Asian Journal of Water, Environment and Pollution, Vol. 19, No. 2 (2022), pp. 17-23.
DOI 10.3233/AJW220019

Determination of Indomethacin Derivative via Xylenol Orange Dye

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Received July 6, 2021; revised and accepted July 19, 2021

Abstract: Indomethacin hydrazide is a very important compound in the medical field prepared recently to reduce the side effects of indomethacin. The role of the drug is as a non-steroidal anti-inflammatory. Therefore, the methods for estimating this important compound are very few. In this study, an easy, inexpensive and fast spectrophotometric method has been proposed for the determination of this compound in various samples. The method is based on two steps, the first step is the oxidising of indomethacin hydrazide with the oxidizing agent N-bromosuxinamide in an acidic medium, where a compound is formed, and gradually shortens the reddish-orange colour of the xylenol orange dye with the steady increase of indomethacin hydrazide in the second step. The orange xylenol dye is of great importance as it is used in removing a lot of pollution damage, especially contamination with metals, where these minerals are complexes with the xylenol dye and are separated and removed. Besides the important use of this dye in the removal of oxidation products of drugs, as in the case of the indomethacin derivative, after it had been converted to the oxidised formula with the aid of oxidising agents. This method obeyed Beer's law at the concentration range from 2-28 μg/mL, Sandall's value was 0.1547 μg^{-2} cm^{-1}, while the molar absorptivity value was 5.77×10^4 L mol^{-1} cm^{-1}. The method was successfully applied to determine the amount of indomethacin hydrazide in different samples.

Key words: Indomethacin, indomethacin hydrazide, N-bromosuxinamide, xylenol orange.

Introduction

Since the very beginning non-steroidal anti-inflammatory drugs (NSAIDs) (Ingale et al., 2012) are being used as antipyretics and pain relieving medicines around the world. Because of their harmful side effects, especially on the stomach and intestines, many doctors are reluctant to treat chronic arthritis. Common NSAIDs are abdominal pain, gastric and duodenal ulcers, diarrhoea, gastrointestinal bleeding and hepatotoxicity, disorders in kidney function and water retention in the body leading to high blood pressure, preventing platelet aggregation to make blood clot, which causes bleeding and also affects the central nervous system. Some of the symptoms are headache, hearing impairment, dizziness and confusion and asthma, rashes, photosensitivity (Amir and Kumar, 2005; Sava et al., 2021). Hydrazones are a class of organic compounds in the basic Schiff family (Sinha, 2013; Zheng et al., 2009).

IndH has been used in a broad screening program in order to identify chemical structures that can inhibit the enzyme phospholipase that produces rate-limiting precursors in the biosynthesis of different types of bioactive lipids involved in inflammatory processes (Amir and Shikha, 2004; Chandra et al., 2010; March, 1977). Inflammation is a physiological reaction that involves cellular and biochemical responses. Therefore, NSAIDs such as indomethacin and its derivatives

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are associated with major defects of gastrointestinal disorders such as gastric ulcers due to direct contact of the free carboxylic group with the gastric mucosa and as a result of reduced production of prostaglandins in tissues. In order to overcome these drawbacks, a new chemical with an excellent anti-inflammatory response and minimal side effects was urgently needed (Bogdan et al., 2005; Hart, 1963).

Indomethacin derivatives have medical uses (Kajal et al., 2014). It has been used to inhibit cancerous tumours such as lung cancer and pancreatic cancer (Shetnev et al., 2019), also it expands the arteries and reduces stomach ulcers by producing high anti-inflammatory and analgesic efficacy while not affecting the stomach lining. The mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs) is their ability to inhibit the action of prostaglandins in the stomach lining through inhibition of cyclooxygenase enzyme (Szczukowski et al., 2020). Other uses of indomethacin derivatives were in the agricultural field, the importance of indomethacin derivatives in this field was used as pesticides and as herbicides (Brien et al., 2005). Indomethacin Hydrazide is a newly developed drug that reduces the side effects of indomethacin, according to recent studies. Indomethacin hydrazide (IndH) is a derivative of the well-known drug indomethacin (Madwar and Madwar, 2018), its chemical name 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) acetohydrazide. Hydrazides are biologically active compounds (Figure 1) **(**Dillon et al., 2003; Peacock and Sadler, 2008).

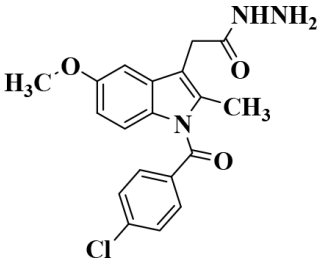

Figure 1: Indomethacin hydrazide (Robert et al., 1996; Marco et al., 2021).

The compound indomethacin hydrazide was prepared in the laboratory by the reaction of indomethacin medication with hydrazine hydrate, as shown in Figure 2 (Amir and Kumar, 2007; Yayoi et al., 2009).

The biological activity of indomethacin derivative NSAIDs (Mloston et al., 2011), including indomethacin, inhibits the secretion of prostaglandins, which perform

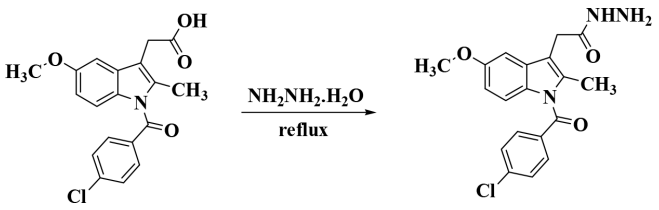

Figure 2: Preperation of Indomethacine derivatives (Darweesh, 2014; Al-Naimi et al., 2018).

several important functions, including protecting the stomach lining from the effect of an acid that the stomach produces to digest food. This means that although NSAIDs reduce inflammation, they also reduce the protection of the stomach lining from acidity and scratching of the stomach wall and causing stomach ulcers. Accordingly, many researchers have resorted to preparing indomethacin derivatives to improve and increase its pharmacological efficacy or to reduce the unwanted side effects of indomethacin and most of the non-steroidal anti-inflammatory drug compounds represented by irritation of the stomach wall (Hamdy et al., 2020; Sharma and Khan, 2003)

In the current study, an easy, accurate and simple spectrophotometric method was proposed for the determination of one of the most important derivatives of the indomethacin drug (Indomethacin hydrazide). This method forms a base on oxidising IndH by N-bromosuxinamide in an acidic medium, then, the resulted product of the oxidation process was then bleached with xylenol orange dye (Belhamel and Draa, 2000), and it was successfully applied for the determination of IndH in various samples.

Research Methodology

Apparatus

Double beam spectrophotometer (JASCOV–630 UV-visible) and 1.0 cm distance plastic cell have been used for all absorbance measurements and final spectrum of indomethacin hydrazide. A double beam spectrophotometer device is a spectral device used to measure the radiated energy in a wide range of the spectrum, including visible region, ultraviolet region and infrared region. This device is mainly dependent on using prisms. The operating principle of a spectrophotometer is based on reducing the optical density of a given light passing through a solution, the decrease or increase is directly proportional to the concentration of the substance in the solution. The pH of the solution was reported using HANA pH meter.

Chemical Solutions

IndH solution, 100 µg.mL^{-1}, was prepared by weighing 0.01 g of IndH, then dissolved in ethanol and making the final volume using distilled water in a 100-mL volumetric flask. An oxidant solution, 0.05% by approximately weighing of 0.05 g of N-Bromosuccinamide (BDH- England), was dissolved in distilled water and the final volume was completed to 100 mL in a volumetric flask. The acid solution, 0.1 N, an appropriate dilution of concentrated hydrochloric acid was diluted with distilled water, in a 100 mL volumetric flask. Dye solution, 1×10^{-3} M, 0.0758 g of xylenol orange dye (Fluka- Switzerland) was prepared in distilled water and the volume was completed to 100 mL volumetric flask.

Results and Discussions

In order to study the optimal conditions for the determination of indomethacin hydrazide, the effect of a number of analytical factors that have a direct effect on the determination of indomethacin hydrazide have been studied in order to apply the suggested method for estimating IndH as it is one of the compounds that have biological, medicinal, and agricultural effects. Therefore, it was decided to apply this method for the determination of IndH in different samples.

Effect of pH

The best medium for the redox process to occur between indomethacin hydrazide and N-bromosuccinamide is the acidic medium. Therefore, several types of acids have been studied with different quantities (0.1-3.0) mL such as acetic acid, sulphuric acid and hydrochloric acid as shown in Figure 3.

As shown in Figure 3, 1.0 mL of 0.1M hydrochloric acid (pH=3.94) was selected as an optimum volume. Four buffer solutions at pH 3.94 were prepared including, KH Phathalate-NaOH, citric acid-sodium citrate, sodium acetate-acetic acid, and succinic acid-NaOH buffer solution and have been chosen for the experiment, and the results are tabulated in Table 1,

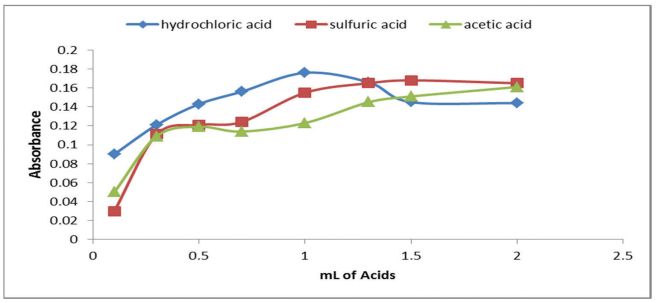

Figure 3: Effect of three acids on the proposed method.

which shows that all buffer solutions decreased the absorbance intensity; therefore, 1.0 mL of hydrochloric acid (pH=3.94) was depending for the subsequent experiment.

Effect of N-Bromosuccinamide

Different quantities of the oxidising agent N-Bromosuccimamide within the range (0.1-3.0) mL were added to 25-mL volumetric flasks containing IndH (50-500) µg, and after adding the optimal amount of the previously studied acid, the xylenol orange reagent was added followed by filling the flasks to make up with distilled water. The distillations were then measured against the blank solution at the wavelength of 583 nm. This study proved that using 0.5 mL of N-Bromosuccimamide gave the best values for absorbance and correlation coefficient (R^2=0.9873) so that this volume of an oxidising agent was selected for the subsequent experiment.

Effect of Xylenol Orange Dye

To check and determine the optimal amount of the reagent xylenol orange, different volumes of xylenol orange ranging from (1-5) mL of 1×10^{-3} M were added to volumetric flasks containing (50-500) µg of indomethacine derivative, 0.5 ml of 0.05% N-Bromosuccimamide and 1.0 mL of 0.1 N hydrochloric acid. According to the results, it was proved that the addition of 2 mL of 1×10^{-3} M xylenol orange gave the best values of absorbance as well as correlation coefficient (R^2 = 0.9972) and this volume of xylenol orange was chosen for the next experiment.

Table 1: Effect of buffer solution

Buffer solution, Ml	*Absorbance/ml of buffer added*			
	KH Phathalate-NaOH	*citric acid-sodium citrate*	*succinic acid-NaOH*	*Sodium acetate-acetic*
1	0.167	0.107	0.096	0.077
2	0.098	0.102	0.074	0.069
3	0.037	0.109	0.071	0.061

Effect of Surfactant

In order to estimate IndH, surface tension agents of various types were added – Cetylpyridinium chloride (positive surfactant), sodium dodecyl sulphate (negative surfactant) and Triton X-100 (neutral surfactant). It was observed from the laboratory results of this study that all of these kinds of surfactants did not give acceptable and satisfactory results or any clear effect on the intensity of absorption of the formed complex or the chosen wavelength, so this study was neglected.

Effect of Order of Addition

The intensity of absorption of the resulting complex was influenced according to the change of the sequence of addition of components of the studied reaction to estimate the drug indomethacin derivative, so several sequences were studied to add the components of the reaction and choose the optimal sequence between them, as shown in Table 2.

Table 2: Sequence of addition of components for the reaction

Reaction components	*Sequence*	*Absorbance*
IndH+NBS+A+XO	I	0.491
IndH+A+NBS+XO	II	0.525
IndH+XO+A+NBS	III	0.111
IndH+NBS+XO+A	IV	0.231
IndH+A+XO+NBS	V	0.128

IndH = indomethacine hydrazide, A = Acid(HCl), NBS = N-Bromosuccimamide, XO = xylenol orange

Time of Oxidation and Stability of the Complex

The studied reaction took 5 minutes to complete the oxidation process and 5 minutes to start stabilising and remained in this for about an hour. These results were obtained from studying the effect of time on the formation and stability of the resulting coloured compound. The study relied on a waiting period of 5 minutes to complete the oxidation process and approximately 5 minutes to measure the stability of the coloured product. This time was selected in subsequent experiments.

Calibration Curve and Final Spectrum

The standard curve of IndH infusions was calculated using a series of clean and dry 25 mL volumetric flasks containing varying amounts of indomethacin derivative within the concentration range (50-700) µg, to which 1 mL of hydrochloric acid was added at a concentration of 0.1 N and 0.5 mL using 0.05% of the oxidisng agent NBS, and after waiting for 5 minutes, 2 mL of xylenol orange reagent was added at a concentration of 1×10^{-3} M. Figure 4 shows the standard curve of IndH according to the proposed method.

Figure 4 illustrated that the proposed method obeyed Beer's law from 2-28 $\mu g.mL^{-1}$, Sandall's value was 0.1547 μg^{-2} cm^{-1}, and the value of molar absorptivity was 5.77×10^{4} L mol^{-1} cm^{-1}. The method was successfully applied to determine the amount of IndH in different samples, and some pharmaceutical preparation of indomethacin. Figure 5 shows the final absorption spectrum of IndH after studying and selecting the optimal conditions for the formation of the resulting colour complex, which was measured at the wavelength of 583 nm. The proposed method has been successful and well applied to various samples.

Accuracy and Precision

To study the accuracy and compatibility of the proposed method, three concentrations of IndH were used, measuring the recovery rate as well as the relative standard deviation ratio, as shown in Table 3.

Nature of the Reaction

In order to determine the reaction ratio between indomethacin derivative and xylenol orange dye, the

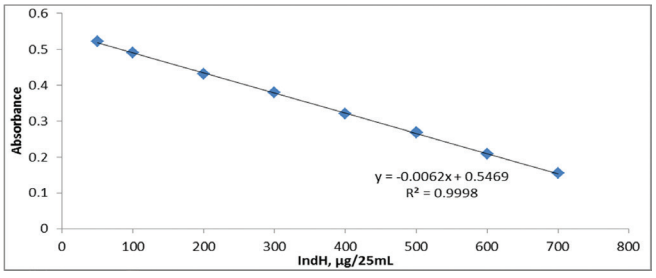

Figure 4: Calibration curve of Indomethacine hydrazide.

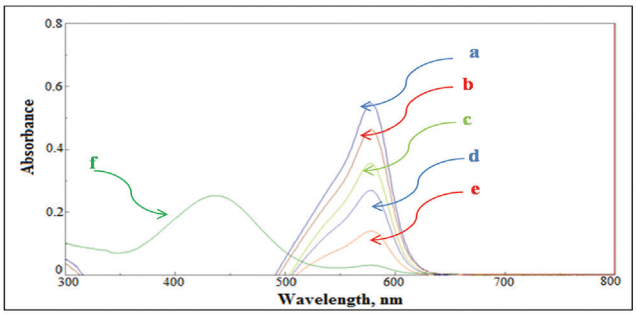

Figure 5: Final spectrum of (a) 50, (b) 100, (c) 300, (d) 500, (e) 700 µg of IndH measured against blank and (f) Blank measured against distilled water.

continuous variables method (Job's method) was used to determine the reaction ratio as shown in Figure 6.

The experiment results as shown in Figure 3 illustrated that the reaction ratio of IndH to xylenol orange dye ratio was 1:2.

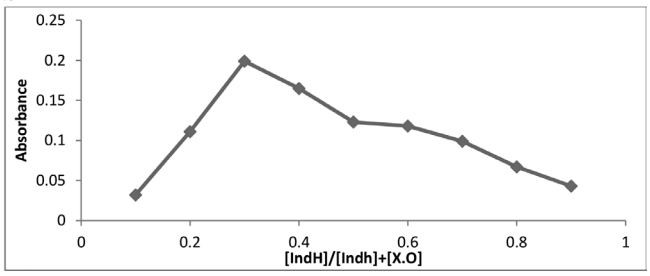

Figure 6: Job's plot for IndH-X.O.

Effect of Interferences

In order to test the efficiency and selectivity of the proposed method, the effect of some exotic additives in the pharmaceutical industries was studied, which were added to improve the taste, colour and aroma of the drug to 100 µg of indomethacin hydrazide and the results are shown in Table 4.

The results in Table 4 show that the studied foreign species did not interfere except toward hydrazine and phenyl hydrazine, which were interacted with xylenol orange or have been oxidised by the oxidising agent.

Table 3: The accuracy and precision of the present method

Amount of IndH taken, µg/25 ml	*Recovery, %*	*µg of IndH measured/25 ml*	*R.E, %*	*RSD, %*
50	98.73	50.09	+ 0.0018	± 0.277
100	99.24	100.36	+ 0.0037	± 0.247
250	99.58	299.41	+ 0.0023	± 0.166

It is evident from Table 3 that the proposed method is reliable.

Table 4: Effect of interferences

Interferences	*Recovery(%) of 100 µg IndH/µg of interference added*			
	100	*300*	*500*	*1000*
Indomethacine	98.43	97.23	98.59	98.24
Hydroxylamine-HCl	97.54	97.22	97.31	97.19
Hydrazine sulphate	80.14	79.32	73.67	60.41
Phenyl hydrazine	77.27	69.45	58.62	51.93
Indometacin morpholinylamide	97.49	98.29	97.23	98.59
Urea	97.77	97.54	97.42	97.28
Menthol	99.83	99.62	99.81	99.93
Thoiurea	97.72	97.69	97.52	97.14
Sodium chloride	99.97	99.91	99.98	99.97
Sodium persulphate	99.73	99.67	99.82	99.62
Sucrose	99.98	99.95	99.89	99.58
Glucose	99.94	99.90	98.87	98.94
Glycerol	99.98	99.98	99.34	99.56
Magnesium sulphate	99.68	99.69	99.62	99.58
Starch	99.98	99.96	99.97	99.95
Lactose	99.93	99.95	98.99	98.92
Boric acid	99.96	99.94	99.96	99.94
Ammonium chloride	99.97	99.96	99.98	99.91
Sodium sulphate	99.98	99.99	99.94	99.90
Calcium sulphate	99.99	99.91	99.99	99.98

Table 5: Application of the present method

IndH, μg	*Recovery(%) of IndH*									
	River water(Tigris in Mosul city/Iraq), ml		*Well water(Al-Qaser village/Mosul-Iraq), ml*		*Sea water Turkey, ml*		*Tap water, ml*		*Indomethacin pure*	
	2	*4*	*2*	*4*	*2*	*4*	*2*	*4*	*100*	*300*
100	99.32	99.32	100.58	100.58	101.49	101.49	99.37	99.37	99.97	99.92
300	100.32	100.32	99.32	99.32	101.63	101.63	99.29	99.42	99.83	99.69
500	99.65	99.65	100.11	100.11	101.72	101.72	99.32	99.32	99.82	99.70

Application of the Method

The present method has been successfully applied to quantify the IndH in various samples, such as river water, well water and tap water, which have been taken from Mosul city-Iraq, while seawater was taken from Turkey. The results tabulated in Table 5 indicate that good recoveries have been obtained.

The analytical variables of the present method is tabulated in Table 6.

Table 6: Analytical variables

Analytical variables	*Present methods*
Reaction	Oxidation-reduction with bleaching
λ_{max} (nm)	583
Reagent	Xylenol orange
Beer's law range ($\mu g.mL^{-1}$)	2-28
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	5.77×10^4
Sandall's sensitivity, μg^{-2} cm^{-1}	0.1547
pH	3.94
Colour stability (minutes)	Red
RSD%	±0.166-±0.277
Method's application	Various samples

Conclusion

A spectroscopic method has been proposed for the determination of one of the most important indomethacin derivatives, which is IndH, as the method is characterised by its sensitivity to some extent and its simplicity, as it is a fast and economical method with reasonable accuracy and precision. The visual parameters and statistical comparison justify this method for application in estimating the routine indomethacin derivative in various samples. Also, the procedure does not include any critical reaction conditions or tedious sample preparation steps. The recommended method is well suited for the assay of IndH in different samples.

The methods for determining IndH are very few. So a spectrophotometric method was suggested to estimate IndH in various samples. The method was based on the oxidation process of IndH with the oxidising agent N-bromosuxinamide in an acidic medium, where a compound is formed, and gradually shortens the reddish-orange colour of the xylenol orange dye with the steady increase of IndH. In addition to the important use of this dye in removing the oxidation products of medicines, as in the indomethacin derivative, after it was converted to the oxidised form using N-bromosuxinamide. This method obeyed Beer's law at the concentration range from 2-28 μg/mL, Sandall's value and molar absorptivity were 0.1547 μg^{-2} cm^{-1}, and 5.77×10^4 $L.mol^{-1}.cm^{-1}$. The method was successfully applied for the determination of IndH in different samples.

Acknowledgement

The authors extend their sincere thanks and gratitude to the University of Mosul, College of Science, Department of Chemistry.

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