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Quality assessment of Victoria 1500 and 2000 capsules a herbal drug product

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ABSTRACT

This study was designed to investigate the quality of Victoria 1500 and 2000 capsules a herbal medication that contain ginseng (major components Rg1&Rb1), royal jelly (marker component is 10-hydroxydec-2-enoic acid (10-HDA)) and yohimbine. The study include determination of major components content, long term stability, determination of elements , studying the microbiological contamination, detection of pesticides residues and mycotoxins. The drugs were stored under different simulated climatic conditions. The reaction rate constant (K), the half-life ($t_{1/2}$) and the activation energies were calculated for ginsenosides Rg1 &Rb1. The activation energies were found to be 10.99 and 10.2($k.cal.mol^{-1}$) for Rg1 &Rb1respectively. The % content of Rg1, Rb1 and 10-HDA of Victoria1500 &2000 capsules after six months storage were found at the lower specifications limit (80-120% of stated amount). For yohimbine were found within allowable limit(90-110%). Studies on the elemental composition confirmed absence of toxic heavy metals. The capsules were found to be free from Microbiological contamination and any pesticide residues. The mycotoxins content were found to be within the allowable limits(800-4300ng/kg). Victoria 1500&2000 capsules were found to comply with the given specifications up to six months storage, thereafter, the samples showed physical &chemical instability with a decrease in Rg1,Rb1 and 10-HDA contents but without effect on yohimbine content.

Introduction

Victoria capsules contains: Royal Jelly, *Panax ginseng*, Bee pollen and Yohimbe bark powder as major ingredients. Royal Jelly is a milky white gelatinous material secreted in the salivary glands of worker bees (*Apis mellifera*) for the sole apparent purpose of stimulating the growth and development of queen bees. Royal Jelly is considered to extend life span and, in general, reinvigorate the body. It is said to increase appetite and general vigor; retard aging; accelerate healing; and strengthen the immune system. It exhibits antibiotic and antiviral properties. Royalisin, a protein isolated from royal jelly was found to possess a strong antibacterial activity against gram-positive bacteria (Supabphol 1995; Fujiwara et al. 1990). 10-hydroxydec-2-enoic acid (10-HDA) has antimicrobial properties. (10-HDA) is used as a maker compound for quality control studies on Royal jelly (Gene and Aslan 1999).

Ginseng is a well-known medicinal herb in traditional Asian medicine. Ginsenosides are active compounds of ginseng and they are usually used as marker compounds for the quality control work on ginseng products. *P. ginseng* contains adaptogens; it is believed to be an anti-aging herb and is a favorite today because of its ability to be used for long-term without much toxic effects on the body. Ginseng possesses anti-cancer properties (Helms 2004; Shin et al. 2000).

Yohimbine is one of the active alkaloids found in Yohimbe bark (*Pausinystalia yohimbe* Pierre) family Rubiaceae. Yohimbine is a selective competitive alpha2-adrenergic receptor antagonist and is used in the treatment of male erectile dysfunction (Anon 1998; Cremona 1998; Nessel 1994). In higher doses yohimbine may lead to rapid heart rate, high blood pressure and anxiety .

Victoria 1500 & Victoria 2000 capsules contain 25 mg ginseng (major components Rg1&Rb1), royal jelly1500 &2000(marker component is 10-hydroxydec-2-enoic acid (10-HDA)) and 25 mg yohimbine, are registered and marketed in Saudi Arabia , Due to the lack of information on the stability & safety of such products, their quality and safety remains questionable. It is thus deemed important to conduct the present research on such products.

Materials and methods

Materials

The pure samples of Ginsenoside Rg1 &Rb1 were purchased from Indofine chemical company ICC (Somerville, NJ 08876 USA), yohimbine, 10-hydroxydec-2-enoic acid (10-HDA)were purchaused from Sigma(St.Louis, Mo, USA). The capsules (Victoria 1500 & Victoria 2000 Batch # 908417 and 908345) were obtained from commercial sources. Methanol, acetonitrile, (all of HPLC grades), hydrochloric acid (Analar), monobasic sodium phosphate, Octane sulfonic acid salt AR grade, potassium dihydrogen phosphate were obtained from BDH (Poole, UK). Cartridge containing 360 mg of octadecylsilyl silica gel for chromatography for solid phase extraction was purchased from Agilent Technologies, Inc., NC, USA. The standard kit used for pesticide residues was purchased from(Kit # 51C/CX) PolyScience Corporation, Chemical Division, Analytical Standards, 6366 Gross Point Road, Niles, IL-60648. USA. RIDASCREEN aflatoxin total screening kit

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(Art. No. 470) manufactured by R-Blopharm AG, Darmstadt, Germany.

Methods

The drugs were stored under different simulated climatic conditions: (25°C ± 1°C, humidity: 75%)chamber 1(CH1); (30°C ± 1°C, humidity: 75%) chamber 2(CH2) and (40°C ± 1°C, humidity 75%) chamber 3 (CH3) for 24 months.

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 X 250 mm, 5µm, flow rate 1ml/min, injector volume 20 µl, wave length 205 nm, 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

Standard solution:

A solution each of 0.5mg/ml of ginsenoside Rg1 and ginsenoside Rb1 reference materials in methanol were prepared.

Preparation of test sample for assay of Rg1 & Rb1 by HPLC:

One gm of the powered 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 60ºC. The residue was taken in 10 ml buffer solution containing 3.5gm of sodium dihydrogen phosphate in 1000 ml of water (Solution A). 5 ml of solution A was applied to the cartridge (clean up procedure 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.

Assay of 10-hydroxydec-2-enoic acid (10-HDA) by High performance liquid chromatography:

The HPLC system used comprised of 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 Symmetry C18, 5 µm, 4.6 x 250 mm (Waters), flow rate 1.5ml/min, injector volume 20 µl, wave length 225 nm, mobile phase composed of Methanol : Water (45 : 55) pH adjusted to 2.5 with H_3PO_4.

Preparation of the standard solution:

Serial solutions of 10-hydroxydec-2-enoic acid (10-HDA) were prepared in methanol at concentrations of 0.05, 0.102, 0.203, 0.305, 0.406, 0.508 mg/ml.

Preparation of test sample for the HPLC assay of 10-HDA:

A quantity of drug equivalent to 1.5 mg of 10-HDA was dissolved by using ultrasonic bath for 30 min. in 10 ml of a mixture of 45 volumes of methanol and 55 volumes of distilled water (pH adjusted to 2.5 with phosphoric acid), then filtered through 0.45 µm.

Assay of Yohimbine in Victoria capsules by High performance liquid chromatography:

The HPLC system used comprised of 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. The seperation was achieved on µ-Bondapack C-18 (Waters), with flow rate of 1.0 ml/min, injection volume 20ul, wavelength 254 nm and mobile phase composed of Methanol : 0.005M Octane sulfonic acid salt (50 : 50).

Preparation of the standard solution:

Serial dilutions were prepared for calibration curve for yohimbine range between 0.05 -0.4mg/ml.

Preparation of test sample:

An accurately weighed powder equivalent to 20 mg of Yohimbine HCl was transferred in to a 50 ml volumetric flask. About 30 ml of distilled water was added; sonicated and shaken for about 10 min., diluted to volume with distilled water then filtered through 0.45 µm.

Stability Studies:

Victoria 1500 & Victoria 2000 capsules and ginsenoside Rg1 and ginsenoside Rb1 standard materials were stored under different simulated climatic conditions: climatic chamber No. 1(CH1) (25°C ± 1°C, humidity: 75%), climatic chamber No. 2(CH2) (30°C ± 1°C, humidity: 75%) and climatic chamber No. 3 (CH3)(40ºC ± 1°C, humidity 75%), for 24 months and analysis was carried after each six months peroid. The rate constant, half-life and activation energy for the this study were calculated.

Study of the Factors Affecting the Degradation of Ginesng

Effect of Strong Acids, Alkalies and temperatures

The effect of strong acids, alkalies and temperatures were studied by treating dilute sample solutions of ginsengs (0.5mg/ml) with both 1 M sodium hydroxide and 1 M HCl. The experiments were conducted at 60± 1°C for 1 hour ,Samples were analysed by the previously described HPLC method.

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 ginseng 0.5 mg/ml was used for the study. Samples were analysed by the previously described HPLC method.

Disintegration time of capsules

This was carraied according to USP Disintegration test.

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.

Preparation of the working standard solutions:

Standard solutions (1mg/ml) 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 HNO_3 to a range of standards that covers the linear range of the element to be determined.

Preparation of test sample for elemental analysis:

1 g of the sample was weighed, dried, ached. The residue was dissolved in 30 ml of 0.1M HNO_3. Blank was treated in the same way as products.

Detection of Pesticides Residues:

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

Extraction and clean up procedures: Various extraction and clean up procedures have been used in the detection of pesticides as described by (Khan et al. 2000, Yoon et al.,1999).

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.

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 (Khan et al. 200, Yoon et al.,1999). 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).

Mycotoxins Determination:

Victoria capsules 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 ELISA (Enzyme linked immunosorbent assay) 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.

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 perroxide) 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.

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*. (Eur.Ph.2012)

Determination of total proteins:

This test was carried on Victoria 1500 capsules, Victoria 2000 capsules, according to the procedure given in: Eur. Ph.7th Edition,

Results and Discussion For victoria 1500&2000 capsules

Introduction

Manufactures of herbal formulations are required to produce products of good homogenicity.On the other hand pharmaceutical analyst is required to develop validated methods of analysis that ensures good quality, safe herbal product that meet the general specifications for such formulations. These general specifications cover, physical, chemical, microbiological and toxicity testes with special stress on stability of the product under certain condition(WHO,USP Herbal compendia 2013). For a herbal product to be liceused and marketed it has to pass these specifications.

In this study we focused on the quality assessment of Victoria 1500&2000 capsules registered in Saudi Arabia.

Method validation for ginsenosides Rg1 &Rb:

Under the chromatographic conditions described, Ginsenosides Rg1 and Rb1 peaks were completely resolved from the sample matrix as compared to the standard Rg1 &Rb1 chromatograms **(Fig.1&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 gisenosides Rg1 and Rb1 are presented in (**Fig. 3&4)**. The correlation coefficient s were 0.9986 and 0.9967 respectively. The RSD% were found to be 0.0014 and 0.574% , limit of dedection and limit of quantatation were 7.5 µg/ml and 30 µg/ml respectively.

Method validation establishes that the method performance characteristics are suitable for the intended use.

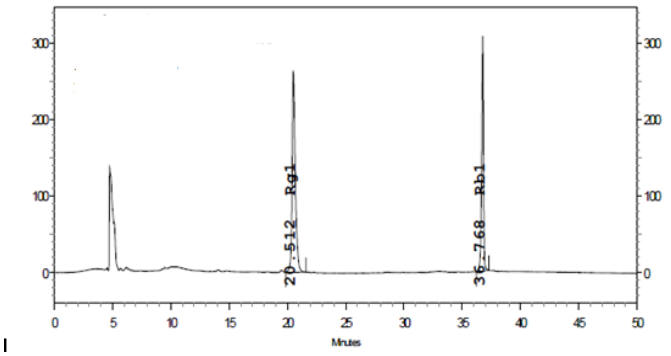

Fig.1 HPLC chromatogramof Ginsenosides Rg1and Rb1 standards

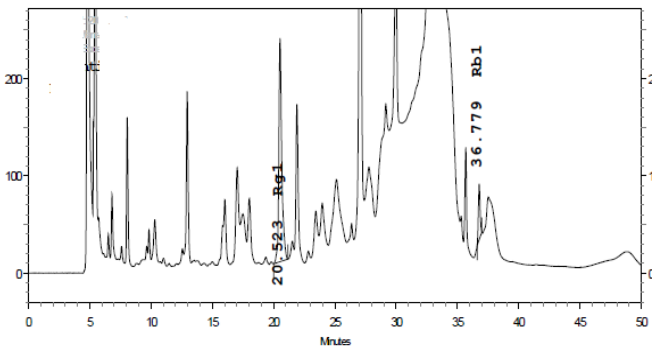

Fig.2 HPLC chromatogram of the contents of Victoria capsules

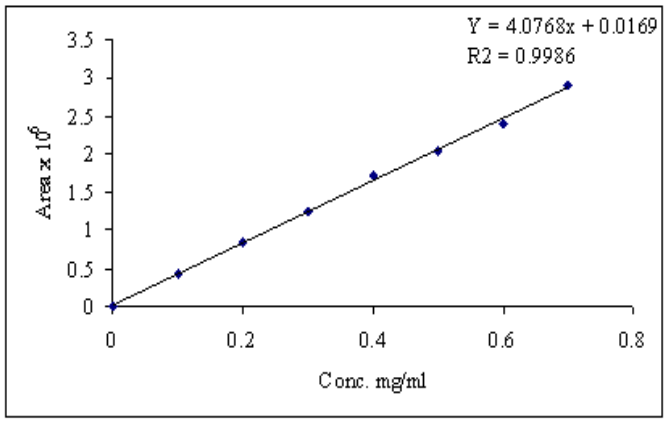

Fig. 3: Calibration curve for Ginsenoside Rg1.

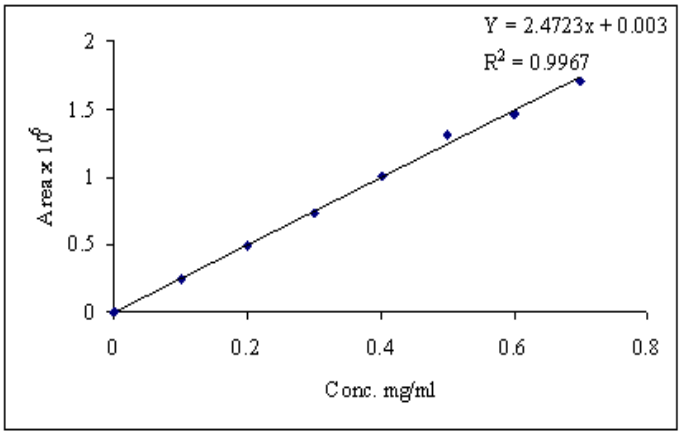

Fig.4: Calibration curve for Ginsenoside Rb1.

Method validation for the quantitative determination of 10-hydroxydec-2-enoic acid (10-HDA):

Table 1. HPLC System suitability parameters for the assay of 10-HDA(RT:21.2 min.)

Theoretical plates	Capacity factor K	Peak Asymmetry	Linearity	Precision	Accuracy
15085	5.32	1.17	Range: 0.05 0.5mg/mL y=5E+07 x +338614 r^2:0.9995	%RSD (n=6) 0.63	Recovery(%RSD) 99.5±0.95%

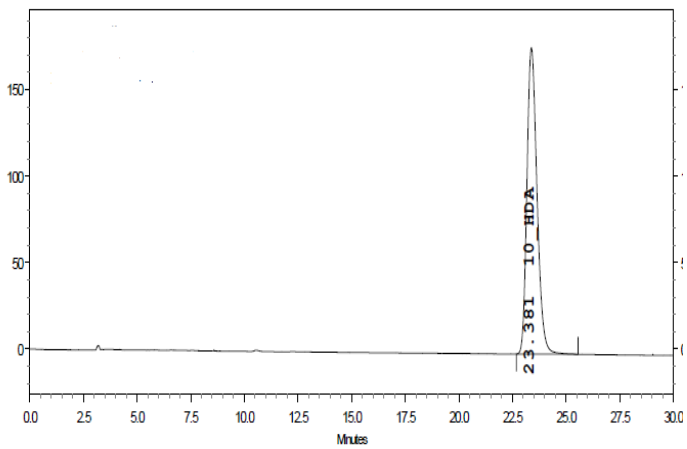

Fig. 5 HPLC chromatogram of 10-HDA standard

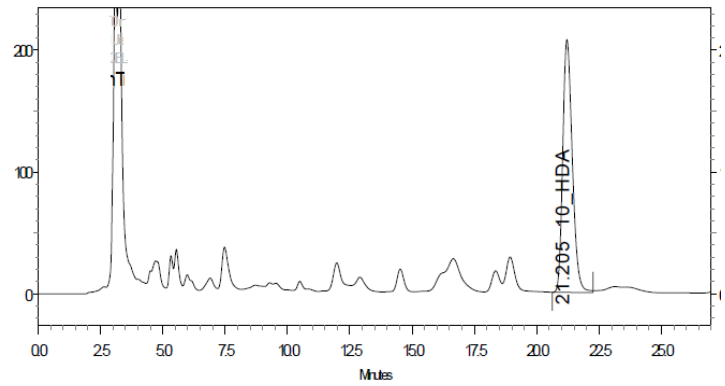

Fig. 6 HPLC chromatogram of 10-HDA in Victoria capsules

Method validation for the quantitative determination of yohimbine

Table 2. HPLC System suitability parameters for assay of yohimbine(RT10.45)

Theoretical plates	Capacity factor K	Peak a symmetry	Linearity	Precision	Accuracy
8567	7.32	1.31	From: 0.05 to 0.4mg/mL y=37.17x-131.52 r^2:0.997	%RSD(n=6) 0.83	Recovery(%RSD) 100.5±0.75%

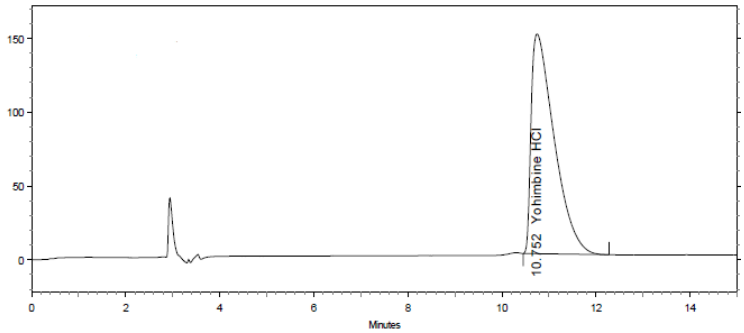

Fig. 7 HPLC Chromatogram of Yohimbine HCL standard

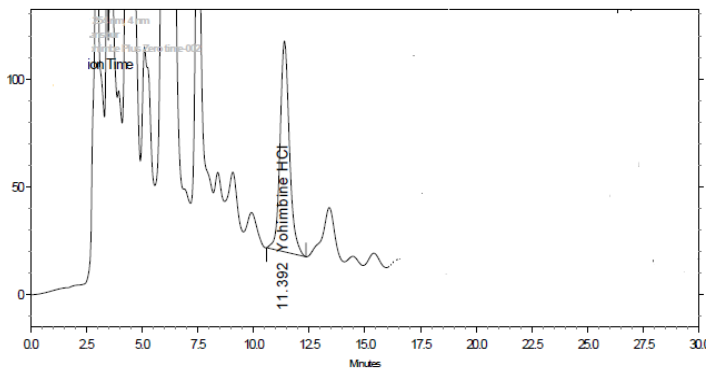

Fig.8. HPLC Chromatogram of Yohimbine HCL in Victoria capsules

Effect of Temperature:

Victoria 1500&2000 capsules were found to comply with the given specifications up to six months storage, although they are at the lower specifications limit and they are considered passing the specification (80-120% of the stated amount).

The results obtained after 6, 12, 18 and 24 months of storage under different conditions, showed an obvious decrease in Rg1 and 10-HDA contents. It is worth mentioning that in all tested samples: after , 12, 18 and 24 months of storage under

different conditions, the Rb1 peak was missing.There was no decrease in yohimbine contents and all the samples remained within the given range (90-110%) (**Tab. 7-8-9-10**).

Table 3: Assay results of 10-HDA and Rg1 in Victoria 1500 capsules at different time intervals and temperatures.

Time	10-HDA			Gensenoside Rg1		
	CH 1	CH 2	CH 3	CH 1	CH 2	CH 3
0	83.3%	83.3%	83.3%	80.23%	80.23%	80.23%
6	82.5%	81.53%	80.83%	80.13%	80.12%	80.1%
12	62.52%	61.52%	51.12%	70.72%	63.22%	59.72%
18	52.26%	50.96%	51.06%	65.06%	55.56%	5246%
24	49.93%	44.13%	43.1%	50%93	49.83%	44.13%

Table 3: Assay results of Rb1 in Victoria 1500 capsules at different time intervals and temperatures.

Time	Gensenoside Rb1		
	CH 1	CH 2	CH 3
0	80.22	80.22%	80.22%
6	80.20%	80.11%	80.02%

Table 4: Assay results of yohimbine in Victoria 1500 at different time intervals and temperatures.

Time	Yohimbine		
	CH 1	CH 2	CH 3
0	104.12%	104.12%	104.12%
6	101.33%	103.90%	99.02%
12	98.37%	98.81%	95.54%
18	97.03%	96.82%	91.86%
24	94.10%	93.37%	91.31%

Table 5 : Assay results of10-HDA & Rg1 in Victoria2000 capsules at different time intervals and temperatures.

Time	10-HDA			Gensenoside Rg1		
	CH 1	CH 2	CH 3	CH 1	CH 2	CH 3
0	90.3%	90.3%	90.3%	81.13%	81.13%	81.13%
6	81.5%	80.53%	80.1%	80.43%	80.33%	80.13%
12	55.52%	62.52%	51.12%	70.43%	62.12%	56.76%
18	52.26%	51.96%	47.06%	64.07%	55.46%	50.36%
24	49.93%	42.13%	38.1%	51%93	44.83%	38.23%

Table 6: Assay results of Rb1 in Victoria 2000 capsules at different time intervals and temperatures.

Time	Gensenoside Rb1		
	CH 1	CH 2	CH 3
0	80.22	80.22%	80.22%
6	80.21%	80.10%	80.05%

Table 6: Assay results of yohimbine in Victoria 2000 at different time intervals and temperatures.

Time	Yohimbine		
	CH 1	CH 2	CH 3
0	110.02%	110.02%	110.02%
6	102.43%	108.30%	98.73%
12	98.37%	99.81%	96.54%
18	94.03%	95.02%	94.86%
24	90.10%	92.37%	91.81%

The standards were stored for 24 months under the specified chambers conditions .They were analysed at intervals of six months each.

Table 7: Assay results of ginsenoside Rg1 and ginsenoside Rb1 standard materials at different time intervals and temperatures

Time	Gensenoside Rg1			Ginsenoside Rb1		
	CH 1	CH 2	CH 3	CH 1	CH 2	CH 3
0	100	100	100	100	100	100
6	95.33	93.90	93.02	96.37	93.55	90.74
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

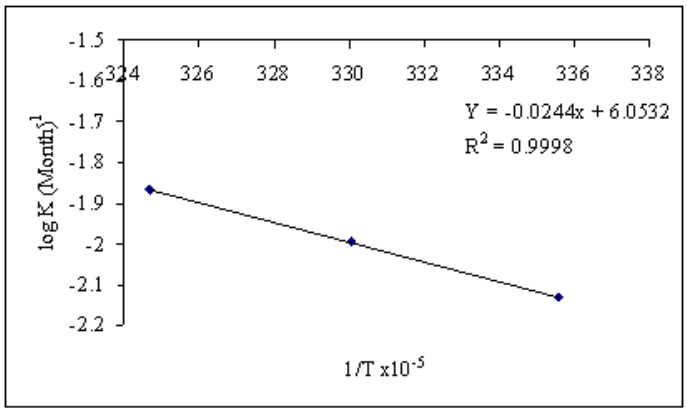

Fig. 9: Arrhenius plot for Ginsenoside Rg1 standard material at different temperatures.

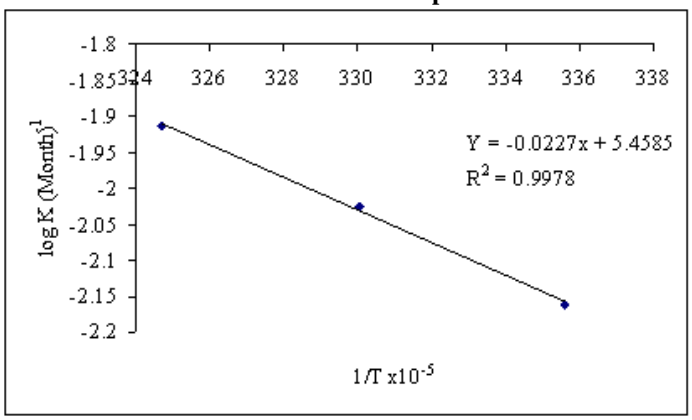

Fig. 10: Arrhenius plot for Ginsenoside Rb1 standard material at different temperatures

Table 8: Effect of temperature on the kinetic parameters of ginsenoside Rg1.

Temp °C	Slope	K(month)	Log K	t ½(month)	E_a(K. cal. Mole)
CH1	0.0032	0.00737	– 2.13	94	11.08
CH2	0.0044	0.011	– 1.99	68.4	
CH3	0.0059	0.0136	– 1.87	51	10.09
		Average activation energy			10.99

From the obtained results presented in (tab.7) and (fig.11&12) it is clear that the ginsenoside Rg1 and ginsenoside Rb1 standards are unstable over the range of 25ºC, 30ºC and 40ºC with humidity 75%. The kinetic parameters for the degradation of ginsenoside Rg1 and ginsenoside Rb1 under different temperatures 25ºC, 30ºC and 40ºC with humidity 75% were found to follow first-order reaction.

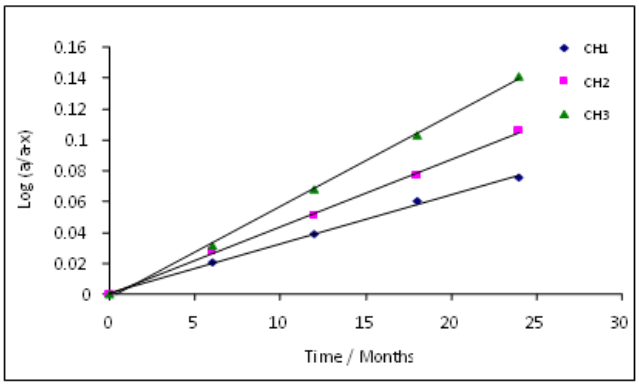

Fig.11: Stability studies on Ginsenoside Rg1 standard exposed to different simulated climatic conditions

Table 9: Effect of temperature on the kinetic parameters of Ginsenoside Rb1 standard material.

Temp °C	Slope	K(month)	Log K	t ½(month)	E_a(K. cal. Mole)
CH1	0.003	0.0069	– 2.16	100.3	10.87
CH2	0.0041	0.0094	– 2.02	73.39	9.52
CH3	0.0053	0.0122	– 1.91	56.7	
		Average activation energy			10.2

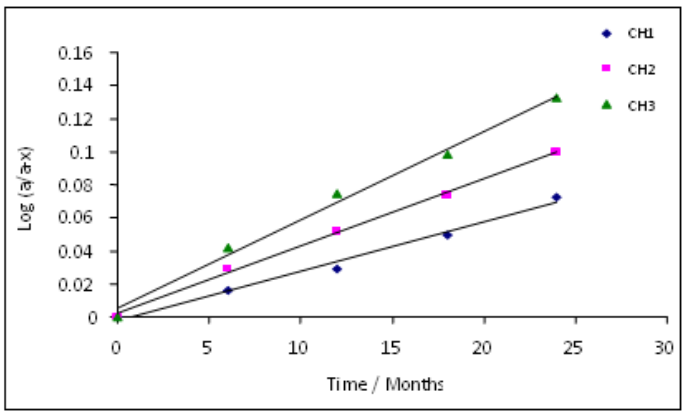

Fig. 12: Stability studies on Rb1 standard material exposed to different simulated climatic conditions.

The semi-logarithmic plot of log (a/a-x) against time (t) were found to be 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. 11), and for Rb1 0.003, 0.0041 and 0.0053 CH1, CH2 and CH3 respectively as shown in (Fig. 12), using the formula for the first-order reaction kinetics (Keaneth, *et al* 1984).

$$\frac{kt}{2.303} = \log \frac{a}{a-x}$$

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 month for CH1, CH2 and CH3 respectively for ginsenoside Rg1 and 100.3, 73.39 and 56.7 month for CH1, CH2 and CH3 respectively for ginsenoside Rb1. The average activation energy was also calculated and was found to be 10.99 kcal/mole and 10.2 kcal/mole for gensenoside Rg1 and gensenoside Rb1 respectively (Tab. 8&9).

Effect of alkalies , acids and phosphate buffer pH7.4 on stability of ginsenosides Rg1 and Rb1

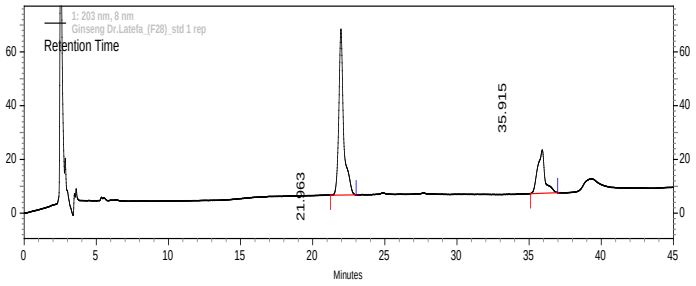

Fig. 13 Chromatogram for control solution

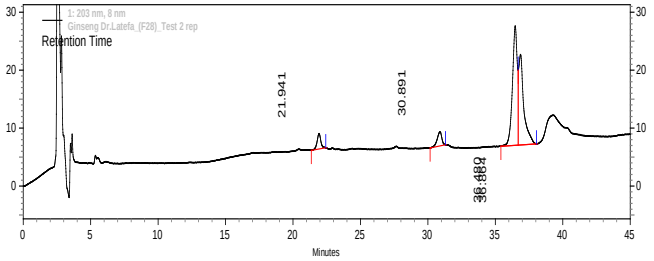

Fig. 14 Chromatogram for acid hydrolysis

It is reported that acid hydrolysis result in degradation of ginseng , causes epimerization ,cyclization and hydration of 20s-protopanaxadiol (s-ppd)and 20s-protopanaxtriol (s-ppt)(Tanaka *et al* .,1972; Han *et al*., 1982). **Figure 14** confirms this result as compared to the control (**Fig.13**)

In this study we confirm the stability of ginseng in alkalies and phosphate buffer pH7.4, as reported before by Yu(Yu *et al*., 2007).

Physical examination:

The samples of victoria 1500&2000 capsules were stored under different storage conditions 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.

Disintegration time was about 10 minutes. Which is suitable enough for absorption before exerting its effect.

Elemental Analysis:

Victoria 1500 capsules were found to contain sodium (2516 µg/g), iron (515µg/g), magnesium(1443µg/g), relatively large amount of potassium (5693 µg/g)and it is rich in calcium (34640µg/g). No others specially heavy metals were found.

Victoria 2000 capsules were found to contain sodium(3551µg/g), iron(576µg/g), magnesium(1411µg/g) and relatively large amount of potassium(7558 µg/g)and it is rich in calcium (23383µg/g). No other s specially heavy metals were found.

Both products therefore, considered a suitable supplement for potassium and calcium.

Detection of Pecticides

Victoria 1500& 2000 capsules were confirmed by mass spectrometry not to possess any pesticide residues.

Aflatoxin results

The mean concentration of total aflatoxin contents (ng/kg) in Victoria 1500 capsules was found to be 3830 ng/kg and for victoria 2000 capsules was 414 ng/kg.

Studies on the detection of mycotoxins demonstrated both products to be within the allowable limits (800 – 4300 ng/kg) of

Saudi Arabian Standards Organization No. 1151/1998 and G.C.C. Organization for Standardization and Methodology No. 841/1997 and USP limits NMT20 ppb.

Microbiological studies:

The results of microbiological studies for both products 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 clearly demonstrate the absence of real microbiological contamination of victoria capsules. The results also indicate that the primary pathogens *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella* were not present in the tested samples of victoria 1500 &2000 capsules.

Total protein

The results of total protein for victoria 1500&2000 over 24 peroid of study were 190 &270 mg/capsule respectively.

In the previous work we reported the freedom of this product from toxicity (Gadkariem etal 2011)

Conclusion

In this study, it was observed that mixing up of unstudied mixtures of different plants might lead to instability of the active costituents in these products, In such mixed products,the possibility of different unknown interactions between various chemical constituents of plants can not be ruled out. This fact was confirmed in this study of Victoria 1500 and Victoria 2000 capsules. Where the instability of victoria 1500&2000 capsules was observed which could be to some interactions with the matrix of the herbal formulation. Although *Panax ginseng is* available as a mixture with many other natural products, however; no results of stability studies of these products are available.

The results obtained for the presence of heavy metals ,microbiological contaminants, pesticides and aflatoxin residues were revealed that both products included in the this study were found to be safe. They were free from toxic heavy metals, pesticides residues, primary pathogens and mycotoxins .

Recommendations

• To improve the quality and stability of a herbal preparation mixing up of scientifically unstudied mixture of plants should be limited. Such mixtures of herbs require more scientific data on each ingredient to justify the claims of the manufacturers.

• There is a possibility that mixing up of different herbs, might increase the chances of interaction of some compounds of one plant with other constituents of another plant present in the mixture.

• To minimize the adverse effects resulted from mixing up of different plants in the preparation of one ‘herbal drug product’ should include the individual stability and toxicity data of each ingredient, along with scientific data about the mixture.

• 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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