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Flavor analysis in a pharmaceutical oral solution formulation using an electronic-nose

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Abstract

Flavors are commonly used in pharmaceutical oral solutions and oral suspensions to mask drug bitterness and to make the formulation more palatable. Flavor analysis during product development is typically performed by human organoleptic analysis, which is often expensive and less objective. A novel approach using a metal oxide sensor-based instrument (electronic-nose) for headspace analysis was explored to replace human sensory perception for consistent qualitative and quantitative analysis of flavors in a pharmaceutical formulation. The use of the electronic-nose technique to qualitatively distinguish among six common flavoring agents (raspberry, red berry, strawberry, pineapple, orange, and cherry) in placebo formulations was demonstrated. The instrument was also employed to identify unknown flavors in drug formulation placebos. Raspberry flavor samples from different lots made by the same manufacturer, as well as freshly prepared and aged samples, were also distinguished by electronic-nose. Therefore, the instrument can potentially be used for identity testing of different flavor raw materials and the flavored solution formulations. The electronic-nose was also employed successfully for quantitative analysis of flavors in an oral solution formulation. The quantitative method might be used to assay the flavor concentration during release testing of the oral solution formulation or to monitor flavor shelf-life in the marketed container. It can also be implemented for packaging selection for the formulation in order to ensure the flavor shelf-life. Chemometric methodologies including principal component analysis (PCA), discriminant factorial analysis (DFA), and partial least squares (PLS), were used for data processing and identification.

Keywords: Flavor analysis; Electronic-nose; Metal oxide sensor; Pharmaceutical; Oral solution; Identification; Stability; Chemometrics

1. Introduction

Flavors are often used in the food industry, and in personal care and pharmaceutical products. During pharmaceutical formulation development for oral solutions and oral suspensions, flavors are commonly included as an important part of the formulations, especially with pediatric formulations, to mask drug bitterness and/or to make the formulations more pleasant. Therefore, it is highly desirable to qualitatively and quantitatively analyze different flavors for formulation development, stability and quality control

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purposes. Flavor analysis during product development is typically performed by human organoleptic analysis by a panel of experts. The use of human panelists for odors is usually accurate, but costly and time-consuming [1]. Furthermore, human sensory panel assessments, which can be adversely affected by external parameters such as illness or fatigue, are usually subjective. The human sensory panel is also limited to odors that are not toxic or obnoxious [2].

Conventional analytical tools such as gas chromatography-based techniques can also provide both qualitative and quantitative analysis for flavors. If the chemical identities for odorants have been determined, GC is very accurate and useful for both quantitative and qualitative analysis. Otherwise, interpretation of chromatograms to accurately represent the odor-active components is a very difficult and challenging problem [3]. These techniques tend to become less reliable as sample complexity increases. An example is the 3-year effort by the USDA to distinguish grain quality based on the analysis of grain odors using headspace GC-mass spectrometry [4]. After analyzing over 300 samples, no relationship between the chemical composition and the odor could be established. Similar problems correlating the results of detailed chemical analysis with organoleptic responses have also been reported [5,6].

In recent years, sensor array-based aroma analysis technology has been developed that complements human sensory analysis. This technology, the so-called "electronic-nose," utilizes an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognizing simple or complex odors [7]. The chemical sensor is usually a small, self-contained integrated system of parts, that, as the result of a chemical interaction or process between the analyte and the device, transforms chemical or biochemical information of a quantitative or qualitative type into an analytically useful signal [4]. Common chemometric methodologies including unsupervised principal component analysis, supervised discriminant factorial analysis and partial least squares are used for the pattern recognition [8]. Among the commercially available instruments, metal oxide semiconductor sensors are most widely used, since they demonstrate a high degree of sensitivity for a range of organic vapors, a wide choice for different selectivity, and offer perhaps the best balance between drift, lifetime, and sensitivity [2]. These types of sensors have also been reported to have good sensor stability and reproducibility [9]. The Fox 4000 electronic-nose used in our work is a metal oxide sensor-based instrument.

While conventional analytical instruments become less effective as sample complexity increases, the electronic-noses usually retain the ability to discriminate closely related samples. The advantages of electronic-noses include high sensitivity and correlation to human sensory panels for many applications [10]. The instrument also demonstrates greater objectivity than human sensory panels and the possibility of standardization, which should increase the reproducibility of the results. Thus, electronic-noses have been widely used in different applications. Many applications in the food industry have been published for flavor or odor analysis of meat, grains, coffee, beer, fruit, and edible oils [5,11–14]. For environmental and safety applications, electronic-noses have been used for water monitoring [15] and wastewater treatment monitoring [16]. Discriminations between different bacterial species or microorganisms have been reported using electronic-noses [17,18]. Some medical applications including breath tests and the diagnosis of urinary tract infections, melanomas, wound infections and neonatal complications have also been reported [19].

This technology has not yet been adopted by the pharmaceutical industry. Even though flavor concentration determination during product release testing is usually not a regulatory requirement, it is an important quality control step for the benefit of consumers. However, there are few very reliable and general quantitation methods that can be used for many different types of flavors for this purpose. Furthermore, the compatibility of flavors and packaging materials is very important for package selection. However, since professional panel flavor analysis is very time-consuming and expensive, it is difficult to fit into the fast moving formulation design process in the pharmaceutical industry. In this paper, we present the results of a feasibility study using the Fox 4000 electronic-nose to complement human sensory perception for consistent qualitative and quantitative analysis of flavors in pharmaceutical formulations.

2. Experimental

2.1. Materials and sample preparation

Artificial raspberry and cherry flavors were purchased from Takasago (Rockleigh, NJ). The natural Strawberry flavor was acquired from F. Hoffmann (La Roche Ltd., Givaudan-roure, Basel, Switzerland). Red berry (artificial), pineapple (artificial), and orange (natural) flavors were all obtained from Virginia Dare (Brooklyn, NY). Sodium citrate USP and citric acid anhydrous were purchased from Tilley Chemical (Baltimore, MD). Sodium butyl hydroxybenzoate (BP) and sodium propyl hydroxybenzoate (USP/BP) were both acquired from Clariant (Nipa Biocides, Mount Holly, NC).

An oral solution placebo was prepared to contain sodium citrate (20.9 mg/ml), sodium propyl hydroxybenzoate (0.225 mg/ml), sodium butyl hydroxybenzoate (0.075 mg/ml), and sodium saccharin (0.1 mg/ml). The pH of the placebo solution was adjusted to 6.8 using citric acid. Using this placebo solution as a diluent, four raspberry flavored placebos (A1, A2, A3, and A5) were prepared at 4 mg/ml using different lots of raw materials received from Takasago (lots 1–4, respectively). A4 was obtained by storing A3 at room temperature for 8 months to evaluate the effect of aging. Placebo samples flavored by cherry, strawberry, red berry, pineapple, and orange (A6–A10, respectively), were prepared to contain 4 mg/ml of each flavor. A placebo diluent sample without any flavor was labeled as A11. Solutions A5 through A11 served as qualitative analysis training standards.

Three samples were prepared by one analyst and analyzed by a second analyst without knowledge of the flavor compositions. Unknowns 1 and 2 contained 4 mg/ml raspberry flavor and red berry flavor, respectively, while unknown 3 contained a mixture of 2 mg/ml strawberry and 2 mg/ml raspberry flavor.

Quantitative analysis training standards were prepared using raspberry flavor from manufacturer's lot 4 in order to generate the calibration curve. The flavor concentrations were 1.01 (B1), 2.01 (B2), 3.00 (B3), 4.04 (B4), and 5.02 (B5) mg/ml, respectively, equivalent to 25–125% of the target concentration (4 mg/ml) of the flavor in the oral solution formulation. Seventy-five ml of solution B4 were transferred into two different potential marketing packages, glass and PET (polyethylene terephthalate) bottles. Solutions in both packages were stressed at 40 and 60 °C for 1 week with 5 °C samples as controls.

2.2. Electronic-nose

All samples were analyzed on a Fox 4000 electronic-nose (Alpha MOS, Toulouse, France) equipped with 18 metal oxide sensors with a headspace autosampler Odorscanner 100. The data were analyzed with the Alpha Soft version 2.1 software. A sensor diagnostics check was performed weekly using the sensor diagnostics kit provided by the manufacturer.

2.2.1. Operating mode and detection mechanism

A diagram of the Fox 4000 electronic-nose is shown in Fig. 1. The samples were sealed in 10 or 20 ml headspace vials and loaded into the autosampler tray. The vial was incubated at 35 °C for 4 min to allow the volatilization of flavor components into the headspace. Then 2 ml of the sample headspace was extracted by the autosampler syringe and flow-injected into the carrier gas flow (synthetic air mixture). The detector includes 18 different metal oxide sensors divided into three chambers. There are three types of sensors: T, P, and LY. Both type T and P are based on tin dioxide (SnO_2), but they have different sensor geometries. LY sensors are chromium titanium oxides ($Cr_{2-x}Ti_xO_{3+y}$) and tungsten oxide (WO_3) sensors [20]. Multiple types of sensors are used in the instrument to ensure adequate sensitivity and selectivity. Odorants first adsorb to the sensors and then react with the metal oxide sensors, depending on the type of sensor and the odorant molecular functionality. The reaction changes the resistances of the sensors, and these changes in sensor resistance are monitored and output as raw signals. The sensors are re-generated to their initial states by reaction with oxygen in the carrier gas after each injection. To simplify the data processing, only the maximum resistance changes of each sensor are used for the analysis.

Chemometric techniques are used to present the data in an understandable graphical format. They provide quick answers and allow evaluation of the relationships between variables and between observations at a glance [21]. Non-supervised analyses, such as principal component analysis (PCA), are used to remove the redundancy of variables and to give a representa-

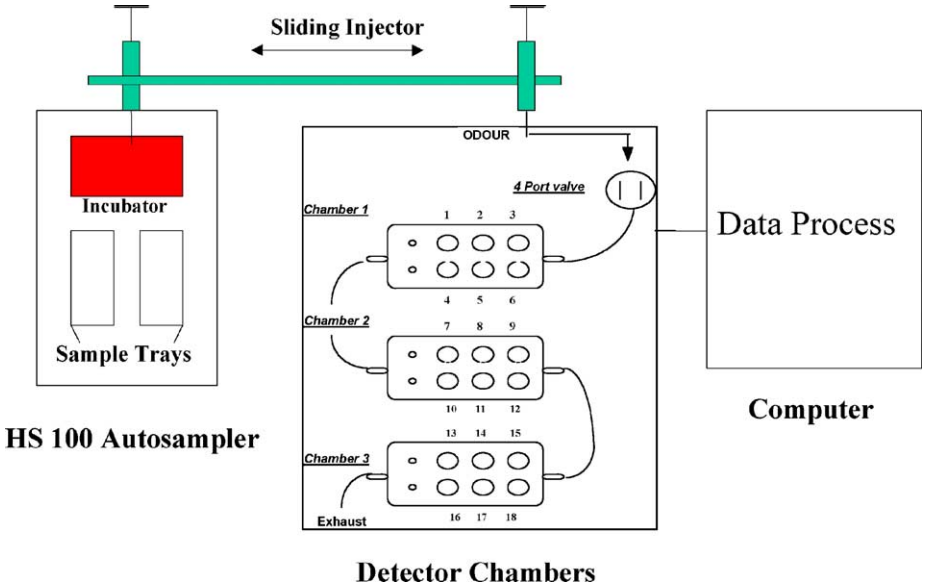

Fig. 1. Electronic-nose instrumental configuration, including autosampler, injector, detector, and data processor.

tive map of the different olfactive areas. The discrimination index indicates the extent of discrimination between samples in the two-dimensional PCA surface [22].[1] Supervised analyses, such as discriminant factorial analysis (DFA), are used to make reliable recognitions for unknown samples [22].

To quantitate the flavor intensity, partial least squares (PLS) analysis is used. Calibration standards are samples covering the range of the constituent to be measured. A calibration curve is hence developed to provide a predictive range. The correlation between actual flavor concentration and electronic-nose measurements is evaluated. A valid model should have a correlation factor above 0.90 [22]. The unknown sample concentrations are then projected on the calibration curve for quantitation.

2.2.2. Analytical conditions

Carrier gas	Synthetic dry air 150 ml/min
Sample preparation	
Quantity of sample in the vial (μl)	1000
Total volume of the vial (ml)	10
Headspace generation	
Headspace generation time (min)	4
Headspace generation temperature (°C)	35
Agitation speed (rpm)	500
Headspace injection	
Injected volume (μl)	2000
Injection speed (μl/s)	2000
Syringe temperature (°C)	40
Acquisition parameters	
Acquisition time (s)	120
Time between injections (min)	10

[1] When the groups are distinct, the discrimination index is positive:

$$\mathrm{Di} = 100 \times \left[1 - \frac{\text{sum of surface occupied by samples}}{\text{total surface to include all samples}}\right]$$

When groups overlap each other, the index is negative:

$$\mathrm{Di} = 100 \times \left[1 - \frac{\text{sum of intersection surface}}{\text{total surface}}\right]$$

Therefore, a higher discrimination index indicates better discrimination.

3. Results and discussion

3.1. Discrimination between flavors and identification of unknown flavors

Five oral solution placebo samples containing cherry (A6), strawberry (A7), red berry (A8), pineapple (A9), and orange (A10) flavors were analyzed, as well as a placebo solution without any flavor (A11). Raspberry flavored samples (A1–A5) were also analyzed and the results were pooled as one sample. Fig. 2 shows the raw signals as resistance changes for 18 sensors as a function of time for raspberry and pineapple samples. The differences between the two patterns are dramatic, and these types of differences between different flavors are used to characterize the flavors. In order to analyze data more efficiently, only the maximum responses from each sensor were used. PCA shows that each of the six flavors is discriminated from the others and from the placebo without any flavor (Fig. 3). The discrimination index is 96 (maximum 100), indicating that a very high degree of discrimination was achieved. According to the statistical model, a successful discrimination model should have an index between 80 and 100.

A model was then built using these different flavor samples as training standards in order to identify unknown flavors, employing DFA. The results are shown in Fig. 4. Depending on the distance between the center of the clusters of the unknowns and the closest clusters of the training map, the recognition

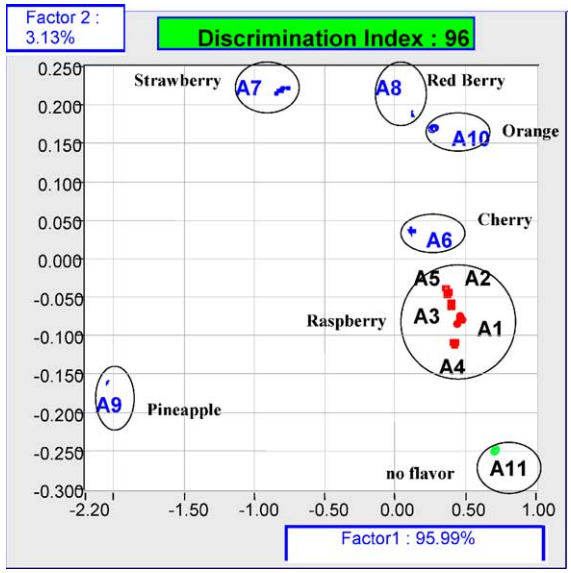

Fig. 3. Principal component analysis of oral solutions with six different flavors: strawberry, red berry, pineapple, raspberry, orange, and cherry, as well as the placebo containing no flavor.

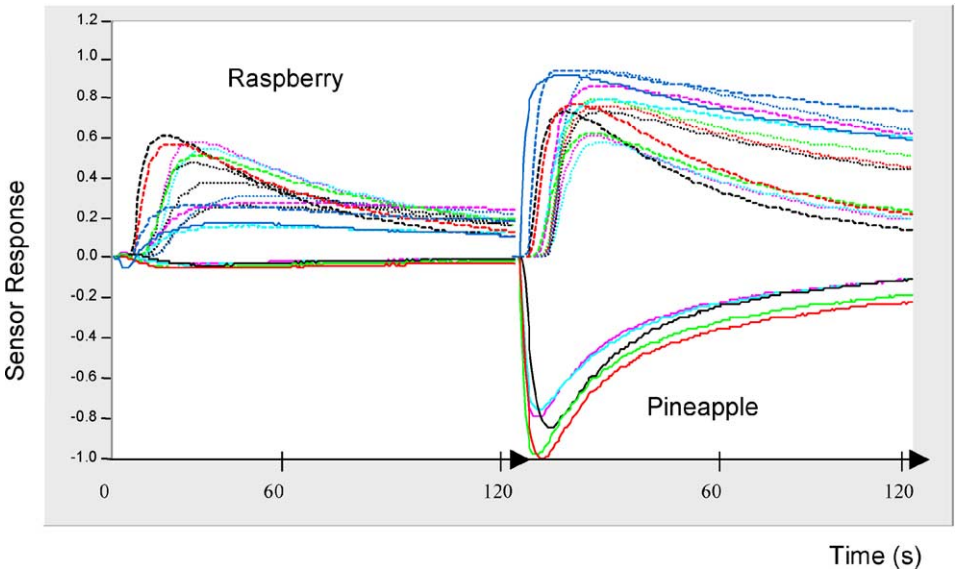

Fig. 2. Raw sensor signals of raspberry (left) and pineapple (right) flavors in oral solution placebo. Sensor response represents $\Delta R/R_0$ where R is resistance.

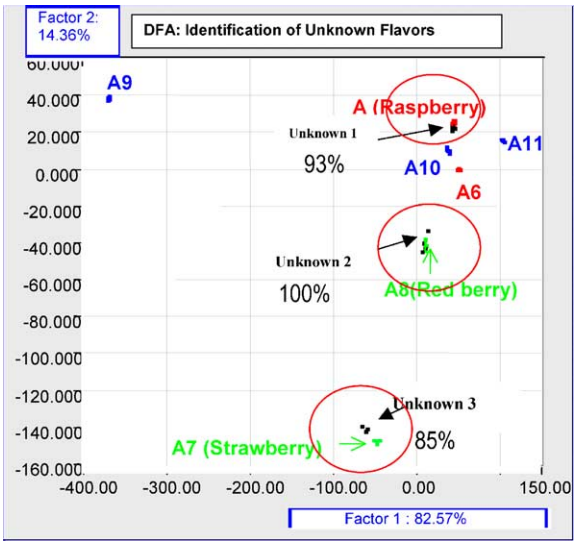

Fig. 4. Unknown flavor (unknown 1–3) identification using discriminant factorial analysis (DFA).

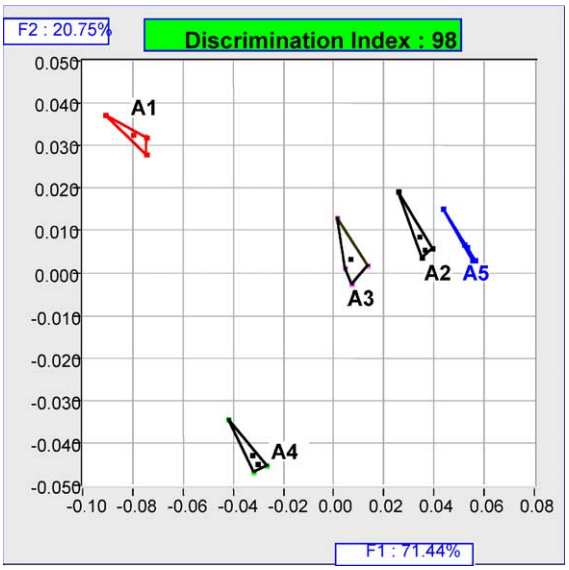

Fig. 5. Principal component analysis (PCA) of raspberry flavors from different lots (A1, A2, A3, and A5), as well as an aged flavor sample (A4).

percentage is calculated to indicate a more or less accurate identification of the various unknown samples. An acceptable unknown identification should have a recognition percentage higher than 90% [22]. Unknown 1, which contains raspberry flavor, was correctly identified to contain raspberry flavor, with 93% recognition score. Unknown 2, which contains red berry flavor, was also correctly identified, with a 100% recognition score. Unknown 3, which was a mixture of raspberry and strawberry flavor, was identified to contain strawberry flavor with a recognition score of 85%, which is below the 90% threshold. When evaluated by human nose, this sample gave the perception of strawberry flavor, though it did not smell exactly the same as the samples containing only strawberry flavor. Therefore, the result from the electronic-nose correlates with the human nose assessment, indicating that the discrimination ability of the instrument is comparable with the human nose. It is possible that strawberry flavor contains stronger or more volatile odorants, which are more easily detected by both electronic-nose and the human nose. In order to identify the true composition of this sample, it would be necessary to include a mixture of both strawberry and raspberry flavors as training standard.

Overall, the discrimination and prediction capabilities of the electronic-nose are very promising. Once a statistical model is built using different flavor standards, the electronic-nose can be used for rapid identity testing for unknown flavors, either as pure flavoring agents or as a constituent of different formulations.

3.2. Raspberry flavor lot-to-lot variation

Five placebo samples containing different lots of raspberry flavors or aged raspberry flavor were also analyzed. Raspberry flavor sample A1 was included in this study because a gas chromatographic identity method for the flavoring agent showed that it had a different peak profile compared to the other three lots (samples A2, A3, and A5) [23]. PCA results (Fig. 5) of electronic-nose analysis also show this lot is significantly different from the other three lots. Therefore, electronic-nose results correlate well with GC results. Sample A4 containing the same lot of raspberry flavor as A3, but having been stored at ambient conditions for 8 months, was also analyzed by electronic-nose. PCA shows that sample A4 is different from A3, indicating the electronic-nose is capable of picking up differences between fresh and aged samples. The human nose evaluation of these two samples verified that the raspberry flavor in the aged sample was weaker. Thus,

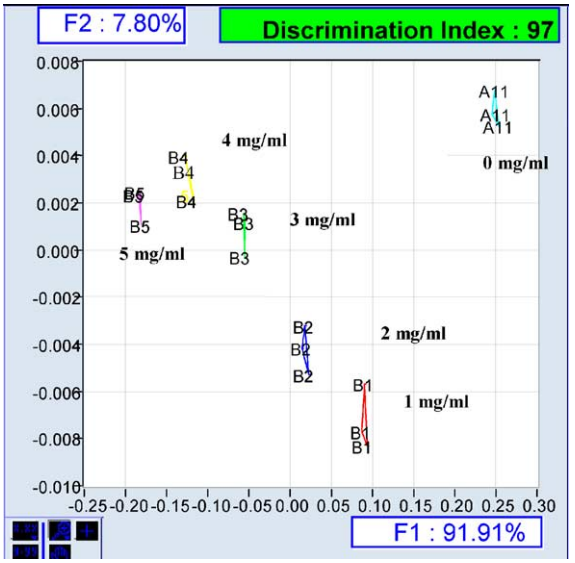

Fig. 6. PCA of raspberry flavor standards at different concentrations in an oral solution formulation, showing the discrimination between these standards.

the storage time and temperature of the formulations might have an impact on the quality of the flavors.

The ability of the electronic-nose to differentiate between flavor lots reflects its sensitivity and selectivity. Utilizing this ability, the electronic-nose could be a useful tool for quality control for accepting or rejecting flavor raw materials from different vendors.

3.3. Quantitative analysis of raspberry flavor in oral solution placebo

To evaluate the possibility of using the electronic-nose for the quantitative analysis of raspberry flavor, formulations containing raspberry flavor at concentrations from 1 to 5 mg/ml were analyzed (four injections for each solution). These concentrations corresponded to 25–125% of target flavor concentration in the oral solution formulation (4 mg/ml). PCA (Fig. 6) shows that the electronic-nose can differentiate all of these standards, as well as the placebo without the flavor.

A calibration curve (Fig. 7A) was generated from these standards using a PLS model. The *X*-axis repre-

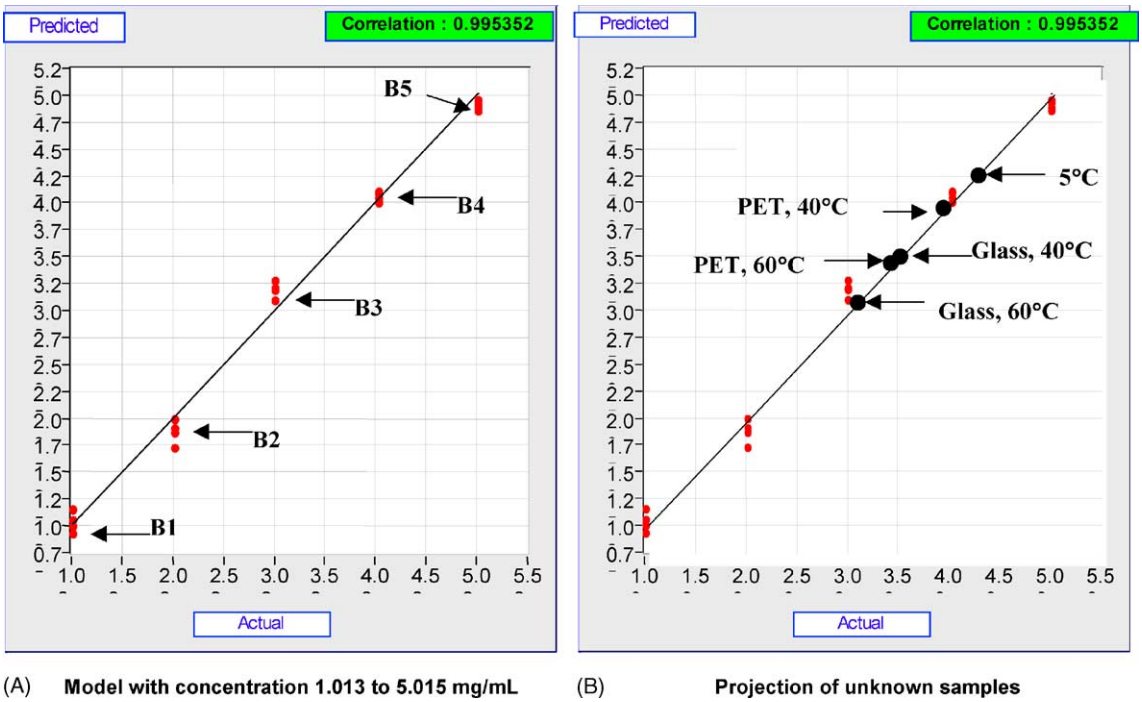

Fig. 7. (A) Calibration curve for the quantitation of raspberry flavor ranging from 1 to 5 mg/ml. (B) Determination of raspberry flavor concentration in PET and glass packages stored at 40 and 60 °C for 1 week, using 5 °C control. The predicted concentrations are shown on the calibration curve.

Table 1
Effects of package and temperature on raspberry favor stability (1 week)

Package	Stress temperature (°C)	Raspberry flavor concentration (mg/ml)	% Claim[a]	S.D. ($n = 3$)
Glass	60	3.10	76.7	6.1
PET	60	3.40	84.2	4.2
Glass	40	3.50	86.6	6.1
PET	40	3.95	97.8	4.9
Control	5	4.20	104.0	0.4

[a] Claim = 4.04 mg/ml of raspberry flavor.

sents the actual flavor concentrations of the standards input to the model, while the *Y*-axis represents the prediction value produced by the model. The linear coefficient of determination $R^2 = 0.9954$ indicates that this calibration model can be used to predict raspberry flavor concentration in unknown samples.

To investigate effects of packaging and temperature on the stability of raspberry flavor in oral solution formulations, samples stored in glass and PET bottles and stressed at 40 and 60 °C for one week were assayed for raspberry flavor concentration, with a sample stored at 5 °C as a control. Each sample was analyzed in triplicate. The results are shown in Table 1 and Fig. 7B. The control sample yielded 104.0% initial of raspberry flavor, indicating that the quantitation is acceptable. Samples stored at 40 and 60 °C yielded lower assays than the 5 °C control, indicating that higher temperature shortens the shelf-life of raspberry flavor. Samples stored at 60 °C exhibited a greater decrease in flavor assay than those at 40 °C. The standard deviation values for three measurements of all the samples ranged from 0.4 to 6.1%, indicating that the reproducibility of the instrument is acceptable for such a flavor analysis. The raspberry assays for samples in PET bottles are 11 and 8% higher than those in glass bottles at 40 and 60 °C, respectively, suggesting that the raspberry flavor is more stable in PET bottles than in glass bottles. This is important information for package selection for the final product. The root cause for the decreased stability of raspberry flavor in glass bottles is still unknown.

Thus, the utility of the electronic-nose for flavor quantitation has been demonstrated. In most cases, the oral solution or suspension can be analyzed directly, or with minimum sample preparation. The electronic-nose methodology can be implemented for the determination of flavor concentration during product release testing. Coupled with a package stability study, it can also be used to study the flavor shelf-life and the compatibility of flavors with market packages under accelerated conditions. These proactive studies will ensure that proper packages are used for the final marketed product. The results can be a valuable supplement to those from other stability studies including stability of the active pharmaceutical ingredient, and may play a critical role in the decision-making process.

4. Conclusions

In this paper, we have presented the results of an evaluation of the application of the Fox 4000 electronic-nose for flavor analysis in a pharmaceutical oral solution. The ability of the electronic-nose to qualitatively distinguish among six common flavors including raspberry, red berry, strawberry, pineapple, orange, and cherry in an oral solution placebo was demonstrated. This indicates that the instrument has adequate selectivity and sensitivity to perform flavor identification in pharmaceutical products. The flavors from the unknown samples were properly identified using the electronic-nose. Raspberry flavor samples from different lots were distinguished using the electronic-nose. The electronic-nose was also able to discern the differences between freshly prepared and aged samples. Therefore, the instrument can be used for identity testing of flavor raw materials and flavored solution formulations. We have also demonstrated the use of electronic-nose instrumentation for quantitative analysis of flavors in an oral solution formulation. The electronic-nose can be used to assay the flavor concentration during release testing, to aid in packaging selection and to monitor flavor stability during shelf-life.

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