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High Pressure Liquid Chromatography of Sterol Benzoates*

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The high pressure liquid chromatographic behavior of forty sterol benzoates on a ZorbaxODS reverse phase column and on a normal phase column of Zorbax SIL is described. The resolution presently attained was at least as good as those previously reported for sterol acetates and free sterols. Analysis with benzoates has the important advantage of a much higher sensitivity of detection. The lower limit of detection of cholesterol benzoate with a UV detector set at 230 nm was of the order of 20 ng, as compared with 20 μg of cholesterol acetate by the use of a refractive index detector. Thus, the present method is extremely valuable for sterol detection, separation, isolation and/or identification

Table I Relatve Retention Times of Sterol Benzoates

Compound	Zorbax ODS Methanol	Zorbax SIL Hexane–CH_2Cl_2 (50:1)
Cholesterol benzoate	1.00	1.00
Cholestanol benzoate	1.12	0.95
Coprostaonl benzoate	0.78	1.05
3-Epicholestanol benzoate	0.88	1.48
3-Epicholesterol benzoate	0.67	1.88
20-Isocholesterol benzoate	0.88	0.96
(20*R*)-20-Isobutyl-5-pregnen-3β-ol benzoate	0.69	1.07
(20*R*)-20-Isopentyl-5-pregnen-3β-ol benzoate	0.83	1.00
(20*R*)-20-Isoheptyl-5-pregnen-3β-ol benzoate	1.22	0.98
(20*R*)-20-Isooctyl-5-pregnen-3β-ol benzoate	1.53	0.95
Campesterol benzoate	1.14	1.00
24-Methylenecholerol benzoate	0.82	1.54
Ergosterol benzoate	0.81	1.72
Sitosterol benzoate	1.29	1.00
Stigmasterol benzoate	1.14	1.04
Fucosterol benzoate	1.00	1.39
Isofucosterol benzoate	1.00	1.47
5α-Cholest-1-en-3β-ol benzoate	0.90	0.91
4-Cholesten-3β-ol benzoate	0.83	1.07

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Compound	Zorbax ODS Nethanol	Zorbax SIL Hexane–CH_2Cl_2 (50:1)
5α-Cholest-6-en-3β-ol benzoate	0.97	1.16
5α-Choest-7-en-3β-ol benzoate	0.97	1.48
5α-Cholest-8(9)-en-3β-ol benzoate	0.93	1.39
5α-Cholest-8(14)-en-3β-ol benzoate	0.89	1.39
5α-Cholest-9(11)-en-3β-ol benzoate	0.86	1.27
5α-Choleet-14-en-3β-ol benzoate	0.95	1.39
(20*E*)-5α-Cholest-20(22)-en-3β-ol benzoate	0.81	1.34
(20*E*)-5,20(22)-Cholestadien-3β-ol benzoate	0.75	1.54
(20*Z*)-5,20(22)-Cholestadien-3β-ol benzoate	0.68	1.17
(22*E*)-5,22-Cholestadien-3β-ol benzoate	0.79	1.17
(22*Z*)-5,22-Cholestadien-3β-ol benzoate	0.71	1.32
(23*E*)-5,23-Cholestadien-3β-ol benzoate	0.75	1.29
(23*Z*)-5,23-Cholestadien-3β-ol benzoate	0.75	1.34
5,24-Cholestadien-3β-ol benzoate	0.78	1.45
5,25-Cholestadien-3β-ol benzoate	0.76	1.40
4,6-Cholestadien-3β-ol benzoate	0.75	1.10
5,7-Cholestadien-3β-ol benzoate	0.84	1.55
6,8(14)-Cholestadien-3β-ol benzoate	0.75	1.75
7,9(11)-Cholestadien-3β-ol benzoate	0.73	1.75
7,14-Cholestadien-3β-ol benzoate	0.66	2.34
8,14-Cholestadien-3β-ol benzoate	0.66	2.30