ANALYSIS OF CITRAL DIMETHYL ACETAL AND OTHER ACETALS BY HPLC[1]

TOBACCO SCIENCE

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Citral dimethyl acetal (CDA) has been patented as a potential cigarette flavorant. This material was applied to cellulose acetate filter tow, which was then analyzed to determine the amounts of both CDA and citral, its hydrolysis product. Unfortunately, the silica substrates used as supports for reversed-phase HPLC columns were sufficiently acidic to cause rapid hydrolysis of CDA and other acetals, thus making the determination impossible. Incorporating a small amount of a basic material in the HPLC solvent prevented hydrolysis. This allowed determination of both the unchanged acetal as well as the aldehyde produced by hydrolysis during aging of the impregnated filter tow. HPLC solvents consisting of 60% acetonitrile and 40% of an aqueous solution of bases, such as 5 mM and 10 mM ammonia or triethylamine, were evaluated. A UV diode array detector was used for HPLC detection of the unsaturated aldehydes citral, benzaldehyde, methacrolein, and senecialdehyde and their dimethyl acetals. Comparisons were made of the relative stability of the acetals on columns packed with C8 and C18 bonded to Nova-Pak, Hypersil, and Zorbax.

Additional key words: Citral dimethyl acetal, cigarette flavor, filter tow plasticizer, HPLC analysis.

INTRODUCTION

Citral dimethyl acetal (CDA) is a useful cigarette flavor, especially when it is incorporated into filter tow (5). In cigarette manufacture CDA can be added to the plasticizer during filter making. Because CDA hydrolyzes to citral on exposure to atmospheric moisture, we were interested in developing a reversed-phase high pressure liquid chromatography (HPLC) method, using ultraviolet (UV) detection, to analyze simultaneously for CDA and citral. We wished to determine these components in both impregnated cellulose acetate filters and in the plasticizer used to impregnate the tow.

Only a few methods of HPLC analysis of acetals using UV detection appear in the literature (2,4). These reports should have discouraged us from considering any method that employed an aqueous solvent in the presence of acidic sites such as occur on reversed-phase columns based on silica. It is well known that even mild acids can catalyze the hydrolysis of an acetal or ketal to its constituent aldehyde or ketone and alcohol.

We found that CDA hydrolysis indeed occurred with silica-based reversed-phase columns using acetonitrile: water (ACN:DZW) eluants. It is common HPLC practice to add amines to the mobile phase to decrease the interaction of basic samples with free silanol groups on the column packing (11). We found that a low concentration of ammonia or triethylamine inhibited the hydrolysis of the acetal and made possible the simultaneous analysis of CDA and citral. Also, we briefly investigated the extent of acetal hydrolysis on different HPLC reversed-phase columns and of other unsaturated acetals. It appears that the extent of acetal hydrolysis might be a good technique to add to the arsenal of methods for characterizing the silanophilic interactions of HPLC stationary phases.

MATERIALS AND METHODS

Materials

Citral, methacrolein, senecialdehyde, and benzaldehyde dimethyl acetal were obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Benzaldehyde came from Eastman Kodak Co. (Rochester, N.Y.). Triethylamine was purchased from Fisher Scientific (Pittsburgh, Pa.). CDA, senecialdehyde dimethyl acetal (SDA), methacrolein dimethyl acetal (MDA), and citral diethyl acetal (CDEA) were prepared at Lorillard Tobacco Company by R.F. Dufresne and M.E. Viso. Estrobond G is a product of Tennessee Eastman (Kingsport, Tenn.). The deionized water (DZW), 18 mohs/cm, was prepared using a Milli-Q_{Plus}

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system, Millipore Corp. (Bedford, Mass.). Acetonitrile (UV grade) was obtained from Baxter Healthcare Corp. (Burdick & Jackson Div., Muskegon, Mich.).

HPLC Equipment

The experimental data were obtained via a Hewlett-Packard model 1090M HPLC equipped with UV diode array detector (UV-DAD), an autosampler and an H-P ChemStation (Pascal version). The acetals were monitored at 210 nm and the α,β-unsaturated aldehydes were monitored at 241 nm.

Columns

The following columns were used (particle diameter, id x length): ODS-Hypersil (5 µm, 4 x 125 mm cartridge from Hewlett-Packard Co., Wilmington, Del.) with a guard cartridge of RP-18 on LiChrospher 100 (5 µm, 4 x 4 mm from Hewlett-Packard Co.), Zorbax Rx-C8 (5 µm, 4.6 x 150 mm from MAC-MOD Analytical, Inc., Chadds Ford, Pa.), Zorbax ODS (5 µm, 4.6 x 250 mm from MAC-MOD Analytical, Inc.), Nova-Pak C18 (4 µm, 5 x 100 mm, from Waters Chromatography Division of Millipore Corp., Milford, Mass.), and Nova-Pak C8 (4 µm, 5 x 100 mm, from Waters Chromatography Division of Millipore Corp.).

Methods

For quantitation of CDA and citral, an isocratic HPLC method was set up using the ODS-Hypersil column (60:40 ACN:DZW with 5mM NH_4OH at 1 mL/min as the mobile phase, and 40°C column temperature). A calibration table was established using 10 µL samples and three levels of mixtures containing both CDA and citral. The areas of the two E/Z citral peaks between 2.8 and 3.0 min were summed in the calibration procedure. The E/Z CDA emerged as a single peak at 5.1 min. The CDA was measured with the UV-DAD signal at 210 nm and citral at 241 nm.

For determining the extent of acetal hydrolysis on the various HPLC columns, the peak areas of both the acetals and the aldehyde hydrolysis products were measured using the 210 nm UV-DAD signal. Before a series of injections of the acetals was made using the autosampler, the column was washed with 60:40 ACN:DZW with 5 mM NH_4OH at 1 mL/min to deactivate the acid sites. After the first injection was made, the mobile phase was switched to 60:40 ACN:DZW, without NH_4OH, to begin the formation of active sites on the column.

RESULTS

Filter Studies

A cigarette filter plasticizer made with CDA was analyzed for CDA and citral after seven months and 34 months aging. After seven months there was 5.0% CDA and 1.0% citral, and after 34 months there was no CDA remaining and 3.1% citral.

One of the early experiments was to determine whether there was any difference in aged filter rods containing CDA when Estrobond G, a tow plasticizer, was used. Two CDA-containing filters, one with and one without Estrobond G, were extracted with 100 mL of ethanol. The extracts were concentrated to 5 mL using a rotary evaporator and analyzed. No CDA remained in either filter extract. The filter extract with the plasticizer contained 24.6 ppm citral and the filter extract without the plasticizer had 19.7 ppm citral.

In another experiment, we prepared a solution of CDA in 95% ethanol. To one-half of the solution we added a cellulose acetate cigarette filter and to the other half of the solution we added a filter and a drop of concentrated ammonia. After the solutions had aged for 50 days they were analyzed by the HPLC method. With the cigarette filter alone, no CDA was detected because it had been hydrolyzed to citral. In the presence of both the filter and ammonia, there was little, if any, hydrolysis of CDA.

Acetal Hydrolysis Studies

During the course of investigating mobile-phase composition, we discovered that if the amine modifier were taken out, the acid sites gradually returned to the column. That is, if 60:40 ACN:DZW alone were pumped, the column gradually recovered its ability to hydrolyze acetals.

In order to compare their resistance to hydrolysis, CDA, BDA, MDA, SDA, and CDEA were repeatedly injected onto an ODS-Hypersil column that was initially deactivated with ammonia in the mobile phase. The solvent was switched to 60:40 ACN:DZW when the first sample of acetal was injected. The initial chromatograms showed only acetal and no aldehyde peaks. After four hours, all of the acetals, except for BDA and MDA, were hydrolyzed to their corresponding aldehydes, and only very small amounts of the acetals got

through the column.

Figure 1 shows how the peak areas of the acetals changed during the sequence of HPLC injections. For comparison, the time for the acetals to hydrolyze to half of their original concentration ($t_{1/2}$) was read from the curves. The $t_{1/2}$ was 15 min for CDEA, 120 min for CDA, and 270 min for SDA.

We also examined five different reversed-phase columns to determine whether they would hydrolyze the acetals, and if so, whether an amine modifier in the mobile phase would inhibit the hydrolysis. Because CDEA was the most easily hydrolyzed acetal, we used its half concentration time to compare the columns.

All of the columns caused CDEA hydrolysis in just under three hours using 60:40 ACN:DZW in the absence of ammonia or triethylamine in the mobile phase. Zorbax Rx-C8 ($t_{1/2}$= 90 min) was the most resistant to regaining its acidity, followed by Nova-Pak C18 ($t_{1/2}$= 58 min), Nova-Pak C8 ($t_{1/2}$= 27 min), ODS-Hypersil ($t_{1/2}$= 17 min), and Zorbax ODS ($t_{1/2}$= 12 min).

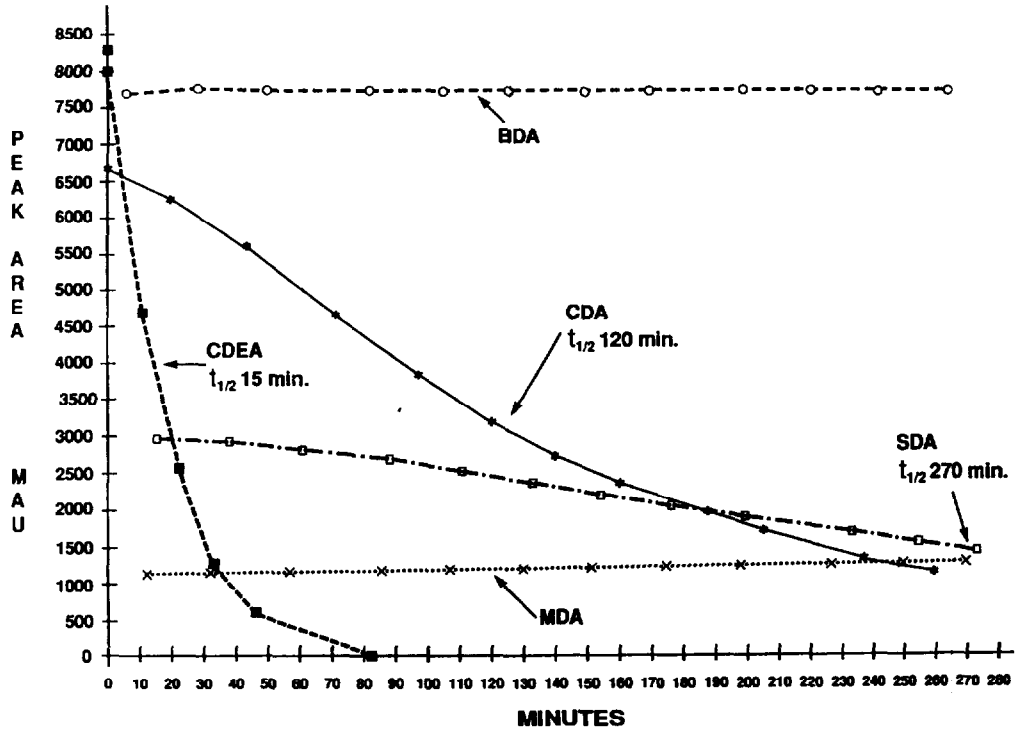
Interestingly, with ammonia present in the mobile phase, E/Z-CDEA separated into two peaks on the Nova-Pak C18 and the Zorbax ODS columns. On the Nova-Pak C8, the Zorbax Rx-C8, and the ODS-Hypersil columns these two isomers were not resolved and only one peak was observed.

DISCUSSION

Disregarding the difference in column dimensions, we noted that the order of the half concentration times ($t_{1/2}$), the rapidity for the ammoniated columns to regain their ability to hydrolyze CDEA, is the reverse order of the acidity of the silica supports as published in the literature (11). Zorbax Rx, with the largest $t_{1/2}$, is the least acidic silica. Zorbax ODS, with the smallest $t_{1/2}$, is the most acidic silica. It did not appear to make any difference whether the bonded phase was C-8 or C-18.

We suggest that this technique of measuring the rate of re-activation of a base de-activated HPLC column with respect to acetal hydrolysis

Figure 1. Activation of ODS-Hypersil HPLC column (4mm x 125mm, at 40° by 60:40 ACN:DZW, 1mL/min) toward hydrolysis of acetals following deactivation by 60:40 ACN:5mM NH_4OH. (BDA = benzaldehyde dimethyl acetal; CDA = citral dimethyl acetal; CDEA = citral diethyl acetal; MDA = methacrolein dimethyl acetal; SDA = senecialdehyde dimethyl acetal; MAU = milli absorbance units.)



might be a very useful tool for further characterizing HPLC columns. Information relating to the activity of unreacted silanol groups on reversed-phase HPLC columns has been of interest for a long time, especially as it relates to analysis of basic compounds and drugs (1,3,6-11).

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