EVALUATION OF ACTIVATED CARBON AS A COMMERCIAL DRUG DISPOSAL PRODUCT USING LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

By

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Abstract

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At present, pharmaceuticals, synthetic organic compounds, and endocrine disrupting compounds are ubiquitous in our environment. These have been introduced by consumers and manufacturers directly and indirectly for decades. There is plenty of published literature addressing the presence of these compounds, the development of newer and more sensitive techniques to detect them at low concentrations, as well as studies showing their effects in the environment and in human health. However, these studies are based on the measurement and detection of these compounds after the fact; and even though some have addressed the need to decrease these pollutants in the environment, procedures to minimize the presence of these compounds in the environment have not been firmly established yet at the consumer level. Consequently, the study of processes to decrease the introduction of these pollutants is well justified.

The purpose of this project was to develop a series of methods and experiments to measure the adsorption capacity of a commercial drug disposal product, composed primarily of activated carbon in an acidified solution. Some sample bottles were obtained commercially and contained a proprietary formula, and some sample bottles containing activated carbon in acidified solution were prepared in house. A

liquid chromatography – mass spectrometry method was developed and applied for the simultaneous quantification of 24 drugs, which included opiates, barbiturates, statins, amphetamine, and benzodiazepine. For this method, 8-oz bottles containing a proprietary composition were loaded with expired or unused prescription drugs by a third party and the residual was quantified. Additionally, this method was used to measure the loading capacity of activated carbon in 8-oz bottle samples and in 1-gal jug samples. In the case of 8-oz samples, the effect of different acids was also investigated. The loading experiment consisted of the addition of a known amount of acetaminophen in solution every 48 hours or longer, followed by the extraction of the supernatant. A 1-gal jug sample was also prepared in house with known amounts of activated carbon and acidified solution. The 1-gal jug sample was loaded with acetaminophen (500 mg) caplets in solid form instead of acetaminophen in solution. In all of these loading experiments, acetaminophen was chosen because it is used in higher doses in comparison to other drugs (codeine, hydrocodone, etc). In the same manner, aliquots from the 1-gal jug sample were extracted prior to the addition of more acetaminophen caplets. Furthermore, a second method was built to study the adsorption capacity for removal of bioactive species (cannabinoids) from plant matter. In this case, 8-oz bottles were prepared in-house with known composition of activated carbon and acidified solution. 8-oz bottles were loaded by a third party and residuals were tested.

The outcome showed that the drug disposal formulations are able to sequester the active ingredient of 24 (solid and liquid forms) drugs and biologically active cannabinoids successfully. Furthermore, the results of studies used to investigate the loading capacity of large volume formulations demonstrated that adsorption is slow, and that the capacity of the formulation was not reached within 48 hours. If allowed to stand longer, the adsorption increased, but further experiments are needed to investigate these results.

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Chapter 1

Introduction and Background

Contaminants in the Environment

The demand for earth's supplies, such as fresh water sources, coal, lumber sources, and food sources has steadily increased with the continuous growth of the human population. In addition, the production, usage and disposal of products have generated concern about their effects on the human race and the environment. Household chemicals, pharmaceuticals, metabolized hormones, pesticides, industrial byproducts waste, and other chemicals consistently find their way to fresh water sources. Consequently, not only the human race is consuming resources at a faster pace, it also is creating waste as much as it consumes its resources.

The presence of these products and byproducts in the environment has raised concerns, such as impairment of reproductive and physiological processes [1-4] and the creation of antibiotic-resistant bacteria [5-7], just to name a few. Antibiotics in particular have been used by our society since the mid 1900's after the discovery of bacteria in the late 1800's [8]. Quinolone and fluoroquinolone antibiotics are the largest quantity antibiotics used in human medicine [9]. Additionally, human and veterinary applications are the main sources of pharmaceuticals in the environment due to the metabolic excretion, improper disposal, or industrial use [10]. Consequently, most municipal sewage contains pharmaceuticals [11]. Unfortunately, studies [12-15] have shown that most of the processes used by water treatment plants (WTPs) are insufficient and are not yet able to eliminate many of these contaminants.

Drug Disposal Needs

Pharmaceuticals enter the environment mainly through human action, either directly (inadequate disposal) or indirectly (excretion through urine and fecal matter). For example, most consumers dispose their unused and expired prescriptions by throwing them in the toilet, sink, or trash. A study, in the United States, found that 7.2% of patients stockpile their expired medications in their homes, only 1.4% returned medications to the pharmacy, while 54% of them disposed medications in the garbage and 35.4% flushed them down the toilet or sink [16]. Another example is the contamination of drinking water with antibiotics from bulk production. A study performed in India and the People's Republic of China effluent from a local

waste water treatment facility showed that all sampled wells were contaminated with more than 1 mg/L of ciprofloxacin, enoxacin, cetirizine, teerbinafine and citalopram. Sampled lakes showed even higher levels of ciprofloxacin (6.5 mg/L), cetirizine (up to 1.2 mg/L), norfloxacin (up to 0.52 mg/L) and enoxacin (up to 0.16 mg/L) [17]. The contamination of the lakes with milligrams per liter of drugs was 100,000 to 1 million times higher than reported levels of fluoroquinolones in surface water in China and the United States contaminated by sewage effluents [9, 18]. As the population continues to grow, the use and need of prescription drugs (antibiotics, over the counter (OTC), analgesics, etc) will rise as well; therefore, better means for effective disposal isnot only needed but required as the human population keeps growing.

Analytical Drug Determinations

Liquid chromatography coupled with atmospheric pressure ionization (API) tandem mass spectrometry is the most commonly used method for the quantification of drugs and other compounds in matrixes biological [19-22] and aqueous [23-25] matrices. Chromatography allows separation, identification, and determination of closely related component from complex mixtures. In liquid chromatography, the sample is dissolved in the mobile phase and is then forced through an immiscible stationary phase, contained in a column. The components of the sample are then able to partition between the mobile and stationary phases to varying degrees. Based on this concept, compounds that interacted strongly with the stationary phase will elute later than those compounds that interacted weakly with the stationary phase. As a consequence, these components are separated into discrete bands or peaks, and can then be analyzed quantitatively and/or qualitatively, with the aid of appropriate detection techniques [26].

Mass spectrometry is a highly specific and sensitive detection technique for liquid chromatography. Formation of gaseous ions is the starting point for mass spectrometry. This formation depends on the method used for ion formation, which fall into two major categories: gas-phase (electron impact, chemical ionization, and field ionization) and desorption (field desorption, electrospray ionization, matrix-assisted desorption-ionization, plasma desorption, etc) sources. In the former, the sample is first vaporized and then ionized, while in the latter, the sample is converted into ions directly. Ion sources are classified as hard and soft sources, where hard ionization sources produce fragment ions with mass-to-

charge (m/z) ratios lower than that of the molecular ion; and soft ionization sources cause little fragmentation.

In the field of liquid chromatography mass spectrometry, soft ionization techniques are most commonly used, and electrospray ionization (ESI) is the most prevalent of these. ESI was first described in 1984 as biomolecule analysis technique. In addition, John B. Fenn shared a portion of the 2002 Nobel Prize in Chemistry for the development of ESI and its applications for the analysis of biomolecules [27]. In general, ESI takes place under atmospheric pressure. A solution of the sample is pumped through a stainless steel capillary needle (maintained at several kilovolts, 3-5 kV) at a rate of a few microliters per minute. A charged spray of fine droplets is created in the atmospheric source region. As droplets becomes smaller due to solvent evaporation, their charge density becomes greater until the Rayleigh limit (surface tension cannot longer support the charge) is reached. Here, Coulombic explosion occurs and the droplet turns into smaller droplets until solvated analyte ions are released from the drops into the gas phase. These are then passed through a desolvation capillary, where residual solvent is liberated from the ionic analytes, and mass analysis can commence [28-32]. A schematic representation of an electrospray source is shown in Figure 1-1.

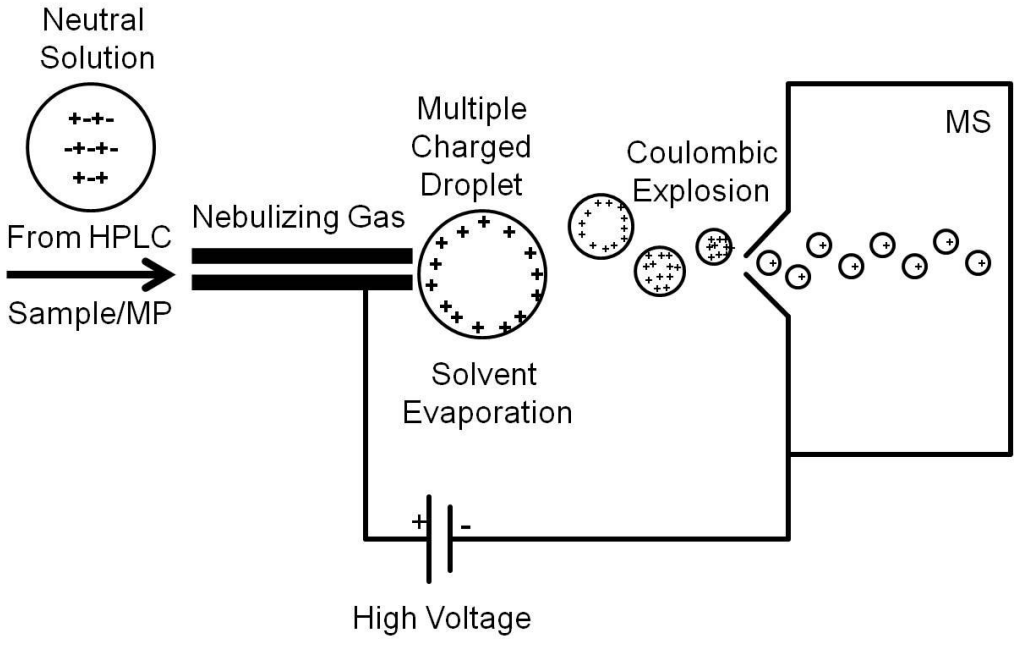

Figure 1-1 Schematic of the electrospray ionization process

After ionization is achieved, the ions are introduced into a mass analyzer (MS). The function of the MS is to separate ions based on their m/z ratios. Once separated, the MS system contains a detector, which converts the beam of ions into an electrical signal by means of a transducer (e.g. an electron multiplier). This electrical signal is then processed, stored, and displayed by a computer. Unlike other detectors, the MS requires a vacuum system to create low pressures of 10-4 to 10-8 Torr. This is because ions are created in the source and can collide with gases on their way to the detector. So in order to avoid unwanted collisions, one must increase the mean-free path (the distance an ions travels without collision) of the ion, which is accomplished by reducing the pressure. A schematic of the mass spectrometer components is presented in Figure 1-2.

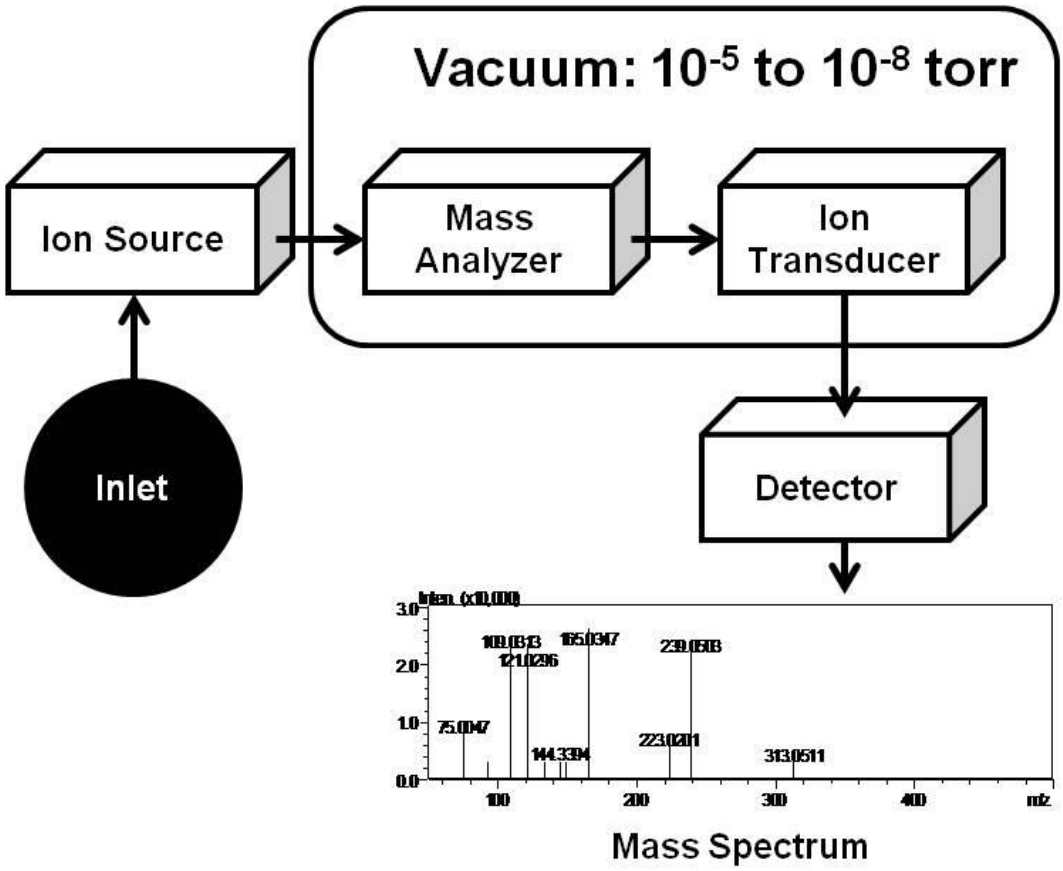

Figure 1-2 Schematic of the components of a mass spectrometer (MS)

The perfect MS would be capable of not only allowing the passage of sufficient number of ions to easily measure ion currents, but also would have the capacity to distinguish minute differences between masses. These two characteristics are difficult to achieve in one instrumental configuration; therefore, the need for more than one MS rises. There are several MS types, such as magnetic sector, double-focusing sector, time-of-flight (TOF), ion trap, and quadrupole mass analyzers. The quadrupole mass analyzer depicted in Figure 1-3 is characterized by its rugged design and high scan rates (<100 ms) [33], high sensitivity and mechanical simplicity – despite its low mass range (maximum m/z is 4000), low mass resolution (R proportional to the square of the ions' number of oscillations) and low limited mass accuracy. The quadrupole is composed of four parallel cylindrical rods that serve as electrodes. Opposing rods (electrodes) are connected electrically, one pair being attached to the positive side of a variable dc source and the other pair to the negative terminal. Additionally, variable radio-frequency ac voltages are applied to each pair of rods. In this device, ions are accelerated into the space between the rods by a potential difference of 5 to 10 V. One pair receives a superimposed positive dc potential (+U) and a time-dependent rf potential. The other adjacent pair of rods receives a negative dc potential (-U) and an rf potential out of phase by 180º. Application of these voltages creates an oscillating field within the rods. So when ions are injected at one end of the quadrupole in the direction of the quadrupole rods (z-direction), separation of these ions of different m/z value is accomplished through the criterion of path stability within the quadrupole field, meaning all ions except for those having a certain m/z value strike the rods and are neutralized; consequently, the quadrupole analyzer is essentially an ion filter [34].

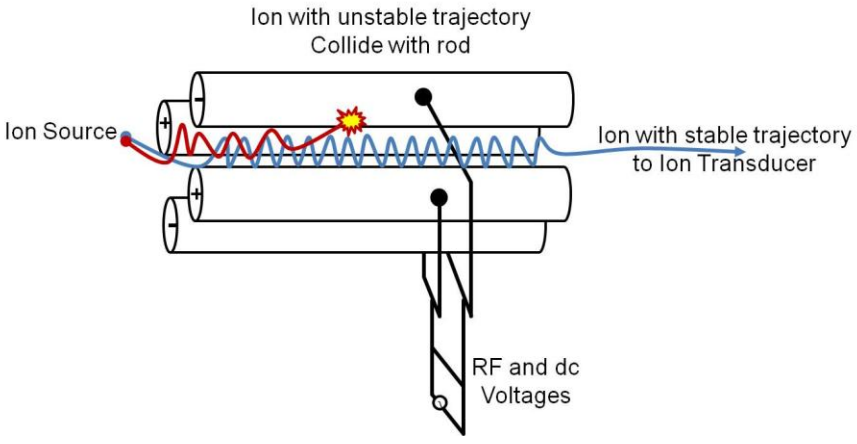

Figure 1-3 Schematic of the quadrupole mass analyzer process

Activated Carbon

Activated carbon (AC) is space enclosed by carbon atoms. This space (porous) has zero electron density and possesses intense van der Waals forces which are responsible for its exceptional ability to adsorb chemical species. These forces will depend on the distance between carbon atoms and the bond arrangement of bonds between carbon and hetero-atoms [35].

The manufacturing process of AC has been tuned to a specific set of parameters so it relies heavily on the consistency of the resources. Some of these resources are different coals, peat, wood, fruit stones and nutshells, such as coconut shells, and some synthetic organic polymers. Combined, these can be used to generate several AC types such as carbon blacks, nuclear graphite, carbon fibers, carbon composite, and electrode graphite. These types of AC differ based on their manufacturing and carbonization process. In the end, these carbon forms are related to graphite lattice in some way or another.

More specifically, carbons are classified in two categories graphitizable and non-graphitizable. These terms were introduced by Rosalind Franklin between 1950 and 1951 [36]; and these carbons are first prepared via carbonization in the solid-, liquid- or gas- phases from organic materials. A solid-phase carbonization occurs in the solid phase and involves structural changes such as atom removal and replacement with a solid lattice that remains rigid through the process creating nongraphitizable carbons (isotropic carbons, carbons when heating above 2000 ºC cannot form three-dimensional x-ray diffraction lines of the graphite lattice). In a liquid-phase carbonization, well-organized solids emerge from the carbonization process and constitute graphitizable (non-porous) carbons (anisotropic carbons when heating above 2000 ºC are capable of producing three-dimensional x-ray diffraction lines of the graphite lattice). Lastly, gas-phase carbonization produces carbon blacks and pyrolytic carbons where porosity is absent. Pyrolytic carbons are highly graphitizable material which is produced by the deposition of carbon atoms from methane or benzene. After the carbonization takes place, activation of the carbon follows. Within this process further porosity, widening of existing porosity, surface modification and carbonization modifications take place. Activation is classified in two processes, chemical and thermal/physical.

Chemical activation involves the co-carbonization with zinc chloride (ZnCl2), phosphoric acid (H3PO4) and potassium hydroxide (KOH). AC is prepared from mainly polymeric type synthetic carbons and lignocellulosic materials. Zinc chloride and phosphoric acid promote the extraction of water molecules from the lignocellulosic structure from the parent material. The KOH mechanism is complex and it involves the disintegration of structure followed by the intercalation and gasification by oxygen from hydroxide.

The preferred source for chemical activation is lignocellulosic material (olive and peach stone). A lignocellulosic material is composed primary of carbon, C, and oxygen, O, (48% and 45%, respectively) and hydrogen, H, (6%). This transformation requires the removal of O and H, which yields around 20-30 wt% lower than the original amount and it's mainly used for the production of granular activated carbons with highly microporosity. The first step is the impregnation step. Here, the reagent (ZnCl2, H3PO4 or KOH) is dissolved in water and mixed with the precursor (lignocellulosic material such as olive and peach stones) and kept at 85 ºC. Then carbonization is carried out under a flow of nitrogen and the resulting carbon is washed to eliminate remaining chemical. Specifically, during chemical activation using ZnCl2, the micropore volume is similar to the volume of ZnCl2 introduced into the particle. This helps ensurethat the microporosity is uniform. Equally in H3PO4 activation, the volume of the micropores developed during activation is similar to the volume of the phosphoric acid and leads to highly activated carbons. Lastly, during the KOH activation mode, the reactant acts after the pyrolysis of the precursor, at temperatures above 700 ºC and the development of porosity is related to the extent of impregnation by KOH. This is because KOH doesn't act as a dehydrating agent on the precursor; it only reacts above 700 ºC, after the formation of the char.

In thermal/physical activation, carbon dioxide (CO2) or steam (H2O) or a combination of these two gases is used to remove carbon atoms, whichwidensthe pore. In this process, carbon atoms are removed by gasification at 800-1000 ºC to avoid ignition and burning. CO2 and H2O extract carbons from the carbon structure according to the following stoichiometric equations:

$$C + CO_2 \rightarrow 2CO \qquad \Delta H = +159 \frac{kJ}{mol}$$

$$C + H_2O \rightarrow CO + H_2 \qquad \Delta H = +117\frac{kJ}{mol}$$

For these endothermic reactions, heat is introduced in to the furnace in a controlled matter to produce the activating gases, such as:

$$CO + \frac{1}{2}O_2 \rightarrow CO_2 \qquad \Delta H = -285\frac{kJ}{mol}$$

$$H_2 + \frac{1}{2}O_2 \rightarrow H_2O \qquad \Delta H = -238\frac{kJ}{mol}$$

Consequently, the activation reactions result in the opening of porosity which was initially inaccessible, the enhancement of micropore volumes and widening of micropores into the mesopore range (narrow microporosity (<0.7 nm), wider microporosity (0.7-2 nm), mesoporosity (2-50 nm) and macroporosity (>50 nm)). The precise interaction between a reacting gas molecule entering into the porosity of a carbon and a molecule of carbon monoxide emerging is still being studied. As a result, activation of carbon is the most important step in the manufacturing of activated carbon because its porosity is the epicenter of adsorption, where the containment of molecules occurs.

AC Applications

Charcoal or AC is as old as Hippocrates, and records show its use in the removal of drugs from the stomach in overdose cases. It was also used in the filters of gas masks worn during World War I for the protection of soldiers against mustard (1,1-thiobis(2-chloroethane)), chlorine (Cl2), and phosgene (COCl2) gases, as well as deodorant [35].

AC is commonly used in water treatment plants (WTC) for the removal of MTBE [37], natural and synthetic organic compounds (SOC) such as pharmaceutically active compounds (PhACs), and endocrine disrupting compounds (EDCs) [38], specifically naproxen and carbamazepine [39]. It has been shown to achieve 90% removal of these contaminants at concentrations of less than 500 ng/L in natural water. Kim et al demonstrated that, in comparison to activated carbon, granular activated carbon (GAC) removed 99% of endocrine disruptor chemicals, pharmaceuticals, flame retardant compounds and hormones from water in WTPs [40]. GAC has been shown to remove dyes (alizarine red-S,bromophenol

blue, malachite green, methyl violet, methylene blue, phenol red, methyl blue and erichrome black-T) from effluents [41] as well. This process is crucial to our environment because over 7 × 105 tons and approximately 10,000 different types of dyes and pigments are produced worldwide annually [42]. In summary, AC use in adsorption applications is extensive and it's shown to be diverse tool for the removal of contaminants in solution. Here, AC in slurry is studied as a disposal product for the disposal of expired and unused drugs.

Chapter 2

Validated Multi-Drug Determination using Liquid Chromatography – Tandem Mass Spectrometry for Evaluation of a Commercial Drug Disposal Product

Abstract

Responsible drug disposal should be considered as important as the manufacture and quality control of drugs. Currently, there are limited established, convenient, and effective means of drug disposal for the consumer. This deficiency creates a gateway to illicit drug use and environmental concerns. Here we evaluated the efficacy of a new drug disposal product. This product claims to sequester up to 100% of the drug's active ingredient making it safe to dispose in landfill waste. High performance liquid chromatography-tandem mass spectrometry was applied to quantify twenty-four drugs (including opiates, barbiturates, statins, amphetamine, and benzodiazepine drugs) in the residual solvent solution from the product following their disposal. Analytes were ionized by electrospray ionization and were quantified by multiple reaction monitoring on a triple quadrupole mass analyzer. Calibration curves were established in the concentration ranges of 0.25 – 7.0 µg/mL and showed good linearity (R^2 ranged from 0.996 to 0.999) for the twenty-four analytes. The limits of detection (LOD) varied from 0.001 to 0.02 µg/mL depending on the drug. Accuracy ranged from 80% to 111% for lowest- and low-level (0.05 to 0.50 µg/mL) and high- and upper-level (2.0 to 10.0 µg/mL) quality control (QC) samples, with a few minor exceptions. Accuracy ranged from 92% to 105% for the mid-level (1.2 µg/mL) QC samples. Precision (CV %) overall varied between 0.2 to 12.7%. In sample bottles tested, where active ingredient of the loaded drug was below the maximum sorption capacity stated on the label, between 98% to >99.9% of the active ingredient was sequestered. Percent active ingredient adsorbed was slightly lower in bottles loaded with active ingredient in excess of label specifications.

Introduction

Unused and expired medications present a risk to consumers and health care institutions. The accumulation of prescription medication creates means for accidental consumption and for illicit use and abuse. In 2013, 57 poison control centers served 3.1 million exposure cases where people came into contact with dangerous or potentially dangerous substances. Approximately half of these cases involved misuse of medications or pharmaceuticals; 93% of human exposures occurred at someone's residence

[43]. Data from the American Association of Poison Control Center (AAPC) 2013 annual statement reported that 50% of the 2.1 million known age case exposure cases (involving analgesics, cosmetics and personal care products, household cleaning substances, sedatives/hypnotics/antipsychotics, and antidepressants) were children aged 5 years old or younger [43].

Many prescription drugs can be very addictive. The National Institute on Drug Abuse (NIDA) Monitoring the Future (MTF) survey found that about 1 in 12 high school student seniors reported past-year nonmedical use of prescription pain relievers in 2010, and 1 out of 20 reported abusing oxycodone [44]. NIDA reported that opioid drugs are the second most abused substance by Americans over 14 years of age. In a 2014 study, 54.4% of nonmedical users obtained opioid pain relievers from friends and relatives for free, while 15.5% bought opioid pain relievers from a drug dealer, friend, or relative [45]. A very common source of nonmedical drugs is friends' and family's medicine cabinets. Therefore, unused and expired drugs (prescription and over-the-counter (OTC)) left in patient's homes have become an unnecessary risk. A way to dispose of these substances is imperative to decrease their misuse.

Unfortunately, pharmaceutical waste disposal has become an increasingly widespread problem. Convenient, accessible, and efficient means have not been firmly established. Pharmaceutical companies have largely left it to the consumer to find the best way to dispose of any unused or expired medications. Currently, more than 2.5 million pounds of prescription medications are left unused by consumers in the United States annually [46]. The Food and Drug Administration (FDA) guidelines, which do not apply to hospitals or pharmacies, advise consumers to take all unused, unneeded, and expired medications out of their original container, mix them with unpalatable items, seal them in a bag, and place them in the trash. Mixing with "kitty litter" appears to be a common recommendation, anecdotally. Kuspis et al. examined how expired medications were disposed by patients and found that 7.2% stockpile them in their homes, only 1.4% returned medications to the pharmacy, while 54% of them disposed medications in the garbage and 35.4% flushed them down the toilet or sink [16]. Even so, greater emphasis should be placed on hospitals as a major source of pharmaceutical pollution [47-49]. The Drug Enforcement Agency (DEA) has not developed clear guidelines for the disposal of controlled substances for DEA registered facilities such as hospitals, pharmacies, and health care facilities. They recommend that any disposal procedure

should involve returning unused medications to the manufacturer, to a reverse distributor, or by destroying them in accordance with federal regulations [50].

Not only have poor standards and means for disposal of expired medications created a doorway to illicit drug use, they have also become a significant source of environmental concern. There are numerous reports of surface and drinking water being contaminated with pharmaceutical components and metabolites [51-54]. As a result, pharmaceutical components are considered ubiquitous in drinking water. For example, Martinez et al. monitored 100 organic contaminants (pharmaceuticals, personal care products, pesticides, and metabolites) in municipal sewage treatment plants. They identified compounds ranging in concentrations from a few ng/L up to µg/L in wastewater; anti-inflammatory and analgesic drugs were the most commonly encountered [55]. Focazio et al. reported that the top five most frequently detected contaminants in surface water were cholesterol (59%, natural sterol), metolachor (51%,herbicide), cotinine (51%, nicotine metabolite), β-sitosterol (37%, natural plant sterol), and 1,7-dimethylxanthine (27%, caffeine metabolite). In ground water, tetrachloroethylene (24%, solvent), carbamazepine (20%, pharmaceutical), bisphenol-A (20%, plasticizer), 1,7-dimethylxanthine (16%, caffeine metabolite), and tri (2-chloroethyl) phosphate (12%, fire retardant) were commonly detected [56]. These studies typically involve the use of liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) to test for the presence of substances in surface water and waste water [10, 57-59] . Furthermore, there are various methods for the detection and quantification of opioids, cannabinoids, benzodiazepines, antidepressants, lefetamine and xenobiotic substances in urine [60-65], as well as the determination of drugs such as heroin, astemizole, morphine, and normorphine in blood plasma [66-69], by HPLC-MS/MS. The analysis of pharmaceuticals in urine and blood are mainly addressed by toxicology and clinical analysis fields.

The objective of this study was to develop and validate an analytical method to determine the performance of a new commercial drug disposal product. An HPLC-MS/MS method based on electrospray ionization and multiple reaction monitoring on a triple quadrupole mass analyzer was developed for the quantification of 24 common prescription and OTC drugs in a single run. Various amounts of drug tablets were loaded into separate drug disposal product bottles, and samples of the

residual product solution were tested for the presence of residual drug compounds. Prescription drugs tested included opiates, barbiturates, statins, amphetamines, and benzodiazepines drugs. The drugs in this study were chosen from those that are prone to misuse and abuse. It was found that when the product's loading capacity (as stated on the product label) was not exceeded, more than 98% (and often >99.9%) of the active ingredient was sequestered. Thus, the product appears to present a viable new means for disposal of unused and expired medications.

Material and Methods

Standards and reagents

Commercially-prepared drug (acetaminophen, metoprolol, warfarin, fentanyl, phentermine, morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone, methylphenidate, meperidine, lorazepam, diltiazem, diazepam and testosterone) standard solutions (1000 µg/mL) were purchased from Cerilliant (Round Rock, TX). Drug standards as pure solids (lisinopril, simvastatin, meloxicam, glipizide and progesterone) were purchased from RT-Corp (Laramie, WY). Lovastatin was purchased from US Pharmacopeial Convention (Rockville, MD) and cyclophosphamide was purchased from Sigma-Aldrich (St. Louis, MO). Standards in solution were stored in the freezer (-4 ºC) until use. LCMS-grade water and methanol were purchased from Honeywell Burdick & Jackson International (Muskegon, MI). LCMS-grade ammonium formate (NH4HCO2) and reagent-grade formic acid were obtained from Sigma-Aldrich (St. Louis, MO).

Quality control samples and calibration standards preparation

A working solution containing all 24 drug standards (each 20 µg/mL) was prepared by mixing the drug standards and diluting with LCMS-grade water. A series of volumetric dilutions were performed using the product matrix solution to obtain calibration standard concentrations of 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 7.0 µg/mL. The product matrix solution was obtained by filtering the supernatant from an unused drug disposal product bottle. Though the exact composition of the liquid is proprietary, it can be reasonably characterized as an acidified aqueous alcohol solution (more information about the product composition is given below). Quality control (QC) samples were prepared at lowest (0.05-0.40 µg/mL), low (0.30-0.50 µg/mL), mid (1.2 µg/mL) high (2.0-7.0 µg/mL) and upper (4.0-10.0 µg/mL)

concentrations in product matrix solution. All these samples were prepared on the day of analysis separated from calibration curve standards.

LC-MS/MS conditions

All measurements were performed on a Shimadzu LCMS-8040 (Shimadzu Scientific Instruments, Columbia, MD) triple quadrupole HPLC-MS/MS instrument. The LCMS-8040 mass analyzer was operated using positive ionization electrospray ionization (ESI) and multiple reaction monitoring (MRM) modes. Source conditions were as follows: Interface voltage, 4.5 kV; nebulizer gas, nitrogen at 3 L/min; heat block temperature, 400 ºC; desolvation line (DL) temperature, 250 ºC; drying gas, nitrogen at 1.5 L/min; collision gas, argon at 230 kPa; and detector voltage, -1.86 kV. MRM event times varied between 0.036 and 0.054 msec. The MRM events optimized for each analyte are summarized in Table 2-1. Dwell time was 15 msec. The drug concentration for the unknowns was obtained by comparison of their respective areas to the equation of the standard curve, constructed by a weighed (1/C) quadratic model using the LabSolution v.5.65 software.

Table 2-1 Retention times, m/z, MRM transitions (ref. ions) and MS parameters for all 24 drug standards

Peak ID#	Drug Name	Ret. Time (min)	m/z	MRM Transitions	Scheduled MRM (min)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Acetaminophen	1.1	151.95>110.05	151.95>65.00-151.95>92.95	0.4 - 1.9	-16	-17; -30; -24	-20; -27; -16
2	Lisinopril	1.6	406.10>84.00	406.10>246.20-406.10>309.25	0.7 - 3.0	-20;-19;-20	-32;-25;-19	-30; -25; -21
3	Oxymorphone	2.4	301.85>284.10	301.85>227.05-301.85>198.00	1.0 - 3.2	-30	-20; -30; -46	-17; -21; -18
4	Morphine	2.5	285.85>164.85	285.85>201.05-285.85>154.85	0.9 - 3.0	-30	-25; -40; -30	-21; -30; -28
5	Phentermine	3.1	149.75>91.10	149.75>65.00-149.75>133.00	2.0 - 3.5	-30	-20; -40; -14	-17; -28; -24
6	Hydromorphone	3.2	285.80>185.00	285.80>157.00-285.80>127.95	2.1 - 3.8	-30	-31; -44; -55	-17; -22; -20
7	Oxycodone	3.9	315.85>298.10	315.85>241.00-315.85>256.00	3.1 - 5.2	-30	-18; -28; -26	-30; -26; -27
8	Codeine	4.0	299.85>128.00	299.85>198.95-299.85>170.90	3.1 - 4.5	-30	-29; -55; -38	-21; -25; -30
9	Metoprolol	4.1	267.85>116.05	267.85>56.05-267.85>74.15	3.0 - 4.5	-30	-18; -27; -21	-21; -10; -30
10	Meloxicam	4.1	352.20>115.00	352.20>140.95-352.20>73.05	3.4 - 4.9	-25; -26; -23	-22; -23; -53	-17; -30; -29
11	Cyclophosphamide	4.4	262.60>141.90	262.60>143.90-262.60>119.95	3.6 - 5.1	-29; -30; -30	-21; -20; -21	-26; -26; -21
12	Methylphenidate	4.4	234.30>84.15	234.30>56.20-234.30>55.20	3.4 - 4.9	-16; -25; -26	-23; -44; -50	-15; -21; -24
13	Hydrocodone	4.6	299.85>198.85	299.85>128.05-299.85>170.85	4.1 - 5.2	-30	-31; -55; -38	-21; -25; -30
14	Warfarin	4.7	309.05>162.95	309.05>250.95-309.05>121.00	4.0 - 5.5	-21	-14; -19; -42	-30; -17; -23
15	Meperidine	4.8	247.85>220.00	247.85>173.95-247.85>70.10	3.6 - 5.1	-30	-21; -19; -29	-23; -18; -30
16	Lorazepam	5.5	322.70>277.00	322.70>304.95-322.70>194.00	4.8 - 6.3	-11; -22; -22	-22; -15; -44	-29; -21; -30
17	Glipizide	5.6	446.15>321.05	446.15>103.10-446.15>286.10	4.8 - 6.3	-22; -22; -16	-13; -25; -43	-22; -30; -18
18	Fentanyl	6.2	336.95>105.05	336.95>187.95-336.95>132.05	5.2 - 6.7	-30	-23; -40; -33	-20; -19; -26
19	Diazepam	6.7	285.20>153.85	285.20>192.90-285.20>221.95	5.9 - 7.3	-30	-31; -27; -26	-20; -30; -23
20	Testosterone	6.7	289.20>97.05	289.20>109.05-289.20>79.05	5.9 - 7.5	-30; -30; -20	-23; -25; -50	-15; -23; -11
21	Diltiazem	6.8	415.00>177.75	415.00>149.95-415.00>109.00	5.9 - 7.4	-30	-34; -45; -55	-16; -22; -16
22	Lovastatin	7.2	405.15>199.10	405.15>285.15	6.4 - 7.9	-20; -29	-12; -14	-30; -14
23	Simvastatin	7.3	419.15>199.15	419.15>285.20-419.15>303.15	6.5 - 8.0	-29; -29; -21	-14; -11; -11	-14; -19; -21
24	Progesterone	8.0	315.15>97.10	315.15>109.10-315.15>79.15	7.2- 8.7	-15; -22; -22	-27; -24; -46	-20; -17; -14

The composition of the mobile phase is an important variable in the sample partition and in ion formation process during electrospray ionization. Thus, four mobile phases were studied and compared; these being water/methanol, 10 mM ammonium formate water/methanol, 0.1% formic acid water/methanol and 10 mM ammonium formate/0.1% formic acid water/methanol. Additionally, injection volumes (1, 3 and 5-µL) and flow rates (200, 400 and 600 µL/min) were evaluated.

Liquid chromatography was performed using a binary solvent delivery system (LC-20AD XR, Shimadzu) and autosampler (SIL-20AC XR, Shimadzu). Mobile phase A was composed of 10 mM ammonium formate (NH4HCO2, pH 6.7) in LCMS-grade water. Mobile phase B was composed of 10 mM NH4HCO2 in LCMS-grade methanol. Standard drugs were eluted with a gradient of 25-99% B over 5.5 min, followed by a 99% B hold for 1 min, and then system re-equilibration at 25% B for 3 min. A flow rate of 400 µL/min was used. The column oven temperature was set to 50 ºC. Chromatographic separations were performed using a RaptorTM Biphenyl (Restek Corporation, Bellefonte, PA) (2.7 µm dp; 100 x 2.1 mm) column (biphenyl bonded phase on a superficially-porous particle). Sample injection volume was 1 µL.

Sample preparation

Sample bottles (8 oz.) of DrugDisposalAllTM were obtained from Disposal Technologies (Keller, TX). Unused, each was approximately half-filled with a slurry of product matrix solution, finely-grained activated carbon, and aquarium pebbles. With addition of pharmaceutical products and shaking, the aquarium pebbles act to break apart formulated drug products and to reduce clumping of the activated carbon. Sample bottles were loaded at Pharmatech Services, LLC (Fort Lauderdale, FL) with drugs (all in tablet form) listed in Table 2-4. Samples were transported to The University of Texas at Arlington in a lapse of 48 hours. Sample bottles were kept at room temperature until sampled. 2-mL aliquots were taken from each bottle and filtered into a standard HPLC autosampler vial using 0.2 µm polytetrafluoroethylene (PTFE) membrane syringe filters. Samples were stored in the freezer (-4 ºC) until analysis. Each sample was diluted as shown in Table 2-4 using LCMS-grade water prior to analysis. All samples were analyzed in triplicate.

Method Validation

This method was validated using guidance from the US FDA Bio-analytical Guidelines [70]. Accuracy, precision (CV %), linearity, limits of detection (LOD) and limits of quantification (LOQ) analysis were performed. Calibration was performed using an external standard approach to generate calibrations curves. These curves had a minimum of 6 calibration points ranging from 0.25 to 7.0 µg/mL. QC samples were prepared daily at low (0.30-0.50 µg/mL), mid- (1.2 µg/mL), and high (2.0-7.0 µg/mL) concentration levels. The concentration depended on the measured limit of quantification for the different drug compounds. Analysis of the LOD and LOQ were performed at 0.05, 0.15, 0.20, 0.30 and 0.40 µg/mL, depending on the sensitivity of the method for a given drug compound; each of these samples was analyzed seven times. The LOD were calculated by multiplying the ratio of the standard deviation, *s*, of peak area at the lowest detectable concentration and the slope of the calibration curve, *m*, by 3; whereas the LOQ was calculated as 10 times the *s/m* ratio. The accuracy for the QC's with the lowest detectable signal was required to be ± 20%.

Additionally, intraday (within 24 hours) and interday (between days) accuracy and precision assays were performed. For the intraday assay, three QC samples (Lower-, mid- and Upper-Levels) were prepared and analyzed in replicates of five (n=5) along with calibration standards. These samples were prepared independently from the calibration standard solutions. In the same manner, additionally three QCs samples (low-, mid- and high-QC) were made and analyzed along with calibration standards (prepared independently from the QC samples) for the interday assessment. Each sample was run three times. This process was repeated for five (5) consecutive days. These results were averaged ($n = 5$) and used to calculate the accuracy (%) and coefficient of variation (CV %). Accuracy and CV% were required to be ± 15% for the low-, mid- and high-QC; while for the lowest- and upper-level QC samples the accuracy and CV % had to be ± 20%.

Results and Discussion

LC-MS/MS analysis

An LC-MS/MS method was optimized for the separation and quantitation of 24 target drug analytes. Here, four mobile phases were compared based on their direct effect on the peak area of all 24

analytes. Peak area parameter was chosen due to its simplicity. The results showed that most of the peak areas of the analyte had an increase in area when the 10 mM ammonium formate in water/methanol was utilized. Additionally, injection volumes (1, 3, and 5-µL) were compared. 1-µL injections resulted in thinner peak widths. Lastly, 400 µL/min flow rate gave higher peak areas than flow rates of 600 µL/min. In comparison to the flow rate of 200 µL/min, the analytes' peak areas were not significantly different. Thus, 400 µL/min was selected due to shorter run time.

Next, MRM transitions (targeting [M+H]+ precursor ions) , voltages, and collision energies were optimized by direct infusion of each drug standard prior to LC-MS/MS method development. A representative separation of all 24 analyte drugs as an extracted ion chromatogram is shown in Figure 1B. Even though codeine and hydrocodone had very similar MRM transitions (299.85>128.00; 299.85>198.95 and 299.85>198.85; 299.85>128.05, respectively), they were well distinguished by the biphenyl stationary phase and eluted at significantly different times (4.0 and 4.6 min, respectively); thus, their independent speciation was successful. The biggest challenge was to elucidate the best mobile phase for the chromatographic separation of all 24 drug standards, which varied significantly in their physicochemical properties. The use of ammonium formate (10 mM) in A and B provided the best compromise between selectivity, retention, and mass spectral response. The final chromatographic method allowed for MRM scheduling to maximize sensitivity. Figure 2-1 shows the extracted chromatograms of a blank sample (A), a 1.0 µg/mL standard mixture in matrix (B), diluted (20 fold) sample 2 (C), and diluted (100 fold) sample 3 (D). The analysis of sample 2 is depicted in Figure 2-1C. Sample 2 was loaded with ten tablets containing 20 mg of methylphenidate each for a total of 200 mg active ingredient; and was loaded with less than the maximum load for the product of 3000 mg. The analysis shows that out of the 200 mg, 1.2 mg of methylphenidate was found free in the aliquot, resulting in an adsorption of 99.4%. On the other hand, Figure 2-1D shows the analysis of a product bottle loaded slightly above the label-stated maximum load for the product (3000 mg). Sample 3 contained ten tablets of 60 mg of codeine and 300 mg acetaminophen each, for a total of 3600 mg of combined active ingredients. It was found that 230.5 mg (10.2 mg for codeine and 220.3 mg for acetaminophen) of combined active ingredients was found to be free in the extracted aliquot, resulting on sorption of 93.6 %

(98.3% for codeine and 92.7% for acetaminophen, see Table 2-4) of combine active ingredients. The percent sequestered by the product for sample #3 was low due to overload.

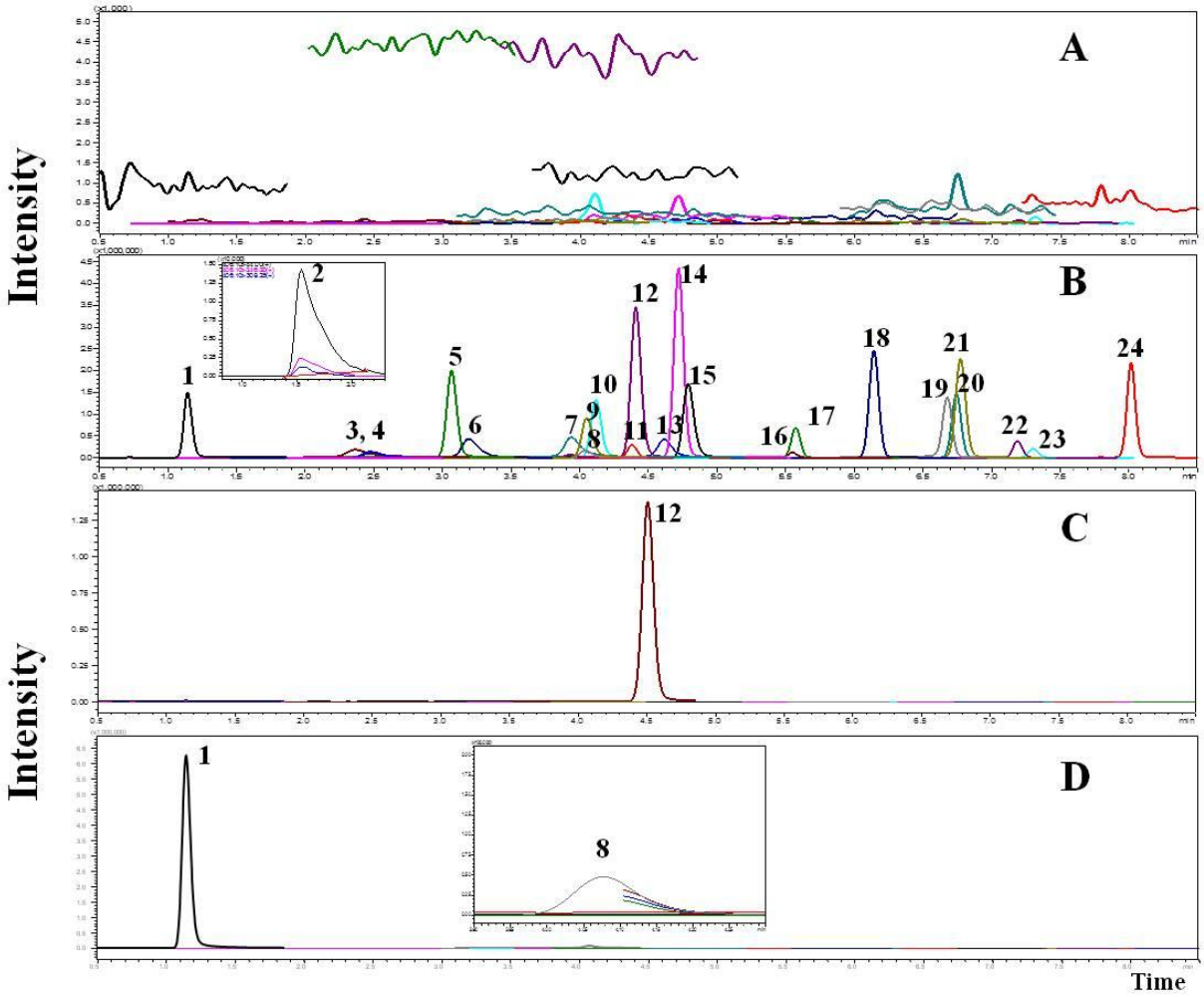

Figure 2-1 Extracted ion chromatograms from: (A) a blank matrix run; (B) a 1.0 µg/mL standard mixture run; (C) diluted (20 fold) sample 2 (loaded with methylphenidate) run; and (D) diluted (100 fold) sample 3 (loaded with codeine/acetaminophen) run

Method Validation

Linearity was assessed based on the R^2 values, and was found to vary from 0.996 to >0.999. For all analytes, the calibration curves were fit to a linear regression using a 1/C weighing method. The accuracy of the method as determined from quality control samples varied from 85% to 111%. Most of these results are in accordance with performance expectations described by the US FDA Bio-analytical Guidelines [70], which states that the error in accuracy (%) should be less than ± 20% for the lowest- and upper-levels (intraday assay) and within ±15% for the other concentrations (low-, mid- and high-levels, interday assay). There were a few exceptions. In the intraday assay, lovastatin (77%) and simvastatin (78%) had accuracies outside the required limits (± 20%) at the upper levels (4.0 µg/mL). While in the intraday assessment, phetermine had an accuracy of 84% (high-level QC, 4.0 µg/mL). For the purpose of the determinations detailed in these studies, this was judged to be sufficient performance. The precision (CV %) for lowest QC, low QC, mid QC, high QC and upper QC samples ranged between 0.7 – 3.9%, 1.2 – 12.7%, 0.3 – 2.6%, 1.8 – 8.4% and 0.1 – 2.0%, respectively. Lastly, the LOD were calculated to be between 0.001 to 0.02 µg/mL; and the LOQ ranged from 0.003 to 0.08 µg/mL. These results are presented comprehensively in Table 2-2 and Table 2–3.

Table 2-2 Statistical results of linearity, LOD, LOQ, intraday accuracy (%) and precision (CV %) for lowest-, mid-, upper-level QCs of 24 drug analytes by LC-MS/MS

					Mean ± STD			
Peak ID#	**Drug Name**	**R^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**Prepared QC (µg/mL)**	**Measured QC (µg/mL)**	**Accuracy (%)**	**Precision (CV %)**
1	**Acetaminophen**	0.999	0.02	0.08	0.4	0.35 ± 0.01	88	2.6
					1.2	1.33 ± 0.01	111	0.8
					4.0	3.59 ± 0.02	90	0.5
2	**Lisinopril**	0.996	0.02	0.05	0.3	0.351 ± 0.004	117	1.2
					1.2	1.18 ± 0.03	99	2.6
					5.0	5.54 ± 0.03	111	0.6
3	**Oxymorphone**	1.000	0.01	0.04	0.2	0.184 ± 0.004	92	2.3
					1.2	1.18 ± 0.02	98	1.3
					4.0	3.41 ± 0.02	85	0.6
4	**Morphine**	0.998	0.01	0.06	0.15	0.14 ± 0.01	93	4.1
					1.2	1.23 ± 0.01	103	0.5
					9.0	7.78 ± 0.05	86	0.6
5	**Phentermine**	0.999	0.01	0.02	0.15	0.170 ± 0.002	114	1.4
					1.2	1.26 ± 0.01	105	0.7
					4.0	3.53 ± 0.01	88	0.4
6	**Hydromorphone**	1.000	0.01	0.02	0.15	0.179 ± 0.002	119	0.9
					1.2	1.2 ± 0.01	100	1.0
					5.0	4.29 ± 0.02	86	0.4
7	**Oxycodone**	0.998	0.01	0.03	0.15	0.142 ± 0.002	95	1.6
					1.2	1.27 ± 0.01	106	0.7
					10.0	8.71 ± 0.05	87	0.6
8	**Codeine**	0.999	0.02	0.06	0.15	0.17 ± 0.01	116	3.7
					1.2	1.23 ± 0.01	103	0.8
					4.0	3.54 ± 0.01	88	0.4

Table 2-2 Continued

9	**Metoprolol**	0.997	0.01	0.03	0.15	0.148 ± 0.004	98	2.4
					1.2	1.28 ± 0.01	107	0.9
					9.0	7.82 ± 0.03	87	0.4
10	**Meloxicam**	0.999	0.01	0.04	0.4	0.331 ± 0.004	83	1.2
					1.2	1.24 ± 0.01	103	0.7
					4.0	3.25 ± 0.01	81	0.4
11	**Cyclophosphamide**	0.999	0.02	0.06	0.15	0.17 ± 0.01	115	3.9
					1.2	1.23 ± 0.02	103	1.4
					4.0	3.54 ± 0.07	88	2.0
12	**Methylphenidate**	1.000	0.001	0.003	0.05	0.0422 ± 0.0004	84	1.1
					1.2	1.21 ± 0.01	101	0.7
					4.0	3.23 ± 0.01	81	0.3
13	**Hydrocodone**	0.998	0.01	0.02	0.4	0.351 ± 0.003	88	0.8
					1.2	1.24 ± 0.01	103	0.7
					10.0	8.88 ± 0.04	89	0.5
14	**Warfarin**	0.998	0.01	0.03	0.15	0.166 ± 0.002	111	1.4
					1.2	1.26 ± 0.01	105	1.0
					4.0	3.39 ± 0.03	85	0.9
15	**Meperidine**	0.998	0.01	0.02	0.15	0.149 ± 0.002	99	1.6
					1.2	1.27 ± 0.01	106	0.4
					10.0	9.82 ± 0.06	98	0.6
16	**Lorazepam**	1.000	0.002	0.06	0.3	0.25 ± 0.01	82	2.3
					1.2	1.26 ± 0.01	105	0.9
					10.0	10.18 ± 0.05	102	0.5
17	**Glipizide**	0.998	0.01	0.02	0.15	0.159 ± 0.002	106	1.1
					1.2	1.26 ± 0.01	105	0.8
					10.0	8.53 ± 0.05	85	0.6

Table 2-2 continued

18	**Fentanyl**	0.997	0.01	0.02	0.15	0.152 ± 0.002	101	1.2
					1.2	1.273 ± 0.005	106	0.4
					7.0	6.16 ± 0.03	88	0.5
19	**Diazepam**	0.998	0.01	0.02	0.15	0.160 ± 0.001	106	0.7
					1.2	1.25 ± 0.01	104	0.7
					9.0	7.45 ± 0.01	83	0.1
20	**Testosterone**	1.000	0.01	0.02	0.15	0.172 ± 0.001	115	0.8
					1.2	1.29 ± 0.01	107	0.7
					5.0	4.31 ± 0.01	86	0.3
21	**Diltiazem**	0.999	0.01	0.03	0.15	0.167 ± 0.002	111	1.3
					1.2	1.23 ± 0.01	102	0.6
					7.0	6.00 ± 0.04	86	0.7
22	**Lovastatin**	0.999	0.01	0.03	0.15	0.153 ± 0.002	102	1.6
					1.2	1.07 ± 0.01	89	0.9
					4.0	3.07 ± 0.01	77	0.5
23	**Simvastatin**	0.999	0.01	0.04	0.15	0.156 ± 0.003	104	2.2
					1.2	1.06 ± 0.01	89	0.5
					4.0	3.11 ± 0.02	78	0.7
24	**Progesterone**	0.998	0.003	0.009	0.15	0.156 ± 0.001	104	0.5
					1.2	1.332 ± 0.005	111	0.3
					10.0	9.41 ± 0.06	94	0.6

Table 2-3 Interday accuracy (%) and precision (CV %) results for low-, mid-, high-level QCs of 24 drug analytes by LC-MS/MS

			Mean ± STD		n = 5 days
Peak ID#	**Drug Name**	**Prepared QC (µg/mL)**	**Measured QC (µg/mL)**	**Accuracy (%)**	**Precision (CV %)**
1	**Acetaminophen**	0.5	0.53 ± 0.01	105	2.7
		1.2	1.22 ± 0.05	102	4.0
		3.0	2.61 ± 0.09	87	3.4
2	**Lisinopril**	0.5	0.55 ± 0.01	111	1.7
		1.2	1.17 ± 0.02	97	1.3
		5.0	5.1 ± 0.1	103	2.3
3	**Oxymorphone**	0.5	0.47 ± 0.02	95	4.5
		1.2	1.26 ± 0.04	105	3.2
		3.0	2.67 ± 0.08	89	3.1
4	**Morphine**	0.3	0.29 ± 0.01	96	2.0
		1.2	1.24 ± 0.02	103	2.0
		4.0	3.40 ± 0.08	85	2.5
5	**Phentermine**	0.5	0.51 ± 0.02	103	3.9
		1.2	1.22 ± 0.06	101	5.1
		4.0	3.4 ± 0.2	84	5.1
6	**Hydromorphone**	0.5	0.51 ± 0.02	102	3.5
		1.2	1.22 ± 0.05	102	3.7
		7.0	5.9 ± 0.3	85	5.6
7	**Oxycodone**	0.5	0.51 ± 0.01	103	1.7
		1.2	1.22 ± 0.02	102	1.5
		5.0	4.34 ± 0.1	87	3.3
8	**Codeine**	0.4	0.40 ± 0.02	99	5.6
		1.2	1.22 ± 0.05	102	4.1
		4.0	3.6 ± 0.1	89	3.8
9	**Metoprolol**	0.5	0.51 ± 0.03	102	5.0
		1.2	1.21 ± 0.06	101	5.1
		4.0	3.5 ± 0.1	86	3.8
10	**Meloxicam**	0.5	0.51 ± 0.05	102	9.1
		1.2	1.25 ± 0.08	104	6.4
		2.0	2.0 ± 0.1	99	5.4
11	**Cyclophosphamide**	0.5	0.49 ± 0.06	97	12.0
		1.2	1.2 ± 0.1	101	10.0
		2.0	1.9 ± 0.1	93	5.5
12	**Methylphenidate**	0.5	0.51 ± 0.01	102	1.7
		1.2	1.22 ± 0.04	102	2.9
		2.0	2.01 ± 0.08	100	4.0

Table 2-3 continued

13	**Hydrocodone**	0.5	0.52 ± 0.03	103	5.2
		1.2	1.25 ± 0.05	104	4.3
		4.0	3.5 ± 0.1	87	3.0
14	**Warfarin**	0.4	0.38 ± 0.05	95	12.7
		1.2	1.24 ± 0.04	103	3.4
		3.0	2.6 ± 0.2	87	6.2
15	**Meperidine**	0.5	0.51 ± 0.02	102	3.1
		1.2	1.21 ± 0.05	101	3.7
		5.0	4.3 ± 0.3	87	6.2
16	**Lorazepam**	0.5	0.50 ± 0.02	100	4.0
		1.2	1.19 ± 0.05	100	4.6
		7.0	6.0 ± 0.4	86	6.4
17	**Glipizide**	0.5	0.51 ± 0.02	103	4.2
		1.2	1.22 ± 0.05	102	4.0
		4.0	3.5 ± 0.1	88	3.2
18	**Fentanyl**	0.5	0.51 ± 0.01	103	1.2
		1.2	1.22 ± 0.03	102	2.1
		4.0	3.6 ± 0.1	89	3.2
19	**Diazepam**	0.5	0.52 ± 0.02	105	3.2
		1.2	1.25 ± 0.02	104	1.6
		4.0	3.4 ± 0.1	86	3.2
20	**Testosterone**	0.5	0.51 ± 0.03	101	6.8
		1.2	1.23 ± 0.02	103	1.9
		3.0	2.56 ± 0.05	85	1.8
21	**Diltiazem**	0.3	0.28 ± 0.03	93	11.0
		1.2	1.23 ± 0.02	103	1.9
		4.0	3.6 ± 0.3	89	8.4
22	**Lovastatin**	0.5	0.45 ± 0.03	91	6.9
		1.2	1.15 ± 0.04	96	3.8
		2.0	1.74 ± 0.09	87	4.9
23	**Simvastatin**	0.5	0.46 ± 0.02	93	3.4
		1.2	1.14 ± 0.05	95	4.0
		2.0	1.75 ± 0.07	88	4.3
24	**Progesterone**	0.5	0.51 ± 0.01	103	1.9
		1.2	1.22 ± 0.03	101	2.6
		5.0	4.3 ± 0.2	86	4.9

Method application

The validated method was used to determine the ability of the DrugDisposeAllTM product to sequester the active ingredient in a series of tests. The measured amounts of free active ingredient in the supernatant of 18 sample bottles containing different drugs compounds were determined as shown in Table 2-3. As we can see, maximum sequestration (more than 99% adsorption) was achieved for those samples (2, 4, 5, 7, 9, 10, 12-14, 16 and 18) where the label-specified loading maximum was not exceeded. On the other hand, when samples were overloaded (samples: 1, 3, 6, 8, 11 and 17), the sequestration of active ingredient diminished and sorption of less than 99% was observed. For example, sample 17 was loaded with 9000 mg of diltiazem and it was determined that 1775 mg was free in solution, achieving 80.3% adsorption of the active ingredient. In sample 6, tablets containing a mixture of hydrocodone and acetaminophen were loaded into the product. The total active ingredient loaded was 7575 mg, which is more than two times the label specification. Total sequestration of 80.9% of the active ingredients was attained, but still 99.1% of the hydrocodone was removed. It may be judged far more important to sequester hydrocodone than acetaminophen, and this was likely achieved based on a higher sorption affinity of hydrocodone for the active charcoal, compared to the more hydrophilic acetaminophen.

Sample 15 was an exception. This sample was loaded with 1085 mg of phentermine, an amount below the specified label indication. The amount sequestered by the product was determined to be 95.5%. An explanation for this very slightly lower performance compared with other trials could be the presence of significant additional excipients in the product formulation, including organic dyes, which might occupy active adsorption sites on the activated charcoal. While this was not found to be a problem with other drugs, phentermine tablets are diet caplets that may contain approximately 35 mg of active ingredient and are generally reported as formulated with blue dye components. Other excipients present in the particular formulation of tablet loaded into this product are unknown. The product could have also been less than optimally mixed when loaded. Overall, it was determined that if the loading of the drug substances tested in this study remained below the label-specified levels, then the vast majority (>95.5%, and usually >99.9%) of the active ingredient was rendered irretrievable.

Table 2-4 Total active ingredient percentage (%) adsorbed results for samples bottles

Sample ID #	Drug A	Drug B	Fold Dilution A	Fold Dilution B	Total A Added (mg)	Total B Added (mg)	Free A in Aliquot (mg)	Free B in Aliquot (mg)	Adsorbed A (%)	Adsorbed B (%)
1	Oxycodone	Acetaminophen	10	2000	100	5000	0.9	386.2	99.1	92.3
2	Methylphenidate		20		200		1.2		99.4	
3	Codeine Phosphate	Acetaminophen	100	1000	600	3000	10.2	220.3	98.3	92.7
4	Fentanyl		1		30.6		<LOQ		>99.9	
5	Meperidine		100		1000		9.9		99.0	
6	Hydrocodone Bitartrate	Acetaminophen	5	10000	75	7500	0.6	1447	99.1	80.7
7	Simvastatin		1		2400		<LOQ		>99.9	
8	Metoprolol Tartrate		1000		6000		929		84.5	
9	Cyclophosphamide		40		2500		10.5		99.6	
10	Warfarin Sodium		1		300		<LOQ		>99.9	
11	Lisinopril		100		4000		785.5		80.4	
12	Lorazepam		1		41.5		<LOQ		>99.9	
13	Hydromorphone HCl		20		472		2.9		99.4	
14	Morphine Sulfate		50		1085		6.12		99.4	
15	Phentermine		100		1177.5		52.6		95.5	
16	Glipizide		4		1000		0.2		>99.9	
17	Diltiazem		2000		9000		1775		80.3	
18	Meloxicam		1		1500		0.6		>99.9	

Conclusion

A precise, accurate, and rapid LC-MS/MS method was developed and validated for the simultaneous quantification of 24 drugs in an acidified medium in less than 10 minutes. While the performance of the product was judged to be high, this is obviously not a fully comprehensive test of all possible candidates for disposal. It would be impossible to test for all drug substances and formulations; however, this study does reveal good performance of the product for a number of drugs that are commonly abused or misused.

The product tested was an 8 oz. bottle meant for retail sales to individual consumers. The label states that once used, the bottle and/or contents can be disposed of in the normal garbage, for landfill. Different states place different regulations on such disposal, but the product's manufacturer has performed independent leachate tests that support this assertion. In fact, activated charcoal is a recommended material for clean up of soil and sludge, according to the US EPA. It is generally understood that once organics are absorbed to activated charcoal, it is extremely difficult to recover them again, unless extremely harsh conditions are used. Larger product formulations would be expected to have greater sorption capacity. As the amount of activated charcoal is increased, the surface area available for sequestration would increase by the square of the amount of that increase. Thus, loading capacities for gallon or five gallon formulations would be expected to support disposal in health care, hospital, and drug diversion settings. Thus, this concept for drug disposal has the potential to aid in the fight against illicit drug use, distributions, and environmental contamination by pharmaceutical substances.

Chapter 3

Loading capacity determination of activated carbon in the presence of acids

The method developed for the determination of 24 drugs was utilized to measure the maximum loading capacity of solid-drug and liquid-drug formulation. Additionally, two different types of acids (acid A and acid B) were compared for the disposal of liquid drugs. For capacity studies on solid drugs, a 1 gallon (1-gal) jug was utilized. To study disposal of liquid drugs, an 8 ounce (8-oz) bottle was used.

Since acetaminophen percentage content is greater in comparison to many other formulated pharmaceuticals, 500-mg acetaminophen caplets were used to determine the loading capacity of the 1-gal jug; and to mimic commercial liquid drug formulation, a saline solution was prepared containing acetaminophen (65 mg/mL).

Material and Methods

Standards and reagents

Commercially-available acetaminophen (1000 µg/mL in methanol) standard solution was purchased from Cerilliant (Round Rock, TX). LCMS-grade water and methanol were purchased from Honeywell Burdick & Jackson International (Muskegon, MI). LCMS-grade ammonium formate (NH_4HCO_2) and reagent-grade formic acid were obtained from Sigma-Aldrich (St. Louis, MO). Kirkland Signature acetaminophen caplets (500 mg active ingredient per pill) were obtained from Costco Wholesale Corporation. Also, acetaminophen solid standard (≥99%) and phosphate buffered saline powder (pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol (200 proof) was obtained from Decon Labs, Inc. (King of Prussia, PA).

A working solution containing only acetaminophen standard (10 µg/mL) was prepared with LCMS-grade water. A series of volumetric dilutions were performed using the product matrix solution to obtain calibration standard concentrations from 0.1 to 2.0 µg/mL. The product matrix solution was obtained by filtering the supernatant from an unused drug disposal product bottle. A quality control (QC) sample was prepared at medium (1.2 µg/mL) concentration in the product matrix solution. Calibration curve solutions were made fresh and analyzed on daily basis.

Sample extraction and preparation

Formulation preparation for solid drug disposal (caplets)

A 1 gallon formulation was made by preparing 16 times the amount of an original proprietary formulation (specified for an 8 oz. bottle) in a 1-gal jug. An emptied 1-gal jug was obtained from Disposal Technologies (Keller, TX). The 1-gal jug was loaded at University of Texas at Arlington (Arlington, TX) with systematically increasing amounts of 500-mg acetaminophen caplets every 48 hours or more and was kept at room temperature until sampled. After addition of the caplets, 1-gal jug was shaken vigorously for a lapse of 5-10 minutes by hand. These amounts are listed in Table 3-1. At the end of the 48-hr period, 1-mL aliquots were removed and filtered into a standard HPLC autosampler vial using 0.2 µm polytetrafluoroethylene (PTFE) membrane syringe filters. A total of 9 samples were acquired. The extracted samples were stored in the freezer (-4 ºC) until analysis. Additionally, 8 samples were extracted every 48 hours without adding more acetaminophen caplets with the purpose to investigate whether an adsorption equilibrium had been reached after 48 hours or not; and if not, to determine when this equilibrium was achieved.

Formulation sample preparation for liquid drug disposal

Empty 8-oz bottles were acquired from Disposal Technologies (Keller, TX). A proprietary formulation was prepared at The University of Texas at Arlington (Arlington, TX), which had a composition similar to that used for solid drug disposal, with exception of the type of acids added. Two bottles were prepared with this liquid-drug formulation, each containing a different type of acid (acid A and acid B) in order to test the efficacy of these two acids individually.

A stock acetaminophen solution was made on-site to mimic the composition of common liquid commercial drug. This solution was used to load 8-oz acid A bottle and 8-oz acid B bottle. Acetaminophen solubility was investigated; since acetaminophen is very soluble in alcohols such as methanol (371.61 g/kg of solvent) and ethanol (232.75 g/kg of solvent), but it has a much lower solubility in water (17.39 g/kg of solvent) [71]. Consequently, the acetaminophen stock solution was made in 40% ethanol in dionized (DI) water containing 0.01M phosphate buffer saline solution (PBS). The addition of ethanol to the solvent was necessary in order to increase the solubility of acetaminophen in 0.01M PBS.

The addition of ethanol resulted in an increase of solubility of acetaminophen in water by three more times, 65.13 g/kg. Thus, a stock solution of 65mg/mL concentration was prepared.

20-mL aliquots of stock acetaminophen solution (65 mg/mL) were loaded to both 8-oz bottles every 48 hours and bottles were shaken energetically by hand for approximately 5 minutes. At the end of the 48-hr cycle, and after significant shaking (5 minutes by hand energetically) and time allotted for settling (15 minutes), 2-mL supernatant was extracted and filtered into a standard HPLC autosampler vial using 0.2 µm polytetrafluoroethylene (PTFE) membrane syringe filters. 2-mL supernatant aliquots were stored in the freezer (-4 ºC) until analysis. Following extraction, 20-mL of acetaminophen solution was added to each 8-oz bottle. A total of 8 extractions were performed. The acquisition of samples by this design is shown in Table 3-3.

LC-MS/MS conditions

All measurements were performed on a Shimadzu LCMS-8040 (Shimadzu Scientific Instruments, Columbia, MD) triple quadrupole HPLC-MS/MS instrument. The LCMS-8040 mass analyzer was operated using positive ionization electrospray ionization (ESI) and multiple reaction monitoring (MRM) modes. Source conditions were as follows: Interface voltage, 4.5 kV; nebulizer gas, nitrogen at 3 L/min; heat block temperature, 400 ºC; desolvation line (DL) temperature, 250 ºC; drying gas, nitrogen at 1.5 L/min; collision gas, argon at 230 kPa; and detector voltage, -1.86 kV. Acetaminophen MRM event time was from 0.626 to 1.626 min. Dwell time was 15 msec. The drug concentration for the unknowns was obtained by comparison of their respective areas to the equation of the standard curve, constructed by a weighed (1/C) quadratic model using the LabSolution v.5.65 software.

Results and Discussion

Method quality control (QC)

A medium QC (1.2 µg/mL) was utilized for quality check purpose and was run 5 times. The accuracy of the method varied from 109-114%. These results are in accordance with performance expectations described by the US FDA Bio-analytical Guidelines [70], which states that the error in accuracy (%) should be less than ±15%. The precision (CV %) for medium QC samples was 2.4%.

Method applications

First, the 24-drug method was used to measure the amount of free acetaminophen in the extracted aliquots obtained from sampling the 1-gal jug every 48 hours or longer. Figure 3-1 shows the extracted chromatogram of an extraction performed on day 29. This ample had been previously loaded with 80,000 mg of acetaminophen caplets.

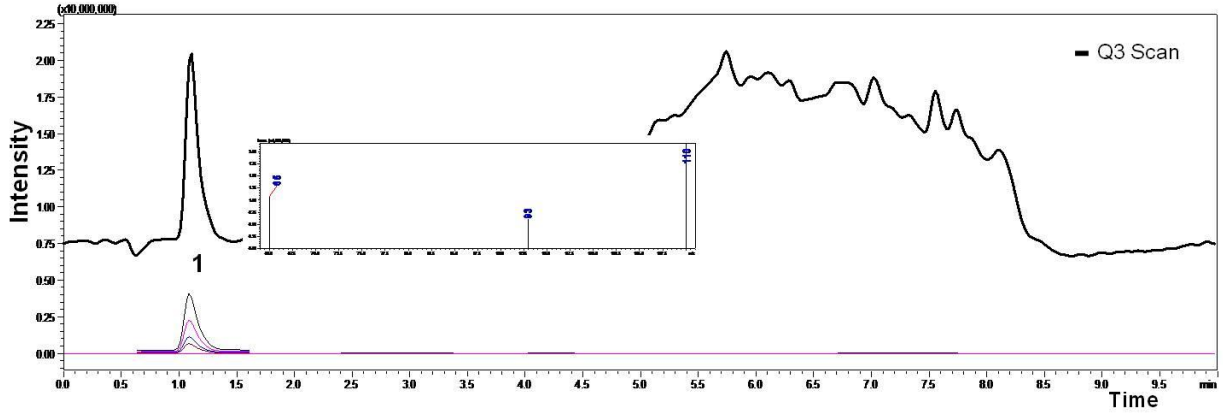

Figure 3-1 Q3 Scan total ion chromatogram and extracted chromatogram for sample JIS_0902 (200 dilution fold) containing a total 80,000 mg of acetaminophen caplets

In more detail, the amount of acetaminophen added in mg to create each sample is shown in Table 3-1 and Figure 3-2. From these results, one can see that as the amount of acetaminophen (mg) was added the amount adsorbed decreased. These results were expected since the porosity within the AC becomes saturated with acetaminophen. However, the maximum loading capacity was significantly exceeded when 80,000 mg of acetaminophen in pill-form was added. The adsorption decreased by one third approximately (92.1% to 62.6%).

Table 3-1 Amounts of 500-mg acetaminophen caplets added every 48 hours to the solid formulation 1-gal jug sample and results for the percentage (%) adsorbed in 48 hours or longer

			Mean ± STD		n = 3
Date	**Added Acetaminophen (mg)**	**Sample ID**	**Measured Acetaminophen (mg)**	**Precision (CV %)**	**Adsorbed (%)**
080215	20000	J1S_0805	481 ± 9	2	97.6
080515	25000	J1S_0807	771 ± 17	2	96.9
080715	27500	J1S_0810	876 ± 5	1	96.8
081015	30000	J1S_0812	1232 ± 90	7	95.9
081215	35000	J1S_0814	1609 ± 69	4	95.4
081415	42500	J1S_0817	3277 ± 109	3	92.3
081715	45000	J1S_0820	3559 ± 260	7	92.1
083115	80000	J1S_0902	29920 ± 584	2	62.6
090215	142500	J1S_0904	85600 ± 2974	3	30.2

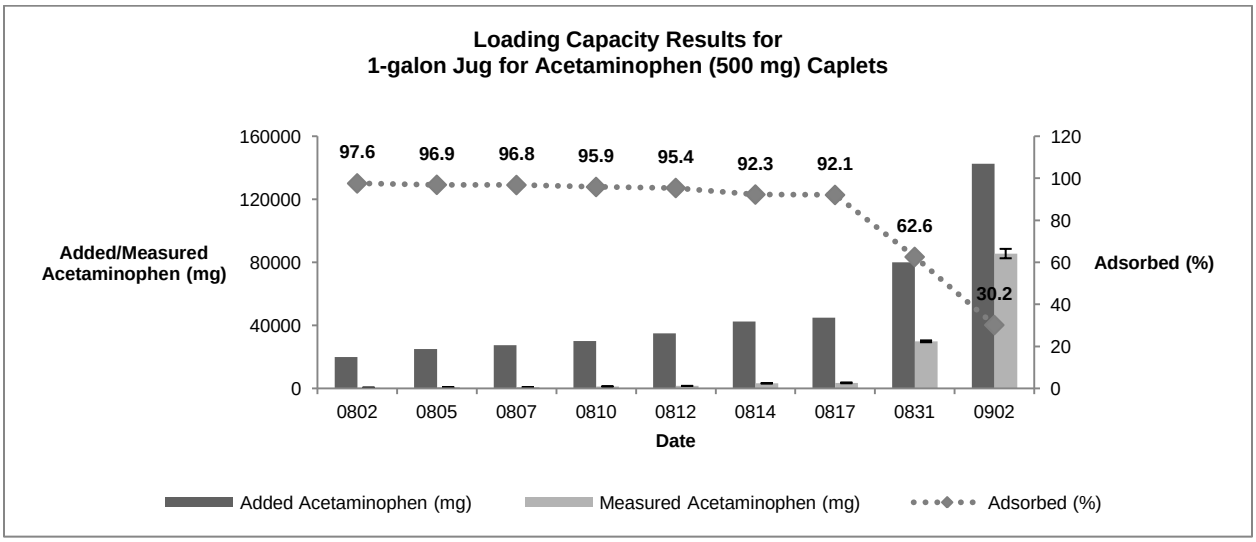

Figure 3-2 Solid-drug formulation results using 500-mg acetaminophen caplets in a 1-gal jug

The extractions (8) that were obtained after 142,500 mg of acetaminophen caplets was added, showed that as slurry was allowed to stand for some additional time period, the adsorption of acetaminophen increased favorably, demonstrating that the loading capacity was reached in the 10th day. Table 3-2 presents the amount of free acetaminophen detected in each extraction.

Table 3-2 Results for adsorption rate over time for 1-gal jug sample containing 142,500 mg of acetaminophen (500-mg) caplets

		Mean ± STD		n = 3
Days	**Sample ID**	**Measured Acetaminophen (mg)**	**Precision (CV %)**	**Adsorbed (%)**
2	J1S_0904	85600 ± 2974	3	30.2
4	J1S_0906	110733 ± 1419	1	22.3
6	J1S_0908	108367 ± 4649	4	23.9
8	J1S_0910	81600 ± 872	1	42.7
10	J1S_0912	35667 ± 830	2	75.0
12	J1S_0914	39160 ± 481	1	72.5
14	J1S_0916	32907 ± 543	2	83.9
16	J1S_0918	35167 ± 509	1	75.3

Here, the amount of free acetaminophen in the extraction increased slightly from day 0 to day 4. After day 4, the amount detected started to decrease. And by day 16, the adsorption capacity seemed to have reached a maximum. Figure 3-3 shows these results in a graphical matter, where the amount detected of free acetaminophen is characterized by bars and the line symbolizes the percentage adsorbed by this formulation.

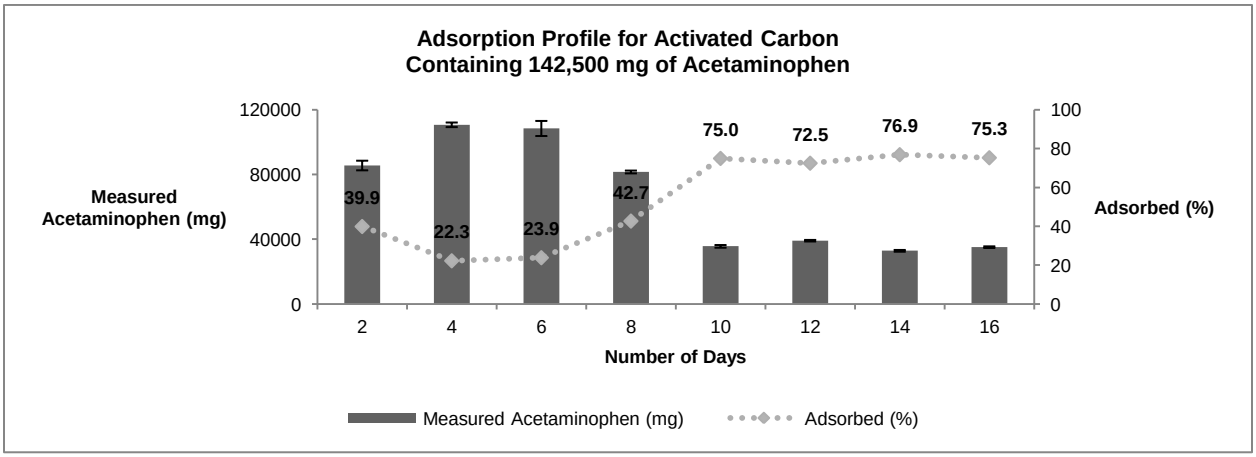

Figure 3-3 1-gal jug adsorption profile results for 142,500 mg of acetaminophen (caplets) over 16 days

Similarly, the 24-drug method was used to test the disposal for liquid drugs using a modified product formulation. Extracted chromatograms are depicted in Figure 3-4. Inserts, in this figure, represent the acetaminophen peak (peak 1) and acetaminophen mass spectra. For samples containing the

acetatmoniophen in saline solution, a solvent peak was detected and recorded, peak S. This peak has not been seen in 1-gal jug extractions. The chromatograms for the total ion count in Q3 scan are similar to 1-gal jug extractions.

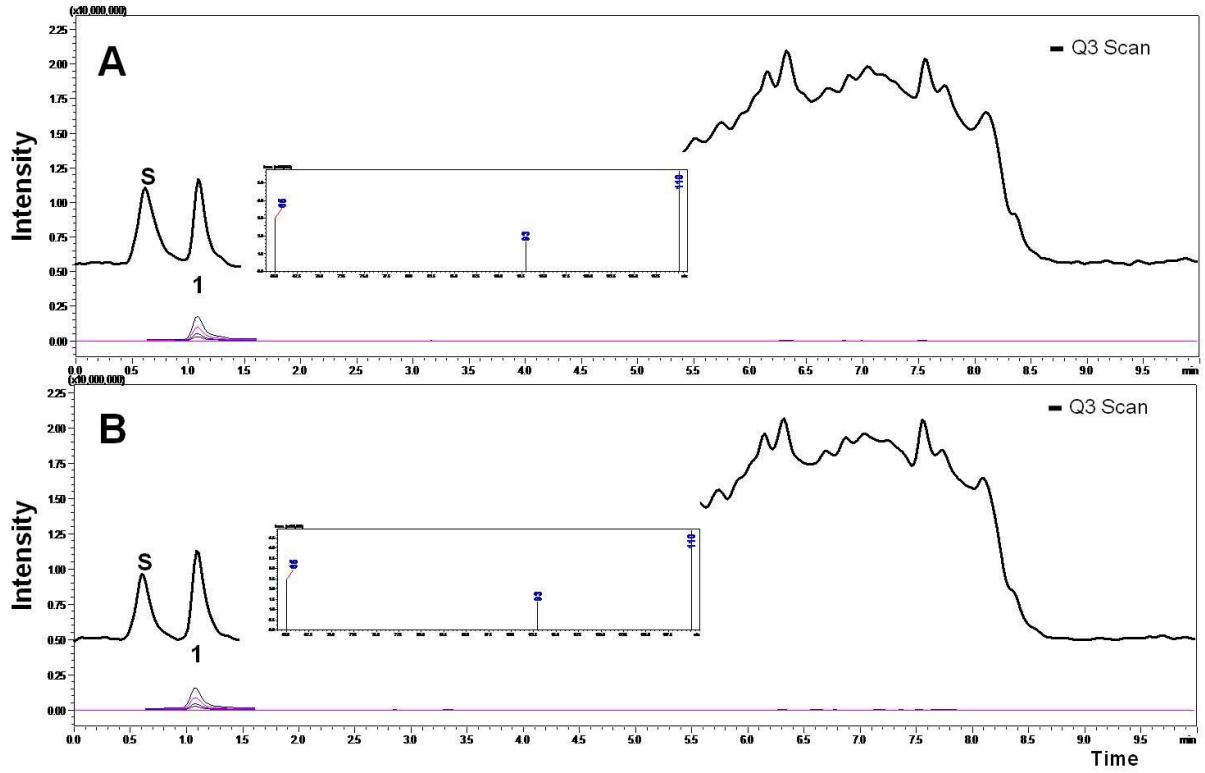

Figure 3-4 Q3-scan total ion chromatogram and extracted chromatograms from samples (diluted 20 folds): (A) AA_0902 and (B) AB_0906. Both samples contained 1,300 mg of acetaminophen at the time of extraction

In this case, one limitation was the size of the bottle (approximately 236 mL); since liquid was added instead of solids the maximum volume of the bottle was reached quickly. So for this experiment, a maximum of 160 mL of 65 mg/mL acetaminophen in 40% methanol in 0.01 M PBS (for a total of 8 additions) was added and is summarized in Table 3-3. Here, the percentage adsorption decreased from 99.8 % to 83.8 % by adding approximately 10 times (10,400 mg) the initial amount (1,300 mg). As expected, as the AC becomes saturated with acetaminophen, the adsorption decreased. The adsorption profile over time (equilibrium between the adsorbate and adsorptive after 48 hours) of the liquid-drug formulation remains yet to be tested.

Table 3-3 Volumes of 65 mg/mL acetaminophen in 40% methanol in 0.01 M PBS added every 48 hours and percentage of adsorbed acetaminophen for 8-oz acid A (AA) and 8-oz acid B (AB) bottles

				Mean ± STD		n = 3
Date Added	**Sample ID**	**Added Acetaminophen (mL)**	**Added Acetaminophen (mg)**	**Measured Acetaminophen (mg)**	**Precision (CV %)**	**Adsorbed (%)**
083115	AA_0902	20	1300	2.6 ± 0.1	4	99.8
090415	AB_0906			4.6 ± 0.9	4	99.6
090215	AA_0904	40	2600	20 ± 6	5	99.2
090615	AB_0908			31 ± 3	5	98.8
090415	AA_0906	60	3900	94 ± 6	7	97.6
090815	AB_0910			126 ± 3	3	96.8
090615	AA_0908	80	5200	204 ± 4	2	96.1
091015	AB_0912			323 ± 22	7	93.8
090815	AA_0910	100	6500	446 ± 8	2	93.1
091215	AB_0914			565 ± 5	1	91.3
091015	AA_0912	120	7800	970 ± 45	5	87.6
091415	AB_0916			882 ± 44	5	88.7
091215	AA_0914	140	9100	1409 ± 95	7	84.5
091615	AB_0918			2122 ± 72	3	76.7
091415	AA_0916	160	10400	1684 ± 167	10	83.8
091815	AB_0920			2978 ± 138	5	71.4

Following, Figure 3-5 illustrates the relation between the added acetaminophen in solution and the percentage adsorbed (%). As the amount added increased the adsorption percentage decreased for each 48-hour extraction. Also, this figure shows that acid A has a slightly higher adsorption percentage than acid B at amounts greater across the range of 1,300 mg to 10,400 mg of acetaminophen.

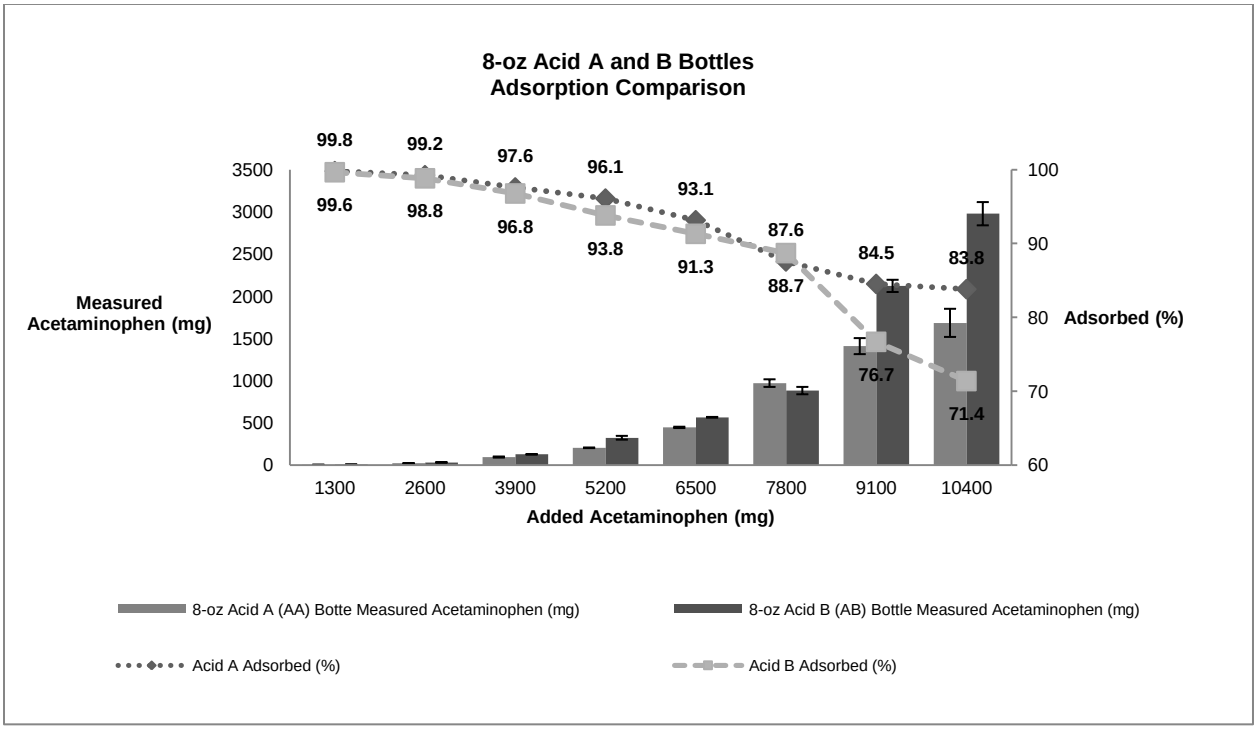

Figure 3-5 Loading capacity comparison results between acid A (AA) and acid B (AB) for liquid-drug formulation using 65 mg/mL acetaminophen in 40% ethanol in 0.01 M PBS added every 48 hours

Conclusions

The methods developed to support the capacity experiments demonstrated excellent performance. Results were reproducible for concentrations within the linearity range (from 0.1 to 2 µg/mL). When comparing the chromatograms of the 1-gal jug extractions with the 8-oz bottles, a solvent peak is present for the former. This might be due to the presence of PBS and ethanol in the extractions. Next, both formulations demonstrated satisfactory adsorption. Even though, acetaminophen is more

hydrophilic than most drugs, and AC is hydrophobic; the formulations showed outstanding adsorption results. Consistency during the extraction process generated reliable data. However, future experiments are needed in other to determine the adsorption profile over time at different loading amounts. As seen for the solid-drug formulation, when first loaded with 142,500 mg of acetaminophen caplets, the adsorbed percentage was only 39.9%. But when the 1-gal jug was allowed to stand for a period of 10 days and was resampled, the amount adsorbed increased, achieving 75% adsorption. For the liquid-drug formulation, acid A showed to have a slightly better outcome than acid B. Therefore, future studies should be continued using acid A. An adsorption profile was not obtained for the liquid-drug formulation. This also should be determined in the future.

Chapter 4

Performance evaluation of a commercial drug disposal product for disposal of Cannabis plant

Introduction

Cannabis sativa L. (hemp) is a dioecious annual flowering plant. Marijuana is the name for the dried leaves and female flowering tops of the hemp plant. Hashish is the resin that originates from the female flower tops. 538 compounds have been identified from this plant. Of these, 108 are identified as cannabinoids and have been classified into 10 main classes (includes tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN)) and 14 subtypes [72]. Cannabinoids are cyclic compounds and most of them are composed of 21 carbons. Cannabinoids can be introduced into the body via smoke, transdermal patch, intravenous injection, oral ingestion, sublingual absorption, vaporization, or rectal suppository. Cannabinoids are then metabolized in the body by the liver. There are two types of cannabionoid receptors: CB1 and CB2. CB1 receptors are located mostly in the central nervous system and peripheral neurons; while CB2 receptors are in the peripheny.

The usage of cannabinoids to treat anxiety and pain as an alternative to synthetic drugs has steadily increased. In fact, marijuana plants has been used as for pain relief, cramps, migraines, convulsion, appetite stimulation, and for attenuation of nausea and vomiting [73]. The rapid progress in understanding the role of cannabinoids in pain mechanism has re-energized its usage for pain management; and as the studies of cannabinoids as pain and anxiety relievers' progress, the need for a means of disposal will rise as many disposal processes for prescription drugs have already risen. Therefore, here we investigate the capacity of a commercial drug disposal product formulation for the disposal of marijuana plant; specifically, the sequestration of biologically active cannabinoids liberated in solutions from the plant material.

Material and Methods

Standards and reagents

Commercially-prepared cannabinoids (Cannabinol-D3, Cannabidiol-D3, and delta-9-tetrahydrocannabinol-D3 (delta-9-THC-D3) at 100 µg/mL and delta-9-tetrahydrocannabinolic acid (delta-9-THC-A) and delta-8-tetrahydrocannabinol (delta-8-THC) at 1000 µg/mL) standard solutions were

purchased from Cerilliant (Round Rock, TX). LCMS-grade water and methanol were purchased from Honeywell Burdick & Jackson International (Muskegon, MI). LCMS-grade ammonium formate (NH4HCO2) and reagent-grade formic acid were obtained from Sigma-Aldrich (St. Louis, MO).

A working solution containing all 5 cannabinoid standards (each 10 µg/mL) was prepared by mixing the drug standards and diluting with LCMS-grade water. A series of volumetric dilutions were performed using the product matrix solution to obtain calibration standard concentrations of 0.10, 0.20, 0.50, 1.0, 2.0 and 4.0 µg/mL. The product matrix solution was obtained by filtering the supernatant from an unused drug disposal product bottle, characterized as an acidified aqueous alcohol solution. A quality control (QC) sample was prepared at medium (1.2 µg/mL) concentration in product matrix solution. All these samples were prepared on the day of analysis.

LC-MS/MS conditions

All measurements were performed on a Shimadzu LCMS-8040 (Shimadzu Scientific Instruments, Columbia, MD) triple quadrupole HPLC-MS/MS instrument. The LCMS-8040 mass analyzer was operated using positive ionization electrospray ionization (ESI) and multiple reaction monitoring (MRM) modes. Source conditions were as follows: Interface voltage, 4.5 kV; nebulizer gas, nitrogen at 3 L/min; heat block temperature, 400 ºC; desolvation line (DL) temperature, 250 ºC; drying gas, nitrogen at 15 L/min; collision gas, argon at 230 kPa; and detector voltage, -1.86 kV. Dwell time was 15 msec and MRM event times were 0.60 min long for all five cannabinoids. The MRM events for each analyte are summarized in Table 4-1. The concentration for the unknowns was obtained by comparison of their respective areas to the equation of the standard curve, constructed by a weighed (1/C) quadratic model using the LabSolution v.5.65 software.

Table 4-1 MS parameters, MRM transitions and calibration curves results for all 5 cannabinoid analytes

Peak ID#	Name	Ret. Time (min)	m/z	MRM Transitions (Ref. Ions)	Scheduled MRM (min)	R^2	Line Equation
1	Delta-9-THC-A	1.31	359.30>341.30	359.30>219.15 359.30>261.25	1.0-1.6	0.999	Y = (2.99596e+006)X + (-45243.3)
2	Cannabidiol-D3	1.34	318.30>196.15	318.30>123.10	1.1-1.7	0.999	Y = (2.67007e+006)X + (-7658.44)
3	Cannabinol-D3	1.75	314.30>223.20	314.30>296.25 314.30>241.20	1.5-2.1	0.999	Y = (5.46560e+006)X + (22055.3)
4	Delta-9-THC-D3	2.01	318.30>196.25	318.30>123.10	1.7-2.3	1.000	Y = (3.19419e+006)X + (12993.4)
5	Delta-8-THC	2.02	315.30>193.25	315.30>123.00 315.30>135.15	1.8-2.4	0.999	Y = (1.76875e+006)X + (6841.85)

For this method, three mobile phases (water/methanol, 10 mM ammonium formate water/methanol and 10 mM ammonium formate/0.1% formic acid water/methanol) were compared. The parameter used to evaluate their effect on the sample was the analytes' peak area. Injection volumes and flow rates were not investigated for this method.

Liquid chromatography was performed using a binary solvent delivery system (LC-20AD XR, Shimadzu Scientific Instruments) and autosampler (SIL-20AC XR, Shimadzu Scientific Instruments). Mobile phase A was composed of 10 mM ammonium formate (NH4HCO2, pH 6.7) in LCMS-grade water. Mobile phase B was composed of 10 mM NH4HCO2 in LCMS-grade methanol. Standards were eluted with a gradient of 90-99% B over 2.75 min, followed by a 99% B hold for 0.25 min, and then a system re-equilibration at 90% B for 2.00 min. A flow rate of 400 µL/min was used. The column oven temperature was set to 50 ºC. Chromatographic separations were performed using a RaptorTM Biphenyl (Restek Corporation, Bellefonte, PA) (2.7 µm dp; 100 x 2.1 mm) column (biphenyl bonded phase on a superficially-porous particle). Sample injection volume was 1 µL.

Sample preparation

Sample bottles (8 oz.) of DrugDisposalAllTM were obtained from Disposal Technologies (Keller, TX) and were half-filled with a slurry of product matrix solution, finely-grained activated carbon, and aquarium pebbles. 8-oz bottles (M1 through M4) were loaded by Disposal Technologies (Keller, TX) with known amounts of marijuana plants. Sample M1 and M2 were loaded with 3.5 g of marijuana plant and sample M3 and M4 were loaded with 7.0 g of marijuana plant. Upon arrival, 8-oz bottles (M1-M4) were kept at room temperature until sampled. 2-mL aliquots were taken from each bottle and filtered into a standard HPLC autosampler vial using 0.2 µm polytetrafluoroethylene (PTFE) membrane syringe filters. Samples were stored in the freezer (-4 ºC) until analysis. Each sample was analyzed in triplicate.

Results and Discussion

Method quality control (QC)

The accuracy and precision were determined by analyzing a QC of medium concentration level (1.2 µg/mL) five times. The accuracy for this level ranged from 101 to 108% for all five cannabinoids. Precision for this medium QC varied from 2.2 to 3.7%. These results are shown in Table 4-2.

Table 4-2 Quality control results for 1.2 µg/mL cannabinoid solution in matrix solution

			Mean ± STD		**n = 5**
Peak ID#	**Analyte Name**	**Prepared QC (µg /mL)**	**Measured QC (µg /mL)**	**Accuracy (%)**	**Precision (CV %)**
1	Delta-9-THC-A	1.2	1.21 ± 0.03	101	2.3
2	Cannabidiol-D3		1.27 ± 0.04	106	3.0
3	Cannabinol-D3		1.26 ± 0.03	105	2.6
4	Delta-9-THC-D3		1.30 ± 0.03	108	2.2
5	Delta-8-THC		1.29 ± 0.03	108	3.7

LC-MS/MS analysis

An LC-MS/MS method was developed for the separation and quantitation of 5 cannabinoid analytes. MRM transitions (targeting [M+H]+ precursor ions), voltages, and collision energies were optimized by direct infusion for each standard prior to LC-MS/MS method development. An extracted chromatogram of all 5 cannabinoid standards along with sample M1 and M3 chromatograms are shown in Figure 4-1. In this figure, even though cannabidiol-D3 and delta-9-THC (peaks 2 and 4 in the chromatogram, accordingly) have similar MRM transitions (318.30>196.15; 318.30>123.10 and 318.30>196.25; 318.30>123.10, accordingly), they eluted at different times (1.4 and 2.1 min, respectively) using the biphenyl stationary phase; therefore they were independently quantify and identify. In addition to the cannabinoid peaks (1-4), a solvent peak (S) was detected. Peak S was seen in all chromatograms for these four samples. The use of 10 mM ammonium formate proves once again to be the most optimal mobile phase for this method (increase of peak area). MRM scheduling maximized methods' sensitivity.

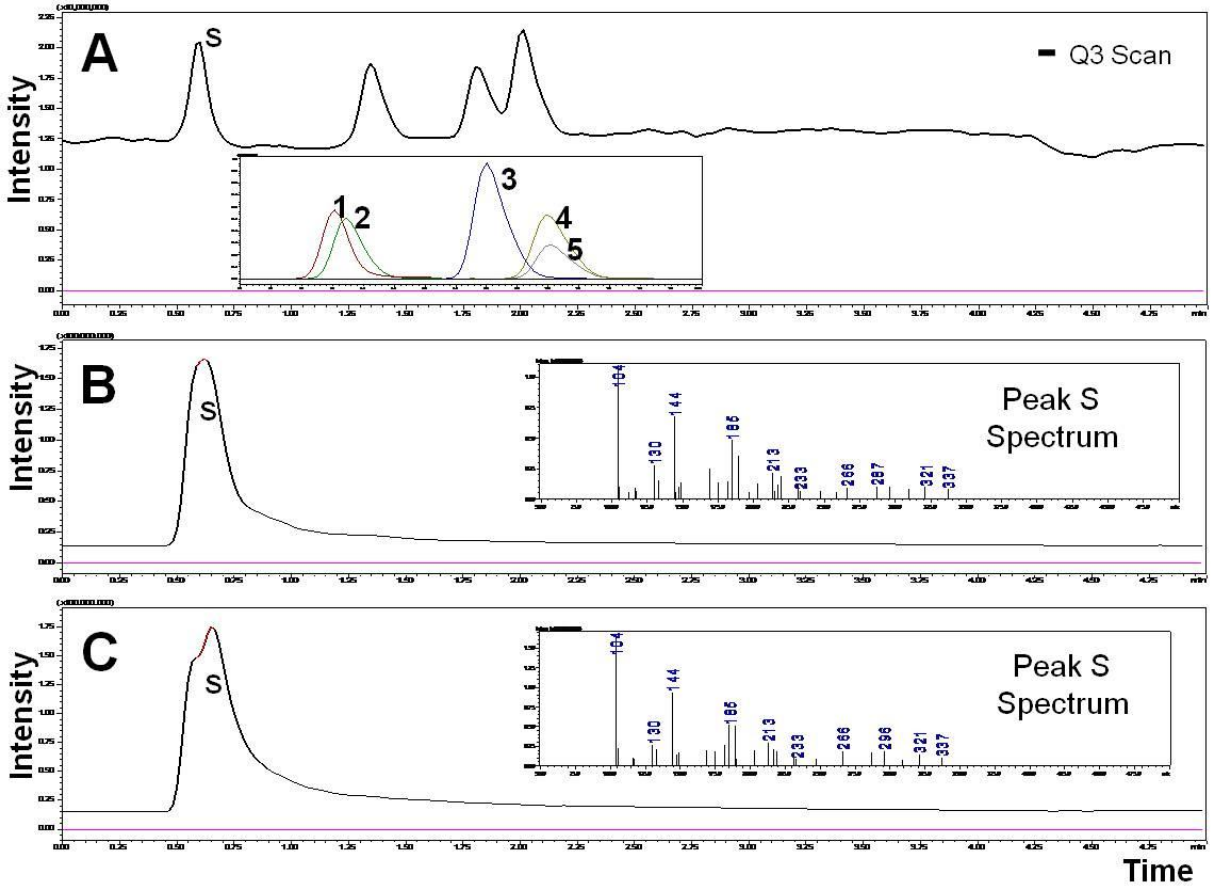

Figure 4-1 (A) Q3-scan mode total ion chromatogram (TIC) and extracted chromatogram of a 1.0 µg/mL (insert) cannabinoid standard mixture run. Q3-scan mode TIC and extracted chromatograms for samples: (B) M1 and (C) M3, loaded with 3.5 g and 7.0 g of Cali Chem-Indica Dominant, respectively

This method was applied to measure the amount of residual THC in the aliquots extracted from the four 8-oz bottles. Very low concentrations of THC (below the limits of detection) were observed in all four samples (M1 through M4). This outcome is summarized in Table 4-3.

Table 4-3 Results of the total active ingredient adsorbed (%) for cannabinoid sample bottles

Sample ID	Cannabinoid Name	Cannabinoid (%)	Plant Added (mg)	Cannabinoid Added (mg)	THC Measured$_{Avg}$ (mg)	Adsorbed (%)
M1	THC	0.15	3500	5.25	<LOD	>99.9
	THC-A	20		700	<LOD	>99.9
M2	THC	0.15	3500	5.25	<LOD	>99.9
	THC-A	20		700	<LOD	>99.9
M3	THC	0.15	7000	10.5	<LOD	>99.9
	THC-A	20		1400	<LOD	>99.9
M4	THC	0.15	7000	10.5	<LOD	>99.9
	THC-A	20		1400	<LOD	>99.9

Conclusion

In conclusion, a rapid, specific and precise LC-MS/MS method was developed for the simultaneous quantitation of five cannabinoids in residual supernatant of a drug disposal product. This method separated 5 cannabinoids under 2.5 minutes. Additionally, quantification was achieved samples of concentrations between 0.1 to 4.0 µg/mL. In summary, it was concluded that DrugDisposeAllTM formulation is capable of sequestering more than 99.9 % of THC and THC-A. Although, further studies are required in order to confirm the capacity of this product to extract the cannabinoids from the leaves and consequently adsorbed them into the activated carbon.

Chapter 5

Summary and Future Work

To extend the knowledge of the formulations (solid and liquid drugs), more experiments should be performed. Thus, for the solid-drug disposal formulation, acid A should be also tested and compared with acid B. Acid A showed better performance in the adsorption process for the liquid-drug disposal formulation. However, the type of sample tested should be kept in mind (acetaminophen caplets vs. acetaminophen powder in solution), since this difference can have an effect on the outcome. Additionally, the solid-drug formulation has so far been tested using caplets; and there is a large scope of compounds that have yet to be investigated such as patches, creams, ointments, capsules, tablets, and suppositories. These compounds have other ingredients that might interfere with or alter the adsorption capacity of this formulation. Moreover, further tuning of the loading capacity experiment for the 1-gal jug is also in need in order to maximize the capability of this product. As seen, this formulation is actively adsorbing over time until it reaches equilibrium. So at this point, the maximum loading capacity has not been established for the solid-drug formulation in a 1-gal jug. The measurements reported here are only after 48 hours and no equilibrium was reached at the time of extraction. Therefore, a similar experiment as the one performed for 142,500 mg sample should be repeated at 50,000 mg, 70,000 mg or 80,000 mg. Since the aim of this product is to be used in hospitals and clinics, the loading capacity of a larger size container (e.g. 5-gal bucket) must also be determined.

In case of the liquid-drug formulation in 8-oz bottles, the adsorption timeline remains to be completed. This experiment will reveal the approximate time it takes for equilibrium between the adsorbate and adsorptive to be reached. Next, future studies should be executed removing or reducing of some of the components, such as the aquarium pebbles. The purpose of the aquarium pebbles are to mechanically disrupt solids and to avoid the formation of clumps in the slurry (activated charcoal in acidified solution). Since the drugs are in the liquid form, there are not any solids that need to be broken; the amount of aquarium pebbles could be reduced. Hence, the right quantify should be determined. Once this formulation is optimized, larger sizes of containers such as 1-gal jug and 5-gal bucket could be tested in the same manner as the solid-drug formulation.

In general, comparison of the formulations' capacity between different types of drugs may be studied further. As has been shown, both of the formulations showed excellent adsorption percentage when acetaminophen (a hydrophilic compound) was used. Greater adsorption percentages should be expected with more hydrophobic compounds. And lastly, a motorized shaker should be obtained and utilized for further testing. Shaking by hand can introduce discrepancies.

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In the past year, Mrs. Waybright has participated in 2015 Annually Celebration of Excellence by Students (ACES), the 48th Annual Meeting-in-Miniature and 39th International Symposium on Capillary Chromatography, where she volunteered to work the front desk. This experience provided her with valuable insight into the coordination and organization that it takes to run a conference event. And Mrs. Waybright plans to continue broadening her analytical skills in the work field.