

# DETERMINATION OF GINSENG SAPONINS BY A STANDARDIZED REFERENCE EXTRACT METHOD

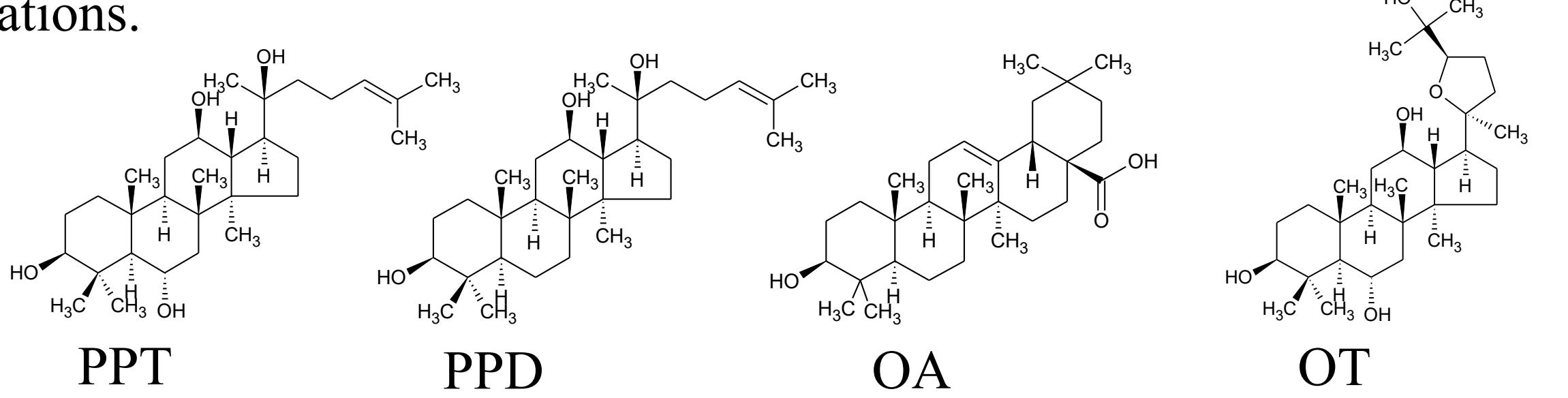
Andrey Stavrianidi<sup>1</sup>, Aleksandra Cvetanovic<sup>2</sup>, Oleg Shpigun<sup>1</sup>

[stavrianidi.andrey@gmail.com](mailto:stavrianidi.andrey@gmail.com)

<sup>1</sup>Chemistry Department, Lomonosov Moscow State University, 119991, Moscow, Russia, Leninskie gory, 1/3.

<sup>2</sup>Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia.

Limited availability of individual standards is a bottleneck for quality control of functional foods, infusions and other products made of medicinal plant materials. The use of standard mixtures or secondary standards is a good option when dealing with complex traditional medicines and preparations.



Ginseng products are typically made of *P. ginseng*, *P. notoginseng*, and *P. quinquefolius* roots and contain several major and dozens minor ginsenosides. These saponins possess a triterpene aglycone (e.g. protopanaxatriol (PPT), protopanaxadiol (PPD), oleanolic acid (OA) and a sugar side chain(s).

## Ginsenoside contents in *P. quinquefolius* extract reference material (XRM)

| Ginsenoside     |                                 | Content, mg/g              |               |                      |
|-----------------|---------------------------------|----------------------------|---------------|----------------------|
| Name            | Ion, m/z                        | HPLC-MS<br>(ME* corrected) | HPLC-MS       | HPLC-UV <sup>†</sup> |
| R <sub>1</sub>  | 977,6 [M+HCOOH-H] <sup>-</sup>  | 0,060 ± 0,004              | 0,056 ± 0,004 | —                    |
| Rg <sub>1</sub> | 845,6 [M+HCOOH-H] <sup>-</sup>  | 1,6 ± 0,1                  | 1,7 ± 0,1     | 1,72                 |
| Re              | 991,6 [M+HCOOH-H] <sup>-</sup>  | 15,5 ± 0,4                 | 17,5 ± 0,5    | 16,85                |
| F <sub>11</sub> | 699,6 [M+HCOOH-H] <sup>-</sup>  | 0,11 ± 0,02                | 0,100 ± 0,02  | —                    |
| Rf              | 845,6 [M+HCOOH-H] <sup>-</sup>  | ≤0,01                      | ≤0,01         | —                    |
| RT <sub>5</sub> | 845,6 [M+HCOOH-H] <sup>-</sup>  | 2,6 ± 0,2                  | 2,7 ± 0,2     | —                    |
| Rh <sub>1</sub> | 683,6 [M+HCOOH-H] <sup>-</sup>  | 0,46 ± 0,03                | 0,42 ± 0,03   | —                    |
| Rg <sub>2</sub> | 829,6 [M+HCOOH-H] <sup>-</sup>  | 2,6 ± 0,2                  | 2,7 ± 0,2     | —                    |
| Rb <sub>1</sub> | 1153,5 [M+HCOOH-H] <sup>-</sup> | 53 ± 3                     | 57 ± 3        | 49,21                |
| Rc              | 1123,6 [M+HCOOH-H] <sup>-</sup> | 13,5 ± 0,9                 | 15 ± 1        | 13,77                |
| Rb <sub>2</sub> | 1123,6 [M+HCOOH-H] <sup>-</sup> | 2,4 ± 0,2                  | 2,7 ± 0,2     | 22,4                 |
| Rb <sub>3</sub> | 1123,6 [M+HCOOH-H] <sup>-</sup> | 2,6 ± 0,2                  | 2,8 ± 0,2     | —                    |
| Rd              | 991,6 [M+HCOOH-H] <sup>-</sup>  | 13,6 ± 0,9                 | 15 ± 1        | 12,17                |
| Ro              | 955,6 [M-H] <sup>-</sup>        | 2,5 ± 0,2                  | 2,8 ± 0,2     | —                    |
| F <sub>2</sub>  | 829,6 [M+HCOOH-H] <sup>-</sup>  | 0,39 ± 0,03                | 0,40 ± 0,03   | —                    |
| Rg <sub>3</sub> | 829,6 [M+HCOOH-H] <sup>-</sup>  | 2,1 ± 0,1                  | 2,2 ± 0,1     | —                    |
| C-K             | 667,6 [M+HCOOH-H] <sup>-</sup>  | 0,16 ± 0,01                | 0,15 ± 0,01   | —                    |
| Rg <sub>6</sub> | 765,6 [M-H] <sup>-</sup>        | 1,4 ± 0,1                  | 1,4 ± 0,1     | —                    |
| Rk <sub>3</sub> | 665,6 [M+HCOOH-H] <sup>-</sup>  | 0,58 ± 0,03                | 0,49 ± 0,03   | —                    |
| F <sub>4</sub>  | 765,6 [M-H] <sup>-</sup>        | 1,5 ± 0,1                  | 1,5 ± 0,1     | —                    |
| Rh <sub>4</sub> | 665,6 [M+HCOOH-H] <sup>-</sup>  | 2,0 ± 0,1                  | 2,1 ± 0,1     | —                    |
| Rk <sub>4</sub> | 765,6 [M-H] <sup>-</sup>        | 1,0 ± 0,1                  | 1,2 ± 0,1     | —                    |
| Rg <sub>5</sub> | 765,6 [M-H] <sup>-</sup>        | 2,0 ± 0,7                  | 2,3 ± 0,8     | —                    |

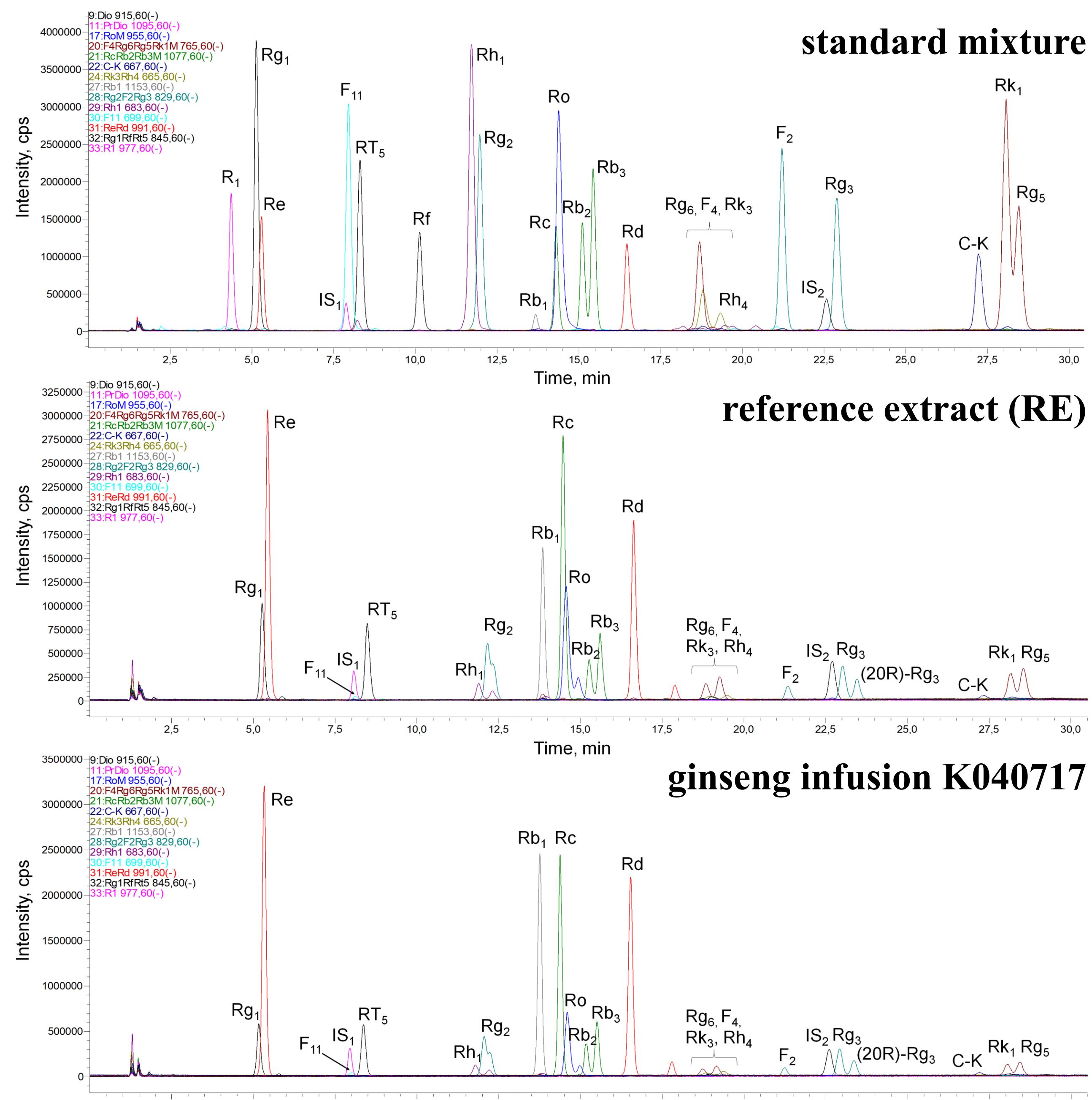
## Quantitative performance of proposed ESM and REM methods

| Ginsenoside     | Method | Linear range (ng/mL) | Calibration equation | Correlation coefficient, r <sup>2</sup> | Limit of detection, ng/mL | Limit of quantification, ng/mL |
|-----------------|--------|----------------------|----------------------|---|---------------------------|--------------------------------|
| R <sub>1</sub>  | ESM    | 4–2000               | y=165645x+262012     | 0,9994                                  | 1                         | 4                              |
|                 | REM    | 4–60*                | y=179572x+40747      | 0,9950                                  | 1                         | 4                              |
| Rg <sub>1</sub> | ESM    | 4–500                | y=275648x+767045     | 0,9990                                  | 1                         | 4                              |
|                 | REM    | 40–1000              | y=251980x+5156951    | 0,9983                                  | 7                         | 40                             |
| Re              | ESM    | 8–2000               | y=150492x+641810     | 0,9993                                  | 3                         | 8                              |
|                 | REM    | 90–3000              | y=136961x+9082332    | 0,9985                                  | 9                         | 90                             |
| RT <sub>5</sub> | ESM    | 4–2000               | y=239252x+334665     | 0,9993                                  | 1                         | 4                              |
|                 | REM    | 20–1500              | y=223866x+4005453    | 0,9972                                  | 5                         | 20                             |
| Rf              | ESM    | 4–2000               | y=152751x+417005     | 0,9993                                  | 1                         | 4                              |
|                 | REM    | —                    | —                    | —                                       | —                         | —                              |
| Rh <sub>1</sub> | ESM    | 4–2000               | y=250844x-194907     | 0,9993                                  | 1                         | 4                              |
|                 | REM    | 10–500               | y=237538x+1019358    | 0,9985                                  | 3                         | 10                             |
| Rg <sub>2</sub> | ESM    | 4–1000               | y=341901x-77504      | 0,9986                                  | 1                         | 4                              |
|                 | REM    | 20–1500              | y=329114x+4750657    | 0,9989                                  | 5                         | 20                             |
| F <sub>11</sub> | ESM    | 4–1000               | y=249772x+73837      | 0,9979                                  | 1                         | 4                              |
|                 | REM    | 10–100               | y=266329x+2388771    | 0,9867                                  | 3                         | 10                             |
| Rb <sub>1</sub> | ESM    | 8–2000               | y=53913x+242394      | 0,9990                                  | 2                         | 8                              |
|                 | REM    | 120–5000             | y=49095x+16125985    | 0,9954                                  | 15                        | 120                            |
| Rc              | ESM    | 4–1000               | y=94266x+40155       | 0,9990                                  | 1                         | 4                              |
|                 | REM    | 100–2800             | y=87776x+6732500     | 0,9987                                  | 9                         | 100                            |
| Rb <sub>2</sub> | ESM    | 4–1000               | y=120756x+119198     | 0,9970                                  | 1                         | 4                              |
|                 | REM    | 15–2800              | y=119557x+1434196    | 0,9954                                  | 4                         | 15                             |
| Rb <sub>3</sub> | ESM    | 4–1000               | y=103593x+79312      | 0,9970                                  | 1                         | 4                              |
|                 | REM    | 20–2800              | y=91598x+2054913     | 0,9975                                  | 5                         | 20                             |
| Rd              | ESM    | 20–2000              | y=157488x+1906387    | 0,9983                                  | 2                         | 8                              |
|                 | REM    | 90–1500              | y=150358x+8147432    | 0,9930                                  | 9                         | 90                             |
| Ro              | ESM    | 120–2000             | y=1603x+157168       | 0,9970                                  | 40                        | 120                            |
|                 | REM    | 60–2000              | y=1653x+264085       | 0,9953                                  | 5                         | 60                             |
| Rg <sub>3</sub> | ESM    | 4–700                | y=261681x+1335378    | 0,9990                                  | 1                         | 4                              |
|                 | REM    | 15–2000              | y=274059x-523459     | 0,9982                                  | 4                         | 15                             |
| F <sub>2</sub>  | ESM    | 4–700                | y=254767x+1679985    | 0,9994                                  | 1                         | 4                              |
|                 | REM    | 4–400                | y=275245x-136550     | 0,9987                                  | 1                         | 4                              |
| C-K             | ESM    | 4–700                | y=105959x-136268     | 0,9973                                  | 1                         | 4                              |
|                 | REM    | 15–150               | y=112460x-1315234    | 0,9960                                  | 4                         | 15                             |
| Rk <sub>3</sub> | ESM    | 20–2000              | y=64899x-3860        | 0,9980                                  | 6                         | 20                             |
|                 | REM    | 20–500               | y=69723x-158940      | 0,9976                                  | 5                         | 20                             |
| Rh <sub>4</sub> | ESM    | 40–2000              | y=11859x-69068       | 0,9980                                  | 10                        | 40                             |
|                 | REM    | 50–2000              | y=12598x-127589      | 0,9962                                  | 7                         | 50                             |
| F <sub>4</sub>  | ESM    | 6–1000               | y=122901x-464264     | 0,9984                                  | 2                         | 6                              |
|                 | REM    | 10–1500              | y=118756x-259842     | 0,9992                                  | 3                         | 10                             |
| Rg <sub>6</sub> | ESM    | 4–1000               | y=123081x-124251     | 0,9983                                  | 1                         | 4                              |
|                 | REM    | 10–1500              | y=128333x-507477     | 0,9987                                  | 3                         | 10                             |
| Rg <sub>5</sub> | ESM    | 4–700                | y=315293x+1985707    | 0,9972                                  | 1                         | 4                              |
|                 | REM    | 15–1200              | y=339844x-1918562    | 0,9981                                  | 4                         | 15                             |
| Rk <sub>1</sub> | ESM    | 8–700                | y=55766x+860032      | 0,9971                                  | 2                         | 8                              |
|                 | REM    | 15–1200              | y=65552x+116933      | 0,9972                                  | 4                         | 15                             |

**Ginseng infusion samples** were purchased from different drug stores and were manufactured by six pharmaceutical companies (Kamelia LLC, Ivanovskaya Pharmaceuticheskaya Fabrika OJSC, Medchimprom OJSC, Tulskaya Pharmaceuticheskaya Fabrika LLC, Vifitekh CJSC, and Yaroslavskaya Pharmaceuticheskaya Fabrika CJSC).

Composition: 100 g of ginseng root in 1 L ethanol:water (70:30, v:v).

**Materials and standards:** Pseudoginsenosides ASB-00031075-005, ChromaDex Int RT<sub>5</sub>, F<sub>11</sub> and Ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, R<sub>e</sub>, R<sub>d</sub>, Rg<sub>1</sub>, R<sub>f</sub>, R<sub>c</sub>, Rg<sub>2</sub>, Rg<sub>3</sub>, Rh<sub>1</sub>, Rh<sub>2</sub>, R<sub>l</sub>, and Compound K (>98%) were obtained from PhytoLab GmbH (Vestenbergsgreuth, Germany). Ginsenosides F<sub>1</sub>, F<sub>2</sub>, Ro and dry *P. quinquefolius* extract (extract reference material, XRM ASB-00031075-005) were from ChromaDex Int. (Irvine, CA, USA).



| Ginsenoside | Reference extract (100 times dilution) | Infusion K050317 (500 times dilution) | | Infusion M010216 (500 times dilution) | | Precision (RSD, %, n = 6) | | Stability (RSD, %, n = 6) |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ME\*, % (80 ng/mL) | ME, % (400 ng/mL) | ME, % (80 ng/mL) | ME, % (400 ng/mL) | Intra-day | | Inter-day | |
| SS | RE | SS |