ELECTRONIC DISCRIMINATION OF MINT PLANTS BASED ON QUARTZ CRYSTAL MICROBALANCE SENSORS

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ABSTRACT

The commonly used methods to test the authenticities of medicinal plants may either require a long and complicated task or would demand for high cost operation. In this study, six AT-cut quartz crystal microbalance (QCM) sensors, leading to the development of electronic nose, was employed as an alternative low cost and reliable method of differentiating commonly used mint plants - yerba buena (Mentha cordifolia opiz.), peppermint (Mentha piperita L.), and spearmint (Mentha spicata L.). The QCM sensors, with gold electrodes on both sides were coated with different sensing materials. Each plant sample was exposed to sensor chamber, with one sensor at a time. The headspace vapors from the incubated sample were carried to the sensor chamber by a stream of nitrogen gas, and the vapors were reversibly adsorbed when in contact with the sensing layer of QCM. The sensor gave a steady response to the headspace vapors, and exhibited good reversibility and reproducibility at an acceptable response time. The integrated response of all the sensors, using polar plot, created an odor graphical profile of each plant. A good discrimination of the plant samples was achieved by Principal Component Analysis (PCA) using the commercial Six Sigma Minitab15 software program.

Keywords: Electronic Nose; Mint Plants; Principal Component Analysis (PCA); Minitab 15; Quartz Crystal Microbalance

INTRODUCTION

Over recent years, the demand for herbal medicine has rise drastically, and the global market for it has been steadily growing (WHO 2003). This increasing popularity has raised concerns on the need for quality standards to ensure the safety, authenticity and efficacy of the herbal product. As a result, quality control guidelines have been formulated (Brinckmann and McIntyre 2006, WHO 2004).

The analysis of the starting plant materials is important in the quality control of herbal medicine (Brinckmann and McIntyre 2006, Joshi et al. 2004). The test methods include sensory inspection, as the first step, in a form of macroscopic and microscopic evaluation. In sensory inspection, the parameters are judged subjectively and substitutes or adulterants which closely resemble the genuine material might not be always detected. Also, the number of replicate samples, which can be evaluated at any time, is limited by the inception of sensory fatigue in the part of analyst.

The sensory inspection may then be followed by chemical profiling to further achieve better authentication of plant. Here, a characteristic chemical pattern for identification is established through instrumental techniques such as high performance liquid chromatography (HPLC) (Springfield et al. 2005), mass spectrometry (MS), infrared spectrometry (IR) (Liu et al. 2006), ultraviolet/visible spectrometry (UV-VIS), and capillary electrophoresis (CE) (Calixto 2000), which can be used alone or in combination. However, these methods are tedious and require expensive instruments.

The potential of electronic nose has been recognized as a novel approach in the quality control of medicinal plants (Baby et al. 2005, Shafiqul 2005). It is based on the analysis of the headspace of the plant material through an array of chemical sensors. The headspace consists of the volatile components present in the botanical sample and can be used to characterize medicinal plants. These compounds interact with the chemical sensors and generate a pattern of responses which can be used to characterize the sample.

One of the chemical sensors that can be used for headspace analysis is the piezoelectric quartz crystal or quartz crystal microbalance (QCM). The volatile compounds of the plants samples interact with a polymer film on the surface of the quartz crystal, and cause the oscillation frequency of the quartz crystal to change. The change in resonant frequency (Δf) is related to the change in the mass loading, described by the Sauerbrey equation (Eq. 1).

$$\Delta f = - Cf \frac{f_0^2}{A} \Delta m \qquad \text{(Eq. 1)}$$

where Δf is the frequency shift (MHz), Δm is the mass change (gram), f_0^2 is the fundamental frequency of quartz crystal (MHz), and A is the area of the quartz plate (cm^2). The sensitivity and the selectivity of the quartz sensor varies with the nature of the polymer coatings, and so that an array of quartz crystals will generate a fingerprint for a plant sample (Gardner and Barlett 1999).

Analysis of the responses obtained from a set of QCM sensors is facilitated using three approaches: a bar graph, a radar plot, and through principal component analysis (PCA). The combined responses of the QCM sensors to the different plant samples can be analyzed using bar graph. A pattern or fingerprint of each plant odor can be generated using a discrimination profile, known as a radar or polar plot. This plot is a two dimensional polar graph that allows the simultaneous display of many variables by plotting each variable along a different radial axis, coming from the origin of the polar plot. Low values are near the center of the polar plot and large values are near the outer circumference (Gardner and Barlett 1999). The last approach is based on principal component analysis (PCA) where the data are preprocessed before analysis. The preprocessing includes standardization done by expressing as deviations from the means; correlations matrices; eigenvalues; eigenvectors and scree plots of the principal components.

In this study, a set of quartz crystal microbalance sensors was used to differentiate mint plants such as yerba buena (*Mentha cordifolia opiz.*), peppermint (*Mentha piperita* L.), and spearmint (*Mentha spicata* L,). Yerba buena, peppermint, and spearmint (under Lamiaceae, the family of mint) are aromatic plants and are commonly used as culinary herbs. In the Philippines, yerba buena is considered of high medicinal values among the three. Peppermint and spearmint are often confused with yerba buena due to their similar features and odors, thus, discrimination is of importance to test authenticity.

Due to unavailability of some equipment for electronic nose system, analysis of headspace VOCs was carried out based on one sensor at a time, instead of doing it with an array of sensors. The sensors' responses were just integrated so as to produce a response profile similar with that of arrayed sensors.

The study did not identify the VOCs in the headspace of the sample. Instead, previous works were used as a guide in explaining the observed behavior. Authentication and discrimination were based on the created profile by each plant material using radar plot. Further discrimination was made using Principal Component Analysis (PCA).

METHODS

Reagents and Materials Preparation

All reagents were prepared from analytical reagent grade chemicals: chloroform (Ajax Laboratory Chemicals); tetrahydrofuran and toluene (Sigma Chemicals Co.).

The polymers used as sensing membranes are polyethylene glycol 6000 (PEG), polyvinyl chloride (PVC), polystyrene (PS), poly(dimethyl)siloxane (PDMS), cyanoacrylate (CA), and ethyl cellulose (EC). The selection of the polymers was based on polarity and inherent adsorption/desorption ability of the polymers when exposed to analyte. The suppliers are as follows: Aldrich Chemical Company Ltd. for PS and EC; Cord Chemicals, Inc.(Bulldog[TM]) for CA; Ajax for PEG-6000; Pioneer (silicone sealant[TM]) for PDMS; and BDH Limited Poole for PVC. The six different sensing films consist of polar, weakly polar, or non-polar polymer solution (0.02 g of polymer dissolved in 10.0 mL solvent): PVC and CA to tetrahydrofuran (THF); PS, PDMS, and EC to toluene (C_7H_8); and PEG to chloroform ($CHCl_3$).

The AT-cut quartz crystal microbalance (4-mm diameter polished gold electrode, 1μm thickness, and with resonance frequency of ~10MHz) was procured from International Crystal Manufacturing Co., Inc., Oklahoma City, U.S.A.

Mature leaves of mint plants – yerba buena (*Mentha cordifolia opiz.*), peppermint (*Mentha piperita L.*), and spearmint (*Mentha spicata L.),* were collected from UP, Los Baňos – Crop Division and authenticated by the University of Santo Tomas Herbarium.

Instrumentation

A schematic diagram of the instrument system is shown in Figure 1. Nitrogen gas, of controlled flow rate, was used as the carrier gas of the headspace volatile compounds from the sample chamber to the sensor chamber. One sensor at a time was exposed per batch of sample. The oscillator enabled oscillation of quartz crystals, and the frequency counter measured the frequency. The data were then processed in the computer.
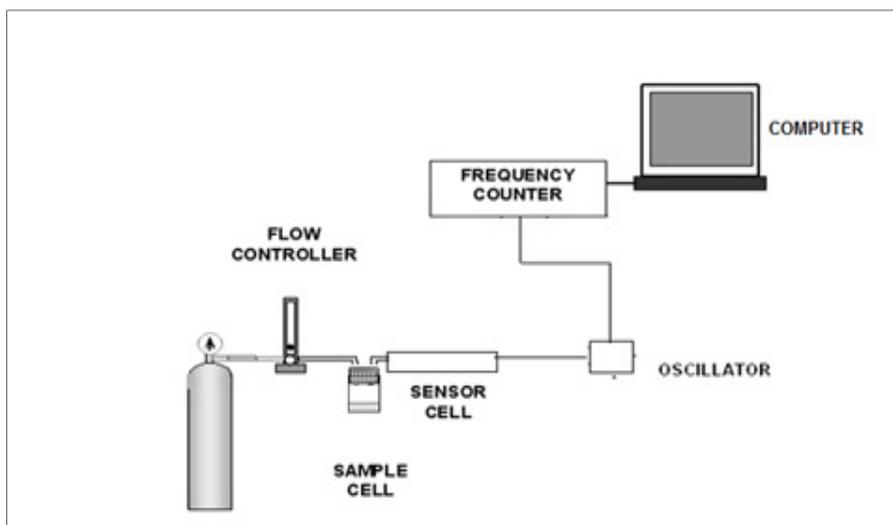

Figure 1. Schematic diagram of the instrumentation set-up

The sample chamber used was an amber bottle of 35mL capacity, with glass tubings connected to the nitrogen line and to the sensor chamber via rubber tubings (Figure 2). The sensor cell was a glass tube with a diameter of 1.5 cm and a length of 8.0 cm, both ends were covered with rubber stoppers. The crystal was positioned horizontally, along with the flow of the entering gas (Figure 3).
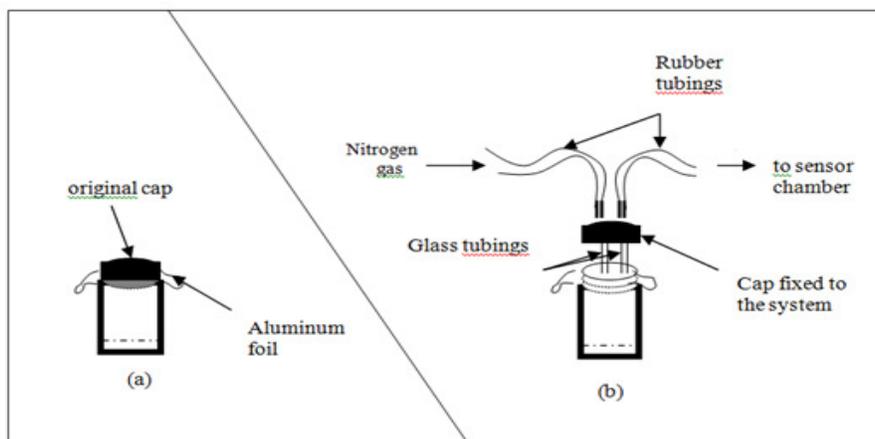
Figure 2.   (a) sample vessel (initially covered with aluminum foil and cap)
(b) sample vessel introduced to the system (original cap is removed
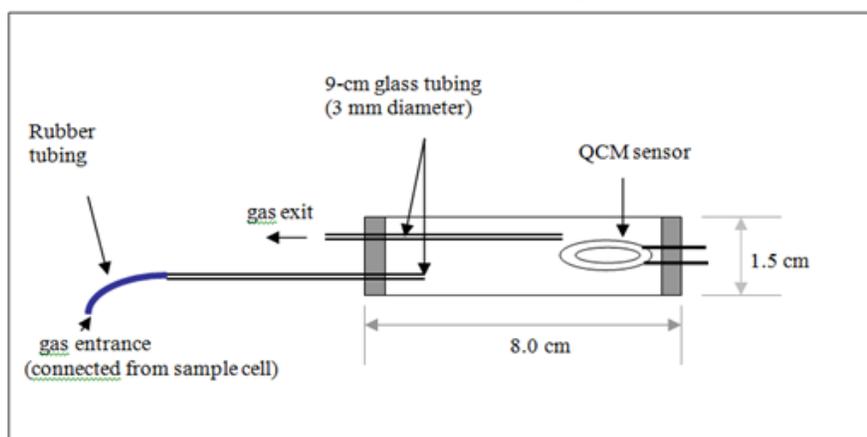and sample vessel is fitted to the cap fixed to the system)

Figure 3. The sensor cell

Sensor preparation

The design of the QCM used for sensor preparation is shown in Figure 4 (Gardner and Barlett 1999). Prior to the coating, the crystal was washed using piranha solution (3:1 H_2SO_4 and H_2O_2), rinsed vigorously with distilled water, air-dried for few minutes, and then exposed to nitrogen gas. The QCM was coated on one side with the sensing material using the drop-coating method. The sensing material was prepared by dissolving 0.0200 g of polymer in 10.0 mL solvent: PVC and CA in THF; PS, PDMS, and EC in toluene; and PEG in $CHCl_3$. The coating was prepared by applying a single 5μL drop of sensing material on top of the crystal's electrode.
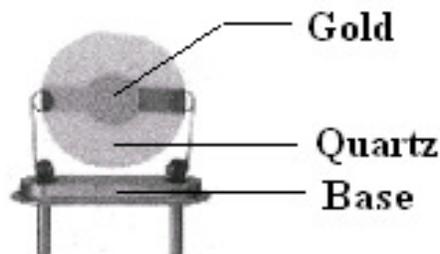

Figure 4. Design of Quartz Crystal Microbalance

Sample Preparation

Crude mature leaves of each plant sample were cut into pieces. About 1.0000 g was placed in 35 mL vial, and covered with aluminum foil with cap. The samples were prepared in triplicates and were incubated under 28^0C ambient temperature, for one hour, at an interval of 30 minutes per replicate, so as to concentrate the volatiles in the headspace of the sample.

Measurement Procedure

Nitrogen gas, at a flow rate of 25mL/min, was passed through the blank cell and the baseline reading was recorded. The sample was then introduced, from which nitrogen gas transported the headspace VOCs to the sensor cell. The reading was monitored until it reached a steady state. Between measurements of replicates, the sensor was purged with nitrogen through a blank cell for cleaning and for the recovery of the response of the sensor. The difference in the steady frequency readings of blank and sample for all the replicates was examined after. The change in resonant frequency (Δf) is related to the change in the mass loading described by the Sauerbrey equation.

RESULT

The typical response of the QCM sensors toward the different plant materials is represented by PEG sensor's response to yerba buena, as shown in Figure 5. A negative frequency shift in the oscillation frequency of the coated crystal occurred when exposed to headspace vapors of the sample. Less than 10 minutes were required for the response of all the sensors to reach a steady state value. The crystal responded reversibly, the frequency reverting to its original value when nitrogen gas was passed through the sensor.
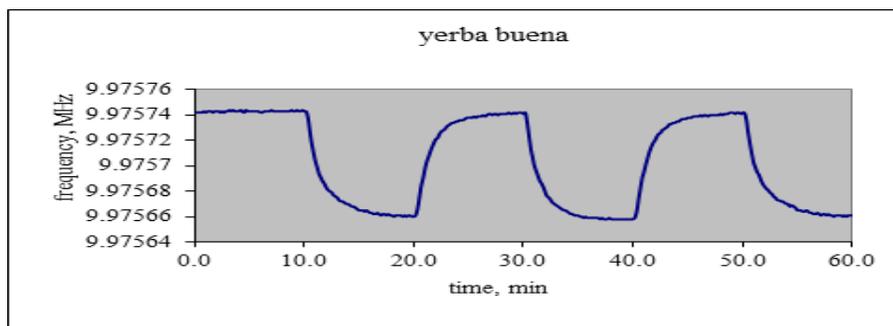

Figure 5. Typical response curve of the repeatability/reversibility of responses for three replicates

The response of all the sensors, as shown in Figure 6, exhibited good repeatability/reversibility, a relative standard deviation of less than 10% being obtained from three successive readings made for each mint plants.
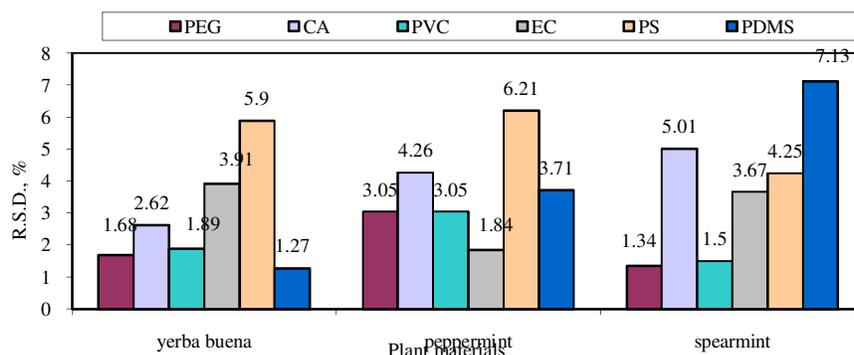

Figure 6. Reversibility of the response of each QCM sensors towards the different plants' VOCs (n = 3)

Each of the sensors gave a unique signal to each plant (Figure 7). Higher responses were observed on the sensors containing PEG, CA, and PVC as the reagent phases. Lower responses with weak polar and nonpolar sensing reagents, such as PS, EC, and PDMS were observed.
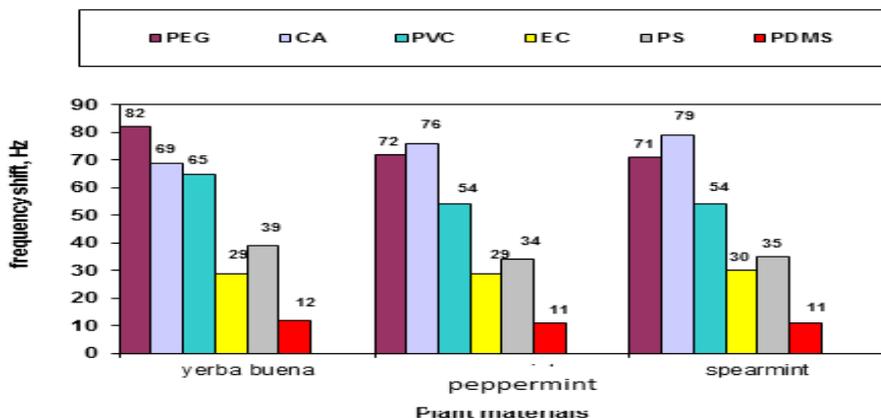

Figure 7. Mean responses of the QCM sensors to the different plant materials (n = 3)

The profiles of the plant odors were established graphically by radar plot (Figure 8). The responses of the six QCM sensors were integrated to create the profiles. Discrimination was not apparent for mints as similar profiles were created.

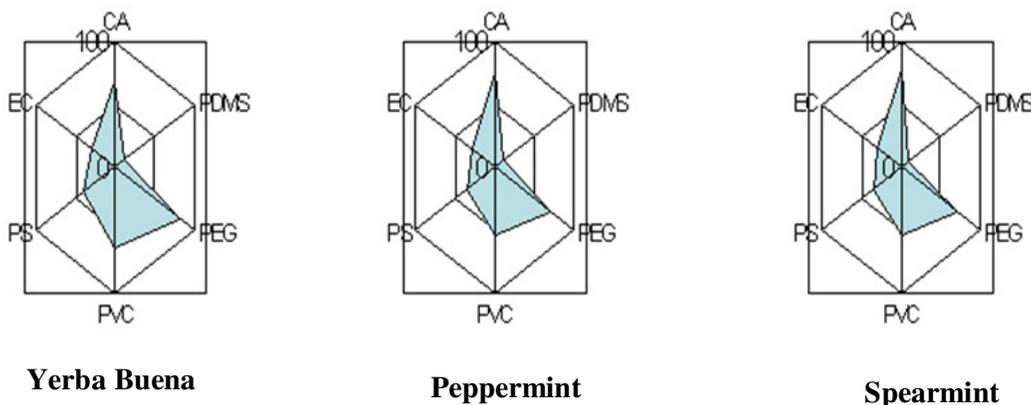**Yerba Buena**   **Peppermint**   **Spearmint**

Figure 8. Odor profiles of the Mint Plants

Better discrimination of the mint plants was obtained using Principal Component Analysis (PCA) (Figure 9), where clear clusters of mint plants (nonoverlapping) are shown. It is a chemometric approach which is a powerful, linear, supervised, nonparametric pattern recognition technique used to discriminate the response of an electronic nose to simple or complex odors (Gardner and Barlett 1999). In this study, the data obtained from the experiment was loaded into a commercial Six Sigma Minitab15 software program as a 6 x 9 data matrix. The data matrix represents the 6 variables (sensors) and the 9 samples (three replicates of three plant samples).

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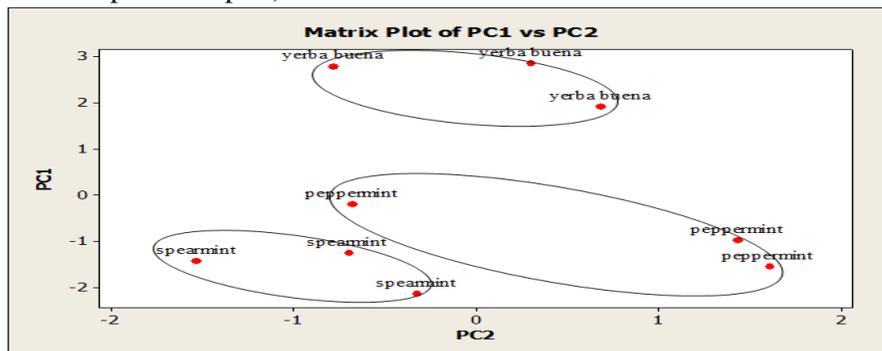

Figure 9. Matrix plot of principal component 1 (PC1) vs principal component 2 (PC2)

PCA is conducted in a sequence of steps where lists of some of the most important information were provided. The number of variables for the analysis is equal to the number of the principal components to be extracted. The principal components account for a maximal amount of variance in the data set. In most cases, the first principal component accounts the relative large and meaningful amounts of variance. The succeeding principal components account for progressively smaller and insignificant amounts. Thus, the first few components (Prin1 and Prin2) are the ones retained for interpretation (Johnson and Wichern 1988).

DISCUSSIONS

In the experiment, six nonconducting polymers were used as sensing membranes. The selection of the polymers were based on polarity (polar, semi-polar, and nonpolar) and inherent adsorption/desorption ability when exposed to analyte. Table 1 shows the list of polymers used in the preparation of sensors. As expected, polar VOCs binded with polar polymers, and the nonpolar interacted with the nonpolar polymers.

Table 1. Sensing materials used in the preparation of sensors

Sensing Material	Chemical Formula/Structure	Polarity
Polyethylene Glycol (PEG)		Highly polar
Cyanoacrylate (CA)		Polar
Polyvinyl chloride (PVC)		Polar
Ethyl Cellulose (EC)	$(C_{12}H_{22}O_5)_n$	Weakly polar
Polystyrene (PS)		Nonpolar
Polydimethylsiloxane (PDMS)		Nonpolar

Polar reagents such as PEG, CA, and PVC gave higher responses since the plants main components are polar, such as menthol and carvone (Singh et al. 1999, URL 2007). However, lower response with weak polar and nonpolar sensing reagents, such as PS, EC, and PDMS were observed due to small fraction of nonpolar volatile oils of the plant.

The responses of the six QCM sensors were integrated to create the profiles of the plant samples (Figure 8). The profiles created for the mints – yerba buena, peppermint, and spearmint were similar. This was expected since the three plants belong to the same family. The directions of the profile discrimination of the plant odors show strong adsorption to PEG, CA, and PVC, as the points are headed to a great extent towards these polar polymers. These can be attributed to the polar principal VOC of each plant (Gardner and Barlett).

For the matrix plot shown in Figure 9, the first two principal components (PC1 and PC2) were used because they explained the most of the variability (0.842 or 84.20%) in the data, as shown in the cumulative value of Table 2. Since the eigenvalues of both components were greater than one (3.9020 and 1.1487), it means they have high distinguishability. The eigenvalue is a measure of the variability of the data. Adding the eigenvalues would result to the same total number of variables being analyzed because each variable contributes one unit of variance to the analysis. Normally, greater eigenvalue corresponds to greater variability (Doble et al. 2003). Thus, the six variables was been effectively reduced to 2 components while maintaining 84.20% of the information.

Table 2. Eigenanalysis (eigenvalue and eigenvector) of the Correlation Matrix of the Six Principal Components

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.9020	1.1487	0.4921	0.3142	0.0993	0.0437
Proportion	0.650	0.191	0.082	0.052	0.017	0.017
Cumulative	0.650	0.842	0.924	0.976	0.993	1.000
Eigenvectors	Principal Components (PC)					
Variable	PC1	PC2	PC3	PC4	PC5	PC6
PEG	0.462	0.249	0.242	0.323	-0.411	- 0.326
CA	0.471	0.015	-0.038	0.536	-0.619	-0.326
PVC	0.462	-0.054	0.206	0.613	0.403	-0.451
EC	-0.036	-0.922	-0.069	0.199	0.011	0.324
PS	0.444	-0.291	0.280	-0.436	-0.513	-0.432
PDMS	0.390	0.034	-0.902	0.053	-0.152	-0.080

Chemometric treatment of the data through Principal Component Analysis resulted in a discrimination of the plant samples. Through this technique, it is possible to identify properly a plant sample, even if it has been shredded or powdered. This capability is useful in the quality control of herbal preparations based on *Yerba Buena*.

ACKNOWLEDGEMENT

REFERENCES

Baby et al., (2005). *Quality c*ontrol of medicinal plants with an electronic nose. *Sensors and Actuators B, 106*: 24-28.

Brinckmann, J. & McIntyre, M. (2006). Proposed Quality Control Guidelines for the EU Directive on Traditional Herbal Medicinal Products: *Implications for Small- to Medium-Sized Enterprises. Herbalgram, 70*, 62-66.

Calixto, J. B. (2000). Efficacy, Safety, Quality Control, Marketing and Regulatory Guidelines for Herbal Medicines (Phytotherapeutic Agents). *Brazillian Journal of Medicinal and Biological Research, 33*: 179-189.

Doble P et al., (2003). Classification of premium and regular gasoline by gas chromatography/mass spectrometry, principal component analysis and artificial neural networks. *Forensic Science International, 132*: 26-39.

Gardner, J.W. & Barlett P. N. (1999). *Electronic Noses: Principles and Applications*, (1st Ed.). New York: Oxford University Press, Inc.

Johnson, R. A. & Wichern, D.W. (1988). Applied Multivariate Statistical Analysis. (2nd ed.). Prentice Hall International edition: 341-342.

Joshi et al., (2004). Molecular markers in herbal drug technology. *Current Science,* 87: 159-165.

Liu, H., Sun, S., Lv, G. & Liang, X. (2006). Discrimination of Extracted Lipophilic Constituents of Angelica with Multi-Steps Infrared Macro-Fingerprint Method. *Vibrational Spectroscopy, 40*, 202-208.

Shafiqul et al., (2005). Transient parameters of a coated quartz crystal microbalance sensor for the detection of volatile organic compounds (VOCs). *Sens. Actuators B, 109*, 238-243.

Singh et al., (1999). Volatile Constituents of Vitex Negundo Leaves. *Planta Medica, 65*, 580-582.

Springfield et al., (2005). Quality Assessment of South African Herbal Medicines by Means of HPLC Fingerprinting. *Journal of Ethnopharmacology, 101*, 75-83.

URL. Botanical.com. *Mints*. Retrieved. (2007). from the World Wide Web http://www.botanical.com/botanical/mgmh/m/mints-39.html#specon.

World Health Organization. (2003). Traditional Medicine (Fact Sheet No. 134) Geneva.

World Health Organization. (2004). Guidelines for the Regulation of Herbal Medicine in the Southeast Asian Region. New Delhi