CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **MAS/36 CRD/6**

Original Language Only

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

36th Session

Budapest, Hungary, 23 – 27 February 2015

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

(Comments of EU, Kenya, Republic of Korea)

EU

The European Union and its Member States (EUMS) would like to make the following comments on the methods of analysis and sampling proposed by CCPVF and CCCF.

CCPFV:

The EUMS support the methods of analysis for canned fruit and for ginseng, as these are methods previously endorsed by CCMAS.

CCCF:

The EUMS support the endorsement of the sampling plans for fumonisins in maize and maize products as proposed by CCCF.

KENYA

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF)

Sampling plans for fumonisins in maize and maize products

We propose the CCMAS to endorse the method, we have no objection since this falls under its Terms of Reference (mandate).

REPUBLIC OF KOREA

Methods for ginseng

Background

The methods of analysis presented in the *Regional Standard for Ginseng Products* (CODEX STAN 295R-2009) were previously endorsed at the 29th session of the CCMAS held in 2008.

At the 27th session of the CCPFV held in 2014, the committee considered the Proposed Standard for Ginseng Products in order to convert the regional standard to a worldwide standard. Noting that there were no outstanding issues, the committee agreed to forward the draft standard to the Codex Alimentarius Commission for adoption at Step 5/8(See REP15/PFV, paragraphs 86 and 87).

At the committee, the Republic of Korea, as Chair of the Electronic Working Group on Ginseng Products, introduced the report of the EWG and highlighted the key issues addressed in the conversion of the regional standard to a worldwide standard, including the methods of analysis and sampling.

The tables for the sampling plans were newly attached as Annexes I and II. For a more concrete and detailed description of the methods of analysis, Annexes A, B and C of the regional standard were replaced by Annexes III (water-insoluble solids), IV (water-saturated n-butanol extracts) and V (identification of ginsenoside Rb1 and Rf). In addition, the Standard Operating Procedures (SOP) for the analysis of moisture

and ash were also attached as References 1 and 2, respectively. These methods of analysis were originally proposed as Type-I in the 27th session of the CCPFV.

Inter-laboratory validation test

The inter-lab validation test was performed, led by the Republic of Korea, in order to convert the status of the methods of analysis from the current Type IV to Type I in the conversion of the regional standard for ginseng products to a worldwide standard. The results are shown in Attachment 1.

The test was performed from October 27 to December 11, 2014; a total of nine laboratories from the Republic of Korea, Japan, and China, all of which are operated according to ISO/IEC 17025:1999, including the KFRI, participated in and performed the test according to the IUPAC-1987 protocol.

The proficiency test materials were dried ginseng and ginseng extract products defined in the standard. Test samples were prepared by making a package purchase by the KFRI, which supervised the efficacy validation test, and then the same sample was distributed to each laboratory. Test methods were conducted according to the analytical methods presented in the draft of the standard (Annex III thr. V and References 1 & 2).

Results and recommendations

As shown in Attachment 1, the results of the Inter-laboratory Validation Test, in which the nine laboratories participated, demonstrated excellent results with RSD values by laboratory for each analytical item given as 2.57~10.70%.

Information on the reliability - including reproducibility, repeatability and specificity, etc. - of the methods of analysis was previously provided as CRD 10 in the 29th CCMAS (For reference, see Attachment 2); and the test results for each item described in the attachment fully prove the validity of the methods of analysis suggested in the standard for ginseng products.

Therefore, the Republic of Korea recommends that this committee endorse the method of analysis for ginseng products as Type-I for moisture, solids, ash and Type II for water-insoluble solids, water saturated n-butanol extracts, identification of ginsenoside Rb1, Rf.

Table1. Method of analysis for ginseng products

PROVISION	METHOD	PRINCIPLE	TYPE	
			PRESENT	PROPOSED
Moisture	AOAC 925.45 B (Dried ginseng) Quantity of sample: 2 g AOAC 925.45 D (Ginseng extract) Quantity of sample: 1.5 g (mixing with 20 g of sea sand)	Gravimetry	IV	I
Solids	AOAC 925.45 B (Dried ginseng) - calculated by subtracting the content of moisture from 100 % Quantity of sample: 2 g AOAC 925.45 D (Ginseng extract)) - calculated by subtracting the content of moisture from 100 % Quantity of sample: 1.5 g (mixing with 20 g of sea sand)	Calculation	IV	I
Ash	AOAC 923.03	Gravimetry	IV	I
Water-insoluble solids	Described in Annex III	Gravimetry	IV	II
Water-saturated n-butanol extracts	Described in Annex IV	Gravimetry	IV	II
Identification of ginsenoside Rb1, Rf	Described in Annex V	TLC or HPLC	IV	II

Attachment 1

<Results of Inter-laboratory Test>

Quality factor	Type of product	Participating laboratories*									Mean	SD	RSD (%)
		1)	2)	3)	4)	5)	6)	7)	8)	9)			
Moisture (%)	Dried Ginseng	10.26	9.60	10.04	10.16	9.65	10.27	10.03	9.46	11.04	10.06	0.47	4.71
	Ginseng Extract	34.49	34.49	34.15	34.06	36.97	36.59	31.16	33.77	34.97	34.52	1.68	4.88
Solids (%)	Ginseng Extract	65.50	65.51	65.85	65.94	63.03	63.41	68.84	66.23	65.03	65.48	1.69	2.57
Ash (%)	Dried Ginseng	4.00	4.00	4.03	4.06	4.05	3.92	3.89	3.93	3.61	3.94	0.14	3.46
Water insoluble solids (%)	Ginseng Extract	1.04	0.93	1.00	1.17	1.24	1.16	1.27	1.16	1.00	1.11	0.12	10.70
Water saturated n-butanol extract (mg/g)	Dried Ginseng	24.16	25.82	27.97	28.88	20.87	27.55	26.03	25.90	30.23	26.38	2.76	10.46
	Ginseng Extract	62.65	71.09	60.37	67.78	61.37	60.44	67.20	62.63	62.95	64.05	3.75	5.85

Identification of ginsenoside**	Rb1	+	+	+	+	+	+	+	+	+	+	+	+
	Rf	+	+	+	+	+	+	+	+	+	+	+	+

* 1) Korea Food Research Institute, Gyeonggi-do Province, Rep. of Korea

2) NongHyup Hansamin co., Ltd., Chungcheongbuk-do Province, Rep. of Korea

3) Korea Ginseng Corp., Daejeon City, Rep. of Korea

4) Kuan Industrial Co., Ltd., Chungcheongnam-do Province, Rep. of Korea

5) National Institute of Horticultural and Herbal Science, Rural Development Administration, Chungcheongbuk-do Province, Rep. of Korea

6) Ilhwa Co., Ltd., Gyeonggi-do Province, Rep. of Korea

7) National Agricultural Products Quality Management Service, Gyeongsangbuk-do Province, Rep. of Korea

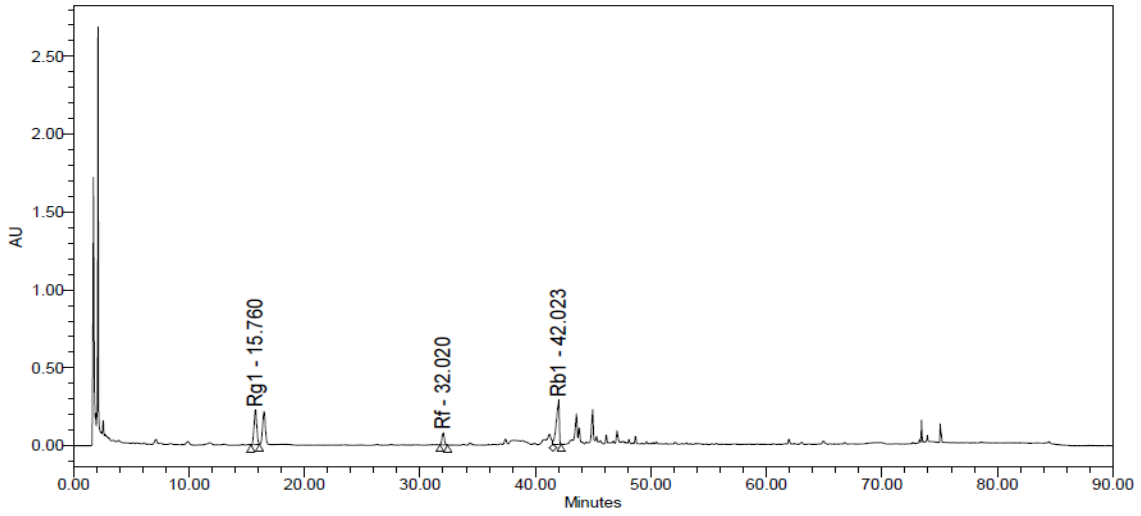
8) Japan Food Research Laboratories, Tokyo, Japan

9) National Ginseng & Deer Products Quality Supervision Inspection Center, Yanji, China

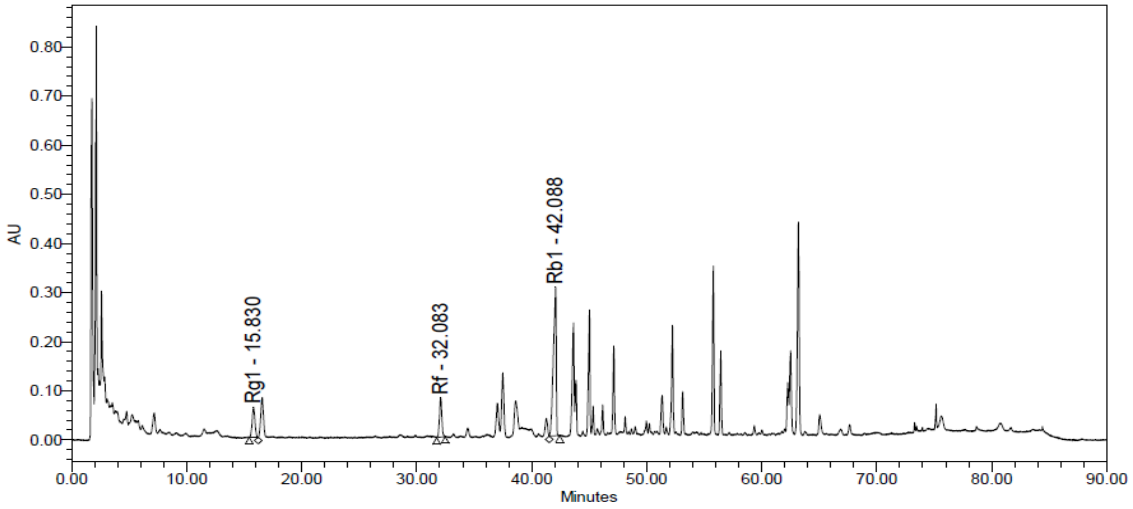
** The identification of ginsenosides is expressed as positive (+)/ negative (-) (Refer to chromatogram a), b) below).

Note: Values are the mean of triplicate analyses.

a) Chromatogram for dried ginseng



b) Chromatogram for ginseng extract



Analytical conditions:

Instrument	Waters Alliance 2695 HPLC system	Mobile phase
Column	4.6 mm id X 25 cm, 5 µm, Discovery c18	
Detector	UV 203 nm	
Flow rate	1.6 mL/min	
Injection volume	20 µℓ	

Time (min)	Distilled water (%)	Acetonitrile (%)
initial	80	20
10	80	20
40	68	32
55	50	50
70	35	65

		72	10	90
		82	10	90
		84	80	20
		90	80	20

Attachment 2

Agenda Item 5 b)

KOREA'S COMMENTS

ON DRAFT STANDARD FOR GINSENG PRODUCTS

Submitted to

THE 29TH SESSION OF

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Background

The 15th session of CCASIA decided to forward the Section on Methods of Analysis and Sampling in the draft standard for Ginseng Products, to CCMAS for their endorsement (See ALINORM 07/30/15 para. 42 and 68). In this regard, Korea has prepared as many additional and supplementary data on the methods as possible.

The following are comments from the government of Korea on the said section, with a focus on Subject B of the document CX/MAS 08/29/6-Add.1 submitted to this 29th session of CCMAS for consideration.

1. Revisions to be made during this session

First of all, from the document CX/MAS 08/29/6-Add.1, Korea wants the revisions in Table 1 below to be made during the 29th session of CCMAS.

Table 1. Draft Standard for Ginseng Product

COMMODITY	PROVISION	METHOD	PRINCIPLE
Ginseng Product	Moisture	AOAC 925.45	~~Vacuum drying~~ *Gravimetry, drying at atmospheric pressure*
Ginseng Product	Solids	AOAC 925.45 and calculated by subtracting the content of water from 100%	Calculation
Ginseng Product	Ash	AOAC 923.03	~~Gravimetry~~ *Furnace, 550 ℃*
Ginseng Product	Water-insoluble Solids	described in Annex A	
Ginseng Product	Water-saturated 1-butanol extracts	described in Annex B	
Ginseng Product	Ginsenosides Rb1 and Rf	described in Annex C	

Here is the justification. As for moisture, AOAC 925.45 does not suggest the method of vacuum drying only but recognizes these all: A. Vacuum Drying B. Drying at Atmospheric Pressure, C. Drying on Pumice Stone and D. Drying on Quartz Sand. Consequently, the Principle in the table above need be amended to 'Gravimetry, drying at atmospheric pressure.' Furthermore, as for Ash, the principle will be more clearly stipulated in 'Furnace, 550℃' rather than in 'Gravimetry.'

2. Supplementary data for verification

Below listed are supplementary data to determine the content of moisture, solids, ash, water-insoluble solids, water-saturated 1-butanol extracts, and ginsenosides Rb1 & Rf.

Moisture

Table 2. Repeatability of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Dried Raw Ginseng	9.93	0.03	0.25	n=12
Dried Steamed Ginseng	7.92	0.03	0.37	n=12
AOAC 925.45 B/sample 5 g/100℃ at Atmospheric Pressure				

Table 3. Content of moisture in different dried ginseng samples

Types of Ginseng Product	Sample	Mean (%)	SD	RSD (%)	Remarks
Dried Raw Ginseng	A	9.05	0.03	0.35	n=12
	B	8.49	0.12	1.36	n=3
	C	8.93	0.09	1.04	n=3
	D	8.52	0.11	1.30	n=3
	E	9.36	0.13	1.35	n=3
	F	10.02	0.04	0.41	n=3
	G	9.93	0.03	0.25	n=12
	H	8.90	0.15	1.68	n=3
	I	9.45	0.21	2.18	n=3
	J	9.75	0.14	1.44	n=3
	K	10.08	0.06	0.63	n=3
	L	10.09	0.00	0.04	n=3
	M	5.66	0.08	1.47	n=12
	N	8.40	0.01	0.16	n=3
	O	7.64	0.03	0.38	n=3
Dried Steamed Ginseng	A	7.92	0.03	0.37	n=12
	B	10.21	0.04	0.41	n=3

	C	9.22	0.04	0.41	n=3
	D	8.14	0.05	0.64	n=3
	E	6.41	0.05	0.79	n=3
	F	5.09	0.08	1.61	n=12
	G	6.12	0.13	2.13	n=3
	H	8.14	0.05	0.64	n=3
	I	6.41	0.05	0.79	n=3
AOAC 925.45 B/sample 5 g/100℃ at Atmospheric Pressure					

Table 4. Reproducibility of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Dried Raw Ginseng	9.26	0.25	2.74	4 Labs.*
Dried Steamed Ginseng	5.30	0.15	2.89	4 Labs.*
AOAC 925.45 B/sample 5 g/100℃ at Atmospheric Pressure				

*The above-mentioned laboratories are officially recognized testing institutions by the KOLAS/APLAC/ILAC.

Solids

Table 5. Repeatability of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Raw Ginseng Extract	68.03	0.48	0.71	n=12
Steamed Ginseng Extract	69.18	0.25	0.36	n=12
AOAC 925.45 B/sample 5 g/100℃ at Atmospheric Pressure/ calculated by subtracting the content of water from 100%				

Table 6. Content of solid in different ginseng extract samples

Types of Ginseng Product		Mean (%)	SD	RSD (%)	Remarks
Raw Ginseng Extract		68.03	0.48	0.71	n=12
Steamed Ginseng Extract	A	69.18	0.25	0.36	n=12
	B	67.71	0.04	0.05	n=3
	C	69.45	0.10	0.15	n=3
	D	68.85	0.06	0.09	n=3
AOAC 925.45 B/sample 5 g/100℃ at Atmospheric Pressure/ calculated by subtracting the content of water from 100%					

Table 7. Reproducibility of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Raw Ginseng Extract	66.60	2.50	3.75	4 Labs.*
Steamed Ginseng Extract	70.97	0.70	0.99	4 Labs.*
AOAC 925.45 B/sample 5 g/100℃ at Atmospheric Pressure/ calculated by subtracting the content of water from 100%				

*The above-mentioned laboratories are officially recognized testing institutions by the KOLAS/APLAC/ILAC.

Ash

Table 8. Repeatability of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Dried Raw Ginseng	3.51	0.03	0.91	n=12
Dried Steamed Ginseng	4.86	0.03	0.69	n=12
AOAC 923.03/sample 3 g/Furnace 550℃				

Table 9. Content of ash in different dried ginseng samples

Types of Ginseng Product	Sample	Mean (%)	SD	RSD (%)	Remarks
Dried Raw Ginseng	A	4.28	0.06	1.33	n=12
	B	3.69	0.04	1.20	n=3
	C	3.85	0.02	0.42	n=3
	D	4.56	0.02	0.55	n=3
	E	3.35	0.04	1.16	n=3
	F	3.45	0.01	0.40	n=3
	G	3.51	0.03	0.91	n=12
	H	3.95	0.02	0.44	n=3
	I	3.12	0.02	0.72	n=3
	J	3.35	0.02	0.45	n=3
	K	3.86	0.03	0.65	n=3
	L	3.76	0.04	0.91	n=3
	M	4.32	0.03	0.61	n=12
	N	4.07	0.02	0.43	n=3
	O	4.09	0.03	0.63	n=3
Dried Steamed Ginseng	A	4.86	0.03	0.69	n=12
	B	4.36	0.01	0.21	n=3

	C	4.06	0.01	0.19	n=3
	D	3.91	0.02	0.55	n=3
	E	3.59	0.01	0.33	n=3
	F	4.76	0.03	0.54	n=12
	G	4.28	0.01	0.26	n=3
	H	4.78	0.01	0.30	n=3
AOAC 923.03/sample 3 g/Furnace 550℃					

Table 10. Reproducibility of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Dried Raw Ginseng	4.31	0.02	0.36	4 Labs.*
Dried Steamed Ginseng	4.21	0.02	0.41	4 Labs.*
AOAC 923.03/sample 3 g/Furnace 550℃				

*The above-mentioned laboratories are officially recognized testing institutions by the KOLAS/APLAC/ILAC.

Water-insoluble solids

Please refer to the document ALINORM 07/30/15, Annex A.

Table 11. Repeatability of the proposed analysis methods

Types of Ginseng Product	Mean (%)	SD	RSD (%)	Remarks
Ginseng Extract	0.87	0.05	5.78	n=6

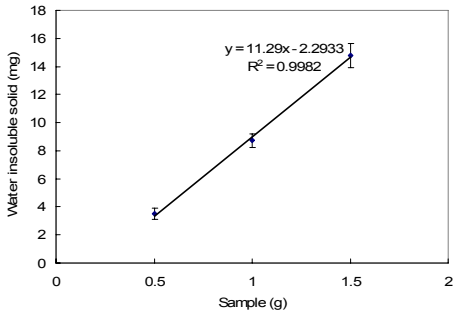


Figure 1. Linearity of proposed analysis methods

Water-saturated 1-butanol extracts

Please refer to Appendix III Annex B to ALINORM 07/30/15 and Appendix I to this CRD.

Table 12. Repeatability of the proposed analysis method for various ginseng products

Types of Ginseng Product		Mean (mg/g)	SD	RSD (%)	Remarks
Ginseng Extract	A	81.9	2.19	2.68	n=6
	B	94.2	2.53	2.69	n=6
Dried Ginseng	A	55.8	0.85	1.53	n=6
	B	42.2	1.76	4.17	n=6

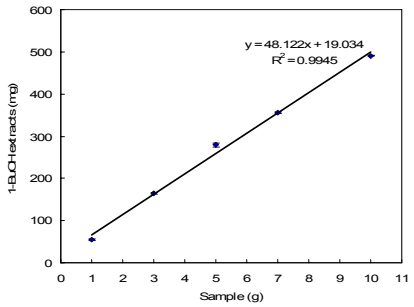


Figure 2. Linearity of proposed analysis methods for dried raw ginseng.

Table 13. . Reproducibility of the proposed analysis methods

Types of Ginseng Product	Mean (mg/g)	SD	RSD (%)	Remarks
Dried Raw Ginseng	43.7	1.92	4.35	3 Labs.

Ginsenosides Rb1 and Rf

Please refer to Appendix III Annex C to ALINORM 07/30/15 and Appendix II to this CRD.

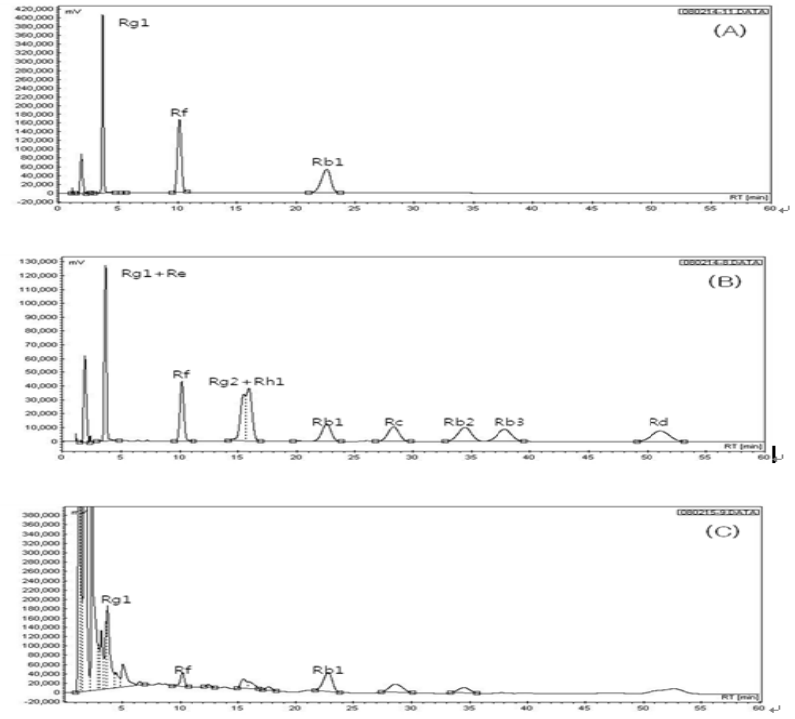


Figure 3. Specificity validation for the proposed HPLC analytical method for ginsenosides Rb1 and Rf deriving from ginseng product: (A) standard solution for the ginsenosides Rb1and Rf (B) standard solution for the commercially available 10 ginsenosides (C) steamed ginseng sample solution. Column: μ-Bondapak C18 (10 ㎛, 3.9×300 mm); Eluent: acetonitrile:water (30:70, v/v) isocratic; Flow rate: 1.5 ml/min; Injection volume: 20 ㎕; Detection wavelength : UV(203nm).

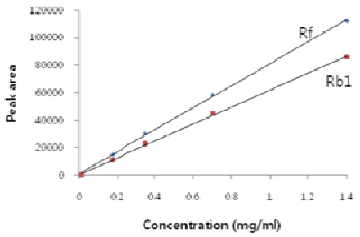


Figure 4. Calibration curves of ginsenoside standards by the proposed HPLC method.

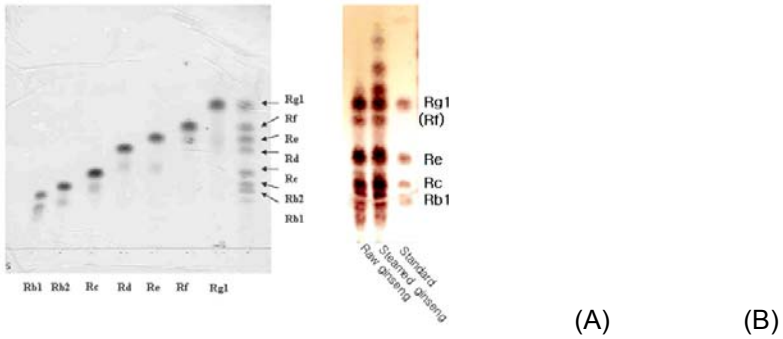
Table 14. LOD and LOQ of the proposed HPLC method

Ginsenoside	LOD (μg/g sample)	LOQ (μg/g sample)
Rf	4.5	13.6
Rb1	22.2	67.4

Table 15. Repeatability of the proposed HPLC method

Types of Ginseng product	Ginsenoside	Mean (mg/g)	SD	RSD (%)	Remarks
Steamed ginseng extract	Rb1	3.45	0.02	0.48	n=5
	Rf	0.60	0.02	3.48	n=5
Dried raw ginseng	Rb1	8.38	0.02	0.27	n=5
	Rf	1.19	0.01	0.71	n=5

Please refer to Appendix III Annex C to ALINORM 07/30/15 and Appendix III to this CRD.



(A) (B)

Figure 5. Specificity validation for the proposed TLC method for ginsenosides Rb1 and Rf deriving from ginseng product: (A) standard solution for the commercially available 7 ginsenosides (B) raw ginseng and steamed ginseng sample solutions. Ginsenosides were separated on the TLC plate with 1-butanol:ethylacetate:water (5:1:4, v/v/v) at 20°C and visualized by 10% (w/v) H_2SO_4 at 110°C for 10 min.

3. Additional appendixes

Korea thinks it will be of great help for understanding what has been considered in the above, to attach three appendixes below.

Appendix I

Determination of Water-Saturated 1-Butanol Extract content

1. SCOPE

This method is recommended for the determination of water-saturated 1-butanol extract content (Crude ginseng saponin fractions) in dried ginseng, ginseng extract, or other products containing ginseng. The content is expressed in percentage (or mg/g sample).

2. PRINCIPLE

After crude saponin fractions are extracted from Ginseng Products by using water saturated 1-butanol, what have been extracted along with the fractions, such as sugars and non-polar lipophilic compounds, shall be eliminated through ethyl ether extraction.

3. REAGENTS AND MATERIALS

Unless otherwise mentioned, all chemical reagents for the analysis must be of certified analytical grade.

3.1 Reagents

3.1.1. Butanol (CAS No. 71-36-3, purity: > 95%)

3.1.2. Diethyl ether (CAS No. 60-29-7)

3.1.3. Methanol (CAS No. 67-56-1)

3.1.4. Distilled water

3.2. Preparation of Water-saturated 1-butanol

Take 1-butanol and distilled water in a separatory funnel in the ratio of 70 to 30 and shake the funnel vigorously to mix these two phases thoroughly. Stand the funnel for 3 hours to let the phases settle and harvest the upper water-saturated 1-butanol layer for further use.

4. APPARATUS

4.1 Analytical balance (accuracy: 0.1 mg)

4.2 Separatory funnel

4.3 Rotary vacuum evaporator

4.4 Drying oven

4.5 Heating apparatus (temperature-adjustable water bath is recommended)

4.6 Extraction apparatus (Erlenmeyer flask with cooling reflux condenser system is recommended)

5. HANDLING OF SAMPLES

Keep dried ginseng products or powdered ginseng samples in a moisture-proof airtight container or desiccator and store them at 20℃ until use.

6. ANALYTICAL PROCEDURE

6.1 Dried ginseng products

6.1.1 Pulverize dried ginseng root in a laboratory grinder. Ginseng powder passed through an 80 mesh standard sieve is recommended for the analysis. However, finished products of ginseng powder, whose size is smaller than 0.120 mm (80 mesh), may be used without grinding.

6.1.2 Place ca 5 g of the powdered ginseng into a 250 ml evaporation flask with a cooling condenser.

6.1.3 Place 50 ml of water-saturated 1-butanol into the flask containing ginseng sample.

6.1.4 Heat the ginseng sample for 1 hour on a water bath under a reflux condenser at a temperature not exceeding 80℃.

6.1.5 After cooling, collect 1-butanol extract through a 250 ml separatory funnel by the aid of filter paper (Whatman No. 2 or its equivalent)

6.1.6 Take up the residue with 50.0 ml of water-saturated 1-butanol in evaporation flask and treat the ginseng sample residue again as mentioned in the above.

6.1.7 Repeat the extracting and filtering procedures (step 6.1.3 – 6.1.5) 2 more times (3 times in total).

6.1.8 Pool the 1-butanol extract through a 250 ml separatory funnel, place 50 ml of distilled water into the funnel, shake vigorously to mix the two phases and stand the funnel until the 1-butanol (upper phase) and water (lower phase) separate clearly from each other.

6.1.9 Collect the 1-butanol layer in an evaporation flask (A) which was dried in advance at 105℃ till it reached constant weight and evaporate & dry it under reduced pressure at a temperature not exceeding 60℃.

6.1.10 Place 50 ml ethyl ether into an evaporation flask containing the evaporated residue, heat the flask in a water bath with a reflux condenser at 46℃ for 30 min and discard the ethyl ether by decanting.

6.1.11 Dry the flask at 105℃ for 30 min and weigh it after cooling in a desiccator (B). Calculate the content of the water-saturated 1-butanol extract according to the following formula:

$$\text{Content of water saturated 1-butanol extract (\%)} = [(A-B)/S] \times 100$$

where

S: weight (g) of sampled ginseng

A: weight (g) of the evaporation flask containing 1-butanol extract residue after removing ethyl ether soluble fractions.

B: constant weight (g) of the empty evaporation flask

6.2 Ginseng extract

Put ca 5 g of ginseng extract concentrate into a 250 ml evaporation flask and dry it under reduced pressure. In the case of extract powder, 2 g sample are enough. The Content of water saturated 1-butanol extract in ginseng extract and extract powder are evaluated using the method described in 6.1.

Appendix II

Identification of ginsenosides Rb1 and Rf in ginseng products using HPLC detection

1. Scope

This method is suitable for the determination of ginsenosides Rb1 and Rf in ginseng products using liquid chromatographic detection. Identify ginsenosides of sample by comparing retention times of peaks with those of the standard.

2. Principles

The basic operating principle of HPLC is to force the analyte through a column of the stationary phase (usually a tube packed with small spherical particles with a certain surface chemistry) by pumping a liquid (mobile phase) at high pressure through the column. The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time and is considered a reasonably unique identifying characteristic of a given analyte.

3. Reagents and Materials

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade.

3.1 Reagents

3.1.1 Ginseng ginsenoside Rb1 (98.0 + %, $C_{54}H_{92}O_{23}$, Fw 1109.29)

3.1.2 Ginseng ginsenoside Rf (98.0 + %, $C_{42}H_{72}O_{14}$, Fw 801.01)

3.1.3 Methanol (HPLC grade)

3.1.4 Acetonitrile (HPLC grade)

3.1.5 Water (HPLC grade)

3.2 Preparation of standard solutions

3.2.1 Ginsenoside stock solution (A)

Weigh ca 2 mg of ginsenoside Rb1 and Rf and make up to 10 mL in a volumetric flask with methanol to give solution (A) of approximate 200 µg/mL and filter through 0.45 µm membrane filter.

4. Apparatus

4.1 Liquid chromatograph with UV detector

4.2 HPLC column: µ-Bondapak C18 (10 ㎛×3.9×300 mm, Waters) column or equivalent

4.3 Analytical balance, capable of weighing to 4 decimal places

5. Procedure

5.1 Test sample

Dissolve the dried 1-butanol extract of Annex B with approximately 10-100 fold volume of methanol, and filter through 0.45 μm membrane filter.

5.2 HPLC operating condition

5.2.1 Detector: UV (203 nm)

5.2.2 Effluent: acetonitrile:water (30:70, v/v) isocratic

5.2.3 Flow rate: 1.5 ml/min

5.2.4 Injection volume: 20 $\mu\ell$

5.2.5 Temp.: room temperature

6. Qualitative analysis - External standard method

6.1 Measure the retention time of the Rb1 and Rf peaks.

6.2 Identify ginsenosides of sample by comparing retention times of peaks with those of the standard

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Appendix III

Identification of ginsenosides Rb1 and Rf in ginseng product using thin layer chromatography

1. Scope

This method is suitable for the identification of ginsenosides Rb1 and Rf in ginseng product using thin layer chromatography. Identify ginsenosides of sample by comparing retention factor (R_f) value of spots with those of the standard

2. Principle

Thin layer chromatography (TLC) is a chromatography technique used to separate chemical compounds. It involves a stationary phase consisting of a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose immobilized onto a flat, inert carrier sheet. A liquid phase consisting of the solution to be separated is then dissolved in an appropriate solvent and is drawn up the plate via capillary action, separating the experimental solution based on the polarity of the components of the compound in question.

3. Reagents and Materials

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade.

3.1 Reagents

3.1.1 Ginseng ginsenoside Rb1 (98.0 + %, $C_{54}H_{92}O_{23}$, Fw 1109.29)

3.1.2 Ginseng ginsenoside Rf (98.0 + %, $C_{42}H_{72}O_{14}$, Fw 801.01)

3.1.3 1-Butanol

3.1.4 Ethylacetate

3.1.5 Methanol

3.1.6 Sulfuric acid

3.2 Preparation of standard solutions

Dissolve standard ginsenoside, such as ginsenoside Rb1 and Rf, in methanol to make 1% solution and filter through 0.45 μm membrane filter.

3.3 Diluted sulfuric acid solution

Dilute sulfuric acid in ethanol to make 10~30% solution

4. Apparatus

4.1 TLC (thin layer chromatography) system

The solvent mixture (ca 50 ml) is usually added into a standard developing tank (inner dimension: length 21 cm; width 9 cm; height 21 cm) lined with filter paper.

5. Procedure

5.1 Test solution

Dissolve the dried 1-butanol extract of Annex B with ca 10 fold volume of methanol, dissolve completely, and filter through 0.45 μm membrane filter.

5.2 TLC process

5.2.1 Spot 2-5 ㎕ of the standard and sample solutions, as indicated in the above, on TLC plate (silica gel), previously dried at 110℃ for 15 minutes in dry oven.

5.2.2 Develop with an 1-butanol:ethylacetate:water (5:1:4, v/v/v) solution. Dry the plates at ambient temperature for a while, and then dry more with a hair dryer.

5.2.3 Spray 10% sulfuric acid or 30% sulfuric acid-ethanol solution over TLC plate. Dry the plates at ambient temperature for a while, and then dry more with a hair dryer.

5.2.4 Visualize the spots in a dry oven at 110℃ for 5-10 minutes

5.2.5 Identify the ginsenosides of ginseng products by comparing the R_f values and colors with those of standard ginsenosides.

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