Int J Legal Med (1997) 110 : 159–163

ORIGINAL ARTICLE

C. Jurado · P. Kintz · M. Menéndez · M. Repetto

Influence of the cosmetic treatment of hair on drug testing

Received: 15 January 1996 / Received in revised form: 23 January 1997

Abstract An important issue of concern for drug analysis in hair is the change in the drug concentration induced by the cosmetic treatment of hair. The products used for this treatment are strong bases and they are expected to cause hair damage. As a result drugs may be lost from the hair matrix or, under conditions of environmental contamination, be more easily incorporated into the hair matrix. We investigated the effects of cosmetic treatment in vivo by analysing hair samples selected from people who had treated their hair by bleaching or dyeing before sample collection. All of the subjects admitted a similar drug consumption during the time period for which the strands were analysed. Samples were viewed under a microscope to establish the degree of hair damage. Treated and untreated portions from each lock of hair were then selected, separated and analysed by standard detection procedures for cocaine, opiates, cannabinoids and nicotine. In all cases the drug content in hair that had undergone cosmetic treatment decreased in comparison to untreated hair. The majority of the mean differences were in the range of 40%-60% (cocaine, benzoylecgonine, codeine, 6-acetylmorphine and THC-COOH). For morphine the mean difference was higher than 60%, and two cases (THC and nicotine) differed by approx. 30%. These differences depended not only on the type of cosmetic treatment, as bleaching produced higher decreases than dyeing, but also on the degree of hair damage i.e. the more damaged the hair, the larger the differences in the concentration levels of drugs.

Key words Hair analysis · Cocaine · Opiates · Cannabis · Nicotine · Cosmetic treatment

C. Jurado (✉) · M. Menéndez · M. Repetto
Instituto Nacional de Toxicología,
Apartado 863, E-41080 Sevilla, Spain

P. Kintz
Institut de Medecine Légale,
11, rue Human, F-67085 Strasbourg, France

Introduction

Interpreting the results from hair analysis for drugs of abuse is difficult, because this is a relatively new methodology and there are many issues that still remain to be answered. One important issue is the stability of drugs in human hair. Many factors are likely to affect the concentration of drugs in hair and thus complicate the interpretation of results from these analyses. These factors include, but are not limited to hair colour or melanin content [1, 2] physicochemical properties of drugs, such as lipophilicity, melanin affinity and membrane permeability [3] structural factors of drugs [4] external contamination [5] and cosmetic treatment of hair [6–11]. Very little has been published about the effects of cosmetic treatment on drug contents in hair. Baumgartner et al. [6] showed that daily shampooing does not significantly affect the drug content in hair. In studies with various hair treatments, Welch et al. [7], found that drug levels can be reduced but probably not eliminated by any reasonable treatment conditions. Blank and Kidwell's studies on external contamination [8] demonstrated that hair treated by dyeing incorporated externally applied drugs. Pötsch and Skopp [9] observed that opiate concentrations declined dramatically after cosmetic treatment (bleaching and permanent waving) and UV exposure, concluding that both the chemical composition of different cosmetic treatments and the methods applied in hair treatment affect drug stability. Cirimele et al. [10] found a decrease in cocaine and opiate concentrations in one bleached hair sample. Finally, Pötsch et al. [11] found that after bleaching and exposure to water or soil for 6 months, drug monitoring of formerly positive hair samples gave negative results.

The purpose of this study was to corroborate the effects of cosmetic treatment on drug contents in hair in vivo, by analysing the difference in cocaine, opiates, cannabis and nicotine concentrations in treated and untreated hair samples. In order to do the study in vivo, the samples were selected from people who had treated their hair before sample collection.

Material and methods

Hair specimens

The study focused on two types of cosmetic treatment i.e. bleaching and dyeing. Hair bleaching consists of the treatment of some locks of hair with the bleaching agent to obtain lighter colour hair, while other locks remain untreated and retain their original colour. The treatment applied in hair dyeing is different whereby all hair strands are cosmetically treated with a colouring formula. For bleached samples the only requirement was that the subject be a drug user; for dyed samples, it was also required that a segment of the hair shaft be untreated. The length of these segments varied from case to case, but in each sample the difference between treated and untreated hair was evident.

For opiates, cocaine and cannabis, the study was performed on hair samples obtained from ten female drug abusers living in southern Spain. Five of them (nr. 1–5) had treated their hair by bleaching and the other five (nr. 6–10) by dyeing. Some of them were multi-drug abusers so that opiates were analysed in six cases (two bleached, nr. 3 and 5, and four dyed hair samples, nr. 6–9),cocaine in seven cases (four bleached, nr. 1–4, and three dyed samples, nr. 7–9), and cannabis in four cases (three from dyeing, nr. 6, 8, 9, and one from bleaching, nr. 4). Nicotine was analysed in eight hair samples (nr. 11–18) collected from French donors who all reported tobacco use but not drug abuse, and had treated their hair by bleaching. Only living subjects were involved in the study and all of them had treated their hair before sample collection.

A questionnaire was also obtained listing the individual histories of drug consumption. To be eligible for a particular hair sample, the subject had to admit regular drug consumption throughout the period of time corresponding to the length of the hair. To corroborate self-reported histories, sectional analyses along the whole hair shaft were performed. Segmentation was different depending on the type of cosmetic treatment. For bleached hair, the shafts were cut into 1-cm segments and each segment was analysed separately. When drug concentrations were similar in all of the segments, those hair samples were considered suitable for analysis. For dyed hair, the strands were cut and separated into two portions at the point where the change between treated and untreated hair was evident. The two portions obtained were subsequently cut into 1-cm segments, and each segment was analysed separately. When drug concentrations were consistent within either the treated or the untreated segments, the sample was considered suitable.

Sampling

Hair samples were cut from the posterior vertex, as close as possible to the scalp. After collection they were sampled differently, depending on cosmetic treatment, in order to separate treated and untreated sections of each lock of hair. For bleached hair, we selected treated and untreated shafts of hair which were placed in separate vials and stored at room temperature until analysis. For dyed hair, sampling included dyed and undyed segments of the same strand of hair, separated at that point closest to the hair root at which the change was evident. From each type of sample we selected the 2-cm segment closest to the point at which they had been cut. They were then stored separately, as previously described for bleached samples.

Analytical methods

Analytical method for opiates, cocaine and cannabis

The method was previously published in detail [12], but the essentials are described below: approx. 50 mg of each type of hair were decontaminated with methylene chloride twice for 15 min at 37° C, and two consecutive hydrolyses were performed in order to extract the three kinds of compounds from the protein matrix. Opiates and cocaine were extracted first with 1ml of 0.1N HCl at 50° C for 18 h in the presence of the deuterated cocaine and opiate compounds as internal standards (Radian, Austin, Tex.). The hair was then filtered and retained for a second hydrolysis. The aqueous fraction was extracted with organic solvent at pH 9.2 and derivatized with 100 µl HFBA and 70 µl HFPOH (Sigma, Madrid, Spain) for 30 min at 60° C. Solutions containing cocaine and opiate substances were evaporated and the residues were redissolved in 100 µl of ethyl acetate. Deuterated cannabis internal standards (Research Triangle Institute, Research Triangle Park, NC) and ten drops of 11.8N KOH were added to the hair retained for a second hydrolysis. After standing for 10 min at room temperature, the aqueous fraction was acidified and the cannabis compounds were extracted with organic solvent. Derivatization was performed with 100 µl of hexane, 60 µl of HFBA and 50 µl of HFPOH at 100°C for 10 min. After drying, derivatized residues containing cannabis were redissolved in 100 µl of hexane. Both derivatized extracts were injected into a Hewlett-Packard 5890 series II gas chromatograph (Palo Alto, Calif.), coupled to a Hewlett-Packard 5971 mass selective detector (MSD) and equipped with an HP-Ultra 1 capillary column (crosslinked methyl-silicone, 25 m × 0.2 mm × 0.33 µm film thickness). The oven temperature was programmed from 60° C (3 min hold) to 280° C at 12° C/min (10 min hold). Injector (splitless 2 min) and interface temperatures were 250° C and 280° C, respectively. The helium carrier flow was 1 ml/min. The MSD was used in the electron impact mode at 70 eV. The electron multiplier voltage was set at 400 V above autotune voltage.

Analytical method for nicotine

About 50–100 mg of hair were decontaminated by washing twice with methylene chloride for 15 min at room temperature and the protein matrix of the hair was destroyed by incubation in 1 ml of 1 M NaOH for 10 min at 100° C [13]. After cooling, the drug was extracted using 5 ml of diethyl ether in the presence of 20 µl of ketamine (1 mg/l) as an internal standard (Parke Davis, Courbevoie, FL). After agitation and centrifugation, the organic phase was removed. A 20-µl aliquot of octanol was then added to ensure nonvolatility of nicotine. After evaporation, the residue was dissolved in 15 µl of methylene chloride, and 1 µl was injected in a model 8500 (Perkin-Elmer, Norwalk, CT, USA) gas chromatograph with an ion-trap detector (ITD). The ITD was operated in the electron-impact mode at 70 eV with an ion source temperature of 200–220° C. The electron multiplier voltage of the detector was set at 1350 V. A fused-silica capillary column (SGE, Austin, Tex.), BP-5 (methylmethylsiloxane), 12 m × 0.22 mm I.D., was used. The flow rate of the carrier gas (helium purity N55) was 1.2 ml/min and the injector port temperature was 250° C. The column oven temperature was programmed from an initial temperature of 60° C (held for 0.9 min) to 280° C at 30° C/min and held at 280° C for the final 1 min. In both procedures the mass spectrometers were operated in the selected ion monitoring mode (SIM). Analytical parameters and procedures were as previously described [12, 13].

Results and discussion

Before analysis, all the hair samples involved in the study were examined for the degree of degradation in the hair fibers by viewing them under a microscope (Nikon Labophot-2 type 104, Tokyo. Japan).

Hair is continuously subjected to natural factors, such as sunlight, weather, water, pollution, etc., which affect and damage the cuticle and hair cosmetic treatments, such as bleaching or dyeing, enhance that damage. The most common bleaching and dyeing formulas are combinations of hydrogen peroxide (H_2O_2) with an agent that basically contains ammonium hydroxide, plus ethanol or natural

Table 1 Cocaine and benzoylecgonine (BE) concentrations (ng/mg) in seven treated and untreated hair samples

Samples	Hair treatment	Cocaine		BE	
		Untreated	Treated	Untreated	Treated
1	Bleaching	53.5	12.8	14.3	4.2
2	Bleaching	58.3	16.4	9.5	3.8
3	Bleaching	8.4	3.2	3.3	1.1
4	Bleaching	7.3	3.3	3.7	1.9
7	Dyeing	7.2	3.3	0.9	0.6
8	Dyeing	202.6	109.9	27.3	16.4
9	Dyeing	13.1	9.1	4.1	2.5

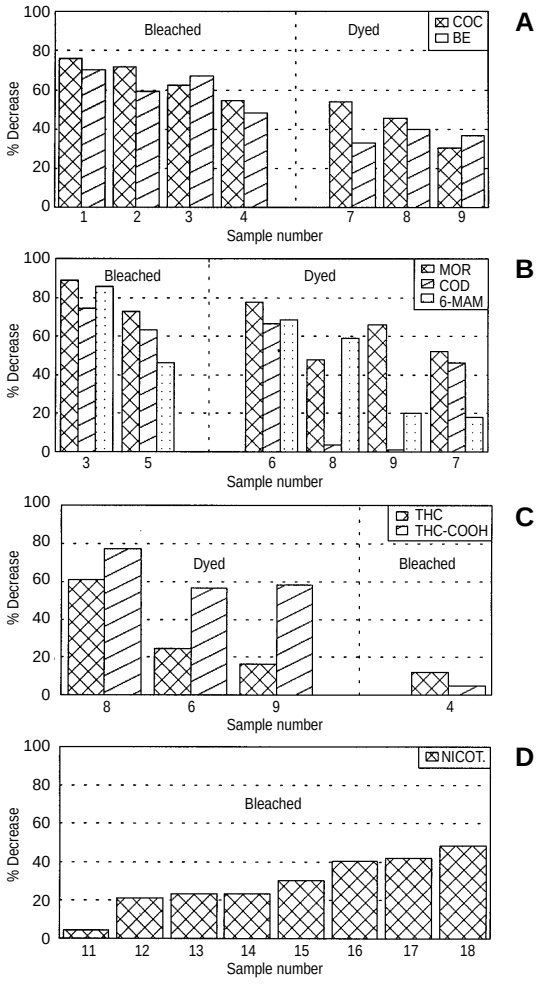

Fig. 1 Decrease (%) in treated and untreated hair samples. **A** cocaine compounds, **B** opiates, **C** cannabis, **D** nicotine

pigments for bleaching or dyeing, respectively. H_2O_2 concentrations vary, depending on the type of treatment desired, being higher for bleaching than for dyeing. The basis of both treatments is similar: ammonium hydroxide opens the scales of the cuticle to facilitate the entry of the other compounds, H_2O_2, a powerful oxidant, attacks the hair pigment and decolorizes the hair, and, in the case of dyeing, the natural pigments contained in the dyeing formula provide the desired colour. All of these circumstances affect the hair fiber, usually producing greater alterations in the cuticle as the distance from the scalp increases.

The hair samples involved in this study were viewed microscopically in order to determine a possible relationship between hair damage and concentration decreases, and to see if the most degraded hair samples also had the highest decreases in drug concentrations. The majority of bleached and dyed samples were microscopically similar and the cuticle was relatively well preserved. Only dyed sample nr. 6 and bleached hair sample nr.10 appeared very damaged: both had split end fibers, irregular, partly broken or, even at times, missing scales. After the microscopical examination, treated and untreated portions from each lock of hair were separated and examined for cocaine, opiates, cannabinoids and nicotine in order to establish differences in the concentrations of these drugs.

Quantitative data for cocaine compounds in the seven hair samples, both treated and untreated, are presented in Table 1. In all cases the concentrations found in treated hair were lower than those found in untreated hair. The differences (Fig. 1A) ranged from 30.6% to 76.0% for cocaine, while benzoylecgonine (BE) differences ranged from 33.3% to 70%. These concentration decreases tended to be higher in bleached hair (mean 66.2% and 61.2% for cocaine and BE, respectively) than in dyed hair (mean 43.4% and 36.6% for cocaine and BE, respectively). Comparing the two analytes tested (cocaine and BE) there was generally good agreement between the decrease for drug and metabolite i.e. cases with the highest decrease in cocaine (sample nr. 1,2 and 3) also had the highest decrease in BE.

The results for morphine, codeine and 6-MAM found in the six cases analysed for opiates are listed in Table 2. A decrease in drug concentrations in all of the treated samples in relation to their respective portions of untreated hair was also detected. The differences (Fig. 1B) ranged from 48.1% to 88.7%, from 1% to 74.4%, and from 17.9% to 85.5% for morphine, codeine and 6-MAM, respectively. As in the cocaine compounds, higher de-

Table 2 Morphine, codeine and 6-MAM concentrations (ng/mg) in six treated and untreated hair samples

Samples	Hair treatment	Morphine		Codeine		6-MAM	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
3	Bleaching	3.00	0.34	3.16	0.81	7.53	1.09
5	Bleaching	3.32	0.91	1.50	0.55	7.29	3.91
6	Dyeing	2.38	0.53	0.63	0.21	1.78	0.56
8	Dyeing	40.46	20.94	13.51	13.00	72.86	30.04
9	Dyeing	2.99	1.01	0.73	0.72	6.13	4.90
7	Dyeing	7.11	3.40	1.10	0.59	17.8	14.6

Table 3 THC and THC-COOH concentrations (ng/mg) in four treated and untreated hair samples

Samples	Hair treatment	THC		THC-COOH	
		Untreated	Treated	Untreated	Treated
8	Dyeing	1.9	0.7	0.7	0.2
6	Dyeing	0.4	0.3	0.2	0.1
9	Dyeing	0.8	0.7	0.5	0.2
4	Bleaching	0.7	0.6	0.5	0.5

Table 4 Nicotine concentrations (ng/mg) in eight treated and untreated hair samples

Samples	Hair treatment	Nicotine	
		Untreated	Treated
11	Bleaching	6.4	6.1
12	Bleaching	10.5	8.3
13	Bleaching	24.2	18.6
14	Bleaching	31.6	24.3
15	Bleaching	24.3	16.9
16	Bleaching	36.6	21.8
17	Bleaching	9.3	5.4
18	Bleaching	8.3	4.3

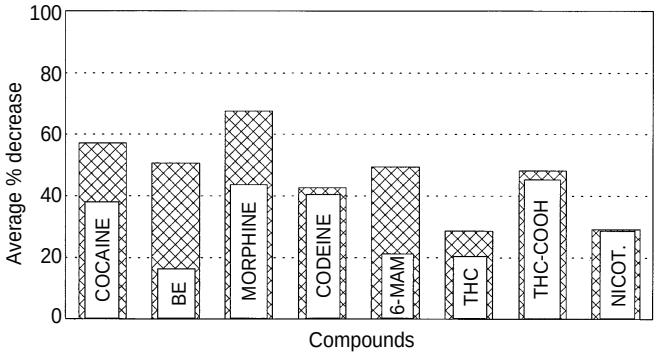

Fig. 2 Average decrease (%) for all of the compounds tested

creases were found in bleached (mean: 80.6%, 68.8%, 65.9% for morphine, codeine and 6-MAM, respectively) than in dyed hair (mean: 61.2%, 29.5%, 41.3% for morphine, codeine and 6-MAM, respectively). A high decrease was found in the dyed sample nr. 6, probably due to the fact that it was an extremely damaged hair sample.

For cannabinoid compounds (Table 3), we analysed the concentrations of THC and THC-COOH in four cases, and found a similar pattern to cocaine and opiates, i.e. the concentrations decreased in all treated samples (the differences ranged from 12.3% to 61.3% and from 5% to 77.6% for THC and THC-COOH, respectively). However, the highest differences (Fig. 1C) were found in the three dyed hair samples (mean 34% and 64.1% for THC and THC-COOH, respectively). In sample nr. 2 from bleached hair, the differences were considerably lower in THC (12.3%) and in THC-COOH (5.0%).

Table 4 shows the concentration levels of nicotine in treated and untreated hair in the eight cases involved in this study and showed a decrease in all treated samples. The differences (Fig. 1D) were less varied than in the other compounds tested (range 4.6%–48.2%) which can be attributed to the samples being more homogeneous (all were treated by bleaching).

Figure 2 summarises the mean differences found in each of the eight drugs tested. The majority were in the range of 40%–60% (cocaine, BE, codeine, 6-MAM and THC-COOH). In morphine, the mean difference was more than 60% and in the cases of THC and nicotine the differences were about 30%.

The data from the present study agree with those reported in earlier publications. However, the majority of the previous studies were performed in vitro, either with spiked hair strands or hair samples previously cut from known drug abusers and subjected to different cosmetic treatment under different conditions. Welch et al. [7] collected hair from cocaine abusers and treated the samples with a dandruff shampoo, an alkaline wave solution, a hair dye, a 30% hydrogen peroxide solution, ethanol or a 1% NaCl solution for a 20-h period. The period of time was much longer than that usually employed in cosmetic treatment. The decreases in cocaine were about 80% in hair treated with hydrogen peroxide and 20% in hair treated with a colour rinse. In the present study the mean cocaine concentration difference in bleached hair was 66.2%, which agreed with the data of Welch et al. [7]. However, our data for dyed hair (43.4%) were greater than those reported by Welch et al. The different results obtained in both studies may be due to the differences in the products used for dyeing as well as the application methods.

Pötsch and Skopp [9] reported a decrease in opiate concentrations in spiked hair strands as well as in hair samples collected from known drug abusers and subsequently treated by bleaching, dyeing or exposure to UV radiation. After bleaching the spiked hair only 2–18% of the initial opiate concentrations were found. In the studies performed on hair from known drug abusers, opiate concentrations in general decreased to a point below the detection limit. In the present study, morphine differences were about 80%, similar to the data of Pötsch and Skopp from spiked hair. Nevertheless, our findings did not agree with those obtained by Pötsch and Skopp in hair from drug abusers. After cosmetic treatment, we always found drugs in detectable and quantifiable concentrations, with one exception, sample nr. 10, in which we detected nei-

Table 5 Drug concentrations (ng/mg) in bleached and unbleached hair (sample nr. 10)

N.D. = Not detected

Samples	Morphine	Codeine	6-MAM	THC	THC-COOH
Unbleached	1.57	1.17	4.32	0.40	0.25
Bleached	N.D.	N.D.	N.D.	N.D.	N.D.

ther opiates nor cannabis in the bleached segment (Table 5). However, it should be noted that not only was it an extremely damaged hair sample, but that the drug contents were also quite low in the untreated portion.

Cirimele et al. [10] were the first to report different opiate and cocaine concentrations in hair samples. They selected and analysed separately bleached and unbleached strands of hair from a female drug addict who had treated her hair with hydrogen peroxide. Drug concentrations were higher in the unbleached than in the bleached hair. Mean differences were 65% and 80% for cocaine and opiate compounds, respectively, which were in accordance with our results.

The present study is the first report on the influence of cosmetic treatment (bleaching and dyeing) on cannabis and nicotine concentrations in hair samples in vivo and the second report on cocaine and opiate concentrations in the same types of samples. High decreases in drug contents were found in hair which had undergone cosmetic treatment.

The differences in drug concentrations in treated and untreated samples are primarily due to the chemical agents involved in bleaching and dyeing, which remove a significant portion of any drugs contained in hair. These products are usually strong basic solutions, and they are expected to cause hair damage. Consequently, drugs are more easily lost from the hair matrix. One of the other contributing factors is the colour of the hair. Several papers have been published on this problem [2, 3, 14, 15] and all agree that darker hair colour favours higher rates of drug incorporation. Drugs are accumulated and bound to the hair melanin, so hair with a high melanin content (black) would incorporate drugs in higher concentrations than hair containing less melanin (e.g. blond) or no melanin at all (albino). As previously explained, bleaching treatment consists of removing hair colour with hydrogen peroxide and leads to loss of melanin granules. Bleached hair, therefore, contains less melanin than unbleached hair and consequently incorporates smaller quantities of drugs.

In conclusion, the current findings confirm the hypothesis that the cosmetic treatment of hair decreases drug concentrations in all treated hair samples. The range of these decreases is, in the majority of the cases, higher in bleached than in dyed hair samples. In addition, this study demonstrates that the more damaged the hair, the larger the differences in the concentration levels of drugs. These data suggest that the effect of cosmetic treatment on hair has to be taken into account when interpreting drug abuse analyses in hair samples, especially in cases of severely damaged hair.

References

1. Harrison WH, Gray RM, Solomon LM (1974) Incorporation of D- amphetamine into pigmented guinea pig hair. Br J Dermatol 91:415–418
2. Reid RW, O'Connor FL, Crayton JW (1994) The "in vitro" differential binding of benzoylecgonine to pigmented human hair samples. Clin Toxicol 32:400–410
3. Nakahara Y, Kikura R, Takahashi K (1995) Hair analysis for drugs of abuse X. Effect of physicochemical properties on incorporation rates into hair. Biol Pharm Bull 18:1223–1227
4. Nakahara Y, Kikura R, Takahashi K (1995) Effect of structural factors on incorporation of drugs into hair. – The incorporation rates of amphetamine analogs. Proceedings of the 1995 International Conference and Workshop in Hair Analysis in Forensic Toxicology. Abu-Dhabi 19–23 November 1995, pp 28–59
5. Mizuno A, Uematsu T, Oshima A, Nakamura M, Nakashima M (1993) Analysis of nicotine content of hair for assessing individual cigarette smoking behavior. Ther Drug Monit 15:99–104
6. Baumgartner WA, Hill VA, Blahd WH (1989) Hair analysis for drugs of abuse. J Forensic Sci 34:1433–1452
7. Welch MJ, Sniegoski LT, Allgood ChC, Habram M (1993) Hair analysis for drugs of abuse: evaluation of analytical methods, environmental issues, and development of reference materials. J Anal Toxicol 17:389–398
8. Blank DL, Kidwell DA (1995) Decontamination procedures for drugs of abuse in hair: are they sufficient? Forensic Sci Int 70:13–38
9. Pötsch L, Skopp G (1996) Stability of opiates in hair fibers after exposure to cosmetic treatment. Forensic Sci Int 81:95–102
10. Cirimele V, Kintz P, Mangin P (1995) Drug concentrations in human hair after bleaching. J Anal Toxicol 19:331–332
11. Pötsch L, Skopp G, Becker J (1995) Ultrastructural alterations and environmental exposure influence the opiate concentrations in hair of drug addicts. Int J Legal Med 107:301–305
12. Jurado C, Giménez MP, Menéndez M, Repetto M (1995) Simultaneous quantification of opiates, cocaine and cannabinoids in hair. Forensic Sci Int 70:165–174
13. Kintz P (1992) Gas chromatographic analysis of nicotine and cotinine in hair. J Chromatogr 580:347–353
14. Rollins DE, Gygi SP, Wilkins DG (1995) Disposition of codeine into hair. Proceedings of the 1995 International Conference and Workshop in Hair Analysis in Forensic Toxicology. Abu-Dhabi 19–23 November 1995, pp 118–135
15. Cone EJ, Darwin WD, Wang Wl (1993) The occurrence of cocaine, heroin and metabolites in hair of drug abusers. Forensic Sci Int 63:55–68