Comparative Hydrolysis Study of Acetylsalicylic Acid and Copper (II)-Acetylsalicylate by RP-HPLC Method

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Summary: The hydrolysis of acetylsalicylic acid (ASA) and copper (II)-acetylsalicylate (CAS) was investigated by using a validated RP-HPLC method. Acetylsalicylic acid was hydrolyzed to (SA), where as CAS was hydrolyzed to ASA, SA, copper salicylate (CS), copper acetate and acetic acid. Comparison of hydrolysis was carried out by monitoring the concentration of SA, one of common hydrolysis product. The hydrolysis study was done in phosphate buffer solution in pH range from 3-11. The study was carried out at constant temperature to evaluate the effect of pH on both drugs. It was found that the hydrolysis rate of both drugs was same at pH value ranging from 3-11 as calculated from the K_{app} and K values. However, there is a slight difference in the degradation of ASA and CAS as revealed from F-test. Results have indicated that CAS first hydrolyzes to acetylsalicylic acid which further hydrolyzes to SA, therefore availability of acetylsalicylic acid is increased.

Keywords: Acetylsalicylic acid, Copper (II)-acetylsalicylate, HPLC, Hydrolysis.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely approved drugs due to their broad spectrum of actions, such as anti-inflammatory, antipyretic, antithrombotic, and analgesic properties. ASA is one of the commonly used NSAIDs and for more than hundred years used for the treatment of rheumatoid arthritis. However, the major problem associated with long term use of ASA is the causation of peptic ulcer [1, 2]. An effort has been made by Sorenson [3] to contradict this unpleasant side effect and find that CAS is more active than ASA and also possesses antiulcer activity. Along with improved anti-inflammatory activity (AI), the antiulcer activities of CAS have now been proven in several research papers. CAS has been recognized as a safe drug for ulcer patients and is used for the treatment of rheumatoid arthritis [4-9]. Recently we have reported the pharmacokinetics of CAS after single dose oral administration in human volunteers to determine the essentially required clinical data, so CAS can be registered as a drug [10]. After iron and zinc, copper is the most abundant essential trace mineral in our body. Since the 1920s, it is considered as an important nutrient [11]. Various copper complexes possess AI activity higher than their parent compounds or ligands. It has been assumed that the active forms of several famous AI drugs are their copper complexes. Rising attention in copper complexes as AI drugs and anti-arthritics is illustrated by a great number of evaluations and symposia published in the last two to four decades [12]. Hydrolysis of Tetrakis-μ-acetylsalicylato-dicopper (II) was reported by using UV/Vis spectroscopy [13]. The present work highlights on the comparison of hydrolysis of ASA and CAS by using validated HPLC method, which is most commonly used technique for the analysis of drugs.

Results and Discussion

Already validated RP-HPLC method was applied to monitor the hydrolysis studies of ASA and CAS at various pH levels keeping the temperature constant. The retention times for CAS, ASA and SA were 2.5, 2.9 and 3.1 min, respectively.

Hydrolysis kinetics was calculated using first and second order kinetic models. The best fits of hydrolysis kinetics are being reported in each case during the study. First and second order reactions equations are given below as Eq. 1 and 2, respectively.

$$-d[A]/dt = k\,[A] \qquad \text{(Eq. 1)}$$

$$-d[A]/dt = k[A][B] \qquad \text{(Eq. 2)}$$

The temperature was maintained at 30 °C and hydrolysis studies were done at different pH values. At pH 3, the mean value of rate of ASA

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hydrolysis calculated is 3.5×10^{-3} mg L^{-1} min^{-1}. Fig. 1 shows the relation between remaining ASA concentration and time at pH 3. The slope of the line is -0.0002 and value of intercept is 1.1814. The negative sign shows decrease in concentration with respect to time. There was slight decrease in concentration of ASA with time and in the same way slight increase in concentration of SA at pH 3. At pH 4, the mean value of rate of ASA hydrolysis calculated is 8.0×10^{-3} mg L^{-1} min^{-1}. Fig. 1 also shows the relation between remaining ASA concentration and time at pH 4. The slope of line is -0.0002 and value of intercept is 1.1895 and negative sign shows decrease in concentration of ASA with respect to time. At pH 4 there was slight decrease in concentration of ASA with time and also slight increase in concentration of SA.

At pH 5, the mean value of rate of ASA hydrolysis calculated is 7.0×10^{-3} mg L^{-1} min^{-1}. The relation between remaining ASA concentration and time at pH 5 is shown in Fig. 1. The slope of line is -0.0003 and the value of intercept is 1.1354 and the relative rates of decrease in concentration of ASA and increase in concentration of SA was very small at pH 5. At pH 6, the mean value of ASA hydrolysis calculated is 2.4×10^{-2} mg L^{-1} min^{-1}. The relation between remaining ASA concentration and time at pH 6 is also shown in Fig. 1. The slope of the line is -0.0003 and intercept is 1.191 and the relative rates of decrease in concentration of ASA and increase in concentration of SA at pH 6 was very slow.

At pH 7, the mean value of ASA hydrolysis calculated is 4.2×10^{-2} mg L^{-1} min^{-1}. Fig. 1 shows the relation between remaining ASA concentration and time at pH 6. The slope of the line is -0.0005 and value of intercept is 1.1448 and relative rates of decrease in concentration of ASA and increase in concentration of SA at pH 7 is very slow. At pH 8, the mean value of ASA hydrolysis calculated is 1.7×10^{-2} mg L^{-1} min^{-1}. The relation between remaining ASA concentration and time at pH 8 is shown in Fig. 1. The slope of the line is -0.0005 and intercept is 1.1891 and relative rates of decrease in concentration of ASA and increase in concentration of SA at pH 8 was slow.

At pH 9, the mean value of ASA hydrolysis calculated is 2.4×10^{-2} mg L^{-1} min^{-1}. Fig. 1 shows relation between remaining ASA concentration and time at pH 9. The slope of the line is -0.0007 and intercept is 1.1905 and relative rates of decrease in concentration of ASA and increase in concentration of SA at pH 9 which was slightly larger than lower pH values. At pH 10, the mean value of ASA hydrolysis calculated is 1.1×10^{-1} mg L^{-1} min^{-1}. Fig. 1 shows relation between remaining ASA concentration and time at pH 10. The slope of the line is -0.0039 and intercept is 1.1625 and relative rates of decrease in concentration of ASA and increase in concentration of SA at pH 10 were faster. At pH 11, the mean value of ASA hydrolysis calculated is 1.5×10^{-1} mg L^{-1} min^{-1}. The relation between remaining ASA concentration and time at pH 11 is shown in Fig. 1. The slope of the line is -0.0339 and value of intercept is 1.1084 and relative rates of decrease in concentration of ASA and increase in concentration of SA with time at pH 11 was very fast.

At pH 3, the mean value of CAS hydrolysis calculated is 1.2×10^{-3} mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 3. The slope the line is -9E-05, negative sign shows decrease in concentration of CAS and value of intercept is 1.1766 and relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 3 was very slow. At pH 4, the mean value of CAS hydrolysis calculated is 1.3×10^{-3} mg L^{-1} min^{-1}. The relation between remaining CAS concentration and time at pH 4 is shown in Fig. 2. The slope of the line is -4E-05, negative sign shows decrease in concentration of CAS and value of intercept is 1.1767 and the relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 4 was very slow.

At pH 5, the mean value of rate of ASA hydrolysis calculated is 4.3×10^{-3} mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 5. The slope of the line is -0.0001 and the value of intercept is 1.1879 and the relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 5 was very small. At pH 6, the mean value of rate of CAS hydrolysis calculated is 5.6×10^{-3} mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 6. The slope of the line is -0.0001 and value of intercept is 1.185 and relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 6 was very slow.

At pH 7, the mean value of rate of CAS hydrolysis calculated is 5.8×10^{-3} mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 7. The slope of the line is -0.0001 and value of intercept is 1.1696 and relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 7 was very small. At pH 8, the mean value of rate of CAS hydrolysis calculated is 6.0×10^{-3} mg L^{-1} min^{-1}. Fig.

2 shows the relation between remaining CAS concentration and time at pH 8. The slope of the line is -0.0001 and value of intercept is 1.1437 and the relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 8 were small. At pH 9, the mean value of rate of CAS hydrolysis calculated is 1.8×10^{-1} mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 9. The slope the line is -0.006 and value of intercept is 1.0997 and relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 9 was very slow.

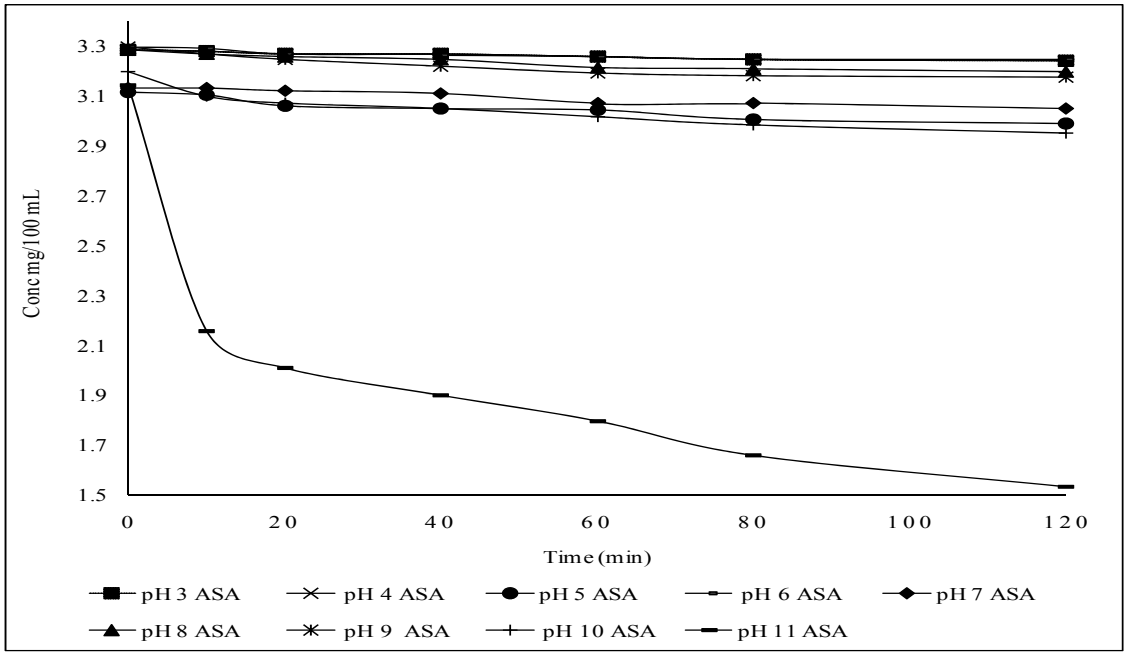

Fig. 1: Concentration of hydrolyzed ASA at various pH vs time.

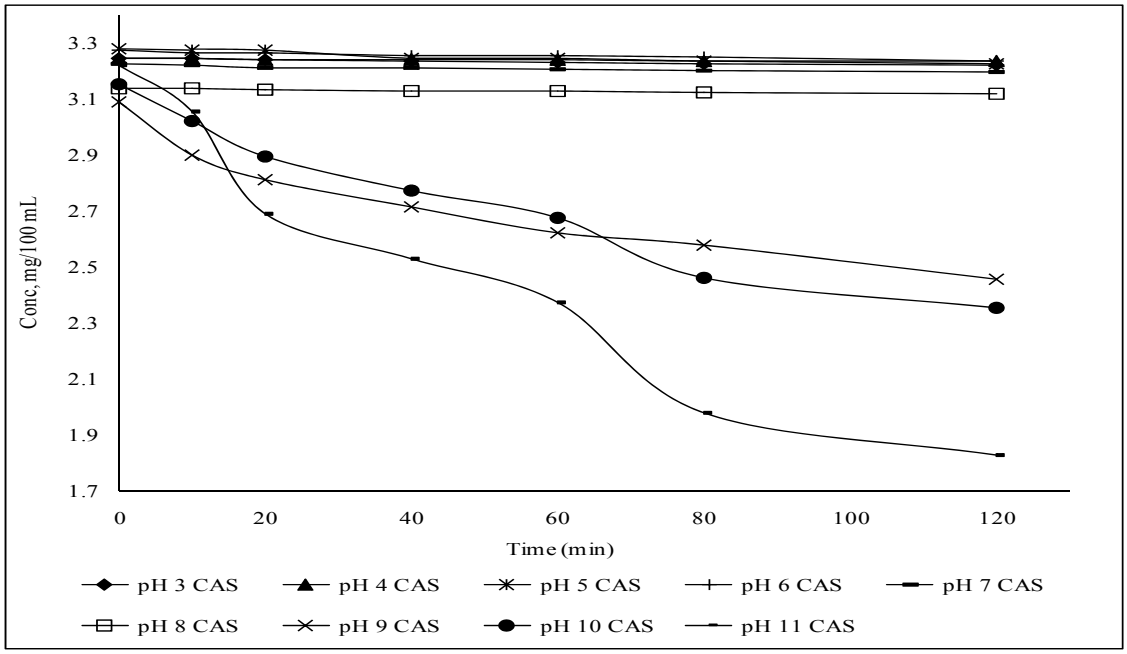

Fig. 2: Concentration of hydrolyzed CAS at various pH vs time.

At pH 10, the mean value of rate of CAS hydrolysis calculated is 6.5×10^{-1} mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 10. The slope the line is -0.0261 and value of intercept is 1.1654 and relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 10 were fast. At pH 11, the mean value of rate of CAS hydrolysis calculated is 1.3 mg L^{-1} min^{-1}. Fig. 2 shows the relation between remaining CAS concentration and time at pH 11. The slope the line is -0.0497 and value of intercept is 1.2028 and relative rates of decrease in concentration of CAS and increase in concentration of SA at pH 11 was very fast.

The temperature of the solution was kept constant and studies was done from pH 3 – 11. It was found that experiments conducted at higher pH values proceeded with a faster rate of decomposition of ASA as well as CAS. At higher pH values, their was more favourable enviroment for the decomposition of ASA and CAS because the decomposition products obtained were acids. Conners *et al* reported that in pH dependent hydrolysis, ASA is more stable at pH 3 and shows very slow degradation at pH 4-8 while at pH 11-12, ASA is immediately hydrolysed [15]. It is important to mention that during persent study, we are reporting HPLC-UV method using a wide range of pH.

Table-1 shows k_{app} and k values of ASA and CAS at different pH values. It was observed that at higher pH values the value of k was large. At larger pH the rate of ASA degradation was fast. However, at lower pH values it was more stable. The rate of ASA degradation at pH 3 was 3.5×10^{-3} mg L^{-1} min^{-1} but at pH 11 it was 1.5×10^{-1} mg L^{-1} min^{-1}. The k_{app} values obtained were more satisfactory. The rate of degradation at pH 3-8 was very small showing zero order kinetics but at higher pH 9-11, there was significant change in concentration was observed showing it to be first order. CAS has also shown the similar behavior as shown by ASA. It also shows more stability at pH 3-8 and fast degradation rate at pH 9-11.

Table-1: k_{app} and k values of ASA and CAS.

	ASA		CAS	
pH	**K_{app} (min^{-1})**	**$k = k_{app} / [OH^-]$ (M^{-1} / min)**	**K_{app} (min^{-1})**	**$k = k_{app} / [OH^-]$ (M^{-1} / min)**
3	**-0.00017**	**-0.0085**	**-0.000033**	**-0.00165**
4	**-0.00022**	**-0.0111**	**-0.000037**	**-0.00185**
5	**-0.00024**	**-0.0123**	**-0.000130**	**-0.00650**
6	**-0.00033**	**-0.0165**	**-0.000171**	**-0.00850**
7	**-0.00042**	**-0.0213**	**-0.000174**	**-0.00870**
8	**-0.00052**	**-0.026**	**-0.000188**	**-0.00940**
9	**-0.00073**	**-0.0365**	**-0.006206**	**-0.31030**
10	**-0.00346**	**-0.1731**	**-0.021523**	**-1.07615**
11	**-0.04225**	**-2.1125**	**-0.042525**	**-2.12625**

It was observed that ASA and CAS were showing same kinetics at each pH value in the range of 3-11. Alich *et al* concluded that pH dependent hydrolysis of ASA and CAS was found to occur at same rate in dilute solutions [13]. Fig. 1 and 2 show the comparison of rates of degradation of ASA and CAS at different pH values. It was observed that at each pH value ASA and CAS had same rates of degradation but there was only slight difference in values of intercepts. Similarly for the comparison of two sets of data, F-Test has been applied on the data and it was observe that there is no significant difference in the two sets, except that CAS initially hydrolyzed to ASA and then ASA hydrolyzed to SA.

Experimental

Reagents and Chemicals

The chemicals used were: methanol (E. Merck), acetic acid (E. Merck), ASA (E. Merck),SA (E. Merck), sodium carbonate anhydrous (E. Merck), cupric sulphate pentahydrate (E. Merck), cupric chloride dihydrate (E. Merck). All the chemicals used were of analytical reagent grade. All of them were used without further purification. CAS and CS were prepared according to a reported method [14].

HPLC

The HPLC system consisted of: LC-10AT VP pump, UV-Vis detector SPD-10A VP, SCL-10A VP system controller all from Shimadzu, Japan. The column used was Shim-Pak ODS 5µm (4.6 × 250 - mm).

Preparation of Mobile Phase

Methanol-acetic acid system was prepared by mixing methanol and acetic acid in a ratio of 20:1. The mobile phase was filtered and degassed before use.

Preparation of Standard ASA Solution

A standard solution was prepared by dissolving, accurately weighed, about 100 mg of ASA RS in 100 mL of mobile phase. Ten mL of this solution was diluted to 100 mL with mobile phase. The solution was filtered through 0.45µ filter and degassed before use.

Preparation of SA Standard Solution

A standard solution was prepared by dissolving, accurately weighed, 100 mg of SA RS in 100 mL of mobile phase. Ten mL of this solution was diluted to 100 mL with mobile phase. The solution

was filtered through 0.45µ filter and degassed before use.

Preparation of CS Standard Solution

A standard solution was prepared by dissolving, accurately weighed, 100 mg of CS in 100 mL of mobile phase. Ten mL of this solution was diluted to 100 mL with mobile phase. The solution was filtered through 0.45µ filter and degassed before use.

Preparation of Standard CAS Solution

A standard solution was prepared by dissolving, accurately weighed, 100 mg of CAS in 100 mL of mobile phase. Ten mL of this solution was diluted to 100 mL with mobile phase. The solution was filtered through 0.45µ filter and degassed before use.

Chromatographic Conditions

The system optimization was carried out by using various compositions of the mobile phase. Separation was achieved under isocratic conditions using a mobile phase of methanol: acetic acid (20:1) at a flow rate 1 mL min^{-1} and UV detection at 294 nm at ambient temperature. The volume of injection was 20 µL.

Preparation of Buffer Solutions

The buffer solutions used for the purpose of determination of rate of hydrolysis are 0.02M Na_2HPO_4 solutions with pH 3-11. Firstly, 0.02 M solution of Na_2HPO_4 was prepared by dissolving 3.56 g of Na_2HPO_4 in 1000 mL of distilled water. Then, 500 mL of 0.02M Na_2HPO_4 solution was taken and orthophosphoric acid was added drop-wise until the required pH was obtained.

Buffer solutions of pH 3-9 were prepared by this method but the solutions of pH 10 and 11 which are highly basic and prepared by adding sodium hydroxide solution (0.02 M). For pH 10 buffer solutions, the 483.2 mL of 0.02 M Na_2HPO_4 was diluted with 16.8 mL of 0.02 M NaOH and similarly for pH 11 buffer 482.6 mL of 0.02 M Na_2HPO_4 diluted with 17.4 mL of 0.02 M NaOH.

Preparation of Sample Solutions of ASA

0.1 g of ASA was weighed accurately, dissolved in 2 mL of methanol by slightly shaking and the volume was marked up with pH 3 buffer solution in 100 mL volumetric flask. One mL of above solution was further diluted up to 100 mL with pH 3 buffer solution. This solution was subjected to HPLC for monitoring the amount of SA generated at this specific pH. The solution was filtered through 0.45µ filter and degassed before injecting into HPLC. All other solutions of ASA at pH 4, 5, 6 7, 8 9, 10 and 11 were prepared according to the procedure given above. ASA hydrolysis generates only SA and its amount at various pH was determined by using above mentioned HPLC-UV method.

Preparation of Sample Solutions of CAS

CAS (0.1 g) was dissolved in methanol (2 mL) by slightly shaking and the volume was marked up with pH 3 buffer solution in 100 mL volumetric flask. One mL of above solution was further diluted up to 100 mL with pH 3 buffer solution. This solution was subjected to HPLC for monitoring the amount of ASA and SA generated at this specific pH. The solution was filtered through 0.45µ filter and degassed before injecting into HPLC. All other solutions of CAS at pH 4, 5, 6 7, 8 9, 10 and 11 were prepared according to the procedure given above. CAS hydrolysis generates ASA, SA and CS and its amount at various pH was determined by using an HPLC method. ASA produced as a result of hydrolysis of CAS was later on also decomposed to SA.

Analysis of Sample Solutions of ASA and CAS at Various pH

The analysis of sample solutions of both ASA and CAS at different pH was performed by using above validated HPLC method.

Conclusion

The RP-HPLC/UV method was successfully validated to study hydrolyses of ASA and CAS. It was concluded from results that the hydrolysis of CAS was slightly slow as compared to ASA. Results have therefore indicated that CAS is slightly more stable towards acidic hydrolysis.

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