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QUANTIFICATION OF VITAMIN B - 12 CONTENT IN MULTIVITAMINS AND ELEMENTS IN MINERAL SUPPLEMENTS IN CHILE, BY TOTAL REFLECTION X-RAY FLUORESCENCE

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ABSTRACT: This manuscript presents the application of a detailed set of analytical technologies to the content of assorted micronutrient supplement and mineral supplement products on the market in the city of Concepcion, Chile. We applied TXRF as a screening technique for the evaluation of 1) The vitamin B-12 content in multivitamins and 2) the elemental composition (P, K, Ca, Mn, Cr, Fe, Cu and Zn) in mineral supplements. The analyzed 16 products (10 sold as pills, 3 as syrups, and 3 as solutions for intramuscular injections), were acquired directly from drugstores without medical prescription. They were processed in 3 different laboratories in order to avoid systematic errors. In the samples we evaluated: 1) The presence of the specified elements, 2) The correlation between the labelled contents and our evaluations; and 3) The possible presence of harmful elements for human health. In this study few anomalies were found: 1) in some products there are significant differences between the concentrations labelled and our evaluations. 2) The absence of cobalt in four of these vitamin B-12 containing products, 3) The presence of lead in a sample for intramuscular injection, 4) Arsenic was found in one of them, and 5) A trace detection of a rare earths element (possibly Ytterbium) in a particular product. The products studied are imported from different countries and they are labelled in their country of origin, so the questions about the safety and efficacy aspects of them could affect not only to Chilean consumers.

INTRODUCTION: Nowadays in society the consumption of multivitamins is common practice because they can be purchased without restrictions. The usage of these products has increased in adults, kids and vegans with the aim of improving health and finding an alternative to include the essential vitamins and minerals in a diet.

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Since these products can be easily acquired, it has been proposed the evaluation of the vitamin B-12 content in multivitamins, and for the determination of the composition in mineral supplements, using the total reflection X-ray Fluorescence (TXRF) technique.

This is a multi-element, sensitive and fast technique, which could be useful for both, pharmaceutical companies and regulatory agencies in order to control impurities in pharmaceutical compounds. Our initial objective is to check that the content of elements labeled in the container matches the one obtained by TXRF. Given the fact that the public policies that regulate this type of products could be deficient, there might be loopholes to the detriment of consumers [1, 2]. In the case of vitamin B-12, the amount of Cobalt declared in the commercial product is related to the content itself of this vitamin by calculating the proportions of molar masses, since one atom of this element is found in the Cyanocobalamin molecule.

In Chile, the National Regulatory Authorities (referred to by its Spanish acronym ARN) carries out activities of medication control and monitoring, along with various entities with legal authority in the subject, such as The Ministry of Health, The Undersecretary of Public Heath (referred to by its Spanish acronym SSP), The Public Health Institute (referred to by its Spanish acronym ISP) and its National Drug Agency Department (referred to by its Spanish acronym ANAMED), and the Regional Ministerial Health Departments in the country [3]. At an international level, two of the most renowned entities in the field are the World Health Organization (WHO) [4] and the Food and Drug Administration (FDA) [5] in the United States.

At a national level, the MINSAL is in charge of establishing health policies that regulate government and private organizations in the medication and food field, among others. In order to enforce these policies, two main organizations stand in the regulation and classification of products: the Undersecretary of Public Health and the Public Health Institute. Each one fulfills a specific task to ensure that every person has the right to health insurance, performing regulatory, normative and overseeing activities that the State of Chile is responsible for. The ISP is the entity responsible for supervising and contributing to the country's public health as the State Technical-Scientific Institution, developing Reference, Monitoring, Authorization and Overseeing functions according to its competences. This overseeing task is performed through several departments, such as ANAMED and the Environmental Health Department.

ANAMED is in charge of controlling pharmaceutical products, cosmetics and other products authorized by law, locally manufactured or imported for its commercialization within the country [6], guarantying quality, efficiency and safety. Its internal structure [7] is organized in eight sub-departments, units and sections [8]. Among the sub-departments we can mention the Sanitary Registration and Authorization sub-department, the Control National Laboratory [9], the Pharmaco-vigilance, the Bio-pharmacy and Bio-equivalence, among others.

Likewise, the Environmental Health Department regulates, supervises and oversees, generating analytic traceable and comparable information that permits an adequate decision-making process in the areas of food and environmental safety, and so on. To achieve this, it relies on the sub-department of Food and Nutrition, which serves as the National Laboratory of Reference in the food area and performs epidemiology vigilance activities, carrying out chemical analyses, in food matrices among others. This department is divided in two sections: Food Chemistry and Nutrition, and Food and Water Microbiology. In addition, it regulates public and private laboratories on the network.

The Sub-Department of Sanitary Registration and Authorization ensures the quality, safety and efficiency of medications and cosmetics, from the investigation stage until further usage. On the other hand, the National Control Laboratory Sub-department performs the quality evaluation of the pharmaceutical products that are subject to sanitary control; the Pharmacovigilance sub-department is in charge of carrying out the detection, evaluation, comprehension and prevention of the possible negative effects associated with the usage of this medications; and finally, the bio-pharmacy and bio-equivalence sub-department authorizes and supervises the facilities where a medication is tested through scientific studies, at both national and international level, in order to exchange it with the comparator product.

When the concentrations of elements present in food supplements are within the limits established [10], the one responsible for its regulation is the Environmental Health Department. Conversely, when these limits are exceeded, for a regular consumer as well as for high-performance athletes, [11, 12] these will be considered as medicaments and they will be supervised by ANAMED, according to the regulations established by the Control National System of Pharmaceutical Products for human consumption [13].

As demand for these products increases, it becomes appropriate the use of techniques that generate information to strengthen the criteria of the health authorities; since they will face multiple challenges in terms of regulation, control, and new norms. In this aspect, we can mention the drug traceability, the evaluation of therapeutic efficiency associated with studies of bio equivalence and the implementation of generic medications, and so on. In the control and monitoring of multivitamin and mineral supplements, the processes already established could be inefficient over the high proliferation of products that are being currently commercialized in the country. During the last two decades fast development in analytic techniques has been observed. Particularly, the TXRF spectroscopy is a fast and efficient technique for multi-element analysis which can be used to perform both qualitative and quantitative determinations for elements with Z > 13.

This technique is based on the study of fluorescence emissions that present characteristic energies of the elements in the sample. When the technique is properly applied, the emission intensity is linearly related with the atomic concentration of the elements in the sample. One of the main characteristics of this technique is the small incidence angle of the X-ray beam which is below a critical value. Given this condition, nearly 100% of the photons of the excitation beam can be reflected, thereby reducing the effect of background noise. Thus, the detection limit of this technique can be decreased to the order of 10^{-9} g/g [14].

TXRF analysis in liquid homogeneous samples is fast, simple and reliable. The specimen obtained from the sample is prepared as a drop residue dried (5 - 50 µL) on a proper pre-cleaned sample carrier. The absolute amount of this film-like deposit is usually below 1 µg and the preparation is usually performed in air. In order to obtain an optimal TXRF analysis, two main prerequisites are required: uniform distribution and minimum height of the residue, which normally has a diameter of 2-6 mm.

To find the composition of an unknown sample, the element intensities obtained from analysis of the spectrum should be corrected by the sensitivity curve. This curve is usually obtained through an analysis of multi-element reference samples of known composition with high accuracy and low uncertainty. Sensitivity curves are provided by manufacturers in modern equipment [15] which remain almost unchanged for the device lifetime. However it is advisable to be adjusted once or twice a year.

This technique shows comparative advantages with spectrophotometric measurements or inductively coupled plasma (ICP), for instance, with one unique measure you can obtain the composition of the sample; it is reliable within a range of compositions of at least three orders of magnitude [16], it is highly sensitive, it is fast, and it is simple to apply. For this reason, TXRF is currently used in a wide variety of disciplines, like Pharmaceutical [17, 18], Environmental, *etc* [19, 20].

In this study we applied the TXRF as a screening technique for the evaluation of the vitamin B-12 content in multivitamins, and for the determination of the composition in mineral supplements. The samples analyzed consisted on 16 products (10 are sold as pills, 3 as syrups, and 3 as solutions for intramuscular injections), which were processed in 3 different and prestigious laboratories in order to avoid systematic errors. In the samples studied we evaluated: 1) the presence of the specified elements, 2) acomparison between the labeled contents and the concentrations obtained by the technique; 3) the possible presence of harmful / pernicious elements for human health.

The resulting information could be useful to the organizations in charge of controlling and monitoring these products [21], according to some international models; [22] and additionally, it could contribute i) to promote the studies related to the bio-equivalence of medications because it makes possible the evaluation of their inorganic chemical profiles; and in case of detecting harmful elements to the detriment of human health, ii) to define legal aspects linked to these products.

In regard to the particular case of vitamin B-12 content, the amount of Cobalt declared in the commercial product is related to the content itself of this vitamin by calculating the proportions of molar masses (because one atom of this element is found in the Cyanocobalamin molecule ($C_{63}H_{88}CoN_{14}O_{14}P$) see **Fig. 1**.

Simultaneously, since the great variety of stock in the market is associated with a wide range in prices that could be set subjectively, in the generated information it is also included the cost of the product, the number of pills, and so on, in order to consider the economic aspects among the variables of interest. Moreover, from an international perspective, doping among athletes happens quite often, supposedly unintended, which is attributable to some inefficiency in the monitoring and control of these products [23]. In this scenario, the usage of fast, simple and reliable techniques could be very useful.

Even though TXRF is a mature technique that had its first trial version in the mid-eighties, currently it is not widely known. On the other hand, at an international level in terms of health evaluation, medications or environment, an extensive specialized bibliography about its applications, can be found [24, 25, 26]. Specifically in the case of Chile we are not aware of the existence of applications related to the characterization of medications or food supplements applying this technique.

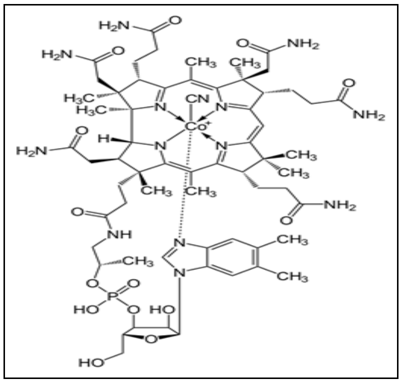

FIG. 1: STRUCTURE OF THE CIANOCOBALAMINE MOLECULE

The elements inside of the red circles can be detected by TXRF. Cobalt determination was chosen because it has higher sensitivity than Phosphorus, by this technique.

Theory: If the residue of the sample is obtained with uniform distribution and minimum height, then the basic equation for TXRF [27] can be expressed as:

$$I_t = K_i I_0 N_0 \omega_i m_i A_i \qquad \text{Eq...1}$$

Being I_i the intensity of the signal produced by the i element. I_t is described based on the following parameters: K_i, a constant that depends on the geometric factors and on the detection efficiency of the energy of the considered line; I_0 is the intensity of the excitement source, N_0, the Avogadro´s number, m_i, the superficial density of the i element with the atomic number Z_i and atomic mass A_i; σ_i is the cross section of the atom (scattering), and ω_i the fluorescence performance.

Due to the sample solution deposited on the reflector is not homogeneous, changes in the distributions of m_i are produced, so just one sample can show significant variations of the total counts in different spectra. To avoid this uncertainty, an internal pattern (STD) is added to the solution. It must be an element that originally is not present in the sample, and whose concentration is well defined. In the data processing, the concentrations of the original elements are referred to the concentration of the included standard. The relationship between the relative intensity of the element STD with respect to each one of the elements i in the sample, can be expressed as:

$$\frac{I_{STD}}{I_i} = \frac{K_{STD} I_o N_o \sigma_{STD} \omega_{STD} A_{STD}}{K_i I_o N_o \sigma_i \omega_i A_i} \cdot \frac{m_{STD}}{m_i} \qquad \text{Eq...2}$$

Due that the relation of m_{STD}/m_i is the same as the relation between the concentrations of these elements in the solution (CSDT/Ci), the Eq. (2) can be written as [1]:

$$\frac{I_{STD}}{I_i} = S_{STD/i} \cdot \frac{m_{STD}}{m_i} = S_{STD/i.} \cdot \frac{C_{STD}}{C_i} \qquad \text{Eq...3}$$

Where $S_{STD/i}$ is the relative sensitivity of the element i related to the internal standard, STD. This relationship is obtained from the sensitivity of detection of an element i, according to:

$$S_i = K_i I_o N_o \sigma_i \omega_i A_i \qquad \text{Eq...4}$$

This sensitivity is different for each element and mainly depends on the fundamental parameters (σ_i and ω_i) and the conditions of measurement (K_i) that generally can be supposed as constant. Generally, this curve is obtained measuring certificated multi-element samples and in recent works, measuring this answer when analyzing the relative intensities of known stoichiometry compounds [29, 30].

In summary, the conversion of the intensity measured I_i into the unknown concentration C_i of the element *i* is obtained from Eq. (2), as:

$$C_i = \frac{I_i}{I_{STD}} \cdot \frac{C_{STD}}{S_{STD/i}} \qquad \text{Eq...5}$$

The intensity I_{STD} and the concentration C_{STD} were already defined.

Experimental:

Reagents: For the calibration of the TXRF spectrometer, eight MERCK ICP single element standard solutions of S, K, Sc, V, Mn, Co, Cu and Ga were used. For the sample preparation as well as for the dilution of the samples, pure water was obtained from a feeder 55 WG with subsequent de-ionization by a Milli-Q SP Reagent Water System (Millipore) which yields ultrapure water with specific resistivity of 18 MΩ.cm. For solid samples digestion MERCK concentrated supra-pure HNO_3 mineral acid was used.

Sample Preparation: For the determination of elemental X-ray line sensitivities for S, K, Sc, V, Mn, Co and Cu, two Multi-Element Solution (MES) standards having elemental concentration of 5 and 10 µg/mL were prepared by mixing the corresponding MERCK single element standards and diluting the resultant solution mixture to the required concentrations, using ~1M nitric acid. Galium was added as internal standard in these solutions with a constant concentration of 5 µg/mL. The TXRF spectra of these standards were measured for 500 s and the relative sensitivities with respect to Ga were determined, as is described in our previous studies [29, 30].

The samples analyzed consisted on 16 products (3 are sold as solutions for intramuscular injections, 10 as pills and 3 as syrups), which were acquired directly from pharmacies (drug stores) without medical prescription, in Concepcion City, Chile.

All of the solid samples (two or three pills, 0.5 - 3 g in weight) were crushed and the protective capsule was removed. Then they were dissolved by a wet chemical digestion utilizing 5 mL concentrated HNO_3 mineral acid, in a hot plate at 75 ºC, for about 24 h, in order to completely transfer the analytes homogeneous liquid samples. Three replicates were made for each sample, since their wet digestion were required to three different specialized Laboratories, established in the Concepcion University, 1) at Institute of Applied Economic Geology - UdeC (L1) [31], 2) Renewable Resources Laboratory - Faculty of Chemical Sciences - Center of Biotechnology - UdeC (L2) [32], and - Laboratory of Animal Nutrition - Department of Pathology and Preventive Medicine - Faculty of Veterinary Sciences- UdeC - Chillan City (L3) [33]. Along with the solid pills, we processed a reference material, a vegetal sample, provided by the International Atomic Agency [34].

In the TXRF technique a complete digestion process is advisable for complete decomposition of the organic, matrix when present, as is the case of products containing vegetables substances, like: Siberian Ginseng, yeast of beer (Saccharomyces Cerevisiae), royal jelly, pollen, cereal germ, Beta-Carotene, *etc*. Prepared samples included Ga in 5 ppm as internal standard, but in a particular case (samples digested at L2) Vanadium at 10 ppm was used as internal standard. In all of the procedures we avoided loss or contamination of the analytes. For the preparation of the blank samples, all the above steps were followed except that milli-Q water was taken in place of solid pills in the beginning. A flow chart for the solid samples preparation procedure is depicted in **Fig. 2**.

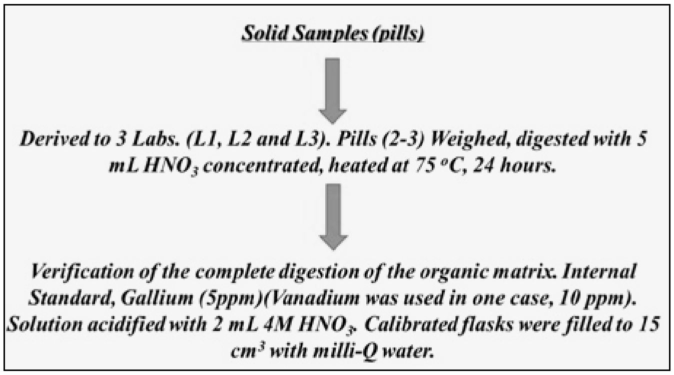

FIG. 2: FLOW CHART OF THE SOLID SAMPLES PREPARATION STEPS FOR THE PROCESSING FOR Co AND MINERAL ANALYSIS BY TXRF

For TXRF specimen preparation 2 - 5 µL aliquots of the digested samples were deposited on cleaned quartz (SiO_2) sample supports and they were dried under an infrared lamp so that a small area sample specimen is formed and adverse effects of non-uniform deposition of the sample if any, is avoided. These specimens were loaded in the TXRF spectrometer and spectra were acquired by signal integration for at least 500 s. The syrups samples and the solutions for intramuscular injections were prepared in a similar way. In each case the acid digestion was applied to 5 mL of the liquid samples.

X-ray Spectrometer: The TXRF instrument used in this study was a S2 Picofox Bruker spectrometer enclosed in an X-ray biological shield. The system includes an X-ray metal-ceramic tube with a molybdenum target operating at 50 W of maximum power, at 50 kV and 1 mA. The system is air-cooled; and includes a multilayer monochromator; a Peltier-cooled high resolution X Flash silicon drift detector (without liquid nitrogen cooling) with a 30 mm^2 active area and an energy resolution less than 140 eV at 100 kcps (Mn Kα line, 5.895 keV). The instrument was controlled by a computer by its Spectra 6.3 software.

RESULTS: In order to obtain better accuracy and precision in the TXRF results, an appropriate internal standard should be defined. This element should have good excitation efficiency and its X-ray lines should not interfering with the elemental X-ray lines of the elements present in the sample. As internal standard, in most of the cases we used Ga in 5 ppm, but in a particular case, (samples derived to L2) Vanadium in 10 ppm was selected.

The Spectra 6.3 software, released by Bruker, was used for the spectral fitting and analysis of the acquired TXRF spectra. The spectra were processed with very good fitting parameters and were used to analyze the trace elements present in it, using the sensitivity values of analytical X-ray lines. The S_i Kα line in the spectra are coming from the quartz sample supports.

The results obtained for vitamin B-12 in a) injectable solution samples are shown in **Table 1**, and the Co and Multi-elemental results in syrups are shown in **Table 4**. For the solid samples (pills, caplets) the results obtained by TXRF for Vitamin B-12 are shown in **Table 2** and for mineral multi elemental quantification are shown in **Table 3**.

TABLE 1: COMPARATIVE RESULTS OF THE COBALT EVALUATION IN INJECTABLE MULTIVITAMINS, DIGESTED IN THE LAB-1 (GEA). FOR EACH PRODUCT AT LEAST THREE DIFFERENT EVALUATION WERE MADE, IN RECIPIENTS WITH DIFFERENT SERIAL NUMBERS

Sample Number	Origin	Cost	Concentration Co in the sample [g/L]	B12			
				Amount Co in the sample [µg]	Quantified [mg/portion]	Labeled [mg/portion]	Quantified / Labeled
1 (L)	Chile	$ 6.294	0.20 ± 0.05	410 ± 21	9.425 ± 0.49	10	94.25 ± 5%
3 (L)	Chile	$ 2.400	0.16 ± 0.04	493 ± 16	11.35 ± 0.03	10	113.5 ± 4%
4 (L)	Mexico	$ 6.890	0.44 ± 0.03	443 ± 20	10.2 ± 0.02	10	101.72 ± 5%

In **Table 1**, we find the data for liquid injectable multivitamins, which has a similar configuration as **Table 3**, cost and origin of each product and concentration as well as quantity present in the sample, quantity per portion, and quantity labelled on the package. From **Table 1**, it can be inferred that in all samples the concentrations quantified by the technique are in full accordance with the labelling. It means that the information provided to the customer is correct.

TABLE 2: COMPARATIVE RESULTS OBTAINED FROM THE MULTIVITAMINS IN PILLS. FOUR SAMPLES WERE ALSO DIGESTED IN OTHER LABORATORIES, IN ORDER TO CORROBORATE SOME ANOMALIES

B-12											
			Lab-1 (GEA)			Lab-2 (BioTec)			Lab-3 (Fac. Vet.)		
Sample Number	Origin	Cost	Mass analized [g]	Quantified [ug/portion]	Labeled [ug/ portion]	Mass analized [g]	Quantified [ug/portion]	Labeled [ug/portion]	Mass analized [g]	Quantified [ug/portion]	Labeled [ug/portion]
1S	Chile	$5,500	*0.98235	0*	0.26	0.6272	0	0.26	1.784	0	0.26
2S	USA	$6,700	2.0974	4.6±0.23	2.09						
4S	Chile	$7,490	2.3977	0.26±0.01	1.57						
5S	Mexico	$12,080	2.0868	6.95±0.35	43.48						
6S	USA	$12,990	3.5764	1.43±0.25	2.17	1.8427	1.48	2.17	2.1573	1.57	2.17
7S	USA	$8,990	0.9827	9.5±0.48	1.04						
8S	USA	$6,986	2.0723	6.06±0.30	2.09						
9S	USA	$15,990	1.0012	0	0.48	3.3157	0	0.48	1.8133	0	0.48
10S	USA	$12,990	1.002	0	0.48	2.779	0	0.48	2.1758	4.79 E-07	0.48

*Averaged Results

As it was indicated in the Introduction, the research was designed to perform a quantification of different brands of multivitamins commonly used. In **Table 2** we observe the data obtained from multivitamins presented in pills. That is, cost, origin, mass of each sample; and with respect to cobalt, concentration presented in the sample, and quantified by the technique in each portion, and the labeling of each pill.

TABLE 3: ELEMENTS IN THE SOLIDS MULTIVITAMINS QUANTIFIED BY TXRF. THE RESULTS ARE COMPARED WITH THE LABELED VALUES IN THE PACKAGING OF THE PRODUCT

L2					L1				L3			
Sample Number	**Mass analized [g]**	**Amount in the mass analized [ug]**	**Quanti-fied [ug/ portion]**	**Labeled [ug/ portion]**	**Mass analized [g]**	**Amount in the mass analized [ug]**	**Quantified [ug/ portion]**	**Labeled [ug/ portión]**	**Mass analized [g]**	**Amount in the mass analized [ug]**	**Quanti-fied [ug/ portion]**	**Labeled [ug/ portion]**
P												
1S	18.427	208554.5	56589.37	N/A	0.9576	1384	0.00935	0.1				
3S	0.6272	1035.7	1651.3	100000								
9S	33.157	146888.03	39856.73	N/A								
10S	29.349	305075	51973.6	10600					1.46	2137	0.001295	10600
Ca												
1S	1.8427	240049.65	65135.31	N/A	0.9576	2232	0.00150298	0.162				
3S	0.6272	1367.63	2180.52	162000								
9S	33.157	240857.77	37074.79	25000					1.6501	141566	0.08423	25000
Mn												
1S	1.8427	48.23	13.08	N/A								
3S	0.6272	70.1	111.76	N/A								
9S	33.157	4417.35	666.13	2000					1.6501	2655.8	0.001609	2000
10S	29.349	10739.78	1829.66	2500					1.46	31.4	1.9029E-5	2500

L2					L1				L3			
Sample Number	**Mass analized [g]**	**Amount in the mass analized [ug]**	**Quanti-fied [ug/ portion]**	**Labeled [ug/ portion]**	**Mass analized [g]**	**Amount in the mass analized [ug]**	**Quantified [ug/ portion]**	**Labeled [ug/ portión]**	**Mass analize d [g]**	**Amount in the mass analized [ug]**	**Quanti-fied [ug/ portion]**	**Labeled [ug/ portion]**
Fe												
1S	1.8427	142.77	38.74	N/A	0.9576	294	5.4333E-05	0.3				
3S	0.6272	112.85	179.92	18000								
9S	33.157	11979.3	1806.45	5000					1.6501	7412.1	4492	5000
10S	29.349	1340.37	228.35	0								
Cu												
9S	33.157	2461.37	371.16	1000					1.6501	1406.6	8524	10000
Zn												
1S	1.8427	0	0	N/A	0.9576	738	1.4217E-05	0.015				
3S	0.6272	17.65	2814	15000								
9S	33.157	17338.82	2614.65	7500					1.6501	9939.4	6000	7500
10S	29.349	85407.07	14550.25	20000					1.46	0.7	4.5	20000
Se												
9S	33.157	200.03	30.76	50					1.6501	54.7	35	50
10S	29.349	382.2	65.12	50								

L2				
Sample Number	**Mass analized [g]**	**Amount in the mass analized [ug]**	**Quanti-fied [ug/ portion]**	**Labeled [ug/ portion]**
K				
3S	0.6272	7077.13	11284	50000
Cr				
9S	33.157	166.45	25.1	50
10S	29.349	119.07	20.28	25

Broadly speaking, we may observe that concentrations quantified by TXRF do not fit values mentioned in labeled packaging, particularly on Cyanocobalamin. In this line, we find multivitamins with varying amounts, considering that they pursue the same aim and function, as in the case of 2(S) = 4.6063 and 6(S) = 32,425 (column N° 7), where the quantified cobalt is approximately eight times smaller. In the column N° 8 we noted as the amount of cobalt varies presented by each brand, where values range from 0.26 to 43,481 µg/portion. The most noticeable case was that of the sample number 1(S) since it completely lacks of cobalt, still being labelled on its packaging. It was also, and contrary to what it was expected, in the case of samples 2(S), 4(S), 5 (S), 6(S), 7(S) and 8(S), it was found that the amount of cobalt was significantly higher to what it was labelled on the respective packages. On the other hand, the Chilean law indicates that the maximum amount of vitamin B-12 in food supplements is 12 µg, which implies that the maximum concentration of cobalt should be 0.522 µg. If we infer this result to column N° 7, we see that only one sample, that is, one brand is within this legal limit, according to quantification by the technique, this sample / brand does not contain cobalt though. In general, this result indicates that the brands sampled should be controlled by ANAMED, *i.e.* as medicaments.

TABLE 4: COMPARATIVE TABLE OF RESULTS OBTAINED FROM SYRUPS MULTIVITAMINS

Sample Number	Origin	Cost	Volume analized [ml]	Amount in Volume analized [ug]	Quantified [ug/portion]	Labeled [ug/portion]
Co						
Sy1	Spain Chile	12.000	5.0	7.525	7.525	2.174
Sy2	Chile	9.500	5.0	0	0	5.0E-4
Sy3	Chile	10.200	5.0	0	0	1.15E-6
P						
Sy2	Chile	9.500	5.0	0.1568	0.1568	N/A
Sy3	Chile	10.200	5.0	502	502	N/A
K						
Sy1	Spain Chile	12.000	5.0	5.375	5.375	N/A
Sy2	Chile	9.500	5.0	28165	28165	N/A
Sy3	Chile	10.200	5.0	3367	3637	N/A
Ca						
Sy1	Spain Chile	12.000	5.0	4.79	4.79	N/A
Sy2	Chile	9.500	5.0	271315	271315	N/A
Sy3	Chile	10.200	5.0	4337	4337	N/A
Cr						
Sy2	Chile	9.500	5.0	72.975	72.975	N/A
Mn						
Sy2	Chile	9.500	5.0	51	51	N/A
Fe						
Sy1	Spain Chile	12.000	5.0	4.325	4.325	N/A
Sy2	Chile	9.500	5.0	776	776	N/A
Sy3	Chile	10.200	5.0	17.5	17.5	N/A
Cu						
Sy3	Chile	10.200	5.0	4.675	4.675	N/A
Zn						
Sy2	Chile	9.500	5.0	49539	49539	N/A
Sy3	Chile	10.200	5.0	4.65	4.65	N/A
Se						
Sy2	Chile	9.500	5.0	170.5	170.5	N/A

In the **Fig. 3**, the number of counts is presented (which represent the number of events that occur in a given time interval) *vs.* energy (in keV). Three random samples corresponding to 1(S), 4(S) and 6(S) are shown. From these samples, strong concentrations of elements such as calcium, iron, etc. are evaluated. These elements are the most representative of the spectrum. Finally, the Gallium observed at 5 ppm is used as internal standard. On the other hand, in sample 6S a small peak can be seen representing the presence of arsenic in this product, which is not labelled in the respective package and its presence is objectionable.

In **Table 3** the mineral multi-elemental quantification in solid pills is shown. The mass of the sample, the amount in the sample of P, Ca, Mn, Fe, Cu, Zn, Se, K and Cr, the amount in each portion recommended and the labelled amount of these elements in the packaging, are shown. On the other hand, the fact that the information on concentrations provided by the brand will be greater to what is required by the regulator, implies that these multivitamins should be regulated as drugs, not as food supplements [2].

In the **Fig. 4**, samples 2(L), 3(L) and 6(L) were chosen as the peaks or traces have the same behavior and / or the same number of counts. The cobalt is the active ingredient of vitamin B-12 and the element to be quantified. In the case of arsenic, it is not a convenient element in these injectable multivitamins. In this case Gallium was again used as internal standard.

On the other hand the presence of Chlorine is typical in this type of injectable solutions. In **Table 4** the Cobalt and mineral multielemental quantification in 3 different syrups is shown. The mass of the sample, the amount in the sample of Co, P, K, Ca, Cr, Mn, Fe, Cu, Zn, and Se, the amount in each portion recommended and the labelled amount of these elements in the packaging, are shown. We notice that in Sy3 sample the amount of Co detected is zero.

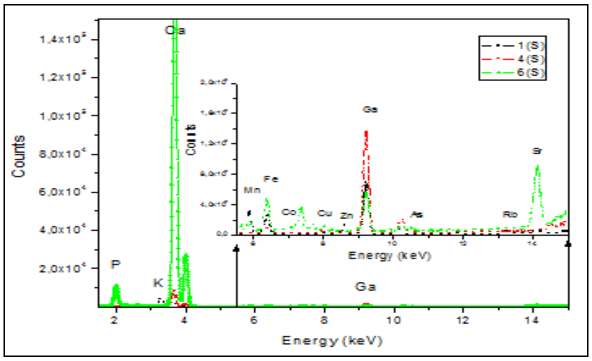

FIG. 3: TXRF SPECTRA OF THREE SOLID SAMPLES (PILLS) ARE SHOWN. SEVERAL ELEMENTS (Co, Zn, Fe, *etc*.) AT DIFFERENT CONCENTRATIONS CAN BE IDENTIFIED

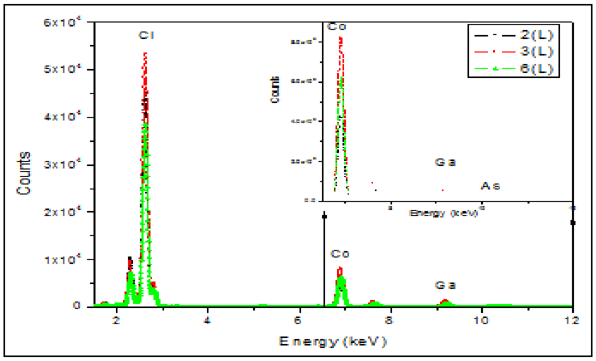

FIG. 4: THE RESULTS OF THREE INJECTABLE MULTIVITAMINS ARE SHOWN. COBALT AND CLORINE ARE THE MAIN ELEMENTS IDENTIFIED, AND ALSO IN A GIVEN SAMPLE ARSENIC WAS QUANTIFIED AT A SMALL LEVEL

CONCLUSION: In this study we applied the TXRF technique for the evaluation of the Vitamin B-12 content in multivitamins, and for the determination of the composition in mineral supplements. The analyzed samples consisted on 16 products (10 are sold as pills, 3 as syrups, and 3 as solutions for intramuscular injections) which were processed in 3 different and prestigious laboratories in order to avoid systematic errors. In the studied samples we evaluated: 1) the presence of the specified elements, 2) the correlation between the labelled contents and the concentrations obtained by the technique; and 3) the possible presence of harmful elements for human health. The technique applied is multi-elemental, simple and fast.

The obtained information could be useful to the pharmaceutical companies and organizations in charge of controlling and monitoring these products and, additionally, it could contribute i) to promote the studies related to the bio-equivalence of medications because it makes possible the evaluation of their inorganic chemical profiles; ii) and in case of detecting harmful elements to the detriment of human health, to define legal aspects linked to these products.

The products analyzed were acquired directly from drugstores without medical prescription, in Concepcion City, Chile. The amount of Vitamin B-12 labeled in the product is related to the cobalt quantified, by calculating the proportions of molar masses, since one atom of this element is found in the Cyanocobalamin molecule. The P, K, Ca, Mn, Cr, Fe, Cu and Zn elements were along with the Co, were simultaneously analyzed.

Since multivitamins products are widely demanded, it becomes opportune the use of techniques that generate information to strengthen the criteria of the health authorities in order to complement the established regulations. These authorities will face multiple challenges in terms of regulation, control, and new norms. In this aspect, we can mention the drug traceability, the studies of therapeutic efficiency associated with studies of bio equivalence and the implementation of generic medications, and so on. In the control and monitoring of multivitamin and mineral supplements, the already established processes could be inefficient over the high proliferation of products that are being currently commercialized in the country.

In the performed process we have identified some anomalies: 1) In some products there are significant differences between the concentrations labelled and our evaluation. According to these results, the classification of these products could be exchanged from medications to food supplements and vice-versa [2]. The variation on their classification could produce also a modification in the Agencies which oversights these products. 2) The absence of cobalt in four of these products, which indicate in their components the vitamin B-12 – three are sold in form of pills and the another is sold as syrup, 3) A trace detection of one element of rare earths (possibly Ytterbium) in a particular product 2(S). The public policies that regulate these products could be incomplete.

Since the great variety of stock in the market is associated with a wide range in prices that could be set subjectively, in the generated information it is also included the cost of the product, the number of pills, and so on, in order to consider the economic aspects among the variables of interest.

If the composition of each studied multivitamin is compared with its respective price, we do not find a direct relationship. In the case of the solid or multivitamin tablets match the lowest cost to the total lack of cobalt. In other cases, both high and multivitamins average has about 20% of indicated cobalt. The case of injectable multivitamins is the most favorable as all samples, since they show around 100% of Cobalt quantified versus indicated. In all cases studied they show varying prices.

Atomic techniques are widely applied in Pharmaceutical and Biomedical Sciences [36, 37, 38]. In the case of TXRF, even though is a mature technique but currently it is not widely known. At an international level in terms of health evaluation, medications or environment, an extensive specialized bibliography about its applications, can be found. Some are for generic evaluations [39]. In the case of Chile, we are not aware of the existence of applications related to the characterization of pharmaceutics products or food supplements applying this technique.

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