ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FLUCONAZOLE AND IBUPROFEN: A REVIEW

Ashutosh Thakur[1], Hemant Kumar Verma[1], Indu[1], Priyankul Palia[1], Dr. Abhishek Soni[1]*

[1]School of Pharmacy, Abhilashi University, Mandi (H.P.), India, 175028

*Corresponding Author- Ashutosh Thakur**

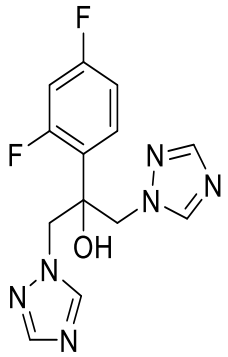
Abstract- The development and validation of analytical methods is an integral aspect of drug discovery and drug substance development in pharmaceuticals. Analytical technique creation and validation are two tasks that are intertwined in the research and development of new therapeutic products, as well as their combination and pharmaceutical quality control. As new medications emerge on a daily basis, the demand for analytical technique development grows, as conventional methods for these compounds are not available in pharmacopoeias. The development of a novel analytical method aids in the enhancement of analysis accuracy, precision, cost, and time consumption. This review article focus on literature findings of method development and validation of Ibuprofen and Fluconazole which will helps in developing new method for the determination of these drugs in bulk and pharmaceutical dosage forms.

Keywords- Ibuprofen, Fluconazole, Method development, HPLC validation.

INTRODUCTION

Fluconazole

Fluconazole is an antifungal drug that is used to treat a range of fungi[1-2]. Candidaasis, blastomycosis, coccidiodomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and pityriasis versicolor are all examples of fungal infections. It's also employed to prevent candidiasis in those who are at high risk, such as those who have had organ transplants, babies born with low birth weight, and persons with low blood neutrophil counts. It's given either orally or by injection into a vein[2].Vomiting, diarrhoea, dermatitis, and elevated liver enzymes are all common adverse effects. Liver issues, QT prolongation, and seizures are all possible serious adverse effects. It should increase the threat of miscarriage during pregnancy, and large doses may cause birth abnormalities [2-3]. Fluconazole reside to the azole antifungal drug class. It is thought to work by altering the fungal cellular level. Fluconazole was first commercially available in 1988 after being patented in 1981[4]. It's listed on the World Health Organization's Essential Medicines List[5]. Fluconazole is a drug that is available as a generic. It was the 182nd most commonly dispensed drug in the United States in 2017, with over three million prescriptions [6-7].



Flg.1- Chemical Structure of Fluconazole

Table.1-Chemical and Physical data

Formula	$C_{13}H_{12}F_2N_6O$
Molar mass	306.271 g·mol^{-1}

Table.2-Pharmacokinetic Data

Bioavailability	>90% (oral)
Protein binding	11–12%
Metabolism	liver 11%
Elimination half-life	30 hours (range 20–50 hours)
Excretion:	kidney 61–88%

Ibuprofen

The Boots Group's research arm developed ibuprofen from propionic acid in the 1960s. Isobutyl (ibu), propionic acid (pro), and phenyl are the three functional groups in the name (fen).It was discovered as a result of 1950s and 1960s research into a safer alternative to aspirin. Stewart Adams led a team that discovered and manufactured the chemical.The medicine was approved as a therapy for rheumatoid arthritis in the UK in 1969, and in the USA in 1974[8]. Ibuprofen is a NSAID that acts as a pain reliever, fever reducer, and inflammation reducer. Menstrual cramps, migraines, and rheumatoid arthritis are examples of such conditions. It can also be used to close the patent ductus arteriosus of a premature baby. It can be administered orally or intravenously[9].When compared with NSAIDs, it may cause a variety of side effects, including gastrointestinal bleeding[10]. It increases the likelihood of developing heart failure, renal failure, and liver failure. At low concentartion, it does not seem to increase the risk of a heart attack[11].

Stewart Adams of Boots UK Limited discovered ibuprofen in 1961, and it was initially marketed under the brand name Brufen. It is listed on the WHO's Essential Medicines List[12.] It's a generic drug that's available [13]. It was the 28th most dispensed drug in the USA in 2017[14-15].

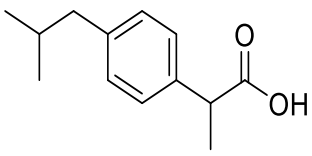


Fig.2- Chemical Structure of Ibuprofen

Table.3-Chemical and Physical data

Chemical Formula	$C_{13}H_{18}O_2$
Molar mass	206.285 g·mol^{-1}

Table.4- Pharmacokinetic Data:

Bioavailability	80–100% (by mouth), 87% (rectal) [16]
Protein binding:	98% [17]
Metabolism:	Liver (CYP2C9)
Metabolites:	ibuprofen glucuronide, 2-hydroxyibuprofen, 3-hydroxyibuprofen, carboxy-ibuprofen, 1-hydroxyibuprofen
Onset of action:	30 min [18]
Elimination half-life:	2–4 h [19]
Excretion:	Urine (95%) [20-21]

METHODS FOR SIMULTANEOUS DETERMINATION OF FLUCONAZOLE AND IBUPROFEN

Shamoushaki et al (2001) revealed the appliance Fluconazole and Ibuprofen Combination: a possible Treatment for Mucosal Candidiasis. Candida species separated from 142 patients with suspected mucosal (oral and vaginal) candidiasis were identified by culture on CHROMagar.Carbohydrate and candida medium assimilation test using the API 20CAUX kitThe broth microdilution method was accustomed evaluate the minimum inhibitory concentration (MIC) of fluconazole alone and together with ibuprofen. Among isolates, 43.7% were identified as Candida species. The very best rate of fluconazole resistance was noticed among C. albicans (50%) isolates. MIC90 of ibuprofen fluconazole combination against C.The MIC90 for Candida albicans isolates was 32 g/mL, which was 8 times below fluconazole alone (MIC90=256 g/mL). Our results expose partial fluconazole resistance among Candida separated from patients with mucosal candidiasis.However, after treatment with the ibuprofen-fluconazole combination, the resistance rate dropped 2.5 times. Further research into the therapeutic potential of the drug combination for the cure of fungal infections is usually recommended [22].

Dinc-Zor et al (2021) simultaneous Determination of Ibuprofen and Additives in Syrup Formulation was improved using an HPLC method. For the development of a fast and efficient HPLC method, multi-response optimization employing the Derringer's desirability function was used to optimise chromatographic separation parameters. For the optimization procedure, the independent variables were 50–60 percent (v/v) for acetonitrile, 5.0–7.0 for pH, and 1.0–2.0 mL/min for mobile phase flow rate. Statistical experimental design was applied to rate the impacts of various variables on the output responses, such as critical resolution between sunset yellow and sodium benzoate and retention time of the last peak indicating method analysis time. For the simultaneous analyses, adequate conditions were acetonitrile:phosphate buffer (60:40, v/v), pH 5.0, and the flow rate of 1.8 mL/min.A light diode detector set at 220 nm was used to monitor the

eluate. The chromatographic analysis took about 3 minutes in total. The suggested approach was successfully used to determine five chemicals in their pharmaceutical formulation [23].

Encarnacao et al (2020) developed and validated a RP-HPLC method for the analysis of paracetamol,olanzapine, ibuprofen and simvastatin during microalgae bioremediation. The method was verified according to the guidelines of the FDA, ICH and Eurachem with respect to system linearity, accuracy, precision, suitability recovery, limits of detection and ruggedness, quantification, selectivity and specificity. The estimated limits of quantitative analysis and detection were, respectively, 0.03 and 0.10 g mL-1 for paracetamol, 0.03 and 0.09 g mL-1 for ibuprofen 0.04 and 0.13 g mL-1 for olanzapine, 0.27 and 0.83 gmL-1 for simvastantin, and 0.05 and 0.14g mL-1 for simvastantin acid. The inter-day and intra-day precision results were within the acceptance limit of related standard deviation (%RSD) of less than 2, and the % recovery was found within the required limits of 80-110%. The developed method is fast, linear, precise, accurate, and robust, and it can be successfully implemented to the measurement of the common pharmaceutical products during microalgae bioremediation [24].

Bashir et al (2020) develop a solid HPLC analysis of fluconazole and voriconazole in rat plasma samples using a phase extraction method. The two broad-spectrum triazole antifungal drugs are fluconazole and voriconazole. The current study describes the method for creating an amino-modified magnetic metal-organic framework. Fluconazole and voriconazole were extracted from rat plasma samples using this substance as a pre-sample treatment sorbent. Different parameters were used to brief the material, which was created using the chemical bonding method. The factors that affect the extraction capacity of the sorbent material were also optimised in this study. Nonspecific interactions were reduced and target drug extraction recoveries were increased in plasma samples due to optimization of solid-phase extraction conditions. For the analysis, the extraction method was combined with the HPLC-UV method. Fluconazole and voriconazole showed excellent linearity (0.1-25 g/mL), detections (0.02, 0.03 g/mL), and quantification limits (0.04, 0.03 g/mL).The maximum recoveries of fluconazole and voriconazole from spiked plasma samples were 86.8 percent and 78.6 percent, respectively, with relative standard deviations of 0.9-2.8 percent and 2.2-3.6 percent. Furthermore, this sorbent material was reused, which was an improvement over single-use commercial sorbent materials. This validated method has the potential to be used for simultaneous drug determination in therapeutic drug monitoring studies as well as routine pharmacokinetic evaluations [25].

Borahan et al (2018.) Simulated gastric phase is a rapid and sensitive reversed phase-HPLC method for determining the presence of ibuprofen and paracetamol in drug samples and their behaviour.Paracetamol is a commonly used fever and pain reliever. Ibuprofen is a commonly used nonsteroidal anti-inflammatory medication. The accurate reversed phase HPLC method was developed in this study for the simultaneous assessment of ibuprofen and PCM. A Phenomenex C18 (250 mm, 4.6 mm, 5 m) column was used to achieve the chromatographic separation. As the mobile phase, 50 milli molar phosphate buffer (pH 7.5) and methanol were used. Paracetamol and ibuprofen had retention times of 5.7 and 10.4 minutes, respectively. The developed method's linearity was set at 0.25 – 250 mg/L, with a correlation coefficient of 0.9998.

Ibuprofen had a limit of detection/quantification value of 0.06/0.19 mg/L and paracetamol had a limit of detection/quantification value of 0.08/0.26 mg/L. The method was used on drug samples such as tablets and suspensions. The calculated concentrations corresponded to the values claimed in their prospectus. The drug samples were tested under simulated gastric conditions to see how the analytes behaved in the human body. Throughout the 210-minute period, there was no change in the retention time of the analyte peak shapes [26].

Han et al (2017) HPLC Method for Simultaneous Determination of Ibuprofen and 17 Related Compounds was developed and validated. For the simultaneous determination of ibuprofen and 17 related compounds, a reversed-phase high-performance liquid chromatography method was developed and validated (chemical process impurities and degradation products) This method could be used to ensure the quality of ibuprofen-containing substances. A variety of chromatographic parameters (column, flow rate, temperature, wavelength, gradient elution, buffer solution, and pH) were assessed. The Agilent ZORBAX Eclipse Plus C18 (250 4.6 mm, 5 m particle size) column at 40 °C was chosen for its separation efficiency and robustness.The column was eluted with a gradient of 10 mM sodium phosphate buffer at pH 6.9 as mobile phase A and acetonitrile as mobile phase B at 1.0 mL min1. The wavelength of the ultraviolet detector was set to 214 nm.According to ICH, guidelines, this method was validated for its system suitability, specificity, linearity, precision, accuracy, sensitivity, robustness, and sample stability. This method was used to examine seven batches of ibuprofen drug products from various manufacturers [27].

El-Fatatry et al (2016) Validated Enantioselective HPLC Method for Determining Ibuprofen Enantiomers in Bulk and Tablet Form It was created a new chiral reversed-phase (RP)-HPLC method with UV detection.Enantioselective resolution of ibuprofen (IBP) was achieved using (3R,4S) -4-(3,5-dinitrobenzamido) -3-(3-(trioxysilyl)-propyl) -1,2,3,4-tetrahydro phenanthrene [(R,R)-Whelk-O2] chiral stationary phase (4.6 mm id 250 mm, 10 m) was tested using a diode array detector at 220 nm with an ethanol–water (30 + 70, v/v) mobile phase containing 100 mM ammonium acetate at a flow rate of 1.3 mL/min.Calibration curves for both IBP enantiomers were linear over the concentration range of 20–180 g/mL.Dexibuprofen (dex-IBP) and levoibuprofen (levo-IBP) had mean percent recoveries SD of 99.74 1.73 and 99.60 0.93, respectively. Intra- and interday precision, expressed as RSD, was not greater than 1.66 percent for dex-IBP and 1.93 percent for levo-IBP.The detection limits for dex-IBP and levo-IBP were 2.09 and 2.06 g/mL, respectively. The method was successfully used to determine dex-IBP in tablet dosage form [28]

Jun et al (2015).developed a method for HPLC simultaneous analysis of ibuprofen and pamabrom Using the same conditions, a selective and simple HPLC method for ibuprofen (IBF) and pamabrom (PAMB) from in bulk and drug product was developed, which was operated on a reverse phase (C18 column) with water (60%) and acetonitrile (40%) solutions at a flow rate of 1.0 mL/min.At 254 nm, IBF was detected at 2.6 minutes and PAMB at 8.0 minutes. In the ranges 125–2000 and 63–100 lg/mL, the correlation coefficient (R2) for IBF was 0.9999 and 0.9998 for PAMB. The intraday precision was less than 1.0 percent RSD. This method was validated in terms of linearity, precision, accuracy, detection limit, and quantitation limit.This method was proposed for the analysis of the fixed dose combination tablet of IBF and PAMB [29].

Davtyan et al (2015) created and validated The intracellular concentration of fluconazole can be determined using a simple RP-HPLC method. A simple and sensitive method based on RP-HPLC was developed to determine FLC intracellular concentration in Candida albicans using tinidazole as an internal standard. Following dichloromethane extraction, chromatographic separation was performed on a Machery-Nagel EC250/2 Nucleodur-100-3 C18 column using gradient elution with a mobile phase of (A) 0.01 M ammonium acetate buffer, pH = 5.00, and (B) acetonitrile. Analytical parameters such as linearity, precision, accuracy, LOQ and robustness were evaluated. At concentrations ranging from 100 to 10000 ng/ml, the method was linear (r = 0.9999). The intraday and interday precisions (relative standard deviation) were both less than 2.79 percent and 2.64 percent, respectively, and the accuracy (relative error) was less than 2.82 percent. The extraction recovery rate ranged between 79.3 and 85.5 percent.The dependable method was successfully applied to an azole-resistance study of C. albicans, and intracellular FLC concentrations were discovered to correlate with a yeast drug susceptibility profile and MIC values. [30]

Eraga et al (2015) developed an HPLC method to analyse the ten ibuprofen brands following official protocols, the drug samples were tested for uniformity of friability, weight, melting point, crushing strength, disintegration, and dissolution. The UV and HPLC methods were used to determine the ibuprofen content.The crushing strength of the drug samples ranged between 6 and 16 kP, while the disintegration time for uncoated tablets ranged between 7.43 and 10.40 min and 3.25–37.32 min for coated tablets. Recrystallized ibuprofen had melting points ranging from 73.5 to 76.0°C and friability values of less than 1%. The amount of ibuprofen released within one hour ranged between 18 and 102 percent, and all brands passed the HPLC test, while two brands failed the active ingredient content in the UV method of assay. The pharmaceutical quality of ibuprofen (400 mg) tablets marketed in Benin City, Niger, varies [31].

Sahoo et al (2015) Response Surface Methodology was used to develop a Chemometric Approach for RP-HPLC Determination of Azithromycin, Secnidazole, and Fluconazole. They demonstrate a chemometric-assisted RP-HPLC method for the simultaneous determination of azithromycin, secnidazole, and fluconazole at a flow rate of 1.2 mL min1 using water: methanol (63:37 percent v=v) as mobile phase.The three drugs were separated on an Xterra RP-18 column (250 4.6 mm ID, 5 mM), and the eluents were detected on a diode array detector at 210 nm wavelength. Using a central composite design, the combined effects of percent methanol and flow rate were investigated and optimised. The percent relative standard deviations for precision study were in the range of 0.2–0.7% and 0.5–1.2% for standard and formulation, respectively, under optimal conditions.The limits of detection for azithromycin, secnidazole, and fluconazole were 12.5, 0.62, and 1.7 mg mL1, respectively, and the limits of quantification were 37.5, 1.86, and 5.1 mg mL1. As a result, the method was applied to direct determination of their presence in commercial formulations (combi-kit) [32].

Belal et al (2014) developed Derivative Spectrophotometric Methods and Micellar HPLC Methods for Determination of Fluconazole and Tinidazole in Biological Fluids and Pharmaceuticals .The concentrations of fluconazole (FLZ) and tinidazole (TNZ) in combined pharmaceutical dosage forms were determined using micellar HPLC and first derivative ultraviolet spectrophotometry..The derivative procedure for FLZ

and TNZ is based on a linear relationship between first derivative amplitude and drug concentration at 220 and 288 nm, respectively. FLZ and TNZ calibration graphs were linear in the 1.5–9.0 mg/mL and 10.0–60.0 mg/mL ranges, respectively. In addition, an HPLC procedure with ultraviolet detection at 210 nm was created. Using an ODS C18 column, the HPLC procedure achieved good chromatographic separation. The mobile phase containing 0.15M sodium dodecyl sulphate, 0.3 percent triethylamine, and 12 percent n-propanol in 0.02M orthophosphoric acid at pH 5.5 was pumped at a flow rate of 1 mL/min.As an internal control, indapamide was used.Over concentration ranges of 1.5–30.0 and 10.0–200.0 mg/mL, the method demonstrated good linearity, with detection limits of 0.36 and 2.70 mg/mL for FLZ and TNZ, respectively, and quantification limits of 1.1 and 8.2 mg/mL.The methods proposed for simultaneous drug analysis in laboratory prepared mixtures, single dosage forms, and co-formulated tablets were successfully applied. The second method was also extended to detect drugs in biological fluids [33].

Singh et al (2014) developed and validated new HPLC methods for estimating fluconazole in various biological fluid simulations Chromatography was performed on an aoctadecylsilane (ODS-3) Hypersil C18 column (250 mm 4.6 mm 5 lm) with water (pH 5.2, adjusted with orthophosphoric acid) and acetonitrile (80:20, v=v) mobile phase at a flow rate of 2.5 mL/min and detection at 260 nm.In GFS, VFS, PB, PBS, and methanol, the calibration curve of standard FLZ was linear in the ranges 0.2–40 lg=mL, 0.1–40 lg=mL, 0.2–40 lg=mL, 0.1–40 lg=mL, and 0.2–50 lg=mL.All of the methods were validated for intra- and inter-day linearity, precision, accuracy, and specificity, and they met the International Conference on Harmonisation (ICH) and United States Food and Drug Administration (USFDA) guidelines [34.]

Jebaliya et al (2013) developed and validated fluconazole analysis method using HPLC and UPLC The most straightforward stability indicating reversed phase.Isocratic HPLC and UPLC methods for determining fluconazole in bulk and solid pharmaceutical dosage form have been developed and validated. SunFire C18 (250 4.5 mm, 5 m particle size) and BEH C18 (100 2.1 mm, 1.7 m particle size) columns were used for HPLC and UPLC, respectively..For HPLC, the mobile phase was Methanol:Water (70:30) and for UPLC, Methanol:Water (55:45 v/v). The isocratic flow rates for HPLC and UPLC were set to 1 mL/min and 0.30 mL/min, respectively.Both the HPLC and UPLC systems were detected at 211 nm with a column oven temperature of 30°C (good elution was obtained at 30°C) and injection volumes of 2 L and 20 L, respectively [35].

Correa et al (2012) HPLC and Derivative UV Spectrophotometry were used to develop and validate a Dissolution Test for Fluconazole Capsules. HPLC and UV first derivative spectrophotometry (UV-FDS) techniques were chosen for quantitative evaluation. Several conditions were evaluated during the development of the release profile.When the most discriminative release profile for fluconazole capsules was obtained, the dissolution test parameters were deemed appropriate. Fluconazole capsules were dissolvable in 900 mL of 0.1 M HCl at 37 0.5 C in baskets spinning at 50 rpm for 30 minutes.According to ICH and USP requirements, the developed HPLC and UV-FDS methods for antifungal evaluation were selective and met the requirements for an appropriate and validated method. Both methods can be useful in

the registration or renewal of new drugs.Cost, simplicity, equipment, solvents, speed, and application to large or small workloads should all be considered when performing routine analysis [36].

Nyola et al (2012) developed a method for RP-HPLC Simultaneous Estimation of Ibuprofen and Famotidine in Pure and Combination Dosage Form A new simple, accurate, precise, and reproducible RP-HPLC method for the simultaneous estimation of ibuprofen and famotidine in tablet dosage forms was developed using a C18 column (Phenomenex, 250 x 4.6 mm, 5 m) in isocratic mode. The mobile phase was made up of 70:20:10 (v/v/v) Methanol, Water, and Phosphate buffer.The detection wavelength was 284 nm, and the flow rate was 1.0 ml/min. The retention times for ibuprofen and famotidine were 3.6 and 7.8 minutes, respectively.For ibuprofen 2-10 g/ml and famotidine 2-10 g/ml, the method was linear over the concentration range.Ibuprofen and famotidine recoveries were found to be 99.037-100.766 percent and 99.703-100.433 percent, respectively. In accordance with ICH guidelines, the method was validated. The described HPLC method was used successfully to analyse pharmaceutical formulations containing combined dosage form [37].

Pina-Vaz et al (1999) Ibuprofen has been shown to have antifungal activity against Candida species, both alone and in combination with fluconazole. Ibuprofen, a nonsteroidal anti-inflammatory drug, was found to have antimicrobial activity against Candida albicans and non-albicans strains. At 10 mg/ml, ibuprofen had a rapid cidal effect on Candida albicans in the exponential growth phase, accompanied by intracellular K+ leak.These findings imply that Candida cells die as a result of direct damage to the cytoplasmic membrane. At 5 mg/ml, ibuprofen inhibited growth but did not kill the yeasts and had no effect on the cytoplasmic membrane. The fluorescent probe FUN-1 was used to assess yeast metabolic vitality, revealing that fungistatic drug concentration-induced growth inhibition was associated with metabolic changes.When ibuprofen and fluconazole were combined, eight of the twelve Candida strains tested positive for synergic activity, including four of the five fluconazole-resistant variants. Fluconazole's MICs for fluconazole-resistant microorganisms were reduced by 2-128 times when combined with ibuprofen. When ibuprofen was combined with all 12 strains studied, MICs for ibuprofen dropped by up to 64-fold.These findings suggest that ibuprofen, alone or in combination with azoles, could be useful in the treatment of candidosis, particularly when administered topically and utilising the drug's antifungal and anti-inflammatory properties [38].

CONCLUSION

In this review article various published analytical methods and their validation for determination of presence of Ibuprofen and Fluconazole either single or in combination with other drugs are reported. These methods are valid, accurate, precise, reliable and reproducible as per validation parameters. New analytical method could be developed from these methods by changing the chromatographic conditions.

REFERNCES

1. Surov AO, Voronin AP, Vasilev NA, Churakov AV, Perlovich GL. Cocrystals of fluconazole with aromatic carboxylic acids: competition between anhydrous and hydrated solid forms Crystal Growth & Design. 2019 Dec 6;20(2):1218-28.

2. Trivedi SS, Lodhi MG, Doifode C A, Raut H. Anti Leishmanial Activities of Some Antidepressant Drugs And Antifungal Drugs. Journal of Advance Research in Pharmacy & Biological Science. 2017 Apr 30;3(4):01-16.

3. US Food and Drug Administration. Fluconazole (Diflucan): Drug Safety Communication-FDA Evaluating Study Examining Use of Oral Fluconazole (Diflucan) in Pregnancy, Safety Alerts for Human Medical Products. Safety Alerts for Human Medical Products. https://www. fda. gov/Safety/MedWatch/Published. 2016 Apr.

4. Heravi MM, Zadsirjan V. Prescribed drugs containing nitrogen heterocycles: an overview. RSC Advances. 2020;10 (72):44247-311.

5. World Health Organization, World Health Organization model list of essential medicines: 21st list 2019. World Health Organization; 2019.

6. Suresh BK, Anusha N, Sravani AB. Analytical method development and validation for the estimation of Mirabegron in pure and its solid dosage form by UV spectrophotometric method. International Journal of Research in Pharmaceutical Sciences and Technology. 2020 Jul 20;1(4):146-50.

7. Fluconazole - Drug Usage Statistics. ClinCalc. Retrieved 11 April2020.

8. Halford GM, Lordkipanidzé M, Watson SP. 50th anniversary of the discovery of ibuprofen: an interview with Dr Stewart Adams. Platelets. 2012 Sep 1;23(6):415-22.

9. Nalini CN. Method Development and Validation for the Simultaneous Estimation of Ascorbic acid, Phenylephrine HCl, Paracetamol and Levocetirizine HCl using RP-HPLC. Research Journal of Pharmacy and Technology. 2020 Apr 30;13(4):1911-6.

10. British Medical Association, Royal Pharmaceutical Society of Great Britain. British National Formulary, London. William Clowes, Beccles, Suffolk. 2014.

11. Chemistry in your cupboard Nurofen. Archived from the original on 5 June 2014.

12. World Health Organization. World Health Organization model list of essential medicines: 21st list 2019.

13. Pishnamazi M, Zabihi S, Jamshidian S, Borousan F, Hezave AZ, Shirazian S. Thermodynamic modelling and experimental validation of pharmaceutical solubility in supercritical solvent. Journal of Molecular Liquids. 2020 Dec 1; 319:114120.

14. Gómez-Oliván LM, editor. Non-Steroidal Anti-Inflammatory Drugs in Water: Emerging Contaminants and Ecological Impact, Springer Nature; 2020.

15. Wong AY, MacKenna B, Morton CE, Schultze A, Walker AJ, Bhaskaran K, Brown JP, Rentsch CT, Williamson E, Drysdale H, Croker R. Use of non-steroidal anti-inflammatory drugs and risk of death from COVID-19: an OpenSAFELY cohort analysis based on two cohorts, Annals of the rheumatic diseases. 2021 Jul 1;80(7): 943-51.

16. Davanzo R, Bua J, Paloni G, Facchina G. Breastfeeding and migraine drugs. European journal of clinical pharmacology. 2014 Nov;70(11):1313-24.

17. Davies N M., Clinical pharmacokinetics of ibuprofen. Clinical pharmacokinetics. 1998 Feb;34(2):101-54.

18. Kelly LE, Sommer DD, Ramakrishna J, Hoffbauer S, Arbab-Tafti S, Reid D, Maclean J, Koren G. Morphine or ibuprofen for post-tonsillectomy analgesia: a randomized trial. Pediatrics. 2015 Feb 1;135(2):307-13.

19. Grosser T, Ricciotti E, FitzGerald G A. The cardiovascular pharmacology of nonsteroidal anti-inflammatory drugs. Trends in pharmacological sciences. 2017 Aug 1;38(8):733-48.

20. Brufen Tablets and Syrup, Therapeutic Goods Administration. 31 July 2012, Archived from the original 2016.

21. Ibuprofen Pregnancy and Breastfeeding Warnings. Drugs.com. Archived from the original on 2017.

22. Shamoushaki T, Fozouni L. Fluconazole and Ibuprofen Combination: A Potential Treatment for Mucosal Candidiasis. Medical Laboratory Journal. 2020 Sep 10;14(5):25-9.

23. Dinç-Zor Ş, Aksu Dönmez Ö. Box-Behnken Design-Desirability Function Approach in Optimization of HPLC Method for Simultaneous Determination of Ibuprofen Along with Additives in Syrup Formulation. Journal of AOAC International. 2021 Jan;104(1):78-83.

24. Encarnac¸aoT, Aguiar A, AtiaPalito C, Pais AACC, Campos MG, Sobral AJFNL, Burrows HD. Development and validation of a RPHPLC method for the simultaneous analysis of paracetamol, ibuprofen, olanzapine, and simvastatin during microalgae bioremediation. MethodsX. 2020.

25. Bashir K, Chen G, Han J, Shu H, Cui X, Wang L, Li W, Fu Q. Preparation of magnetic metal organic framework and development of solid phase extraction method for simultaneous determination of fluconazole and voriconazole in rat plasma samples by HPLC. Journal of Chromatography B. 2020 Sep 1; 1152:122201.

26. Borahan T, Unutkan T, Şahin A, Bakırdere S. A rapid and sensitive reversed phase HPLC method for simultaneous determination of ibuprofen and paracetamol in drug samples and their behaviorsin simulated gastric conditions. Journal of separation science. 2019 Feb;42(3):678-8.

27. Han Z, Lu L, Wang L, Yan Z, Wang X. Development and validation of an HPLC method for simultaneous determination of ibuprofen and 17 related compounds. Chromatographia. 2017 Sep;80(9):1353-60.

28. El-Fatatry HM, Mabrouk M M, Hammad SF, E l-Malla S F. A validated enantioselective HPLC method for determination of ibuprofen enantiomers in bulk and tablet dosage form. Journal of AOAC International. 2016 May 1;99(3):604-11.

29. Jun H S, Kang J S, Park J S, Cho C W. Simultaneous analysis of ibuprofen and pamabrom by HPLC. Journal of Pharmaceutical Investigation. 2015 Dec;45(6):555-60.

30. Davtyan TK, Melikyan LA, Nikoyan NA, Aleksanyan HP, Grigoryan N G. Development and validation of Simple RP-HPLC Method for intracellular determination of fluconazole concentration and its application to the study of Candida albicans azole resistance. International journal of analytical chemistry. 2015 Dec 9;2015

31. Eraga SO, Arhewoh MI, Chibuogwu RN, Iwuagwu MA. A comparative UV− HPLC analysis of ten brands of ibuprofen tablets. Asian Pacific Journal of Tropical Biomedicine. 2015 Oct 1;5(10):880-4.

32. Sahoo DK, Sahu PK. Chemometric approach for RP-HPLC determination of azithromycin, secnidazole, and fluconazole using response surface methodology. Journal of Liquid Chromatography & Related Technologies. 2015 Apr 3;38(6):750-8.

33. Belal F, Sharaf El-Din MK, Eid MI, El-Gamal RM. Micellar HPLC and derivative spectrophotometric methods for the simultaneous determination of fluconazole and tinidazole in pharmaceuticals and biological fluids. Journal of chromatographic science. 2014 Apr 1;52(4):298-309.

34. Singh A, Sharma PK, Majumdar DK. Development and validation of new HPLC-methods for estimation of fluconazole in different simulated biological fluids: a comparative study. Journal of Liquid Chromatography & Related Technologies. 2014 Feb 25;37(4):594-607.

35. Jebaliya H, Patel M, Jadeja Y, Dabhi B, Shah A, A comparative validation study of fluconazole by HPLC and UPLC with forced degradation study. Chromatography Research International. 2013.

36. Corrêa JC, Duarte Vianna-Soares C, Salgado HR. Development and validation of dissolution test for fluconazole capsules by HPLC and derivative UV spectrophotometry. Chromatography Research International. 2012 May 14;2012.

37. Nyola N, Jeyabalan GS. Simultaneous estimation of ibuprofen and famotidine in pure and combination dosage form by RP-HPLC. Journal of Applied Pharmaceutical Science. 2012 May 1;2(5):79.

38. Pina-Vaz C, Sansonetty F, Rodrigues AG, Martinez-De-Oliveira J, Fonseca AF, Mårdh PA. Antifungal activity of ibuprofen alone and in combination with fluconazole against Candida species. Journal of medical microbiology. 2000 Sep 1;49 (9):831-40.