AOAC SMPR® 2020.014

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 industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. Approved by AOAC, AOAC SMPRs may be used for method development, are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠, 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). The most common analytes are listed in Table 2. The list is not exhaustive and not all compounds on the list may be present in all GEWR products. All compounds detected above the limit of quantitation (LOQ) must be identified and quantitated.

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.

4 Definitions

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

Recovery.—Fraction or percentage of analyte that is measured when the test sample is analyzed using the entire method.

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 repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratories by different operators on different instruments. The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

5 Method Performance Requirements

See Table 1.

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* (2019) *Official Methods of Analysis of AOAC INTERNATIONAL,* 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www. eoma.aoac.org/app_f.pdf)

Validation studies should include at least four 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* (2019) *Official Methods of Analysis of AOAC INTERNATIONAL,* 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www. eoma.aoac.org/app_f.pdf)

9 Maximum Time-to-Results

None.

Developed by AOAC Working Group on Glycerol Esters of Wood Rosins (GEWR). Approved by AOAC GEWR stakeholders on December 15, 2020.

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Table 1. Method performance for neutral components from GEWR

	Monoterpene and sesquiterpene neutrals	Diterpene and high MW neutrals
LOQ, %	0.005[a]	0.01[a]
Analytical range, %	0.005–1[a]	0.01–1[a]
Recovery, %	80–110	90–107
RSD_r, %	6	5.3
RSD_R, %	8.8	8

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

Table 2. Potential GEWR neutral components from EFSA 2018 opinion

Possible Neutral Components by Class	Molecular Weight (Monoisotopic)	Repeating Molecular Weight (a)	Component Type	Expected SPE Fraction (b)
Monoterpene Neutrals (c)				
p-Cymene	134.1096		Monoterpene hydrocarbon	D
α-Pinene	136.1252	a	Monoterpene hydrocarbon	D
Limonene	136.1252	a	Monoterpene hydrocarbon	D
Methyl chavicol (estragole)	148.0888		Monoterpene hydrocarbon	D
Borneol	154.1358	b	Monoterpene alcohol	F, G
α-Terpineol	154.1358	b	Monoterpene alcohol	F, G
trans -Dihydro α terpineol	156.1514	c	Monoterpene alcohol	F, G
cis -Dihydro-α-terpineol	156.1514	c	Monoterpene alcohol	F, G
Longifolene (d)	204.1878		Sesquiterpene	D
Diterpene Neutrals				
Dehydrodehydroabietane	266.2035		Diterpene hydrocarbon	D
Dehydroabietane	268.2191		Diterpene hydrocarbon	D
Abietadiene	270.2348	d	Diterpene hydrocarbon	D
Palustradiene	270.2348	d	Diterpene hydrocarbon	D
Pimaradiene	270.2348	d	Diterpene hydrocarbon	D
Dihydroabietane	272.2504		Diterpene hydrocarbon	D
Methyl Dehydrodehydroabietate	312.2089		Methyl ester of rosin	D
Methyl Dehydroabietate	314.2246		Methyl ester of rosin	D
Methyl Abietate	316.2402		Methyl ester of rosin	D
Methyl Dihydroabietate	318.2559		Methyl ester of rosin	D
Dehydrodehydroabietal	282.1984		Diterpene aldehyde	F
Dehydroabietal	284.2140	e	Diterpene aldehyde	E
Abietal	286.2297	f	Diterpene aldehyde	E
Communal	286.2297	f	Diterpene aldehyde	E
Isopimaral	286.2297	f	Diterpene aldehyde	E
Neoabietal	286.2297	f	Diterpene aldehyde	E
Palustral	286.2297	f	Diterpene aldehyde	E
Pimaral	286.2297	f	Diterpene aldehyde	E
Dihydropimaral	288.2453	g	Diterpene aldehyde	E
18-Nordehydrodehydroabietol	270.1984		Diterpene nor-alcohol	F, G
18-Nordehydroabietol	272.2140		Diterpene nor-alcohol	F, G
18-Norabietol	274.2297	h	Diterpene nor-alcohol	F, G
18-Norisopimarol	274.2297	h	Diterpene nor-alcohol	F, G
18-Norpalustrol	274.2297	h	Diterpene nor-alcohol	F, G
18-Norpimarol	274.2297	h	Diterpene nor-alcohol	F, G
18-Nordihydroabietol	276.2453		Diterpene nor-alcohol	F, G
Dehydrodehydroabietol	284.2140	e	Diterpene alcohol	F, G
Dehydroabietol	286.2297	f	Diterpene alcohol	F, G
Abietol	288.2453	g	Diterpene alcohol	F, G
Communol	288.2453	g	Diterpene alcohol	F, G
Isopimarol	288.2453	g	Diterpene alcohol	F, G
Neoabietol	288.2453	g	Diterpene alcohol	F, G
Palustrol	288.2453	g	Diterpene alcohol	F, G
Pimarol	288.2453	g	Diterpene alcohol	F, G
Sandaracopimarol	288.2453	g	Diterpene alcohol	F, G
Dihydroabietol	290.2610		Diterpene alcohol	F, G
Rosin Diol of Dehydroabietic Acid	302.2246		Diterpene di-alcohol	G
Rosin Diol of Abietic Acid	304.2402		Diterpene di alcohol	G
High Molecular Weight Neutrals				
Dehydroabietic Acid Ester of Borneol	436.3341		Monoterpene alcohol ester of rosin (e)	D, E
Abietic Acid Ester of Borneol	438.3498	i	Monoterpene alcohol ester of rosin	D, E
Isopimaric Acid Ester of Borneol	438.3498	i	Monoterpene alcohol ester of rosin	D, E
Palustric Acid Ester of Borneol	438.3498	i	Monoterpene alcohol ester of rosin	D, E
Pimaric Acid Ester of Norneol	438.3498	i	Monoterpene alcohol ester of rosin	D, E
Dihydroabietic Acid Ester of Borneol	440.3654		Monoterpene alcohol ester of rosin	D, E
Dehydroabietic Acid + Linoleyl Alcohol (18:2)	548.4593		Fatty alcohol ester of rosin (f)	D, E
Dehydroabietic Acid + Oleyl Alcohol (18:1)	550.4750	j	Fatty alcohol ester of rosin	D, E
Dehydroabietic Acid + Stearyl Alcohol (18:0)	552.4906	k	Fatty alcohol ester of rosin	D, E
Abietic Acid + Linoleyl Alcohol (18:2)	550.4750	j	Fatty alcohol ester of rosin	D, E
Abietic Acid + Oleyl Alcohol (18:1)	552.4906	k	Fatty alcohol ester of rosin	D, E
Abietic Acid + Stearyl Alcohol (18:0)	554.5063		Fatty alcohol ester of rosin	D, E
Dehydroabietic Acid + Dehydroabietol	568.4280	l	Diterpene alcohol ester of rosin (g)	D, E
Dehydroabietic Acid + Abietol	570.4437	m	Diterpene alcohol ester of rosin	D, E
Dehydroabietic Acid + Dihydroabietol	572.4593	n	Diterpene alcohol ester of rosin	D, E
Abietic Acid + Dehydrodehydroabietol	568.4280	l	Diterpene alcohol ester of rosin	D, E
Abietic Acid + Dehydroabietol	570.4437	m	Diterpene alcohol ester of rosin	D, E
Abietic Acid + Abietol	572.4593	n	Diterpene alcohol ester of rosin	D, E

(a) Repeating molecular weights (monoisotopic masses) are indicated by letter.
(b) Fractions from solid-phase extraction (SPE) procedure discussed in Section 3 of this report.
(c) Trace quantities of related monoterpenes not listed here may also be present in rosin.
(d) A sesquiterpene ($C_{15}H_{24}$) - others also possible.
(e) Other resin acid isomers and monoterpene alcohols may also react to form esters - these are representative of this group.
(f) Other resin acid isomers and fatty alcohols may also react to form esters - these are representative of this group.
(g) Other resin acid isomers and diterpene alcohols may also react to form esters - these are representative of this group.