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Note

Comparison of high-performance liquid chromatographic, gas chromatographic—mass spectrometric, and fluorometric methods for the determination of homovanillic acid and 5-hydroxyindoleacetic acid in human cerebrospinal fluid

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A number of analytical methods have been employed to determine 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) in cerebrospinal fluid (CSF). These acid metabolites have been measured using fluorometry [1—9], gas chromatography—mass spectrometry (GC—MS) [10—13], and liquid chromatography with flow-through fluorometric [14—17], amperometric [18, 19] or combined fluorometric/amperometric [20] detectors. Relatively few of the procedures have been compared to alternative methods in a systematic fashion. The HPLC—fluorometric methods for 5HIAA have been compared to a GC—MS technique [17] and an amperometric method [20],

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and one of the fluorometric procedures for 5HIAA has been compared to a GC—MS procedure [21]. Two studies have compared the fluorometric determination of HVA to GC—MS assays [21, 22]. Here we present a four-way comparison of HPLC, fluorometric, and GC— MS methods for the determination of 5HIAA and HVA in human CSF.

METHODS

Lumbar CSF was obtained from subjects after administration of probenecid. Fluid from the first approx. 12 ml was mixed and 2—3-ml aliquots frozen without addition of preservative. Samples were stored at —80°C and analysed within two months.

HPLC

The CSF sample was briefly centrifuged (1 min, ca. 10,000 *g*) and 10—25 μl then injected directly into the HPLC system. The compounds of interest, along with tyrosine and tryptophan, were separated on a 10-μm average particle size 30 × 0.39 cm μBondapak C_{18} reversed-phase column. HVA was detected amperometrically while 5HIAA was measured with both amperometry and fluorometry [20].

GC—MS

One-ml samples were extracted with ethyl acetate after acidification and the addition of 100 ng/ml of dideuterated 5HIAA and HVA. The organic phase was removed and evaporated in a stream of nitrogen. The residue was then reacted with 100 μl of pentafluoropropanol—pentafluoropropionic anhydride (20:80). Following evaporation under nitrogen the residue was dissolved in 50 μl of hexane and 2—5 μl injected onto a ca. 2 m 3% OV-17 column. After separation and selected monitoring of ions derived from the proteo and dideutero (HVA-d_2 and 5HIAA-d_2) species, quantitation was performed by interpolation from a standard curve constructed from injection of known amounts of proteo and d_2 standards (0—500 ng d_0/100 ng d_2) [12]. The method was performed in two different laboratories (GC—MS-1 and GC—MS-2) using similar sample preparation procedures. Finnigan 3300 GC—MS instruments (electron impact mode) were used in both laboratories.

Fluorometric

The fluorometric (FLUOR) methods for 5HIAA [1, 6] and HVA [5, 10] both involve acidification, extraction into an organic solvent, and back-extraction. Fluorescence of 5HIAA is mesaured after addition of acid. HVA is determined fluorometrically after oxidation to a dimeric fluorophore using ferricyanide.

Samples were run in duplicate for all methods, except for the HPLC assay where the fluorometric and amperometric 5HIAA values were averaged. Samples were collected over a 14-month period and run with the normal workload of the laboratories.

RESULTS AND DISCUSSION

In Tables I and II the methods are compared, with the population means, correlation values and average percent differences being listed. Fig. 1a—f presents selected correlation diagrams obtained from the cross-comparisons.

TABLE I

STATISTICAL ANALYSIS OF CSF 5HIAA COMPARISON

Figure	Methods (y vs. x)	Average % difference	Mean y/x	r	Equation ($y = mx + b$)		n
					m	b	
1a	FLUOR vs. HPLC	16.5	106 /106	0.85	0.66	37	38
	GC—MS-1 vs. HPLC	18.2	97.6/ 92.4	0.74	0.75	29	14
	GC—MS-2 vs. HPLC	19.4	107 /100	0.65	0.67	40	13
	GC—MS-1 vs. FLUOR	17.7	97.6/100	0.71	0.70	27	14
	GC—MS-2 vs. FLUOR	16.3	107 /103	0.68	0.67	38	13
	GC—MS-1 vs. GC—MS-2	17.4	99.5/107	0.74	0.72	22	11
1b	HPLC FLUOR vs. EC	8.48	111 /115	0.95	0.94	10	33

TABLE II

STATISTICAL ANALYSIS OF CSF HVA COMPARISON

Figure	Methods (y vs. x)	Average % difference	Mean y/x	r	Equation ($y = mx + b$)		n
					m	b	
1c	FLUOR vs. HPLC	30.8	171/223	0.77	0.67	21	38
1d	GC—MS-2 vs. HPLC	11.1	232/223	0.94	1.04	0.50	37
	GC—MS-1 vs. HPLC	10.8	191/198	0.87	0.77	37.8	18
1e	GC—MS-1 vs. GC—MS-2	8.11	191/199	0.96	0.94	3.6	18
	GC—MS-1 vs. FLUOR	22.8	191/166	0.72	0.66	81	18
1f	GC—MS-2 vs. FLUOR	34.8	232/171	0.69	0.88	82	38

For the determination of 5HIAA good agreement was observed between the population means (see Table I); however, the average percent differences of 16—19% and the low correlation coefficients (0.65—0.85) indicate only fair agreement across methods for individual samples. The small range of values for the samples (50—150 ng/ml) — and the small number of samples analysed by the GC—MS methods — account in part for the low r values. The high average percent difference between the two GC—MS methods (17.4%) and the high correlation (0.95) and low average absolute percent difference (8.48%) seen when the HPLC—amperometric and HPLC—fluorometric values were compared (Fig. 1b) suggest that the HPLC value is the most accurate estimation. This is further supported by the high correlation (0.99) of the HPLC—fluorometric and —amperometric methods for 5HIAA obtained in a previous comparison [20]. It should be pointed out this conclusion regarding the relative accuracies of the 5HIAA determinations is meant to apply to this study only.

For the analysis of HVA (Table II and Fig. 1c—f) the group means determined by fluorometry were significantly ($p < 0.01$) lower than those determined by HPLC (171 vs. 223), or the GC—MS methods (166 vs. 191 and 171 vs. 232). This underestimation of HVA has been previously reported [21, 22]

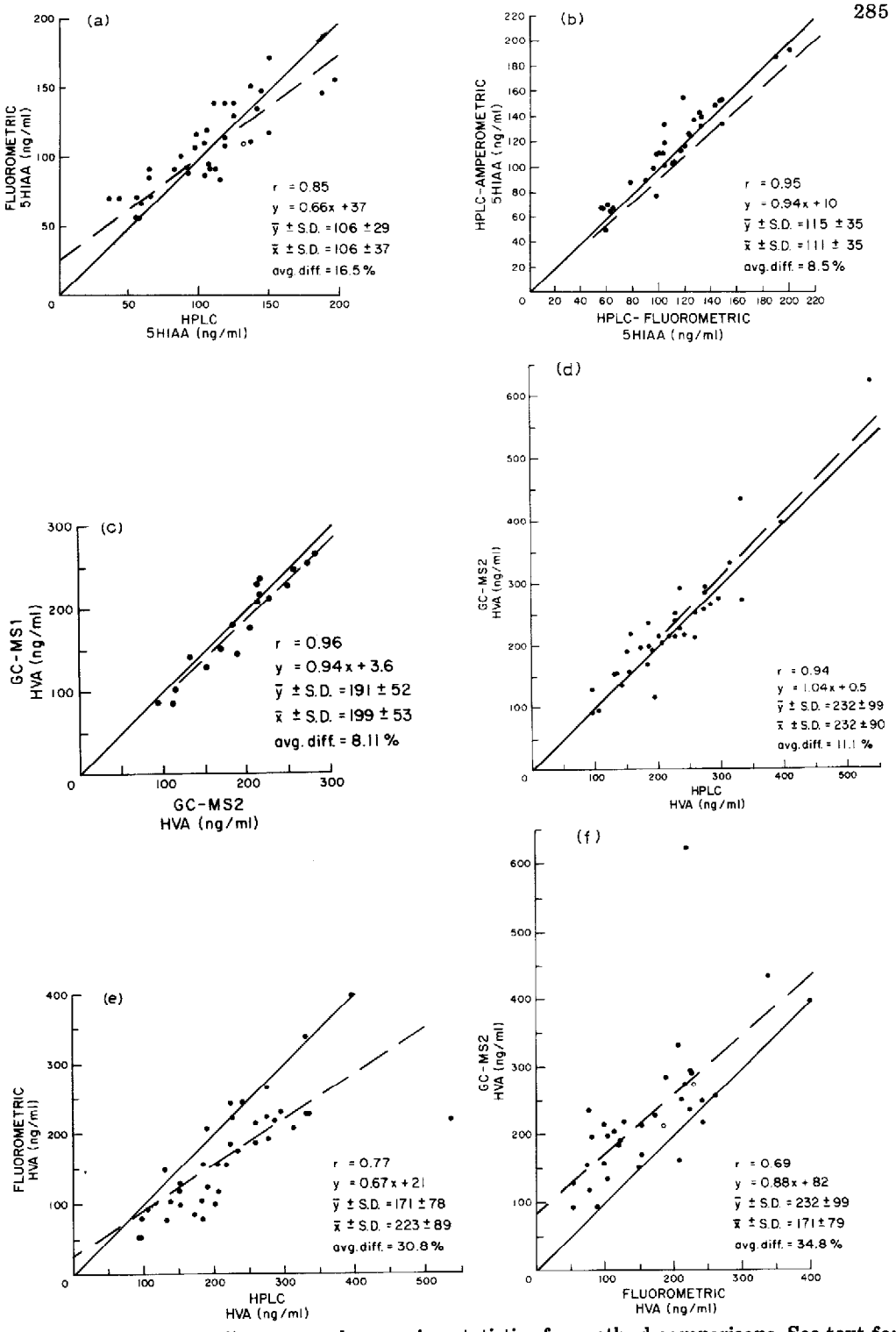

Fig. 1. (a—f) Scatter diagrams and regression statistics for method comparisons. See text for abbreviations and details of methods used. Points plotted as open circles comprise two samples. The regression line has been plotted as a broken line, while the solid line is the line of identity ($x = y$).

and has been suggested to be due to inefficient conversion of HVA to the dimeric fluorophore. The HPLC and GC—MS methods for HVA compared favorably to one another with population means differing by less than 5% and average absolute individual sample differences of 8—11%. While most of the 5HIAA and HVA levels were significantly elevated above the normal adult range (10—100 ng/ml) due to the administration of probenecid we believe the results of a comparison in an untreated population would be largely similar. However, the higher detection limits obtained for HVA and 5HIAA using the fluorometric (FLUOR) methods might compromise their use for less concentrated samples.

CONCLUSION

We have compared three different instrumental techniques for the determination of HVA and 5HIAA in human CSF. Good agreement between the HPLC and GC—MS methods was observed for HVA, as measured by correlation coefficients, population means and average individual sample differences. The fluorometric method for HVA gave significantly lower values and was less correlated. Population means for 5HIAA were similar, however agreement across methods was only fair for individual samples.

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