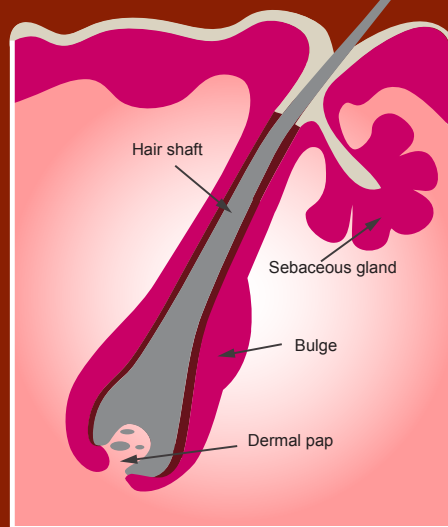




Hair Follicle



EFFECTS OF HAIR BLEACHERS IN THE ANALYSIS OF AMPHETAMINE(S) AND BATHSALT DRUGS

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INTRODUCTION

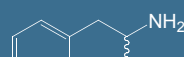
In the area of hair analysis, one of the potential issues facing analysts is treatment of hair samples with bleaching agents. These compounds act to change the color and morphology of the hair samples while growing *in situ*. They may also have a deleterious effect on changing (reducing) the actual concentrations of drugs such as amphetamines and synthetic cathinones in hair samples. This change may impact greatly upon the interpretation of the toxicology offered by a forensic toxicologist.

This group of agents may cover a diverse range of substances such as bleach (aqueous sodium hypochlorite), peroxide (aqueous hydrogen peroxide) or highly basic solutions such as ammonium hydroxide solutions. In this study the effect of treating fortified hair samples with these bleaching agents, then extracting them via a modified hydrolysis procedure was evaluated in a methodology employing both mixed mode SPE, and LC-MS/MS techniques. The data presented demonstrates the effect of simple aqueous "bleaching" agents may have upon treated samples.

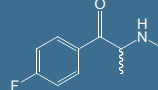
MATERIALS AND CHEMICALS

Amphetamine, methamphetamine, MDA, and MDMA (and d5 analogues), Butylone, Ethylone, Flephedrone, Mephedrone, Methylone, Methedrone, Mephedrone-d3, Methcathinone, Methyleneoxypropylvalerone (MDPV) and Pyralerone were supplied as liquid standards in methanol (1 mg/ mL) from Lipomed (Cambridge, MA). Acetonitrile, acetic acid (glacial), ammonium hydroxide, isopropanol, methylene chloride, methanol, phosphate buffer (pH 6), and sodium hydroxide were obtained from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained from Acros Chemicals (via Fisher Scientific). All chemicals were of ACS quality. Solid phase extraction columns (CSDAU206 (6mL, 200 mg)) were obtained from UCT Inc., (Bristol, PA).

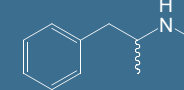
Amphetamine:



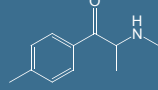
Flephedrone:



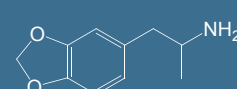
Methamphetamine:



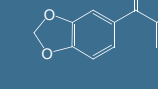
Mephedrone:



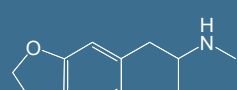
Methyleneoxyamphetamine (MDA):



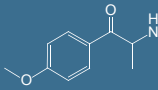
Methylone:



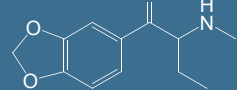
Methylenedioxyethamphetamine (MDMA):



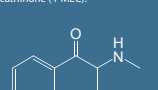
Methedrone:



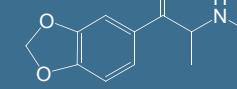
Butylone:



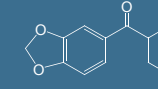
Methcathinone (4-MEC):



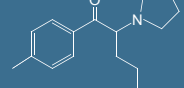
Ethylone:



Methyleneoxypropylvalerone (MDPV):



Pyralerone:



ANALYTICAL METHOD:

Samples of decontaminated hair (10 mg) containing amphetamines and bathsalt type drugs (amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxyethamphetamine (MDMA), butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypropylvalerone (MDPV) and pyralerone) were treated with 10% aqueous sodium hypochlorite solution (Bleach), 3% aqueous hydrogen peroxide solution, or 3% aqueous ammonium hydroxide solution for 2 hours before being removed, washed and dried. The samples were then digested in 0.1 M NaOH (containing deuterated analogues) for 0.5 hour at room temperature. Each solution was adjusted to pH 6 with 0.1 M phosphate buffer (4 mL) and applied to a conditioned SPE column. The samples were extracted on CLEAN SCREEN® DAU206 SPE columns. After loading the sample, the sorbent was washed with deionized water, acetic acid (0.1 M), and methanol (3 mL of each, except 1 mL for acetic acid). Each SPE column was dried and eluted with 3 mL of a solvent consisting of methylene chloride/isopropanol/ ammonium hydroxide (78:20:2). After elution, 200 µL of mobile phase was added to the collection tube. The samples were then evaporated to the mobile phase for analysis by LC-MS/MS in positive multiple reaction monitoring (MRM) mode.

INSTRUMENTAL CONDITIONS:

Liquid chromatography was performed in gradient mode employing a 50 x 2.0 mm C₁₈ analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% for re-injection. The total run time for each analysis was less than 5 minutes.

Tandem mass spectrometry was performed in using positive multiple reaction (MRM) mode. The following transitions were monitored (quantification ions underlined): Amphetamine m/z: 136.1 to 91.0, 65.0, Amphetamine-d₅: m/z 141.1 to 124.0, 93.0, Methamphetamine: m/z 150.1 to 91.1, 119.1 Methamphetamine-d₅: m/z 155.2 to 92.1, 121.2, MDA: m/z 180.2 to 163.1, 105.1, MDA-d₅: m/z 195.2 to 168.1, 110.1, MDMA: m/z 194.2 to 163.1, 105.1, MDMA-d₅: m/z 199.2 to 165.1, 106.8, Butylone: m/z 222.1 to 174.2, 204.2, Ethylone: m/z 222.1 to 174.2, 204.2, Flephedrone: m/z 182.1 to 164.2, 149.1, Mephedrone: m/z 178.1 to 145.1, 160.1, Methylone: m/z 208.1 to 160.1, 132.1, Methedrone: m/z 194.1 to 176.2, 161.1, Methylethylcathinone (4-MEC): m/z 192.1 to 174.2, 144.1, Methyleneoxypropylvalerone (MDPV): m/z 276.2 to 135.1, 126.1 and Pyralerone: m/z 246.2 to 105.1, 175.2, respectively.

RESULTS:

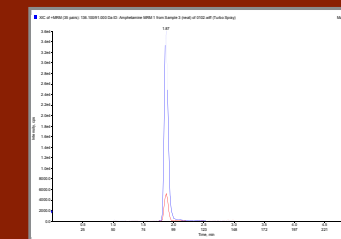
The limits of detection/ quantification for the SPE method were determined to be 0.05 ng/ mg and 0.1 ng/ mg, respectively for the amphetamines (amphetamine, methamphetamine, MDA, and MDMA) and synthetic cathinones (butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypropylvalerone (MDPV) and pyralerone). The method was found to be linear from 0.1 ng/ mg to 10 ng/ mg (r²>0.999). Recoveries of amphetamines/bathsalts were found to be greater than 90%. Interday and Intraday analysis of amphetamines/bathsalts were found to < 7% and < 10%, respectively.

Results of hair treated by various bleaching agents

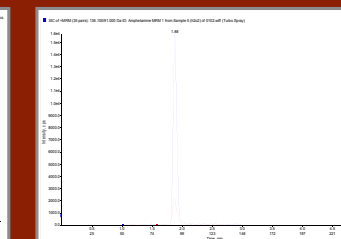
Compound	Control Hair (Area)	Bleach Area	% Loss	Peroxide Area	% Loss	Amm-OH Area	% Loss
Amphetamine	3E+05	2.2E+04	92.7	1.6E+05	46.7	3.5E+04	88.3
Methamphetamine	1E+05	1.4E+04	86.0	4.8E+04	52.0	1.05E+04	89.5
MDA	2.8E+05	3E+03	98.9	8.9E+04	68.2	3.3E+04	88.2
MDMA	5.3E+05	4.2E+03	99.2	1.2E+04	97.7	3.9E+03	99.3
Butylone	2.4E+05	0	100.0	3.3E+03	98.6	0	100.0
Ethylone	3.5E+05	0	100.0	3.0E+03	99.1	0	100.0
Flephedrone	2.4E+05	1.3E+03	99.5	3.0E+03	98.8	1.8E+04	92.5
Mephedrone	2.8E+05	1.1E+03	99.6	5.0E+03	98.2	0	100.0
Methylone	5.5E+05	1.0E+03	99.8	7.7E+03	98.6	8.4E+02	99.8
Methedrone	2.6E+05	8E+02	99.7	3.8E+03	98.5	4.0E+02	99.8
MDPV	9.5E+03	0	100.0	0	100.0	0	100.0
Pyralerone	5.7E+03	0	100.0	0	100.0	0	100.0
Methcathinone	3.0E+04	0	100.0	0	100.0	0	100.0

CHROMATOGRAMS OF HAIR TREATMENT

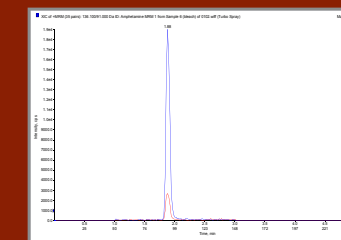
1. Untreated hair containing Amphetamine



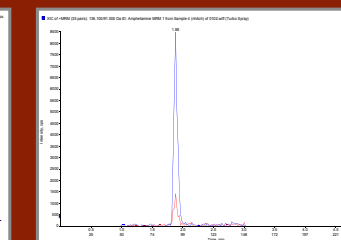
2. Hair containing Amphetamine treated with Bleach (Sodium Hypochlorite Solution (aq))



3. Hair containing Amphetamine treated with Hydrogen Peroxide solution (aq)



4. Hair containing Amphetamine treated with Ammonium Hydroxide solution (aq)



CONCLUSION

The use of the information given in this new procedure for the analysis of amphetamine and synthetic cathinones will be of great use to analysts in the field of forensic hair analysis as it demonstrates the use of SPE/LC-MS/MS to provide valuable data from about the effects of bleaching agents in hair analysis.

QR Code



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