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Supporting Information

Plasma Levels and Distribution of Flavonoids in Rat Brain after Single and Repeated Doses of Standardized *Ginkgo biloba* Extract EGb 761®

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Method validation

Results of the method validation for brain and plasma samples are summarized in **Table 1S**.

Specificity

Blank samples of brain and plasma did not show any signal at the retention times for quercetin, kaempferol, and isorhamnetin, supporting the specificity of the developed method for both types of matrixes (see **Figure 9**).

Linearity

The coefficients of determination (R^2) of the plasma or brain response versus concentration curves (n=3) were determinate and statistically analyzed. The values were all above 0.99 for the three aglycones, indicating linearity over the calibration range of 0.125-250 ng/mL for kaempferol and isorhamnetin in both plasma and brain, and for quercetin over the calibration range of 0.125-500 ng/mL in plasma and 2.5-500 ng/mL in brain.

Lowest limit of quantification

In plasma, the lowest limit of quantification (LLOQ), defined as the lowest concentration of analytes measured with acceptable accuracy and precision (R.S.D. ≤ 15%), was 0.125 ng/mL for the three aglycones, quercetin, kaempferol, and isorhamnetin. In brain tissue, the LLOQ was found to be 2.5 ng/mL for quercetin and 0.125 ng/mL for kaempferol and isorhamnetin.

Recovery

The relative recovery in the linear concentration range was evaluated by comparing the response of the brain and plasma samples spiked before extraction with the response of the brain and plasma samples spiked at the same nominal concentration after extraction. Curves of the recovered amount of spiked samples in function of the nominal concentration were

generated for each analyte (n = 3). The slopes of these curves represent the fractions of the recovered analyte (mean recovery) in the linear concentration range. The recovery over the whole concentration range was constant in all cases, justifying therefore the use of calibration curves for quantification.

Accuracy and precision

For the evaluation of accuracy curves of the obtained mean, concentrations were plotted against the respective nominal values for each analyte over the whole dynamic range and the slopes and intercepts were statistically analized (n=3). If accuracy is given, the slope of this plot must be equal to the unit (admitted null hypothesis H_o). For the three investigated aglycones, the null hypothesis was true at a confidence level of 95% [39], verifying thus the accuracy of the method. In addition, the intercept was statistically analyzed, and no difference with zero was found, which means that the method does not present systematic errors.

The precision was evaluated by determining both: the reproducibility, which is the closeness of agreement among the results obtained with the same method under different conditions, and the repeatability, which refers to the agreement among successive measurements using the same sample. The reproducibility was expressed as the RSD calculated for three calibration curves in the whole dynamic range obtained on three consecutive days for each analyte and was under 15% for the three aglycones. Repeatability was evaluated by calculating the RSD. of three spiked samples extracted independently at the concentration levels in the whole dynamic range and analyzed under the same conditions on the same day. The values for the three analytes were also under 15%. All values obtained for repeatability and reproducibility were within the limits recommended for bioanalytical methods.

Stability

Stability studies indicated that analytes are stable in plasma and brain in processed samples over a period of 48 hours at room temperature and for at least two weeks at -20 °C after one and two freeze-thaw cycles.

Table 1S Validation criteria for the determination of quercetin, kaempferol, and isorhamnetin in brain and plasma matrixes

	Brain			Plasma		
	Quercetin	Kaempferol	Isorhamnetin	Quercetin	Kaempferol	Isorhamnetin
Specificity	Yes	Yes	Yes	Yes	Yes	Yes
Linear Range (ng/mL)[a]	2.5 - 500	0.125 - 250	0.125 - 250	0.125 - 500	0.125 - 250	0.125 - 250
LLOQ (ng/mL)	2.5	0.125	0.125	0.125	0.125	0.125
Recovery	78.6%	73.2%	69.1%	82.0%	98.5%	98.2%
Accuracy	99.7%	99.9%	99.5%	95.7%	99.6%	99.0%
Precision (Repeatability)[b]	7.0%	7.0%	6.4%	1.4%	6.2%	8.4%
Precision (Reproducibility)[b]	9.3%	9.4%	7.3%	9.2%	10.3%	12.7%
Stability	>48 h	>48 h	>48 h	>48 h	>48 h	>48 h
Freeze-Thaw cycles	2	2	2	2	2	2

[a]The coefficients of determination were $R^2 > 0.99$ for all the analytes in both matrixes.

[b]Values expressed as relative standard deviation (RSD).

Table 2S Distribution of *Ginkgo* flavonoid conjugates, determined as corresponding aglycones, in rat brain after eight daily doses of 600 mg/kg or 100 mg/kg of EGb 761®[a]

	Quercetin		Kaempferol		Isorhamnetin	
	100 mg/kg	600 mg/kg	100 mg/kg	600 mg/kg	100 mg/kg	600 mg/kg
Hippocampus (H)	< LLOQ	1300	27	1112	1154	1941
Striatum (S)	< LLOQ	1167	< LLOQ	1278	1298	1986
Frontal cortex (FC)	< LLOQ	1233	398	1089	1235	1874
Cerebellum (C)	< LLOQ	1180	< LLOQ	974	1259	2008
Rest (R)	< LLOQ	66	203	287	199	263

[a] Values are expressed as amount of the respective aglycone per weight of brain protein (ng/g). Each value represents the mean of six samples per brain region.