CONFERENCE CONTRIBUTION

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Virginia Tobacco Leaves (CTA-VTL-2) – new Polish CRM for inorganic trace analysis including microanalysis

Abstract Preparation and certification of a new Polish reference material (CTA-VTL-2) for inorganic trace analysis including microanalysis is described. Virginia Tobacco Leaves of Bulgarian origin were hand picked, dried, comminuted and sieved at conditions preventing contamination of the material with metals. Ca. 13 kg of the material with particle size $\phi \leq 80$ μm was prepared. Certification was based on world-wide interlaboratory comparison in which 60 laboratories from 18 countries participated. Data evaluation was done using an approach which includes rejection of outliers by concurrent use of four different outlier tests followed by calculation of overall means, confidence intervals etc. Several criteria were employed to qualify or disqualify the obtained overall mean as a certified or information value, respectively. 33 elements were certified and information values were established for 10 others. Particle size distribution of CTA-VTL-2 was investigated microscopically. The modal particle diameter was in the range of 15–35 μm. Extensive homogeneity checking by neutron activation analysis revealed that the material is sufficiently homogeneous for mg sample sizes.

Introduction

Reliable measurements in analytical chemistry require the use of certified reference materials (CRMs) to check the performance of laboratories, analysts, new methods etc. CRMs are especially important in trace analysis where differences in results provided by individual laboratories may sometimes reach several orders of magnitude [1, 2].

Natural matrix CRMs are usually inhomogeneous on a microscopic scale and apparent homogeneity is achieved by grinding, sieving and mixing so that a sufficiently large number of individual particles is present in a subsample taken for analysis.

CRMs currently available from various producers have usually homogeneity guaranteed for sample weights of 100–250 mg [3–6] and sometimes even as high as 0.5–1.0 g [7–8]. This does not necessarily mean that smaller subsamples of these materials will show distinct inhomogeneity, but as the relevant information is not available they should not be used below the sample mass recommended by the manufacturer.

On the other hand some microanalytical techniques such as EDXRF, PIXE, SS-AAS use in fact smaller sample masses than those mentioned above and in this case no CRMs are practically available. Other techniques like e.g. INAA also have capability to use smaller sample masses than 100 mg. Therefore the search for CRMs which would be suitable for microanalytical techniques is of vital importance [9].

In this paper preparation and certification of a new Polish reference material Virginia Tobacco Leaves (CTA-VTL-2) for inorganic trace analysis including microanalysis is described.

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Collection, preparation and testing of the material

The general strategy was similar to that employed when preparing CTA-OTL-1 CRM [3]. Fresh tobacco leaves of the variety "Virginia" were hand-picked, dried first in the air and then at 40°C and comminuted by hand in agate mortars with agate pestles. All operations were performed at conditions minimizing the possibility of contamination of the material with metals. The ground leaves were sieved through an 80 μm all plastic nylon sieve and collected in a nylon bag.

Dry Tobacco Leaf powder (ca. 13 kg) was homogenized for several hours in a 110 L polyethylene (PE) drum placed in a specially constructed homogenizer. Preliminary homogeneity testing done by determining Fe, Ca and K in several subsamples by X-ray fluorescence (XRF) did not indicate any significant inhomogeneity.

The material was distributed in 25 g portions (future CRM) into 150 mL PE bottles with screw cap and in 10 g portions into

PE envelopes which were subsequently heat-sealed (intercomparison samples). The distribution was performed with the aid of a teflon scoop with adjustable volume constructed in our laboratory, and PE or teflon vessels which were previously cleaned with acids of spectral purity and washed with bidistilled water according to the procedure recommended by Mart [10].

Several drying procedures were tested. It was found that drying at 85°C gives very reproducible results without any sign of material decomposition. The recommended procedure for moisture determination is 24 h drying at 85°C and doing it on separate subsample (not that taken for analysis).

To ensure long-term stability of the new CRM the whole lot after distribution into containers was sterilized by electron beam radiation (energy 13 MeV) from linear accelerator LAE-13/9. The dose was approximately 30 kGy.

Study on particle size of CTA-VTL-2

Particle size measurements were performed using Mastersizer $X^©$ (Malvern Instruments Ltd.) at Chemistry Unit, IAEA Laboratories Seibersdorf. The samples suspended in water-detergent solution gave unexpectedly high results (median diameter between 98 and 125 μm, modal value between 164 and 174 μm). Measurements repeated with the same instrument but using a dry powder feeder yielded markedly different results: median particle diameter 46–50 μm, modal value 79–85 μm. In addition some of the distributions were distinctly bimodal with the smaller peak corresponding to approximately 20–30 μm and the second larger peak corresponding to ca. 83 μm. Both series of results seemed doubtful, especially taking into account that the whole lot of CTA-VTL-2 was sieved through an 80 μm sieve.

Microscopic examination revealed that particles of this material have strong tendency to aggregate. To minimize this effect and obtain samples suitable for microscopic particle size measurements, the suspension of the material in water was agitated in an ultrasonic bath for 30 min. A drop was transferred onto the glass plate and dried in air. Martin's diameter (arithmetic mean of the maximum distance between opposite sides of a particle and a distance in perpendicular direction [11]) of 100–200 particles chosen at random were measured, using the microscope with × 800 magnification. A typical histogram is presented in Fig. 1. (see Supplementary material). The measurements were repeated several times yielding quite reproducible results and confirming that most numerous were particles with the diameter in the range of 15–35 μm, while the particles with Martin's diameter ≥ 70 μm (having fiber form) were extremely rare.

Estimation of homogeneity of the material and its suitability for microanalytical techniques

From a simplified model assuming that the material consists of only two kinds of spherical particles with different percent content of the analyte [12–14], one can conclude that subsampling error is directly proportional to $r^{3/2}$ (where r is particle radius) and inversely proportional to subsample mass, m.

Final homogeneity checking of CTA-VTL-2 as a function of sample mass was performed by instrumental NAA, analyzing 6 samples of a given mass taken from different containers chosen at random from the whole population and comparing the results with these obtained by analyzing 6 similar samples from a seventh container.

The results for Ba, Co, Cr, Cs, Eu, Fe, Hf, Rb, Th and Zn in the two series were compared by Fisher's test and t-test [15]. Five series of analyses for nominal sample masses of 100 mg, 50 mg, 30 mg, 5 mg and 1 mg, respectively, were performed and the results are shown in Tables 1 and 2 (see Supplementary material). In all cases $F \leq F_{0.95}$ and $t \leq t_{0.05}$.

Considering that the contents of elements used in homogeneity checking span nearly five orders of magnitude (Eu: 0.033 mg/kg; Fe: 1083 mg/kg) it follows that the material can be considered homogeneous down to sample masses of the order of one milligram.

The number, n, of spherical particles, in the mass m of the material is given by:

$$n = \frac{3\,m}{4\,\pi\,r^3\,d}$$

where d is material density.

The density of CTA-VTL-2 determined using a pycnometer and n-hexane was found to be 0.635 g cm^{-3}. Assuming that the most frequent Martin's particle diameter of approximately 25 μm (cf. Fig. 1) can be used as a *quasi-spherical diameter* in Eq. (1), the number of particles in 1 mg sample is 1.9×10^5, so it seems large enough to assure satisfactory homogeneity.

Good homogeneity of CTA-VTL-2 in 10 mg samples was also confirmed by Zhang et al. [16] using INAA. For such techniques as micro PIXE, in which analytical information is obtained from sample masses of the order of single micrograms or even lower [17], the number of individual particles of CTA-VTL-2 involved will be of the order of hundreds which may be too small to secure good homogeneity. Even in this case however, the observed scatter of results for most elements did not exceed 10–25% [17].

Interlaboratory comparison and data evaluation

The world-wide interlaboratory comparison on the determination of trace elements in CTA-VTL-2 was organized in the years 1989–92 with a few results supplied at a later date. Sixty laboratories from 18 countries took part contributing 4100 bits of chemical information (1057 laboratory means) on 57 elements. Neutron activation analysis (NAA), atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), and X-ray fluorescence (XRF) were the most frequently used methods. Details are given in Table 3 (see Supplementary material).

The method of data evaluation was essentially the one used earlier, however, with some refinements [3, 5, 18–21]. The essence of the method consists in concurrent use for the population of laboratory means of the four criteria for rejection of outliers (i.e. those of Dixon, Grubbs, coefficient of skewness and coefficient of kurtosis, each at a significance level of 0.05) followed by calculation of the overall mean of results, remaining after outlier rejection, and its confidence interval. This is, however, not a purely statistical approach, as the inseparable part of this method of assigning certified and information values are qualification criteria based on long time experience which are fully described in a recent Report [20].

Certified and information values

Table 4 presents certified and information values for elements in CTA-VTL-2. One can note that quite an impressive number of 4 major and 29 trace elements could be certified and in addition information values for 10 elements were given. It is worth noting that certified values could be assigned to several elements like Ce, Cs, La, Li, Sm, Tb, Th and U for which very few biological CRMs seem to exist.

Table 4 Certified and information values for CTA-VTL-2

Major elements		Trace elements			
Element	Concentration wt %	Element	Concentration μg/g (ppm)	Element	Concentration μg/g (ppm)
Certified values					
Ca	3.60 ± 0.15	As	0.969 ± 0.072	Mo	2.01 ± 0.15
Cl	0.743 ± 0.028	Ba	42.7 ± 6.6	Ni	1.98 ± 0.21
K	1.03 ± 0.04	Br	14.3 ± 1.4	P	2204 ± 78
Mg	0.510 ± 0.023	Cd	1.52 ± 0.17	Pb	22.1 ± 1.2
		Ce	1.91 ± 0.29	Rb	48.6 ± 2.3
		Co	0.429 ± 0.026	Sb	0.312 ± 0.025
		Cr	1.87 ± 0.16	Sm	0.157 ± 0.022
		Cs	0.515 ± 0.046	Sr	110 ± 12
		Cu	18.2 ± 0.9	Tb	0.022 ± 0.005
		Fe	1083 ± 33	Th	0.378 ± 0.031
		Hf	0.118 ± 0.022	U	0.163 ± 0.008
		Hg	0.048 ± 0.009	V	4.00 ± 0.42
		La	1.01 ± 0.10	W	0.233 ± 0.063
		Li	23.0 ± 1.9	Zn	43.3 ± 2.1
		Mn	79.7 ± 2.6		
Information values					
S	0.669	Al	1682	Si	4590
		Eu	0.033	Ta	0.033
		Na	312	Ti	76.3
		Sc	0.268	Yb	0.080
		Se	0.188		

Auxiliary actions associated with the certification process

Although our methodology of data evaluation has proved its merits over the period of nearly twenty years [3, 5, 18–22] and was adopted by other groups of workers [23, 24] it is always tempting to have independent confirmation of the validity of the "recommended" or "certified" data. Over the past years a work on devising "very accurate" and "definitive" (in a sense of definition by Uriano and Gravatt [25]) methods by radiochemical NAA for selected elements was carried out in this Laboratory. This enabled direct verification of certified data at least for some elements.

The certified value for cobalt: 429 ± 26 μg/kg is in excellent agreement with the result: 425 ± 38 μg/kg obtained by "definitive" method [26, 28]. Similarly the certified value for cadmium: 1.52 ± 0.17 mg/kg agrees well with our result: 1.39 ± 0.11 mg/kg obtained by the recently published "very accurate" method [27]. Also the certified value for nickel: 1.98 ± 0.21 mg/kg is in a good agreement with the result: 2.11 ± 0.18 mg/kg obtained by a recently developed "very accurate" method [28].

The certified value for uranium: 163 ± 8 μg/kg is in excellent agreement with our result by "very accurate" method: 164 ± 8.6 μg/kg [29]. And the certified value for molybdenum: 2011 ± 151 μg/kg is in a good agreement with our value by very accurate method: 1933 ± 141 μg/kg [29]. It can be concluded that for all these elements the results obtained by "definitive" or "very accurate" methods afforded additional assurance as to the validity of as-

CERTIFIED vs. EXPERIMENTAL DATA
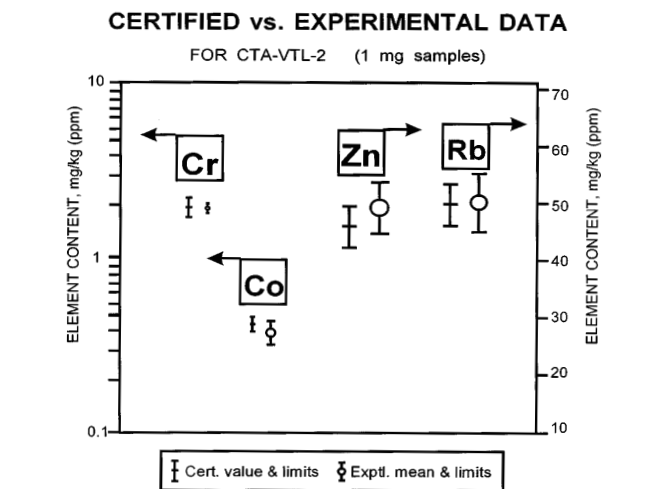FOR CTA-VTL-2 (1 mg samples)

Fig. 2 Comparison of experimental values for Co, Cr, Rb, and Zn and their confidence limits as obtained by analyzing 1 mg samples of CTA-VTL-2 by INAA (circles with bars), with the certified values and their confidence limits (horizontal lines with bars)

signed certified and "information" values obtained using our method of data evaluation.

To confirm the suitability of CTA-VTL-2 for microchemical work, several elements were quantitatively determined in 1 mg samples by INAA, and the means and their confidence intervals were compared with the certified values and their confidence limits [30]. Some data are shown in Fig. 2, demonstrating good accuracy and preci-

sion of the measurements. It can be concluded that CTA-VTL-2 may be used as a CRM for microchemical work provided that analytical information is obtained from sub-samples with masses not markedly lower than 1 mg.

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