Journal of Chromatography, 392 (1987) 464–469
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 382

Note

Separation and determination of steroid isomers on amino-bonded silica by conventional and overpressurized thin-layer chromatography

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(First received October 31st, 1986; revised manuscript received December 23rd, 1986)

Amino-bonded silica gel is a chemically modified polar sorbent. Amino-bonded layers can be prepared from bare silica gel by treatment of 3-aminopropyltriethoxysilane (3APTS)[1–4] and are also available commercially. The properties of commercially available amino-bonded silica gel plates were described by Jost and Hauck[5,6], who used their for the separation of ionizable substances such as nucleotides and sulphonic acids with aqueous eluents and for the separation of steroids and phenolic compounds using organic solvents as the mobile phase. The retentions of steroids and barbiturates in different mobile phases containing organic solvents on amino-bonded and normal silica gel chromatographic plates have recently been compared[7].

In this work, steroid isomers were separated on amino-bonded and bare silica layers, using conventional and overpressurized[8,9] development methods. The applicability of some commonly used spray reagents on the amino-bonded layer was also investigated.

EXPERIMENTAL

Pre-coated HPTLC NH_2 $F_{254}s$ (Merck No. 15647, 10 × 10 cm) and HPTLC silica gel F_{254} (Merck No. 5642, 10 × 20 cm) chromatographic plates were used for thin-layer chromatographic (TLC) and overpressurized TLC (OPTLC) separations, respectively. The developments were performed in a normal unsaturated chamber (N_{us}) and by OPTLC using a Chrompres 10 instrument (Labor-MIM, Esztergom, Hungary). The mobile phase compositions are given in Table I.

The structures of the steroids investigated are shown in Fig. 1. These materials were supplied by Chemical Works of Gedeon Richter (Budapest, Hungary) in the highest available quality. Samples of 0.5–2 µg were applied to the layer.

The chromatograms were evaluated with an Opton KM-3 chromatogram spectrophotometer in the remission mode at 254 nm. The components of the spray reagents were obtained from Merck (Darmstadt, F.R.G.) and Reanal (Budapest, Hungary).

Quantitative measurements

Conventional thin-layer chromatography. Sample solutions of 0.001% were prepared with chloroform and 5 µl of the test solutions were spotted on the plates in

0021-9673/87/$03.50

TABLE I

MOBILE PHASES USED FOR THE SEPARATION OF ISOMERS

Sample	*Mobile phase (v/v)*
Budesonide	Chloroform–diethyl ether–methyl ethyl ketone (4:3:3)
Norgestimate, Norgestrel oxime	*n*-Hexane–methyl ethyl ketone–diethylamine (8:1:1)
6β-Hydroxynorgestimate	Chloroform–acetone (9:1)

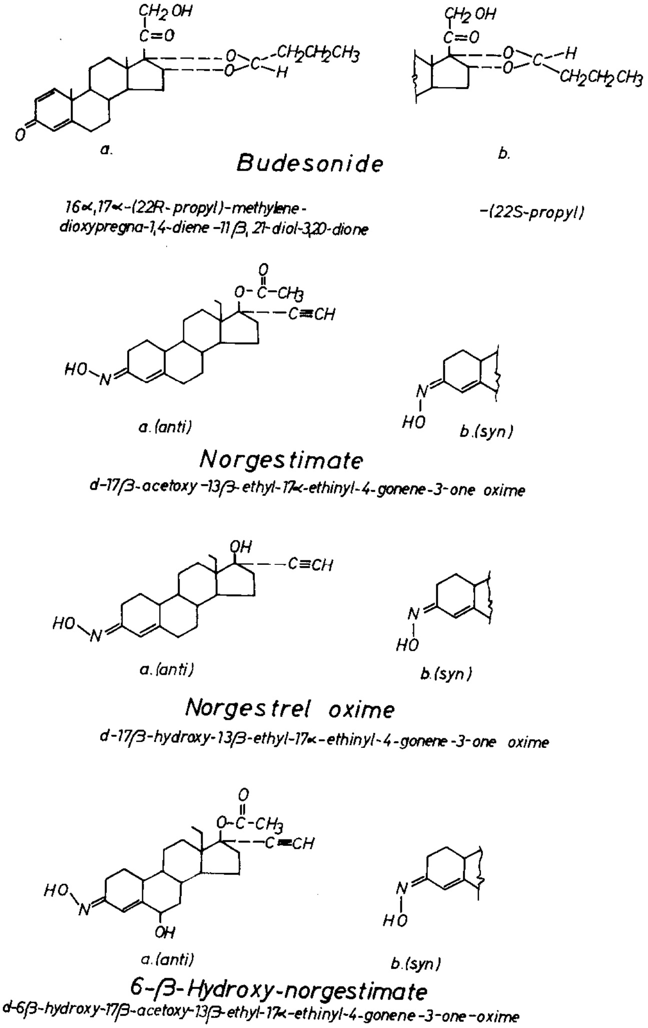


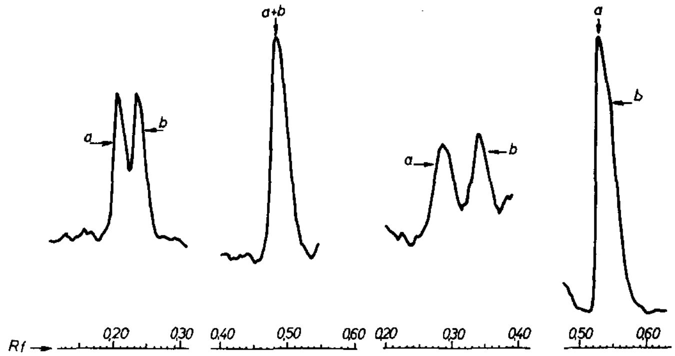
Fig. 1. Structures of the steroids investigated.

TABLE II

CHROMATOGRAPHIC PARAMETERS FOR SEPARATED ISOMERS IN DIFFERENT CHAMBER SYSTEMS

Mobile phases as in Table I. $k' = (1 - R_F)/R_F$.

Stationary phase	*Chamber type*	*Budesonide*			*Norgestimate*			*Norgestrel oxime*			*6β-Hydroxy-Norgestimate*		
		$R_{F(a)}$	$R_{F(b)}$	k'_a/k'_b	$R_{F(a)}$	$R_{F(b)}$	k'_a/k'_b	$R_{F(a)}$	$R_{F(b)}$	k'_a/k'_b	$R_{F(a)}$	$R_{F(b)}$	k'_a/k'_b
HPTLC NH_2 $F_{254}S$	N_{us}	0.21	0.24	1.18	0.33	0.29	1.20	0.28	0.23	1.29	0.20	0.30	1.71
HPTLC silica gel F_{254}	N_{us}	0.49	0.49	1.00	0.27	0.24	1.17	0.25	0.22	1.18	0.20	0.29	1.63
HPTLC NH_2 $F_{254}S$	OPTLC	0.29	0.34	1.25	0.39	0.35	1.18	0.25	0.20	1.33	0.23	0.34	1.72
HPTLC silica gel F_{254}	OPTLC	0.53	0.54	1.03	0.33	0.30	1.14	0.23	0.20	1.19	0.22	0.32	1.66



Chamber type	N_{us}	N_{us}	OPTLC	OPTLC
Stationary phase	HPTLC NH_2 F_{254s}	HPTLC silica gel F_{254}	HPTLC NH_2 F_{254s}	HPTLC silica gel F_{254}
Running distance	9cm	9cm	about 16cm (with overrunning	16cm
Running time	26min	23min	13min	11min
R_f -values	0.21 0.24	0.49	0.29 0.34	0.53 0.54

Fig. 2. Separation of Budesonide isomers by HPTLC on silica gel F_{254} and NH_2 $F_{254}s$. Eluent: chloroform–diethyl ether–methyl ethyl ketone (4:3:3, v/v).

1-cm streaks. After drying the plates, the chromatograms were developed to a distance of 9 cm. The dried chromatoplates were evaluated by spectrodensitometry in the remission mode at 254 nm.

The isomer ratios were calculated by comparing the corresponding peak areas of each pair of isomers.

Overpressurized thin-layer chromatography. Similarly prepared test solutions were developed in a pressurized ultramicro chamber using a Chrompres 10 instrument with a flow-rate of 1–1.5 cm/min and a membrane pressure of 1 MPa. The chromatograms were developed to a distance of 16 cm and then evaluated as described above. The phase systems (plate and mobile phase compositions) are given in Tables I and II.

High-performance liquid chromatography (HPLC). A Liquochrom OE-312 chromatograph (Labor-MIM) equipped with a variable-wavelength UV detector, a loop-type injector and an electronic integrator was used. The separations were per-

TABLE III
DETERMINATION OF TWO ISOMERIC FORMS OF NORGESTIMATE BY OPTLC AND HPLC

No. of sample	*Anti/syn ratio*	
	OPTLC	*HPLC*
1*	1.41 ± 5.1%	1.42 ± 2.1%
2	1.42	1.45
3	1.43	1.46
4	1.47	1.47
5	1.43	1.33

* Mean values and relative standard deviations were determined from seven parallel runs.

formed on a LiChrosorb Si 60, 10 μm pre-packed column (250 × 4.6 mm I.D.) (Chrompack, Middelburg, The Netherlands). The eluent was *n*-hexane–isopropanol (98:2) at a flow-rate of 2 cm^3/min.

The chromatogram was recorded at 254 nm. A 20-μl volume of a 0.01% solution of norgestimate prepared with the eluent was injected on to the column. Concentrations were calculated by comparison of corresponding peak areas.

RESULTS AND DISCUSSION

A comparison was made between the separations of isomer pairs on amino-bonded and bare silica gel sorbents, using a normal unsaturated chamber (N_{us}) and OPTLC. The R_F values and the selectivity factors (k'_a/k'_b) are given in Table II.

The separations are better and the selecitivity factors are higher on amino-bonded silica gel. With Budesonide (Fig. 2) the two isomers can only be separated on amino-bonded silica gel. The separation can be improved by using OPTLC.

The *anti/syn* ratios for Norgestimate samples were also determined and a comparison of the results obtained by OPTLC and HPLC is given in Table III.

Although the chromatograms were evaluated by spectrodensitometry, the applicability of different spray reagents on amino-bonded silica gel was also investigated. Acidic spray reagents applicable for the development of steroid compounds can be used effectively on this sorbent material. However, reagents that react with nitrogen-containing compounds uniformly colourize the amino-bonded layer, and the chromatograms cannot be evaluated visually. The results are summarized in Table IV.

From these results, it can be concluded that reagents based on reactions with nitrogen should not be used with this sorbent owing to their very strong interaction with the bonded amino groups.

TABLE IV

APPLICABILITY OF SPRAY REAGENTS ON AMINO-BONDED SILICA GEL PLATE

Spray reagent	*Number according to Stahl*[10]	*Applicability**
Sulphuric acid	241.A	+
Phosphoric acid	208.A	+
p-Toluenesulphonic acid	251	+
Molybdophosphoric acid	168.B	+
Tetrazolium blue	247	+
Antimony(III) chloride	15	+
Chlorine–tolidine	42	–
Iodine	141	–
Ninhydrin	178	–
Dragendorff's reagent	95	+
Dragendorff's reagent	97	–

* +, The reagent can be used; –, the reagent cannot be used.

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