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QUALITATIVE AND QUANTITATIVE ANALYSIS OF DITERPENE RESIN ACIDS BY GLASS CAPILLARY GAS–LIQUID CHROMATOGRAPHY*

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SUMMARY

Wall-coated open-tubular glass capillary columns are useful for the qualitative gas–liquid chromatographic analysis of diterpene resin acid methyl ester mixtures. The elution characteristics of 77 compounds on six liquid stationary phases (Silar 10C, BDS, SP-2330, SP-1000, SE-54 and SE-30) are given. For most applications, two stationary phases, one polar and the other non-polar, will suffice. The reproducibility of quantitative data was found to be unacceptable when typical size samples were injected into an inlet splitter by an automatic injector. However, by injecting large samples (over 20 ng per component on to the column) reliable quantitative data are obtainable.

INTRODUCTION

The gas–liquid chromatography (GLC) of diterpene resin acid methyl esters is an important procedure in the study of extractives, oleoresin, rosin and tall oil. Although the esters can range in molecular weight from about 300 to 375 daltons, most of those found in pines and other conifers are related isomers within a molecular weight range of 10 daltons. Separation of many of the isomers by packed column GLC has been achieved with polar liquid stationary phases. Retention data for a large number of diterpene resin acid methyl esters on DEGS and SE-30/EGiP packed columns have been reported as part of a compilation of spectral data[1]. More recent developments in GLC of resin acid esters with packed columns have been reviewed by Zinkel and Engler[2].

The use of wall-coated open-tubular (WCOT) capillary columns for the analysis of resin acids has been attempted by several workers. In 1965, Sandermann and Weissman[3] used stainless-steel WCOT columns coated with BDS and with SE-30 for the GLC of resin acid methyl esters. Although no chromatographic conditions were

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given, one illustration (apparently for a BDS column) showed a resolution that was no better than for a 6-ft. packed column. Partial resolution of the difficult palustrate–levopimarate pair was demonstrated by Weissmann and Simatupang[4] in an analysis of an Indonesian rosin on a 50-m stainless-steel ethylene glycol phthalate WCOT column. From their published chromatogram it can be estimated that the column gave about 20,000 theoretical plates. Hasson and Kulkarni[5] used stainless-steel WCOT columns coated with Apiezon N and with Versamid 930 for analysis of rosin fluxes. Palustrate and isopimarate were resolved on Versamid 930 but not on Apiezon N. The palustrate–levopimarate separation was not considered because levopimarate is not a component of rosin flux; however, levopimarate is extensively decomposed on packed Versamid columns. Claeys[6] used stainless-steel Silar 10C WCOT columns for the analysis of resin acids in kraft mill effluent but did not address the problem of on-column isomerization of abietadienoic acid esters such as methyl palustrate and methyl levopimarate.

Retention data of several resin acids from tall oil have been reported by Holmbom et al.[7] for BDS and Apiezon L coated stainless-steel WCOT columns and for OV-101 (a methyl silicone) and SP-1000 (a substituted polyethylene glycol terephthalate) on glass WCOT columns. Holmbom[8] later included glass WCOT columns coated with BDS, Apiezon L, SE-30 and Silar 10C. Complete palustrate–levopimarate resolution was not achieved even with the Silar 10C column, and high on-column losses of levopimarate are evident in the data. Recently, Mayr et al.[9] described the chromatography of mixtures of resin acids on glass WCOT columns by temperature programming. FFAP (free fatty acid phase) was shown to separate methyl palustrate and methyl levopimarate; however, methyl isopimarate was not well resolved from methyl palustrate.

All of these attempts to use WCOT capillary columns for the analysis of resin acids have had limited objectives. This work has determined the retention characteristics of a large number of resin acid methyl esters for several columns coated with stationary phases spanning the range of available polarities. This will facilitate selection of columns appropriate for various applications. The quantitative reproducibility of WCOT glass capillary column GLC was evaluated using the response factors for the resin acids common in pines.

EXPERIMENTAL

A Hewlett-Packard Model 5840A gas chromatograph equipped with an 18835B inlet splitter and a flame-ionization detector was used. Retention times and areas of peaks were measured through the data system of the gas chromatograph. WCOT glass capillary columns were obtained from the following sources: SE-30, SE-54 and SP-1000 (a Carbowax derivative similar to FFAP) from J&W Scientific. (Orangevale, CA, U.S.A.), BDS (butane-1,4-diol succinate) and Silar 10C from Altech Assoc. (Deerfield, IL, U.S.A.) and SP-2330 (68% cyanopropyl silicone) from Supelco (Bellefonte, PA, U.S.A.). The helium carrier gas flow-rate was set at ca. 30 cm/sec. Column temperature (see footnotes in Table I) was optimized to resolve the commonly found pine resin acid methyl esters (resolution of the palustrate–levopimarate pair was the criterion) while maintaining reasonable analysis times (elution of methyl pimarate in less than 20 min). For the determination of response factors, resin acid

methyl esters were purified by the rigorous methods required in preparing reference standards[1].

RESULTS AND DISCUSSION

Qualitative GLC with WCOT glass capillary columns

Nearly all of the resin acids derived from pine and other conifers belong to four basic diterpene skeletal classes: abietane, pimarane, isopimarane and labdane (Fig. 1). This classification by skeletal types has been used to organize the retention characteristics of 77 resin acid esters as obtained with six stationary phases (Silar 10C, BDS, SP-2330, SP-1000, SE-54 and SE-30; see Table I). These stationary phases were chosen to span the range of liquid phase polarity available. In practice we find that a polar and a non-polar stationary phase are adequate for most applications. Either stationary phase can resolve all the components commonly found in pines, but the two phases are complementary. The polar stationary phase provides good resolution of the common resin acids, but the analysis time may extend to several hours when oxygenated derivatives are present. When oxygenated components are encountered, the non-polar stationary phase reduces analysis times to within reasonable limits. As discussed below, the non-polar SE-30 will resolve all the common resin acids if the
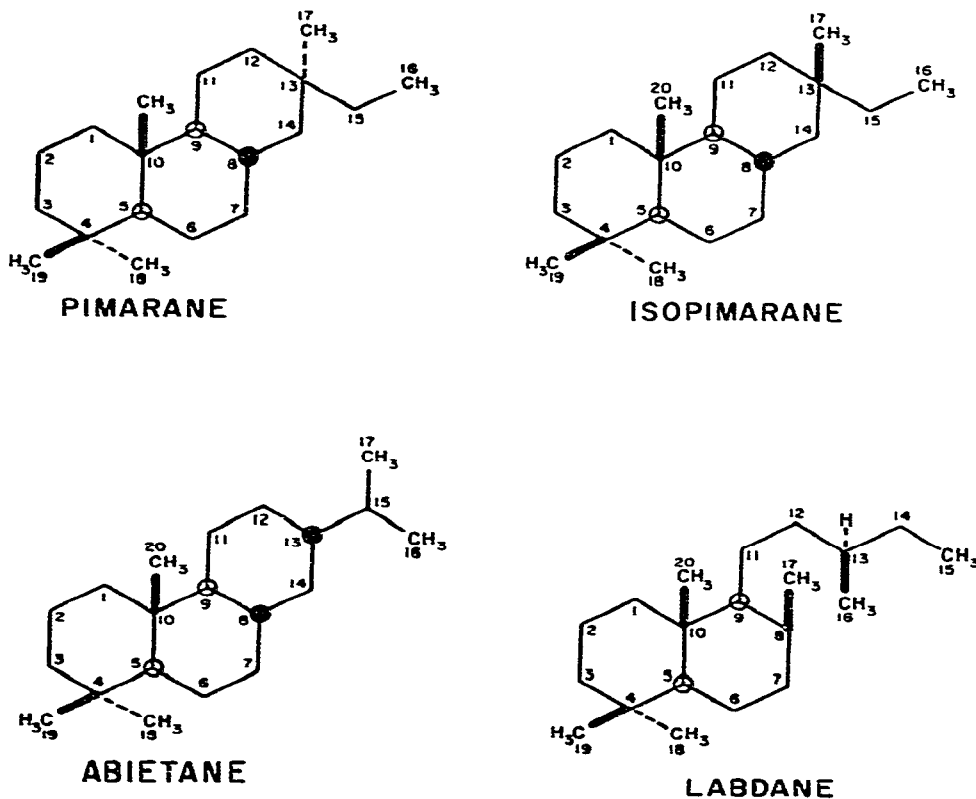

Fig. 1. Parent hydrocarbon structures for common diterpene resin acids (for nomenclature, see refs. 10 and 11).

TABLE I

RETENTION CHARACTERISTICS OF DITERPENE RESIN ACID METHYL ESTERS ON GLASS CAPILLARY GLC COLUMNS

Retentions relative to methyl pimarate.

Systematic name*	Common name	Silar 10C** (230°C)	BDS*** (190°C)	SP2330 † (190°C)	SP1000 †† (200°C)	SE-54 ††† (190°C)	SE-30 † (190°C)	SE-30 †† (170°C)
Abietane skeleton;								
18-Abietanonate		1.041	1.111	1.098	1.119	1.309	1.319	1.373
13β-Abietan-18-oate		1.012	0.996	1.039	0.986	1.183	1.195	1.212
8α,13β-Abietan-18-oate		1.096	1.202	1.240	1.206	1.379	1.377	1.418
9β,13β-Abietan-18-oate		1.044	1.081	1.087	1.090	1.264	1.281	1.313
7-Abietan-18-oate		1.387	1.551	1.528	1.520	1.508	1.477	1.544
13β-Abiet-7-en-18-oate		1.329	1.389	1.409	1.362	1.381	1.349	1.392
8-Abieten-18-oate		1.026	1.133	1.181	1.141	1.249	1.242	1.287
13β-Abiet-8-en-18-oate		1.082	1.220	1.188	1.229	1.334	1.323	1.371
8(14)-Abieten-18-oate		1.094	1.162	1.158	1.180	1.297	1.300	1.335
13β-Abiet-8(14)-en-18-oate		1.172	1.187	1.217	1.190	1.292	1.287	1.316
13-Abieten-18-oate		1.031	1.123	1.090	1.134	1.211	1.211	1.234
13(15)-Abieten-18-oate		1.431	1.565	1.583	1.560	1.556	1.504	1.570
7,13,15-Abietatrien-18-oate	Abietate	1.897	2.182	2.155	2.071	1.697	1.603	1.703
8,12-Abietadien-18-oate		1.506	1.792	1.768	1.779	1.564	1.476	1.581
8,13-Abietadien-18-oate	Palustrate	1.252	1.396	1.397	1.379	1.287	1.247	1.301
8,13(15)-Abietadien-18-oate		1.597	1.938	1.870	1.932	1.719	1.638	1.760
8(14),13(15)-Abietadien-18-oate	Neoabietate	2.225	2.505	2.571	2.469	2.036	1.892	2.032
13β-Abietu-7,9(11)-dien-18-oate		1.450	1.654	1.637	1.622	1.432	1.368	1.438
8(14),12-Abietadien-18-oate	Levopimarate	1.320	1.354	1.421	1.352	1.282	1.242	1.279
8,11,13-Abietatrien-18-oate	Dehydroabietate	2.117	2.274	2.447	2.098	1.506	1.362	1.439
8,11,13-Abietatrien-19-oate	Callitrisate	1.734	1.808	1.944	1.680	1.303	1.216	1.260
5β-Abieta-8,11,13-trien-18-oate		1.321	1.158	1.358	1.080	0.923	0.885	0.882
5β-Abieta-8,11,13-trien-19-oate		1.539	1.605	1.740	1.511	1.195	1.119	1.164
7(13),15-Abietatrien-18-oate		3.840	4.308	4.829	3.864	2.150	1.866	2.047
6,8,11,13-Abietatetraen-18-oate		2.499	2.662	2.959	2.421	1.501	1.326	1.414
8,11,13,15-Abietatetraen-18-oate		3.884	4.299	4.842	3.863	2.152	1.889	2.045
20-nor-Abieta-5,7,9-trien-18-oate		2.566	2.754	3.139	2.531	1.570	1.358	1.445
12α-Methoxy-7,13-abietadien-18-oate	12-Methoxyabietate	3.414	4.108	4.398	3.689	2.323	2.342	2.660
12α-Acetoxy-7,13-abietadiene-18-oate		-	7.91	8.85	6.98	3.950	3.370	3.957
7-Oxo-8,11,13-abietatrien-18-oate		12.91	12.20	17.86	9.36	3.726	2.829	3.217

Pimarane skeleton:

Compound		1	2	3	4	5	6	7
18-Pimaranoate		0.930	0.912	0.944	0.903	1.087	1.109	1.117
8α-Pimaran-18-oate		1.183	1.171	1.224	1.156	1.309	1.317	1.344
8-Pimaren-18-oate		0.893	0.900	0.929	0.902	1.039	1.045	1.061
8(14)-Pimaren-18-oate	Dihydropimarate	0.946	0.953	0.959	0.962	1.097	1.115	1.160
8,15-Pimaradien-18-oate		0.990	0.981	1.016	0.968	0.966	0.953	0.961
8(14),15-Pimaradien-18-oate	Pimarate	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Isopimarane skeleton:

Compound		1	2	3	4	5	6	7
18-Isopimaranoate		0.998	1.012	1.024	1.000	1.179	1.322	1.308
8α-Isopimaran-18-oate		1.154	1.130	1.177	1.115	1.275	1.286	1.223
7-Isopimaren-18-oate	Dihydroisopimarate	1.293	1.350	1.370	1.327	1.341	1.319	1.366
8-Isopimaren-18-oate		0.879	0.874	0.906	0.891	1.013	1.015	1.032
8(14)-Isopimaren-18-oate		0.966	0.988	0.988	1.004	1.129	1.145	1.159
7,15-Isopimaradien-18-oate	Isopimarate	1.426	1.470	1.500	1.406	1.241	1.201	1.230
8,15-Isopimaradien-18-oate		0.913	0.889	0.922	0.873	0.879	0.875	0.868
8(14),15-Isopimaradien-18-oate	Sandaracopimarate	1.121	1.122	1.142	1.099	1.065	1.057	1.065
ent-7,15-Isopimaradien-19-oate	Oblongifolate	1.058	1.098	1.065	1.073	1.026	1.019	1.022

Labdane skeleton:

Compound		1	2	3	4	5	6	7
8(17)-Labdene-15,18-dioate	Pinifolate	3.590	4.353	4.837	3.665	2.625	2.358	2.730
8(17),E-13-Labdene-15,18-dioate	Dehydropinifolate	5.80	7.60	8.20	6.02	3.558	3.073	3.603
8(17),E-13-Labdadiene-15,19-dioate	Agathate	4.94	6.23	6.79	5.10	3.158	2.766	3.214
8(17)-Labdene-15,19-dioate	Dihydroagathate	3.073	3.637	4.090	3.101	2.308	2.129	2.407
15-Hydroxy-8(17)-labden-19-oate	Imbricataloate	—	—	—	—	2.480	2.090	2.405
15-Oxo-8(17)-labden-19-oate	Imbricatalate	4.002	3.325	4.753	2.675	1.698	1.539	1.639
15-Methyl-15-oxo-8(17)-labden-19-oate	(Epiimbricataloate)	4.639	4.025	5.57	3.219	2.132	1.932	2.144
15-Oxo-8(17)-labden-18-oate	(Acetoxyisocupressate)	4.741	4.128	5.82	3.220	1.950	1.737	1.895
15-Acetoxy-8(17),E-13-labdadien-19-oate		4.575	5.85	6.55	5.29	3.431	3.040	3.521
8(17),E-12,14-Labdatrien-19-oate	Communate	1.242	1.287	1.358	1.196	1.082	1.061	1.100
8(17),E-12,14-Labdatrien-18-oate	(Epicommunate)	1.451	1.559	1.645	1.438	1.239	1.228	1.267
8(17),13(16),14-Labdatrien-19-oate	Myrceocommunate	0.889	0.866	0.909	0.835	0.811	0.815	0.816
8,13β-Epoxy-14-labden-19-oate	Manoyl oxide ester	1.302	1.186	1.339	1.079	0.987	0.969	0.974
8,13β-Epoxylabdan-19-oate		1.124	1.074	1.154	1.010	1.091	1.095	1.104
8,15-Epoxy-14-labden-19-oate	Epimanoyl oxide ester	1.276	1.214	1.300	1.132	1.065	1.052	1.062
8(17),13-Labdadien-16,15-olid-19-oate	Pinusolid	—	—	—	—	7.99	4.970	6.22
15,16-Epoxy-8(17),13(16),14-labdatrien-19-oate	Lambertianate	2.463	2.511	2.767	2.240	1.438	1.313	1.397
8(17),E-13-Labdadien-15-oate	Anticopalate	1.346	1.482	1.472	1.323	1.286	1.278	1.319
3-Oxo-8(17),E-13-labdadien-15-oate	Ketoanticopalate	10.81	9.77	14.28	6.59	3.041	2.474	2.807
3-Acetyl-8(17),E-13-labdadien-15-oate	Acetylanticopalate	8.03	10.77	11.83	7.90	4.693	4.037	4.919

TABLE I (continued)

Systematic name*	Common name	Silar 10C** (230°C)	BDS*** (190°C)	SP2330† (190°C)	SP1000†† (200°C)	SE-54††† (190°C)	SE-30† (190°C)	SE-30†† (170°C)
Others:								
14S,17-Cyclolabda-8(17),12-dien-18-oate	Strobole	1.600	1.697	1.728	1.673	1.453	1.399	1.446
14S,17-Cyclolabd-8(17)-en-18-oate		1.392	1.423	1.466	1.388	1.355	1.306	1.358
14S,17-Cyclolabd-13-en-18-oate		1.738	1.934	1.47	1.935	1.804	1.828	1.828
14S,17-Cyclolabdan-18-oate		1.092	1.109	1.130	1.093	1.244	1.254	1.277
8βH,14S,17-Cyclolabdan-18-oate		1.455	1.508	1.551	1.496	1.588	1.568	1.621
2α-[2'-(m-Isopropyl)phenyl)ethyl]-1β,3α-dimethylcyclohexanecarboxylate	Secodehydro-abietate	0.881	0.811	0.947	0.772	0.794	0.792	0.802
15,16-Epoxy-3,13(16),14-clerodatrien-18-oate	Hardwickate	4.370	4.657	5.28	3.836	2.116	1.885	2.068
ent-13α,16-Cyclolntisan-19-oate		1.032	0.947	0.984	0.945	0.989	0.993	0.981
12'-Methoxy-8,11,13-podocurpatrien-19-oate	Podocarpate (methyl ether)	3.863	3.388	4.304	2.900	1.397	1.195	1.250
Happlopappate		7.19	8.80	10.12	6.78	3.823	3.231	3.837
ent-5-Epiclerodu-3,13E-dien-15-oate		18.36	10.49	20.56	7.43	2.406	1.751	1.882
13-Ketopodocarp-8(14)-en-18-oate								
ent-16-Kauren-19-oate		1.499	1.353	1.449	1.306	1.191	1.162	1.160

* See refs. 10 and 11.
** 10-m Silar 10C column, 230°C, u = 30.8 cm/sec, t'_{plm} = 4.16 min.
*** 10-m BDS column, 190°C, u = 30.3 cm/sec, t'_{plm} = 14.03 min.
† 10-m SP2330 column, 190°C, u = 30.3 cm/sec, t'_{plm} = 2.52 min.
†† 10-m SP1000 column, 200°C, u = 33.3 cm/sec, t'_{plm} = 8.37 min.
††† 10-m SE-54 column, 190°C, u = 30.3 cm/sec, t'_{plm} = 8.55 min.
† 10-m SE-30 column, 190°C, u = 30.3 cm/sec, t'_{plm} = 7.82 min.
†† 10-m SE-30 column, 170°C, u = 28.7 cm/sec, t'_{plm} = 18.12 min.

column temperature is reduced from 190 to 170°C; this, of course, increases the analysis time, and it is more practical to carry out the analysis on the two stationary phases.

The retention data (for BDS and SE-30) from Table I for pimarate, sandaraco-pimarate, levopimarate, palustrate, isopimarate, abietate, dehydroabietate and neoabietate were compared with those reported by Holmbom et al.[7]. The relative retention data for BDS agreed to within 1 % even though the column temperatures differed by 5°C. Good agreement was also found for the SE-30 data when we extrapolated the 170 and 190°C retention data to 200°C and compared these data with those reported for 200°C by Holmbom et al. Holmbom et al. reported that palustrate and levopimarate are not resolved at 200°C on SE-30. Indeed, our data show that while this pair is completely resolved at 170°C, the retention times are predicted to converge at 200°C.

Although data for comparison are limited[2,13], capillary retention characteristics of diterpene resin acid methyl esters generally parallel those from packed columns containing the same stationary phases. For the polar stationary phases (Silar 10C and BDS) the order of elution from the capillary columns remains the same as from the packed column, but the separation factors from the capillary columns increase progressively with retention time.

Zinkel et al. have discussed the considerable difficulty in the separation of methyl isopimarate and methyl anticopalate, which have nearly identical retention characteristics on three stationary phases (packed columns) of different polarities. However, these two compounds were resolved on all six of the capillary columns tested.

In packed column GLC analysis of the methylated acidic fraction[14] of oleoresin or extractives, the most perplexing problem has been the resolution of the palustrate–levopimarate pair. This resolution was accomplished only on the polar

TABLE II

RESPONSE CHARACTERISTICS OF DITERPENE RESIN ACIDS ON GLASS CAPILLARY COLUMNS COATED WITH SE-30 AT LOW LOADING

Sample range 3–10 ng applied to the column (i.e., injection of 0.3–1.0 μg in 1 μl of n-hexane with a 100:1 split).

Resin acid methyl ester	F_c^* for SE-30			
	Average	No. of data points	Range	Standard deviation (σ)
Abietate	0.986	23	0.835–1.147	0.056
Dehydroabietate	0.965	9	0.927–1.008	0.024
Isopimarate	0.996	16	0.948–1.038	0.018
Neoabietate	0.993	10	0.904–1.153	0.083
Levopimarate	0.989	17	0.951–1.058	0.024

* F_c = correction factor $= \dfrac{A_s W_x}{W_s A_x} = 1/F$ (where F = response factor); when the response of a component X is multiplied by F_c it is corrected to equivalence with the reference component S (in this instance S is pimarate).

stationary phase Silar 10C[2]. However, concurrent with the resolution of palustrate–
levopimarate on Silar 10C, the resolution of neoabietate and dehydroabietate was
lost. Hence the analysis of a typical mixture of resin acid methyl esters requires GLC
using at least two packed columns. On the 10-m glass capillary column coated with
SE-30, palustrate and levopimarate can be resolved by lowering the column tempera-
ture from 190 to 170°C. Resolution can be effected on the polar stationary phases
(BDS and Silar 10C). At 230°C neoabietate and dehydroabietate do not converge on
Silar 10C WCOT glass columns.

Caution must be used in applying the data in Table I for the identification of
compounds. For example, a peak found on SE-30 at 190°C at r_{pim} = 1.323 should not
necessarily be interpreted as resulting from 13β-abiet-8-en-18-oate. There are four
other compounds (18-abietanoate, 6,8,11,13-abietatetraen-18-oate, 8α-isopimaran-
18-oate and 7-isopimaren-18-oate) that elute within the range of uncertainty of the
retention data ($= r_{pim} \pm 0.005$). In this example, one could apply BDS at 190°C for a
clear resolution of identity.

Quantitation by glass capillary GLC

Capillary GLC will provide useful retention data at very low sample levels.
However, obtaining reliable quantitative data with less than 10–20 ng applied to the
column (i.e., injection of 1–2 μg in 1 μl of n-hexane with a 100:1 split) is difficult. For
example, the response data in Table II were obtained by introducing 3–10 ng of each
component on to the column using an automatic sampling device injecting into an
inlet splitter. At the 3σ confidence level, the data in Table II indicate that the error
may range from 6 to 25%. A similar lack of reproducibility was recently reported by
Lanza et al.[15] for the analysis of fatty acid methyl esters on a cyanosilicone stationary
phase. Grob and Neukom[16] have also experienced a lack of precision with vaporizing
injectors and recommend a cold on-column injection technique as the solution to the
problem. Later, Grob and Rennhard[17] recommended a manual injection technique
wherein the syringe needle is pre-heated for several seconds before the sample is
injected from the syringe barrel. This technique was applied to the analysis of diter-
pene resin acid esters, and the precision of response data was maintained at levels

TABLE III

COMPARISON OF DETECTOR RESPONSE RELATED TO SAMPLE SIZE

The sample consisted of methyl pimarate and dehydroabietate in approximately a 1:1 mixture.

Sample size (ng)	Average F_c^*	Standard deviation (σ)
500	0.975	0.009
350	0.982	0.011
175	0.966	0.004
85	0.973	0.007
45	0.978	0.004
20	0.983	0.006
10	1.021	0.090

* F_c is the correction factor, which is multiplied by the peak area of dehydroabietate to give an equal
area response per unit weight of both resin acid esters.

TABLE IV

QUANTITATIVE CORRECTION FACTORS FOR METHYL ESTERS OF RESIN ACIDS ON A 10-m GLASS CAPILLARY COATED WITH SE-30 AT HIGH SAMPLE LOADING

Ester*	F_c	Standard deviation (σ)
Isopimarate	0.999	0.011
Levopimarate	1.025	0.007
Palustrate	1.062	0.004
Dehydroabietate	1.001	0.007
Abietate	0.997	0.010
Neoabietate	1.018	0.010

* The reference compound is pimarate.

down to less than 10 ng applied to the column. Unfortunately, this injection technique cannot be reproduced by most autosamplers.

When using an autosampler with the inlet splitter, accurate results may be obtained by introducing samples of greater than 20 ng per component on to the column (Tables III and IV). It is clear from Table III that the relative response by this method is linear for amounts of sample from 20 to 500 ng. The reproducibility of the response for several resin acid methyl esters is shown in Table IV. This method of quantitation is much easier than the on-column method described by Grob and Neukom[16], and the standard deviations show that the reproducibility is better.

Obviously, for meaningful quantitative results the components must endure the chromatographic process without isomerization or loss. A useful test of packed column activity is the isomerization of certain abietadienoic acid esters (levopimarate and palustrate). On-column transformation is recognized by the elevation of the baseline following the levopimarate and palustrate peaks, the elevation extending through the elution of neoabietate[13]. With capillary columns it is more difficult to observe this transformation because the peaks are usually displayed on the chart at greater attenuation and more closely grouped than with packed columns. Consequently, the baseline deviation is not so apparent. Therefore, it is imperative that response factors are regularly monitored.

Table IV lists the correction factors (reciprocal of response factors) of several of the more common pine resin acid methyl esters, relative to pimarate as determined on the SE-30 column at 190°C. Because the correction factors fall within the range of experimental error of the method, normalization of peak areas will suffice for most analyses.

CONCLUSION

WCOT glass capillary GLC is superior to packed column GLC for the qualitative analysis of diterpene resin acid mixtures. Tentative identification of components may be accomplished more rapidly and with greater certainty than with packed columns.

When samples of less than 10–20 ng per component are introduced on to the capillary column from an inlet splitter, specific injection techniques are necessary for reliable quantitation. Most automatic injectors cannot produce these techniques.

However, it is possible to achieve accurate quantitation by glass capillary GLC using an automatic injector and inlet splitter if samples of adequate size (greater than about 20 ng per component) are used. When the samples involved have widely varying component concentrations, some components will overload the column. As a result of overloading, the analysis must often be repeated at different sampling levels in order to obtain complete retention data and quantitative analysis. However, this usually takes less time than carrying out several analyses on two or more packed columns.

NOTE ADDED IN PROOF

Mediantsev et al.[18] recently claimed to have optimized the resolution of a mixture of common resin acid methyl esters chromatographed on a 50 m × 0.3 mm glass column coated with a 1:1 mixture of EGS–DEGS. However, they did not resolve the levopimarate–palustrate pair as would be expected considering our success with the slightly less polar BDS liquid phase on a 10-m column.

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