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Comparison of four homologous retention index standard series for qualitative gas chromatography of nitrogenous acidic and neutral drugs

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Abstract

Four homologous retention index standard series, the alkylmethylhydantoins, alkylhydantoins, alkylmaleimides and alkylbis(trifluoromethyl)phosphine sulphides, were evaluated for the screening of blood samples for acidic and neutral drugs on Ultra 2 and HP-1701 capillary columns over a six-month period. An index series consisting of actual drug substances was used as a standard of comparison. All the series produced high precision, and the precision differences between the series were rather small. Considering the limitations of the other series, the alkylmethylhydantoins and alkylhydantoins turned out to be the most feasible internal retention index standard series in the present dual column setting.

Keywords: Retention indices; Alkylmethylhydantoins; Alkylhydantoins; Alkylmaleimides; Alkylbis(trifluoromethyl)phosphine sulphides; Drugs; Drug screening; Capillary gas chromatography

1. Introduction

Capillary gas chromatography continues to be one of the essential techniques in the screening for drugs and poisons in biological material. Although identification methods relying on the relative retention time are common, a variety of more advanced methods based on retention indices have been published during the last decade [1–3]. Less attention has been paid to the structure of index standards although this matter is of paramount importance when using selective detectors and when high precision is sought [4].

In recent studies from this laboratory, an homologous 1,4-benzodiazepine index standard series was described for benzodiazepine drug screening [5] and four homologous index standard series were described for the screening of basic drugs [6]. In the present study, the precision of identification of nine acidic and neutral test drugs was investigated on Ultra 2 and HP-1701 columns using four homologous secondary index standard series (I^*), three of which are new, and with a reference I^* series consisting of acidic and neutral drugs. The study was performed over a six-month period with continuous loading with autopsy blood extracts.

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2. Experimental

2.1. Gas chromatography

The gas chromatograph was a Micromat HRGC 412 (HNU-Nordion, Helsinki, Finland) with two nitrogen–phosphorous 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. Two analytical columns entered the injector through a two-hole ferrule. Automated injections were performed with a CTC A200S (CTC Analytics, Zwingen, Switzerland) autosampler using a 1-μl apparent injection volume. The autosampler was set to take the *I** standards in ethyl acetate from a separate vial prior to the sample.

The fused-silica capillary columns were Ultra 2 (5% phenylmethylsilicone) (Hewlett-Packard, Wilmington, DE, USA), 25 m×0.32 mm with 0.17 μm film thickness and HP-1701 (14% cyanopropylphenylmethylsiloxane) (Hewlett-Packard), 30 m×0.32 mm with 0.15 μm film thickness. The carrier gas was helium with a column head pressure of 120 kPa and 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. In the evaluation study, 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 8 min.

2.2. Other apparatus

^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian (Palo Alto, CA, USA) Gemini-200 Fourier transform NMR spectrometer. The samples were dissolved in deuterochloroform. The internal reference was tetramethylsilane.

Mass spectra were recorded with a Hewlett-Packard 5972 series mass selective detector combined with a 5890 series II gas chromatograph (GC–MS), using an electron energy of 70 eV.

2.3. Standards and test drugs

The 3-alkyl-1-methylhydantoins (MH series) and the 3-alkylhydantoins (H series) were prepared by alkylating the corresponding 1-methylhydantoin (Aldrich, Steinheim, Germany) and hydantoin (Aldrich). The mixture of 1-methylhydantoin or hydantoin (2.63 mmol), potassium carbonate (1.45 mmol) and alkyl halide (Aldrich) (2.63 mmol; with RI, R=CH_3, C_2H_5, $C_4H_9\cdots C_{12}H_{25}$; with RBr, R=$C_{14}H_{29}$, $C_{16}H_{33}\cdots C_{22}H_{45}$) in dry dimethylformamide was stirred for 24 h. With MH1–MH14 and H1–H14 the reaction was carried out at room temperature and with MH16–MH22 and H16–H22 at 80°C. The solvent was removed in vacuo and the residue was partitioned between dichloromethane and water. MH1, MH2, H1 and H2 were dissolved in dichloromethane because of their remarkable solubility in water. Finally the organic phase was removed in vacuo. Yields ranged from 57–85%. Structures were confirmed by GC–MS, and the structure of MH4 also by ^{1}H- and ^{13}C-NMR. ^{1}H-NMR (MH4): δ (ppm) 0.93 (3H, triplet, bytyl CH_3), 1.33 (2H, sextet, CH_2 gamma to N), 1.59 (2H, quintet, CH_2 beta to N), 3.00 (3H, singlet, NCH_3), 3.47 (2H, triplet, NCH_2), 3.80 (2H, singlet, $COCH_2N$). ^{13}C-NMR (MH4): δ (ppm) 13.63, 19.98, 29.60, 30.20, 38.82, 51.64, 157.01, 169.94. MS (MH4): *m/z* 170 (41%) [M+], 141 (20), 128 (55), 115 (100), 99 (88), 56 (36). MS (H4): *m/z* 156 (14%) [M+], 114 (41), 101 (100), 85 (43), 56 (36). The structure of H4 was confirmed by methylating the amide group by the method of Johnstone and Rose [7] but using sodium methoxide as base. Identical mass spectra to MH4 were obtained.

The N-alkylmaleimides (MI series) were synthesized by a modification of the method of Braish and Fox [8]. Maleic anhydride (Aldrich) (51 mmol) was suspended in 100 ml of dichloromethane. Alkylamine (Aldrich) (51 mmol) was added at 0°C (butyl to dodecyl) or at room temperature (tetradecyl to octadecyl) and the mixture was stirred for 20 h at room temperature. The mixture was cooled in an ice bath and one drop of dimethylformamide was added, followed by slow addition of oxalyl chloride (Aldrich) (53.5 mmol). The ice bath was removed and stirring was continued for 8 h. The solvent was removed in vacuo to remove the excess of oxalyl chloride. The residue was dissolved in 60 ml of dichloromethane, triethylamine (51 mmol) was added, and the mixture was stirred for 1 h. The organic layer was washed with 50 ml of 1 *M* HCl

solution, dried over $MgSO_4$, and dichloromethane was removed in vacuo. The products were purified by flash chromatography on silica gel using cyclohexane–acetone (90:10, v/v) for MI4–MI14, 85:15 for MI16, and first 85:15 and then 90:10 for MI18. Yields were 30% (MI18)–67% (MI4). Compounds were identified by GC–MS and MI4 was identified also by ^{1}H- and ^{13}C-NMR. ^{1}H-NMR (MI4): δ (ppm) 0.83 (3H, triplet, CH_3), 1.22 (2H, sextet, CH_2 gamma to N), 1.48 (2H, quintet, CH_2 beta to N), 3.43 (2H, triplet, NCH_2), 6.62 (2H, singlet, CH=CH). ^{13}C-NMR: δ (ppm) 13.92, 20.27, 30.90, 37.93, 134.37, 171.23. MS (MI4): m/z 153 (30%) [M^+], 111 (55), 110 (100), 98 (17), 82 (34), 54 (20).

The alkylbis(trifluoromethyl)phosphine sulphides (M series) were from HNU-Nordion. The drugs (DR series and the test drugs) were obtained from various pharmaceutical companies.

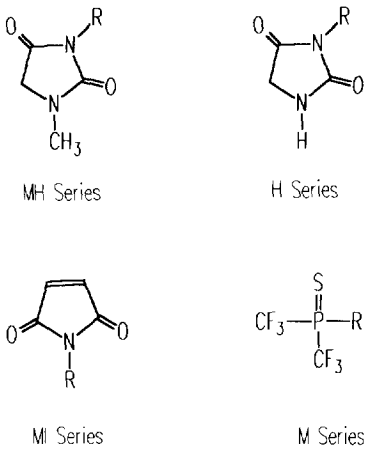

Fig. 1. Structures of the *I** standard series (R=alkyl).

2.4. Sample preparation

Whole blood (1 ml), fortified with the test drugs, was transferred to a centrifuge tube (10 mm I.D.), saturated ammonium chloride solution (0.5 ml) was added, and the mixture was shaken. The sample was extracted with ethyl acetate (0.5 ml) in a vortex-mixer for 5 min and centrifuged, and an aliquot of the organic phase (100 μl) was transferred to an autosampler vial.

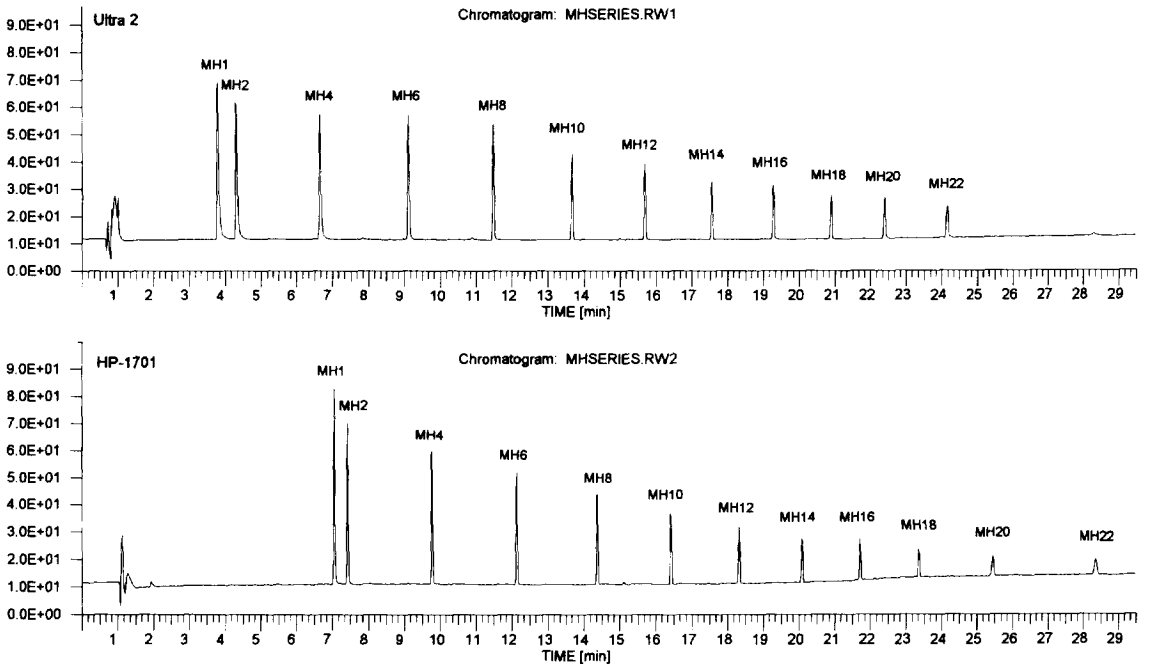

Fig. 2. MH-series *I** standards (MH1: R=methyl etc.) chromatographed on Ultra 2 and HP-1701 columns using a linear temperature program from 70°C to 290°C (held for 8 min).

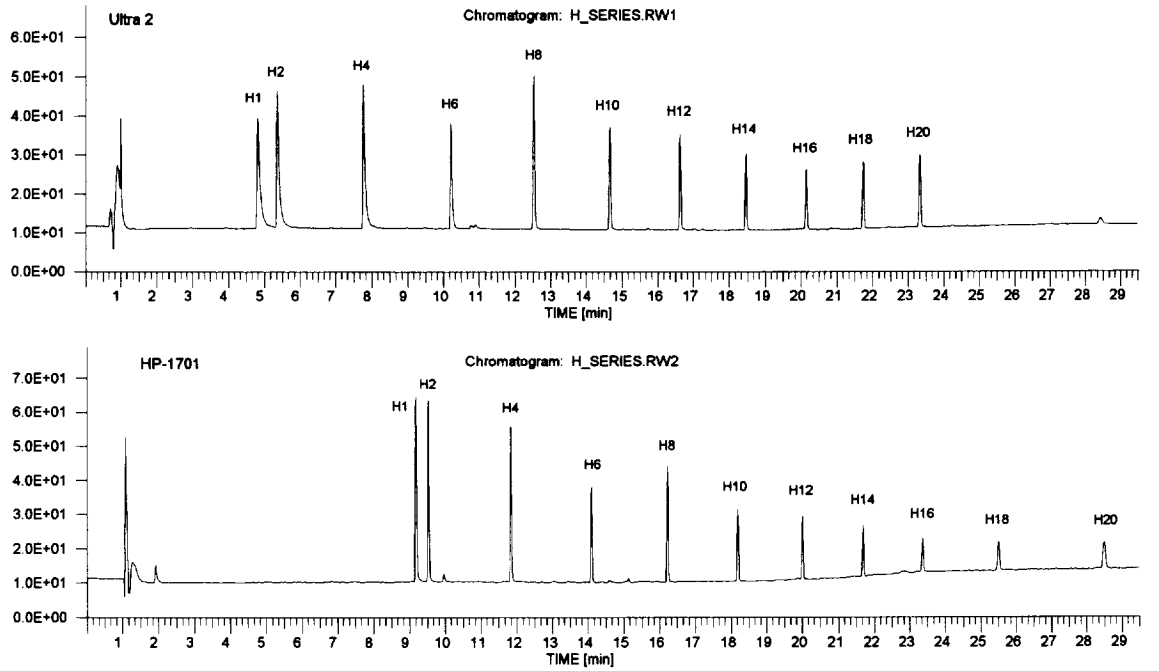

Fig. 3. H-series *I** standards (H1: R=methyl etc.) chromatographed on Ultra 2 and HP-1701 columns using a linear temperature program from 70°C to 290°C (held for 8 min).

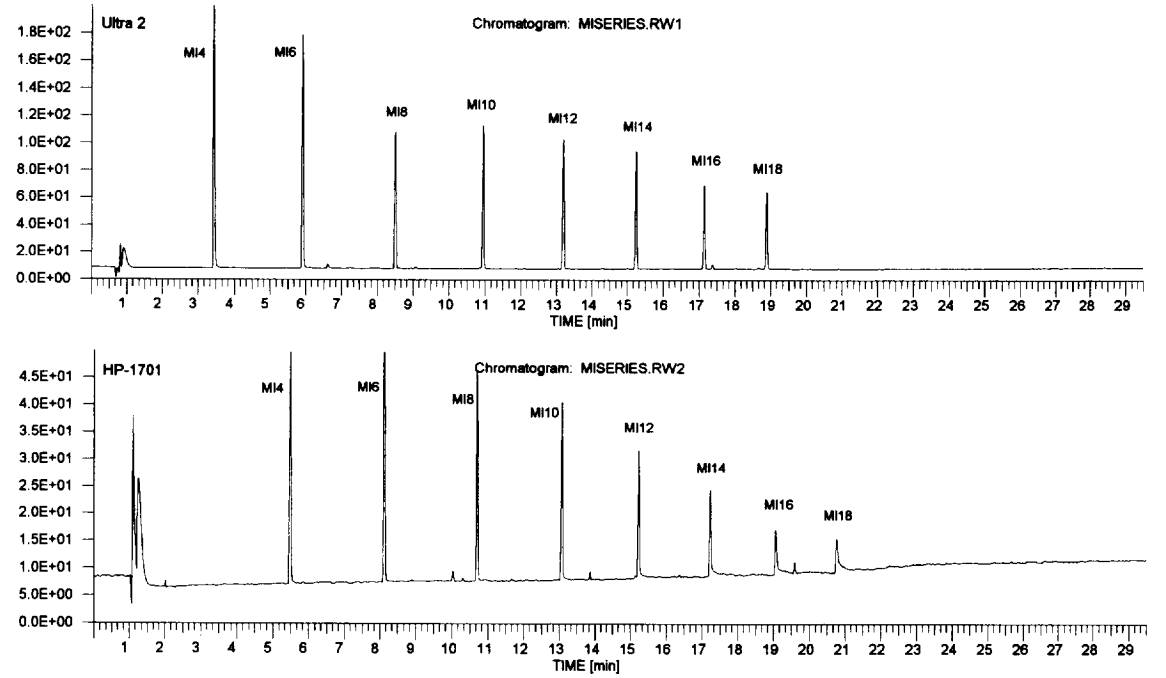

Fig. 4. MI-series *I** standards (MI4: R=butyl etc.) chromatographed on Ultra 2 and HP-1701 columns using a linear temperature program from 70°C to 290°C (held for 8 min).

2.5. Measurement of retention indices

The data processing was performed with SC-Workstation 3.0 (Sunicom, Helsinki, Finland), which is similar to Micman 5.1 (HNU-Nordion). The program automatically assigned the I^* standards by a pattern recognition algorithm; the I^* standards were identified as members of a pattern rather than individual peaks. After that the program calculated the I^* values of other peaks by linear interpolation using absolute retention times, compared the values with library data and reported the identified compounds for both columns. For the MH, H, MI and M series standards, the alkyl chain carbon number multiplied by 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 structures of the present homologous I^* series are shown in Fig. 1. The design of the series was based on the fact that many relevant acidic and neutral drugs contain a hydantoin moiety or a related structure. The M series was originally designed for the analysis of pesticides and chemical warfare agents [9] but has been utilized also in the analysis of acidic and neutral drugs [10]. A series containing the therapeutic drugs ethosuximide, amobarbital, cyclobarbital, primidone and flunitrazepam was chosen as a reference.

Fig. 2, Fig. 3 and Fig. 4 show the chromatographic behaviour of the MH, H and MI series under linear temperature programmed conditions. The peak shapes are fairly symmetrical, except for the long chain MI standards which, unexpectedly, show tailing on HP-1701. The methyl and ethyl derivatives of the MH and H series elute close to each other, which is probably due to the dominant hydantoin nucleus. This phenomenon has been observed earlier with alkylbenzenes [11] and alkylbenzodiazepines [5].

Table 1 shows the precision of the I^* values of the nine test drugs using different I^* methods. The values were obtained from twenty-five separate runs by each I^* method within a six-month period using a

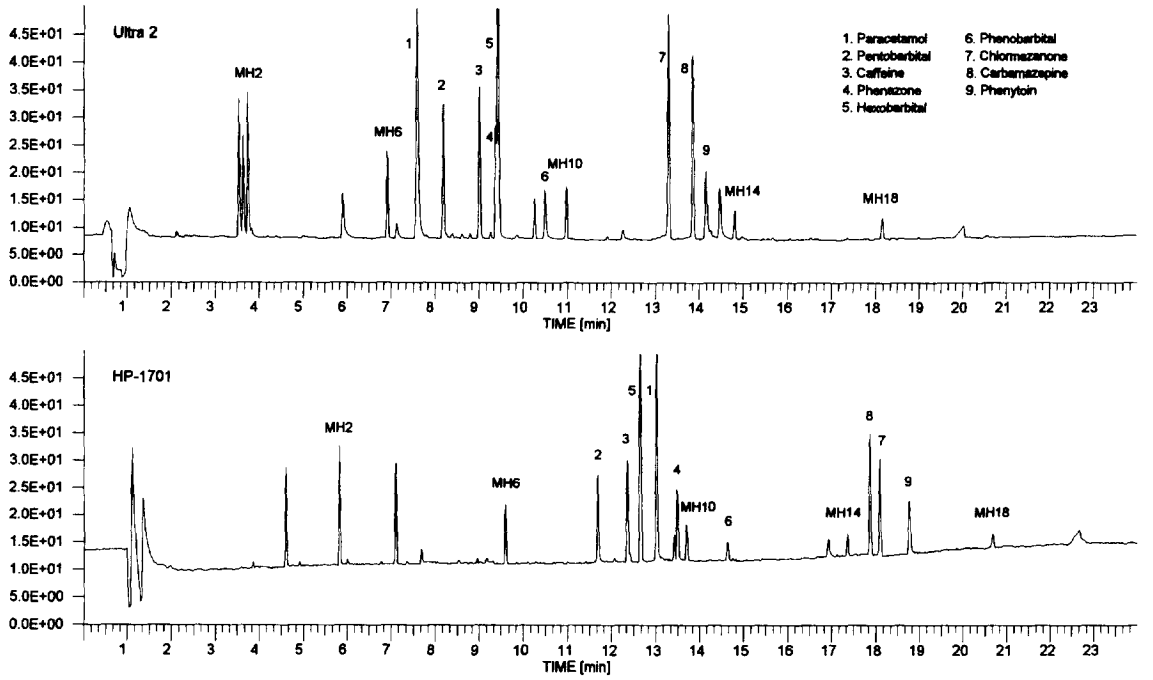

Fig. 5. Chromatograms of the test drugs in blood, the extract co-injected with selected MH series I^* standards on Ultra 2 and HP-1701 columns. Peaks: 1=paracetamol (20 mg/l); 2=pentobarbital (5 mg/l); 3=caffeine (2 mg/l); 4=phenazone (5 mg/l); 5=hexobarbital (3 mg/l); 6=phenobarbital (10 mg/l); 7=chlormezanone (5 mg/l); 8=carbamazepine (5 mg/l); 9=phenytoin (10 mg/l).

Table 1
Precision of I^* values of test drugs with use of different I^* standard series[a]

Test drug	MH Series[b]		H Series[c]		MI Series[d]		M Series[e]		DR Series[f]	
	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.(%)
Paracetamol										
Ultra 2	676.0	0.50	583.2	0.47	918.6	0.42	1266.6	0.30	459.3	0.23
HP-1701	930.9	0.12	758.8	0.08	1250.1	0.20	1680.5	0.26	746.3	0.12
Pentobarbital										
Ultra 2	729.2	0.30	638.8	0.26	977.1	0.28	1321.6	0.18	491.8	0.04
HP-1701	804.9	0.11	630.1	0.27	1122.4	0.04	1542.1	0.12	669.5	0.03
Caffeine										
Ultra 2	809.4	0.08	718.0	0.17	1059.0	0.06	1405.4	0.08	540.9	0.24
HP-1701	867.4	0.09	694.0	0.04	1185.7	0.16	1608.1	0.23	707.6	0.10
Phenazone										
Ultra 2	843.1	0.11	751.2	0.22	1092.6	0.07	1440.6	0.09	561.4	0.27
HP-1701	969.7	0.27	798.5	0.27	1289.4	0.31	1724.8	0.35	770.0	0.22
Hexobarbital										
Ultra 2	848.6	0.07	756.7	0.11	1098.1	0.06	1446.3	0.06	564.9	0.16
HP-1701	895.7	0.04	722.9	0.07	1214.4	0.12	1640.4	0.19	724.8	0.05
Phenobarbital										
Ultra 2	956.8	0.26	863.5	0.17	1205.8	0.20	1558.5	0.18	631.2	0.09
HP-1701	1101.4	0.03	922.0	0.07			1862.8	0.17	843.9	0.03
Chlormezanone										
Ultra 2	1242.5	0.06	1145.3	0.09	1483.6	0.06	1859.7	0.06	796.4	0.12
HP-1701	1479.1	0.28	1295.7	0.25			2267.3[g]	0.32	1045.6	0.02
Carbamazepine										
Ultra 2	1301.1	0.07	1205.1	0.12	1543.3	0.06	1921.4	0.07	830.1	0.12
HP-1701	1454.0	0.21	1272.8	0.17			2241.1[g]	0.28	1033.7	0.03
Phenytoin										
Ultra 2	1335.9	0.18	1240.9	0.15	1579.1	0.17	1958.1	0.14	850.2	0.10
HP-1701	1570.7	0.09	1379.2	0.07			2363.0[g]	0.15	1089.0	0.11
Mean										
Ultra 2		0.18		0.20		0.15		0.13		0.15
HP-1701		0.14		0.14		0.17		0.23		0.08

[a] Based on 25 measurements of blood extracts over a six-month period.[b] 3-Alkyl-1-methylhydantoins (MH2, MH6, MH10, MH14, MH18).[c] 3-Alkylhydantoins (H2, H6, H10, H14, H18).[d] N-Alkylmaleimides (MI6, MI10, MI14, MI16, MI18); data for late eluting test drugs on HP-1701 missing due to bad peak shapes of I^* standards.[e] Alkylbis(trifluoromethyl)phosphine sulphides (M8, M12, M16, M20, M22).[f] Drugs: ethosuximide, amobarbital, cyclobarbital, primidone, flunitrazepam.[g] Elutes after the last index standard.

single pair of columns. During this time, the columns were loaded additionally with fourteen autopsy blood extract injections per day. On the unpolar Ultra 2 column, all the I^* methods produced a good average precision, and the precision differences between the methods were small. On the medium polar HP-1701 column, a good average precision was also obtained but the differences were slightly larger: the drug series method was the most precise, the M series method was the least precise, and the other methods in between did not show much mutual difference.

The MH and H methods produced the best performance with the present dual column setting. The long chain MI series standards suffered from poor chromatography, and the M series standards exceeding M22 are not easily available because of synthetic reasons. The drug series can not be used as an internal I^* series, i.e. coinjected with each sample, as the members of the series are commercially available drugs. Fig. 5 shows the separation of the test drugs extracted from blood using the MH series method. This method has been in routine use in the authors' laboratory for one year and it has allowed precise intralaboratory screening for a variety of acidic and neutral drugs using a window size of less than ±5 units on the present scale for most drugs in a 44-compound library.

The present retention parameters are not strictly based on the retention index theory by Kováts [12] but they are calculated using secondary standards to obtain high intralaboratory precision for a limited group of compounds encountered in daily routine screenings. However, the present indices can be easily transformed to Kováts I values by simple

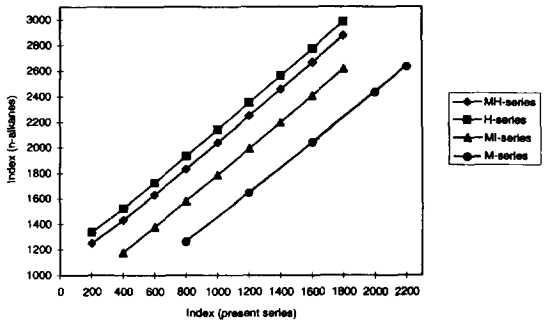

Fig. 6. Relationship between the present I^* standards and n-alkanes on Ultra 2.

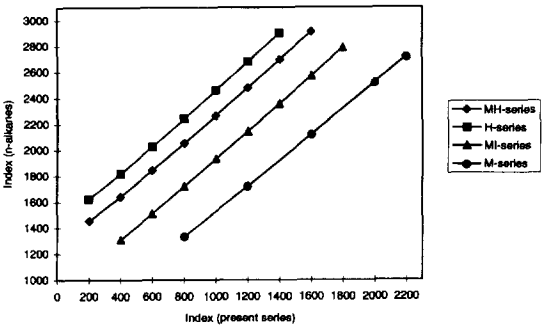

Fig. 7. Relationship between the present I^* standards and n-alkanes on HP-1701.

calibration graphs (Fig. 6 and Fig. 7) for searching larger interlaboratory libraries.

It has been stated that alkane derivatives as index standards cannot compete with actual drug index standards in search of high precision of identification in drug screening [4,13]. However, recent results have shown that carefully designed internal alkane derivatives offer better precision than external drug standards [14]. The present three series are the first internal I^* series designed for the screening of acidic and neutral drugs.

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