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Chromatographic method verification by means of three-dimensional, multivariate visualization

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ABSTRACT

Using free-fatty-acid model systems, principal-component analysis as automated in Justice Innovations' Chrom Perfect and Echo Data's DataMax software was used to correlate, visualize, and verify the relationships of nine system-suitability parameters. Many-dimensional data were mapped by relaxing the usual 90° axes and by using non-orthogonal vectors. The arccosines of the correlation coefficients were used as the vector angles.

Two types of matrices were non-orthogonally mapped: (1) chromatography system-suitability parameters as the vectors and chromatography components as the objects; (2) chromatography parameters for each component as the vectors and methods as the objects. The first type of matrix was found to be suitable for clustering similar chromatography parameters, for visualizing a single chromatogram, for comparing replicate results, and for verifying expected chromatography correlations.

The second type of matrix had the same results as a chromatographic response function in that a chromatogram was reduced to a single value, or coordinate in the case of a non-orthogonal map. Practical applications include the visualization of multidetector, multiwavelength, multicolumn data. Two examples were demonstrated: (1) chromatography method evaluation by non-orthogonally mapping a 4 × 35 matrix representing resolution, theoretical plates, tailing factor, α, and capacity factor for a seven-component mixture; (2) identification of optimum concentration, time-course effects, and replicate reproducibility using a 180 × 6 matrix representing 180 multicomponent chromatograms. In each case, Echo Data's DataMax software was found to facilitate more rapid screening than currently possible by the use of computerized statistical analysis alone.

INTRODUCTION

Computerized mathematical approaches which simultaneously account for all chromatography parameters and which improve on the near inability of the human mind to understand the complex relationships between six chromatography parameters have been described by Guiochon and co-workers [1,2]. Chromatography visualization techniques have typically been limited to orthogonal plotting of one variable for each dimension thus restricting

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the number of variables that can be simultaneously viewed to three. Brown and co-workers [3,4] have described a visualization technique that maps many-dimensional data in a three-dimensional reduced space by the use of non-orthogonal vectors. The usual 90° axes between vectors are relaxed and instead the arccosines of the correlation coeffients are used as the angles.

The objective of our investigation was to examine whether multivariate, non-orthogonal mapping as described by Brown *et al.* [5] and as automated in Echo Data's DataMax computer software could be useful in visualizing the relationships in multicomponent chromatography data. The suitability of non-orthogonal mapping as applied to chromatography data was evaluated using a seven-component model system. Six free-fatty acids typically found in cow rumen and an internal standard were separated by one temperature-programmed and by four isothermal gas chromatography methods. Isothermal gas chromatography with flame ionization detection was chosen for this initial confirmatory study because of the ease of determining column void volumes. Berridge [6] has noted that computerized capacity factor (k') calculations are limited by the difficulty of precisely determining column void volume and that a technique which could accurately substitute retention time for k' would be useful. The gas chromatography methods were designed solely to test the visual accuracy of DataMax software using typical system-suitability parameters and were not intended to be rigorous contributions.

The temperature-programmed data were derived from ongoing *in vitro* bovine ruminal studies by Kung and Hession [7] evaluating whether bacterial inoculations in lieu of antibiotic treatment could enhance ruminal fermentations by increasing concentrations of acetic and propionic acids. Ionophore lysocellin, a divalent polyether antiboitic, has been shown to improve daily gain and feed efficiency when fed to growing cattle presumably by increased concentrations of short-chain fatty acids [8]. The heretofore unpublished results presented in this paper included 180 chromatograms consisting of 5 replicated treatments, 2 protocols (5 bacterial concentrations, 12 and 24 h fermentation times, 2 diets, 4 replicates injected twice) and corresponding zero-hour controls (5 bacterial concentrations, 2 diets, 2 injections). A large data set of this type generally requires time-consuming, computerized statistical analysis [9] for interpretation. A visual technique which could rapidly screen and verify biological activity would be highly useful.

EXPERIMENTAL

The gas chromatography system consisted of a Hewlett-Packard 5890 Series II gas chromatography (Valley Forge, PA, USA) equipped with a HP 7673A autosampler, flame ionization detector (250°C), and the Justice Innovations' Chrom Perfect data system (Palo Alto, CA, USA). The test mixture consisted of seven short-chain, free-fatty acid standards (Fisher Scientific, Pittsburgh, PA, USA) dissolved in 5% metaphosphoric acid in the following concentrations: 50 m*M* acetic acid, 30 m*M* propionic acid, 4 m*M* isobutyric acid, 8 m*M* butyric acid, 4 m*M* isovaleric acid, 4 m*M* *n*-valeric acid and 4 m*M* isocaproic acid as the internal standard.

Samples (1 μl) were injected on a Hewlett-Packard Carbowax 20M capillary column (10 m × 0.53 mm I.D., 1.33 μm film thickness) using a 7:1 split ratio with an injection temperature of 200°C. Methods 1–4 were identical except for the following variations in isothermal oven temperature and duration time: 100°C (10 min), 90°C (10 min), 80°C (12 min) and 70°C (25 min) for methods 1, 2, 3 and 4, respectively. For the 13.5-min temperature-programmed method, the initial temperature of 70°C was held for 1 min and ramped to 100°C at 5°/min. Helium carrier gas was set at 10.6 ml/min at 90°C.

Chromatographic results in ASCII format were prepared using Justice Innovations' ReportWrite-Plus and RESULTS software, and the multivariate, non-orthogonal maps were prepared using Echo Data's DataMax software (Orem, UT, USA) on an 80486DX microcomputer (PC Science, Morris Plains, NJ, USA), equipped with Microsoft MS-DOS 5.0 and a Hewlett-Packard LaserJet IIP printer. The 180 samples in the temperature-programmed study were prepared using the methodology of Kung *et al.* [8] and consisted of 20 replicated treatment groups (160 chromatograms representing 5 bacterial concentrations, 12 and 24 h fermentation time, 2 diets, 4 replicates injected twice) and of 10 unreplicated controls (20 chromatograms representing 5 bacterial concentrations, 2 diets, zero fermentation time, injected twice).

The following nine system-suitability parameters specified by the US Pharmacopeia [10] and by the Food and Drug Administration [11], were automatically calculated by the Chrom Perfect and ReportWritePlus computer software using the equations described by Justice [12]: resolution (RS), α (ALPHA), theoretical plates (PLATES), 10 and 5% peak tailing factors (TF5%, TF10%), 10 and 5% peak skew (Skew 10%, Skew 5%), peak half-width (HFWD), and capacity factor, k' (K'). Normalized values for the nine system-suitability parameters were non-orthogonally mapped by the DataMax software using the principal-component equations described by Brown and Fluckiger [13]. For the temperature-programmed method, component concentrations for 180 chromatograms were merged into a single ASCII file by the RESULTS software described by Justice [14] and were non-orthogonally mapped by the DataMax software.

RESULTS

System-suitability parameters

In Fig. 1, nine system-suitability parameters for each of the seven components separated by the 100°C isothermal method were non-orthogonally mapped using the 7 × 9 matrix, correlation calculations, and X–Y–Z object coordinates given in Table

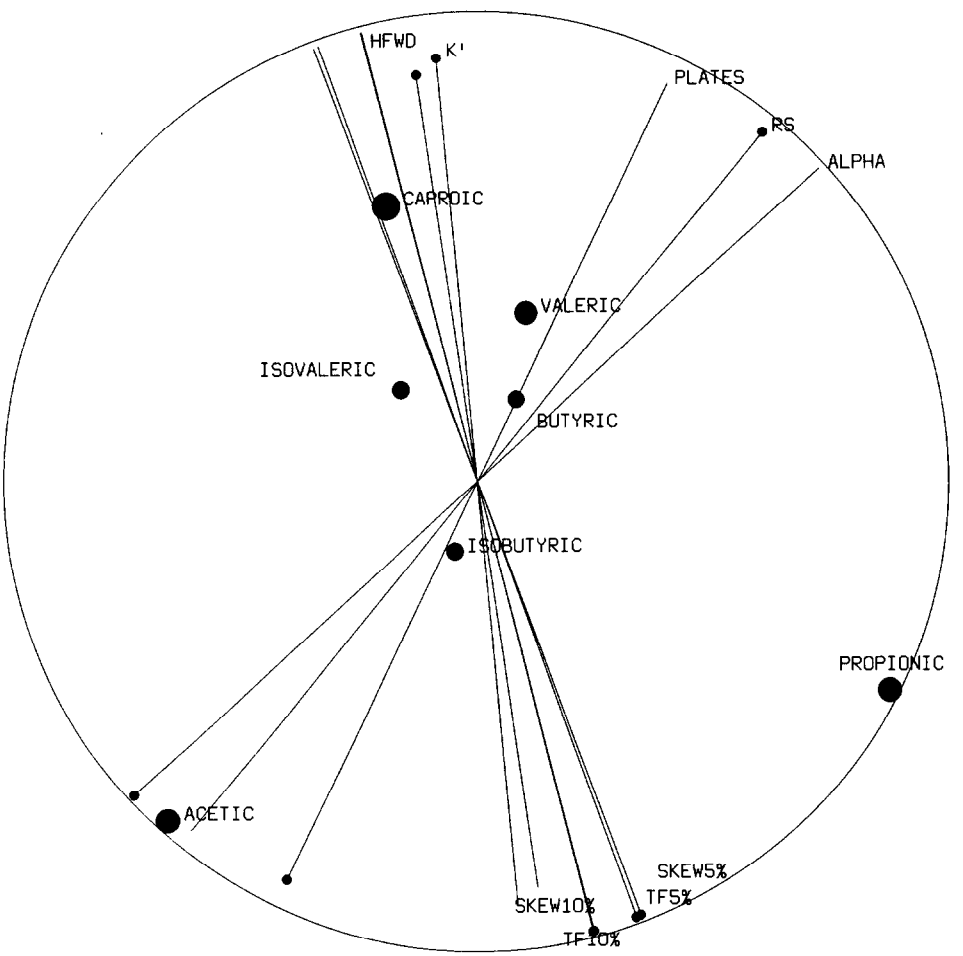

Fig. 1. System-suitability parameters using capacity factor. Non-orthogonal map of 100°C isothermal gas chromatography method as described in Experimental section using 7 × 9 matrix and correlation calculations in Table I. The ninth vector was k' (capactiy factor).

TABLE I

SYSTEM-SUITABILITY PARAMETERS FOR 100°C METHOD

7 × 10 matrix and correlation calculations for 100°C isothermal gas chromatography method visualized in Figs. 1 and 2. The ninth vector was capacity factor and retention time for Figs. 1 and 2, respectively.

NAME,	RS,	ALPHA,	PLATES,	TF10%,	TF5%,	SKEW10%,	SKEW5%,	HFWD,	K',	RT,
ACETIC ,	0.00,	0.00,	3834.0,	1.41,	1.42,	1.82,	1.84,	0.029,	4.17,	0.77,
PROPIONIC ,	6.02,	1.52,	6195.0,	1.45,	1.51,	1.90,	2.03,	0.033,	6.28,	1.09,
ISOBUTYRIC ,	2.58,	1.16,	6622.0,	1.26,	1.27,	1.52,	1.53,	0.036,	7.28,	1.24,
BUTYRIC ,	5.72,	1.36,	7328.0,	1.14,	1.18,	1.27,	1.37,	0.045,	9.89,	1.63,
ISOVALERIC ,	4.14,	1.24,	7218.0,	1.13,	1.16,	1.26,	1.32,	0.055,	12.22,	1.98,
VALERIC ,	7.00,	1.43,	7353.0,	1.13,	1.16,	1.27,	1.32,	0.075,	17.39,	2.76,
CAPROIC ,	6.88,	1.41,	7033.0,	1.06,	1.09,	1.11,	1.18,	0.107,	24.50,	3.83,

Total variance accounted for:
In one dimension: 75.659%
In two dimensions: 91.760%
In three dimensions: 99.256%

Correlations between Attributes:

	RS	ALPHA	PLATES	TF10%	TF5%	SKEW10%	SKEW5%	HFWD	K'
RS	1.0000	0.8944	0.7984	-0.5365	-0.4494	-0.5354	-0.4372	0.6520	0.6945
ALPHA	0.8944	1.0000	0.9031	-0.4698	-0.3880	-0.4689	-0.3800	0.4369	0.4979
PLATES	0.7984	0.9031	1.0000	-0.7570	-0.7012	-0.7547	-0.6954	0.5226	0.5916
TF10%	-0.5365	-0.4698	-0.7570	1.0000	0.9938	0.9998	0.9915	-0.7839	-0.8171
TF5%	-0.4494	-0.3880	-0.7012	0.9938	1.0000	0.9931	0.9998	-0.7653	-0.7945
SKEW10%	-0.5354	-0.4689	-0.7547	0.9998	0.9931	1.0000	0.9907	-0.7838	-0.8164
SKEW5%	-0.4372	-0.3800	-0.6954	0.9915	0.9998	0.9907	1.0000	-0.7631	-0.7919
HFWD	0.6520	0.4369	0.5226	-0.7839	-0.7653	-0.7838	-0.7631	1.0000	0.9964
K'	0.6945	0.4979	0.5916	-0.8171	-0.7945	-0.8164	-0.7919	0.9964	1.0000

Attribute Coordinates: Comunalities:

Attribute Name	X	Y	Z	1 D	2 D	3 D	Total
RS	-0.74	0.61	-0.21	0.549	0.373	0.044	0.9657
ALPHA	-0.66	0.73	0.11	0.442	0.532	0.012	0.9864
PLATES	-0.85	0.41	0.33	0.716	0.168	0.112	0.9958
TF10%	0.96	0.24	-0.15	0.915	0.060	0.023	0.9975
TF5%	0.93	0.34	-0.16	0.859	0.113	0.027	0.9996
SKEW10%	0.96	0.25	-0.15	0.914	0.060	0.022	0.9964
SKEW5%	0.92	0.35	-0.17	0.852	0.120	0.028	0.9991
HFWD	-0.87	-0.13	-0.48	0.749	0.016	0.231	0.9959
K'	-0.90	-0.08	-0.42	0.813	0.007	0.177	0.9966

Object Coordinates:

Data Point Name	X	Y	Z
ACETIC	0.720	0.659	-0.194
PROPIONIC	0.446	-0.873	-0.199
ISOBUTYRIC	0.150	0.049	0.311
BUTYRIC	-0.176	-0.084	0.353
ISOVALERIC	-0.195	0.163	0.274
VALERIC	-0.357	-0.106	-0.100
CAPROIC	-0.587	0.193	-0.446

I. Parameter axes were based on interparameter correlations. Since k' (12 o'clock in Fig. 1) positively correlated with peak width at half height (0.9964), both parameters were plotted on nearly identical axes. Axes scales were based on normalized differences from the mean with high values corresponding to axes labels. For example, the high, midpoint and low values for resolution at 2 o'clock in Fig. 1 were: -1, 0 and $+1$ corresponding to 9.2, 4.6 and 0, respectively. The data point with the largest deviance from the mean was plotted at either the high or low ends of the axis. Clockwise starting at 1 o'clock, plates, resolution and α were highly correlated and were plotted on similar axes. The four asymmetry factors at 5 o'clock were highly correlated to each other and were negatively correlated to HFWD and k'. The negative correlation was visually represented by plotting asymmetry factors nearly 180° from HFWD as determined by the arccosines of the correlation coefficients. By relaxing the usual 90° orientation, all nine parameters were visualized in three dimensions.

In Fig. 2, normalized numerical values for each component were visualized by the use of orthogonal lines from propionic acid (4 o'clock) to each of the nine vectors. Propionic acid exhibited high asymmetry, low retention time and HFWD, and nearly mean plates, resolution and α. Although retention

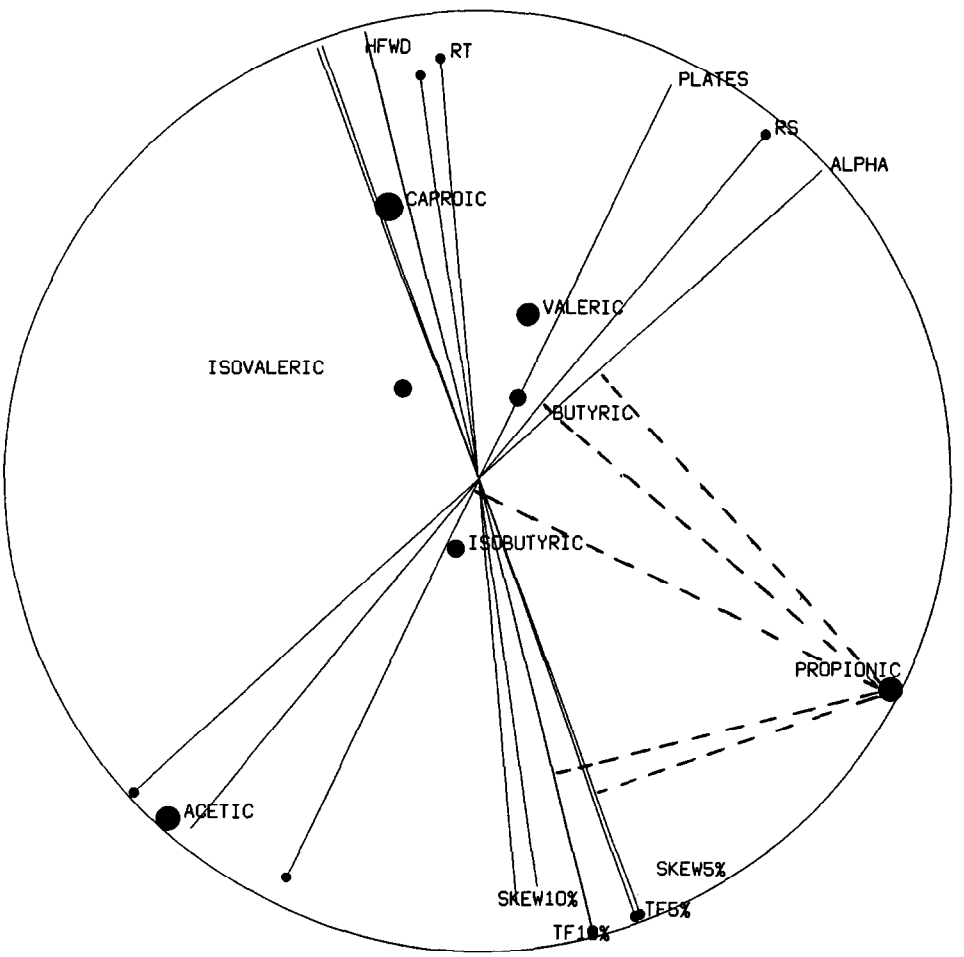

Fig. 2. System-suitability parameters using retention time. Non-orthogonal map of 100°C isothermal gas chromatography method as described in experimental section using 7 × 9 matrix in Table I. The ninth vector was retention time (RT). The perpendicular dotted lines from propionic acid (4 o'clock) to each of the nine vectors aid in visualizing the normalized numerical value for each component.

time was substituted for k' as the ninth vector, Figs, 1 and 2 were nearly identical because of the use of normalized data.

Method optimization

The utility of this visualization approach for method optimization was investigated in Fig. 3 in which resolution and plates for four isothermal temperatures for seven peaks were non-orthogonally mapped using the 4 × 14 matrix in Table II. Resolution values for all four methods were highly correlated and were plotted on the same axes at 1 o'clock. Plates for each peak were not correlated and were plotted on axes ranging from 2 o'clock to 5 o'clock. M1–100 DEGREES (7 o'clock) exhibited the lowest resolution for all peaks and the lowest plates for acetic and propionic acids. M3–80 DEGREES (2 o'clock) exhibited the highest plates for acetic and propionic acids and nearly mean values for all other parameters. M4–70 DEGREES and M3–80 DEGREES appeared to exhibit optimum resolution for this limited test.

In Fig. 4, a 4 × 35 matrix consisting of five optimization parameters (resolution, plates, α, k' and tailing factor at 10% peak height) for seven components was non-orthogonally mapped. k', α and res-

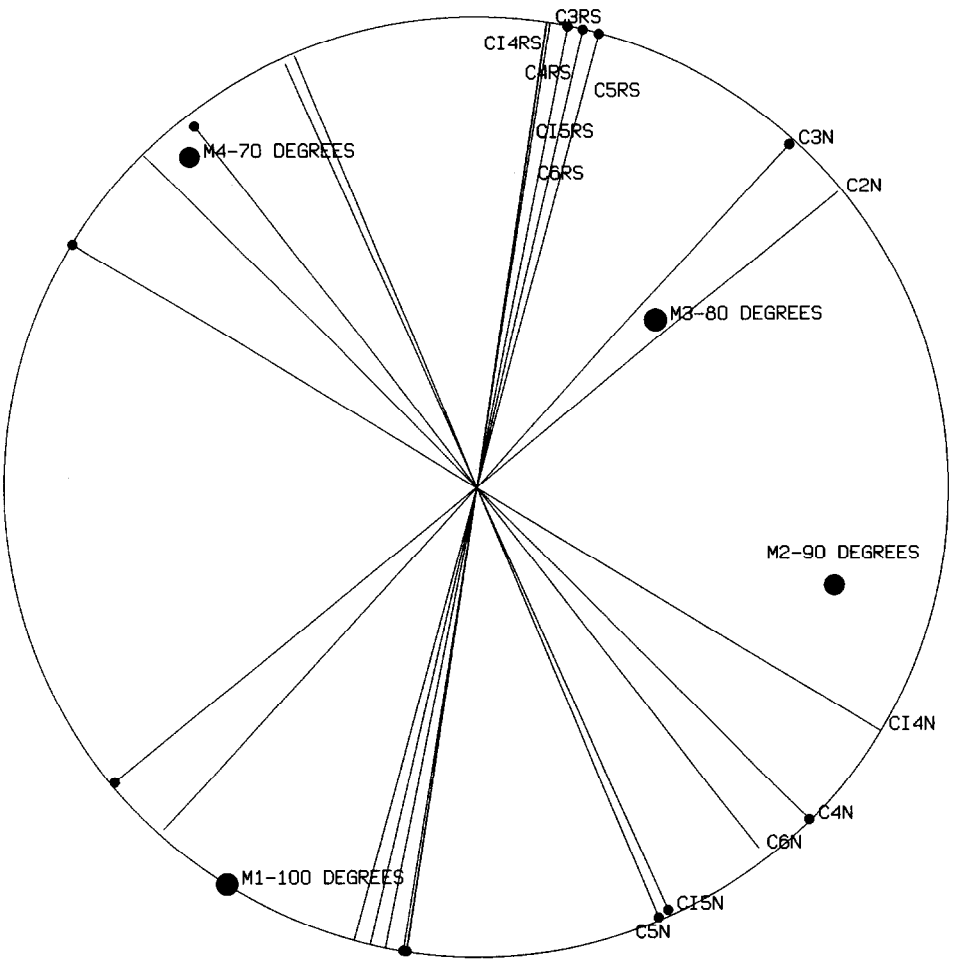

Fig. 3. Method comparison of plates and resolution. Non-orthogonal map comparing four isothermal gas chromatography methods 100°C (M1–100 DEGREES), 90°C (M2–90 DEGREES), 80°C (M3–80 DEGREES), 70°C (M4–70 DEGREES) methods using 4 × 14 matrix and correlations in Table II representing resolution (C2RS, C3RS, CI4RS, C4RS, CI5RS, C5RS, C6RS) and theoretical plates (C2N, C3N, CI4N, C4N, CI5N, C5N, C6N) for acetic, propionic, isobutyric, butyric, isovaleric, valeric and isocaproic acids, respectively. Fields with zero values (C2RS) were not plotted.

TABLE II

METHOD COMPARISON OF PLATES AND RESOLUTION

4 × 14 matrix and correlations representing resolution (C2RS, C3RS, CI4RS, C4RS, CI5RS, C5RS, C6RS) and theoretical plates (C2N, C3N, CI4N, C4N, CI5N, C5N, C6N) for acetic, propionic, isobutyric, butyric, isovaleric, valeric and isocaproic acids, respectively, comparing four isothermal gas chromatography methods: 100°C (M1–100 DEGREES), 90°C (M2–90 DEGREES), 80°C (M3–80 DEGREES), 70°C (M4–70 DEGREES). Visualized in Fig. 3.

"VFARSPL.DME",

"",	"C2RS",	"C3RS",	"CI4RS",	"C4RS",	"CI5RS",	"C5RS",	"C6RS",	"C2N",	"C3N",	"CI4N",	"C4N",	"CI5N",	"C5N",	"C6N",
"VFA100D",	0.0000,	6.0200,	2.5800,	5.7200,	4.1400,	7.0000,	6.8800,	3834.5000,	6195.3000,	6621.9000,	7328.3000,	7218.0000,	7352.9000,	7032.7000,
"VFA90D",	0.0000,	8.3800,	3.2700,	6.5100,	4.6100,	7.6200,	7.2900,	6661.4000,	7206.8000,	7174.7000,	7611.6000,	7041.9000,	7197.4000,	7644.1000,
"VFA80D",	0.0000,	9.6300,	3.6500,	6.9400,	4.8900,	7.9500,	7.5600,	6426.1000,	7543.2000,	6709.8000,	7221.2000,	6623.4000,	6739.0000,	6350.6000,
"VFA70D",	0.0000,	10.2700,	3.8200,	7.1500,	4.9200,	7.9700,	7.6000,	5699.9000,	7027.6000,	6052.3000,	6502.2000,	5709.1000,	5968.9000,	5618.8000,

Total variance accounted for:
In one dimension: 74.497%
In two dimensions: 98.903%
In three dimensions: 100.000%

Correlations between Attributes:

	C3RS	CI4RS	C4RS	CI5RS	C5RS	C6RS	C2N	C3N	CI4N	C4N	CI5N	C5N	C6N
C3RS	1.0000	0.9999	1.0000	0.9943	0.9915	0.9958	0.7415	0.8171	-0.3784	-0.5905	-0.8310	-0.8480	-0.6586
CI4RS	0.9999	1.0000	1.0000	0.9956	0.9928	0.9969	0.7441	0.8225	-0.3736	-0.5851	-0.8261	-0.8438	-0.6575
C4RS	1.0000	1.0000	1.0000	0.9946	0.9917	0.9962	0.7402	0.8175	-0.3797	-0.5911	-0.8308	-0.8481	-0.6608
CI5RS	0.9943	0.9956	0.9946	1.0000	0.9993	0.9994	0.7815	0.8683	-0.3086	-0.5209	-0.7746	-0.7965	-0.6177
C5RS	0.9915	0.9928	0.9917	0.9993	1.0000	0.9975	0.8039	0.8839	-0.2740	-0.4909	-0.7541	-0.7761	-0.5883
C6RS	0.9958	0.9969	0.9962	0.9994	0.9975	1.0000	0.7599	0.8527	-0.3402	-0.5481	-0.7928	-0.8146	-0.6440
C2N	0.7415	0.7441	0.7402	0.7815	0.8039	0.7599	1.0000	0.9500	0.3381	0.0914	-0.2712	-0.2895	0.0079
C3N	0.8171	0.8225	0.8175	0.8683	0.8839	0.8527	0.9500	1.0000	0.1853	-0.0330	-0.3594	-0.3917	-0.1952
CI4N	-0.3784	-0.3736	-0.3797	-0.3086	-0.2740	-0.3402	0.3381	0.1853	1.0000	0.9647	0.8059	0.8001	0.9198
C4N	-0.5905	-0.5851	-0.5911	-0.5209	-0.4909	-0.5481	0.0914	-0.0330	0.9647	1.0000	0.9325	0.9267	0.9486
CI5N	-0.8310	-0.8261	-0.8308	-0.7746	-0.7541	-0.7928	-0.2712	-0.3594	0.8059	0.9325	1.0000	0.9984	0.8981
C5N	-0.8480	-0.8438	-0.8481	-0.7965	-0.7761	-0.8146	-0.2895	-0.3917	0.8001	0.9267	0.9984	1.0000	0.9105
C6N	-0.6586	-0.6575	-0.6608	-0.6177	-0.5883	-0.6440	0.0079	-0.1952	0.9198	0.9486	0.8981	0.9105	1.0000

Attribute Coordinates:

Attribute Name	X	Y	Z
C3RS	-0.99	0.16	0.03
CI4RS	-0.99	0.16	0.02
C4RS	-0.99	0.16	0.02
CI5RS	-0.97	0.23	-0.04
C5RS	-0.96	0.27	-0.03
C6RS	-0.98	0.20	-0.05
C2N	-0.62	0.77	0.12
C3N	-0.73	0.67	-0.16
CI4N	0.52	0.85	0.05
C4N	0.71	0.70	-0.07
CI5N	0.90	0.40	-0.16
C5N	0.92	0.38	-0.11
C6N	0.77	0.60	0.23

Communalities:

1 D	2 D	3 D	Total
0.976	0.025	0.001	1.0000
0.973	0.026	0.000	1.0000
0.975	0.024	0.001	1.0000
0.944	0.054	0.002	1.0000
0.927	0.072	0.001	1.0000
0.957	0.040	0.002	1.0000
0.390	0.594	0.016	1.0000
0.528	0.448	0.025	1.0000
0.271	0.727	0.002	1.0000
0.501	0.495	0.004	1.0000
0.812	0.163	0.025	1.0000
0.841	0.148	0.011	1.0000
0.591	0.356	0.053	1.0000

Object Coordinates:

Data Point Name	X	Y	Z
VFA100D	0.844	0.535	-0.026
VFA90D	0.212	-0.758	0.092
VFA80D	-0.352	-0.384	-0.124
VFA70D	-0.704	0.607	0.059

olution values for all four methods were highly correlated and were clustered on axes at 11 o'clock, 12 o'clock, and 1 o'clock, respectively. Tailing factors for each of the seven components were not correlated and were plotted on axes ranging from 11 o'clock (C2T) to 8 o'clock (C5T). For example, M1–100 DEGREES (7 o'clock) exhibited the highest tailing factor for *n*-valeric and isobutyric acids. Using this expanded test, five optimization parameters for each of the seven components were simultaneously evaluated. M3–80 DEGREES (2 o'clock) still appeared to be the best compromise separation in that it exhibited nearly mean values for all five optimization parameters.

Concentration–activity comparisons

Similar types of matrices were used in Figs. 5 and 6 to nonorthogonally visualize a multicomponent data set too large to interpret without the use of computerized statistical analyses. The objective was to visually determine if bacterial inoculation in lieu of antibiotic treatments could enhance ruminal fermentations by increasing concentrations of acetic and propionic acids.

In Fig. 5, mean component amounts for 5 bacterial concentrations, 2 diet protocols (12-h and 24-h fermentations each) and for ten corresponding 0-h controls were non-orthogonally mapped using the 30 × 6 matrix in Table III. All of the treatments with the highest bacterial concentration (treatment 9 as indicated by 24H9, 24M9, 12H9, and 12M9 labels) exhibited a significant visual deviation and positioned in the quadrant between 1 and 2 o'clock. The corresponding 0–h controls for treatment 9 (OH9 and OM9 at 4 o'clock) were similarly shifted from the other controls which were bunched at 5 o'clock. Although this observation is under investi-

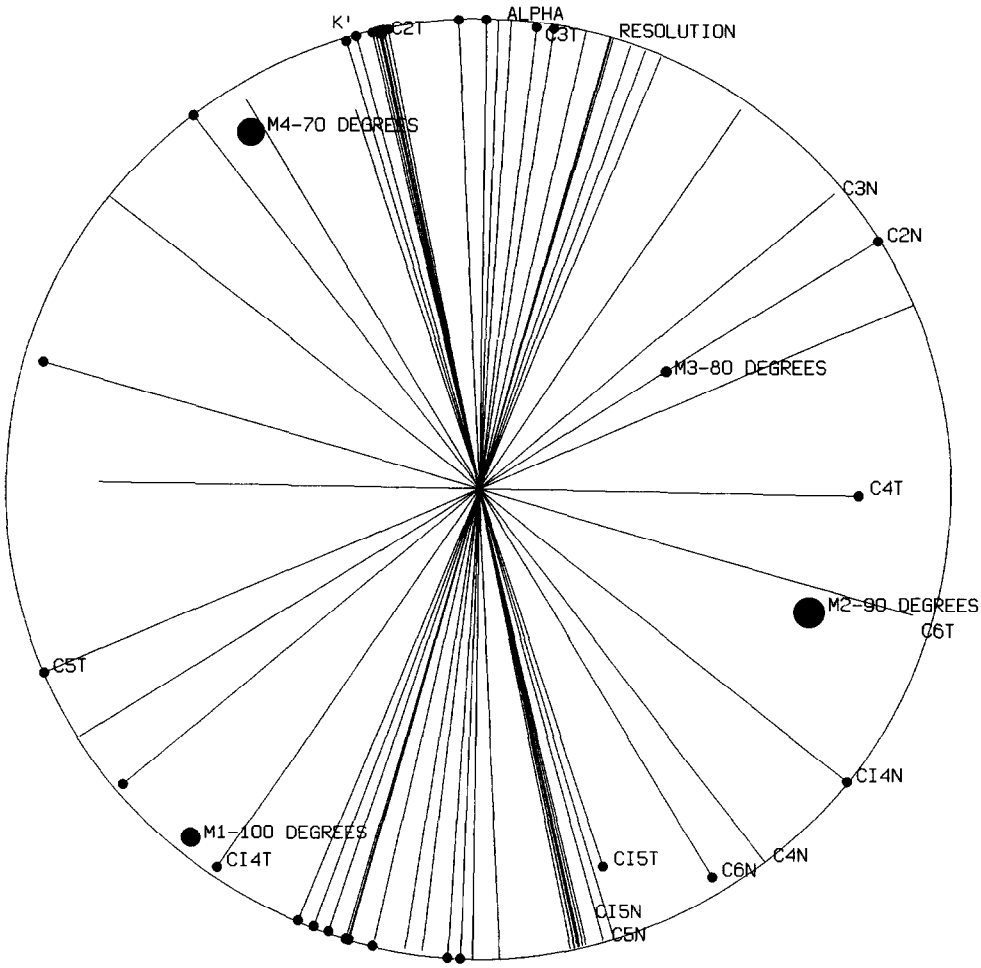

Fig. 4. Method comparison of plates, resolution, tailing factor, α and k'. Non-orthogonal map comparing four isothermal gas chromatography methods 100°C (M1–100 DEGREES), 90°C (M2–90 DEGREES), 80°C (M3–80 DEGREES), 70°C (M4–70 DEGREES) methods using 4 × 35 matrix representing resolution (C2RS, C3RS, CI4RS, C4RS, CI5RS, C5RS, C6RS), theoretical plates (C2N, C3N, C14N, C4N, C15N, C5N, C6N), tailing factor at 10% peak height (C2TF, C3TF, CI4TF, C4TF, CI5TF, C5TF, C6TF), α (C2A, C3A, CI4A, C4A, CI5A, C5A, C6A), capacity factor (C2K, C3K, CI4K, C4K, CI5K, C5K, C6K) for acetic, propionic, isobutyric, butyric, isovaleric, valeric and isocaproic acids, respectively. Fields with zero values (C2RS and C2A) were not plotted.

gation, it appears that treatment 9 initially elevated the concentrations of acetic and propionic acids. The concentration-time-diet clustering in Fig. 5 was interesting in that the concentrations of acetic and propionic acid appeared to increase most significantly after 12-h fermentation and were not apparently affected by diet.

In Fig. 6, replicate results for all 20 treatments (5 bacterial concentrations, 2 fermentation times, 2 diets each, 4 replicates injected twice) and the corresponding 0-h controls (5 bacterial concentration, 2 diets, injected twice) were non-orthogonally mapped using a 180 × 6 matrix representing 180 chromatograms. Replicate reproducibility for treatment 9 can be visualized at 1 and 2 o'clock. The only significant visual outliers occurred at 5 o'clock and corresponded to duplicate injections of a 12-h fermentation sample (vfa. 79A and vfa. 80A, diet 1, no bacterial inoculation). The other 3 replicates for this treatment all exhibited higher concentrations of acetic acid.

Non-orthogonal visualization was found to be a useful tool for screening biologically active treatment protocols as well as for identifying anomalous

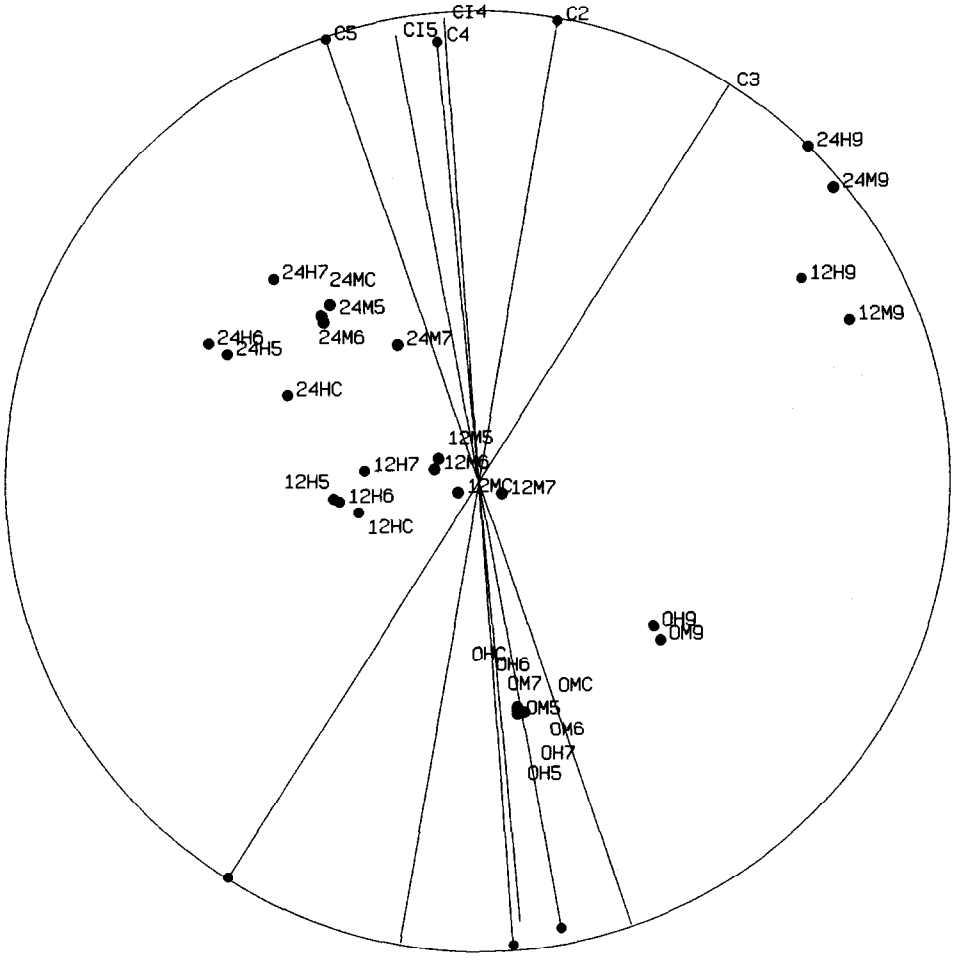

Fig. 5. Comparison of mean bacterial treatments. Non-orthogonal map comparing mean values for 5 bacterial inoculation concentrations: after 0-h fermentation (0HC, 0H5, 0H6, 0H7, 0H9), 12-HR (12HC, 12H5, 12H6, 12H7, 12H9), and 24-h fermentation (24HC, 24H5, 24H6, 24H7, 24H9) for acetic, propionic, isobutyric, butyric, isovaleric and valeric acids (C2, C3, CI4, C4, CI5, C5, respectively) at 2 diets (M substituted for second diet) using 30 × 6 matrix in Table III.

outliers. Computerized statistical analysis verifying these visual observations will be reported in future publications.

CONCLUSIONS

Using free-fatty acid model systems, multivariate visualization as automated in Echo Data's Data-Max software was used to correlate, visualize, and verify the relationships of chromatography parameters. Two types of matrices were plotted: (1) chromatography parameters as the vectors and components as the objects (Figs. 1 and 2); (2) chromatography parameters for each component as the vectors and methods or chromatograms as the objects (Figs. 3–6). The first type of matrix was shown to be suitable for visualizing the results for a single chromatogram, for clustering similar parameters such as skew and tailing factors, for verifying chromatography relationship such as the correlation between α and resolution, and for comparing replicate results.

The second type of matrix was shown to have the same result as a mathematically-derived chromatographic response function in that an entire chromatogram was reduced to a single value, or to a coordinate in the case of a non-orthogonal map.

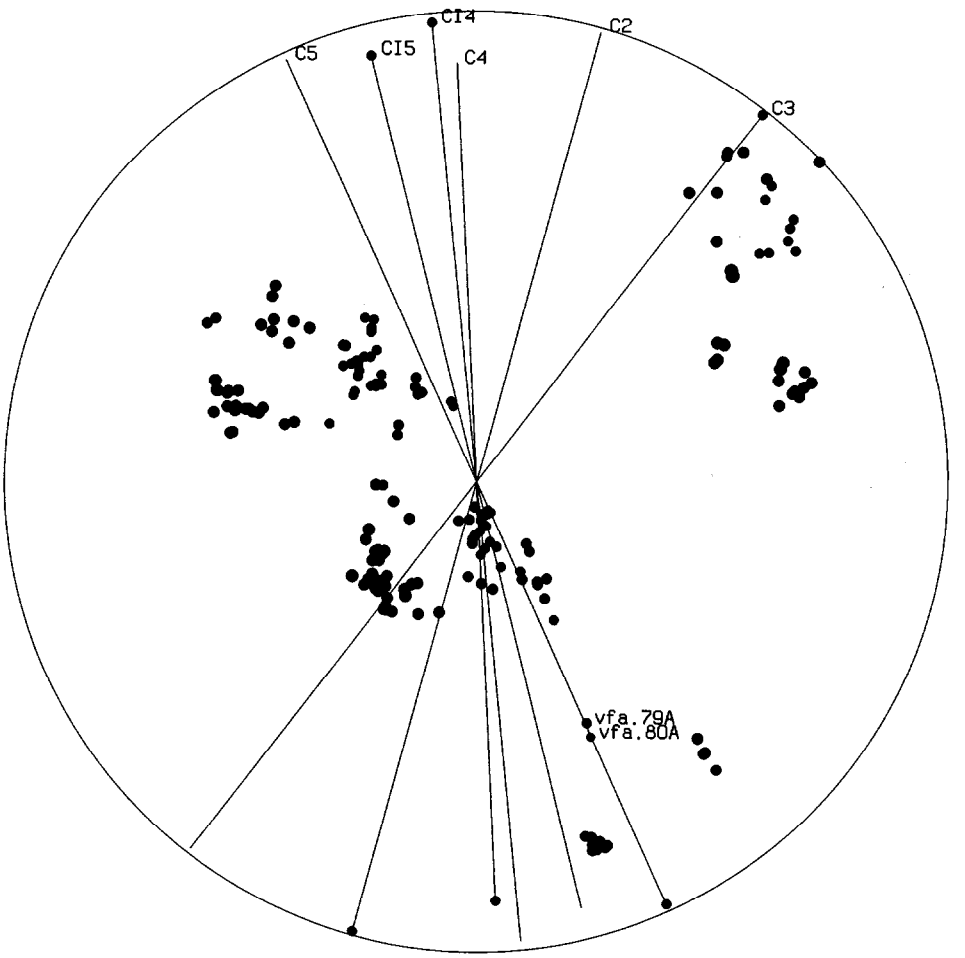

Fig. 6. Replicate comparison for 180 chromatograms. Non-orthogonal pendicular plot comparing replicated data for 5 bacterial inoculation concentrations for 2 diet protocols after 0-h, 12-h, and 24-h fermentation for acetic, propionic, isobutyric, butyric, isovaleric and valeric acids (C2, C3, CI4, C4, CI5, C5, respectively) using 180 × 6 matrix representing 180 chromatograms. 12-h and 24-h treatments were replicated 4 times, 2 injections each. 0-h fermentation were unreplicated, 2 injections each.

TABLE III

COMPARISON OF MEAN BACTERIAL TREATMENTS

30 × 6 matrix comparing mean values for 5 bacterial inoculation concentrations (C, 5, 6, 7, 9), 2 diet protocols (H and M), after 0-h, 12-h and 24-h fermentations (0, 12, 24) for acetic, propionic, isobutyric, butyric, isovaleric and valeric acids (C2, C3, CI4, C4, CI5, C5, respectively). Visualized in Fig. 5.

"BACTERIAL INOCULATION 12 & 24 HR MEANS",

"",	"C2",	"C3",	"CI4",	"C4",	"CI5",	"C5",
"OHC",	24.0100,	6.6100,	0.6300,	5.2300,	0.7700,	0.6000,
"OH5",	24.0500,	6.6000,	0.6300,	5.2200,	0.7700,	0.6000,
"OH6",	24.1400,	6.6800,	0.6400,	5.2800,	0.7800,	0.6100,
"OH7",	23.6900,	6.6900,	0.6300,	5.1800,	0.7700,	0.6000,
"OH9",	33.0900,	16.9300,	0.7700,	5.7200,	0.8300,	0.6300,
"OMC",	23.9800,	6.5900,	0.6300,	5.2600,	0.7800,	0.6100,
"OM5",	23.2400,	6.4600,	0.6300,	5.1500,	0.7700,	0.6000,
"OM6",	24.2700,	6.7000,	0.6200,	5.2500,	0.7700,	0.6100,
"OM7",	23.7300,	6.6800,	0.6300,	5.1500,	0.7800,	0.6000,
"OM9",	32.1400,	16.5800,	0.7500,	5.5400,	0.8000,	0.6200,
"12HC",	36.3400,	13.9200,	0.8500,	8.1900,	1.0700,	1.4900,
"12H5",	36.9700,	13.9100,	0.8600,	8.3800,	1.1000,	1.5700,
"12H6",	36.7500,	14.0400,	0.8500,	8.8300,	1.0800,	1.5700,
"12H7",	38.6500,	18.1200,	0.8700,	9.8300,	1.1000,	1.6900,
"12H9",	56.9000,	52.0200,	1.0500,	12.3700,	1.2900,	1.6800,
"12MC",	42.1200,	17.8900,	0.8200,	12.2200,	0.9300,	1.5300,
"12M5",	44.5900,	18.7100,	0.8800,	12.6500,	0.9800,	1.6000,
"12M6",	43.1200,	18.3200,	0.8600,	12.6900,	0.9700,	1.5900,
"12M7",	42.7100,	20.3300,	0.8200,	10.9000,	0.9400,	1.5100,
"12M9",	56.4300,	51.1500,	0.9700,	12.4500,	1.1500,	1.6700,
"24HC",	44.1300,	19.4500,	0.9400,	11.5700,	1.2200,	2.0000,
"24H5",	45.8100,	19.8800,	0.9900,	12.7200,	1.2700,	2.2200,
"24H6",	46.3600,	19.7000,	0.9800,	14.1200,	1.2700,	2.2800,
"24H7",	51.2100,	27.1800,	1.0500,	15.7900,	1.3300,	2.3700,
"24H9",	68.5200,	60.4600,	1.1000,	19.4700,	1.3700,	2.1200,
"24MC",	50.7200,	24.0400,	0.9900,	19.0300,	1.1700,	2.1100,
"24M5",	52.3900,	24.9700,	1.0000,	19.1100,	1.1700,	2.1600,
"24M6",	50.3600,	24.0400,	0.9700,	18.8800,	1.1600,	2.1300,
"24M7",	50.4700,	25.7800,	0.9800,	15.5600,	1.1700,	1.9500,
"24M9",	67.9600,	58.3400,	1.0300,	20.0700,	1.2000,	2.1600,

Total variance accounted for:
In one dimension: 88.367%
In two dimensions: 96.122%
In three dimensions: 99.280%

Correlations between Attributes:

	C2	C3	CI4	C4	CI5	C5
C2	1.0000	0.9108	0.9513	0.9199	0.8786	0.8694
C3	0.9108	1.0000	0.7917	0.7217	0.7211	0.6195
CI4	0.9513	0.7917	1.0000	0.8952	0.9657	0.9448
C4	0.9199	0.7217	0.8952	1.0000	0.8266	0.9045
CI5	0.8786	0.7211	0.9657	0.8266	1.0000	0.9458
C5	0.8694	0.6195	0.9448	0.9045	0.9458	1.0000

Attribute Coordinates:				Comunalities:			
Attribute Name	X	Y	Z	1 D	2 D	3 D	Total
C2	-0.98	0.17	-0.07	0.961	0.030	0.004	0.9949
C3	-0.84	0.54	0.06	0.707	0.288	0.004	0.9979
CI4	-0.99	-0.07	0.10	0.972	0.005	0.009	0.9861
C4	-0.94	-0.09	-0.33	0.876	0.007	0.112	0.9953
CI5	-0.95	-0.17	0.25	0.901	0.030	0.061	0.9921
C5	-0.94	-0.32	-0.00	0.886	0.105	0.000	0.9905

(Continued on p. 12)

TABLE III *(continued)*

Object Coordinates:

Data Point Name	X	Y	Z
OHC	0.489	-0.093	-0.016
OH5	0.489	-0.093	-0.016
OH6	0.478	-0.081	-0.010
OH7	0.491	-0.092	-0.014
OH9	0.308	-0.372	0.030
OMC	0.484	-0.081	-0.012
OM5	0.494	-0.081	-0.013
OM6	0.490	-0.098	-0.019
OM7	0.488	-0.086	-0.009
OM9	0.337	-0.387	0.017
12HC	0.066	0.258	0.086
12H5	0.037	0.311	0.097
12H6	0.043	0.298	0.075
12H7	-0.024	0.245	0.066
12H9	-0.430	-0.687	0.148
12MC	0.023	0.045	-0.089
12M5	-0.050	0.087	-0.067
12M6	-0.028	0.096	-0.075
12M7	0.026	-0.050	-0.050
12M9	-0.341	-0.788	0.063
24HC	-0.184	0.407	0.086
24H5	-0.272	0.535	0.089
24H6	-0.294	0.574	0.051
24H7	-0.431	0.435	0.055
24H9	-0.711	-0.703	0.011
24MC	-0.352	0.336	-0.116
24M5	-0.375	0.317	-0.117
24M6	-0.338	0.332	-0.121
24M7	-0.291	0.173	-0.029
24M9	-0.624	-0.756	-0.100

Unlike the chromatographic response functions, data were not simplified and all parameters were simultaneously considered. Practical applications include the visualization of multidetector, multiwavelength, multicolumn data. Two examples were demonstrated: (1) evaluation of four chromatography methods by non-orthogonally mapping a 4 × 35 matrix representing resolution, theoretical plates, tailing factor, α and capacity factor for a 7-component mixture; (2) identification of optimum concentration, time-course effects, and replicate reproducibility by non-orthogonally mapping a 180 × 6 matrix representing 180 multicomponent chromatograms. In each case, non-orthogonal visualization was found to facilitate more rapid screening than currently possible by the use of computerized statistical analysis alone.

In summary, non-orthogonal mapping was found to be a useful and accurate technique for visualizing chromatography data because of the high correlations inherent in most chromatography data. This technique is a useful addition to the mathematical approaches described by Guiochon and coworkers [1,2] in which chromatography decisions were based on simultaneous consideration of all measurable variables. Additional chromatographic applications and areas for research include: (1) multiwavelength, multidetector and multicolumn forensic fingerprinting; (2) pattern recognition of complex environmental pollutants such as polychlorinated biphenyls; (3) structure–activity and structure–reactivity screening; (4) identification of significant process parameters; (5) quality control and replicate comparison of multicomponent samples in the petroleum, perfume, agricultural and food industries. Automated computer processing of separation parameters makes this technique feasible for fast, on-line monitoring of refinery, polymer, medicinal and other chemical processes. The observation that retention time could be substituted for capacity factor resolves the concern expressed by Berridge [6] of computerized determination of column void volumes.

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