Analysis of Body Odor Using the zNoseÒ

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Electronic Noses

Conventional electronic noses (eNoses) produce a recognizable response pattern using an array of dissimilar but not specific chemical sensors. Electronic noses have interested developers of neural networks and artificial intelligence algorithms for some time, yet physical sensors have limited performance because of overlapping responses and physical instability. eNoses cannot separate or quantify the chemistry of aromas.

A new type of electronic nose, called the zNose®, is based upon ultra-fast gas chromatography, simulates an almost unlimited number of specific virtual chemical sensors, and can produce high-resolution two-dimensional olfactory images based upon aroma chemistry. The zNose® is able to perform analytical measurements of volatile organic vapors and odors in near real time with part-per-trillion sensitivity. Separation and quantification of the individual chemicals within an odor is performed in seconds. Using a patented solid-state mass-sensitive detector, picogram sensitivity, universal non-polar selectivity, and electronically variable sensitivity is achieved. An integrated vapor preconcentrator coupled with the electronically variable detector, allows the instrument to measure vapor concentrations spanning 6+ orders of magnitude. In this paper a portable zNose®, shown in Figure 1, is used to assess underarm body odor sampling methods.

Figure 1- Portable zNoseÒ technology incorporated into a handheld instrument

How the zNose™ Quantifies the Chemistry of Odors

A simplified diagram of the zNose™ system shown in Figure 2 consists of two sections. One section uses helium gas, a capillary tube (GC column) and a solid-state detector. The other section consists of a heated inlet and pump, which samples ambient air. Linking the two sections is a "loop" trap, which acts as a preconcentrator when placed in the air section (sample position) and as an injector when placed in the helium section (inject position). Operation is a two step process. Ambient air (odor) is first sampled and organic vapors collected (preconcentrated) on the trap. After sampling the trap is switched into the helium section where the collected organic compounds are injected into the helium gas. The organic compounds pass through a capillary column with different velocities and thus individual chemicals exit the column at characteristic times. As they exit the column they are detected and quantified by a solid state detector.

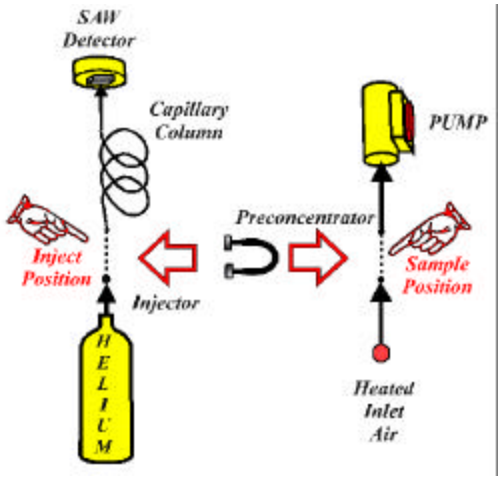

Figure 2- Simplified diagram of the zNose™ showing an air section on the right and a helium section on the left. A loop trap preconcentrates organics from ambient air in the sample position and injects them into the helium section when in the inject position.

An internal high-speed gate array microprocessor controls the taking of sensor data which is transferred to a user interface or computer using an RS-232 or USB connection. Odor chemistry, shown in Figure 3, can be displayed as a sensor spectrum or a polar olfactory image of odor intensity vs retention time. Calibration is accomplished using a single n-alkane vapor standard. A library of retention times of known chemicals indexed to the n-alkane response (Kovats indices) allows for machine independent measurement and compound identification.

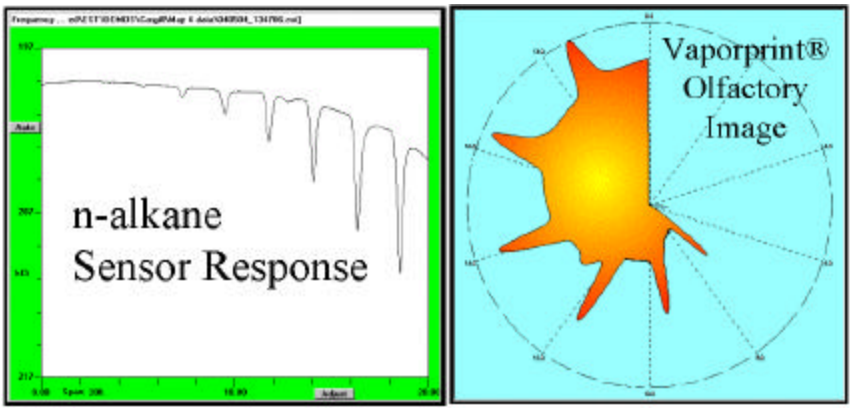

Figure 3- Sensor response to n-alkane vapor standard, here C6-C14, can be displayed as sensor output vs time or its polar equivalent olfactory image.

Chemical Analysis (Chromatography)

The time derivative of the sensor spectrum (Figure 3) yields the spectrum of column flux, commonly referred to as a chromatogram. The chromatogram response (Figure 4) of n-alkane vapors (C6 to C14) provides a set of reference retention times. Graphically defined regions, shown as red bands, provide a method dependent reference time base against which subsequent chemical responses can be compared and indexed. As an example, a response midway between C10 and C11 would have a retention time index of 1050.

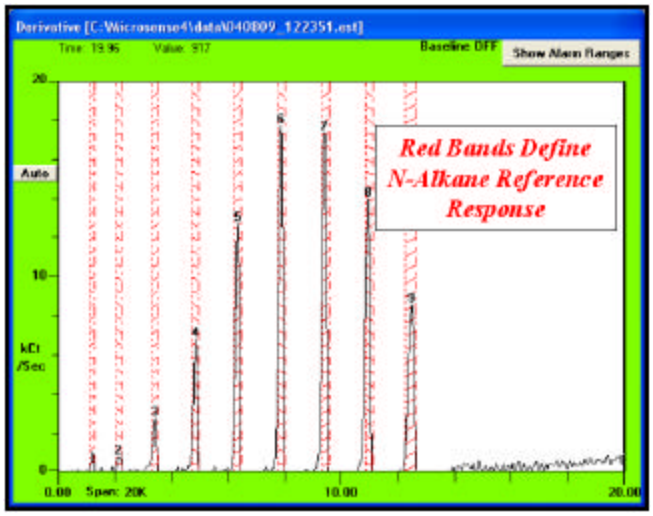

Figure 4 - Chromatogram of n-alkane vapors C6 to C14).

Chemistry of Body Odor

Sweat glands (apocrine glands) secrete a substance that is the major non-food/drink related cause of body odor. This substance, which contains protein, carbohydrates, and lipids, is quickly attacked by bacteria. Sweat itself doesn't actually smell, the odor is caused by the action of bacteria. Special microscopic glands (sweat glands) in the deep layer of the skin produce sweat by filtering fluid and salts out of the blood and secreting this fluid through small tubes in the skin (sweat ducts) that empty out into small pores at the top layer of the skin (the stratum corneum).

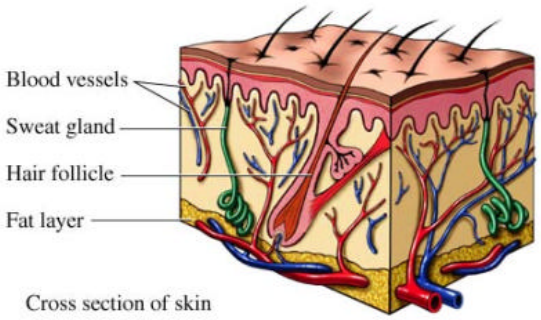

Figure 5- Cross-section of skin and sweat glands.

A GC-MS analysis of the headspace composition of fresh sweat revealed ethanol (15.1% of the total amount of volatiles trapped), acetic acid (10.9%), and 3-hydroxy-2-butanone (9.5%) as the most abundant compounds; a wide range of ethyl esters was present as well. None of the ethyl esters was detected in the headspace collections from incubated sweat, while the relative amounts of ethanol, acetic acid, and 3-hydroxy-2-butanone were strongly reduced. After bacterial action the showed indole (27.9%), 1-dodecanol (22.4%), and 3-methyl-1-butanol (10%) were present in high amounts, while they were absent or present in only minor amounts in the headspace collections from fresh sweat. Geranyl acetone (6%) and 6-methyl-5-hepten-2-one (1.9%) were relatively abundant in both the fresh and incubated headspace samples as well. Other stinky compounds, such, as (E)-3 -methyl-2-hexenoic acid, can also be produced by bacteria. Another compound, 3-methyl-3-sulfanylhexan-1-ol has also been identified as a component of underarm sweat. These compounds produce such intense odors even at extremely low concentrations in sweat is a result of their unusually low detection threshold.

Thus the typical odor associated with the human armpit can contain a complex cocktail of odorous substances (Table I), including various hormonal derivatives, deodorants and antiperspirants, volatile fatty acids, and sulfur-containing compounds all mixed together and forming the odor perceived by our sense of smell.

Table I- Typical Chemicals Found in Body Odor

aluminium chlorohydrate	Antipersperant
aluminum chloride hexahydate	Antipersperant
acetylcholine	Neuro-transmitter
4-ethyloctanoic acid	odoriferous
trans-3-methyl-2-hexenoic acid	odoriferous
HMHA	odoriferous
3-methyl-3-sulfanylhexan-1-ol	odoriferous
ethanol	odoriferous
acetic acid	odoriferous
3-hydroxy-2-butanone	odoriferous
indole	odoriferous
1-dodecanol	odoriferous
3-methyl-1-butanol	odoriferous
geranyl acetone	odoriferous
6-methyl-5-hepten-2-one	odoriferous

No one yet knows whether the chemical profile of our sweat is truly unique although the odor can vary substantially from person to person. In general the chemistry of fresh sweat, before bacterial action takes place, is remarkably the same. As an example, vertically offset chromatograms of fresh sweat from exercising men and women are shown in Figure 6 and show much the same chemistry.

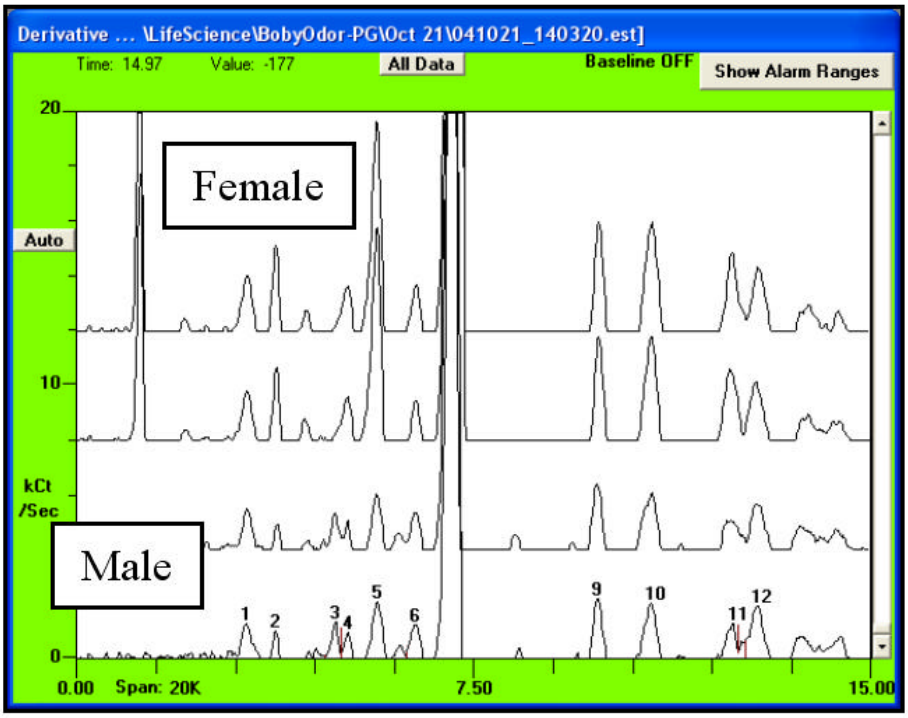

Figure 6- Offset chromatograms of fresh sweat from men and women show many of the same chemicals.

Odor can be influenced by the mix of bacteria that colonize a given individual's body and by the composition of the sweat produced. Sweat composition can be influenced by the combined effect of the foods last eaten and the physical or psychological body state as well. Foods, such as garlic, onion, and asparagus, are known to impart a characteristic smell to body secretions. Certain disease states, such as renal failure and ketoacidosis, are also known to change body odor and produce a characteristic smell.

Body Odor Testing Methods

Testing body odor as perceived by humans can be a time consuming and complex endeavor requiring the expertise of trained 'sniffers' as shown in Figure 7. Because the human sense of smell is selective and does not respond equally to every chemical present, it must judge odor characteristics in human terms, which can be very different from that produced from a full chemical analysis of the odor.

Figure 7- Testing Armpit odor by trained 'sniffers'.

The zNose® is a new quantitative testing tool for in-situ odor analysis and compliments rather than replaces evaluations by human test panels. With the instrument as just another member of the test panel, measurements of the concentration of odor producing chemicals can provide valuable real time information.

To illustrate its use, samples of underarm odor were collected and tested using absorbent Q-tip and wipe pads for comparison. Subjects for whom a deodorant was applied to only the left armpit were used. An absorbent was held under each arm to collect sweat for 1, 5, and 10 minutes. Subjects were tested twice, once at the beginning (TO) and again 5 hours later (T5). For each type of absorbent 12 samples were collected as shown in Table II.

Table II- Samples Collected

	Sample Collection			
	TO		T5	
	Left Arm	Right Arm	Left Arm	Right Arm
1 min	Deodorant	No Deodorant	Deodorant	No Deodorant
5 min	Deodorant	No Deodorant	Deodorant	No Deodorant
10 min	Deodorant	No Deodorant	Deodorant	No Deodorant

Samples were placed in septa sealed vials and vial odors tested. Several analysis methods are illustrated in Figure 9. The chemical composition of an odor sample is shown in the 15-second chromatogram. Compound peaks are labeled and their concentration tabulated. Graphically defined virtual chemical sensors (red bands in chromatogram) highlight specific odor producing compounds, which can more simply be displayed and interpreted without a chromatogram.

Figure 8- GC methods used to analyze odors.

Testing Samples Without Deodorant (Right Arm)

The concentration of compounds in samples collected from the right arm of subjects did not contain deodorant and were much lower in concentration. This allowed the sensitivity of the instrument to be maximized without overloading. Vapors from septa-sealed 40-milliliter vials were sampled and preconcentrated for 30 seconds followed by a 20-second chromatographic analysis using a DB-624 column temperature programmed 40°C to 160°C at 5°C/second. A detector temperature of 10°C was used to detect and quantify the concentration of volatile organic compounds as they eluted from the GC column.

Shown in Figure 9 are vertically offset chromatograms comparing vial odors from samples taken 5 hours apart. Letters identify compound peaks and their concentration in counts is listed. The retention time of each compound is given in Kovats indices relative to the retention time of n-alkanes. The additional compounds present in the sample odors taken after 5 hours are pronounced and presumably due to bacterial action.

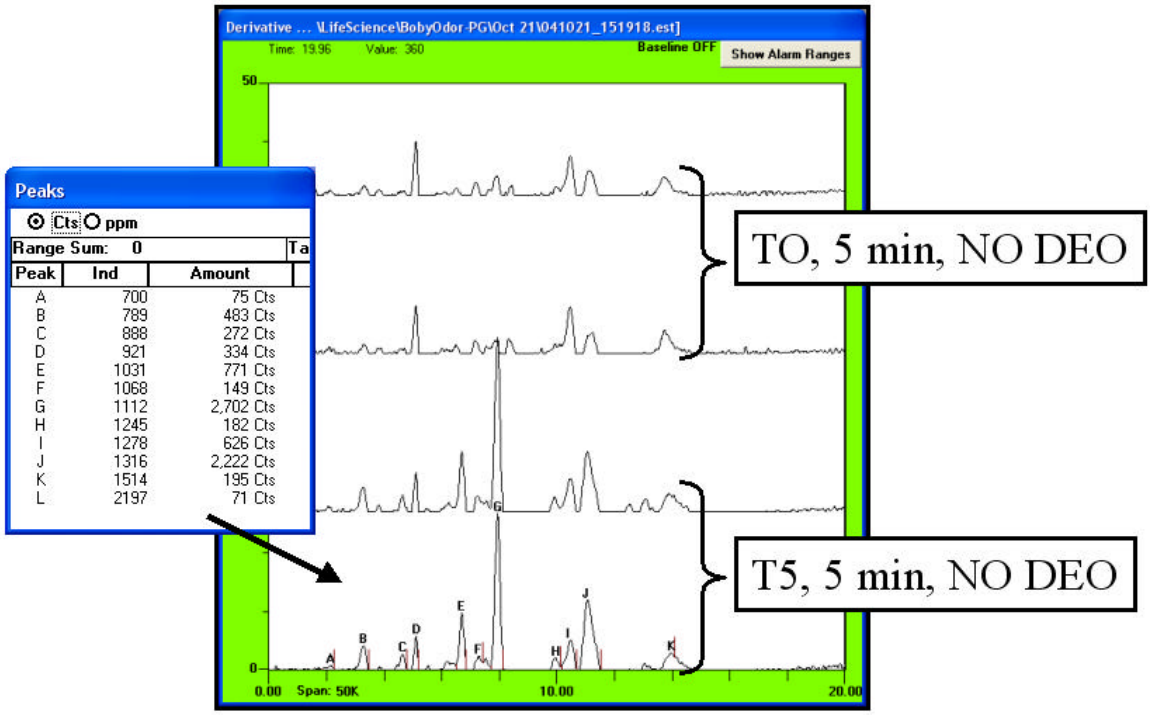

Figure 9- Vertically offset chromatograms comparing initial odor chemistry (TO) subjects with odor chemistry collected form subjects 5 hours later (T5).

Testing Samples With Deodorant (Left Arm)

The concentration of compounds in samples collected from the left arm of subjects contained deodorant and of much higher concentration than the odor causing compounds. In effect, for the instrument, the deodorant compounds dominated the odor chemistry and this can be clearly seen in the detector signal and Vaporprint® olfactory image shown in Figure 10.

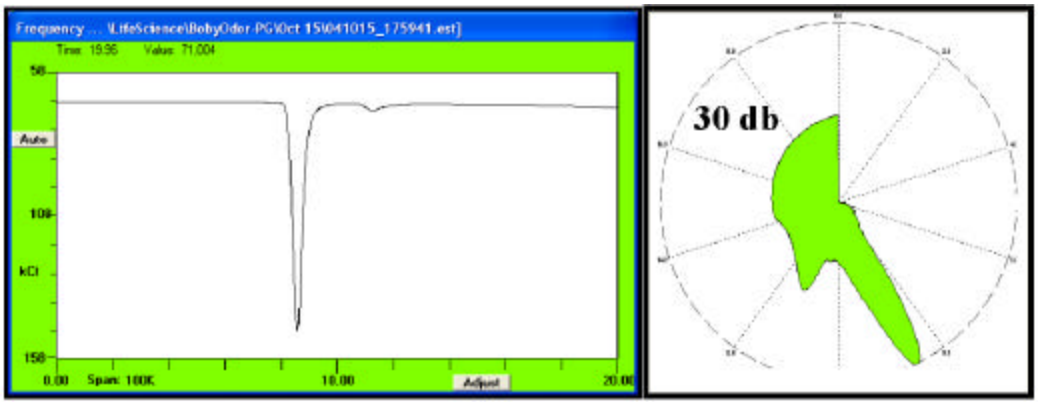

Figure 10- Sensor response and olfactory image of underarm odor with deodorant

Chromatograms of odors from Q-tip and Wipe absorbents containing just deodorants are shown in Figure 11. In addition to the main deodorant compound (index=1144) there were several other low concentration compounds that were produced. The concentration and number of these potentially interfering peaks were greater in odors from wipes than from Q-tips and may be caused by the absorbent material itself rather than the deodorant.

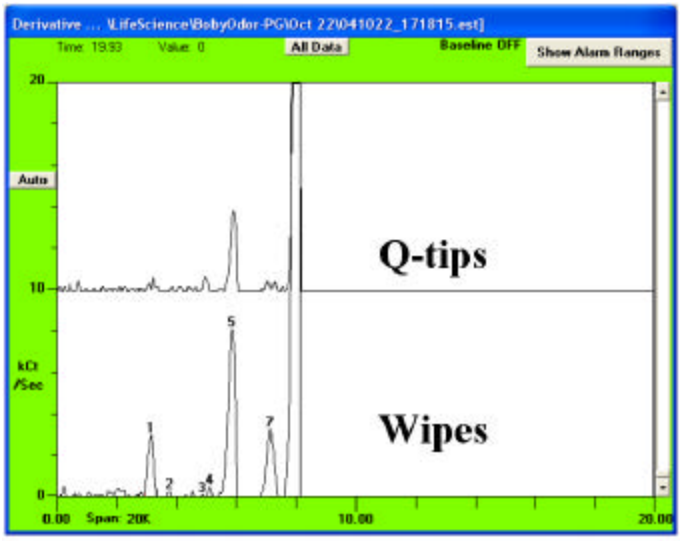

Figure 11- Chromatograms of deodorant alone using Q-tips and Wipes.

Using the variable sensitivity of the SAW detector it was possible to prevent overloading by raising the temperature of the detector. The concentration in counts of the main deodorant compound as a function of detector temperature is shown in figure 12.

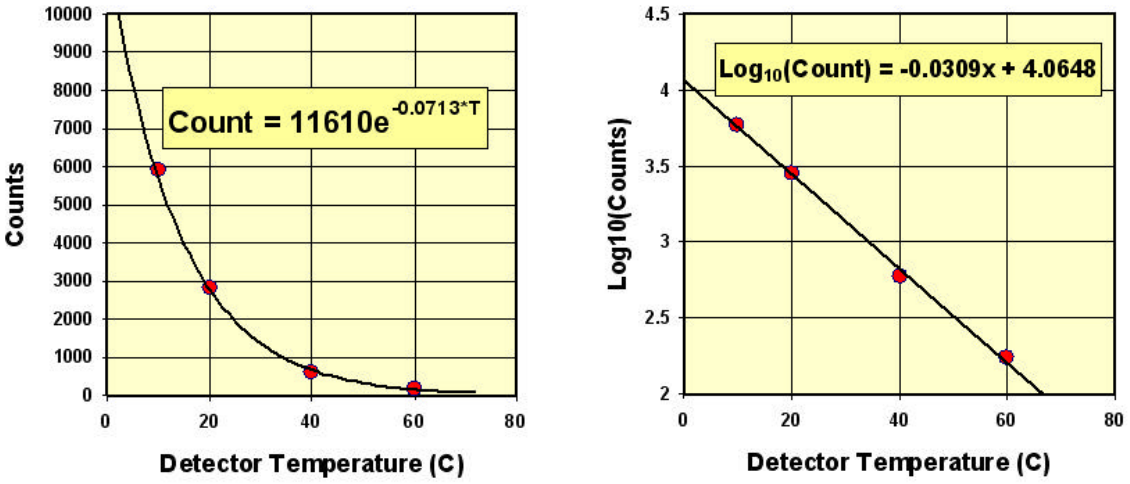

Figure 12- The exponential temperature dependence of SAW detector allows electronically variable sensitivity to be achieved over a wide range of vapor concentrations.

Using a combination of vapor sampling (preconcentration) time and detector temperature, it was possible to measure the chemistry of samples containing deodorant. The approach is illustrated in Figure 13. With a short 10-second vapor sample and a 20°C detector there is no overloading and the high boiling point compounds with retention times greater than the main deodorant compound are easily analyzed. With a longer 30-second sample time and lower detector temperature (10°C), the lower boiling point and more volatile compounds, which have retention times below the main deodorant peak, can be measured. In the later case the main deodorant peak overload the detector and prevents detection of compounds with retention time. Overloading caused no damage to the detector, which completely recovered after a short 15 second, 150oC, heat cycle at the end of each measurement.

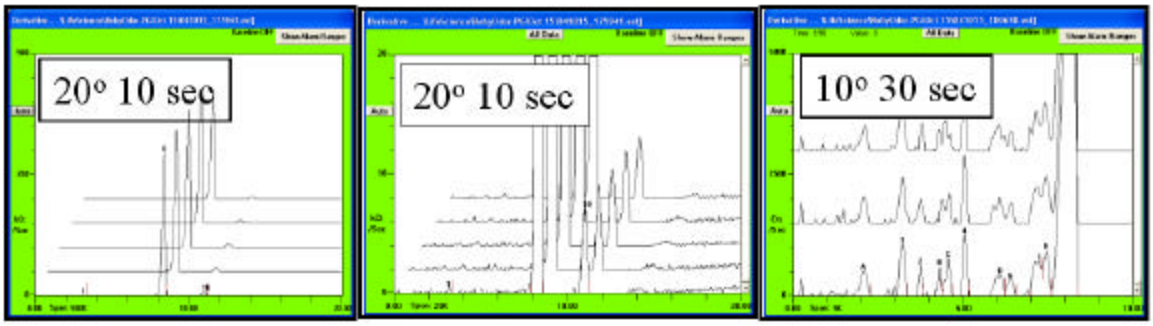

Figure 13- Adjusting sample time and detector temperature allows measurement of samples containing high concentration deodorant compounds.

Testing Results with Q-Tip Sampling

Offset chromatogram of vial odors from Q-tips held in place for 1,5, and 10 minutes are shown in Figures 14-17. For these comparisons maximum sensitivity using a 30 second sample time and a 10°C detector was used. Some variation in initial (TO) samples without deodorant (Figure 14) can be seen while TO samples with deodorant (Figure 15) show a more consistent increase in concentration with sample time.

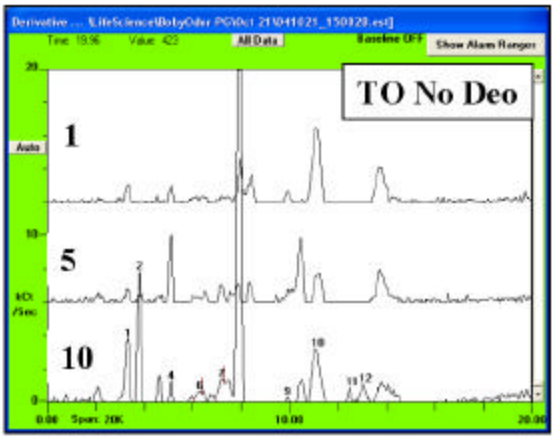

Figure 14

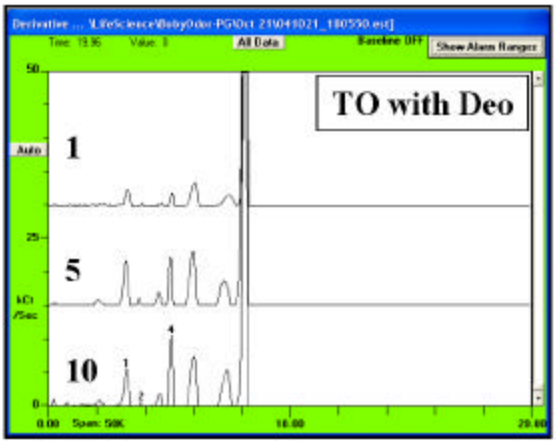

Figure 15

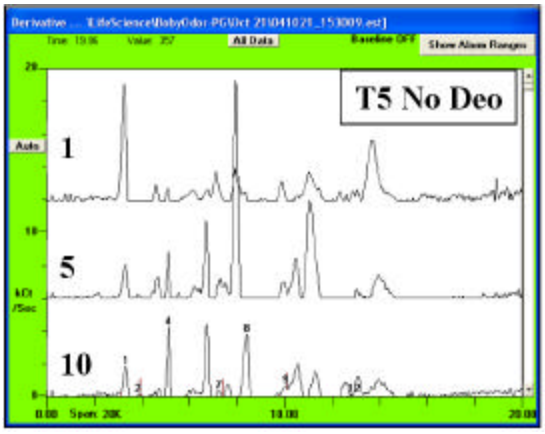

Figure 16

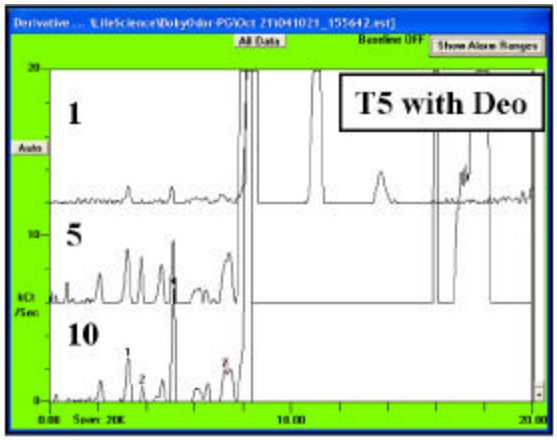

Figure 17

Odors from samples taken 5 hours later (T5) also show variations not consistent with increasing absorption time. For example odors without deodorant (Figure 16) show 10-minute exposure concentrations are less than with a 5-minute exposure time. Similar inconsistency can be seen in odors from samples with deodorant (Figure 17).

The cause of the variation in absorption efficiency of Q-tips is unknown. A possible cause may be related to the small size of the Q-tip, which may make it somewhat site specific.

Testing Results with Wipe Sampling

Offset chromatograms of vial odors from wipe pads held in place for 1,5, and 10 minutes are shown in Figures 18-21. All chromatograms used the same method and are plotted with the same amplitude scale as previously used for Q-tips. Generally there was very little increase in amplitude with increasing sample exposure time. Also, samples from the right arm without deodorant (Figures 18 and 20) show presence of the main deodorant compound indicating cross contamination has occurred.

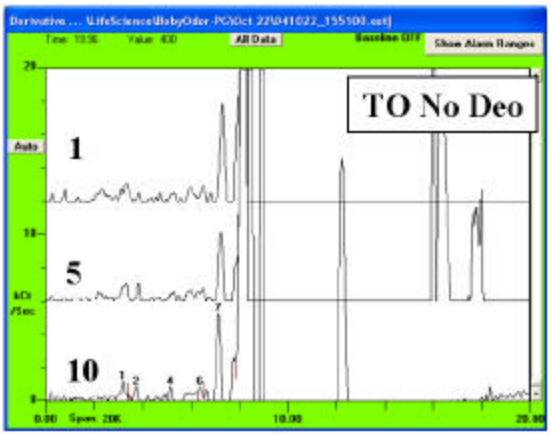

Figure 18

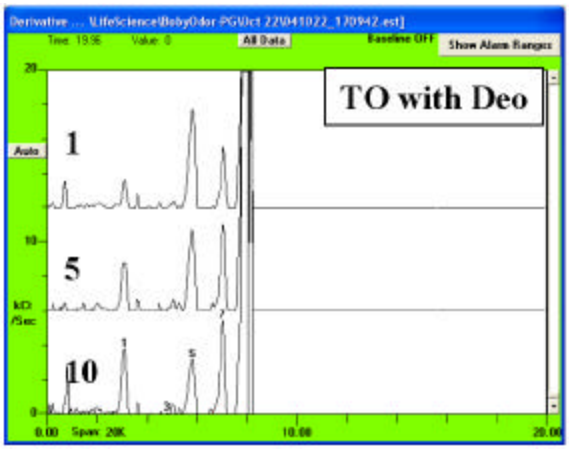

Figure 19

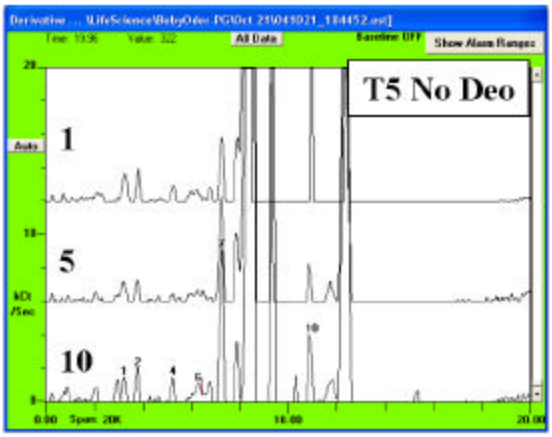

Figure 20

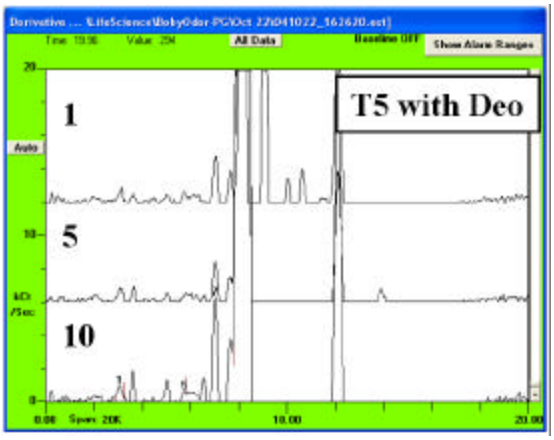

Figure 21

The concentration of volatile compounds from vials containing wipes was much lower than the concentration of the same volatiles collected with Q-tips. The increased concentration of interfering compounds from the wipes, previously described in Figure 11, can also be seen, especially in Figure 19.

Although wipes may collect more sample from a larger area, they also released less of the chemicals into the test vials and hence produced a lower concentration vapor for measurements. In addition they produce their own volatile compounds which may interfere with quantification of chemicals associated with body odor.

Summary

Odors associated with the human armpit can contain a complex cocktail of odorous substances, including various hormonal derivatives, deodorants and antiperspirants, volatile fatty acids, and sulfur-containing compounds all mixed together and forming the odor perceived by our sense of smell. Testing body odor can be a time consuming and complex endeavor requiring the expertise of trained 'sniffers'. Because the human sense of smell is subjective and does not respond equally to every chemical present, it must judge odor characteristics in human terms, which can be very different from that produced from a full chemical analysis of the odor

The zNose® is a new quantitative testing tool for in-situ odor analysis and compliments rather than replaces evaluations by human test panels. With this portable instrument measurements of underarm odor and quantification of the chemicals produced can be performed at the odor source in real time. However, in this paper samples of underarm odor were first collected using Q-tips and cotton pads or wipes. The samples were then placed in sealed vials and the resulting vapors produced within the vials tested using the zNose®.

Q-tips produced higher concentration chemical vapors with fewer interfering chemicals than samples collected using cotton pads. Cotton pads also showed a tendency to become cross-contaminated so additional care may need to be taken in their use. However, Q-tips showed concentration variations, which were uncorrelated with collection time. This suggests that Q-tips only sample a small-localized area when placed under the arm and therefore results may be location dependent.

Future work might be to use a Q-tip to swab the entire underarm area as an alternative approach to collecting samples of underarm odor. Also, chemical standards of known odor producing chemicals might be used to calibrate the zNose® and establish target compounds to aid the analysis of underarm odors.

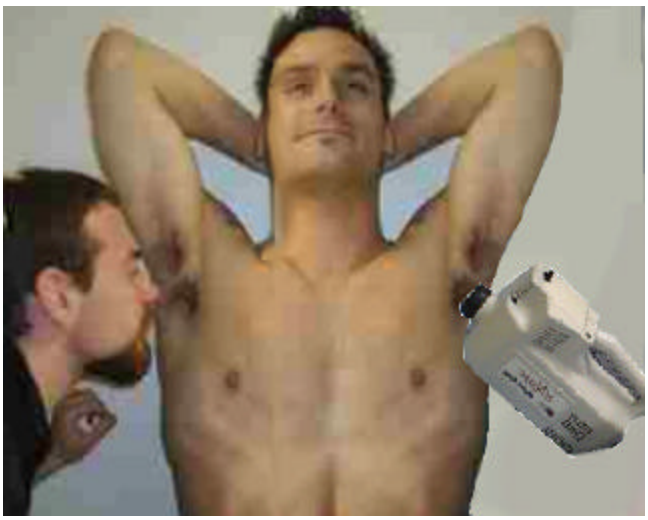

Figure 22- Two different but complimentary methods of analyzing armpit odors.