

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

**SUDAN UNIVERSITY OF SCIENCE
AND TECHNOLOGY**

College of Graduate Studies

Stability Study of Artesunate

دراسة ثبات عقار الارتيسونيت

**A thesis Submitted in Partial
Fulfillment for the requirements
of the degree of Master of
Science in Chemistry**

By

Elsadig Hassan Rudwan

(BSc, Higher Diploma Chemistry)

Supervisor

Dr. Ahmed Elsadig Mohamed Saeed

Dedication

**To the soul of my father, my mother,
my wife and my sons**

Acknowledgment

I am particularly appreciative to:

Dr. Ahmed Elsadig Mohamed Saeed, who encourage supervisor guidance and also providing new ideas and unique scientific environment.

Amipharma Laboratories for laboratory facilities and all my colleagues at the department of quality control.

My best friend Elsadig Hassan khamis for fruitful discussion, words of encouragement and a very pleasant social environment.

Mis: mona shouna for her good assistance.

ABSTRACT

In this work the stability of artesunate in solid form and liquid form towards high temperature and direct sunlight, was investigated and analyzed by using different analytical methods such as high performance liquid chromatography UV spectroscopy and potentiometric titration.

1. The reaction rate of artesunate was pH and temperature dependent. Artesunate stability varies as a function of pH and temperature.
2. All the investigated pharmaceutical excipients were found to increase the thermal instability at 70 °C in aqueous media. The tendency of which to increase the thermal stability was in the following order: methyl paraben, Propyl paraben, talcum powder, sodium benzoate, povidon and aerosil respectively
3. Artesunate was affected when exposed to UV radiation in solid and liquid form. The photothermal reaction rate of artesunate in aqueous media and solid state showed first order reaction kinetics.
4. The result reveals that temperature above 40 °C affected the stability of artesunate when the test solution was exposed to direct sunlight, in both liquid form and solid form.

5. The hydrolysis of artesunate by sodium hydroxide 2M, yielded different products which compared to the hydrolysis in hydrochloric acid 2M.

6. With increased temperature, the rate of decomposition was increased.

7. At low pH value as 2 and 3, artesunate was more hydrolysed than in neutral pH 7.0 were stable.

8. At high pH values, decomposition of artesunate was rapid both at room temperature and in sunlight.

9. Titration methods were not adequate for determination of artesunate in presence of 0.05M NaOH as hydrolysis product like succinic acid would be produced.

10. High performance liquid chromatography (HPLC) was found to be stability indicating method of analysis of artesunate and its related substances.

الْخُلَاصَة

فى هذا العمل تمت دراسة ثبات عقار الأرتوسينيت فى اتجاه درجة الحرارة العالية وضوء الشمس المباشر ، باستخدام اجهزه الكروماتقرافيا السائل عاليه الاداء والطيف المرئ والمعائيرات المجهاديه واضحت النتائج الأتى:

1- معدل تفاعل الأرتوسينيت يعتمد فى ثباته على الأس الهيدروجينى pH ودرجة الحرارة

2- كل المصوغات الصيدلانية التي بحثت إتضح إنها تؤثر فى زيادة ثبات عقار الأرتوسينيت عند درجة حرارة 70 درجة مئوية وكان مسلكها فى زيادة ثبات الأرتوسينيت ضد التطل الحرارى على النحو التالى
ميثيل برابين- ، بروبييل برابين- ، بدرة التلك وبنزوات الصوديوم والبوليفيدون وأخيرا الإيروسيل .

- 3- الأرتوسنيت عندما يتعرض للأشعة البنفسجية وضوء الشمس المباشر-
فى الحالة الصلبة والسائلة أظهر حركية تفاعل من الرتبة الأولى .
- 4- أظهرت النتائج أن ارتفاع درجة الحرارة أعلى من 40 درجة مئوية
يؤثر على ثبات عقار الأرتوسينيت فى الحالتين الصلبة والسائلة عند
تعرضه لأشعة الشمس المباشرة.
- 5- تحلل الأرتوسينيت فى محلول هيدروكسيد الصوديوم 2 مولارى
يعطى منتجات مختلفة مقارنة لتحلل الأرتوسينيت فى وسط حامضى مثل
حمض الهيدروكلوريك 2 مولارى.
- 6- عند زيادة درجة الحرارة يزداد معدل التفكك الحرارى للأرتوسينيت .
- 7- وضح جلياً أنه عند قيمة الأس الهيدروجيني pH من 2 و 3 كان
الأرتوسينيت أكثر تحللاً منه فى الوسط المتعادل ذو الأس
الهيدروجيني 7 pH .
- 8- عند قيم الأس الهيدروجيني العليا إتضح أن التفكك كان سريعاً فى
درجة حرارة الغرفة ودرجة حرارة الشمس المباشرة .
- 9- ثبت عدم جدوى تقدير كميات الأرتوسينيت بالمعايرة عند إستخدام
محلول هيدوكسيد الصوديوم 0.05 مولارى نسبة لتكوين منتجات
التحلل القاعدى وكمثال لذلك حامض الساكسينيك .

10- ثبت أن طريقة التحليل بواسطة كروموتوجرافيا السائل ذات الأداء
العالي طريقة دالة على الثبات ويمكن إستخدامها فى تحليل
الأرتوسينيت الكمي والمواد الناتجة عن التحلل الحرارى و الضوئى .

Table of Contents

Table of contents	Page
Dedication	II
Acknowledgment	III
English Abstract	IV
Arabic Abstract	V
List of content	VIII
List of Tables	XII
List of Figure	XIII
List of Abbreviation	XVI

List of content

CHAPTERONE	page
1. Introduction	1
1.1. General principle	1
1.2. Definition of terms	2
1.2.1. Accelerated stability testing	2
1.2.2. Bracketing	2
1.2.3. Drug substance	3
1.2.4. Dosage form	3
1.2.5. Batch lot	3
1.2.6. Excipients	3
1.2.7. Climatic Zone	4
1.2.8. Mean kinetic temperature	4
1.2.9. Real time (long-term) studies	5
1.2.10. Shelf life	5
1.2.11. Stability	

5	
1.2.12. Stability test	
6	
1.2.13. Stability indicating method	
6	
1.2.14. Supporting stability data	
6	
1.2.15. Storage condition tolerance	
6	
1.3. Drug substance	
6	
1.3.1. Stress testing	
6	
1.3.2. Testing frequency	
7	
1.3.3. Storage condition	
8	
1.3.4. Stability commitment	
9	
1.4. Drug product	
10	
1.4.1. In the developing phase	
10	
1.4.2. Design of stability testing	
11	
1.4.3. Shelf life	
12	
1.5. General case	
13	

1.16. Drug stability	
14	
1.7. Chemical degradation	
14	
1.7.1 Solvolysis	
14	
1.7.2. Oxidation	
15	
1.7.3 .Photrolysis	
15	
1.7.4. Polymerization	
15	
1.7.5. Optical isomerization	
15	
1.8. Physical degradation	
15	
1.8.1. Polymorphism	
15	
1.8.2. Vaporization	
16	
1.8.3. Water loss	
16	
1.8.4. Absorption of water	
16	
1.9. Factor influencing degradation	
16	
1.9.1. Temperature	
16	
1.9.2. Solvents	

17	
1.9.3. Moisture (Atmospheric)	
17	
1.10. Additives	
17	
1.10.1 Buffer salts	
17	
1.10.2. Surfactant	
18	
1.10.3. Complexing agent	
18	
1.10.4. Antioxidant	
18	
1.11. Storage	
19	
1.12. Literature Review	
20	
1.12.1. Artemisinin and its derivative	
21	
1.12.2. Biopharmaceutical information of Artemisinin	
22	
1.12.3. Mode of action of artemisinin	
22	
1.12.4. Biosynthesis of artemisinin	
23	
1.12.5. Pharmacokinetic of artemisinin	
25	
1.12.6. Artesunate	
25	

1.12.7. Chemical name	26
1.12.8. Pharmacokinetic of artesunate	28
1.12.9. Pharmacodynamic of artesunate	29
1.12.10. Synthesis of artesunate	32
1.13. Aim and objectives	33
CHAPTER TWO	
2. Material and instrument	35
2.1. Chemical and material	35
2.2. Instruments	36
2.3. Method of analysis and experimental results	37
2.3.1. Melting point determination	37
2.3.2. PH value	38
2.3.3. Specific optical rotation	38
2.3.4. Infra red spectroscopy of artesunate	38
2.3.5. Method of determination artesunate by HPLC	40

2.3.6. Related substance of artesunate	
41	
2.3.7. Titrometric method of determination artesunate	
44	
2.3.8. Ultraviolet spectroscopy of determination artesunaate	45
2.3.9. The effect of pH on UV spectrum of artesunate	
46	
2.3.10. The effect of solvents on UV spectrum of artesunate	48
2.3.11. The effect of pH on artesunate at room temperature	51
2.3.12. The effect of pH on artesunate to direct sunlight	52
2.3.13. The effect of pH on hydrolysis of artesunate	
55	
2.3.14. The effect of some excipients in the reaction rate of thermal decomposition of artesunate at 70 °C	
57	
2.3.15. The effect of temperature on artesunate stability	58
2.3.16. The effect of daylight on the stability of artesunate in solid state	
61	
2.3.17. The effect of daylight on the stability of artesunate	
in liquid state	
63	
2.3.18. The effect of UV radiation on artesunate	

64

2.3.19. The effect of dark room on artesunate

66

2.3.20. Decomposition of artesunate in aqueous media
in

direct sunlight

67

CHAPTER THREE

Discussion

68

Conclusion

75

Recommendation

76

CHAPTER FOUR

References

77

List of Tables

Table1.1.		Climatic	zone
4			
Table	1.2	Storage	condition
9			
Table1.3.		Testing	plan
12			
Table2.1.		Physical	properties of artesunate
38			
Table	2.2	Infra	red of artesunate
39			
Table	2.3	Related	substance and assay
43			
Table	2.4	Titration	results
45			
Table	2.5	UV	assay of artesunate
46			
Table	2.6	The effect	of pH on artesunate
47			
Table2.7.	Preparation of approximate universal buffer solutions		
(pH 2 to12)	48		
Table	2.8	The effect	of solvents on artesunate
49			
Table	2.9	The effect	of pH on artesunate at room temprature
51			
Table	2.10.	The effect	of pH on arteunate at direct daylight

52

Table 2.11. Hydrolysis of artesunate in alkaline media
54

Table 2.12. Hydrolysis of artesunate in acidic media
55

Table 2.13 The effect of some excipients on the rate of thermal
decomp

osition of artesunate

57

Table 2.14. Decomposition of arteunate at high temperature
58

Table 2.15. The effect of temperature on artesunate in solid form
60

Table 2.16. The effect of direct sunlight on artesunate in solid
form 62

Table 2.17. The effect of direct sunlight on artesunate in liquid
form 63

Table 2.18. The effect of UV radiation on artesunate in solid form
64

Table 2.19. The effect of UV radiation on artesunate in liquid form
65

Table 2.20. The effect of dark room on artesunate in liquid form
66

Table(2.21) Artesunate in solid form at dark room for 6 months
67

Table(2.22) Artesunate decomposed at daylight in liquid form
67

List Of Figures

Figure 1.1.	Artemisinin and its derivatives	21
Figure 1.2.	Biosynthesis of artemisinin	24
Figure 1.3.	Artesunate	25
Figure 1.4.	Synthesis of artesunate	31
Figure 2.1.	IR spectrum of artesunate reference standard	40
Figure 2.2.	IR spectrum of artesunate test sample	40
Figure 2.3	Chromatogram of test sample solution of artesunate	42
Figure 2.4.	Chromatogram of artesunate working standard	43
Figure 2.5	Chromatogram of related substance of artesunate solution A	44
Figure 2.6.	Chromatogram of related substance of artesunate solution B	44
Figure 2.7.	Pure artesunate reacted with sodium hydroxide 0.05M	45
Figure 2.8.	The effect of pH on artesunate at UV spectrum	47
Figure 2.9.	The effect of solvents on artesunate spectrum	50
Figure 2.10.	Pure artesunate spectrum in methanol, ethanol and acetonitrile	50
Figure 2.11.	The effect of pH on artesunate at room temperature	

52

Figure 2.12. The effect of pH and direct sunlight on artesunate
53

Figure 2.13. Chromatogram of pure artesunate reference standard
54

Figure 2.14. Chromatogram of basic degradation of artesunate
55

Figure 2.15. Chromatogram of pure artesunate reference standard
56

Figure 2.16. Chromatogram of acid degradation of artesunate
56

Figure 2.17. The effect of pharmaceutical excipients of reaction rate in stability of artesunate at 70 °C
58

Figure 2.18. Chromatogram of pure AS standard
59

Figure 2.19 Chromatogram of artesunate test sample in stability chamber 40°C

59

Figure 2.20 The effect of temperature in stability of AS in liquid form
61

Figure. 2.21 The effect of daylight on AS over 12 weeks solid form
62

Figure 2.22 The effect of daylight in stability of AS in liquid form
64

Figure 2.23 The effect of UV radiation on AS in liquid form at 254 nm
65

Figure 2.24 The effect of dark room in stability of AS in liquid form

List of abbreviation

As	Artesunate
DHA	Dihydroartimisinin
ART	Artemisinin
BP	British pharmacopeia
USP	United state pharmacopeia
FT	Fourier transformation
IR	Infrared
HPLC	High performance liquid chromatography
PP	Propyle paraben
MP	Methyl paraben
UV	Ultraviolet light
VIS	Visible light
WHO	World health organization
m.p	Melting point
λ_{\max}	Maximum wavelength of absorption
API	Active pharmaceutical ingredient