Evaluation of Poly S-179 as a stationary phase for the gas–liquid chromatography–mass spectrometry of bile acid methyl ester acetates

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Summary The stationary phase Poly S-179 has been found to offer distinct advantages over the previously reported SP-525 for the gas–liquid chromatographic separation of bile acid methyl ester acetates. Relative retention times of these bile acid derivatives are compared on the two phases.

Supplementary key words relative retention times

We have previously reported the use of the aromatic hydrocarbon SP-525 (Supelco, Inc., Bellefonte, PA) for the gas–liquid chromatographic–mass spectrometric analysis of bile acid methyl ester acetates (1). It has come to our attention that, due to its sensitivity to oxidation, this stationary phase is no longer available from the manufacturer. We therefore would like to report the use of a new phase, Poly S-179, which offers several advantages over SP-525 and has replaced the latter phase for this kind of analysis in our laboratory.

Poly S-179 is a polymer derived from polyphenyl ether sulfones. Its excellent thermal stability between 200 and 400°C and the earlier reports of low column bleed (2, 3) led us to examine the suitability of this phase for mass spectrometric use. We confirmed the lack of background ions in the diagnostic range for bile acids: a newly conditioned column coated with 1.0% Poly S-179 exhibits a negligible background spectrum, with no detectable ions above *m/e* 207, when the column is heated to 325°C.

Experimental

Poly S-179 was obtained as a 1.0% coating on 100/120 mesh Gaschrom Q (Applied Science Laboratories, Inc., State College, PA). The material was packed in a glass column (183 cm × 1 mm) and conditioned overnight at 325°C with a stream of

Abbreviations: RRT, relative retention time; GLC, gas–liquid chromatography; CI, chemical ionization; MS, mass spectrometry.

nitrogen. The thermal stability of the phase permits the conditioning of the column at temperatures well above its normal operating temperature but well below the maximum temperature of the phase. This procedure produces a well-conditioned column that gives good gas–liquid chromatographic separation and reproducible retention times for the bile acid derivatives. The elevated temperature used for conditioning does not affect the column life.

TABLE 1. Comparison of relative retention times of bile acids as methyl ester acetates on SP-525 and Poly S-179[a]

Functional Groups of 5β-cholanoic Acid	SP-525 RRT[b]	Poly S-179 RRT[c]
C_{24} 5β 12α OAc Δ3	0.31	0.28
C_{24} 5β 12=0		0.52
C_{24} 5β 7=0		0.59
C_{24} 5β 3α OAc Δ11	0.70	0.65
C_{24} 5β 3α OAc (Lithocholic acid)	0.71	0.67
C_{24} 3β Δ5		0.83
C_{24} 5β 3α OEt 7α OAc 12α OAc	0.915	0.92
C_{24} 5β 7=0 12α OAc		0.93
C_{24} 5β 3α OAc 12 OAc Δ8(14)	0.86	0.97
C_{24} 5β 3=0	0.75	0.99
C_{24} 5β 3α OAc 12α OAc (Deoxycholic acid)	1.00	1.00
C_{24} 5β 3α OMe 7α OAc 12α OAc	0.925	1.02
C_{24} 5α 3=0	0.93	1.14
C_{24} 5α 3α OAc 12α OAc (Allodeoxycholic acid)	1.14	1.22
C_{24} 5β 3α OAc 7α OAc Δ11	1.26	1.36
C_{24} 5β 3α OAc 7α OAc (Chenodeoxycholic acid)	1.30	1.38
C_{24} 5β 3=0 12α OAc	1.14	1.46
C_{24} 5α 3β OAc 12α OAc	1.45	1.52
C_{24} 5β 7=0 12=0		1.62
C_{24} 5β 7=0 12α OH		1.85
C_{24} 5β 3α OAc 7α OAc 12α OAc (Cholic acid)	1.62	1.87
C_{24} 5β 3α OAc 6α OAc (Hyodeoxycholic acid)	1.82	1.87
C_{24} 5β 3β OAc 7α OAc 12α OAc	1.65	1.88
C_{24} 5β 3α OAc 6β OAc	1.69	1.91
C_{24} 5β 3α OAc 7β OAc (Ursodeoxycholic acid)	1.93	2.02
C_{24} 5α 3=0 12α OAc	1.62	2.14
C_{24} 5α 3α OAc 7α OAc 12α OAc (Allocholic acid)	1.83	2.15
C_{24} 5β 3=0 7α OAc	1.62	2.16
C_{24} 5β 3α OAc 12=0	2.02	2.29
C_{24} 5β 3α OAc 6α OAc 7 OAc (Hyocholic acid)	2.36	2.45
C_{24} 5β 3α OAc 7=0	1.95	2.47
C_{24} 5β 3α OAc 12=0 Δ9(11)	2.11	2.62
C_{24} 5β 3α OAc 6β OAc 7α OAc (α-Muricholic acid)	2.26	2.66
C_{24} 5β 3α OH 7α OH		2.74
C_{24} 5β 3=0 7α OAc 12α OAc	1.82	2.89
C_{24} 5β 3α OAc 7=0 12α OAc	2.40	3.24
C_{24} 5β 3α OAc 6β OAc 7β OAc (β-Muricholic acid)	3.10	3.85
C_{24} 5β 3α OAc 7α OAc 12α OH	(3.59)	3.87
C_{24} 5β 3α OAc 7α OAc 12=0	3.12	4.18
C_{24} 5α 3=0 12=0	2.42	4.22
C_{24} 5β 3=0 6=0	2.80	4.95

[a] Run on a Varian 1400 gas chromatograph using a glass column (183 cm × 1 mm) packed either with 0.5% SP-525 on 100/200 mesh Gaschrom Q, temperature at 240°C, or with 1.0% Poly S-179 on 100/120 mesh Gaschrom Q, temperature at 250°C. Injector and detector maintained at 280°C; N_2 flow rate 15 ml/min.
[b] Retention time relative to deoxycholate at 665 sec.
[c] Retention time relative to deoxycholate at 825 sec.

TABLE 2. Relative recoveries of bile acid methyl ester acetates on 1% Poly S-179[a]

Bile Acid	Weight (μgm)	Area (%)	Response Factor
Lithocholate	2.18	25.36 ± 0.62	1.016 ± 0.024
Deoxycholate	2.28	24.77 ± 0.45	0.949 ± 0.018
Chenodeoxycholate	1.98	22.90 ± 0.30	1.011 ± 0.013
Cholate	2.30	26.98 ± 0.85	1.025 ± 0.032

[a] Bile acids analyzed as a mixture. Data represents an average of three analyses.

Bile acid standards previously used to evaluate SP-525 (1) were examined on a Varian 1400 series gas chromatograph (Varian Associates, Palo Alto, CA) using Poly S-179 columns conditioned as stated. Relative retention times were determined in triplicate, using the deoxycholate derivative as a standard.

Results and discussion

Poly S-179 exhibits separation characteristics similar to those obtained using SP-525, as shown in **Table 1.** In addition, the greater stability of the phase assures that long column life and reproducible measurements of retention time can be obtained using this phase. Over a two month period, with a variety of columns and samples at the same nominal temperature and flow rate, the absolute retention time of the deoxycholic acid derivative ranged from 13.60 to 14.37 min, with a standard deviation of 0.27 min. On a single day, the standard deviation was 0.08 min. The column is usually operated at temperatures between 250 and 275°C, and at these temperatures the column performance is maintained for a long period of time. Columns have been used for over 9 months in our laboratory. (Due to the fragility of the glass columns and the nature of the samples analyzed, the absolute lifetime of the phase is difficult to assess.)

The relative recoveries of several bile acid methyl ester acetate derivatives on 1.0% Poly S-179 are shown in **Table 2**. The response factors obtained for these samples indicate that the phase gives excellent correlation between the peak area response and the weight of sample applied to the column. The number of plates obtainable on this phase, as calculated by averaging the results from several columns, was fairly low, i.e., approximately 650, indicating a rather low column efficiency and a HETP (height equivalent to a theoretical plate) of 2.8. However, the most outstanding property of the phase is not its efficiency but its thermal stability and low column bleed for gas–liquid chromatographic–mass spectrometric work.

It has been shown that Poly S-179 is similar to

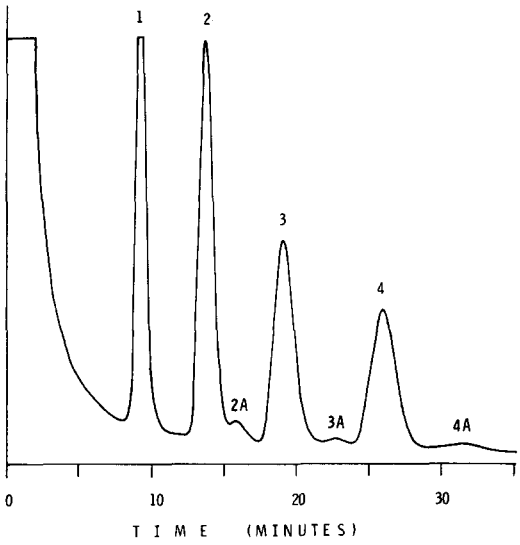

Fig. 1. Gas–liquid chromatographic trace of bile acid methyl ester acetates on Poly S-179; conditions same as listed in Table 1: lithocholic (*1*), deoxycholic (*2*), chenodeoxycholic (*3*), and cholic acids (*4*). Peaks *2A*, *3A*, and *4A* are unidentified.

SP-525 in its separation of bile acid methyl ester acetates. However, while SP-525 facilitates the complete separation of the cholate and ursodeoxycholate derivatives, this separation is somewhat diminished on Poly S-179. In addition Poly S-179 fails to resolve the hyodeoxycholate and cholate derivatives, and the 7-keto-lithocholate derivative can be seen to overlap that of hyocholate. However, the use of the phase for the identification of bile acids using GLC–MS and/or characterization of bile acid containing samples using CI–MS-selected ion monitoring techniques is possible because any confusion that may exist in the identification of these compounds can easily be resolved by the differences in their mass spectra. Poly S-179, in fact, may be preferable in these kinds of analyses to a phase such as OV-225 (5), which gives superior resolution of most of the bile acid methyl ester acetates, but which cannot resolve the hyodeoxycholate and ursodeoxycholate derivatives, both of which give similar mass spectra. Poly S-179 also offers a distinct advantage over another newly reported stationary phase, PPE-20 (4), because baseline separation of the cholate and chenodeoxycholate derivatives can be obtained (**Fig. 1**). This separation permits the use of elevated temperatures, so that certain bile acid analyses can be completed in 30 min or less.

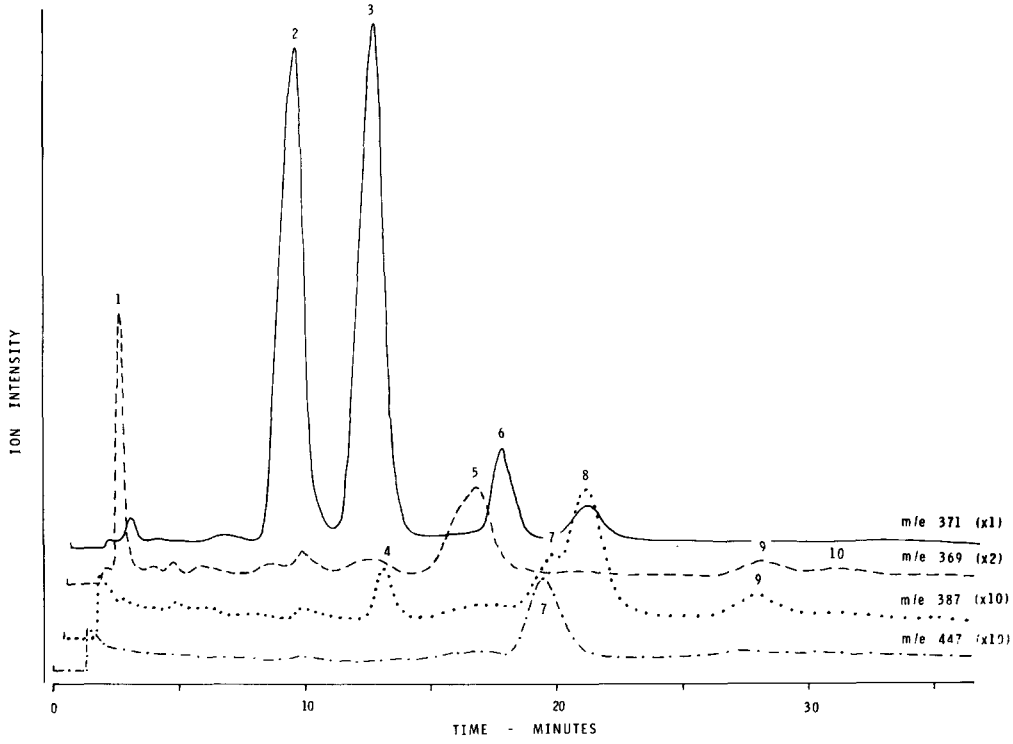

Fig. 2. Selected ion monitoring trace of bile acids in human bile (as the methyl ester acetates) obtained on 1% Poly S-179 at 265°C, 12 ml/min. He flow using GLC-isobutane CIMS on a Biospect quadrupole mass spectrometer (Scientific Research Instruments, Inc., Baltimore, MD). The partial analysis exhibits the following peaks: *1*, cholesterol; *2*, deoxycholate; *3*, chenodeoxycholate; *4*, 3-keto-deoxycholate; *5*, cholate; *6*, ursodeoxycholate; *7*, 12-keto-deoxycholate; *8*, 7-keto-chenodeoxycholate; *9* and *10*, identity not confirmed.

An example of the use of Poly S-179 for gas–liquid chromatographic–mass spectrometric analysis is illustrated in the partial examination of a sample of bile acids from human duodenal bile (**Fig. 2**). The selected ion monitoring traces for three groups of bile acids are shown: *m/e* 371 represents the diacetoxy bile acids, *m/e* 369 the triacetoxy compounds, and *m/e* 387 and 447 the monoacetoxy, monoketo compounds. Additional mass ions were monitored to obtain the complete characterization of the sample.

In conclusion, we believe that no one liquid phase has yet been found that has all the properties required for every type of bile acid analysis. Some liquid phases offer excellent GLC separation but give high column bleed, which limits their use for mass spectrometric work; some phases enable a rough bile acid analysis to be completed in an extremely short period of time but give incomplete separation of important bile acids which prohibits their use for quantitation by either gas–liquid chromatography or mass spectrometry; some phases give exceptional separation of the major bile acids which makes them preferable for the measurement of bile acid kinetics, but their use is limited by poor column stability, and so forth. The reported phase offers another choice for the analysis of bile acids. The use of Poly S-179 is practical because it provides the thermostability and low column bleed needed for gas–liquid chromatographic–mass spectrometric applications and because it offers excellent and reproducible separation of the bile acids as their stable, easily prepared methyl ester acetate derivates.

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