Journal of Chromatography, 596 (1992) 285–289
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 012

Short Communication

Estimation of tobacco blend compositions using closed-loop stripping analysis and stepwise multiple linear regression and partial least-squares techniques

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(First received October 14th, 1991; revised manuscript received January 14th, 1992)

ABSTRACT

Multiple linear regression and partial least-squares techniques were applied to closed-loop stripping analysis results for flavours from tobacco blends. The merits of this approach for the determination of blend compositions were evaluated.

INTRODUCTION

Closed-loop stripping analysis (CLSA) was introduced by Grob [1] in 1973, and has been developed for the determination of volatile organic compounds at the ng/l level in potable water [1–4]. CLSA in a slightly modified version can also be applied to volatile organic compounds in solid biological materials [5,6]. The closed system consists of a membrane pump, a thermostated sample compartment and an active carbon filter. The volatile components from the sample are transported continuously by a stream of (pre-heated) gas to the carbon filter where they are adsorbed. The adsorbates are eluted from the filter with a suitable solvent, and are analysed by gas chromatography (GC).

Heinzer *et al.* [6] applied CLSA to divide virginia tobaccos into three quality classes on the basis of headspace GC fingerprints, using principal component analysis and discriminant analysis. The carbon trap was eluted with carbon disulphide and cold on-column injection was applied without a pre-column. Heinzer *et al.* reported that the performance of the GC column irreversibly diminished and ascribed this problem to contamination by a relatively large amount of stripped fatty acids.

The aim of this work was to evaluate the GC analysis of tobacco blends for the purpose of production and taste quality control. First, the CLSA–GC method applied by Heinzer *et al.* [6] was modified to overcome the problem of column contamination. Second, multiple linear regression (MLR) and partial least-squares (PLS) techniques [7–10] were applied for calibration (and cross-validation).

0021-9673/92/$05.00

EXPERIMENTAL

Chemicals and characterization of blends

N-Methylbis(trifluoroacetamide) (MBTFA) and Deriva-sil were supplied by Chrompack (Middelburg, Netherlands) and carbon disulphide, dichloromethane and methanol by J. T. Baker (Deventer, Netherlands).

Batches of burley, virginia and oriental tobacco were subjected to a single casing treatment. EXTO is an "expanded" tobacco [11]; the expanded stems are the (treated) main veins of virginia tobacco leaves. The water content of these tobacco samples was determined with ISO Method 6488. Next, the samples were milled to fine powders. A total of ten calibration blends (code 01–10) and five individual tobaccos (code 11–15) were prepared on a dry weight basis. The compositions of the blends were chosen at random within practical ranges: burley 17.7–51.6%, virginia 8.6–32.1%, oriental 7.2–25.4%, EXTO 4.3–33.0% and stems 1.0–10.7%.

Apparatus and procedures

The experiments were conducted with a CLSA system according to Grob (see Fig. 1a), equipped with a glass sample compartment for solid samples and a filter holder (Brechbühler, Schlieren, Switzerland). Throughout 1 g of blend was analysed. The temperatures of the sample compartment and the heating block were 37 ± 1 and 40°C, respectively. The flow-rate of the stripping gas (air) was within the range 1.0–2.5 ml/min (according to the manufacturer's specification), and the strip time was 30 min. The filter holder contained 1.5 mg of finely powdered active carbon between stainless-steel screens. The solutes were quantitatively eluted from the filter with two 1-μl volumes of dichloromethane by moving the plunger of an injection syringe (see Fig. 1b) up and down ten times. The eluates were stored in closed 100-μl vials at −18°C. The filter was regenerated by consecutively sucking through 2 ml of carbon disulphide, methanol and dichloromethane, and was dried under vacuum.

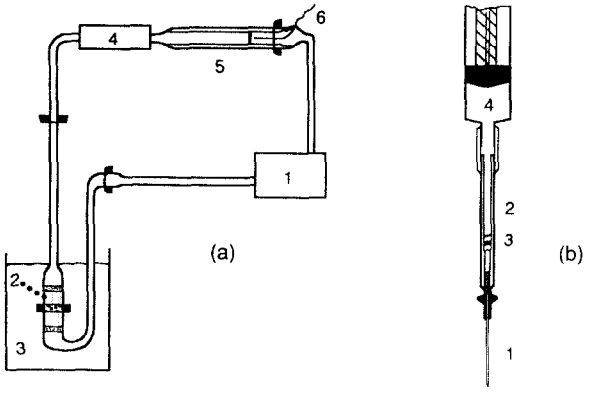

Fig. 1. (a) Scheme of the CLSA apparatus for solid samples. 1 = Membrane pump; 2 = sample compartment; 3 = thermostated water-bath; 4 = heating block; 5 = filter holder; 6 = thermocouple. (b) Design for elution from the carbon filter. 1 = Stainless-steel needle; 2 = filter holder; 3 = carbon filter; 4 = syringe (2 ml) with plunger.

The analyses were executed with an HP 5890 gas chromatograph, equipped with a split injector, a flame ionization detector and an HP 3396 integrator (Hewlett-Packard, Amstelveen, Netherlands). The quartz insert of the split injector was filled with tightly compressed glass-wool to minimize peak broadening. The splitting ratio was 1:10 and the sample size was 5 μl. These conditions were about optimum as far as minimum solute deterioration in the hot injector, amount of sample entering the GC column and extent of contamination of the GC column by strongly retained solutes are concerned. The applied CP Sil-8-CB fused-silica capillary (50 m × 0.25 mm I.D., 95% dimethyl–5% phenylsiloxane, layer thickness 0.23 μm) and the empty fused-silica precolumns (1.5 m × 0.25 mm I.D.) were supplied by Chrompack (Middelburg, Netherlands). After 50 injections the analytical column was flushed through with methanol and the precolumn was replaced. The flow-rates of the carrier gas (helium), septum purge gas, vent gas, make-up gas, hydrogen and air were 1.3 (at 60°C), 3.6, 14, 38, 36 and 270 ml/min, respectively. The injector and detector temperatures were 275 and 300°C, respectively. The oven temperature was linearly programmed from 60°C (injection) to 235°C at 2.5°C/min.

To avoid possible decomposition of thermo-labile constituents [6], the extracts were treated with MBTFA, giving trifluoroacetyl derivatives of primary and secondary amines [12]. Derivasil was applied to convert hydroxyl, amido and amino groups to trimethylsilyl derivatives [13]. These reactions were conducted in either carbon disulphide or dichloromethane.

Ten blends and five individual tobaccos were examined. Each of these was analysed twice by CLSA–GC, giving a total of 30 data series, each consisting of 42 relative peak area data (denoted as the 42/30 set)[a]. To validate the results obtained with MLR and PLS, 28 data series were used for calibration, whereas the two remaining (randomly chosen) data series were used to compute the corresponding single-blend compositions [7]. This procedure was repeated till all 30 blend compositions were computed. Mean-blend compositions were calculated from the results for two single compositions of blends with identical composition. In general, a large number of calibration samples was required for MLR. Therefore, stepwise Max R multiple linear regression [14] was applied in this work. In this technique, more or less characteristic peaks for the various tobaccos are selected, which reduced the required amount of input data. In PLS the number of calibration samples must be at least equal to the number of components to be determined. In general, a larger number is recommended for complex samples in order to compensate for unexpected sources of variation of a chemical and/or statistical nature. The stepwise Max R MLR software (SAS) was supplied by the SAS Institute (Hilversum, Netherlands), and the PLS software (Unscrambler II) by Oliemans, Punter & Partners (Utrecht, Netherlands).

RESULTS AND DISCUSSION

The derivatization reactions appeared to result in the formation of unsoluble residues after evaporation of the solvent. Such evaporation tests were also applied to the untreated extracts. Those in carbon disulphide gave a persistent, unsoluble precipitate on the vial wall after heating at 200°C.

This was not observed when dichloromethane was used as the solvent. Therefore, dichloromethane was used, and derivatization was not applied. The CLSA–GC procedure outlined above appeared to be adequate. Up to 50 samples could be analysed without significant decline of the column performance.

Inspection of the chromatograms revealed that all the blends examined had 8 resolved peaks (higher than ten times the noise level) in common. The relative net retention times of these peaks were calculated, and the peak areas formed the basic 81/30 data set. First, the repeatability of the CLSA–GC results was determined by analysing a representative blend six times. The relative standard deviations (R.S.D.) ranged from 3 to 140%, the lower ones corresponding to large peaks, and the large ones to small peaks, as expected. The median R.S.D. was 11.4%.

In order to improve the precision of the data input, relative peak areas were calculated and R.S.D. values were recalculated. Next, data with R.S.D. $>12\%$ were not used in the statistical analyses. In addition, peaks having similar relative peak areas in two or more individual tobaccos were omitted to eliminate redundant information from the data base. In this manner 42 peaks were selected. The mean and the median of the R.S.D. data of the relative peak areas were $7.5 \pm 2.7\%$ and 8.0%, respectively. Thus, $R.S.D._a = 8\%$ may be considered as the estimate of the analytical precision (*i.e.*, including sub-sampling errors) of the 42/30 data set.

The impact of the correlation between the relative peak area data for the five individual tobaccos was evaluated with PLS. The 42/30 data set was used. Mean results from computed single compositions (as outlined under Experimental) are given in Table I. If no correlation were to occur between the data series for the tobaccos and the experimental errors were to be negligible, a composition of about 100% would be predicted for each of the tobaccos. This is approximately the case, except for virginia lamina and stems, which cannot be distinguished. This can be ascribed to the fact that the examined stems originated from virginia tobacco leaves. Thirty-seven out of 42 relative peaks area data for these tobaccos appear to be correlated ($r = 0.9256$). The data input for these constituents cannot simply be combined to overcome this problem, because the concentrations of virginia and stems strongly determine the taste of a blend. As percentage compositions are computed, it needs no comment that correlation effects also have an adverse impact on the other results in Table I. Therefore, it will be examined whether such effects can be diminished by omitting the data series for the individual tobaccos, which results in the 42/20 data set.

In order to examine the quality of the PLS results,

[a] The data input is available from the authors on request.

TABLE I

MEAN PLS RESULTS FOR INDIVIDUAL TOBACCOS, USING THE 42/30 DATA SET

Code	Real (R) and computed (C) compositions (%)									
	Burley		Virginia		Oriental		EXTO		Stems	
	R	C	R	C	R	C	R	C	R	C
11	100	85.8	0	1.6	0	5.4	0	2.6	0	4.6
12	0	−5.3	100	88.0	0	11.3	0	0.3	0	5.7
13	0	−15.8	0	11.4	100	98.0	0	3.4	0	3.0
14	0	−10.8	0	1.4	0	4.6	100	99.1	0	5.7
15	0	36.5	0	114.6	0	−78.4	0	−26.1	100	53.4

mean-blend compositions were calculated from single-blend results, using the 42/30 data set (as outlined under Experimental). For each of the five blend constituents these results were compared with the real compositions. The largest positive and negative deviations thus obtained are presented as range limits in Table II. These ranges are a measure of the predictive reliability. This reliability appears to improve when the 42/30 instead of the 81/30 data set is used, but no further improvement is achieved with the 42/20 data set. Obviously, correlation effects persist in the 42/20 data set.

The predictive precision was calculated for each blend constituent as the square root of the pooled variance of the two computed results for blends of identical composition. These precision data are given in Table II. The average precision for the five blend constituents appears to improve from 7.1% for the 81/30 set to 4.4% for the 42/30 and 42/20 sets.

PLS and MLR results for two single blends and mean results from single blends with an identical composition are given in Table III. The results from the 81/30 data set appear to be poor, as expected, whereas those from the 42/30 and 42/20 data sets are equivalent. On average, the MLR and PLS methods appear to give similar predictive reliabilities. However, note that the sum of the MLR results is not equal to 100%, which is a weak point in this method. Further, it is illustrated in Table III that poor predictions can incidentally be obtained for single-blend data (for instance, the burley content of blend 07.1, computed with PLS from the 42/30 data set); the mean result for burley in the 07 blend is more accurate. Using the 42/30 and 42/20 dats sets, the mean deviation from the real percentages is $\pm 3.0\%$. It is noted that for taste quality control absolute deviations of $\leqslant 2\%$ are desirable.

Finally, it will be estimated to what extent correla-

TABLE II

MEAN PLS RESULTS FOR THE PREDICTIVE RELIABILITY (RANGE) AND PRECISION, USING THE 81/30, 42/30 AND 42/20 DATA SETS

Set	Parameter	Burley	Virginia	Oriental	EXTO	Stems
81/30	Range	−7.4; 7.0	−17.9; 16.0	−6.7; 4.2	−3.9; 5.2	−9.0; 7.6
	Precision	7.4	10.3	4.5	5.1	4.0
42/30	Range	−4.5; 7.9	−7.2; 6.0	−4.4; 3.9	−5.0; 9.6	−7.2; 3.5
	Precision	6.7	4.0	3.0	4.9	1.4
42/20	Range	−4.1; 2.9	−4.6; 7.4	−6.4; 4.8	−5.7; 10.6	−7.4; 4.5
	Precision	2.7	3.3	6.4	6.0	1.6

TABLE III

PLS AND MLR RESULTS FOR THE BLENDS 03.2 AND 07.1, AND MEAN RESULTS FOR THE 03 AND 07 BLENDS, USING THE 81/30, 42/30 AND 40/20 DATA SETS

Data input	Real and computed compositions (%)				
	Burley	Virginia	Oriental	EXTO	Stems
Composition 03	26	23.9	26.0	18.9	4.6
Blend 03.2					
81/30 PLS	21.9	33.8	23.9	15.1	5.3
42/30 MLR	33.8	12.8	22.0	20.0	5.0
42/30 PLS	30.8	18.8	26.4	17.7	6.3
42/20 PLS	26.4	20.8	26.0	22.7	4.1
Blends 03					
42/30 PLS	22.1	23.7	24.0	23.0	7.4
42/20 PLS	25.8	23.0	22.2	23.6	5.4
Composition 07	31.1	10.7	22.9	31.7	3.6
Blend 07.1					
81/30 PLS	41.4	−17.0	18.2	42.2	15.2
42/30 MLR	30.6	18.9	17.0	29.4	2.0
42/30 PLS	18.5	16.6	21.6	35.2	8.1
42/20 PLS	32.5	16.0	19.0	27.1	5.4
Blends 07					
42/30 PLS	29.1	15.0	22.0	28.1	5.9
42/20 PLS	31.6	18.1	21.0	26.0	3.4

tion between the relative peak-area data for the various tobaccos adversely affected the predictive precision of the PLS results. First, it is assumed that R.S.D.$_a$ = 8% for all blend constituents. Second, it is assumed for convenience that the analysis errors propagate unaltered into the final results (which is probably too pessimistic an assumption). On this basis, the standard deviations, s_a, can be calculated from the real composition data given in Table III. It was verified with the Kolmogorov–Smirnov test [15] that the differences between the computed and real percentages for single-blend results (using the 42/30 and 42/20 data sets) do not deviate significantly from a normal distribution. Combination with the precision data (here denoted as s_p) obtained from the 42/30 data set presented in Table II gives s_c/s_p $[\geqslant (1 - s_a^2/s_p^2)^{\frac{1}{2}}] \geqslant 0.9$, where s_c is the standard deviation due to correlation effects. Therefore, it can be concluded that correlation effects, rather than analytical precision, determine the predictive precision (and reliability) of the PLS (and MLR) results for tobacco blend analysis.

CONCLUSIONS

Tobacco flavours can be analysed satisfactorily with the CLSA–GC technique if split injection is applied and dichloromethane is used to elute these compounds from the carbon filter. The median R.S.D. of the relative areas of 42 selected peaks is 8%, which is acceptable in ultra-trace analysis.

CLSA–GC in combination with PLS can be successfully applied to identify individual tobaccos (burley, virginia, oriental and EXTO), but virginia tobacco leaves cannot be distinguished from its stems owing to the correlation between the peak area data. Correlation effects predominantly determine the reliability and precision of the computed blend composition. Using a 42/30 (or a 42/20) data set, and PLS or stepwise MLR, the means of computed results for two identical blends provide more reliable estimates and show, on average, a deviation of ±3.0% from the real percentages of the blend constituents. The mean precision of the computed percentages is 4.4%.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. M. Gerritsen (University of Nijmegen, Netherlands) for the execution of the PLS computations, and to Drs. A. Spijkers (Philip Morris Holland) for his contribution to the MLR computations.

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