

INVESTIGATION OF STABILITY OF KOREAN GINSENG IN HERBAL DRUG PRODUCT

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ABSTRACT

This study was designed to investigate long term stability of K-Gin capsules, a herbal medication that contains korean ginseng. Stability studies ensuring the maintenance of product quality, safety and efficacy throughout the shelf life are considered as pre-requisite for the acceptance and approval of any pharmaceutical product. Conducting long term stability studies following the guidelines issued by ICH, WHO and USP. The drug was stored under different climatic conditions: (25±1°C, humidity: 75%) Chamber 1(CH1); (30±1°C, humidity: 75%) Chamber 2(CH2) and (40±1°C, humidity 75%) Chamber 3(CH3) for 24 months, evaluation of capsules chemically (ginsenosides Rg1 and Rb1), physically and microbiologically, besides determination of certain elements including heavy metals, detection of pesticides residues and mycotoxins. The activation energies were found to be 10.99 and 10.2 (k.cal.mol⁻¹) for Rg1 and Rb1 respectively. The calculated t_{1/2} values for Rg1 were found to be 94 months in (CH1), 68.4 months in (CH2) and 51 months in (CH3). For Rb1 were found to be 100.3 months in (CH1), 73.39 months in (CH2) and 56.7 months in (CH3). Capsules assay results for Rg1 after 24 months period of study were found to be 98.93% in (CH1), 97.13% in (CH2) and 96.43% in (CH3). For Rb1 96.93% in (CH1), 96.83% in (CH2) and 96.13% in (CH3). Studies on the elemental composition confirmed absence of toxic heavy metals. Microbiological studies revealed no significant changes in total viable aerobic count of bacteria and fungi. The capsules were found to be free from any pesticides residues and the mycotoxins content was 723 ng kg⁻¹. The methods involved in this study covered, High Performance Liquid Chromatography (HPLC), Atomic Absorption Spectroscopy (AAS), Gas Chromatography-Mass Spectrometry (GC-MS) and ELISA. The results were found to be within the allowable limits stated in the guidelines. From this study K-Gin capsules were proved to be stable chemically and physically under studied storage conditions for 24 months.

Keywords: Ginsenosides Rg1 and Rb1, Determination, Stability, Activation Energy, Elements

1. INTRODUCTION

Ginseng is a well-known medicinal herb in traditional Asian medicine and is considered an adaptogen. *Panax ginseng* C.A. Meyer (Araliaceae), grows in China and Korea and more than 30 ginseng saponins (ginsenosides) have been isolated as the main biologically active constituents that include anti-carcinogenic, anti-diabetic and anti-inflammatory effects, as well as cardiovascular

protection and neuroprotection (Zhang *et al.*, 1996; Yun *et al.*, 2001; Joo *et al.*, 2005; Jung *et al.*, 2005).

As cancer is quickly becoming the leading cause of death in the world, many reports have focused on chemoprevention trials with *Panax ginseng*. Most of the pharmacological actions of ginseng are attributed to a variety of ginsenosides, which are triterpenoid saponins (Attele *et al.*, 1999; Huang *et al.*, 2005). The physiological and medicinal effects of the various

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ginsenosides differ and can even be oppositional (Sengupta *et al.*, 2004; Joo *et al.*, 2005). Since ginsenosides produce effects that differ from one another and a single ginsenoside initiates multiple actions, the overall pharmacology of ginseng is complex. Thus, ginseng extracts have been studied to examine the final activity of a wide range of biological actions (Tsang *et al.*, 1985; Nishino *et al.*, 2001; McElhaney *et al.*, 2004).

Korean Ginseng contains adaptogens that have been shown to return the body's system levels back to normal by equalizing body's system levels and has been used for the following: To lower cholesterol, increase metabolism, increase energy, stimulate the immune system, alleviate fatigue, reduce nervousness and reduce stress on the body (Bucci, 2000).

Korean ginseng is officially registered in Saudi Arabia for its anti-diabetic and anti-inflammatory effects, as well as cardiovascular protection with a recommended dose of 700 mg twice daily. Stability data is not reported in the official registration files of Korean ginseng. Therefore, the present study was designed to investigate the stability of Korean ginseng capsules, besides carrying the general chemical, physical and microbiological quality control measurements for it.

2. MATERIALS AND METHODS

2.1. Materials

The pure sample of Ginsenoside Rg1 and Rb1 was purchased from Indofine chemical company ICC (Somerville, NJ 08876 USA). The commercial capsules (K-Gin Capsules 700 mg Batch # 908345) were obtained from commercial sources. Methanol, acetonitrile, (all of HPLC grades), hydrochloric acid (Analar), monobasic sodium phosphate, potassium dihydrogen phosphate were obtained from BDH (Poole, UK). Cartridge containing 360 mg of octadecylsilyl silica gel for chromatography was purchased from Agilent Technologies, Inc., NC, USA. The standard kit used for pesticide residues was: Kit # 51C/CX, PolyScience Corporation, Chemical Division, Analytical Standards, 6366 Gross Point Road, Niles, IL-60648. USA. RIDASCREEN aflatoxin total screening kit (Art. No. 470) manufactured by R-Biopharm AG, Darmstadt, Germany.

2.2. Methods

2.2.1. HPLC For Determination of Ginsenosides Rg1 and Rb1

Analysis were carried out using a Shimadzu LC-10ADVP pumps, SIL-10ADVP auto injector, CTO-10ADVP column oven, DGU-14A degasser, SPD-

M10AVP diode array detector and SCL-10AVP system controller, Separation was achieved on a reverse phase C18 hypersil BDA 4×250 mm, 5 μm, flow rate 1 mL min⁻¹, injector volume 20 μL, wave length 205 nm and mobile phase composition:

Acetonitrile (A) and (B) water for gradient elution	
Time (min.)	Concentration of B(%)
0-14	5
14-21	10
21-23	14
23-55	20
55-60	10
60-80	5

2.2.2. Standard Solution

A solution each of 0.5 mg mL⁻¹ of ginsenoside Rg1 and ginsenoside Rb1 reference materials in methanol were prepared.

2.2.3. Preparation of Test Sample

1 gm of the powdered drug was weighed extracted with 70 mL of 50% (v/v) solution of methanol, boiled in water bath under reflux for one hour. Then cooled centrifuged, evaporated to dryness under reduced pressure at a temperature not exceeding 60°C. The residue was dissolved in 10 mL buffer solution containing 3.5 gm of sodium dihydrogen phosphate in 1000 mL of water (solution A). Five ml of solution A was applied to the cartridge (clean up procedure using solid phase extraction containing 360 mg of octadecylsilyl silica gel for chromatography), eluted with 20 mL of water, followed by 15 mL of 30% (v/v) methanol. The elute was discarded and then the cartridge was eluted with 20 mL of methanol. The methanol extract was evaporated to dryness. The residue was redissolved in 2 mL of methanol before injection.

2.3. Elemental Analysis

Atomic absorption spectroscopy was performed using Shimadzu AA-6800 (AAS) with graphite and flame auto switch over mode, ASC 6100 auto sampler and HVG1 hydride generator was used. The amount of each element was determined by using appropriate lamp. The content of the respective element was calculated by using a calibration graph. Range of wavelengths: 190 to 900 nm.

2.3.1. Preparation of the Working Standard Solution

Standard solutions (1 mg mL⁻¹) of Pb, Hg, Na, Ca, Cr, Cd, As, K, Mg, Cu, Mn, Fe obtained from Sigma-

Aldrich Chemie GmbH, Germany were used to prepare the standard solutions. Standard solutions were diluted, with 0.1M HNO₃ to a range of standards that covers the linear range of the element to be determined.

2.3.2. Preparation of Test Sample

One g of the sample was weighed, dried, ached. The residue was dissolved in 30 mL of 0.1M HNO₃. Blank was treated in the same way as product.

2.4. Stability Studies

K-Gin capsules and Ginsenosides Rg1 and Rb1 standards were stored under different simulated climatic conditions: Climatic chamber No. 1 (25±1°C, humidity: 75%), climatic chamber No. 2 (30±1°C, humidity: 75%) and climatic chamber No. 3 (40±1°C, humidity 75%), for 24 months. The samples were studied every six months and subjected to different analysis.

2.5. Study of the Factors Affecting the Degradation of Ginesing

2.5.1. Effect of Strong Acids, Alkalies and Temperature

The effect of strong acids, alkalies and temperature were studied by treating dilute samples solutions of ginsenosides Rg1 and Rb1 (0.5 mg mL⁻¹ each) with both 1 M sodium hydroxide and 1 M HCl. The experiments were conducted at 60±1°C for 1 h, Samples were analysed by the previously described HPLC method.

2.5.2. Effect of Phosphate Buffer pH 7.4

The catalytic effect of phosphate buffer pH 7.4 (plasma pH) at 60±1°C for one hour was studied. The same concentration of ginsenosides Rg1 and Rb1v (0.5 mg mL⁻¹ each) was used for the study. Samples were analysed by the previously described HPLC method.

2.6. Disintegration Time of Capsules

The test was carraied according to USP Disintegration test.

2.6.1. Detection of Pesticides Residue

Different isomers of pesticides were checked: Dichlorodiphenyl Trichloroethane (DDT), Benzene Hexachloride (BHC), Lindan, Heptachlor, Methoxychlor, Chlorolane, Toxaphene, Endosulfan, Tetrachlorodiphenylethane (TDE), Dieldrin and Endrin.

2.6.2. Extraction and Clean up Procedures

Various extraction and clean up procedures have been used in the detection of pesticides as described by (Yoon *et al.*, 1999; Khan *et al.*, 2001).

2.6.3. Pesticides Standards

The standard kit used for comparison was.

Kit # 51C/CX, PolyScience Corporation, Chemical Division, Analytical Standards, 6366 Gross Point Road, Niles, IL-60648. USA.

The standards included in the kit were: DDT, BHC, Lindan, Heptachlor, Methoxychlor, Chlordane, Toxaphene, Endosylfan, TDE, Dieldrin and Endrin.

2.6.4. Recovery Experiment

The samples were extracted with hexane. The extracts were cleaned up by Solid Phase Extraction (SPE), using Florasil columns and hexane and hexan/CH₂Cl₂ (17: 3) as eluents. The standard was processed following the same procedure (Yoon *et al.*, 1999; Khan *et al.*, 2001). The eluates were analyzed by GC-MS under the following conditions:

Column: (1). RTX-5, 30 M, 0.25 mm ID, 0.25 µm (5% diphenyl 95% dimethyl polysiloxane, Thames Restek UK Ltd. (2). RTY-35 MS, 30 M, 0.25 mm ID, 0.25 µm (35% dephenyl-65% dimethyl polysiloxane, Thames Restek UK Ltd.

Instrument/Detector: GC-MS-pp5050 Shimadzu Electron impact ETP. Carrier gas: He Inj. Temp.: 250°C, Det. Temp.: 250°C, Oven: 50 to 310°C (10°C per minute rise).

2.7. Microbiological Studies

The drug sample was tested using the standard protocol for the total viable aerobic count of bacteria and fungi to check the possible presence of *Escherichia coli*; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella*. (CE, 2004)

2.8. Mycotoxins Determination

K-Gin capsule was tested in duplicate for the aflatoxins content, which are naturally occurring mycotoxins. A quantitative evaluation of aflatoxin were carried out through antigen antibody reaction using Enzyme Linked Immunosorbent Assay (ELISA) technique on minilyser (fully automated system), modified Tecan RSP 5051 by Tecan company using RIDASCREEN aflatoxin total screening kit (Art. No. 470) manufactured by R-Blopharm AG, Darmstadt, Germany.

2.8.1. Test Procedure

The tested samples were finely ground and shaken with 70% (v/v) methanol and filtered. The aflatoxin standards, samples dilutions, enzyme labeled aflatoxin (enzyme conjugate) and anti-aflatoxin antibodies were added in the wells in the microtiter strips which were coated with specific antibodies to mouse antibodies. Free and enzyme labeled aflatoxin competed for the antibody binding sites of the anti-aflatoxin antibodies, which themselves were bound simultaneously by the capture antibodies on the microtiter plate. Any unbound enzyme conjugate was removed in a washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) were added to the wells and incubated. Bound enzyme conjugate converted the colorless chromogen into a blue product.

By the addition of the stop reagent, the color changed from blue to yellow. The measurement was made photometrically at 450 nm (optical reference wavelength ≥ 600 nm). The absorption is inversely proportional to the aflatoxin concentration in the sample.

3. RESULTS

3.1. Method Validation

3.1.1. Linearity

3.1.2. Precision

Results of six injections from standard of ginsenoside Rg1 and ginsenoside Rb1 (0.5 mg mL^{-1}) were found to be $99.73 \pm 0.27\%$ and $100.3 \pm 0.57\%$ respectively ($X \pm \text{RSD}\%$, $n = 6$).

3.2. Accuracy (Recovery %)

For Rg1 at 0.5, 2.5 and 30 mg mL^{-1} were found to be $99.73 \pm 0.27\%$, $100.13 \pm 0.7\%$ and $99.36 \pm 0.54\%$ respectively ($X \pm \text{RSD}\%$, $n = 6$).

For Rb1 at 0.5, 2.5 and 30 mg mL^{-1} were found to be $100.3 \pm 0.57\%$, $99.8 \pm 0.61\%$ and $100.4 \pm 0.87\%$ respectively ($X \pm \text{RSD}\%$, $n = 6$).

3.3. Elemental Analysis

K-Gin capsules were found to contain sodium ($2718 \mu\text{g g}^{-1}$), magnesium ($235 \mu\text{g g}^{-1}$) and relatively large amount of potassium ($15095 \mu\text{g g}^{-1}$) and it is rich in calcium ($30831 \mu\text{g g}^{-1}$). No other specially heavy metals were found.

3.4. Effect of Temperatures

The effect of temperatures were presented in the following tables and figures

3.5. Effect of Alkalies, Acids and Phosphate Buffer pH7.4 on Stability of Ginsenosides Rg1 and Rb1

3.5.1. Physical Examination

The K-Gin powder and shell were examined over the period of 24 months. The powder retained its brown color and no distinctive odor was noticed except in CH2 and CH3 after 24 months where the color changed to dark brown. The capsule shell remained transparent and colorless over the same period except for samples in CH2, CH3 condition after 24 months the capsules powder was observed change to hard cylindrical lumps.

Disintegration time was found to be between 5 and 6 min.

3.6. Microbiological Studies

The results of microbiological studies for K-Gin revealed no significant changes in total viable aerobic count of bacteria and fungi. The total count of viable aerobic bacteria and fungi was found to be less than 10 colony forming unit/gm (fcu) after 24 month period of study, regardless of storage conditions. The results also indicate that the primary pathogens *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* were not present in the tested samples of K-Gin capsules.

3.7. Aflatoxin Results

The mean concentration of total aflatoxin contents in K-Gin capsules was found to be 723 ng kg^{-1} .

4. DISCUSSION

4.1. Introduction

It is a general belief even among health professionals that herbal product formulations are safe used without limitation. People thus believe that these formulations are among the over counter preparations that can be sold freely; however for quality control complete assessment of such preparations should be investigated covering physical, chemical, microbiological, toxicological and therapeutic stability to insure evidence-based safety (Thakur *et al.*, 2011). Accordingly this study was carried to one type of herbal product.

The quality control of herbal products forms one of the difficulties for the analysts due to their complex matrix. Methods used for the assays of active constituents in these preparations should be precise,

accurate and stability indicating, therefore proper validation of these methods should be carried. HPLC forms one of the best methods that can be applied for such assay. Any results obtained should be properly evaluated to insure safe, non toxic herbal product.

4.2. Method Validation

Under the chromatographic conditions described, ginsenosides Rg1 and Rb1 peaks were completely resolved **Fig. 1 and 2**. Calibration curves constructed from the peak areas and the corresponding ginsenosides concentrations, in each calibration standard were linear from 0.1 to 0.7 mg mL⁻¹. The mean slope and intercept for the different calibration curves of ginsenosides Rg1 and Rb1 are presented in **Fig. 3 and 4**. The correlation coefficients were 0.9986 and 0.9967 respectively. The RSD% were found to be 0.0014 and 0.574%, limit of detection and limit of quantitation were e 7.5 and 30 µg mL⁻¹ respectively.

Method validation establishes that the method performance characteristics are suitable for the intended use, as shown in **Table 1**.

4.3. Elemental Analysis

K-Gin capsules were found to contain sodium, magnesium and relatively large amount of potassium and it is rich in calcium, this is in agreement with the finding reported by (Jackson *et al.*, 2004). No other elements especially heavy metals were found. The herb therefore is considered a suitable supplement for K and Ca.

4.4. Effect of Temperature

From the results obtained it is noticed that ginsenoside Rg1 and ginsenoside Rb1 in K-Gin capsules are stable over the range of 25, 30 and 40°C with humidity 75%. Ginsenoside Rg1 and ginsenoside Rb1 in K-Gin capsules undergoes slight decomposition and lose about 0.17, 0.47 and 1.17% for ginsenoside Rg1 and 0.17, 0.67 and 1.47% for ginsenoside Rb1 after six months at CH1, CH2 and CH3 respectively. The rate of decomposition increases more and it reaches around 1.07, 2.87 and 3.57% for ginsenoside Rg1; and 3.07, 3.17 and 3.87% for ginsenoside Rb1 after 24 months storage in CH1, CH2 and CH3 respectively **Table 2**.

However, it is worth noting that the ginsenoside Rg1 and ginsenoside Rb1 standards were unstable as raw materials over the range of 25, 30 and 40°C with humidity 75%. Ginsenoside Rg1 and ginsenoside Rb1, both, undergo slight decomposition under these

conditions, ginsenoside Rg1 loses about 4.67, 6.1 and 6.98% and ginsenoside Rb1 loses about 3.63%, 6.45% and 9.96% after six months storage in CH1, CH2 and CH3. The rate of decomposition increases with time. The loses reached around 15.8 21.6 and 26.5% for ginsenoside Rg1 and around 15.25, 20.51 and 26.25% for ginsenoside Rb1 respectively **Table 3**. The kinetic parameters for the degradation of ginsenoside Rg1 and ginsenoside Rb1 under different temperatures 25, 30 and 40°C with humidity 75% were found to follow first-order reaction. The semi-logarithmic plot of log (a/a-x) against time (t) were found to give straight line with slopes of 0.0032, 0.0044 and 0.0059 under CH1, CH2 and CH3 conditions respectively for ginsenoside Rg1 as shown in **Fig. 7** and for Rb1 0.003, 0.0041 and 0.0053 CH1, CH2 and CH3 respectively as shown in **Fig. 8**, using the formula for the first-order reaction kinetics $\frac{kT}{2.303} = \log \frac{a}{a-x}$ (Connors, *et al.*, 1986). The

reaction rate constant (K) was found to be 0.00737, 0.011 and 0.0136 month⁻¹ for samples from CH1, CH2 and CH3 respectively for ginsenoside Rg1 and 0.0069, 0.0094 and 0.0122 month⁻¹ for CH1, CH2 and CH3 respectively for ginsenoside Rb1. The half-life time $t_{1/2}$ was found to be 94, 68.4 and 51 months for CH1, CH2 and CH3 respectively for ginsenoside Rg1 and 100.3, 73.39 and 56.7 months for CH1, CH2 and CH3 respectively for ginsenoside Rb1 **Table 4 and 5**.

A graph of the logarithm rate constant (K) against the reciprocal of temperature 1/T is rectilinear with a slope of -Ea/2.303R and an intercept at 1/T = log A (Arrhenius plot) (**Fig. 5 and 6**), from which the activation energy was calculated and was found to be 10.99 kcal mole⁻¹ and 10.2 kcal mole⁻¹ for ginsenoside Rg1 and ginsenoside Rb1 respectively as shown in **Table 4 and 5**.

4.5. Effect of Alkalies, Acids and Phosphate Buffer pH7.4 on Stability of Ginsenosides Rg1 and Rb1

It is reported that acid hydrolysis of ginseng result in degradation of ginseng, causes epimerization, cyclization and hydration of 20s-protopanaxadiol (s-ppd) and 20s-protopanaxatriol (s-ppt) (Han *et al.*, 1982). **Fig. 10** confirms this result as compared to the control **Fig. 9**.

While ginseng was found unstable in acid, however it is stable in alkalies and phosphate buffer pH 7.4. The findings in this study is confirm with the results obtained by Yu *et al.* (2007).

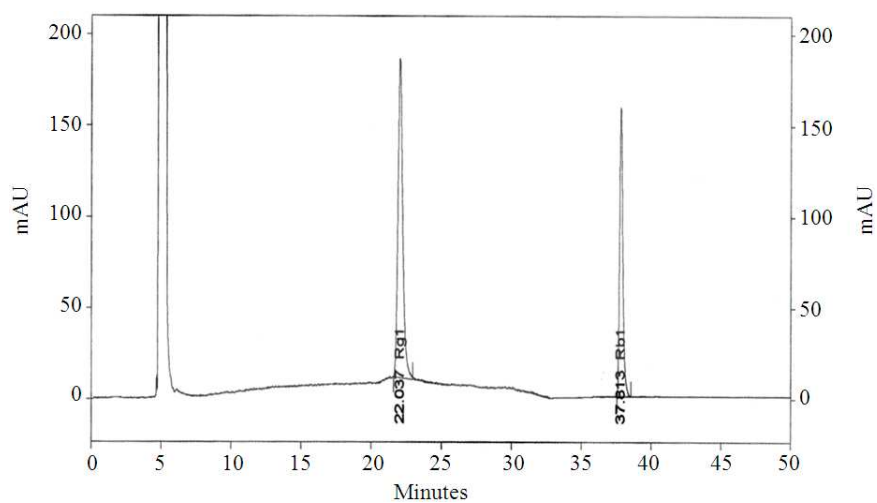


Fig. 1. A typical HPLC chromatogram of ginsenoside Rg1 and Rb1 standard

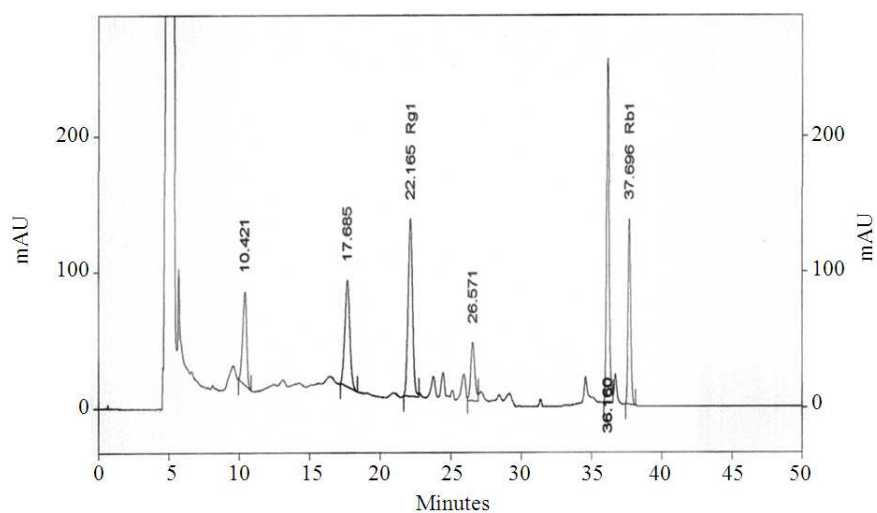


Fig. 2. A typical HPLC chromatogram of the contents of K-Gin capsules

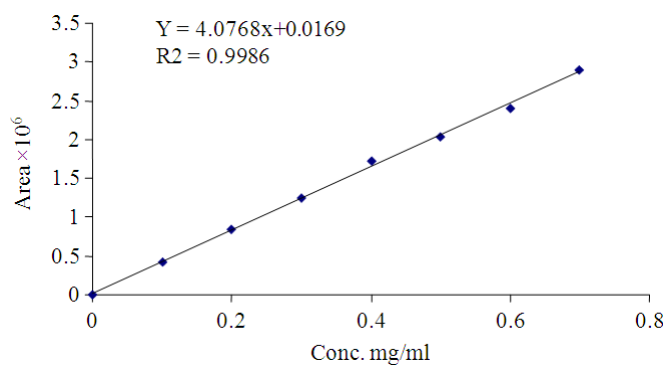


Fig. 3. Calibration curve for Ginsenoside Rg1

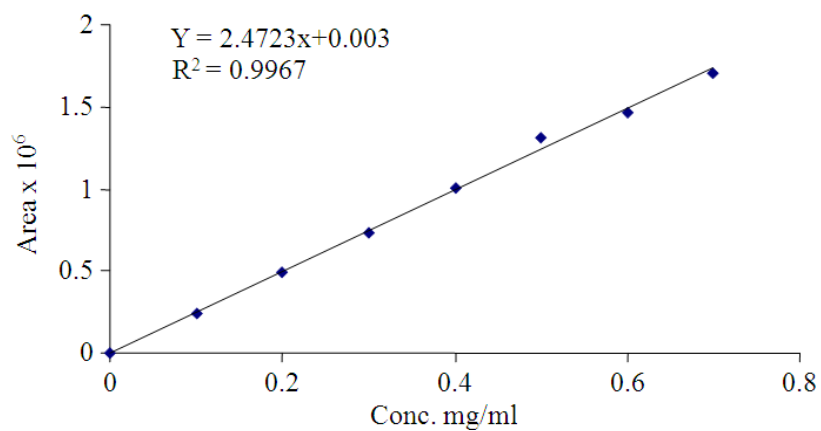


Fig. 4. Calibration curve for Ginsenoside Rb1

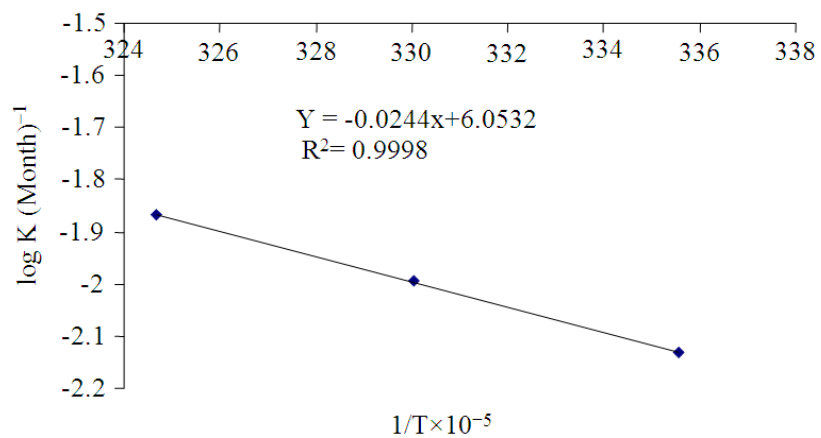


Fig. 5. Arrhenius plot for Ginsenoside Rg1 standard at different temperatures

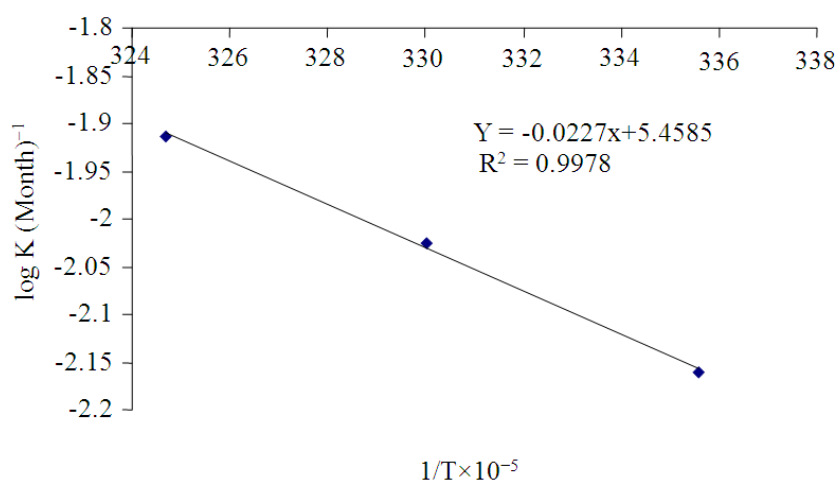


Fig. 6. Arrhenius plot for ginsenoside Rb1 standard at different temperatures

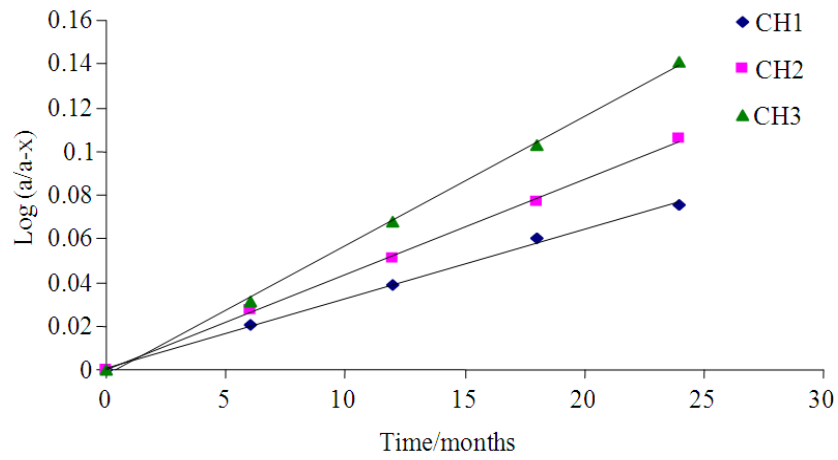


Fig. 7. Stability studies on Ginsenoside Rg1 standard exposed to different simulated climatic conditions

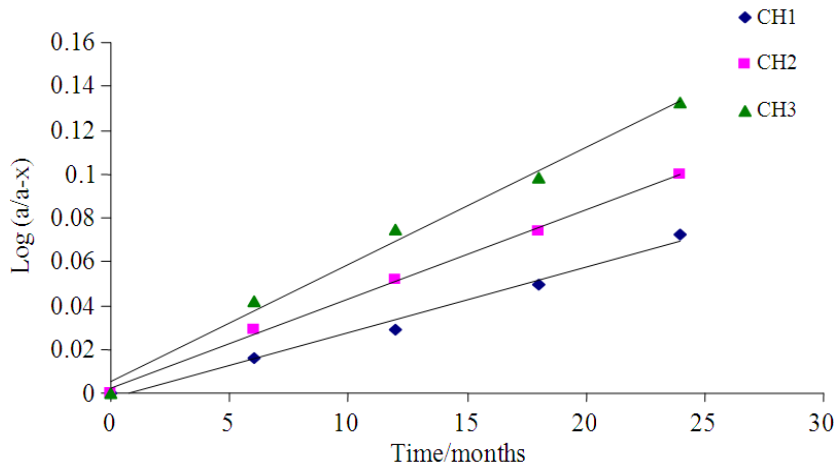


Fig. 8. Stability studies on Rb1 standard exposed to different simulated climatic conditions

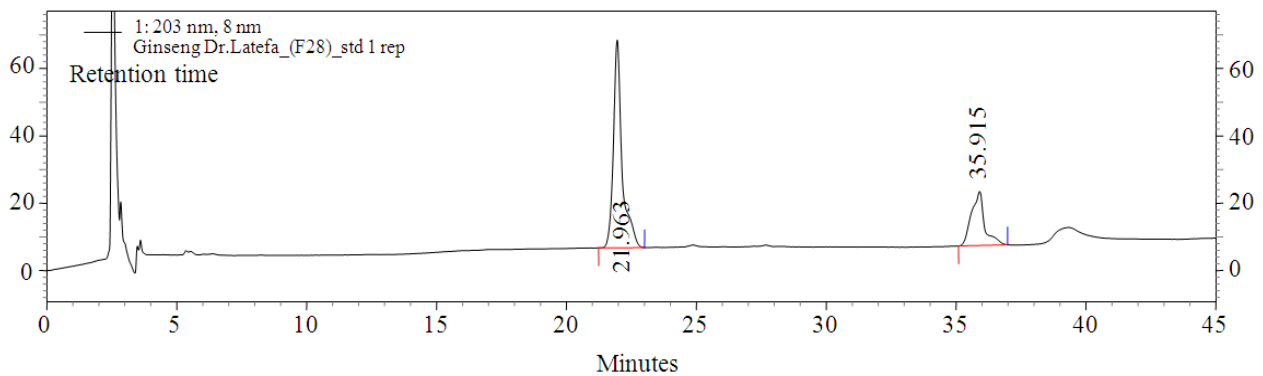


Fig. 9. Chromatogram for control solution

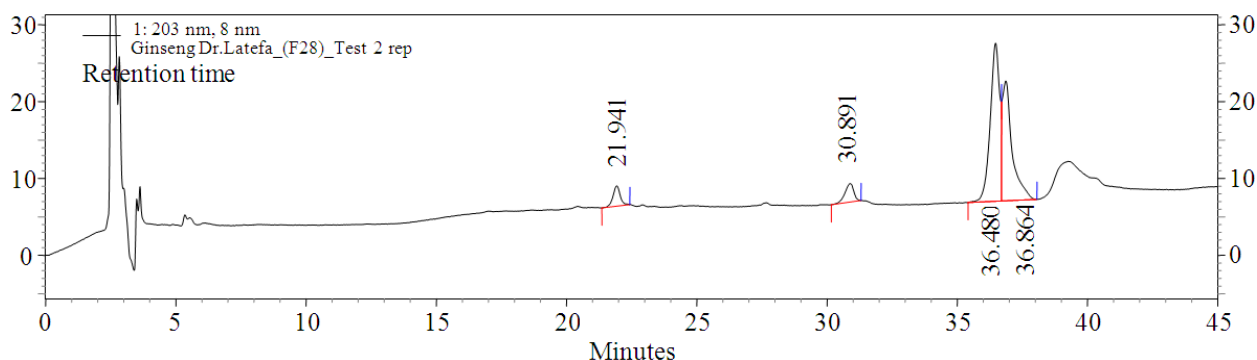


Fig. 10. Chromatogram for acid hydrolysis

Table 1. System suitability parameters for the assay of Rg1 and Rb1

Name	RT	Theoretical plates	Capacity K factor	Resolution	Asymmetry
Rg1	22.1	20127	3.4		1.07
Rb1	37.8	13077	6.56	11.57	1.7

Table 2. Assay percentage results of K-Gin capsules at different time intervals and temperatures

	CH1	CH2	CH3	CH1	CH2	CH3
0	100.5	100.5	100.5	99.98	99.98	99.98
6	99.83	99.53	98.83	99.83	99.33	98.53
12	99.52	98.52	98.12	97.72	98.22	97.72
18	99.26	97.96	97.06	97.06	97.56	96.46
24	98.93	97.13	96.43	96.93	96.83	96.13

Table 3. Assay percentage results of ginsenoside Rg1 and ginsenoside Rb1 standard materials at different time intervals and temperatures

Time	Ginsenoside Rg1			Ginsenoside Rb1		
	CH1	CH2	CH3	CH1	CH2	CH3
0	100.1	100.1	100.1	100	100	100
6	95.33	93.90	93.02	99.83	99.33	98.53
12	91.37	88.81	85.54	93.55	88.73	84.18
18	87.03	83.82	78.86	89.21	84.03	79.74
24	84.10	78.37	72.31	84.75	79.49	73.75

Table 4. Effect of temperatures on the kinetic parameters of ginsenoside Rg1 standard material

Temp °C	Slope	K(month)	Log K	t ^{1/2} (month)	E _a (K. cal. mole)
CH1	0.0032	0.00737	-2.13	94	
CH2	0.0044	0.011	-1.99	68.4	11.08
CH3	0.0059	0.0136	-1.87	51	10.9
Average activation energy					10.99

Table 5. Effect of temperatures on the kinetic parameters of Ginsenoside Rb1 standard material

Temp °C	Slope	K(month)	Log K	t ^{1/2} (month)	E _a (K. cal. mole)
CH1	0.003	0.0069	-2.16	100.3	
CH2	0.0041	0.0094	-2.02	73.39	10.87
CH3	0.0053	0.0122	-1.91	56.7	9.92
Average activation energy					10.99

4.6. Physical Examination

The capsules retained their appearance throughout the studies and the disintegration time was found between 5 and 6 min, which is suitable enough for quick contents absorption within short time to exert their action.

4.7. Pesticides Residues

K-Gin capsules were found to be free from any pesticide residues, this is in agreement with the finding reported by (Durgnat *et al.*, 2005).

4.8. Microbiological Studies

The results demonstrate absence of microbiological contamination in K-Gin capsules, this is in agreement with the finding reported by (Jackson *et al.*, 2004)

4.9. Aflatoxin Contents

The result of aflatoxin contents in k-Gin capsules was compared with the specification limits outlined in the Saudi Arabian Standards Organization No. 1151/1998 and G.C.C. Organization for Standardization and Methodology No. 841/1997 and USP limits which is ranging from 800-4300 ng kg⁻¹, NMT 20ppb, respectively and the result was within the allowable limits.

In a previous work we reported the freedom of the product from toxicity (Gadkariem *et al.*, 2010).

5. CONCLUSION

Assessment of the stability, quality and safety of finished herbal drug products is an important and challenging issue for health professionals.

The results obtained revealed that the drug product included in the current study was found to be safe on several aspects. They were free from toxic heavy metals, pesticides residues and primary pathogens. It is worth mentioning that in all these drug product mycotoxins was lower than the allowable limits.

The stability results obtained during the current study supported the rule of two years shelf life for K-Gin registered in Saudi Arabia.

6. RECOMMENDATION

According to our findings, This type of rigorous investigations should be carried out for all herbal products sold in pharmacies or herbal shops. Future research in such preparations should include cilinical feedback to insure the safety of such preparations.

In cases where the manufacturers claim and apply for increase in the shelf life of their product (more than two years), they have to provide more data regarding the safety and stability of their product, covering the desired period of time, in the drug registration files. Because of the complex nature of the herbal drugs, their recommended storage conditions must be included and specified on the drug container.

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