AOAC SMPR 2013.001

Standard Method Performance Requirements for Hypericins, Hyperforins, and Flavonoids in St. John's Wort (*Hypericum perforatum*) and Other *Hypericum* spp.

1 Applicability

Determination of hypericins, and/or hyperforins, and/or flavonoids [According to Herbal Drugs and Phytopharmaceuticals (3rd Ed.), the main flavonoids in St. John's wort are hyperoside, rutoside, and the biflavones I3, II8-biapigenin, and amentoflavone. Quercetin is also present. (http://www.ncbi.nlm.nih.gov/pubmed/11842341)] in St. John's wort (*Hypericum perforatum*) and other *Hypericum* spp. in powdered extracts, tablets, hard-shell capsules, and liquid alcohol extracts.

2 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable. It is acceptable to have a different analytical method for each class of analytes.

3 Definitions

Limit of quantitation (LOQ).—The minimum analyte concentration for which quantitative results may be obtained with 95% confidence.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r), or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R) or % reproducibility relative standard deviation (%RSD_R).

Recovery.—The fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range, and a protocol to demonstrate suitability.

6 Reference Material(s)

Use an appropriate Certified Reference Material (CRM) where available.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*^{SM}.

8 Maximum Time-to-Result

Analysis time must be less than the established stability time of the analytes in solution.

Approved by the AOAC Stakeholder Panel on Strategic Foods Analytical Methods (SPSFAM). Final Version Date: March 13, 2013. Effective Date: March 14, 2013.

Table 1. Method performance requirements

Performance parameter		Target analyte		
		Hypercin	Hyperforin	Flavonoids
Analytical range[a], %		0.05–1	0.05–10	0.05–10
Limit of quantitation (LOQ)[a], %		≤0.02	≤0.02	≤0.02
Repeatability (RSD_r), %	0.05 to ≤1	≤5	≤5	≤5
	1 to ≤5	NA	≤3	≤3
	5 to ≤10	NA	≤3	≤3
Recovery[a], %	0.05 to ≤1	95–105	95–105	95–105
	1 to ≤5	NA	97–103	97–103
	5 to ≤10	NA	98–102	98–102
Reproducibility (RSD_R), %[b]	0.05 to ≤1	≤8	≤8	≤8
	1 to ≤5	NA	≤5	≤5
	5 to ≤10	NA	≤4	≤4

[a] % (w/w) for the starting material prior to sample preparation.

[b] RSD_R calculated as 1.2*$PRSD_R$ where $PRSD_R = 2C^{-0.15}$, where C is the mass fraction of the lower limit of each range, i.e., C = 0.0005 for the 0.05 to <1% range. $PRSD_R$ is the predicted relative standard deviation. Information on the $PRSD_R$ can be found in Annex D of Appendix F: Guidelines for Standard Method Performance Requirements in the *Official Methods of Analysis of AOAC INTERNATIONAL*, 19th Ed. (2012).