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USE OF DUAL-COLUMN FUSED-SILICA CAPILLARY GAS CHROMATOGRAPHY IN COMBINATION WITH DETECTOR RESPONSE FACTORS FOR ANALYTICAL TOXICOLOGY

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SUMMARY

Retention and detector response factor data have been given for 188 compounds on the DB1 capillary column using a dual nitrogen—phosphorus and flame ionization detection system. Factors affecting the detector response factor parameter in a dual-capillary column system have been discussed showing its advantage in drug screening.

INTRODUCTION

Recent developments in fused-silica capillary column manufacture have resulted in the increased use of capillary gas chromatography (GC) as a drug-screening technique in analytical toxicology [1—4]. The improved chromatography of the capillary column provides an excellent screening technique for drugs or poisons isolated from tissue extracts. The improvement in resolution in capillary GC can be tempered somewhat by the increased complexity of chromatogram generally experienced by the analyst in comparison to previous packed-column techniques. A recent report by Perrigo et al. [4] described the reproducibility of retention indices obtained from a capillary column system. The analyst still has the problem of differentiating between compounds with similar retention indices. These similarities in retention index occur between drug compounds themselves or between drugs and other products resulting from the extraction procedure.

It has been the practice in our laboratory with packed-column GC to use a variety of detectors and columns to handle various screening and quantitation problems [4—6]. Methods of dual-column screening have been reported using

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columns of differing polarity for drug identification [7, 8]. The relative ease with which two capillary columns can be inserted into a single injection port makes dual-column screening quite appealing.

The furter characterization of drugs in terms of retention time and a relative detector response ratio was reported by Baker [9]. Seventy-one drugs were characterized by their retention time on a packed 3% OV-17 column and by their relative response on a nitrogen-selective detector and a flame ionization detector. Caffeine was used as the internal standard for the detector response factors. This characterization of response ratio was successfully used to differentiate drugs having similar retention times.

This report discusses our recent investigations of using detector response factors (DRF values) in combination with retention indices (RI), achieved by temperature-programmed capillary GC, for improved toxicological analyses. Matched DB1 capillary columns are used to provide retention data with a concurrent determination of DRF values. Accordingly, the discriminating power [10, 11] for this combined approach was examined and tested.

EXPERIMENTAL

Equipment

A Hewlett-Packard Model 5880A gas chromatograph (Avondale, PA, U.S.A.), equipped with a flame ionization and a nitrogen—phosphorus detector was used to obtain the data in this report. The columns used were Durabond fused-silica DB1, 15 m × 0.32 mm I.D. with a film thickness of 0.25 μm (J & W Scientific, Rancho Cordova, CA, U.S.A.). DB1 is a bonded methyl polysiloxane equivalent phase that has been marketed to substitute for SE-30, OV-1 or SP-2100. Two closely matched columns were obtained by breaking a 30-m column in half.

The chromatograph was operated in the split mode, 10:1 using helium as the carrier and make-up gas to the nitrogen—phosphorus detector; make-up gas flow-rate was 20 ml/min.

The carrier gas linear velocity used was 29 cm/sec. This was slightly higher than the optimum velocity required for maximum column efficiency. The septum purge rate was 1 ml/min. Gas flow-rates to the nitrogen—phosphorus detector were hydrogen 4 ml/min, air 50 ml/min and to the flame ionization detector, hydrogen 20 ml/min, air 270 ml/min. The injection port temperature was 250°C and the injection port liner contained a 2-cm plug of 3% OV-101. The standard temperature programme used was 8°C/min from 120°C to 280°C with a 5-min hold at the upper temperature level. The detector temperature was 300°C.

The dual-column configuration was accomplished by inserting two 15-m columns into the same injection port. A good seal was obtained using a graphite ferrule with a slightly enlarged single hole. Retention times were matched on each column by injecting a test mixture and breaking off a small portion of the column having the later elution times until values were within ± 0.02 min. Once standard conditions had been set up the carrier gas flow-rate was adjusted slightly to keep eluting standards within ± 0.05 min of a reference value.

Method

The retention indices in Table I were determined by linear interpolation to retention time (t_R) values for hydrocarbons run under standard conditions as previously described [4]. The reference hydrocarbon t_R values used have been listed in Table II.

TABLE I

RETENTION INDICES (RI) AND DETECTOR RESPONSE FACTOR (DRF) DATA

Compound	RI (DB1)	DRF (ACB)	Compound	RI (DB1)	DRF (ACB)
Cyclopentamine	1085	4.02	Tryptamine	1681	3.80
Amphetamine	1118	1.62	Talbutal	1689	0.50
Methamphetamine	1173	2.08	Amobarbital	1697	0.45
Tropine	1183	3.24	Salol	1702	0.00
Ethosuximide	1193	0.44	Pentobarbital	1716	0.48
Arecoline	1195	3.72	Pethidine	1730	1.82
Tranylcypromine	1198	2.42	Norpethidine	1749	1.89
Fenfluramine	1220	1.68	Methohexital	1756	1.36
Mephentermine	1243	1.77	Meprobamate	1762	0.04
Phenylpropanolamine	1308	1.90	Caffeine	1768	11.7
Nicotine	1326	3.80	Secobarbital	1769	0.46
Chlorphentermine	1338	1.07	Pheniramine	1788	2.85
Ethinamate	1349	0.056	Alphaprodine	1788	1.52
Ephedrine	1350	2.38	Butrylaminophenol	1790	1.12
Pseudoephedrine	1360	2.14	Glutethimide	1806	0.20
Tyramine	1371	2.88	Prilocaine	1811	2.73
Hydroxyamphetamine	1404	1.94	Hexobarbital	1831	1.60
Salicylamide	1405	0.054	Ethoheptazine	1836	1.78
Metharbital	1417	1.96	Thiopentobarbital	1837	2.24
Phenmetrazine	1419	2.21	Carisoprodol	1847	1.99
Hordenine	1432	2.68	Diphenhydramine	1849	1.76
Methylenedioxyamphetamine	1443	1.98	Lidocaine	1854	2.66
Barbital	1465	0.69	Methylphenobarbital	1869	1.40
Tolazoline	1471	3.34	Aminopyrine	1879	5.54
Methyprylon	1497	1.41	Thiamyal	1886	2.11
Nikethamide	1497	4.39	Azapetine	1917	1.17
Benzocaine	1523	1.39	Theophylline	1917	14.1
3,4-Dimethoxyamphetamine	1537	1.62	Orphenadrine	1924	1.60
Chlorprenaline	1560	2.14	Phenyltoloxamine	1926	1.85
Allobarbital	1575	0.48	Phenobarbital	1928	0.52
Ibuprofen	1594	0.00	Butallylonal	1944	0.50
Aprobarbital	1594	0.56	Tripellenamine	1961	4.18
Methsuximide	1597	1.14	Methapyrilene	1965	4.74
Phenylephrine	1606	4.05	Pemoline	1968	1.45
Phensuximide	1607	1.44	Chlorpheniramine	1985	2.87
Bethanidine	1618	4.91	Aminochlorobenzophenone	1994	1.00
Acetaminophen	1631	1.43	Metoprolol	2023	2.20
Butabarbital	1634	0.55	Heptabarbital	2032	0.42
Butethal	1641	0.51	Mepivacaine	2041	2.73
Methoxymethylenedioxyamphetamine	1662	2.02	Oxytheophylline	2052	11.2
Mescaline	1663	2.19	Brompheniramine	2082	3.11
			Dicyclomine	2091	1.56
			Nomifenison	2108	2.07

(Continued on p. 84)

TABLE I (continued)

Compound	RI (DB1)	DRF (ACB)	Compound	RI (DB1)	DRF (ACB)
Methaqualone	2115	1.72	Hexahydrocannabinol	2407	0.00
Dextromethorphan	2116	1.49	Butacaine	2436	2.57
Aminodichlorobenzo-phenone	2119	1.00	Grey stopper artifact	2457	0.00
			Nordiazepam	2459	2.04
Methadone	2131	1.37	Tetrahydrocannabinol	2471	0.00
Propranolol	2136	1.94	Chlorpromazine	2474	2.82
Alverine	2137	1.50	Acetylcodeine	2480	1.47
Procyclidine	2154	1.62	Oxycodone	2483	1.80
Primidone	2159	2.87	Monoacetylmorphine (06)	2491	1.65
Hyoscyamine	2174	1.58	Oxethazine	2494	4.83
Cocaine	2175	1.79	Thebacon	2498	1.50
Propoxyphene	2178	1.33	Methotrimeprazine	2511	2.53
Amitriptyline	2179	1.50	Clobazam	2514	2.44
Atropine	2183	1.77	Norpropoxyphene amide	2527	1.29
Nortriptyline	2191	1.57	Trimethoprim	2534	5.33
Procainamide	2193	5.38	Cannabinol	2538	0.00
Trimipramine	2204	2.55	Nalorphine	2542	1.60
Imipramine	2205	2.40	Prenylamine	2546	1.26
Zimelidine	2206	3.17	Phenacaine	2546	2.15
Medazepam	2207	2.49	Temazepam	2554	2.27
Doxepin	2210	1.55	Midazolam	2559	3.36
Fluopromazine	2212	3.01	Bromazepam	2563	4.05
Tetracaine	2212	3.90	Flunitrazepam	2572	3.23
Desipramine	2217	2.52	Chloroquine	2600	4.33
Nordoxepin	2219	1.68	Amoxapine	2600	4.50
Norzimelidine	2223	3.28	Diamorphine	2602	1.51
Benzhexol	2226	1.55	Prazepam	2624	2.02
Protriptyline	2226	1.38	Hydroxyethylflurazepam	2630	2.33
Triprolidine	2236	2.88	Nimetazepam	2640	3.71
Benactyzine	2249	1.72	Naloxone	2644	1.92
Halazepam	2250	2.26	Trifluoperazine	2662	4.01
Promethazine	2254	2.61	Cinchocaine	2693	3.69
Carbamazepine	2259	1.01	Fentanyl	2701	2.06
Bupivicaine	2267	2.30	Nitrazepam	2714	2.61
Antazoline	2280	4.29	Flurazepam	2763	3.42
Trimeprazine	2283	2.87	Quinine	2773	2.73
Scopolamine	2286	1.93	Chlordiazepoxide	2778	3.55
Phenytoin	2289	0.85	Clonazepam	2795	2.89
Oxazepam	2293	2.25	Bisacodyl	2814	1.12
Benztropine	2302	1.56	Hydroxyzine	2874	3.85
Maprotiline	2315	1.41	Doxapram	2874	2.75
Levallorphan	2330	1.40	Alprazolam	2910	3.44
Cyproheptadine	2333	1.33	Haloperidol	2921	2.17
Phenylbutazone	2344	1.80	Diltiazem	2927	3.15
Codeine	2348	1.60	Triazolam	3008	3.70
Dihydrocodeine	2357	1.65	Meclozine	3030	3.08
Cannabidiol	2375	0.00	Etorphine	3033	1.23
Lorazepam	2375	2.31	Dimethothiazine	3050	4.54
Clomipramine	2397	2.47	Cholesterol	3081	0.00
Hydrocodone	2401	1.70	Strychnine	3109	2.36
Diazepam	2404	2.53	Thioridazine	3117	1.84
Desalkylflurazepam	2405	1.94	Noscapine	3168	1.49
Morphine	2406	1.71			

TABLE II

STANDARD RETENTION TIMES

Hydrocarbon RI value	t_R (min)	Hydrocarbon RI value	t_R (min)
1100	1.34	2200	12.21
1200	1.74	2300	13.30
1300	2.30	2400	14.34
1400	3.08	2500	15.35
1500	4.04	2600	16.32
1600	5.13	2700	17.26
1700	6.31	2800	18.17
1800	7.53	2900	19.04
1900	8.74	3000	19.88
2000	9.93	3100	20.76
2100	11.09		

The DRF values listed in Table I were calculated from the relative detector response of a compound X, nitrogen—phosphorus detection/flame ionization detection (NPD/FID), as compared to internal standard 2-amino-5-chlorobenzophenone (ACB). The formula for these calculations is

$$\text{DRF (ACB)} = \frac{\text{NPD area X/FID area X}}{\text{NPD area ACB/FID area ACB}}$$

Standards used for injection were made up in ethanol, methanol, or hexane to a concentration of 5—15 mg per 100 ml. Two to four runs of each drug compound were made with standard solutions of ACB, caffeine and prazepam to provide retention index and average DRF values. Some compounds that gave complex chromatograms and were not included in Table I owing to uncertainty as to the cause of these effects were tolbutamide, warfarin and carbromal, as well as the desmethyl metabolites of propoxyphene and chlordiazepoxide.

RESULTS AND DISCUSSION

Careful consideration was given to the choice of standard for DRF determinations. The data in Table III demonstrate the levels of precision calculated using four test compounds (nicotine, caffeine, ACB, and prazepam) for ten drugs with divergent retention indices. Although all four compounds gave good precision, ACB was chosen as the reporting standard for the following reasons: reluctance to co-inject caffeine with case material was expressed by some analysts queried in this regard owing to that compound's potential significance; ACB eluted in an appropriate mid-range position, chromatographically; ACB is readily available as a chemical compound and has proven stable in ethanol at room temperature for a period of at least one month; ACB has potential use as a DRF standard for the electron-capture detector.

As commented on by Baker [9], a distinction should be made between the level of reproducibility expected in short-term (i.e. daily) or long-term test

TABLE III

TEST STANDARDS FOR DRF CALCULATION (n = 15)

Test compound	RI	DRF using nicotine		DRF using caffeine		DRF using ACB		DRF using prazepam	
		Average	C.V. (%)	Average	C.V. (%)	Average	C.V. (%)	Average	C.V. (%)
Fenfluramine	1220	0.440	0.8	0.147	1.5	1.78	2.4	0.881	4.3
Nicotine	1326	—	—	0.333	2.0	4.04	2.9	1.99	4.3
Clorprenaline	1560	0.531	5.3	0.177	3.5	2.14	2.8	1.06	2.8
Pethidine	1730	0.469	1.7	0.156	0.6	1.90	1.4	0.939	3.5
Caffeine	1768	3.00	2.0	—	—	12.1	1.1	6.00	3.2
Diphenhydramine	1849	0.450	2.4	0.150	0.7	1.82	1.0	0.902	3.1
ACB	1994	0.247	3.1	0.082	1.1	—	—	0.495	2.4
Methaqualone	2115	0.424	2.7	0.141	3.4	1.71	2.7	0.847	3.3
Amitriptyline	2179	0.380	3.1	0.127	1.3	1.54	0.7	0.749	2.3
Carbamazepine	2259	0.256	4.6	0.085	2.4	1.03	1.5	0.513	1.4
Diazepam	2404	0.648	3.8	0.216	2.1	2.62	1.4	1.30	2.0
Prazepam	2624	0.500	5.3	0.167	3.4	2.02	2.5	—	—
Cinchocaine	2693	0.992	5.4	0.330	3.3	4.00	2.3	0.198	1.4
Triazolam	3008	0.971	7.2	0.324	5.4	3.92	4.5	1.94	3.6

TABLE IV

LONG-TERM VARIATION IN DRF VALUES

n = 75, over a ten-month period.

	Average	S.D.	C.V. (%)	Range
Area NPD/FID for caffeine	117	39	33	67—220
DRF for caffeine, using ACB reference	11.69	0.38	3.3	10.55—12.29
Area NPD/FID for ACB	10.1	3.4	34	5.5—19

situations. A comparison of DRF precision for data collected over a ten-month period for repeated injections of ACB and caffeine is shown in Table IV. The data presented in Tables III and IV are both representative of the level coefficient of variation (C.V., %) experienced when testing is carried out over a long-term period. In the single-test situation (i.e. runs on the same day) the choice of a standard may be optimized to easily produce a C.V. value of less than ± 3% for tested compounds. However, in the longer term or in comparisons of inter-laboratory data bases, a variation of 5—10% is likely to be more prudent when using the DRF parameter for drug screening. The absolute variation in individual NPD/FID response ratios for ACB and caffeine as presented in Table IV indicates the requirement of an internal standard for DRF calculations. Changing the bead does not affect DRF. The effect on concentration on DRF values was also briefly studied. A summary of this information in Table V shows there is little difference within injection of 5—1000 ng.

Discriminating power (DP) calculations have been used to demonstrate the benefits of various combinations of search data in the identification of drug compounds [10]. A DP calculation tests each member of a data set against all other members using designated error factors or search windows. For example, a peak with RI = 2100 would be considered unresolved from compounds eluting in the RI range 2080—2120 if the search window was set at ± 20 RI

TABLE V

EFFECT OF CONCENTRATION ON DRF VALUES

Compound	DRF (ACB) average values for ng injected		
	5—15	50—150	> 1000
Fenfluramine	1.69	1.68	1.74
Nicotine	3.82	3.80	4.21
Clorprenaline	2.19	2.14	2.14
Phensuximide	1.36	1.44	1.45
Pethidine	1.80	1.82	1.97
Caffeine	11.3	11.7	11.5
Diphenhydramine	1.72	1.76	1.81
Methaqualone	1.62	1.72	1.64
Propoxyphene	1.30	1.33	1.45
Amitriptyline	1.50	1.50	1.51
Carbamazepine	1.05	1.01	1.01
Diazepam	2.79	2.53	2.42
Prazepam	1.95	2.02	1.91
Cinchocaine	3.99	3.69	3.75
Sample size (n)	3—5	8—12	3—5

units. The number of matches or unresolved members in the data base is then used to calculate DP as follows:

$$DP = 1 - 2M/n(n-1)$$

where M = number of matches; n = sample size. A DP value of 1.0 implies resolution of all compounds in the data base.

The value of DRF calculation using varied error factors for comparison is shown in Table VI, using a data sample of 188 compounds. The number of matches (M) is included to give a better perspective of the results.

The number of possible matches in a data base of this size is 17 578 if there was zero discriminating power. Because the variation in DRF value must be expressed as a percentage, rather than as an absolute number, a slightly

TABLE VI

DISCRIMINATING POWER (DP) CALCULATIONS

Sample size n = 188; M = number of matches.

RI values			Combination RI + DRF (ACB) values		
Error factor + RI units	M	DP	Error factor ± DRF (%)	M	DP
30	598	0.9659	10	142	0.9919
30	598	0.9659	5	73	0.9958
10	194	0.9890	10	50	0.9972
10	194	0.9890	5	21	0.9988
5	106	0.9940	10	28	0.9984
5	106	0.9940	5	9	0.9995

different approach was taken than for the determination of error factors for retention indices. The percentage error factors for DRF values listed in Table VI assume that the error is present in both values under test for discrimination. For example, two compounds which have DRF values of 1.00 and 1.50 are considered discriminated at the ± 10% level but not discriminated at the ± 20% level: i.e. 1.0 ± 10% = range 0.90—1.10; 1.5 ± 10% = range 1.35—1.65, indicating no match (or resolution is achieved); 1.0 ± 20% = range 0.80—1.20; 1.5 ± 20% = range 1.20—1.80, indicating a match where peaks are not resolved.

The combined DP values shown in Table VI compare favorably to combination values obtained from (packed) GC columns of differing polarity [11]. A range of error factors for both RI and DRF parameters has been calculated and is shown in Table VI for purposes of comparison and to show the benefits of increasing precision for RI and DRF calculations. The combined DP values of RI and DRF demonstrate the usefulness of the detector response qualifier in data base searching. A search window of ± 5 RI units in combination with a DRF variation of ± 10% has been routinely used in our laboratory. Future work in the authors' laboratory will involve the use of the electron-capture detector in a DRF context, as well as the choice of a secondary screening and quantitation column for further compound discrimination.

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