Study Protocol

Evaluation of Volcano® Vaporizer for the efficient emission of THC, CBD, and CBN and the significant reduction and/or elimination of total particulate matter (TPM) and Tar components (various organic compounds in TPM that absorb UV radiation) obtained from NIDA.

Study Sponsor(s):	Multidisciplinary Association for Psychedelic Studies (MAPS) 3 Francis Street Belmont, MA 02478
Client No.:	1311619
	California NORML 2215-R Market St. #278 San Francisco CA 94114
Client No.:	14151813

Screening Range(s): TBD	
Test Chemical: Marijuana	
Lot #: TBD	**CAS No.:** NA
Comments:	
Test Sponsor Signature:	**Date:**
Test Sponsor Signature:	**Date:**

To be completed by Chemic Laboratories, Inc.

Testing Facility:	Chemic Laboratories, Inc. 480 Neponset Street-Bldg. 7 Canton, MA 02021
Dose: NA	
Proposed Experimental Dates (Start):	**(termination):**
Study Director **Date:**	**Manager, Quality Assurance Unit:** **Date:**

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1. Purpose

This protocol is intended to provide guidance for the conduct of several analytical investigations the development of rapid-onset, non-smoked cannabinoid delivery systems, as recommended by the Institute of Medicine in its report, "Marijuana and Medicine" (1999). In particular, this protocol is intended to assess a vaporizer device known as the Volcano®. It has been demonstrated in previous experiments that the act of vaporization (i.e., $\triangle$T NMT 220^{o}C) versus combustion (i.e. $\triangle$T NLT 240^{o}C) significantly reduces the production of Tar, total particulate matter (TPM) and polynuclear aromatics (PNAs) while delivering a comparable concentration of cannabinoid components. Data obtained as a result of the proposed study design will provide quantitative analytical data associated with the precision, accuracy and robustness of vapor delivery, as expressed at differing temperatures of vaporization. Furthermore data obtained will be provided to Dr. Donald Abrams of USCF to be utilized where applicable in association of Investigational New Drug Application # 68,057 and Investigational New Drug Application # 72,987.

The Volcano® device has been demonstrated to collect vaporized cannabinoid analytes (under controlled conditions) while minimizing and/or eliminating the production of Tar TPM, and PNAs. Analytical data generated during the conduct of the proposed experimentation is anticipated to provide sufficient results such that the assertions associated with the functional outcome of the Volcano® operation can be commented upon. Those assertions evaluated will include (1) Dose precision: The measure of delivery variability (e.g., closeness of fit) from the mean value. (2) Dose accuracy: The measure of delivered dose to the collection device as directly compared to the recovered concentration obtained from solvent extracted material. (3) Device robustness: The outcome of experimental data when system temperatures are deliberately modified. (4) Device efficacy: The comparative isolation and delivery of cannabinoids and medically active terpenoids while reducing and/or eliminating the production of Tar, TPM and PNAs. (5) THC, CBD and terpenoid delivery: The comparative efficiency of delivery for THC ,CBD, and terpenoids using sample from a single source (i.e., NIDA). Experiments will be repeated at different temperatures so as to investigate the relationship between outcome variables and temperature.

2. Introduction:

Investigations of THC, CBD, CBN, PNA, TPM, terpenoid and Tar components evolved by use of vaporizer methodologies will be completed using a device known as a Volcano®. The device will be “loaded” with approximately 200 mg of finely screened, homogeneous marijuana (provided by a pre-determined DEA-regulated source), in which the % moisture has been previously determined using moisture balance methods (attachment 1). Upon loading

of the device (figure 1) the marijuana will be heated to approximately 180-200 °C. Device parameters (temperature and airflow) will be monitored using validated thermocouples and flow meters and the data duly recorded. In previous completed studies, a vaporization/volatilization temperature range of 180-200°C was utilized. It is reported that volatilization of cannabinoids can occur at temperatures as low as 140°C . In an effort to optimize THC delivery with the device, experiments will be repeated at 140-160°C and 160-180°C. It is noted that the device manufacturer documents the temperature accuracy of the device as ± 1.5°C Evolved vapor will be collected in the device collection trap five times sequentially (i.e., five individual "balloon" samples) (n=5). Subsequently, the vapor entrapped by the device will be transferred to either a methanol collection trap (figure 2) or a volatile gas trap (Fisher part # 11-134-190, or equivalent). This process of vapor evolution, collection, and transfer (in-order to generate precision information associated with the Volcano®) will be completed on three (3) consecutive aliquots of screened product (e.g. sample n = 15).

Additionally, to determine the cannabinoid baseline concentration, robustness of the device used for multiple product sources, and the quality, and relative percentage of available cannabinoids (e.g., THC, THCA, CBD, CBN) delivered, experiments will be repeated using standard NIDA marijuana (two sample lots). To measure the concentrations of THC and CBD solvent/Soxlett extraction of extracted analytes of the two raw material products prepared in triplicate (e.g., n=6) will be completed. Final solvent extracts will be assayed using High Performance Liquid Chromatograph-Diode Array-Mass Spectrometry HPLC-DAD-MS) and the extract concentration, and subsequently product potency w/w, will be quantitatively determined using methods of external and internal standardization. External standardization will be completed using high purity references standards of THC, CBD, and CBN of known concentration, while internal standardization will utilize a known concentration of a suitable compound subjected to complete sample extraction therefore accommodating the extraction efficiency of the Target analytes. Direct comparison to the recovered concentration of Target analytes captured during the previously described Volcano® experiments will be documented.

The samples (n=6) collected and solubilized in the methanol trap will subsequently be assayed by a quantitative High Performance Liquid Chromatograph-Diode Array-Mass Spectrometry (HPLC-DAD-MS) analytical method. The samples collected and solubilized in the gas trap will subsequently be assayed by a quantitative Gas Chromatography-Mass Spectrometry (GC-MS) analytical method. The samples collected via glass fiber filter will subsequently be assayed by quantitative gravimetric analysis. This analysis will be used to investigate differences in the vapor of the two different marijuana samples due to their differing consistencies. Additionally the remaining material (following heating and vapor evolution) will be extracted using solvent/ Soxlett extraction techniques and assayed as previously described. Final data collected will be used to in Mass Balance calculations by assessing the total volaitilized and collected analytes versus that left behind in the plant material and subsequently extracted via Soxhlet extraction.

All peaks isolated by means of HPLC-DAD-MS will be evaluated for their MS spectral characteristics and reported according to reporting parameters set forth within this document.

Parameters evaluated:
1) Chromatographic peak resolution
2) Chromatographic peak purity (where applicable)
3) Extraction efficiency (i.e., CV% between multiple samples (6 replicates))
4) Sample concentrations from Volcano® (multiple extractions (15 replicates) ensuring complete recovery)

This protocol is offered and to meet the requirements of cGMP: 21 CFR Part 160.

3. Application:

To characterize the extractable profile over multiple vapor sample collections (n = 15) in Marijuana using vaporizer technology and analyzing the resulting extract by HPLC-DAD-MS, GC-MS, and TPM

4. Reagents, Materials, and Equipment:

A. Water, ASTM Type II
B. Methanol (Chromatographic grade or equivalent)
C. Acetonitrile (Chromatographic grade or equivalent)
D. Toluene (Chromatographic grade or equivalent)
E. Volcano® (Vapormed 78532, Tuttlingen, Germany)
F. 250 mL Volatile gas trap (Fisher part # 11-134-190, or equivalent)
G. Methanol collection trap
H. GAST vacuum pump
I. Tygon® tubing
J. Assorted volumetric glassware (flasks, pipettes, gas tight syringes, etc)
K. Assorted sample analysis vials
L. Assorted sized sized sieve
M. Sartorius Analytical balance (or equivalent)
N. Sartorius Top loader balance (or equivalent)
O. Traceable thermocouple
P. High Performance Liquid Chromatograph-Diode Array-Mass Spectrometer (Hewlett Packard model 1100)
Q. Analytical C18 end capped HPLC column (or equivalent)
R. Marijuana (provided by a pre-determined DEA-regulated source).

5. Standards:

A. Tetrahydrocanbinol (THC) (Aldrich cat # J2753, or equivalent)
B. Canabidiol (CBD) (Aldrich cat # C6395, or equivalent)
C. Canabinol (CBN) (Aldrich cat # C6888, or equivalent)
D. Tetrahydrocanbinol-acid (THCA)
E. Delta-9-Tetrahydrocannabinol-d3 (internal reference standard)
F. Polynuclear-aromatic (PNA) mixed standard (Chem Service, Inc. cat # SP-Chemic-1AMZ, or equivalent)

Note: All reference standards are used on an “as-received” basis

6. Materials & Methods

I. Review the procedure (where appropriate) supplied or approved by the test Sponsor.

II. Inventory any supplies needed, i.e., chromatographic columns, solvents etc.

III. Analyte extraction using a Soxlett extraction system

A. Install triplicate Soxlett extraction units in a suitable fume hood providing airflow of approximately 100 CFM according to appropriate standard operating parameters
B. Finely (removing any hard core material) screen a minimum of 2 samples each (approximately 1 g of marijuana) obtained from NIDA using a suitable sized sieve (all performed in triplicate) thus ensuring a homogeneous test sample.
C. Determine and record the percent moisture content in the prepared marijuana according to Chemic Laboratories standard operating procedure 4.62 (all performed in triplicate).
D. Quantitatively transfer separately 200 ± 5 mg screened marijuana to the Soxlett extraction thimbles in triplicate (n=6).
E. Attach an appropriately sized round bottom flask containing 250 mL of methanol.
F. Heat (to refluxing temperature) the Soxlett extraction system using a controlled heating mantel such that the gentle refluxing of the system is maintained for a period of approximately 4 hours.
G. Remove the heat and allow the system to reduce to ambient room temperature.
H. Remove the methanol collection flask, being careful to rinse the system to remove residual material into the round bottom flask.
I. Quantitatively transfer 1.00 mL of delta-9-Tetrahydrocannabinol-d3 internal reference standard to the round bottom flask.
J. Subject the sample to roTary evaporation until approximately 10 mL of final extract is obtained.
K. Quantitatively transfer the solution to a 50 mL volumetric flask and bring to final volume with methanol.
L. Transfer a 1.5 mL volume to two individual, appropriately sized actinic glass analysis vials with hermetically sealed Teflon® lined closures.
M. The extract solutions are assayed according to HPLC-DAD-MS parameters identified within the study protocol.
N. Quantitative analysis and resultant data is obtained and the available dose mass of the Target analytes is determined.
O. The mean and coefficient of variations is determined and reported. It is anticipated that extraction precision will be established as ± 2% from the mean recovered concentration.

IV. Analyte extraction using Volcano® Precision determination

A. Install the Volcano® (figure 1) in a suitable fume hood providing airflow of approximately 100 CFM.
B. Attach the traceable thermocouple to the vaporizer device such that the operating temperature may be determined and “calibrated” to the device settings.

C. Finely screen approximately 1 g of marijuana (e.g., obtained from NIDA) using a suitable sized sieve and store in individual actinic glass container/closures.
D. Determine and record the percent moisture content in each prepared marijuana sample according to Chemic Laboratories standard operating procedure 4.62 (Attachment 1).
E. Quantitatively transfer 200 ± 5 mg screened marijuana to the vaporizer device according to the manufacturer's instructions (Attachment 2).
F. Attach the vaporizer trap according to the manufacturer's instructions.
G. Turn on the vaporizer unit for the maximum time allowed sufficient to fill the collection trap (time to fill is observed and recorded).
H. Remove the collection trap and transfer the vapor, via vacuum, to a suitable methanol trap containing 50.0 mL methanol (time to transfer is observed and recorded).
I. Quantitatively transfer 1.00 mL of delta-9-Tetrahydrocannabinol-d3 internal reference standard to the 50 mL methanol, yielding an internal standard concentration of ≈200 µg/mL.
J. Repeat procedure G, H, & I sequentially four additional consecutive times, being sure to transfer the vapor to individual solvent traps (e.g., n= 5 samples each product)
K. Transfer 1.5 mL volumes to individual, appropriately sized actinic glass analysis vials with hermetically sealed Teflon® lined closures.
L. The remaining solutions (e.g. approx 48.5 mL) of methanol are transferred to individual appropriately sized actinic glass bottles with hermetically sealed Teflon® lined closures and stored securely under refrigerated conditions.
M. The collection experiment (procedural steps E through L) are repeated two additional consecutive times <u>varying the output energy</u> to the device (i.e., lowest heat setting, middle heat setting and highest heat setting). Therefore a total of 15 vaporized samples are collected.
N. The collection experiment (procedural steps E through L) are repeated two additional consecutive times <u>varying the vacuum</u> by a minimum of 0.5X and 1.5X to the collection device. Therefore a total of 15 vaporized additional samples are collected.
O. The collection experiment (procedural steps E through L) are repeated two additional consecutive times varying the vapor collection time by a minimum of 0.5X and 1.5X. Therefore an additional 15 vaporized samples are collected.
P. The extract solutions are assayed according to HPLC-DAD-MS parameters identified within the study protocol.
Q. Quantitative analysis and resultant data is obtained and the available dose mass of the Target analytes is determined.
R. The mean and coefficient of variations is determined and reported. A precise dose delivery system will be concluded if the precision (CV) outcome is NMT ± 10% from the mean value.
S. Dose accuracy will be determined by direct comparison of resultant quantitative data between the mean recovered concentrations of Target analytes obtained via Soxlett extraction versus Target analytes obtained via vaporization.

V. <u>TAR, Total Particulate Matter (TPM), and PNA</u>

In order to simulate collection of TPM as described in the Federal Trade Commission (FTC) method for burning cigarettes, the Volcano® device will be modified such that a

custom fit adapter equipped with a pre-weighed (W_I) glass-fiber filter of defined pore size (Figure 3) will be attached to the device valve prior to attachment of the balloon.

A. Quantitatively transfer 200 ± 5 mg screened marijuana to the vaporizer device according to the manufacturers instructions (Attachment 2) inclusive of the pre-weighed glass fiber filter.
B. Turn on the vaporizer unit for the maximum time allowed sufficient to fill the collection trap (time to fill is observed and recorded).
C. Determination of TPM: The glass-fiber filter will be removed form the adapter, weighed (W_F), washed with a variety of solvents (polar→ nonpolar), and dried under nitrogen stream to a constant weight (W_I). TPM will be recorded as (W_F-W_I). The combined washes will be reserved for HPLC-DAD-MS analysis.
D. Thermogravimetric analysis: TGA will be used to assess the volatility and thermal stability of TPM (dry weight). TPM weight loss will be measured as heating temperature is increased.
E. Spectrophotometric determination of Tar: Aliquots of vapor emitted by the device and subsequently collected in the methanol collection trap, as well as combined washes of the TPM-impregnated glass-fiber filter will be will be subjected to HPLC-DAD analysis for quantification of UV-absorbable components.
F. GC-MS determination of PNA: Aliquots of vapor emitted by the device and subsequently collected in the methanol collection trap, as well as combined washes of the TPM-impregnated glass-fiber filter will be will be subjected to GC-MS analysis for quantification of PNA components.

7. Standard Preparation

Prior to analysis, consideration of the type of instrumental calibration is discussed with the study sponsor. The use of internal standardization as well as external standardization may be employed.

Prepare reference material stock solutions (where appropriate) in duplicate containing approximately 10.0 mg/mL of each reference standard in methanol as described below. Analytical grade material should be used when available. The two categories of chemical reference standards available are USP/NF (i.e., reference standards which do not need characterization) and non-compendia standards that are purchased through a chemical supplier. These standards should be of the highest purity available and be characterized (i.e. be accompanied with a certificate of analysis and/or purity).

A. Reference Stock Preparation: HPLC-DAD-MS

Delta-9-Tetrahydrocannabinol-d3 Stock Solution: Approximately 1.00 g of Delta-9-Tetrahydrocannabinol-d3 reference standard is quantitatively transferred to a 100 mL volumetric flask and solubilized with approximately 50 mL methanol. Upon complete dissolution of the delta-9-Tetrahydrocannabinol-d3, the solution is brought to final volume with methanol. The final concentration of the internal reference stock solution is approximately 10.0 mg/mL

Approximately 10 mg (e.g. 0.36 mL of 28 mg/mL reference stock solution) of THC reference standard is quantitatively transferred to a 10.0 mL volumetric flask and

solubilized with approximately 5 mL methanol. Upon complete dissolution of the analyte the solution is brought to final volume with methanol. The final concentration of this stock solution is approximately 1.0 mg/mL THC.

Approximately 10.0 mg of THCA reference standard is quantitatively transferred to a 10.0 mL volumetric flask and solubilized with approximately 5 mL methanol. Upon complete dissolution of the analyte the solutions is brought to final volume with methanol. The final concentration of this stock solution is approximately 1.0 mg/mL THCA.

Approximately 10.0 mg of CBN reference standard is quantitatively transferred to a 10.0 mL volumetric flask and solubilized with approximately 5 mL methanol. Upon complete dissolution of the analyte the solutions is brought to final volume with methanol. The final concentration of this stock solution is approximately 1.0 mg/mL CBN.

CBD reference standard is purchased pre-solubilized at a final concentration of approximately 1.0 mg/mL and used as received during preparation of subsequent quantitation standards.

B. Mixed Reference Standard Solutions: HPLC-DAD-MS

Mixed reference standards are prepared as described below:

Target Compound	Stock Conc. (mg/mL)	Initial Volume (µL)	Final Volume (mL)	Standard Conc. (µg/mL)
THC	1	100	10	10.0
CBD	1	100		10.0
CBN	1	100		10.0
THCA	1	100		10.0
Delta-9-Tetrahydrocannabinol-d3	10	75		75.0
THC	1	200	10	20.0
CBD	1	200		20.0
CBN	1	200		20.0
THCA	1	200		20.0
Delta-9-Tetrahydrocannabinol-d3	10	75		75.0
THC	1	400	10	40.0
CBD	1	400		40.0
CBN	1	400		40.0
THCA	1	400		40.0

Delta-9-Tetrahydrocannabinol-d3	10	75		75.0
THC	1	800	10	80.0
CBD	1	800		80.0
CBN	1	800		80.0
Delta-9-Tetrahydrocannabinol-d3	10	75		75.0
THC	1	1600	10	160
CBD	1	1600		160
CBN	1	1600		160
THCA	1	1600		160
Delta-9-Tetrahydrocannabinol-d3	10	75		75.0

Transfer approximately 1.5 mL of each reference to appropriately sized actinic analysis vials and hermetically seal with Teflon® lined caps.

Proper standard solution preparation is demonstrated by a coefficient of variance (CV) of ≤ 5 % for triplicate injections of each standard solution.

C. Reference Stock Preparation: GC-MS
PNA reference stock solution prepared in toluene at a concentration of 2,500 µg/mL is obtained from Chem Service Inc. West Chester, PA 19381-0599 and includes the following analytes:

Naphthalene	Acenaphthylene	Phenanthrene
Acenaphthene	Fluorene	Pyrene
Anthracene	Chrysene	Benzo(a)pyrene
1,2-Benzanthracene	Benzo(k)fluoranthene	Benzo(b)fluoranthene
1,1,2-Benzoperylene	1,2,4,6-Dibenzanthrancene	Indeno [1,2,3-c,d]pyreneFlouranthene

D. Mixed Reference Standard Solutions: GC-MS
Quantitation and LOD reference standards are prepared as described below:

PNA Stock	Stock Conc. (mg/mL)	Initial Volume (µL)	Final Volume (mL)	Standard Conc. (µg/mL)
Quant std.	2500	500	10.0	125
LOD std.	2500	3.00	10.0	2.30

8. Initial Instrumental Conditions

A. HPLC-DAD-MS

Analysis using a reverse phase analytical separation column and an isocratic aqueous-organic solvent mobile phase (25% 0.1% TFA in ASTM type II water, 75% Acentonitrile) over 60 min, including a mobile phase flow rate of 1.00 mL/min, diode array monitoring signals of 205 and 254 nm, MS detector in Total ion chromatography mode scanning from 50 – 1000 AMU

B. GC-MS

Proposed methodology will include but not be limited to: direct injection GC-MS analysis using an DB-XLB analytical separation column (J&W 122-1232, or equivalent: 30M X 250µm X 0.30 µm film), and a thermo-gradient of 110^{o}C to 320^{o}C over 53 min, ramp @ 5^{o}C/ min. Injection and detection port temperatures, split flow ratio, carrier gas and make up gas flow rates, and injection volume (i.e., 1-5 µL) as appropriate. MS detector: Single ion monitoring mode

9. Evaluation Criteria

A. Equilibrate the HPLC-DAD-MS, GC-MS with the analytical column and instrumental conditions until a stable system baseline is achieved. Demonstrate stabilized system by injecting control extraction solvent to evaluate the HPLC system for any artifact peaks. If artifact system peaks are observed at greater than 3X the noise, the appropriate system maintenance must be performed.

B. Linearity – A minimum of five different concentrations (calibration working standards) of the each reference standard (injected in duplicate prior to and following sample assays; n=4 for each concentration) must be used to produce a standard calibration curve. A linear or other appropriate functional relationship that yields a $R^2 \geq 0.985$ for the calibration standards demonstrates that quantitative data may be generated using this regression methodology.

C. The relative standard deviation (RSD) of the replicates of each reference standard concentration is determined as follows: RSD = (Standard Deviation/Mean Area Response)*100. The RSD between the replicate standard injections should be NMT 10%.

D. Standard Response Factors

Calculate Relative Response Factors (RRF) for each reference standard as follows:

Relative Response Factor = Standard Concentration / Peak Area Standard

The CV for the calculation RRF of the standards should be NMT 7.5%.

Integrate all unknown peaks that have an area greater than the lowest detectable concentration standard.

Report the results of each preparation of the samples.

10. Records To Be Maintained

Records to be maintained include, but are not limited to, correspondence and other documents related to the interpretation and evaluation of data, as well as all raw data and documentation generated as a result of the study. A copy of the draft and final reports are maintained with the raw data. The data will include documentation describing (but not limited to), the following:

A. Sample preparation procedures, including all reagents and equipment.
B. Stock solution preparation.
C. Instrumental conditions and actual analysis.
D. Method statistical calculations.
E. Clearly labeled chromatograms, labeled with the study number, sample ID, date of analysis and initials of the analyst.

11. Data Archival

All data is archived in Chemic Laboratories permanent archive as per SOP # 1.15

12. References

"Marijuana and Medicine" (1999): (Rick/ Dale add full reference)

21 CFR Part 210/211, Pharmaceutical Industry GMPs

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Text on Validation of Analytical Procedures*. 10/27/94.

Guidance for Industry Analytical Procedures and Method Validation Chemistry, Manufacturing, and Control Documentation, Draft Guidance, August 2000.

Figure #1

Volcano®

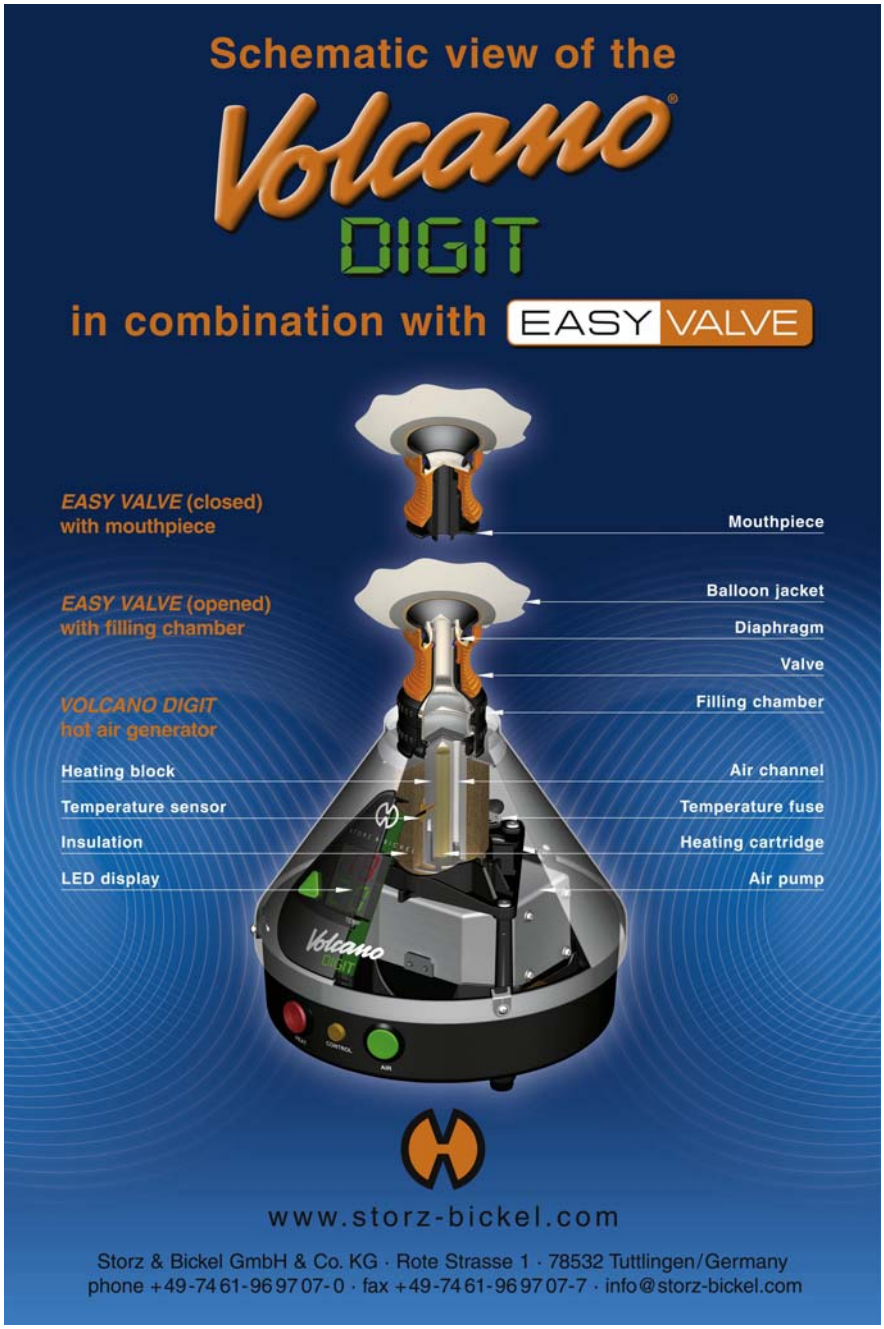


Figure #2

Methanol Collection Trap

Attachment #1

Standard Operating Procedure 4.62
(Uncontrolled Copy)

Operation, Maintenance, and Calibration of OHAUS MB45 Moisture Analyzer

1. Purpose

To provide an outline procedure for the operation, maintenance and calibration of the OHAUS MB45 Moisture Analyzer and to meet the requirements of:

cGMP: 21 CFR Part 211.160
21 CFR Part 820.70
GLP: TSCA – 40 CFR Part 792.8
FIFRA – 40 CFR 160.81
FDA – 21 CFR Part 58.81
OECD – Section 7

2. Application

The OHAUS MB45 Moisture Analyzer performs measurements based on the gravimetric principle, i.e. the moisture is determined from the weight loss of a sample dried by heating. The Moisture Analyzer comprises two instruments: a precision balance and a dryer unit. The halogen dryer unit ensures fast heating of the sample.

This SOP is applicable to the equipment and procedures for the operation, maintenance and calibration of the OHAUS MB45 Moisture Analyzer, serial number 1121051485.

3. Procedures

A. Installation:

1. Do not install the unit near drafts, air conditioning or heating vents, vibrations, or magnetic fields. Install the unit on a level work surface with at least one meter of clearance above.

2. Lift the cover straight up and install the heat shield in the base of the heating chamber.

3. Install the draft shield element (only one position possible) on top of the heat shield.

4. Install the pan support into position. Turn the pan support until it engages. In the locked position, the rear arm of the pan support points directly towards the rear of the analyzer.

5. Adjust the leveling feet at the rear of the Moisture Analyzer until the air bubble in the indicator is centered. The level indicator is located under the cover towards the rear of the analyzer. [NOTE: The instrument should be leveled each time the location is changed.]

6. Connect the power cord supplied to the three-pin connector located at the rear of the Moisture Analyzer and to a power source. The unit will become operational as soon as power is applied. The display will remain on until the On/Off button is pressed. [NOTE: The moisture analyzer is on at all times when connected to a power source. It is the display that may be turned on and off.]

7. Allow the unit to warm up for at least 30 minutes to enable to it to adapt to the ambient conditions.

B. Setup:

1. Press the **Test Menu** button, TEST LIBRARY is displayed. When starting a new test, press the **Enter** button. The screen will change to TEST PARAMETERS.

2. The last test entered will appear and can be changed to a new test. Press the **Enter** button.

3. Using the arrow buttons, enter a test name or identifying number and press the **Enter** button.

4. Using the arrow buttons, scroll to PROFILE and press the **Enter** button. Scroll to highlight the desired drying profile and press **Enter**. The drying profile contains four settings:
Standard – The oven heats to the final temperature, which remains constant for the entire drying time.
Fast – The oven heats past the final temperature for a set amount of time, and then cools to the final temperature for the remainder of the drying time.
Ramp – The temperature and time elapsed between the start of drying and the attainment of the final temperature is set.
Step – The temperature and hold time is defined for each step of the drying process.

5. Using the arrow buttons, scroll to DRY TEMP and press the **Enter** button. Using the arrow buttons, enter the desired drying temperature and press the **Enter** button. The drying temperature can be set from 50 to 200°C.

[NOTE: At temperatures above 180°C, a time limit becomes active. The higher the temperature, the shorter the time until the instrument starts to lower the temperature. If you are working with temperatures above 180°C, wait 2 to 3 minutes with the instrument open between individual measurements to ensure good reproducibility and avoid overheating of the instrument.]

6. Using the arrow keys, scroll to SWITCH OFF and then press the **Enter** button. Then scroll to highlight the desired switch-off profile and press **Enter**. If using the "Auto Free" switch-off profile, use the arrow buttons and enter key to enter the weight and time parameters. Switch-off defines when the instrument ends the drying. The menu offers four different switch-off criteria:
Manual – The measurement process continues until the **Stop** button is pressed.
Timed – The measurement continues until the preset drying time has elapsed.
Auto – Based on a weight loss per unit of time. As soon as the mean weight loss is less than a preset value (<1 mg in 30 seconds, <1 mg in 60 seconds, or <1 mg in 90 seconds) during a specified time, the instrument considers drying as complete and automatically discontinues the measurement process.
Auto Free – Based on a user defined weight loss per unit of time, as described above. (1 to 10 mg, and 10 to 120 seconds)

7. Using the arrow buttons, scroll to TARG. WGT and press the **Enter** button. Target weight is used when previous samples have been tested and the target weight is known. Target weight is a reminder to use a consistent sample size. Using the arrow buttons, define the target weight a press the **Enter** button.

8. To save the test parameters, use the arrow buttons, scroll to SAVE TEST and press the **Enter** button. Using the arrow buttons, select YES or NO and press the **Enter** button. Scroll to select a library number and press **Enter.** If the library number has been previously assigned, "OK TO OVERWRITE" will be displayed. Scroll to select YES or NO and press **Enter**. Press the **Display** button to return to the main display.

C. Operation:

1. The moisture analyzer is in the standby mode when connected to a power source. Use the On/Off button to turn the display on and off during use. The unit needs no warm up time, provided it was in the standby mode (turned off by the power switch).

2. Open the cover on the analyzer. Place the empty sample pan in the pan handler. This is possible without tilting the sample pan if inserted in the pan handler from the side directly below the round flange.

3. Place the pan handler in the sample chamber. Ensure that the tongue of the pan handler fits exactly in the slot of the draft shield element. The sample pan must lie flat in the pan handler.

4. Press the **Tare** button. The unit will be set to zero. A new display appears with instructions.

5. Place the sample in the sample pan. For assistance in sample preparation, consult the instrument manual.

6. Close the cover.

7. Press the **Start/Stop** button. The analyzer starts the drying and measurement process, using the previously entered parameters.

8. A running real time display illustrates the drying process which includes the test ID, set temperature, type of switch-off, initial weight, current temperature in the chamber, actual elapsed time of test, and current moisture content.

9. The **Display** button on the front panel, when pressed repeatedly, accesses each of the five displays during the run mode and cycles through each display.

10. The test automatically stops at the end of the set time. If you want to end the test sooner, press the **Start/Stop** button.

11. Carefully remove the sample pan handler from the drying area. Let the pan and sample cool before removal.

12. To remove the sample pan from the pan handler, lift the pan slightly from below and pull it sideways out of the pan handler. If you no longer need the sample and pan, you can simply tilt the pan handler until the pan slides out.

13. The last display from the test remains on the screen until you press the **Tare** button. This sets the analyzer to zero. The display returns to the first test display, ready to repeat the test.

14. To leave the test mode, simply press any other button on the unit.

15. Data may be displayed during or after a test by pressing the display button. Pressing this button repeatedly scrolls between the screens.

16. Results may be displayed in units of % moisture, % solids, % regain, or grams.

17. Using the arrow buttons, scroll to RESULT and press the **Enter** button. Using the arrow buttons, scroll to highlight the desired result units and press the **Enter** button. Results may also be displayed in custom units. Consult the manual for assistance in developing and entering custom units.

D. Calibration:

Note: These calibration procedures are performed prior to each days use and are documented in the raw data.

1. Temperature:

   a) A temperature kit is needed to perform the calibration. If the unit has been recently used, allow at least 30 minutes before performing the calibration.

   b) Press the **Setup** button. Using the arrow buttons, scroll to TEMP CAL. Press the **Enter** button. You are now prompted to remove the pan handler and pan support. Replace the pan handler and place a temperature calibration unit on the pan handler. [NOTE: The unit will not calibrate with the pan support in place.]

c) Press the **Enter** button to initiate the temperature calibration process. Follow the screen prompts throughout the process. The dryer unit is heated to 100°C. The dryer temperature and count down period are displayed on the screen.

d) After 15 minutes, read the temperature through the inspection window. Use the left/right arrow buttons to highlight ACCEPT NEW CAL, then press the **Enter** button.

e) The dryer now heats to the second temperature (160°). After 15 minutes, read the temperature through the inspection window. Use the left/right arrow buttons to highlight ACCEPT NEW CAL, then press the **Enter** button. The display returns to ANALYZER setup.

f) Remove the calibration unit and replace the pan support and pan handler to their proper positions.

2. Weight:

a) The moisture balance is span calibrated using a 20 g weight.

b) Clear the pan handler, put the sample pan in place, and close the cover.

c) Press the **Setup** button. Using the arrow buttons, scroll to WEIGHT CAL. Press the **Enter** button. Follow the screen prompts.

d) Place the required weight on the sample pan and close the cover.

e) Follow the screen prompts. The screen indicates if the calibration was successful.

f) Press the **Display** button to return to the display. To abort, press the **Start/Stop** button.

Maintenance:

1. Disconnect the instrument from the power supply before cleaning.

2. Open the cover and remove the pan handler, sample pan, draft shield, and heat shield from the instrument .

3. Using a soft, lint-free cloth, clean the exterior of the instrument and the drying compartment components with a mild cleaning agent. Never use abrasive cleaning agents or solvents. Ensure that no liquid enters the interior of the instrument. Replace the components after cleaning.

4. With the cover open, check the protective glass and the temperature sensor for debris which could impede the operation. If the glass appears dirty, clean the surface facing the compartment with a commercial glass cleaner. If the sensor is dirty, clean using a mild cleaning agent.

5. If the inside of the glass is dirty, open the cover and remove the four cover screws. Remove the glass holder and glass from the cover. Clean with a commercial glass cleaner on both sides.

6. The air inlet of the fan is located at the rear of the instrument and its exterior should be cleaned to free it from dust deposits.

DATE EFFECTIVE: _____

APPROVALS:

_____
Author

_____
Manager,
Quality Assurance/Quality Control

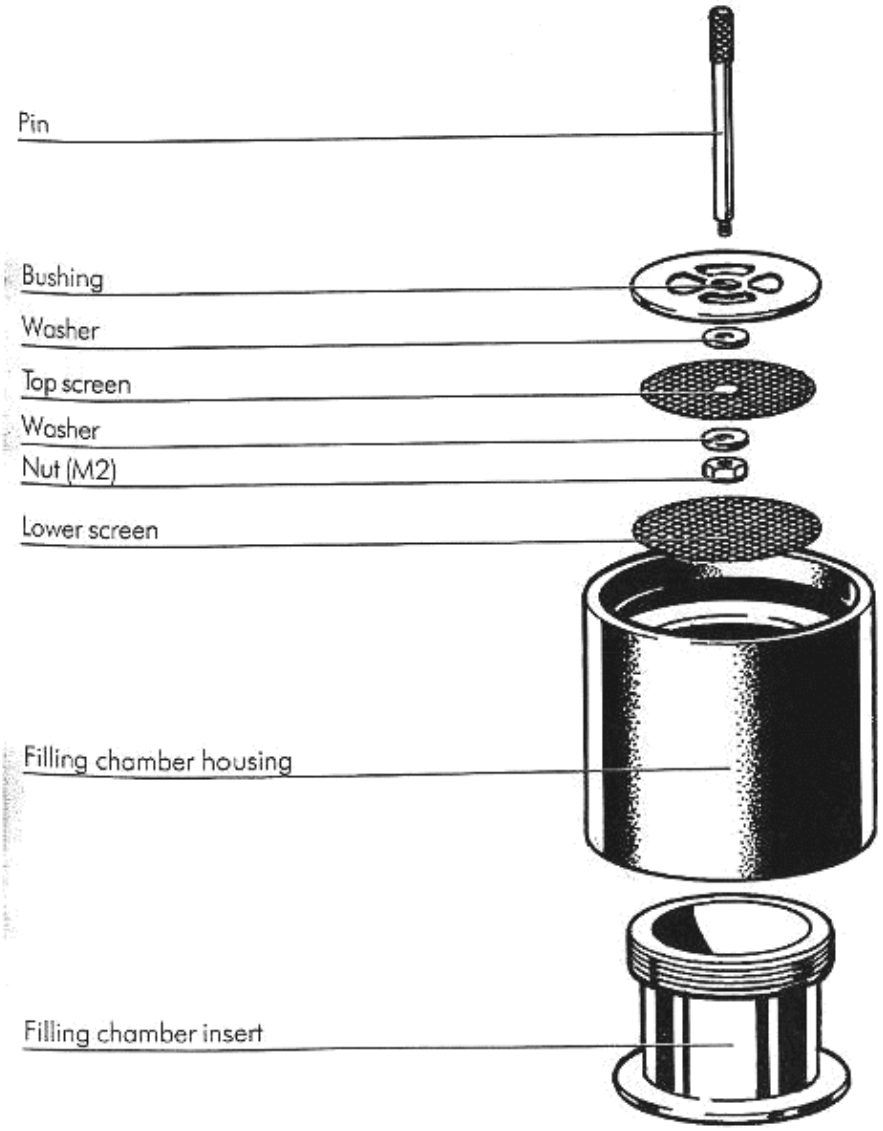
_____
Director of Analytical Services

Attachment #2

Disassembly of the filling chamber:

The pin with the top screen and bushing can easily be removed all in together from the filling chamber. If necessary, the screen can be unscrewed from the pin. The lower screen will be pushed up out of the filling chamber.

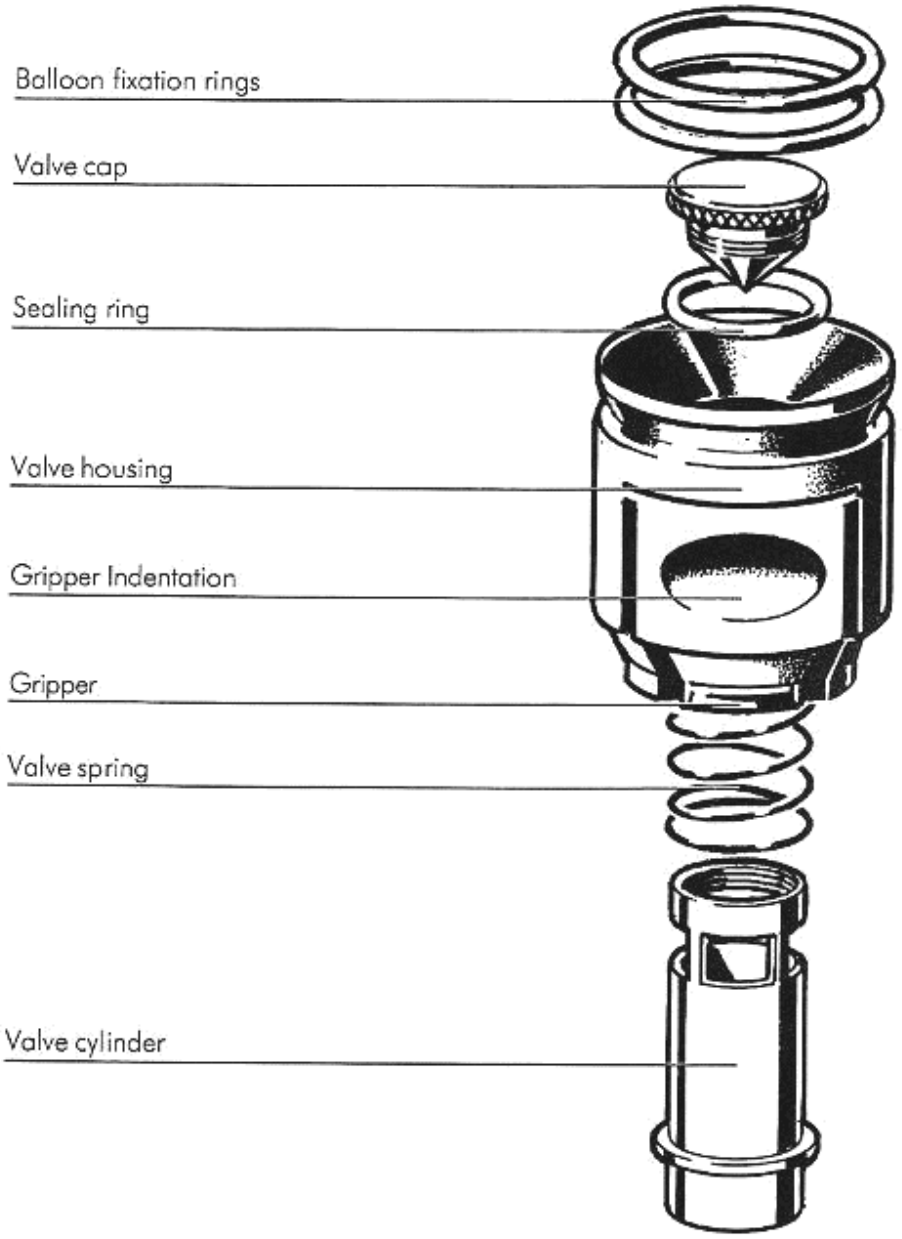
Filling chamber housing and filling chamber insert do not need to be screwed apart for cleaning purposes.
Reassembly in reverse sequence.



Disassembly of the valve

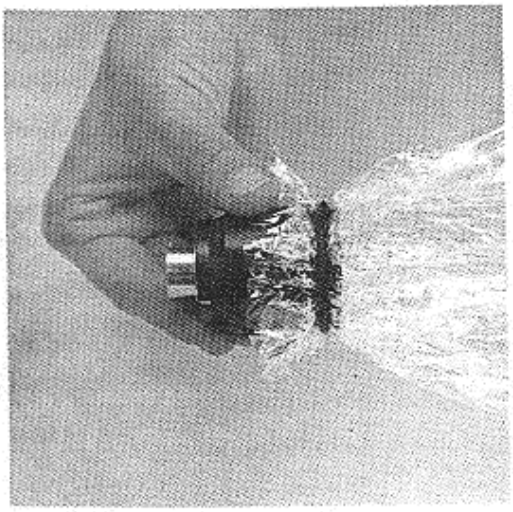
Take the balloon including the fixation rings off. Insert your left index finger into the open end of the valve cylinder. Push the valve cylinder out until you can unscrew the valve cap. Remove the valve cap and sealing ring.

Pull the valve cylinder along with the valve spring out of the valve housing. Reassembly in reverse sequence.

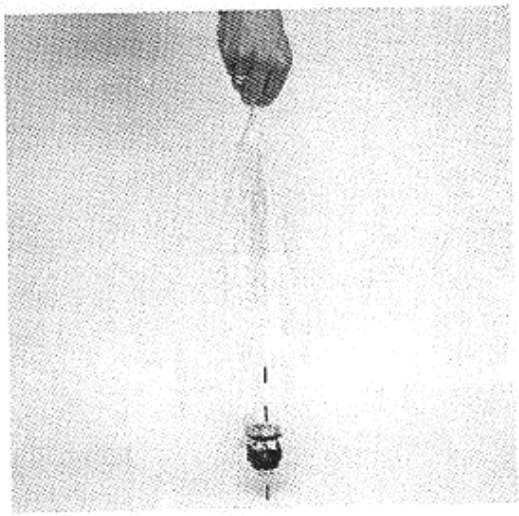


Attachment of the balloon to the valve

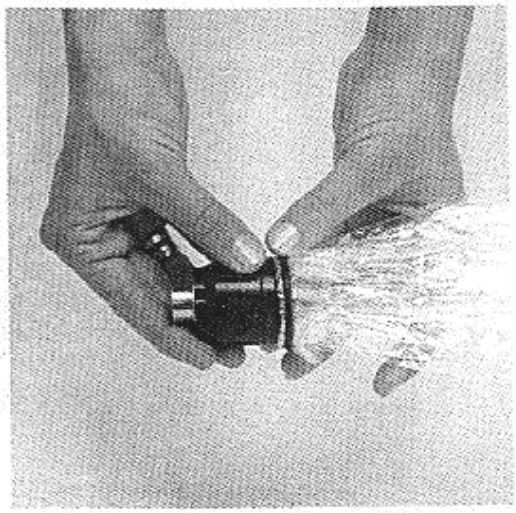
Cut a piece approximately 50-60 cm long and tie it closed at one end using the polyester band included in the package of oven tubes. As the opening of the tube is much wider than the diameter of the valve housing, the oven tube must be folded. To do this pull one of the balloon fixation rings approximately 3 cm over the open end of the oven tube and then pull the first balloon fixation ring with the oven tube over the collar of the valve until it fits into the intended notch. Adjust the folds around the valve collar equally making certain that the valve hangs straight down when the oven tube is held at the end. Bend the remaining end of the tube back. Secure the oven tube by rolling the second balloon fixation ring over both the valve and the first balloon



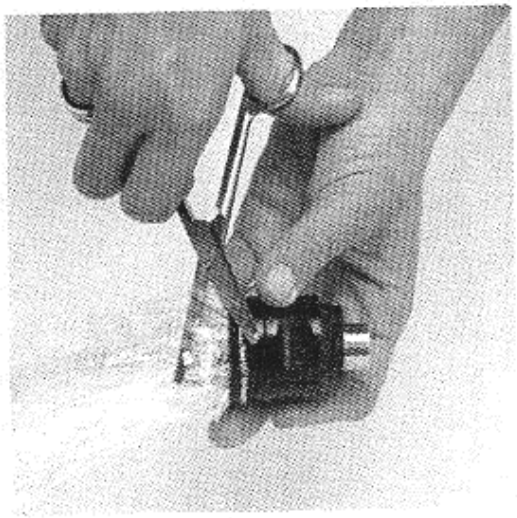
Attaching the oven tube to the valve



Valve shall hang vertical



Securing the oven tube with the second ring



Trimming the excess of the oven tube

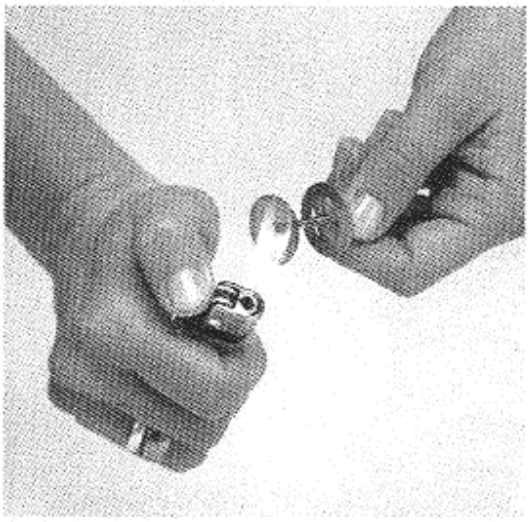
fixation ring so that the first balloon fixation ring is no longer visible and the remaining end of the oven tube is towards the back. If needed, the excess oven tube material can be trimmed away using a pair of scissors, making certain that a hole is not poked into the balloon.

Always use a tasteless, heatproof and foodsafe oven bag, as is used in the food industry, and as is available in nearly every drug store and supermarket. Recommandation: Melitta Toppits Oven tube type "Extra Wide".

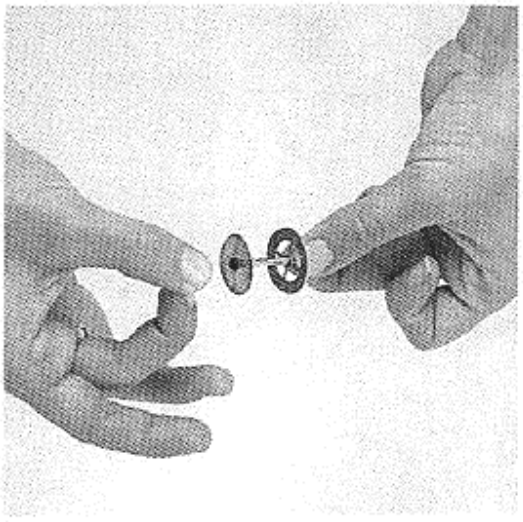
Should you not be able to find such oven tubes, they can of course be ordered directly from us. If you take care of it, the balloon could be used more than 100 times. We recommend though to replace the balloon with a new one after a maximum of 50 uses, respectively after one week at the latest, due to taste reasons.

Screens

Screens obstructed with plant materials can easily be cleaned when you burn the screen using the flame of a lighter and then snip the ashes out of the screen with your index finger.



Burning out the screen



Snipping out the ashes

Attachment #3

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Method Information

MAPS
THC, CBN, CBD Analysis
LC-DAD-MS (Electrospray)

Method Change History

Operator	Date	Change Information
CS	9/20/2002 11:11:22 AM	
CS	9/23/2002 9:57:51 AM	added THC int/calib table
CS	9/23/2002 10:00:24 AM	added CBD int/calib. table
CS	9/23/2002 10:01:19 AM	added CBN int/calib. table

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	off
Save GLP Data:	on
Post-Run Cmd/Macro:	off
Save Method with Data:	skipped - no ACQ running

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

```
=====================================================================
                  1100 High Pressure Gradient Pump 1
=====================================================================

Control
    Column Flow            :      1.000 ml/min
    Stoptime               :        60.00 min
    Posttime               :      Off

Solvents
    Solvent A 1            :      100.0 % (25% 0.1% TFA in H2O, 75% AcN)
    Solvent B 1            :      Off

PressureLimits
    Minimum Pressure       :        0 bar
    Maximum Pressure       :      400 bar


Auxiliary
    Maximal Flow Ramp      :      100.00 ml/min^2
    Compressibility A      :       50*10^-6/bar
    Minimal Stroke A       :      Auto
    Compressibility B      :      115*10^-6/bar
    Minimal Stroke B       :      Auto

Store Parameters
    Store Ratio A          :      Yes
    Store Ratio B          :      Yes
    Store Flow             :      Yes
    Store Pressure         :      Yes


Agilent 1100 Contacts Option
============================

    Contact 1              :      Open
    Contact 2              :      Open
    Contact 3              :      Open
    Contact 4              :      Open


=====================================================================
                  Agilent 1100 Diode Array Detector 1
=====================================================================

Signals

    Signal  Store  Signal,Bw   Reference,Bw  [nm]
      A:     Yes     205  16      425  10
      B:     Yes     254  16      425  10
      C:     No      210   8      360 100
      D:     No      230  16      360 100
      E:     No      280  16      360 100

Spectrum
    Store Spectra          :      All
    Range from             :           205 nm
    Range to               :           400 nm
    Range step             :          2.00 nm
    Threshold              :          1.00 mAU

Time
    Stoptime               :      As pump
    Posttime               :      Off

Required Lamps
```

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

```
        UV lamp required       :      Yes
        Vis lamp required      :      Yes

    Autobalance
        Prerun balancing       :      Yes
        Postrun balancing      :      No
        Margin for negative Absorbance: 100 mAU

    Peakwidth                  :        >  0.1 min
    Slit                       :             4 nm

    Analog Outputs
        Zero offset ana. out. 1:             5 %
        Zero offset ana. out. 2:             5 %
        Attenuation ana. out. 1:          1000 mAU
        Attenuation ana. out. 2:          1000 mAU


    Agilent 1100 Contacts Option
    ============================

        Contact 1              :      Open
        Contact 2              :      Open
        Contact 3              :      Open
        Contact 4              :      Open


    ========================================================================
                            Mass Spectrometer Detector
    ========================================================================


    General Information
    ------- -----------

    Use MSD                  : Enabled
    Ionization Mode          : API-ES
    Tune File                : atunes.tun
    StopTime                 : asPump
    Time Filter              : Enabled
    Data Storage             : Condensed
    Peakwidth                : 0.07 min
    Scan Speed Override      : Disabled


    Signals
    -------

    [Signal 1]

    Polarity                 : Positive
    Fragmentor Ramp          : Disabled

    Scan Parameters

       Time |      Mass Range     |Frag- | Gain|Thres-| Step-
      (min) |   Low    |  High    |mentor| EMV | hold | size
     -------|----------|----------|------|-----|------|-------
        0.00     50.00   1000.00    50    2.0      20  0.10


    Spray Chamber
    ----- -------

    [MSZones]

    Gas Temp                 : 350 C                 maximum 350 C
```

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

DryingGas	: 13.0 l/min	maximum 13.0 l/min
Neb Pres	: 60 psig	maximum 60 psig
VCap (Positive)	: 3500 V	
VCap (Negative)	: 3500 V	

FIA Series

FIA Series in this Method : Disabled

Time Setting
Time between Injections : 0.80 min

Agilent 1100 Column Thermostat 1

Temperature settings

Left temperature	:	25.0°C
Right temperature	:	Same as left
Enable analysis	:	When Temp. is within setpoint +/- 0.8°C
Store left temperature	:	Yes
Store right temperature	:	No

Time

Stoptime	:	As pump
Posttime	:	Off

Column Switching Valve : Column 1

Integration Events

Default Integration Event Table "Event"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ADC"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Detector Default Integration Event Table "Event_FLD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Signal Specific Integration Event Table "Event_MSD1SPC"

Event	Value	Time
Initial Slope Sensitivity(Full Scan)	1.000	Initial
Initial Peak Width(Full Scan)	0.250	Initial
Initial Slope Sensitivity(Cond. Scan/SIM)	0.100	Initial
Initial Peak Width(Cond. Scan/SIM)	0.050	Initial
Initial Area Reject	0.000	Initial
Initial Height Reject	5.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_VWD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ECD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_MWD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Signal Specific Integration Event Table "Event_MSD1TIC"

Event	Value	Time
Initial Slope Sensitivity	200000.000	Initial
Initial Peak Width	0.250	Initial
Initial Area Reject	100000.000	Initial
Initial Height Reject	100000.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_MSD"

Event	Value	Time
Initial Slope Sensitivity	200000.000	Initial
Initial Peak Width	0.100	Initial
Initial Area Reject	200000.000	Initial
Initial Height Reject	10000.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_DAD"

Event	Value	Time
Initial Slope Sensitivity	20.000	Initial
Initial Peak Width	0.200	Initial
Initial Area Reject	150.000	Initial
Initial Height Reject	60.000	Initial
Initial Shoulders	OFF	Initial

Apply Manual Integration Events: No

Advanced Baseline : No

Peak Top Type : parabolic interpolation

Calibration Table

Calib. Data Modified : 9/23/2002 10:01:05 AM

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: DAD1 A, Sig=205,16 Ref=425,10
Signal 2: DAD1 B, Sig=254,16 Ref=425,10
Signal 3: MSD1 TIC, MS File

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
7.190	1	1	0.00000	0.00000	0.00000			CBD
9.450	1	1	0.00000	0.00000	0.00000			CBN
11.140	1	1	0.00000	0.00000	0.00000			THC

Peak Sum Table

No Entries in table

TOPLEVEL PARAMETERS

Method Information For: D:\MSDCHEM\1\METHODS\PNASIMDT.M

Method Sections To Run:

(X) Save Copy of Method With Data
( ) Pre-Run Cmd/Macro =
(X) Data Acquisition
(X) Data Analysis
( ) Post-Run Cmd/Macro =

Method Comments:
residual solvent msd

END OF TOPLEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet: GC
Injection Source: GC ALS
Mass Spectrometer: Enabled

HP6890 GC METHOD

OVEN
Initial temp: 110 'C (On)
Initial time: 1.00 min
Maximum temp: 320 'C
Equilibration time: 0.50 min
Ramps:

#	Rate	Final temp	Final time
1	5.00	320	10.00
2	0.0(Off)		

Post temp: 0 'C
Post time: 0.00 min
Run time: 53.00 min

FRONT INLET (SPLIT/SPLITLESS)
Mode: Splitless
Initial temp: 280 'C (On)
Pressure: 11.20 psi (On)
Purge flow: 30.5 mL/min
Purge time: 2.00 min
Total flow: 34.4 mL/min
Gas saver: On
Saver flow: 15.0 mL/min
Saver time: 2.50 min
Gas type: Helium

BACK INLET (PURGED PACKED)
Initial temp: 280 'C (On)
Pressure: 2.84 psi (On)
Gas type: Helium

COLUMN 1
Capillary Column
Model Number: J&W 122-1232
DB-XLB
Max temperature: 320 'C
Nominal length: 30.0 m
Nominal diameter: 250.00 um
Nominal film thickness: 0.30 um
Mode: constant pressure
Pressure: 11.20 psi
Nominal initial flow: 1.0 mL/min
Average velocity: 38 cm/sec
Inlet: Front Inlet
Outlet: MSD
Outlet pressure: vacuum

COLUMN 2
Capillary Column
Model Number: Restex 85825
RTX-5 widebore
Max temperature: 340 'C
Nominal length: 30.0 m
Nominal diameter: 530.00 um
Nominal film thickness: 5.00 um
Mode: constant flow
Initial flow: 2.6 mL/min
Nominal init pressure: 2.84 psi
Average velocity: 24 cm/sec
Inlet: Back Inlet
Outlet: Back Detector
Outlet pressure: ambient

FRONT DETECTOR (NO DET)

BACK DETECTOR (NPD)
Temperature: 200 'C (Off)
Hydrogen flow: 3.0 mL/min (Off)
Air flow: 60.0 mL/min (Off)
Mode: Constant makeup flow
Makeup flow: 5.0 mL/min (On)
Makeup Gas Type: Helium
Adjust offset: 30.00
Electrometer: On
Bead: Off
Equilibration time: 0.00

SIGNAL 1
Data rate: 20 Hz
Type: back detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: back detector
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
Derive from back detector

COLUMN COMP 2
Derive from back detector

THERMAL AUX 1
Use: MSD Transfer Line Heater
Description: 5973N MSD
Initial temp: 320 'C (On)
Initial time: 0.00 min

#	Rate	Final temp	Final time
1	0.0	(Off)	

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

7673 Injector

Front Injector:

Sample Washes	2	
Sample Pumps	4	
Injection Volume	3.0	microliters
Syringe Size	10.0	microliters
PostInj Solvent A Washes	4	
PostInj Solvent B Washes	0	
Viscosity Delay	0	seconds
Plunger Speed	Fast	
PreInjection Dwell	0.00	minutes
PostInjection Dwell	0.00	minutes

Back Injector:
No parameters specified

MS ACQUISITION PARAMETERS

General Information

Tune File	: atune.u
Acquistion Mode	: SIM

MS Information

Solvent Delay	: 3.50 min
EM Absolute	: False
EM Offset	: 0
Resulting EM Voltage	: 1588.2

[Sim Parameters]

GROUP 1

Group ID	: 1							
Resolution	: Low							
Plot 1 Ion	: 128.0							
Ions/Dwell In Group	(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)		
	(128.0,	100)	(136.0,	100)	(152.0,	100)		
	(154.0,	100)	(166.0,	100)	(178.0,	100)		
	(188.0,	100)	(202.0,	100)	(228.0,	100)		
	(252.0,	100)	(276.0,	100)	(278.0,	100)		

[MSZones]

MS Quad	: 150 C	maximum 200 C
MS Source	: 230 C	maximum 250 C

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: D:\MSDCHEM\1\METHODS\PNASIMDT.M

Percent Report Settings

Sort By: Signal

Output Destination
    Screen: No
    Printer: Yes
    File: No

Integration Events: AutoIntegrate

Generate Report During Run Method: Yes

Signal Correlation Window: 0.020

Qualitative Report Settings

Peak Location of Unknown: Apex

Library to Search	Minimum Quality
DEMO.L	0

Integration Events: AutoIntegrate

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: No

Quantitative Report Settings

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: No

PNASIM1
Calibration Last Updated: Thu Jun 06 13:55:56 2002

Reference Window: 2.00 Minutes
Non-Reference Window: 1.00 Minutes
Correlation Window: 0.10 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

---

1) Acenaphthalene ( )

Ret. Time 6.24 min., Extract & Integrate from 5.74 to 6.74 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

2) Acenaphthene ( )

Ret. Time 6.51 min., Extract & Integrate from 6.01 to 7.01 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

3) Fluorene ( )

Ret. Time 7.39 min., Extract & Integrate from 6.89 to 7.89 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

4) Anthracene ( )

Ret. Time 9.00 min., Extract & Integrate from 8.50 to 9.50 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

5) Phenanthrene ( )

Ret. Time 9.08 min., Extract & Integrate from 8.58 to 9.58 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

6) d-Naphthalene ( )

Ret. Time 3.89 min., Extract & Integrate from 3.39 to 4.39 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

7) d-Anthracene ( )

Ret. Time 9.02 min., Extract & Integrate from 8.52 to 9.52 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

---

8) Fluoranthene ( )

Ret. Time 11.14 min., Extract & Integrate from 10.64 to 11.64 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

```
Lvl ID   Conc ( )  Response
1        not used for this compound

Qualifier Peak Analysis ON
Curve Fit: Linear
---------------------------------------------------------------------------

 9)  Pyrene                                        ( )

Ret. Time  11.52 min., Extract & Integrate from  11.02 to  12.02 min.

Signal       Rel Resp.   Pct. Unc.(rel)    Integration
Tgt     TIC                                *** AUTO ***

Lvl ID   Conc ( )  Response
1        not used for this compound

Qualifier Peak Analysis ON
Curve Fit: Linear
---------------------------------------------------------------------------

10)  Chrysene                                     ( )

Ret. Time  14.41 min., Extract & Integrate from  13.91 to  14.91 min.

Signal       Rel Resp.   Pct. Unc.(rel)    Integration
Tgt     TIC                                *** AUTO ***

Lvl ID   Conc ( )  Response
1        not used for this compound

Qualifier Peak Analysis ON
Curve Fit: Linear
---------------------------------------------------------------------------

11)  Benzo[a]anthracene                           ( )

Ret. Time  14.57 min., Extract & Integrate from  14.07 to  15.07 min.

Signal       Rel Resp.   Pct. Unc.(rel)    Integration
Tgt     TIC                                *** AUTO ***

Lvl ID   Conc ( )  Response
1        not used for this compound

Qualifier Peak Analysis ON
Curve Fit: Linear
---------------------------------------------------------------------------

12)  Benzo[b]fluoranthene                         ( )

Ret. Time  18.69 min., Extract & Integrate from  18.19 to  19.19 min.

Signal       Rel Resp.   Pct. Unc.(rel)    Integration
Tgt     TIC                                *** AUTO ***

Lvl ID   Conc ( )  Response
1        not used for this compound

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```

Qualifier Peak Analysis ON
Curve Fit: Linear
---

13) Benzo[k]fluoranthene ( )

Ret. Time 18.88 min., Extract & Integrate from 18.38 to 19.38 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1	not used for this compound		

Qualifier Peak Analysis ON
Curve Fit: Linear
---

14) Benzo[a]pyrene ( )

Ret. Time 20.35 min., Extract & Integrate from 19.85 to 20.85 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1	not used for this compound		

Qualifier Peak Analysis ON
Curve Fit: Linear
---

15) Indeno[1,2,3-cd]pyrene ( )

Ret. Time 27.51 min., Extract & Integrate from 27.01 to 28.01 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1	not used for this compound		

Qualifier Peak Analysis ON
Curve Fit: Linear
---

16) Dibenzo[a,h]anthracene ( )

Ret. Time 27.90 min., Extract & Integrate from 27.40 to 28.40 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1	not used for this compound		

Qualifier Peak Analysis ON
Curve Fit: Linear
---

17) Benzo[g,h,i]perylene ( )

Ret. Time 29.40 min., Extract & Integrate from 28.90 to 29.90 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt TIC			*** AUTO ***

Lvl	ID	Conc ( )	Response
1		not used for this compound	

Qualifier Peak Analysis ON
Curve Fit: Linear

END OF DATA ANALYSIS PARAMETERS

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Attachment #4

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Method Information

MAPS
THC, CBN, CBD Analysis
LC-DAD-MS (Electrospray)

Method Change History

Operator	Date	Change Information
CS	9/20/2002 11:11:22 AM	
CS	9/23/2002 9:57:51 AM	added THC int/calib table
CS	9/23/2002 10:00:24 AM	added CBD int/calib. table
CS	9/23/2002 10:01:19 AM	added CBN int/calib. table

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	off
Save GLP Data:	on
Post-Run Cmd/Macro:	off
Save Method with Data:	skipped - no ACQ running

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

```
=====================================================================
                  1100 High Pressure Gradient Pump 1
=====================================================================

Control
    Column Flow            :      1.000 ml/min
    Stoptime               :        60.00 min
    Posttime               :      Off

Solvents
    Solvent A 1            :      100.0 % (25% 0.1% TFA in H2O, 75% AcN)
    Solvent B 1            :      Off

PressureLimits
    Minimum Pressure       :        0 bar
    Maximum Pressure       :      400 bar


Auxiliary
    Maximal Flow Ramp      :      100.00 ml/min^2
    Compressibility A      :       50*10^-6/bar
    Minimal Stroke A       :      Auto
    Compressibility B      :      115*10^-6/bar
    Minimal Stroke B       :      Auto

Store Parameters
    Store Ratio A          :      Yes
    Store Ratio B          :      Yes
    Store Flow             :      Yes
    Store Pressure         :      Yes


Agilent 1100 Contacts Option
============================

    Contact 1              :      Open
    Contact 2              :      Open
    Contact 3              :      Open
    Contact 4              :      Open


=====================================================================
                  Agilent 1100 Diode Array Detector 1
=====================================================================

Signals

    Signal  Store  Signal,Bw   Reference,Bw  [nm]
      A:     Yes     205  16      425  10
      B:     Yes     254  16      425  10
      C:     No      210   8      360 100
      D:     No      230  16      360 100
      E:     No      280  16      360 100

Spectrum
    Store Spectra          :      All
    Range from             :           205 nm
    Range to               :           400 nm
    Range step             :          2.00 nm
    Threshold              :          1.00 mAU

Time
    Stoptime               :      As pump
    Posttime               :      Off

Required Lamps
```

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

```
        UV lamp required       :      Yes
        Vis lamp required      :      Yes

    Autobalance
        Prerun balancing       :      Yes
        Postrun balancing      :      No
        Margin for negative Absorbance: 100 mAU

    Peakwidth                  :        >  0.1 min
    Slit                       :             4 nm

    Analog Outputs
        Zero offset ana. out. 1:             5 %
        Zero offset ana. out. 2:             5 %
        Attenuation ana. out. 1:          1000 mAU
        Attenuation ana. out. 2:          1000 mAU


    Agilent 1100 Contacts Option
    ============================

        Contact 1              :      Open
        Contact 2              :      Open
        Contact 3              :      Open
        Contact 4              :      Open


    ========================================================================
                            Mass Spectrometer Detector
    ========================================================================


    General Information
    ------- -----------

    Use MSD                  : Enabled
    Ionization Mode          : API-ES
    Tune File                : atunes.tun
    StopTime                 : asPump
    Time Filter              : Enabled
    Data Storage             : Condensed
    Peakwidth                : 0.07 min
    Scan Speed Override      : Disabled


    Signals
    -------

    [Signal 1]

    Polarity                 : Positive
    Fragmentor Ramp          : Disabled

    Scan Parameters

       Time |      Mass Range     |Frag- | Gain|Thres-| Step-
      (min) |   Low    |  High    |mentor| EMV | hold | size
     -------|----------|----------|------|-----|------|-------
        0.00     50.00   1000.00    50    2.0      20  0.10


    Spray Chamber
    ----- -------

    [MSZones]

    Gas Temp                 : 350 C                 maximum 350 C
```

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

DryingGas	: 13.0 l/min	maximum 13.0 l/min
Neb Pres	: 60 psig	maximum 60 psig
VCap (Positive)	: 3500 V	
VCap (Negative)	: 3500 V	

FIA Series

FIA Series in this Method : Disabled

Time Setting
Time between Injections : 0.80 min

Agilent 1100 Column Thermostat 1

Temperature settings

Left temperature	:	25.0°C
Right temperature	:	Same as left
Enable analysis	:	When Temp. is within setpoint +/- 0.8°C
Store left temperature	:	Yes
Store right temperature	:	No

Time

Stoptime	:	As pump
Posttime	:	Off

Column Switching Valve : Column 1

Integration Events

Default Integration Event Table "Event"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ADC"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

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Detector Default Integration Event Table "Event_FLD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Signal Specific Integration Event Table "Event_MSD1SPC"

Event	Value	Time
Initial Slope Sensitivity(Full Scan)	1.000	Initial
Initial Peak Width(Full Scan)	0.250	Initial
Initial Slope Sensitivity(Cond. Scan/SIM)	0.100	Initial
Initial Peak Width(Cond. Scan/SIM)	0.050	Initial
Initial Area Reject	0.000	Initial
Initial Height Reject	5.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_VWD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ECD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_MWD"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Signal Specific Integration Event Table "Event_MSD1TIC"

Event	Value	Time
Initial Slope Sensitivity	200000.000	Initial
Initial Peak Width	0.250	Initial
Initial Area Reject	100000.000	Initial
Initial Height Reject	100000.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_MSD"

Event	Value	Time
Initial Slope Sensitivity	200000.000	Initial
Initial Peak Width	0.100	Initial
Initial Area Reject	200000.000	Initial
Initial Height Reject	10000.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_DAD"

Event	Value	Time
Initial Slope Sensitivity	20.000	Initial
Initial Peak Width	0.200	Initial
Initial Area Reject	150.000	Initial
Initial Height Reject	60.000	Initial
Initial Shoulders	OFF	Initial

Apply Manual Integration Events: No

Advanced Baseline : No

Peak Top Type : parabolic interpolation

Calibration Table

Calib. Data Modified : 9/23/2002 10:01:05 AM

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Method: D:\HPCHEM\1\METHODS\MAPS40.M of 9/23/2002 10:01:19 AM

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: DAD1 A, Sig=205,16 Ref=425,10
Signal 2: DAD1 B, Sig=254,16 Ref=425,10
Signal 3: MSD1 TIC, MS File

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
7.190	1	1	0.00000	0.00000	0.00000			CBD
9.450	1	1	0.00000	0.00000	0.00000			CBN
11.140	1	1	0.00000	0.00000	0.00000			THC

Peak Sum Table

No Entries in table