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Chemometric Tool of Chromatographic Pattern Recognition for the Analysis of Complex Mixtures

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Abstract □ A chemometric tool was developed for the analysis of complex mixtures such as crude drugs by the method of pattern recognition. Pattern recognition was accomplished by a multiple reference peak identification method and three kinds of outlier statistics. This tool was tested on the analysis of synthetic mixtures.

Keywords □ Pattern recognition, analysis of complex mixture

The crude drugs are complex mixtures consisted of numerous detectable and undetectable compounds. Nonetheless, the usual chemical analysis used for crude drugs has been focused on the quantitation of an unique and/or major compound, but it is usually true that the results of analysis may distort the real nature of crude drug as a complex mixture. Therefore, this kind of multivariate chemical system should be analyzed by the multivariate analytical method[1-5]. A system of pattern recognition made of conventional chromatograph and personal computer has been developed.

Modern chromatograph has powerful resolution even for the complex mixtures, showing combinations of pattern consisted of well-resolved peaks. These patterns are as specific as they can be used as a fingerprint of a certain crude drugs[6,7] A chromatographic data processing system was designed for personal computer which is connected to the conventional chromatograph *via* analogue/digital convertor and digital output device[8] Using this system, the digitized chromatogram can be used for the identification and quantitation of complex mixtures of compounds. The analysis of unknown complex mixtures may be complicated by interference with one or more peaks by other compounds that ordinarily are not the part of the reference complex mixture. These interfering peaks may be rejected statistically by the methods of outlier detection. To accomplish this kind of data processing on-line, two softwares were develped and the system was applied for the analysis of the mixtures of essential oils.

Hewlett-Packard 5890 series II gas chromatograph used was equipped with a flame ionization detector and interfaced to IBM/PC compatible personal computer *via* analog/digital convertor. A fused silica capillary column (0.2 mm i.d., 25 m long) coated with Carbowax 20 M was used. Reference lemon oil (Charabot, France) was mixed with eau de cologne (Pacific Chemical Co., Korea) to make synthetic complex mixtures. This synthetic mixture was subjected to steam distillation using Dean and Stark-type trap[9]. The n-hexane of the trap was used to concentrate the essential oils in the aqueous condensate. Acetophenone (Sigma, U.S.A.) dissolved in n-hexane was used as internal standard. The concentration of acetophenone as an internal standard was 0.3% in the final solution. The conditions for capillary gas chromatograph were as following: FID and injector temperature, 250℃ ; column oven temperature, 50℃ for initial 10 min and raised upto 200℃ (r=5℃/min). The sample injection volume was 2.0 μl and split ratio was 50 : 1.

Two softwares made for the experiment are the main program named [ChroMan] and the chromatographic data acquisition program named [Ch-

roAQ]. Using this data acquisition system, the chromatograms can be acquired in the digital form for the data processing. The chromatographic data were averaged for every 0.2 second and this averaged data were collected in a data file on magnetic disk. The main program, [ChroMan], made for the pattern recognition has a sub-program, [FindPeak], for the usual function of a digital integrator[10,11]. In addition to this, the Savitzky-Golay polynomials were used for the noise reduction of chromatogram[12]. The relative retention time and relative peak area of chromatographic peaks were calculated in reference to the retention time and peak area of internal standard peak. The pattern recognition system constructed for the actual application of the method uses the retention data provided from peak recognition system for the matching of chromatographic peaks of the standard mixture and those of the complex mixtures of question by the method of nearest neighbors[13]. Then, the ratios of areas of matched peaks are calculated and subjected to the statistical treatment for the outlier rejection using Dixon's 'Q Test', the two-sided Grubbs' test or Huber's elimination rule[14-19]. The simple arithmetic mean and the weighted mean with the weighting values which were determined by the area ratio of the peaks on the standard chromatogram were calculated from the remaining data of ratios of areas. The concentration of standard mxture in the complex mixtures are calculated from these mean values. All these procedures have been incorporated as sub-programs of [ChroMan]. The program supports graphic cards to show chromatograms on graphical with 11 types of display and this graphical

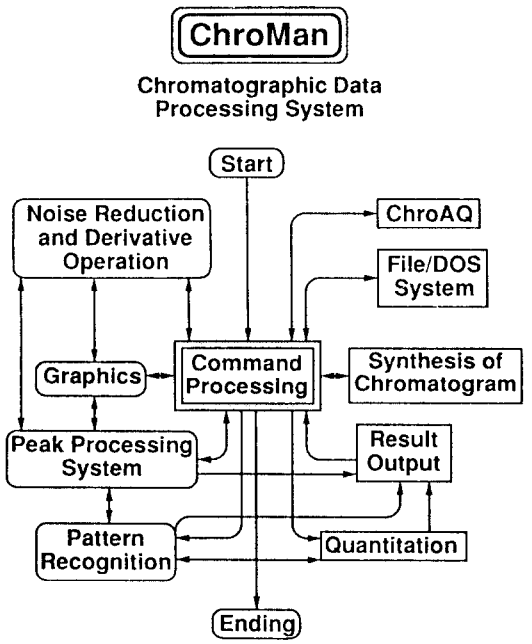

Fig. 1. Connection between sub-programs of [ChroMan].

screen can be hardcopied on printers. Automatic peak picking is available. The connection between the sub-program of [ChroMan] is shown in Fig. 1.

The outlier peaks of the chromatogram are resulted from the inteference with one or more peaks by other compounds that normally are not the components of the standard essential oils. The detection and rejection of outliers among the ratio values calculated from matched peaks was accomplished by three kinds of statistical methods. The results of statistical treatments of data were compared to

Table I. Recovery (%) of lemon oil in binary mixtures with eau de cologne

Sample*	Simple Mean			Weighted Mean		
	Dixon	Grubbs	Huber	Dixon	Grubbs	Huber
A	138.1	130.9	108.5	104.6	104.6	102.7
B	113.2	101.6	98.4	96.5	94.5	96.9
C	129.2	129.2	108.3	105.1	105.1	102.6
D	117.8	117.4	112.5	95.3	94.7	94.7
E	128.1	120.5	110.9	101.9	101.5	99.8
Mean(n=5)	125.3	119.9	107.7	100.7	100.1	99.3
S.D.	9.9	11.7	5.5	4.5	5.2	3.5

*Lemon oil (0.20 m*l*) was mixed with 2.0 m*l* (sample A), 3.0 m*l* (sample B), 4.0 m*l* (sample C), 5.0 m*l* (sample D) or 6.0 m*l* (sample E) of eau de cologne, respectively.

those of one another. Lemon oil itself is a kind of complex mixture, showing complicated multiple peaks. The mixture of lemon oil and eau de cologne is much more complicated. Table I shows the result of analysis of binary mixtures. Although the chromatogram of mixture is much complicated, the amount of lemon oil in the mixture was calculated with reasonable accuracy. The calculation using weighted mean showed better result than that using simple mean. Among three methods used for outlier rejection, the Huber's elimination rule was superior one, showing smaller value of standard deviation.

As a conclusion, the pattern recognition system made of the chromatographic data acquisition system, [ChroAQ], and the data processing system, [ChroMan], which utilize the digital chromatogram provided by [ChroAQ], can be used effectively for the quantitative analysis of complex mixtures.

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