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Evaluation of Indomethacin Stability

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A B S T R A C T

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that is most often used to treat inflammation and pain due to rheumatic diseases. Forced degradation is carried out to save time in carrying out contamination analysis on pharmaceutical preparations. Forced degradation is carried out by providing extreme exposure conditions. The aim of this research is to determine whether indomethacin is stable under various degradation conditions such as acid, base, oxidation, photolysis and temperature. The HPLC method is the main choice because it can simultaneously separate compounds, identify compounds and measure concentrations. The mobile phase used was formic acid: acetonitrile (55:45), C18 column with a flow rate of 1.5 ml/minute and a wavelength of 240 nm. The results of this research show that the validation of Indomethacin meets the validation requirements which include selectivity, linearity, precision, accuracy, LOD, LOQ, and resilience. Indomethacin stability tests under acidic, alkaline, oxidation, photolysis and temperature conditions found that Indomethacin was unstable under all conditions.

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INTRODUCTION

Indomethacin (Figure 1) with the chemical name 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. Indomethacin is FDA approved for acute pain, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, bursitis, gout arthritis, and patent ductus arteriosus (Pacifici, 2013). Indomethacin as a pharmaceutical preparation needs to be kept safe from the presence of contamination. Indomethacin contamination is one of the elements that can affect the quality of medicinal products. Contaminants that appear during storage of pharmaceutical products must be identified to ensure the quality of the drug preparation. Forced degradation is carried out in order to save time when conducting contamination analysis in pharmaceutical preparations. Forced degradation is carried out by providing extreme exposure conditions. After exposure to extremes, analysis is then carried out to identify contamination. The amount of contamination in the form of degradation results is generally very small so sensitive analysis methods are needed. Comparison standards for contamination are difficult to obtain so that the analysis is carried out by forced degradation under various conditions (Blessy *et al*., 2013).

British Pharmacopoeia 2013 determined 4-chlorobenzoic acid and 5-methoxy-2-methyl acid indolacetate acid as Indomethacin contamination and have been identified as contaminants also by *Ali et al*., 2015. In the Indonesian Pharmacopoeia Edition VI also mentioned Indomethacin contamination, namely 4-chlorobenzoate and acid 5-methoxy-2-methyl indolacetic acid (Depkes, 2020). 4-chlorobenzoic acid (Figure 2) and 5-methoxy-2-methyl indolacetate acid (Figure 3) are degradation products of indomethacin under hydrolytic conditions. Indomethacin with its two degradation products should be monitored because it causes hepatotoxicity (Kougioumtzoglou *et al*., 2015).

Stability tests are performed to show how the quality of a drug or medicinal product changes over time under the influence of various environmental factors such as acids, bases, oxidation, temperature, and humidity. Stability testing can determine recommended storage conditions, retesting periods, and expiration dates (Blessy *et al*., 2013).

Previous research conducted Kougioumtzoglou *et al*., 2015 which discusses the Indomethacin stability test using the RP-HPLC method with the detector used is a UV-Vis detector at a wavelength of 240 nm with a stationary phase of column C18, (250 mm x 4.6 mm) and its mobile phase acetonitrile and orthophosphoric acid 0.5% (50:50, v / v) with a flow rate of 1.5 ml / minute. The results of the stability test obtained are that Indomethacin is stable in acidic conditions and unstable in alkaline conditions.

The analytical method used for the validation of Indomethacin and its contamination uses HPLC. The HPLC method is the main choice because it can simultaneously separate compounds, identify, measure compounds, and can measure concentrations. The mobile phase used is formic acid: acetonitrile (55:45), column C18 with a flow rate of 1.5 ml / minute and a wavelength of 240 nm. The purpose of this study is to determine whether indomethacin is stable in acid, alkaline, oxidation, photolysis and temperature.

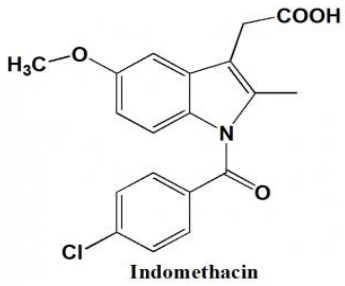

Figure 1. Structure of Indomethacin

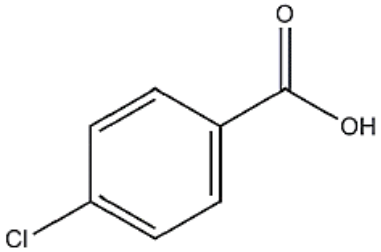

Figure 2. Structure of 4 chlorobenzoic acid

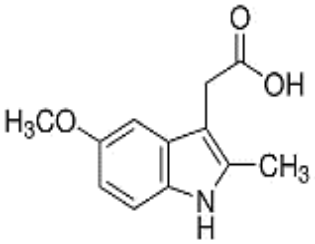

Figure 3. Structure of 5 methoxy 2 methyl 3 indolacetic acid

THEORETICAL REVIEW

Indomethacin

Indomethacin (Figure 1) with the chemical name 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. Indomethacin is FDA approved for acute pain, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, bursitis, gout arthritis, and patent ductus arteriosus (Pacifici, 2013). In the Indonesian Pharmacopoeia Edition VI, it is also mentioned that Indomethacin is contaminated with compounds similar to A Indomethacin and compounds similar to B Indomethacin, namely 4-chlorobenzoate and acid acid acid acid 5-methoxy-2-methyl indoleacetic acid (Ministry of Health, 2020). 4-chlorobenzoic acid and acid acid acid acid 5-methoxy-2-methyl indoleacetic acid are degradation products of Indomethacin under hydrolytic conditions. Indomethacin with its two degradation products must be monitored because it causes hepatotoxicity (Kougioumtzoglou *et al*., 2015).

Indomethacin's mechanism of action is by inhibiting prostaglandin synthesis. Prostaglandins are produced primarily by the enzyme cyclooxygenase (COX), and they are important mediators of inflammation, fever, and pain. They also maintain renal function, GI mucosa, and platelet activity. COX-1 is involved in the production of thromboxane A2 (a critical mediator of platelet aggregation); thus, inhibition of this enzyme is likely responsible for the antiplatelet effects of NSAIDs. COX-1 is responsible for maintaining the GI mucosa, while COX-2 is upregulated in inflamed tissues and produces prostaglandins responsible for inflammation, fever, and pain. Consequently, COX-2 selective NSAIDs may have fewer GI-related side effects. Prostaglandin E2 relaxes smooth muscle and inhibits closure of the ductus arteriosus. In premature infants with respiratory distress syndrome, the ductus arteriosus fails to close, resulting in a patent ductus arteriosus (PDA) due to relatively high concentrations of prostaglandin

E2. Uncorrected PDA can cause differential cyanosis. Indomethacin, by inhibiting PGE2 synthesis, is useful in PDA closure (Lucas, 2016, Harish *et al*,. 2021).

Indomethacin Contamination

Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) that is commonly used to reduce inflammation, pain, and fever. Like other pharmaceutical compounds, indomethacin can degrade over time, leading to the formation of Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) that is commonly used to reduce inflammation, pain, and fever. Like other pharmaceutical compounds, indomethacin can degrade over time, leading to the formation of

Stability

The stability of drug products is divided into chemical stability and physical stability. Physical factors such as heat, light, and humidity, may cause or accelerate chemical reactions, so that in determining chemical stability, physical stability must also be determined (Attwood and Florence, 2011). Stability of pharmaceutical preparations is one of the most important criteria for good production results. Instability of drug products can result in a decrease to loss of efficacy, the drug can become toxic or there is a change in the appearance of the preparation (color, odor, taste, consistency and others) which has detrimental consequences for users (Attwood and Florence, 2011). Forced degradation is a process that involves the degradation of drug products and drug substances under conditions that are more severe than accelerated conditions and thereby produces degradation products that can be studied to determine the stability of the molecule. Drug substance degradation ranging from 5-20% is still acceptable as a reasonable limit for chromatographic validation. A recommended minimum list for forced degradation testing should include acid and base hydrolysis, thermal degradation and, oxidation (Blessy *et al*., 2013).

METHODOLOGY

Equipment and Materials

This research was carried out using an AS-4050 HPLC (Jasco) equipped with an autosampler and auto injector with a UV detector. CAPCEL PAK C18 UG 120 S-5 5µm 4.6 mm x 150 mm column (Osaka Soda), Indomethacin reference standard was obtained from Bpom RI, while 4-chlorobenzoic acid was purchased from Aldrich Chemicals. HPLC grade formic acid, acetonitrile, HCl, NaOH, H2O2 were purchased from Merck, Germany.

Chromatographic conditions

The system used is stationary phase in the form of liquid chromatographic column column CAPCEL PAK C18 UG 120 S-5 5µm 4.6 mm x 150 mm (Osaka Soda), mobile phase formic acid 0.1%: acetonitrile with a ratio of 55:45, flow rate 1.5 mL/min. The UV detector was set at 240 nm.

Preparation of Standard stock solutions

The standard solution of indomethacin is made by taking a solution of formic acid: acetonitrile with a ratio of 55:45 then set it to pH 8 with the addition of NaOH 0.2 N then put 10 mg of Indomethacin and 10 mg of 4 chlorobenzoic acid into a flask measuring 10 ml then ad kan with a solution of formic acid: acetonitrile (55:45) pH 8 ad 10 ml so that a concentration of 1000 ppm is obtained and then sonicated for 30 minutes.

Selectivity

Selectivity is made by taking 100 ppm from a standard solution of Indomethacin with 3 replications. Acceptance criteria are assessed by looking at the existence of another peak with a resolution that enough.

Linearity

The linearity of Indomethacin and 4 chlorobenzoic acid is made by diluting the parent Indomethacin solution and 4 chlorobenzoic acid 1000 ppm into several concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, and 600 ppm in a 10 ml measuring flask, each measuring area. A calibration curve is created between responses to the concentration is then calculated equation regression, correlation coefficient, variation coefficient linear regression.

Forced degradation studies

The forced degradation test was performed at a concentration of 600 ppm. After degradation at set intervals, the aliquot portion of this solution is diluted with the mobile phase to produce a target concentration. Periodic time intervals are determined based on the sensitivity of the drug to stress agents. The various conditions under in which the stress studies are conducted are described below.

Acid hydrolysis

Preparation of acid hydrolysis stability solution is taken 2.5 ml of standard solution of Indomethacin added 1 ml of HCl 1 N. Take 10 µL of acid degradation samples analyzed in optimized chromatography.

Base hydrolysis

Preparation of alkaline hydrolysis stability solution is taken 2.5 ml of Indomethacin standard solution added 1 ml of 0.2 N NaOH. Take 10 µL of base degradation sample analyzed in optimized chromatography.

Oxidation

Preparation of oxidation stability solution taken 2.5 ml of Indomethacin standard solution added 1 ml of H2O2 concentration of 5%, kept at a temperature of ± 30oC for 3 hours. Take 10 µL of acid degradation samples analyzed in optimized chromatography.

Photolytic degradation

Photolysis stability solution is taken 2.5 ml of Indomethacin standard solution and then placed in a place exposed to sunlight for 3 hours. Take10 µL of acid degradation samples analyzed in optimized chromatography.

Thermal degradation

Preparation of thermal degradation is taken 2.5 ml of Indomethacin standard solution then add 1 ml of aqua pro HPLC then heated at 70 o, 80o and 90o in a hotplate for 3 hours. 10 µL of degradation samples were analyzed in optimized chromatography.

Data Analysis

The output data results from the HPLC (High Performance Liquid Chromatography) instrument will be processed using Microsoft excel 2016 built-in from Windows 10 which includes degradation test results using Microsoft excel to obtain % decrease in levels or loss of Indomethacin levels. Calculation by dividing the sample area after exposure by the area before exposure multiplied by 100%.

RESULTS

Selectivity

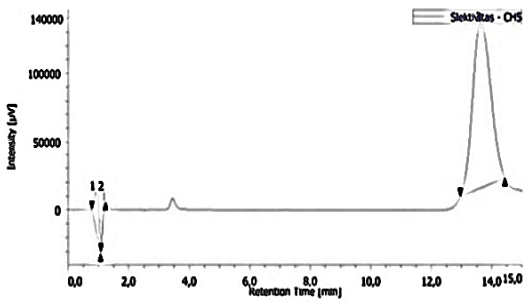

Figure 4. Indomethacin Standard Chromatogram

In figure 4 is the result of the BPFI standard Indomethacin chromatogram, the retention time obtained is 13,637 minutes.

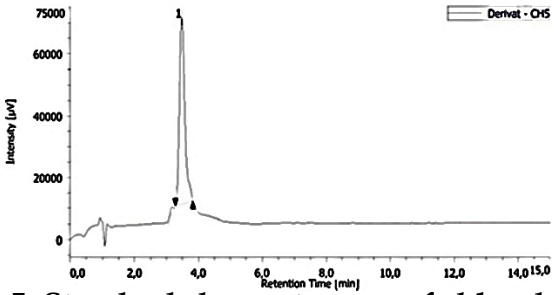

Figure 5. Standard chromatogram of chlorobenzoic acid 4

Figure 5 shows chromatogram results of Indomethacin contamination, 4-chlorobenzoic acid, at a retention time of 3,480 minutes.

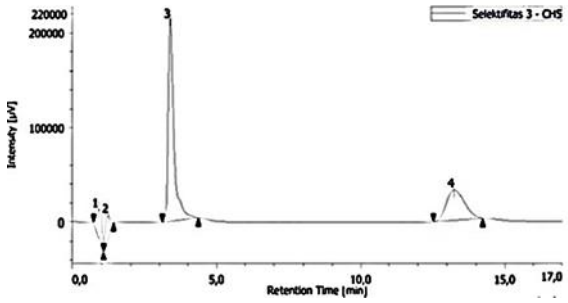

Figure 6. Chromatogram Indomethacin and Chlorobenzoic Acid 4

In figure 6 there are 4 peaks, namely the retention time obtained for 4-Chlorobenzoic acid solution is 3,390 minutes and the standard solution of Indomethacin BPFI is 13,260 minutes, Indomethacin standard solution and its contamination, namely 4-chlorobenzoic acid, each can be identified because it has a comparison standard, while for indomethacin contamination, 5 methoxy acid 2 methyl indolacetate is still not certain to be identified because it is still difficult to obtain material from the contamination, but it can still be estimated, namely comparing with previous studies. From the chromatogram shown in figure 10, it is evident that the analyzed compounds are completely separated from each other (Rs = 13.486), meaning that the Rs obtained is more than 1.5, which shows that this method is selective and can be used for identification of Indomethacin as well as for testing the stability of Indomethacin and its contamination.

Based on research conducted by Kougioumtzoglou et al., 2015 which discusses the Indomethacin stability test using the RP-HPLC method, chromatograms are obtained as below:

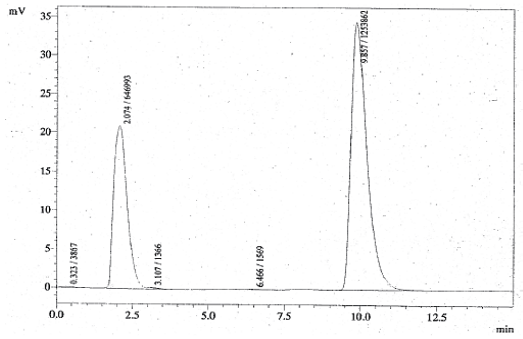

Figure 7. Indomethacin chromatogram spiked with 4 chlorobenzoic acid

In figure 7 above using the reverse phase HPLC system or RP-HPLC mobile phase acetonitrile and orthophosphoric acid 0.5% (50:50, v / v), the retention time of chlorobenzoic acid 4 is 2.074 minutes and the retention time of Indomethacin is 9.857 minutes.

Based on research conducted by Pai & Sawant, 2017 which discusses the RP-HPLC application method to determine Indomethacin and its contaminants, chromatograms are obtained as below:

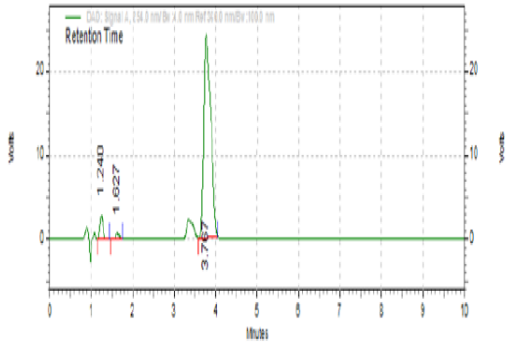

Figure 8. Indomethacin chromatogram spiked with 4 chlorobenzoic acid and 5 methoxy-2-methyl indolacetate acid

In figure 8 above using the reverse phase HPLC system or RP-HPLC with mobile phase methanol mobile phase: acetonitrile: sodium acetate buffer pH 3, 10:50:40% v/v, 5-methoxy-2-methyl indolacetate acid retention time is 1,240 minutes, The retention time of chlorobenzoic acid 4 is 1.627 minutes and the retention time of Indomethacin is 3.767 minutes. From research conducted by Pai & Sawant, 2017 chromatogram based on the order of retention time first appeared, namely 5-methoxy-2-methyl indolacetate acid contamination followed by 4 chlorobenzoic acid was 1.627 and finally Indomethaccin standard.

From the comparison of studies using the same system as researchers, namely using the reverse phase HPLC system, it can be estimated that the chromatogram in the figure of the second peak 10 with a retention time of 1.233 minutes is 5 methoxy acid contamination 2 methyl indolacetate 5, the third peak with a retention time of 3.155 is 4 chlorobenzoic acid and a retention time of 13.260 is Indomethacin, the reason why there is a difference in retention time between previous studies and those studied by the author is because of differences in the brand of HPLC used, detector, mobile phase, flow rate and wavelength used. In research conducted by Kougioumtzoglou et al., 2015 using mobile phase acetonitrile and orthophosphoric acid 0.5% (50:50, v / v), The detector used is a UV-Vis detector at a wavelength of 240 nm and a flow rate of 1.5 ml per minute.

Linearity

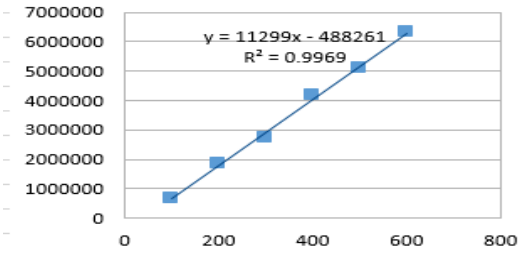

Figure 9. Calibration curves for linearity determination for Indomethacin

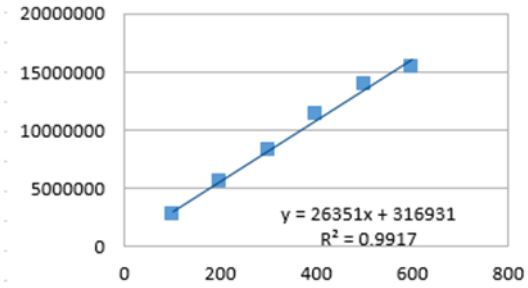

Figure 10. Calibration curves for linearity determination for 4 chlorobenzoic acid

The measurement results show that the greater the concentration of the standard solution of Indomethacin measured, the greater the area obtained. This is because at higher concentrations, the concentration level of Indomethacin compounds is also higher. Based on the results of the determination of the linearity of Indomethacin and Chlorobenzoic Acid 4 above, each r = 0.9984 and r = 0.9958 were obtained. The criterion for the acceptability of the r value in the linearity test according to the United States Pharmacopeia is ≥0.99, which means the results of a linearity test performed in accordance with the conditions of acceptability according to the United States Pharmacopeia. Based on the results of the linearity test obtained shows that there is a linear relationship between the standard levels of Indomethacin and Chlorobenzoic Acid 4 with the area obtained.

Stability Studies

This Indomethacin Stability Test uses a solution with a concentration of 600 ppm which is tested with acid, base, oxidation, photolysis and temperature conditions of 70°, 80° and 90°.

Acid hydrolysis

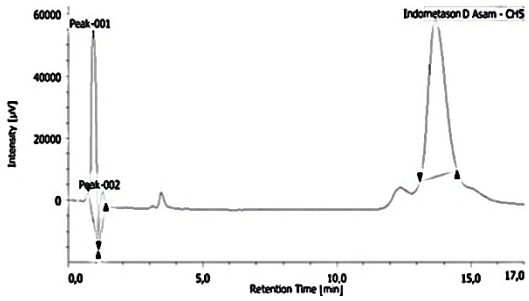

Figure 11. Indomethacin chromatogram results on Acid hydrolysis

Based on the results of the chromatogram with acidic conditions in figure 11 above, it was found that Indomethacin was degraded by 76.38%. In figure 10 there are 3 peaks that appear, the first peak appears at a retention time of 0.910 minutes, peak 2 appears at a retention time of 1.257 minutes which is estimated to be 5 methoxy acid contamination 2 methyl indolacetate and finally the peak

of Indomethacin which appears at a retention time of 13.677 minutes. Indomethacin is a nonpolar compound. In this study, it was found that Indomethacin is unstable under acidic conditions.

Base hydrolysis

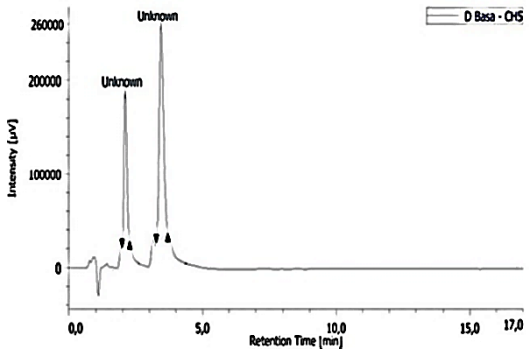

Figure 12. Indomethacin chromatogram results on base hydrolysis

Based on the results of the chromatogram with alkaline conditions in figure 12, it was found that Indomethacin was degraded because the peak could not be read the retention time, but there were 2 other peaks that appeared, namely peak 1 at a retention time of 2,093 minutes and peak 2 appeared at a retention time of 3,440 minutes. In this study, it was found that Indomethacin is unstable in alkaline conditions.

Oxidation

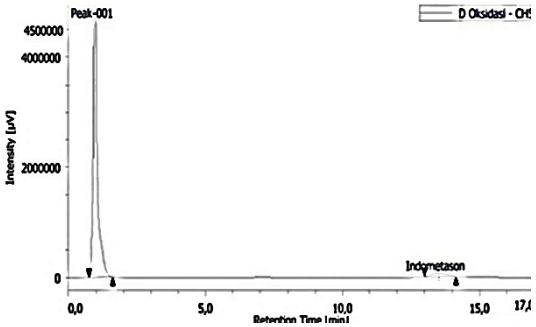

Figure 13. Indomethacin chromatogram results on oxidation

Based on the results of the chromatogram with oxidation conditions in figure 14 above, it was found that Indomethacin was degraded as much as 75.15%. In the picture above there are 2 peaks that appear, the first peak appears at a retention time of 0.970 minutes which is estimated to be a peak of 5 methoxy acid 2 methyl indoleasetate, peak 2 appears at a retention time of 13.503 minutes. In this study, it was found that Indomethacin is unstable under oxidation conditions.

Photolytic degradation

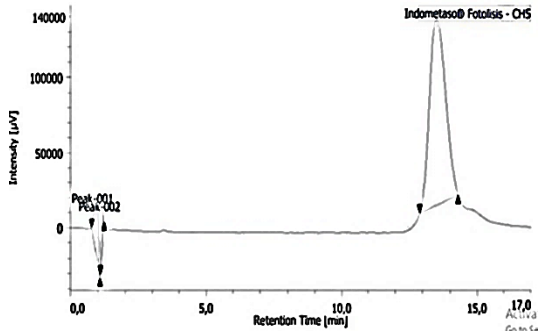

Figure 16. Indomethacin degradation with photolysis

Based on the results of the chromatogram with photolysis conditions in figure 16 above, it was found that Indomethacin was degraded by 33.91%. In the picture above there are 3 peaks appearing, peak 1 appears at a retention time of 0.923 minutes, peak 2 appears at a retention time of 1.180 minutes which is estimated to be the peak of 5 methoxy acid 2 methyl indoleasetate and peak 3 appears at a retention time of 13.510 minutes which is the peak of Indomethacin. In this study, it was found that Indomethacin is unstable under photolysis conditions.

Thermal degradation 70°C

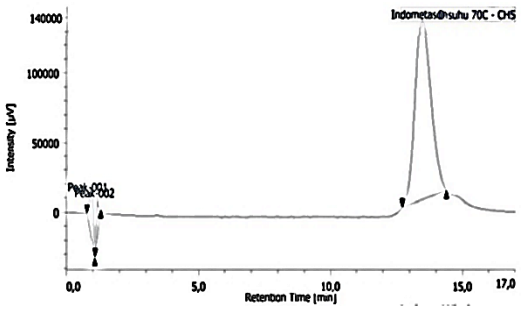

Figure 17. Indomethacin degradation with thermal 70°C

Based on the results of the chromatogram with temperature conditions of 70°C in figure 17 above, it was found that Indomethacin was degraded by 26.16%. In the picture above there are 3 peaks that appear, peak 1 appears at a retention time of 0.917 minutes, peak 2 appears at a retention time of 1.177 which is estimated to be 5 methoxy 2 minutes indoleacetic acid and peak 3 appears at a retention time of 13.460 minutes which is the peak of Indomethacin. In this study, it was found that Indomethacin is unstable at 70 ° C.

Thermal degradation 80°C

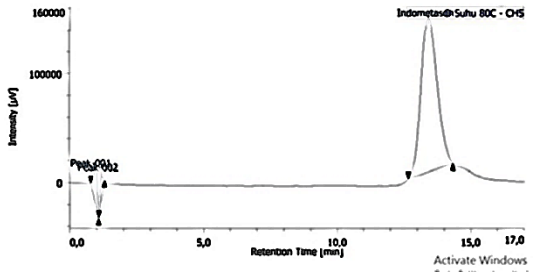

Figure 18. Indomethacin degradation with thermal 80°C

Based on the results of the chromatogram at 80°C in figure 18 above, it was found that Indomethacin was degraded by 18.52%. In the picture above there are 3 peaks that appear, peak 1 appears at a retention time of 0.917 minutes which is estimated to be 2 amino 5 methoxybenzoic acid, peak 2 appears at a retention time of 1.177 minutes which is estimated to be 5 methoxy 2 indoleacetic acid and peak 3 appears at a retention time of 13.417 minutes which is the peak of Indomethacin. In this study, it was found that Indomethacin is unstable at 80°C.

Thermal degradation 90°C

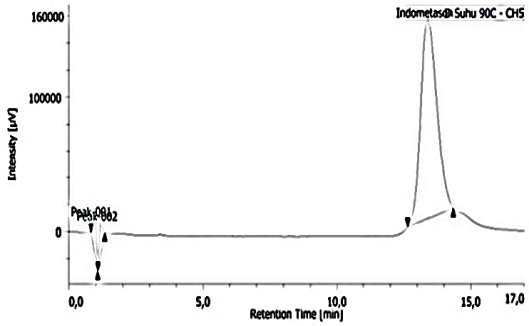

Figure 19. Indomethacin degradation with thermal 90°C

Based on the results of the chromatogram at 90°C in figure 19 above, it was found that Indomethacin was degraded by 14.95%. In the picture above there are 3 peaks that appear, peak 1 appears at a retention time of 0.947 minutes estimated at 2 amino 5 methoxybenzoic acid, peak 2 appears at a retention time of 1.163 minutes estimated 5 methoxy 2 indoleacetic acid and peak 3 appears at a retention time of 13.393 minutes which is the peak of Indomethacin.

CONCLUSIONS AND RECOMMENDATIONS

Indomethacin Stability Test under acid, base, oxidation, photolysis, temperature 70 °C, temperature 80 °C and temperature 90 °C found that Indomethacin was unstable in all conditions carried out, namely acid, base, oxidation, photolysis, temperature 70 °C, temperature 80 ° C and temperature 90 °C.

FURTHER STUDY

For further research, Indomethacin 5 methoxy 2 indoleacetic acid contamination can be added to ensure whether the unread peaks in HPLC are 5 methoxy 2 indoleacetic acid contamination or not.

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