Journal of Chromatography, 183 (1980) 391—401
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 632

DETERMINATION OF HOMOVANILLIC, ISOHOMOVANILLIC AND VANILLYLMANDELIC ACIDS IN HUMAN URINE BY MEANS OF GLASS CAPILLARY GAS—LIQUID CHROMATOGRAPHY WITH TEMPERATURE-PROGRAMMED ELECTRON-CAPTURE DETECTION

JASBIR CHAUHAN and ANDRÉ DARBRE*

Department of Biochemistry, University of London King's College, Strand, London WC2R 2LS (Great Britain)

(First received February 28th, 1980; revised manuscript received May 16th, 1980)

SUMMARY

The determination of urinary homovanillic, isohomovanillic and vanillylmandelic acids as their trifluoroacetylhexafluoroisopropyl ester derivatives by glass capillary gas—liquid chromatography has been studied. It was shown that even with high column efficiencies a single peak—single compound relationship could not be assumed and for reliable quantitation it was necessary to check determinations with a second gas—liquid chromatography column.

INTRODUCTION

Many methylated phenolic compounds derived from aromatic amino acids and catecholamines have been discovered in urine. Synthetic and degradative routes have been published [1]. Homovanillic (HVA), isohomovanillic (IHVA) and vanillylmandelic (VMA) acids have been widely studied because of their clinical importance in cases of neuroblastoma, Parkinsonism and neural chest tumours and other conditions [1,2]. Various techniques have been used for their determination such as colorimetry [3], fluorimetry [4, 5], spectrophotometry [6], thin-layer chromatography [7—9], paper chromatography [10], high-performance liquid chromatography [11—15], gas chromatography [16—36] and mass fragmentography [2,37—39]. Mass fragmentography is probably the best method for specifically determining small quantities of these metabolites in biological fluids, because it can eliminate interference due to other compounds, but the method is only available to a limited number of workers.

Gas—liquid chromatography (GLC) with the electron-capture detector (ECD) offers a method of high sensitivity, but problems arise because of the multiplicity of peaks obtained with urine samples. Thus it is necessary to analyze samples on different liquid phases in order to confirm the purity and identity of compounds represented by individual peaks. With GLC—ECD the nature of the metabolites shown on the chromatogram can also be inferred.

Urine samples show large differences between the retention times of a range of compounds and temperature programming is necessary in order to obtain satisfactory peaks for both quickly and slowly migrating compounds. The ECD is normally used in conjunction with isothermal conditions with packed columns, but more recently capillary columns have been introduced [40—46].

We report here on the determination of HVA, IHVA and VMA in human urine using glass capillary gas chromatography with temperature programming and ECD. This work was developed for the particular study of urine in high-pressure neurosyndrome.

EXPERIMENTAL

Materials

Vanillylmandelic acid (VMA), homovanillic (HVA) and bis(4-hydroxy-3-methoxyphenylglycol) piperazine salt (HMPG) were obtained from Sigma (St. Louis, MO, U.S.A.), homovanillyl alcohol (HMPE) from Aldrich (Milwaukee, WI, U.S.A.), 3-(*p*-hydroxyphenyl)propionic acid (*p*-OHPPA) and *p*-hydroxyphenylacetic acid (*p*-OHPAA) from R.N. Emanuel (Wembley, Great Britain), hexafluoroisopropanol (HFIP), trifluoroacetic anhydride (TFAA), *p*-hydroxydiphenyl (*p*-OH-diphenyl), ethyl acetate, sodium chloride and hydrochloric acid were obtained from BDH (Poole, Great Britain). Dexsil 300GC was from Analabs (North Haven, CT, U.S.A.), OV-101 and SP-2250 were from Chromatography Services (Wirral, Great Britain), OV-225 was from Phase Separations (Queensferry, Great Britain) and polypropylene sebacate (PPSeb) from Griffin and George (Alperton, Great Britain). Glass 1-ml ampoules were obtained from Baird and Tatlock (Chadwell Heath, Great Britain). Isohomovanillic acid (IVHA) was a gift from Roche Products (Welwyn Garden City, Great Britain).

Gas chromatography

Gas chromatography was carried out with a Hewlett-Packard Model 7620A gas chromatograph fitted with a 2 mCi ^{63}Ni electron-capture detector. Peak areas were determined with a Hewlett-Packard integrator Model 3370A. Capillary columns were drawn with a Hupe-Busch machine from 1.5 m of Pyrex glass tubing (9 mm O.D. and 3 mm I.D.) to give 75 m of coiled length. This was coated with 5% Chromosorb R and 5% liquid phase by a single step method and 12.5-m lengths at each end were removed and discarded. Sample volumes were usually restricted to 0.3 μl by Hamilton syringe injected directly into the lumen of glass-lined tubing. The columns gave approximately 1400—2500 theoretical plates/m with the HVA peak.

Preparation of samples

It was necessary to standardise the method for urine samples. The creatinine content of urine was determined and a volume of urine [47] was then taken (usually 0.7—1.5 ml) which contained 1.15 mg of creatinine. This was made up to 2.0 ml with distilled water. Excess sodium chloride to saturate (500 mg) was added, followed by 50 µl of 6 *M* hydrochloric acid (pH < 1) and a known quantity of internal standard, HMPE or *p*-OHPPA. The solution thus obtained was extracted three times with 1-ml volumes of ethyl acetate. A similar method was shown with [^{14}C]HVA to give 99.9 ± 1.9% extraction [21]. From the combined ethyl acetate extracts 0.25-ml aliquots were transferred to 1-ml ampoules. These were taken to dryness in vacuo over phosphorus pentoxide. The dried residue from urine samples was derivatized with 100 µl of a mixture of HFIP—TFAA (1:1, v/v) in sealed ampoules at 100°C for 1 h. The ampoule was cooled and the excess of reagents removed under a stream of nitrogen. The dried residue was dissolved in 150 µl of ethyl acetate and 0.3 µl was injected onto the GLC column. It was found that pure HVA, IHVA and VMA (500 pmol to 15 nmol) were completely derivatized with HFIP—TFAA (3:1, v/v) at 100°C in 15 min, but no change was seen with incubation for 2 h.

RESULTS AND DISCUSSION

It is advisable to use as low an injection port temperature as possible consistent with good peak efficiency particularly for heat-sensitive compounds. An inlet temperature of 270°C was used. Although there was substantial loss of hippuric acid at this temperature, no decomposition products were observed on the chromatogram. Below 250°C reduced responses were obtained due to inadequate volatilization of the sample.

Fig. 1 shows the response curves for different compounds (pmol) against the peak area response. The linear dynamic range was approximately 100 for each compound under the conditions quoted. It is possible to increase the linear range by increasing the pulse interval. But increasing the pulse interval led to increased size of dips in the baseline with corresponding peak integration difficulties. It was found that the size of the dips varied with the liquid phase and we do not attribute the dips to a dirty detector as suggested previously [48].

The derivatization procedure was complicated by the presence of carboxylic, phenolic and alcoholic groups on the compounds being studied. Recoveries were dependent on the amount derivatized as well as the ratio of reagents used.

Variable results due to leakage were obtained when using glass-stoppered test-tubes and Reactivials [27]. Sealed glass ampoules were used.

The effect of changing the HFIP:TFAA ratio six-fold from 1:2 to 3:1 showed no effect on the derivatization of HVA and IHVA but VMA in urine showed low recoveries with TFAA in lower ratio than 1:1 (v/v).

The time course of the reaction for VMA, HVA and IHVA in urine using a reaction mixture of HFIP—TFAA (1:1, v/v) showed that the reaction at 100°C was completed in 15 min, but no losses were incurred by incubation for a period of 90 min. These results agree with other workers who used HFIP—TFAA (1:2, v/v) at 100°C for 1 h for HVA and IHVA [27]. HVA and IHVA were derivatized with HFIP—TFAA (1:3, v/v) at 65°C for 15 min [28] but in a later paper [39] HFIP—TFAA (1:2, v/v) incubation at 75°C for 60 min was used. We were unable to obtain good yields for VMA at 75°C. Similarly we were unable to use the conditions (75°C, 90 min) or reagent ratio alcohol:anhydride (1:10, v/v) described for the pentafluoropropionyl-pentafluoropropyl ester derivative of HVA [34]. Hippuric acid always gave a big detector response

TABLE I

RELATIVE RETENTION DATA FOR METABOLITES RELATIVE TO HMPE ON DIFFERENT LIQUID PHASES

GLC conditions as in Fig. 1.

Liquid phase	Initial temperature (°C)	°C/min	HMPE (min)	*p*-OHPAA	VMA	HVA	IHVA	HA	HMPG
Dexsil 300GC	110	4	16.38	0.53	0.67	0.76	0.79	0.84	0.97
OV-101	100	4	12.95	0.57	0.79	0.83	0.86	0.88	1.04
OV-225	115	4	12.75	0.49	0.67	0.70	0.73	0.88	1.09
SP-2250	100	4	12.52	0.46	0.58	0.73	0.77	0.82	0.91
QF-1	115	4	12.05	0.56	0.84	0.80	0.82	0.87	1.20
GE SE-54	105	4	12.05	0.55	0.74	0.82	0.85	0.87	1.03
Dexsil 300GC + OV-17 (2:1, w/w)	100	4	17.79	0.52	0.62	0.71	0.75	0.78	0.87
OV-17 + OV-101 (1:1, w/w)	100	4	13.39	0.52	0.68	0.78	0.81	0.85	0.96
PPSeb	125	*	26.14	0.17	0.21	0.47	0.54	**	***

*Isothermal.
**Not observed.
***Not run.

TABLE II

RELATIVE MOLAR RESPONSE VALUES OF METABOLITES RELATIVE TO HMPE TAKEN AS 1.0 ON DIFFERENT LIQUID PHASES

GLC conditions as in Fig. 1.

Compound	Dexsil 300GC ($n = 5$)	OV-101 ($n = 4$)	SP-2250 ($n = 5$)	QF-1 ($n = 5$)	GE SE 54 ($n = 4$)	Dexsil 300GC + OV-17 (2:1, w/w) ($n = 4$)	OV-17 + OV-101 (1:1, w/w) ($n = 4$)
p-OHPAA	2.51 ± 0.12	2.62 ± 0.21	2.58 ± 0.11	2.41 ± 0.07	2.69 ± 0.13	2.51 ± 0.08	2.62 ± 0.11
VMA	4.56 ± 0.27	4.42 ± 0.29	4.87 ± 0.21	4.34 ± 0.15	4.42 ± 0.16	4.62 ± 0.21	4.34 ± 0.18
HVA	3.51 ± 0.21	3.45 ± 0.12	3.58 ± 0.11	3.35 ± 0.12	3.55 ± 0.12	3.57 ± 0.08	3.47 ± 0.08
IHVA	2.79 ± 0.18	2.65 ± 0.03	2.83 ± 0.13	2.66 ± 0.07	2.79 ± 0.08	2.70 ± 0.11	2.67 ± 0.09

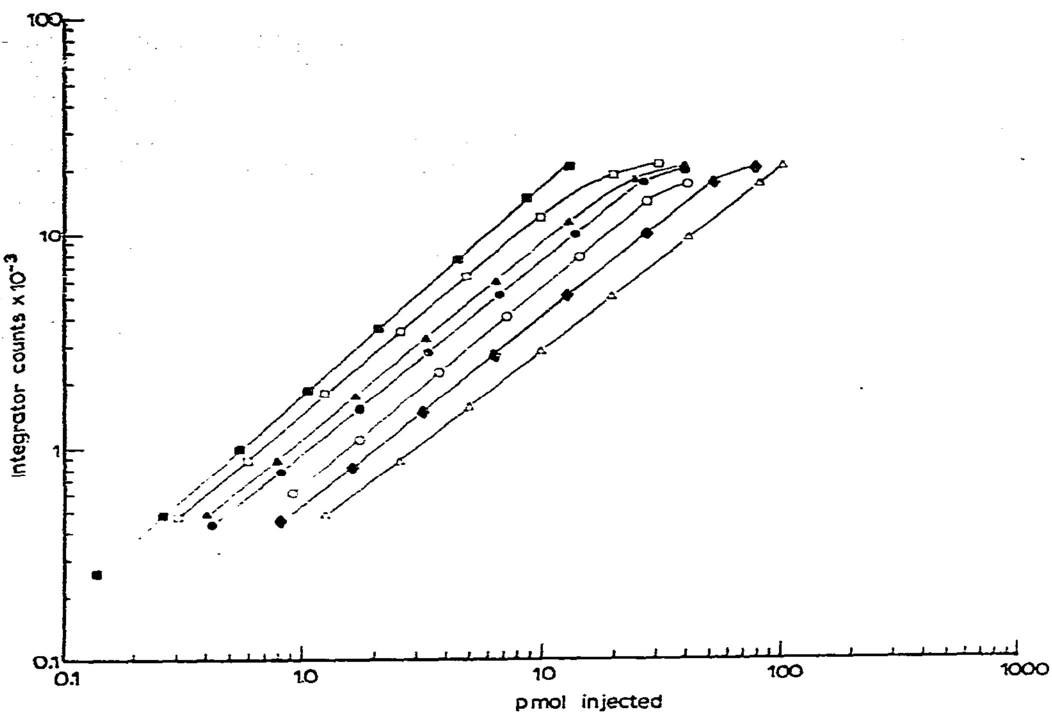

Fig. 1. Response—concentration curve for trifluoroacetyl-hexafluoroisopropyl ester derivatives. Preparation of sample: see Experimental. GLC conditions: 50 m × 0.27 mm I.D. coated with 5% Chromosorb R and 5% SP-2250 SCOT column; carrier gas, hydrogen at a flow-rate of 3 ml/min; make-up gas, argon—methane (90:10) flow-rate, 50 ml/min; temperatures, detector, 290°C; injector, 275°C; column, 100°C programmed at 4°C/min; pulse interval, 15 μsec; attenuation, 2 × 10^2; sample size, 0.3 μl. ■ = HMPG, □ = VMA, ▲ = HVA, ● = IHVA, ○ = *p*-OHPAA, ◆ = hippuric acid, △ = HMPE.

and in order to minimize this the derivatization reaction was standardized at 100°C for 60 min with HFIP—TFAA (1:1, v/v). This loss of hippuric acid was not investigated. Additional peaks corresponding to benzoic acid or glycine were not observed on the chromatogram.

The recoveries of metabolites added to urine samples were as follows: VMA, 95 ± 4.5%; HVA, 96 ± 3.8%; IHVA, 98 ± 3.5% (n = 4). Using the same derivatives with GLC determination recoveries from urine were reported as follows: VMA, HVA and IHVA 95—105% [39].

Choice of stationary phase

Relative retention times of six metabolites on nine different liquid phases are given in Table I. All columns with the exception of PPSeb were temperature-programmed and baseline separation was obtained for all the compounds listed. Exceptionally VMA had a longer retention time than HVA and IHVA with QF-1 liquid phase, although this did not occur with other polar stationary phases OV-225 and PPSeb.

The relative molar response (RMR) values of the metabolites were determined with different liquid phases (Table II). This work was done because it had been reported that recoveries varied with the liquid phase. Using the same derivatives with ECD, recoveries were reported of 79 ± 6% for HVA on a 3.8% SE-30 column, 65 ± 4% with a 2% SE-52 column and 65 ± 5% with a 2% QF-1 column [27]. VMA showed the highest standard deviation. Comparing liquid phases the RMR values showed some variation for all the metabolites but these may be attributed to the inherent problems with ECD where many critical parameters such as gas flow-rate and temperature have been specified [48—53].

Fig. 2a shows the separation of seven metabolite derivatives on a SP-2250 SCOT column (1420 theoretical plates/m) in about 12 min. Approximately a 15-fold reduction in quantity injected gave the same peak height as with a packed column in shorter time and with improved resolution. Fig. 2b shows the

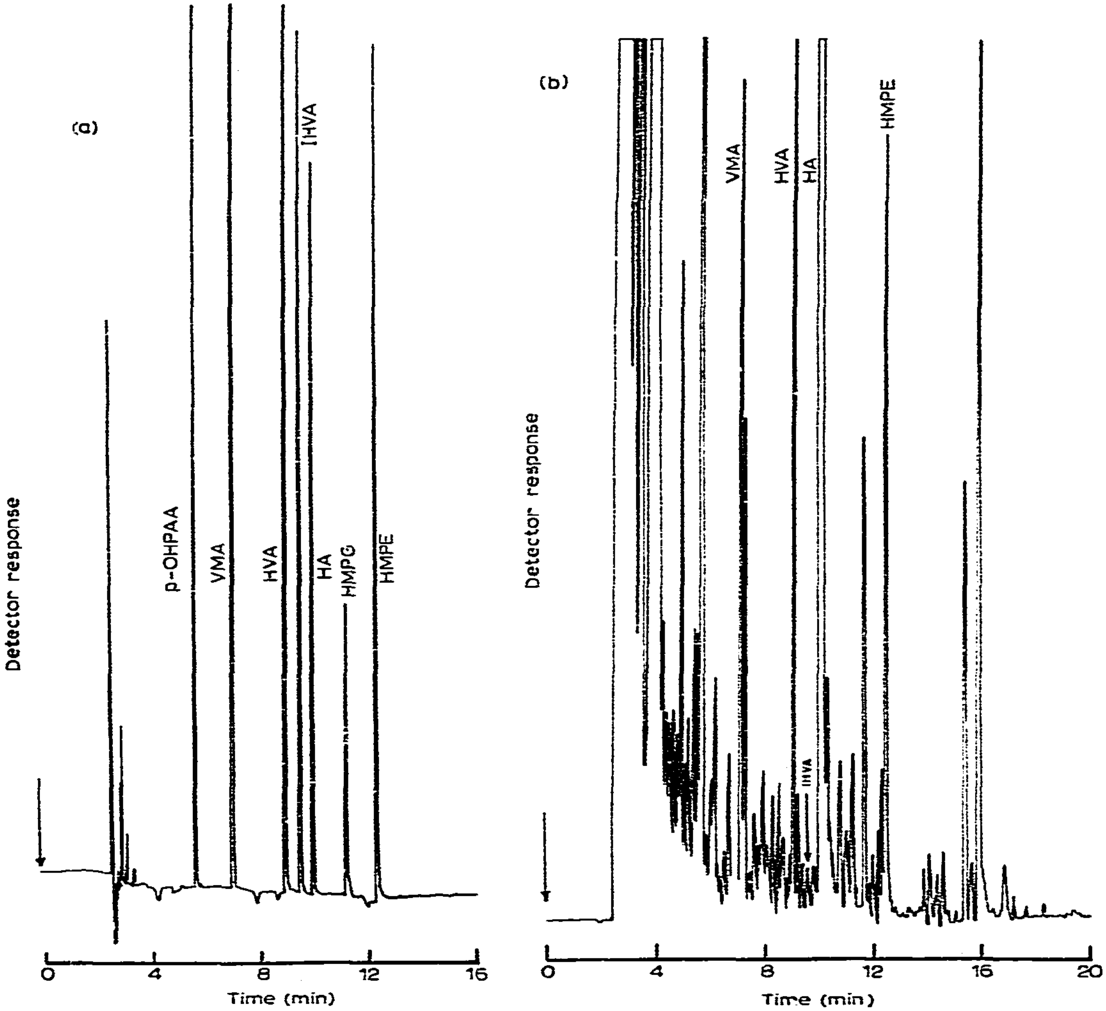

Fig. 2. Separation of trifluoroacetyl-hexafluoroisopropyl ester derivatives on SP-2250 SCOT column. GLC conditions as in Fig. 1. (a) Standard compounds injected: *p*-OHPAA, 4.6 pmol; VMA, 3.4 pmol; HVA, 4.3 pmol; IHVA, 4.5 pmol; hippuric acid (HA), 8.8 pmol; HMPG, 1.4 pmol; HMPE, 13.3 pmol. (b) Urine sample, with internal standard HMPE.

TABLE III

URINARY CONCENTRATIONS (nmol/ml) OF VANILLYLMANDELIC, HOMOVANILLIC AND ISOHOMOVANILLIC ACIDS DETERMINED ON DIFFERENT LIQUID PHASES

GLC conditions as in Fig. 1. $n = 3$.

Liquid phase	VMA	HVA	IHVA
OV-101	4.4	21.3	3.2
OV-225	18.5	22.9	*
QF-1	*	20.3	*
Dexsil 300GC	4.7	21.7	0.6
SP-2250	9.4	42.4	0.5
OV-101 + OV-17	4.8	19.6	*
Dexsil 300GC + OV-17	4.9	19.8	5.7

*Not determined owing to interfering peaks.

chromatogram obtained for the metabolites in a urine sample. The VMA peak was only partially resolved from some unknown peak. Changing the temperature programme did not improve the resolution.

Because of the multiplicity of peaks with urine samples and the possibility of overlap, quantitative recoveries were determined with seven different liquid phases (Table III). VMA gave spuriously high values with OV-225 and SP-2250 because there were obscured peaks with compounds having the same retention times. HVA gave an average value of 20.9 ± 1.26 nmol/ml urine on six liquid phases, but SP-2250 (as shown in Fig. 2b) gave the very high value of 42.4 nmol/ml urine because of an unseen interfering peak. The peak for IHVA was always very small and adjacent peaks made accurate integration difficult.

Fig. 3a shows the separation of standard metabolites on an OV-101 SCOT column and Fig. 3b shows the peaks obtained with a urine sample. Reliable values for VMA and HVA were obtained on this column but interference with the IHVA peak on this column resulted in a spuriously high value (see also Table III). Thus OV-101 could only be used to determine HVA and VMA but not IHVA in urine samples. HMPE was used as internal standard with this stationary phase [28,39]. It was reported that free HMPE occurred naturally in rat urine [54], but none was detected in human urine by these authors or in this work.

The liquid phases OV-225, GE SE-54 and QF-1 gave a satisfactory separation of standard metabolite derivatives, but they were not satisfactory for urine samples. The separation of the standard metabolites on Dexsil 300GC is shown in Fig. 4a and a urine sample in Fig. 4b. HMPE could not be used as an internal standard on this column because of interference by an unknown compound with a retention time of 16.5 min in Fig. 4b. Instead, *p*-OHPPA and *p*-OH-diphenyl were used as internal standards. A constant peak area ratio for these two standard compounds was a good indication that no urine metabolite was present having the same retention times. The values for VMA, HVA and IHVA (Table III) indicated that on Dexsil 300GC there were no interfering peaks leading to spuriously high values. But for IHVA this was not always true and it was necessary to analyze each urine sample on two columns as a check on accuracy of quantitation.

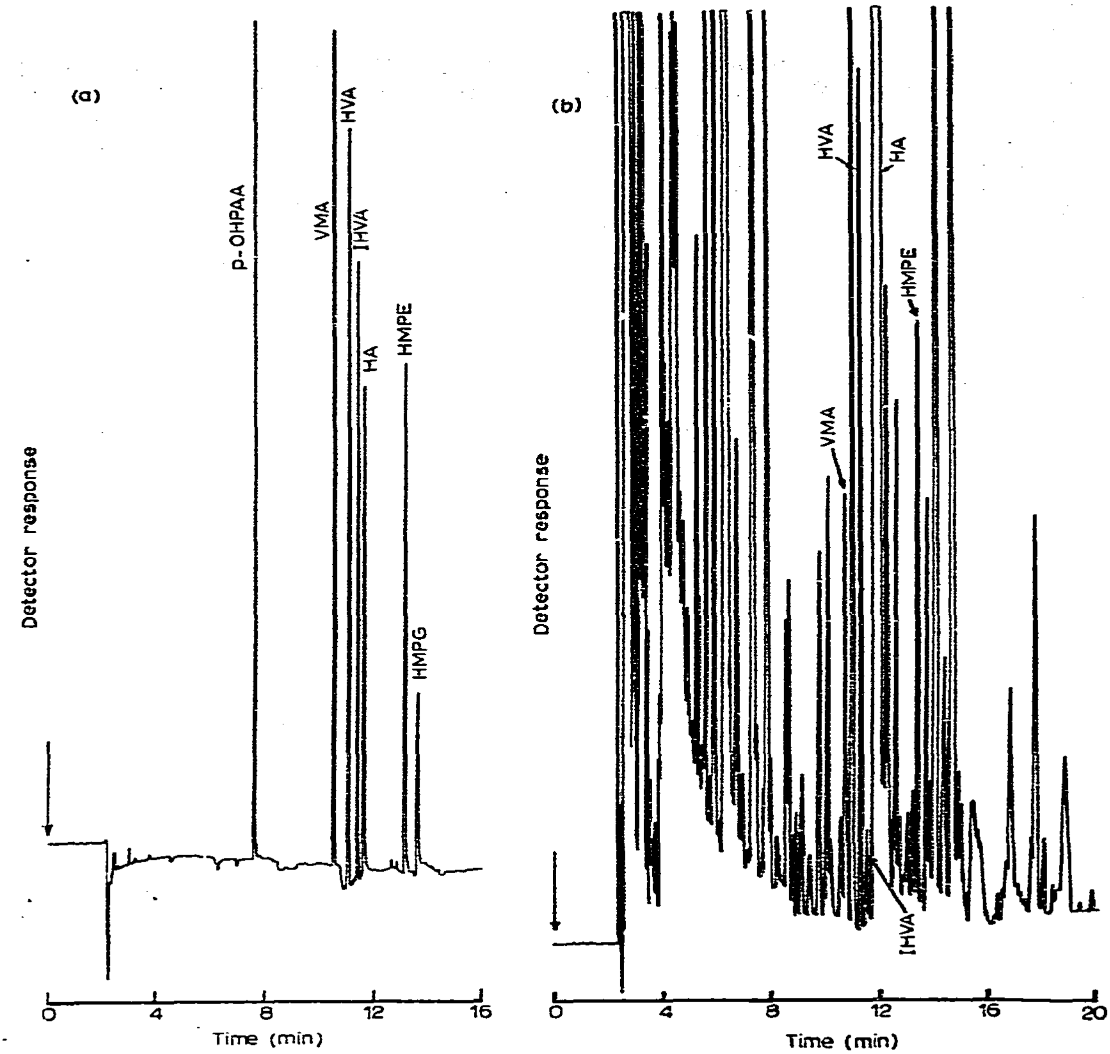

Fig. 3. Separation of trifluoroacetyl-hexafluoroisopropyl ester derivatives on OV-101 SCOT column. GLC conditions as in Fig. 1. (a) Standard compounds; (b) urine sample with internal standard HMPE.

Table IV shows the analysis of VMA, HVA and IHVA in five urine samples of male healthy volunteers obtained with two different GLC columns. The results are given as nmol per ml urine, μg per mg creatinine and mg/day in order to enable full comparison to be made with published results. There is general agreement for the VMA and HVA results on the two columns. IHVA determined on Dexsil 300GC shows values of 0.44—1.01 nmol/ml in urine, whereas on OV-101 the values are falsely high 0.99—3.85 nmol/ml (as expected, see Table III). Our results agree with other published values for HVA (4.05 mg/day) and IHVA (0.1 mg/day) obtained by mass fragmentography [39], HVA (3.30 ± 1.08 S.D. μg per mg creatinine) by GLC—ECD [21], HVA (4.45 ± 0.62 S.E.M. mg/24 h) by fluorimetry [55] and HVA (5.4 mg/24 h) and IHVA (0.05

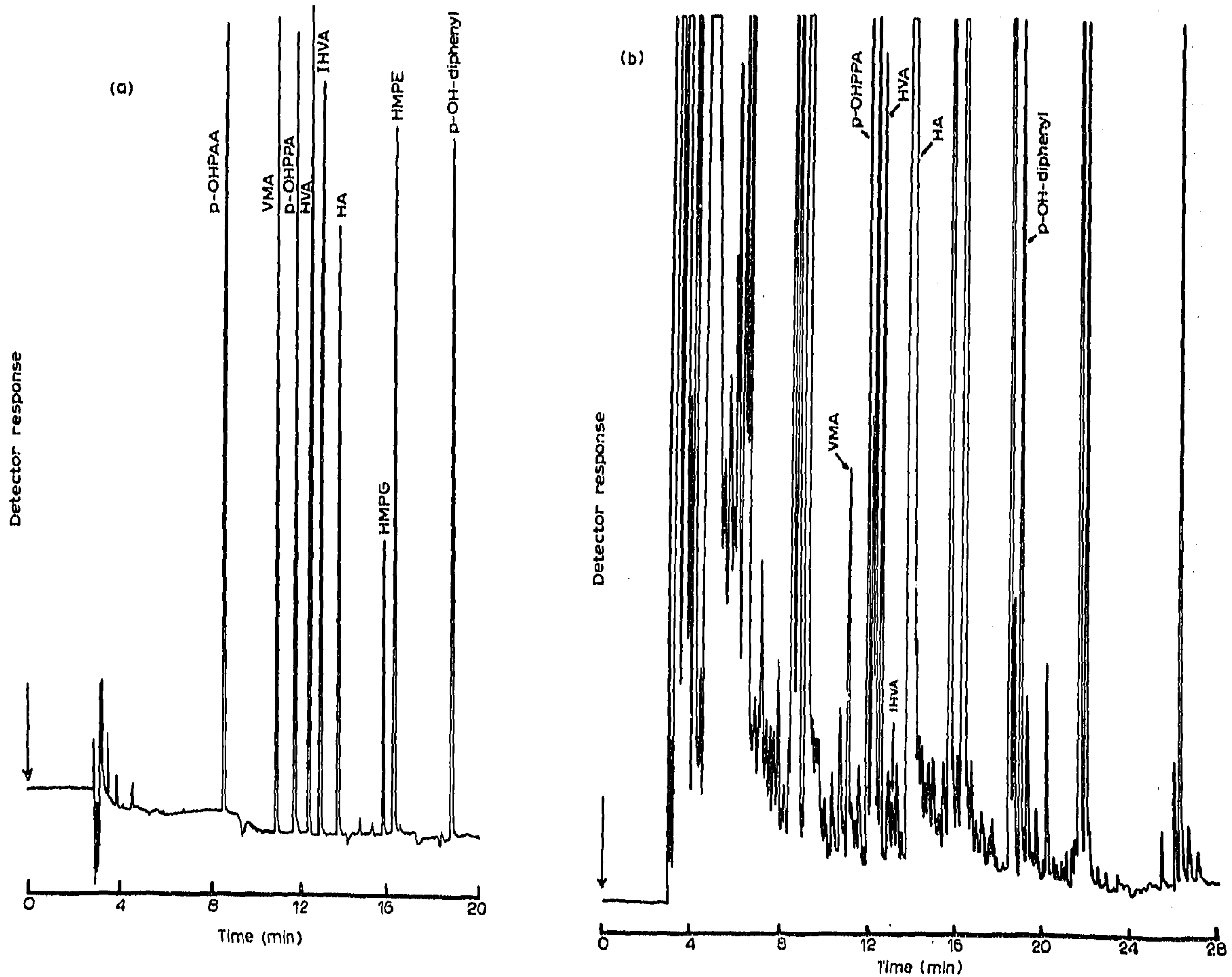

Fig. 4. Separation of trifluoroacetyl hexafluoroisopropyl [illegible]

TABLE IV

VANILLYLMANDELIC, HOMOVANILLIC AND ISOHOMOVANILLIC ACIDS IN URINE DETERMINED WITH TWO GLC LIQUID PHASES

Compound	No.	OV-101			Dexsil 300GC		
		nmol/ml	µg/mg creatinine	mg/day	nmol/ml	µg/mg creatinine	mg/day
VMA	1	15.19	1.73	2.03	13.57	1.54	1.81
	2	13.99	1.86	3.04	15.12	2.01	3.28
	3	5.98	1.43	2.43	5.28	1.27	2.15
	4	14.46	1.89	3.43	15.30	2.00	3.63
	5	10.74	2.02	2.83	9.77	1.85	2.57
HVA	1	33.59	3.51	4.13	33.81	3.54	4.16
	2	39.36	4.81	7.85	40.86	5.00	8.15
	3	16.02	3.53	5.99	15.54	3.42	5.81
	4	44.69	5.37	9.75	44.28	5.33	9.66
	5	23.25	4.03	5.62	25.76	4.65	6.23
IHVA	1	2.38	0.24	0.29	0.59	0.05	0.06
	2	2.36	0.28	0.47	0.85	0.10	0.17
	3	0.99	0.21	0.37	0.44	0.09	0.16
	4	3.85	0.46	0.84	1.01	0.12	0.22
	5	1.83	0.31	0.44	0.65	0.11	0.15

mg/24 h) by paper chromatography and colorimetry [56].

Grob [57] has pointed to the problem of single peaks which may be due to several compounds. This work confirms the real need to check on single peaks obtained from urine samples even with high-efficiency capillary columns. We have tried to meet criticism that methods which are acceptable with pure metabolites should also be applicable to urine samples [58,59].

ACKNOWLEDGEMENT

The authors thank the Ministry of Defence (A.M.T.E.) for financial support.

REFERENCES

1 F.A.J. Muskiet, D.C. Fremouw-Ottevangers, G.T. Nagel, B.G. Wolthers and J.A. de Vries, Clin. Chem., 24 (1978) 2001.

2 F.A.J. Muskiet, H.J. Jeuring, G.T. Nagel, H.W.A. de Bruyn and B.G. Wolthers, Clin. Chem., 24 (1978) 1899.

3 J.J. Pisano, J.R. Crout and D. Abraham, Clin. Chim. Acta, 7 (1962) 285.

4 F. Geissbühler, Clin. Chim. Acta, 26 (1969) 231.

5 Z. Kahane and P. Vestergaard, Clin. Chim. Acta, 35 (1971) 49.

6 F.W. Sunderman, Jr., P.D. Cleveland, P.D. Law and F.W. Sunderman, Amer. J. Clin. Pathol., 34 (1960) 293.

7 I. Sankoff and T.L. Sourkes, Can. J. Biochem. Physiol., 41 (1963) 1381.

8 J.A. Preston, Clin. Chem., 13 (1967) 19.

9 J.S. Annino, M. Lipson and L.A. Williams, Clin. Chem., 11 (1965) 905.

10 F. Zoghbi, J. Emerit, J. Fermanian, O. Bousquet, J.C. Legrand, A. Sarrazin and P. Desgrez, Biomedicine, 27 (1977) 37.

11 A. Yoshida, M. Yoshioka, T. Yamazaki, T. Sakai and Z. Tamura, Clin. Chim. Acta, 73 (1976) 315.

12 A. Yoshida, M. Yoshioka, T. Tanimura and Z. Tamura, J. Chromatogr., 116 (1976) 240.
13 I. Molnar and Cs. Horváth, Clin. Chem., 22 (1976) 1497.
14 J.G. Flood, M. Granger and R.B. McComb, Clin. Chem., 25 (1979) 1234.
15 T.G. Rosano and H.H. Brown, Clin. Chem., 25 (1979) 550.
16 C.M. Williams and M. Greer, Clin. Chim. Acta, 11 (1965) 495.
17 D.D. Clarke, S. Wilk and S.E. Gitlow, J. Gas Chromatogr., 4 (1966) 310.
18 Z. Kahane, J.H. Mowat and P. Vestergaard, Clin. Chim. Acta, 26 (1969) 307.
19 T.J. Sprinkle, A.H. Porter, M. Greer and C.M. Williams, Clin. Chim. Acta, 25 (1969) 409.
20 S. Addanki, E.R. Hinnenkamp, R.L. Gombos and J.F. Sotos, Clin. Chem., 18 (1972) 691.
21 S.W. Dziedzic, L.M. Bertani, D.D. Clarke and S.E. Gitlow, Anal. Biochem., 47 (1972) 592.
22 S.W. Dziedzic, L.B. Dziedzic and S.E. Gitlow, J. Lab. Clin. Med., 82 (1973) 829.
23 M. Cagnasso and P.A. Biondi, Ital. J. Biochem., 23 (1974) 345.
24 S.W. Dziedzic and S.E. Gitlow, J. Neurochem., 22 (1974) 333.
25 N. Narasimhachari, K. Leiner, J.M. Plaut and R.L. Lin, Clin. Chim. Acta, 50 (1974) 334.
26 E. Watson and S. Wilk, Anal. Biochem., 59 (1974) 441.
27 J.M.D. Pearson and D.F. Sharman, Brit. J. Pharmacol., 53 (1975) 143.
28 S. Yoshiue, M. Yoshioka and Z. Tamura, Chem. Pharm. Bull., 23 (1975) 3040.
29 S. Addanki, E.R. Hinnenkamp and J.F. Sotos, Clin. Chem., 22 (1976) 310.
30 W. Bernhardt and B. Weisner, J. Clin. Chem. Clin. Biochem., 14 (1976) 443.
31 A. Lun, R.R. Hacker, R.G. Brown and J.F. Hurnik, Anal. Biochem., 73 (1976) 267.
32 S.K. Wadman, D. Ketting and P.A. Voûte, Clin. Chim. Acta, 72 (1976) 49.
33 M:A. Brewster, D.H. Berry and M. Moriarty, Clin. Chem., 23 (1977) 2247.
34 J. Hyttel, J. Neurochem., 28 (1977) 227.
35 M.A. Brewster and D.H. Berry, Clin. Chem., 24 (1978) 843.
36 D.S. Walter, P.W. Dettmer, K. Taylor, G.M. Shilcock and A. Cowan, J. Neurochem., 30 (1978) 929.
37 B. Sjöquist, B. Lindström and E. Änggård, J. Chromatogr., 105 (1975) 309.
38 B. Sjöquist and B. Johansson, J. Neurochem., 31 (1978) 621.
39 S. Takahashi, M. Yoshioka, S. Yoshiue and Z. Tamura, J. Chromatogr., 145 (1978) 1.
40 M. Novotny, M. Maskarinec, A. Steverink and R. Farlow, Anal. Chem., 48 (1976) 468.
41 S. Nygren and P.E. Mattsson, J. Chromatogr., 123 (1976) 101.
42 G. Schomburg, R. Dielmann, H. Husmann and F. Weeke, J. Chromatogr., 122 (1976) 55.
43 E. Evrard, C. Razzouk, M. Roberfroid, M. Mercier and M. Bal, J. Chromatogr., 161 (1978) 97.
44 C. Razzouk, E. Evrard, G. Lhoest, M. Roberfroid and M. Mercier, J. Chromatogr., 161 (1978) 103.
45 H. Brötell, N.-O. Ahnfelt, H. Ehrsson and S. Eksborg, J. Chromatogr., 176 (1979) 19.
46 F.A. Fitzpatrick, D.A. Stringfellow, J. Maclouf and M. Rigaud, J. Chromatogr., 177 (1979) 51.
47 P.B. Hawk, B.L. Oser and W.H. Summerson, Practical Physiological Chemistry, 12th ed., J. & A. Churchill, London, 1947, p. 839.
48 W.A. Aue and S. Kapila, J. Chromatogr. Sci., 11 (1973) 255.
49 P. Devaux and G. Guiochon, J. Chromatogr. Sci., 7 (1969) 561.
50 P. Devaux and G. Guiochon, J. Gas Chromatogr., 5 (1967) 341.
51 H.J. van de Wiel and P. Tommassen, J. Chromatogr., 71 (1972) 1.
52 W. Jennings, Gas Chromatography with Glass Capillary Columns, Academic Press, New York, San Francisco, London, 1978, p. 84.
53 M. Verzele and P. Sandra, J. High Resol. Chromatogr. Chromatogr. Commun., 2 (1979) 303.
54 F. Karoum, H. Lefèvre, L.B. Bigelow and E. Costa, Clin. Chim. Acta, 43 (1973) 127.
55 E.R.B. Smith and H. Weil-Malherbe, Clin. Chim. Acta, 35 (1971) 505.
56 E. Comoy and C. Bohuon, Clin. Chim. Acta, 35 (1971) 369.
57 K. Grob, Chromatographia, 8 (1975) 423.
58 A. Lanser, K. Oldenziel, C. Pronk and H.C. Lequin, Clin. Chim. Acta, 50 (1974) 293.
59 H.-U. Melchert and H. Hoffmeister, Clin. Chem., 24 (1978) 842.