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Effect of the shampoo Ultra Clean on drug concentrations in human hair

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Abstract The influence of the special shampoo Ultra Clean (Zydot Unlimited, Tulsa, Oklahoma) on the results of hair analyses was investigated. Hair samples from persons (n = 14) with a known history of drug abuse were collected at autopsy. The hair samples were divided into separate strands which were analyzed both after washing with Ultra Clean and without treatment. Hair analyses were performed by methanol extraction under sonication, purification by solid phase extraction and GC/MS in SIM mode according to routine procedures for tetrahydrocannabinol (THC), cocaine, amphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDE), heroin, 6-monoacetylmorphine (6-MAM), morphine, codeine, dihydrocodeine and methadone. All drugs originally present in the hair fibers were still detected after a single application of Ultra Clean. However, a slight decrease in drug concentrations could mostly be observed e.g. cocaine (n = 10) –5%, 6-MAM (n = 12) –9%, morphine (n = 12) –26%, THC (n = 4) –36%. The findings clearly demonstrated that drug substances had not been sufficiently removed from human hair by a single Ultra Clean treatment to drop their concentrations below the limit of detection of the analytical method applied.

Key words Hair · Drug testing Hair analysis · Manipulations · Shampoo effect

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Introduction

Hair testing for drugs of abuse has been established as a routine method in drug monitoring [1–3]. Since a positive drug test is usually connected with considerable legal or economic consequences, e.g. the loss of the driving license, drug abusers try to attain negative test results. Besides complete shaving, common manipulations are bleaching or hair dyeing. Ultra Clean, a commercially available hair care product, is recommended to remove "medications, chemical build-up and other unwanted impurities from within the hair shaft". The manufacturer (Zydot Unlimited, Tulsa, Oklahoma) offers a "money back guarantee" if the product should fail. The aim of the present study was to investigate the effect of Ultra Clean on drug concentrations in human hair samples obtained from chronic drug abusers. The hair samples were analyzed after washing with Ultra Clean and without any treatment. Hair analysis was performed by a methanol/sonication extraction procedure [4, 5] followed by a further purification step using solid phase extraction.

Material and methods

Hair samples

Hair samples from persons (n = 14) with a known history of drug abuse were collected at autopsy. The hair samples were collected from the vertex posterior region. A strand (about 5 mm in diameter) was cut as close as possible to the scalp, fixed with string and enveloped. Root and tip of the hair strand were marked. The fibers were investigated for morphological parameters including hair color. The samples were stored under dry conditions at room temperature until analysis.

Sample treatment

Each of the 14 hair samples was divided by length into 4 strands, 2 of which were treated with Ultra Clean prior to analysis. The remaining two hair strands were subjected to analysis without any pretreatment. The cleansing product Ultra Clean, consisting of shampoo, purifier and conditioner (see Table 1), was applied according to the directions for use given by the manufacturer. The

Table 1 Ingredients of the cleansing product Ultra Clean

Components	Ingredients
Shampoo (tube 1)	Sodium laureth sulfate, aloe vera, cocamidopropyl betaine, cocamid DEA, sodium PCA, tetrasodium EDTA, panthenol, citric acid, sodium thiosulfate, methylparaben, DMDM hydantoin, sodium chloride, fragrance, coloring
Purifier (tube 2)	Aloe vera, propylene glycol, EDTA, potassium sorbate, methylparaben, carbomer-940, triethanolamine, coloring
Conditioner (tube 3)	Aloe vera, geranium maculatum, comfrey, grapefruit juice, hydrolized animal protein, N-octadecanol, cetyl trimethyl ammonium bromide, N-hexadecyl alcohol, methylparaben, propylparaben, fragrance, coloring)

hair was first wetted and half of the shampoo was applied for 3 min (step 1). Second the purifier was thoroughly distributed on the hair and left to work for 3 min. Subsequently, the remainder of the shampoo was applied again for 3 min (step 3) before the conditioner was used in the fourth step. After each step the hair was rinsed well with warm water. Shampoo, purifier and conditioner were applied and distributed on the hair strands with a small brush. The hair samples were finally air dried.

Hair extraction

The proximal 4 cm of the hair strands were used for analysis. The hair segment was placed in a polypropylene vial and washed for 5 min each with water (5 ml), acetone (5 ml) and hexane (5 ml). After drying, the hair was cut into small pieces of about 1 mm and 40–80 mg was used for analysis. The hair was transferred to a polypropylene vial and 4 ml methanol and 100 µl of the internal standard (IS) mixture (2 ng/µl of amphetamine-D_{11}, MDA-D_5, MDMA-D_5, MDE-D_6, cocaine-D_3, morphine-D_3, codeine-D_3 and methadone-D_9; 0.2 ng/µl of THC-D_3) were added. The closed vial was sonicated for 4 h at 50 °C.

Purification

The methanol was evaporated and the residue was dissolved in 7 ml of phosphate buffer (0.1 M, pH 6) containing 400 mg of bovine serum albumin. The mixture was then applied to a solid phase extraction column (Bakerbond SPE C18, 500 mg), which had been conditioned by flushing with 2 ml of methanol and 2 ml of phosphate buffer (0.1 M, pH 6). The column was rinsed with 1 ml of 0.1 M acetic acid and dried for 10 min under vacuum. THC was first eluted with 3 ml dichloromethane/acetone (1:1; v/v), followed by elution of amphetamines, opiates and cocaine with 3 ml dichloromethane/2-propanol/ammonia (40:10:1, v/v/v). Both extracts were evaporated under a slight stream of nitrogen at 30 °C.

Derivatization

THC was methylated with methyl iodide. First 150 µl of a mixture of dimethylsulfoxide and 60% aqueous tetrabutylammonium hydroxide solution (98:2, v/v) was added to the extract. Subsequently, 50 µl methyl iodide was added and the mixture was vortexed. After 5 min at room temperature,350 µl 0.1 N hydrochloric acid was added. The methylated THC (THC-Me) was then extracted with 2 × 1 ml isooctane. The organic layer was separated and the solvent evaporated at 30 °C in a slight nitrogen stream. For GC/MS analysis the dry residue was dissolved in 50 µl of water-free ethyl acetate.

Amphetamines and opiates were derivatized by adding 50 µl of pentafluoropropionic anhydride (PFPA) to the extract and the mixture was incubated at 70 °C for 30 min. The excess pentafluoropropionic anhydride was evaporated at room temperature in a slight nitrogen stream. For GC/MS analysis the dry residue was dissolved in 50 µl of water-free ethyl acetate.

GC/MS analysis

For GC/MS analysis of amphetamines, opiates and cocaine a HP-5 MS capillary column was used. The carrier gas was He (constant flow: 1 mL/min), the injection volume 1 µl (splitless injection), the injector temperature 250 °C and the transfer line temperature 280 °C. The oven temperature program was 2 min isothermally at 60 °C, 40 °C/min to 170 °C, 8 °C/min to 270 °C and 13 min isother-

Table 2 Ions measured in SIM-mode and retention times of the analyzed compounds as well as the respective internal standards (IS)

Compound	Ions	Retention time
Amphetamine-D_{11}-PFP (IS)	m/z = 128 (target), 194, 292	5.37 min
Amphetamine-PFP	m/z = 91, 118 (target), 190, 281	5.40 min
MDA-D_5-PFP (IS)	m/z = 136 (target), 167, 330	7.20 min
MDA-PFP	m/z = 135 (target), 162, 190, 325	7.22 min
MDMA-D_5-PFP (IS)	m/z = 208 (target), 344	8.10 min
MDMA-PFP	m/z = 135, 162, 204 (target), 339	8.12 min
MDE-D_6-PFP (IS)	m/z = 224 (target), 359	8.41 min
MDE-PFP	m/z = 135, 162, 218 (target), 353	8.44 min
Methadone-D_9 (IS)	m/z = 78 (target), 303, 318	12.31 min
Methadone	m/z = 72 (target), 223, 294, 309	12.37 min
Cocaine-D_3 (IS)	m/z = 85, 185 (target), 306	12.98 min
Cocaine	m/z = 82, 182 (target), 303	12.99 min
Morphine-D_3-2PFP (IS)	m/z = 417 (target), 580	13.56 min
Morphine-2PFP	m/z = 414 (target), 415, 577	13.58 min
Dihydrocodeine-PFP	m/z = 282 (target), 445	13.86 min
Codeine-D_3-PFP (IS)	m/z = 285 (target), 448	13.97 min
Codeine-PFP	m/z = 284, 300, 447 (target)	14.00 min
6-MAM-PFP	m/z = 204, 361, 414 (target), 473	14.71 min
Heroin	m/z = 268, 310, 327 (target), 369	16.98 min
THC-D_3-Me (IS)	m/z = 331 (target), 316, 248	14.55 min
THC-Me	m/z = 328 (target), 313, 285, 245	14.58 min

Table 3 Summary of results

	No. of positive findings	Intra-assay CV (%)	Mean vealue of change in concentration (%)	Median of change in concentration (%)
THC	4	8	–36 (d)	–51 (d)
Amphetamine	6	10	–41 (d)	–51 (d)
MDA/MDMA/MDE	6	7*	–9 (d)	–11 (d)
Cocaine	10	13	–5 (n)	–20 (d)
Heroin	6	20	–19 (n)	–51 (d)
6-MAM	12	21	–9 (n)	–18 (n)
Morphine	12	12	–26 (d)	–22 (d)
Codeine	13	11	–30 (d)	–27 (d)
Dihydrocodeine	7	26	11 (i)	–32 (d)

* mean value for MDA, MDMA, MDE; d = decrease; i = increase; n = no significant effect

mally at 270 °C. EI ionization (70 eV) was used. The ions listed in Table 2 were measured in the selected ion monitoring (SIM) mode (dwell time per ion: 30 ms). For analysis of THC the same GC/MS conditions were applied. The measured ions for THC-Me and THC-D_3-Me are also listed in Table 2.

Quantification and validation data

For quantification the peak areas of the ions specified as "target" (see Table 2) were used. Quantification was based on peak area ratios relative to the respective internal standard (IS). A 5-point calibration curve was obtained by measuring spiked hair samples (100 mg) containing 10, 50, 100, 200 and 400 ng of the analytes. The calibrations were linear in the range tested and the correlation coefficients were > 0.99 for all compounds. The intra-assay precision was determined by analyzing five spiked hair samples (100 mg) containing 200 ng of each drug in one series. The intra-assay coefficients of variation (CV) ranged from 7% to 26% (listed in Table 3). Inter-assay precision data were obtained from analyses of the spiked hair samples (200 ng), performed on six different days. The day-to-day coefficients of variation (CV) ranged from 8% to 29% and the limit of detection (signal-to-noise ratio = 3) was < 0.01 ng/mg for all compounds. Each test series included a drug-free hair sample as negative control.

Instrumentation and reagents

Instrumentation: Gas chromatograph HP 6890 with auto-sampler (Hewlett-Packard, Palo Alto, Calif.), mass-spectrometer HP 5973 (Hewlett-Packard), capillary column HP-5 MS (30 m, 0.25 mm internal diameter, 0.25 µm film thickness; Hewlett-Packard). For sonication the Elma T 460/H ultrasonic bath (Elma, Singen, Germany) was used, the 50 ml polypropylene vials with screw caps were purchased from Falcon (Lincoln Park, N.J.). All solvents and reagents were analytical grade. Methanol, acetone, hexane, ethyl acetate, dichloromethane, isooctane, ammonia, dimethylsulfoxide, tetrabutylammonium hydroxide, acetic acid and 2-propanol were purchased from E. Merck (Darmstadt, Germany), methyl iodide, pentafluoropropionic anhydride and bovine serum albumin from Sigma-Aldrich (Deisenhofen, Germany), solid phase extraction columns from Mallinckrodt Baker (Griesheim, Germany) and all drug standard solutions as well as deuterated compounds from Radian (Austin, Tex.).

Table 4 Changes in THC concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.03	0.04	35
0.04	0.01	–78
0.57	0.28	–51
0.61	0.29	–51

Table 5 Changes in amphetamine concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.11	0.05	–55
0.12	0.07	–44
0.13	0.15	16
0.34	0.17	–48
0.74	0.33	–56
0.94	0.39	–59

Results

It has been already pointed out that each of the 14 hair samples was divided into 4 strands. Two strands were treated with Ultra Clean prior to analysis, whereas the remaining two strands were analyzed without any treatment. This procedure made it possible to run the test series in duplicate producing two independent analytical results for each sample of treated and untreated hair. For evaluation of the analytical data obtained, the mean values of the two independently determined drug concentrations were used to reduce the influence of statistical effects. Therefore all concentrations listed in Tables 4–12 under "Without treatment" and "After Ultra Clean" are mean values of two independently determined values.

The drug concentrations in the untreated hair samples were compared to those measured in the corresponding samples after washing with Ultra Clean. The relative change in concentration after Ultra Clean was given as a percentage of the concentration in the untreated sample. Only those changes which exceeded the intra-assay coefficient of variation (%) were considered to be a decrease or increase. No effect was assumed if the relative change was in the range of the intra-assay coefficient of variation.

Table 3 summarizes the results and the respective intra-assay coefficients of variation (CV) of the tested compounds are listed. The mean values and medians of changes in concentration due to Ultra Clean treatment were calculated from the percentage differences given in Tables 4–12. Decreases in concentration after Ultra Clean are marked by "(d)", increases by "(i)" and no significant

Table 6 Changes in MDA, MDMA and MDE concentration

Compound	Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
MDMA	0.02	0.03	61
MDA	0.10	0.10	4
MDA	0.10	0.03	–71
MDE	0.34	0.38	14
MDMA	0.37	0.27	–25
MDMA	0.46	0.30	–36

Table 7 Changes in cocaine concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.08	0.18	120
0.33	0.11	–66
0.65	0.20	–69
2.24	1.18	–47
2.29	2.77	21
2.30	4.25	84
3.94	0.35	–91
4.31	6.07	41
7.59	5.63	–26
23.75	20.19	–15

Table 8 Changes in heroin concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.07	0.02	–75
0.13	0.26	100
0.42	0.56	34
0.44	0.22	–50
0.46	0.14	–69
0.52	0.25	–52

Table 9 Changes in 6-MAM concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.03	0.08	139
0.07	0.02	–71
0.10	0.02	–76
0.16	0.29	80
0.29	0.42	45
0.31	0.08	–76
1.10	0.90	–19
2.67	1.56	–42
7.01	7.18	2
12.17	5.15	–58
18.32	15.47	–16
21.59	18.00	–17

changes by "(n)". The number of positive findings in the untreated samples for the tested compounds is also given in Table 3. All drugs were also detected in the corresponding hair samples which were treated by Ultra Clean prior to analysis, so that no difference in the qualitative analytical results could be observed. The influence of Ultra Clean on methadone concentrations was not evaluated because only two of the hair samples were positive.

The detailed results of the concentration changes of THC, amphetamine, MDA, MDMA, MDE, cocaine, heroin, 6-MAM, morphine, codeine and dihydrocodeine are presented in Tables 4–12. The methylenedioxyamphetamine derivatives MDA, MDMA and MDE were combined for the evaluation of Ultra Clean effects on their concentrations (Table 6). Therefore the average intra-

Table 10 Changes in morphine concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.04	0.03	–10
0.06	0.07	5
0.06	0.05	–20
0.06	0.02	–62
0.08	0.07	–8
0.24	0.14	–45
0.25	0.21	–15
0.29	0.15	–47
0.63	0.59	–5
2.03	1.52	–25
2.54	1.49	–41
13.27	8.33	–37

Table 11 Changes codeine in concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.03	0.02	–48
0.04	0.01	–61
0.05	0.02	–52
0.05	0.04	–27
0.07	0.03	–59
0.10	0.08	–24
0.16	0.06	–63
0.20	0.15	–23
0.42	0.36	–14
0.61	0.79	29
1.13	1.20	6
4.32	3.43	–21
6.14	4.22	–31

Table 12 Changes in dihydrocodeine concentration

Without treatment (ng/mg)	After Ultra Clean (ng/mg)	Difference (%)
0.06	0.02	–68
0.14	0.05	–61
0.31	1.08	253
0.32	0.45	42
0.40	0.10	–76
30.52	35.53	16
53.55	36.45	–32

assay coefficient of variation of MDA, MDMA and MDE (7%) was used for the evaluation as increase or decrease. A relatively small decrease was found for these compounds (mean value: –9%, median: –11%). This must be considered as a result of the relatively large increase of 61% in the MDMA concentration observed in one sample. No distinct tendency was found for cocaine (Table 7). Whereas 6 of the 10 cocaine positive samples showed decreasing concentrations, in 4 cases considerable increases up to 120% could be observed. Remarkable is an enormous increase of dihydrocodeine up to 253% observed in one sample (Table 12). It is obvious that this particular large increase raised the mean value of the changes in dihydrocodeine concentrations up to 11%.

Discussion

The mean values of the percentage changes in concentration (Table 3) showed a decrease after Ultra Clean treatment for all tested compounds with the exception of dihydrocodeine which had an average increase of 11%. However, the average decreases of cocaine, heroin and 6-MAM concentrations could not be considered as significant because they were in the range of the respective intra-assay coefficients of variation, although the median values listed in Table 3 clearly demonstrate a general tendency towards decreasing concentrations after Ultra Clean treatment. The median values of all compounds, except 6-MAM, exhibit a distinct decrease in concentration exceeding the respective intra-assay coefficient of variation. Even the median value of the changes in dihydrocodeine concentrations showed a decrease of –32%. The considerable differences between mean values and medians for cocaine, heroin and dihydrocodeine are obviously caused by particular samples with high increases in concentration after Ultra Clean treatment. In one sample the cocaine concentration was 0.08 ng/mg without treatment and 0.18 ng/mg after Ultra Clean, corresponding to an increase of 120% (Table 7), heroin increased from 0.13 to 0.26 ng/mg (100%) in one case (Table 8) and an large increase in dihydrocodeine concentration of 253% (0.31 to 1.08 ng/mg) was found in another sample (Table 12). Generally decreases in the range of about –5 to –50% were observed after washing with Ultra Clean compared to the untreated samples.

Increased concentrations after Ultra Clean treatment were mostly found in hair samples containing relatively small amounts of drug (see Tables 4–10), e.g. for THC +35% at a concentration of 0.03 ng/mg without treatment and 0.04 ng/mg after washing, MDMA +61% (0.02/0.03 ng/mg), cocaine +120% (0.08/0.18 ng/mg) or 6-MAM +139% (0.03/0.08 ng/mg). Therefore, it has to be assumed that increased concentrations were mainly caused by fluctuations of the analytical results, since especially at low concentration levels the scattering of analytical measurements has a larger influence on the obtained result than at higher levels [6]. However, it has to be taken into account that in these particular samples drug substances may have possibly penetrated the hair fiber during the intensive washing procedure like other small molecules [7].

The extent of the general decrease in concentration was different for the particular compounds. For example THC and amphetamine showed relatively large average decreases of –36% or –41%, respectively, whereas the average decrease for cocaine was only –5 %. In consideration of the relatively small number of 14 samples this might be statistical effects, but this finding could possibly be a result of different sites and strengths of binding of the particular analytes to the various morphological hair components [8]. It is known that cocaine in particular exhibits a high melanin affinity and a considerable tendency for incorporation into hair [9, 10]. In the present study, correlations between change in concentration and the individual color or type of hair could not be established.

In conclusion the major result of this study was that all drugs originally present in the tested hair samples were still detectable after the application of Ultra Clean. Therefore, our findings clearly demonstrated that the drugs had not been sufficiently removed by a single Ultra Clean treatment to drop their concentration below the limit of detection of the analytical method applied. Overall, although a general tendency towards a decrease in concentration could be observed, the "special purifying effect" of this product might possibly not have exceeded those obtained by any "usual" shampoo.

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