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Date: December 31, 1999
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1 SCOPE OF APPLICATIONS

1.1 This method describes the extraction and determination of phenolic compounds in the sidestream (SS) tobacco smoke by reversed phase high performance liquid chromatography (HPLC).

1.2 Applicable to the trapping and quantitation of phenolic compounds in sidestream tobacco smoke on the sidestream glass fibre filter disc (pad) and impinger only.

2 NORMATIVE REFERENCES

2.1 American Society for Testing and Materials (ASTM) D 1193-77 – Standard Specification for Reagent Water, Version 1977.

2.2 Health Canada Test Method: T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

4.1 10 equidistant ports of a standard 20 port linear smoking machine are reconfigured with the BAT (British American Tobacco) fishtail chambers and flow-controlled vacuum pumps.

4.2 Cigarettes or other tobacco products are smoked beneath the fishtail chambers and the smoke is swept up the chimney at the rate of 3 L/minute.

4.3 The total particulate matter (TPM) from the sidestream smoke is collected on a pad at the top of the chimney. The filtered puff is then bubbled through an impinger containing 100 mL of 1 % acetic acid.

4.4 After smoking two cigarettes*, the sidestream pad is placed in a glass-stoppered Erlenmeyer flask that contains the impinger solution and 2 X 20 mL rinses of the BAT fishtail chamber and is extracted by wrist-action shaking.

*For other tobacco products, select a number such that breakthrough does not occur.

4.5 An aliquot of the TPM extract is then syringe filtered and subjected to reversed-phase gradient high performance liquid chromatography (HPLC).

4.6 Phenols are monitored using selective fluorescence detection and quantified by comparison to an external standard calibration.

4.7 The sample generation and analysis should be completed in one day.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

5.1 Equipment needed to perform conditioning as specified in T-115.
5.2 Equipment needed to perform marking for butt length as specified in T-115.
5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
5.4 Glass fibre filter holders.
5.5 Glass fibre filter discs (pads), 44 mm in diameter, with no more than 5 % acrylic type binder.
5.6 Analytical Balance capable of measuring to at least four decimal places.
5.7 Wrist Action Shaker.
5.8 Vacuum Pumps.
5.9 Flow meters.
5.10 Fishtail Chambers - BAT (10).
5.11 Glass Impingers with frits and cooling jackets - 10 X 10", capacity 250 mL.
5.12 Cooling Bath.
5.13 250 mL Erlenmeyer flasks with ground glass stoppers.
5.14 Volumetric flasks 10 mL, 25 mL and 50 mL, Actinic Red.
5.15 Glass Micropipettes - assorted volumes (100, 150, 300, 400, 500, 800, 1000, and 2000 µL).
5.16 Glass Transfer Pipettes - 1, 2, 5, 6, 7, 8, and 20 mL.
5.17 Glass Graduated Measuring Cylinders 25 mL and 50 mL.
5.18 Erlenmeyer flasks with ground glass joints 50 mL, Actinic Red.
5.19 High Pressure Liquid Chromatography System consisting of:

5.19.1 Solvent Delivery System - tertiary gradient pump.
5.19.2 Refrigerated Autosampler with 20 µL sampling loop.
5.19.3 Programmable Wavelength Spectrofluorometer.
5.19.4 Column Temperature Modifier.
5.19.5 Cooling Bath.
5.19.6 Data collection system.
5.19.7 RP18e 250 mm X 4 mm and 5 µm Column with 10 mm X 4 mm guard column.

6 REAGENT AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade quality.

6.1 Syringe Filters 0.45 µm PVDF.
6.2 Disposable syringes.
6.3 Disposable Glass Pasteur Pipettes.
6.4 Rubber Bulbs.
6.5 Autosampler vials, screw caps and septa.
6.6 Masking Tape.
6.7 Aluminum Foil.
6.8 Methanol - Distilled in Glass (DIG).
6.9 Acetonitrile – DIG.

6.10 Isopropanol (IPA) – DIG.
6.11 Ethanol – DIG.
6.12 Acetic Acid - HPLC grade.
6.13 Octanol > 99 % purity.
6.14 Type I water (meets ASTM D 1193 specification).
6.15 Hydroquinone > 99 % purity.
6.16 Resorcinol > 99 % purity.
6.17 Catechol > 99 % purity.
6.18 Phenol > 99 % purity.
6.19 m-Cresol > 99 % purity.
6.20 p-Cresol > 99 % purity.
6.21 o-Cresol > 99 % purity.

7 PREPARATION OF GLASSWARE

7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

8.1 Prepare 4 L fresh 1 % acetic acid solution in Type I water (40 mL diluted up to 4 L) and test by HPLC for contamination.

9 PREPARATION OF STANDARDS

9.1 Primary (1°) Phenol Standards (See Appendix 1)

9.1.1 Weigh 25 mg of the following phenols (Hydroquinone, Resorcinol, Catechol, Phenol, m-Cresol, p-Cresol and o-Cresol) into individual 25 mL volumetric flasks and make up to the mark with fresh 1 % acetic acid solution.

9.1.2 Concentrations will be in the range of 1.0 mg/mL. Prepare fresh primary phenol stock standards every 10 working days.

9.2 Secondary (2°) Phenol Standard Solutions (See Appendix 1)

9.2.1 Take appropriate volumes of the 1° Phenol Standards and dilute to 10 mL with 1 % acetic acid.

9.2.2 Prepare 2° phenol stock standards fresh with each new primary stock standards.

9.3 Tertiary (3°) Phenol Solution (See Appendix 1)

9.3.1 Take corresponding volumes of each phenol solution and add to a single 50 mL volumetric flask. Dilute up to the mark with 1 % acetic acid.

9.3.2 Prepare phenol working stock solution fresh every five working days.

9.4 Phenol Working Standards

9.4.1 Take appropriate volumes (0.100 to 7.5 mL) of the Tertiary (3°) Stock Phenol solution and dilute to 10 mL with 1 % acetic acid to give calibration standards with appropriate phenol concentrations.

9.4.2 Transfer to autosampler vials.

9.4.3 Phenol calibration standards are prepared fresh every five working days.

9.5 Phenol Spiking Solution for laboratory fortified blanks (LFB)

9.5.1 Add selected volumes of the phenol stock standards together in a 50 mL volumetric flask and make up to the mark with 1 % acetic acid.

9.5.2 Prepare phenol spiking solution fresh every five working days.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product shall be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

12.2.2 Assemble the Phenol sidestream apparatus as per the diagram.

12.2.3 Raise chimney level to highest position (loading position).

12.2.4 The sidestream impinger is loaded with 100 mL of 1 % acetic acid plus two drops of octanol and the impinger jackets are connected to a cooling bath at 10 °C.

12.2.5 Install the weighed sidestream filter pad assembly at the top of the fishtail chamber and place impingers with tops connected to coarse frits onto the rear section of smoke machine.

12.2.6 Tubing from impinger front (internal stem connection) attaches to SS filter pad holder and from impinger rear (bulb) to vacuum pump.

12.2.7 Calibrate the vacuum pumps to draw at the rate of 3 L/minute. Record the flow meter settings.

12.2.8 Attach the mainstream filter pad holders to the corresponding port on the smoking machine.

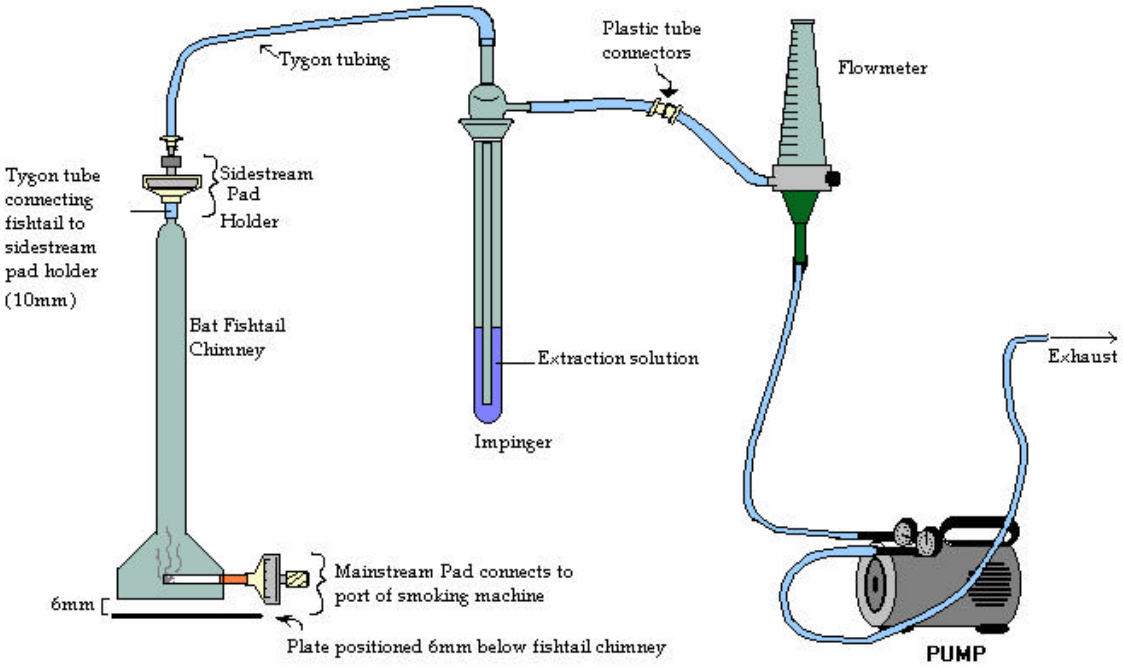


FIGURE 1a : SIDESTREAM APPARATUS

13 SAMPLE GENERATION

Note: It is important to ensure that at least 50 to 100 mg of TPM has been deposited on the sidestream filter pad before proceeding with the analysis.

13.1 Using the vacuum bar, install the first test cigarette to be smoked in position below the fishtail in the 10 calibrated ports. Gently insert the cigarette into the cigarette holder to the marked butt length.

13.2 Turn on the sidestream pumps (3 L/minute) at the beginning of the lighting procedure at t minus 30 seconds.

13.3 Light the cigarette (on the first puff) and initiate the puff count according to the following schedule:

13.3.1 Normal lighting procedure is 15 second warm-up beginning at t-18 seconds followed by five seconds ignition. (Three seconds prior to puff plus the two seconds puff).

13.4 Lower the fishtail assembly over the cigarette to a position of 6 mm above a plate that is beneath the cigarette. Do not allow the cigarette to touch the chimney. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.

13.5 The test cigarettes are smoked to the previously marked standard butt length. Extinguish and remove from beneath the BAT fishtail chamber.

13.6 The pump continues for an additional 30 seconds to sweep any residual smoke up to the sidestream pad.

13.6.1 The smoking process is repeated for the second cigarette.

13.6.2 Smoking is terminated when the final test cigarette has been consumed to the predetermined end mark.

13.6.3 At the end of the smoking process raise the chimney and disassemble the sidestream apparatus.

13.6.4 Re-weigh and record the “after smoking” weights of the sidestream filter holders.

14 SAMPLE ANALYSIS

14.1 Extraction of filter pads

14.1.1 One run consists of 10 samples (pads). Process 10 samples at a time but not more than two runs or 20 samples per day. Do not smoke more than can be analyzed in a 24 hours period. Hydroquinone is especially temperature and time sensitive.

14.1.2 Remove the sidestream pad, fold in half and in half again with the “clean” side facing out. Grasp with a pair of clean tweezers, and wipe the holder. Place the pad into a 250 mL Erlenmeyer flask.

14.1.3 Add the 100 mL of impinger solution to the Erlenmeyer flask.

14.1.4 Rinse the fishtail chimney with 2 X 20 mL of fresh 1 % Acetic Acid. Use a glass rod to free up any debris on the chimney. Add the chimney washings to the Erlenmeyer flask for a total volume of 140 mL.

14.1.5 Place a piece of 1” masking tape over the ground glass stopper to hold it in place.

14.1.6 Prepare an Laboratory Reagent Blank (LRB) with each day’s smoking as follows to demonstrate that interference from the analytical system, glassware, and reagents are not present.

14.1.6.1 LRB: Add one blank filter pad from the smoking room to a clean 250 mL Erlenmeyer flask, add 140 mL of 1 % Acetic Acid solution and stopper.

14.1.7 Prepare an Laboratory Fortified Blank (LFB) with each day’s smoking as follows to determine whether there is any loss of analyte as a result of the analytical process.

14.1.7.1 LFB: Add one blank filter pad from the smoking room to a clean 250 mL Erlenmeyer flask, add 139 mL of 1 % acetic acid plus 1 mL of phenol spiking solution and stopper.

14.1.8 Wrap flasks with tin foil completely. Clamp flasks onto armature of wrist action shaker and agitate 30 minutes. The pad should be disintegrated once agitation is complete.

14.1.9 After shaking, syringe-filter the smoke extract directly into autosampler vials in duplicate. Rinse the vial first, discard the rinse; and then fill to minimize headspace.

14.1.10 After shaking, prepare an Laboratory Fortified Matrix (LFM) using a standard control brand with each day of smoked samples:

14.1.10.1 Attach a 0.45 µm syringe filter to a disposable syringe and filter the smoke extract directly into 10 mL volumetric flasks that have been preloaded with 1 % acetic acid and the phenol spike as necessary to dilute the smoke extract to 10 mL. Mix the volumetric flask well and then using a Pasteur pipette, fill autosampler vials in duplicate. Rinse vial first and then fill to minimize head space.

14.1.11 The LRB and LFB are syringe-filtered directly into autosampler vials.

14.1.12 Place the vials in a vial file and store at 4 °C, protected from light, until instrument analysis takes place.

14.1.13 A run log is then generated to record the total time samples are at room temperature from smoking to the end of analysis.

Note: It is very critical that analysis be completed in minimal time without interruption as the samples will decompose with prolonged exposure at room temperature.

14.2 Instrument Analysis: HPLC Equipment

14.2.1 High Pressure Liquid Chromatography System consisting of:
14.2.2 Solvent Delivery System - ternary gradient pump.
14.2.3 Refrigerated Autosampler with 20 µL sampling loop.
14.2.4 Programmable Wavelength Spectrofluorometer at Gain 100, ATTN 8.
14.2.5 Slit Width: Ex. 18 nm, Em 18 nm.
14.2.6 Wavelength Profile:

Time Initial	Excitation (nm)	Emission (nm)
0.0	304	338
5.5	274	298
32.0	274	298
33.5	304	338

14.2.7 Cooling Bath with column temperature modifier attachment.

14.3 Chromatographic Conditions (Reversed Phase Analysis)

14.3.1 Column Temperature: 20 °C.

14.3.2 Mobile Phase: Reagents.

14.3.2.1 Solvent A: Prepare 2 L of 1 % Acetonitrile, 1 % Acetic Acid, 1 % IPA filter and degas. (UHP Helium sparged).

14.3.2.2 Solvent B: Prepare 2 L of 28 % Acetonitrile, 1 % Acetic Acid, 1 % IPA filter and degas. (UHP Helium sparged).

14.3.2.3 Solvent C: Acetonitrile (UHP Helium sparged).

14.3.3 Sample Wash: Solvent A.

14.3.4 Mobile Phase: Gradient.

Flowrate: 1.5 mL/minute.

Time (minutes)	Composition		
0.0	100 % A	0 % B	0 % C
5.0	100 % A	0 % B	0 % C
15.0	75 % A	25 % B	0 % C
20.0	25 % A	75 % B	0 % C
28.0	0 % A	100 % B	0 % C
30.0	0 % A	0 % B	100 % C
32.0	0 % A	0 % B	100 % C
34.0	95 % A	0 % B	5 % C
Method End Action (Equilibrate 10 minutes).	100 % A	0 % B	0 % C

14.3.5 Sample vials are loaded onto the autosampler such that every 10th vial is a standard solution and in such quantities that the total analysis time does not exceed 24 hours.

14.3.6 Twenty µL of each sample vial is injected onto the HPLC. Elution pattern should be similar to **Figure 1**.

14.4 Calculations

14.4.1 Construct a Calibration Curve

14.4.2 Twenty µL of each calibration standard is injected onto the HPLC column and analyzed as per the chromatographic conditions. Do in duplicate. Elution pattern should be similar to **Figure 2**.

14.4.3 Determination of Response Factor

14.4.3.1 A calibration curve of the various hydroxybenzene compounds is prepared by plotting the concentration of the standards versus their respective peak areas.

14.4.3.2 Determine the Response Factor from the calibration curve.

14.5 Sample Quantification

14.5.1 The amount of the various phenolic compounds in smoke samples is quantified by the external standard method.

14.5.2 The identification of peaks is by comparison of retention times with standards, and the spiking of smoke samples.

14.6 Determination of Phenol Deliveries in µg/cigarette

14.6.1 Hydroxybenzene [µg/cigarette] = $\frac{\text{Peak Area}}{\text{Resp Factor}} \times \frac{\text{DF}}{\text{No. of Cigarettes}}$.

where DF is the dilution factor.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See **Figure 1, 2.**

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should also include:

A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, trapping reagents, filter pads, and analyzer effects.

A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss.

15.2.2 A standard run as a sample to verify the calculation process and validate the calibration.

15.3 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary stock Phenol standards are prepared fresh weekly.

15.4.2 All work standards, and reagents are prepared fresh weekly.

15.4.3 All samples are analyzed as soon as they are smoked and within 24 hours.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

16.1 The number of cigarettes does not have to be altered for intense smoking.

17 REFERENCES

17.1 Risner, C.H. and Cash, S.L. “A High Performance Liquid Chromatographic Determination of Major Phenolic Compounds in Tobacco Smoke”, *Journal of Chromatographic Science*, p. 28, 1990.

17.2 Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113: p. 1509-1513.

APPENDICES

Appendix 1: Calibration Standards

(a): Phenol Standards

Phenol										
Hydroquinone										

Phenol Working Standards +

Label	5	10	100	200	350	500	750	1000
Vol (mL) Tertiary	0.050	0.100	1.000	2.000	3.500	5.000	7.500	10.000
Phenol	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]
Hydroquinone	0.04811	0.09623	0.96228	1.92456	3.36798	4.81140	7.21710	9.62280
Resorcinol	0.00466	0.00931	0.09314	0.18628	0.32599	0.46570	0.69854	0.93139
Catechol	0.02208	0.04415	0.44154	0.88308	1.54539	2.20770	3.31155	4.41540
Phenol	0.05386	0.10771	1.07712	2.15424	3.76992	5.38560	8.07840	10.77120
m-Cresol	0.01327	0.02653	0.26532	0.53064	0.92862	1.32660	1.98990	2.65320
p-Cresol	0.00695	0.01390	0.13900	0.27799	0.48649	0.69498	1.04247	1.38996
o-cresol	0.00960	0.01920	0.19198	0.38396	0.67193	0.95990	1.43986	1.91981
m+p-Cresol	0.02022	0.04043	0.40432	0.80863	1.41511	2.02158	3.03237	4.04316

\+ In 1% (v/v) Acetic Acid in single 10mL volummetric flasks

(c): Spiking Solution

Phenol	LFB Spiking Solution ***						LFM Spike ++			
	Stock Level	Stock [mg/mL]	Volume (mL)	Dilute to Vol (mL)	Spike [ug/mL]	Analyzed [ug/mL]	Volume Spike (mL)	Dilute to Vol (mL)	Spike [ug/mL]	Analyzed [ug/mL]
Hydroquinone	Primary	0.96228	1.0		38.4912	0.76982			19.24560	1.92456
Phenol	Primary	1.07712	0.6	25.0	25.85088	0.51702	5.0	10.0	12.92544	1.29254
o-cresol	Secondary	0.04800	1.4		2.68773	0.05375			1.34387	0.13439

*** In 1% (v/v) Acetic Acid in a single 25mL volummetric flask
++ In 1% (v/v) Acetic Acid in a single 10mL volummetric flask

Figure 1: Chromatogram of a Typical Phenol Calibration Standard

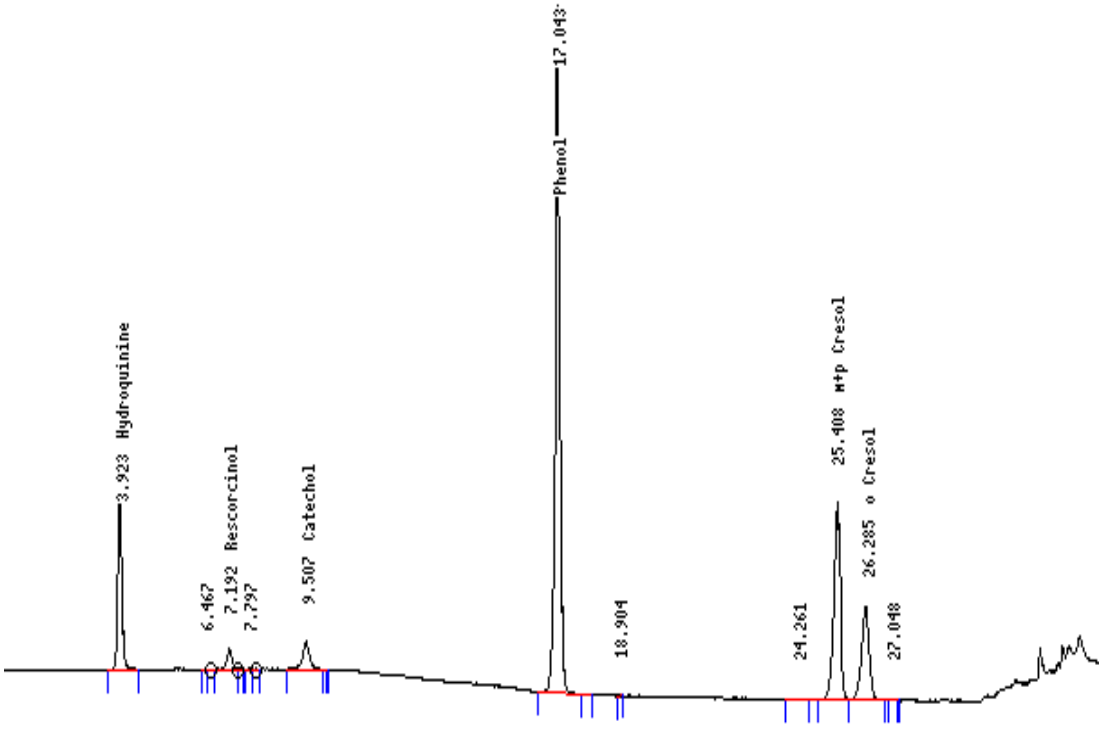


Figure 2: Chromatogram of The Analysis of Sidestream TPM for Hydroxybenzenes.

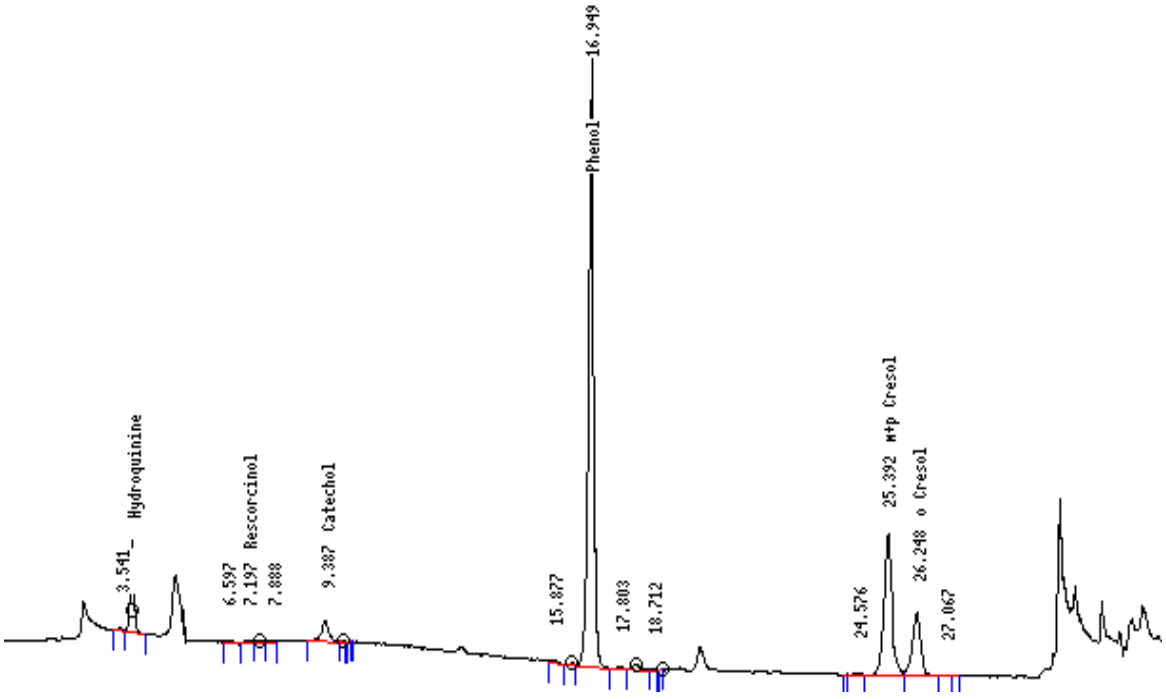
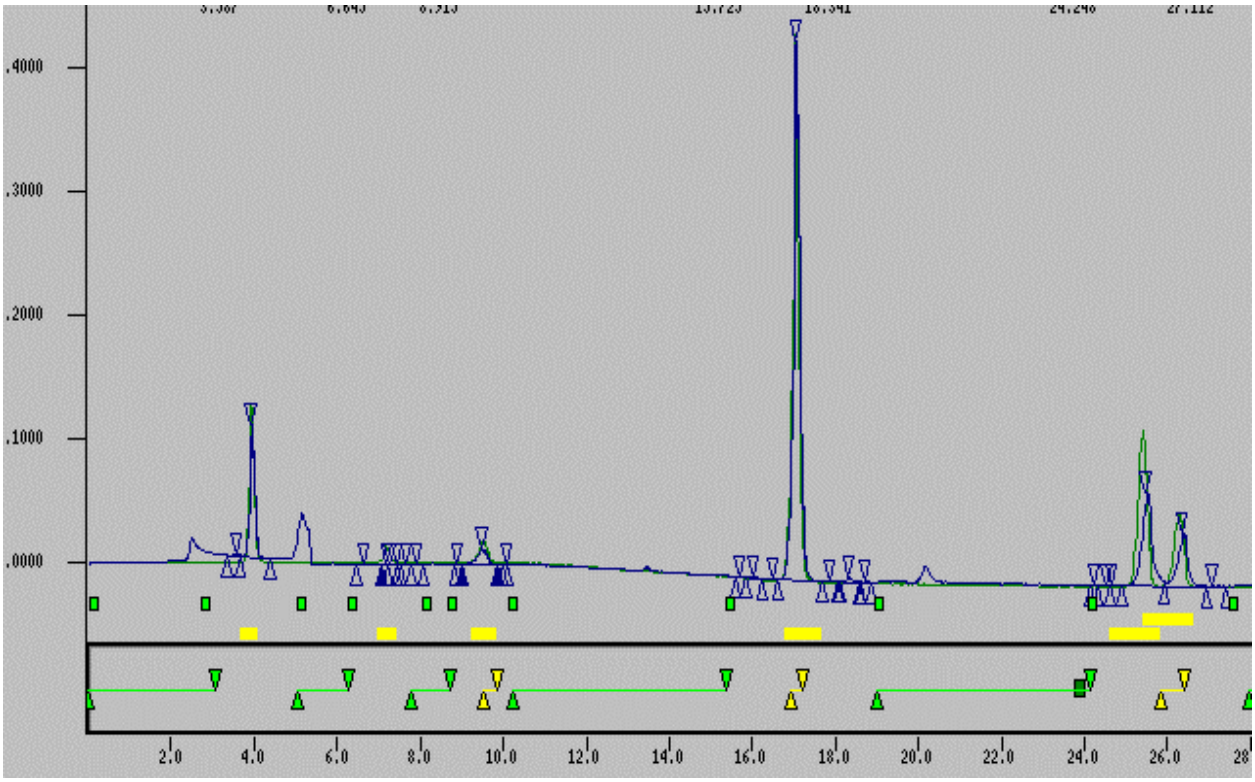


Figure 3: Overlay of Control Cigarette Phenols and Calibration Standard



Appendix 4a: Hydroquinone Calibration Curve

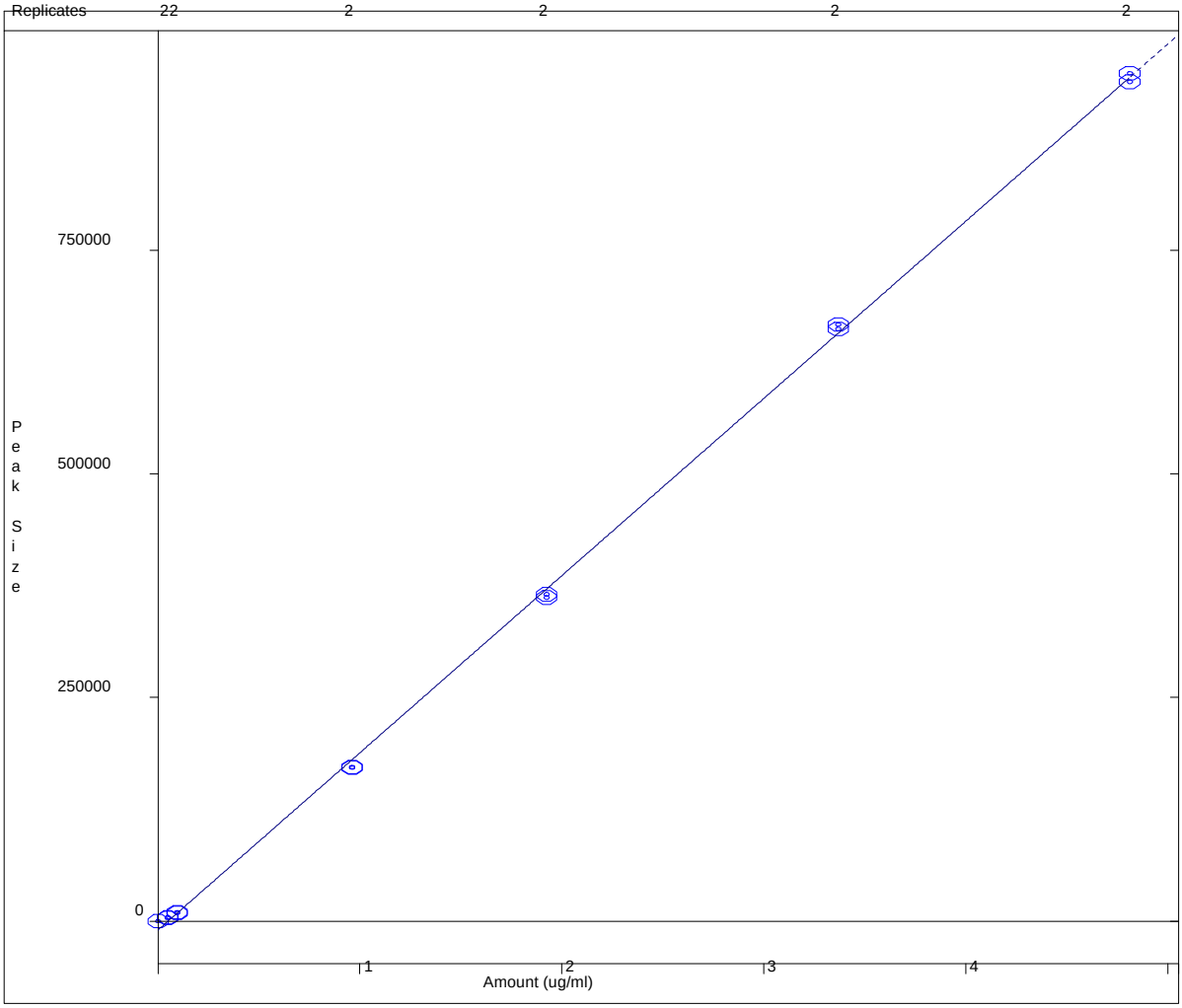
Calibration Curve Report
File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

Hydroquinine

External Standard Analysis
Curve Type: Linear
Origin: Include
y = +1.980434e+005x -9.836358e+003

Resp. Fact. RSD: 31.64%
Corr. Coef.(R^2): 0.999628

Replicates	2	2	2	2	2



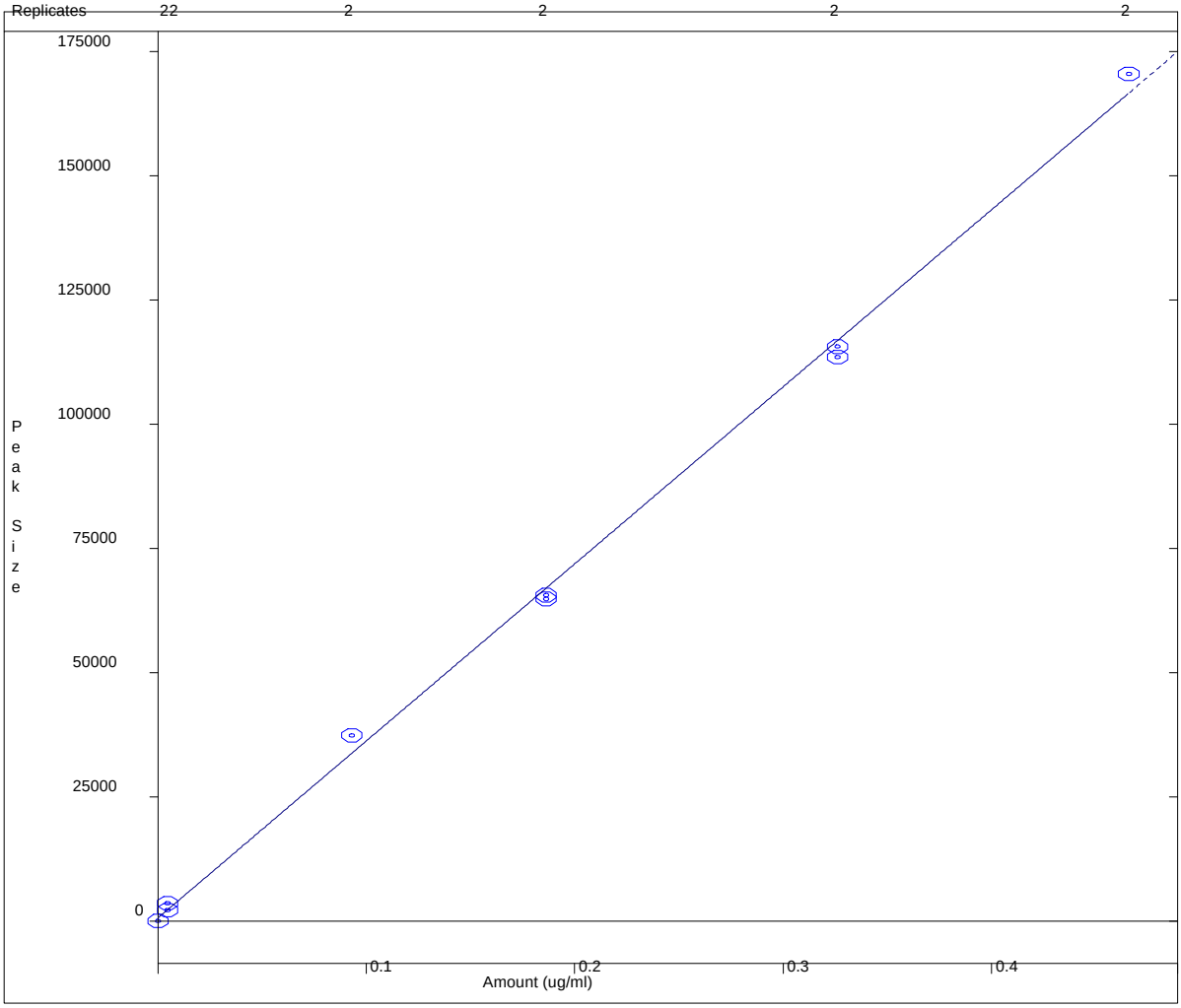
Appendix 4b: Resorcinol Calibration Curve

Calibration Curve Report
File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

Rescorcinol

External Standard Analysis
Curve Type: Linear
Origin: Include
y = +3.564001e+005x +5.952439e+002

Resp. Fact. RSD: 33.80%
Corr. Coef.(R^2): 0.998306



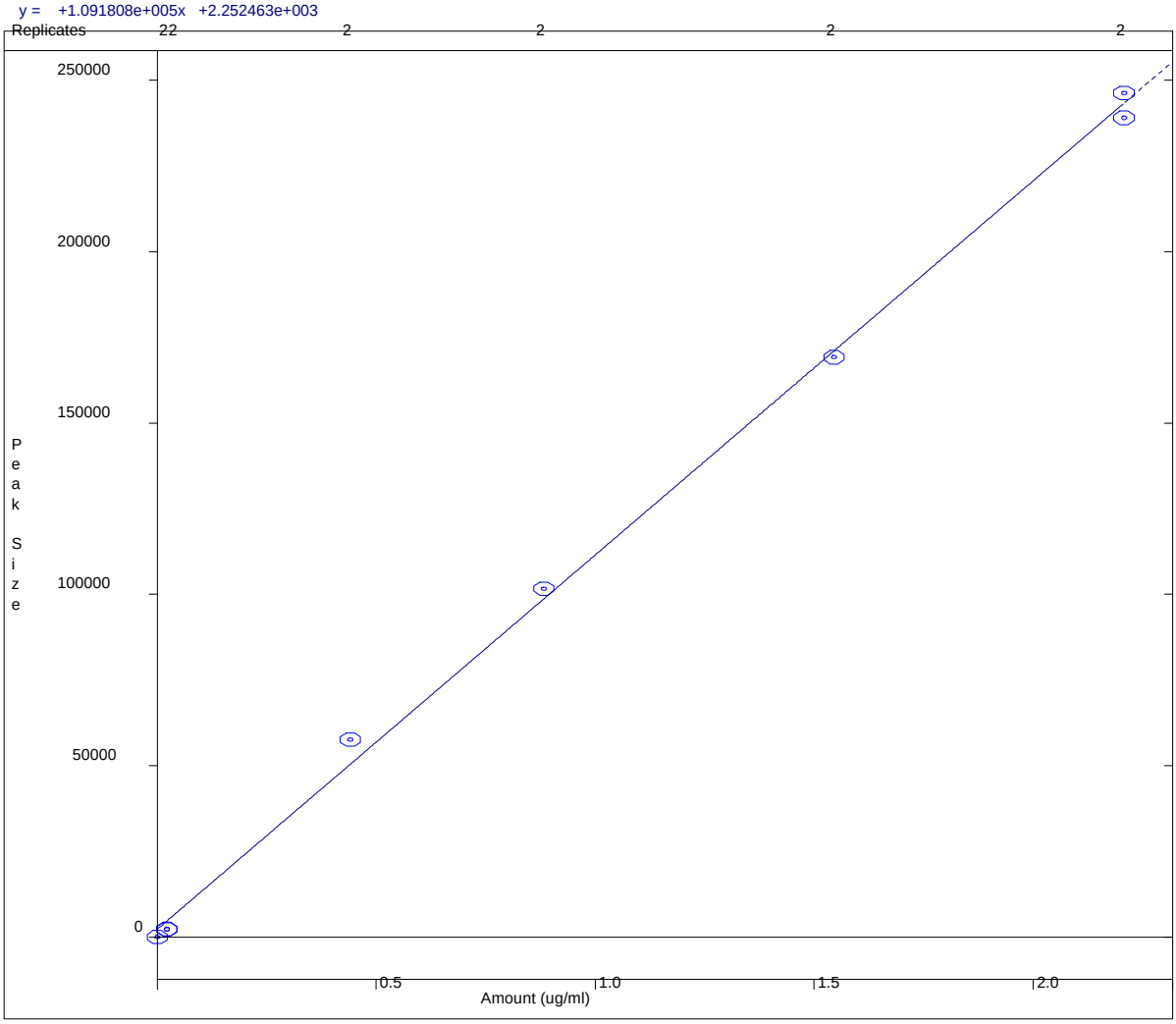
Appendix 4c: Catechol Calibration Curve

Calibration Curve Report
File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

Catechol

External Standard Analysis
Curve Type: Linear
Origin: Include
y = +1.091808e+005x +2.252463e+003

Resp. Fact. RSD: 8.326%
Corr. Coef.(R^2): 0.998611



Appendix 4d: Phenol Calibration Curve

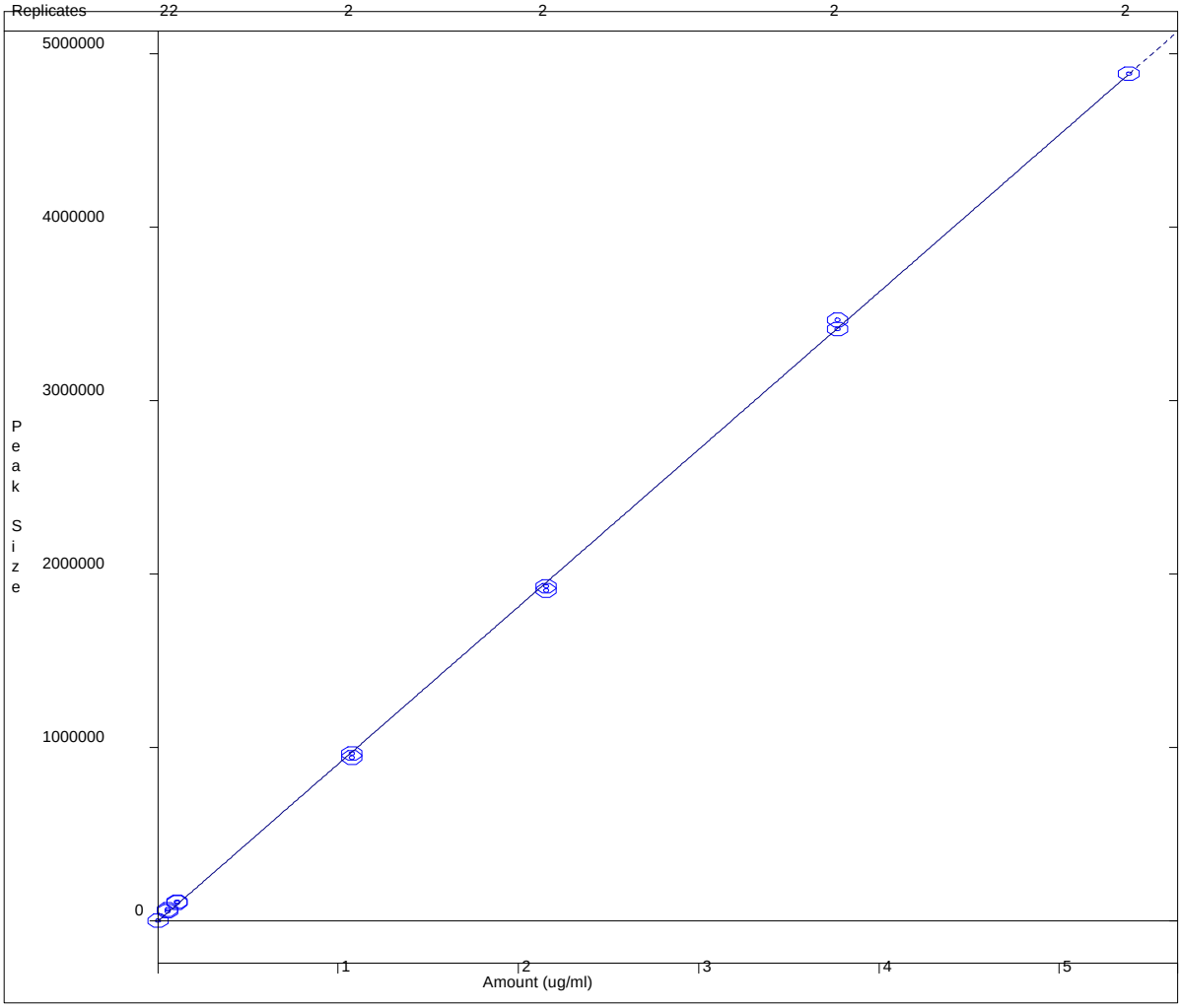
Calibration Curve Report
File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

Phenol

External Standard Analysis
Curve Type: Linear
Origin: Include
y = +9.074024e+005x -3.767191e+003

Resp. Fact. RSD: 10.50%
Corr. Coef.(R^2): 0.999767

Replicates 2 2 2 2 2



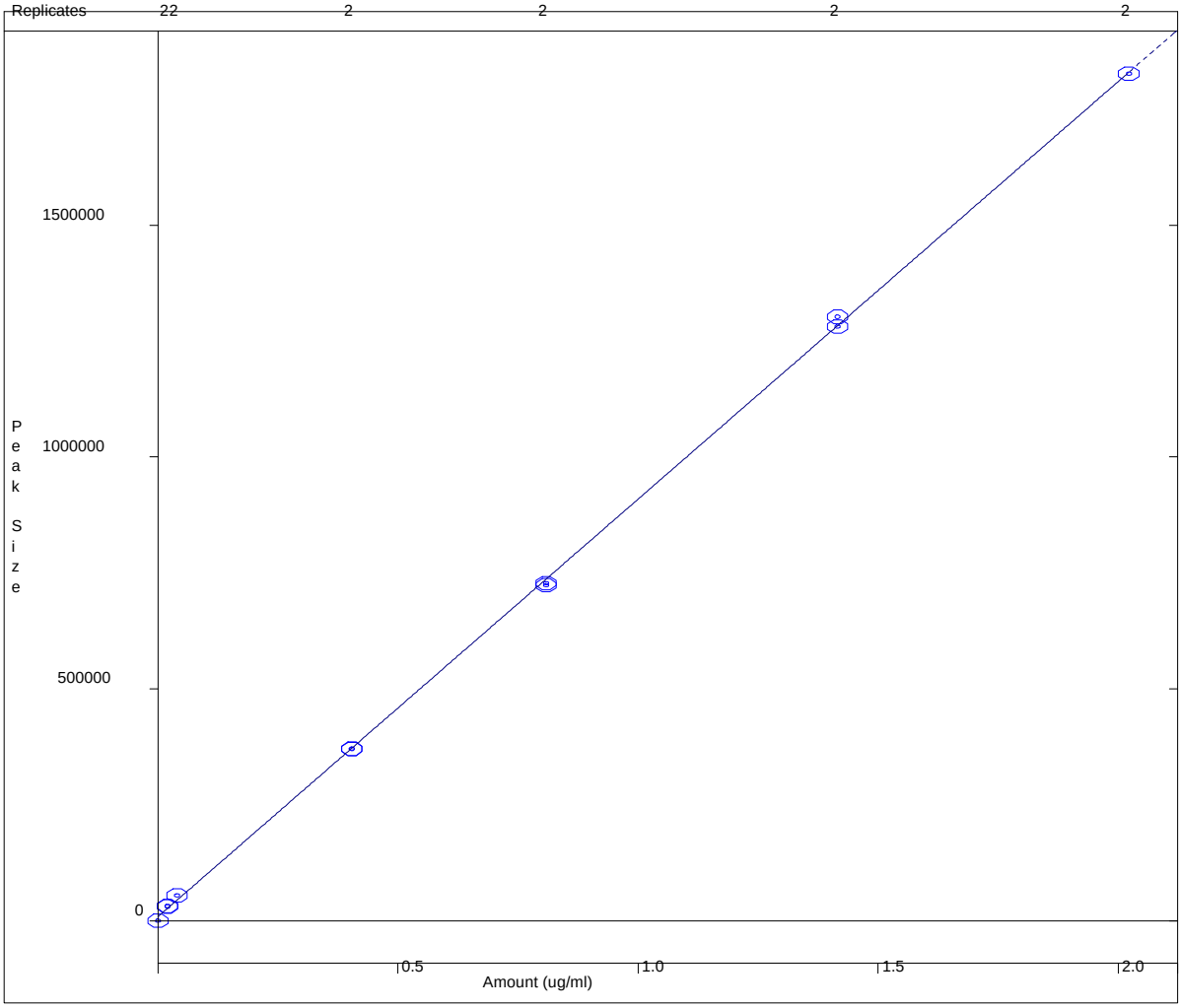
Appendix 4e: m+p Cresol Calibration Curve

Calibration Curve Report
File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

m+p Cresol

External Standard Analysis
Curve Type: Linear
Origin: Include
y = +9.009191e+005x +8.426607e+003

Resp. Fact. RSD: 26.32%
Corr. Coef.(R^2): 0.999778



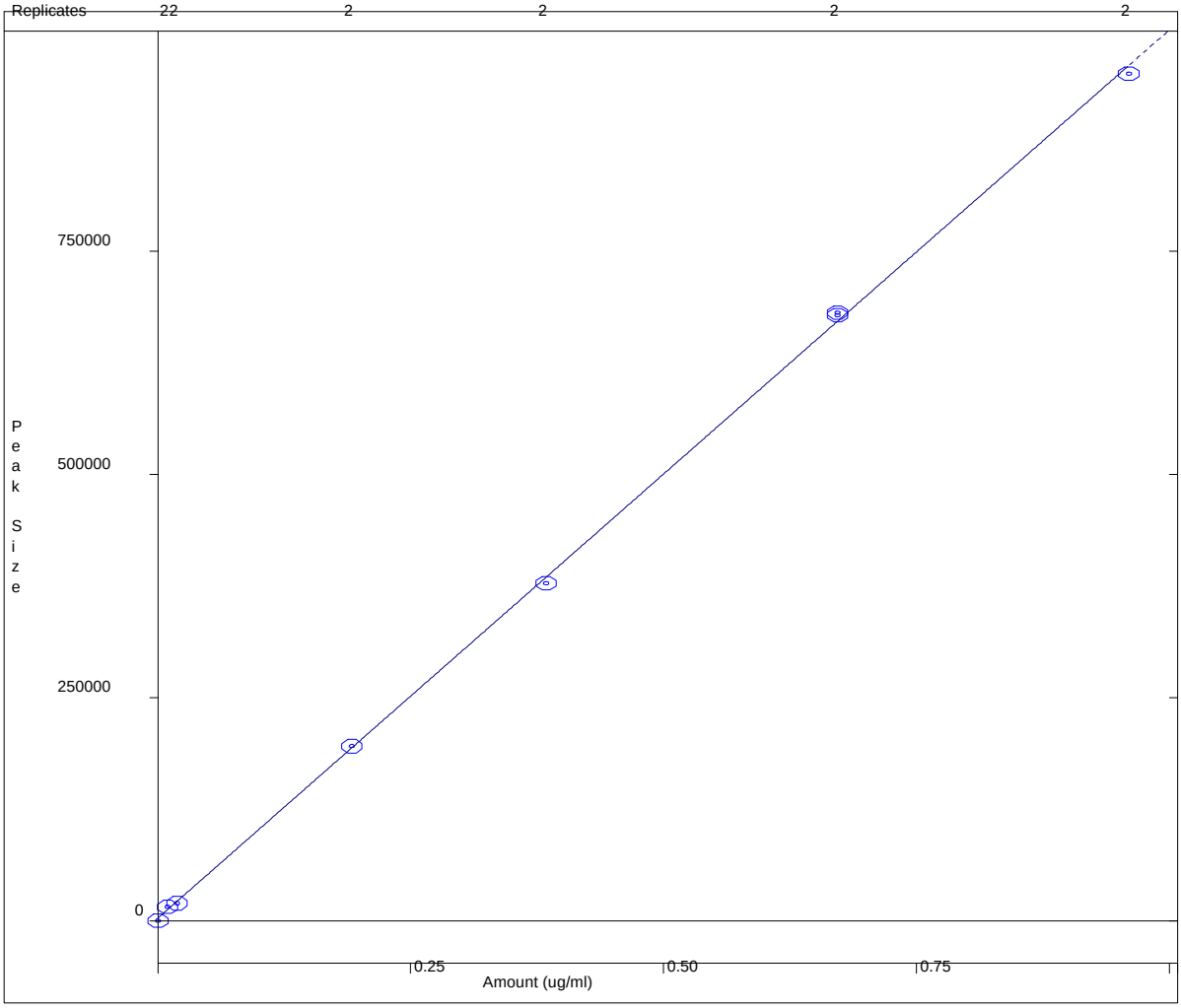
Appendix 4f: o-Cresol Calibration Curve

Calibration Curve Report
File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

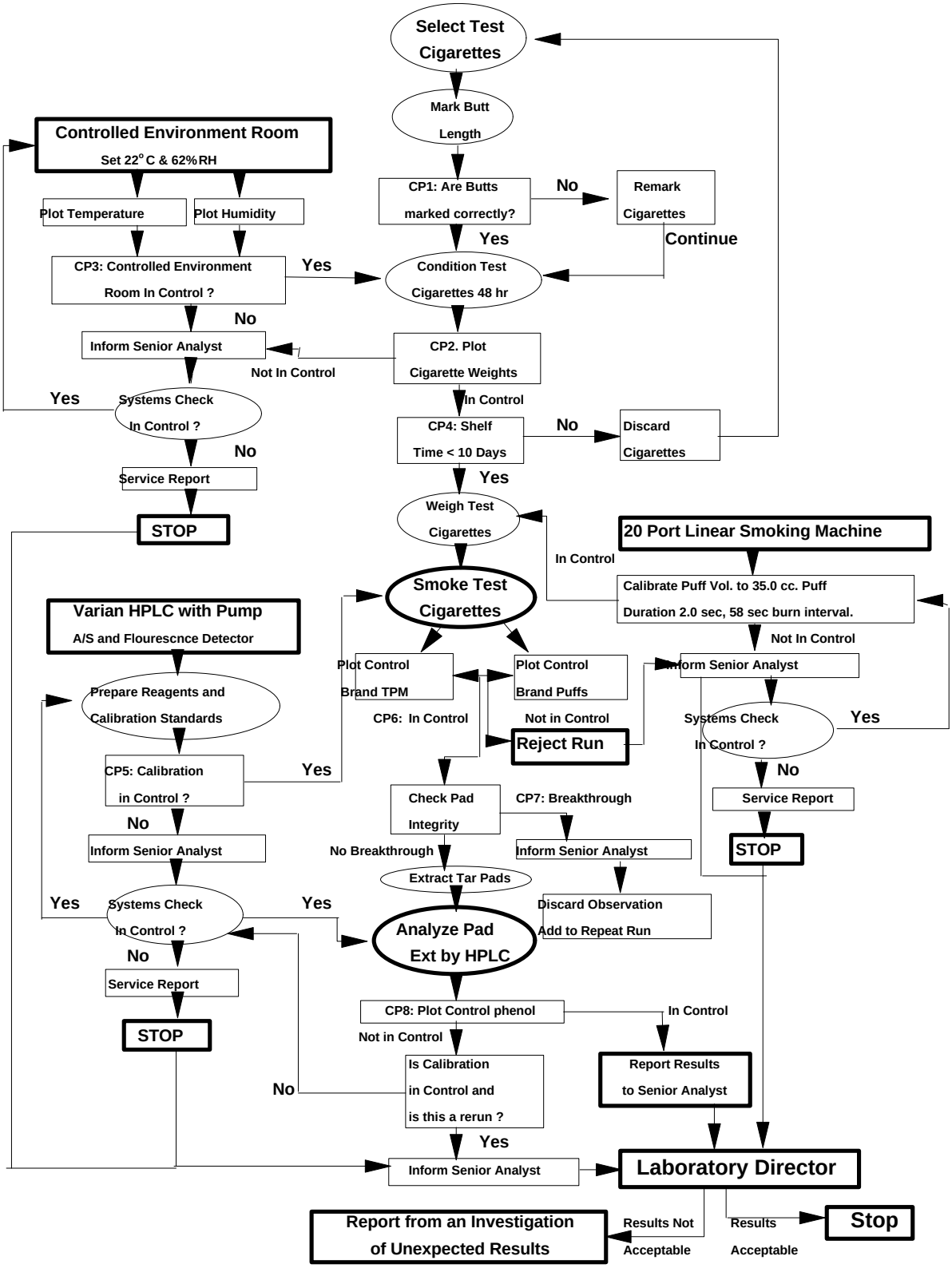
o Cresol

External Standard Analysis
Curve Type: Linear
Origin: Include
y = +9.947791e+005x +2.906716e+003

Resp. Fact. RSD: 21.22%
Corr. Coef.(R^2): 0.999678



Appendix 5: Phenols Process Control Flow Diagram



Appendix 6a: Laboratory Reagent Blanks (LRB) from a Recent Sidestream Study

Sample Description	Hydroquinoine (ug/ml)	Resorcinol (ug/ml)	Catechol (ug/ml)	Phenol (ug/ml)	m+p Cresol (ug/ml)	o-Cresol (ug/ml)
Day1_LRBa	Not Det	0.0112	0.0260	0.0113	0.0218	0.0003
Day2_LRBa	Not Det	0.0107	0.0235	0.0113	Not Det	0.0076
Day3_LRBa	Not Det	0.0139	0.0468	0.0136	Not Det	0.0044

Sample Description	Hydroquinoine (ug/cig)	Resorcinol (ug/cig)	Catechol (ug/cig)	Phenol (ug/cig)	m+p Cresol (ug/cig)	o-Cresol (ug/cig)
Day1_LRBa	Not Det	0.112	0.260	0.113	0.218	0.003
Day2_LRBa	Not Det	0.107	0.235	0.113	Not Det	0.076
Day3_LRBa	Not Det	0.139	0.468	0.136	Not Det	0.044
Average	Not Det	0.119	0.321	0.121	0.218	0.041

Appendix 6b: Laboratory Fortified Blanks (LFB) from a Recent Sidestream Study

Results are reported on a Per cent Recovered Basis

Sample Description	Hydroquinoine (ug/ml)	Phenol (ug/ml)	o-Cresol (ug/ml)
Day1_LFBa	73.40	94.00	114.23
Day2_LFBa	83.10	96.75	74.23
Day3_LFBa	90.23	97.25	125.21
Average	82.24	96.00	104.56

Appendix 6c: Laboratory Fortified Blanks (LFM) from a Recent Sidestream Study

Results are reported on a Per Cent Recovered Basis

Sample Description	Hydroquinoine (ug/ml)	Phenol (ug/ml)	o-Cresol (ug/ml)
R04_P12_LFMa	98.76	93.75	95.84
R08_P09_LFMa	99.02	102.82	102.76
R12_P17_LFMa	100.78	109.32	124.93
Average % LFM Recovered	99.52	101.96	107.85

Appendix 7: Minimum Detection Limit (MDL) and Limit of Quantitation (LOQ) for Sidestream Phenols.

Phenols	Standard 1 Hydroquinoine (ug/ml)	Standard 2 Resorcinol (ug/ml)	Standard 2 Catechol (ug/ml)	Standard 1 Phenol (ug/ml)	Standard 2 m+p Cresol (ug/ml)	Standard 2 o-Cresol (ug/ml)
	0.0512	0.0092	0.0543	0.0427	0.0355	0.0215
	0.0510	0.0069	0.0598	0.0474	0.0342	0.0193
	0.0493	0.0052	0.0557	0.0465	0.0356	0.0229
	0.0514	0.0071	0.0619	0.0474	0.0354	0.0211
	0.0490	0.0068	0.0570	0.0484	0.0351	0.0179
	0.0543	0.0054	0.0506	0.0489	0.0368	0.0182
	0.0544	0.0056	0.0439	0.0424	0.0366	0.0202
	0.0497	0.0059	0.0472	0.0424	0.0357	0.0205
	0.0506	0.0071	0.0485	0.0451	0.0353	0.0221
	0.0538	0.0073	0.0628	0.0452	0.0347	0.0189
Average	0.0515	0.0067	0.0542	0.0456	0.0355	0.0203
Std Dev	0.0020	0.0012	0.0065	0.0025	0.0008	0.0017
Coeff of Var	3.9	17.9	11.9	5.4	2.2	8.3
MDL (ug/ml)	0.0061	0.0036	0.0194	0.0074	0.0023	0.0050
MDL (ug/cig)	0.122	0.071	0.387	0.149	0.047	0.100
LOQ (ug/ml)	0.0203	0.0119	0.0646	0.0248	0.0078	0.0167
LOQ (ug/cig)	0.406	0.238	1.291	0.496	0.156	0.335

Appendix 8: Kentucky Reference cigarette (1R4F) Yields

Sidestream Yield Summary for Kentucky Reference 1R4F (Brand 507)

Analyte	Mean	Units	Std. Dev.	Coeff. Var.
Phenolic grp				
Hydroquinone	116	ug/cig	17.3	14.9%
Resorcinol	0.806	ug/cig	0.568	70.5%
Catechol	93.4	ug/cig	13.9	14.9%
Phenol	247	ug/cig	25.7	10.4%
mp_cresol	72.9	ug/cig	10.1	13.8%
o_cresol	34.7	ug/cig	5.82	16.8%