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Research Article

Studies of Food & Drug Interaction with Leflunomide

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Abstract

Leflunomide is a well-known rheumatoid arthritis drug. The study's main objective was to evaluate the food and drug interaction studies of leflunomide/visible spectrophotometer technology. A previously developed selective, observant, and exact UV spectroscopic approach for quantitative analysis of leflunomide was applied here. Stock solutions of 1 mMole (0.027 g dissolved in 100ml volumetric flask in methanol) were prepared and diluted to concentration ranges of 0.01 to 0.055 mMole using the same solvent for the leflunomide assay. 50 g mL^{-1} solutions of leflunomide were prepared to study the potential interaction between leflunomide and fruit juices (apple, orange, and grey fruit). Individually, these solutions were diluted in an equimolar ratio and maintained on a water bath at 37±5°C for three hours. The UV/visible spectrophotometer was being used to evaluate these solutions. Although leflunomide and juices remained constant throughout the experiment, the interaction spectra of all juices with the drug showed that the availability of leflunomide was changed in the presence of all juices. In methanol, leflunomide used to have a maximum absorbance in the ultraviolet region at (259nm), but after interaction with fruit juices (apple, orange, and grey fruit), complex formation was indicated by a change in spectrum shape, pattern, and maximum absorbance with an increase in percentage availability (113.90 -139.64%) as the interaction began, λmax shifted to 271 nm. There was a clear interaction between leflunomide and these juices that could change the drug's pharmacological effects. Leflunomide's maximal absorbance in methanol was found to be 259 nm, its spectra changed following interaction with fruit juices, indicating that a complex was formed. Co-administration of these medications should be avoided, as a result.

Keywords

Leflunomide, Methanol, Food-drug Interaction, Absorbance Maxima, Fruit Juices

1. Introductions

Drug release, absorption, distribution, metabolism, and/or excretion can all be affected by the simultaneous intake of both food and medication. Therefore, food-drug interactions are one of the biggest obstacles to oral drug delivery. In contrast to the wide range of reasons for pharmacokinetic (PK) food-drug interactions, pharmacodynamic (PD) food-drug interactions are caused by the interaction of certain drugs with

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certain beverages and foodstuffs. Food-drug interactions and their pharmacokinetic processes have been extensively studied in recent years. Every intake of food or drink changes the physiological conditions in the human gastrointestinal tract. Different foods and drinks affect the processes of drug absorption, distribution, metabolism, and/or elimination. Understanding these processes is important in order to be able to predict and avoid food-drug interactions. Several research groups have looked into a variety of factors that potentially result in food-drug interactions. Food-drug interactions can be classified as pharmacokinetic (PK) or pharmacodynamic (PD) based on the results of these studies. Several research groups have looked into a variety of mechanisms that may play a role in food-drug interactions. Based on these findings, a distinction can be made between food-drug interactions that are pharmacokinetic (PK) and/or pharmacodynamic (PD) [2]. A specific interaction between a drug and a component of the food results in a distinct pharmacological effect, which is known as a pharmacodynamic food-drug interaction. The "cheese response," which is caused by the mediation of tyramine, a component of cheese or uncooked sausages, and inhibitors of the enzyme monoaminoxidase such as tranylcypromine, is a well-known example of this sort of food-drug interaction [3]. Leflunomide (LEF) [N-(4-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide] is the new non-steroidal anti-inflammatory derivative. Figure 1 shows the chemical composition of Leflunomide [4].

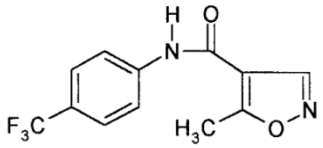

Figure 1. *The chemical structure of Leflunomide.*

Several methods for leflunomide determination and quantification of raw materials, tablets, and serum have been developed and validated previously. A mixture of acetonitrile, methanol, and water (40: 20: 20) was used by Pawinski as the mobile phase. A high column temperature (70 ℃) was necessary for the procedure [5]. Previously, the drug-drug interaction of levocetirizine (5 mg tablet) and atenolol (100 mg tablet) was investigated, with both drugs being introduced to the dissolution apparatus in simulated gastric juice (pH 1), pH 4, pH 7.4, and pH 9 at 37 ℃ at zero time, and the absorbance maxima of both drugs at the corresponding wavelength being measured. At each set of experiments, graphs were generated for drug availability percentage (%) vs. time. In the presence of atenolol, the availability percentage (%) of levocetirizine in buffers with pH mimicking gastric pH 4, pH 7.4, and pH 9 was 436.78 percent, 376.90 percent, and 436.78 percent, respectively [6]. Another study evaluated health claims data on prescription drugs from a nationwide database to analyze potential DDIs (drug-drug interactions). The Lexi-Interact Module was used as the interaction reference source. A logistic regression model was used to assess the impact of patient-specific variables on the risk of potentially clinically meaningful DDIs [7]. Since hydroxychloroquine has previously been widely used with different indications and its safety has been demonstrated in humans, it was commonly used around the globe, including in Turkey, for treating potentially fatal cases of COVID-19, and it is still being used in many countries. However, in the eight months following the onset of the outbreak, published studies on the effect of hydroxychloroquine/chloroquine in cases of SARS-CoV-2, its place in COVID-19 treatment processes, and, in particular, its undesirable cardiotoxic effects in cases of COVID-19 have necessitated a re-evaluation of hydroxychloroquine's role in treating this disease [8, 9]. A flow injection analysis (FIA) of leflunomide via UV-detection is described in another publication. An aqueous solution of ethanol (25 percent, v/v) was shown to be the best carrier solvent. The optimal parameters for determining leflunomide were a flow rate of 0.8 mL/min 21 and a detection wavelength of 260 nm. The proposed approach was tested in pharmaceutical tablets containing leflunomide, and excellent results were obtained. UV spectrophotometry findings were compared to the results produced by this method. There was no significant variance between the methods [10]. The UV spectrophotometric method is used to study possible interactions such as drug-drug interaction, drug-food or drug-metal interactions, when co-administered simultaneously.

To the best of our knowledge, there is no study on the determination of Leflunomide in pharmaceutical preparations, and no reports have been published concerning the interaction of food and nutrients with this drug using UV–spectrophotometry. This research aims to study the food-drug interaction of Leflunomide and to evaluate the possible food-drug and drug-drug interactions of Leflunomide by using a UV-spectrophotometer.

Previous studies have extensively reported the mechanisms of food–drug interactions and their pharmacokinetic implications [1]. Recent pharmacogenetic and epigenetic investigations have highlighted genetic and methylation-site interactions influencing leflunomide response in rheumatoid arthritis patients [11]. Therapeutic drug monitoring studies emphasize the importance of monitoring leflunomide levels to optimize immunosuppressive therapy and minimize toxicity [12]. Experimental studies have demonstrated leflunomide-induced metabolic effects, including weight loss mediated through alterations in amino acid synthesis pathways [13]. Other investigations have evaluated the biological effects of leflunomide–metal complexes, reporting changes in ROS, TNF, and neurotransmitter levels in rheumatoid arthritis models [14]. Pharmacokinetic studies have also assessed herb–drug interactions involving leflunomide, highlighting the need for caution during co-administration with herbal products [15]. Toxicological studies have reported leflunomide-associated nephrotoxicity mediated via TGFβ-related signaling pathways [16]. Additionally, recent reviews have summarized updated knowledge

on food and drug interactions in rheumatoid arthritis therapy, reinforcing the clinical relevance of interaction studies [17].

2. Materials and Method

2.1. Chemicals

The standard Leflunomide from Sigma was obtained (St. Louis, MO, USA). The preparation of prescription tablets for Aravaw, a result of Aventis Pharma A.S. (Istanbul, TR), was obtained from a nearby drug store containing 20 mg of active material. Other chemicals were of an analytical quality, and Merck Co. supplied them (Darmstadt, G). Using all Pyrex glass devices, distilled water and ethanol used for the preparation of the solutions were produced in our laboratory.

2.2. Apparatus

UV spectrophotometer was performed by a system consisting of a model (SPECORD 200 PLUS) for the determination of leflunomide interaction with food, a model (HH-4D) thermostat water bath container for providing constant temperature throughout the experiment, a model (GS-DS120) sonication for dissolving the drug, and a weighing machine of a model number (ADV220) for weighing the drug.

2.3. Preparation of the Solutions

The best solvent used for these experiments was an aqueous solution of 250 ml of solvent in which 20% methanol (200ml) and 80% water (800ml) was made in a volumetric flask. 100ppm Stock solution of leflunomide was prepared, in which 0.01g of the drug was taken. The necessary dilutions were made from that solvent and stock solution for precision, linearity, and consistency studies.

2.4. Analysis of Pharmaceutical Formulation

Ten Arava tablets of 20 mg leflunomide were weighed in a weighing machine, the total weight of each tablet was calculated, and then ground in a mortar and pestle. A sufficient amount of drug powder equivalent to the average weight of the content of the tablet was accurately weighed, and 20% methanol was added to dissolve the active material. It was sonicated for 3 minutes in a sonicator.

2.5. Calibration Curve Studies

For the leflunomide assay, stock solutions of 1 mMole (0.027 g was dissolved in 100ml volumetric flask in methanol) were prepared and diluted to a concentration range of 0.01 to 0.055 mMole using the same solvent. A pop-up scan of stock solutions was run in the region of 200-700 nm against the reagent blank. The λ_{max} was observed and recorded at 259 nm. Calibration curve of absorbance against concentration, obeyed Beer's law, Figure 2 was plotted, and molar absorptivities were also calculated from these observations.

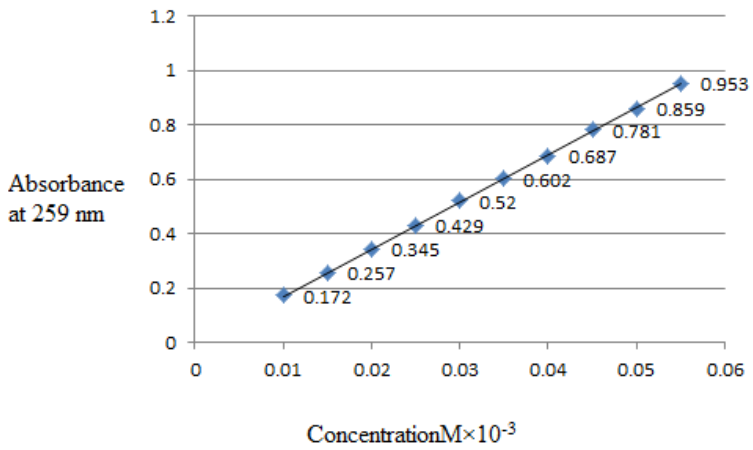

Figure 2. *Reference standard of leflunomide in methanol.*

2.6. Leflunomide Interaction Studies with Fresh Juices

To study the possible interaction of leflunomide with the mentioned juices, we also ran leflunomide and juices as controls under the same environment (i.e., for three hours at 37 ± 5 ℃). It has been observed that leflunomide and juices remain stable throughout the experiment (Table 1, Figure 3, & Table 2), but interaction spectra of all juices with the drug show that the availability of leflunomide was affected in the presence of

all juices.

Table 1. Quantitation of leflunomide-metal complexes by UV/visible spectrophotometric method.

Drug/juice complex	λmax (nm)	Absorbance (nm) Average
Leflunomide	259	0.28644
Leflunomide- grey fruit juice	271	1.806
Leflunomide- apple juice	271	0.77675
Leflunomide- orange juice	271	1.916

Table 2. UV-visible leflunomide spectra and leflunomide juice spectra after interaction.

TIME	ABSORBANCE (nm) leflunomide	Grey fruit	Apple	Orange
0 min	0.2864	1.57	0.724	1.67
15 min	0.2866	1.58	0.73	1.79
30 min	0.288	1.59	0.77	1.88
1 hr	0.2859	1.6	0.792	1.99
1.5 hrs	0.28	1.64	0.788	1.99
2 hrs	0.2863	1.66	0.8	2.06
2.5 hrs	0.2863	1.6	0.8	1.99
3 hrs	0.2861	1.59	0.81	1.96

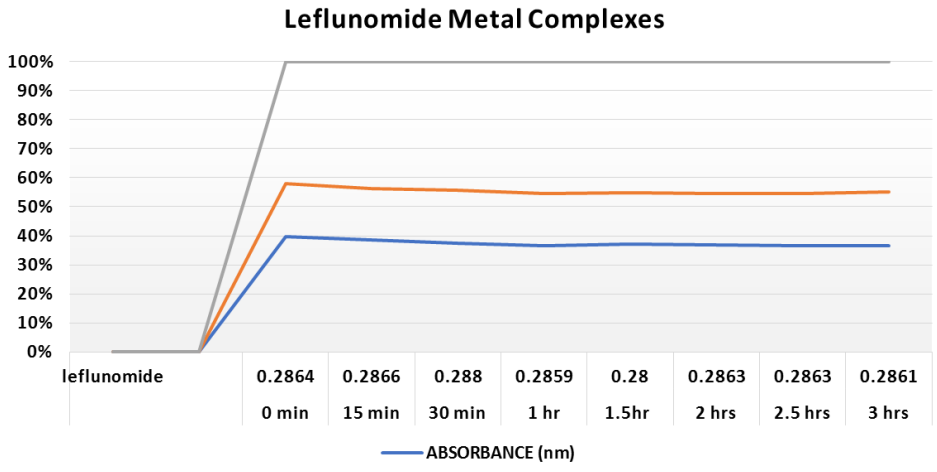

Figure 3. Leflunomide-metal complexes by UV/visible spectrophotometric method.

3. Result

Leflunomide gave maximum absorbance only in the ultraviolet region at 259 nm in methanol but after interaction with fruit juices (apple, orange and grey fruit), complex formation was indicated by the change in spectrum shape, pattern, and maximum absorbance with an increase in percentage availability (113.90 - 139.64%); As the interaction started, lambda max shifted towards 271 nm. In case of leflunomide-grey fruit interaction, maximum interaction takes place at 2 hours, with

the rise in absorbance from 0.286nm to 1.806 nm. In case of leflunomide-apple interaction, maximum interaction takes place at 1 hr with the rise in absorbance from 0.286nm to 0.776 nm. In case of leflunomide-orange interaction, maximum interaction takes place at 2 hours with the rise in absorbance from 0.286nm to 20.6 nm as shown in Figure 4. All this evidence showed the clear interaction of these juices with leflunomide, which can alter the drug's pharmacological properties too.

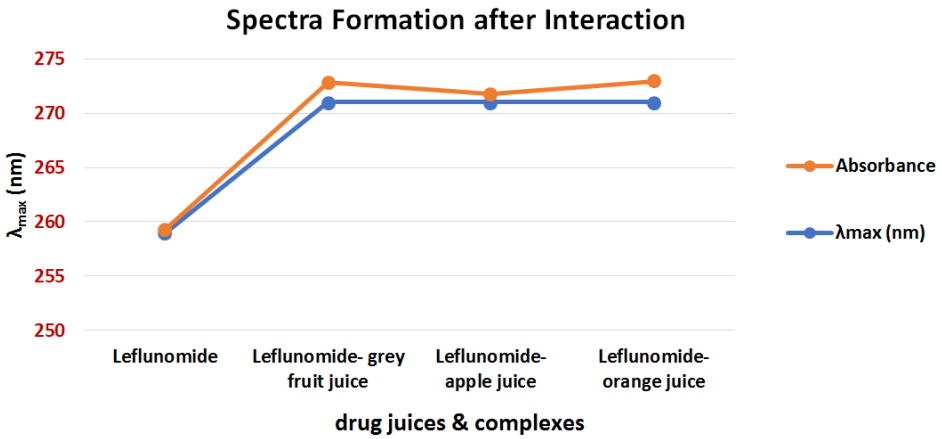

Figure 4. *Formation of spectra after the interaction of leflunomide and leflunomide juices.*

4. Discussion

UV/visible spectrophotometer technique, being of its simplicity, holds a very key place in the field of analytical chemistry, even to analyse dosage formulation's constituents. A selective, perceptive, hasty, and precise UV spectroscopic method, which was developed previously for quantitative analysis of leflunomide, was used here. This method does not involve tedious sample preparation and is employed for studies.

5. Conclusion

Based on this study, it is concluded that the interaction of leflunomide with fruit juices (apple, orange, and grey fruit) results in complex formation, as indicated by changes in the spectrum shape, pattern, and maximum absorbance, with an increase in percentage availability. Therefore, co-administration of this drug should be avoided.

Abbreviations

UV	Ultraviolet
PD	Pharmacodynamic
PK	Pharmacokinetic
DDI	Drug–Drug Interaction
FIA	Flow Injection Analysis
LEF	Leflunomide
mMole	Millimole
ppm	Parts per Million
λmax	Maximum Wavelength
nm	Nanometer
°C	Degree Celsius
v/v	Volume by Volume
MO	Methanol
H_2O	Water
pH	Potential of Hydrogen

Conflicts of Interest

There are no conflicts of interest.

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