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A Liquid Chromatography Procedure for Analysis of Nicotine on Cellulose Acetate Filters *

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SUMMARY

A procedure has been developed for filter analysis whereby the cellulose acetate is dissolved in acetonitrile to release any trapped nicotine. Dissolving the filter eliminates time consuming steam distillation or solvent extraction steps and assures that the recovery of nicotine is complete. After the filter is dissolved, the cellulose acetate is precipitated by addition of an amine-phosphate buffer and an aliquot of the filtered solution is analyzed by high-pressure liquid chromatography (HPLC).

Two methods of HPLC analysis are described. In both cases the separation is achieved on a cyano-bonded silica column and detection is by ultraviolet absorption at 254 nm. Different mobile phases are used in the two methods. In the first procedure, a diethylamine-phosphate buffer at pH 7.56 is used while in the second procedure, a dimethylamine-phosphate buffer at pH 3.00 is used. Analytical results are equivalent for both chromatographic methods, but the second procedure may offer extended analytical column life. Results of a study relating the structure of the amine in the mobile phase to nicotine retention are presented.

The amount of nicotine trapped on cellulose acetate filters during smoking was determined with increasing intervals between smoking and analysis. These results demonstrate that nicotine is stable on filters and previous problems of analysis were caused by difficulty in removal from aged filters.

ZUSAMMENFASSUNG

Es wird über eine neue Methode zur Untersuchung von Zigarettenfiltern berichtet, bei der das gesamte im Filter retinierte Nicotin durch Lösen des Celluloseacetates in Acetonitril freigesetzt wird. Das Auflösen des Filters erspart zeitaufwendige Wasserdampfdestillations- oder Lösungsmittelextraktionsschritte und gewährleistet eine vollständige Rückgewinnung des Nicotins. Nach dem Auflösen des Filters wird das Celluloseacetat durch Zugabe eines Amin/Phosphat-Puffers ausgefällt. Ein aliquoter Teil der filtrierten Lösung wird mittels Hochdruckflüssigchromatographie (HPLC) analysiert.

Die Hochdruckflüssigchromatographie ist auf zweierlei Art durchführbar. In beiden Fällen erfolgt die Trennung an einer cyangebundenen Silikasäule und die UV-Detektion bei einer Wellenlänge von 254 nm. Als mobile Phase wird im einen Fall ein Diethylamin/Phosphat-Puffer mit einem pH-Wert von 7,56 und im anderen Fall ein Dimethylamin/Phosphat-Puffer mit einem pH-Wert von 3,00 verwendet. Die Analyseergebnisse sind bei beiden Chromatographie-Varianten gleich, wobei die Säule bei der letztgenannten Art etwas länger benutzt werden kann. Es wurde untersucht, welche Bedeutung die in der mobilen Phase verwendete Aminstruktur für die Nicotinretention hat; die Ergebnisse werden dargelegt.

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Die im Celluloseacetatfilter retinierte Nicotinmenge wurde in Abhängigkeit vom Zeitintervall zwischen Abrauchen und Analyse bestimmt. Es zeigte sich, daß das Nicotin im Filter stabil ist und daß in früheren Untersuchungen aufgetretene Probleme darauf zurückzuführen sind, daß es sich bei gealtertem Filter schwerer extrahieren läßt.

RÉSUMÉ

Ce rapport concerne une nouvelle méthode d'examen des filtres de cigarettes qui permet de libérer toute la nicotine retenue dans le filtre par dissolution de l'acétate de cellulose dans l'acétonitrile. La dissolution du filtre évite d'avoir recours à des procédés demandant beaucoup plus de temps, comme l'entraînement à la vapeur d'eau ou l'extraction par solvant, tout en garantissant la récupération intégrale de la nicotine. Après la dissolution du filtre, l'acétate de cellulose est précipité par addition d'une solution tampon amino-phosphatée. Une partie aliquote de la solution filtrée est analysée par chromatographie en phase liquide à haute pression (HPLC).
La chromatographie en phase liquide à haute pression peut être effectuée de deux façons. Dans les deux cas, la séparation est réalisée dans une colonne de silice cyanoliée, et la détection UV à une longueur d'onde de 254 nm. On utilise comme phase mobile dans un cas un tampon de phosphate de diéthylamine à un pH de 7,56 et, dans l'autre, un tampon de phosphate de diméthylamine à un pH de 3,00. Quelle que soit la méthode employée, les résultats de l'analyse sont équivalents; la colonne peut servir un peu plus longtemps dans le cas de la seconde méthode. Une étude, dont les résultats sont présentés, a porté sur la relation existant entre la structure de l'amine utilisée dans la phase mobile et la rétention de la nicotine.
La quantité de nicotine retenue dans le filtre d'acétate a été déterminée en fonction de l'intervalle de temps séparant le fumage de l'analyse. Il en résulte que la quantité de nicotine contenue dans le filtre est stable et que les problèmes qui s'étaient posés lors d'examens effectués dans le passé, étaient imputables au fait qu'il est difficile d'extraire la nicotine de filtres ayant vieilli.

INTRODUCTION

A major motivation for development of this procedure was to determine if nicotine collected on cellulose acetate filter material during smoking is stable.
Nicotine is an important constituent of tobacco and tobacco smoke. Because of this importance numerous analyses for this alkaloid have been developed. Procedures for measurement of leaf and smoke nicotine are well established and their accuracy and reliability well known. Analyses of nicotine deposited on cellulose acetate filters are less well developed; however, these measurements are important for studying filtration efficiency and human smoking. Procedures used for determination of filter nicotine include solvent extraction, steam distillation, or a combination of these methods. Most of these procedures require removing nicotine from the physically intact filter. They are time consuming and sometimes unreliable. For instance, *Ohnishi* and co-workers (1) have reported that nicotine collected on butt filter tips is not completely extracted by isopropyl alcohol. However, in a model experiment in which pure nicotine was adsorbed by a filter tip, nicotine was completely extracted. They suggested that some substance in cigarette smoke is interfering with this extraction. In our laboratory, we found that the age of the filter butts prior to extraction with an organic solvent is a major variable. Butts extracted soon after smoking give higher nicotine values than samples aged before extraction. Two reasons were proposed for this difference. Either nicotine is unstable on filter material or it migrates into the cellulose acetate fiber and becomes more difficult to remove upon aging. If migration into the filter tow is the problem, an analysis which dissolves the cellulose acetate would give more consistent results. A *Coresta* procedure has previously been published whereby cellulose acetate filters are dissolved in acetone prior to steam distillation for the analysis of nicotine (2). Also, *Curran* and *Miller* (3) have removed ^{14}C-labeled materials from filters prior to scintillation spectrometry analysis by dissolving the cellulose acetate in a methylene chloride - methanol solution. This was done to insure that removal of the labeled material from the filter was complete.

EXPERIMENTAL

Sample Preparation

The filter butts from five cigarettes are placed in a 125 ml Erlenmeyer flask. To this flask is added 50 ml of acetonitrile (Burdick and Jackson, distilled in glass). The flask is shaken on a mechanical shaker until the cellulose acetate is dissolved. Fifty milliliters of a 0.08 M diethylamine-phosphate buffer is added to the dissolved filter solution. If other buffers are used as the mobile phase in the HPLC, they replace the 0.08 M diethylamine-phosphate buffer for sample preparation. The combined acetonitrile-buffer solution is shaken on a mechanical shaker until precipitation of the cellulose acetate is complete.
The suspension is filtered first through folded filter paper (Schleicher and Schnell (No. 588)) and a 4 ml aliquot of this filtrate is further filtered through a Millipore Model FHLP 01300 (0.5 µm) disposable filter. A 15 µl aliquot of this solution is analyzed by HPLC.
Estimated time for sample preparation is 10—15 minutes. However, a number of samples may be prepared simultaneously.

Figure 1.
Filter butt nicotine analysis with a mobile phase of 80% 0.08 M diethylamine-phosphate buffer and 20% acetonitrile.

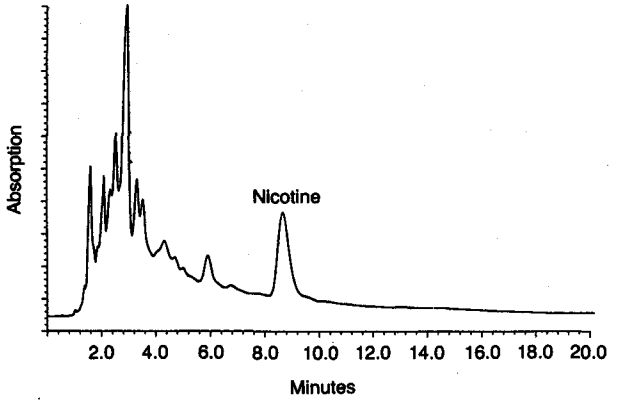
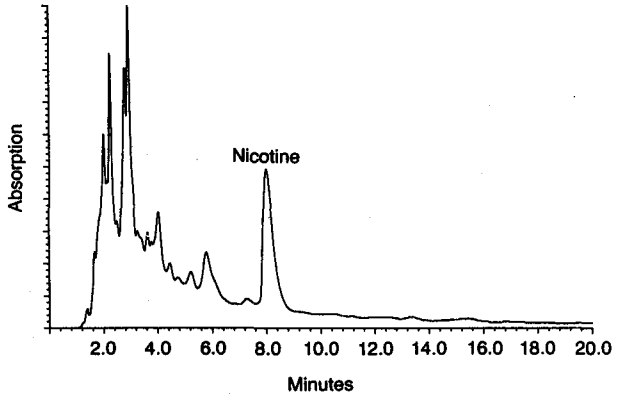


Figure 2.
Filter butt nicotine analysis with a mobile phase of 70% 0.01 M dimethylamine-phosphate buffer and 30% acetonitrile.



Nicotine Standard Preparation

An external standard is prepared by addition of 3.776 mg nicotine (Eastman, 99% pure) to a 50 ml acetonitrile solution of five unused cellulose acetate filters. Addition of the buffer and filtration are the same as in sample preparation.

Preparation of 0.08 M diethylamine-phosphate buffer at pH 7.56: Thirty ml of diethylamine (Aldrich, reagent grade) is added to 3.5 l of distilled water. This solution is adjusted to pH 7.56 (4) by addition of 85% phosphoric acid (Mallinckrodt, ACS grade).

Preparation of 0.01 M amine-phosphate buffer at pH 3.0: The procedure described below is generalized for the preparation of most amine-phosphate buffers. For example, if a 0.01 M dimethylamine buffer is desired, then dimethylamine would be used as the amine in the procedure.

Weigh one-tenth mole of amine into a 100 ml flask and add 70 ml of distilled water. Adjust the pH to 2.5 with 85% phosphoric acid and dilute to 100 ml with water to prepare a 1.0 M stock solution. The 0.01 M amine-phosphate buffer is prepared by diluting 10 ml of the stock solution to one liter with distilled water. This procedure produces a buffer of pH 3.0 when dimethylamine is the amine used.

HPLC Conditions for 0.08 M Diethylamine-Phosphate Buffer at pH 7.56:

Pump:	Waters Associates (6000A)
Column:	Waters Associates (RCM-CN: 10 µm, 8 mm inside diameter)
Mobile phase:	80% 0.08 M diethylamine-phosphate buffer at pH 7.56 and 20% acetonitrile
Flow rate:	2.0 ml / min
Detector:	fixed wavelength at 254 nm (UV)
Injection volume:	15 µl
Analysis time:	12 minutes

HPLC Conditions for 0.01 M Dimethylamine-Phosphate Buffer at pH 3.0:

Mobile phase:	70% 0.01 M dimethylamine-phosphate buffer at pH 3.0 and 30% acetonitrile

All other chromatographic conditions are the same.

Examples of chromatograms for filter butt nicotine analysis with the two different mobile phases are shown in Figures 1 and 2.

RESULTS AND DISCUSSION

Sample Preparation

In many ways, the development of the sample preparation procedure is based upon conditions necessary for the HPLC analysis. For instance, it is desirable that the sample be in a solution similar to the HPLC mobile phase. When the amine-phosphate buffer is added to the sample, the cellulose acetate is precipitated from solution and removed by filtration. Water can be used in place of the buffer to precipitate the cellulose acetate, but an unfilterable gel is formed. Precipitation and removal of the cellulose acetate are important for protection of the chromatographic system, but it also appears that the combination of acetonitrile and amine-phos-

Table 1.
Comparison of nicotine recoveries from solutions of acetonitrile and acetonitrile plus diethylamine-phosphate buffer.

Sample No.	Acetonitrile	Acetonitrile plus buffer
	nicotine (mg/filter)	nicotine (mg/filter)
1	0.60	0.68
2	0.77	0.91
3	0.43	0.68
4	0.53	0.74
5	0.47	0.79
6	0.50	0.91
7	0.54	0.71
8	0.52	0.80
9	0.54	0.68
10	0.42	0.82
Mean	0.53	0.77
Relative standard deviation (%) *	18.7	11.6

* Relative standard deviation (%) = standard deviation × 100 / mean.

phate buffer gives higher recovery of nicotine than acetonitrile alone. This is shown by the results in Table 1.

The Liquid Chromatography Procedure

Development of the liquid chromatographic procedure for analysis of filter butt nicotine is based upon earlier work on tobacco alkaloids by *Piade* and *Hoffmann* (4). They used reverse-phase chromatography on an octadecylsilane-modified silica column with a mobile phase consisting of acetonitrile and a 0.07 M triethylamine-phosphate buffer at pH 7.56. We found that for filter nicotine analysis a cyano-bonded silica column and solvent system consisting of acetonitrile and 0.08 M diethylamine-phosphate buffer at pH 7.56 give more symmetrical chromatographic peaks.

Quantitation is achieved by an external standard method in which a known amount of nicotine is added to a solution of five unused filters dissolved in 50 ml acetonitrile. Blanks have been run on unused filters to insure that there is no interfering material from that source. Also, experiments have been performed which show that, when nicotine is injected into unused filters, the recovery is complete. Detector response is linear over the range of 0.1—100 mg nicotine per sample.

Although no problems in column life have been experienced, the 7.56 pH of the mobile phase is dangerously close to the column limit of pH 8.0 recommended by the column manufacturer. Waters Associates (5) have published procedures for analysis of amines with a mobile phase of acetonitrile and di-*n*-butylamine-phosphate buffer at pH 3.0. This system does not work for filter butt analysis because the nicotine is not well separated from other smoke constituents which absorb at 254 nm. A study of nicotine retention versus the structures of amine used in the buffer showed that the elution of nicotine relative to other smoke constituents can be modified. Results of this study are shown in Table 2.

Table 2.
Retention of nicotine versus structure of amine used in a mobile phase of 0.01 M amine-phosphate in 80% water and 20% acetonitrile at pH 3.0.

Amine	Retention time of nicotine (min)
Di-*n*-butyl	2.39
Triethyl	4.42
Diethyl	6.67
Dimethyl	10.10
Ammonium phosphate	15.89

Of course, the mechanism by which nicotine is retained in these chromatographic systems is quite complex and not well understood, but the bulk of the amine hydrocarbon group may interfere with interactions between the sample and surface of the column material. Thus, as the amine hydrocarbon group becomes smaller, interaction between nicotine and column material increases and retention is longer. As a result of this study, dimethylamine-phosphate buffer was found to give excellent separation of nicotine from other smoke components in filter nicotine analysis. Details of sample preparation are the same for both buffers except in one procedure 0.08 M diethylamine-phosphate at pH 7.56 is used while in the other procedure 0.01 M dimethylamine-phosphate buffer at pH 3.0 is used.

Analysis of filter butt nicotine is equivalent with either of the amine-phosphate buffer systems described. Because of its lower pH, the acidic buffer may offer an improvement in analytical column life, but this has not been demonstrated in practice. For the remaining discussion, data are presented that have been generated from both procedures. Since the procedures are equivalent, no effort is made to differentiate the results based upon analysis method.

Analytical Results

To establish reproducibility of the chromatographic analysis, one sample of filter butts was analyzed five times. These results are shown in Table 3. These data show that the details of the chromatographic analysis including injection, chromatography, and detection are very reproducible. Variation in the analysis of filter butt nicotine from unselected cigarettes was established by smoking 50 cigarettes under standard smoking conditions and dividing the butts into 10 samples. Results of this analysis are shown in Table 4. They show that much more variation exists in the amount of filter nicotine due to sample non-uniformity than from the chromatography. To reduce sample variability, 50 cigarettes selected in the pressure drop range of 129—144 mm water at a flow rate of 17.5 cm^3/s were

Table 3.
Reproducibility of chromatographic analysis.

Analysis No.	Nicotine/filter (mg)
1	0.704
2	0.689
3	0.701
4	0.691
5	0.696
Mean:	0.695 mg
Standard deviation:	0.009 mg
Relative standard deviation (%) *:	1.29

* Relative standard deviation (%) = standard deviation × 100 / mean.

Table 4.
Analysis of filter butt nicotine from unselected cigarettes.

Analysis No.	Nicotine/filter (mg)
1	0.796
2	0.779
3	0.727
4	0.727
5	0.607
6	0.619
7	0.671
8	0.734
9	0.719
10	0.649
Mean:	0.700 mg
Standard deviation:	0.060 mg
Relative standard deviation (%) *:	8.57

* Relative standard deviation (%) = standard deviation × 100 / mean.

Table 5.
Analysis of filter nicotine from pressure drop selected cigarettes.

Analysis No.	Nicotine/filter (mg)
1	0.733
2	0.727
3	0.652
4	0.699
5	0.680
6	0.731
7	0.671
8	0.681
9	0.709
10	0.686
Mean:	0.697 mg
Standard deviation:	0.028 mg
Relative standard deviation (%) *:	4.02

* Relative standard deviation (%) = standard deviation × 100 / mean.

Table 6.
Analysis of nicotine on aged filter butt samples.

Age	Nicotine/filter * (mg)
Fresh, room (25 °C)	0.70 ± 0.03
1-day, room (25 °C)	0.69 ± 0.03
3-day, room (25 °C)	0.73 ± 0.04
6-day, room (25 °C)	0.71 ± 0.03
30-day, room (25 °C)	0.71 ± 0.04
30-day, freezer (—25 °C)	0.72 ± 0.04

* Average of triplicate samples.

smoked and the filter butts analyzed. These data are shown in Table 5. The values in this table show that about one half of the sample variation for filter butt nicotine can be eliminated by pressure drop selection of the cigarettes. Undoubtedly, further improvement in sample variability could be made by weight as well as pressure drop selection of test cigarettes; however, this has not been necessary for our current studies.

Nicotine on Aged Filter Butt Samples

Earlier studies with a non-dissolving solvent extraction procedure showed that fresh filter butts give consistently higher levels of nicotine than similar aged samples. It was unknown whether nicotine is not stable on cellulose acetate containing other smoke constituents or whether the nicotine is merely more difficult to extract because of migration into the filter tow. Reaction with other smoke constituents might also make the aged filter butt nicotine more difficult to extract. To investigate the stability of filter butt nicotine, a sample of pressure-drop selected cigarettes (122—144 mm water) was smoked under standard Federal Trade Commission (FTC) smoking conditions. Butts were collected, randomized, and analyzed in triplicate after periods of one, three, six and thirty days at room temperature. Samples were also stored in capped vials at —25 °C for thirty days. Results of these analyses are shown in Table 6. These values show conclusively that nicotine trapped on cellulose acetate filters during the smoking of a cigarette is stable at room temperature. Interestingly enough, even though the 30-day room and freezer samples ranged in color from dark brown to light tan, the level of nicotine was the same. This color difference would indicate that smoke compounds other than nicotine are not stable for a long period. Problems that have been encountered with the analysis of aged filter butts by other methods apparently are due to incomplete extraction of the nicotine.

Variation of Butt Nicotine with Puff Volume

To further illustrate the utility of the method, a study was made of nicotine collected on the filter for ciga-

Table 7.
Filter butt nicotine at various puff volumes.

	Puff volume (ml)		
	20	35	65
Filter nicotine (mg/filter):			
Brand A	0.64 ± 0.04	0.77 ± 0.05	1.07 ± 0.08
Brand B	0.62 ± 0.03	0.77 ± 0.04	1.10 ± 0.08
Smoke nicotine (mg/cigarette):			
Brand A	0.68 ± 0.01	1.04 ± 0.02	1.53 ± 0.04
Brand B	0.72 ± 0.03	1.12 ± 0.03	1.67 ± 0.02
Filter efficiency (%):			
Brand A	48	43	41
Brand B	46	41	40

rettes smoked under standard smoking conditions of 2-second puff duration at an interval of once a minute but at puff volumes of 20, 35 and 65 milliliters. Results of these analyses are shown in Table 7. Quite obviously, these data show that smoke nicotine increases with puff volume. Changing puff volume may also affect filter efficiency (6). A greater part of the nicotine is retained by the filter at lower puff volumes. However, for the products tested, changing the puff volume from 35 to 65 ml did not greatly affect filter efficiency. Apparently, changes in filter efficiency are greatest at puff volumes less than 35 milliliters.

The data in Table 7 demonstrate at least one problem which may be encountered while attempting to relate the amount of nicotine a smoker receives to filter butt nicotine. Since filter efficiency changes with puff volume, the size of the smoker's puff can make considerable difference in the relationship between filter butt and smoke nicotine. Other human smoking parameters which may be important in the relationship between filter butt and smoke nicotine include puff frequency, duration, butt length, puff shape, and non-uniform frequency. All of these factors combine to make the prediction from filter butt analysis of nicotine received by a smoker only an approximation. More refined measurements of smoking parameters are necessary to correct filter butt values before accurate prediction of smoke nicotine can be made.

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