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Differentiation of Commercial Polyester Fibers Using Two-Dimensional High-Performance Liquid Chromatography (HPLC) and Multivariate Pattern Recognition Techniques

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ABSTRACT: Six types of colorless commercial poly(ethylene terephthalate) polyester fibers (PET) from different manufacturers were investigated using two-dimensional high-performance liquid chromatography (HPLC). As to their peak structure and area distribution, their chromatograms differed only insignificantly. Therefore a possible use of multivariate pattern recognition techniques was checked for a PET fiber identification based upon a data set obtained through HPLC analysis. The results show that the application of cluster methods (dendrogram, display mapping, and potential density method) enables a classification into different manufacturers.

KEYWORDS: forensic science, fibers, synthetic fibers, chromatographic analysis, two-dimensional HPLC, pattern recognition

The analysis of man-made fibers is an important part of criminal investigations, because such fibers often present important clues in crimes [*1–6*]. Comparative examinations are often necessary and only small sample sizes are available in most of the cases. Therefore, for the purpose of forensic fiber analysis, only those analytical techniques are considered that promise a maximum information output in the face of small sample sizes (micrograms). That is why industrial analytical procedures which serve to identify the finish, surface coating, fiber type, or the manufacturing process involved in general are not suited for criminological purposes. While the optical appearance offers enough distinctive features for natural fibers, an analytical assessment of man-made fibers is more difficult.

Recently, numerous articles on fiber analysis have been published in the literature dealing with the application of microscopic [*7–12*], physical [*13,14*], chemical [*15,16*], and particularly instrumental [*17–28*] analytical methods. These techniques allow several man-made fibers to be easily identified according to the qualitative properties resulting from their macromolecular component. Through infrared spectroscopic fiber study [*29*] and specialized high-performance liquid chromatography (HPLC) methods [*30*], it is even possible to examine monofilaments. A thorough survey of the main techniques used in examining fibers for criminological purposes together with comments and a bibliography has been compiled by Fong [*31*].

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Identification difficulties have always occurred when similar fibers from different manufacturers were analyzed. Therefore the applicability of two-dimensional HPLC to polyester fiber analysis was tested.

Although it is characteristic of chromatographic techniques that the material tested is destroyed in the process, this disadvantage is compensated for by the fact that chromatographic methods provide information which cannot be obtained by using other techniques. The information obtained can even be increased by combining two or more chromatographic techniques based on different characteristics of separation (multidimensional chromatography) [*32*]. In addition, chromatographic and spectrographic methods may be used together, allowing to identify the separate components [*33*].

This work deals with the analysis of low-molecular weight constituents. Our approach is based upon the fact that the polymers used for fiber manufacturing generally contain low-molecular weight substances, which are added either intentionally (fiber upgrading) or nonintentionally. In the latter case, as they are a byproduct of synthesis, these substances are mainly oligomers. Their concentration is rather low and depends strongly on the course of reaction and the processes that follow. Therefore differences in the production processes at the manufacturing level should lead to distinctive features in each manufacturer's final product.

Consideration, however, must be given to the fact that the univariate measuring and differentiation methods used for chromatographic data assessment do not always lead to satisfactory results, especially when fiber samples show only slight qualitative differences. In addition, modern HPLC techniques produce a relatively large amount of data within a relatively short period of time, which can be difficult to process and interpret rapidly. But with the introduction of computer-aided multivariate pattern recognition, it has become possible to analyze such data in less time and permits to extract information in a large data sets in an automatic way. Several authors [*34–36*] reported the mathematical and methodological fundamentals of multivariate pattern recognition techniques.

We report the combined application of size exclusion liquid chromatography (SEC) and reversed-phase chromatography (RPC) for the analysis of low-molecular weight compounds in similar PET fibers from different manufacturers. Mathematical cluster analysis algorithms (dendrogram, display mapping, and potential density methods) are applied to the data and a search is made for the presence of natural clusters.

Samples and Sample Preparation

Six types of PET fibers (A–F) from four manufacturers were selected from the large number of PET fibers on the market. They were tested by a method combining the SEC/RPC techniques. More specific information on the fiber samples is given in Table 1.

To prepare the solution of 1.5% used for analysis, three samples of each type of fiber were weighed (375 μg) and dissolved in 25 μL reagent-grade 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Fluka AG, Switzerland).

TABLE 1—*PET fibers analyzed.*

Designation	Manufacturer	Fiber Type	Sample No.
A	Leinefelde/Eichsfeld	Crimped Yarn (DIOLEN)	1–3
B	Höchst-Faserwerke	Flock Type 210 (TREVIRA)	4–6
C	Höchst-Faserwerke	Flock BW-Type (TREVIRA)	7–9
D	CFW Premnitz	Flock W-Type (GRISUTEN)	10–12
E	CFW Premnitz	Flock BW-Type (GRISUTEN)	13–15
F	CFW Guben	Flock (GRISUTEN)	16–18

Unsoluble fillers were removed by centrifuging. A total of 18 SEC/RPC tests (three samples from each A–F) were made.

Equipment and Chromatographic Techniques

SEC and RPC Examinations

Examinations were performed using an HP 1090A HPLC device (Hewlett Packard) with built-in diode array detector (DAD) and multichannel integrator (DPU). The configuration embraced a ternary DR-5 pump system, an auto-injector, and an HP 2225/7470 printer/plotter combination. Separating columns were an RT 250-7 Hibar prepacked column filled with LiChrogel PS 20 (10 μm) for SEC, and an RT 250-4 Hibar prepacked column filled with LiChrosorb RP-18 (5 μm) (Merck, Germany) for RPC.

For chromatographic SEC and RPC conditions see Table 2.

The chromatographic conditions for the two methods of separation were chosen in accordance with the characteristic properties of the samples (solubility, polarity, molar weight, UV-detectability). General rules for optimizing HPLC were observed [*37*].

Solvents

Prior to their use, the reagent-grade chromatographic solvents dichloromethane (DCM), methanol (MeOH) (Laborchemie Apolda, Germany), and deionized water (W) were distilled twice in a 60-cm Vigreux column and then filtered through a 0.5-μm millipore filter.

SEC/RPC Off-Line Coupling

For separating low-molecular-weight compounds of $M < 20\,000$ g/mol from polymers, the SEC elugram was cut in the range of t_R = 5.6 to 6.6 min (fractionating volume 1 mL). Figure 1 shows the SEC cut made of all samples (hatched area) and the peaks resulting from subsequent off-line reverse-phase chromatography.

The SEC fractions were evaporated under nitrogen atmosphere in microvessels fitted

TABLE 2—*Chromatographic conditions of SEC and RPC analyses (DCM dichloromethane, W water, MeOH methanol, HFIP 1,1,1,3,3,3-hexafluoro-2-propanol).*

	SEC	RPC
Separating column	LiChrogel PS 20 (250 mm × 7 mm)	LiChrosorb RP-18 (250 mm × 4.6 mm)
Particle size	10 μm	5 μm
Sample concentration	1.5% (HFIP)	SEC fraction evaporated to 100 μL (preparative mode)
Solvent A	. . .	W
Solvent B	. . .	MeOH/DCM 85/15 (v/v)
Solvent C	DCM	. . .
Gradient	. . .	70%–100%/B/8 min 100%/B/3 min 100%– 70%/B/3 min
Injection volume	5 μL (75 μg) (analytically) 20 μL (300 μg) (preparatively)	20 μL of SEC fraction
Flow	1 mL/min	1 mL/min
Column temperature	30°C	40°C
UV detection	254 nm	254 nm

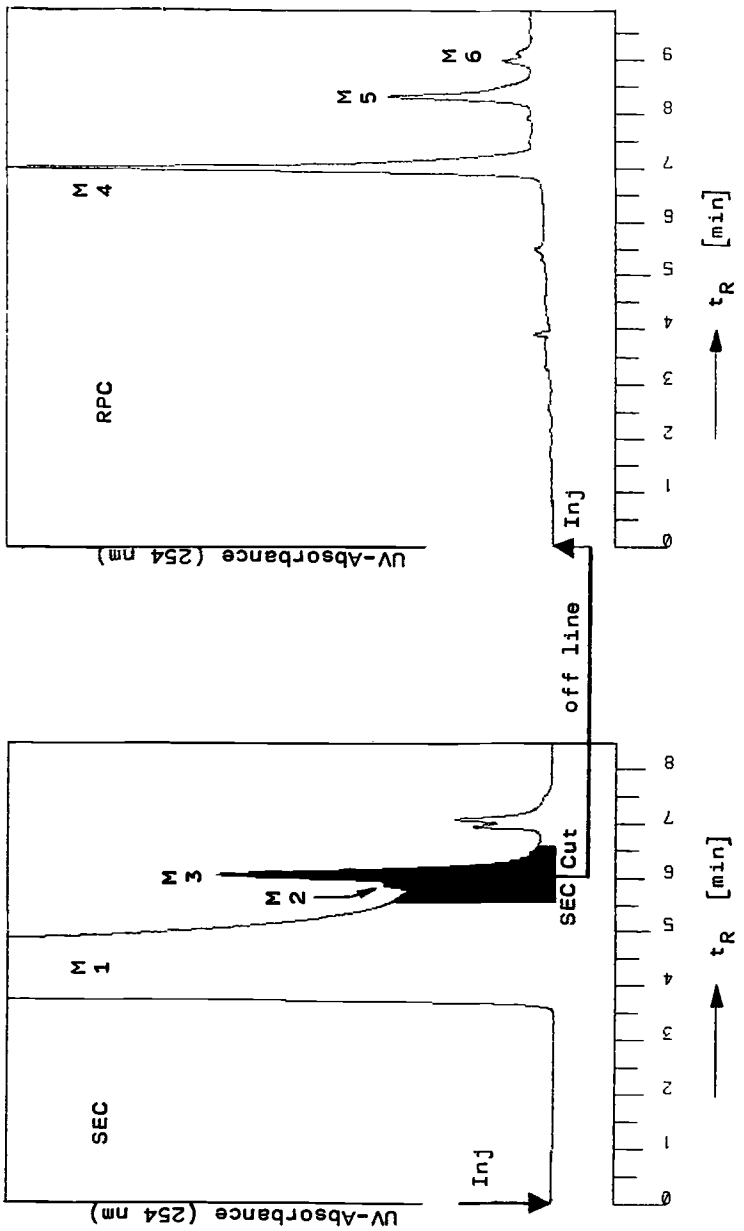

FIG. 1—*SEC cut for PET Sample 10 (GRISUTEN) and features (M1-M6) derived from SEC/RPC sample analysis for pattern recognition.*

with cooling condenser, and the residues were dissolved in 100 μL of RPC eluent. A 20-μL sample was injected into the column for RP analysis.

Each of the 18 samples examined in this study was analyzed 5 times by SEC/RPC combination. Mean values for the retention time as well as for peak area were obtained for both SEC and RPC methods.

Table 3 shows the mean retention time t_R (Columns 1 and 5) and mean relative peak area (Columns 3 and 7) for Sample 10, which serves as a typical example of analysis data. The relative standard deviation values ($\sigma_{rel.}$) for t_R are depicted in Columns 2 and 6, and $\sigma_{rel.}$ values for relative peak area are given in Columns 4 and 8.

Acquisition of Data

Figure 2 shows chromatograms typical of the samples examined. The chromatograms of two arbitrary samples, 5 (TREVIRA) and 17 (GRISUTEN), do not differ from each other significantly. However, the mean relative peak areas (%) recorded at 254 nm (M1–M6) served as features of the specimens.

Table 4 shows the man values of the peak areas recorded for the different fiber samples (A–F) for three measurements each. The peak areas of the overlapping peaks are determined by a curve-fitting procedure (tangent method). The peak identity was checked by checking the time of retention (Table 3) and by recording the UV spectra within the range of wavelengths between 200 and 400 nm by means of the diode array detector (Fig. 3).

Since 18 analysis were made, the set of data comprised the results of 18 individual tests, each supplying 6 characteristic features (18 × 6 data). Thus every fiber sample examined was characterized by a six-dimensional vector of characteristic features. The data were autoscaled and then subjected to a principal component analysis (PCA), a hierarchical and a potential-density clustering [*34–36*].

Evaluations were carried out using a PC 1715 personal computer and available BASIC programs.

Results and Discussion

The results from several cluster methods depicted in Figs. 4 and 5 can be used to identify the interior structure of the measured data and the existence of natural clusters. In particular, the dendrogram mapping [*34–36*] resulting from the hierarchic clustering (Fig. 4) shows a clear separation of two classes (A,B,C and D,E,F), represented by DIOLEN/TREVIRA and GRISUTEN, respectively. The manufacturers are situated in West Germany and East Germany.

The display technique (Fig. 5), based upon the principal component analysis (PCA mapping) [*34,36*], provides similar results. The first two principal components make up 95% of the total variance, so that the two-dimensional mapping contains nearly all the information produced in the HPLC analysis.

The results of the potential-density clustering technique [*35*] (CLUPOT) were entered in the display mapping by rimmed cells, with only those clusters marked which showed stability over a wide range of the smoothing factor [*35*]. CLUPOT separates Classes D and E from Class F, so that an additional distinction between Premnitz and Guben GRISUTEN fibers could be made.

Finally, it must be pointed out that the procedure described should be looked upon as a method which may be used in addition to the well-established methods used in forensic fiber analyses. It should be used in certain cases, for example, when there are larger amounts of fibers (300 μg). We are aware that more research input should be invested to be able to draw a general conclusion.

TABLE 3—*Mean retention time, mean relative peak areas, and relative standard deviations for Sample 10 using SEC/RPC off-line combination.*

	SEC					RPC			
	t_R (min)		Rel. Area (%)			t_R (min)		Rel. Area (%)	
	$\bar{x}$	σ rel (%)	$\bar{x}$	σ rel (%)		$\bar{x}$	σ rel (%)	$\bar{x}$	σ rel (%)
Peak	1	2	3	4	Peak	5	6	7	8
M1	4.10	0.78	95.99	0.07	M4	7.0	0.08	76.08	0.38
M2	5.80	0.33	0.91	1.21	M5	8.3	0.06	19.84	1.61
M3	5.99	0.35	3.10	1.84	M6	8.9	0.06	4.08	4.14

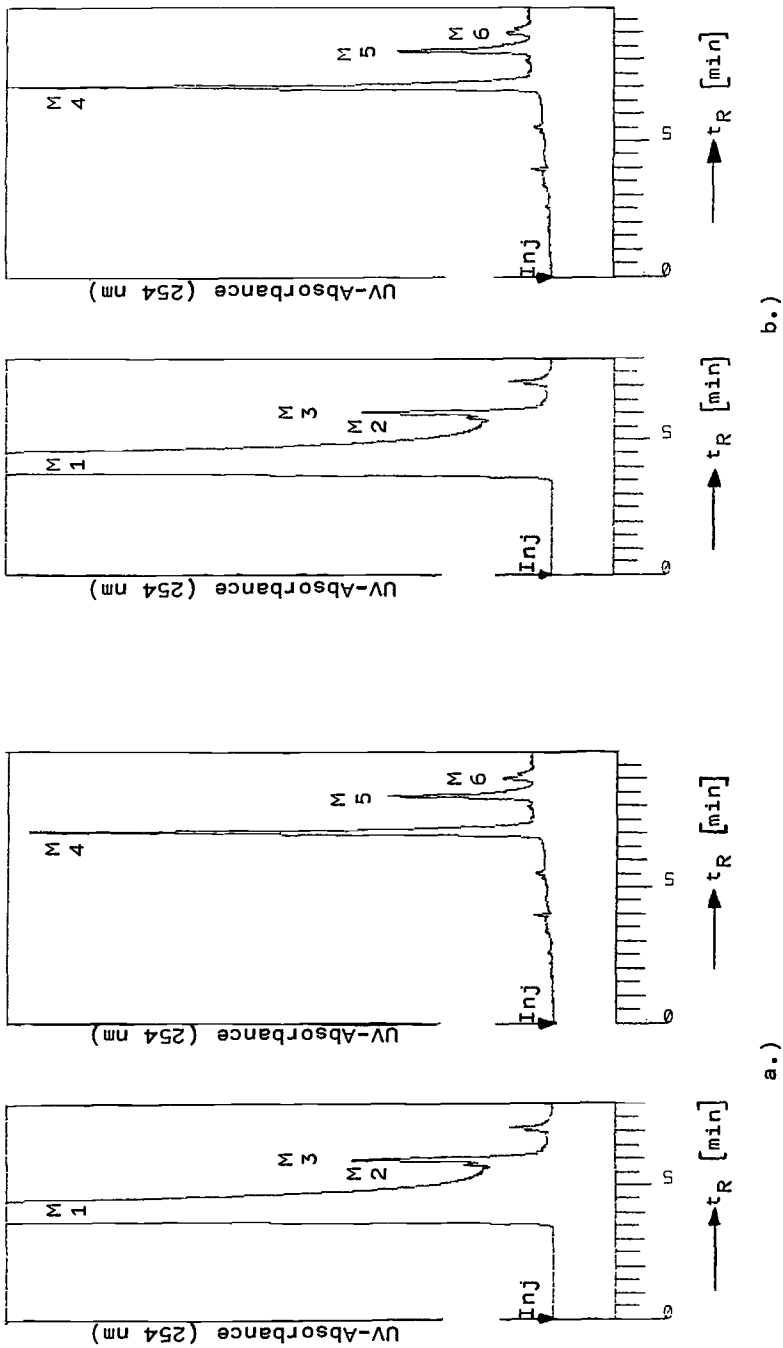

FIG. 2—*Typical SEC/RPC chromatograms of PET samples (for chromatographic data see Table 4).* (a) *Sample 5 (TREVIRA),* (b) *Sample 17 (GRISUTEN).*

TABLE 4—*SEC/RPC data for PET fibers of different manufacturers.*

Peak:		Mean Rel. SEC Areas (%)			Mean Rel. RPC Areas (%)		
t_R (min):		M 1	M 2	M 3	M 4	M 5	M 6
PET	Sample	4.1	5.8	6.0	7.0	8.3	8.9–9.1
A	1–3	95.78	0.95	3.27	73.14	21.17	5.69
B	4–6	95.73	0.95	3.31	74.03	20.70	5.27
C	7–9	95.68	0.95	3.37	75.02	19.91	5.07
D	10–12	95.99	0.91	3.10	76.08	19.84	4.08
E	13–15	95.98	0.91	3.11	77.03	18.76	4.21
F	16–18	95.73	0.92	3.29	77.13	18.61	4.26

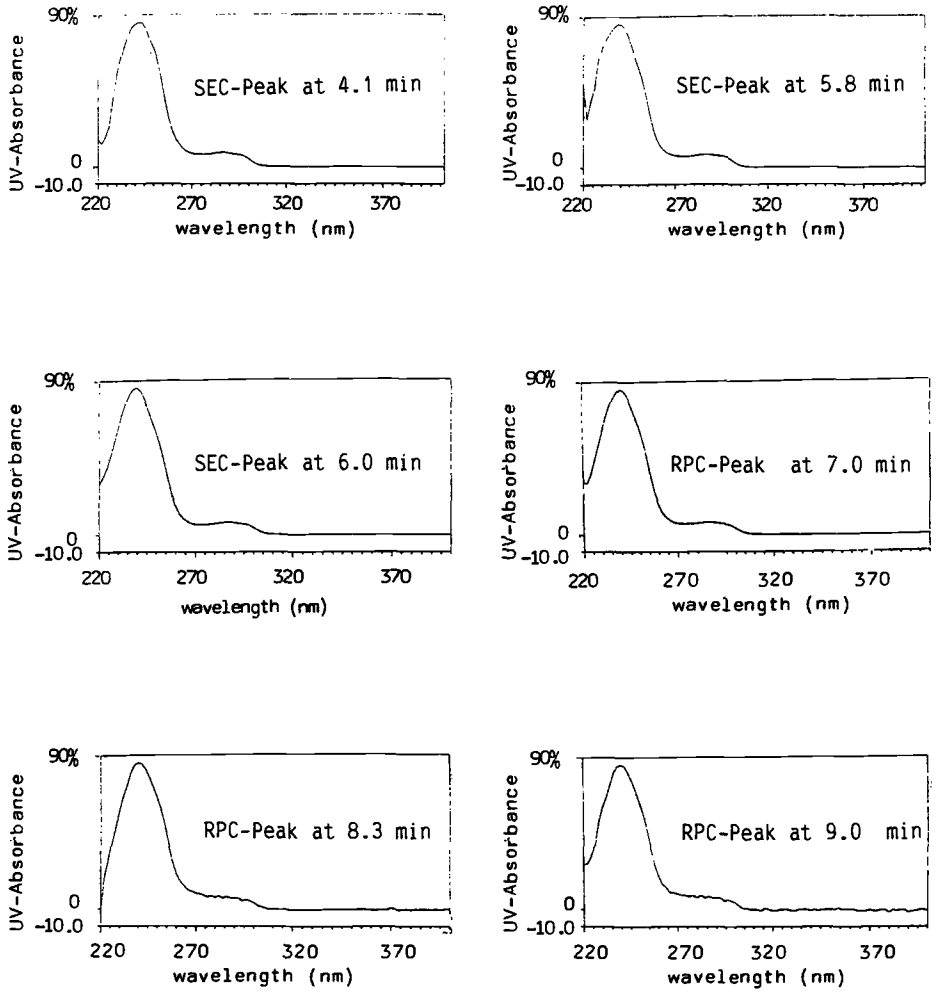

FIG. 3—*UV-spectra for peak-identification illustrated by fractions of Sample 10 (GRISUTEN).*

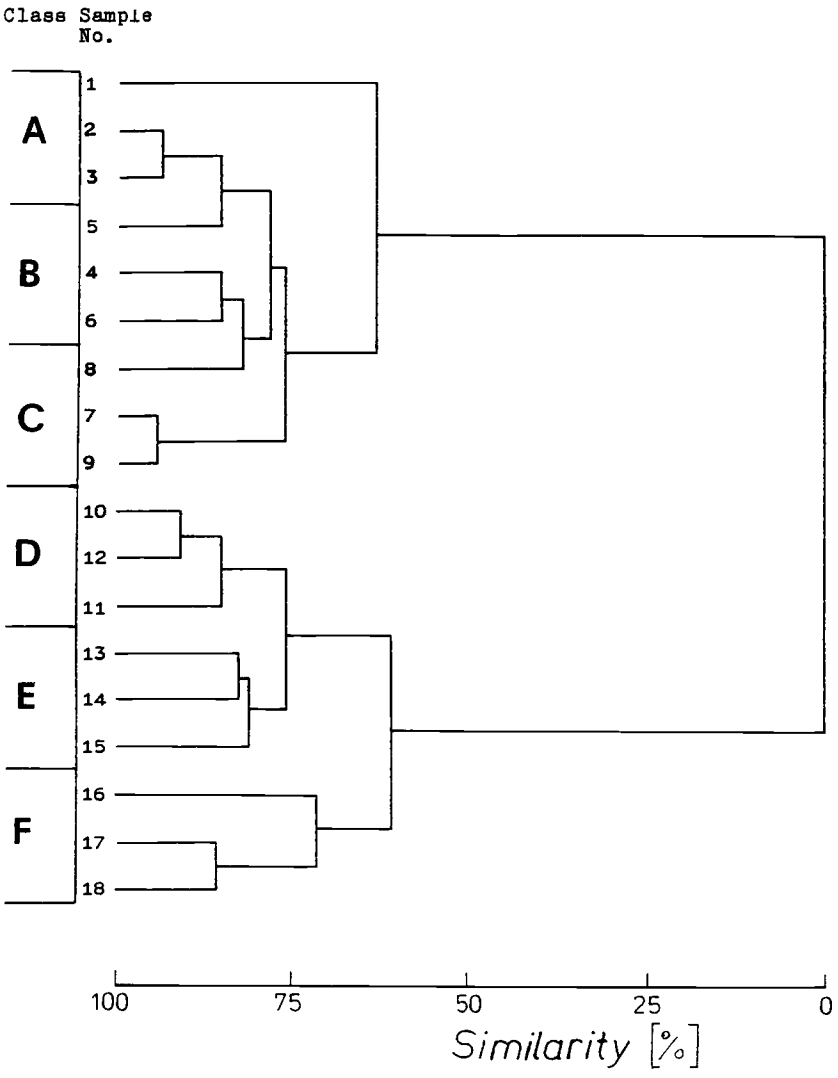

FIG. 4—*Dendrogram chart.*

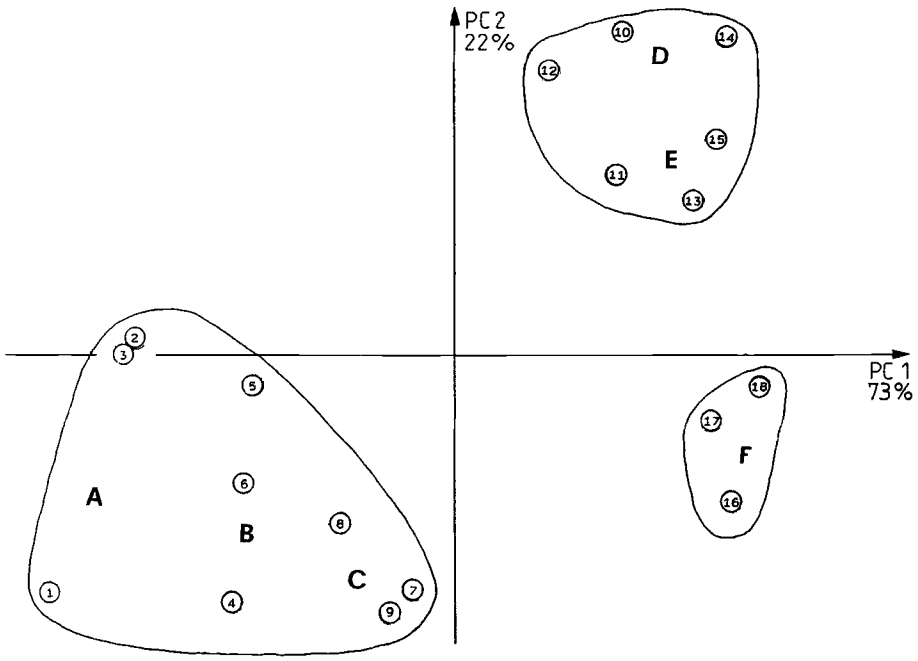

FIG. 5—*Principal component analysis (PCA).*

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