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GAS CHROMATOGRAPHIC IDENTIFICATION OF COMMON DRUGS BY THEIR MULTIPLE PEAKS AND THOSE OF THEIR TRIMETHYLSILYL DERIVATIVES

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SUMMARY

The extent to which common drugs produce multiple peaks in gas chromatography on an OV-17 column at an injection port temperature of 300°C and temperature programme from 120 to 270°C or at an isothermal temperature of 300°C was studied. Forty-six out of 116 drugs tested produced more than one peak either as the parent compounds or as their trimethylsilyl derivatives. The retention times provide a useful means of identification.

INTRODUCTION

In gas chromatography (GC) dual columns[1,2] or even three columns[3] have sometimes been utilized although, for basic drugs, the use of more than one stationary phase is reported to be of very limited value for identification purposes[4]. Many compounds, moreover, may have the same retention times for a given set of conditions and need the use of gas chromatography–mass spectrometry (GC–MS) for more reliable identification[2,5].

Generally, published GC retention data record only single values for each compound[1–3,6] and only in rare instances is more than one retention value given for a few odd compounds[4–5,7–10].

GC of derivatized compounds, principally by silylation to give trimethylsilyl derivatives, has been used for identification purposes[6,11] and, although the possibility of more than one derivative being formed has been suggested[12], only a few compounds have been reported to produce more than one peak[13].

This study was undertaken to explore the extent to which common drugs produce more than peak and the feasibility of using their retention times and those of their silylated derivatives for identification.

EXPERIMENTAL

The instrument used was a Perkin-Elmer F17 gas chromatograph with a 3% OV-17 Gas-Chrom Q (100–120 mesh) glass column (2 m × $\frac{1}{4}$ in. × 3 mm I.D.) and a

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flame-ionization detector (FID). The carrier gas was nitrogen at a flow-rate of 30 ml/min and the hydrogen and air flow-rates were 30 and 450 ml/min, respectively. The injection port and detector temperatures were 300°C and the oven temperature was programmed from 120 to 270°C at 10°C/min and held at 270°C for 16 min. For isothermal work on compounds not eluted by temperature programming, the injection port, detector and oven temperatures were 300°C. A linear recorder with a chart speed of 30 cm/h and an input voltage of 5 mV was used. Retention times were read manually.

Pure samples of the drugs were obtained from the Singapore Pharmaceutical Department, manufacturers, the British Pharmacopoeia Commission (Reference Substances), the United States Pharmacopoeia (Reference Standards) and the United Nations Narcotics Division of the World Health Organization (International Chemical Reference Substances).

Approximately 1% solutions were prepared fresh in either analytical-reagent grade chloroform, ethanol or 50% ethanol. For silylation, about 0.5 mg of the compound was mixed with about 0.1 ml of chloroform and 0.1 ml of bis(trimethylsilyl)trifluoracetamide (Merck, Darmstadt, G.F.R.) in a 60 × 7 mm tapered-rimmed test-tube, in duplicate, and heated in an air-oven at 80°C for about 1.5 h. Silylated products should not be diluted as precipitation may occur. If the solution is found to be too strong the reaction should be repeated with a smaller amount of the compound.

Volumes of 0.5–2 μl of the standard solution and the silylated mixture (or its supernatant liquid if undissolved solids remained) were each injected on to the column. If the height of the main peak was outside the range of 4–7 in. the run was repeated with an adjusted volume of solution and, if necessary, adjustment of the attenuation.

As the silylated derivatives could undergo hydrolysis on standing[8], they were injected within 1–2 h of their formation.

RESULTS AND DISCUSSION

The retention times of 116 compounds, with temperature programming, of the parent compounds and their silylated derivatives are listed in Table I. Table II lists similar data obtained under isothermal conditions for compounds that are not eluting with temperature programming. A plus or a minus sign denotes the presence of absence of a peak, indicated by the retention time in the neighbouring column. Peaks with heights below 10% of that of the highest peak have been omitted to allow for impurities and artifacts.

An analysis of the data in Tables I and II is given in Table III. By defining that two peaks are distinct when their retention times differ by more than 1.5 min, 35 compounds and the stilylated derivatives of 11 others have more than one distinct peak, making a total of 46 (39.6%) of the 116 compounds tested. This does not take into account another 19 compounds that gave single distinct peaks of their silylated derivatives with retention times different from those of their parent compounds.

Hence, under selected conditions the gas chromatograph can play a useful role in identifying a large number of compounds by multiple peak formation as the parent compounds and/or as their silylated derivatives. The phenomenon of multiple peak

TABLE I

GAS CHROMATOGRAPHIC RETENTION TIMES OBTAINED WITH TEMPERATURE PROGRAMMING

Compound	*Retention time (min)**	
	Parent compound	*Silylated derivative*
Adephenine hydrochloride	18.9	+
Allobarbital	10.8	+
Amethocaine hydrochloride	19.0	+
Aminoglutethimide	22.0	+
Amitriptyline hydrochloride	17.2	+
Amphetamine sulphate	3.6	+
Antazoline hydrochloride	4.0	–
	13.3	+
	14.0	+
	–	17.5
Atropine sulphate	15.4	–
	–	16.0
	–	16.7
	–	18.0
Benzhexol hydrochloride	–	16.4
	17.1	+
Buclizine hydrochloride	2.8	+
	6.1	+
	10.4	+
	11.8	+
	12.9	+
	13.4	+
	14.4	–
	19.2	+
	28.2	–
Butobarbitone	11.2	+
Caffeine	14.5	+
Carbemazepin	15.2	+
	22.6	+
Chlordiazepoxide	–	2.0
	–	14.2
	–	16.6
	–	24.6
	–	27.3
Chloroquin sulphate	14.2	+
	16.1	+
	17.6	+
Chlorpheniramine maleate	2.3	–
	–	4.8
	14.9	+
Chlorphentermine hydrochloride	6.6	+
Chlorpromazine hydrochloride	26.2	+
Clomipramine	16.8	+
Cocaine hydrochloride	18.2	+
Codeine phosphate	–	4.4
	20.6	–
	–	21.5
	22.8	–

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TABLE I *(continued)*

Compound	*Retention time (min)**	
	Parent compound	*Silylated derivative*
Cyclizine hydrochloride	15.8	+
Dextroamphetamine sulphate	3.8	+
	–	5.0
Dextromethoprhan hydrobromide	17.5	+
Diazepam	25.4	+
Diethylcarbamazine citrate	2.4	–
	8.2	+
	–	10.0
Diphenhydramine hydrochloride	13.6	+
Diphenidol hydrochloride	19.3	+
	23.0	+
Diphenoxylate hydrochloride	16.2	+
Disopyramide	14.5	+
	–	15.4
	18.0	+
	–	25.0
Ephedrine hydrochloride	2.6	–
	–	5.8
	6.7	–
	7.0	–
Ergometrine maleate	–	5.3
Ergotamine tartrate	–	8.4
Emetine hydrochloride	–	12.8
	14.3	+
	19.0	+
Guanethidine sulphate	–	2.0
Homatropine hydrobromide	–	15.6
	16.8	–
Hydralazine hydrochloride	10.4	+
	–	13.8
	–	14.0
	–	14.3
	17.7	–
Hydroxyamphetamine hydrochloride	8.4	+
	12.0	+
	–	13.2
Hydroxyzine hydrochloride	10.8	+
	13.2	+
	19.8	+
Hyoscine N-butyl bromide	17.5	+
	–	19.2
	20.8	+
	–	22.6
Hyoscine hydrobromide	17.8	–
	–	18.8
	21.0	–
Imipramine hydrochloride	15.0	+
	18.0	+

TABLE I *(continued)*

Compound	*Retention time (min)**	
	Parent compound	*Silylated derivative*
Iproniazid phosphate	–	4.5
	–	5.0
	10.8	+
Isoniazid	11.2	+
	12.0	–
Isopropamide iodide	–	15.6
	16.8	+
	18.6	+
	19.2	+
	23.2	+
	23.8	+
Isopropylhexidine hydrochloride	3.9	+
	–	4.9
	–	8.7
Lignocaine hydrochloride	13.6	+
Lorazepam	24.6	+
Maprotiline hydrochloride	20.8	+
	–	22.2
Medazepam	19.8	+
Meprobamate	–	3.2
	–	7.7
	–	8.2
	13.7	+
Mepyramine maleate	1.7	–
	–	5.2
	18.6	+
Mepyrylcaine hydrochloride	–	4.8
	10.8	+
Methadone hydrochloride	16.8	+
Methaqualone	18.6	+
Methimazole	12.0	+
Methoxyphenamine hydrochloride	9.2	+
Methylampheatamine hydrochloride	4.1	+
Metronidazole	–	10.8
	12.4	–
Morphine hydrochloride	–	21.7
Naphazoline nitrate	17.2	+
	–	18.8
	–	19.6
Narcotine	13.5	+
Nalorphine hydrobromide	24.2	+
Neostigmine bromide	13.6	+
Nortriptyline hydrochloride	18.3	+
	–	19.6
Oxazepam	21.8	+
Oxyphenbutazone	2.3	–
	2.8	–
	11.6	–
	–	29.4

(Continued on p. 120)

TABLE I *(continued)*

Compound	*Retention time (min)**	
	Parent compound	*Silylated derivative*
Oxyphencyclimine hydrochloride	10.2	+
	14.1	–
	26.6	–
Oxyphenonium bromide	10.2	+
	16.8	–
	17.6	–
Paracetamol	–	12.0
	12.8	–
Pethidine hydrochloride	12.0	+
Phenacetin	12.1	+
Phenalzine sulphate	1.6	+
	1.8	+
	–	3.8
	–	4.6
	–	5.5
	7.6	–
	–	8.9
	–	10.0
	16.4	–
	–	16.8
Phendimetrazine tartrate	8.0	+
Phenformin	–	3.5
	–	3.9
	4.4**	–
	–	8.0
	11.7	+
	–	15.4
	–	16.0
	–	17.4
	22.8	–
Phenoxybenzamine hydrochloride	14.4	+
	17.4	+
	18.0	+
Pentazocine hydrochloride	–	16.6
	18.4	–
Phenylephrine hydrochloride	–	9.4
	13.0	–
	13.4	–
	13.8	–
Phenylpropanolamine hydrochloride	7.3	–
	–	6.0
	–	6.6
Phenytoin sodium	24.6	+
Physostigmine salicylate	8.0	+
	–	12.6
	14.0	+
Piperazine citrate	–	1.8
	–	10.3

TABLE I *(continued)*

Compound	*Retention time (min)**	
	Parent compound	*Silylated derivative*
Prilocaine	15.4	+
Procaine hydrochloride	16.0	+
Prochlorperazine dimaleate	–	5.2
Promethazine hydrochloride	16.6	+
	19.0	+
Propantheline bromide	10.8	+
	–	14.1
	–	19.0
	19.6	+
Propranolol hydrochloride	–	15.7
	17.0	–
Propylhexidrine	3.6	+
	+	8.9
Propylthiouracil	15.6	+
Quinidine sulphate	–	25.1
Quinine sulphate	–	26.1
Thebaine	28.2	+
Theophylline	16.9	+
Tolbutamide	12.6	+
Tranylcypromine sulphate	–	5.5
	–	7.6
	–	8.2
	–	18.4
Tripelennamine hydrochloride	14.9	+
Triprolidine hydrochloride	15.4	+
	18.8	+
	22.6	+
	26.5	+
Trifluoperazine tartrate	15.8	+
	30.0	+
Xylocaine hydrichloride	13.8	+

* A + or – sign indicates the presence or absence of a peak at the corresponding retention time.
** Does not always appear.

formation should, or course, be avoided as much as possible, especially in trace analysis work, in order to achieve optimum detection and quantitative results of drugs, but when they do occur it would be useful to know their number.

The retention times listed in Tables I and II should be used only as a guide as it may be difficult to reproduce exactly the chromatographic conditions of another laboratory[3]. Also, the data in these tables are limited to specific instrument parameters and operating conditions. Most important, the number of decomposition peaks formed will depend on the type of support used, its activity and age and perhaps also on the size of column. It is therefore important that selected standards should always be chromatographed when confirming the identity of an unknown compound.

The results also emphasize the dearth of multiple peaks in published data. The highest number of compounds with more than one peak that has been reported is only fourteen[7], and this was in a list of 570 retention data. Twelve compounds out of the fourteen had not even been tested in this study.

TABLE II

GAS CHROMATOGRAPHIC RETENTION TIMES OBTAINED UNDER ISOTHERMAL CONDITIONS

*Compound**	*Retention time (min)***	
	Parent compound	*Silylated derivative*
Bromazepam	7.0	+
	9.3	+
Brucine	Nil	Nil
Cinchocaine hydrochloride	6.0	+
Chlordiazepoxide	2.0	+
	6.1	+
Clonazepam	3.6	+
	11.4	–
	13.0	–
	14.9	+
Ergometrine maleate	–	6.0
Ergotamine tartrate	3.4	–
	4.0	–
Flurazepam	8.0	+
Flunitrazepam	7.2	+
Guanethidine sulphate	Nil	Nil
Heroin hydrochloride	7.0	+
	8.8	+
Morphine hydrochloride	–	3.1
	–	3.5
Narceine hydrochloride	Nil	Nil
Nitrazepam	2.8	+
	11.7	+
Papaverine hydrochloride	12.4	+
Pholcodine	–	3.2
	–	3.7
Pilocarpine nitrate	2.4	+
Piperazine citrate	Nil	Nil
Prochlorperazine dimaleate	3.2	+
	–	13.0
Quinidine sulphate	–	4.8
Quinine sulphate	–	4.8
Reserpine	Nil	Nil
Strychnine hydrochloride	–	25.0
	–	28.4
Tranylcypromine sulphate	–	2.0
	–	2.4
	–	2.5
	2.8	+
Tubocurarine hydrochloride	Nil	Nil
Yohimbine hydrochloride	Nil	Nil

* Compounds that do not elute with temperature programming.

** A + or – sign indicates the presence or absence of a peak at the corresponding retention time.

To confirm that the phenomenon of multiple peaks was not due to sample inpurity, international standards were tested under similar GC conditions and, except for minor peaks for two compounds, there was corroboration of the data in all instances.

Combined GC–MS was carried out on hyoscine hydrobromide and oxyphenbutazone using a Hewlett-Packard HP 5985B instrument, in order to investigate the

TABLE III

ANALYSIS OF DATA IN TABLES I AND II

	No. of compounds					
	Parent compound			*Silylated derivative*		
	Temperature programming	*Isothermal*	*Total*	*Temperature programming*	*Isothermal*	*Total*
Single peak	59	6	65	70	6	76
Multiple peaks	32	4	36	29	6	35
No peaks			15			5
Distinct* multiple peaks	31	4	35	1	–	1**
Parent compound single peak; silylated derivative:						
Single peak with distinot retention time	–	–	–	12	1	13
Multiple distinct peaks	–	–	–	6	–	6
Parent compound no peaks; silylated derivatives:						
Single peak with distinct retention time	–	–	–	6	–	6
Multiple distinct peaks	–	–	–	3	2	5
Parent compound single peak; silylated derivative identical peak***	40	5	45	–	–	–

* Retention time difference between two peaks is more than 1.5 min.

** Excludes compounds with the same retention time as the parent compound and 11 others with distinct retention times which have been included elsewhere in the table.

*** Presumably no reaction had taken place.

nature of the products formed. The ionization voltage was 70 eV and the ion source temperature was 200°C. The chromatograph was fitted with a 6 ft. × 2 mm I.D. glass column packed with 3% OV-1 on Chromosorb W HP (100–200 mesh) and interfaced to the mass spectrometer by a jet separator maintained at 275°C. The injection temperature was 275°C and the oven temperature was programmed from 120 to 270°C at 10°C/min and held at 270°C for 10 min. The carrier gas was helium at a flow-rate of 30 ml/min. The EI mode was used for both the compounds. The results obtained are given in Table IV.

It can be seen that although oxyphenbutazone remains intact with the direct insertion probe, it undergoes complete decomposition in the gas chromatograph, forming at least three products which bear hardly any resemblance to the parent compound. With hyoscine the two peaks correspond to unchanged hyoscine and hyoscine with the loss of a molecule of water, probably between the α-hydroxy and β-hydrogen atoms. No doubt for other compounds other reactions could take place, but their elucidation is beyond the scope of this study.

Higher injection port temperatures are expected to produce greater decomposition and when lower temperatures, for example 200°C[3], are used the absence of multiple retention times can be expected. However, at 300°C, their absence or almost complete absence[2,4,5], except in a few instances[12,14], remains unexplained. Due importance to subsidiary peaks had probably not been accorded, although this study has demonstrated their usefulness in confirming the identity of compounds.

TABLE IV

RESULTS OBTAINED BY GC–MS

Compound	*Mol.wt.*	*DIP*/GC*	*Retention time (min)*	*m/e*
Oxyphenbutazone	324.37	DIP	–	55 65 77 93 107 121 135 199 268 324
		GC	0.97**	51 64 74 91 119
			8.07***	55 64 80 93 106 119 165 217
			11.40***	52 65 80 93 107 119 135 148 161 177 187 204 231
Hyoscine	303.35	GC	11.18***	51 68 77 94 103 120 138 154 285
			12.70**	55 65 77 94 108 120 138 154 303

* Direct insertion probe.
** Major peak.
*** Minor peak.

CONCLUSION

With injection port and detector temperatures of 300°C and an OV-17 column, a large number of common drugs produce characteristic peaks as the parent compounds or as their trimethylsilylated derivatives, and thereby afford a means of identification.

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