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Standard Method Performance Requirements **(SMPRs) for Characterization and Quantitation of Neutral Components from Glycerol Esters of Wood Rosin (GEWR)**

Intended Use: Global reference method

1. Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are drafted by AOAC working groups composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. Approved by AOAC, AOAC SMPRs may be used for method development and are used by AOAC expert review panels in their evaluation of validation study data for method being considered for *Performance Tested Methods*^{SM} or AOAC *Official Methods of Analysis*^{SM} and can be used as acceptance criteria for verification at user laboratories.

2. Applicability

Characterization and quantitation of neutral components of glycerol esters of wood rosin (GEWR) from *Pinus halepensis*, *Pinus brutia*, *Pinus palustris*, and *Pinus elliottii* (and potentially other pine species).

3. Analytical Technique

A GC method, which could also be performed by GC × GC, that measures the analyte of interest and meets the following method performance requirements is/are acceptable. The most common analytes are listed in Table 2, but all peaks of neutral components present above the LOQ in the final analysis must be identified and quantitated.

4. Definitions

Limit of quantitation (LOQ).—LOQ is the lowest level of analyte in a test sample that can be quantified at a specified level of precision.

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

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratory by different operators on different instruments. 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 analyte that is measured when the test sample is analyzed using the entire method.

5. Method Performance Requirements

Table 1. Method Performance Table for Neutral Components from GEWR

	Monoterpene and sesquiterpene neutrals	Diterpene and high MW neutrals
Limit of quantitation (LOQ)	0.005%[a]	0.01%[a]
Analytical range	0.005–1%[a]	0.01–1%[a]
Recovery	80–110%	90–107%
Repeatability (RSD_r)	6%	5.3%
Reproducibility (RSD_R)	8.8%	8%

[a]Percent refers to the concentration of a molecule in the GEWR product, e.g., 1% = 0.01 g molecule X per g GEWR.

6. System Suitability Tests and/or Analytical Quality Control

(a) Suitable methods will include blanks and appropriate check standards.
(b) Retention time should be stable to ±1%.
(c) Method developer should provide proof of identity and purity of peaks, preferably by MS. NIST Mass Spectral Library can be helpful.
(d) Method developer should explain how response factors are estimated when a direct standard is not available.
(e) Purity of the internal standard should be >95%.

7. Validation Guidance

Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www. eoma.aoac.org/app_f.pdf)

Validation studies should include at least 4 GEWR products produced from different pine species to include, but not be limited to, *Pinus halepensis*, *Pinus brutia*, *Pinus palustris*, and *Pinus elliottii*.

8. Reference materials

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

9. Maximum Time-to-Results

None.

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Common Name	CAS Number	IUPAC Name
Monoterpenes		
alpha-pinene	80-56-8	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl
beta-pinene	127-91-3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene
limonene (dipentene)	138-86-3	Cyclohexene, 1-methyl-4-(1-methylethenyl)
beta-phellandrene	555-10-2	Cyclohexene, 3-methylene-6-(1-methylethyl)
trans-dihydro-alpha-terpineol	5114-00-1	Trans- α , α ,4-trimethylcyclohexanemethanol
cis-dihydro-alpha-terpineol	7322-63-6	Cis- α , α ,4-trimethylcyclohexanemethanol
beta-terpineol	138-87-4	Cyclohexanol, 1-methyl-4-(1-methylethenyl)
estragole (methyl chavicol)	140-67-0	Benzene, 1-methoxy-4-(2-propen-1-yl)-
alpha-terpineol	98-55-5	3-Cyclohexene-1-methanol,α,α,4-trimethyl-
borneol	507-70-0	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1R,2S,4R)-rel
camphene	79-92-5	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene
para-cymene	99-87-6	Benzene, 1-methyl-4-(1-methylethyl)
terpinolene	586-62-9	Cyclohexene, 1-methyl-4-(1-methylethylidene)
allocimene	673-84-7	2,4,6-Octatriene, 2,6-dimethyl
Sesquiterpenes		
longifolene	475-20-7	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)
caryophyllene	87-44-5	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (1R,4E,9S)
Diterpenes		
pimaradiene	1686-56-2	(4*aS*,4*bS*,7*S*,10*aS*)-7-ethenyl-1,1,4*a*,7-tetramethyl-3,4,4*b*,5,6,9,10,10*a*-octahydro-2*H*-phenanthrene
palustridiene	41577-36-0	(4*aS*,10*aS*)-1,1,4*a*-trimethyl-7-propan-2-yl-2,3,4,5,6,9,10,10*a*-octahydrophenanthrene
dehydroabietane	19407-28-4	(4*aS*,10*aS*)-1,1,4*a*-trimethyl-7-propan-2-yl-2,3,4,9,10,10*a*-hexahydrophenanthrene
abietadiene	35241-40-8	(4*aS*,4*bR*,10*aS*)-1,1,4*a*-trimethyl-7-propan-2-yl-2,3,4,4*b*,5,6,10,10*a*-octahydrophenanthrene
pimarinal (pimaral)	472-39-9	(1*R*,4*aR*,4*bS*,7*S*,10*aR*)-7-ethenyl-1,4*a*,7-trimethyl-3,4,4*b*,5,6,9,10,10*a*-octahydro-2*H*-phenanthrene-1-carbaldehyde
palustrinal (palustral)	13508-03-7	1,4*a*-dimethyl-7-propan-2-yl-2,3,4,5,6,9,10,10*a*-octahydrophenanthrene-1-carbaldehyde
isopimarinal (isopimaral)	N/A	
dehydroabietinol (dehydroabietol)	3772-55-2	[(1*R*,4*aS*,10*aR*)-1,4*a*-dimethyl-7-propan-2-yl-2,3,4,9,10,10*a*-hexahydrophenanthren-1-yl]methanol
palustrinol (palustral)	21414-53-9	(1,4*a*-dimethyl-7-propan-2-yl-2,3,4,5,6,9,10,10*a*-octahydrophenanthren-1-yl)methanol
elliotinal	N/A	

abietinal (abietal)	6704-50-3	(1*R*,4*aR*,4*bR*,10*aR*)-1,4*a*-dimethyl-7-propan-2-yl-2,3,4,4*b*,5,6,10,10*a*-octahydrophenanthrene-1-carbaldehyde
neoabietinal (neoabietal)	19898-57-8	(1*R*,4*aR*,4*bS*,10*aR*)-1,4*a*-dimethyl-7-propan-2-ylidene-3,4,4*b*,5,6,9,10,10*a*-octahydro-2*H*-phenanthrene-1-carbaldehyde
pimarinol (pimarol)	1686-59-5	[(1*R*,4*aR*,4*bS*,7*S*,10*aR*)-7-ethenyl-1,4*a*,7-trimethyl-3,4,4*b*,5,6,9,10,10*a*-octahydro-2*H*-phenanthren-1-yl]methanol
sandaracopimarinol (sandaracopimarol)	24563-84-6	[(1*R*,4*aR*,4*bS*,7*R*,10*aR*)-7-ethenyl-1,4*a*,7-trimethyl-3,4,4*b*,5,6,9,10,10*a*-octahydro-2*H*-phenanthren-1-yl]methanol
isopimarinol (isopimarol)	1686-64-2	[(1*R*,4*aR*,4*bS*,7*S*,10*aR*)-7-ethenyl-1,4*a*,7-trimethyl-3,4,4*b*,5,6,8,10,10*a*-octahydro-2*H*-phenanthren-1-yl]methanol
elliotinol (communol)	10178-31-1	[(1*S*,4*aR*,5*S*,8*aR*)-1,4*a*-dimethyl-6-methylidene-5-[(2*E*)-3-methylpenta-2,4-dienyl]-3,4,5,7,8,8*a*-hexahydro-2*H*-naphthalen-1-yl]methanol
abietinol (abietol)	666-84-2	[(1*R*,4*aR*,4*bR*,10*aR*)-1,4*a*-dimethyl-7-propan-2-yl-2,3,4,4*b*,5,6,10,10*a*-octahydrophenanthren-1-yl]methanol
neoabietinol (neoabietol)	640-42-6	[(1*R*,4*aR*,4*bS*,10*aR*)-1,4*a*-dimethyl-7-propan-2-ylidene-3,4,4*b*,5,6,9,10,10*a*-octahydro-2*H*-phenanthren-1-yl]methanol
Other		
3,5-dimethoxystilbene	21956-56-9	1,3-dimethoxy-5-[(*E*)-2-phenylethenyl]benzene
beta-sitosterol	83-46-5	(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol
campesterol	474-62-4	(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol