertility activity in albino rats. *Int. J. Morphol.* 27(1):173-182.

Kamath J. V. and Rana A. C. (2002): Preliminary study on antifertility activity of Calotropis procera roots in female rats. *Fitoterapia,* 73(2): 111-115.

Keshri G., Kumar S., Kulshreshtha, D. K., Rajendran S. M. and Singh M. M. (2008): Postcoital interceptive activity of Wrightia tinctoria in Sprague–Dawley rats: a preliminary study. *Contraception,* 78: 266–270.

Keshri G., Oberoi R. M., Lakshmi V., Pandey K. and Singh M. M. (2007): Contraceptive and hormonal properties of the stem bark of Dysoxylum binectariferum in rat and docking analysis of rohitukine the alkaloid isolated from active chloroform soluble fraction. *Contraception,* 76: 400–407.

Khushalani H., Tatke P. and Singh K. (2006): Antifertility activity of dried flowers of Woodfordia fruticosa kurz.(Short Communication). *Indian Journal of Pharmaceutical Sciences*, 68(4): 528-529.

Lakshmi V., Kumar R., Agarwal S.K. and Dhar J.D. (2006): Antifertility activity of Piper longum Linn. in female rats. *Natural Product Research*, 20(3): 235–239.

Lilaram and Nazeer A.R. (2012): Effect of ethanolic seed extract of Caesalpinia bonducella on female reproductive system of albino rat: a focus on antifertility efficacy. *Asian Pacific Journal of Tropical Disease*, S957-S962.

Madhavan V., Kumar B. H. P., Murali A. and Yoganarasimhan S. N. (2009): Antifertility activity of Drosera burmannii. *Pharmaceutical Biology*, 47(2): 128–131.

Makonnen E., Zerihum L., Assefa G. and Rostom A. A. (1999): Antifertility activity of Ricinus communis seeds in female guinea pigs. *East. Afr. Med. J.*, 76(6): 335-337.

Mandal R. and Dhaliwal P. K. (2007) Antifertility effect of Melia azedarach Linn. (dharek) seed extract in female albino rats. *Indian J Exp Biol*. 45(10): 853-860.

Mishra M., Gautam R. K. and Mathur R. (2011): Evaluation of antifertility potential of Calotropis gigantea linn. in female albino rats. *J. of Pharm. Res. and opinion*, 1(3): 92-93.

Monsefi M., Ghasemi M. and Bahaoddini A. (2006):The effects of Anethum graveolens L. on female reproductive system of rats. *DARU* 14 (3): 131-135.

Morovati M., Mahmoudi M., Ghazi-Khansari M., Khalil Aria A. and Jabbari L. (2008): Sterility and abortive effects of the commercial Neem (Azadirachta indica A. Juss.) extract NeemAzal- T/S® on female rat (Rattus norvegicus). *Turk J Zool* 32: 155-162.

Murugan V., Shareef H. R., Rama Sarma G. V. S., Ramanathan M. and Suresh B. (2000): Anti-fertility activity of the stem bark of Alangium salviifolium (Linn. f.) wang in wistar female rats. *Indian J Pharmacol*, 32: 388-389.

Mutreja A., Agarwal M., Kushwaha S. and Chauhan A. (2008): Effect of Nelumbo nucifera seeds on the reproductive organs of female rats. *Iranian Journal of Reproductive Medicine.* 6(1): 7-11.

Nayanatara A.K., Alva A., Kottari S., Soofi A.A., Rejeesh E. P., Bhagyalakshmi K., Shetty S.B., Kini R.D. and Pai S.R. (2012): Effect of Cynodon dactylon extract on estrous cycle and reproductive organs in female wistar rats. *International Journal of Analytical, Pharmaceutical and Biomedical Sciences.* 1(3): ISSN: 2278-0246.

Nisreen M., Dissi A. L., Salhab A. S. and Al-Hajj H. A. (2001): Effect of Inula viscosa leaf extract on abortion and implantation in rats. *J Ethnopharmacol*, 77: 117-121.

Nivsarkar M., Patel M., Padh H., Bapu C. and Shrivastava N., (2005): Blastocyst implantation failure in mice due to "nonreceptive endometrium": endometrial alterations by Hibiscus rosa-sinensis leaf extract. *Contraception,* 71: 227–230.

Okoko I. E., Osinubi A. A. A., Olabiyi O.O., Kusemiju T. O., Noronha C.C. and Okanlawon A.O. (2010): Antiovulatory and anti-implantation potential of the methanolic

extract of seeds of Abrus precatorius in the rat. Antifertility Effects of Abrus, *endocr pract*.16(4) : 554-560

Oluyemi K. A., Okwuonu U. C., Baxter D. G. and Oyesola T. O. (2007): Toxic effects of methanolic extract of Aspilia africana leaf on the estrous cycle and uterine tissues of wistar rats. *Int. J. Morphol*., 25(3): 609-614.

Orihuela P. A. and Ishiyama V. (2006): Postcoital ingestion of the aqueous extract of Erythrina falcata Benth prevents pregnancy in the mouse. *Contraception,* 73(3): 307-310.

Oyesola T. O., Oyesola O. A. and Okoye C. S. (2010): Effect of aqueous extract of Aspilia africana on reproductive functions of female wistar rats. *Pakistan journal of Biological sciences*, 13(3): 126 -131.

Padmashali B., Vaidya V. P., Vagdevi H. M. and Satyanarayana N. D. (2006): Antifertility efficacy of the plant Balanites roxburghiiin (Balanitaceae) the in female rats. *Indian J. Pharm. Sci.*, 68(3): 347-351.

Purohit A. and Daradka H. M. M. (1999): Antiandrogenic efficacy of Curcuma longa (50%EtOH extract) with special emphasis on testicular cell population dynamics. *Indian Drugs*, 36:142–143.

Ranjan P.M., Manjushree M., Sabita M. and Samira S. (2013): Antifertility effect of alcoholic stalk extract of Piper betel Linn. on female albino rats. *International Research Journal of Pharmacy.* 4 (1): 218-220.

Ravichandran V., Suresh B., Sathishkumar M. N., Elango K. and Srinivasan R. (2007): Antifertility activity of hydroalcoholic extract of Ailanthus excelsa (Roxb): An ethnomedicines used by tribals of Nilgiris region in Tamilnadu. *Journal of Ethnopharmacology,* 112: 189–191.

Roop J. K., Dhaliwal P. K. and Guraya S. S. (2005): Extracts of Azadirachta indica and Melia azedarach seeds inhibit folliculogenesis in albino rats. *Brazilian Journal of Medical and Biological Research*, 38: 943-947

Sakila S., Begum N., Kawsar S., Begum Z. A. and Zoha M. S. (2009): Relationship of anti-fertility effects of Andrographis paniculata and hormonal assay in female rats. Bangladesh *Journal of Medical Science*, 8: 1-2.

Salman S., Kumbasar S., Ozgen U., Erdogan F. and Suleyman H. (2009): Contraceptive effects of Onosma armeniacum on embryo implantation in rats. *Cell membranes and free radical research*. 1(3) : 90-94.

Sani U. M. and Sule M. I. (2007): Anti-fertility activity of methanol extracts of three different seed varieties of Ricinus communis linn (euphorbiaceae). *Nig. Journ. Pharm. Sci.*, 6(2): 78 – 83.

Savadi R. V. and Alagawadi K. R. (2009): Antifertility activity of ethanolic extracts of Plumbago indica and Aerva lanata on albino rats. International Journal of Green Pharmacy. 3(3): 230-233.

Savadi R. V., Alagawadi K. R. and Darade S. S. (2009): Antifertility activity of ethanolic

extract and its n-hexane and benzene fractions of Heliotropium indicum leaves on albino rats. *Journal of Pharmacy Research*, 2(5): 927-930.

Sharanabasappa A., Vijayakumar and Saraswati B. and Patil B. (2002): Effect of Momordica charantia seed extracts on ovarian and uterine activities in albino rats. *Pharmaceutical Biology*, 40(7): 501–507.

Sharangouda and Patil S.B. (2008): Estrogenic activity of petroleum ether extract of seeds of Citrus medica on immature albino rats. *IJGP*, 2(2): 91-94.

Sharma J.D., Sharma L. and Yadav P. (2007): Antifertility efficacy of Piper betle Linn. (Petiole) on female albino rats. *Asian J. Exp. Sci*., 21(1): 145-150.

Sheeja E., Joshi S.B. and Jain D.C. (2009): Antiovulatory and estrogenic activity of Plumbago rosea leaves in female albino rats. *Indian J Pharmacol*. 41(6): 273-277.

Shivalingappa H., Satyanarayan N. D., Purohit M. G., Sharanabasappa A. and Patil S.B. (2002): Effect of ethanol extract of Rivea hypocrateriformis on the estrous cycle of the rat. *Journal of Ethnopharmacology*, 82: 11-17.

Shivalingappa H., Satyanarayan N.D. and Purohit M.G. (2001): Anti-implantation and pregnancy interruption ef□cacy of Rivea hypo-crateriformis in the rat. *Journal of Ethnopharmacology* 74, 245 Á/249.

Singh S.P. and Singh S.P. (2013): Antifertility effects of Solanum xanthocarpum seeds on female albino rats, *Journal of Applied and Natural Science*. 5(1): 153-156.

Srivastava S. R., Kesarwani S.,Keshri G. andSingh M. M. (2005): Evaluation of contraceptive activity of a mineralo-herbal preparation in Sprague-Dawley rats. *Contraception,* 72(6): 454-458.

Srivastava S. R., Keshri G., Bhargavan B., Singh C. and Singh M. M. (2007): Pregnancy interceptive activity of the roots of Calotropis gigantea Linn.in rats. *Contraception,* 75: 318– 322.

Tafesse G., Mekonnen Y. and Makonnen E. (2005): In vivo and in vitro anti-fertility and anti-implantation properties of Leonotis ocymifolia in rats. *Afr. J. Trad. CAM,* 2(2): 103-112.

Thakare V. N., Kothavade P. S., Dhote V. V. and Deshpande A. D. (2009): Antifertility Activity of Ethanolic Extract of Allium cepa Linn in Rats. *International Journal of PharmTech Research*, 1(1): 73-78.

Thakur S., Bawara B., Dubey A., Nandini D., Chauhan N. S. and Saraf D.K. (2009): Effect of Carum carvi and Curcuma longa on hormonal and reproductive parameter of female rats. *International Journal of Phytomedicine* 1: 31-38.

Thakur S., Bawara B., Dubey A., Nandini D., Chauhan N. S. and Saraf D.K. (2009): Effect of Carum carvi and Curcuma longa on hormonal and reproductive parameter of female rats. *International Journal of Phytomedicine* 1: 31-38.

Trishna D., Shanti Bhushan M., Mrinmoy B., Mohanty J.P. and Dibyendu S. (2010): Evaluation of phytochemical screening and anti-fertility activity of Curcuma Aromatica Salisb. *International Journal of Pharmaceutical Sciences and*

Research 1 (3) : 18-22.

Valsala S. and Karpagaganapathy P. R. (2002): Effect of Mimosa pudica root powder on oestrous cycle and ovulation in cycling female albino rat, Rattus norvegicus. *Phytother Res*., 16(2): 190-2.

Vasudeva N. and Sharma S. K. (2006): Post-coital antifertility activity of Achyranthes aspera Linn. Root. *Journal of Ethnopharmacology*, 107: 179-181.

Vasudeva N. and Sharma S. K. (2007): Post-coital antifertility activity of Hibiscus rosa-sinensis Linn. roots. Eivd Based Complement Alternat med., 5(1): 91-94.

Yadav N.P. and Chanotia C.S. (2009): Phyto chemical and pharmacological profile of leaves of Aegle marmelos Linn. *The pharma Review*,144-149.

Yadav R. and Jain G. C. (1999): Antifertility effect of aqueous extract of seeds of Cassia fistula in female rats. *Adv. Contracept*., 15 (4): 293-301.

Yadav R. and Jain G. C. (2013): Effect of petroleum ether extract of Cassia fistula seeds on uterine histoarchitecture of ovariectomized female rats. *Indian Journal of Fundamental and Applied Life Sciences.* 3(1): 167-174.

Yadav R. and Jain G. C. (2006): Postcoital antifertility effect of petroleum ether extract of Curcuma longa rhizome in female rats. *Asian J. Exp. Sci*., 20(1): 201-208.

Yakubu M.T., Akanji M. A., Oladiji A.T., Olatinwo A.W.O., Adesokan A. A., Yakubu M. O., Owoyele R. N, R. M, B. V., Sunmonu T. O. and Ajao M. S. (2008): Effect of Cnidoscolous aconitifolius (Miller) I.M. Johnston leaf extract on reproductive hormones of female rats. *Iranian Journal of Reproductive Medicine* 6(3): 149-155.

Zade V., Wikhe M., Dabhadkar D. and Pare S.(2012): Antifertility effect of alcoholic and aqueous extracts of Dolichandrone Falcata leaves on estrous cycle of female albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4(3) : 462-465.

Adv. Pharmacol. Toxicol. Vol. 25 (2) 2024, 69-78
ISSN - 0973 - 2381 SILVER JUBILEE VOLUME

IN-VITRO COMPARATIVE EVALUATION OF BRANDED AND GENERIC FUROSEMIDE40MG TABLET

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In the pharmaceutical field, quality is a very important issue due to get safe and therapeutically active agent . In-vitro evaluation is one of the authentic way to ensure the quality, bioavailability as well as optimum therapeutic activity of drug. The main therapeutic activity of Furosemide tablet is diuretic and also utilized for treating high blood pressure and oedema. This study aimsto compare two different kinds of furosemide 40mg tablets, where one type is sale as generic name and another type is sale as branded product in Indian market, to verify if they meet their claims according to Indian Pharmacopoeia (IP) limits for such products. The quality of furosemide or frusemide in the tablet formulations was determined by testing batches of furosemide tablets sold in India for characteristics such as weight uniformity, hardness, disintegration, friability, and an assay based on spectro photometry. Furosemide 40 mg tablet batches have been tested for furosemide content and found to be within the acceptable range established by the Indian Pharmacopoeia.

INTRODUCTION

When taken orally, furosemide tablet is a powerful loop diuretic that works on the kidneys to enhance water loss in the body. It's chemically related to anthranilic acid. Edema caused by illnesses including high blood pressure, kidney failure, liver failure, heart failure, may be treated with furosemide tablets. This medication has the potential to increase urinary water loss and decrease renal electrolyte reabsorption. The tablet form of furosemide, which has a rapid onset but short duration of action, has been used successfully in both children [M. M. Parker, J. A. Hazelzet, and J. A. Carcillo (2004)] and adults. In therapeutic circumstances when a medication with a stronger diuretic capability is needed, frusemide tablets are very useful. Patients who are unable to take oral medication or who are in emergency clinical situations may also be administered furosemide by intravenous or intramuscular injection'[Arafat M, Ahmed Z, Arafat O(2017)].

IUPACname :4-Chloro-N-furfuryl-5-sulfamoylanthranilicacid[Benet LZ.(1979)]
Formula :C12H11ClN2O5S

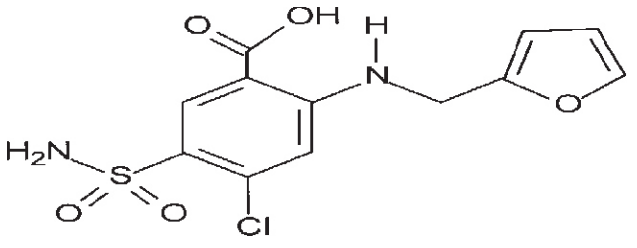

Fig. 1: Structure of Furosemide

Molar mass :330.74g·mol- 1

Physical appearance : White slightly yellow crystalline solid

Presence of ring: Furan, phenyl
Number of chiral centers : Not present
General uses of Furosemide tablets are

· Treatment of edema related to Congestive Heart Failure [Arafat M(2015)],renal diseases along with liver cirrhosis,.

· Management of mild to severe hypertension and pulmonary edema [Mohamed M, Siaan KAA, Almarzouki A, Anwair MA(2015)]

Side Effects of Furosemide tablets are Fainting, Allergic reactions, Dehydration,Drymouth,Musclecramps,Photosensitivity,Stomachcramps, Loss of appetite, Lowblood potassium and sodium levels, Diarrhea, Fatigue, Dizziness, Vomiting, Blurred vision, and Nausea [Dires T.A (2005), Murthy, K. S. Ghebre-Sellassie, I Murthy, K. S. Ghebre-Sellassie, I(1993), Murtaza, G. Khan, S. A., Naham-ul-Haq, M. Hussain(2014), Lindenberg, M. Kopp, S. Dressman, J. B.(2004)].

MATERIALS AND METHOD

Materials:- 10 strips of generic Frusemide tablets (40 mg) were collected from the local hospital of South Midnapore in West Bengal and10 strips of LASIX 40MG TABLET which contain Frusemide 40 mg as label claim, were collected from the local retail shop of South Midnapore in West Bengal. All other ingredients were taken as an analytical grade.

Methodology: Frusemide tablets (40 mg) are physically inspected and found to be correctly

strip packed and intact with the correct product information, including the name, manufacturing and expiration dates strength, manufacture country, pack size, and National Agency for Food and Drug Administration (NAFDAC) registration number. Furo A [26] is the label for the generic 40 mg Frusemide tablets, while Furo B is the label for the LASIX 40MG TABLET [Granero, G. E. Longhi, M. R. Mora, M. J. Junginger (2010)]. Forty (40) pills from each brand were chosen at random and used for the following analyses:

Table 1: List of materials required for evaluate Furosemide tablet

GlassApparatus	Chemicals	Equipments
1000 ml,100 ml and 50 ml Volumetric flask	Ethanol	Digital Weight Balance
500 ml & 100 ml Measuring Cylinder	Distilled water	Hardness tester
Glass rods	4-dimethylaminobenzaldehyde	FriabilityTester
250 ml &500 ml Beaker	Sodiumhydroxide	Disintegrator
Motor and pestle	Potassium di-hydrogen phosphate	Dissolution machine
Filterpaper No.1		UV-VIS Spectrophotometer
5 ml & 10ml Pipette		

Identificationtest

1)When examined in the range of 220nm to 360nm, thefinal solution obtained in the Assay shows three absorption maxima at about 228nm, 271nm, and 333nm. The ratio of the absorbance at the maximum at about 228nm is 0.52 to 0.57.

2)Shake a quantity of the powdered tablets containing 80mg of frusemide with 10 ml of ethanol(95%), filter, and evaporate the filter/filtrate to dryness. Dissolve 25mg of the residue obtained in 2.5ml of ethanol (95%) and add 2ml of 4-dimethylamino benzaldehyde reagent, a green color is produced which changesto deep red[Qureshi, S. A. McGilveray, I. J.(1998)].

Uniformity of weight test

Twenty pills were chosen randomly from the strip-packed blisters of each batch. A digital analytical balance (Wensar®, India) was used to weigh the tablets both individually and in bulk [Azoulay P(2002)]. The average, variation coefficient, and standard deviation weights were calculated [Maggio, R. M. Castellano, P. M. Kaufman, T. S.(2008)].

Hardness test

The hardness/crushing strength of ten furosemide pills was measured utilizing a Mosanto® [30] tablet hardness tester, and the results varied widely across batches (Monsanto, India)[Yong, K. H. Simionato, L. D. Calvo, R. G. Mattei, M. B. Segall, A. I.(2014)]. For each batch, we calculated the average crushing strength, variation coefficient and standard deviation.

Fig. 2 : Mosanto® tablet hardness tester(Monsanto, India)

Friability test

A total of twenty pills from each brand were utilised in the study [Abdelbary G, Prinderre P, Joachim J, Reynier J, Piccerelle P.(2005)]. Tablets were dedusted by blowing air on them, then weighed before being rotated at 25rpm for 4minutes in a Veego twin drum tablet friability test device (Veego®, India). Friability (F) was determined by dividing the tablets' pre-test weight (Wo) by their post-test weight (Wf) using equation below. For each batch, two separate analyses were performed.

$F = [Wo-Wf/Wo] \times 100\%$

Fig. 3 : Veego twin drum friability test apparatus (Veego®, India)

Disintegration test

To conduct the analysis, six furosemide pills were picked at random from both the generic and brand-name samples. Each tablet was placed on a glass disc and then placed into the cylindrical hole of one of the disintegration tester's (Veego® VTD-D double basket, India) six holes. 500 mL of 0.1 N NaOH at 37 ± 0.5°C were placed in each beaker. Time was recorded as six pills were allowed to disintegrate and travel through the mesh. There were three sets of calculations done. Each reading's mean and standard deviation were calculated. The sample of furosemide pills was collected in the same way[Anwar S, Fell JT, Dickinson PA(2005)].

Dissolution test

The release of furosemide's active pharmacological component from various batches was studied by analysing their dissolving profiles (API). We employed Labtronics® LT-720 six-station dissolving apparatus (Labtronics®, India) [Ascion FJ, Kirking DM, Gaither CA, Welage LS.(2001)]. Phosphate buffer (pH 5.8) has been added to each flask and 37 ± 0.5°C was

Fig. 4 : Veego twin drum friability test apparatus (Veego®, India)

the maintained temperature while 50 rpm was the paddle speed for 60 minutes. At predetermined intervals, 5 mL samples were taken from each flask containing one pill from each batch.

After each removal, the removed samples were replaced with five (5) mL of dissolving fluid kept at 37 ± 0.5°C. To quantify concentrations, we used the previously established calibration curve and a LABTRONICS model LT- 2201 spectrophotometer calibrated at 271 nm to analyse filtered samples.

Fig. 5 : Six-station dissolution test apparatus (Veego®, India)

Preparation of phosphate buffer solution:

Dissolve 6.8gm of potassium dihydrogen phosphate in1000ml of water and adjusted to pH 5.8 using 10M potassium hydroxide solution[38].

Assay

Weigh and powder twenty (20) tablets. Mix 0.1 g of furosemide tablet powder with 150 cc of 0.1 M sodium hydroxide for 10 minutes. Filter after adding enough 0.1M sodium hydroxide to make 250 ml. The absorbance of a solution that has been diluted from 5ml to 200ml with 0.1M sodium hydroxide peaks at around 271nm. Using a specific absorbance of 580 at 271 nm, determine the amount of frusemide ($C_{12}H_{11}ClN_2O_5S$) in a pill.

Fig. 6 : UV-VISspectrophotometer (Labtronics®, India)

Preparation of 0.1Msodium hydroxide solution:

Dissolve 4.2 gm of sodium hydroxide in sufficient carbon dioxide free water to produce 1000ml.

RESULTS AND DISCUSSION

The results of the physical tests conducted on the items are listed in Table 2. All products were appropriately strip-packaged, with the correct API potency, and use-by dates.

Product name, NAFDAC registration date of manufacturing and expiration, batch number, and Manufacturer's address, pack size, were all printed large on the packets holding the strip packs. Nothing had been tampered with, and all items were undamaged.

Table 2: Some relevant information on the package of two kinds of frusemide tablets

Sample Code/batch	Manufactur--ing Country	Manufactur--ing Date	Expiry date	NAFDAC status	Tablet strength
FUROA	India	06/2022	05/2024	Registered	40mg
FUROB	India	01/2023	12/2025	Registered	40mg

Table3: Uniformity of Weight of two kinds of frusemide tablets

Tablet No.	Weight of Generic table ts	Weight of Branded tablets
Tab1	0.13 gm	0.16 gm
Tab2	0.12 gm	0.16 gm
Tab3	0.13 gm	0.16 gm
Tab4	0.13 gm	0.16 gm
Tab5	0.13 gm	0.16 gm
Tab6	0.13 gm	0.16 gm
Tab7	0.12 gm	0.16 gm
Tab8	0.13 gm	0.16 gm
Tab9	0.12 gm	0.16 gm
Tab10	0.13 gm	0.16 gm
Tab11	0.13 gm	0.16 gm
Tab12	0.13 gm	0.16 gm
Tab13	0.12 gm	0.16 gm
Tab14	0.13 gm	0.16 gm
Tab15	0.13 gm	0.16 gm
Tab16	0.12 gm	0.16 gm
Tab17	0.13 gm	0.16 gm
Tab18	0.12 gm	0.16 gm
Tab19	0.13 gm	0.16 gm
Tab20	0.13 gm	0.16 gm
Average weight of tablet	0.127 gm	0.160 gm

Tablet No	Weight of Generic Tablets	Weight variation of generic tablets =Weight of each tablet – average weight of tablets(0.127 gm)	Weight of Branded tablets	Weight variation of branded tablets =Weight of each tablet – average weight of tablets(0.160gm)
Tab1	0.13 gm	0.003	0.16 gm	0
Tab2	0.12 gm	0.007	0.16 gm	0
Tab3	0.13 gm	0.003	0.16 gm	0
Tab4	0.13 gm	0.003	0.16 gm	0
Tab5	0.13 gm	0.003	0.16 gm	0
Tab6	0.13 gm	0.003	0.16 gm	0
Tab7	0.12 gm	0.007	0.16 gm	0
Tab8	0.13 gm	0.003	0.16 gm	0
Tab9	0.12 gm	0.007	0.16 gm	0
Tab10	0.13 gm	0.003	0.16 gm	0
Tab11	0.13 gm	0.003	0.16 gm	0
Tab12	0.13 gm	0.003	0.16 gm	0
Tab13	0.12 gm	0.007	0.16 gm	0
Tab14	0.13 gm	0.003	0.16 gm	0
Tab15	0.13 gm	0.003	0.16 gm	0
Tab16	0.12 gm	0.007	0.16 gm	0
Tab17	0.13 gm	0.003	0.16 gm	0
Tab18	0.12 gm	0.007	0.16 gm	0
Tab19	0.13 gm	0.003	0.16 gm	0
Tab20	0.13 gm	0.003	0.16 gm	0

So, upper % deviation = (Average weight of 20 tablets- upper weight) x 100/Average weight of 20 tablets
In case of generic furosemide tablet upper % deviation = (0.127-0.13) x 100/ 0.127= 2.36 %
In case of branded furosemide tablet upper % deviation = (0.160-0.16) x 100/ 0.160= 0 %
Again, lower % deviation = (Average weight of 20 tablets- lower weight) x 100/Average weight of 20 tablets
In case of generic furosemide tablet upper % deviation = (0.127-0.12) x 100/ 0.127= 5.51 %
In case of branded furosemide tablet upper % deviation = (0.160-0.16) x 100/ 0.160= 0 %

Table5: Hardness of two kinds of frusemide tablets

SlotNo	Hardness of Generic tablets	Hardness of Branded tablets
Slot1	3.40	3.45
Slot2	3.40	3.50
Slot3	3.30	3.60
Average Hardness	3.36	3.52

Table 6: Friability of two kinds of frusemide tablets

Tablet	(Friability of tablets)F=[Wo-Wf/Wo]x100 %
20 generic tablets	0.79 %
20 branded tablets	0.31 %

Here,
Wo of generic tablets = 2.54 gm

Wf of generic tablets = 2.52 gm
Wo of branded tablets = 3.20 gm
Wf of branded tablets=3.19gm

Table7:Disintegration of two kinds of frusemide tablets

Sample	Serial number	Result	Average disintegration time
Branded tablets	Tablet 1	8min 12sec	8min 15sec
	Tablet 2	8min 15sec	
	Tablet 3	8min 17sec	
Generict ablets	Tablet 1	6min 15sec	6min 18sec
	Tablet 2	6min 18sec	
	Tablet 3	6min 20sec	

Table 8 : Dissolution of two kinds of frusemide tablets

Time(min)	FuroA (generic tablet) in %	FuroB(branded tablet) in %
0	0	0
1	23.4	29.6
2	45.9	48.8
3	49.7	54.0
4	53.6	59.8
5	58.8	64.1
6	61.7	67.5
7	65.5	70.8
8	70.8	76.1
9	77.5	81.8
10	85.2	89.0

Identification as per mathod 1

Table 9: Spectrophotometric reading for Branded Tablet

Wave length in nm	Absorbance
228	0.085
271	0.047
333	0.039

So, 271 : 228 =0.047 : 0.085
=0.047 / 0.085
=0.5529 =0.5373

Table10: Spectrophotometric reading for Generic Tablet

Wave length in nm	Absorbance
228	0.067
271	0.036
333	0.028

So,271 : 228 = 0.036 : 0.067
=0.047 / 0.085
=0.036 / 0.067

Average weight	Sample taken	Absorbance
0.16gm=160mg	0.032 gm = 32 mg	0.047

Method No. 2 :- Both kinds of furosemide tablet powder comprising 40mg were mixed with 10 ml of 95% ethanol, shaken, filtered, and then evaporated to dryness. Green turns to deep crimson when 25 mg of the resulting residue is dissolved in 2.5 ml of ethanol (95 percent) and 2 ml of the 4-dimethylamino benzaldehyde reagent is added. Thus the sample changes to deep red which means it contains ultimate quality of furosemide.

Here this identification test of furosemide tablet complies with both generic and branded sample of furosemide.

Table11: Data for For Branded frusemide tablets

Average weight	Sample taken	Absorbance
0.127 gm=127mg	0.02 gm = 20 mg	0.036

Calculation:-
1000 x 0.036 x 250 x 200 x 127 / 100 x 580 x 20 x 5
= 39.4138 mg
=39.4138 x 100 / 40
= 98.53 %

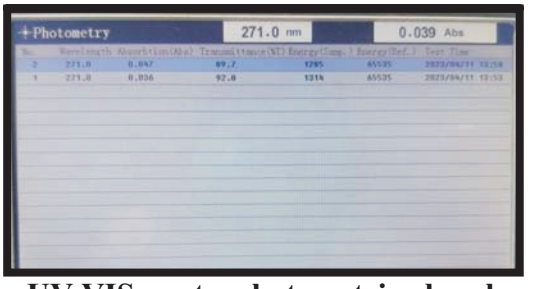

Fig. 7 : UV-VISspectrophotometric absorbance of both kind of furosemide tablets

CONCLUSION

Results from physical testing of both generic and brand-name 40-mg furosemide pills indicated that both were authentic and had not been tampered with. The findings of the other tests used to evaluate the tablets were also within the range established by the Indian Pharmacopoeia. All batches passed the disintegration test since the values obtained for all slots of samples were less than 15 minutes, as specified by the Indian Pharmacopoeial set limit for uncoated tablets (IP, 2022). As a result, the pharmaceutically active component would dissolve within 60 minutes after intake. This, as one would think, would improve the oral absorption of the medicine by a factor of around 20%.This expectedly would aid absorption of the80% drug after oral administration. The branded and generic tablets of furosemide are acceptable as per the result. So we can get therapeutic value from both tablets.

REFERENCES

M. M. Parker, J. A. Hazelzet, and J. A. Carcillo. (2004) Pediatric considerations. Critical Care Medicine. Nov ; 32(11): SUPPL. doi: 10.1097 / 01. CCM. 0000145904. 97821.0D.

Arafat M, Ahmed Z, Arafat O. (2017)Comparison between generic drugs and brand name drugs from bioequivalence and the rmoequivalence prospective. *Int J Pharm Pharm Sci*.;9:1–4.

Benet LZ. (1979)Pharmacokinetics/pharmacodynamics of furosemide in man: a review. J

Pharmacokinetics. Biopharm. 1979;7:1–27.

Arafat M. (2015)Approaches to achieve an oral controlled release drug delivery system using polymers: a recent review. *Int J Pharm Pharm Sci*. ;7:16–21.

Mohamed M, Siaan KAA, Almarzouki A, Anwair MA.(2015) Evaluation of the pharmaceutical quality of some furosemide tablet brands. Int J Pharm Chem Sci. ;4(2):238–246.

Dires T. A (2005) comparative in-vitro evaluation of anti hypertensive drugs products from local market. Addis Ababa University.

Murthy, K. S. Ghebre-Sellassie, I.(1993) Current perspectives on the dissolution stability of solid oral dosage forms. *J. Pharm. Sci.* ; 82: 113–126. DOI: 10. 1002 /jps. 2600820202.

Murtaza, G. Khan, S. A., Naham-ul-Haq, M. Hussain, I. (2014) Comparative evaluation of various solubility enhancement strategies for furosemide. *Pak. J. Pharm. Sci.* ; 27: 963–973.

Lindenberg, M. Kopp, S. Dressman, J. B. (2004) Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *Eur. J. Pham. Biopharm*. 58: 265– 278. DOI: 10.1016/j.ejpb.2004.03.001.

Granero, G. E. Longhi, M. R. Mora, M. J.Junginger. (2010) Biowaiver monograph for immediate release solid oral dosage forms: Furosemide. *J. Pharm. Sci*. ; 99: 2544–2556. DOI: 10.1002/jps.22030.

Qureshi, S. A. McGilveray, I. J. (1998)Assessment of pharmaceutical quality of furosemide tablets from multinational markets. *Drug Dev. Ind. Pharm.* ; 24: 995–1005. DOI: 10.3109/03639049809089943.

Maggio, R. M. Castellano, P. M. Kaufman, T. S. (2008)(A new principal component analysis-based approach for testing "similarity" of drug dissolution profiles. *Eur. J. Pharm. Sci*. 34: 66–77. DOI: 10.1016/j.ejps..02.009.

Yong, K. H. Simionato, L. D. Calvo, R. G. Mattei, M. B. Segall, A. I. (2014)Comparison of dissolution profiles of furosemide tablets available in the Argentinian Market. *J. App. Sol. Chem. Model.* ; 3: 186–193. DOI: 10.6000/1929-5030.2014.03.04.1

Abdelbary G, Prinderre P, Joachim J, Reynier J, Piccerelle P. (2005)Determination of the in vitro disintegration profile of rapidly disintegrating tablets and correlation with oral disintegration. Int. J Pharm. ; 292: 29 – 41.

Anwar S, Fell JT, Dickinson PA .(2005) An investigation of the disintegration of tablets in bio relevant media. Int J pharm. ; 290:121 –7.