Stability Studies Of andrographolide

1.Stability Test Methods:

Introduction:

The purpose of stability testing is to provide evidence on how the quality of andrographolide varies with time under the influences of temperature and humidity and to establish recommended storage conditions, retest periods and shelf life.

Responsibility:

QA/QC Director is responsible for the stability study program and is also responsible for the stability samples analyzed as well as the maintenance of the program records.

Procedure and Design of Stability Studies:

Long Term Studies:

The samples of andrographolide are closed tightly in polyethylene bags, sealed in PE bags and packaged in aluminum containers. They are stored under long-term conditions of 252℃ temperature and 60±5% humidity conditions according to ICH/FDA guidelines. The three consecutive process validation batches and one batch every year are sampled and placed on long term stability testing. After the initial analysis, these samples will be analyzed at intervals of 3, 6, 9, 12, 18, 24 and 36 months. The results to date demonstrate that andrographolide is stable at 25±2℃temperature and 60±5% humidity conditions.

Accelerated Studies:

The samples of andrographolide are placed in polyethylene bags, sealed in PE bags, packaged in aluminum containers and stored at 40±2℃ and 75±5% humidity conditions. The three consecutive batches are taken for testing. After the initial analysis, these samples are analyzed at 1, 2, 3 and 6 months according to ICH Guidelines. The results to date demonstrate that andrographolide is stable at 40±2℃ temperature and 75±5% humidity conditions.

2.Stability Test Results

The following pages summarize the Accelerated and Long Term Stability Testing results available to date for three batches of andrographolide. As more data becomes available, we commit to forward this data so that it may be included as annual updates to the Product Drug Master File.

Shaanxi Guanjie Technology Co., Ltd.
andrographolide Accelerated Stability Study

Batch No: 20161012 Temperature: 40±2℃ Humidity: 75±5% Mfg. Date: 10/12/2016
Container: Duplicates commercial container (PE & PE coated Al bags in Aluminum canister)

Test Frequency	Appearance	Identity by UV	Loss on Drying	Assay (Andrographolide) (by HPLC)
Specs	To Pass	complies	≤1.0%	≥98.0%
Initial		complies	0.13	98.4
1M		complies	0.05	98.2
2M		complies	0.12	98.9
3M		complies	0.18	98.1
6M		complies	0.34	98.1

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Shaanxi Guanjie Technology Co., Ltd.
andrographolide Accelerated Stability Study

Batch No: 20161020 Temperature: 40±2℃ Humidity: 75±5% Mfg. Date: 10/20/2016
Container: Duplicates commercial container (PE & PE coated Al bags in Aluminum canister)

Test Frequency	Appearance	Identity by UV	Loss on Drying	Assay (Andrographolide) (by HPLC)
Specs	To Pass	complies	≤1.0%	≥98.0%
Initial		complies	0.10	98.1
1M		complies	0.21	98.2
2M		complies	0.14	98.0
3M		complies	0.26	98.3
6M		complies	0.05	98.6

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Shaanxi Guanjie Technology Co., Ltd.
andrographolide Accelerated Stability Study

Batch No: 20161028 Temperature: 40±2℃ Humidity: 75±5% Mfg. Date: 10/28/2016
Container: Duplicates commercial container (PE & PE coated Al bags in Aluminum canister)

Test Frequency	Appearance	Identity by UV	Loss on Drying	Assay (Andrographolide) (by HPLC)
Specs	To Pass	complies	≤1.0%	≥98.0%
Initial		complies	0.05	98.2
1M		complies	0.12	98.4
2M		complies	0.05	98.3
3M		complies	0.14	98.4
6M		complies	0.23	98.3

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Shaanxi Guanjie Technology Co., Ltd.
andrographolide Long Term Stability Study

Batch No: 20161012 Temperature: 25±2℃ Humidity: 60±5% Mfg. Date: Mfg. Date: 10/12/2016
Container: Duplicates commercial container (PE & PE coated Al bags in Aluminum canister)

Test Frequency	Appearance	Identity by UV	Loss on Drying	Assay (Andrographolide) (by HPLC)
Specs	To Pass	Complies	≤1.0%	≥98.0%
Initial		Complies	0.20	98.2
3M		Complies	0.06	98.3
6M		Complies	0.21	98.1
9M		Complies	0.02	98.3
12M		Complies	0.11	98.2
18M		Complies	0.08	98.1
24M		Complies	0.18	98.4
36M		Complies	0.25	98.3

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Shaanxi Guanjie Technology Co., Ltd.
andrographolide Long Term Stability Study

Batch No: 20161020 Temperature: 25±2℃ Humidity: 60±5% Mfg. Date: Mfg. Date: 10/20/2016
Container: Duplicates commercial container (PE & PE coated Al bags in Aluminum canister)

Test Frequency	Appearance	Identity by UV	Loss on Drying	Assay (Andrographolide) (by HPLC)
Specs	To Pass	Complies	≤1.0%	≥98.0%
Initial		Complies	0.05	98.2
3M		Complies	0.12	98.3
6M		Complies	0.05	98.2
9M		Complies	0.18	98.1
12M		Complies	0.32	98.2
18M		Complies	0.32	98.2
24M		Complies	0.14	98.5
36M		Complies	0.13	98.6

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Shaanxi Guanjie Technology Co., Ltd.
andrographolide Long Term Stability Study

Batch No: 20161028 Temperature: 25±2℃ Humidity: 60±5% Mfg. Date: Mfg. Date: 10/28/2016
Container: Duplicates commercial container (PE & PE coated Al bags in Aluminum canister)

Test Frequency	Appearance	Identity by UV	Loss on Drying	Assay (Andrographolide) (by HPLC)
Specs	To Pass	Complies	≤1.0%	≥98.0%
Initial		Complies	0.20	98.2
3M		Complies	0.23	98.3
6M		Complies	0.25	98.2
9M		Complies	0.05	98.0
12M		Complies	0.24	98.1
18M		Complies	0.12	98.2
24M		Complies	0.02	98.4
36M		Complies	0.05	98.1

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3.Degradation Study

The purpose of the degradation study is to determine if the HPLC method used by our facility for product release and stability studies will detect impurities and as a result is stability indicating. Two lots on stability, CT990123, CT990124 were chosen and samples from these lots were subjected to:

a. Sodium Hydroxide, 0.00001 N
b. Hydrochloric Acid, 0.04 N
c. Hydrogen Peroxide, 10%
d. Heat at 60℃
e. Ultraviolet light

The equipment used for this study was:

- HPLC: Waters 600, USA
- Ultrasonic De-gasifier: JL-120, Shanghai, China
- Column: Bondapak C_{18}, 3.9mm x 300mm, Waters, USA
- Water Bath
- Ultraviolet lamp
- Analytical Balance
- 100mL Measuring Flasks
- 10mL Pipettetes

The study was performed as follows:

1.Place 0.4g of andrographolide in a 100mL measuring flask and add acetonitrile to a total volume of 100mL.
2.Place 10.0mL of the solution from Step 1 in five separate 100mL measuring flasks.
   A. Add 20mL of 0.00001N NaOH to the first two flasks, cover and place in a boiling water bath for one hour.
   B. Add 20mL of 0.04N HC1 to the second flasks, cover and place in a boiling bath for one hour.
   C. Add 10mL of 10% Peroxide to the third pair of flasks; shake for 2 minutes and allow to stand for 30 minutes.
   D. Heat the fourth pair of flasks to 60℃ and maintain this temperature for seven days.
   E. Place the fifth pair of flasks under an ultraviolet lamp for seven days.
3.Prepare a reference standard for each sample by placing 15mg of andrographolide in a 100mL measuring flask and q. s. to 100mL with reagent grade solvent.
4.To adjust, the following is necessary.
   A. Use 20mL of 0.04N HC1 to neutralize the 0.00001N NaOH in the first two flasks.
   B. Use 20mL of 0.00001N NaOH to neutralize the 0.04N HC1 in the second two flasks.

C. Adjust the volumes of all five flasks to 100mL with mobile phase after the required conditions have been met.

5.Check all 12 flasks on the HPLC for a minimum of 30 minutes for each run to demonstrate that the method is stability indicating. The operating conditions or the HPLC are the same as those defined in **Quantitative Analysis of andrographolide by HPLC**. andrographolide CT020123, CT020124 as used in this degradation study.

Conclusion

The degradation study demonstrates that andrographolide is most stable when exposed, 0.04N Hydrochloric Acid, 0.00001N Sodium Hydroxide and Heat at 60℃, in that order. andrographolide is more unstable when exposed to peroxide, UV light. We can, therefore, conclude that the HPLC method used by Shaanxi Guanjie Technology Co., Ltd. will detect degradants for both stability studies and finished product testing/release. The method is, therefore, stability indicating.