Qualitative analysis of color additives in hair conditioner products by UPLC with Photo Diode Array detection

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ABSTRACT

A test portion of hair conditioner product is diluted in tetrahydrofuran (THF) then applied to an acidified BAKERBOND® Wide-Pore carboxylic acid solid-phase extraction cartridge (CBX SPE) for concentration and cleanup. The column is washed with water and methanol and the color is eluted with ammonium hydroxide in methanol. The eluate is evaporated to dryness and re-constituted in methanol for qualitative analysis by UPLC with Photo Diode Array (PDA) detection. Seven commonly used color additives in hair conditioners—FD&C Yellow Nos. 5 and 6, FD&C Red Nos. 4 and 40, FD&C Blue No. 1, D&C Red No. 33, and D&C Orange No. 4—were evaluated in control hair conditioner using THF as the extractant. Additionally, eight retail products containing multiple color additives were extracted on a qualitative basis using this method.

INTRODUCTION

In 2016, hair conditioner was among the top 25 health and beauty care product categories in the United States (US) and generated sales of more than $2 billion. (1) Hair care has a long history dating to ancient Egypt, but the first modern hair conditioner, *Brillantine*, was presented at the 1900 *Exposition Universelle* in Paris by perfumer Édouard Pinaud. (2) His pomade-like hair product was intended to soften men's hair, including beards and mustaches. Today's hair conditioners, usually viscous, non-greasy liquids, are used to decrease friction, minimize frizz and tangle, improve the ease with which hair is managed and produce a healthy shine. (3, 4) A cross-sectional view of most hair fibers would show a center core (medulla), surrounded by the cortex which contains color pigments, and an outer-most layer, called the cuticle, which resembles overlapping scales. (4, 5) When these "scales" lay flat, the cuticle takes on a smooth appearance, causing light reflection and giving the preferred shiny appearance. (3, 4) Hair conditioners are typically acidic (pH of approx. 3.5) and contain anti-static and lubricating substances that assist the hair "scales" to lay flat. These lubricating substances can be divided into 5 main groups: polymers (including silicones), oils, waxes, hydrolyzed amino acids, and cationic polymers. (4) Conditioners also frequently contain surfactants, which can be anionic, cationic, amphoteric, or nonionic in nature (4, 6), though the precise mechanisms of action of charged surfactants on the hair are still under investigation. (7) The presence of various surfactants, particularly those that are charged, may interfere with chemical extraction and clean-up procedures.

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Even though hair conditioners are used with water, and, therefore, seemingly water-soluble, typical color additives (water-soluble dyes such as FD&C Blue No. 1 and FD&C Yellow No. 5) added to conditioners are not easily extracted using the methods in Colors Manual 2016. (8) Neutral or acidified methanol are ineffective at extracting dyes from the emulsified hair conditioner products possibly due to surfactants or the lubricating substances intended to make hair more manageable.

Interestingly, tetrahydrofuran (THF) was found to dissolve or demulsify the hair conditioner products tested. THF has also been utilized in the chromatographic separation of surfactants in hair conditioner products. (6) Following the demulsification, the solution was applied to a 6-mL solid-phase extraction cartridge (CBX SPE) that had been pre-treated with an acidified solution, for concentration and cleanup as described in Colors Manual 2016. This analysis scheme has been successful in detecting commonly used certifiable color additives at or below approximately 1 ppm in food and cosmetic products. (8) Seven common color additives in hair conditioners—FD&C Yellow Nos. 5 and 6, FD&C Red Nos. 4 and 40, FD&C Blue No. 1, D&C Red No. 33, and D&C Orange No. 4—were evaluated in control hair conditioners using THF as the extractant. Eight commercial retail products containing one or more of these color additives were extracted and analyzed on a qualitative basis using this method.

EXPERIMENTAL

Equipment

SPE cartridges: (CBX) J.T. Baker BAKERBOND® Wide-Pore carboxylic acid disposable SPE columns, 6-mL (Part # JT7217-6 through VWR Intl, Radnor, PA) [Recommend no substitution].

Laboratory Equipment: Nitrogen Evaporator with temperature-controlled water bath (Organomation Associates, Inc. or equivalent); Refrigerated centrifuge, capable of accelerating 15- & 50- mL tubes to 10,000 rpm (Thermo Fisher RC6+ or equivalent); Sonicator (Model# 8892, Cole-Parmer or equivalent); Vortex mixer; SPE vacuum manifold; 5-5,000 µL manual pipettors.

Laboratory Consumables: Syringe filters: Pall 13-mm disposable PTFE 0.2 µm pore size (Part # 4542, VWR Intl); 15- & 50-mL disposable, conical-bottom, graduated, polypropylene tubes with caps (#352097 and #352098, respectively, Becton-Dickinson, Franklin Lakes, NJ); 12 x 75 mm disposable glass tubes (#47729-570, VWR Intl).

Solvents/Reagents: Deionized water (≥18.2 MΩ·cm conductivity); High-purity chromatographic and spectrophotometric grade water (UPLC-MS grade), methanol, acetonitrile, and concentrated hydrochloric acid (HCl, 12 N) (Sigma Aldrich, St. Louis, MO, or equivalent); Ammonium hydroxide (NH_4OH, 28-30% as NH_3) (Part# BDH3014, VWR Intl); Ammonium acetate (EMD or equivalent).

Instrument Parameters

Instrument: Waters UPLC (Ultra Performance Liquid Chromatography) Acquity system, with photodiode array detector (270 nm-790 nm)
Instrument software: Empower 3 Support ID EM8JN01725; Database ID: 2412999898
Column: Waters UPLC BEH 1.7 micron, 2.1 x 50 mm, Part # 186002350
Mobile phase A: 100 mM ammonium acetate (pH 6.5, acidified with 5% HCl) in 99:1 water:acetonitrile
Mobile phase B: 75% acetonitrile / 25% methanol
Injection volume: 1 to 2 µL, vial compartment maintained at 26 °C
Flow rate: 0.400 mL/min (constant)

Parameters for Gradient System 1 used for the analysis of water-soluble dyes.

Time (min)	%A	%B	Curve/Rate
Initial	100	0	--
0.50	100	0	6
12.0	0	100	6
15.0	0	100	6
15.1	100	0	11

Standard Preparation

Reference standards were received from the Center for Food Safety and Nutrition (CFSAN), Division of Color Certification and Technology, as well as purchased from Sigma-Aldrich (St. Louis, MO), Spectrum Chemical (New Brunswick, NJ), and other vendors. For this analysis, several color additives were investigated based on dyes noted in commercial conditioner hair products, which included: Yellow 5, Yellow 6, Red 4, Red 33, Red 40, Blue 1, and Orange 4. For stock standard solutions, accurately weigh each standard powder individually into 15-mL polypropylene tubes. Stock solution concentrations: 500 µg/mL to 1,000 µg/mL. Water-soluble dyes were dissolved in water except for D&C Red No. 33 which was prepared in a 1:1 solution of methanol and water. To prepare the fortification standard, pipette 0.500 mL of each stock standard into a 15-mL polypropylene centrifuge tube. Calculate each concentration based on the final dilution (in the case of seven standards for a final volume of 3.5 mL). Apply cap and mix using a vortex mixer.

One level with six replicates was evaluated. The fortified control spikes were prepared as follows: Three commercial products which did not list color additives were used for spiking: Suave® Professionals Color Care Conditioner and Suave® Professionals Moroccan Infusion (Unilever, Englewood Cliffs, NJ) and Pantene® Conditioner (Procter & Gamble, Cincinnati, OH). The fortification standard was prepared by combining 0.5 mL of each stock standard solution, with a final volume of 3.5 mL for the seven standards. Example concentration: for Yellow 5, 1,086 µg/mL x 0.5 mL/3.5 mL = 155 µg/mL. Calculation of fortification level (ppm): Standard concentration (µg/mL) x spike volume (mL) / weight of sample (g). For example: for Yellow 5, 155 µg/mL x 0.050 mL / 2 g = 4 µg/g (ppm). The levels of fortification (2.0 g conditioner spiked with 0.050 mL mixed standard) ranged as follows:

Fortification Mixed Standard	Concentration, µg/mL	Level of fortification (ppm)
FD&C Yellow No. 5	155	4
FD&C Yellow No. 6	134	3
D&C Red No. 33 (Acid red 33)	89	2
FD&C Red No. 40	86	1
FD&C Red No. 4 (Ponceau SX)	74	2
FD&C Blue No. 1	72	2
D&C Orange No. 4 (Orange II)	115	3

For the UPLC standard solution, a 0.050 mL aliquot of the mixed standard (containing all seven dyes) was diluted into 0.500 mL methanol, then filtered prior to injection. For a more visual recovery comparison of the UPLC standard and fortified control, use the same aliquot (0.050 mL) of standard solution for both the UPLC standard and spiking volume, and make the final dilution 0.500 mL methanol for both. The standard and spiked control product will essentially have the same concentration in the final solutions.

The seven color additives were selected based on a review of current retail products. The retail products tested declared the following color additives:

Retail Product	Color Additives Listed
Herbal Essences® "Totally Twisted"	Red 33 and Blue 1
Suave® "Honey Infusion"	Yellow 5 and Red 33
VO5® "Moisture Milks"	Red 40, Red 4, and Orange 4
VO5® "Herbal Escapes"	Yellow 5 and Blue 1
Suave® "Green Apple"	Yellow 5 and Blue 1
Suave® "Strawberry Pink"	Yellow 5 and Red 33
Suave® "Blue"	Red 33 and Blue 1
Import Sample Conditioner	Yellow 5 and Blue 1

PROCEDURE

Sample/Control Preparation: Weigh approximately 2 g ± 0.1 g hair conditioner (emulsified) into a 15-mL disposable polypropylene tube. Add 3 mL THF to sample, apply screw cap and vigorously mix using a vortex mixer for approximately 1 min. The product should completely demulsify, eliminating the need to heat or centrifuge the product extract. The dissolved product should appear translucent. If the product appears cloudy, add an additional 1 mL THF.

CBX Column Prep/Loading: Prepare a CBX SPE column on a vacuum manifold by passing 3 mL methanol through column, followed by 1 mL 1% HCl in methanol. Apply the entire extract to the SPE and allow it to pass through column at approximately 5 drops/sec.

Column Wash/Elution: Wash SPE column with one column-volume (approx. 3 mL) methanol, two column-volumes water, followed by one column-volume methanol. Use slight vacuum to remove remaining methanol from column prior to elution, but column should not be completely dry. Remove column from vacuum manifold and elute color by passing 3.5 mL 10% ammonium hydroxide in methanol through column into a 12x75 mm disposable glass tube using slight air pressure applied to the top of the SPE column. Evaporate eluate under nitrogen in an approximately 50 °C water bath to dryness, then re-suspend residue in 0.5 mL or 1.0 mL methanol, based on color intensity. Pass solution (as well as all control extracts, standards, etc.) through a 0.2 micron PTFE syringe filter prior to injection on UPLC.

RESULTS AND DISCUSSION

The extraction procedure for liquid products (such as shampoos and body creams) in Colors Manual 2016 (8) was found to be problematic when applied to hair conditioners. Generally, color additives are easily extracted into methanol from liquid, water-soluble products. Figure 8 displays three tubes of hair conditioner product (VO5® "Moisture Milks" which listed Red 4, Red 40, and Orange 4) extracted in methanol, acidified methanol, and THF, respectively. Methanol alone produced more emulsion in the product that was not separated through centrifugation. When acidified methanol was used instead, minimal color was extracted from the conditioner (the majority of the color remained in the precipitate), and the supernatant was difficult to pass through the CBX SPE cartridge. Tetrahydrofuran was found to dissolve the emulsified conditioners unlike any other solvent tested (acetone, a similar polar aprotic solvent, did not produce the same effect). The dissolved product not only flowed easily through the CBX SPE cartridge, but more importantly, did not significantly affect the retention of the color additives on the SPE cartridge. If the sample extract remained slightly opaque, an additional 1 mL of THF was added. Neither heating nor centrifugation was required. Once the extract was applied to the CBX SPE cartridge, it could be washed with any number of solvents -- in this case, methanol and water followed by additional methanol. Application of 10% NH_4OH in methanol appeared to completely elute the color additives from the cartridge. Based on visual comparison of the UPLC chromatography, the majority of the fortified-control color additives were adequately recovered except for Orange 4. Although its recovery was below 50%, the spectral match for the spiked-control product was acceptable for this qualitative analysis. Furthermore, retail products which declared Orange 4 were successfully characterized as containing that dye using this method modification.

Figure 1 shows the UPLC chromatogram for a hair conditioner control (Pantene®) used for the fortification recovery information. No interferences were observed for this product or for the other control conditioners tested. Figures 2 and 3 display the UPLC chromatograms for the standard mix (alone) and a spiked conditioner, respectively. Visually comparing the mixed standard injection and fortified control (both are equivalent in concentration), it is evident that D&C Orange No. 4 produced the lowest recovery (at 3 ppm), though it was well above the detectable limit. Figure 4 shows the chromatogram for the VO5® conditioner product that declared Red 40, Red 4, and Orange 4 as color additives. All three of the dyes were identified in the product based on retention time and comparison of their spectra to library spectra of reference standards (Figure 5). Identification of a color additive required a retention time match to a reference standard within 5% and for their absorbance maxima to be within 5 nm of each other. Figure 6 displays the chromatogram of a Suave® conditioner which listed Yellow 5 and Blue 1 as color additives. Both dyes were detected in this product and identity confirmed using reference standards (Figure 7).

Conditioners were found to be very different than other water-soluble cosmetic liquids such as shampoos. For this reason, this new extraction procedure is offered as an alternative to methods described for water-soluble liquids in Colors Manual 2016.

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Figure 1. Commercial hair conditioner (Pantene) control which did not declare color additives.

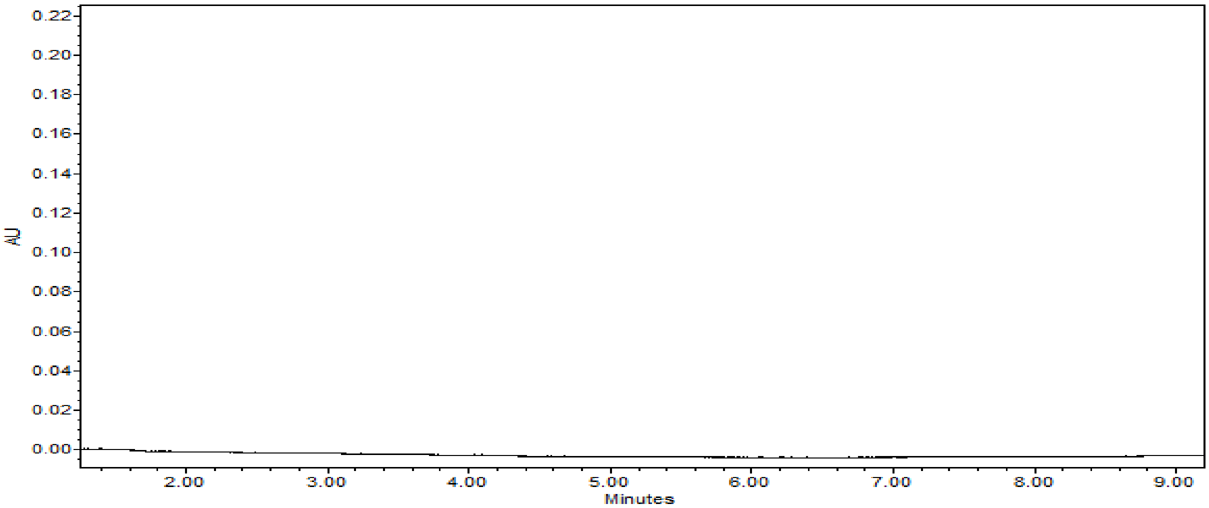


Figure 2. Mixed standard showing peak retention of FD&C No. Yellow 5 (1.700 min), FD&C Yellow No. 6 (2.717 min), D&C Red No. 33 (3.048 min), FD&C Red No. 40 (3.120 min), FD&C Red No. 4 (4.334 min), FD&C Blue No. 1 (4.621 min) and D&C Orange No. 4 (5.379 min).

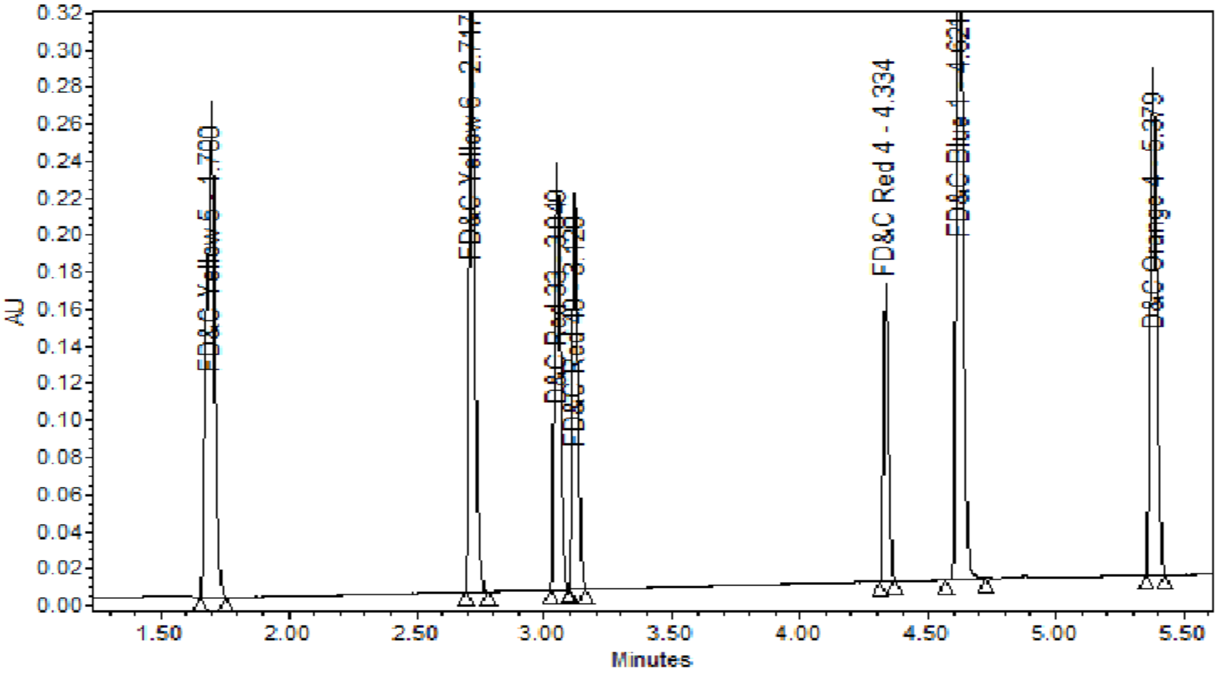


Figure 3. Fortified conditioner (spike) showing peak retention of FD&C Yellow No. 5 (1.706 min), FD&C Yellow No. 6 (2.716 min), D&C Red No. 33 (3.047 min), FD&C Red No.40 (3.118 min), FD&C Red No. 4 (4.336 min), FD&C Blue No.1 (4.619 min) and D&C Orange No. 4 (5.379 min).

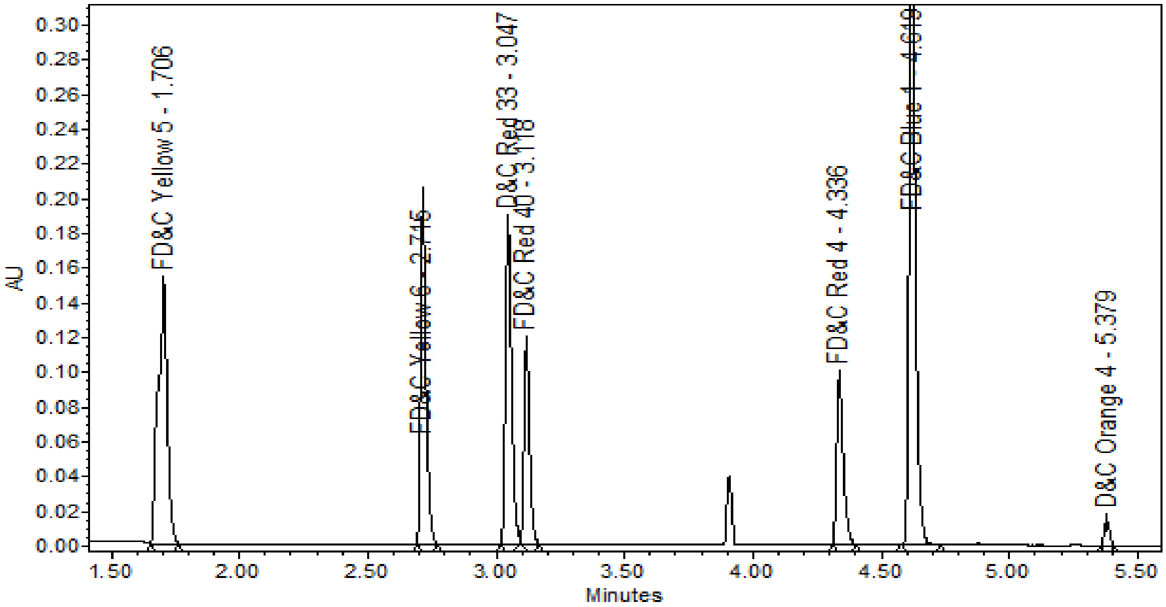


Figure 4. VO5® conditioner product that listed Orange 4, Red 4, and Red 40 color additives on its label. Detected in the product: FD&C Red No.40 (3.116 min), FD&C Red No. 4 (4.331 min), and D&C Orange No. 4 (5.377 min).

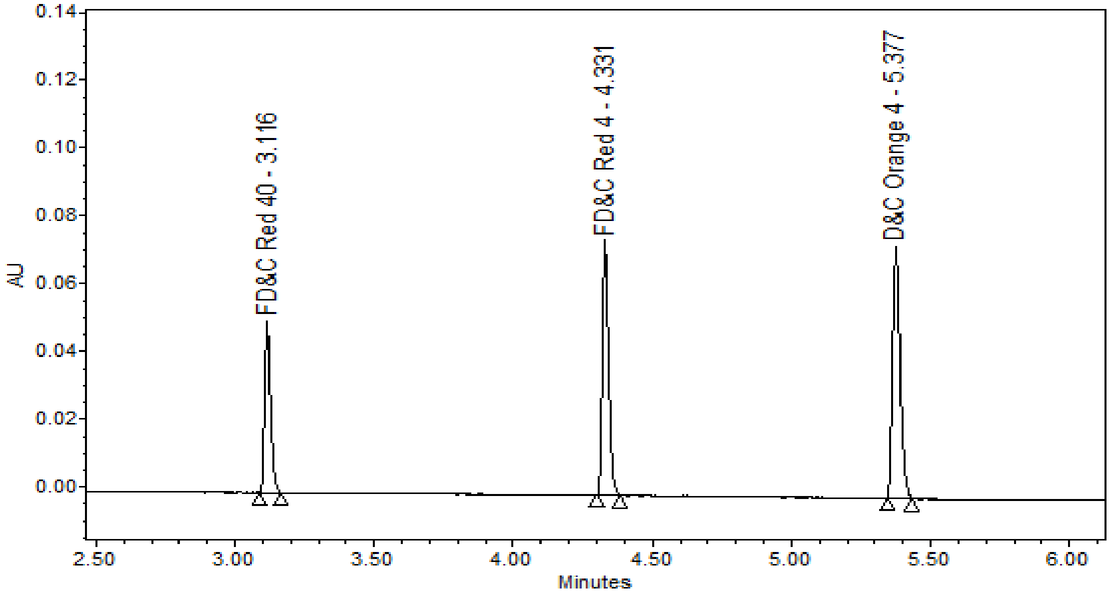
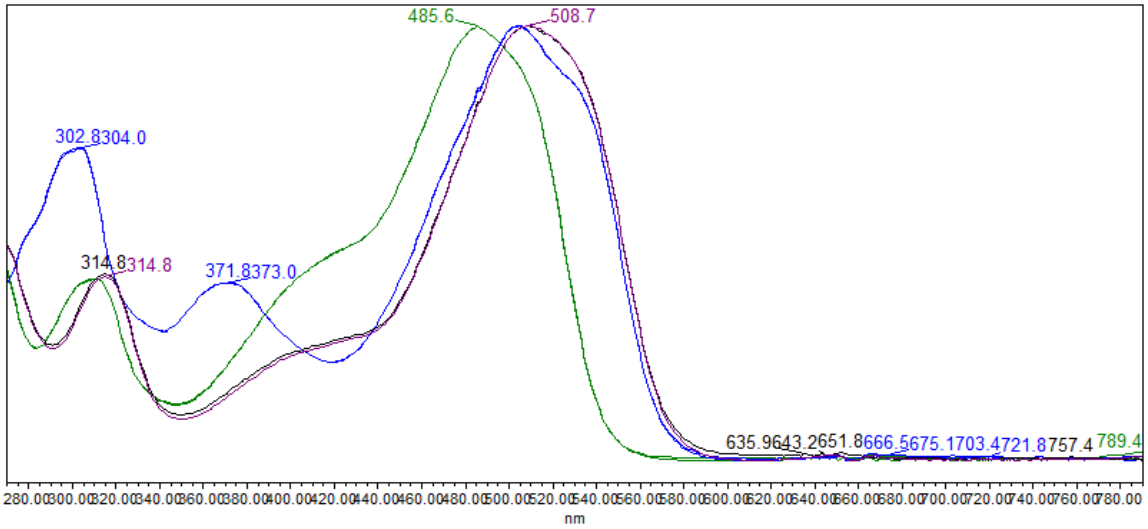


Figure 5. VO5® conditioner product containing Red 40, Red 4, and Orange 4. UV/Vis spectra of the three color additives successfully matched the absorbance maxima of reference spectra within ±5 nm and had retention times within 5% of the retention times of reference standards. Green spectrum: Orange 4 (Abs. max at 485.6 nm); Blue spectrum: Red 4 (Abs. max at 505.0 nm); Purple spectrum: Red 40 (Abs. max at 508.7 nm).



Retention times (in minutes); First value is observed RT; Second is that of comparable reference standard. X-axis ranges from 280 – 780 nm.

Figure 6. Chromatogram of Suave® "Totally Twisted" conditioner which declared Red 33 and Blue 1 on its label. Both D&C Red No.33 (3.011 min) and FD&C Blue No. 1 (4.618 min) were detected in product by this method.

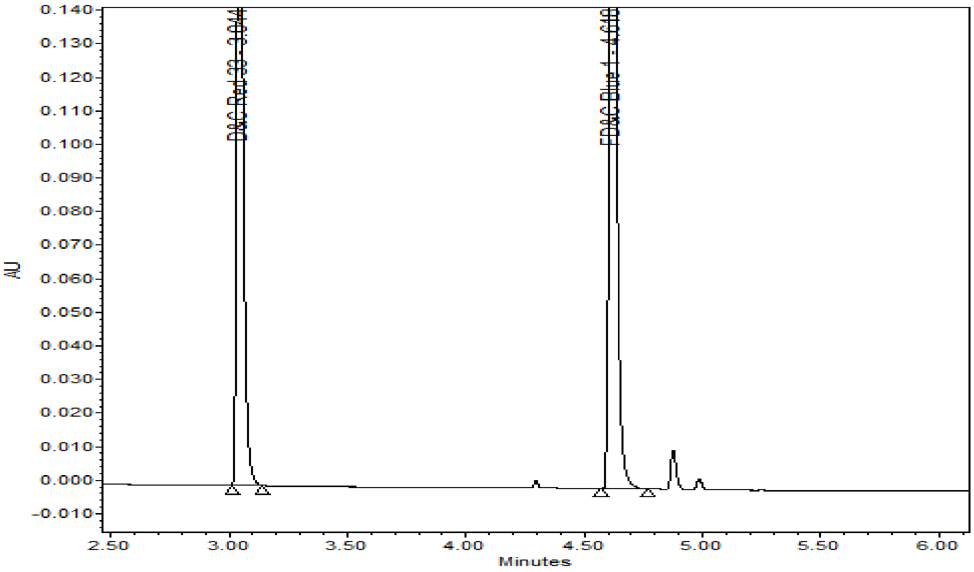
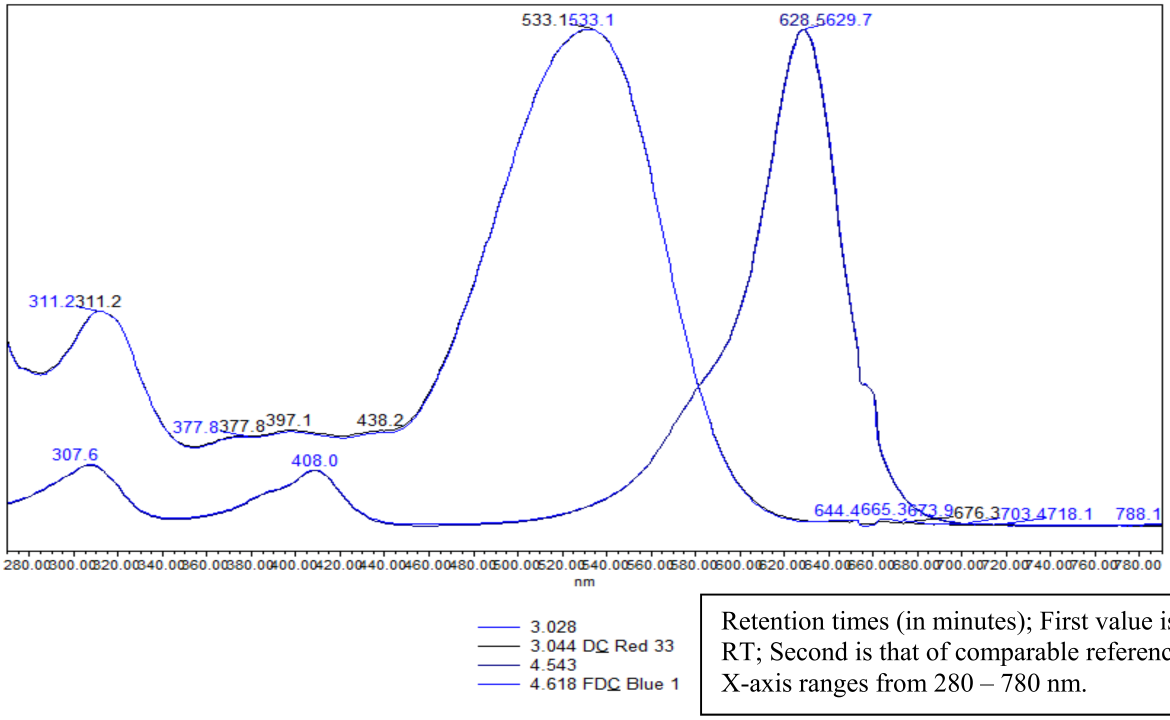


Figure 7. Suave® "Totally Twisted" conditioner. UV/Vis spectra of the three color additives successfully matched the absorbance maxima of reference spectra within ±5 nm and had retention times within 5% of reference standards. Shown are spectral overlays of the product and related reference standards. Detected in the product were: Red 33 (Abs. max at 533.1 nm) and Blue 1 (Abs. max at 629.7 nm).



Retention times (in minutes); First value is observed RT; Second is that of comparable reference standard. X-axis ranges from 280 – 780 nm.

Figure 8. Comparison of extractants after centrifugation: Tube 1 ("MeOH") displays the product further emulsified with methanol. Tube 2 ("Acid/MeOH") shows a majority of the color additives remaining in precipitate. Tube 3 ("THF") displays an extract that is completely transparent (additional 1 mL of THF added to product.)