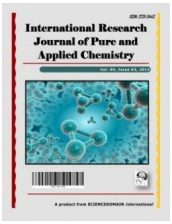

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Stability and Kinetics Studies Using an RP- HPLC-UV Method Developed for Assays of Salvianolic Acid A Degradation as a Therapeutic

Were L. L. Munyendo[1,2*], Lilechi D. Baraza[3] and George Sowayi[2]

[1]Department of Chemistry and Biochemistry, Moi University, P.O.Box 3900-30100, Eldoret, Kenya.
[2]Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, P.O.Box 190-50100, Kakamega, Kenya.
[3]Department of Pure and Applied Chemistry, Masinde Muliro University of Science and Technology, P.O.Box 190-50100, Kakamega, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Author WLLM designed the study, mobilized resources, engaged in laboratory activities and wrote the first draft of the manuscript. Authors LDB and GS wrote the protocols and managed the analyses of the study. All authors read, reviewed and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the stability of Salvianolic acid A (SAA), a promising cardiovascular drug candidate. Additionally avail an archetype in-vitro therapeutic monitoring from SAA degradation profile for characteristic oral gavages.
Study Design: Experimental by laboratory analysis.
Place and Duration of Study: Department of Chemistry & Biochemistry, Moi University; Department of Pure & Applied Chemistry and Department of Medical Laboratory Sciences of Masinde Muliro University of Science & Technology, between June 2013 and September 2014.
Methodology: Drug stability was studied according to ICH guidelines. A stability-indicating

**Corresponding author: E-mail: munyendollw@yahoo.com;*

HPLC method was developed and validated; mimicking the systemic environs SAA degradation kinetics was then evaluated.
Results: SAA degradation followed first-order kinetics with rate constant increasing from 0.0099 h^{-1} at 313 K to 0.08044 h^{-1} at 363 K. The $t_{0.5}$ was between 70.0 and 9.8 hours while $t_{0.9}$ was between 10.6 and 1.5 hours within 313 K to 363K temperature range. Activation energy was 39.56 $KJmol^{-1}$. A V-shaped pH-rate profile was observed with maximum stability at pH4.0. Degradation was rapid in hydrogen peroxide solution and in the simulated gastro-intestinal fluids. SAA exhibits high stability at pH 4.0, hence the suggested optimum pH for processing.
Conclusion: This study provides sufficient physicochemical data depicting SAA to be of intermediate stability. Therefore availing an imperative basis for selecting suitable dosage forms and expected kinetics during therapeutic SAA monitoring.

Keywords: Salvianolic acid A; stability; therapeutic; degradation; kinetics.

1. INTRODUCTION

Salvianolic acid A (SAA); (2R)-3-(3,4-Dihydroxyphenyl)-2-[(E)-3-[2-[(E)-2-(3,4-dihydroxyphenyl) ethenyl]-3,4-dihydroxyphenyl] prop-2-enoyl] oxypropanoic acid, is a major component of the Danshen extract of *Salviae miltiorrhiza* [1]. It is obtained as a result of hydrolysis of the ester linkages of Salvianolic Acid B (SAB), the most abundant and bioactive member of the salvianolic acids in Danshen. Published works also suggest that SAA is a decarboxylation product of Lithospermic Acid (LA), which is a hydrolysis product of SAB [2].

In recent years, there has been growing interest in SAA due to its potent bioactivities and extensive application in alternative medicinal preparations [3-5]. As major water-soluble components of *S. miltiorrhiza*, SAA, SAB and Rosmarinic Acid (RA) are now widely accepted in western countries especially in the treatment of cardiovascular disorders [6]. The problem is that, therapeutic drug monitoring for this cardiac medication highlights a narrow therapeutic window. This could be due to degradation products and metabolites that usually manifest as overdose causing vomiting, diarrhea, confusion, visual disturbances, and cardiac arrhythmias [7]. Preparations of SAA as a danshen injection have been reported to be very unstable in distilled water [8]. SAA is also not found to be listed in most of the recognized Pharmacopoeias and there is little information regarding the degradation kinetics and quantification of SAA in bulk.

There is need for pharmaceutical scientists to avail SAA in conventional dosage formulations for use in the management of cardiovascular cases. Prior to this formulation however; it is essential that certain fundamental physical and chemical properties of the drug molecule and derivatives are determined. This information dictates many of the subsequent events and approaches in dosage formulation development. Additionally assessment of the mode of degradation is important for a medication.

This study therefore covered the development of a stability-indicating HPLC method with an ultraviolet detector for quantification of SAA and its degradation products or metabolites. The method was utilized to study solid state stability of SAA under influence of strong UV light, relative humidity and dry heat. Stability of SAA in Simulated Gastro-Intestinal Fluid (SGIF) was then evaluated according to the guidelines of International Conference on Harmonization (ICH) [9,10]. In this paper the influence of temperature, pH and oxidation on the degradation kinetics is also reported.

2. MATERIALS AND METHODS

2.1 Materials Chemicals and Instruments

Salvianolic acid A (98.78% pure) was a kind donation by Prof. Zhou Jianping of China Pharmaceutical University, Nanjing China. Methanol (HPLC grade) was purchased from Yuwang Group Company, acetonitrile (HPLC grade) from Shanghai Lingfeng Chemical Reagent Co. Ltd China. All the other chemicals and solvents were typically analytical grade and supplied by Sinopharm Chemical Reagent Co. Ltd China.

The HPLC analytical work was done at Moi University Chemical Engineering Laboratory on a LC–10AT Liquid Chromatography system with SPD–10A UV-Vis detector (Shimadzu), using a Hedera™ ODS-2 RP-18 column (250 mm x 4.6

mm, 5 µm) and 20 µL variable loop injector. The flow rate was 0.5 $\mathrm{mL \cdot min^{-1}}$ and UV detection at 286 nm. Other instruments included BS 124s electronic balance (d=0.0001 g, Sartorius, Germany); PHS 25B digital pH meter (Suntex, Taiwan); Dri-bath oven (XMTB, Shanghai China), HH-8 electrothermal thermostatic water-jacket (Guo Q, China).

2.2 Validation of the Method

RP-HPLC method was developed and validated according to ICH guidelines [9]. The HPLC system comprised of a Shimadzu LC-10AT pump, a SPD-10A UV detector, on a Hedera™ ODS-2 RP-18 column (250 mm x 4.6 mm, 5 µm) column thermostated at 25°C and detection carried out at 286 nm. The mobile phase consisted of mixture of acetic acid in water solution (1 ml/100 ml v/v): Methanol: Acetonitrile (35:50:15, v/v/v) to which $CaCl_2$ (0.5 mol.L^{-1}) was added. System suitability parameters were evaluated using 5 $\mathrm{mg \cdot mL^{-1}}$ SAA in the mobile phase.

2.2.1 Linearity and range

To establish linearity and range, stock solution of SAA (100 $\mu\mathrm{g \cdot mL^{-1}}$) in methanol was diluted to achieve two sets of standard solutions in the ranges 15 – 75 $\mu\mathrm{g \cdot mL^{-1}}$ and 20 – 100 $\mu\mathrm{g \cdot mL^{-1}}$. Correlation coefficients (R^2) from plot of Peak area against the concentrations was then utilized to select the best range.

2.2.2 Accuracy and precision

Relative Standard Deviation (RSD) was used to establish precision while the accuracy by determination of analytical recoveries. This entailed intra-day and inter-day evaluations by analysis of SAA at three concentration levels (15 $\mu\mathrm{g \cdot mL^{-1}}$, 45 $\mu\mathrm{g \cdot mL^{-1}}$ and 75 $\mu\mathrm{g \cdot mL^{-1}}$) in quality control samples (n=9) on the same day and on three consecutive validation days.

2.2.3 Limits of quantification and detection

The Limits of quantification and detection were calculated by the simultaneous analysis of SAA in prepared solutions and using standard deviations of pre-determined analytical recoveries [10].

2.2.4 Robustness

To determine the robustness of the method, experimental conditions were purposely altered. Effect of the flow rate on peak resolution was studied by changing stepwise from a flow rate of 0.9 $\mathrm{mL \cdot min^{-1}}$ to 1.1 $\mathrm{mL \cdot min^{-1}}$ while mobile phase composition remained the same. Effect of the column temperature on the resolution was also studied at 25°C, 45°C and 65°C.

2.3 Stability Studies

2.3.1 Solid state SAA stability

This was carried out according to guidelines of ICH [10]. To evaluate the solid state stability, SAA powder (5 mg) was spread as a thin layer on unprotected open aluminum foil and subjected to following treatments.

- Dry heat – triplicate samples were placed in a dry bath incubator at 333K.
- High Humidity – triplicate samples were stored at 273 K under 92.3% and 75% relative humidity achieved using saturated potassium nitrate and sodium chloride solutions respectively, in closed desiccators without direct light.

HPLC analysis was then carried out on the 0^{th}, 5^{th} 10^{th} and 15^{th} day.

Three batches each 5 mL of SAA solution prepared in methanol (5 $\mathrm{mg \cdot mL^{-1}}$) in Pyrex glass bottle and secured with transparent parafilm were illuminated with strong UV light (4500 lx) at 15 cm from the source (Philips F4T5/D fluorescent lamp) in a 20 cm x 15cm x 15cm chamber. Every 5 days 1 mL of solution from each batch was sampled and diluted to 10 mL with methanol before analysis of SAA concentration by HPLC.

The percentage of SAA remaining was expressed as follows:

$$\text{Remaining percentage } (\%) = \frac{C_t}{C_0} * 100 \qquad (1)$$

where C_0 is the concentration of SAA at day 0 and C_t is the concentration of SAA remaining at the t^{th} day.

2.3.2 Stability study of SAA in Simulated Gastro-Intestinal Fluids (SGIF)

Simulated Gastric Fluid (SGF) was prepared with 2 g of sodium chloride, 3.2 g of pepsin (385 units/mg), 7 mL of hydrochloric acid (37% HCl to adjust pH to 1.2) and completed with water to 1

L. While pre-concentrated Simulated Intestinal Fluid (SIF) free of Na-TC and lecithin comprised 10.3 g of sodium dihydrogen phosphate, 12.4 g of sodium chloride, 35 mL of sodium hydroxide solution 1 N, 7 g of sodium bicarbonate (to adjust pH to 6.5 when mixed with SGF in a ratio of 50/50; v/v) and completed with water to 1 L. SAA (5 mg) powder was then introduced into 10 mL of the Simulated Gastric Fluid (SGF) in a vessel, to which 10 mL of pre-concentrated Simulated Intestinal Fluid (SIF) was added after 55 minutes. This resulted into a two-fold dilution of the initial pre-concentrated SIF achieving desired concentration ratios for a SGIF.

For stability studies, the SAA in SGIF solution was incubated in a water bath maintained at 310 K (body temperature) from which aliquots of 0.5ml were sampled at different time intervals: 5, 15, 30 and 55 minutes in SGF and 60, 70, 85, 120, 150, 180, 240, 360, 480, 960 and 1440 minutes in SIF replenishing with 1 ml fresh media each time. The sampled solution was diluted with equal volume of methanol filtered through a 0.45 µm pore-sized microporous membrane before HPLC analysis for SAA content. All the experiments were carried out in triplicates.

2.4 Degradation Studies

SAA solution was dissolved in de-ionized water ($5\ \mathrm{mg \cdot mL^{-1}}$) to study the effect of temperature on degradation while for the effect of pH on degradation the solution was prepared in various buffer solutions. Samples were then sealed in screw-topped test tubes and put in a thermostated bath at predetermined temperatures. Sample solution of 1 mL each was periodically withdrawn during the kinetics run, then diluted to 10 mL with methanol and analyzed within 2 hours.

2.4.1 Effect of temperature on degradation

Effect of temperature on SAA was studied at 313K, 323K, 333K, 343K, 353K and 363K. The degradation rate kinetics was then evaluated by plotting log of concentration of drug remaining versus time. The kinetics parameters as apparent order, degradation rate constant (k), $t_{0.5}$ and $t_{0.9}$ were determined from the slopes of the straight lines at each temperature obtained by plot of equation (2).

$$\mathrm{Inc_t} = \mathrm{lnc_0} - \mathrm{k_{obs}} \quad (2)$$

where c_t is the time-dependent concentrations and c_0 concentration of SAA at 0 time respectively, k_{obs} is the observed rate constant of the first order degradation.

The kinetic parameters were then estimated using the following expressions;

$$\mathrm{k\ (h^{-1})} = \frac{\mathrm{lnC_0 - ln\ C_t}}{\mathrm{t_0 - t_t}} \quad (3)$$

$$\mathrm{t_{1/2}\ (hrs)} = \frac{\mathrm{ln\ 0.5}}{\mathrm{-k}} \quad (4)$$

$$\mathrm{t_{0.9}\ (hrs)} = \frac{\mathrm{ln\ 0.9}}{\mathrm{-k}} \quad (5)$$

The equation 6 below was used to determine the Arrhenius activation energy (Ea),

$$\mathrm{ln\ k} = \mathrm{ln\ A} - \frac{\mathrm{Ea}}{\mathrm{RT}} \quad (6)$$

where R is the universal gas constant and T is the absolute temperature in Kelvin.

Salvianolic acid A activation energy was calculated by plotting ln k against 1/T then applying the Arrhenius expression with the ideal gas constant (R= 8.314 $\mathrm{Jmol^{-1}K^{-1}}$).

2.4.2 Effect of pH on degradation

To evaluate the effect of pH on degradation, SAA solutions were prepared at a concentration of 5 $\mathrm{mg \cdot mL^{-1}}$ in buffer solutions (China Pharmacopoeia, 2005) of pH range 1.2 to 8.0 (1 M). Solutions were then incubated at 333 K in a thermostated water bath. Aliquots were taken at appropriate time intervals and analyzed immediately for SAA concentration after diluting with methanol.

2.4.3 Oxidation in hydrogen peroxide

For oxidation, 5 $\mathrm{mg \cdot mL^{-1}}$ SAA solutions were prepared in 0.3%, and 3% (v/v) hydrogen peroxide in water and stored at 25ºC. Aliquots (1 mL) were taken at appropriate time intervals, diluted to 10 mL with methanol before HPLC analysis to evaluate oxidation in hydrogen peroxide

2.5 Statistical Analysis

SPSS version 16.0 for Windows was used for linear regression and other statistical analysis. A value of $P < 0.05$ was considered statistically

significant. Data for multiple comparisons was analyzed by One-Way and Two-Way ANOVA.

3. RESULTS AND DISCUSSION

3.1 Validation of Method

A chromatographic method was developed by rational selection of mobile phase composition. Calcium chloride was effectively used as mobile phase additive which improved peak resolution and shortened retention time. The Ca^{2+} ions as electron acceptors, coordinates well with groups such as COOH, OH and NH_2 in the mobile phase resulting in alteration of the retention characteristics. The relative maximum absorption was realized at 286nm in the UV spectrum. Well resolved peaks were realized at 5.9 minutes for pure SAA while the degraded SAA displayed various peaks and the partially degraded SAA peak appeared at 5.5 minutes (Fig. 1A, 1B and 1C).

3.1.1 Linearity

Linearity was established in the range 15 $\mu g \cdot mL^{-1}$ to 75 $\mu g \cdot mL^{-1}$ with triplicates of the concentrations 15, 30, 45, 60 and 75 $\mu g \cdot mL^{-1}$. This fitted a straight line with the equation

$$A = 1.00024 * 10^{-5} * C + 51486 * 10^{-4} \quad (7)$$
$$R^2 = 0.9999, (n = 5)$$

where A is the Peak Area and C the concentration of SAA

The correlation coefficient (R^2) exceeded 0.999; illustrating a high linear dependence in the tested range.

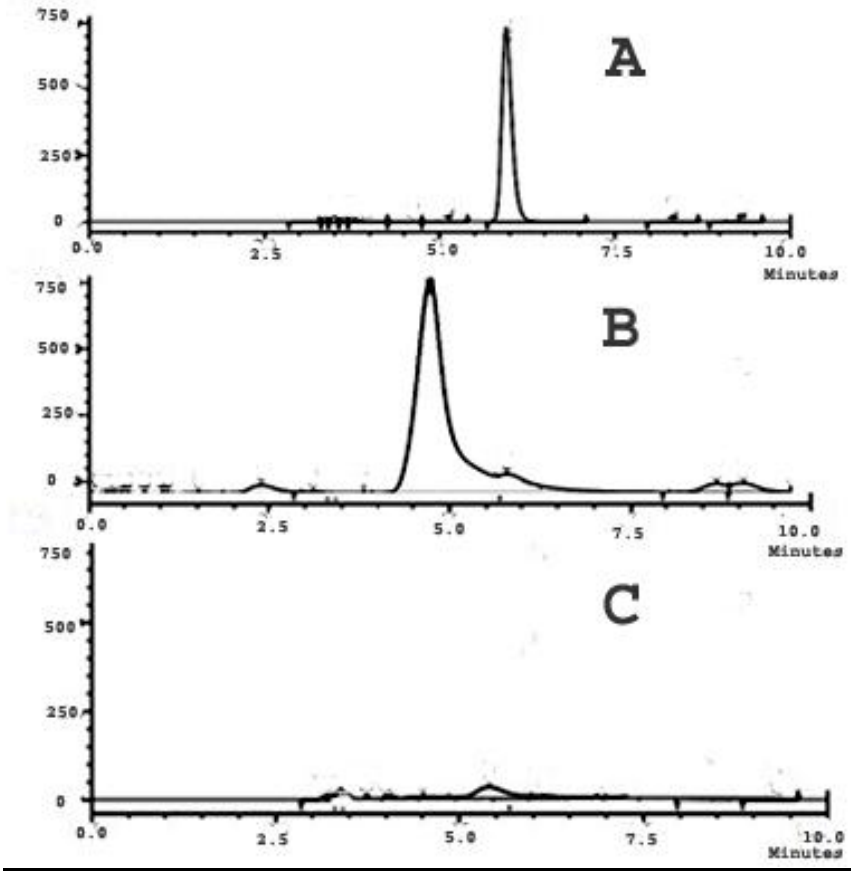


Fig. 1. Chromatographs obtained by HPLC Assays (A = Pure SAA; B = Degraded SAA; and C = Partially Degraded SAA)

3.1.2 Accuracy and precision

Precision expressed as recoveries for the inter/intra-day were from 95.23% to 99.77% at all the concentration levels (Table 1) with all R.S.D. values quantitatively less than 2.5%. Results of statistical analysis shown in Table 2 confirmed the high levels of accuracy and precision hence good repeatability. This indicated that the variation in analysis between days and within days (different concentrations) was not significant at the 95% level of confidence, allowing for satisfactory determinations of kinetics parameters using the developed method.

3.1.3 Limits of quantification and detection

The lowest concentration of analyte that could be determined [10], Limit Of Quantification (LOQ) was found to be 1.48 $\mu g \cdot mL^{-1}$ while the Limit Of Detection (LOD) was 0.44 $\mu g \cdot mL^{-1}$. This was accordingly 3.3 times lower than the LOQ thus conforming to the recommendations of ICH.

3.1.4 Robustness

Variation of chromatographic conditions did not result in significant change in values of the peak area. Variation of the flow rate from 0.75 ml/min to 1.0 ml/min resulted in only shortening of the retention time but not the peak area likewise 0.5 ml/min increased the retention time with same proportionality but no change in peak area. Column temperatures, usually affects dissolution of analyte in the mobile phase; elution at 25°C, 45°C or 65°C didn't result in recognizable changes in assay values. Robustness of the method was thus confirmed hence it could be utilized for routine analysis of SAA as an API and the degradation products as impurities in bulk.

3.1.5 Selectivity

Selectivity of the method was confirmed from the observed chromatographs. There was high resolution of the pure drug peak which was quite symmetric (Fig. 1A). For the degraded SAA, selectivity of method was also illustrated as some of the degradation product had a different retention time (2.5 min) from that of the pure drug and even partial degradation could be detected (Fig. 1C).

3.2 Stability of Salvianolic Acid A

3.2.1 Solid state SAA stability

Under conditions of strong UV light (4500lx), the colour of SAA solution gradually turned from yellow to brown indicating degradation to different products. Percentage of SAA reduced to only 27.3% (Table 3). This degradation is reminiscent formation of oxidation products by photolytic reactions. The SAA in powder form was reduced to 93.1% following exposure to 333 K dry heat for 15 days. This indicated decomposition of SAA at high temperatures.

Table 1. Recovery results for inter-day and intra-day analysis

Concentration ($\mu g \cdot mL^{-1}$)	Day 1	Day 2	Day 3	Mean	S.D.	R.S.D %
15	95.23	95.57	96.51	95.77	0.663	0.69
45	97.30	98.77	98.89	98.32	0.885	0.90
75	99.14	99.20	99.31	99.22	0.086	0.09
mean	97.22	97.85	98.24			
S.D.	1.956	1.983	1.510			
R.S.D %	2.01	2.03	1.54			

Table 2. Statistics results for SPSS analysis for variation between-subjects

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.870E14[a]	4	1.217E14	7.587E4	.000
Day	1.699E10	2	8.494E9	5.293	.007
Concentration	4.870E14	2	2.435E14	1.517E5	.000
Total	2.118E15	81			
Corrected Total	4.871E14	80			

[a]R Squared = 1.000 (Adjusted R Squared = 1.000); Dependent Variable = Peak Area
Analysis of variance to establish significance of inter-day and inter-concentration variations

In high humidity conditions at room temperature, SAA powder absorbed a substantial amount of water. There was a decrease in amount of SAA to 75.7% and 76.2% for 92.5% RH and 75% RH respectively after 15 days. Reduction in amount of solid SAA under high humidity indicated a hygroscopic nature and instability of SAA resulting in decomposition in humid environments. These findings formed a basis for study of the degradation kinetics involved.

3.2.2 Stability of SAA in simulated gastro-intestinal fluids (SGIF)

Incubation of SAA in the SGF was characterized with a rapid reduction in SAA concentration (Fig. 2) with time and an apparent gradual colour change to brown. An up to 85% drop in concentration was registered between 0 and 55 min and further gradual reduction. Whatever the medium (SGF or SIF) it was evident that there was gross break down of the drug, within 55 minutes in the SGF, and continued disintegration in SIF. This revealed SAA instability in the Gastrointestinal Tract (GIT), hence the need for consideration of protection of the drug by a drug delivery system against the gastric environment.

These further illustrated the need for evaluation of the kinetics involved in order to generate substantial information and important therapeutic monitoring creteria for an oral delivery system.

3.3 Degradation Kinetics Studies

The correlation coefficients (R^2) of the semi-logarithmic plots of drug remaining versus time were all above 0.99, indicating degradation of SAA followed first-order kinetics.

The effect of temperature on SAA degradation was illustrated by the plot of ln (C_t/C_o) against time, the degradation process could be described to take the apparent first-order kinetics straight-line behaviour (Fig. 3). k, $t_{0.5}$ and $t_{0.9}$ were calculated at 313 K, 323 K, 333 K, 343 K, 353 K and 363K, (Table 4), the rate constant increased from 0.0099 h^{-1} at 313K to 0.08044 h^{-1} at 363 K. The $t_{0.5}$ was between 70.0 and 9.8 hours while $t_{0.9}$ was between 10.6 and 1.5 hours within 313 K to 363 K temperature range. SAA activation energy determined from the Arrhenius plot (Fig. 4) was 39.56 $KJmol^{-1}$. The k values are realized to increase with temperature and the corresponding $t_{0.5}$. Such direct proportionality between k value and temperature is characteristic first order degradation kinetics under influence of temperature [11-13].

Table 3. Results for Stability Testing of SAA

	Remaining percentage of SAA			
Treatment	**Day 0**	**Day 5**	**Day 10**	**Day 15**
Strong UV light (4500lx)	100.0±0.03	54.0±0.05	33.8±0.01	27.3±0.02
Dry Heat (333k)	100.0±0.03	96.0±0.00	95.7±0.01	93.1±0.07
High humidity (92.5% R.H.)	100.0±0.03	93.8±0.03	81.9±0.01	75.7±0.01
High humidity (75% R.H.)	100.0±0.03	91.0±0.00	77.8±0.02	76.2±0.00

Mean ± Relative Standard Deviation (R.S.D.), in triplicate

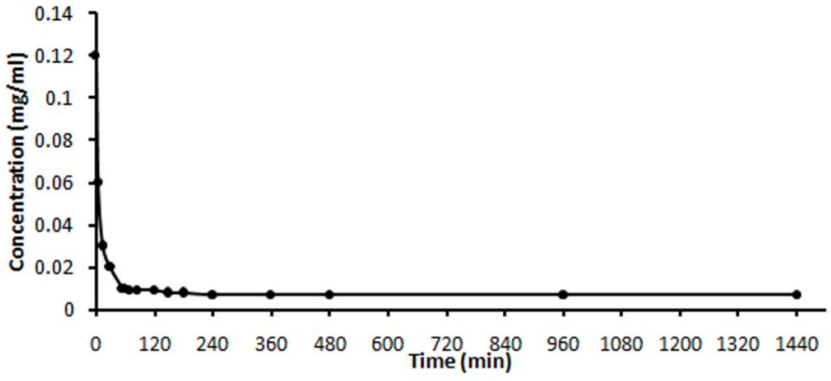


Fig. 2. Concentration of SAA in SGIF with time

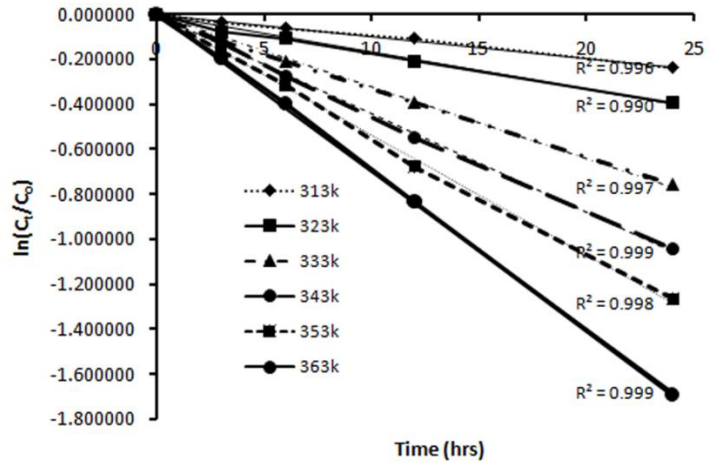


Fig. 3. First-order plots for the degradation of SAA at various temperatures
313k RSD= ±0.024; 323k RSD= ±0.020; 333k RSD= ±0.086; 343k RSD= ±0.066

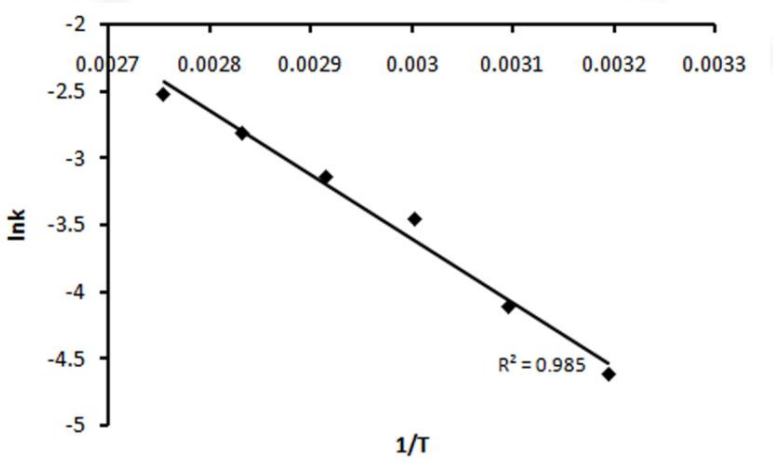


Fig. 4. Arrhenius plot for Ea determination

Table 4. Calculated first-order degradation rate constant k, $t_{0.5}$ and $t_{0.9}$ in solution

Temperature (k)	k (h^{-1})	$t_{0.5}$ (hrs)	$t_{0.9}$ (hrs)
313	0.0099	70.0	10.6
323	0.01633	42.4	6.5
333	0.03154	22.0	3.3
343	0.04342	16.0	2.4
353	0.0595	13.2	2.0
363	0.08044	9.8	1.5

Calculated values using expressions for first – order kinetics parameters (equations 3 - 5)

The degradation of SAA in buffer solutions over pH 1.2 to pH 8.0 at 333 K gave plots with correlation coefficients (R^2) above 0.99, indicating that degradation followed first-order kinetics (Fig. 5). A V-shaped pH-rate profile of SAA was observed with maximum solution stability at around pH 4.0 (Fig. 6) having an estimated degradation constant of 0.06246 h^{-1} and half life of 11.09 hours at 333 K. At pH less than 3.5 the rate of degradation falls gradually while a rapid degradation rate is then encountered from pH 4.0 to pH 5.0. Above pH 5.0 the rate of degradation increases stepwise with increase in pH value. At about the region pH 5 to pH 7 the curve exhibits an inflection. Such inflections are usually observed in regions where the pKa of the substrate falls, when the reactivity of the protonated and unprotonated are sufficiently different [14]. It could therefore be suggested to be the pH range at which the pKa of SAA falls.

The degradation rate as a result of each species on SAA could therefore be expressed as;

$$k'_{(obs)} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_{BH}[BH] + k_B[B^-] \quad (8)$$

where k_0 is the first-order degradation rate constant in water, and k_H, k_{OH}, k_{BH} and k_B are second-order rate constants for degradation products of SAA influenced by protons, hydroxyl ions, acid form of buffer [BH] and basic form of buffer [B^-] respectively.

Instant degradation of SAA was noted upon dissolving in hydrogen peroxide solution. This is explained from the nature of SAA chemical structure and supported also by previous studies [15].

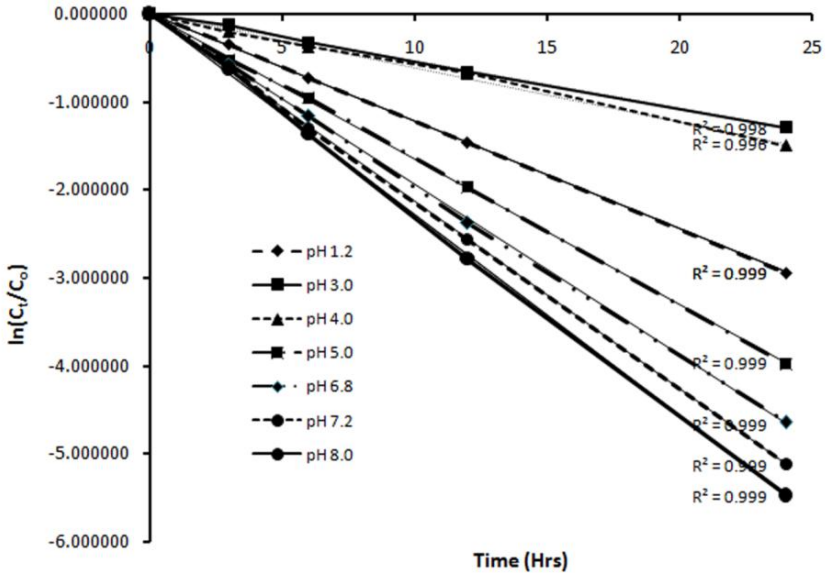
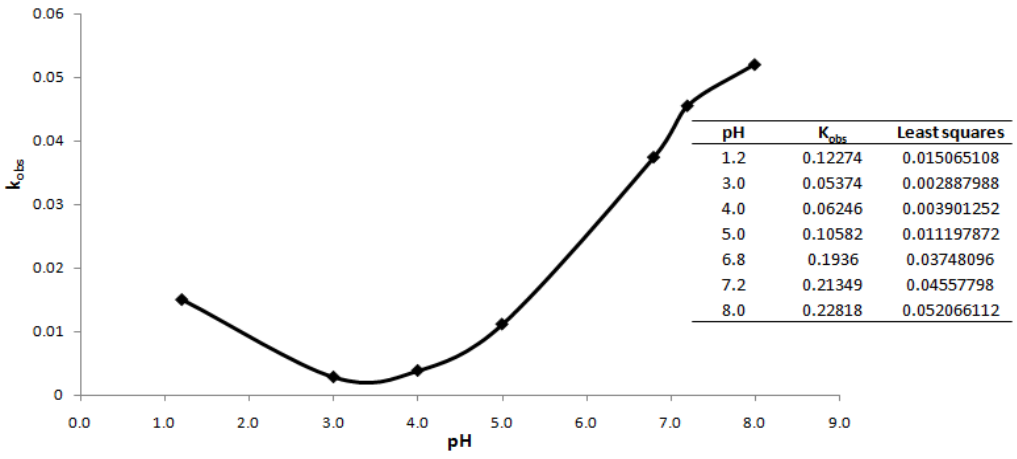


Fig. 5. First-order plots for the degradation of SAA at various pH values

pH 1.2 RSD= ±0.034; pH 3.0 RSD= ±0.040; pH 4.0 RSD= ±0.046; pH 5.0 RSD= ±0.016; pH 6.8 RSD= ±0.034; pH 7.2 RSD= ±0.023; pH 8.0 RSD= ±0.055



pH	K_{obs}	Least squares
1.2	0.12274	0.015065108
3.0	0.05374	0.002887988
4.0	0.06246	0.003901252
5.0	0.10582	0.011197872
6.8	0.1936	0.03748096
7.2	0.21349	0.04557798
8.0	0.22818	0.052066112

Fig. 6. pH – rate (k_{obs}/hr) profile for SAA at 333k (plot of pH against Least squares of K_{obs})

4. CONCLUSION

Salvianolic acid A and its degradation products could be effectively separated and detected by the developed and validated RP-HPLC-UV method. SAA undergoes degradation under thermal stress, high humidity and strong UV light; it is instantly oxidized in hydrogen peroxide solution. SAA is also quite unstable in the simulated gastro-intestinal fluids. The degradation in aqueous media followed first order kinetics. The activation energy of SAA suggests a kinetic degradation model of intermediate sensitivity to temperature.

Salvianolic acid A exhibited high stability at around pH 4.0; it therefore suggested optimum pH for processing and release from the dosage delivery system. Development of the drug into self emulsifying tablets stabilized by Medium Chain Triglycerides (MCT) that form emulsions in biological systems could be a promising formulation strategy.

Considering the Rule of 5 [16]; from the structural point of view, SAA falls short of only a single criteria in possessing more than 5 H-bond donors. Nevertheless results from the Rule of 5 calculations are only indicative. The results in this paper therefore, avails physicochemical properties data necessary for a desirable formulation strategy to achieve oral absorption of SAA.

CONSENT

It is not applicable.

ETHICAL APPROVAL

No ethical considerations were made as there was no laboratory animals' use in the entire study

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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