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Development of a Thin Layer Chromatography Method for the Separation of Enantiomers Using Chiral Mobile Phase Additives

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ABSTRACT

Resolution of enantiomeric controlled substances is a problem for forensic chemists as one enantiomer is usually controlled, while the other enantiomer is not. Enantiomeric substances that are of forensic interest include dextromethorphan and levomethorphan, dextropropoxyphene and levopropoxyphene, and dextro-methamphetamine and levo-methamphetamine. The objective of this project was to develop an inexpensive and simple method for enantiomer determinations as an alternative to using mixed crystal test methods, polarimetry or more expensive instrumental methods. Microcrystal tests may be used to differentiate these substances; however, these tests are difficult, time-consuming and can involve elaborate extraction schemes to purify the substances of interest. Alternatively, polarimetry may be used; however large sample sizes and pure samples are required. Instrumental methods such as Gas Chromatography (GC), Capillary Electrophoresis (CE) and High Performance Liquid Chromatography (HPLC) may also be used for certain separations, if these instruments are available. The Controlled Substances Section of the Virginia Department of Forensic Science (VADFS) evaluated the use of chiral mobile-phase additives (CMAs) in Thin Layer Chromatography (TLC) as an alternative method of enantiomer determination. While TLC is not a novel technique, its use as a method for resolving stereoisomeric controlled substances has not been widely explored. The use of β-cyclodextrin, hydroxypropyl-β-cyclodextrin and vancomycin as CMAs were evaluated for their effectiveness in performing enantiomeric separations on drug substances using both reverse phase (RP) and normal phase (NP) TLC. The use of chiral TLC plates and microcrystalline cellulose TLC plates was also briefly explored. While much has been learned about the use of chiral mobile phase additives in TLC, an effective and consistently reproducible method has not been developed.

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Executive Summary

The National Institute of Justice (NIJ) sought research and development projects to enhance the detection and analysis of controlled substances for criminal justice purposes. Specifically requested were projects focusing on novel chemical methods for the resolution of stereoisomeric controlled substances. The Controlled Substances section of the Virginia Department of Forensic Science (VADFS) evaluated the use of chiral mobile-phase additives (CMAs) in Thin Layer Chromatography (TLC) as an alternative method of enantiomer determination. Some drug enantiomers have vastly different effects from one another and one form may have a higher control status than the other, so a simple method of differentiating them was desired. Having the ability to quickly and easily differentiate between the enantiomers of various drugs and pharmaceuticals would be a valuable tool for any laboratory performing controlled substance analysis. A more efficient and cost effective method to differentiate stereoisomers could aid in managing the ever increasing backlog situations that affect many laboratories by reducing the amount of time spent by examiners on such determinations. While TLC is not a novel technique, its use as a method for resolving enantiomeric controlled substances had not been widely explored. The use of CMAs in TLC to differentiate amino acid enantiomers, as well as some drug compounds, had been reported in the literature[1-7] but little had been reported concerning drugs of forensic interest. Enantiomeric substances that were of forensic interest to differentiate included dextromethorphan and levomethorphan, dextropropoxyphene and levopropoxyphene, and dextro-methamphetamine and levo-methamphetamine.

Presently, VADFS uses a mixed crystal test method for enantiomer determination of methorphan but this method has proven to be difficult or ineffective for some types of samples. The objective of this project was to develop an inexpensive and simple method for enantiomer

determinations as an alternative to using mixed crystal test methods or more expensive instrumental methods. Other methods that may be used for chiral determinations, such as polarimetry, high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE), may not be practical for widespread use within the forensic community due to the need for specialized equipment, as well as budgetary and spatial constraints that many laboratories face.

For this project, VADFS evaluated the use of β-cyclodextrin (BCD), hydroxypropyl-β-cyclodextrin (HPBCD), and the macrocyclic antibiotic vancomycin for their effectiveness as CMAs in both reverse phase (RP) and normal phase (NP) TLC for the differentiation of certain enantiomeric drug substances. These substances had previously been demonstrated in the literature to have chiral selective properties. Both standard and high performance reverse phase plates were evaluated, as well as chiral TLC plates and microcrystalline cellulose TLC plates. The goal of this project was to find a simple, fast, cost effective alternative for enantiomer determination that any forensic laboratory would be able to use.

The majority of the project involved the development of enantiomeric separation methods using TLC with BCD, HPBCD or vancomycin as the CMA. Mobile phases were prepared using aqueous solutions of BCD, HPBCD or vancomycin mixed with an organic modifier (OM), such as methanol or acetonitrile. Molar concentrations of the CMAs were varied, as were percentages of the OM, and each mobile phase preparation was evaluated for its ability to separate the enantiomers of the compounds of interest. Adjustments were made to the pH of the mobile phases using formic acid, trifluoroacetic acid, ammonium hydroxide, sodium hydroxide or diethylamine. Buffers were also used to make pH adjustments. Sodium chloride was added to mobile phases in those instances where it was necessary to stabilize the stationary phase on the TLC plate. Methods were attempted using mobile phases with no chiral selector as well as

mixtures of chiral selectors. Two mobile phases were attempted for some methods; some utilizing two solutions with different chiral mobile phase additives and others containing chiral selector in only one of the mobile phases. It was presumed that more than one mobile phase may be required to perform enantioselective separation of all of the selected drug compounds, so the initial focus of the project was on the separation of the methorphan enantiomers.

It was intended that the retention factor (Rf) would be calculated for each of the compounds of interest in each mobile phase. However, due to long development times and heavy tailing, it was difficult to obtain accurate Rf values. More often than not, Rf values were not obtained. Visual records of many results were instead kept by making photocopies of the TLC plates. It was also intended that the successful method(s) would be validated by determining limit of detection, selectivity, repeatability and ruggedness of the method(s). As the replication of successful results was an issue, any attempted method validation never progressed beyond evaluating reproducibility of results.

Experiments with BCD were largely unsuccessful. At best, slight separations of the methorphan enantiomers were observed that would not be sufficient to separate components of a racemic mixture. The limited solubility of BCD in water made it difficult to work with. For concentrations above 0.01M, a saturated solution of urea was required to achieve dissolution.

Mobile phases utilizing HPBCD generated results that were slightly better, although the best results that were observed initially (utilizing a 0.1M solution of HPBCD as the mobile phase) could not be replicated once fresh methorphan standard solutions were prepared. Mobile phases that consisted of 17:3 acetonitrile: 1mM HPBCD in deionized water, at a pH of 8, demonstrated significant separation of levomethorphan base and dextromethorphan HBr. However, when both standards were run in their base form, no separation was achieved. Treating the standards with HCl, in order to create an HCl salt, resulted in greater elution of the

standards but did not affect separation. Further examinations of similar mobile phases, utilizing various TLC plate types, did not result in significant separation.

Initially, studies using vancomycin were limited due to difficulties in visualizing the developed plate. While the numerous functional groups that vancomycin possesses are a factor in it's effectiveness as a chiral selector, they also impede the visualization of results by reacting with the visualization reagents themselves. Utilizing much lower concentrations of vancomycin in the mobile phase alleviated this issue and vancomycin ended up exhibiting the most promise as a CMA for these separations. Successful results were achieved using a 2.5mM aqueous solution of vancomycin, with a mobile phase composition of 85% acetonitrile, on Whatman KC-18F reversed phase plates. The R_f difference was 0.37 between the spots. The separation was observed when one drop of 1.0M HCl was added to 0.2mL of the 2 mg/mL methorphan base standards, effectively creating the methorphan HCl salt. Unfortunately, successful separation of the methorphan enantiomers occurred only about 25% of the time. Separation was never observed when the standards were combined in a racemic mixture, which was always present as a single spot adjacent to levomethorphan run alongside. The successful vancomycin mobile phase was always slightly opaque or cloudy and separation seemed dependent on the relative cloudiness of the mobile phase. Further experimentation around this semi-successful mobile phase did not result in method optimization.

Mobile phases were prepared that used mixtures of organic modifier to alleviate the tailing that was observed with acetonitrile alone as the organic modifier. Mixtures of chiral selector in a single mobile phase were also tried, as were mobile phases that contained no chiral selector. Methods were attempted which utilized two mobile phases run consecutively with drying in between. Some of these two mobile phase methods utilized a different chiral selector in each mobile phase while others contained no chiral selector in one of the mobile phases. For

other methods, plates were pre-treated by development of the plate in a chiral mobile phase prior to spotting the analyte on the plate. The plates were developed again in a different mobile phase. None of these method variations resulted in successful separation of the methorphan enantiomers.

While the focus of this project was on the use of CMAs in TLC for enantiomer determinations, limited experiments were also done with chiral TLC plates and with microcrystalline cellulose TLC plates, which are reported to have some chiral selectivity on their own. The chiral TLC plates were predominantly used with mobile phases that did not contain any chiral selector, while the microcrystalline cellulose plates were used more extensively both with and without CMAs present. Neither type of plate was found to have success.

The enantiomers of methamphetamine and propoxyphene were sparingly worked with due predominantly to difficulties in visualizing the analyte spots on the developed plates. The presence of chiral selector in the mobile phase seemed to impair the analytes response to various visualization techniques, especially at higher concentrations of chiral selector. When results were able to be visualized, no separation was observed for either set of enantiomers. Lowering the concentration of chiral selector in the mobile phase did improve visualization of the results but did not result in significant separation of the enantiomers.

Methanol and acetonitrile were the most commonly used organic modifiers in this study. In general, mobile phases prepared with acetonitrile resulted in greater tailing of analytes than those that were prepared with methanol. However, less elution was observed for mobile phases containing methanol as compared to acetonitrile. Mobile phases that combined methanol and acetonitrile did result in increased elution while reducing tailing, but ultimately did not affect separation.

The presence of a chiral selector in the mobile phase was a factor in poor visualization of results. While methorphan typically exhibits strong response to both iodoplatinate and UV, its response to each was weakened in the presence of a chiral selector. Neither propoxyphene nor methamphetamine produces strong responses to these visualization techniques without chiral selector present, although propoxyphene was visualized more often than methamphetamine in the limited experiments that were performed. Mobile phases that contained lower concentrations of chiral selector promoted better visualization of the analytes but incomplete complexation was observed as a result of this decrease.

Ultimately, a successful TLC method for the determination of methorphan, methamphetamine or propoxyphene enantiomers was not found. While promising results were observed, they could not be consistently replicated. As BCD is nearly insoluble in water, it was extremely difficult to remove from the glassware and this may have resulted in the false separation of the methorphan enantiomers and the lack of reproducibility. HPBCD was much more soluble in water; therefore glassware contamination was not an issue. Although successful differentiation of methorphan enantiomers using HPBCD in CE had been previously performed, it is apparent from this research that methorphan does not complex with HPBCD and BCD in an enantioselective manner under TLC conditions. Methorphan simply may not interact with hydrophobic inner cone area of the cyclodextrins or it may be that the size and structure of the methorphan molecule sterically hinders interaction. As the other enantiomeric compounds investigated in the literature are much smaller and less rigid than methorphan, it is plausible that steric hindrance is a factor in the lack of successful results. While inconsistent, vancomycin was the most successful chiral mobile phase additive for producing separation of the methorphan enantiomers. Difficulties encountered with visualization required that the concentration of

vancomycin in the mobile phase be limited to 0.05M or below. This limited how much could be done with vancomycin as a chiral selector.

The mobile phases containing chiral selector mixtures did not enhance complexation and were instead a hindrance to elution of the analytes. The two system method in which plates were pre-treated with a chiral selector seemed to work best lower concentrations as those above 5mM often resulted in strange plate development patterns.

Investigation of other potential CMAs could lead to a successful method and should be considered. Investigation of γ-cyclodextrin and its derivatives may be worthwhile, as the size of the β-cyclodextrin cavity may have played a role in the inability to differentiate the methorphans. Vancomycin showed the most promise in this study and is widely used as a chiral selector for HPLC. Not much is known about how molecules complex with vancomycin other than that it has many functional groups with which molecules could interact. Further research using vancomycin or another macrocyclic antibiotic for TLC separations may be worthwhile, particularly in concert with different stationary phases that were employed in this study, such as diphenyl or ethyl reverse phase plates, if available. In addition, other methods of visualization could be explored to eliminate the problems experienced with viewing the results and allow for further investigation of the differentiation of the propoxyphene and methamphetamine enantiomers.

While dissemination of the project results to a much larger field was intended, the lack of successful results did inhibit publication and presentation. Progress reports of the project were presented at both 2010 NIJ Grantees Meeting at the annual meeting of the American Academy of Forensic Science in Seattle, WA and in-house at the 2011 VADFS Controlled Substances section meeting in Glen Allen, VA. In order to, a discussion of the project was presented at the May 2012 meeting of the Mid-Atlantic Association of Forensic Scientists.

I. Introduction

a. Statement of the problem

Enantiomers of drugs can have very different pharmacological, pharmacokinetic and metabolic behavior, thus a few drug enantiomers are controlled under legal statutes in only one enantiomeric form. At VADFS, the most commonly encountered need for enantiomer determination involves cases where a form of methorphan is present. Dextromethorphan is widely used as a non-prescription antitussive and is not a scheduled substance. Conversely, levomethorphan is a potent narcotic listed in Schedule II of the Controlled Substances Act. While its identification in marked pharmaceutical preparations is straightforward, dextromethorphan is often encountered as a cutting agent in heroin samples, in mimic tablets sold as "Ecstasy" or in submissions of unknown syrups. Levomethorphan is not commercially available in the United States and therefore it would be unusual for a forensic laboratory to encounter it.[8] However, for accurate reporting purposes, the enantiomeric form of the drug must be determined. The enantiomers of propoxyphene and methamphetamine would also be of forensic interest to differentiate. In addition, there are many new pharmaceutical preparations that consist of only one enantiomeric form of a drug. Forensic laboratories may have an increasing need to perform enantiomer determinations if this trend persists.

Currently, VADFS uses the mixed crystal test technique for the enantiomeric determination of methorphan. The advantages of this method are its simplicity and sensitivity. However, when using this method, it can be difficult to obtain an exact match between sample and standard because of impurities, concentration differences, and formation of different crystal forms (polymorphism). In some instances, extractions may be performed but they are not always effective in isolating the methorphan. In addition, while training and experience is helpful, crystal tests are somewhat of an art form that is dependent on individual skill. Interpretation is

subjective, thus it is required to have verification by a second scientist or a photographic record of the test.

Other methods of enantiomer determination have been reported including polarimetry, CE[8,9], HPLC[9-11] and GC. The inherent problems with each of these techniques are expense and, in some cases, lack of available space for additional instrumentation. Also, the amount of time required to perform the analysis can be substantial because samples must be run individually. Polarimetry requires large sample sizes and pure samples. Chiral columns for HPLC are expensive and some CMAs used with HPLC have a high absorbance background that interferes with the detection of separated enantiomers.[10] GC can be used either with chiral selective columns or by the formation of chiral derivatives. Substances such as methorphan, a tertiary amine, are not suitable for reaction with chiral derivatizing agents. In addition, some chiral derivatizing agents, such as n-Trifluoroacetyl-l-prolylchloride (l-TPC), are subject to degradation even with refrigerated storage. Chiral columns are relatively expensive and more than one chiral phase may be required to perform all desired separations. While most laboratories do have gas chromatographs available, few can afford to dedicate one instrument for stereoisomeric separations only, so valuable laboratory time would be lost by having to change GC columns when stereoisomeric determinations are necessary. In contrast, TLC is a technique that is fast, easy, inexpensive and is available to all laboratories. It is also efficient because it allows multiple samples to be run simultaneously.

b. Literature citations and review

Literature suggests that the use of cyclodextrins (CDs) or their derivatives as CMAs in thin layer chromatography is useful in stereoisomer differentiation.[1-5,10-15] The interaction of the analyte molecules with the CDs is dependent on such factors as their polarity, hydrophobicity, size, and spatial arrangement. The advantages of using CDs in chiral separations is that CDs are stable over a wide pH range, nontoxic, resistant to light, and mostly UV-transparent in the range commonly used for chromatographic detection.[12] Chromatographic separation using CDs in the mobile phase is largely based on the formation of inclusion complexes. The size of the CD is a factor in chiral recognition and can result in different enantioselectivities.[9]

In order to form an inclusion complex, the drug compound must be of a certain size and needs to enter the cavity of the CD at least partially. The cavity itself is nonpolar which enables it to interact with the hydrophobic part of the analyte. If this portion of the molecule is larger than the cavity opening, it will not be possible to form the inclusion complex. In contrast, if the hydrophobic portion of the molecule is much smaller than the opening then chiral resolution may not occur. The opening of the cavity is more polar and can form hydrogen bonds with the polar portion of the analyte. These interactions can also influence enantioselectivity if the polar portion of the molecule is at or near the chiral center. Thus, the cavity allows for preferential inclusion of one of the enantiomers which leads to chiral discrimination of the two enantiomers. Factors such as pH, temperature and composition of the mobile phase can also affect the ability to form CD inclusion complexes.[12]

Derivatization of CDs can increase their solubility in the mobile phase as well as enhance enantioselectivity.[12] The latter is presumed to be due to changes in the ability of the analyte molecule to form hydrogen bonds with hydroxyl groups at the mouth of the CD cavity.[5,12] Care must be taken not to use a derivatized CD with too many substituents, as this can block inclusion

complexation. Hydroxypropyl-β-cyclodextrin with 0.6 M substitution has been reported to be effective for stereoisomeric separations.[3]

Macrocyclic antibiotics, such as vancomycin, provided another avenue that could be explored for use as CMAs. Compounds in the glycopeptide class of macrocyclic antibiotics, to which vancomycin belongs, have been used with much success as chiral selectors in HPLC and CE.[11,16] Vancomycin itself has been reported as a successful CMA for enantiomeric separations using both normal phase[11] and reverse phase TLC.[6] Compared to the cyclodextrins, the macrocyclic antibiotics are a relatively new class of chiral selector, but have shown promising results, exhibiting a high degree of selectivity for numerous compounds. They possess a number of stereogenic centers and functional groups that allow multiple interactions with chiral molecules. They can interact by hydrophobic, dipole-dipole, π–π interactions and hydrogen bonding as well as steric repulsion.[7,13,16] Hydrophilic groups, as well as ionizable groups, provide good solubility in aqueous solutions. A number of ionizable groups are present, which controls their charge and affects their chiral recognition. Therefore, pH is an important factor to consider when working with these types of molecules.[16]

The glycopeptide class of macrocyclic antibiotics is characterized by the "basket" shape formed by the fused macrocyclic rings in the aglycon portion of the molecule.[7,16] The basket typically consists of three or four fused macrocyclic rings composed of linked amino acids and substituted phenols. The difference in the macrocyclic antibiotics is seen in the number and type of carbohydrate moieties attached to the aglycon basket. These are free to rotate and may exhibit various orientations.[16]

While the macrocyclic antibiotics have not been used extensively for TLC, their success as chiral selectors in CE and HPLC warranted some investigation of their applicability to our purposes.

c. Statement of hypothesis or rationale for the research

As BCD and HPBCD have been widely used in the literature to peform chiral separations using TLC, HPLC and CE, both of these substances were logical choices to attempt to differentiate the enantiomers of interest. Lurie and Cox were successful in their differentiation of dextro- and levo- methorphan using CE, demonstrating that a stereoselective interaction between HPBCD and the methorphan enantiomers can occur.[8]

Vancomycin is a relative newcomer to the world of chiral separations but has been quite successful as a chiral selector in HPLC and CE.[11,16] While it's use as a CMA for TLC applications has not been widely explored, it's success in performing these separations using other techniques adds merit to the further investigation of vancomycin as a chiral selector for TLC separations.

The use of CMAs as opposed to chiral stationary phases (CSPs) was the preferred method for stereoisomeric separations using TLC. They are much less expensive than using a chiral stationary phase and there are relatively few commercially available chiral TLC plates, none of which are impregnated with CDs or macrocyclic antibiotics. The use of chiral selectors in the mobile phase instead of in the chiral stationary phase allowed the concentration of the chiral selector to be easily adjusted and allowed the option of different organic modifiers in the mobile phase to effect separation.

II. Methods

a. Materials

Methorphan standard solutions were made with levomethorphan from Cerilliant (Round Rock, TX), dextromethorphan from USP (Rockville, MD) and Dextromethorphan HBr from LaRoche (Nutley, NJ). Uniplate RPSF, HPTLC-RP18F and Avicel F microcrystalline cellulose TLC plates were obtained from Analtech (Newark, DE), as were Uniplate Silica Gel GHLF normal phase plates. Fluka aluminum-backed, normal phase plates were obtained from Sigma-Aldrich (St. Louis, MO). Whatman KC-18F reversed phase plates and EMD Chemicals aluminum-backed normal phase plates were obtained from VWR (Westchester, PA). Sodium chloride, potassium iodide, and iodine crystals were obtained from Mallinckrodt (St. Louis, MO). β-CD solutions were made with Calbiochem β-cyclodextrin (99.6% purity; LaJolla, CA), which was obtained from VWR. Pharmaceutical grade Trappsol® hydroxypropyl-β-cyclodextrin (Cyclodextrin Technologies Development, Inc.), hydrochloric acid, diethylamine (DEA), chloroform, and ammonium hydroxide were obtained from Fisher Scientific or Acros Organics (a part of Thermo Fisher Scientific) (Fairlawn, NJ). Urea was provided by Baker (99.7% purity; Phillipsburg, NJ) and trifluoroacetic acid (TFA) was from Burdick and Jackson (Muskegon, MI). Vancomycin and platinic chloride were obtained from Sigma-Aldrich (St. Louis, MO). EMD HPLC grade acetonitrile and EMD Omisolv methanol were provided by VWR. Deionized water was provided on site. Formic acid (FA) was from EM Science (Gibbstown, NJ). Cylindrical and rectangular TLC chambers and saturation pads were acquired from Analtech, a Mettler Toledo AL104 balance was used, and VWR International capillary tubes were used.

b. Procedures

For the mobile phase, β-CD was used with varying molar concentrations, from 0.5mM to 0.2M, dissolved in deionized water. Urea was added in a saturated amount to the aqueous β-CD solutions to increase solubility. HP-β-CD was also used with varying molar concentrations, from 0.5mM to 0.42M, dissolved in deionized water without urea. Dissolving β-CD and HP-β-CD into aqueous solutions was time consuming, often taking hours for the higher concentrations or sometimes resulting in an unusable white and cloudy mixture. Vancomycin concentrations were varied as well, from 0.5mM to 0.05M, in aqueous solutions. Acetonitrile was used as an organic modifier in the mobile phase. The amount of acetonitrile varied from 20%-85% of the mobile phase depending on the concentration of the chiral selector. Methanol was also used as an organic modifier in the mobile phase, varying from 10%-70% depending on chiral selector concentration. Sodium chloride (0.6M) was added to mobile phases composed of more than 50% aqueous solution in order stabilize the reversed phase TLC plate binder. Either formic acid or trifluoroacetic acid was added to the mobile phase dropwise to make the mobile phase more acidic. 10% ammonium hydroxide was added in drop quantities to make the mobile phase more basic. Diethylamine and sodium hydroxide were also used. Varying concentrations, ranging from 2:1 to approximately 15:1, of chloroform and methanol were explored as mobile phases, as was a 1:1 mobile phase of ethylacetate in hexane and a 25:5:1 solution of ethyl acetate/acetone/ammonium hydroxide. The latter mobile phases did not utilize any CMA.

The reversed phase and normal phase TLC plates were spotted using 5 μL capillary tubes. The dextromethorphan and levomethorphan standard solutions were originally prepared as 2 mg/mL solutions and were later diluted to 1 mg/mL. Both concentrations were used for TLC plate runs and compared. Standard solutions of levopropoxyphene, propoxyphene, d-methamphetamine, and d,l-methamphetamine were also prepared as 2 mg/ mL solutions.

Approximately 6mL of the mobile phase was added to the round TLC chamber in which the plate was developed. Blotter paper was sometimes used to assist in chamber saturation. The development chamber was allowed to equilibrate for at least 5 minutes before the TLC plate was added. The length of time it took for a TLC plate to develop ranged from a few minutes to a few hours, proportional to the chiral selector concentration. Methods were also evaluated using a two system procedure. Before the standards were applied, the plate was run in a mobile phase with the chiral selector. Once the solvent front reached the top of the plate, the plate was removed from the chamber, allowed to dry, and the methorphan standards were spotted on the plate. The plate was then run for a second time in a mobile phase of the same composition as the first but without the chiral selector. Other two mobile phase systems were tried that utilized a second mobile phase of a different composition than the first.

Once the TLC plate had been removed from the chamber and allowed to dry, visualization was done using short wave UV light, an iodine chamber, or a series of visualization sprays including iodoplatinate reagent, ceric sulfate, ninhydrin, potassium permanganate, Marquis, or Erhlich's reagent. Under short wave UV light (254nm), methorphan quenched the Fluorescein dye in the stationary phase and appeared as a dark spot on the bright green background. Plates were placed in an iodine chamber, a large glass container with iodine crystals at the bottom for approximately 10-15 minutes. The volatile iodine turned the methorphan a yellowish-brown color, compared to the light yellow plate. When visualizing with the iodoplatinate reagent, the plate was first lightly sprayed with 6N HCl solution. The iodoplatinate reagent was then sprayed onto the surface of the plate, turning the methorphan a light purple against a pink background. If methorphan could not be visualized or was a faint color, ceric sulfate was used after iodoplatinate to enhance visualization. The remaining visualization sprays were used intermittently, often with unsuccessful results.

Once the methorphan enantiomers were visualized, the R_f value was calculated if separation occurred. Often streaking or tailing occurred which made calculating the R_f value difficult. If tailing occurred, the mobile phase was changed slightly in order to correct the tailing and produce a tighter spot during plate development, which was rarely successful.

III. Results

a. Statement of Results

BCD

In varying concentrations, BCD was used as a chiral selector in the mobile phase. Since the solubility of BCD is 0.017M in water, it was not necessary to use a saturated solution of urea for concentrations lower than 0.01M in order to achieve dissolution. Several mobile phases were made using 1mM BCD both with and without urea to investigate if any effect was had on the development of the plates. Using both reversed phase and normal phase plates, there was no significant difference between the mobile phases that contained urea and those that did not.

Initial studies were performed using either Analtech RPSF Uniplates or Analtech Avicel microcrystalline cellulose plates with either 0.10M or 0.13M BCD in varying ratios with methanol. The results are displayed in Table 1. Using the RPSF plates, slight separation was observed for only one of these mobile phases, a 10:6:1.5 mixture of methanol, 0.1M BCD and formic acid. The observed separation is so slight that it would not be sufficient to separate the components of a racemic mixture of methorphan. No spots were visualized on the microcrystalline cellulose plates. The standards used for these early studies were dextromethorphan HBr and levomethorphan base, which were easily visualized with little to no tailing observed. The Rf values were not calculated in most cases due to the long elution times observed and the plates were often not removed before the solvent front had reached the top of

the plate. One mobile phase utilizing a 17:3 ratio of acetonitrile to 1mM BCD in water was attempted during the initial studies, with the result listed in Table 5. Slight separation was observed, however the solution was cloudy indicating that BCD may have been precipitating out.

In later studies, compiled in Table 2, BCD was used in the mobile phase with various stationary phases. Low concentrations of BCD were used in mobile phases with acetonitrile as the organic modifier, as Taha et al. utilized in their separation of cetirizine.[11] The lower concentrations resulted in varied complexation with the methorphan enantiomers, which were both in the base form. To ensure that complexation was occurring, a plate was developed in a mobile phase without BCD and the standards did not elute up the plate. It was common for dextromethorphan base (2mg/mL in methanol) to be visualized as two spots, indicating incomplete complexation. In contrast, levomethorphan base (2mg/mL in methanol) fully complexed and was visualized next to the higher of the two dextromethorphan spots. Decreasing the concentration of the drug standard to 1mg/mL resulted in full complexation, with only one spot visualized, however separation of the two enantiomers did not occur.

As the concentration of BCD in the mobile phase was increased from 1mM to 3mM and 5mM, no significant separation was observed. Once the concentration of BCD reached 5mM, it became necessary to adjust the mobile phase composition because BCD was precipitating out of solution. The general mobile phase composition utilized by Taha et al. (17:3 acetonitrile: BCD)[11] could no longer be used and the amount of acetonitrile was decreased. For higher concentrations, such as 0.05M and 0.1M, the acetonitrile to BCD mobile phase ratio could not exceed 50% acetonitrile. This was not an issue when using methanol as the organic modifier.

Both reversed phase and normal phase plates were used for the lower concentrations of BCD. Methorphan base did not complex with BCD on the reversed phase plates and was always visualized at or near the origin, exhibiting a greater affinity for the stationary phase at lower

concentrations of BCD. Conversely, the normal phase plates facilitated full complexation between methorphan and BCD, however no significant separation occurred for any mobile phases. When a blank mobile phase (no BCD) was used, neither enantiomer moved from where it was spotted, indicating that complexation did occur in the presence of BCD. The aluminum backed normal phase plates facilitated good elution of the methorphan resulting in compact spots with minimal tailing, however no significant separation occurred. For the microcrystalline cellulose normal phase plates it was necessary for the concentration of methorphan to be 1mg/mL in order to prevent the stationary phase from becoming saturated. On these plates methorphan traveled with the solvent front to the top of the plate with no visualized separation.

Higher concentrations of BCD were slightly more successful than the lower concentrations. Slight separation was produced between dextromethorphan and levomethorphan with 0.05M BCD on the reversed phase plates, both traveling approximately 2cm. However, no separation occurred when the racemic mixture was developed. The reversed phase plates also exhibited tailing, which made the calculation of an R_f value for either methorphan enantiomer difficult. Methorphan traveled approximately 5cm on the aluminum-backed normal phase plates but without enantiomeric separation. Higher concentrations of BCD did not develop differently than 0.05M and did not affect enantioselectivity between dextromethorphan and levomethorphan.

Numerous mobile phase ratios and organic modifier amounts were investigated. The most successful mobile phases for BCD were composed of 50% acetonitrile or 30-50% methanol. Higher acetonitrile amounts tended to induce tailing but slight separation still occurred. Methanol did not exhibit as much tailing as acetonitrile, however methorphan did not elute as far up the plate with methanol as the organic modifier. Mixtures of organic modifiers in the mobile phase were also attempted. A mobile phase composed of 50% acetonitrile, 35%

BCD, and 15% methanol produced spots with slight tailing, approximately 3 cm from the origin, but without separation. Tailing was predominantly observed on the reverse phase plates compared to the normal phase plates. The amount of acetonitrile was increased in the mobile phase up to 85%; however this was only possible with low concentrations of BCD. The two mobile phase method, which involved precoating the plates with the chiral selector, did not facilitate separation.

The pH of the mobile phase was also varied. Armstrong et al. stated that a pH of 4 or 7 was optimal for separation[4], while Taha et al. performed successful separation at a slightly more basic pH.[11] BCD mobile phases were run at pH's of 4, 6, 7, 8, and 10 in order to evaluate the effect of pH on plate development. Formic acid was used to lower the pH and 10% ammonium hydroxide was used to raise the pH. The mobile phase solutions existed at a pH of 6 before adjustment. With more acidic mobile phases, methorphan tended to travel further without tailing but no separation occurred. Using the aluminum-backed normal phase plates, at a pH of 7 or 8, the results varied according to BCD concentration. The increased pH resulted in increased elution distance when the BCD concentration was low, but had no significant effect when the BCD concentration was above 0.05M. At a pH of 10, no movement was visualized on both the reversed phase and normal phase plates; indicating that complexation between BCD and the methorphan enantiomers did not occur.

HPBCD

HPBCD was successful at achieving partial separation of the methorphan enantiomers. Results for HPBCD are displayed in Tables 3, 4 and 5. The concentration of HPBCD varied from low (0.5mM, 1mM, 3mM, 5mM, and 0.05M) to high (0.1M, 0.2M, 0.35M, and 0.42M) in accordance with concentrations listed in the literature. Taha et al. separated cetirizine enantiomers with 1mM HPBCD.[11] Armstrong et al. separated amino acids and some drug

enantiomers using 0.2M and 0.4M HPBCD.[4] As HPBCD is much more soluble in water than β-CD, higher concentration solutions were easier to prepare. No concentration above 0.4M was recorded in the literature, due to high viscosity of the resulting solutions and long development times, so 0.42M was the highest concentration explored in this research.

Early studies utilizing HPBCD as the chiral selector were done using the Analtech RPSF Uniplates and focused on the higher concentration range. Baths were prepared to a selected ratio and then adjusted with additional OM or HPBCD solution to evaluate the effect of each on the efficiency of the mobile phase. No significant effects were observed due to increases in amount of either component. In general, if separation was observed it was either not reproducible or was so slight that one would not be able to distinguish components of the racemic mixture. Significant separation was only achieved using a 0.1M solution with no OM present and was not reproducible upon the preparation of fresh standard solutions. Brief studies were done early on which utilized the microcrystalline cellulose plates, however no spots were visualized. Later studies utilized the lower range of concentrations and a wider range of plate types.

Significant separation was observed between dextromethorphan HBr was levomethorphan base when utilizing the 17:3 acetonitrile:1mM HPBCD mobile phase on Analtech GHLF normal phase. A weak spot was also observed in the dextromethorphan standard at the same elution level as the spot in the levomethorphan standard. It was initially surmised that the dextromethorphan standard may not be racemically pure, however base extraction of the two standards resulted in matching spots for dextromethorphan and levomethorphan base. These results indicated that the salt form of the drug had more affinity for the mobile phase than the base form and that the dextromethorphan HBr salt partially converted to the base form. The conversion of methorphan base to an HCl salt prior to analysis was explored and it was determined that adding a couple of drops of HCl to the standard solution was

the optimal method. Although Taha had performed separations using normal phase plates,[11,14,15] it seemed that in this situation more successful results might be achieved using reverse phase plates which exhibited less affinity for methorphan than the normal phase plates.

Later HPBCD mobile phases were run on both reversed phase and normal phase plates. The reversed phase plates were developed using each of the HPBCD concentrations. Generally, at the lower concentrations of HPBCD, methorphan traveled only 1-1.5cm up the plate and heavy tailing was a problem on the reversed phase plates. At the higher concentrations of HPBCD, the reverse-phased plates still exhibited tailing, however slight separation was visualized using 0.1M HPBCD. The aluminum-backed normal phase plates were also used with each of the HPBCD concentrations. The lower concentrations produced tighter spots and methorphan traveled approximately 3cm up the plate but no significant separation occurred. In general, the normal phase plates exhibited less tailing. Incomplete complexation occurred with the normal phase plates similar to BCD. Dextromethorphan, especially at 2mg/mL, developed two spots while levomethorphan complexed fully and traveled with the higher dextromethorphan spot. This was solved by reducing the methorphan standard solution to a 1mg/mL concentration.

Similar mobile phases were used with HPBCD as were used with BCD. The most successful mobile phases, in terms of partial separation, were 50-60% acetonitrile, 50-65% methanol, and 4:2:1, 4:2:2, and 8:8:2 acetonitrile:$HPBCD_{aq}$:methanol. All of the partial separations exhibited heavy tailing so calculating the R_f values proved difficult. As the amount of methanol increased in the mobile phase, the distance traveled by the methorphans increased and the amount of tailing decreased. When acetonitrile was the only organic modifier, heavy tailing occurred and thus mixed mobile phases containing both acetonitrile and methanol were used. The mixed organic modifier corrected most of the tailing, however full separation of the

enantiomers was not achieved. The two mobile phase system method was unsuccessful as well at separating the enantiomers.

The changes in the mobile phase pH for HPBCD were also similar to BCD. The pH of the mobile phase was varied from 3-10 using formic acid and 10% ammonium hydroxide. At a pH of 3-4, the enantiomers traveled further up the plate and had less tailing. No significant difference was observed in methorphan travel between the pH ranges of 6-10.

Vancomycin

Initial work with vancomycin was limited, then abandoned, due to difficulties encountered while visualizing the analyte on the TLC plates post-development. Interference occurs due to the functional groups present in vancomycin reacting with conventional visualization methods such as ultraviolet (UV) light, acidified iodoplatinate spray and potassium permanganate spray. Table 6 displays the mobile phases and concentrations of vancomycin used in these early studies. Vancomycin was later re-visited as a CMA using much lower concentrations in the mobile phase. It ultimately was the most successful CMA used for separation of the methorphan enantiomers. Table 7 lists the mobile phases with lower concentrations of vancomycin that were attempted prior to the development of the successful mobile phase and concentration. Table 8 shows the successful separation data and the alterations made to the mobile phase, vancomycin concentration, and pH in order to increase robustness. At a concentration of 2.5mM and with a mobile phase composition of 85% acetonitrile, separation of dextromethorphan and levomethorphan was observed on the Whatman KC-18F reversed phase plates, with an R_f difference of 0.37 between the spots. Both spots were compact, with no tailing, and were visualized using an iodine chamber or acidified iodoplatinate reagent. The separation was not observed for methorphan base but occurred with one drop of 1.0M HCl was added to 0.2mL of the 2 mg/mL standards, effectively creating the methorphan HCl salt. The

robustness of this separation was extremely low; occurring approximately 25% of the time and never when the standards were combined in a racemic mixture. When run alongside the single enantiomers , the racemate was observed as a single spot next to levomethorphan.

Because of the inconsistency in achieving separation many of the experimental parameters were varied. These included mobile phase composition, vancomycin concentration, and mobile phase pH. The mobile phase composition was adjusted around the successful composition noted above, with ranges of 5-20 mL for acetonitrile and 3-5 mL for the vancomycin solution. Methanol was added in 1-3 mL amounts. No separation was observed as a result of these changes. The two system method for plate development was also attempted using vancomycin, with no resulting separation. The vancomycin concentration was also varied from 1mM to 0.05M, but was ineffective in achieving separation. Slight separation was observed at 2mM but no separation occurred at the other concentrations. Also, at concentrations beyond 3mM, vancomycin precipitated out of the mobile phase as a white solid. The pH was adjusted between 2.5-8 using formic acid, trifluoroacetic acid, and 10% ammonium hydroxide. Varying the pH from 6 had no effects on separation, however less tailing occurred at 2.5-4 pH.

The vancomycin aqueous solution composition was changed by adding a buffer in order to increase consistency of the separation. Three buffers were prepared: potassium hydrogen phthalate and HCl (pH=2.2), KCl and HCl (pH=2.2), and NaCl and HCl (pH=2.2). The mobile phase composition was 17:3 acetonitrile: buffer solution with vancomycin. Buffers containing HCl were investigated as mobile phase components to determine if methorphan base would be converted to the salt form in situ, thus eliminating a step in the preparation of the standard. This was unsuccessful. A more consistent enantioselective complexation was not achieved with the additional HCl in solution and separation between the enantiomers did not occur.

No chiral selector

A few experiments were done in the absence of any chiral selector, these are documented in Table 9. Initially, mobile phases that are used regularly by VADFS for normal phase TLC was used to test the efficacy of the visualization methods with the reverse phase plates after development. Some separation was noted with the 9:1 chloroform: methanol mobile phase, so the experiments were carried further to investigate whether or not this separation could be optimized by modifying the solvent ratio. No successful separation resulted from these mobile phases. Experiments without CMAs were also done using the Chiralplates and Avicel F microcrystalline cellulose plates that predominantly utilized mobile phases that had been reported by the respective manufacturers for these plates. Neither the Chiralplates nor the microcrystalline plates affected separation under these conditions.

Two mobile phase methods

As noted above, some separation of the methorphan enantiomers was observed after development of the reverse phase plates with 9:1 chloroform: methanol. When modification of the solvent ratio did not result in a more significant separation, further experiments were performed utilizing two mobile phases. Initially TLC plates were developed in one mobile phase, allowed to dry, and then developed again in a second mobile phase. The results of these experiments are detailed in Table 10. Slight separation was observed when the 9:1 chloroform: methanol mobile phase was followed by a mobile phase containing 10:1:0.5 methanol: 0.2M HPBCD: formic acid. However, this separation was not significant enough to differentiate components of a racemic mixture. Further modifications of the two mobile phase method were unsuccessful.

Later experiments with two mobile phase systems utilized one mobile phase containing chiral selector and the other simply deionized water in combination with an organic modifier at

the same ratio. These systems predominantly utilized HPBCD as the chiral selector, although both BCD and vancomycin were used as well. The results of these experiments, which are listed in Table 11, were unsuccessful.

Chiral selector mixtures were also used with the two mobile phase system, these experiments are documented in Table 12. The plate was precoated with HPBCD and run again in a vancomycin mobile phase after the racemic standard had been spotted.. Conversely, the plate was precoated with vancomycin and run a second time in HPBCD. Reverse phase plates (Whatman) and aluminum-backed normal phase plates (Fluka) were used, the pH remained at six, and the mobile phases used were 17:3 acetonitrile to aqueous chiral selector. No separation was observed with either of the racemic mixtures, base form and HCl salt, at any of the chiral selector concentrations (1mM, 5mM, and 10mM). The HCl salt racemic methorphan standard had heavy tailing on the reverse phase plate but had a tight spot on the normal phase plate, in both instances traveling approximately 6cm up the plate. The base methorphan only traveled 1cm on both plates and did not tail.

Single Mobile Phase Chiral Selector Mixtures

Vancomycin, HPBCD, and BCD were added to the mobile phase in mixtures. Whatman KC-18F reversed phase, Fluka and Whatman aluminum-backed normal phase, and Uniplate cellulose normal phase plates were used. Table 13 displays the results of mixing two chiral selectors in the mobile phase. For the Whatman reverse phase plates, the methorphan enantiomers did not travel from the origin except for in 17:3:3 acetonitrile:HPBCD:BCD which traveled 1cm without separating. For both types of normal phase plates, the enantiomers traveled with the solvent front to the top of the plate without separating.

Methamphetamine and Propoxyphene enantiomers

Limited studies were done on the separation of the propoxyphene and methamphetamine enantiomers due to difficulties experienced in visualizing the results post-development. Early experiments were performed utilizing higher concentrations of chiral selector and the presence of the chiral selector in the mobile phase seemed to impair the response of the analyte, which is already weak for these compounds. When the results were able to be visualized, no separations were observed. The results of these experiments are compiled in Table 14. Brief experiments were later attempted using the mobile phases with a 17:3 ratio of acetonitrile: cyclodextrin solution. These are detailed in Table 4. No separation of either set of enantiomers resulted, and visualization was again difficult.

b. Tables

Table 1. Early Mobile Phases Utilizing BCD as Chiral Selector

Levomethorphan base and Dextromethorphan HBr standards

A: acetonitrile; M: methanol; CD: cyclodextrin; FA: formic acid; DEA: diethylamine

a: 1.5 mL FA; b: 2 mL NaCl; c: 5 mL NaCl; d: 1mL FA; e: 0.14 mL DEA; f: 0.42 mL DEA

Plate	Ratio		CD Concentration	Separation	Tailing	Comments
Uniplate RPSF reverse phase	M:CD[a,b]	10.5:3	0.13M	No	No	
	M:CD[a,b]	10:6	0.13M	No	No	
	M:CD[a,b]	10:1	0.13M	No	No	
	M:CD[b]	10:3	0.13M	No	very slight	spots are oblong
	M:CD[b]	10:3	0.13M	No	very slight	Rf = 0.512 (d) Rf = 0.451 (l/d,l)
	M:CD[b]	5:3	0.13M	No	No	
	M:CD[b]	20:3	0.13M	No	No	
	M:CD[c]	10:3	0.13M	No	No	
	M:CD[a]	10:2	0.13M	No	No	
	M:CD[a,b]	10:3	0.10M	No	No	
	M:CD[a,b]	10:6	0.10M	No	No	
	M:CD[a,b]	10:1	0.10M	No	No	
	M:CD[b]	10:6	0.10M	No	slight	
	M:CD[a]	10:6	0.10M	slight	No	
	M:CD[a,b]	10:3	0.10M	No	No	
Avicel F Microcrystalline cellulose	M:CD[d]	7:2	0.10M	no spots visualized		
	A:CD[d]	7:2	0.10M	no spots visualized		
	M:CD[e]	4:1	0.10M	no spots visualized		
	M:CD[f]	4:1	0.10M	no spots visualized		

Table 2. Later Mobile Phases Utilizing BCD as Chiral Selector

Levomethorphan base and Dextromethorphan base standards

A: acetonitrile; M: methanol; CD: cyclodextrin; a: formic acid; b: ammonium hydroxide; c: 2mL 0.6M NaCl

Plate	Ratio	Ratio	CD Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F	1:1	A:CD	0.05M[c]	6	Slight	3cm	yes
			0.1M[c]	6	No	3cm	yes
			0.15M[c]	6	No	3cm	yes
		M:CD	0.05M	6	No	2cm	yes
			0.1M	6	No	2cm	yes
			0.15M	6	No	2cm	yes
			0.05M	3.5[a]	Slight	2cm	yes
			0.1M	3.5[a]	No	3cm	yes
			0.15M	3.5[a]	No	3cm	yes

Table 2. (continued)

Plate	Ratio	Ratio	CD Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F	2:1	M:CD	0.05M	3.5[a]	No	3cm	yes
			0.1M	3.5[a]	No	3cm	yes
			0.15M	3.5[a]	No	3cm	yes
			0.05M	6	No	2cm	slight
			0.1M	6	No	2cm	slight
			0.15M	6	No	2cm	slight
	17:3	A:CD	1mM	3.5[a]	No	3cm	yes
			3mM	3.5[a]	No	3cm	yes
			5mM	3.5[a]	No	3cm	yes
			1mM	6	No	2cm	yes
			3mM	6	No	2cm	yes
			5mM	6	No	2cm	yes
			1mM	8[b]	No	2cm	yes
			3mM	8[b]	No	2cm	yes
			5mM	8[b]	No	2cm	yes
			1mM	10[b]	No	2cm	yes
			3mM	10[b]	No	2cm	yes
			5mM	10[b]	No	2cm	yes
	3:7	A:CD	1mM	6		no movement	
			3mM	6		no movement	
			5mM	6		no movement	
	1:3	A:CD	0.05M[c]	6	No	2cm	yes
			0.1M[c]	6	No	2cm	yes
			0.15M[c]	6	No	2cm	yes
		M:CD	0.05M[c]	6	No	2cm	yes
			0.1M[c]	6	No	2cm	yes
			0.15M[c]	6	No	2cm	yes
	10:3	M:CD	0.05M[c]	6		no movement	
Uniplate Avicel microcrystalline cellulose	7:2	M:CD	0.1M	6	No	8cm	Yes
	7:2	M:CD	0.15M	6	No	8cm	Yes
	5:4	M:CD	0.1M	6	No	8cm	Yes
Fluka and EMD Aluminum backed Silica gel	1:1	A:CD	0.05M	6	No	5cm	none
		M:CD	0.05M	6	No	5cm	none
	2:1	M:CD	0.05M	6	No	6cm	none
			0.1M	6	No	6cm	none
			0.15M	6	No	6cm	none
			0.05M	3.5[a]	No	6cm	none
			0.1M	3.5[a]	No	6cm	none
			0.15M	3.5[a]	No	6cm	none
	1:6	M:CD	0.05M	6	No	1cm	none

Table 2. (continued)

Plate	Ratio	Ratio	CD Concentration	pH	Separation	Distance Traveled	Tailing
Uniplate RPSF and Uniplate HPTLC-RP18F	17:3	A:CD	1mM	6	not visualized		
			3mM	6	not visualized		
			5mM	6	not visualized		
	1:1	A:CD	3mM	6	not visualized		
			0.1M	6	not visualized		
			0.2M	6	not visualized		

Table 3. Early Mobile Phases Utilizing HPBCD as Chiral Selector

Levomethorphan base and Dextromethorphan HBr standards

A: acetonitrile; M: methanol; CD: cyclodextrin; FA: formic acid; TFA: trifluoroacetic acid

Plate	Ratio		CD Conc.	pH	Adjustments	Separation	Tailing	Comments
Uniplate RPSF	10.5:5:1.5	M:CD:FA	0.1M	2		No	No	
						No	No	Rf =0.704
				2	3 mL + 1 mL M	No	No	
				2	3 mL + 1 mL CD	No	No	
				3	1 mL + 3 mL CD	No	No	
	10.5:5:0.5	M:CD:FA	0.1M	3		No	No	
				3	3 mL + 1 mL M	No	No	
				3	3 mL + 1 mL CD	No	No	
				2	2 mL + 2 mL CD	Slight	No	
				3	1 mL + 3 mL CD	Slight	No	
				3	3 mL + 1 mL CD	No	No	
				2	2 mL + 2 mL CD	No	No	
				3	1 mL + 3 mL CD	No	No	
	10.5:5:0.5	M:CD:FA	0.15M	3		No	No	
						No	No	Rf =0.686
				3	1 mL + 3 mL CD	No	No	
				3	2 mL + 2 mL CD	No	No	
				2.5	3 mL + 1 mL CD	No	No	
				2.5	3 mL + 1 mL M	No	No	
				3	2 mL + 2 mL M	No	No	
	2:2:0.5	M:CD:FA	0.1M	2.5		No	No	
	1:3:0.5	M:CD:FA	0.1M	2.5		No	No	
	1.5:2.5:0.5	M:CD:FA	0.1M	2.5		No	No	
	1:4:0.5	M:CD:FA				No	No	
	0.6:3:0.5	M:CD:FA				No	No	
	0.5:3:0.5	M:CD:FA				No	No	
	1:1	M:CD	0.1M	3		No	No	
	1:3	M:CD	0.1M	3		No	No	
	1:2	M:CD	0.1M	3		No	No	

Table 3. (Continued)

Plate	Ratio	CD Conc.	pH	Adjustments	Separation	Tailing	Comments
Uniplate RPSF (continued)	10.5:5:0.5 M:CD:FA	0.08M					
				3 mL + 1 mL CD	No	No	
				2 mL + 2 mL CD	No	No	
				1 mL + 3 mL CD	No	No	
				1 mL + 3 mL M	No	slight	
				1 mL + 2 mL M	No	No	
				2 mL + 2 mL M	No	No	
				2 mL + 1 mL M	Yes	No	
				3 mL + 1 mL M	Yes	No	
				2 mL + 1 mL M	No	No	
				3 mL + 1 mL M	No	No	
	6:6:0.5 M:CD:FA	0.08M					
				3 mL + 1 mL CD	No	No	
				2 mL + 2 mL CD	No	No	
				1 mL + 3 mL CD	No	No	
	10.5:5:0.5 M:CD:FA	0.12M			No	No	
				3 mL + 1 mL CD	No	No	
				2 mL + 1 mL CD	No	No	
				1 mL + 3 mL CD	slight	No	
				1 mL + 3 mL M	No	slight	
				1 mL + 2 mL M	No	slight	
				2 mL + 2 mL M	No	No	
				2 mL + 1 mL M	No	No	
				3 mL + 1 mL M	No	No	
	10.5:5:0.5 M:CD:FA	0.2M			No	No	
				3 mL + 1 mL CD	No	No	
				2 mL + 2 mL CD	slight	No	
				1 mL + 3 mL CD	No	No	
				1 mL + 3 mL M	No	No	
				1 mL + 2 mL M	No	No	
				2 mL + 2 mL M	No	No	
				2 mL + 1 mL M	No	No	
				3 mL + 1 mL M	No	No	
	10.5:5:0.5 M:CD:FA	0.25M			No	No	
				1 mL + 3 mL M	slight?	No	
				1 mL + 2 mL M	No	No	
				2 mL + 2 mL M	No	No	
				2 mL + 1 mL M	No	No	
				3 mL + 1 mL M	No	No	

Table 3. (Continued)

Plate	Ratio	CD Conc.	pH	Adjustments	Separation	Tailing	Comments
Uniplate RPSF (continued)	10.5:5:0.5 M:CD:AA	0.2M			No	No	
				1 mL + 3 mL M	No	No	
				1 mL + 2 mL M	No	No	
				2 mL + 2 mL M	No	No	
				2 mL + 1 mL M	No	No	
				3 mL + 1 mL M	No	No	
	10:5:0.5 M:CD:TFA	0.2M			No	No	
	20:5:0.5 M:CD:FA	0.2M			No	No	
	10:1:0.5 M:CD:FA	0.2M			slight	No	
					slight	No	
	10:3:0.5 M:CD:FA	0.2M			No	No	
	2:0.5:0.5 M:CD:FA	0.2M			No	Yes	
	10:5:0.5 A:CD:FA	0.2M			No	slight (lvm)	cloudy
					No	slight (lvm)	
					No	slight (all)	Rf = 0.781
	20:5:0.5 A:CD:FA	0.2M			No	slight (dxm)	cloudy
	10:1:0.5 A:CD:FA	0.2M			No	slight	cloudy
	10:3:0.5 A:CD:FA	0.2M			No	No	Rf = 0.52
	20:0.5:0.5 A:CD:FA	0.2M			No	No	Rf = 0.168
	10.5:2:0.5 M:CD:FA	0.12M		2 mL + 1 mL M	No	No	Rf = 0.709
	6:6:0.5 M:CD:FA	0.08M			No	No	Rf = 0.662
	CD only	0.1M			Yes	Yes	multiple runs
	CD only	0.1M			No	Yes	fresh stds
	CD only	0.09M			No	slight	fresh stds
Avicel F microcrystalline cellulose	10.5:5:1.5 M:CD:FA	0.1M			no spots visualized		
	10.5:5:0.5 M:CD:FA	0.15M			no spots visualized		

Table 4. Early Mobile Phases Utilizing 17:3 Acetonitrile:Aqueous Solutions

A: acetonitrile; CD: cyclodextrin

a: pH adjusted by addition of NH_4OH; b: pH adjusted by addition of trifluoroacetic acid

	Propoxyphene enantiomers						
Uniplate GHLF	**Mobile Phase**	**CD**	**Conc.**	**pH**	**Separation**	**Tailing**	**Comments**
normal phase	17:3 A:CD[a]	HPBCD	1mM	8	slight		poor visualization

Table 4. (continued)

	Methorphan enantiomers						
Plate	**Mobile Phase**	**CD**	**Conc.**	**pH**	**Separation**	**Tailing**	**Comments**
Uniplate GHLF normal phase	17:3 A:H20 17:3 A:H20	none none		5-6 5-6	 slight	 at bottom	pretreatment
	17:3 A:CD 17:3 A:H20	BCD none	1mM	5-6 5-6	 slight	 at bottom	pretreatment, cloudy
	17:3 A:CD 17:3 A:H20	HPBCD none	1mM	5-6 5-6	 slight	 at bottom	pretreatment
	17:3 A:CD[a]	HPBCD	1mM	8	yes		dxm hbr, lvm base
	17:3 A:CD[a]	HPBCD	1mM	8	yes		dxm hbr, lvm base (fresh)
	17:3 A:CD	HPBCD	1mM	6	no	slight	base ext'd stds
	17:3 A:CD[a]	HPBCD	1mM	8	no	slight	base ext'd stds
	17:3 A:CD	HPBCD	1mM	6	no	no	base ext'd stds treated w/ conc HCl (1-2 drops)
	17:3 A:CD[a]	HPBCD	1mM	8	slight	no	base ext'd stds treated w/ conc HCl (1-2 drops)
	17:3 A:H20	HPBCD	none	6-7	yes	no	
	17:3 A:CD[a] 17:3 A:H20	HPBCD none	1mM	8 6-7	 no	 no	pretreatment dxm has two spots?
	17:3 A:CD[b]	HPBCD	1mM	5	no	no	stds prepared by adding HCl to previously prepared stds
	17:3 A:CD	HPBCD	1mM	5	no	no	
	17:3 A:CD[a]	HPBCD	1mM	8-9	no	no	

Table 4. (continued)

	Methamphetamine enantiomers						
	Mobile Phase	**CD**	**Conc.**	**pH**	**Separation**	**Tailing**	**Comments**
Uniplate GHLF normal phase	17:3 A:H20 17:3 A:H20	none none		5-6 5-6	 no	 yes	pretreatment
	17:3 A:CD 17:3 A:H20	BCD	1mM	5-6 5-6	 no	 yes	pretreatment, cloudy
	17:3 A:CD 17:3 A:H20	HPBCD	1mM	5-6 5-6	 no	 yes	pretreatment visualized w/ KMnO4 only (slow)

Table 5. Later Mobile Phases Utilizing HPBCD as Chiral Selector

Levomethorphan base and Dextromethorphan base standards

A: acetonitrile; M: methanol; CD: cyclodextrin; a: formic acid; b: ammonium hydroxide; c: 2mL 0.6M NaCl

Plate	Ratio	Ratio	CD Conc.	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F	17:3	A:CD	1mM	6	No	1cm	yes
			3mM	6	No	1cm	yes
			5mM	6	No	1cm	yes
			1mM	10[b]		no movement	
			5mM	10[b]		no movement	
			1mM	3.5[a]	No	3cm	yes
			3mM	3.5[a]	No	3cm	yes
			5mM	3.5[a]	No	3cm	yes
			0.1M	6	No	1cm	yes
			0.2M	6	No	1cm	yes
			0.35M	6	No	1cm	yes
			0.42M	6	No	1cm	yes
	3:7	A:CD	1mM	3.5[a]	No	no movement	
			3mM	3.5[a]	No	no movement	
			5mM	3.5[a]	No	no movement	
			3mM	5[a]	No	no movement	
	8:5	A:CD	0.1M[c]	6	slight	4cm	yes
			0.35M[c]	6	No	no movement	
	2:10	A:CD	1mM[c]	6	No	no movement	
			3mM[c]	6	No	no movement	
			0.35M	6	No	no movement	
			0.35M[c]	6	No	4cm	yes
	4:1	A:CD	0.35M[c]	3.5[a]	No	4cm	slight
	3:5	A:CD	0.35M	6	No	no movement	
			0.42M[c]	6	No	1cm	yes

Table 5. (continued)

Plate	Ratio	Ratio	CD Conc.	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F (continued)	2:1	M:CD	1mM	6	No	3cm	yes
			5mM	6	No	3cm	yes
			0.1M	6	slight	3cm	yes
			0.2M	6	No	none	
			0.1M	3.5[a]	No	3cm	yes
	1:1	M:CD	5mM[c]	6	No	1cm	slight
			0.2M	3.5[a]	No	1cm	yes
	4:1	M:CD	1mM[c]	6	No	1cm	slight
	2:1	M:CD	1mM[c]	6	No	1cm	slight
	4:1	M:CD	0.1M[c]	9.5[b]	No	no movement	
			1mM[c]	6	No	3cm	yes
			3mM[c]	6	No	3cm	yes
	6:1	M:CD	5mM[c]	6	No	3cm	yes
			1mM[c]	6	No	3cm	yes
			3mM[c]	6	No	3cm	yes
	10:1	M:CD	5mM[c]	6	No	3cm	yes
			1mM[c]	6	No	1cm	slight
			3mM[c]	6	No	1cm	slight
	1:1	M:CD	5mM[c]	6	No	1cm	slight
	4:1	M:CD	0.35M[c]	6	No	4cm	yes
	10:3	M:CD	0.35M[c]	6	No	1cm	yes
			0.1M[c]	3[a]	No	6cm	none
			0.2M[c]	3[a]	No	6cm	none
			0.35M[c]	3[a]	No	6cm	none
			0.42M[c]	3[a]	No	4cm	none
	4:2:1	A:CD:M	0.1M[c]	6	slight	4cm	yes
	4:2:1	M:CD:A	0.1M[c]	6	No	3cm	yes
	4:2:2	A:CD:M	0.1M[c]	6	slight	4cm	yes
	4:2:2	M:CD:A	0.1M[c]	6	No	4cm	yes
	8:5:2	A:CD:M	0.1M[c]	6	No	3cm	slight
	8:5:2	M:CD:A	0.1M[c]	6	No	3cm	slight
	10:2:1	M:A:CD	1mM	6	No	1cm	slight
			3mM	6	No	1cm	slight
			5mM	6	No	1cm	slight
	8:8:2	A:CD:M	0.1M[c]	6	slight	3cm	slight

Table 5. (continued)

Plate	Ratio	Ratio	CD Conc.	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F (continued)	10:5:2	M:CD:A	1mM	6	No	no movement	
			3mM	6	No	no movement	
			5mM	6	No	no movement	
			1mM	3.5[a]	No	4cm	slight
			5mM	3.5[a]	No	4cm	slight
	10:5:4	A:CD:M	1mM	6	No	no movement	
			3mM	6	No	no movement	
			5mM	6	No	no movement	
			1mM	3.5[a]	No	4cm	slight
			3mM	3.5[a]	No	4cm	slight
			5mM	3.5[a]	No	4cm	slight
	5:5:2	A:CD:M	1mM	3.5[a]	No	4cm	yes
			3mM	3.5[a]	No	4cm	yes
			5mM	3.5[a]	No	4cm	yes
	10:5:4	M:CD:A	1mM	3.5[a]	No	1cm	yes
			3mM	3.5[a]	No	1cm	yes
			5mM	3.5[a]	No	1cm	yes
	10:2:2	CD:A:M	3mM[c]	6	No	no movement	
			5mM[c]	6	No	no movement	
	8:5:2	A:CD:M	0.35M[c]	6	No	4cm	yes
		A:CD:M	0.35M[c]	3.5[a]	No	4cm	slight
	3:3:1	A:CD:M	0.35M	6	No	no movement	
	7:5:2	A:CD:M	0.42M[c]	3.5[a]	No	4cm	yes
Fluka and Whatman aluminum backed silica gel	8:5:2	A:CD:M	0.2M	6	No	6cm	none
			0.35M	6	No	6cm	none
			0.35M	4[a]	No	4cm	none
	10:3	M:CD	0.1M	3.5[a]	No	6cm	yes
			0.2M	3.5[a]	No	6cm	yes
			0.35M	3.5[a]	No	6cm	yes
	17:3	A:CD	1mM	6	No	6cm	none
			3mM	6	No	6cm	none
			3mM	8[b]	No	6cm	none
	1:6	M:CD	3mM	6	No	1cm	none
			0.1M	6	No	2cm	yes
			0.2M	6	No	2cm	yes
	3:1	A:CD	5mM	6		no movement	
Uniplate Avicel microcrystalline cellulose	7:2	M:CD	0.1M	6	No	8cm	Yes
	7:2	M:CD	0.15M	6	No	8cm	Yes
	5:4	M:CD	0.1M	6	No	8cm	Yes

Table 5. (continued)

Plate	Ratio	Ratio	CD Conc.	pH	Separation	Distance Traveled	Tailing
Uniplate RPSF and Uniplate HPTLC-RP18F	17:3	A:CD	1mM	6	not visualized		
			3mM	6	not visualized		
			5mM	6	not visualized		
	1:1	A:CD	3mM	6	not visualized		
			0.1M	6	not visualized		
			0.2M	6	not visualized		

Table 6. Early Mobile Phases Utilizing Vancomycin as Chiral Selector in 0.6M NaCl solution

Levomethorphan base and Dextromethorphan HBr standards

A: acetonitrile; M: methanol; V: Vancomycin; FA: formic acid; TFA: trifluoroacetic acid; DEA: diethylamine

Plate	Ratio	Vancomycin Concentration	pH modifier	Separation	Tailing
Uniplate RPSF	2:10 A:V	0.025M	none	not visualized	
	2:2 A:V	0.012M	none	no	no
	2:2 A:V	0.012M	1 mL FA	no	no
	2:2 A:V	0.012M	3 mL FA	no	no
	2:2 A:V	0.012M	1 mL TFA	no	no
	2:2 A:V	0.012M	1 mL 4N NaOH	no	slight
	2:2 A:V	0.012M	1 mL DEA	no	slight
HPTLC-RP18F	2:2 A:V	0.012M	none	not visualized	
Chiralplate	2:2 A:V	0.012M	none	no	no

Table 7. Later Mobile Phases Utilizing Vancomycin as Chiral Selector

Levomethorphan base and Dextromethorphan base standards

A: acetonitrile; M: methanol; V: vancomycin
a: formic acid; b: 10% ammonium hydroxide; c: 2mL 0.6M NaCl; d: 10% trifluoracetic acid; e: base standard; f: 1.0M HCl salt standard

Plate	Ratio		Vancomycin Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F	1:1	A:V	2mM[c]	6		no movement	
			2mM[c]	3.5[a]	No	2cm	Yes
			0.01M[c]	6	No	1cm	Yes
			0.025M[c]	6	No	1cm	Yes
			0.05M[c]	6	No	1cm	Yes
		M:V	2mM[c]	6		no movement	
	1:2	A:V	2mM[c]	6		no movement	
		M:V	2mM[c]	6		no movement	
	1:2	A:V	2mM[c]	6		no movement	
		M:V	2mM[c]	6		no movement	
	2:1	A:V	2mM	6		no movement	
		M:V	2mM	6		no movement	
	4:3:4	A:V:M	2mM	6		no movement	
	3:1	A:V	2mM	6		no movement	
		M:V	2mM	6		no movement	
	8:5:2	A:V:M	2mM	6		no movement	
Fluka and EMD aluminum backed silica gel	1:1	A:V	2mM	6	No	4cm	No
		A:V	2mM	3.5[a]	No	4cm	No
		M:V	2mM	6	No	4cm	No
	1:2	A:V	2mM	6	No	5cm	No
		M:V	2mM	6	No	5cm	No
	4:3:4	A:V:M	2mM	6	No	4cm	Yes
	3:1	A:V	2mM	6	No	4cm	Yes
		M:V	2mM	6	No	4cm	Yes
	8:5:2	A:V:M	2mM	6	No	4cm	No
	17:3	A:V	1mM	6	No	5cm	No
			2mM	6	No	5cm	No

Table 8. Later Mobile Phases Utilizing Vancomycin as Chiral Selector

Levomethorphan and Dextromethorphan standards

A: acetonitrile; M: methanol; V: vancomycin

a: formic acid; b: 10% ammonium hydroxide; c: 2mL 0.6M NaCl; d: 10% trifluoracetic acid; e: base standard; f: 1.0M HCl salt standard

Plate	Ratio		V Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F	17:3	A:V	1mM[e]	6	No	5cm	Yes
			2mM[e]	2[a,d]	No	5cm	Yes
			2mM[e]	3.5[a,d]	No	5cm	Yes
			2mM[e]	6	No	5cm	Yes
			2mM[e]	8[b]	No	5cm	Yes
			2mM[e]	10[b]	No	5cm	Yes
			2mM[f]	2[a,d]	No	5cm	No
			2mM[f]	3.5[a,d]	Yes	D=4.6cm,L=2.6cm	No
			2mM[f]	6	Yes	D=6cm,L=3.4cm	No
			2.5mM[e]	2[a,d]	No	5cm	Yes
			2.5mM[e]	3.5[a,d]	No	5cm	Yes
			2.5mM[e]	6	No	5cm	Yes
			2.5mM[e]	8[b]	No	5cm	Yes
			2.5mM[e]	10[b]	No	5cm	Yes
			2.5mM[f]	2[a,d]	No	5cm	No
			2.5mM[f]	3.5[a,d]	Yes	D=5cm,L=2.4cm	No
			2.5mM[f]	6	Yes	D=4.4cm,L=2.1cm	No
			2.5mM[f]	8[b]	No	5cm	Yes
			2.5mM[f]	10[b]	No	5cm	Yes
			3mM[f]	2[a,d]	No	5cm	Yes
			3mM[f]	3.5[a,d]	No	5cm	Yes
			3mM[f]	6	No	5cm	Yes
			3mM[f]	8[b]	No	5cm	Yes
			3mM[f]	10[b]	No	5cm	Yes
			5mM[f]	6	No	5cm	Yes
	17:4	A:V	2mM[f]	6	No	5cm	Yes
	16:3	A:V	2mM[f]	6	No	5cm	Yes
	16:4	A:V	2mM[f]	6	No	5cm	Yes
	15:4	A:V	2mM[f]	6	No	5cm	Yes
	15:5	A:V	2mM[f]	6	No	5cm	Yes
	17:3:1	A:V:M	2mM[f]	6	No	2cm	Yes
	17:3:2	A:V:M	2mM[f]	6	No	2cm	Yes
	17:3:3	A:V:M	2mM[f]	6	No	2cm	Yes
Uniplate RPSF and Uniplate HPTLC-RP18F	17:3	A:V	2.5mM[e]	6		not visualized	
			2.5mM[f]	6		not visualized	

Table 9. Mobile Phases Without Chiral Selector Added

Levomethorphan base and Dextromethorphan HBr standards

ACN: acetonitrile; C: CHCl3; M: methanol; EA: ethyl acetate; A:acetone;

PLATE	Mobile Phase	Separation	Tailing	Observations
Uniplate RPSF reverse phase	9:1 C:M	slight	yes	racemic mixture streaked the length of both
	9:1 C:M	slight	yes	taller plates, similar results
	9:1 C:M	no	no	diluted stds, racemic mix is slightly oblong
	5:1 C:M	no	slight	
	15:1 C:M	slight	slight	racemic mixture didn't separate
	2:1 C:M	yes?	yes	lvm/racemic mix streak, exhibit diffuse spot matching dxm
	2:1 C:M	no	slight	oblong, diffuse, poor visualization (dxm)
	3:1 C:M	no	slight	oblong, diffuse, poor visualization (dxm)
	4:1 C:M	no	slight	oblong, diffuse, poor visualization (dxm)
	25:5:1 EA:A:NH4OH	no	no	little to no movement
HPTLC-RP18F	9:1 C:M	no	no	faint spots with UV only
	18:1 NH3sat'd C:M	no	no	faint spots with UV only
	100:1.5 M:NH4OH	no	no	faint spots with UV only
	25:5:1 EA:A:NH4OH	no	no	little to no movement
Chiralplate	2:2:8 M:H20:ACN	no	yes	poor visualization
	5:5:3 M:H20:ACN	no	faint	poor visualization
	1:8 M:H20	no spots visualized		
	5:1:1 A:MeOH:H20	no spots visualized		
	25:5:1 EA:A:NH4OH	no	no	little to no movement
Avicel F microcrystalline cellulose	20% MeOH in H2O	no	heavy	runtime less than 1/2 hr, nothing visible w/ UV, purple streaks on pink w/ acidified iodoplatinate, streaks travel further and become more diffuse as MeOH increases
	30% MeOH in H2O	no	heavy	
	40% MeOH in H2O	no	heavy	
	50% MeOH in H2O	no	heavy	

Table 10. Methods Utilizing Two Mobile Phases

Levomethorphan base and Dextromethorphan HBr standards

ACN: acetonitrile; MeOH: methanol; V: Vancomycin; FA: formic acid; TFA: trifluoroacetic acid; DEA: diethylamine

Plate	Mobile phases	Separation	Tailing
Uniplate RPSF reverse phase	9:1 CHCl3:MeOH	yes	yes
	10:1:0.5 MeOH:0.2M HPBCD:FA	yes	no
	18:1 NH3sa'td CHCl3:MeOH	no	no
	10:1:0.5 MeOH:0.2M HPBCD:FA	no	no
	100:1.5 MeOH:NH4OH	no	no
	10:1:0.5 MeOH:0.2M HPBCD:FA	no	no
	10:1:0.5 MeOH:0.2M HPBCD:FA	nr	nr
	9:1 CHCl3:MeOH	no	no
	9:1 CHCl3:MeOH	yes	yes
	10.5:3:1.5 MeOH:0.13M HPBCD:FA	no	no

Table 10. (continued)

Plate	Mobile phases	Separation	Tailing
Uniplate RPSF reverse phase	0.2 M HPBCD 9:1 CHCl3:MeOH	pretreated no spots visualized	 slight (UV only)
	2:2 0.12 M HPBCD:MeOH 9:1 CHCl3:MeOH	pretreated no spots visualized	
	0.13 M BCD 9:1 CHCl3:MeOH	nr no spots visualized	Nr
	2:2:2 0.13M BCD:MeOH:0.6 M NaCl 9:1 CHCl3:MeOH	nr no	Nr Yes
	9:1 CHCl3:MeOH 2:2 0.2 M HPBCD:ACN	nr no	Nr Slight
	9:1 CHCl3:MeOH 2:2 0.13 M BCD:ACN	nr no	Nr Slight

Table 11. Additional Methods Utilizing Two Mobile Phases

Levomethorphan and Dextromethorphan base standards

A: acetonitrile; M: methanol; CD: cyclodextrin; V: vancomycin; W: water

a: formic acid; b: ammonium hydroxide; c: 2mL 0.6M NaCl

Plate	Ratio	Ratio	Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F	1:6 1:6	M:CD M:W	3mM HPBCD[c]	6 6	 No	 2cm	 Yes
	1:6 1:6	M:CD M:W	0.1M HPBCD[c]	6 6	 No	 2cm	 Yes
	1:6 1:6	M:CD M:W	0.42M HPBCD[c]	6 6	 No	 not visualized	 Yes
	1:6 1:6	M:CD M:W	0.1M BCD[c]	6 6	 No	 1cm	 Yes
	1:1 1:1	M:CD M:W	3mM HPBCD[c]	6 6	 No	 2cm	 Yes
	1:1 1:1	M:CD M:W	0.1M HPBCD	6 6	 No	 2cm	 Yes
	1:1 1:1	M:CD M:W	0.42M HPBCD[c]	6 6	 No	 not visualized	 Yes
	1:1 1:1	M:CD M:W	0.1M BCD[c]	6 6	 No	 1cm	 Yes
	1:1 1:1	A:CD A:W	1mM HPBCD[c]	6 6	 slight	 4cm	 Yes
	1:1 1:1	A:CD A:W	5mM HPBCD[c]	6 6	 slight	 4cm	 Yes
	1:1 2:1	A:CD A:W	3mM HPBCD[c]	6 6	 No	 2cm	 Yes

Table 11. (continued)

Plate	Ratio	Ratio	Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F (continued)	1:1 2:1	A:CD A:W	5mM HPBCD[c]	6 6	No	2cm	Yes
	1:1 3:1	A:CD A:W	1mM HPBCD[c]	6 6		no movement	
	1:1 3:1	A:CD A:W	3mM HPBCD[c]	6 6		no movement	
	1:1 10:3	A:CD M:W	3mM HPBCD[c]	6 3.5[a]	slight	2cm	none
	1:1 10:3	A:CD M:W	5mM HPBCD[c]	6 3.5[a]	slight	2cm	none
	17:3 17:3	A:CD A:W	1mM HPBCD	6 6	No	4cm	Yes
	17:3 17:3	A:CD A:W	3mM HPBCD	6 6	No	4cm	Yes
	17:3 17:3	A:V A:W	1mM V	6 6	No	4cm	Yes
	17:3 17:3	A:V A:W	3mM V	6 6	No	4cm	Yes
	17:3 17:3	A:CD A:W	1mM BCD	6 6	No	2cm	Yes
	17:3 17:3	M:CD M:W	5mM HPBCD	6 6	No	3cm	Yes
	17:3 17:3	M:CD M:W	0.1M HPBCD	6 6	No	3cm	Yes
Fluka and EMD aluminum backed silica gel	1:6 1:6	M:CD M:W	3mM HPBCD	6 6	No	6cm	none
	1:6 1:6	M:CD M:W	0.1M HPBCD	6 6	No	2cm	none
	1:6 1:6	M:CD M:W	0.42M HPBCD	6 6	No	1cm	Yes
	1:6 1:6	M:CD M:W	0.1M BCD	6 6	No	2cm	Yes
	1:1 1:1	M:CD M:W	3mM HPBCD	6 6	No	6cm	none
	1:1 1:1	M:CD M:W	0.1M HPBCD	6 6	No	2cm	none
	1:1 1:1	M:CD M:W	0.42M HPBCD	6 6	No	1cm	Yes
	1:1 1:1	M:CD M:W	0.1M BCD	6 6	No	2cm	Yes
	1:1 1:1	A:CD A:W	1mM HPBCD	6 6	No	6cm	none
	1:1 1:1	A:CD A:W	5mM HPBCD	6 6	No	6cm	none

Table 11. (continued)

Plate	Ratio	Ratio	Concentration	pH	Separation	Distance Traveled	Tailing
Fluka and EMD aluminum backed silica gel (continued)	1:1 2:1	A:CD A:W	3mM HPBCD	6 6	No	5cm	none
	1:1 2:1	A:CD A:W	5mM HPBCD	6 6	No	5cm	none
	1:1 3:1	A:CD A:W	1mM HPBCD	6 6	No	2cm	none
	1:1 3:1	A:CD A:W	3mM HPBCD	6 6	No	2cm	none
	1:1 10:3	A:CD M:W	3mM HPBCD	6 3.5[a]	No	5cm	none
	1:1 10:3	A:CD M:W	5mM HPBCD	6 3.5[a]	No	5cm	none
	17:3 17:3	A:CD A:W	1mM HPBCD	6 6	No	6cm	none
	17:3 17:3	A:CD A:W	3mM HPBCD	6 6	No	6cm	none
	17:3 17:3	A:V A:W	1mM V	6 6	No	6cm	none
	17:3 17:3	A:V A:W	3mM V	6 6	No	6cm	none
	17:3 17:3	A:CD A:W	1mM BCD	6 6	No	6cm	none
	17:3 17:3	M:CD M:W	5mM HPBCD	6 6	No	6cm	none
	17:3 17:3	M:CD M:W	0.1M HPBCD	6 6	No	2cm	Yes

Table 12. Methods Utilizing Two Mobile Phases With Different Chiral Selectors

A: acetonitrile; M: methanol; CD: cyclodextrin; V: vancomycin; W: water

a: formic acid; b: 10% NH_4OH; c: 2mL 0.6M NaCl; d: 10% trifluoracetic acid; e: base standard; f: 1.0M HCl salt standard

Plate	Ratio	Ratio	Concentration	pH	Separation	Distance Traveled	Tailing
Whatman KC-18F (reverse phase)	17:3 17:3	A:CD A:V	1mM HPBCD 1mM V	6 6	No	6cm[f],1cm[e]	Yes
	17:3 17:3	A:V A:CD	1mM V 1mM HPBCD	6 6	No	6cm[f],1cm[e]	Yes
	17:3 17:3	A:V A:CD	5mM V 5mM HPBCD	6 6	No	6cm[f],1cm[e]	Yes
	17:3 17:3	A:CD A:V	5mM HPBCD 5mM V	6 6	No	6cm[f],1cm[e]	Yes
	17:3 17:3	A:CD A:V	10mM HPBCD 10mM V	6 6	No	6cm[f],1cm[e]	Yes
	17:3 17:3	A:V A:CD	10mM V 10mM HPBCD	6 6	No	6cm[f],1cm[e]	Yes

Table 12. (continued)

Plate	Ratio	Ratio	Concentration	pH	Separation	Distance Traveled	Tailing
Fluka aluminum backed (normal phase)	17:3	A:CD	1mM HPBCD	6			
	17:3	A:V	1mM V	6	No	6cm[f],1cm[e]	None
	17:3	A:V	1mM V	6			
	17:3	A:CD	1mM HPBCD	6	No	6cm[f],1cm[e]	None
	17:3	A:CD	5mM HPBCD	6			
	17:3	A:V	5mM V	6	No	6cm[f],1cm[e]	None
	17:3	A:V	5mM V	6			
	17:3	A:CD	5mM HPBCD	6	No	6cm[f],1cm[e]	None
	17:3	A:CD	10mM HPBCD	6			
	17:3	A:V	10mM V	6	No	6cm[f],1cm[e]	None
	17:3	A:V	10mM V	6			
	17:3	A:CD	10mM HPBCD	6	No	6cm[f],1cm[e]	None

Table 13. Mobile phases Utilizing Mixtures of Chiral Selectors

Levomethorphan and Dextromethorphan base standards, all runs at pH = 6

A: acetonitrile; M: methanol; CD: cyclodextrin; V: vancomycin; a: formic acid; b: ammonium hydroxide; c: 2mL 0.6M NaCl

Plates	Ratio	Ratio	Concentration	Separation	Distance Traveled	Tailing
Whatman KC-18F	12:3:3	A:HPBCD:BCD	5mM, 1mM		no movement	
		M:HPBCD:BCD	5mM, 1mM		no movement	
	14:5:1	A:HPBCD:BCD	5mM, 1mM		no movement	
	10:3:3	M:HPBCD:BCD	5mM, 1mM		no movement	
	5:3:3	M:HPBCD:BCD	5mM, 1mM[c]		no movement	
		A:HPBCD:BCD	0.05M, 0.05M[c]		no movement	
		A:HPBCD:BCD	0.1M, 0.1M[c]		no movement	
	15:3:3	M:HPBCD:BCD	5mM, 1mM		no movement	
	5:2:2	A:HPBCD:BCD	5mM, 1mM		no movement	
			5mM, 5mM		no movement	
			1mM, 5mM		no movement	
			0.05M, 0.1M		no movement	
			0.5mM, 1mM		no movement	
			0.5mM, 5mM		no movement	
		M:HPBCD:BCD	0.05M, 0.1M		no movement	
	3:2:2	A:HPBCD:BCD	0.05M, 0.1M		no movement	
	17:3:3	A:HPBCD:BCD	5mM, 1mM	No	1cm	Yes
	1:1:1	A:HPBCD:V	5mM, 5mM		no movement	
	3:1:3	A:HPBCD:V	5mM, 5mM		no movement	
	3:3:1	A:HPBCD:V	5mM, 5mM		no movement	
Fluka and EMD aluminum backed silica gel	12:3:3	A:HPBCD:BCD	5mM, 1mM	No	8cm	No

Table 13. (continued)

Plates	Ratio	Ratio	Concentration	Separation	Distance Traveled	Tailing
Uniplate Avicel microcrystalline cellulose	10:3:3	M:HPBCD:BCD	5mM, 1mM	No	8cm	No
	5:3:3	M:HPBCD:BCD	5mM, 1mM	No	8cm	No
	15:3:3	M:HPBCD:BCD	5mM, 1mM	No	8cm	No
	5:2:2	A:HPBCD:BCD	5mM, 1mM	No	8cm	No
	5:2:2	A:HPBCD:BCD	5mM, 5mM	No	8cm	No
	5:2:2	A:HPBCD:BCD	1mM, 5mM	No	8cm	No
	1:1:1	A:HPBCD:V	5mM, 5mM	No	8cm	No
	3:1:3	A:HPBCD:V	5mM, 5mM	No	8cm	No
	3:3:1	A:HPBCD:V	5mM, 5mM	No	8cm	No

Table 14. Mobile Phases Utilized with Methamphetamine and Propoxyphene

ACN: acetonitrile; M: methanol; CD: cyclodextrin; FA: formic acid; EA: ethyl acetate; A:acetone; DEA: 33% diethylamine

a: 0.5mL FA; b: 1.5mL FA; c:1mL FA; d: 7mL CD; e: 0.14 mL DEA; f: 0.42 mL DEA

					Methamphetamine		Propoxyphene	
Plate	**RATIO**		**CD**	**Conc.**	**Separation**	**Tailing**	**Separation**	**Tailing**
Uniplate RPSF reverse phase	M:H2O:ACN	5:5:3	none	none	no spots visualized		slight	slight
	M:CD[a]	10:1	HPBCD	0.20M	no spots visualized		no spots visualized	
	M:CD[b]	10.5:3	BCD	0.13M	no spots visualized		no	no
	EA:A:NH4OH	25:5:1	none	none	no spots visualized		no	slight
	M:CD[b,d]	10.5:3	BCD	0.13M	no spots visualized		only one visualized	
	M:CD	5:5	HPBCD	0.25M	no spots visualized		no spots visualized	
	M:CD	40:60	HPBCD	0.08M	no*	yes	no*	yes
	M:CD	30:70	HPBCD	0.08M	no spots visualized		no spots visualized	
	M:CD	30:70	HPBCD	0.12M	no spots visualized		no spots visualized	
	M:CD	40:60	HPBCD	0.12M	no spots visualized		no spots visualized	
	M:CD	20:80	HPBCD	0.12M	no spots visualized		no spots visualized	
	M:CD	25:75	HPBCD	0.12M	no spots visualized		no spots visualized	
	M:CD	25:75	HPBCD	0.20 M			no spots visualized**	
	M:CD	40:60	HPBCD	0.20M	no spots visualized		no spots visualized	
	M:CD	30:70	HPBCD	0.20M	no spots visualized		no spots visualized	
	M:CD	35:65	HPBCD	0.20M	no spots visualized		no spots visualized	
	M:CD	20:80	HPBCD	0.20M			never developed fully**	
	M:CD	20:80	HPBCD	0.25M	no*	slight	no*	slight
	M:CD	25:75	HPBCD	0.25M	no*	slight	no*	slight
	M:CD	30:70	HPBCD	0.25M	no spots visualized		no*	slight
	M:CD	35:65	HPBCD	0.25M	no spots visualized		no*	slight
	M:CD	40:60	HPBCD	0.25M	no spots visualized		no*	slight
HPTLC-RP18F	M:CD	20:80	HPBCD	0.25M	never developed fully, discarded			
	M:CD	25:75	HPBCD	0.25M				
	M:CD	30:70	HPBCD	0.25M				
	M:CD	35:65	HPBCD	0.25M				
	M:CD	40:60	HPBCD	0.25M				

Table 14. (continued)

					Methamphetamine		Propoxyphene	
Plate	**RATIO**		**CD**	**Conc.**	**Separation**	**Tailing**	**Separation**	**Tailing**
Avicel Microcrystalline cellulose	M:CD[b]	10.5:5	HPBCD	0.10M	no spots visualized		no spots visualized	
	M:CD[a]	10.5:5	HPBCD	0.15M	no spots visualized		no spots visualized	
	M:CD[c]	7:2	BCD	0.10M	no spots visualized		no spots visualized	
	ACN:CD[c]	7:2	BCD	0.10M	no spots visualized		no spots visualized	
	M:CD[e]	4:1	BCD	0.10M	no spots visualized		no spots visualized	
	M:CD[f]	4:1	BCD	0.10M	no spots visualized		no spots visualized	

*only observed with UV

**ran overnight

IV. Conclusions

a. Discussion of Findings

The most predominantly used organic modifiers in this study were methanol and acetonitrile, which was consistent with the literature. Throughout the literature, the most successful enantiomeric separations were achieved using reversed phase plates which necessitated using a polar mobile phase. The mobile phase composition and chiral mobile phase additive concentrations were chosen based on the values investigated by other research. Generally speaking, mobile phases prepared with methanol resulted in more compact spots and less streaking than those that were prepared with acetonitrile. However, mobile phases containing acetonitrile promoted elution to a greater extent than those containing methanol. Utilizing mixtures of methanol and acetonitrile increased elution while reducing streaking, but ultimately did not affect separation.

Chiral selectors interfered with visualization of results. Methorphan typically has a strong response to both iodoplatinate and UV, but its response to each was weakened in the presence of a chiral selector. Both propoxyphene and methamphetamine are not typically strong responders to these visualization techniques and were unable to consistently be visualized in the

presence of a chiral selector. Propoxyphene was visualized more often than methamphetamine in the limited experiments that were performed. Mobile phases with lower concentrations of chiral selector promoted better visualization of the compound of interest. However, incomplete complexation also resulted from the decrease in concentration.

For both cyclodextrins, any separations that occurred were not reproducible. BCD was extremely difficult to remove from the glassware because of its insolubility in water and as a result, glassware contamination was a factor in false separation of the methorphan enantiomers and lack of reproducibility. HPBCD was much more soluble in water and resulted in less contamination issues.

Early studies performed with levomethorphan base and dextromethorphan HBr resulted in separation when utilizing 17:3 ACN:1mM HPBCD at pH 8 with NH_4OH. However, upon base extracting the standards, no separation was achieved and not much elution from the origin occured. Treating base standards with HCl to create the salt form resulted in greater elution but was not consistent in affecting separation.

Although Lurie demonstrated successful differentiation of methorphan enantiomers using HPBCD in CE[8], it is apparent from this research that methorphan does not complex with HPBCD and BCD in an enantioselective manner under TLC conditions. This could be due to methorphan not interacting with the hydrophobic inner cone area of the cyclodextrins or it may simply be steric hindrance because of size and structure of the methorphan molecule. When comparing the structure of methorphan with the other enantiomeric compounds investigated in the literature, it becomes plausible that steric hindrance is the cause as the other compounds are much smaller and less rigid than methorphan.

The chiral selector mixtures did not enhance complexation and were instead a hindrance to methorphan movement on the plate. Since no movement was observed at pH=6, the pH of the

mobile phase was not varied because of the negative impact of two chiral selectors. The two system method that involved pre-coating the plates with a chiral selector seemed to work best using concentrations less than 5mM. Higher concentrations often resulted in strange plate development patterns.

Vancomycin was the most successful chiral mobile phase additive for producing enantiomeric separation, although inconsistently. Visualization of methorphan was troublesome because the multiple functional groups on vancomycin react with iodoplatinate, ceric sulfate, and the I_2 vapors. Therefore, the concentration of vancomycin could not exceed 0.05M. The successful vancomycin mobile phase was always slightly opaque or cloudy. Each time the mobile phase was made, the opaqueness seemed to vary slightly and the separation seemed dependent on the appearance of the mobile phase. Even though the solution was cloudy, vancomycin did not come out of solution as a white precipitate as was observed with other mobile phases. Although not much is known about how molecules complex with vancomycin, it has many functional groups with which molecules could interact. Further research using vancomycin or another macrocyclic antibiotic for TLC separations may be worthwhile.

b. Implications for policy and practice

The ability to quickly and easily differentiate between the enantiomers of various drugs and pharmaceuticals would be a valuable tool for any laboratory performing controlled substance analysis. Because of the differences in the legal consequences due to one stereoisomeric form of a drug having a higher control status than another, it is imperative to be able to differentiate them. A more efficient, cost effective method to differentiate stereoisomers has the potential to aid in coping with the ever increasing backlog situations in many laboratories by reducing the

time spent on such determinations. Unfortunately, a suitable TLC method was not developed to serve this purpose.

c. Implications for further research

Investigation of other potential CMAs could lead to a successful method. As the size of the β-cyclodextrin cavity may have played a role in the inability to differentiate the methorphans, it may be worthwhile to investigate the use of the larger γ-cyclodextrin and its derivatives. Vancomycin is widely used as a chiral selector for HPLC and showed the most promise in this study. It could be further investigated using different stationary phases or mobile phase compositions. The reverse phase plates employed in this study were of the octadecyl variety (Whatman KC18F, Uniplate HPTLC-RP18F), or were of a similar nature (Uniplate RPSF). There may be potential for additional studies using diphenyl or ethyl reverse phase plates, if these types of plates become available. Diphenyl plates were unable to be located or purchased from VADFS's usual suppliers and an order that was placed for KC2F (ethyl) plates was not filled by the supplier due to lack of availability. Other methods of visualization could be explored to eliminate the problems experienced with viewing the results and allow for further investigation of the differentiation of the propoxyphene and methamphetamine enantiomers.

V. References

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VI. Dissemination of Research Findings

It was intended that results from this project would be widely shared with the forensic science community. VADFS had planned to seek publication in scientific journals such as the *Journal of Forensic Science* or the *Microgram Journal*. VADFS staff also intended to attend annual meetings of forensic organizations to present information about using CMAs in TLC to separate and differentiate enantiomers and diastereomers of controlled substances. However, due to the fact that a suitable chiral mobile phase was not found, there have been no publications or presentations as to the results of this project. The progress of the project was presented at the 2010 NIJ Grantees Meeting at the annual meeting of the American Academy of Forensic Science in Seattle, WA. An updated progress report was presented in-house at the 2011 VADFS Controlled Substances section meeting in Glen Allen, VA. As the authors felt that it was important to disseminate even unsuccessful results to the forensic community, the work was presented at the May 2012 meeting of the Mid-Atlantic Association of Forensic Sciences in Ellicott City, MD.