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Chemical Straightening as a Source of Analytical Variability in Hair Testing: A Dual-Analyte Case Report

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ABSTRACT

Hair analysis is increasingly used in forensic toxicology; however, result interpretation may be influenced by cosmetic hair treatments. Although the effects of bleaching, dyeing, and thermal straightening have already been evaluated, data on permanent chemical hair straightening are scarce, particularly under in vivo conditions. This case report evaluates the impact of an alkaline-based permanent hair straightening procedure on the determination of an endogenous compound, gamma-hydroxybutyrate (GHB), and an exogenous drug, alprazolam, in hair. Hair samples were collected from a 39-year-old woman before and 1 month after undergoing chemical hair straightening based on ammonium thioglycolate. Adjacent hair strands corresponding to identical temporal windows were analyzed. GHB was quantified in 0.5-cm hair segments using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method, whereas alprazolam was analyzed in 2-cm segments using a validated LC-MS/MS method for drugs of abuse and psychoactive pharmaceuticals. All analyzed segments were positive for GHB in both samples. After the straightening procedure, endogenous GHB concentrations showed a pronounced increase compared with pretreatment levels, with fold changes ranging from approximately 6 to 43 in corresponding segments. In contrast, alprazolam concentrations in segments corresponding to the reported period of drug intake decreased by approximately 1.7- to 2-fold following chemical straightening. This case report demonstrates that permanent chemical hair straightening can produce marked and analyte-dependent effects on hair drug concentrations. These findings emphasize the importance of systematic documentation of cosmetic hair treatments and careful interpretation of hair analysis results in forensic casework, particularly when analyte concentrations are low.

1 | Introduction

The interpretation of the results obtained from the analysis of a hair sample can be complex, due to the multitude of factors that must be taken into account: hair color (melanin has a positive effect on the incorporation of many substances, especially basic compounds) and hair type (thickness, porosity, and structure), the degree of hair damage and the resulting susceptibility to external contamination, the type of substance analyzed, and the influence of cosmetic hair procedures that may damage the internal structure of the hair [1–3].

The effects of cosmetic treatments such as bleaching, hair dyeing, or permanent waving have been described in the literature. However, there are very few studies on the consequences of permanent chemical hair straightening.

This treatment has gained popularity in recent years, as it provides several advantages to users: long-lasting results (up to several months, depending on the rate of hair growth), low-maintenance hair styling, reduced frizz, and improved hair manageability. It is mainly chosen by women with curly or wavy hair or hair prone to frizz.

Permanent hair straightening involves the application of chemical products that break disulfide bonds in the hair to achieve long-lasting straightening. These products can be broadly classified into two types: alkaline relaxers and acid-based straighteners. Alkaline treatments include two distinct subtypes: hydroxide-based relaxers (lye or no lye), typically employed for afro-textured hair and formulated with sodium hydroxide, guanidine hydroxide, or lithium hydroxide, and thioglycolate-based straighteners, commonly used in European and Asian markets for curly, wavy, or frizzy hair based on ammonium thioglycolate. Acid-based straighteners include products based on glyoxylic acid, formaldehyde-releasing agents, or keratin-based formulations. Following chemical application, thermal straightening is used to reshape the hair into a straight configuration, and finally, a neutralizer is applied to reform the bonds and "lock in" the straight shape [4].

Given the increasing prevalence of this practice, especially among the female population, it is essential to characterize its impact on the detection of different substances in hair analysis. Understanding how these cosmetic treatments affect analyte concentrations is crucial for the correct interpretation of results, as their application may lead to reduced concentrations or even false-negative results.

In this context, we present a case in which permanent hair straightening was performed, allowing the evaluation of its impact on the detection of both an endogenous compound, gamma-hydroxybutyrate (GHB), and an exogenously administered drug, alprazolam.

2 | Case Description

A 39-year-old woman with long, wavy, and brown hair reported occasional insomnia previously treated with alprazolam (0.25 mg/day), which she had discontinued approximately 5 months prior to sample collection. She had no known comorbidities and reported no use of tobacco, alcohol, or drugs of abuse.

The woman expressed interest in undergoing a permanent hair straightening and voluntarily agreed to donate a hair sample prior and after the procedure. A hair sample measuring 14.5 cm was collected before the procedure from the posterior vertex region (Strand A). After sample collection, she attended a hairdressing center where a commercial alkaline-based permanent straightening treatment known as "Japanese thermal reconditioning" and based on ammonium thioglycolate (Schwarzkopf Professional) was performed. One month after the treatment, a second hair sample consisting of an adjacent strand measuring 15.5 cm was collected (Strand B).

Both samples were submitted to the Toxicology Service of the Institute of Forensic Sciences (Universidade de Santiago de Compostela), where two analytical determinations were performed, one for the determination of GHB and another for the determination of a panel of drugs of abuse and pharmaceuticals.

2.1 | GHB Analysis

GHB analysis was performed using a previously published and fully validated method [5]. Briefly, hair samples were segmented into 0.5-cm sections and decontaminated twice with dichloromethane (DCM). After decontamination, 10 mg of each segment was pulverized and incubated with Milli-Q water and the internal standard, followed by sonication and centrifugation. The resulting extracts were evaporated, reconstituted in acetonitrile:methanol (3:1, v/v), and analyzed by LC-MS/MS. Chromatographic separation was achieved using an Acquity UPLC H-Class system (Waters Corporation, Milford, MA, United States) equipped with a CORTECS UPLC HILIC column (2.1 × 100 mm, 1.6 μm) maintained at 40°C, with 10-mM ammonium acetate (pH 6.0) and acetonitrile (ACN) as mobile phases. Detection was carried out using a Xevo TQ-XS triple quadrupole mass spectrometer (Waters Corporation), operating in negative electrospray ionization mode (ESI). The calibration range was 0.5–50 ng/mg.

To evaluate basal GHB levels in the female subject and to confirm the absence of exogenous administration, the entire hair strands (A and B) were analyzed. All hair segments were processed and analyzed simultaneously under identical analytical conditions. As there was an approximate 1-month interval between the collection of both strands, Segment 1 of the strand collected before chemical hair straightening (Strand A) corresponds temporally to Segment 3 of the strand collected after the straightening procedure (Strand B).

2.2 | Drugs of Abuse and Pharmaceuticals Analysis

Drugs of abuse and psychoactive pharmaceuticals were analyzed using a previously published and fully validated method, with some modifications [6]. The method allows the identification of 35 licit and illicit drugs, including cannabinoids, opioids, stimulants, benzodiazepines, hypnotics, and antidepressants.

Hair samples were segmented into 2-cm sections and decontaminated three times with DCM. After decontamination, 25 mg of each segment was pulverized and incubated overnight in methanol at 50°C with the internal standards. The samples subsequently underwent liquid–liquid extraction with hexane:ethyl acetate (55:45, v/v), followed by solid-phase extraction using reverse-phase cartridges. The final extracts were evaporated and reconstituted in initial mobile phase prior to analysis by LC-MS/MS. Chromatographic separation was performed using an Acquity UPLC H-Class system equipped with an ACQUITY UPLC HSS T3 column (2.1 × 50 mm, 1.6 μm), maintained at 35°C. Mobile phases consisted of 2-mM ammonium formate with 0.1% formic acid and ACN. The injection volume was 10 μL, with a total run time of 15 min at a flow rate of 0.417 mL/min. Detection was carried out using a Xevo TQ-XS triple quadrupole mass spectrometer operating in ESI+. The calibration range was from 0.5–20 to 2000–20,000 pg/mg, depending on the analyte.

Based on the reported history of alprazolam use and its discontinuation approximately 5 months prior to the collection of Strand A, hair segment selection was restricted to the period

corresponding to documented drug intake. Accordingly, a 6-cm hair section corresponding to the last 6 months of alprazolam treatment before discontinuation was selected and subsequently subdivided into three consecutive 2-cm segments for analysis. For Strand B, collected 1 month later, the corresponding 6-cm section representing the same retrospective time window was selected by considering the estimated hair growth occurring between collections (approximately 1 cm/month). This ensured that both strands corresponded to the same months of reported alprazolam intake. Strand B was likewise subdivided into three consecutive 2-cm segments. All hair segments were analyzed simultaneously within the same analytical batch.

3 | Results

GHB was detected in all segments of both hair strands. Alprazolam analysis was restricted to the segments corresponding to the reported period of intake, and the drug was detected in all segments analyzed. Before the chemical hair straightening treatment, endogenous GHB concentrations ranged from 0.019 to 0.165 ng/mg ($n = 29$ segments of 0.5 cm each) as shown in Table 1, whereas alprazolam concentrations ranged from 2.2 to 3.0 pg/mg ($n = 3$ segments of 2 cm each) as shown in Table 2. After the straightening procedure, GHB concentrations ranged from 0.81 to 2.22 ng/mg ($n = 31$ segments of 0.5 cm each), whereas alprazolam concentrations ranged from 1.3 to 1.5 pg/mg ($n = 3$ segments of 2 cm each).

As shown in Figure 1, marked fluctuations in GHB concentrations were observed between corresponding hair segments before and after the straightening treatment, with an increase ranging from approximately 6- to 43-fold. In contrast, alprazolam concentrations showed a decrease after the straightening procedure, ranging from 1.7- to 2-fold, as illustrated in Figure 2.

4 | Discussion

Although the effect of various cosmetic hair treatments on hair analysis has been described in the literature, the evaluation of hair straightening procedures remains limited. Most available studies have focused on thermal straightening rather than on chemical straightening treatments and have been conducted exclusively under in vitro conditions [3, 7–10]. Within this context, several authors have investigated the impact of thermal straightening, namely, the exposure of hair to high temperatures and pressure, on the determination of different substances.

Ettlinger et al. [7] investigated the effect of thermal straightening on ethyl glucuronide (EtG) concentrations in hair. Their results showed marked variability, with EtG concentrations increasing by up to 50.9% in 8 of 15 samples, whereas decreases of up to 79.3% were observed in the remaining 7 samples. A relationship between changes in EtG concentration and hair color was identified, with increases observed in dark hair and decreases in light-colored hair. In a subsequent study by the same author [8], the effect of thermal straightening on the detection of cocaine, benzoylecgonine (BE), cocaethylene, and cannabis markers (Δ9-tetrahydrocannabinol [THC] and cannabinol [CBN]) was assessed. Seventeen hair samples previously positive for cannabis and seven positive for cocaine were subjected to thermal straightening. A decrease in THC was observed in 11 of the 17 samples, whereas an increase in CBN was detected in 15 samples, 2 of which had been negative in the initial analysis, consistent with the thermal conversion of THC to CBN. In all cocaine-positive samples, a reduction in cocaine concentration was observed, accompanied by an

TABLE 1 | Segmental analysis of GHB concentrations (ng/mg) in hair samples obtained before (Strand A) and after (Strand B) chemical straightening treatment.

Strand A segments	[GHB] (ng/mg)	Strand B segments	[GHB] (ng/mg)
		1	0.856
		2	0.855
1	0.033	3	0.880
2	0.025	4	0.931
3	0.019	5	0.837
4	0.041	6	0.858
5	0.031	7	0.819
6	0.064	8	0.960
7	0.073	9	0.818
8	0.082	10	0.912
9	0.104	11	0.806
10	0.145	12	0.951
11	0.133	13	0.976
12	0.079	14	0.933
13	0.054	15	0.833
14	0.148	16	0.905
15	0.158	17	0.953
16	0.108	18	1.379
17	0.116	19	1.017
18	0.107	20	1.216
19	0.095	21	1.061
20	0.123	22	1.551
21	0.114	23	1.741
22	0.108	24	2.036
23	0.165	25	1.491
24	0.107	26	1.952
25	0.085	27	1.577
26	0.098	28	2.216
27	0.109	29	1.918
28	0.111	30	1.371
29	0.110	31	1.355

TABLE 2 | Segmental analysis of alprazolam concentrations (pg/mg) in hair samples obtained before (Strand A) and after (Strand B) chemical straightening treatment.

Strand A segments	[Alprazolam] (pg/mg)	Strand B segments	[Alprazolam] (pg/mg)
A1	3	B1	1.5
A2	2.7	B2	1.5
A3	2.2	B3	1.3

increase in BE. Additionally, a decrease in cocaethylene was detected in four of these samples, three of which had been negative prior to straightening.

Gerace et al. [3] evaluated the effect of hair damage caused by three cosmetic procedures, bleaching, dyeing, and thermal straightening, on the in vitro incorporation of cocaine and BE from an external solution. In this study, all treated hair samples tested positive for cocaine, whereas BE was not detected. Notably, higher cocaine concentrations were measured in bleached and dyed hair compared with untreated hair, highlighting the role of

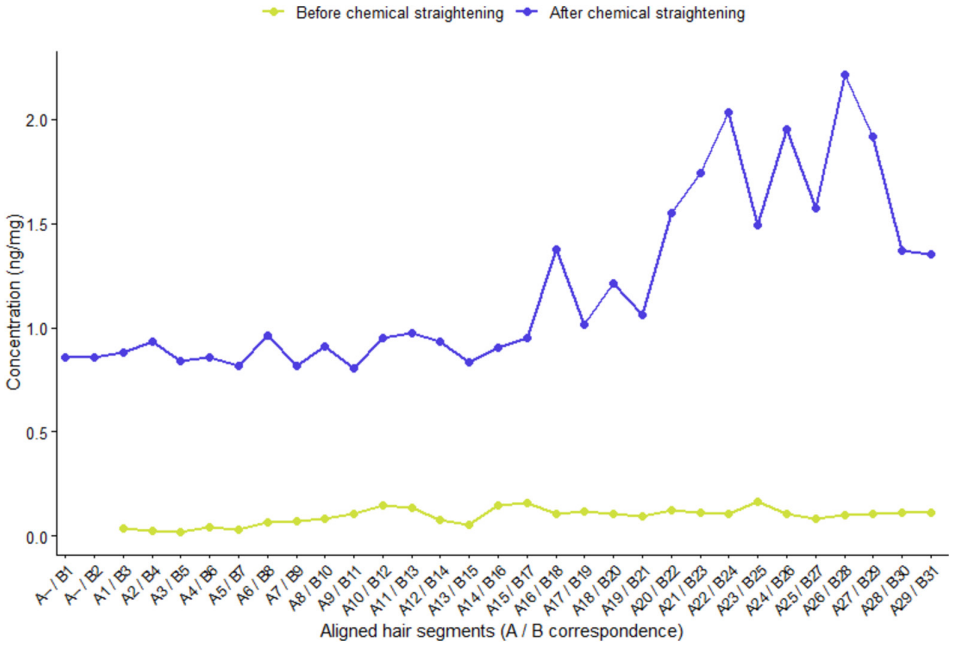

FIGURE 1 | Segmental GHB profile before and after chemical hair straightening.

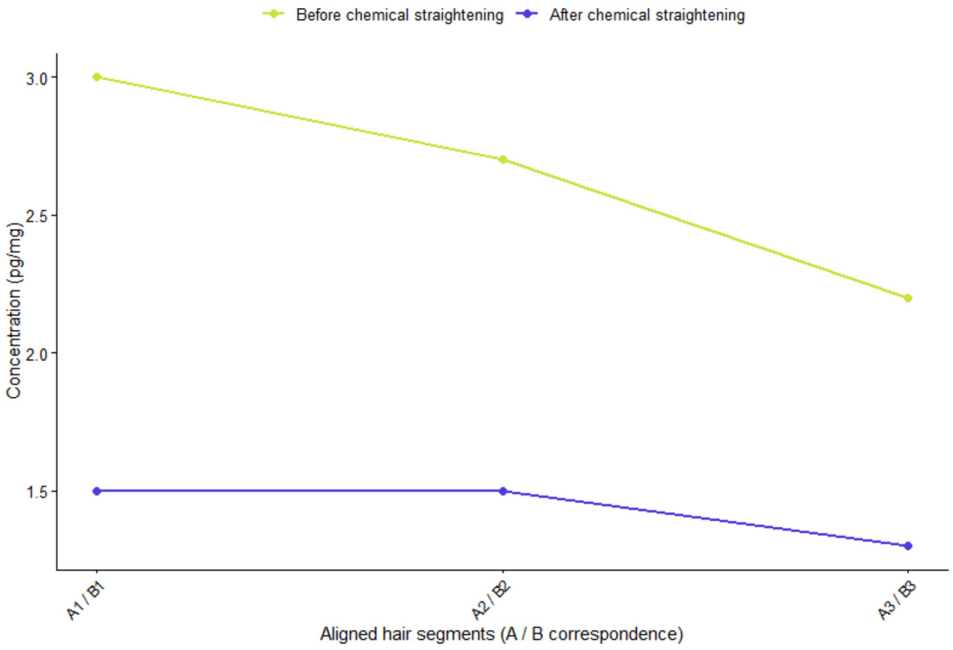

FIGURE 2 | Segmental alprazolam profile before and after chemical hair straightening.

hair damage in facilitating external drug incorporation. In contrast, thermally straightened hair showed cocaine levels comparable to those of untreated hair, indicating that heat-induced damage alone did not significantly enhance cocaine incorporation through external contamination.

Ramírez Fernández et al. [9] conducted an in vitro study to investigate the influence of thermal straightening and bleaching on the determination of endogenous GHB. Their results demonstrated that longer straightening times and consequently greater heat exposure led to increased losses of endogenous GHB. Moreover, the combination of bleaching and thermal straightening resulted in a significant reduction in endogenous GHB levels.

Regarding chemical hair straightening, Pritchett and Phinney [10] examined its impact on the detection of THC, phencyclidine, phenylcyclohexyl piperidine, cocaine, BE, and cocaethylene, although the straightening procedure was performed in vitro. A decrease in the concentration of all analytes was observed, with only 6%–67% of the concentrations measured in untreated hair remaining after straightening. Among the analytes studied, cocaethylene was the most affected by this cosmetic procedure.

Evidence regarding the influence of in vivo chemical hair straightening remains scarce. To date, only the abstract of an oral presentation delivered at the 2024 annual meeting of the International Association of Forensic Toxicologists (TIAFT) has addressed this issue [11]. In that study, the effect of keratin-based hair straightening on caffeine and endogenous GHB was evaluated. Endogenous GHB concentrations decreased from 1.2 to 4.1 ng/mg prior to treatment to 0.8 to 1.3 ng/mg after straightening, which contrasts with the findings of the present case report. It should be noted, however, that the chemical formulations used differed between studies. The authors attributed the decrease in GHB to the incorporation of hydrolyzed keratin into the hair shaft, increasing hair mass and thereby producing a dilution effect. In contrast, caffeine concentrations increased after treatment, from 1.2–9.2 to 1.3–9.3 ng/mg, which was explained by the presence of caffeine as an ingredient in the straightening product and its possible incorporation into the hair during the procedure.

In the present case report, chemical hair straightening was associated with divergent effects on the concentrations of the two analytes investigated. Endogenous GHB concentrations showed a marked increase after the straightening procedure, in contrast to previously published studies reporting decreased GHB levels following cosmetic hair straightening treatments [9, 11]. Notably, the type of straightening procedure differed between studies, with Nowak et al. [11] reporting the use of an acid-based keratin straightening treatment, whereas the subject in the present study underwent an alkaline straightening procedure. Differences in chemical composition and pH between straightening methods may therefore contribute to the observed discrepancies.

Although the underlying mechanisms cannot be elucidated within the scope of this case report, several factors may contribute to this finding, including parent drug-metabolite transformations or altered analyte accessibility due to hair damage following chemical treatment. Both GHB and its glucuronide conjugate have been detected in human hair at endogenous levels, even in individuals without exogenous exposure [12], and glucuronide conjugates are known to be chemically labile, with potential hydrolysis influenced by pH and temperature [13]. In addition, chemical hair straightening induces structural damage to the hair shaft, which may increase analyte accessibility and extraction efficiency [14], an effect that could be particularly relevant in distal hair segments already affected by cumulative environmental and cosmetic damage [15]. In contrast, the observed decrease in alprazolam concentrations after straightening is consistent with most previous literature describing reduced levels of exogenous drugs in cosmetically treated hair [7–11] and falls within the range of expected effects associated with chemical hair damage.

It should be noted that the validated LOQ of the analytical method for GHB is 0.5 ng/mg. In the present case, all concentrations measured in Strand A were below this threshold, although most values were above the LOD (0.1 ng/mg). Therefore, these results should be interpreted with caution from a quantitative standpoint. Consequently, the reported values should not be considered precise determinations of endogenous levels but rather indicators of intraindividual relative changes observed under identical analytical conditions.

This case report was conducted using an in vivo approach, which provides complementary information to in vitro studies investigating the impact of cosmetic treatments on drug concentrations in hair. Although in vitro experimental designs allow strict control of experimental variables, direct comparison between treatments on the same hair matrix, and mechanistic investigation of analyte stability, they may not fully reproduce the complexity of cosmetic procedures performed in routine hairdressing practice, which typically involve sequential chemical application, thermal exposure, neutralization, and washing steps carried out under variable conditions. In addition, only one type of permanent chemical hair straightening procedure (alkaline-based, ammonium thioglycolate formulation) was evaluated. Therefore, the observed analyte-dependent effects cannot be generalized to other straightening techniques, such as hydroxide-based relaxers or acid-based/keratin treatments, which differ substantially in chemical composition and pH. Further studies, including other straightening methods and larger sample sets, would help to better characterize the influence of cosmetic straightening procedures on analyte concentrations in hair.

5 | Conclusion

The increased use of permanent hair straightening highlights the need to better understand its impact on hair drug testing. In the present case report, in vivo chemical hair straightening was associated with divergent effects on analyte concentrations, with a marked increase in endogenous GHB levels and a decrease in alprazolam concentrations. These findings indicate that chemical hair straightening may significantly affect the measured concentrations of both endogenous and exogenous compounds, potentially leading to false-negative or false-positive results.

Currently, only a very limited number of studies have investigated the effects of chemical straightening on analyte concentrations

in hair, and further research is therefore warranted. In addition, systematic documentation of cosmetic treatments should be performed to improve result interpretation, particularly in cases involving single drug exposures or drug-facilitated crimes, where analyte concentrations are commonly low.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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