SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF DEXTROMEHTORPHAN IN HAIR AFTER EXPOSURE TO COSMETIC TREATMENT

by Amy Avirett

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ABSTRACT

SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF DEXTROMEHTORPHAN IN HAIR AFTER EXPOSURE TO COSMETIC TREATMENT

by

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Degree: B.S. in Chemistry: Biomedical Emphasis

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A high-performance liquid chromatography method has been developed for the determination of dextromethorphan in hair. The separation and quantitation are achieved on a phenyl column (4.6 x 150 mm) using a mobile phase of 80:20% v/v 6.25 mM sodium phosphate buffer (pH 3.0) and acetonitrile at a flow rate of 1.0 mL/min with UV detection at 226nm. The retention time for dextromethorphan is within four minutes. The method showed linearity for dextromethorphan in the 0.20-1.00 mg/mL range. The sensitivity of the method was determined to be a concentration of 0.025 mg/mL or 25 μg/mL. The inter-day RSDs ranged from 0.86 to 9.60%. A solid-phase extraction method has been developed for the extraction of dextromethorphan from hair using a Strata C18 cartridge. This method yielded an

average percent recovery of 80.84% on blank, untreated hair. The SPE-HPLC was also employed to determine the effects of cosmetic treatment on the concentration of dextromethorphan in hair. Commercially available bleach (Sun-In®) and hair dye (Clairol® Natural Instincts Loving Care #75) were applied in vitro to hair strands spiked with the dextromethorphan drug. In these hair samples, the drug levels had been reduced but distinct tendencies could not be observed. Further research and investigations must be conducted in order to conclude the effects of such cosmetic treatment on the concentration of dextromethorphan in hair.

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Introduction

Over the counter drug abuse is becoming an increased problem among adults as well as children in our society. Research shows that one out of ten teens from across the country and of all backgrounds, have abused cough medicine to get high (1). Even infants can be affected from this type of drug abuse. The common cold medicine Triaminic recently removed its Infant and Toddler Thin Strips from over the counter shelves, due to the number of accidental child overdoses. Triaminic contains dextromethorphan as its active ingredient (2).

Dextromethorphan is an antitussive (cough suppressant) found in over 120 over the counter medicines (3). Some examples include Alka-Seltzer Plus Cold & Cough Medicine, Coricidin HBP Cough and Cold, Dayquil LiquiCaps, Dimetapp DM, Robitussin cough products, Sudafed cough products, Triaminic cough syrups, Tylenol Cold products, Vicks 44 Cough Relief products and Vicks NyQuil LiquiCaps (1). Pure dextromethorphan occurs as a powder made up of white crystals, but it is generally administered via syrups, tablets, or lozenges. Dextromethorphan is a central nervous system depressant. As a result, experiments are also being conducted for psychological applications as well as pain relief (3).

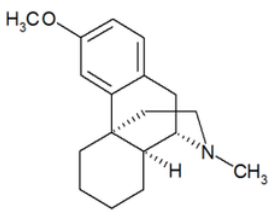

Figure 1-Chemical structure of dextromethorphan

Due to its extensive and easy availability and cheap price, dextromethorphan is one of the most commonly abused drugs. When abused, dextromethorphan acts as a dissociative hallucinogenic drug and produces symptoms of euphoria common to the street drug PCP (3). Dextromethorphan abusers report a heightened sense of perceptual awareness, altered time perception, and visual hallucinations. The typical clinical presentation of intoxication involves hyper excitability, lethargy, slurred speech, sweating and hypertension. Abusers of dextromethorphan describe the following four dose-dependent "plateaus:" (4).

Plateaus	***Dose (mg)***	***Behavioral Effects***
1st	100-200	Mild stimulation
2nd	200-400	Euphoria and hallucinations
3rd	300-600	Distorted visual perceptions Loss of motor coordination
4th	500-1500	Dissociative sedation

Table 1-Four dose-dependent "plateaus" of dextromethorphan abuse

When taken in excess amounts, dextromethorphan can be extremely dangerous or even deadly. It is a central nervous system depressant, so high levels can literally cause the brain to cease normal functioning. Not only are high levels of dextromethorphan dangerous, but cold medicines usually contain combinations of other drugs which can also be hazardous, including acetaminophen (an analgesic pain reliever), guaifenesin (an expectorant), ephedrine or pseudoephedrine (stimulants), and chlorpheniramine maleate (an antihistamine with anticholinergic and sedative

side effects). Of particular concern is the ingredient acetaminophen, which can result in severe liver damage when taken in excessive quantities (5).

Over the counter drugs, such as dextromethorphan, present a problem for drug testing. Since the drug is commonly used in combination with other drugs and easy to flush out of the body, it is often hard to determine from standard drug tests if it has been abused. A solution to this problem is drug testing using extraction from hair. Drugs stay in hair for an extended period of time, and it is impossible to flush them out. Drug testing using hair is highly accurate, efficient, and has a quick turnover rate. Hair grows approximately 0.5 inch (1.27cm.) a month, so it is possible to determine when the drug was consumed. Body hair and even hair follicles can be used if head hair has been shaven (6). Although liquids cannot be used to flush drugs out of hair, it is possible that cosmetic treatment, such as hair dyes and bleaches could alter drug concentrations. The effects of cosmetic treatment on drug concentration have been previously studied. In a study conducted on the influence of bleaching on stability of benzodiazepines in hair, results showed that the concentrations of all drugs decreased in bleached hair in comparison with non treated hair (7). Another study that evaluated the stability of opiates in hair fibers after exposure to cosmetic treatment, also noted a decrease in concentration. In the spiked hair, only 2-18% of the starting solution was detected after bleaching and 20-30% could be detected after perming (8). More research in this area will help establish the validity of drug testing using hair.

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A HPLC method for the determination of dextromethorphan will first be established, followed by a Solid Phase Extraction method for the extraction of dextromethorphan from hair. After the validation of both methods, the stability of dextromethorphan in hair after exposure to cosmetic treatment will be tested by treating the spiked hair with a bleach solution (sun-in®) as well as a hair dye solution (Clairol® Natural Instincts Loving Care #75). Factors such as the retention time of dextromethorphan in HPLC determination, linearity, sensitivity and RSD of the method, peak size and shape, percent recovery after solid phase extraction, and degradation patterns of dextromethorphan will also be considered.

Experimental

2.1. Preparation of HPLC Determination Method

In order to evaluate the concentration of dextromethorphan, a reverse-phase HPLC method for its determination in solution needed to first be established. Many trials and errors were conducted before an effective method was produced using a Shimadzu LC-20AT HPLC. This method included a polar mobile phase composed of 80% 6.25mM sodium phosphate buffer solution at pH 3.00 and 20% acetonitrile. The buffer was prepared by the following calculation:

$$\frac{6.25\text{mmol of sodium phosphate}}{1\text{L}} \times \frac{1\text{mol}}{1000\text{mmol}} \times \frac{268.07\text{g sodium phosphate}}{1\text{mol}} = 1.68\text{g in 1L}$$

Therefore, 1.68g of 6.25mmol sodium phosphate must be added to water in order to form a final volume of 1 liter of solution. The pH of this solution was then brought

down to pH 3.00 by the use of HCl. A phenyl column (4.6 x 150 mm) served as the non-polar stationary phase of determination. The flow rate was set at 1 mL/min, and the UV detector was set at a wavelength of 226nm. Using this HPLC method, the concentration of a solution containing an unknown sample of dextromethorphan can be determined based on the size of peak produced. The retention time of the dextromethorphan peak using this method was approximately 3.3 minutes.

*See Appendix-A for photos of the HPLC Method

2.2. Preparation of Solid Phase Extraction Method

After an HPLC method had been finalized, a solid phase extraction method had to be prepared. Solid phase extraction of dextromethorphan was completed on a Varian Vacuum Manifold using a Strata C18 cartridge. This method also involved a strenuous trial and error process.

0.300g of standard hair obtained from a generous volunteer was carefully weighed and then cut into small pieces using scissors to form a powdered texture. This was performed so that the dextromethorphan would become incubated into the cortex of the hair not just on the outer coat. The hair was then spiked using a combination of a specific known concentration of dextromethorphan and 6.25mM sodium phosphate buffer (pH 3.00). The final volume of the solution equaled approximately 10 mL. The hair was incubated in the dextromethorphan solution overnight or for several days before solid phase extraction was performed. After

incubation but prior to extraction, the pH of the solution was changed to a pH of 7.00 using NaOH. The dextromethorphan solution was then filtered off of the sample and replaced with 10 mL of distilled water. This insured that only the dextromethorphan that was incubated within the cortex of the hair was extracted not the dextromethorphan left in the solution. The distilled water provided the sample with a blank solution from which no products could be extracted.

Solid phase extraction was then performed on the hair solution utilizing the following method:

1.) Cartridge Conditioning

2 x 3mL of methanol followed immediately by 3mL of distilled water

**DO NOT ALLOW CARTRIDGE TO RUN DRY!

2.) Sample Addition

Add up to 50 mL of sample

Aspirate sample completely from cartridge

Air-dry cartridge under vacuum for 3 to 4 minutes

3.) Cartridge Wash

2 sequential 0.5mL volumes of distilled water

4.) Analyte Elution—Use a clean tube

2 sequential 0.5mL volumes of methylene chloride

Following the extraction procedure, the extract was immediately injected into the HPLC apparatus, and the concentration of dextromethorphan was determined using

the HPLC determination method. The area of the peak was compared to the area of the standard peak for the concentration originally incubated into the hair. Finally percent recovery of the solid phase extraction method was determined.

*See Appendix-B for photos of the Solid-Phase Extraction Method

2.3. Preparation of Hair Dye and Bleach Samples

A method was determined to test the stability of dextromethorphan in hair dye and bleach. 1.00mg/mL dextromethorphan standards were prepared to determine the effect of hair dye and bleach solely on the drug. 100µL of dextromethorphan at concentration of 10mg/mL was added to 1000µL of 6.25mM sodium phosphate buffer pH 3.00 in order to form a drug concentration of 1.00mg/mL. Next 10µL of Clairol® Natural Instincts Loving Care #75 hair dye was added to one standard and 10µL of Sun-In® bleach was added to a second standard. These standards were injected using the HPLC determination method, and their peak area sizes were monitored over a two week period.

In order to test the stability of dextromethorphan in hair after exposure to cosmetic treatment, cosmetic treatment was performed on the hair prior to extraction. Commercially available bleaching and dying formulations, Sun-In® and Clairol® Natural Instincts Loving Care #75, were applied to two separate 0.300g samples of hair according to the manufacturer's instruction. After being exposed to cosmetic treatment, the hair was blow dried. The dried hair was once again cut to form a

powder and incubated in a 1.00mg/mL dextromethorphan/buffer solution with a final volume of 9900µL. After adequate incubation, the previously described solid phase extraction method was performed on the treated hair followed by the HPLC determination method. The peak size was determined and compared to that of the standard. Percent recoveries were also calculated and compared to the percent recoveries determined from untreated hair extractions.

Results and Discussion

3.1. HPLC Determination of Dextromethorphan in Solution

The previously described HPLC method provided chromatograms with a steady baseline for many concentrations of the drug dextromethorphan ranging from 0.025 mg/mL or 25 µg/mL to 1.00mg/mL. The method proved to be linear, sensitive and precise.

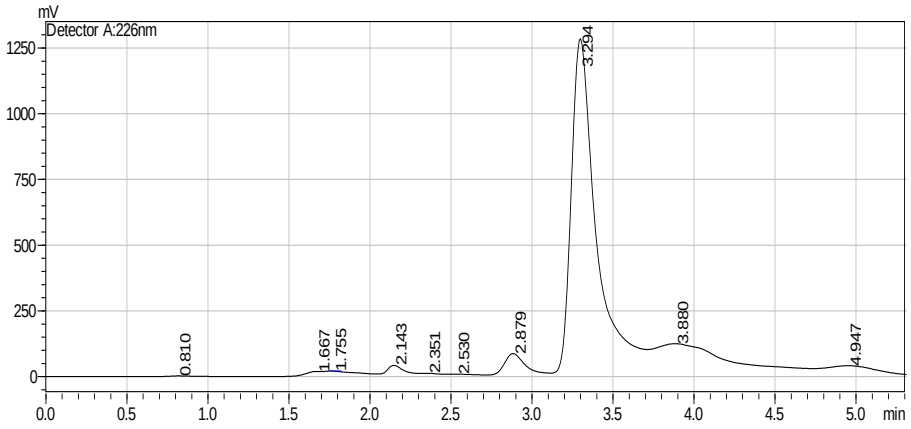

Figure 2- Chromatogram of a 1:10 dilution (1.00mg/mL) of dextromethorphan.

In order to test the linearity of the method, 0.10g of dextromethorphan was dissolved in 10mL of buffer creating a standard with a concentration of 1.00g/mL. Five different dilutions ranging from a 1 to 10 dilution to a 1 to 50 dilution were made. Each dilution was injected three times in order to determine the average absorbance peak concentration. The results are as follows:

Dilution	***Concentration***	***Average Peak Area***
1:10	1.00 mg/mL	9,017,468.33
1:20	0.50 mg/mL	5,924,312.66
1:30	0.33 mg/mL	4,496,044
1:40	0.25 mg/mL	3,822,878.33
1:50	0.20 mg/mL	3,405,657.66

Table 2- HPLC results used to determine the linearity of the method

The concentration of dextromethorphan and the area of the absorbance peak were plotted in order to test the linearity of the method. The standard curve formed from this procedure could also be used to determine unknown drug concentrations when the absorbance peak area of the sample is known or has been determined.

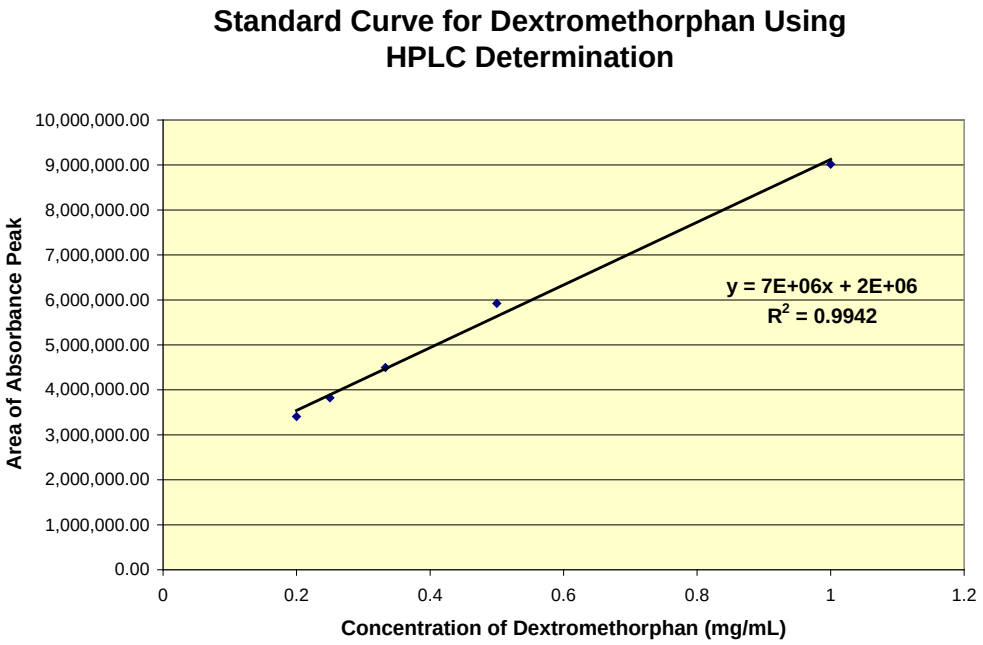

Figure 3- Standard curve to test linearity for dextromethorphan using dilutions 1:10 through 1:50

According to the R^2 of the standard curve (0.9942), this method produces linear results that are reliable and could be used to determine an unknown concentration.

The sensitivity of the HPLC method was also tested. Using the limit of detection formula the lower limit was determined to be a concentration of 0.025 mg/mL or a 1 to 400 dilution. In order to test this hypothesis, a 1:100 dilution was injected followed by a 1:200 dilution. The peak absorbance area of the 1:100 dilution was about twice that of the 1:200 dilution. This means that the 1:200 dilution results are consistent and accurate. As a result, a 1:400 dilution was injected. The results were about half of the results from the 1:200 dilution, meaning that these results are

also consistent and accurate. In order to ensure that this was the lowest possible limit of detection, a 1:800 dilution was injected. The baseline of the chromatogram was extremely unstable indicating that results are not accurately attainable at this concentration. This proved that the limit of detection is a 1:400 dilution or a dextromethorphan concentration of 0.025mg/mL.

Dilution	***Concentration***	***Average Peak Area***
1:100	0.100 mg/mL	1,487,277.33
1:200	0.050 mg/mL	854,951.66
1:400	0.025 mg/mL	489,746

Table 3-HPLC results used to determine the sensitivity of the method

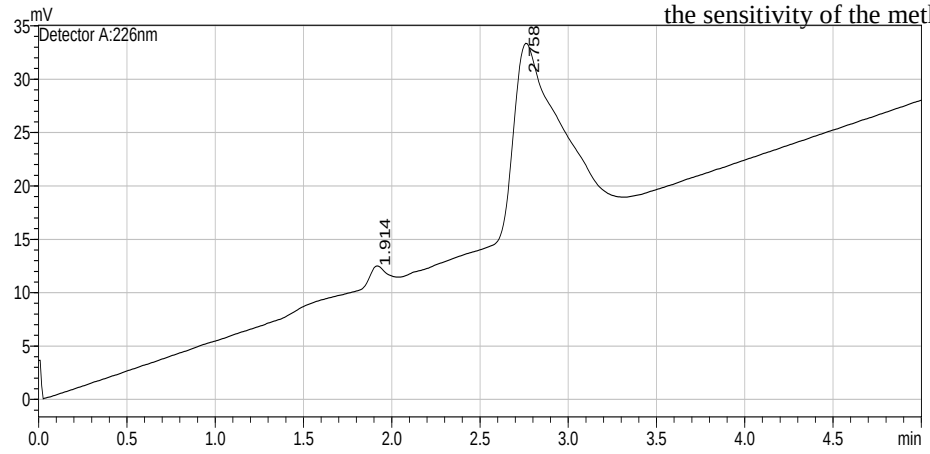

Figure 4- Chromatogram of a 1:800 dilution (0.0125mg/mL) of dextromethorphan. The baseline is extremely unstable indicating the concentration is too small to be accurately measured using this method.

To obtain inter-day precision data for the HPLC determination method, the % Relative Standard Deviation (RSD) values were determined for the data that was used to construct the standard curve. For each concentration, three different tests or injections were performed during the same day. % RSD is used to measure the precision of the method; therefore, the smaller the % RSD value, the more precise the data. The RSDs for this method ranged from 0.86 to 9.60% and were calculated using the following formula:

$$\% \text{ RSD} = \frac{\text{Standard deviation of three injections}}{\text{Average peak area of the injections}} \times 100$$

Dilution	***Concentration***	***% RSD***
1:10	1.00 mg/mL	3.77
1:20	0.50 mg/mL	6.64
1:30	0.33 mg/mL	9.60
1:40	0.25 mg/mL	1.34
1:50	0.20 mg/mL	0.86

Table 4- Inter-day precision data for the HPLC method of dextromethorphan determination

3.2. Solid Phase Extraction of Dextromethorphan From Hair

The previously described solid phase extraction method was performed on hair and the results analyzed. The solubility of dextromethorphan in distilled water was unknown; therefore, it was not known whether the wash step actually washed away with it some of the analyte or if it washed away only the contaminants remaining in the cartridge. As a result tests had to be conducted in order to determine the effectiveness of the wash step. One method utilized the wash step and one did not. After the completion of several tests, it was evident that the wash step was effective in the recovery of a higher percentage of dextromethorphan. Without the wash step many extractions yielded immeasurable results resulting in the percent recovery not being able to be calculated. Of those that did yield measureable results, the percent recovery was only 38.40%.

Dilution	***Concentration***	***Peak Area***	***Percent Recovery***
1:100	0.10 mg/mL	618,390.41	9.59%
1:100	0.10 mg/mL	7,751,837	56.12%
1:15	0.66 mg/mL	5,751,196	49.49%

Table 5- Solid phase extraction recovery without utilizing wash step

When utilizing the wash step, percent recoveries were much higher. They ranged from 67.62% to 92.91% with an average percent recovery of 80.84%. This data indicates that the solid phase extraction method is much more effective when the wash step is utilized.

Dilution	***Concentration***	***Peak Area***	***Percent Recovery***
1:20	0.50mg/mL	5,960,140	67.62%
1:10	1.00mg/mL	9,352,389	92.91%
1:10	1.00mg/mL	8,298,450.5	82.00%

Table 6- Solid phase extraction recovery utilizing the wash step

Through numerous tests, it was also determined that for the most accurate results the extracted analyte should be injected immediately following extraction into the HPLC determination method. When allowed to evaporate overnight, the recovery was significantly lower. This indicates that some of the dextromethorphan concentration evaporates with the solution.

3.3. Stability of Dextromethorphan in Hair Dye and Bleach

The stability of dextromethorphan in hair dye and bleach was tested overtime by incubating 10μL of Clairol® Natural Instincts Loving Care #75 hair day and Sun-In® bleach in two different 1.00mg/mL dextromethorphan standards. The standards were injected into the HPLC determination method a total of four times over a

fourteen day time period, and the average peak areas were compared. By comparing the absorbance peak areas to the peak area determined on day one, the concentrations of dextromethorphan, as well as the percent of dextromethorphan remaining were determined. For both the hair dye and the bleach samples, the peak areas decreased on day 3 as well as day 10 injections, but on day 14, both the hair dye and the bleach peak areas increased. This would theoretically mean that the concentration of dextromethorphan in the solution increased between day 10 and day 14. Since this does not seem to be a practical conclusion, further degradation tests need to be conducted in order to conclude why the peak areas of both samples increased on this day. One possibility may be that as the dextromethorphan degrades, one of the degradation products co-eludes under the original dextromethorphan peak. If this is true, then the combined peaks may make the area of the peak larger than if it was only the original dextromethorphan peak.

Date	***Average Area***	***Concentration***	***Percent***
3/24/08	14,103,247	1.00mg/mL	100%
3/27/08	11,595,707	0.82 mg/mL	82%
4/3/08	11,080,467.5	0.79 mg/mL	79%
4/7/08	14,086,025.5	0.99 mg/mL	99%

Table 7-Stability of dextromethorphan in hair dye solution

Date	***Average Area***	***Concentration***	***Percent***
3/24/08	13,791,154	1.00mg/mL	100%
3/27/08	13,771,292	0.99 mg/mL	99%
4/3/08	9,891,240.5	0.72 mg/mL	72%
4/7/08	13,944,878.5	1.01 mg/mL	101%

Table 8-Stability of dextromethorphan in bleach solution

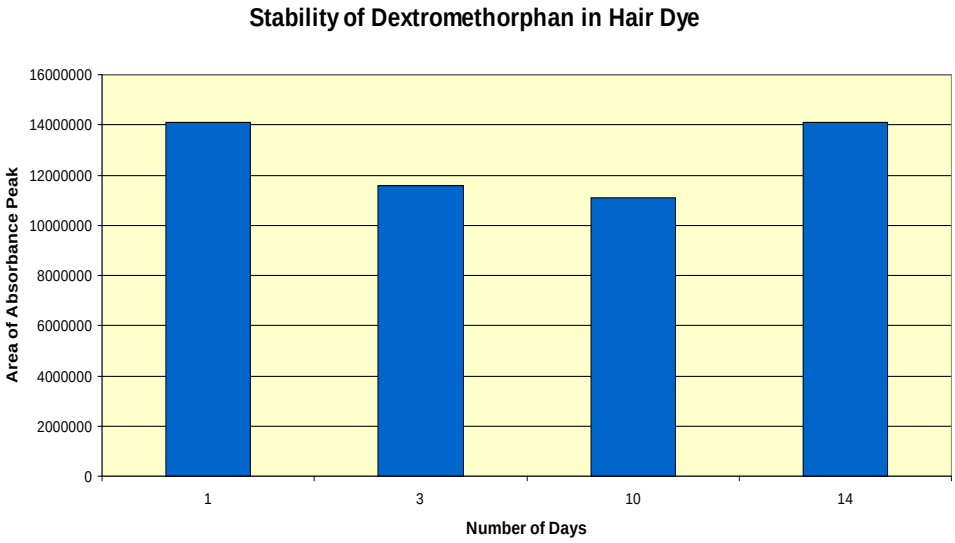

Figure 5- Graph displaying the stability of dextromethorphan in hair dye

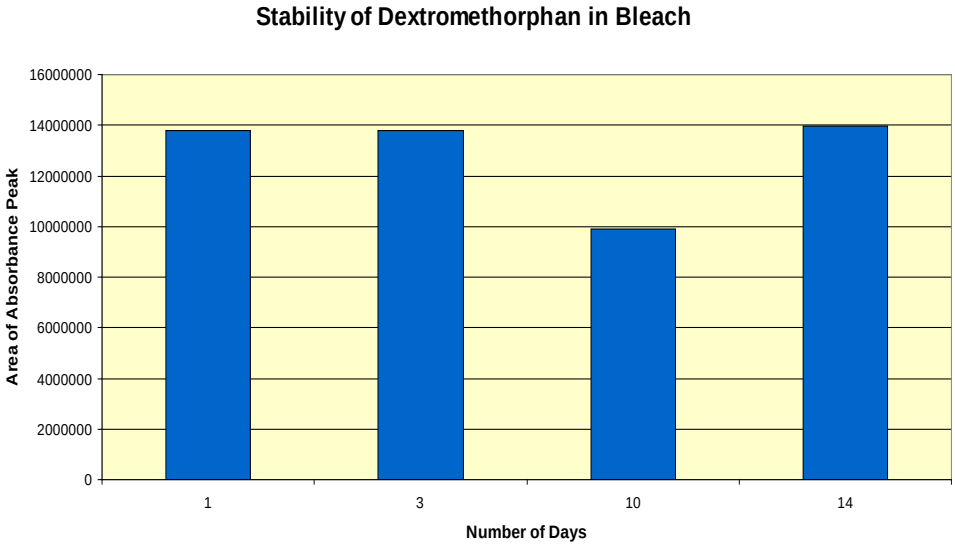

Figure 6- Graph displaying the stability of dextromethorphan in bleach

In order to test the stability of dextromethorphan in hair after exposure to cosmetic treatment, the hair was treated prior to being incubated in the drug solution and prior to extraction. The same solid phase extraction method that was performed on untreated hair was performed on the treated hair, and the eluent was immediately injected into the HPLC determination method. Due to a limited amount of time, only one test of the stability of dextromethorphan in hair after exposure to hair dye and bleach was performed. The bleach solution yielded a 14.95% recovery, while the hair dye solution yielded immeasurable results. Further tests must be conducted in order to determine whether the presence of cosmetic treatment actually lowers the concentration of dextromethorphan this significantly or if some other factors altered the results. Future tests could also reveal whether the method needs to be refined to take into account the chemical structure of the hair dye and bleach.

3.4. Recommended Future Applications

The research conducted thus far on this topic is only the very foundation of what can be discovered in the future. The possibilities for expansion on the topic of stability of drugs in hair after exposure to cosmetic treatment are vast in number. The research completed at this time provides future researchers with the basic methods of HPLC determination and solid phase extraction and allows them the freedom to test many new areas related to the degradation of dextromethorphan in cosmetics. One important application is to test the degradation patterns of dextromethorphan by

loading the sample. This can be done by exposing the drug to high levels of bleach, which will quickly degrade the sample, and injecting it frequently to determine the shape, appearance, and size of degradation peaks. This method can be used to answer the previously asked question of why the peak area of dextromethorphan that had been exposed to cosmetics increased after fourteen days. Was a degradation peak under the original dextromethorphan peak? If so, how large was this peak, and how large was the original dextromethorphan peak? If cosmetics cause the concentration of dextromethorphan to decrease, they likely degrade the drug in same way. Therefore, knowing the identities and locations of the degradation products of dextromethorphan will be vital in future experiments where the drug has been exposed to cosmetic treatment.

Another future application is the actual validation of whether cosmetic treatment has an affect on dextromethorphan concentration in hair. Cosmetic treatment includes hair dyes, bleaches, perms, shampoos, and relaxers. This can only be accomplished after the degradation patterns of dextromethorphan have been firmly identified. The solid phase extraction method may have to be altered due to the composition of the degradation products or the composition of the cosmetic treatments. Many trials will have to be conducted in order to determine whether this change is necessary, and if necessary what specific changes should be made.

4. Conclusion

The proposed HPLC method and solid-phase extraction method for the determination of dextromethorphan in hair in this study have the advantage of simplicity, precision, and accuracy. The methods use simple reagents, with minimum sample preparation procedures, encouraging their application in routine analysis. The HPLC method yielded inter-day RSDs that ranged from 0.86 to 9.60% indicating its precision. The solid phase extraction method had an average percent recovery of 80.84% on blank, untreated hair indicating its accuracy. In addition to providing simple methods for routine use, this study laid the foundation for more in-depth experimentation to come.

Using the methods developed in this study, degradation patterns of dextromethorphan in cosmetic treatments can be determined by loading the sample, and finally validation of whether cosmetic treatment has an affect on dextromethorphan concentration in hair can be concluded. Based on the results of previous experiments as well as this study, it is probable that cosmetic treatments do decrease the measurable drug concentration found in hair. If this is true, it might be possible for a drug abuser to use hair treatments to decompose or eliminate drugs from hair completely. Further studies on the validity of hair analysis should definitely be performed prior to its application in forensic toxicology.

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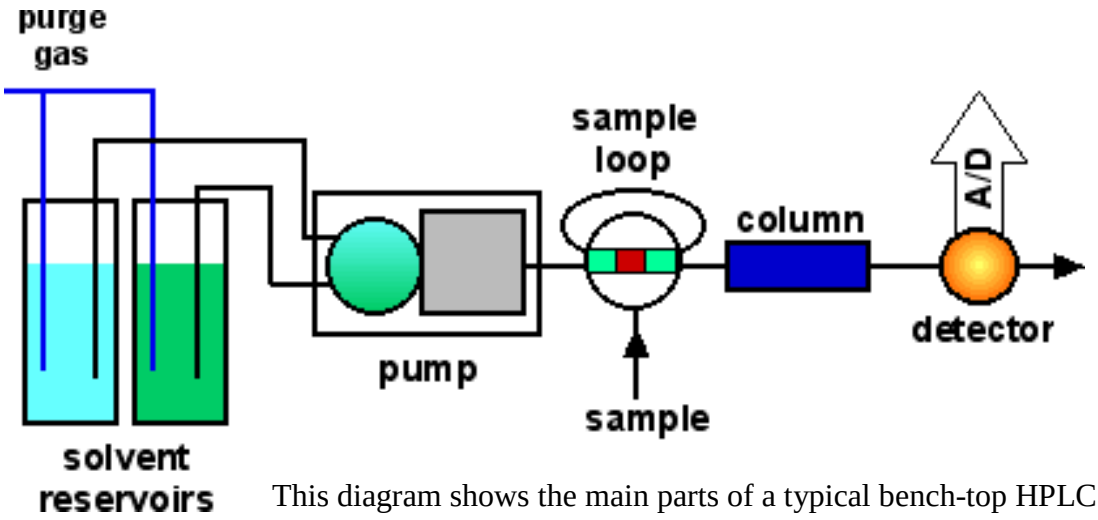
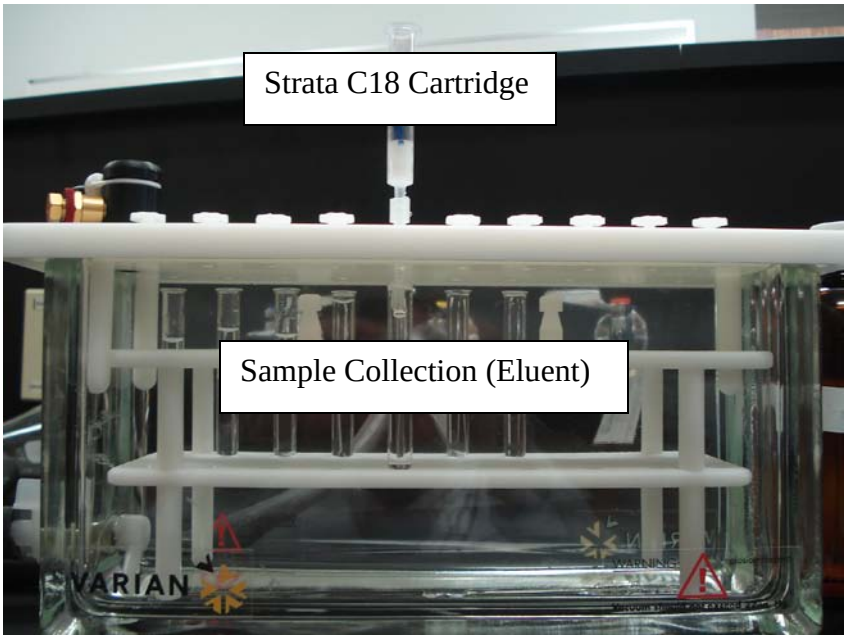
APPENDIX-A



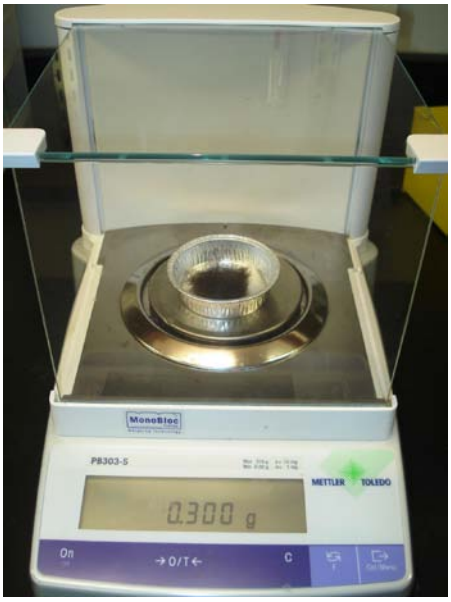
This diagram shows the main parts of a typical bench-top HPLC system (9).

The actual Shimadzu LC-20AT HPLC system that was used in this study.

APPENDIX-B

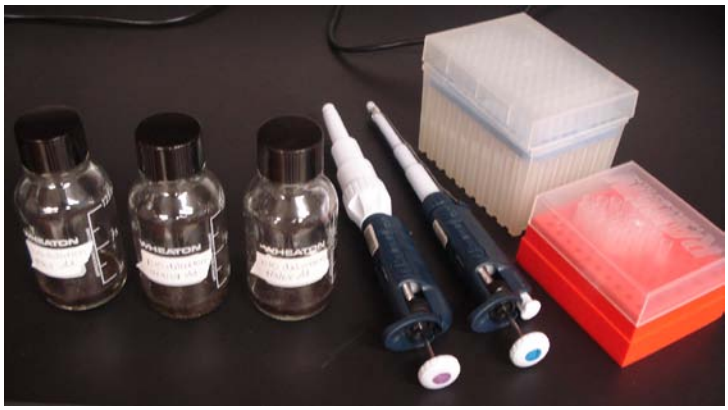


The actual Varian SPE Vacuum Manifold used to perform extractions of dextromethorphan from spiked human hair.



0.300g of hair was weighed

Hair was cut with scissors to form a powder



Hair was incubated in a specific known concentration of the dextromethorphan solution.

The pH of the solution was adjusted to 7.00

The dextromethorphan solution was filtered off of the hair and replaced with 10mL of distilled water.

The solid phase extraction method was employed on the hair in order to elute the dextromethorphan.