

## **AN EVALUATION OF ILLICIT STIMULANTS AND METABOLITES IN WASTEWATER EFFLUENT AND THE WISCONSIN RIVER ALONG THE CENTRAL WISCONSIN RIVER BASIN**

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**Abstract.** The goals of the study were to develop a method for extracting and quantifying illicit stimulants and metabolites, methamphetamine, amphetamine, cocaine, and benzoylecgonine from wastewater effluent and surface water grab samples, and evaluate Central Wisconsin wastewater treatment plant's (WWTP) removal efficiency of compounds of interest. The method created used HLB solid-phase extraction (SPE) cartridges to extract substances of interest and High Performance Liquid Chromatography tandem Mass Spectrometry (HPLC/MS/MS) for quantification and qualification. All four wastewater effluent samples and three Wisconsin River samples had quantifiable concentrations of at least one analyte. Conclusions derived from the study were: The method created is effective for separating, quantifying, and identifying amphetamine, cocaine, and benzoylecgonine from wastewater effluent and surface water grab samples, and each illicit stimulant and metabolite analyzed in this study were all quantified in wastewater effluent, indicating these compounds have the ability to survive WWTP.

**Key words:** narcotics, wastewater, wastewater treatment, WWTP, liquid chromatography, HPLC, mass spectrometry (MS), solid-phase extraction, SPE, cocaine, methamphetamine, amphetamine, benzoylecgonine, environmental chemistry, Wisconsin River

### **INTRODUCTION**

Methamphetamine, amphetamine, and cocaine are schedule II illicit substances under the rules and regulations of the United States Drug Enforcement Administration and present various health implications to humans [United States... 2011]. Amphetamine-type stimulants (ATS) are the second most frequently used drugs, with an estimated 53 million users of ATS worldwide in 2010 [Hughes et al. 2013]. Amphetamine and benzoyleco-

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gnine are the main urinary metabolites of methamphetamine and cocaine, respectively [Zuccato et al. 2006]. Although the negative physiological effects of methamphetamine, amphetamine, and cocaine are well known, there is little research in the literature about the health effects of benzoylecognine on either humans or other organisms. One study found an association between fatalities and cocaine/cocaine metabolites as result of using cocaine in conjunction with fentanyl, but no well-established evidence of direct health effects of benzoylecognine alone was found during literary review [Hull et al. 2007]. Subsequent to the use of either methamphetamine or cocaine, both the parent drug and metabolites are excreted in urine. Substances that have unknown impacts on biota and environmental settings are considered pollutants of emerging concern [Boles and Wells 2010]. These pollutants include narcotics and their metabolites, as well as pharmaceuticals being discharged in WWTP effluent, thus forming complex mixtures in surface waters [Phillips et al. 2010].

Several studies have been performed on recreational substances in wastewater and in environmental systems with different intended study objectives. A majority of these studies have emphasized epidemiology and have evaluated quantities of illicit substances in wastewater in respect to many different variables: day of the week sampled, WWTP processes, wastewater flow variability, and the socio-economic status of the city served by the treatment plant [Boles and Wells 2010]. One objective of the present study is to develop a method for detecting and quantifying illicit stimulants and metabolites of interest in treated wastewater effluents and river water samples. The other objective is to evaluate whether the substances of interest can survive WWTP processes common to Wisconsin. The hypotheses are established as:

$H_0$ : No quantifiable concentrations of substances of interest can survive the WWTP process and therefore, are not measurable in either treated wastewater effluent or in the Wisconsin River.

$H_a$ : There is at least one sample site that contains a quantifiable concentration of at least one substance of interest in treated wastewater effluent, or in the Wisconsin River, implying that these substances can survive WWTP.

## METHODS

Sample collection was conducted on 8 November 2013 and 9 November 2013, a weekend, as illicit stimulants and metabolites of this study are typically found in wastewater at higher concentrations on weekends [Boles and Wells 2010, Reid et al. 2011]. Samples were taken from twelve sites in four cities, three sample sites for each city. The four cities that samples were taken from were (all in Wisconsin, USA):

1. Merrill, Lincoln County.
2. Wausau, Marathon County.
3. Stevens Point, Portage County.
4. Wisconsin Rapids, Wood County.

The three sampling sites in each city were chosen because of their proximity to local WWTP (Fig. 1). A sample upstream of the wastewater effluent discharge, a treated wastewater effluent sample, and a sample downstream of the wastewater effluent discharge

were taken in each city. Grab samples were taken at each site with one liter amber glass vials. The samples were stored in these amber vials in a refrigerator at approximately 4°C in the dark. The samples were split further into H<sub>2</sub>SO<sub>4</sub> preserved and unpreserved samples. The analytical results of the preserved and unpreserved samples were compared to evaluate extraction efficiency.

Solid-phase extraction (SPE) was used to isolate and concentrate intended analytes. The SPE method used was influenced by and followed the same procedure as an SPE method used in a Spanish study evaluating pharmaceuticals and hormones in wastewater [Pedrouzo 2011]. Solid-phase extraction cartridges used were 500 mg Waters, Inc. HLB cartridges. HLB cartridges were initially conditioned with 10 mL methanol and 10 mL Optima water. Subsequently, 100 mL of sample was loaded onto the HLB cartridge at a rate of 10 mL per minute. After loading, the cartridge was washed with 5% methanol and dried for 10 minutes with nitrogen gas. Finally, 5 mL of 100% methanol was used to elute the cartridge to extract substances of interest. Extracts were concentrated down below 1 mL using nitrogen gas, then diluted back to 1.0 mL using a mixture of acidified water, 100% methanol, and internal standard to achieve a matrix of 90% acidified water and 10% methanol. Deuterated internal standards of each compound of interest, purchased from Grace Analytical, were used for all analytes except for cocaine. For cocaine analysis, deuterated internal standard for benzoylecgonine was used.

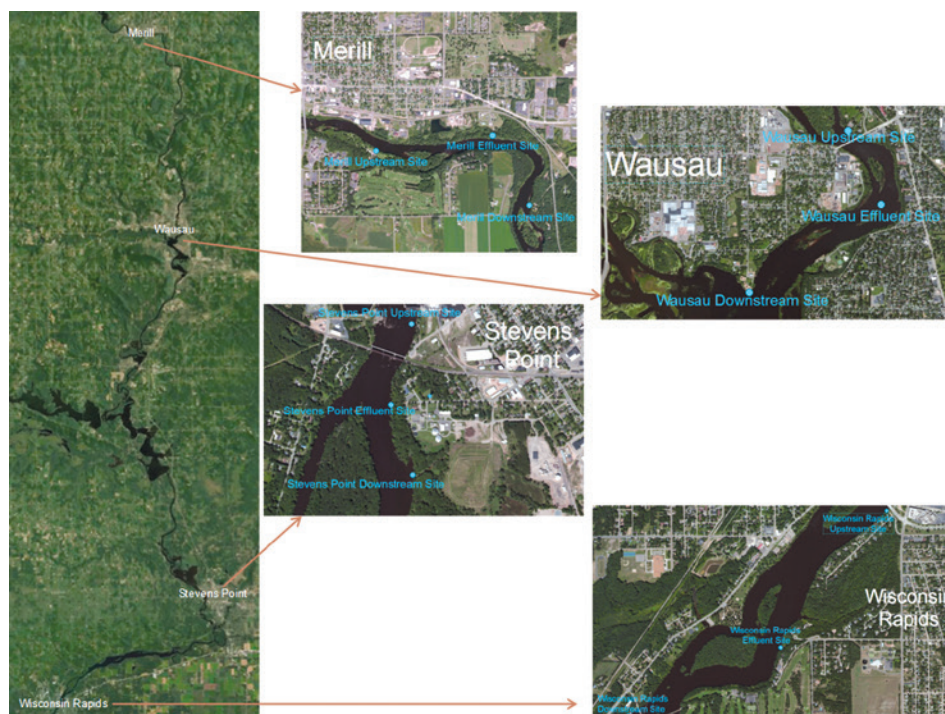


Fig. 1. Sample Locations

An Agilent 6430 high-performance liquid chromatograph triple-quadrupole mass spectrometer was used to separate, identify, and quantify the illicit stimulants and metabolites. The mass spectrometer was optimized prior to analysis to achieve the maximum quantities of precursor and product ions for each analyte. The mass spectrometer operating conditions that were constant for all analytes were an electrospray ionization source (ESI), gas temperature at 350°C, gas flow at a rate of 10 L/minute, nebulizer set to 45 psi, and positive polarity usage (see Table 1 for analyte-specific specifications). A six-point calibration curve was established for each analyte. The calibration standard solution concentrations were of 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 µg/L. A minimum R<sup>2</sup> value of 0.990 was used. If necessary, one calibration standard was eliminated to achieve an R<sup>2</sup> value greater than or equal to 0.990. A signal-to-noise ratio of 3 or greater was used as the Minimum Detection Limit (MDL) for this study. Using an MDL equivalent to a signal-to-noise ratio of 3 is a common, easy method for establishing an MDL [Ahrer 2001].

Table 1. Analyte specific parameters of the mass spectrometer for the illicit stimulant method.

Substance	Precursor ion (unit/Enh)	Product ion (unit/Enh)	Dwell	Frag (V)	CE (V)	Retention Time (Min)
Cocaine	304.1	182.2	25	105	20	5.23
Benzoyllecognine	290.1	168.1	25	100	26	5.45
Benzoyllecognine IS	293.1	171.1	25	115	26	5.45
Methamphetamine	150.1	119.1	20	80	13	3.67
Methamphetamine IS	159.1	125.2	25	80	10	3.67
Amphetamine	136.1	119.2	20	66	9	3.5
Amphetamine IS	142.1	125.2	20	66	9	3.5

## RESULTS

The concentrations obtained from the analysis of the preserved samples were chosen to be reported, because all of these concentrations were greater than those of their unpreserved counterpart samples (other than amphetamine in the Wausau effluent samples). The literature also supports preserving samples for extraction to achieve better recoveries [Boles and Wells 2010]. Reported concentrations in Table 2 are the final concentrations after back-calculating for the original concentration in the initial volume collected. All wastewater effluents analyzed had quantifiable concentrations of at least one analyte of interest. Three Wisconsin River sample sites, Wausau downstream, Stevens Point upstream, and Stevens Point downstream had quantifiable concentrations of analytes (table 2). Stevens Point upstream and downstream had quantifiable concentrations of cocaine and benzoyllecognine, whereas Wausau downstream had a quantifiable concentration of only benzoyllecognine. The Wausau effluent sample had a concentration of amphetamine above the MDL according to the integrated values of the chromatogram observed, but in reality should not have been quantified (discussion and Fig. 2). Reported methamphetamine results are only conservative estimates because co-eluting ions were observed (see discussion and Fig. 3–5)

Table 2. Concentrations of each narcotic/metabolite of interest at each sample site. Sites with analyte concentrations less than the MDL and that had no distinguishable peaks in chromatograms were omitted.

Sample Site	Amphetamine Sample (ng/L)	Methamphetamine Sample (ng/L)	Cocaine Sample (ng/L)	Benzococaine Sample (ng/L)
Merril Effluent	< 1.0	**23.1	4.9	7.7
Wausau Effluent	*2	**46.0	12.5	88.7
Wausau Downstream	< 1.0	< 5.0	< 5.0	4.7
Stevens Point Upstream	< 1.0	< 5.0	3.9	< 5.0
Stevens Point Effluent	12.8	3.9	18.9	74.4
Stevens Point Downstream	< 1.0	< 5.0	5.1	10.3
Wisconsin Rapids Effluent	< 1.0	**7.7	17.5	56.9

\*\* Multiple, non-Gaussian peaks cast doubt on quantifiable presence.

\*\* Conservative estimate of concentration based on integrating only peak that matched retention time with valid qualifier ion ratios.

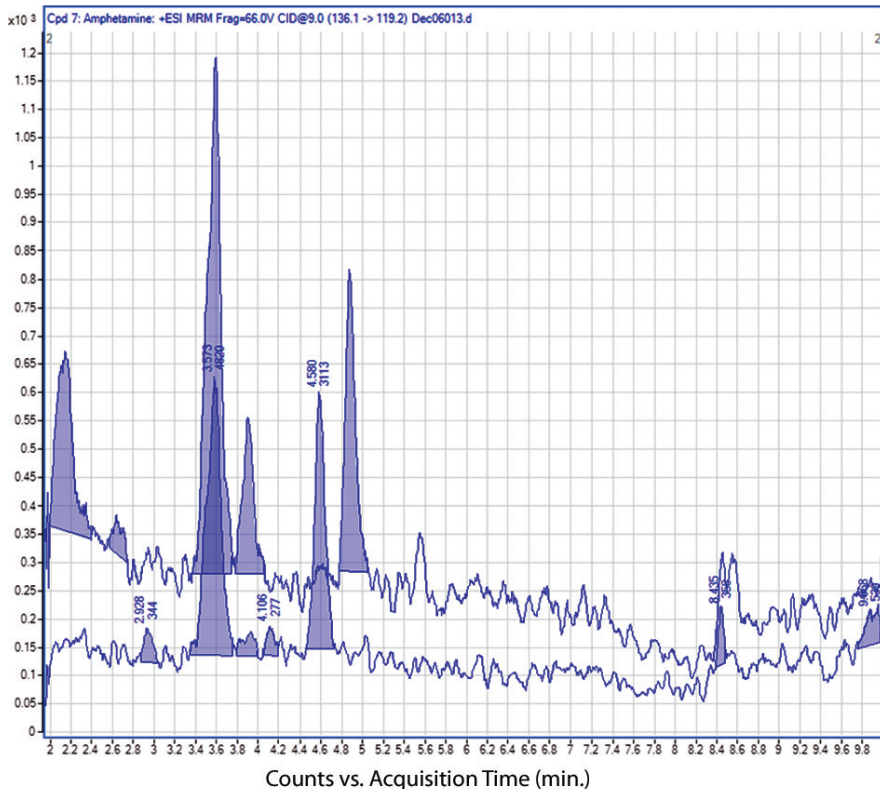


Fig. 2. Chromatogram of amphetamine for the Wausau effluent. The integrated area that was calculated encompassed all the highlighted peaks



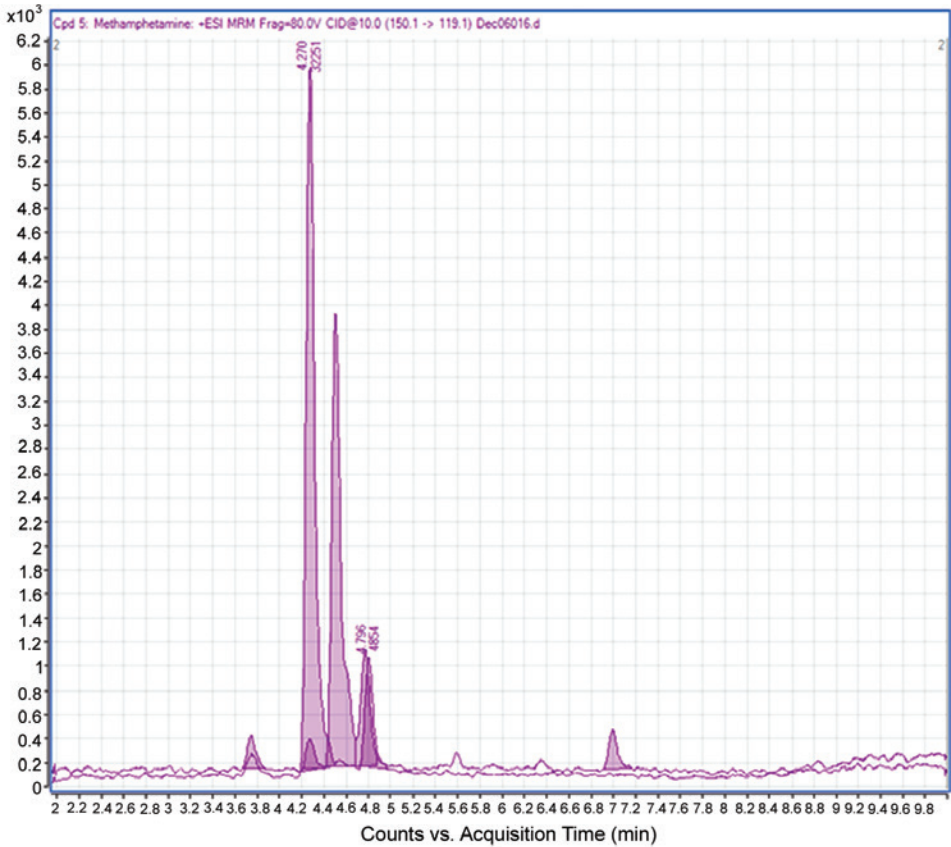


Fig. 3. Methamphetamine chromatogram for the Stevens Point effluent

## DISCUSSION

As a result of the quantifiable concentrations of narcotics and metabolites in more than one sample site, the null hypothesis has been rejected in favor of the alternative. Grab samples were taken on a weekend in November, creating bias. The bias from weekend sampling was intended to increase the probability of finding illicit stimulants and metabolites of interest. Therefore, any conclusions relating to substance survivability in wastewater treatment should not be extrapolated to other times of the week or other seasons. Any attempt to calculate mass loading based on these calculations would create biased loading estimates in relation to the particular level of use of substances and wastewater flow at the exact time of sample collection. For these reasons, a mass loading could not be calculated for this study. Future investigations of these stimulants in wastewater and surface water should include: multiple polar organic compound integrative samplers (POCIS, passive sampling devices) in wastewater effluents and receiving water bodies to confirm mass loading and to evaluate wastewater treatment efficiency in removing these substances based on the season, time of the week, level of use, and effluent flow.

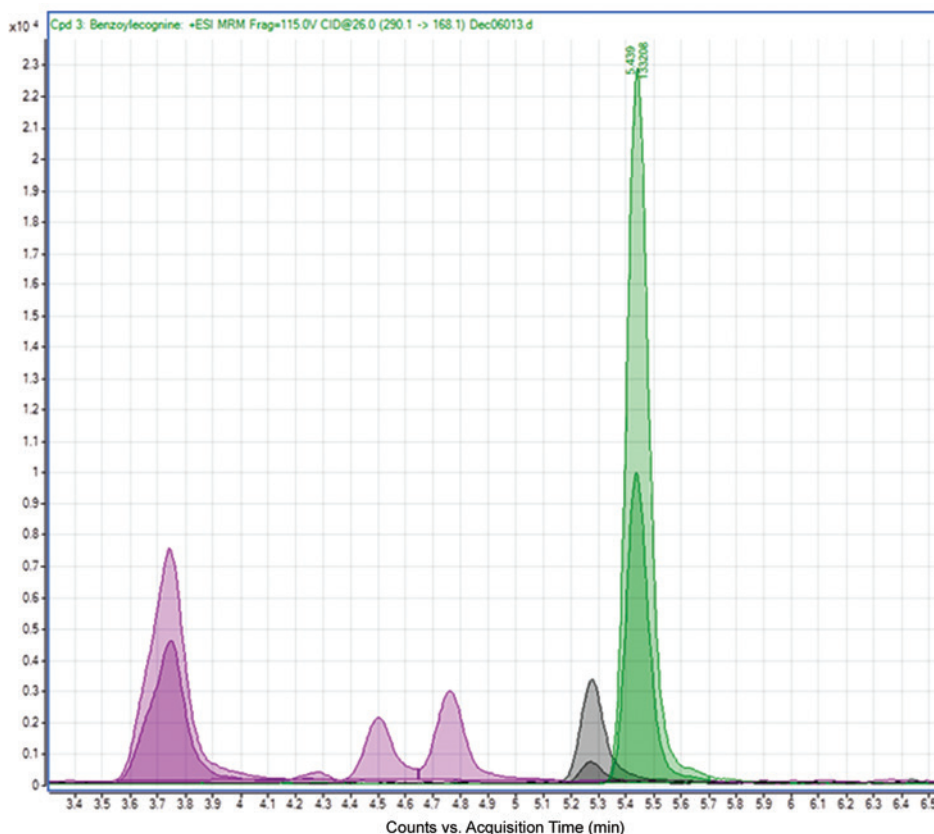


Fig. 4. Methamphetamine, cocaine, and benzoylceognine chromatograms for the Wausau effluent. Co-eluting ions were observed for methamphetamine

Yet another reason for not calculating mass loads of narcotics and metabolites was that some analytes had questionable quantities, as indicated by their chromatograms. Some quantifiable concentrations of narcotics and metabolites had implications or manual integrations for more accurate estimations had to be performed. This occurred solely with the analytes amphetamine and methamphetamine, where noise was integrated or co-eluting ions were observed. In the Wausau effluent, a supposed final concentration of 2.0 ng/L was measured via Agilent integrative software; however, the chromatogram for this sample depicts multiple, non-Gaussian peaks with no qualifier ions matching in acquisition time. Therefore, this concentration of amphetamine is presumed to be invalid for reporting (Fig. 2). When methamphetamine was quantifiable, co-eluting ions were observed (Fig. 3–5). Co-eluting ions observed had the same mass as methamphetamine, but had different acquisition times, indicative of possible other compounds fragmenting into the same ion masses. As recommended by the U.S Environmental Protection Agency, methods for quantifying substances should avoid co-eluting ions in chromatograms, as co-eluting ions may cause ramifications regarding identification or quantification. Co-eluting ions are particularly a concern when overlapping of peaks are observed [United States... 2003].

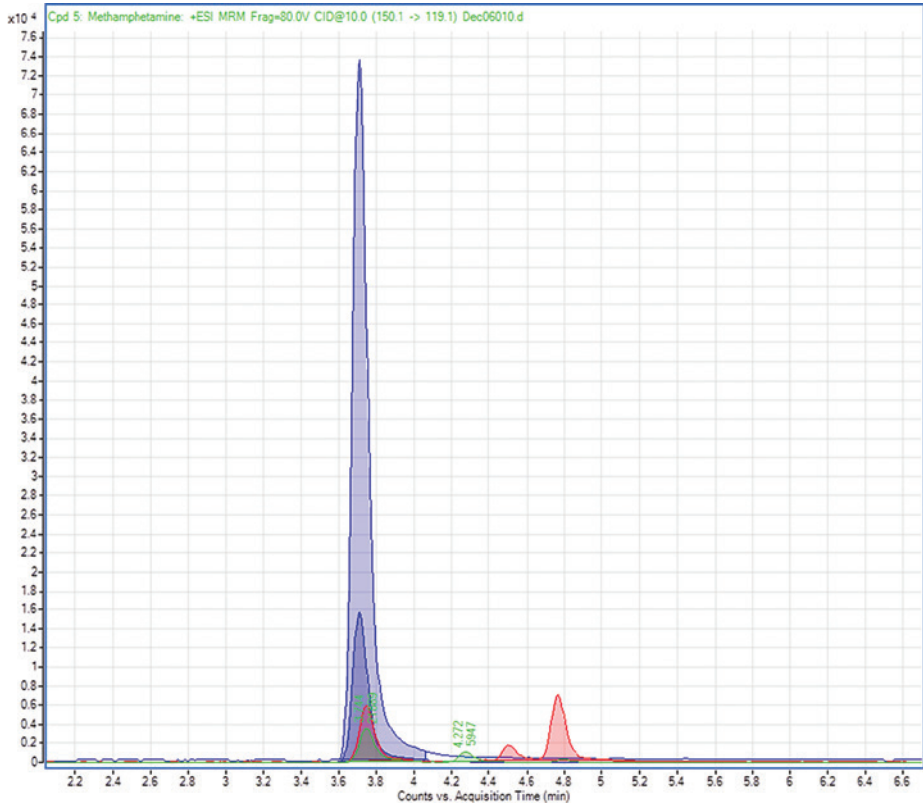


Fig. 5. Chromatogram of methamphetamine and methamphetamine internal standard

Further use of this method to quantify methamphetamine should include a clean-up procedure on samples to eliminate co-eluting ions with the same mass as the methamphetamine ion. As a result of co-eluting peaks observed, this study made conservative estimations of methamphetamine concentrations by only integrating peaks that matched the known acquisition time, had correct masses for product and precursor ions, and correct qualifier ion ratios (Table 2). Despite observed interferences, the presence of methamphetamine is not questionable, as methamphetamine chromatograms were compared with methamphetamine internal standard chromatograms. As a result of this comparison, matching acquisition times and similar qualifier ion ratios to deuterated internal standard were observed (Fig. 5). Despite limitations to the quantification of methamphetamine, identifying the presence of methamphetamine and conservative estimations of methamphetamine above the MDL still shows evidence of methamphetamine surviving wastewater treatment.

There are several suggestions for future research and use of this method. Spiked samples and duplicates in preserved samples should be analyzed in future studies. Also, the method presented in this study was effective for identifying and quantifying cocaine, benzoylecgonine, and amphetamine. For future work, the method should be modified to include a clean-up procedure when attempting to identify and quantify methamphetamine. Both



the method of wastewater treatment and the efficiency of the WWTP in eliminating these substances influence the concentration of illicit stimulants and metabolites being discharged in the effluent from treatment facilities [Boles and Wells 2010]. Therefore, further studies should be performed in evaluating WWTP efficiency by sampling untreated wastewater influent and comparing concentrations of stimulants and metabolites to treated effluent samples. Also, POCIS sampling should be conducted to accurately establish the mass loading of illicit stimulants and metabolites from treated effluent into bodies of surface water over time. By establishing the mass load of a treatment plant, one can further evaluate and comprehensively investigate the impact of treated wastewater effluents on receiving surface water bodies. Also, future studies may want to integrate sample matrix adjustment techniques into this method to aid in amplifying analyte peak areas [Ahrer et al. 2001].

## **CONCLUSIONS**

As a result of the findings of this study, it has been concluded that the narcotics and metabolites observed, as pollutants of emerging concern, are able to survive the wastewater treatment process typical of central Wisconsin and are able to be quantified in treated wastewater effluents and river samples. However, there are a multitude of variables that encompass this problem and further, similar investigations in other locations should be conducted to help in understanding these variables. Conclusions regarding the method established are: the method is effective for extracting, identifying, and quantifying amphetamine, cocaine, and benzoylecognine, but not methamphetamine, in treated wastewater and surface water. Co-eluting ions should be eliminated for this method to be effective when analyzing for methamphetamine in these matrices. The conclusion regarding WWTPs' ability to eliminate these substances was that these substances typically survive standard WWTP processes in central Wisconsin and that WWTP processes need to be modified in order to decrease concentrations of these substances below measurable concentrations.

## **ACKNOWLEDGMENTS**

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## **REFERENCES**

- Ahrer, W., Scherwenk, E., Buchberger, W. (2001). Determination of Drug Residues in Water by the Combination of Liquid Chromatography or Capillary Electrophoresis with Electrospray Mass Spectrometry. *J. Chromatogr. A.*, 910, 69–78.
- Boles, T.H., Wells, M.J.M. (2010). Analysis of Amphetamine and Methamphetamine as Emerging Pollutants in Wastewater and Wastewater-impacted Streams. *J. Chromatogr. A.*, 1217(16), 2561–568.

- Hughes, J., Ayoko, G., Collett, S., Golding, G. (2013). Rapid Quantification of Methamphetamine: Using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Chemometrics. *PLoS ONE*, 8(7), e69609.
- Hull, M.J., Juhascik, M., Mazur, F., Flomenbaum, M.A., Behonick, G.S. (2007). Fatalities Associated with Fentanyl and Co-administered Cocaine or Opiates. *J. Forensic Sci.*, 2, 1383–1388.
- Pedrouzo, M., Borrull, F., Pocurull, E., Marcé, R.M. (2011). Presence of Pharmaceuticals and Hormones in Waters from Sewage Treatment Plants. *Water Air Soil Pollut.*, 217(14), 267–281.
- Phillips, P.J., Smith, S.G., Dana W. Kolpin, D.W., Zaugg, S.D., Buxton, H.T., Furlong, E.T. (2010). Method Description, Quality Assurance, Environmental Data, and other Information for Analysis of Pharmaceuticals in Wastewater-Treatment-Plant Effluents, Streamwater, and Reservoirs, 2004–2009. U.S. Geological Survey Open-File Report 1102.
- Reid, M., Harman, Ch., Grung, M., Thomas, K.V. (2011). The Current Status of Community Drug Testing Via the Analysis of Drugs and Drug Metabolites in Sewage. *Norsk Epidemiologi*, 21 15–23. Print.
- United States Department of Justice (2011). Drug Enforcement Administration (DEA). Drugs of Abuse. By M.M. Leonhart. Web. 29 Oct. 2013, [http://www.justice.gov/dea/pr/multimedia-library/publications/drug\\_of\\_abuse.pdf](http://www.justice.gov/dea/pr/multimedia-library/publications/drug_of_abuse.pdf).
- United States Environmental Protection Agency (EPA) (2003). Method 8000c. Determinative Chromatographic Separations (General Users). Web. 22 Dec. 2014, [http://www.epa.gov/solidwaste/hazard/testmethods/pdfs/8000c\\_v3.pdf](http://www.epa.gov/solidwaste/hazard/testmethods/pdfs/8000c_v3.pdf).
- Zuccato, E., Chiabrando, Ch., Castiglioni, S., Bagnati, R., Fanelli, R. (2006). Estimating Community Drug Abuse by Wastewater Analysis. *J. Environm. Sci. Technol.*, 40, 357–363.

## OCENA NIELEGALNIE STOSOWANYCH METABOLITÓW W ŚCIEKACH DOPROWADZANYCH DO OCZYSZCZALNI ŚCIEKÓW RZEKI WISCONSIN USYTUOWANEJ W PUNKCIE CENTRALNYM ZLEWNI TEGO CIEKU

Celem artykułu jest przedstawienie opracowanej metody ekstrakcji i oznaczania ilościowego nielegalnych używek i metabolitów, metamfetaminy, amfetaminy, kokainy i benzoylcegoniny w oczyszczalniach ścieków i wody oraz oceny skuteczności usuwania tychże w oczyszczalni ścieków w Central Wisconsin. Przedstawiona metoda wykorzystuje ekstrakcję do fazy stałej (SPE) HLB wyodrębniając substancje docelowe przez wysokosprawną chromatografię cieczową przy zastosowaniu spektrometrii masowej (HPLC / MS / MS). Wszystkie próbki ścieków pochodzą z rzeki Wisconsin. Wniosek główny pochodzące z przeprowadzonych badań to ten, że metoda przedstawiona pozwala na skuteczne oddzielenie, kwantyfikację i identyfikację amfetaminę, kokainę i benzoylcegonine w ściekach i wodach powierzchniowych.

**Słowa kluczowe:** narkotyki, oczyszczanie ścieków, oczyszczalnia ścieków, chromatografia cieczowa, HPLC (wysokosprawną chromatografię cieczową), spektrometria masowa (MS), ekstrakcja do fazy stałej (SPE), kokaina, metamfetamina, amfetamina, benzoylcegonina, chemia środowiska, rzeka Wisconsin

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