Kinetic Stability Study of Selected Hydroxamic Acids Using HPLC/UV

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ABSTRACT

The kinetics of the acid and base hydrolysis of salicylhydroxamic acid (SHAM) and O-acetyl-salicylhydroxamic acid (OAc-SHAM) were studied using High Performance Liquid Chromatography with UV-Detection (HPLC-UV). The effect of temperature and pH values on the hydrolysis was investigated. It was found that both SHAM and OAc-SHAM were more stable in basic media (high pH values) and at lower temperatures; whereas all reactions were found to follow pseudo-first order kinetics. Reaction rate constants (k) were calculated at different temperatures and pH values. The general trend was that as pH values of the solution increase (basic media), the rate constant decreases indicating a slow hydrolysis rate for both acids. Also, low hydrolysis rates were observed with decreasing the temperature in all cases. The oxidation was also investigated with a solution of 1.0 % hydrogen peroxide (H_2O_2) as the oxidizing agent. OAc-SHAM was found to be insoluble in the H_2O_2 solution. While SHAM was almost hydrolyzed in two hours at room temperature.

Keywords: Hydroxamic acid, kinetics, hydrolysis, HPLC/UV.

INTRODUCTION

Hydroxamic acids refer to a class of organic compounds of the general formula RC (=O) N (R') OH, this type of acids is much weaker in acidity than the structurally related carboxylic acids[1]. Hydroxamic acids have a variety of applications in biology and medicine. Oxyhydroxamic acid was the first hydroxamic acid discovered in 1869, whose discoverer was Lossen, but studies on these compounds have first started in the 1980's [2].

These compounds possess antibacterial and antifungal properties and are selective inhibitors for a variety of enzymes such as peroxidases[3-5] ureases[6-8], matrix metalloproteases[9-13] hydrolases[14], cyclooxygenases[15, 16], lipooxygenases[9], and peptide deformilases[17]. This makes hydroxamic acids ideal candidates for drug design. Hydroxamic acids also represent a wide spectrum of bioactive compounds that have a hypotensive[18], anticancer[19-22], anti malarial[23], and anti-tuberculosis[24] properties. They have been identified as key functional groups of potential chemotherapeutics targeting cardiovascular diseases, HIV, and Alzheimer disease[25,26]. Moreover, their physiological effects have been attributed to their particular affinity for metals[27]. Hydroxamate ions are powerful metal chelating agents[28]. Hydroxamic acids usually bind to metal ions through the two oxygen atoms, but also other binding modes are possible [29]. Hydroxamic acids are also known for their ability to release Nitrous Oxide (NO) which is known for its role in physiological processes[17].

SHAM is a member of this family known for its pharmaceutical applications. It prevents the formation of calcium oxalate stones in kidneys[30]. SHAM also prevents the formation of phosphate stones by inhibiting urease enzyme activity. The splitting of urea to ammonia and carbon dioxide is then catalyzed by the urease

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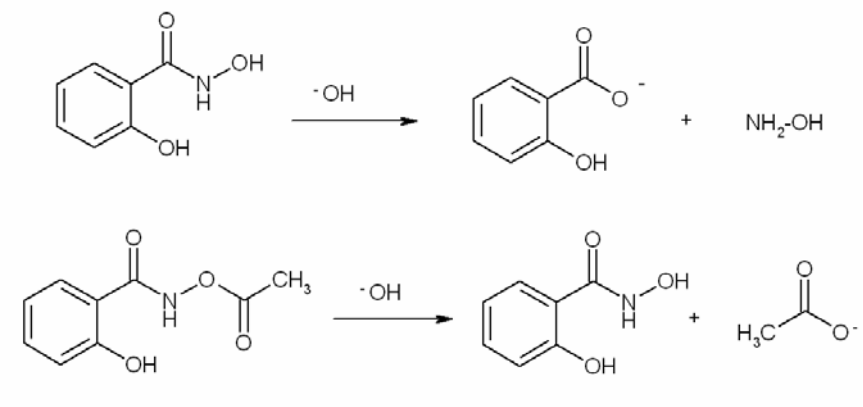
enzyme in cases of urinary tract infection. By inhibiting urease activity, SHAM reduces ammonia formation and retains urea acidic. It also reduces serum uric acid and reduces the incidence of ureate and uric stones[31].

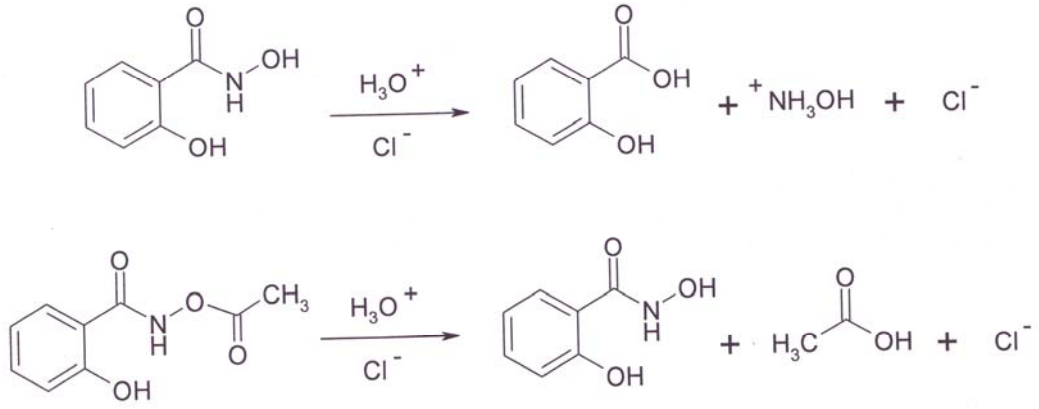
OAc-SHAM is a pale yellow crystal which has an inhibitory effect ten-fold greater than aspirin on prostaglandin H-synthase[32]. Since hydroxamic acid group is a much weaker acid than the carboxylic acid group which is present in aspirin, it should cause less irritation to the mucus layer covering the gastric system.

It is important to know the extent of hydrolysis of SHAM, because packing forms are important to consider since the drug degradation in the body depends on factors such as pH and temperature.

Basic hydrolysis of SHAM and OAc-SHAM can be represented by the following equations[33].



The acidic hydrolysis of SHAM and OAc-SHAM can be represented by the following equations[33]:



In this paper, the extent of hydrolysis of SHAM and OAc-SHAM was investigated at different parameters like time, temperature and pH, using High Performance Liquid Chromatography with UV detection (HPLC/UV).

RESULTS AND DISCUSSION

The stability of SHAM under acidic and basic conditions at different temperatures was investigated. A calibration curve was constructed for SHAM

concentration versus peak areas in the HPLC chromatogram in neutral conditions and at room temperature (25°C). The linear range was found to be in the range of 1-10 ppm. The validity of the calibration curve was tested at the beginning and at the end of each experiment to ensure the absence of any hydrolysis at that pH value and also to ensure the reproducibility of the results. SHAM was hydrolyzed in buffer solutions of pH 4.0, 6.0, and 8.0 at 40°C, 50°C, and 60°C, respectively. At any given pH value, the reaction was found to follow a pseudo first order kinetics. Figures (1-3) show plots for the concentration versus time at pH values of 4.0, 6.0, and 8.0, respectively. The rate constant (k) was calculated using the slope of the plot of the natural logarithm of the concentration versus the temperature assuming a first order reaction. At pH 4.0, the rate constants k were found to be 0.0118, 0.0129, and 0.0144 min^{-1} at 40°C, 50°C, and 60°C, respectively, and the results are shown in Figure (1). At pH 6.0, the rate constants were found to be 0.0047, 0.0070, and 0.0098 min^{-1} at 40ºC, 50°C and 60°C, respectively, and the results are shown in Figure (2). At the last pH value of 8.0, the rate constants k were found to be 0.0008, 0.0050, and 0.0060 min^{-1} at 40°C, 50°C, and 60°C, respectively, as shown in Figure (3). From the obtained results, one can conclude that SHAM is more stable in basic media than in acidic, and increasing the pH will result in less hydrolysis (more stability) of SHAM. Increasing temperatures resulted in increasing the rate of hydrolysis.

Figure (4) displays the room temperature data obtained for the hydrolysis of SHAM in 1.0 M sodium hydroxide and in 1.0 M hydrochloric acid. The results emphasize the high stability at basic conditions compared to acidic media.

In a manner similar to SHAM, OAc-SHAM showed high stability towards increasing the pH values and decreasing the temperature. The degradation of OAc-SHAM was found to follow pseudo first order kinetics. At pH 8.0, the rate constants were 0.0078, 0.0089 and 0.0121 min^{-1} at 40°C, 50°C, and 60°C, respectively. While at pH 9.0, the rate constants were 0.0049, 0.0076, and 0.0092 min^{-1} at 40°C, 50°C, and 60°C, respectively. Finally, at pH 10.0 the rate constants were 0.0045, 0.0074, and 0.0086 min^{-1} at 40°C, 50°C, and 60°C, respectively. Hydrolysis of OAc-SHAM in 1.0 M of NaOH and in 1.0 M HCl was also investigated and the results showed that OAc-SHAM has higher stability in basic media.

Oxidation of SHAM was studied using 1.0 M hydrogen peroxide. The experiments revealed that oxidation has been almost completed after two hours. Figure (5) shows the amount of SHAM left after the oxidation of 10.0 ppm solution. The kinetic investigation of OAc-SHAM oxidation in hydrogen peroxide was not possible because it is not soluble in the oxidizing agent H_2O_2. At a relatively moderate temperature (40 ̊C), the stability of SHAM and OAc-SHAM increases as the pH increases. i.e. less is hydrolyzed in more basic media.

Finally, the importance of hydroxamic acids in the drug industry mandated the study of their stability in acidic, basic, and oxidizing media at various temperatures. Our results showed that both SHAM and OAc-SHAM were more stable in basic media rather than in acidic media. It is recommended that the storage and dissolution of these acids must be done at low temperatures and in basic media (high pH) in order to minimize their degradation, which would lead to the increasing their shelf-time.

General Experimental

All chemicals used in this work were of analytical grade (A.R) and were used as received without further purification. HPLC-grade solvents were filtered through a 0.45 μm Nylon membrane filter and then sonicated before they were used. HPLC-grade Acetonitrile used for the mobile phase was from TEDIA/ USA. Salicylhydroxamic acid (99% pure) was purchased from ALDRICH/ Germany, while the O-acetylsalicylhydroxamic acid was synthesized in our laboratory according to the method described by O'Brien *et al.*[4]. NMR and IR data for the yielded compound were utilized to prove the correct product with very good purity. Sodium hydroxide pellets, hydrochloric acid & hydrogen peroxide (50%) were all purchased from GCC (Gailand Chemical Company)/ U.K. Salts used for the preparation of the phosphate buffer

were: di-sodium hydrogen orthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$) from BDH/England and Potassium di-hydrogen phosphate (KH_2PO_4) which was purchased from Peking Chemical Works/ China.

A Beckman HPLC injector with a 20 µL loop connected to a Beckman 114 M solvent delivery pump is used to deliver the eluent through a RP-C18, (25cm x 4.6mm, 5µm column) (Supelco). A Varian Prostar UV detector 325 module was used for detecting the concentration of the hydroxamic acids. For adjusting the pH of the buffers, an Eutech PC 300 pH meter Instrument Cyberscan (Singapore) with an accuracy of ± 0.01 pH units was used. All masses were measured using a four digit analytical balance. IR spectra were acquired using a Precisa XB120 A/ FTIR spectrometer (Switzerland), Thermonecolit (nexes 670). Phosphate buffers were prepared according to methods documented in the literature[34].

For all measurements, the mobile phase composition was (80:20%) (v/v) acetonitrile: water, the same solution was used for preparing standard solution and for the consequent dilutions. The pump flow rate was 0.5 mL/min. The UV detector was set at λ=300 nm and λ=298 nm for the SHAM and the OAc-SHAM, respectively, and with an attenuation of 0.1 AUFS.

A 2.50 mg from each of SHAM and OAc-SHAM were dissolved in 25.0 mL (100 ppm) of the buffer and kept in a water bath at a constant temperature of the hydrolysis reaction (40, 50 or 60 °C). Every ten minutes, 1.0 mL was taken from the stock solution, diluted with the mobile phase and immediately injected onto the HPLC column under the above mentioned conditions.

The resulting peak areas were compared to those resulted from a calibration curve constructed from standard concentrations, and then the corresponding concentrations of the SHAM or the OAc-SHAM left were calculated. Figures (6) and (7) represent a typical HPLC chromatogram for SHAM and OAc-SHAM solutions, respectively.

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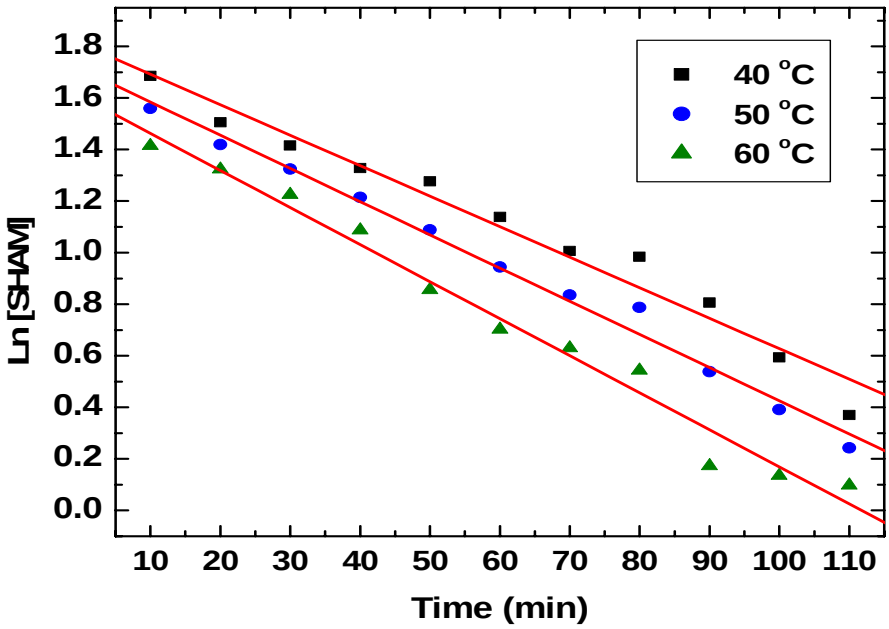

Figure 1. The hydrolysis of SHAM in a phosphate buffer solution of pH = 4

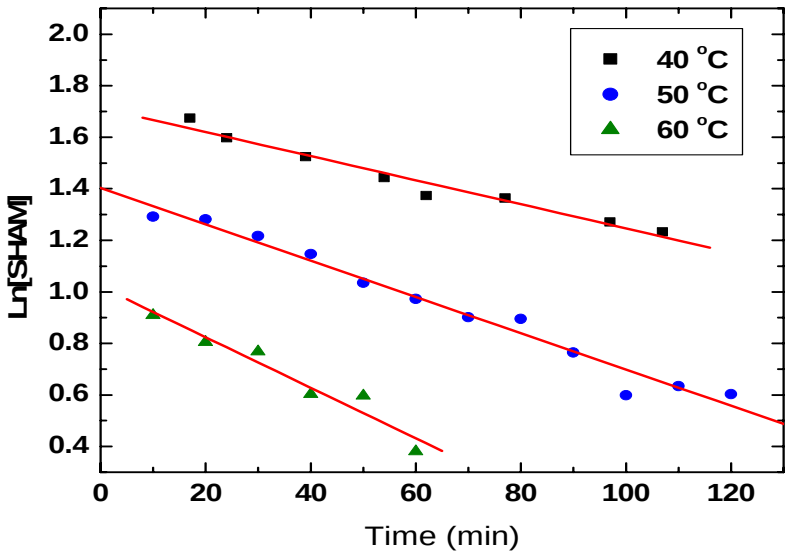

Figure 2. The hydrolysis of SHAM in a phosphate buffer solution of pH = 6

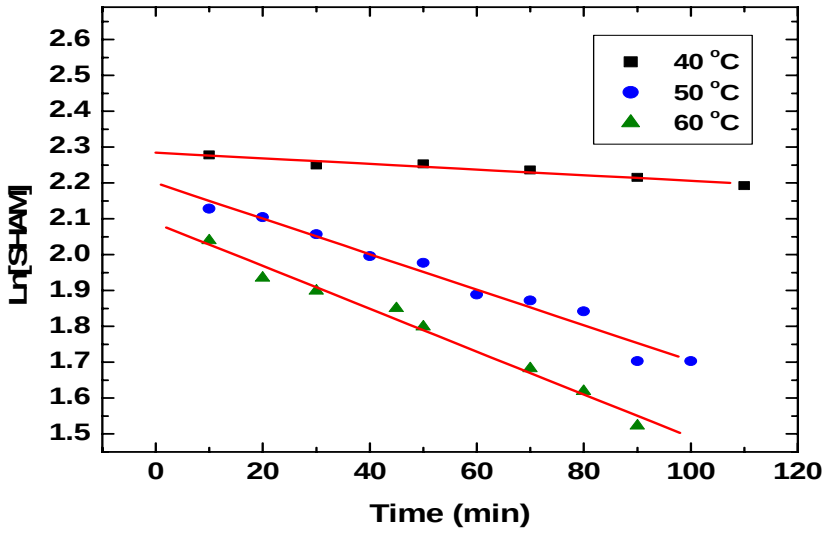

Figure 3. The hydrolysis of SHAM in a phosphate buffer solution of pH = 8

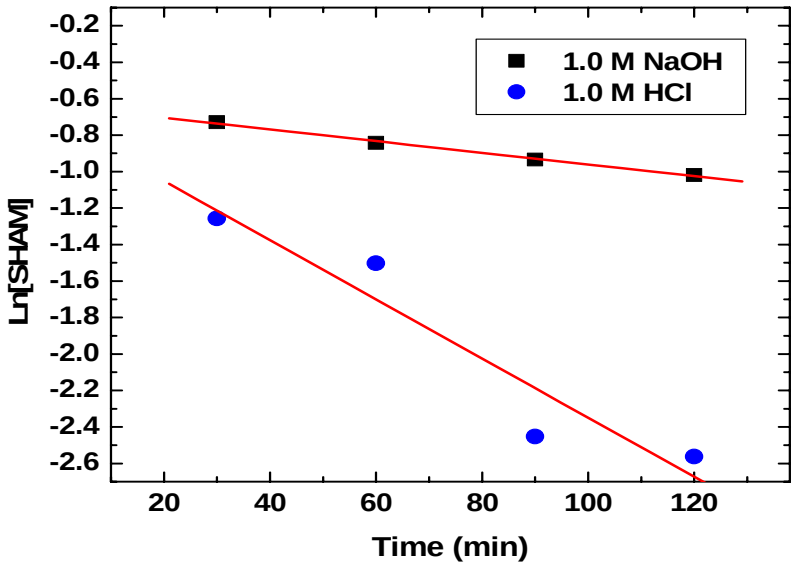

Figure 4. The hydrolysis of SHAM in 1.0 M NaOH and in 1.0 M HCl

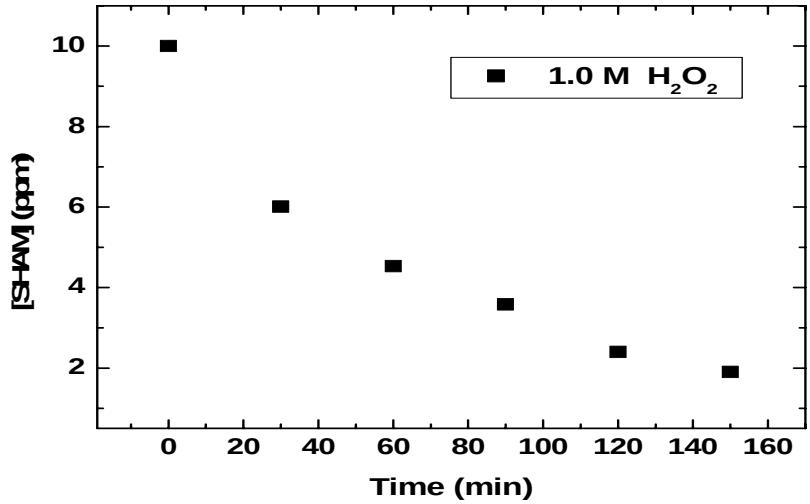

Figure 5. The amount of SHAM left after the oxidation of 10.0 ppm solution in H_2O_2

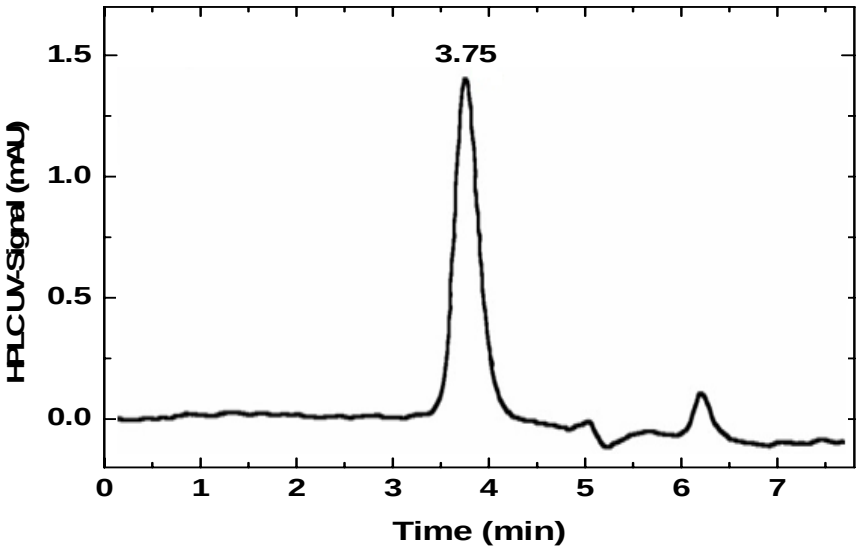

Figure 6. A typical HPLC Chromatogram for SHAM

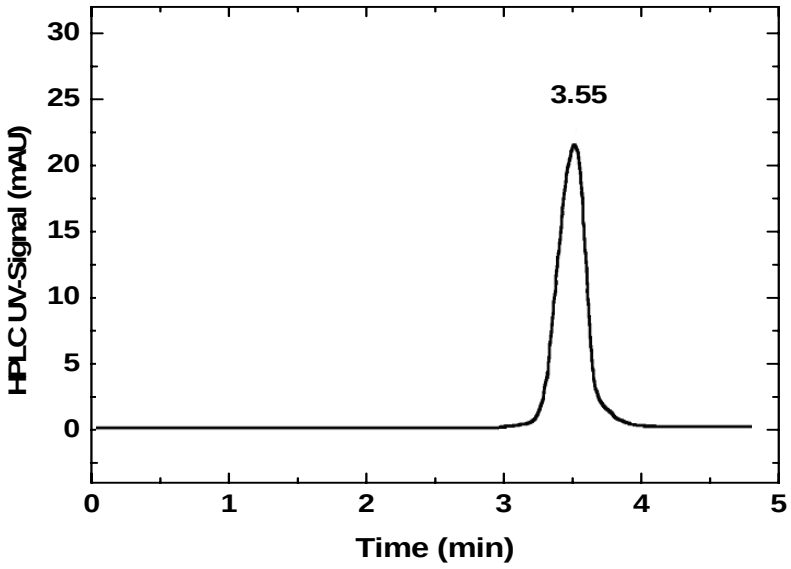

Figure 7. A typical HPLC Chromatogram for OAc-SHAM

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دراسة الحركية والثباتية لأحماض هيدروكزاميه مختارة باستخدام الكروماتوغرافيا السائلة ذات الكفاءة العالية والمكشاف فوق البنفسجي

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ملخـص

تمت دراسة التفاعلات الحركية لتميه الـ Slicylhydroxamic acid (SHAM) و-O-acelyl (OAc-SHAM) salicylhydroxamic acid في الظروف الحمضية والقاعدية وذلك باستخدام الكروماتوغرافيا السائلة ذات الكفاءة العالية والقياس فوق البنفسجي. تمت متابعة التميه على درجات حرارة وحموضة مختلفة. ولقد أظهر كلا المركبين ثباتاً أكثر عند درجات الحرارة المنخفضة وعند قيم حموضة عاليه حركياً، وكانت جميع التفاعلات من الدرجة الأولى وتم حساب ثابت السرعة لها. وبشكل عام كلما زادت قيمة الحموضة انخفض ثابت السرعة للمركبين في الوسط القاعدي، وارتفعت قيمة ثابت السرعة مع زيادة درجات الحرارة في جميع الظروف التي تمت الدراسة فيها. لقد تم أيضا دراسة تأكسد الـ SHAM بالعامل المؤكسد فوق أكسيد الماء (محلول 1.0%) وتم أكسدة معظم المركب في خلال ساعتين بينما لا يوجد ذائبية للمركب OAc-SHAM في نفس العامل المؤكسد.

الكلمـات الدالـة: الأحماض الهيدروكزامية، التفاعلات الحركية، التميه، الكروماتوغرافيا السائلة ذات الكفاءة العالية/ القياس فوق البنفسجي.

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