QUALITY CONTROL OF GINSENG PREPARATIONS BY MEANS OF HPLC: A SAFE METHOD FOR THE PHARMACEUTICAL INDUSTRY

K.H. Rückert
Pharmaton Ltd., Lugano-Bioggio, Switzerland

Abstract

By means of numerous examples the practical possibility is demonstrated, which HPLC offers at the quality control of Ginseng-preparations.

The method is not only suitable for quali-quantitative evaluation of finished products, but also for in-process controls at production of Ginseng specialities.

From the examination of various German products which are on the European market, it results, that the contents of Ginsenosides fluctuate strongly.

The mechanisms are shown, which could cause destruction of the Ginsenosides at the processing of Ginseng roots. The guiding principles for the production of standardized Ginseng preparations are defined.

Standardized products: A demand for the pharmaceutical industry

For biosynthesis of its components (ginsenosides), the ginseng plant, like any other plant, follows a natural cycle, which is strongly influenced by many factors (for instance nature of soil, humidity of soil and air, sun conditions, age of plant). For this reason the ginsenoside content varies from plant to plant. From the numerous pharmacological works it is seen clearly that various ginseng root preparations do not always give the same pharmacological effect. According to KAKU et al. (2) these discrepancies are due to the various methods of manufacture of the preparations, in which the active ingredients—ginsenosides—can vary greatly in quantity and proportion. Therefore the fluctuating content of ginsenosides in the plant justifies the use of standardized products as the standardized Ginseng Extract G 115®.

In Table I and II the results of the HPLC—quantitative determinations of the ginsenosides—are indicated (12).

From these values it can be seen that there are products on the European markets, the content of which varies from one lot to another and some products do not contain any ginseng at all.

How can the ginsenosides be destroyed

The Ginsenosides, like all plant glycosides, are easily destroyable. The general degradation mechanism takes place, as generally for glycosides, according to the reaction

$$\text{Glycoside} \longrightarrow \text{Aglycon} + \text{Sugar}$$

But the degradation mechanism of the Ginsenosides is not only caused through cleavage of

Table 1. Results of quantitative determinations: Content of ginsenosides from commercial products.

	% content								
	Rg_1	Re	Rf	Rg_2	Rb_1	Rc	Rb_2	Rd	Total
G115 standardized ginseng extract, Pharmaton Ltd., Lot No. 14879	0.548	0.352	0.270	0.065	1.338	0.714	0.567	0.286	4.140
G115 standardized ginseng extract, Pharmaton Ltd., Lt No. 19269	0.946	0.586	0.285	0.110	0.976	0.603	0.425	0.267	4.198
G115 standardized ginseng extract, Pharmaton Ltd., Lot No. 199028L	0.733	0.539	0.238	0.142	1.195	0.682	0.443	0.229	4.201
G115 S standardized ginseng extract, Pharmaton Ltd., Lot No. 09701002	0.733	1.453	0.317	0.168	1.776	1.555	1,028	0.553	7.583
G115 S standardized ginseng extract, Pharmaton Ltd., Lot No. 107901003	0.893	1.442	0.317	0.188	1.842	1.618	1.081	0.572	7.953
G115 S standardized ginseng extract, Pharmaton Ltd., Lot No. 107901004	0.769	1.149	0.301	0.200	1.743	1.507	1.028	0.515	7.212
Ginseng root, in hard gelatin capsules, Company B, Lot No. 13262	0.268	0.174	0.062	0.024	0.244	0.127	0.101	0.065	1.065
Ginseng root, in hard gelatin capsules, Company B, Lot No. 15453	0.257	0.271	0.080	0.031	0.335	0.249	0.187	0.087	1.497
Ginseng root, in tablets, Company C, Lot No. B 514 C	0.060	0.126	0.024	0.020	0.185	0.171	0.104	0.053	0.743
Ginseng root, in hard gelatin capsules, Company D, Lot No. 4305	0.269	0.155	0.075	—	0.281	0.190	0.142	0.069	1.181
Ginseng root, in hard gelatin capsules, Company E, Lot No. 7050816	0.318	0.195	0.092	0.056	0.460	0.285	0.201	0.063	1.670
Ginseng extract, in hard gelatin capsules, Company F, Lot No. M-1	0.132	0.086	0.033	—	0.136	0.106	0.063	0.042	0.598
Ginseng fluid extract, Company G, Lot No. 2076	0.141	0.066	0.029	—	0.230	0.161	0.120	0.093	0.840
Ginseng root, Company H, Lot No. 97021	0.433	0.269	—	—	0.351	0.270	0.195	0.152	1.670
Ginseng root, Company H, Lot No. 97022	0.194	0.199	—	0.058	0.362	0.206	0.142	0.114	1.275
Ginseng root, Company I, Lot No. 1097001	0.283	0.152	0.105	—	0.449	0.285	0.213	0.171	1.658
Ginseng root, Company I, Lot No. 2097001	0.239	0.199	0.098	—	0.526	0.333	0.195	0.190	1.780
Ginseng root, in hard gelatin capsules, Company L, Lot No. 68128	—	—	—	—	—	—	—	—	—
Ginseng extract, Company M, Lot No. Ep 50905	0.005	0.012	—	—	0.383	0.397	0.354	0.457	1.608
Panax quinquefolium root, Company N, Lot No. PG 004	0.239	1.043	—	—	0.263	0.063	—	0.095	1.703

O-glycosidic bound sugar. Also a cyclisation of the side chain at C-20 of the Aglycon takes place. The final products of the degradation reactions are Panaxadiol and Panaxatriol.

Two significant reasons exist, which can cause destruction of the Ginsenosides during production of Ginseng preparations:

—The influence of enzymes
—The influence of heat

Enzymes

Enzymes are essential for life of the Ginseng plant. While the plant is living, these enzymes are separated from other ingredients (hence they are in other compartments). At leaving or immediate grinding of the harvested roots, the cellular walls are destroyed (and therefore the compartimentation). The enzymes can now come into contact

Table 2. Results of quantitative determinations: therapeutic daily dose of ginsenosides in the standardized product GINSANA®

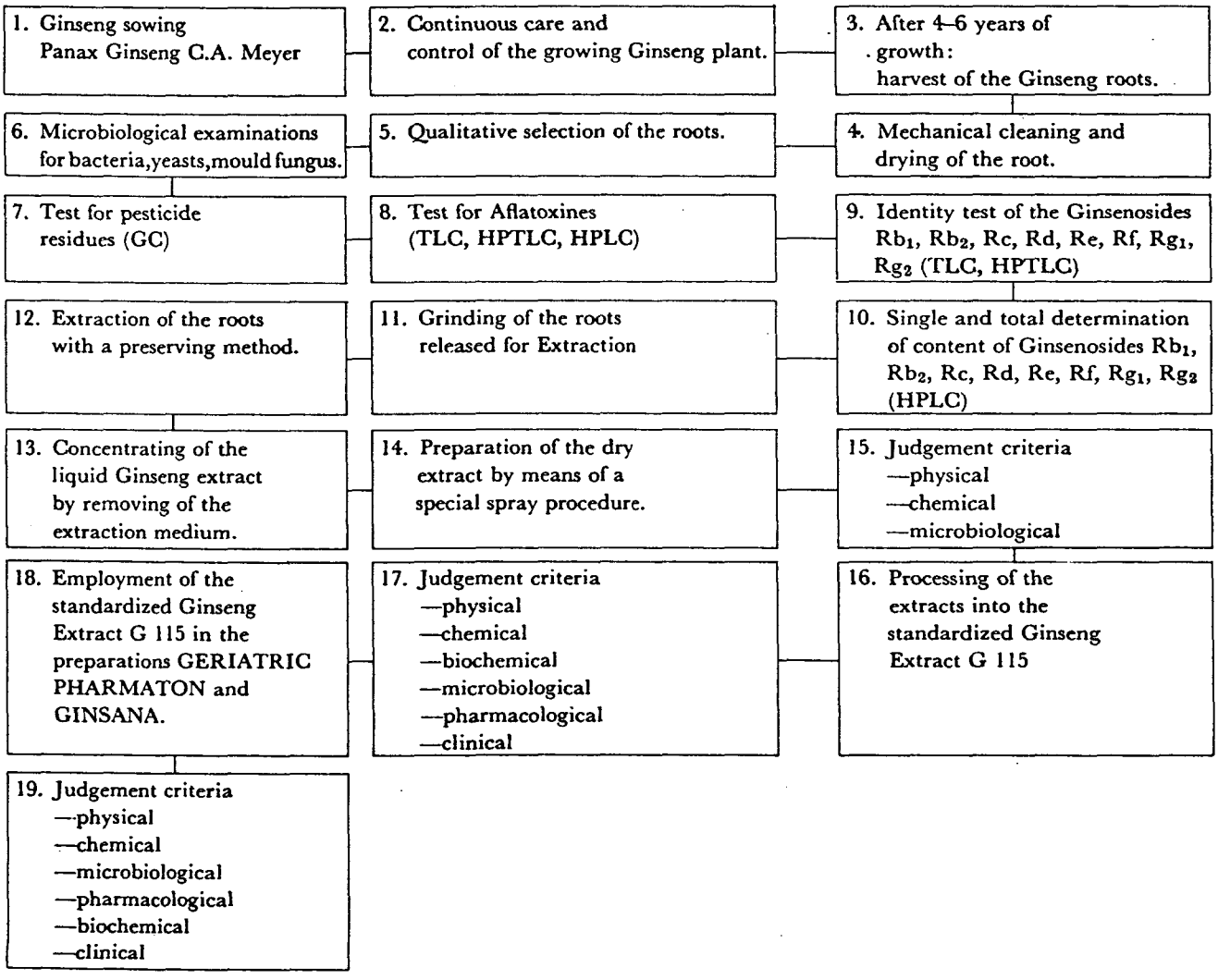
	mg								
	Rg_1	Re	Rf	Rg_2	Rb_1	Rc	Rb_2	Rd	Total
GINSANA®, soft gelatin capsules, Ginseng Products Ltd., Lot No. 201/150	1.166	0.910	0.476	0.272	2.148	1.110	0.708	0.382	7.152
GINSANA®, soft gelatin capsules, Ginseng Products Ltd., Lot No. 201/151	1.308	1.196	0.626	0.156	1.930	0.952	0.744	0.382	7.294
GINSANA®, soft gelatin capsules, Ginseng Products Ltd., Lot No. 201/152	1.660	1.078	0.578	0.132	1.908	1.016	0.780	0.382	7.534
GINSANA®, soft gelatin capsules, Ginseng Products Ltd., Lot No. 201/153	1.574	1.196	0.422	0.090	1.820	1.016	0.708	0.342	7.168

with the Ginsenosides, and therefore their destruction (hydrolysis) sets in. The easiest way to inactivate the enzymes, is to dry the roots immediately after harvesting. The roots lose their water, which is essential for life of the enzymes.

Heat

A careful temperature treatment of the Ginseng roots and preparations made thereof, is the basic condition not to destroy the Ginsenosides. As for all plant glycosides, temperatures above 40°C

Table III.



should be avoided. The employment of spray-drying, which also gave very efficient results at thermolabile substances like antibiotics, enables us to produce extracts, the ingredients of which remain intact.

Schematic growth of a standardized ginseng product

As visible from Table III
The development of the standardized Ginseng Extract G 115® and the pharmaceutical specialities made thereof, such as GINSANA® and GERIATRIC PHARMATON®, needed the collaboration of scientists of various fields, from pharmacognosy, chemistry, biochemistry, pharmacology, galenical up to clinical pharmacology.

Quality control for the standardized ginseng extract G 115

To consider only one aspect of this drawing (one cannot enter into all details here), we would like to mention the judgement criteria, which we apply for quality control of the standardized Ginseng Extract G 115®. They can be seen on the following Table IV.

Table IV.

1. Appearance
2. Colour
3. Odour
4. Taste
5. Solution 2% in water
6. Colour of the 2% solution
7. Determination of reaction (pH)
8. Loss at drying
9. Extractable substances with ethanol of 70%
10. Reducing sugars
11. Combustion residue
12. Composition of the combustion residue (atomic absorption)
13. Identity test of the Ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, (TLC, HPTLC).
14. Single and total content of the Ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, (HPLC).
15. Pesticide residues (GC)
16. Aflatoxines (TLC, HPTLC, HPLC)
17. Microbiological examinations (bacteria, yeasts and mould fungus)

Aim of all these tests is, to create a product for the doctor and his patient, the composition of which and therewith also its effect is constant.

Summary

The possibility of producing a standardized Ginseng extract can be summarized with the following 4 points:
A) Carefully selected raw material
B) Standardized extraction method in order to guarantee a total extraction of the ginsenosides and to assure that they are not destroyed or altered during the extraction process
C) Mixing of the obtained extracts to one standardized extract
D) Manufacturing of the standardized extract to an optimal galenical formulation
By means of the HPLC-method we can precisely control the successive steps A, B, C to D. The HPLC-method enables not only the assessment of the various ginseng drug preparations, but also the in-process-control for the production of standardized ginseng extracts, e.g. the standardized ginseng extract G 115® and the quality control of the final products, e.g. GINSANA®.

Chairman: Now the time is open to discussion.

Questioner: In this symposium, we have seen many interesting effects of ginseng but they were done with unidentified extracts or different saponin concentration and in some cases, the investigators even did not know exactly the composition of the substance which was tested. Therefore, I am happy to hear there is really any standardized extracts of which all active ingredients can be analysed quantitatively. My question now, since this G115 extract has equivalently certain unique procedure. I wonder whether some ginseng research institutes or universities are using this extract for their researches.

Rückert: Well, in Europe, and in the U.S. some institutes and universities have already this extract and are doing tests. Also the National Cancer Institute in the U.S. they have this material there, and recently the Chinese University of Hong Kong, they have included one

of their cancer tests and toxicological study was made. We of course have some other tests running. By the way, if any institute or participant of this symposium speakers would be interested to, let's say to have some pharmacological, clinical test substances I think made available to them.

Questioner: May I have one question and one comment? Do you have any experimental evidence for the production of free panaxadiol or free panaxatriol from ordinary product of ginseng?

Rückert: Do you mean whether we also produce isolated substances, maybe the ginsenoside or isolated pure substances. Is that what you mean?

Questioner: My question is that "Did you find any free panaxadiol or panaxatriol in the extract of ordinary ginseng product?

Rückert: You said that enzyme hydrolysis probably result in the production of free panaxadiol or free panaxatriol or aglycon. You know I am not the analyst. Maybe Dr. Soldati is able to answer this question.

Soldati: We could not find free panaxadiol or panaxatriol in the extract. It cannot be possible. There is one question to Prof. Shibata. Did you find any free panaxadiol or free panaxatriol from the extract?

Shibata: It depends what kind of extract you use. My very old experience, I never come across to find the panaxadiol, panaxatriol in the extract and these compounds were obtained by the acid hydrolysis.

Soldati: You mean that you obtained the panaxadiol or panaxatriol only by mineral acid hydrolysis?

Shibata: We are studying on the very early stage of experiment. I could not find panaxadiol or panaxatriol in the extract.

Soldati: Your experience is quite reasonable. I could not detect any panaxadiol or panaxatriol in the extract. This is the experimental evidence. I made this work in my laboratory. I searched in the ginseng roots products. If you take ginseng roots in not good dried and you make an analysis of this ginseng roots, you can observe the hydrolysis products, panaxadiol or panaxatriol.

Shibata: I observed free protopanaxadiol or triol but never panaxadiol or panaxatriol. Never cyclization occur by hydrolysis.

Soldati: Of course it is important not to have this destruction product in the extract. We hope that the saponins in the extract are intact and not the artifacts in the extracts, because of that we are in research of the presence. We don't hope there are panaxadiol or panaxatriol. However, it's possible to find panaxadiol or panaxatriol in the extracts. Because sometimes if the alcoholic degree is not so high for the extraction, the glycosidase are very active and very stable at the temperature and at the alcoholic degrees. The glycosidase in the plants are active in this condition and you can obtain certain hydrolytic products during 2–3 hours of the extraction. Probably in the ordinary extraction procedure of the products it is difficult to obtain enough level of the products because generally the extraction time is very short. But in the case of the time is very long, you can get partially the hydrolytic products.

Shibata: In my experience, aglycon was produced only in the presence of acid at high temperature.

Soldati: Yes, but you can obtain, I am sure, if you treat the ginseng roots dried. We worked for two to three hours you obtain a very strong modification of the picture of the ginsenoside and you can obtain the monoglycoside all in different position and also the free aglycons. Not in big quantity but in certain quantity it is possible to obtain.

Han: Let's discuss in the other place. I have one comment. You analyzed the ginseng by experience and detected and quantitatively estimated the content of ginsenoside Rb_1, Rb_2, and Rg, Rg_2 and you estimated the total content by some of them but I think that you must consider the following point. For example, in Korea, we drink it after ginseng boiled and we administer by oral route in gastric acid, all ginsenoside is destroyed to prosapogenin. I have some tried and may I show you the structure of prosa-

pogenin. Please allow me to show that.

Chairman: We have a few minutes and I want you to make briefly and Please say in two minutes.

Han: Those are ginsenoside found in Korean ginseng. The structure was established by Shibata. Characteristic is all ginsenoside have C-20 group. If C-20 group was eliminated, then all sapogenin is simplified to a few prosapogenin. Next slide is the thin layer graph which was obtained at the gastric acid hydrolysis on the physiological condition. A1, A2, A3 means prosapogenin obtained at the gastric acid hydrolysis on the physiological condition. A1, A2, A3 were produced from ginsenoside Rg_1. C1, C2, C3 were produced from ginsenoside Re. E1, E2, E3 were produced from ginsenoside Rg_1. All protopanaxadiol group ginsenosides produce same E1, E2, E3 in gastric acid. The hydrolysis rate within two hours showed that Rg_1 was decomposed almost 90% within 30 min., almost 62% was destroyed and remained only 37.9% in 2 hours. Two hour incubation is correspond to retention in the gastric sac. Similar data were obtained with ginsenoside Rg_1 and Re. We isolated the prosapogenin A1, A2, A3, and A1 was very unstable and A2, A3 were very stable. Therefore we could determine the structure by carbon and hydrogen analysis. Final conclusion, all ginsenoside were decomposed by gastric acid to prosapogenin A2, C2 or A3, C3. A2, C2 are decomposed from C-20 group and A3 or C3 is the product, produced by the hydrolysis on the side chain. This is gastric acid decomposed product. Then I wish to have one comment. Before you draw your conclusion, you must consider that the ginseng product might be destroyed to prosapogenin. Therefore, you must analyse the content of prosapogenin all-together. This is my comment.

Soldati: Can I give you the answer? I was in the laboratory and I must give you the answer. We could analyze quantitatively, not qualitatively but we made only the thin layer chromatography and we saw this hydrolysis product. About the destruction in the acetic acid of the drug, we had also the same result. With all ginsenoside the hydrolysis with different rate it depends on the sugar moiety but another aspect you have two aspect mechanism in the drug. You have a destruction mechanism with acetic acid but we have also an adsorption of the body and this two mechanism go together and you have also at the same time destruction but also an adsorption. You cannot say all destroyed because the body does absorb our normally medicament there remains an hour in the stomach and in 50 minutes you have very good absorption. We have also the same result. This is interesting for me to see that.

References

1. Sticher O. and F. Soldati: Planta medica **36**, 30 (1979)
2. Kaku T., T. Miyata, T. Uruno, I. Sako and A. Kinoshita: Arzneimittel-Forschung **25**, 539 (1975)
3. Wagner H.: Apoth. Journal **11**, 38 (1979)
4. Petkov V.: Arzneim.-Forschung **28**, 388 (1978)
5. Schole J., G. Marisch, H.P. Sallmann: Belastung, Ernährung und Resistenz- Fortschritte in der Tierphysiologie und Tierernährung 9, Hamburg, Berlin, 1978, Verlag P. Parey
6. Bittles A.M., J. Fulder, E.C. Grant and M.R. Nicholls: Gerontology **25**, 125 (1979)
7. Food and Drug Research Laboratories: A 90-Day Feeding Study of Ginseng Extract G 115 Pharmaton to purebred beagle dogs. FDRL-Report, September 26, 1978, Waverly Research Center, USA
8. Food and Drug Research Laboratories: Evaluation of the Savety of Ginseng Extract G 115 Pharmaton Following DietaryAdministration through two Generations, FDRL-Report, November 15, 1978, Waverly Research Center, USA
9. Dörling E., A.M. Kirchdorfer and K.H. Rückert: Notabene medici, in print (1980)
10. Schmidt U., I. Kalbe, F.M. Schulz and G. Brüschke: XI. International Congress of Gerontology, Tokyo, August 20–25, 1978
11. Quiroga, H.A. and A.E. Imbriano: Orientation Médica: **28**, 1208, 86 (1979)
12. Soldati F. and O. Sticher: Planta medica, in print (1980)