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Comparison of four homologous retention index standard series for gas chromatography of basic drugs

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Abstract

Four homologous retention index standard series with amine structure were evaluated for the screening of blood samples for basic drugs on NB-54 and DB-1701 capillary columns over a six-month period. An index series consisting of actual drug substances was used as a standard of comparison as it produced the most precise index values. The precision differences between the homologous series were generally small. On the intermediate-polarity column NB-1701, the 4-fluoroaniline series produced the most precise index values, whereas on the low-polarity column NB-54 the trialkylamine series was the most precise. These differences were thought to be caused by the polarity and basicity properties of the standards.

1. Introduction

Screening of body fluids or tissues for drugs is an essential part of clinical toxicology, post-mortem forensic toxicology, human-performance forensic toxicology and forensic urine drug testing. In broad-scale drug screening, the technique which has proven to be particularly efficient is capillary gas chromatography with the use of retention indices (*I*) [1–3]. While conventional *n*-alkane based *I* libraries serve well in inter-laboratory exchange of chromatographic data, these standards are not precise enough for daily routine screening for basic drugs, because of the large structural difference between the standards and the analytes. Additionally, *n*-alkanes are not compatible with the selective detectors required in drug screening. In the present study, the precision of identification of seven basic test drugs was investigated on NB-54 and DB-1701 columns using four homologous amine *I* standard series, three of which are new, and with a reference *I* series consisting of basic drugs. The study was performed over a six-month period with continuous loading with autopsy blood extracts.

2. Experimental

2.1. Standards and test drugs

The N,N-dialkyl-4-fluoroanilines (FA series), the N,N-dialkyl-4-fluorobenzylamines (FB series) and the N,N-dialkylbenzylamines (BA series) were prepared by alkylating the corresponding 4-fluoroaniline (Aldrich, Steinheim, Germany), 4-fluorobenzylamine (Aldrich) and

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benzylamine (Aldrich), respectively. The mixture of amine (0.5 mmol), alkyl halide (1 mmol) and potassium carbonate (1.2 mmol) in 7 ml acetone–water (6:1, v/v) was heated under reflux for 24 h. The solvent was removed in vacuo and the residue was partitioned between dichloromethane and water. The dichloromethane phase was concentrated in vacuo, and the crude products were purified by preparative high-performance liquid chromatography (HPLC). The trialkylamines (TA series) were from Eastman-Kodak (Rochester, NY, USA) and the drugs (the DR series and the test drugs) were obtained from various pharmaceutical companies.

2.2. Apparatus

The gas chromatograph was a Micromat HRGC 412 (HNU-Nordion, Helsinki, Finland) with two nitrogen–phosphorus detectors. The Grob type split/splitless injector fitted with a 1-ml quartz liner was operated in the splitless mode. Silanized glass wool was used in the liner. Automated injections were performed with a CTC A200S (CTC Analytics, Zwingen, Switzerland) autosampler using a 1.5-μl apparent injection volume. The autosampler was set to take the *I* standards in butyl acetate from a separate vial prior to the sample.

HPLC was performed with a Waters (Milford, MA, USA) 501 pump, a Rheodyne (Cotati, CA, USA) 7125 injector equipped with a 1-ml loop, and a Waters 481 variable-wavelength UV–Vis absorbance detector.

2.3. Sample preparation

Whole blood (1 ml) was transferred to a centrifuge tube (10 mm I.D.), Tris buffer (1 *M*, pH 11, 0.3 ml) was added, and the mixture was shaken. The sample was extracted with butyl acetate (0.3 ml) in a vortex mixer for 2 min and centrifuged, and an aliquot of the organic phase (100 μl) was transferred to an autosampler vial.

2.4. Chromatographic conditions

The fused-silica capillary columns were NB-54 (5% phenyl, 1% vinylmethylpolysiloxane) (HNU-Nordion) and DB-1701 (7% phenyl, 7% cyanopropylmethylpolysiloxane) (J & W Scientific, Folsom, CA, USA), both 15 m × 0.32 mm I.D. with 0.25 μm film thickness. Uncoated deactivated fused-silica precolumns (HNU-Nordion) of 5 m × 0.32 mm I.D. were connected to the analytical columns. The carrier gas was helium with a flow-rate of about 2.5 ml/min at 70°C for each column. The injector and detector temperatures were 270 and 290°C, respectively. The splitless time was 0.7 min. The oven temperature was initially held at 70°C for 0.7 min, increased by 20°C/min to 140°C, then increased by 10°C/min to 290°C, and held at the final temperature for 9.5 min.

A 300 mm × 7.8 mm μPorasil silica column (Waters) was used in HPLC. For the purification of the FA series standards, the mobile phase was first cyclohexane–dichloromethane–diethylamine (90:10:0.05, v/v), and finally (240:10:0.125, v/v) for the separation of the remaining alkyl halides from FA8-FA14. The FB and BA series standards were purified using first the mobile phase cyclohexane – dichloromethane – diethylamine (360:10:0.2, v/v) and finally (720:0.5:0.2, v/v).

2.5. Measurement of retention indices

The data processing was performed with Micman 5.0 software (HNU-Nordion). The programme was set to automatically identify the retention index standards by pattern recognition and to report the linear (polygonal) retention indices of the identified test drugs. For the FA, FB, BA and TA series standards (Fig. 1), the single alkyl chain carbon number multiplied by

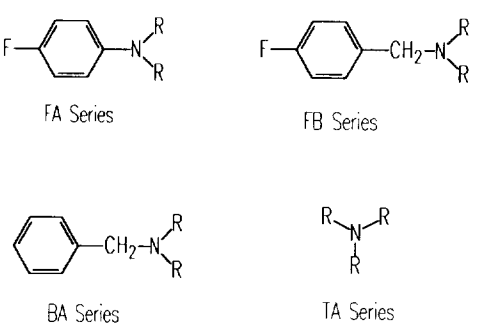

Fig. 1. Structures of the *I* standard series (R = alkyl).

100 was used as the I value. The DR series standards' absolute retention times in seconds were used as the I values.

3. Results and discussion

The chromatographic behaviour of the seven test drugs extracted from blood and co-injected with the four different I standards series is shown in Figs. 2–6. In the design of the FA, FB and BA series, chromatographic properties similar to basic drugs were sought by including amino and phenyl groups in the structures. The FA and FB standards were designed also to be compatible with both nitrogen–phosphorus and electron-capture detection. The TA series had been used earlier in the screening for basic drugs by gas chromatography [4]. The DR series, which acted as a reference, consisted mainly of commercially available drugs and was modified from an earlier study by Franke et al. [5] by choosing four drugs out of fourteen and by adding phenethylamine. The figures show that each homologous series produces well-shaped peaks on NB-54 and DB-1701 columns, and their elution ranges satisfactorily cover the elution range of toxicologically important basic drugs. When a linear temperature programme was used, all the four homologous series showed a fairly linear elution behaviour.

Table 1 shows the precision of identification of the seven test drugs using different I methods. The values were obtained from twenty separate runs by each I method within a six-month period. During this time, the columns were loaded additionally with fourteen autopsy blood extract injections per day, and two pairs of analytical columns representing different lots were used. Table 1 indicates that the DR series method is more precise than the homologous series methods, as could be expected from the results of earlier investigations [5]. However, according to an established concept, the maximum control of deviations in column performance, temperature programme and carrier gas flow can be obtained by co-injecting the I standards with every sample [6,7]. The DR series, consisting mainly of commercially available drugs, is thus unusable for this type of analysis.

The four homologous I series methods differed from each other in precision. The FA series method was the best on the intermediate-polarity column DB-1701 and the TA series method on the low-polarity column NB-54. The FB series method was slightly better than the BA method on DB-1701 but the situation was opposite on NB-54. Thus the ability of the present I methods to provide precise identification of basic drugs appears to depend on the polarity and basicity of the standards and on column polarity. The FA series standards are fairly polar but essentially non-basic. The FB and BA series standards are less polar than the FA standards, being moderately basic but still less basic than the analytes. The TA series standards are fairly non-polar but they are of the same basicity as the analytes. The DR series fulfils the conditions of both polarity and basicity.

The present retention parameters, although called I values, are not strictly based on the retention index theory by Kováts [8]. The values are calculated by linear (polygonal) interpolation with several standards to obtain high intralaboratory precision for a limited group of drugs encountered in daily routine screenings. The absolute I values themselves are not aimed to be universally valid but may depend on e.g. the temperature programme used. However, in case of an unidentified compound, the intralaboratory retention parameters can be converted to Kováts I values by a simple calibration graph.

4. Conclusions

The drug series (DR series) method proved to be superior to the homologous series methods in terms of precision in the chromatographic identification of basic drugs, as expected. However, due to the DR series standards' usage as drugs, the series can not be co-injected with each sample to obtain the maximum control of chromatographic deviations. Comparison of the four homologous series methods revealed that on

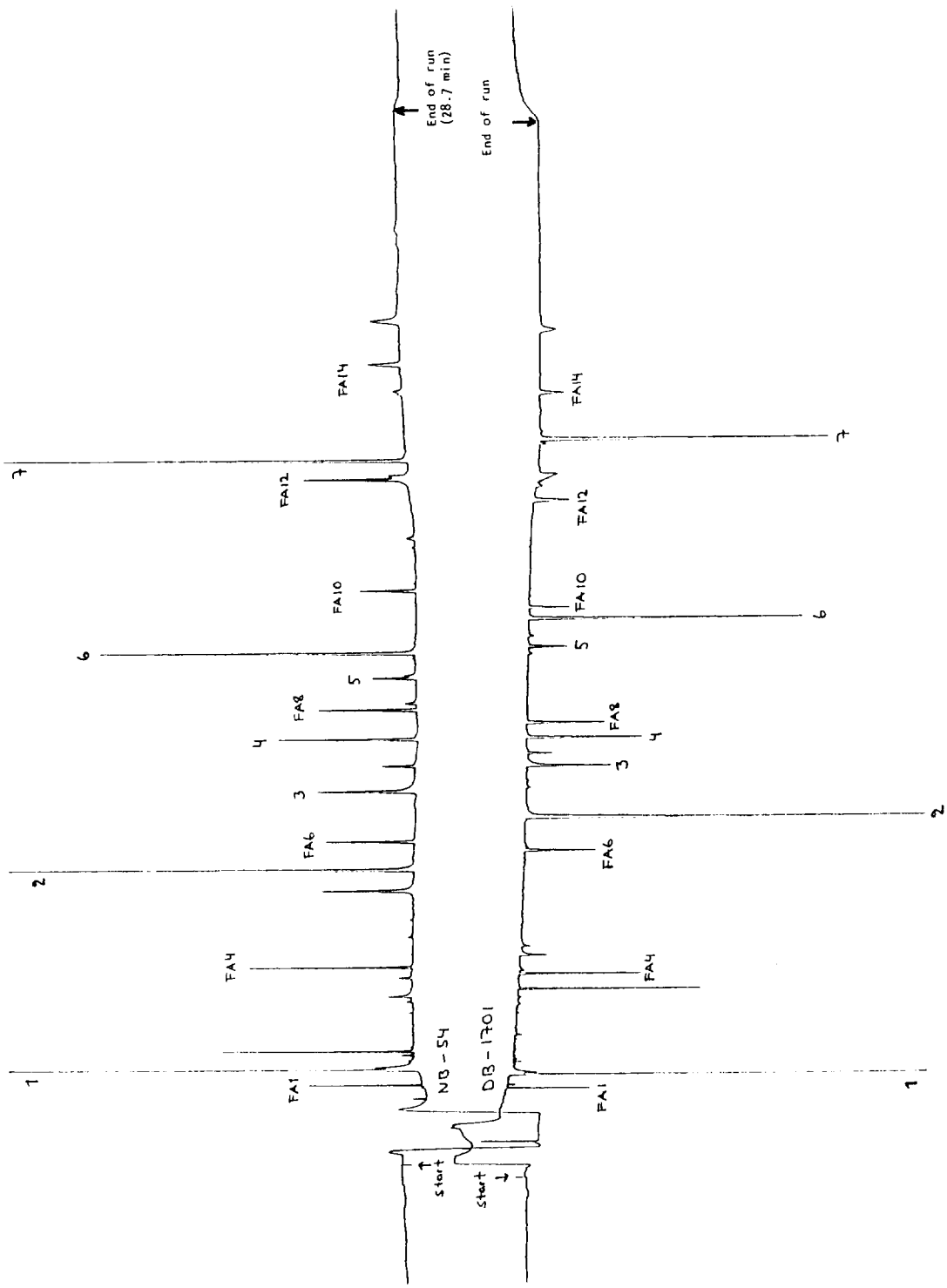

Fig 2. Chromatogram of the test drugs (1–7) co-injected with the FA series *I* standards (FA1: R = methyl etc.). Chart speed, 1 cm/min.

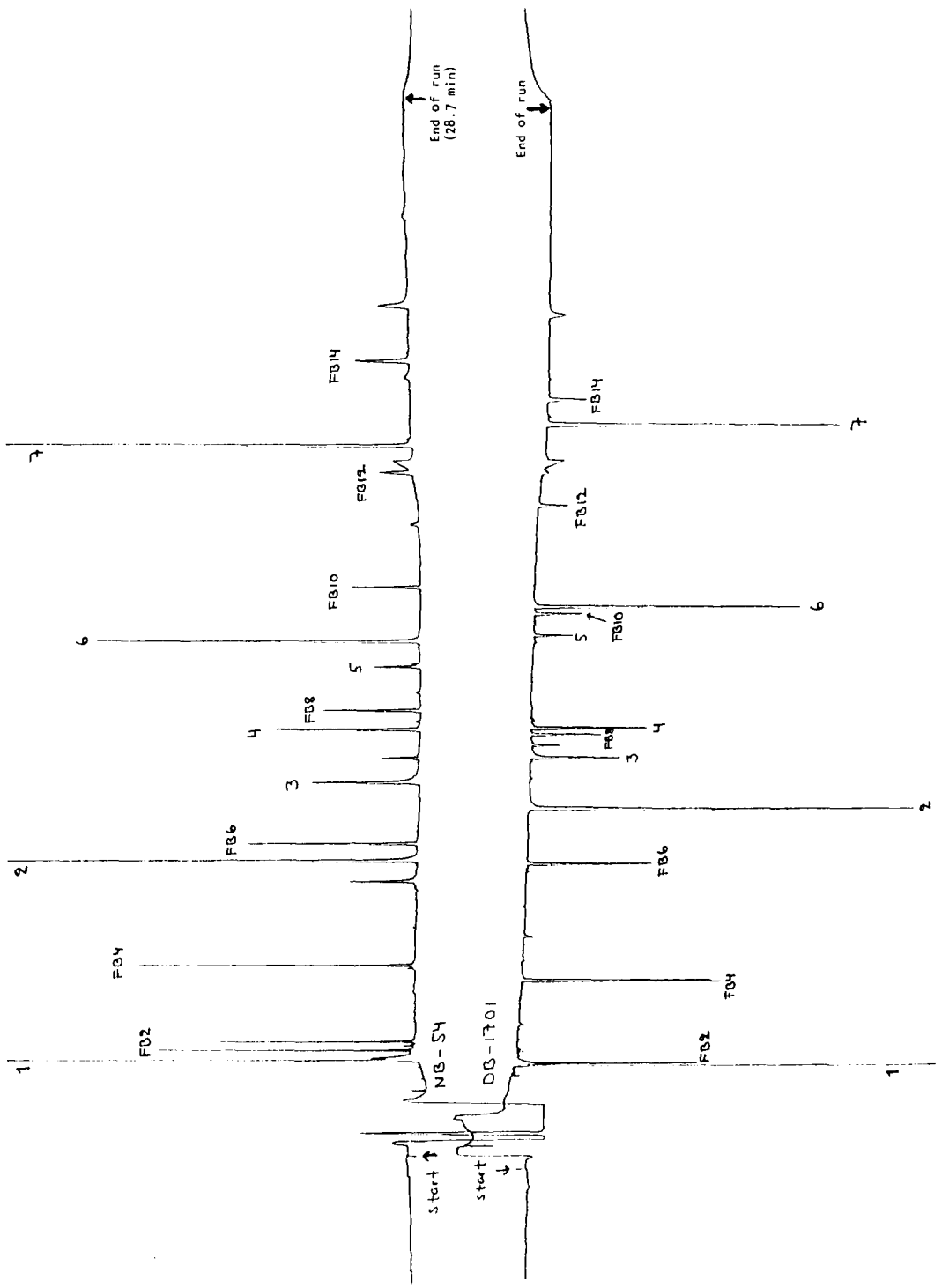

Fig. 3. Chromatogram of the test drugs (1–7) co-injected with the FB series *I* standards (FB2: R = ethyl etc.). Chart speed, 1 cm/min.

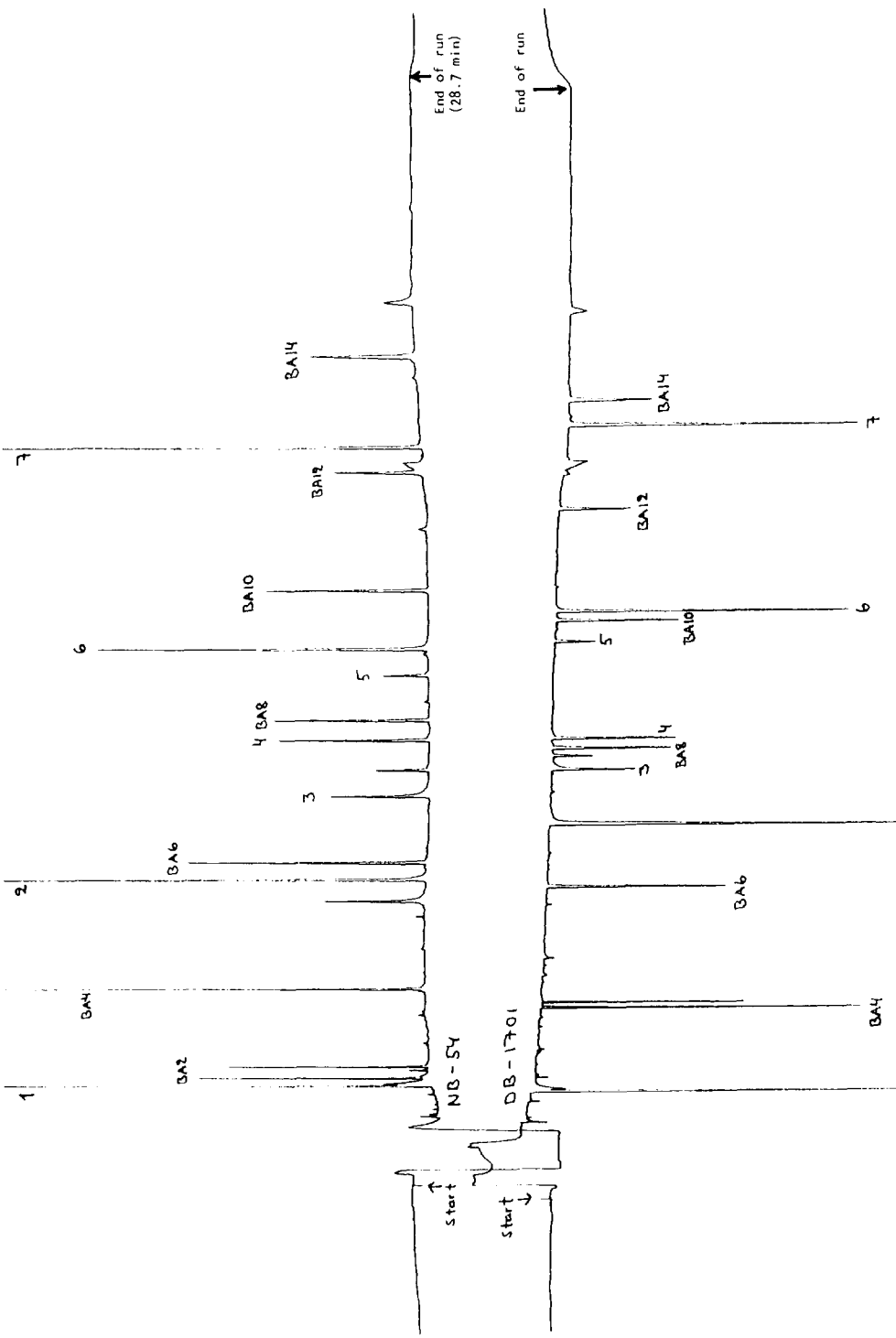

Fig. 4. Chromatogram of the test drugs (1–7) co-injected with the BA series *I* standards (BA2: R = ethyl etc.). Chart speed, 1 cm/min.

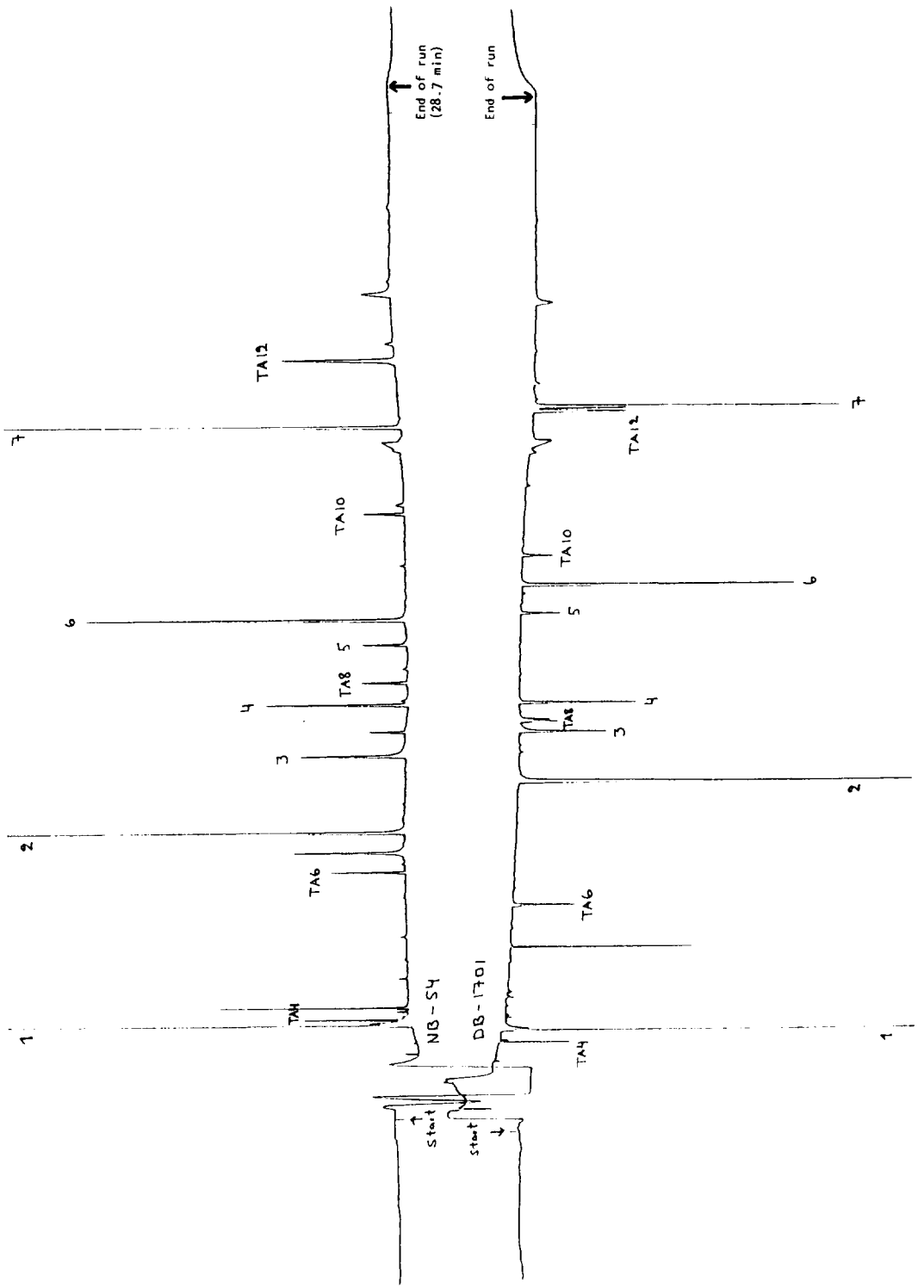

Fig. 5. Chromatogram of the test drugs (1–7) co-injected with the TA series *I* standards (TA4: R = butyl etc.). Chart speed, 1 cm/min.

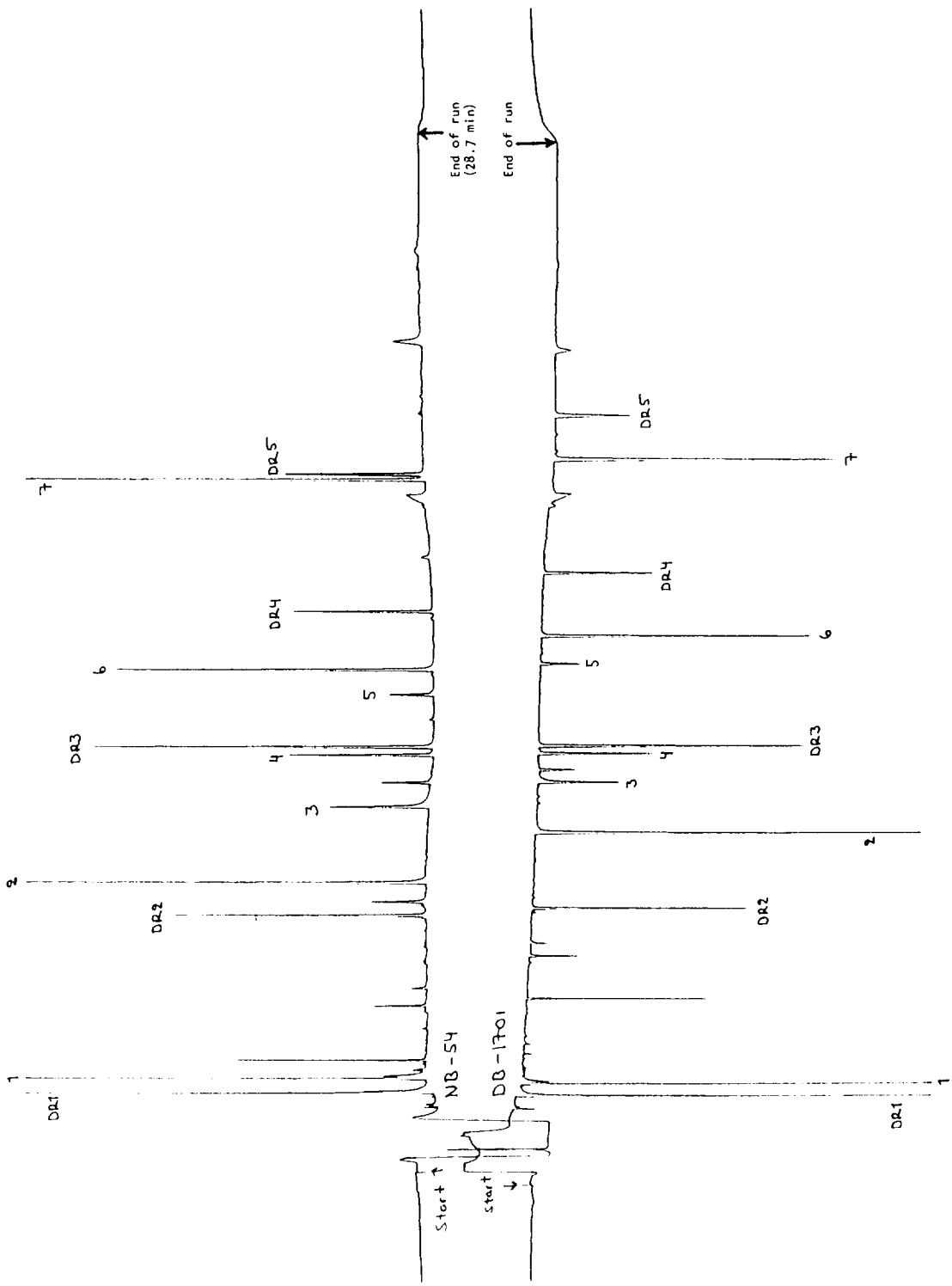

Fig. 6. Chromatogram of the test drugs (1–7) co-injected with the DR series *I* standards (see Table 1, footnote e). Chart speed, 1 cm/min.

Table 1
Precision of identification of test drugs with use of different retention index standard series

Test drug	FA Series[a]		FB Series[b]		BA Series[c]		TA Series[d]		DR Series[e]	
	Mean *I*	R.S.D. (%)	Mean *I*	R.S.D. (%)	Mean *I*	R.S.D. (%)	Mean *I*	R.S.D. (%)	Mean *I*	R.S.D. (%)
(1) Phentermine										
NB-54	138.9	0.431	180.2[f]	0.733	183.8[f]	0.564	372.8[f]	1.190	159.6	0.534
DB-1701	136.5	0.235	195.7[f]	0.201	202.0[g]	0.584	417.6	0.102	188.9	0.209
(2) Caffeine										
NB-54	552.2	0.650	569.6	0.650	571.1	0.615	639.7	0.470	490.1	0.486
DB-1701	653.0	0.339	686.7	0.352	692.0	0.352	735.4	0.376	628.3	0.199
(3) Metoprolol										
NB-54	675.0	0.243	692.3	0.258	692.6	0.247	721.6	0.282	622.1	0.161
DB-1701	733.5	0.257	766.7	0.237	771.4	0.239	790.2	0.308	714.5	0.098
(4) Amitriptyline										
NB-54	753.7	0.323	770.9	0.308	770.5	0.278	775.5	0.303	708.9	0.057
DB-1701	778.3	0.161	812.1	0.164	816.8	0.171	823.4	0.272	762.5	0.021
(5) Codeine										
NB-54	849.7	0.500	867.8	0.478	866.5	0.446	843.4	0.429	809.2	0.179
DB-1701	935.0	0.322	967.5	0.312	970.9	0.324	933.8	0.322	916.9	0.069
(6) Dibenzepine										
NB-54	892.5	0.394	909.9	0.379	908.4	0.349	873.5	0.352	851.6	0.059
DB-1701	984.5	0.327	1017.8	0.363	1021.1	0.371	968.2	0.318	964.9	0.061
(7) Thioridazine										
NB-54	1230.3	0.119	1244.9	0.276	1240.0	0.215	1109.2	0.169	1168.0	0.171
DB-1701	1314.2	0.140	1354.3	0.160	1355.8	0.167	1212.1[h]	0.328	1282.3	0.402
Mean[i]										
NB-54		0.372		0.392		0.358		0.334		0.186
DB-1701		0.258		0.265		0.271		0.321		0.142

Based on 20 measurements of blood extracts over a six-month period.
[a]N,N-Dialkyl-4-fluoroanilines.
[b]N,N-Dialkyl-4-fluorobenzylamines.
[c]N,N-Dialkylbenzylamines.
[d]Trialkylamines.
[e]Drugs: phenethylamine, methyl phenidate, trimipramine, prazepam, strychnine.
[f]Elutes before the first index standard.
[g]Coelutes occasionally with the first index standard.
[h]Elutes after the last index standard.
[i]Excluding the values of phentermine (see footnotes f and g).

DB-1701 the 4-fluoroaniline series (FA series) produces the best results, whereas the trialkylamine series (TA series) is feasible on NB-54. However, the precision differences between the homologous series is small. Obviously, further studies are required to create a non-drug series producing high precision simultaneously on both columns.

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