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Carbon and Nitrogen Analysis of Soil Samples

--Manuscript Draft--

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Science Education Title Carbon and Nitrogen Analysis of Soil Samples

Overview: Elemental Analysis is a method used to determine elemental composition of a material. In soils, we are particularly interested in the amount of nitrogen and carbon in the samples. The Flash EA 1112 instrument oxidizes the sample with a catalyst through combustion in a high temperature chamber. The products of combustion are then reduced to N_2 and CO_2 and detected with a thermal conductivity detector.

Unlike other methods for total nitrogen determination (Kjeldahl method) and total carbon determination (Walkley-Black, Heanes or Leco methods), the Flash EA 1112 does not use toxic chemicals and is therefore a much safer method to use.

Principles: The soil samples are placed in a tin disc and dropped into the oxidation reactor via an auto sampler where it is burned in an oxygen environment at 900°C in the presence of an oxidation catalyst. The carbon in the sample is converted to carbon dioxide and the nitrogen is converted to nitrogen gas and some nitrogen oxides.

$C + O_2 \rightarrow CO_2$
$4N + x\ O_2 \rightarrow N_2 + 2\ NO_x$

Helium gas carries these products into a second reaction tube filled with copper that reduces the nitrogen oxides to nitrogen gas and removes excess oxygen. This is completed at 680°C.

$NO_x \rightarrow N_2 + CuO$
$O_2 + Cu \rightarrow CuO$

The gas stream then flows through a filter filled with magnesium perchlorate to remove any water vapor before the stream reaches the gas chromatograph column, which is at room temperature.

The N_2 will exit the GC column first at about 110 s and then the CO_2 will exit at about 190 s. Using a standard curve created using aspartic acid, the %N and %C in the soil sample can be determined.

Procedure

1. Preparation of Soil Samples

    1.1. Dry soil samples at 60°C for 48 h.

Comment [AK1]: The title somewhat concerns me, because only carbon and nitrogen are analyzed. "Elemental Analysis" can involve spectroscopic methods like XRF spectroscopy, which can give you information about a broad spectrum of elements. It's not clear if the instrumentation used in this experiment is limited to carbon and nitrogen analysis, but if it is, I recommend that the title be made more specific and allude to carbon and nitrogen only rather than "elemental analysis".

Comment [maw2]: The instrument we use can do HCNOS. Since we mainly use it for soil and/or biomass testing, we really are only interested in C and N. It is common to call this "Elemental Analysis" to distinguish it from measuring, say, nitrate, but I can change it to Carbon and Nitrogen with no hesitation.

1.2. Pass the soil through a 2x2 mm sieve.

1.3. Put approximately 5g of the soil into the ball mill grinder and grind for 2 min. It is important to get a homogeneous sample since your sample size will be very small.

1.4. Put milled soil into a small container and store in a desiccator until ready to use.

2. Setting up the instrument parameters

2.1 Turn on the Flash EA 1112 instrument in the back by flipping the switch up.

2.2 Turn on the computer.

2.3 Double click on the Eager 300 icon to start the software program that runs the instrument.

2.4 Double click on the NC Soils icon to open the method that runs the instrument setup for soils.

2.5 Heat up the instrument by opening up the "Edit Elemental Analyzer Parameters" and clicking on the "Send" button. The parameters should be as follows (See Figures 1 – 3):

a. Temperatures: Left = 900°C, Right = 680°C, Oven 50°C

b. Gas flow: Carrier = 130 ml/min, Oxygen = 250 ml/min, Reference = 100 ml/min

c. Cycle Runtime = 360 s

d. Sampling Delay = 12 s

e. Oxygen Injection End = 5 s

f. Detector = Filament On

2.6 Create a sample table by clicking on "Edit Sample Table" and then "Fill Sample Table." Change the filename to today's date. Input the number of samples you plan to run, including the standards and blanks. Then click on "Replace" to replace the last sample table that was created with your new sample table.

3. Creating a standard curve

   3.1 Using forceps, remove one tin disc from the pack and mold it into a cup shape using the special apparatus. Avoid touching the tin disc with your fingers to avoid transferring oils from your fingertips. (See Figures 4 – 5)

   3.2 Using forceps, place the tin disc on the microbalance and zero the balance.

   3.3 Using forceps, remove the tin disc from the microbalance and using a microspatula, place approximately 1 mg of Aspartic Acid standard into the tin disc.

   3.4 Weigh the tin disc with the Aspartic Acid standard on the microbalance. Enter this weight into the data table in the Eager 300 software on the computer.

   3.5 Seal up the tin disc with the forceps so that none of the aspartic acid standard will spill out of it. Place the tin package into the autosampler. (See Figure 6)

   3.6 Repeat steps 3.1 – 3.5, putting approximately 5 mg of Aspartic Acid standard into the tin disc.

   3.7 Repeat steps 3.1 – 3.5, putting approximately 7.5 mg of Aspartic Acid standard into the tin disc.

   3.8 Repeat steps 3.1 – 3.5, putting approximately 10 mg of Aspartic Acid standard into the tin disc.

4. Loading the autosampler with soil samples

   4.1 Using forceps, remove one tin disc from the pack and mold it into a cup shape using the special apparatus. You should not touch the tin with your fingers to avoid transferring oils from your fingertips.

   4.2 Using forceps, place the tin disc on the microbalance and zero the balance.

   4.3 Remove the tin disc from the microbalance and place approximately 50 mg of the homogenized soil into the tin disc using a microspatula.

   4.4 Weigh the tin disc with soil sample on the microbalance. Enter this weight into the data table in the Eager 300 software on the computer.

   4.5 Seal up the tin disc using the forceps so that the soil is contained. Transfer the tin package to the autosampler tray.

   4.6 Repeat steps 4.1 – 4.5 for all of your samples. It is recommended to run triplicate trials of each sample. A triplicate experiment is considered a good rule of thumb to rule out experimental errors.

5. Running the samples

5.1 When the appropriate temperatures have been reached on the instrument, the green "Temperature Ready" light will turn on. At the bottom of the screen on the computer, it will also say "Ready for Analysis."

5.2 Before starting your sample run, click on "File" and "Save Method" to save the data you just input. It is recommended that you save the method with your last name and the date.

5.3 To begin the run, click on the green arrow and push "Start Now."

5.4 It will take approximately 6 min per sample to run.

5.5 After the run is complete, you can see the results by clicking on "Recalculation" then "Summarize Results."

Representative Results A chromatogram for each sample is produced showing the amount of nitrogen and carbon in the sample (Figure 7).

The area of the curve of each of the peaks from the sample chromatogram are compared to the standard curves (Figures 8 and 9), and the amount of nitrogen and carbon in the sample is calculated. Based on the weight of the original sample, the %N and %C is calculated (Figure 10).

Applications The Carbon to Nitrogen (C:N) ratio in soil is a ratio of the mass of carbon to the mass of nitrogen in the soil sample. The C:N ratio of soil and anything put on the soil (like crop residue cover) can affect crop residue decomposition and nutrient cycling. Soil microorganisms have a C:N ratio of approximately 8:1. To maintain this ratio, they must acquire their carbon and nitrogen from the environment. However, since some of the carbon the microorganisms acquire must be used as a source of energy in addition to what it needs for body maintenance, the microorganisms require a C:N ratio of approximately 24:1. If leaf litter or soil cover with a C:N ratio of HIGHER than 24:1 is placed on the soil (example corn stover with a C:N ratio of 57:1), the microorganisms will be required to use nitrogen from the soil in order to decompose the litter material. This results in a nitrogen deficit in the soil. If leaf litter or soil cover with a C:N ratio of LOWER than 24:1 is placed on the soil (example alfalfa hay with a C:N ratio of 13:1), there will be some nitrogen remaining after the decomposition of the litter material. The Elemental Analyzer method can not only be used to determine the C:N ratio of the soil samples, but can also be used to determine the C:N ratio in plant materials, such as tree leaves and crop residue. This information is important for farmers in order to help them decide what type of crop cover to use. The C:N ratio of the crop residue added to cover the soil influences how quickly the residue will decompose. This has implications for whether or not the soil is protected for the desired length of time. In addition the C:N ratio of the crop residue can lead to a release of nutrients to the soil (if higher than 24:1) or immobilization of soil nitrogen (if lower than 24:1).

Comment [AK3]: The authors will need to agree to download and use screen recording software for the software demonstration portions of this submission.

Comment [maw4]: I'm ok with this. Do we need to get some kind of permission from the company?

Comment [DM5]: Aaron, I'm working with the authors to address this issue. We'll have it ironed out prior to scripting. I'll update you at our next meeting.

Comment [AK6]: I think the practical use of this information is a bit understated here. It seems like elemental analysis is important to help determine the appropriate amount of leaf litter or soil cover to ensure healthy soil with an optimal ratio of C:N.

Comment [AK7]: So this last sentence includes the kind of information we typically want for applications content. Can the authors elaborate on how information gained from elemental analysis of tree leaves and crop residue is of significance to horticulturalists, arborists, and agriculturalists.

Comment [DM8]: This yellow-highlighted section is what they added in response to your comments above Aaron.

Legend

Figure 1: Flash EA 1112 instrument Screen 1 (step 2.5.a)

Figure 2: Flash EA 1112 instrument Screen 2 (step 2.5.b-e)

Figure 3: Flash EA 1112 instrument Screen 3 (step 2.5.f)

Figure 4: Removing a tin disc with forceps (step 3.1)

Figure 5: The tin disc molded into a cup shape using the apparatus (step 3.1)

Figure 6: The tin package being placed into the autosampler (step 3.5)

Figure 7: Chromatogram showing nitrogen and carbon levels

Figure 8: The area of the curves and peaks from the sample chromatogram compare to standard curves 1

Figure 9: The area of the curves and peaks from the sample chromatogram compare to standard curves 2

Figure 10: Calculation of %N and %C, based on the weight of the original sample.

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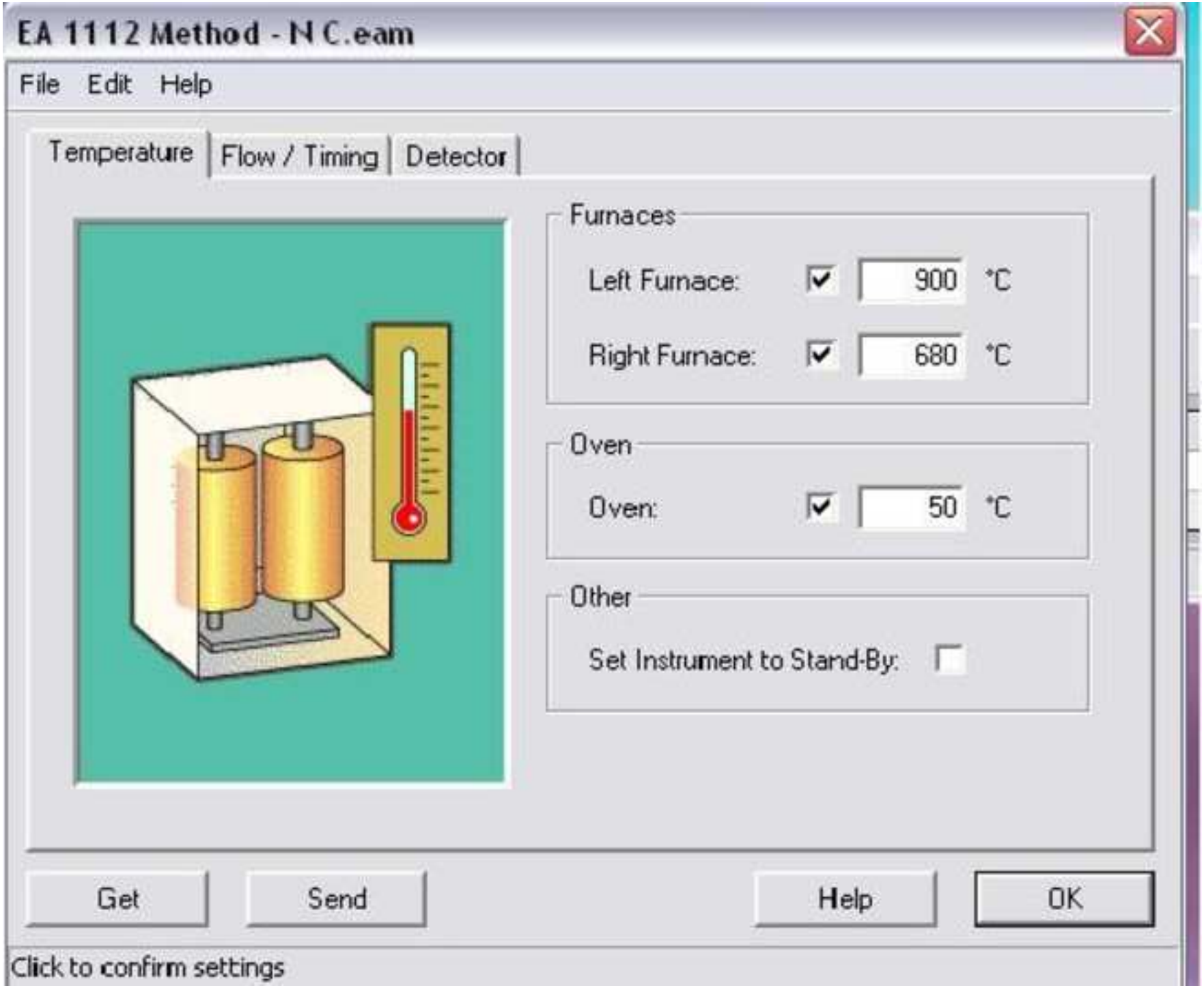

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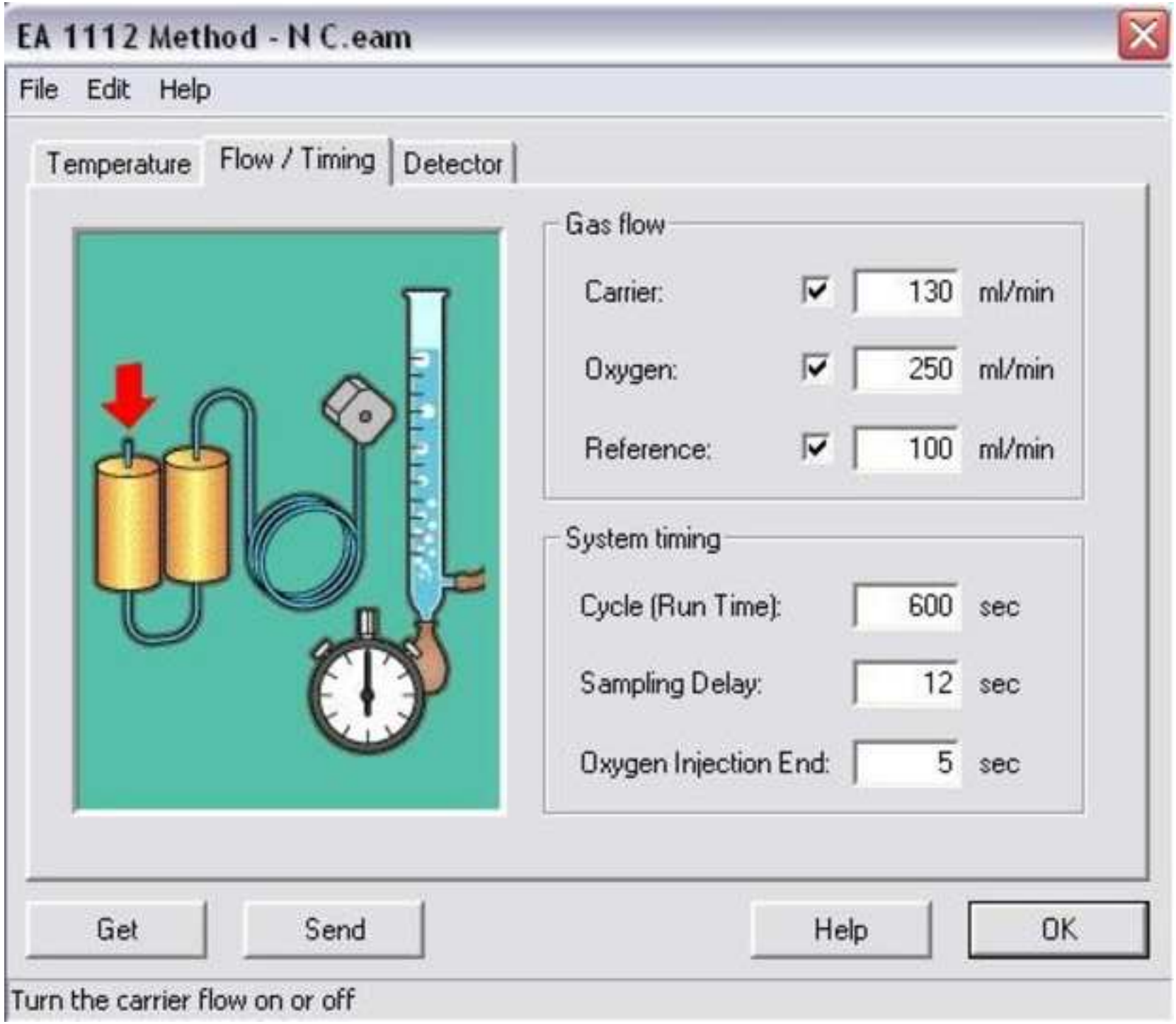

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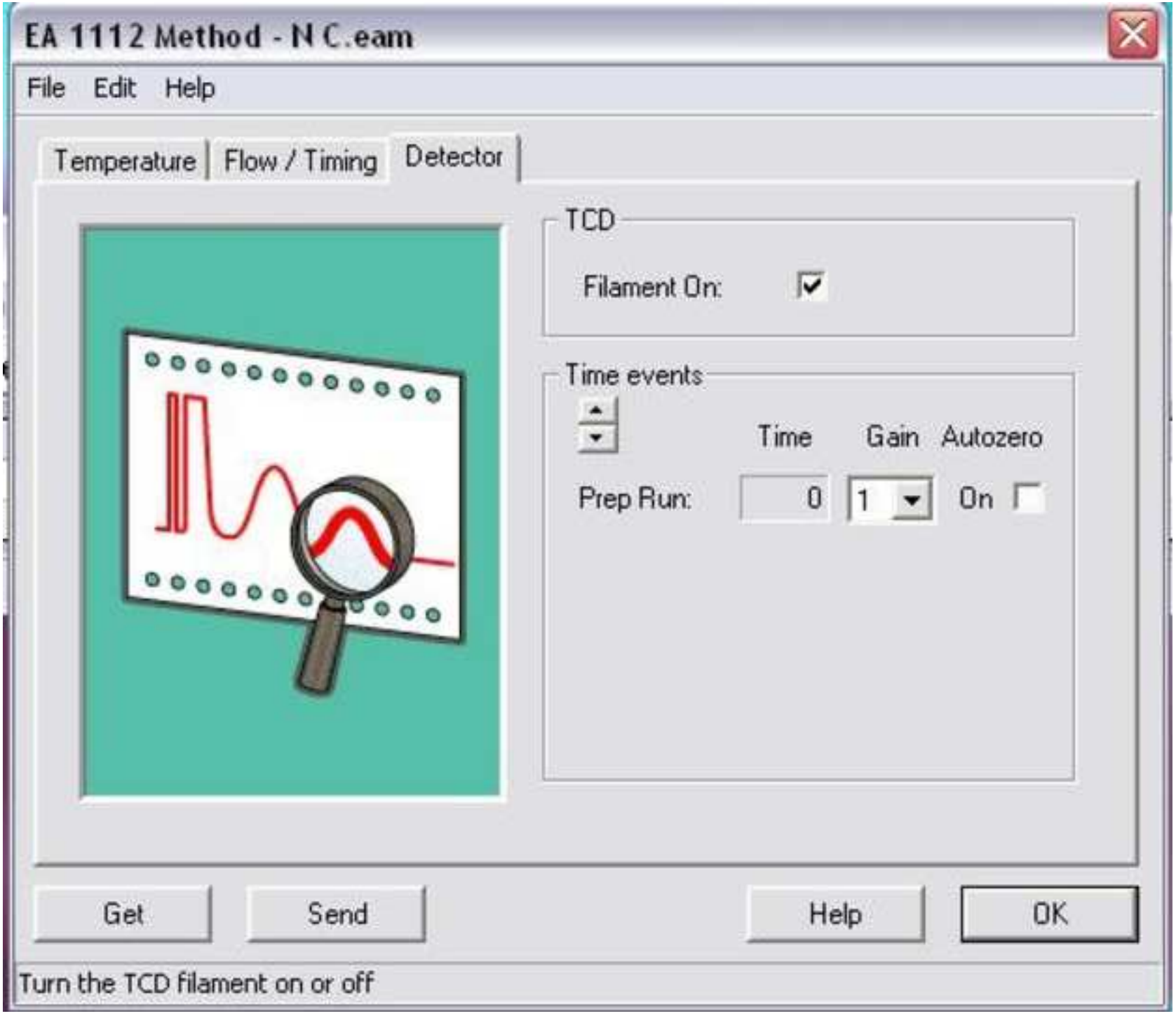

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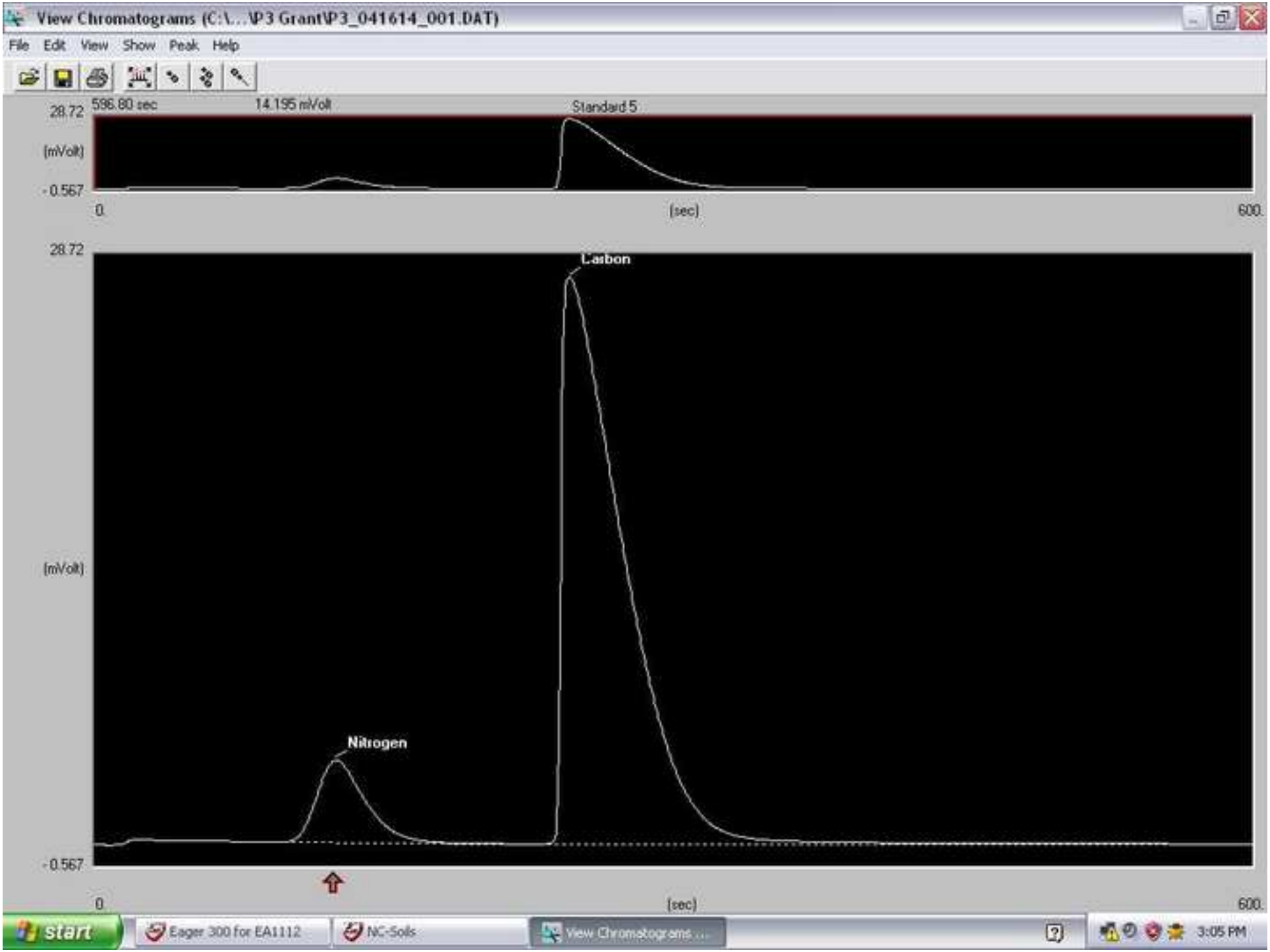

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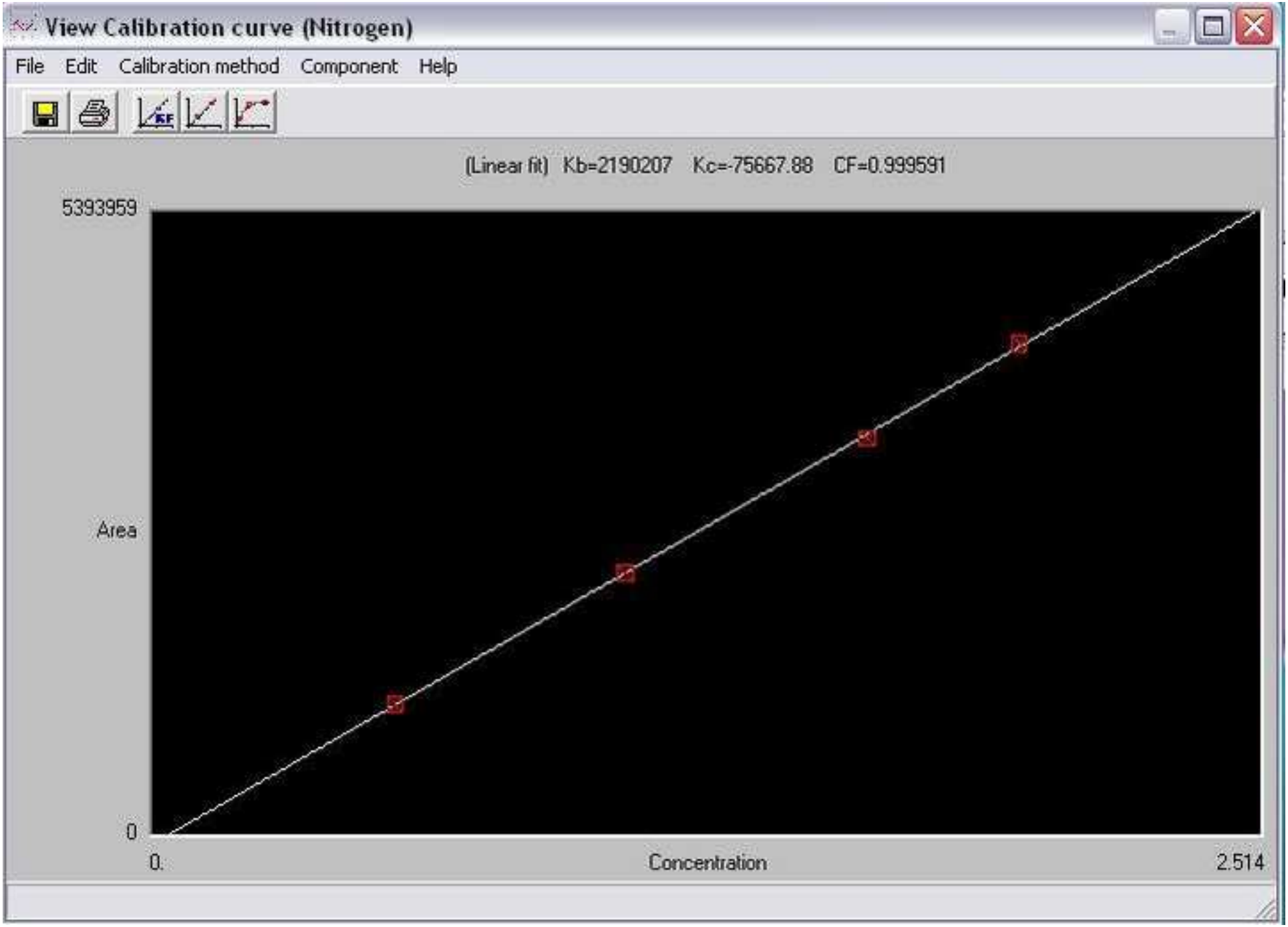

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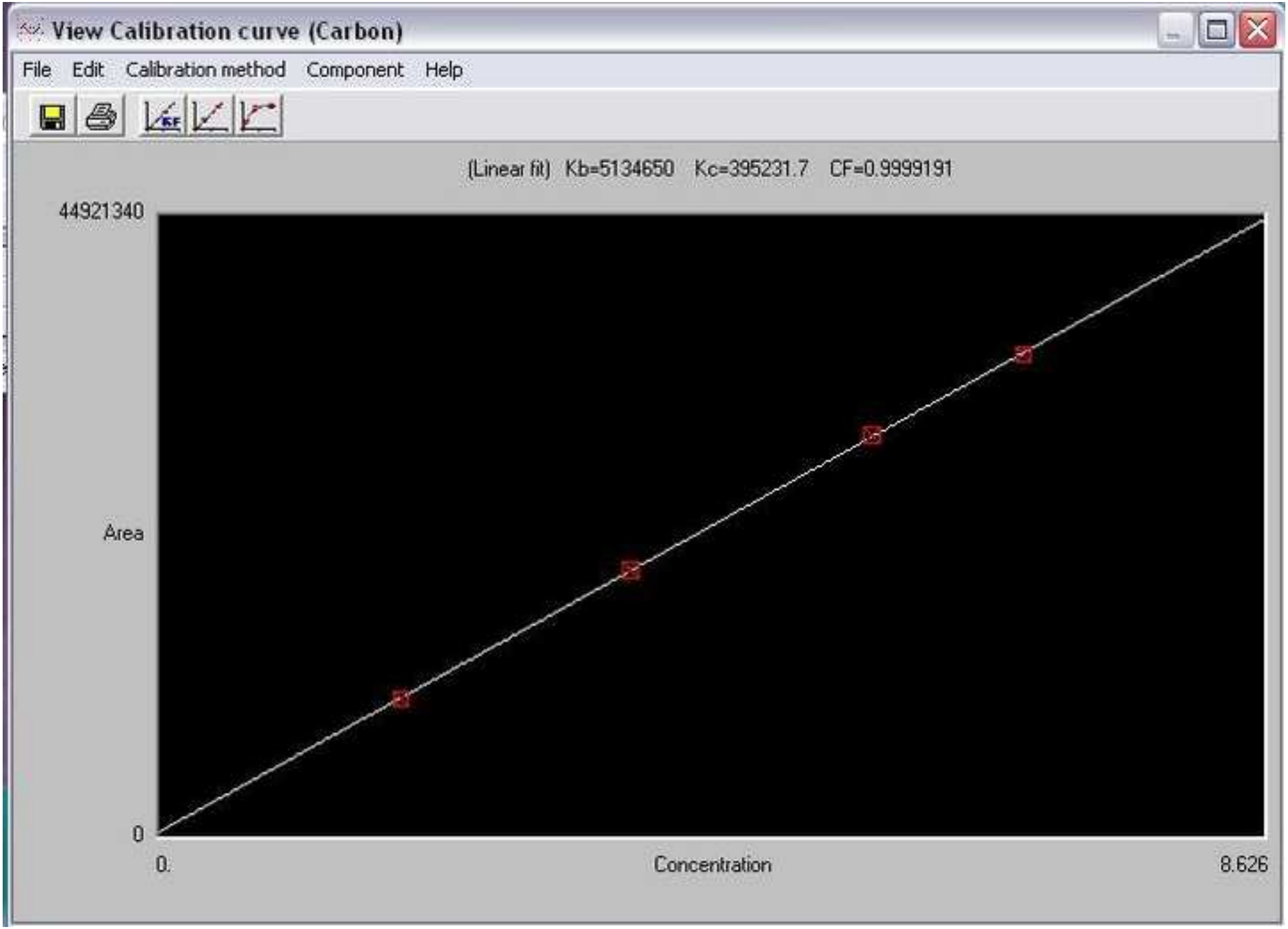

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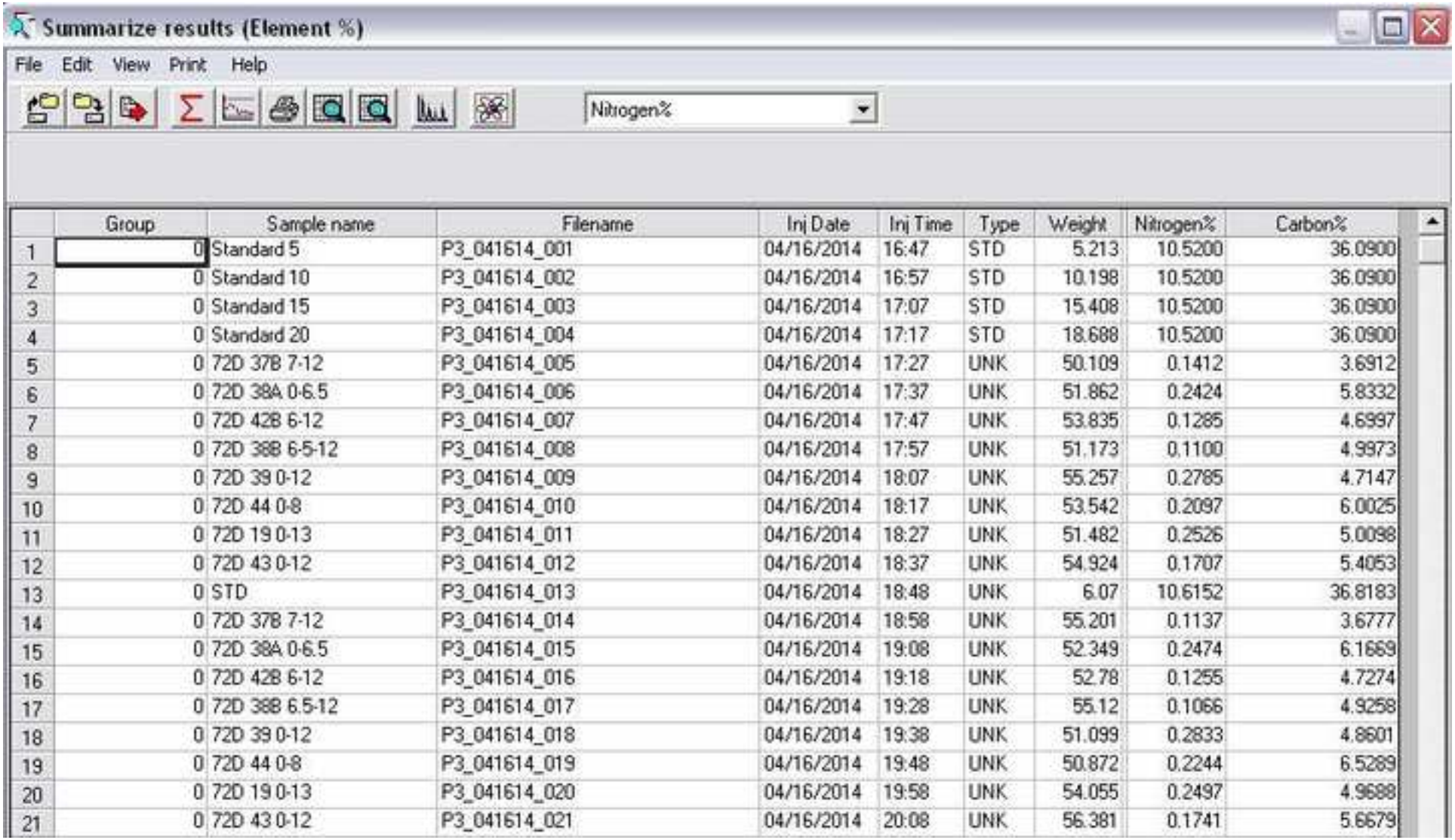

	Group	Sample name	Filename	Inj Date	Inj Time	Type	Weight	Nitrogen%	Carbon%
1	0	Standard 5	P3_041614_001	04/16/2014	16:47	STD	5.213	10.5200	36.0900
2	0	Standard 10	P3_041614_002	04/16/2014	16:57	STD	10.198	10.5200	36.0900
3	0	Standard 15	P3_041614_003	04/16/2014	17:07	STD	15.408	10.5200	36.0900
4	0	Standard 20	P3_041614_004	04/16/2014	17:17	STD	18.688	10.5200	36.0900
5	0	72D 37B 7-12	P3_041614_005	04/16/2014	17:27	UNK	50.109	0.1412	3.6912
6	0	72D 38A 0-6.5	P3_041614_006	04/16/2014	17:37	UNK	51.862	0.2424	5.8332
7	0	72D 42B 6-12	P3_041614_007	04/16/2014	17:47	UNK	53.835	0.1285	4.6997
8	0	72D 38B 6-5-12	P3_041614_008	04/16/2014	17:57	UNK	51.173	0.1100	4.9973
9	0	72D 39 0-12	P3_041614_009	04/16/2014	18:07	UNK	55.257	0.2785	4.7147
10	0	72D 44 0-8	P3_041614_010	04/16/2014	18:17	UNK	53.542	0.2097	6.0025
11	0	72D 19 0-13	P3_041614_011	04/16/2014	18:27	UNK	51.482	0.2526	5.0098
12	0	72D 43 0-12	P3_041614_012	04/16/2014	18:37	UNK	54.924	0.1707	5.4053
13	0	STD	P3_041614_013	04/16/2014	18:48	UNK	6.07	10.6152	36.8183
14	0	72D 37B 7-12	P3_041614_014	04/16/2014	18:58	UNK	55.201	0.1137	3.6777
15	0	72D 38A 0-6.5	P3_041614_015	04/16/2014	19:08	UNK	52.349	0.2474	6.1669
16	0	72D 42B 6-12	P3_041614_016	04/16/2014	19:18	UNK	52.78	0.1255	4.7274
17	0	72D 38B 6.5-12	P3_041614_017	04/16/2014	19:28	UNK	55.12	0.1066	4.9258
18	0	72D 39 0-12	P3_041614_018	04/16/2014	19:38	UNK	51.099	0.2833	4.8601
19	0	72D 44 0-8	P3_041614_019	04/16/2014	19:48	UNK	50.872	0.2244	6.5289
20	0	72D 19 0-13	P3_041614_020	04/16/2014	19:58	UNK	54.055	0.2497	4.9688
21	0	72D 43 0-12	P3_041614_021	04/16/2014	20:08	UNK	56.381	0.1741	5.6679