

*Electronic  
Sensor  
Technology*

## **The chemistry of black tea aroma**

**By**

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**Electronic Sensor Technology**

[www.zNose.com](http://www.zNose.com)

**abstract**

**A new electronic nose technology based upon ultra-high speed gas chromatography, called the zNose™, quantifies the chemistry of black tea aroma with precision and accuracy in seconds. In this paper the results of testing 20 samples of blended black tea with the zNose™ is described. Test methods for tea include chromatographic analysis using Kovats indices and n-alkane vapor standards, a virtual chemical sensor array specific to black tea, principal chemical component analysis, and VaporPrint™ olfactory images. The zNose™ can monitor quality online and even has the ability to calibrate itself using proprietary zNose™ perfumes.**



**zNose™ ...THE NEED FOR SPEED**

# Testing Tea Samples with the zNose<sup>®</sup>

## Chemistry of Tea

Tea contains a diverse mix of hydrocarbons both volatile and non-volatile<sup>1,2</sup>. Of interest are the volatile compounds listed in Table I together with their chemical formula, molecular weight, and published Kovats indices<sup>3</sup> (KI) relative to n-alkanes. Most are aldehydes and alcohols spanning the range C5 to C16. Kovats indices for a db5 column indicate retention times span the range C6 to C14 relative to the retention times of the straight chain alkanes. It should be clear that the aroma of tea is a very complex chemical mixture indeed.

Table I- Volatile Hydrocarbons in Tea

KI-db5	Name	Odor	Formula	MW
0650	3-methyl Butanal	malty	C5H10O	86.1
0653	2-methyl Butanal	roasted cocoa	C5H10O	86.1
0764	1-pentanol	balsamic	C6H12O	88.1
0775	1-penten-3-one	pungent	C5H8O	84.1
0801	hexanal	cut grass	C6H12O	100.2
0806	2-methyl-propanal	pungent	C4H8O	72.1
0851	hexanol	resinous	C6H14O	102.2
0902	(Z)-4-heptenal	biscuit	C7H12O	112.2
0903	heptanal	fatty	C7H14O	114.2
0968	benzaldehyde	almond	C7H6O	106.1
0979	1-octen-3-one	mushroom	C8H14O	126.2
0982	1-octen-3-ol	mushroom	C8H16O	128.2
1006	octanal	soapy	C8H16O	128.2
1039	benzyl alcohol	sweet	C7H8O	108.1
1053	phenylacetaldehyde	hawthorne	C8H8O	120.1
1100	(Z,Z)-3,6-nonadienal	fatty	C8H14O	154.2
1100	linalool	lemon	C10H18O	154.3
1118	2-phenylethanol	honey	C8H10O	122.2
1154	(E,Z)-2,6-nonadienal	waxy	C8H14O	138.2
1201	2,4-nonadienal	watermelon	C8H14O	138.2
1234	methyl salicylate	peppermint	C8H8O3	152.1
1275	geraniol	rose	C10H18O	154.2
1284	(Z,Z)-2,4-decadienal	orange	C10H16O	152.2
1297	(E,Z)-2,4-decadienal	tallow	C10H16O	152.2
1386	b-damascenone	rose	C13H20O	190.1
1317	(E,E)-2,4-decadienal	fried	C10H16O	152.2
1410	vanillin	vanilla	C8H8O3	152.1

<sup>1</sup> <http://www.ars-grin.gov/duke/>

<sup>2</sup> <http://www.teatalk.com/science/compounds.htm>

<sup>3</sup> <http://www.nysaes.cornell.edu/flavornet/>

## Calibrating the zNose n-Alkane Standards

Testing of tea aroma requires that the retention time scale of the zNose be calibrated using standard headspace vapors containing C6-C14 n-alkanes. The zNose<sup>®</sup> is able to speciate and quantify the concentration of these compounds in seconds using a proprietary SAW detector. The SAW detector produces a stable frequency whose deviation is directly proportional to the concentration of the individual n-alkanes as shown in Figure 1.

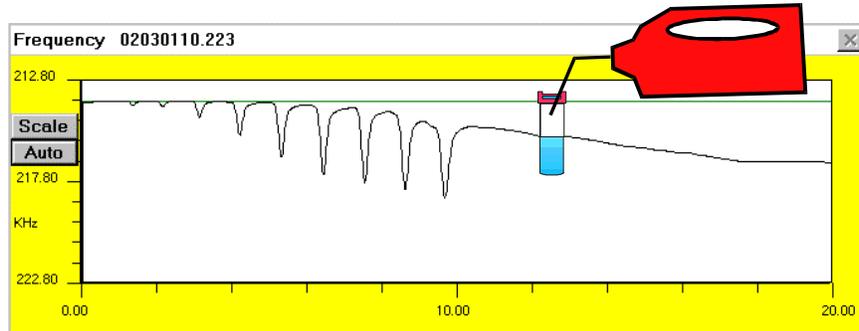


Figure 1- SAW detector frequency response to n-alkanes C6-C14 using 10ps2a1b method, 10 second sample, 60°C detector.

Retention time is defined by the flux (derivative) of the sensor response and is shown for replicate measurements of n-alkane vapors in Figure 2. Microsense software simultaneously displays the sensor frequency and derivative-of-frequency which is equivalent to a conventional chromatogram. All hydrocarbons C6 to C14 can be analyzed in less than 10 seconds.

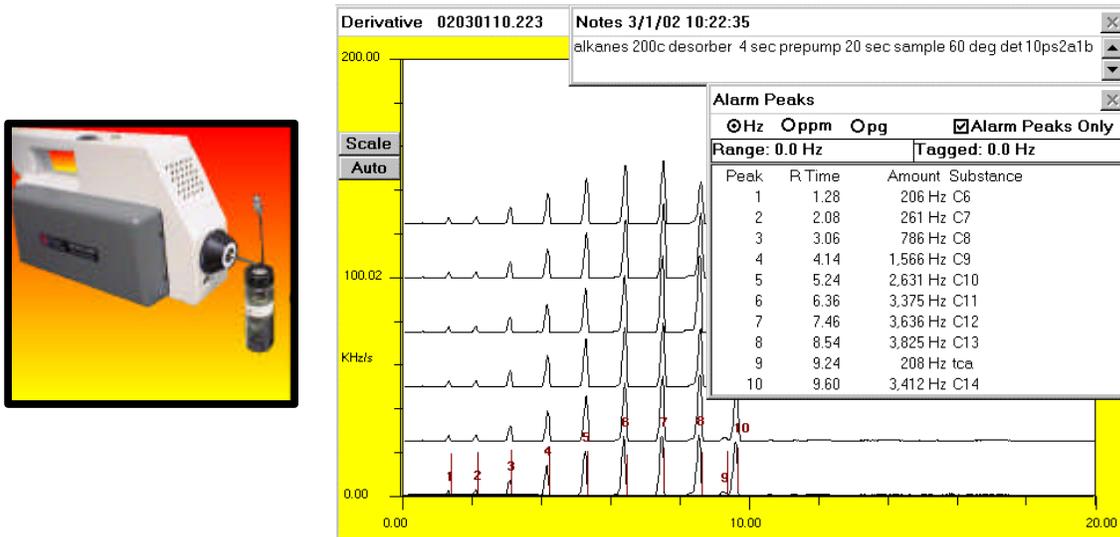


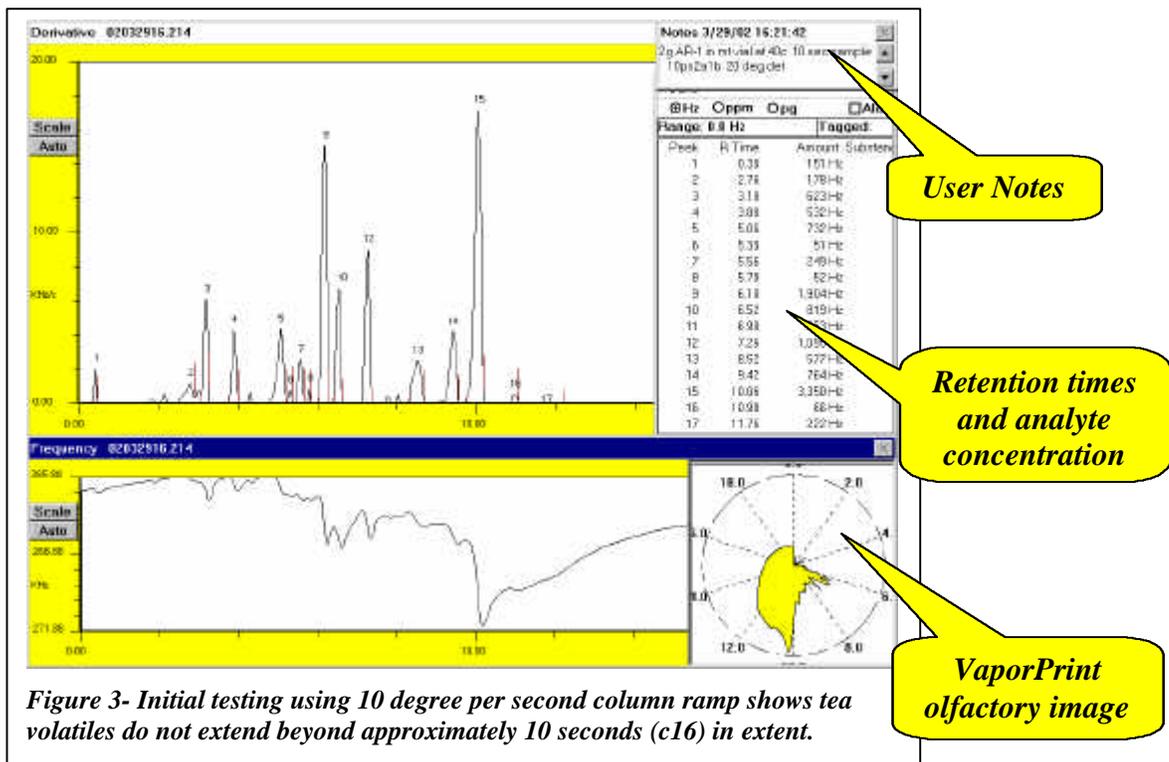
Figure 2- Six replicate analyses (vertically offset) performed on n-alkane vapors. The retention times are identified and the frequency deviation (concentration) for each listed for the most recent (bottom) test.

## Test Method

The zNose<sup>®</sup> is an ultra-fast chemical analyzer with 10 second speed and picogram sensitivity. It is equipped with an internal sampling pump and tenax trap capable of sampling inlet vapors at 30 ccm. To measure the headspace chemistry of liquid or dry tea samples were placed in 40 mL septa sealed vials. A two-zone temperature controlled vial heater is used to maintain the samples at a constant temperature for testing repeatability and accuracy. For testing tea samples, bottom and top zones were held at 40°C and 50°C respectively.



The entire testing process takes approximately 1 minute per sample. A vapor sample is collected and preconcentrated by inserting a side-ported sampling needle through the septa. The preconcentrated vapors are then injected and analyzed as shown in Figure 3. The zNose<sup>®</sup> software provides a wide range of testing and sampling methods. These are called GC methods and each method is designed to achieve a specific level of sensitivity, selectivity, and speed. A high speed method used to analyze tea aroma produces the chemical spectrum shown in Figure 3.



## GC Method for Tea Aroma

The resolving power of the measurement can be increased by changing the GC method e.g. decreasing the speed of the measurement. Sensitivity of the measurement can also be increased by preconcentrating for a longer time and decreasing the detector temperature. Using a slower method and temperature profile (5°C/second) all of the hydrocarbons in tea can be analyzed over the range C6 to C16 with good resolution in 20 seconds. A c6-c14 alkane standard vapor calibration is shown in Figure 4.

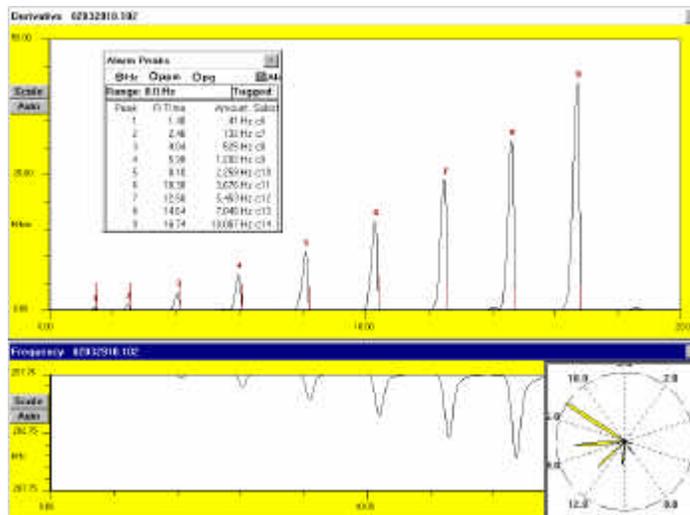


Figure 4- Response of zNose to c6-c14 n-alkanes vapor standards using a 5ps2a1b GC method

The zNose functions much like a hydrocarbon spectrum analyzer with retention time proportional to alkane number or molecular weight of the analytes. This can be seen in the tabulation of retention time and plot of retention time vs alkane number shown in Figure 5.

<i>n</i> -Alkane	Retention Time (s)
6	1.40
7	2.46
8	4.04
9	5.98
10	8.10
11	10.30
12	12.50
13	14.64
14	16.74

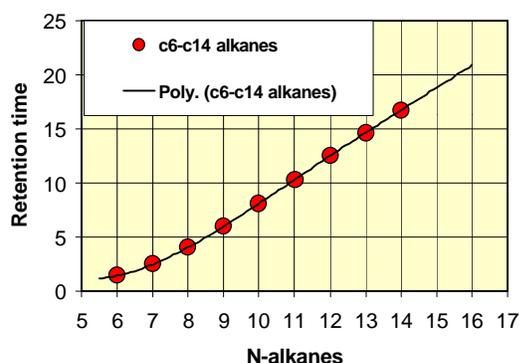


Figure 5- Retention time vs alkane number.

## Tea Analysis Techniques

A number of different techniques can be used to characterize and quantify the chemistry contained within tea aroma e.g. quality. A chromatographic analysis using the tea method (5ps2a1b) settings is shown in Figure 6. In this analysis the n-alkane response is overlaid in red so as to allow easy determination of the retention time of the tea chemicals relative to that of the n-alkanes.

Using a 20 second analysis, approximately 29 individual chemicals can be resolved in this sample. Their retention time, concentration (detector counts), and Kovats indice are tabulated in Figure 7.

Peak No.	Retention Time (s)	Counts (Hz)	Kovat Indices
1	0.48	231	513
2	0.94	199	557
3	1.46	56	606
4	2.22	83	677
5	2.72	397	716
6	4.14	533	805
7	4.46	3007	822
8	5.68	2269	885
9	6.46	470	923
10	7.4	192	967
11	8	2001	995
12	8.42	50	1015
13	8.88	780	1035
14	9.34	1132	1056
15	10.22	6021	1096
16	10.9	2189	1127
17	11.46	2012	1153
18	12.38	3344	1195
19	12.92	386	1220
20	13.78	348	1260
21	14.52	539	1294
22	15.02	260	1318
23	15.7	154	1350
24	16.04	218	1367
25	16.68	2524	1397
26	17.82	8556	1451
27	19.54	1991	1533
28	20.1	54	1560
29	20.62	550	1585

Figure 7- Kovats indices for tea analytes

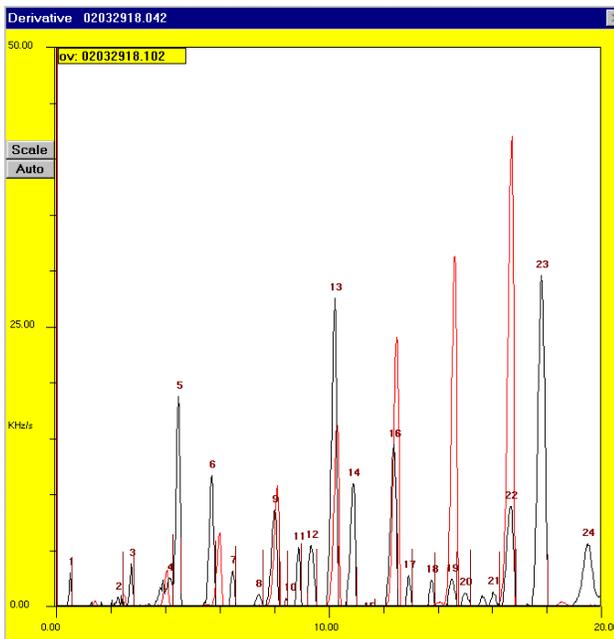


Figure 6- Increased showing approximately 29 distinct hydrocarbons present in tea samples.

Since concentration is proportional to the detector counts, this is plotted for each compound detected vs it's Kovats indices in Figure 8.

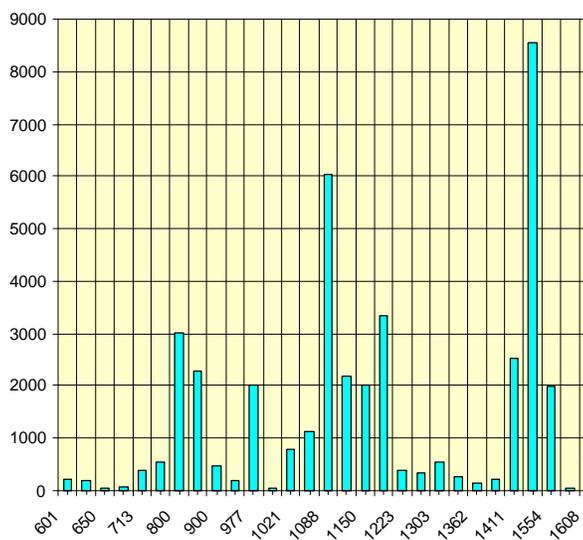
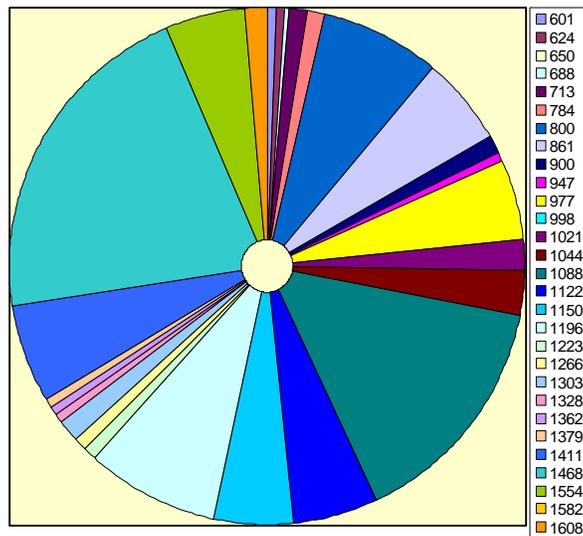


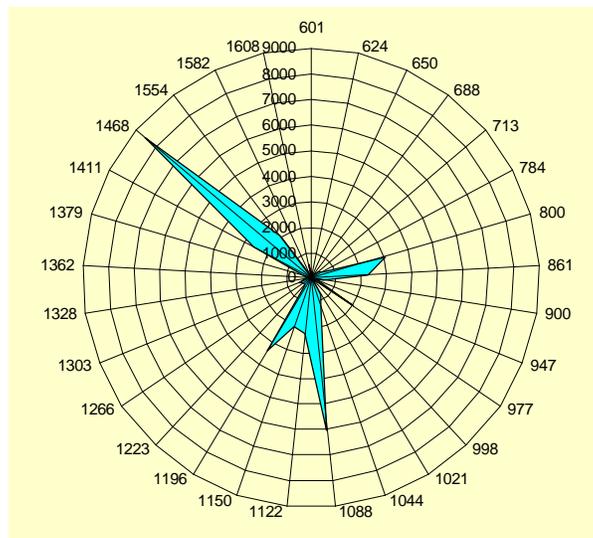
Figure 8- Detector counts vs Kovats Induce for tea

## Principal Component Analysis

The individual chemicals present constitute a reduced set of numbers defining the principal chemical components of the tea aroma. These can be used to perform principal component analysis against holistic variables like taste by recognizing the pattern of their concentrations. One example is a plot of the concentration of principal components vs their Kovats indices using a color wheel (Excel doughnut plot) shown in Figure 9. Alternately a polar display (Excel radar plot) of concentration vs Kovat indices shown in Figure 10 can be used. These patterns can readily be analyzed by commercially available multi-variant analysis software, e.g. SAS.



*Figure 9- Principal component analysis using color wheel response vs Kovats indices. Indexing begins and ends at top position of wheel.*



*Figure 10- Principal component analysis using radar plot vs Kovats indices. Indexing begins and ends at top position of plot*

## Virtual Chemical Sensors and VaporPrint<sup>®</sup> olfactory images

Another useful tool for monitoring the quality of tea using its aroma chemistry is to define a set of virtual chemical sensors. Each sensor monitors the concentration of a specific chemical within the tea aroma. Although the zNose<sup>®</sup> contains a single physical sensor, system software can create hundreds of virtual chemical sensors based upon retention time windows.

An example based upon tea vapors is shown in Figure 11. Virtual chemical sensors are specific to each chemical and allow the user to set alarms and be notified when these alarm levels are exceeded.

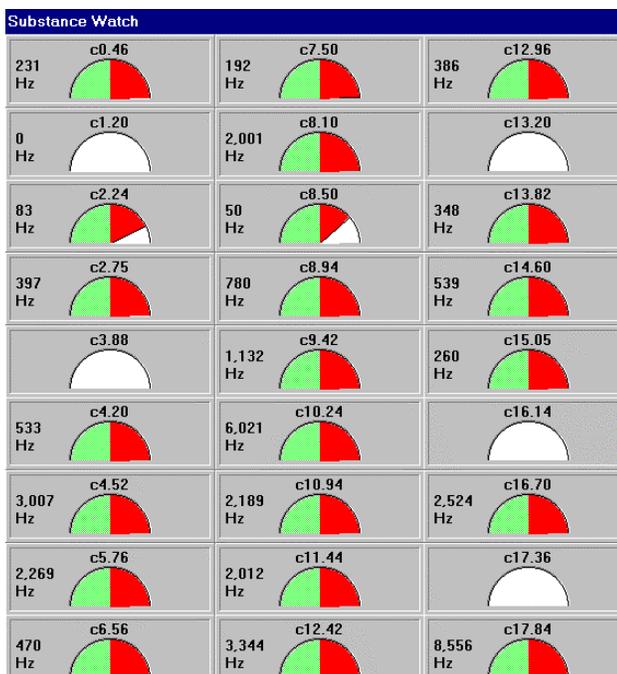


Figure 11- Virtual chemical sensors defined by alarms and calibration standards allow alarms to be set to monitor quality of tea aroma based upon chemical concentrations.

Another approach unique to the zNose<sup>®</sup> is to use visual 2-dimensional olfactory images, called VaporPrints<sup>®</sup>. Using retention time as the angular variable and the SAW detector response as the radial variable the olfactory image for one tea sample is shown in Figure 12. This approach provides a high resolution visual image which displays the aroma chemistry in its entirety. Skilled observers can frequently judge quality by observing only this image.

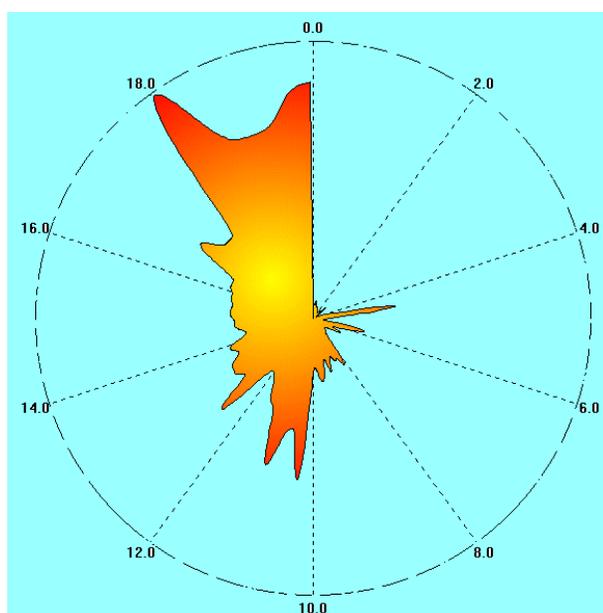
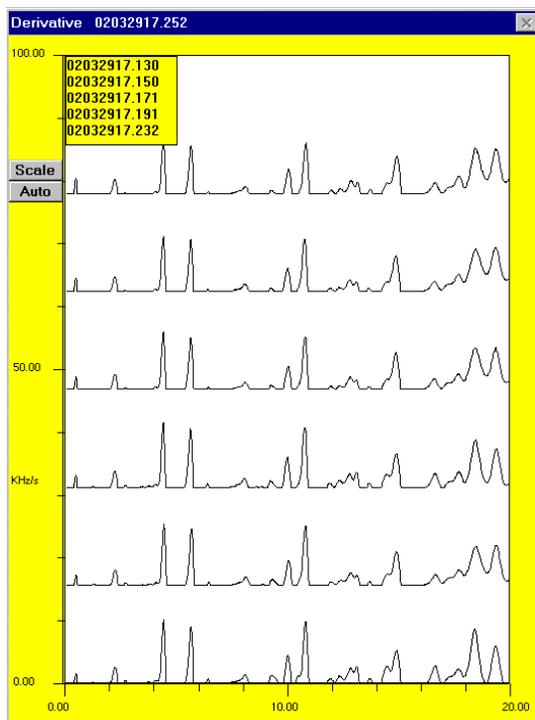


Figure 12- High resolution 2-dimensional olfactory images formed from SAW sensor signal and retention time.

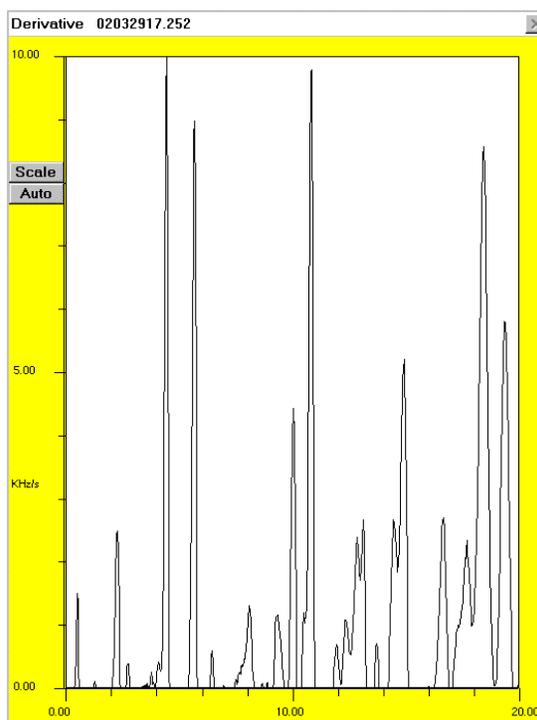
## Headspace Chemistry of Dry and Liquid Tea (Sample AR-1)

The concentration of volatile organics from dry tea is proportional to the vapor pressure of the individual analytes and the surface area of the tea particles. The headspace concentration of volatile organics from water containing dissolved tea is determined by the vapor pressure of the analytes and their solubility in water. Liquid Tea was produced using AR-1 sample. The tea was prepared using 2 grams of tea grounds in 20 mL water measured into a 40 mL vial and equilibrated at 40°C for 5 minutes. Shown in Figure 13 are offset replicate chromatograms showing the chemistry of the headspace vapors from liquid tea. Reproducibility or precision of the measurement was excellent as can be seen in the identical nature of the chromatograms.

Shown in Figure 14 is a more detailed view of the liquid tea aroma and its chemistry. The number of compounds resolved was the same as in the dry tea, however their relative concentrations were altered because each analyte exhibited a different Henry's constant (= Vapor pressure/solubility).



*Figure 13- Replicate chromatograms showing reproducibility and repeatability of liquid tea test method.*



*Figure 14- Liquid tea (40°C) chromatogram using 5ps10a1b method, 30°C detector, and a 15 mL vapor sample. (2g/20 mL water in 40 mL vial)*

A comparison between dry and liquid tea chromatograms is shown in Figure 15. In this figure the top chromatogram (dry tea) is offset for easier viewing and it is clear that the solubility of certain analytes (RT=8, 8.6, 10, 12 & 18), results in their being considerably lower in concentration than that of dry tea. However, in other cases (RT=2.2, 4.5, 5.5, 10.5, 14.8 & 18.2), the opposite is true and being soluble in water allows extraction of the compound from the tea matrix and increases its concentration in the headspace vapors.

The headspace vapors in dry and liquid tea samples can also be overlaid for comparison. In Figure 16 the dry tea chromatogram (RED) is overlaid with liquid (BLACK). In this case bipolar nature of the chromatogram is also displayed (clipping=Off) so every small inflection of the derivative response can be viewed. It is clear in either case that the compounds are the same and that they just have different concentrations. Hence, the aroma of liquid tea is different from dry tea, yet both display the same chemicals.

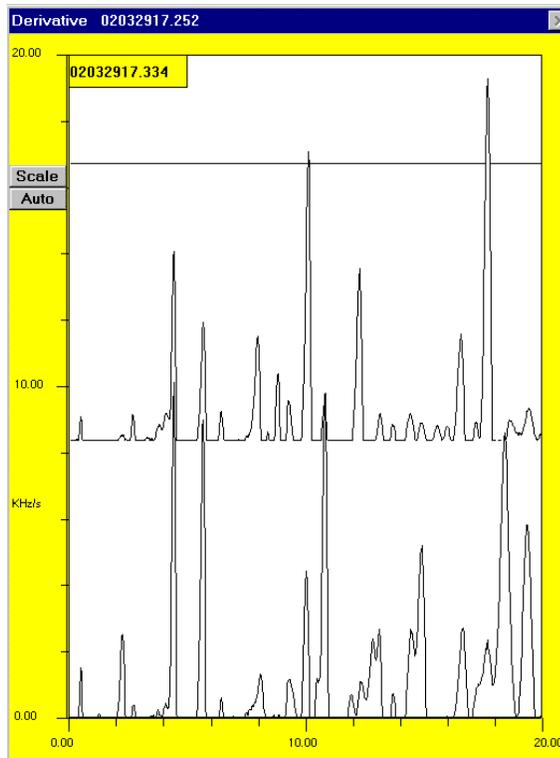


Figure 15- Comparing liquid (bottom) vs dry (top) tea samples

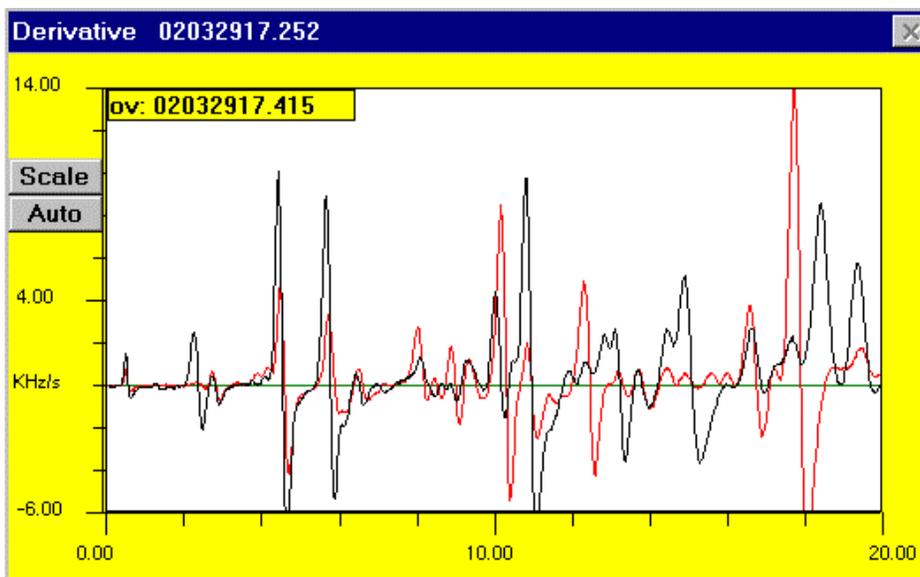


Figure 16- Liquid tea response derivative vs dry overlay in RED.

## Dry Tea Aroma vs Amount of Tea Placed in Vial (AR-1)

Headspace vapors were tested using different amounts of dry tea in vials held at 40°C. The concentration of individual analytes as a function of tea placed in the vial is shown in Figure 17. Concentration increased with tea mass for all analytes detected but not always in direct linear proportion to the amount of tea in the vial. The concentration of a select number of analytes is plotted in Figure 18. In general the more volatile compounds displayed a linear relationship while the less volatile compounds exhibited a saturation effect.

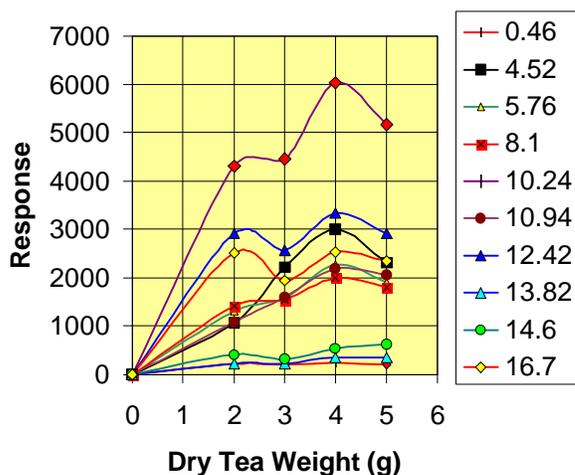


Figure 18- Detector counts vs the amount of dry tea in vial at 40°C.

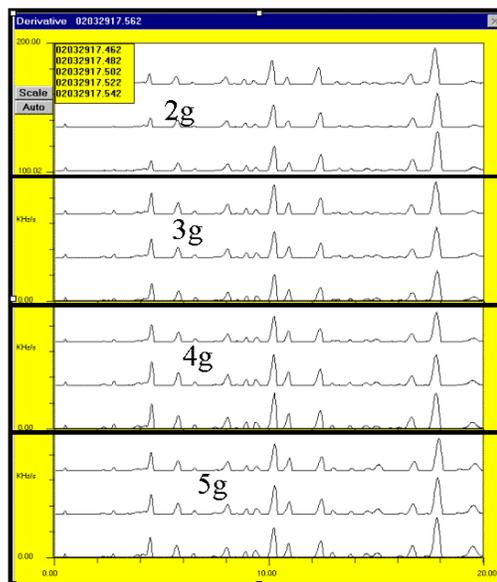


Figure 17- Chromatograms for differing amounts of dry tea in vials. Each triplicate measurement demonstrates precision of the method and provides an average concentration for all analytes detected.

## Evaluation of 20 Different Dry Tea Samples

Five (5) grams of tea were placed in 40 mL septa sealed vials and thermostated in a 2-zone vial heater at 40°C bottom and 50°C top zone. Using a 5ps2a1b method, 20°C detector and 30 second sample time, 15 mL of headspace vapors were preconcentrated and analyzed with the zNose<sup>®</sup>. Each sample was analyzed in triplicate and the Kovats indices evaluated by also including an analysis of n-alkane standard vapor as shown in Figure 19.

The response from the 3<sup>rd</sup> analysis run in each triplicate measurement series are compared as offset traces in Figure 20 for samples AR-1 through AR-6. In similar fashion Figures 21-23 show chromatograms for samples AR-7 through AR-20.

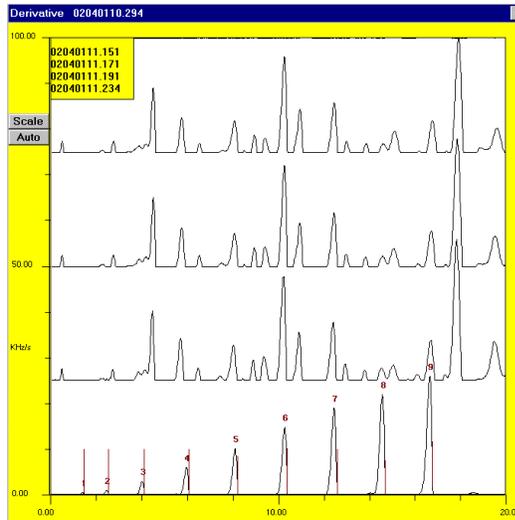


Figure 19- Each sample was measured in triplicate followed by blanks and n-alkane runs to insure quality of measurement.

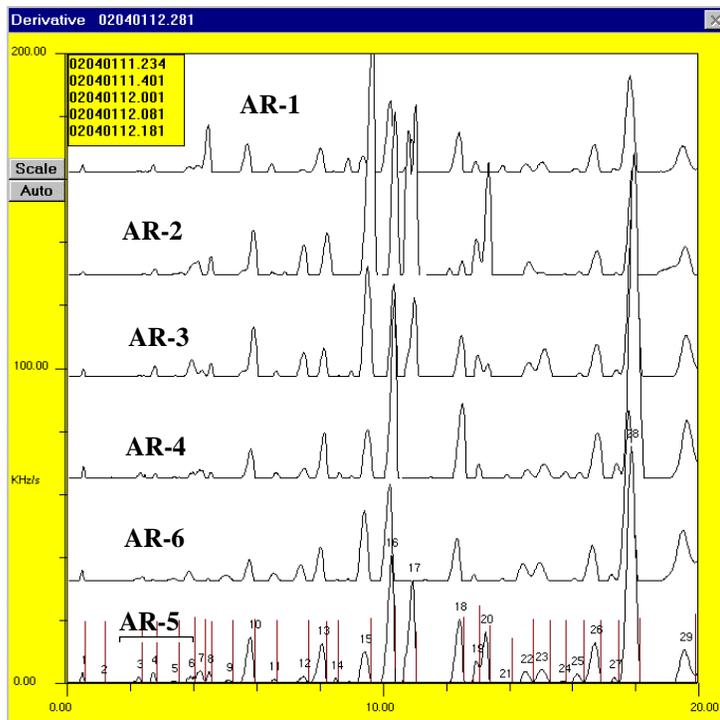


Figure 20- Chromatograms for Samples AR-1 to AR-6. Each analysis represents 3<sup>rd</sup> run of triplicate measurement.

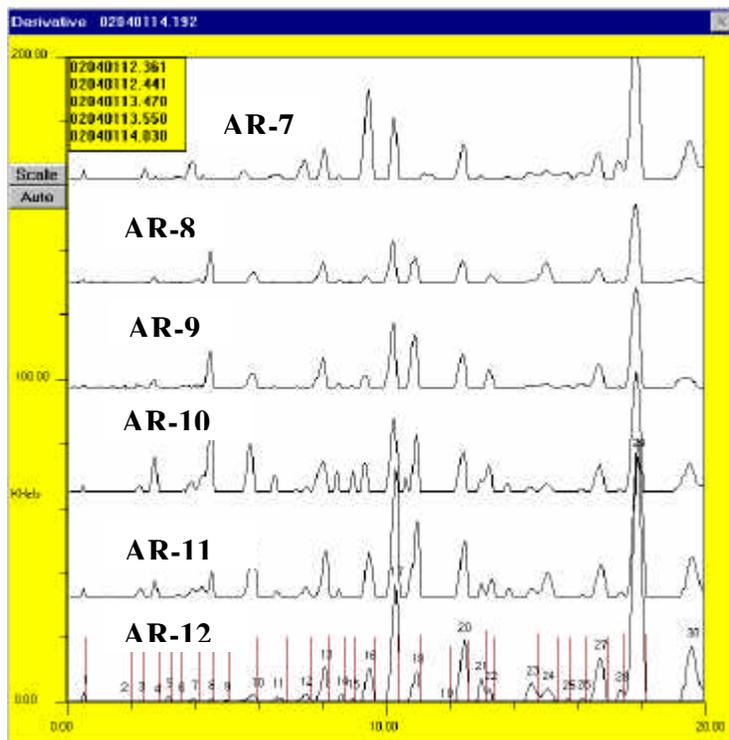


Figure 21- Chromatograms of sample AR-7 to AR-12.

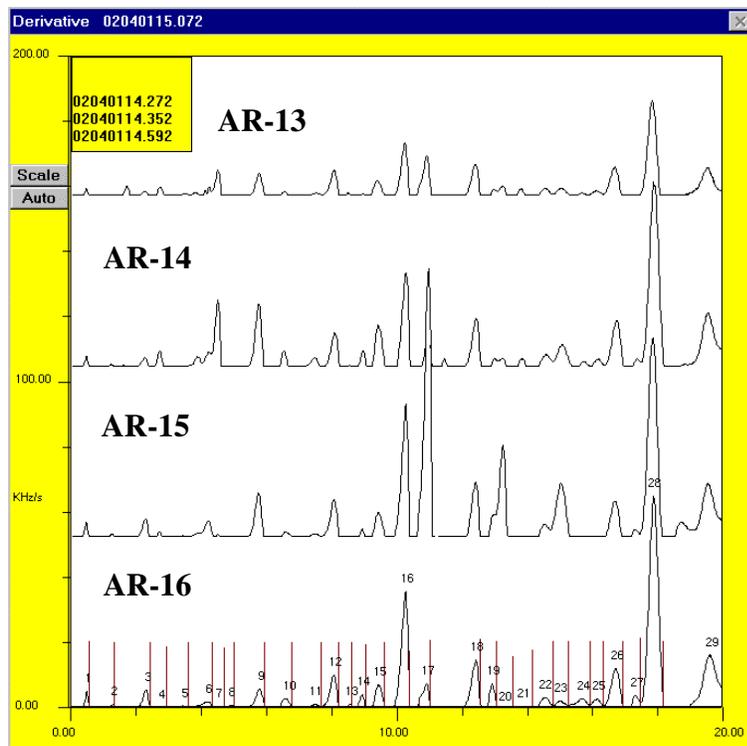


Figure 22- Chromatograms of samples AR-13 to AR-16

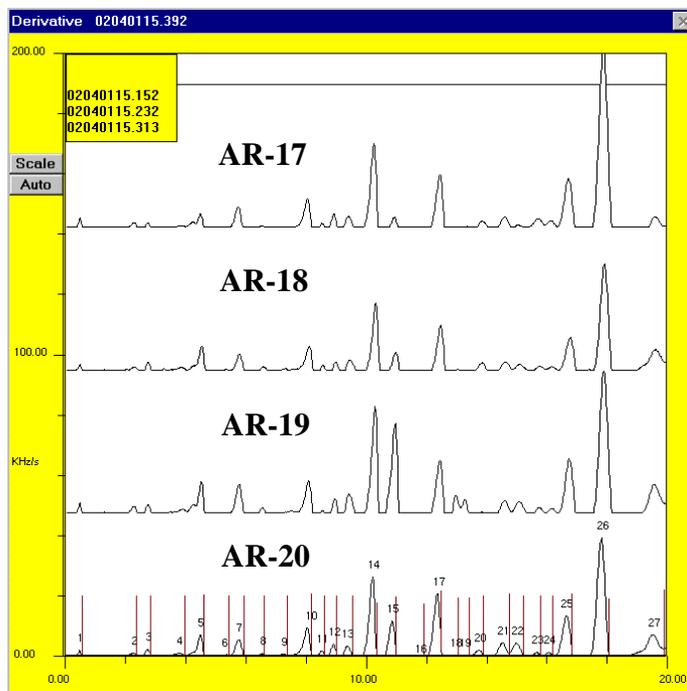


Figure 23- Chromatograms of samples AR-17 to AR-20

After completing testing of all samples the original AR-1 sample was again heated to 40°C and re-tested. The zNose® response with the original result overlaid in RED is shown in Figure 24. Curiously, with the exception of only three compounds the original vapor concentration was exactly the same. Why these three compounds increased in concentration may be the result of heating of the tea matrix over time.

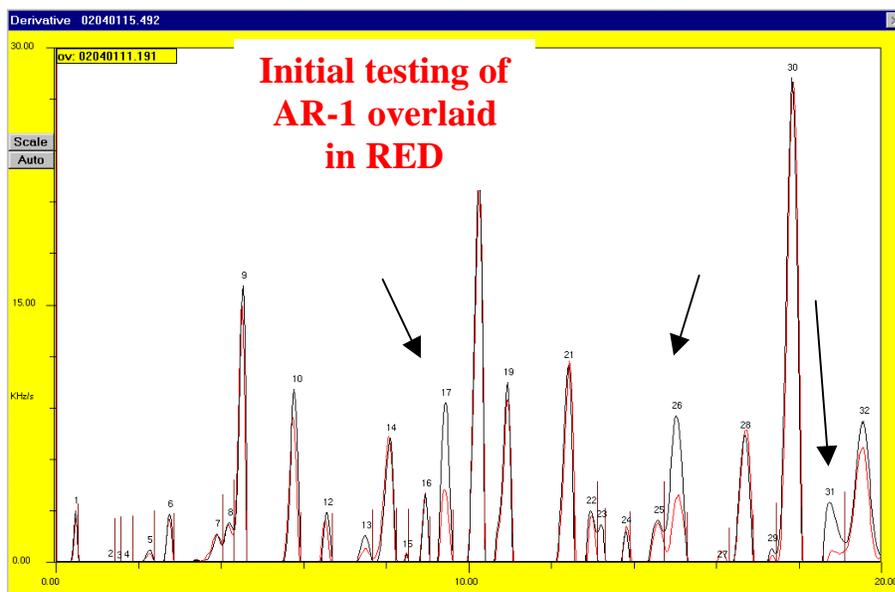
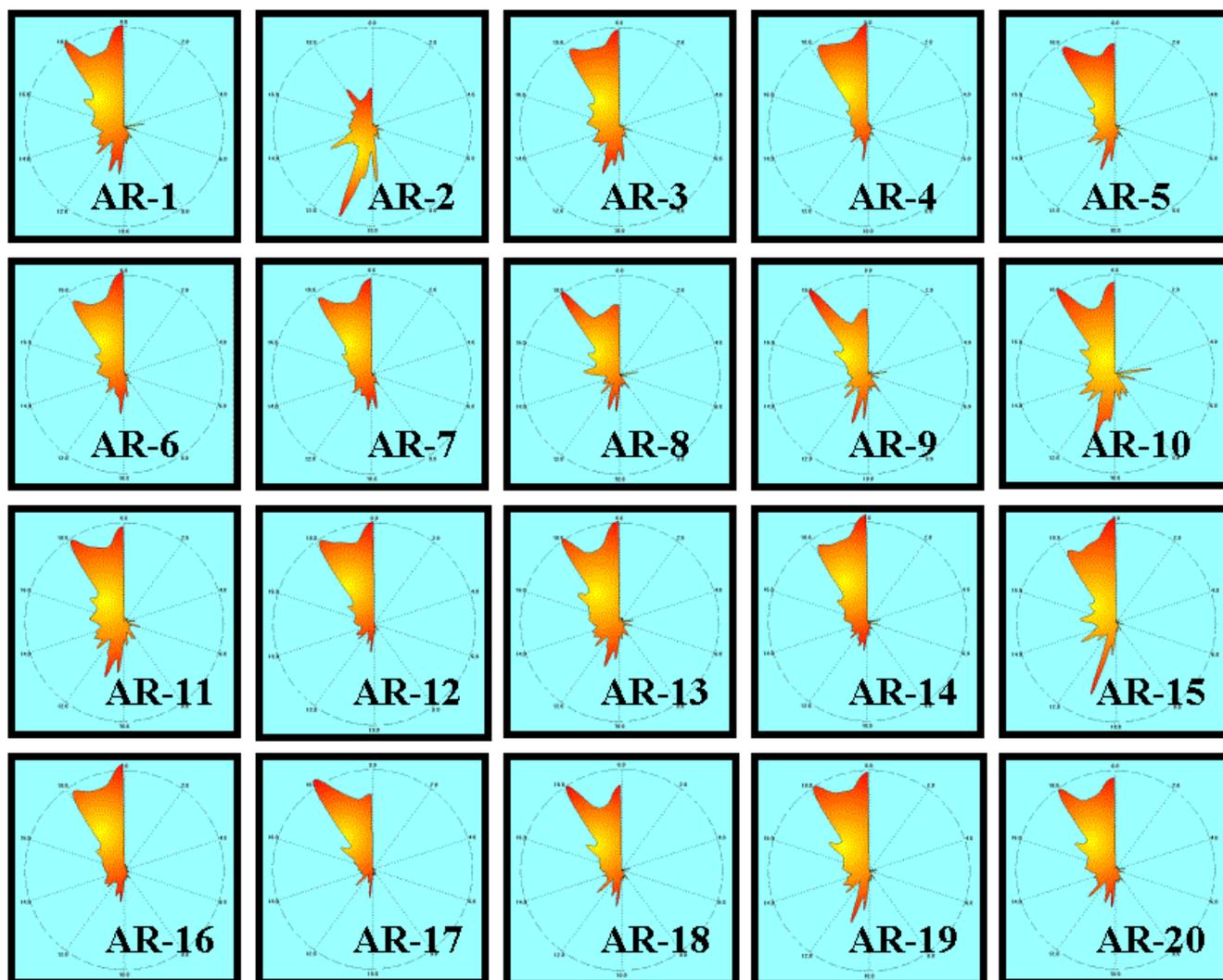


Figure 24- Re-testing and comparing original with a new (current) analysis of sample AR-1.

## Comparison Using VaporPrint<sup>®</sup> Olfactory Images

Whereas chromatographic results show the trees of the forest, olfactory images known as VaporPrints<sup>®</sup> display the forest of trees in its entirety. Olfactory images display aroma concentration with retention time as the angular variable. Because of our acute visual perception skills and abilities in recognizing subtle changes in 2-dimensional figures and patterns, olfactory images provide human operators with a useful tool for comparing the chemistry of fragrances, odors, and aroma. An example is the display of Figure 25 showing a gallery of VaporPrint<sup>®</sup> images for the 20 different tea samples. Clearly sample AR-2 was an exception to the normal pattern. Nevertheless the basic pattern can be seen to undergo many different variations on the basic theme represented in sample AR-1.



*Figure 25- Comparison using olfactory images normalized to highest concentration analyte.*

## Summary and Conclusions

A new instrument, the zNose<sup>®</sup>, is providing chemists and quality control personnel with a fast and accurate tool for measuring the quality of a wide variety of foods and beverages. Based upon ultra-high speed chromatography, the instrument is able to speciate and quantify the concentration of nearly 30 or more individual compounds in seconds. With a zNose<sup>®</sup>, over 300 tea samples can be evaluated in an 8 hour period.

In addition to standard chromatographic methods, high resolution olfactory images, called VaporPrints<sup>®</sup>, allow quality to be assessed based upon the chemical odor chemistry in its entirety. These 2-dimensional images are particularly useful to humans because of their ability to recognize and differentiate visual images. In effect the olfactory response is transformed into a visual response.

An evaluation of 20 blind tea samples was carried out using headspace vapors from dry tea. Water extracted tea was also tested and found to contain the same chemicals but a much altered concentration profile. The testing of dry tea is simpler and quicker since no extraction process is required. However, some compounds are enhanced by extraction and for these the liquid extraction method may be a more appropriate approach.

Identification of compounds was performed indirectly. The individual chemicals in the tea samples were identified by their retention times compared to that of n-alkanes (Kovats Indices). Recommended future work would be to evaluate the Kovats indices for chemicals known to be present in tea. These findings would then be compared to the Kovats indices of the compounds observed in the tea samples.