The Detection of Date Rape Drug Residues Using X-Ray Diffraction

Emily A. Walsh
Forensic Science Department
Dr. Virginia M. Maxwell

Abstract

A predominant problem investigators and forensic scientists encounter is difficulty in the detection of date rape drugs in a drug facilitated sexual assault (DFSA) victim's system, more specifically their blood, hair, and urine. After a matter of hours, these fast acting drugs have little chance of being detected with modern toxicological techniques due to their rapid metabolism by the body. Proving the use of these drugs can be very hard to establish due to the challenge of detection. This project utilized X-ray diffraction (XRD) in order to detect and identify date rape drugs on various materials. The date rape drugs used for the purposes of this project were Gamma-Hydroxybutyric acid (GHB), Chloral Hydrate, Ketamine, Flunitrazepam (Rohypnol), and MDMA (Ecstasy). XRD has been implemented in many forensic science laboratories due to low cost, versatility, and the non-destructive nature of analysis. The focus of this research was to evaluate the use of XRD for the detection of date rape drug residues on clothing and in containers, such as those typically submitted as evidence in suspected DFSAs.

Introduction

Numerous studies have been conducted on the prevalence of sexual assault on college campuses. The Campus Sexual Assault (CSA) Study conducted by the National Institute of Justice provides statistics about these assaults against college-aged women, indicating that 19% of survey participants had experienced sexual assault since entering college, a 3% increase from the 16% of participants who had experienced sexual assault before entering college. Since entering college, one out of five undergraduate women has experienced completed or attempted sexual victimization. Drug facilitated sexual assault (DFSA) was an area of focus for the study. Drugs classified as date rape drugs are utilized for the purposes of DFSA due to their colorless and odorless properties. 5.3% of CSA study participants reported being given a drug, most likely GHB or Rohypnol, without their knowledge or consent. Due to low rates of reporting, DFSA and any type of sexual assault most likely occur in greater rates than observed through research. (1)

Date rape drugs affect the body in many different ways, often magnified when used in conjunction with alcohol. Date rape drugs are fast acting depressants which cause drowsiness, decreased anxiety, and mental impairment, taking effect within 10 to 30 minutes of consumption. The Drug Enforcement Administration (DEA) has categorized substances into schedules constructed upon their accepted medical uses and potential for dependency and abuse, for the purposes of regulating these substances. The schedules run one through five (I-V) and encompass a wide range of medical uses as well as abuse rates. A drug that has no medial use, a high potential for abuse, and severe dependence is considered schedule I. Oppositely, a schedule V drug has a low potential for abuse and many accepted medical uses. GHB and MDMA are schedule I controlled substances. Ketamine is schedule III and Rohypnol and Chloral Hydrate are schedule IV. (2)

The inability to detect the existence of a date rape drug in a victim's urine, blood stream, or hair has been a major problem in suspected DFSA cases. The difficulty detecting these drugs stems from a property called half-life. Half-life is the period of time needed for the body to metabolize half the drug, resulting in the quantity of the drug in the body to fall to half its value. After this point the drug will continue to be metabolized and decay at an exponential rate. The half-life of GHB, as well as its analog GBL, is 30 to 60 minutes and the effects of the drug last from 3 to 6 hours. The effects of Rohypnol will peak at around 2 hours, but the drug's half-life is between 18 to 26 hours. The half-life of Ketamine, 2.5 hours, occurs when the drug's effects are most intense. Chloral hydrate's effects last several hours with a half-life of 8 to 10 hours. Within 30 to 45 minutes the effects of MDMA occur and will last from 4 to 6 hours. Average doses of the drugs vary due to their effects on the body. GHB is often used in 1 to 5 gram doses whereas Rohypnol is used in 0.5 to 2 mg doses. Common doses for Ketamine, Chloral Hydrate, and MDMA are 100 to 200 mg, 500 mg, and 50 to 150 mg, respectively. (2) (3)

The technique of X-ray diffraction is commonly used in the field of forensic science for the purpose of analyzing solid material evidence from minute trace amounts to large complex mixtures with several different compositions. The method has great versatility because crystalline structure is the only requirement for analysis. Materials composed of metallic, organic, and inorganic compounds can be analyzed using this technique. (4) XRD is non-destructive to the materials being analyzed, which permits the ability of carrying out further analysis of the materials by other methods. The decreasing cost of the instrument has made X-ray diffractometers a popular choice to implement in forensic science laboratories for evidence examinations. Drug research using XRD has been able to identify drugs within mixtures, as well as their analogs, or forms of the drug after it has been metabolized. This power of identification is useful for forensic science analyses. (5)

The theory behind X-ray diffraction can be explained by the operative equation: $n\lambda = 2d \sin\theta$, commonly known as the Bragg Equation , where n is the order of a reflection, λ the wavelength, d the distance

between parallel lattice planes, and θ the Bragg angle. More specifically, the Bragg angle is the angle between the incident beam and a lattice plane. (6)

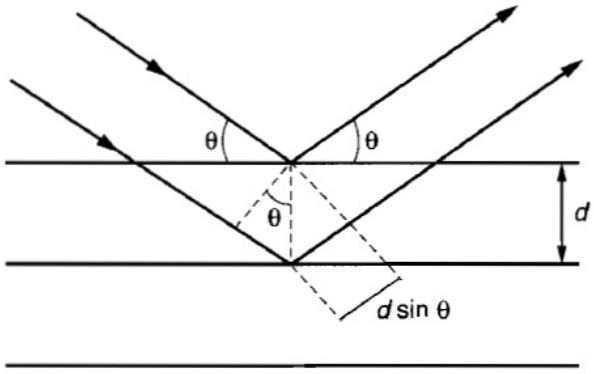

Figure 1: Diagram of Bragg's Law (6)

The principle behind X-ray diffraction involves the crystallinity of a sample scattering x-rays. The x-rays produced by the instrument are passed through the sample and the ordered arrangement of atoms scatter the x-rays. The constructive interference created by the scattered beams moving in phase with each other ultimately produces a diffracted beam. (7) The ability to distinguish one sample from another comes from each compound having a characteristic crystal structure, resulting in distinct peak positions. This unique diffraction pattern is directly influenced by the geometry of the crystal lattice. (6) Diffraction patterns are influenced by both the intensity and angle of the diffracted beams gathered by the detector component of the instrument. (8) When the sample diffracts the x-rays, that are then detected by the instrument, the angle at which they were diffracted is what produces unique diffraction peak patterns.

Methods and Materials

The instrument used for this project was the Rigaku MiniFlex II Desktop X-ray Diffractometer. The software utilized for analyzing the obtained spectra was MDI Jade 9.

Figure 2: The X-ray Diffractometer used for this project.

A standard silicone test was performed to ensure the instrument was working properly, every day before samples were analyzed. Preliminary samples of the following were run to gain familiarity with the instrument: a business card, two brands of baby powder, two brands of duct tape, milk thistle, aspirin, ibuprofen, acetaminophen, comet, baking powder, and confectionary sugar. A technique of preparing samples for future use was developed by practicing with the powdered samples. Most powders could be transferred directly into the 2 x 2 cm well on a zero background sample holder and made flush with the top of the well using a glass microscope slide. Other samples, like the milk thistle, aspirin, ibuprofen, and acetaminophen, need to be prepared differently. The waxy coating on these samples was stripped away using a razor blade and the remaining substance was ground into a powder using a mortar and pestle. The preliminary samples were analyzed with XRD from the angles of 2.000 to 90.000 degrees with run times of 10.000 deg/min and 5.000 deg/min.

Four fabric types: denim, white cotton, polyester, and grey cotton (90%) and polyester (10%), were cut to cover the 2 x 2 cm well on a zero background sample holder and mounted using cellophane tape. Individual spectra of the fabric samples were obtained with the parameters of the angles of 2.000 to 90.000 degrees and run times of 10.000 deg/min and 5.000 deg/min. The fabrics were then soaked in water to remove any surfactants and air dried. Spectra were obtained of the fabric samples again and peak tables of all fabric runs were printed using the MDI Jade 9 software.

Pure date rape drug samples (GHB, Ketamine, Chloral Hydrate, Rohypnol, and MDMA) were loaded into the zero background holder with a small circular well about 2 mm in diameter using toothpicks. The previously determined sample preparation was used for loading the holder, but only the Chloral Hydrate needed to be ground into a powder because of its large and irregular crystal structure. Spectra were obtained with the same parameters of 2.000 to 90.000 degrees and run times of 10.000 deg/min and 5.000 deg/min. Multiple runs for each of the date rape drugs at those parameters, as well as longer run times (2.000 deg/min) were run to demonstrate reproducibility. The spectra were analyzed for peaks using the Jade software.

Two dry mixtures of confectionary sugar and drug (Ketamine and Chloral Hydrate) were prepared to be 10% drug mixtures to simulate the high sugar content of many mixed drinks. More specifically, 0.09 g confectionary sugar and 0.01g of drug were weighed out and placed in a small centrifuge tube. After spectra of the two mixtures were obtained using the same parameters, peak tables were found using Jade. These peak tables were then compared to the pure drug and confectionary sugar peak tables found earlier, in order to assign the mixture peaks as being from the drug or sugar.

Pastes of the 10% drug mixtures were created by adding a single drop of water to the centrifuge tubes containing the dry mixtures. The Ketamine paste was used in further testing by smearing it on the four fabric samples. These were then mounted on the zero background holder

and analyzed using XRD with the same parameters and peak tables were created using Jade.

A mixture of 5 mL ethanol and 0.0028 g Ketamine was prepared in a 10 mL falcon tube. A few drops of the mixture was pipetted onto a flat zero background holder. This was left out to evaporate. Once a residue was formed, analysis with XRD with the same parameters was carried out and peak tables obtained with Jade.

Results

Fabrics analyzed with XRD give diffraction patterns of only a few (3 to 5) peaks because of the uniformity in the structure of the material. This minimal diffraction pattern is shown by the denim (cotton) spectrum in Figure 3.

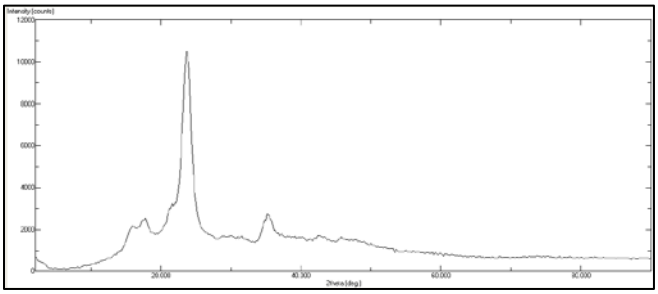

Figure 3: Spectrum of Denim Fabric.

Each of the date rape drugs as well as the confectionary sugar sample analyzed with XRD yielded clear diffraction patterns with numerous peaks as shown in Figures 4 and 5. These background spectra were utilized in later peak comparison and assignment of peaks in the mixture samples.

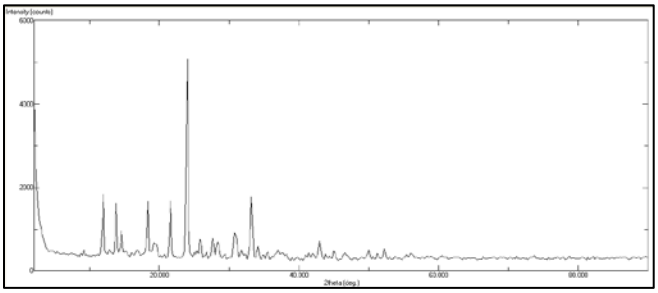

Figure 4: Spectrum of Ketamine.

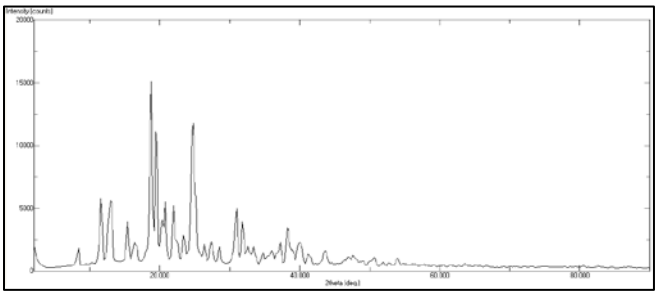

Figure 5: Spectrum of Confectionary Sugar.

The dry mixture samples and the paste samples transferred onto the fabrics yielded more complex spectra due to the multiple components of the samples. Examples of these spectra are shown in Figures 6 and 7. Despite this complexity, XRD had the power to analyze these multifaceted mixtures resulting in being able to distinguish between the individual component diffraction patterns. Coupled with the Jade software, XRD was able to provide enough data to assign to the individual peaks to the component in which it originated for all the mixture samples. The dry 10% drug mixtures yielded peaks that could be attributed to the confectionary sugar, but more importantly the peaks for the constituent drugs were unchanged and visible. The same could be concluded for the paste on the fabrics.

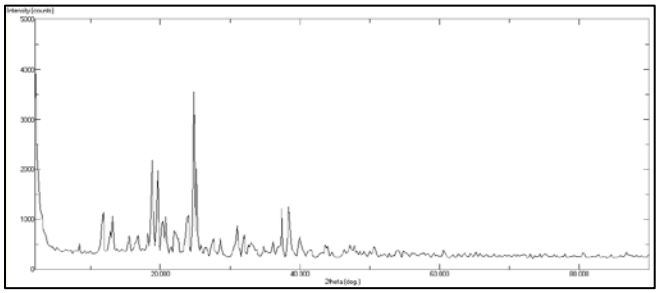

Figure 6: Spectrum of 10% Mixture of Ketamine and Confectionary Sugar.

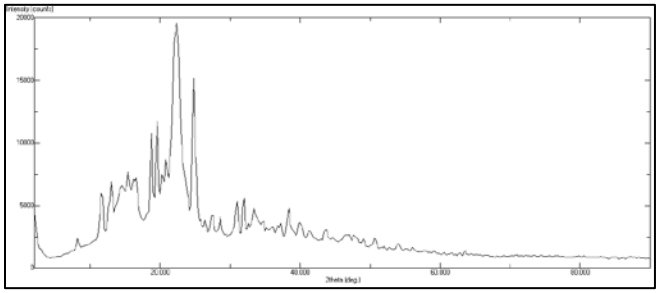

Figure: 7: Spectrum of Denim Fabric with 10% Ketamine and Confectionary Sugar Paste.

When the residue, seen in Figure 8, from the evaporation of the Ketamine and ethanol mixture was completed, consequently leaving a crystal structure on the zero background holder, the XRD was able to analyze the residue. Through visual comparison of the diffraction pattern as seen in Figure 9 with the pattern seen in Figure 4, Ketamine was concluded to be present in the sample.

Figure 8: Ketamine and Ethanol Mixture Residue.

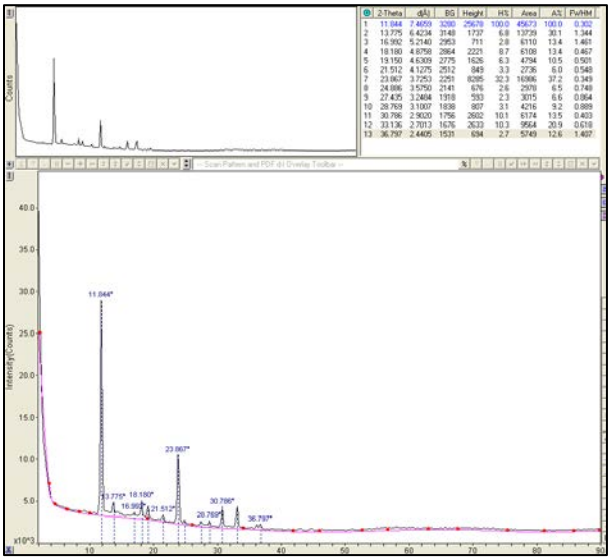

Figure 9: Spectra and Peak Table analysis of the Ethanol and Ketamine Mixture using the MDI Jade 9 Software.

The presence of Ketamine was further validated using the Jade software and comparing the peak positions of the sample to the Ketamine sample. These comparison results are shown in Figure 10.

Corresponding Peak Values					
Denim	Confectionary Sugar	Ketamine	10% Ketamine Mixture	Denim and Paste	Ethanol and Ketamine Mixture
2-Theta	2-Theta	2-Theta	2-Theta	2-Theta	2-Theta
16.003	8.331	9.189	8.356	8.227	11.844
17.786	11.597	11.945	11.712	11.619	13.775
23.624	12.955	13.814	13.079	12.999	16.992
30.015	15.403	16.827	15.499	14.599	18.180
31.573	16.445	18.373	16.686	15.405	19.150
35.217	18.779	19.270	18.832	16.554	21.512
42.641	20.695	21.590	19.600	18.801	23.867
45.815	22.026	23.976	20.661	19.587	24.866
	23.441	25.794	23.890	22.377	27.435
	24.792	27.668	24.904	24.821	28.769
	27.386	30.873	26.517	27.463	30.786
	28.556	33.203	27.548	28.598	33.136
	30.934	35.555	28.616	31.961	36.797
	31.849	37.000	30.972	33.437	
	33.367	39.788	31.985	37.066	
	34.798	41.373	33.000	38.378	
	35.974	42.981	34.899	40.027	
	37.116	45.028	36.155	43.663	
	38.300	46.621	37.350	44.614	
	40.006	49.970	38.440	46.631	
	41.295	46.621	40.037	50.658	
	43.632	49.970	41.497	54.010	
	47.616	51.225	43.676	63.607	
	50.563	52.227	46.420		
	51.906	53.824	47.217		
	53.975	56.024	50.622		
	56.037	60.594	54.025		

Figure 10: Chart of Peak Values from the Spectra shown in Figures 3-8.

Discussion

The use of XRD for the purposes of this project was validated by the results explained best in Figure 10. The chart clearly indicates that Ketamine peaks were detected amongst all the other components within each individual mixture sample. Figure 7 demonstrates how complex XRD spectra can be. Visual comparison of Figure 7 with the spectra from Figures 3-5 was not sufficient in order to find the peaks that represent the presence of Ketamine. Utilizing the MDI Jade 9 software, peak tables validated the outcome that Ketamine was detected in the mixtures using XRD.

The ethanol and Ketamine mixture was prepared so that it simulated a beverage that had been “spiked” with the date rape drug. A common dosage of Ketamine, in a standard 12 fl. Oz., beverage is about 200 mg. This ratio was then exercised in 5 mL ethanol by adding the calculated amount of Ketamine. After evaporation, the Ketamine was able to recrystallize in the residue left behind. The Ketamine was then successfully detected using XRD and the Jade Software, as seen in Figure 9, which demonstrates how the software was utilized to further analyze all the samples. The peak positions, indicated above the peaks, were found by setting a background, which is the pink line. The software used threshold, intensity cutoff, and range to accurately pick and assign peak values.

Even though these successful results were concluded, it is also important to comment on the methods tried that lacked results. It was discovered that GHB would not work for the purposes of this project due to its hygroscopic property. An initial spectrum of the pure drug was unable to be obtained because the drug did not maintain its crystalline structure when exposed to the atmosphere and therefore was not able to be detected using XRD. Small amounts (traces – 0.01 g) of the drugs were consumed by testing when made into the various mixtures. Due to limited supply of Rohypnol and MDMA only initial spectra of the pure drugs were able to be obtained. No further testing was completed with those two drugs. Chloral Hydrate yielded results for the dry mixture with confectionary sugar, but when made into a paste the drug would not recrystallize. This indicated a further problem with its detection and nothing further was tested.

The spectra in Figures 3-7 and 9, as well as the table in Figure 10, are only a summation of the work completed for this project. Additional spectra and similar conclusions for other samples were also obtained. Ketamine was detected on the three other fabric samples containing the paste through analysis with XRD and the application of peak picking and peak table comparison with the MDI Jade 9 software.

Conclusion

The technique of X-ray Diffraction was successfully employed to detect date rape drugs in pure form for this project. XRD and the MDI Jade 9 software was also utilized to detect Ketamine in various mixtures. Supplementary knowledge about date rape drugs was gained when research problems were encountered. This project was able to demonstrate and give validity to the use of XRD for the purposes of detecting date rape drugs and residues.

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Biography

Emily Walsh, originally from Bolton, Connecticut, is a senior at the University of New Haven. She is double majoring in Forensic Science and Chemistry. She will be completing her Honors thesis project with Dr. Maxwell entitled: The Detection of Malicious Contaminants in Flour-based Food Products Using Fourier Transform Infrared Spectroscopy. Outside of her research studies, Emily is a general chemistry, organic chemistry, quantitative analysis, and instrumental analysis teaching assistant through the Chemistry department at UNH. Her future goal for after graduation is to attend graduate school to study Food Science.