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INVESTIGATION INTO THE YELLOWING ON AGING OF SABRIL® TABLET CORES

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ABSTRACT

Uncoated Sabril® tablet cores under long term storage become slightly discolored from the initial white to a yellowish off-white color. In order to ensure the aesthetics of the product, Sabril® tablet cores are film coated with an opaque white coating. The nature of this yellowing reaction was of interest even though discolored tablets showed no significant loss of potency on assay. Excipient compatibility studies showed that the vigabatrin active in Sabril® mixed with Avicel® (microcrystalline cellulose) in the presence of moisture also became off-colored when stressed at elevated temperatures.

The nature of the discoloration in aged Sabril® core tablets was investigated. Chromatographic and spectroscopic data indicate that the source of this color comes from the Maillard Reaction between vigabatrin and Avicel® which results in a multitude of products analogous to "browning reactions" of food products. A gravimetric determination of the leached colored products from 7 year old Sabril® core tablets gave a residue of less than 0.1% relative to vigabatrin. Furthermore, based on spectroscopy, most of this residue was found to be povidone, an excipient in the tablets which was isolated along with the colored substances.

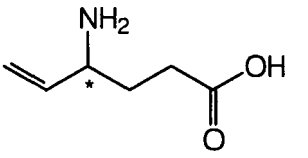
Therefore, the colored products identified in the core of Sabril® tablets stored for an extended period of time represent only minor impurities. Their formation through aging arises via the Maillard Reaction and would only constitute a matter of aesthetics. The latter problem is avoided by the currently employed film coating process.

Similar reactions could be predicted for other drugs having amine functional groups if they are formulated with microcrystalline cellulose or reducing sugars.

INTRODUCTION

Uncoated Sabril® tablet cores under long term storage become slightly discolored from the initial white to a yellowish off-white color . In order to ensure the aesthetics of the product, Sabril® tablet cores are film coated with an opaque white coating. The nature of this yellowing reaction was of interest even though discolored tablets showed no significant loss of potency on assay . Excipient compatibility studies showed that the vigabatrin active in Sabril® mixed with Avicel® (microcrystalline cellulose) in the

presence of moisture also became discolored when stressed at elevated temperatures. However, no significant loss of assay strength was detected.



VIGABATRIN

The following study was performed to understand the nature of this yellowing reaction.

MATERIALS

Vigabatrin and stressed Sabril® core tablets were obtained from internal supplies. Avicel® was obtained from FMC (Philidelphia, PA). Povidone was obtained from International Specialty Products (Wayne, NJ). The solvents used, methanol and water, were liquid chromatographic grade. The C-18 Sep-Pak™ cartridges were obtained from Waters Associates (Milford, MA). An authentic sample of Compound II was prepared in-house.

METHODS

Isolation of Colored Product from Vigabatrin and Avicel® Mixtures

An Avicel® - vigabatrin mixture was prepared, as part of an excipient compatibility study, containing 10 g vigabatrin, 2.82 g Avicel® and 641 mg water. This mixture was stored at 60°C for 3 months. A 5 g portion of this stressed sample was mixed with 50 mL of water. The resulting colored yellow-tan supernatant solution was passed through a C-18 Sep-Pak™. The majority of the colored species was isolated from the solution onto the C-18 Sep-Pak™. The Sep-Pak™ was thoroughly rinsed with water (the colored species remained on the Sep-Pak™). After the water washing, methanol was used to elute the colored species from the Sep-Pak™. The methanol solvent was removed under a stream of nitrogen gas. The colored residue (sample 1) was obtained. A second sample (sample 2) was also isolated similarly and a similarly colored residue was obtained. To obtain additional stressed material 10 g of vigabatrin, 2.8 g of Avicel® and 5.0 mL of water were stored at 60°C for 14 weeks. This material was treated as above to isolate the colored species and the third colored isolate sample (sample 3) was collected.

Isolation of Colored Products in Aged Sabril® Core Tablets

A batch of core tablets stored at 25-30°C for seven years was used. Each Sabril® core tablet contains 500 mg vigabatrin, 140.69 mg Avicel®, 13.9 mg sodium starch glycolate, 18.3 mg povidone and 5.09 mg magnesium stearate.

Fifty 500-mg Sabril® core tablets were disintegrated into 200 mL of water. The supernatant liquid was decanted and collected after the residue settled (by centrifugation). The residue was leached with another 200 mL of water, the mixture centrifuged and the supernate decanted. The decanted supernates were then filtered through a sintered glass filter. The filtered supernates were then passed through a C-18 Sep-Pak™ to collect the colored product as summarized below.

The Waters C-18 Sep-Pak™ was prewashed with 10 mL of methanol and then 20 mL of water using a 20-mL syringe. Portions of the supernatant liquid containing the colored extract from the core tablets were processed through the Sep-Pak™. After ca. 60 mL of the liquid was passed through the Sep-Pak™, the Sep-Pak™ was washed with ca. 40 mL of water and the colored products eluted with ca. 5 mL of methanol. The methanol eluted fraction was saved. The Sep-Pak™ was rewashed with ca. 20 mL of water and then more of the supernatant liquid was processed. This was repeated until all of the supernatant liquid was processed.

The collected methanol fractions were stripped of methanol under a nitrogen stream. The residue was dissolved into a small amount of 50/50 v/v methanol/water and transferred to a tared vial. The solvent was then evaporated under a nitrogen stream and the residue was weighed.

HPLC Analysis

The HPLC analysis conditions used are given below. A Waters Model 374 gradient HPLC equipped with an Applied Biosystems Inc. 757 detector was used. Data acquisition was performed using a CALS system (Beckman).

Column:	C-18 Vydac 218 TP 54 (250 x 4.6 mm).
Mobile phase:	60 min. linear gradient. 100% A to 100% B.
	Solvent A: 0.1% v/v trifluoroacetic acid in water.
	Solvent B: 0.1% v/v trifluoroacetic acid in 50/50 v/v acetonitrile/water.
Flow rate:	1 mL/min.
Detection:	at λ = 290 nm.
Sample Injection:	See chromatograms.

SPECTROSCOPY

NMR Spectroscopy

Proton NMR spectra were obtained using Varian VXR-300 or Unity-300 NMR spectrometers. The spectra were obtained in CD_3OD at 25°C and are referenced versus tetramethylsilane (TMS).

Mass Spectrometry

Chemical ionization mass spectra were acquired using a Finnigan TSQ 700 mass spectrometer. Methane was used as the reagent gas at a manifold pressure of 6×10^{-6} Torr. Samples were introduced via the direct exposure probe at a heating rate of 20 mA/sec. The mass range was scanned from 65 to 850 amu at one second intervals. For deuterium oxide chemical ionization helium was bubbled through the deuterium oxide in the GC oven set at 90°C to get the deuterium oxide into the source. Argon was used as the makeup gas with the manifold pressure adjusted to 5×10^{-5} Torr.

MS/MS daughter ion spectra were acquired at collision energies of -10, -20, and -30 eV with argon as the collision gas. Daughter ion spectra were acquired for m/z 331 and 328. Fast atom bombardment (FAB) mass spectra and accurate-mass data were acquired using a VG ZAB2-SE mass spectrometer. Glycerol, m-nitrobenzyl alcohol, and triethanolamine were used as FAB matrices. The accurate-mass data were acquired in the FAB ionization mode at a resolution of 10,000. The matrix ions were used as the internal accurate-mass calibrant.

Infrared Spectroscopy

Infrared spectra were acquired using a Perkin Elmer Model 1800 FT-IR spectrophotometer or Mattson Galaxy 5020 FT-IR spectrophotometer. The povidone standard spectrum was acquired as a potassium bromide pellet and the spectrum of the residue from the isolated color fraction of Sabril® core tablets was acquired as a thin film pressed between two potassium bromide plates.

RESULTS AND DISCUSSION

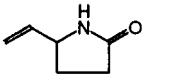
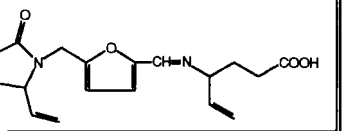
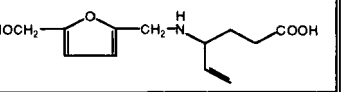
From preliminary work with aged Sabril® core tablets which had yellowed, it was known that much of the color could be leached from the tablets using either methanol or water. Some additional work showed that the colored substance was somewhat lipophilic and could be isolated on the C-18 bonded phase of a packed C-18 bonded silica cartridge such as a Waters C-18 Sep-Pak™. Of equal importance, were the earlier excipient compatibility studies which showed that in the presence of moisture and heat, vigabatrin and Avicel® would discolor to yield a yellowish-brown mixture. This allowed for the quick generation of a colored product free from other tablet excipients. This colored product mixture could then be used as a supply source for the isolation of the colored Avicel®-vigabatrin reaction product.

The first attempts to isolate this colored product were on the Avicel®-vigabatrin excipient compatibility sample. The residues of the isolated colored species were analyzed by spectroscopy. Both the methane chemical ionization (CI) and the FAB mass spectral data for the first isolated residue (sample 1) indicated it was composed of multiple components. Table 1 summarizes the mass spectral data for the vigabatrin related degradation products observed in either the CI or FAB or both mass spectra. Based on possible quasi-molecular ions observed in the mass spectra, accurate-mass and daughter ion mass spectra were acquired to assist in proposing structures for the various components in this mixture. Data for each possible quasi-molecular ion is summarized and is presented for the components labeled as Compounds I-VI.

Compound I was identified as the lactam which is a dehydration product of vigabatrin. The lactam was observed only in the CI spectrum and may result from further degradation of the sample in the ion source.

The proposed structure of Compound II is shown in Table 1 as well as the mass spectral evidence which supports this structure. Another experiment used to determine the structure was deuterium oxide CI. This experiment determined the number of

TABLE 1

Compd.	Protonated Molecular Ion/ Elemental Formula*	CI Daughter Ions MS/MS	Proposed Structure
I	112	---	
II	331 $C_{18}H_{23}N_2O_4$	313, 285, 220 219, 152, 124	
III	328 $C_{18}H_{22}N_3O_3$	310, 292, 282 268, 216	None
IV	440 $C_{24}H_{30}N_3O_5$	---	None
V	240 $C_{12}H_{18}NO_4$	---	
VI	553	---	None

*Obtained from molecular ion data and HRMS, respectively.

exchangeable protons. In the deuterium oxide chemical ionization mass spectrum of this sample the ion at m/z 331 in the methane CI mass spectrum, which is the protonated molecular ion for Compound II, was shifted to m/z 333. This two amu shift is consistent with Compound II having one exchangeable proton and the formation of the deuterium adduct. A standard for Compound II was prepared and the CI MS/MS daughter ion mass spectrum is consistent with the daughter ion spectrum of m/z 331 obtained for the first isolated residue. The chromatography of Compound II is discussed elsewhere in this report.

Although no structure has been proposed for Compound III, this degradation product is related to only vigabatrin and does not contain the saccharide portion. The $C_{18}H_{21}N_3O_3$ elemental formula may correspond to three vigabatrin molecules minus three water molecules with an additional loss of six hydrogen atoms resulting in a compound with a total of ten rings and/or double bond equivalents. This compound will be highly conjugated and therefore may be colored.

No structure has been proposed for Compound IV, but it may be related to Compound II. Based on the elemental formula Compound IV contains the elemental composition of Compound II with an additional C_6H_7NO. This is consistent with the

displacement of a hydrogen atom with the lactam (C_6H_8NO). Compound IV was observed only in the FAB mass spectrum.

The proposed structure of Compound V is shown in Table I and is based on the molecular ion data and the elemental formula derived from accurate-mass peak matching. This component of the mixture was also observed only in the FAB mass spectrum. Compound V was also found to be a significant degradation product in the interaction of vigabatrin and 5-hydroxymethyl-2-furfuraldehyde at 60° C. Data for this sample was used in proposing the structure for Compound V.

Compound VI has quasi-molecular ions at m/z 553 ($M+H^+$) for positive ion FAB and m/z 551 ($M-H^-$) for negative ion FAB. No structure has been proposed for this degradation product.

Since an insufficient amount of the first isolated color residue (sample 1) was available for NMR, the second isolated colored residue (sample 2) was obtained and used to acquire the NMR data. A FAB mass spectrum of sample 2 showed it has a similar profile to that of sample 1. No additional CI nor MS/MS data were acquired on sample 2. Both samples were isolated identically from the Avicel®-vigabatrin compatability mixture.

The ^1H NMR spectrum of the second isolated colored residue sample 2 in CD_3OD is shown in Figure 1. The spectrum is typical of a mixture of many components. While specific assignments cannot be made several regions are qualitatively identified. Farthest downfield, near 9.4 ppm, several singlets are observed consistent with aldehyde protons. In the region between 7 and 8 ppm several multiplets are observed consistent with the shift expected for substituted vinyl protons. Between 5 and 6 ppm the larger resonances are consistent with the three vinyl protons of a mono-substituted ethylene (such as is present in vigabatrin or many of the proposed reaction products). The complexity of this region, as for the other portions of the spectrum, is consistent with a many component mixture. Similarly, the aliphatic region is consistent with the aliphatic protons of vigabatrin and the complexity indicates many components.

It became apparent from the spectral data on the isolates that they were composed of multiple components. In order to estimate the number of components and their relative magnitude, gradient HPLC analysis was performed. The third isolated sample (sample 3) was shown by FAB-MS to be similar to the above. This sample was dissolved in methanol to give an approximate 10 mg/mL solution and 100 µL of this solution was chromatographed using the chromatographic conditions outlined in the METHODS section. The chromatogram obtained is shown in Figure 2. This chromatogram shows one large spiked hump centered at a retention time of ca. 32 minutes. The nature of this "hump" was further explored as to whether this "hump" was a single poorly chromatographing compound or a large series of compounds eluting over a range of retention times. To sort this out, three additional 200 µL injections of this solution were chromatographed. During each chromatogram, two fractions were collected; one from 15-30 minutes and one from 30-50 minutes. These fractions were blown down to dryness under nitrogen. The early (15-30 minute) fraction and the later (30-50 minute) fraction were redissolved into ca. 1 mL of methanol and 200 µL of the respective samples were chromatographed. The resulting chromatograms in Figure 3 show that they are not similar and that each is composed of multiple components. Therefore, the "hump" seen in Figure 2 for the chromatogram of sample 3 is a result of a large series of compounds and not a result of poor chromatography.

Similarly, a 2-mL sample of the synthesized Compound II (4 mg/mL in methanol) was chromatographed. This compound gives a sharp, single peak at ca. 29 minutes. It is likely that this compound is just one of the many compounds present in sample 3 and the other samples.

It is not unexpected that mixtures of Avicel® and vigabatrin would produce multiple compounds. Avicel® (microcrystalline cellulose) is a polysaccharide with the general

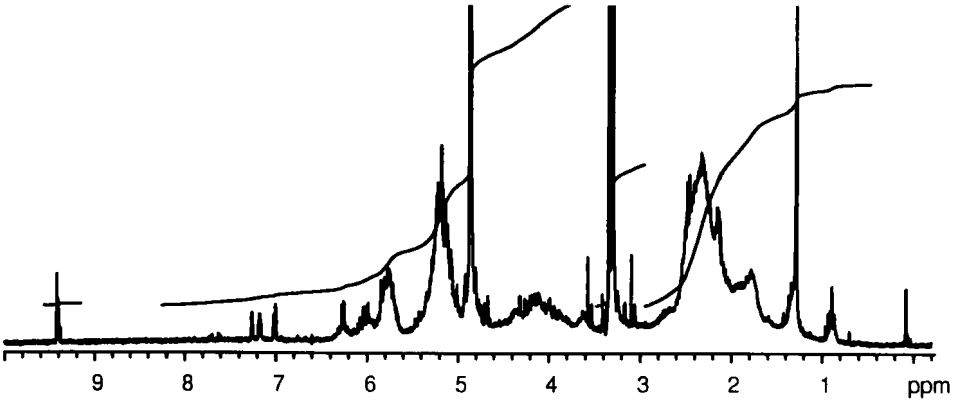


FIGURE 1

^{1}H NMR Spectrum of Sample 2 in CD_3OD

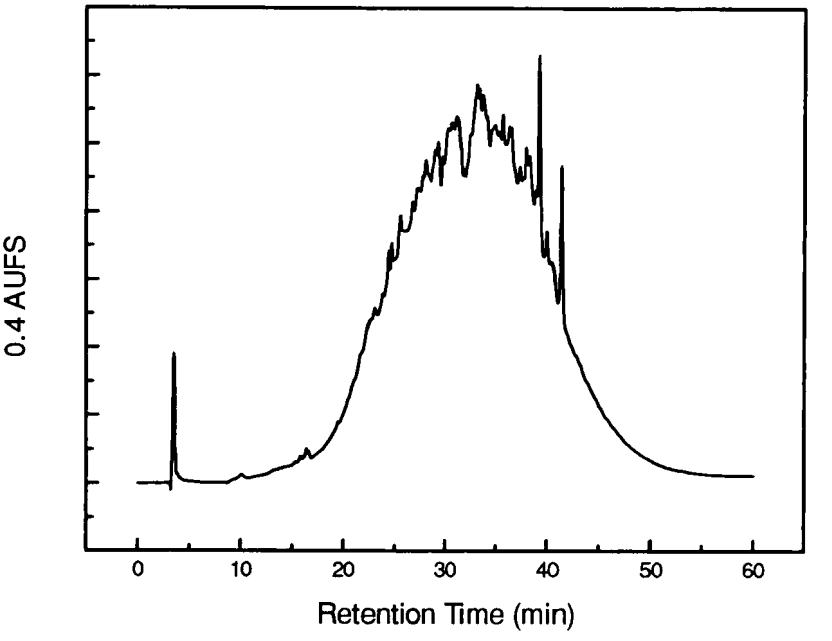


FIGURE 2

HPLC Chromatogram of Isolated Colored Residue Sample 3

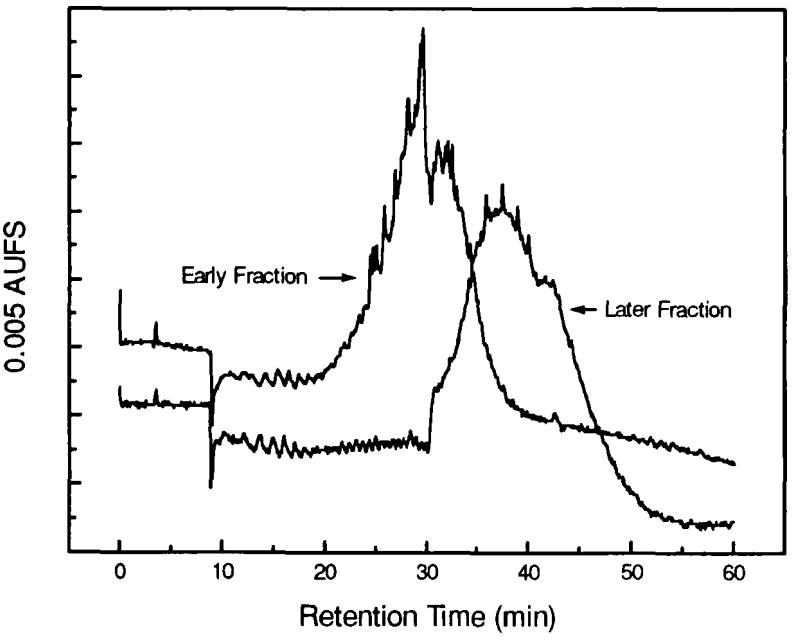


FIGURE 3

HPLC Chromatograms of Subfractions of Sample 3

formula of $(C_6H_{10}O_5)_n$ and therefore capable of forming many homologous products. In addition, vigabatrin is racemic and would be expected to form diastereomers with the optically pure microcrystalline cellulose. Whereas, the above reasons may help explain the formation of multiple products, it is still difficult to understand from where the color would originate, as most of these simple degradation products would not be expected to be colored. The source of the color is most likely the subsequent reactions which take place as part of the well known Maillard Reaction (1-5), also known as the "nonenzymatic browning reaction", which is common in food chemistry. The Maillard reaction can take place between reducing sugars and amines or amino acids.

The first step of this reaction results in the formation of a glycosylamine. The glycosylamine can then rearrange via an Amadori rearrangement (1) to form a 1-amino-1-deoxyketose which can then react further to form dicarbonyl compounds. These can then react with additional amines or amino acids to form advanced Maillard reaction products. The advanced Maillard reaction products usually adsorb in the UV and on standing, react further with amines and other carbonyl compounds to form brown polymeric fluorescent compounds called melanoidins, the structures of which are poorly understood (1). A pictorial representation of the Maillard reaction sequence is shown in reference 1.

The HPLC (1) and the capillary zone electrophoresis (5) profiles of the reaction products typically show a wide range of compounds with varying degrees of polarity. This is in agreement with our findings for the third isolated sample in Figure 2. Compound II, which was identified by mass spectrometry, is a component of this sample

and would be expected to result from the early "Amadori Steps" of the Maillard reaction. It could result from a direct reaction of vigabatrin with either glucose polymers or with 5-hydroxymethyl-2-furfuraldehyde which is a known glucose degradation product (6).

It became obvious that it would be impossible to isolate and identify all of the color bearing components from the reaction of vigabatrin with Avicel®. To obtain a gravimetric estimate on the level of these colored bearing components in aged Sabril® tablets, a study on aged Sabril® core tablets was performed. The majority of the color from seven year old Sabril® tablet cores stored at controlled room temperature was leached and isolated as outlined in the METHODS section. The weight of the resulting residue, 21.6 mg, was 0.09% relative to the 25.0 g of vigabatrin in the original Sabril® core tablets.

The colored residue obtained was analyzed by spectroscopy . The proton NMR and IR spectra are consistent with the primary component being povidone. A sample of authentic povidone was obtained and confirmed this conclusion. Povidone is present in the tablet formulation at the ca. 3% level. The FAB mass spectrum of this colored isolate sample did not have any significant ions attributable to vigabatrin degradation.

CONCLUSIONS

The nature of the discoloration seen in aged Sabril® core tablets was investigated. Chromatographic and spectroscopic data indicate that the source of this color comes from the Maillard Reaction between vigabatrin and Avicel® which results in a multitude of products analogous to "browning reactions" of food products. A gravimetric determination of the leached colored products from 7 year old Sabril® core tablets gave a residue of less than 0.1% relative to vigabatrin. Furthermore, based on spectroscopy, most of this residue was found to be povidone from the tablets which was isolated along with the colored substances.

Therefore, the level of colored products seen in Sabril® core tablets appears to be very low. This color formation on aging is a result of the Maillard Reaction and mostly an issue of aesthetics. The problem of aesthetics is overcome by the present film coating process.

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