

# A simple method for water discrimination based on an light emitting diode (LED) photometer

Alexandre Fonseca, Ivo M. Raimundo Jr. \*

*Instituto de Química, Universidade Estadual de Campinas, UNICAMP, Cx Postal 6154, 13084-971 Campinas, Brazil*

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## Abstract

This work describes the use of a multi-LED photometer for discrimination of mineral water samples, employing chromogenic reagents and chemometric techniques. Forty-five water samples (including 7 different brands of mineral water and samples of deionised, distilled and tap waters) were analysed in a monosegmented flow system, using three different chromogenic reagents (murexide, PAR and eriochrome black T) in a pH 10.0  $\text{NH}_3/\text{NH}_4^+$  buffer in separate injections. Measurements were performed at 470, 500, 525, 562, 590, 612, 636 and 654 nm. Analyses were carried out using PCA, employing data sets including absorbance values obtained with one, two or all three reagents, which comprise 8, 16 or 24 variables, respectively. The best result was obtained with the data set from murexide and eriochrome black T, providing a clear distinction between 9 groups (distilled and deionised waters were classified in the same group). Based on the loading values, it was possible to select four wavelengths (470, 500, 590 and 654 nm) that provided a similar discrimination. With the use of these four LED, an HCA was performed, providing discrimination between 8 groups at a similarity level of 0.88. A model based on SIMCA allowed correctly classifying 94% of the samples. The discrimination between different groups is due to the metal ion contents in the water samples, mainly calcium and magnesium. Therefore, the use of common complexing reagents, such as murexide and eriochrome black T, a multi-LED photometer and chemometric techniques provide an easy and simple method for water discrimination.

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## 1. Introduction

Chemometric methods, such as principal component analysis (PCA), hierarchical cluster analysis (HCA), soft independent modelling of class analogy (SIMCA), discriminant analysis (DA) and neural Networks have been employed for characterisation and classification of a great variety of samples. The discrimination of soils [1], pen inks [2], fuels [3], wines [4], olive oils [5], milk [6], coconut water [7] and waters [8] are amongst some examples of application of these methods, which are of great interest in agronomy, archaeology, food engineering, medicine, environmental chemistry and forensic science.

Pattern recognition, as these methods are commonly known, is based on the composition of the samples and/or their chemical-physical properties, such as pH, density, viscosity and so on.

Different techniques have been employed to provide data for pattern recognition, including gas chromatography [9], mass spectrometry [10], atomic spectrometry [11], fluorescence spectrometry [12] and spectrophotometry [13]. Frequently, these techniques provide large data sets, as it seems to be necessary to obtain enough information from the samples to allow performing proper classifications. Recently, arrays of sensors have been proposed for classification purposes of different kind of samples. They are known as electronic tongues [14] or electronic noses [5], according to their application in liquids and gaseous media, respectively. In these kinds of arrays, the sensors do not necessarily have to be extremely selective, as discrimination is often accomplished due to differences in the responses of each sensor to the analytes present in the samples. The main drawback of these kinds of sensor arrays, especially the electronic noses, is that the temperature must be controlled within a narrow range, in order to provide reproducible data for multivariate calibration.

UV-Vis-NIR spectrophotometry is a useful tool for pattern recognition, as spectrophotometers, multichannel or Fourier

\* Corresponding author. Tel.: +55 19 3521 3136; fax: +55 19 3521 3023.  
E-mail address: [ivo@iqm.unicamp.br](mailto:ivo@iqm.unicamp.br) (I.M. Raimundo Jr.).

transform-based, can provide large data sets in short periods of time, with good resolution, detectivity and accuracy. Although the discrimination process can be performed by employing the whole spectra (which can contain values obtained in hundreds of wavelengths), usually a variable selection is carried out in order to eliminate the wavelengths which carry no information or even add noise to the classification model. In addition, algorithms have been proposed for variable selection, also aimed at minimising correlated variables, providing more robust and parsimonious models [15]. Considering these aspects, a multi-LED photometer seems to be a simple alternative for the realisation of pattern recognition, as measurements at a few wavelengths can provide enough information for obtaining a proper model for classifications.

In recent years, mineral water has becoming more popular in many countries. This fact can be confirmed by the number of brands of mineral water offered in the supermarkets. As a consequence, there is an increased concern regarding the quality of mineral waters as well as the possibility of falsification by addition of tap water. The chemical composition of a mineral water depends on the soil from which it was extracted. Physical and chemical parameters are used to identify a mineral water, which are printed on the label of the commercialised bottle, for instance, pH, conductivity, dissolved total solids, and sodium, calcium, magnesium, chloride, bicarbonate and sulphate contents, to mention a few commonly listed ions. Obviously, these parameters are useful for water discrimination when employing multivariate analysis (PCA and HCA), as proposed by Caselli et al. [11]. However, this approach presents as a principal disadvantage the necessity of performing many measurements, based on atomic spectrometry (for metal ions) and ion chromatography (for anions), which are time consuming and expensive. As an alternative, Versari et al. [8], employing PCA, HCA and DA, carried out a classification of mineral waters based on the information reported on the labels of the commercial bottles, which is not a recommended procedure as the values expressed on these labels are average values and do not represent the real concentration of the metal ions in the sample. Recently, electronic tongues have been also proposed for classification purposes. Martínez-Máñez et al. [14] employed an array of 12 electrodes (thick films of  $\text{RuO}_2$  10  $\Omega/\text{sq}$ ,  $\text{RuO}_2$  1  $\text{M}\Omega/\text{sq}$ , C, Ag, Ni, Cu, Au, Pt, Al, and small sticks of Sn, Pb and graphite) for classification of six Spanish mineral waters, tap waters and osmotised water, which was accomplished with PCA and neural networks. Moreno et al. [16] constructed an array of ISFET sensors and interdigitated platinum electrodes (for measurements of pH, conductivity, redox potential,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Cl}^-$ ), which was applied to classification of 13 brands of mineral water based on PCA, HCA and SIMCA approaches. Gallardo et al. [17] developed an array of nine electrodes based on PVC membranes, selective to  $\text{H}^+$  (2 different ionophores),  $\text{Li}^+$ ,  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and two “generics” (with non-specific ionophores), for classification of 23 water samples (natural mineral, natural sparkling mineral,  $\text{CO}_2$  added mineral, gas added mineral and tap waters, as well as lemonade) by employing PCA. Proper classifications were obtained with these electronic tongues, which present as a drawback

the possibility of sensor poisoning as well as their useful lifetime.

Recently, we have proposed a photometer based on an array of eight LED, which was successfully applied to the simultaneous determination of  $\text{Zn(II)}$  and  $\text{Cu(II)}$  in pharmaceutical and metal alloy samples, by employing multiple linear regression [18]. The LED photometer possesses as main characteristics simplicity, low cost, low power consumption and portability, which make it useful for on-site measurements. Although it is not as versatile as a spectrophotometer, there are a great variety of LED in the market (they are available from UV to NIR region of the spectrum), allowing the construction of a dedicated instrument for a specific application. Therefore, in the present work, the use of this photometer as a tool for discrimination of waters is described. They were mixed with a chromogenic reagent (murexide, PAR or eriochrome black T) at pH 10.0 ( $\text{NH}_3/\text{NH}_4^+$  buffer) for obtaining the spectral data set. A monosegmented flow system, employing a simultaneous multiple injection approach [19], was used to facilitate the mixing of the solutions and to avoid sample dispersion, allowing to scan all LED of the array while the sample zone is inside the flow cell. Discriminations and classifications of 45 water samples (including mineral, tap, deionised and distilled waters) were performed by employing principal component analysis (PCA), hierarchical clustering analysis (HCA) and soft independent modelling of class analogy (SIMCA).

## 2. Experimental

### 2.1. Reagents and solutions

Analytical grade reagents and deionised water were employed for preparation of all solutions. A 0.32  $\text{mol L}^{-1}$  ammonium buffer solution was prepared by mixing proper amounts of concentrated ammonia solution and ammonium chloride salt and adjusting the pH to 10.0. Solutions of 4-(2-pyridylazo)resorcinol (PAR) and eriochrome black T (EBT) were prepared by dissolution of appropriate quantities of the respective salts to obtain a concentration of 0.30  $\text{mmol L}^{-1}$ . An ammonium purpurate (murexide) solution at the same concentration was prepared in 10% (v/v) aqueous ethanol. Chromogenic reagent solutions were prepared just before use. Mineral water samples were acquired in a local market and were analysed without any pre-treatment.

## 3. Instrumentation

All measurements were carried out with a multi-LED photometer constructed in our laboratory [18], equipped with eight LED (maximum emissions at 470, 500, 525, 562, 590, 612, 636 and 654 nm) as light sources and a photodiode (RS 308-067) as detector. Optical fibres were employed to guide the light to and from the flow cell (10 mm optical path). Software written in Microsoft VisualBasic 3.0 was employed to control the photometer with the aid of a parallel interface PCL-711S (Advantech), furnished with a 12-bit A/D converter. Measurements were performed by sequentially turning on/off all the LED

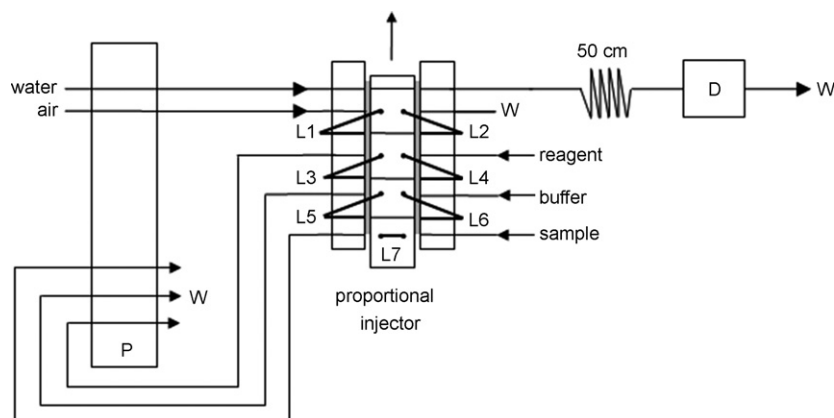


Fig. 1. Diagram of the monosegmented flow system employed to manage the solutions. (P) Peristaltic pump; (W) waste; (L1–L7) injection loops; (D) flow cell. See text for details.

of the array, while the sample was passing through the flow cell.

#### 4. Procedure

The monosegmented flow system employed to manage the solutions is shown in Fig. 1. The simultaneous multiple injection approach [19] was adopted as a means of mixing water sample and chromogenic reagent solutions. A peristaltic pump (Ismatec MP-13 R) was employed to impel carrier fluid (deionised water) and air (that fills loops L1 and L2, 50  $\mu$ L each) and to aspirate chromogenic reagent solution (loops L3 and L4, 40  $\mu$ L each), buffer solution (loops L5 and L6, 50  $\mu$ L each) and sample solution (that fills loop L7, 50  $\mu$ L). When the proportional injector was placed in the injection position, the monosegment containing the solutions (water sample, buffer and reagent) was homogenised while it was pumped through the reaction coil (50 cm long, 0.7 mm i.d.), towards the detection cell (10 mm optical path). While the sample monosegment was passing through the cell, the photometer was able to perform 10 measurements for each LED (80 acquisitions), which were then averaged to obtain the signal intensities.

#### 5. Data set, data pre-treatment and calculations

The variables employed in the calculations were the signal intensities acquired with each LED of the array, employing the three chromogenic reagents. Thus, the original data set is described by a matrix which has 135 rows and 24 columns. The 135 rows represent the samples (triplicate measurements for all 45 water samples), while the 24 columns represent the variables (signal intensities obtained with each 8 LED employing 3 chromogenic reagents). It is important to emphasise that 45 samples were studied, although three replicate measurements were inserted in the data set, as a means of observing signal variations due to instrumental noise. The chemometric studies were performed with all samples, while the number of variables was altered in order to evaluate the performance of each reagent. The raw data were mean-centred before performing the chemometric studies. The Unscram-

bler 7.5 (CAMO) was employed to run PCA and SIMCA, and Pirouette 2.11 (Infometrix) was used for HCA calculations.

#### 6. Results and discussion

The objective of pattern recognition is to find the similarities and differences among many samples, based on a set of previously measured properties. With this approach it is possible to enhance knowledge about a data set by means of natural groupings of different samples (unsupervised pattern recognition) or by constructing a model able to classify new samples (supervised pattern recognition) [20]. When the number of parameters (variables) measured for each sample is small (3 or less) and each of them possesses information with high discriminatory character, similarities and differences can be easily observed by plotting the measured variables for each sample in a three-dimension graph. Unfortunately, this is not the usual case and pattern recognition based on UV-Vis-NIR spectroscopy requires tens or even hundreds of variables, even though an algorithm for variable selection is often employed in order to eliminate wavelengths that introduce noise and redundancies in the models, leading to a more robust and parsimonious classification [15]. In addition, electronic tongues have proved that good discriminations can be performed by employing few variables (around 10) [14,16,17].

For a discriminatory analysis, it is fundamental that the measured parameters (variables) reflect the singular characteristics

Table 1  
Characteristics of the mineral water samples as shown on the labels of the bottles

Fountain	Samples	[Ca <sup>2+</sup> ] (mg L <sup>-1</sup> )	[Mg <sup>2+</sup> ] (mg L <sup>-1</sup> )	Hardness (mg L <sup>-1</sup> )
Levíssima	7	0.57	0.35	0.92
Leve	5	7.03	2.84	9.86
Purified	4	10.40	4.80	15.20
Ycuara	6	6.22	9.06	15.28
São Bernardo	4	13.23	4.96	18.19
Água Santa	7	16.40	8.34	24.74
N. S. Aparecida	3	39.90	12.90	52.80

of the sample sets that are under observation. In the present work, mineral water samples acquired in the local market present noticeable differences in the concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions, as indicated by their respective labels, whose values are listed in Table 1. The total concentration of these ions gives the hardness of the water, which can be determined by complexometric titration with standard EDTA solution. In this titration, murexida and EBT are usually employed for end point indication, as they form complexes with these metal ions, whose colours are different from those of the free indicators. Therefore, in the present work, murexide and EBT were used in the photometric analysis of waters, aimed at obtaining information regarding their hardness, which was the basis of the classification. In addition, PAR was also used, as a means of acquiring information about others metal ions, such as  $\text{Zn(II)}$  and  $\text{Mn(II)}$ .

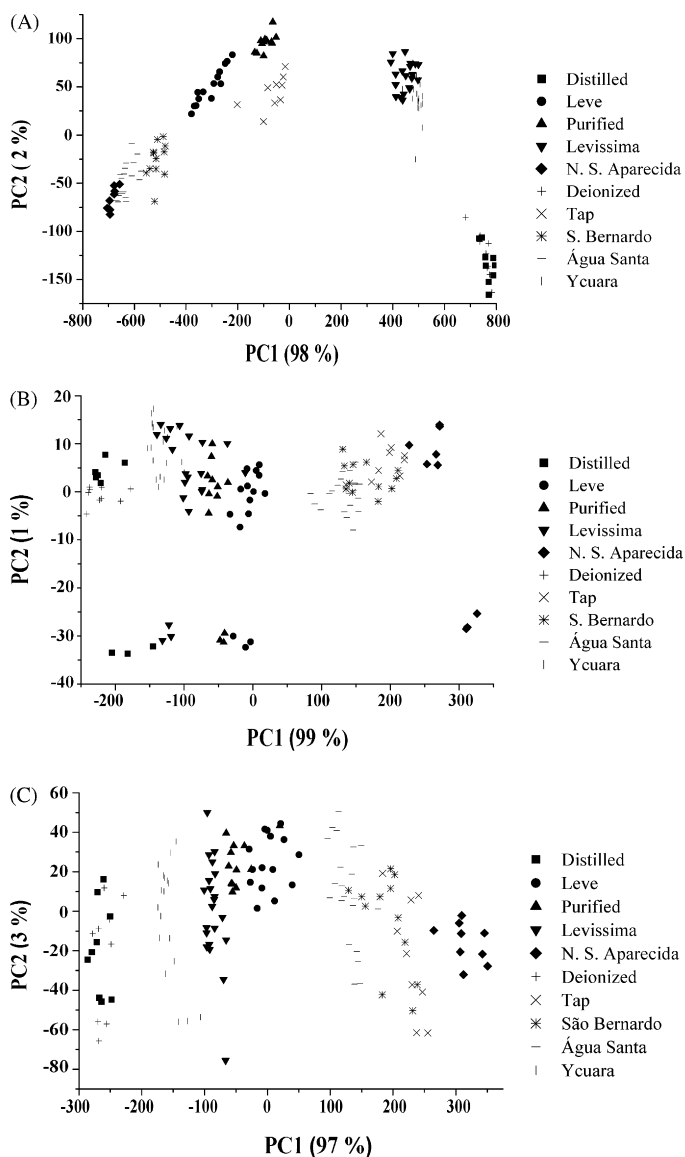


Fig. 2. Scores graphs for PCA performed with spectral data obtained with eriochrome black T (A), PAR (B) and murexide (C) acquired datas. Values in parenthesis indicate the explained variance of the principal component.

Mineral water samples from 6 different sources (6 different brands) and one mineralised water (purified water to which salts were added) were analysed. In order to improve the variability of the samples (due to different weather conditions, that is, rainy and dry periods), waters with different bottling dates were acquired, covering periods of two (minimum) to seven (maximum) months for the same brand of water. Three samples each of deionised, distilled and tap waters were also included in the study, aimed at observing the performance of the method, which would indicate its capability of identifying adulteration of mineral waters.

Initially, exploratory analyses based on PCA were performed, by employing data sub-sets obtained with measurements using each one of the chromogenic reagents (135 samples and 8 variables in each case). The raw data were previously mean-centred and the obtained models were validated with full cross-validation approach. This study showed that three principal components were sufficient to virtually explain all the variance of the data set and, therefore, this number of PC was employed throughout the work.

Fig. 2 shows the results obtained for each complexing agent, based on the score graphs for the two first PC, by employing signal intensities of all eight LED of the photometer. It can be seen from the graphs that it is possible to identify many groups of samples, formed basically along PC1, which explains more than 96% of the total variance in all cases. The distinction between these groups is more pronounced for the analysis performed

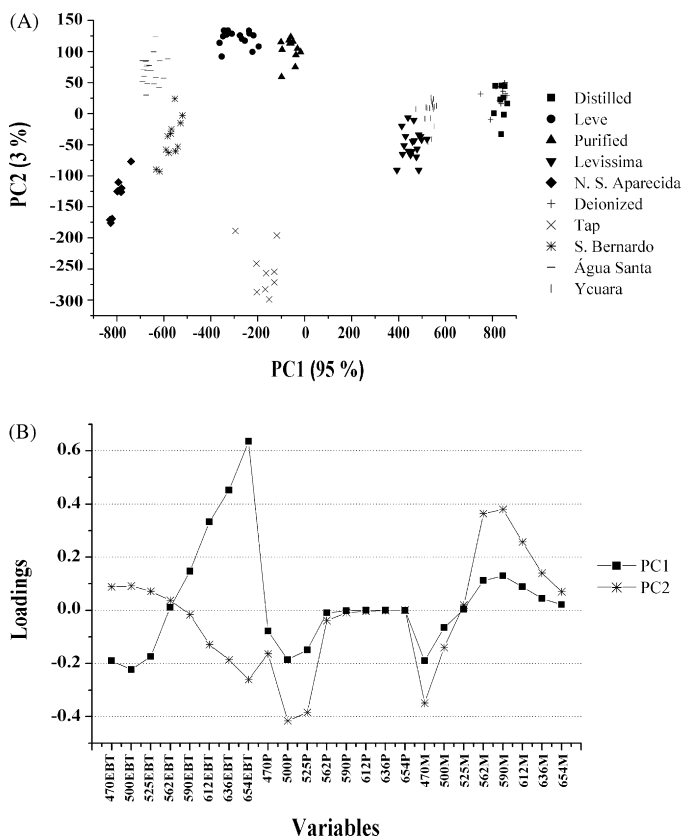


Fig. 3. Scores (A) and loadings (B) graphs for PCA performed with combined data obtained with all reagents. Values in parenthesis indicate the explained variance of the principal component.

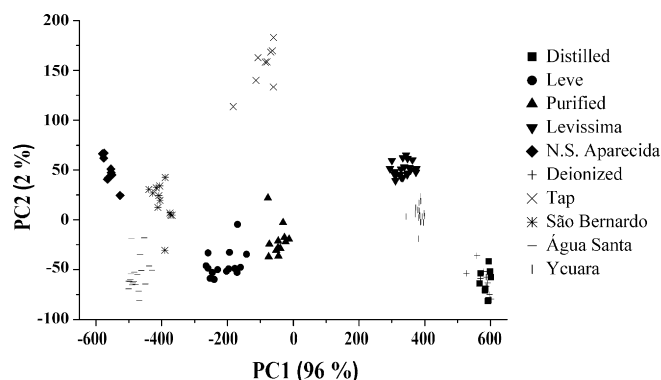


Fig. 4. Scores graphs for PCA performed with the combined data for EBT (500 and 654 nm) and murexide (470 and 590 nm). Values in parenthesis indicate the explained variance of the principal component.

with EBT and murexide, which can be explained based on the fact that these reagents are more sensitive to the alkaline earth metal ions  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  than is PAR. It can also be noted that these reagents provide different grouping for the samples; for example, in graph 2a “Leve”, “Purified” and “Tap” water samples are clearly separated, while in Fig. 2C, “Ycuara” and “N.S. Aparecida” are better grouped.

Due to these observations, exploratory analyses with PCA were also performed combining the data obtained with all three reagents (24 variables) and those obtained with murexide and EBT (16 variables), employing the same conditions above described. In both cases, a better discrimination among the sample groups was observed, as a result of the use of a number of variables higher than 8. Nine groups were formed along PC1 and PC2, distinguishing the water samples according to their sources, as can be seen in Fig. 3A. As expected, samples of deionised and distilled waters formed a single group, as their metal ion contents are virtually null. In addition, PC1 tends to distinguish the groups according to the hardness of the water

Table 2

Data sets employed for model construction and prediction in the SIMCA classification

Fountain	Samples	Model	Prediction
Levissima	7	4	3
Leve	5	3	2
Purified	4	3	1
Ycuara	6	3	3
São Bernardo	4	3	1
Água Santa	7	4	3
N. S. Aparecida	3	2	1
Tap	3	2	1
Distilled	3	2	1
Deionised	3	2	1

samples, from “N.S. Aparecida” to deionised/distilled waters. This behaviour is not strictly obeyed, as the values of hardness were taken from the labels of the water bottles and may not represent the correct values.

Fig. 3B shows the graph of loadings for each variable employed for this classification. It can be noted that most of the PAR variables does not have a significant influence in the calculation of both PC, except for wavelengths of 500 and 525 nm that affect PC2. Although these two variables have some influence on the PC calculation, none PAR variables were employed in the subsequent studies. The graph of loadings also shows that the most important variables for murexide are 470 and 590 nm, while for EBT are 500 and 654 nm. A PC analysis performed with those four variables produced the classification shown in Fig. 4, in which all the nine groups are clearly separated. PC analysis carried out by using 16 variables (murexide and EBT plus all LED) and eight variables (the four above mentioned wavelengths and both reagents) provided results similar to those shown in Fig. 4.

A hierarchical cluster analysis (HCA) was also performed, as a means of confirming if the data provided are sufficient for a

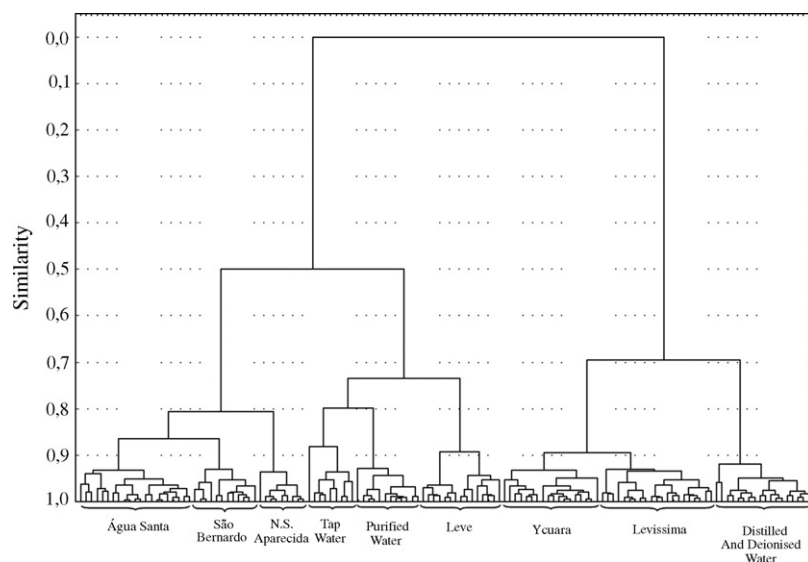


Fig. 5. Dendrogram for HCA performed with combined data obtained with EBT (500 and 654 nm) and murexide (470 and 590 nm). Mean-centred data, Euclidian distances and complete connexion.



Table 3

Results obtained for SIMCA classification at 95% of confidence level

Fountain	Number of replicates	Replicates included in its own class	Replicates included in a wrong class	Replicates not classified	Classification rate (%)
Levíssima	9	8	0	1	89
Leve	6	6	0	0	100
Purified	3	3	0	0	100
Ycuara	9	7	0	2	78
São Bernardo	3	3	0	0	100
Água Santa	9	9	0	0	100
N. S. Aparecida	3	3	0	0	100
Tap	3	3	0	0	100
Distilled and deionised	6	6	0	0	100

good discrimination. HCA is a statistical method for exploratory analysis different from PCA, in which the samples are grouped according to the distances between them. These distances can be determined in different ways [20], being employed in similarity ( $S$ ) calculations, according to the equation:

$$S_{ab} = 1 - \frac{d_{ab}}{d_{\max}},$$

where  $d_{ab}$  is the distance between samples a and b, and  $d_{\max}$  is the largest distance found between all samples. If the  $S$  value is near zero, the samples are very dissimilar, while the opposite is valid if the value is close to one. A bi-dimensional graph, called a dendrogram, is then constructed based on the  $S$  values, making visible the grouping of the samples.

Fig. 5 shows the dendrogram obtained with a HCA carried out with the above mentioned four variables, employing mean-centred data, Euclidian distances and complete connexion [20]. At a similarity level of 0.88, eight groups are formed, which are counted by the number of vertical lines that cross the horizontal line at this level. The groups are similar to those obtained with PCA, except for the samples from the “Ycuara” and “Levíssima” sources, which belong to the same group. At a similarity of 0.90, excluding one tap water sample, ten groups are formed; the samples “Ycuara” and “Levíssima” are in different groups, as expected, while the “Leve” samples are also separated, which does not occur in the PC analysis. Although there is not a level of similarity able to discriminate the samples as PCA does, it can be affirmed that the information provided by the four variables is sufficient for a good discrimination by employing both methods.

For SIMCA classification, the 45 water samples were randomly divided into two groups; one for constructing the classification model (28 samples) and one for prediction (17 samples), respectively, as indicated in Table 2. As each water sample was injected in the flow analyser in triplicate, the total data set consisted of 135 values (84 for calibration and 51 for prediction). All SIMCA models were constructed by employing mean-centred data, the four selected variables and full cross-validation. The number of latent variables was determined based on the validation error, by using the default settings of the software The Unscrambler 7.5. As SIMCA classification is based on PCA, a single model was constructed for deionised and distilled water samples, instead of one model for each class.

Table 3 shows the results obtained with SIMCA classification at a confidence level of 95%. As can be seen, SIMCA did not correctly classify three injected water samples (1 Levíssima and 2 Ycuara), providing a classification rate of 94%. These samples were not classified in any of the nine classes of the model, providing three errors of type-I (object not included in its own class) and no error of type-II (object included in a wrong class), results that indicate the good performance of the instrument.

## 7. Conclusions

A simple photometer, based on an array of eight LED, is a useful tool for performing discrimination of mineral waters. The method employs measurement with two complexing agents (murexide and eriochrome black T), generating analytical signals that are proportional to the metal ion contents, mainly  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , in the water samples. PCA, HCA and SIMCA approaches are able to correctly discriminate or classify water samples according to their sources, demonstrating that a complete characterisation of the samples, employing atomic spectrometry, to perform this task is not necessary. Finally, the monosegmented flow system provides an ease and rapid way to process the samples, avoiding dispersion, which would impair the precision of the measurements, as many data points must be obtained when the sample zone is passing through the flow cell.

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