

Subpart B—TOBACCO

26 CFR 601.311: IMPOSITION OF TAXES; REGULATIONS

Method for sequential solvent extractions for differentiating cigars and cigarettes. See ATF Proc. 76-2, below.

27 CFR 270.11: MEANING OF TERMS

(Also 275.11, 290.14, 290.15, 295.11, 26 CFR 601.311)

The Bureau publishes an amplified method for sequential solvent extractions used in differentiating cigars and cigarettes. ATF Proc. 73-5 superseded in part.

ATF Proc. 76-2

SECTION 1. PURPOSE.

This ATF Procedure describes the sequential solvent extraction method now employed by ATF in making distinctions between cigars and cigarettes as defined in 26 U.S.C. 5702. This method is essentially the same as the method first announced in ATF Procedure 73-5, ATF C.B. 1973, 142, except that the instructions are amplified and the standards have been redetermined on a residue-free solvents basis.

SEC. 2. BACKGROUND.

.01 Several factors are considered by the Bureau in ruling on the taxable category of smoking products wrapped in other than natural leaf tobacco, as discussed in ATF Ruling 73-22, ATF C.B. 1973, 89. To aid in the evaluation of these factors the Bureau employs various laboratory

and consumer smoking tests, which are described in ATF Procedure 73-5. Some industry members have stated that the description in that Procedure for the sequential extraction test is not adequately specific for it to be effectively employed in their laboratories. Another observation made about the extractive procedure was that it failed to take into account the possibility of varying residues from the solvents themselves, and consequently the results of the tests would be erroneous to the extent the solvent residue in each subsequent test differed from the solvent residue present when the standards were run. We agree that both of these observations are valid.

.02 Accordingly, (a) the cigar and cigarette standards have been redetermined on a residue-free solvents basis, using quantities of filler tobaccos still on hand from the standards determination as published in ATF Proc. 73-5, (b) the method has been modified to include the distillation of corrective blanks for each solvent used, and (c) the description of the method as included in this Procedure has been significantly amplified from that previously published.

.03 Another comment made about the sequential extraction method was that because of the small size of the sample (1 gram), slight procedural errors can cause unreliable results and consequently the method does not have adequate precision for

reproducibility by industry laboratories. To factually determine reproducibility between chemical laboratories the Association of Official Analytical Chemists has agreed to conduct a collaborative study of the method. However, the Bureau believes that with the expanded instructions below any properly equipped and staffed laboratory will be able to reasonably duplicate the precision achieved by the ATF laboratory in applying this slightly modified method.

SEC. 3. PURPOSE AND NATURE OF THE EXTRACTIVE METHOD.

The sequential solvent extraction method was developed to aid the Bureau in making cigar/cigarette distinctions for tax purposes, as required for administration of 26 U.S.C. Chapter 52. It is based on cigar and cigarette standards as represented by the filler tobaccos of typical cigars and typical cigarettes obtained from the retail market, which have been subjected to sequential extraction with six successive solvents of increasing extractability. The resultant standards are shown in Tables I and II. Subsequently, filler tobaccos of unknown character are subjected to the same extractive method and the results mathematically compared with the extractives of the standards to determine the degree to which the unknown tobaccos approximate the typical cigar and cigarette extractive patterns. Similarly, the extracts of reconstituted cigar wrapper material are compared to the cigar tobacco standard to determine the degree to which the reconstituted product approximates natural cigar tobacco.

SEC. 4. SOLVENTS AND EQUIPMENT

.01 Solvents

Petroleum ether (30° - 58°C), American Chemical Society (ACS) reagent grade

Tetrahydrofuran, ACS reagent grade

Acetonitrile, ACS reagent grade

95% Ethanol, U.S. Pharmacopea grade

Dioxane-20% water by volume, ACS reagent grade dioxane and demineralized water with antifoam added, as described in "Preparation of Solvents", Section 5 below

Water, demineralized, with antifoam added, as described in Section 5 below

.02 Apparatus and equipment

Balance, analytical—semimicro (Type H-15, Mettler Instrument Corp., Princeton, N.J.)

Bottle, wash—polyethylene

Demineralizing cartridge, double—research model

Desiccator

Evaporator, rotary—Rotovapor

Extraction apparatus—Soxhlet standard, with vapor trail portion of thimble receptacle insulated with single thickness of 1" x 1/32" asbestos tape, applied wet. No other portion of the apparatus is insulated. (See figure 1.)

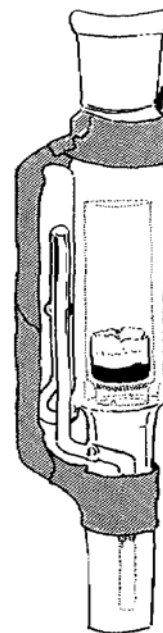


FIGURE 1

PROPER INSULATION OF SOXHLET APPARATUS

Extraction thimble—glass, 25mm x 85mm, coarse porosity fritted disc
 Extraction heaters—six unit electric (Cat. No. 65500, GCA/Precision Scientific, Chicago, Ill.)
 Flask, drying—glass, 250 ml round bottom
 Flask, receiving—glass, 125 ml flat bottom
 Funnel, 45mm diameter
 Mill, cutting—Wiley, intermediate (cutting chamber 40 mm inside diameter x 20 mm depth) with 20-mesh screen
 Oven, convection
 Pipette—with rubber bulb
 Regulator, water pressure (installed between water supply and Soxhlet condensers)
 Stopcock—3-way (inserted between rotary evaporator and water aspirator vacuum pump—used to break vacuum before disconnecting 250 ml round-bottom flasks)
 Vacuum, house
 Vacuum pump—water aspirator
 Valve, needle
 .03 Supplies
 Chips, boiling—chemically inert carbon. (Todd Scientific Corp., Cedars, Pa.)
 Cotton—sterile grade medicinal
 Desiccant—indicating silica gel
 Antifoam—(Anti-Foam No. 820, Scientific Industries, Inc., Springfield, Mass.)

SEC. 5. PREPARATION OF SOLVENTS

All solvents except water are distilled in glass within 24 hours of use. Petroleum ether and tetrahydrofuran are distilled under normal atmosphere. Acetonitrile, ethanol, and dioxane are distilled either under normal atmosphere or under reduced pressure using water aspirator vacuum controlled with the aid of a needle valve. When distilling dioxane, if the tap water is very cold its flow through the condenser is slowed to prevent dioxane crystallization. Water is purified by passing through the research model double demineralizing cartridge resulting in water

equivalent to triple distilled. One drop of freshly shaken antifoam is added for each 100 ml water. This water is used in the dioxane-20% water combination and as the water solvent.

SEC. 6. PREPARATION OF SAMPLES

If the tobacco sample is filler from a manufactured product, accumulate about 150 grams from products randomly selected from those available. Place filler tobaccos in a hopper and thoroughly mix by hand before pulverizing. Pulverize about 50 grams of the tobacco sample in the Wiley intermediate cutting mill with a 20-mesh screen on the delivery tube. To minimize fines the tobacco is ground in the natural moisture state (about 14 percent). After pulverizing, cap the receiving jar and mix thoroughly by hand tumbling. Dry the sample to constant weight by placing in the convection oven for about 72 hours at $103^{\circ} \pm 2^{\circ}$ C. In addition to moisture removal this also drives off low volatiles. Heat in the oven overnight at $103^{\circ} \pm 2^{\circ}$ C the clean extraction thimbles and 250 ml round-bottom flasks which are to be later used in drying the petroleum ether extract: After heating, transfer to desiccator containing indicating silica gel, where the thimbles should remain for about 2 hours until they cool to room temperature. Transfer one gram of the pulverized moisture-free tobacco sample to a tared glass thimble and weigh. Insert a small cotton plug, about 30mm x 30mm, in the thimble on top of the sample and again weigh thimble and contents. Place thimble into thimble receptacle portion of Soxhlet apparatus, and connect 125 ml receiving flask containing a fresh boiling chip. Repeat for each additional sample to be run. (From three to six replicate samples are run simultaneously.)

SEC. 7. EXTRACTIONS.

.01 *Petroleum ether*

To create a vapor seal at the joint connecting the Soxhlet apparatus and the receiving flask, moisten the male joint of the Soxhlet apparatus with a drop of demineralized water (without antifoam), and connect to the receiving flask with a twisting motion. Add 100 ml of freshly distilled petroleum ether slowly to the thimble within the Soxhlet apparatus, which should cause two to three zero solvent trips. Set control on heating units to "15" and bring solvent to boil, adjusting control as necessary to achieve a trip cycle rate of one each three minutes. Occasionally it is necessary to add a measured quantity of petroleum ether through the top of the condenser to replace loss by evaporation. Extract for 24 hours, counting from the time of the first trip after bringing to boil. (During this time clean, dry overnight in the oven at $103^{\circ} \pm 2^{\circ} \text{C}$, and store in the dessicator, the 250 ml round-bottom flasks to be later used in drying the tetrahydrofuran extract.) After petroleum ether extraction for 24 hours turn off heaters and let cool about 30 minutes until at room temperature. Transfer extraction solvent in thimble, thimble chamber, and in the receiving flask to a tared 250-ml round-bottom flask. Remove the excess solvent from the thimble using a bulb and pipette, and then remove the last traces of petroleum ether by placing the bottom of the thimble into a funnel containing a plug of cotton and connected to a vacuum line. Remove residue from the receiving flask and wash down small traces of water condensate in the condenser by directing 2 or 3 ml of freshly distilled tetrahydrofuran from a wash bottle into the top of the vertically held water condenser joined to the receiving

flask (figure 2), adding this washing to the 250 ml round-bottom flask.

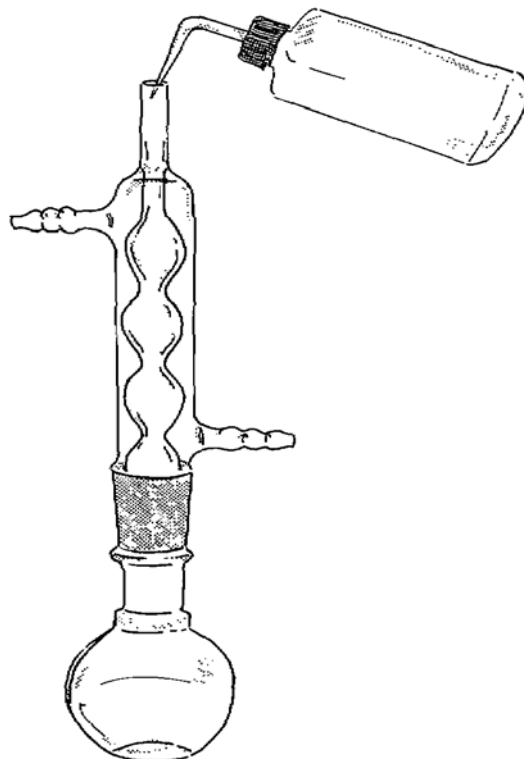


FIGURE 2

PROCEDURE FOR TETRAHYDROFURAN RINSE FOLLOWING PETROLEUM ETHER EXTRACTION

(Return thimble containing tobacco sample to the Soxhlet apparatus, and replace the boiling chip in the receiving flask with a fresh one.) Remove solvent from the extract in the 250 ml round-bottom flask with rotary evaporator. Bleeder stopcock is either closed or left to bleed in a small amount of air after having been so set at a previous water extraction, as described in .04 below. Make sure the tip of the rotary evaporator is free from residue, and then, on the evaporator in a tared 250 ml round-

bottom flask vacuum distill to dryness a 100 ml petroleum ether corrective blank (making appropriate mathematical adjustments if petroleum ether was added to some samples because of evaporation.) Place the drying flasks containing extract and corrective blank in the oven overnight (at least 10 hours) at $103^{\circ} \pm 2^{\circ}$ C. Place in desiccator for at least two hours or until flasks reach room temperature, and then weigh. Calculate the percentage of extract on a residue free basis as follows:

$$\% \text{ Extract} = \frac{100(\text{Weight of extract} - \text{Weight of corrective blank})}{\text{Weight of sample}}$$

Example:
 $\% \text{ Petroleum Ether} = \frac{100(0.0480 - 0.0013)}{1.0842} = 4.31$

.02 *Tetrahydrofuran*

Moisten the male joint of the Soxhlet apparatus with a drop of demineralized water (without antifoam), and connect to the receiving flask with a twisting motion. Add 100 ml of freshly distilled tetrahydrofuran slowly to the thimble within the Soxhlet apparatus, which should cause two to three zero solvent trips. Set control on heating units to "60" and bring solvent to boil, adjusting control as necessary to achieve a trip cycle of one each four minutes. Occasionally it is necessary to add a measured quantity of tetrahydrofuran through the top of the condenser to replace loss by evaporation. Counting from the time of the first trip after bringing to boil, extract for 24 hours and then turn off heaters. (During this time prepare 250 ml round-bottom flasks for use in drying the acetonitrile extract). Let cool about 30 minutes until at room temperature. Transfer extraction solvent in thimble, thimble chamber, and in the receiving flask to a tared 250 ml round-bottom flask, using a bulb and pip-

ette to achieve maximum removal from the thimble. Remove residue from the receiving flask using a wash bottle and 2 or 3 ml of demineralized water (without antifoam), adding this washing to the 250 ml round-bottom flask. Remove the traces of the water wash from the receiving flask with a wash bottle and 2 or 3 ml of freshly distilled acetonitrile and add this also to the 250 ml round-bottom flask. (Return thimble containing tobacco sample to the Soxhlet apparatus, replace the boiling chip in the receiving flask with a fresh one, and connect the flask and apparatus.) Remove solvent from the tobacco extract in the drying flask with rotary evaporator. Make sure the tip of the rotary evaporator is free from residue, and then in a tared 250 ml round-bottom flask vacuum distill to dryness a 100 ml tetrahydrofuran corrective blank (making appropriate mathematical adjustments if tetrahydrofuran was added to some samples because of evaporation). Proceed with drying, weighing, and calculating in the same way as with petroleum ether in step .01 preceding.

.03 *Acetonitrile, 95% Ethanol, and Dioxane-20% Water*

Sequentially using freshly prepared acetonitrile, 95% ethanol, and dioxane-20% water, slowly add 100 ml of solvent to the thimble within the Soxhlet apparatus, which should cause two to three zero solvent trips. Set control on heating units to "70" for acetonitrile and "80" for ethanol and dioxane-water, and bring solvent to boil. Adjust control as necessary to achieve a trip cycle of one each six minutes. Counting from the time of the first trip after bringing to boil, extract for 24 hours and then turn off heaters. (During this time prepare 250 ml round-bottom flasks to be used in drying the extract of the fol-

lowing solvent.) Let cool about 30 minutes until at room temperature. Transfer extraction solvent in thimble, thimble chamber, and in the receiving flask to a tared 250 ml round-bottom flask, using a bulb and pipette to achieve maximum removal from the thimble. Remove residue from the receiving flask using a wash bottle and 2 or 3 ml of demineralized water (without antifoam), adding this washing to the drying flask. In the case of acetonitrile and ethanol extracts, remove the traces of the water wash from the receiving flask using a wash bottle and 2 to 3 ml of the solvent to be used in the following extraction, and add this also to the 250 ml round-bottom flask. (Return thimble and contents to the Soxhlet apparatus, replace the boiling chip in the receiving flask with a fresh one, and connect the flask and apparatus.) Remove solvent from the tobacco extract in the 250 ml round-bottom flask with rotary evaporator. Make sure the tip of the rotary evaporator is free from residue, and then in a tared 250 ml round-bottom flask distill to dryness a 100 ml solvent corrective blank, and proceed with drying, weighing, and calculating in the same way as with petroleum ether in step .01.

.04 Water

Add 100 ml of demineralized water containing one drop of antifoam slowly to the thimble within the Soxhlet apparatus, which should cause two to three zero solvent trips. Set control on heating units to "100" and bring solvent to boil, adjusting control as necessary to achieve a trip cycle of one each ten minutes. Counting from the time of the first trip after bringing to boil, extract for 24

hours and then turn off heaters. Let cool about 30 minutes until at room temperature. Transfer extraction solvent in the thimble, thimble chamber, and in the receiving flask to a tared 250 ml round-bottom flask, using a bulb and pipette to achieve maximum removal from the thimble. Remove residue from the receiving flask with a wash bottle and about 5 ml of demineralized water (without antifoam), and with an aluminum spatula to aid in removing any possible charred solid residue, adding this washing to the 250 ml round-bottom flask. Remove solvent from the tobacco extract in the 250 ml round-bottom flask with rotary evaporator, making sure the aqueous extract does not go to complete dryness. To avoid excess foaming which can occur with highly fermented tobaccos, adjust bleeder stopcock to bleed just enough air through the attachment nipple to insure continued condensation of water vapor on the spiral condenser. This setting of the bleeder valve is left unchanged for all water extract and water blank evaporation, and is optionally so maintained for evaporation of all solvent extracts and corrective blanks. The 3-way stopcock between the water aspirator and the rotary evaporator is then used to cut off and to break the vacuum. If the water extract does go to dryness, bleed a small amount of water into the vacuum system through the attachment nipple and evaporate to low volume. Make sure the tip of the rotary evaporator is free from residue, and then in a tared 250 ml round-bottom flask vacuum distill to dryness a 100 ml water corrective blank. Proceed with final drying, weighing, and calculating in the same way as with petroleum ether in step .01.

.05 Summary of Extractions

<i>Solvent</i>	<i>Approximate Heat Setting</i>	<i>Trip Cycle in Minutes</i>	<i>Ending Wash for Receiving Flask</i>	<i>Special Instructions</i>
Petroleum Ether	15	3	2 to 3 ml tetrahydrofuran	Use drop of water to connect receiving flask. Sometimes necessary to add petroleum ether to replace evaporation loss. Solvent remaining in thimble extracted with vacuum. Water wash not used. Tetrahydrofuran wash done thru condenser. If additional petroleum ether used make mathematical adjustments to corrective blank results.
Tetrahydrofuran	60	4	2 to 3 ml demineralized water followed by 2 to 3 ml acetonitrile	Use drop of water to connect receiving flask. Sometimes necessary to add tetrahydrofuran to replace evaporation loss, in which case make mathematical adjustments to corrective blank results.
Acetonitrile	70	6	2 to 3 ml demineralized water followed by 2 to 3 ml 95% ethanol	
95% Ethanol	80	6	2 to 3 ml demineralized water followed by 2 to 3 ml dioxane-20% water	
Dioxane-20% Water	80	6	2 to 3 ml demineralized water	
Water	100	10	5 to 6 ml demineralized water, with aid of aluminum spatula to remove possible charred solid residue	Do not take to complete dryness on evaporator. Bleed in small amount of air and use 3-way stopcock to break vacuum. (Sample is discarded after this solvent.)

SEC. 8. STATISTICAL EXPRESSION OF RESULTS.

.01 The manner in which the extractives data are statistically expressed is presently under study by the Bureau. As a result it is possible there will be some change in the

mathematical expressions of the comparisons between the standards and unknowns. However, we do not believe this would change the basic character of the comparisons nor result in any significant differences in interpretive results. Consequently, reconstituted tobacco wrapper material

or tobacco filler which meets minimal expectations under the present procedure for statistical interpretations should similarly be acceptable under any alternative manner of interpretation that might be adopted. The present procedure for expression of the sequential extractive data is described in the following two paragraphs.

.02 The percentage of tobacco character of wrapper material is determined by comparing the replicate sample mean total corrected dry weight extractives with the comparable value (46.54%) for typical cigars as shown in Table I. The formula for this computation is:

$$\% \text{ Tobacco Character} = \frac{100(\text{Total extract from sample wrapper})}{46.54}$$

.03 The cigar (non-cigarette) character of filler tobaccos is computed from five different combinations of the six dry-weight extractives, the most important of which is the combination of the extractives from tetrahydrofuran, acetonitrile, and ethyl alcohol. The computations for cigar (non-cigarette) character are based on the differences between the corrected dry weight extractives of typical cigars and typical cigarettes, using in the formulas the average values for cigar and cigarette extractives for the particular combinations of solvents being compared, as shown in Tables I and II.

The formula is expressed as:

$$\% \text{ Cigar character} = \frac{100(\text{Mean cigarette extract—Replicate sample mean extract of unknown})}{\text{Mean cigarette extract—Mean cigar extract}}$$

The five formulas used are:

a. Tetrahydrofuran, Acetonitrile, and Ethyl Alcohol—

$$\% \text{ Cigar character} = \frac{100(28.32 - \text{unknown})}{11.40}$$

b. Petroleum Ether, Tetrahydrofuran, Acetonitrile, and Ethyl Alcohol—

$$\% \text{ Cigar character} = \frac{100(32.43 - \text{unknown})}{12.04}$$

c. Tetrahydrofuran, Acetonitrile, Ethyl Alcohol, and Dioxane-Water—

$$\% \text{ Cigar character} = \frac{100(35.07 - \text{unknown})}{11.94}$$

d. Petroleum Ether, Tetrahydrofuran, Acetonitrile, Ethyl Alcohol, and Dioxane-20% Water—

$$\% \text{ Cigar character} = \frac{100(39.18 - \text{unknown})}{12.58}$$

e. All six extracts—

$$\% \text{ Cigar character} = \frac{100(55.87 - \text{unknown})}{9.33}$$

SEC. 9 EFFECT ON OTHER DOCUMENTS.

ATF Procedure 73-5, ATF C.B. 1973, 142, is superseded insofar as it relates to the sequential extractive method as described in Sections 7.08 and 8.02 through 8.04, and Tables I and II, of that Procedure.

SEC. 10. INQUIRIES.

Any inquiries concerning this ATF Procedure should refer to its number and be addressed to the Director, Attention RIS, Bureau of Alcohol, Tobacco and Firearms, Washington, DC 20226.

SEQUENTIAL DIFFERENTIAL SOLVENT EXTRACTIONS

Table I (Typical Cigars)

Sample	% Petroleum Ether Extract	% Tetrahydrofuran Extract	% Acetonitrile Extract	% Ethyl Alcohol (95%) Extract	% Dioxane-Water (20%) Extract	% Water Extract	% Total Extractives
76804F	3.41	2.34	5.57	8.61	5.68	17.77	43.38
76805F	3.98	3.26	3.54	8.89	5.92	18.12	43.71
76806F	4.73	2.85	3.39	8.67	4.66	19.68	43.98
76807F	2.64	2.19	4.79	12.34	5.26	17.98	45.20
76808F	3.77	3.04	6.14	8.96	6.02	19.94	47.87
76809F	3.63	3.56	3.61	11.26	5.81	21.31	49.18
76810F	4.21	2.52	2.98	9.89	6.79	26.13	52.52
76811F	2.26	2.93	5.39	8.28	8.07	18.81	45.74
76836F	2.93	2.21	5.32	9.36	6.29	20.56	46.67
76837F	3.79	2.80	2.71	10.47	5.81	20.83	46.41
76838F	3.67	2.70	3.70	12.39	5.92	18.19	46.57
76839F	2.65	2.76	4.76	8.76	8.39	19.90	47.22
Range	2.26-4.73	2.19-3.56	2.71-6.14	8.28-12.39	4.66-8.39	17.77-26.13	43.38-52.52
Mean	3.47	2.76	4.33	9.82	6.22	19.94	46.54
Std. dev.	0.72	0.41	1.14	1.46	1.07	2.29	2.56
Rel. std. dev., %	20.75	14.86	26.33	14.87	17.20	11.48	5.50

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SEQUENTIAL DIFFERENTIAL SOLVENT EXTRACTIONS

Table II (Typical Cigarettes)

Sample	% Petroleum Ether Extract	% Tetrahydrofuran Extract	% Acetonitrile Extract	% Ethyl Alcohol (95%) Extract	% Dioxane-Water (20%) Extract	% Water Extract	% Total Extractives
76787F	4.26	2.19	6.55	16.82	6.27	17.36	53.45
76788F	4.35	2.36	8.73	15.85	6.85	17.68	55.82
76789F	4.04	2.23	8.38	17.82	6.80	15.12	54.39
76790F	4.64	2.02	9.68	15.95	6.13	15.20	53.62
76791F	4.50	2.80	7.48	18.84	6.56	17.36	57.54
76792F	5.59	2.59	7.79	17.89	7.85	15.89	57.60
76793F	4.10	2.41	5.63	20.31	7.98	16.08	56.51
76794F	3.44	2.18	7.28	19.39	6.17	17.33	55.79
76795F	3.56	2.62	6.09	19.14	6.84	16.72	54.97
76796F	3.51	3.02	6.24	19.16	6.28	16.22	54.43
76797F	4.03	2.81	7.07	17.46	6.41	17.88	55.66
76798F	4.56	2.68	8.42	17.89	6.30	17.19	57.04
76799F	4.21	2.39	8.16	19.20	6.69	18.84	59.49
76800F	3.72	2.39	9.46	17.54	7.17	17.59	57.87
76801F	3.64	3.25	7.27	18.98	6.32	16.32	55.78
76802F	3.97	2.46	5.21	20.81	7.44	15.80	55.69
76803F	3.69	2.56	6.99	18.89	6.61	15.48	54.22
Range	3.44-5.59	2.02-3.25	5.21-9.68	15.85-20.81	6.13-7.98	15.12-18.84	53.45-59.49
Mean	4.11	2.53	7.44	18.35	6.75	16.71	55.87
Std. dev.	0.54	0.32	1.27	1.38	0.57	1.05	1.65
Rel. std. dev., %	13.14	12.65	17.07	7.52	8.44	6.28	2.95

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27 CFR 275.11: MEANING OF TERMS

Method for sequential solvent extractions for differentiating cigars and cigarettes. See ATF Proc. 76-2, above.

27 CFR 290.14: CIGARS

Method for sequential solvent extractions for differentiating cigars and cigarettes. See ATF Proc. 76-2, above.

27 CFR 290.15: CIGARETTES

Method for sequential solvent extractions for differentiating cigars and cigarettes. See ATF Proc. 76-2, above.

27 CFR 295.11: MEANING OF TERMS

Method for sequential solvent extractions for differentiating cigars and cigarettes. See ATF Proc. 76-2, above.