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CASE REPORT

CRIMINALISTICS

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Forensic Investigation of Formaldehyde in Illicit Products for Hair Treatment by DAD-HPLC: A Case Study

ABSTRACT: The illegal use of formalin (commercial formaldehyde) in cosmetic products harms the health of individuals exposed to this substance. Over the last years, the commercial availability of these products, especially those containing irregular dosage of formaldehyde, has increased in Brazil. This work analyzes some products for hair treatment available in the Brazilian market and verifies their safety. The adopted analytical methodology involved sample derivatization with 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography with ultraviolet detection (UV–VIS) at $\lambda = 365$ nm. The limit of quantification is $2.5 \times 10^{-3\%}$ w/w, and the recovery tests were around 93%. Some of the samples contained high and illegal formaldehyde levels ranging from 9% to 19% (w/w) and others presented suitable concentrations of the analyte. On the basis of the results, this work discusses the efficiency and practicality of this analytical method for forensic purposes.

KEYWORDS: forensic science, formaldehyde, hair straightening, high-performance liquid chromatography, forensic chemistry, 2,4-dinitrophenylhydrazine

Formalin consists of formaldehyde in aqueous solution. The usual commercial concentration is about 37% w/w. It serves as an antibacterial and preservative in some foods and products such as antiseptics, medicines, and cosmetics. Even though some formulations do not directly employ formaldehyde, they contain substances that may release it, as in the case of the degradation of some common materials like polyethylene glycol, which is present in some cosmetics (1–4).

Although formaldehyde occurs in these common matrixes, it is considerably dangerous to the human health. The International Agency for Research on Cancer (IARC) has observed a positive association between exposure to formaldehyde and sinonasal cancer. IARC has also evaluated how formaldehyde relates to cancer of the nasopharynx and leukemia (5–7).

Exposure to formaldehyde air concentrations of about 0.4–3 μg/mL irritates the nose, throat, and eyes and increases tearing. The literature states that formaldehyde is dangerous to life and health at 20 ppm (8).

Formaldehyde toxicity has called for regulations; ANVISA—Agencia Nacional de Vigilância Sanitária (Brazilian Health Surveillance Agency)—allows its use in cosmetics at the maximum concentration of 0.2% for preservative purposes. However, its strengthening effect in hair has led to illegal use of this substance in creams, shampoos, lotions, and pomades (3,9).

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In Brazil, hair products are among the cosmetics with the fastest growing market. Because of the high cost of the permanent straightening process, many consumers seek low-priced substitutes like the so-called progressive hair straightening. Regardless of the regulatory actions taken by the official health agencies, the use of homemade and unregistered creams containing formalin has become a common practice in Brazil (4). Due to this fact, there is a lack of chemical composition labeled in these products.

Another context for Brazilian cosmetics for hair treatment involves the untrue information registered in commercial products whose labels contain a "formaldehyde free" inscription. Actually, some of them present unacceptably concentrations of this substance (10).

Forensic chemistry applies science to ensure law enforcement. Hence, the quantification of formaldehyde in cosmetic products is within the forensic context, because its unsuitable use has legal consequences for those who produce the cosmetics and for victims that present health damages. Indeed, this case study was developed after a suspect of intoxication in some people by the exposure to high concentration of the substance.

The quantification of formaldehyde in cosmetics and its risk assessment has been studied worldwide, and it is possible to observe the same fact related to its concentration. Then, the development of different methodologies for this kind of analysis highlights, and it might include alternative techniques such as ^{1}H NMR spectroscopy and various procedures for sample preparation (10,11,12).

It is possible to determine formaldehyde by different methodologies, including the reaction between 2,4-dinitrophenylhydrazine (DNPH) and the carbonyl group present in the structure of alde-

hydes and ketones. The reaction and the complex as its final product are illustrated in Fig. 1. The latter reaction allows the analysis of this group in different matrixes, such as polymers, fuels, and cosmetics followed via chromatographic techniques (13–16).

High-performance liquid chromatography (HPLC) and gas chromatography have found wide application in analytical chemistry, because these techniques are robust, and accurate, and enable quantification of low levels of the target analyte. Their versatility combined with their sensitivity is applicable for forensic purposes even in the case of complex matrixes (17). In terms of routine analysis in police laboratories, instrumental chromatographic and spectrometric techniques figure as the most employed in forensic analysis (18).

Moreover, the literature reports the use of DNPH derivatization for analyses in cosmetics by HPLC as a specific, sensitive, and selective methodology (10).

Okumura et al. (14) have reported on a simple methodology to derivatize acetaldehydes with DNPH in fuels. Here, we have employed this procedure to analyze commercial samples of hair cosmetics and to determine the formaldehyde concentration in their composition, as well as the experimental conditions described by Wu et al. (3) More specifically, the aim of this work was to detect the presence of formalin in seized commercial samples of hair strengthening lotions and to quantify this substance using DNPH derivatization and HPLC. We will show that this methodology is potentially applicable in industries and police laboratories, due to its reliable accuracy and sensitivity parameters.

Method

All the reagents, such as methanol and H_2SO_4, were analytical grade and were purchased from Aldrich Chemical Company (Milwaukee, WI). The methanol used as mobile phase was HPLC grade and was obtained from Mallinckrodt (Xalostoc, Mexico). Water was demineralized in a Milli-Q Water System (Millipore, CA). 2,4-Dinitrophenylhydrazine (Merck—Darmstadt, Germany) was purified by three successive recrystallizations from methanol. The formaldehyde standard was supplied by Sigma-Aldrich Company (St. Louis, Missouri, USA) in ACS grade.

Chromatographic Parameters

For all the assayed samples, aldehyde was derivatized with DNPH (15), followed by HPLC analyses on the Shimadzu LC 10ADVP (Kyoto, Japan) coupled to a binary pump with UV–VIS detection (SPD-10AVP) at a wavelength of 365 nm.

A Shimadzu ODS 4.6 × 250 mm 10 μm column was used. The mobile phase was 80:20 methanol/water at a flow rate of 1.0 mL/min. For these conditions, a 20-μL loop was established, and the chromatographic run was conducted under isocratic condition.

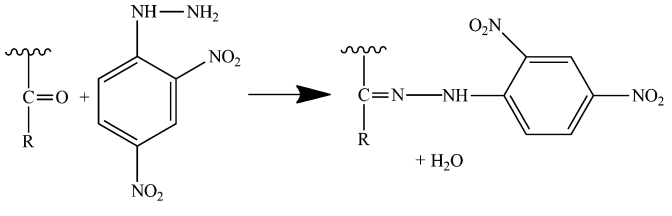

FIG. 1—*Schematic representation of the mechanism for the treatment of the carbonyl group present in the structure of aldehydes and ketones with 2,4-dinitrophenylhydrazine (DNPH), where R = H, or alkyl.*

DNPH Solution, Standard Solutions, and Sample Derivatization

The DNPH solution (0.4%) was obtained by dissolving DNPH (0.2 g; ca. 1.0 mmol) in 50 mL of methanol.

The standard solutions were prepared by dissolving weighed amounts of pure formaldehyde–DNPH, according to procedure reported on literature, when the matrix of interest were fuels (14,19). Aliquots of these solutions were diluted with methanol, and the analytical curve was prepared for concentrations ranging from 2.1 to 184.6 μg/mL.

The formaldehyde–DNPH compound was synthesized by means of the well-known reaction between carbonyl compounds and DNPH in acidic medium. This reaction protonated the carbonyl group (19). To this end, a solution containing 50 mL of DNPH solution 0.4% and 20 mL of H_2SO_4 0.05 mol/L was prepared. Next, the solution containing 40 mg of the sample dissolved in 30 mL of methanol was added. The resulting solution was stirred at room temperature for 30 min. Sample derivatives were filtered through a 0.45-μm filter from Millipore and injected into the HPLC system.

Analysis of Hair Strengthening Lotion Samples

Three different commercial hair strengthening lotions of the same brand name but with distinct fabrication dates were analyzed to determine formaldehyde in their composition. These products were suspected to contain formaldehyde at higher concentrations than those allowed by legislation, especially when some professionals who had worked with these lotions in the city of Ribeirão Preto-SP city presented signs of toxicity.

All the samples were supplied in a blank plastic recipient, as a blank lotion. The products had not passed their end date yet. Their labels did not inform about their chemical composition or the professional responsible for their production.

Other two commercial samples of different brand were analyzed: a sealer lotion (SL) and a lotion for curly hair (LH). Both of them had "formaldehyde free" inscription in their labels and are currently found in Brazilian markets.

In all the samples, an excess of DNPH was added and in this way, even unknown concentrations of formaldehyde—which might be in high concentration—could react and produce the final complex for chromatographic detection.

Results and Discussion

Optimization of Instrumental Conditions

Verification of the best analyte/signal ratio between absorbances at different wavelengths—340, 353, 367, 373 nm—and 397 nm led us to fix detection at 365 nm. The optimized mobile phase, flow rate, and injection volume were 80:20 methanol/water, 1.0 mL/min, and 20 μL, respectively.

Analytical Parameters for Quantification

Plotting of the peak area against the analyte concentration furnished the analytical curve for formaldehyde–DNPH, described by the equation $y = 1.01 \times 10^5 x - 9.09 \times 10^4$ (see insert in Fig. 2) where y is represented in u.A. as the chromatographic response, and x in μg/mL in formaldehyde–DNPH. The linear

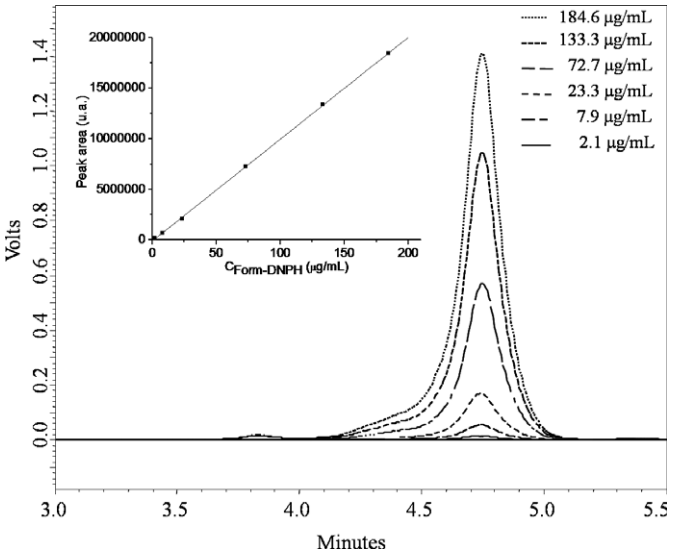

FIG. 2—*HPLC chromatogram of formaldehyde–DNPH at different concentrations. Experimental conditions: see Experimental section. Detection wavelength = 365 nm. The calibration curve is shown as the insert.*

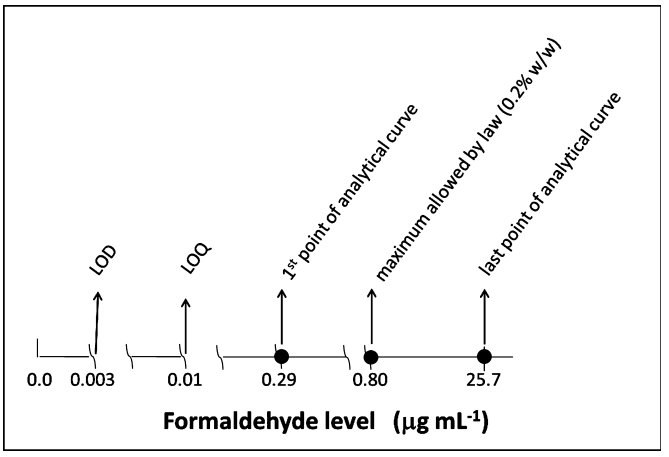

FIG. 3—*A comparison among the formaldehyde levels covered by the analytical curve. LOD, limit of detection; LOQ, limit of quantification.*

correlation coefficient was 0.9993 for formaldehyde–DNPH concentration ranging from 2.1 to 184.6 µg/mL. This work range showed that it should be easy to determine formaldehyde within a long interval of concentrations with good sensitivity and linearity. The recovery experiment for this assay was about 93%, calculated after the role analysis, from a standard solution of formaldehyde–DNPH with a known concentration.

Considering the limit of detection (LOD) as the lowest level of analyte that gives a measurable response and the limit of quantification (LOQ) as the minimum concentration of analyte that provides an accurate response for quantification, statistic treatment provides $LOD = 3 \times \sigma/m$ and $LOQ = 10 \times \sigma/m$, being m the sensibility given by angular coefficient of the equation and σ the standard deviation obtained by linear fitting applied to calibration curve database (20). Calculation of these parameters furnished $LOD = 7.7 \times 10^{-4\%}$ w/w and $LOQ = 2.5 \times 10^{-3\%}$ w/w. It is important to mention that these results come from LOD and LOQ in 0.022 and 0.074 µg/mL for the species formaldehyde–DNPH, respectively. Considering that each µg of formaldehyde–DNPH corresponds to 0.14 µg of formaldehyde in a sample, we can therefore report LOD and LOQ for formaldehyde in 0.003 and 0.010 µg/mL, respectively. Figure 3 illustrates a comparison among LOD, LOQ, the maximum amount of formaldehyde authorized by law, and the work range employed in this methodology.

Hence, it is possible to use the proposed method to determine all the formaldehyde–DNPH derivatives at low levels. Indeed, the next step of this work employed this method to determine formaldehyde in samples of hair strengthening lotions.

Sample Analysis

Using the optimized experimental conditions previously defined, we treated three samples of hair strengthening lotion with DNPH, as described in the experimental section, and then we analyzed 20-µL aliquots of the resulting samples by HPLC. Figure 4 shows a characteristic chromatographic separation obtained for free DNPH (t_r = 3.8 min) and for the formaldehyde–DNPH species (t_r = 4.8 min). This signal for

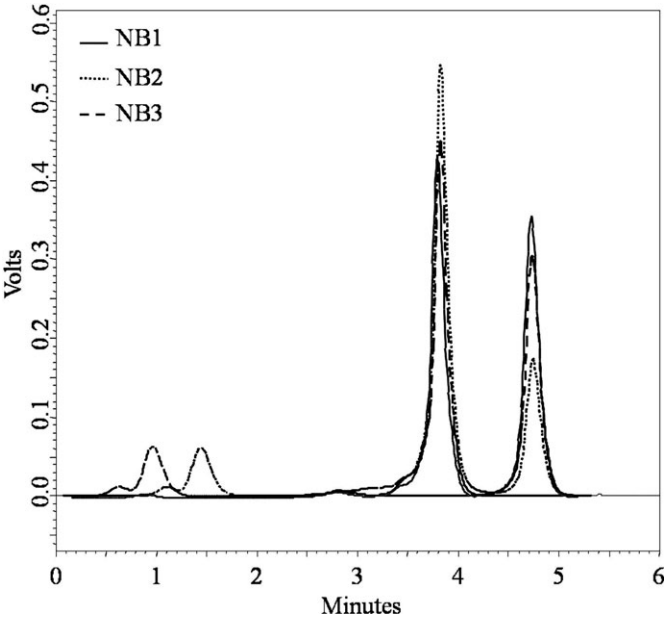

FIG. 4—*HPLC chromatogram of hair strengthening samples after derivatization with 2,4-dinitrophenylhydrazine. Chromatographic peaks at 3.8 and 4.8 min correspond to free DNPH and Form-DNPH species, respectively. Experimental conditions: see Experimental section. Detection wavelength = 365 nm. NB1, NB2, and NB3 = investigated samples.*

free DNPH guaranteed the occurrence of the reaction of all formaldehyde in the samples.

Formaldehyde occurred in all the commercial samples; the analytical curve method provided the content of formaldehyde–DNPH in the samples. Table 1 lists the results obtained in these samples within the interval of uncertainty. As mentioned before, in Brazil, the maximum allowed formaldehyde concentration is 0.2%.

Fortunately, samples named as SL and LH showed very low concentrations of formaldehyde, as an indicator of suitable products for consuming. Both of the samples are found in Brazilian market and are commonly used. The methodology was able to quantify very low values of the complex formaldehyde–DNPH.

On the other hand, some of the samples of cosmetics contained between 9% and 19% of formaldehyde. This value revealed that the production process of these products was not

TABLE 1—*Results obtained from HPLC analyses of the hair strengthening samples.*

Sample	% w/w of Formaldehyde	*n*	CV (%)
NB1	18.4	3	4.2
NB2	9.2	3	3.9
NB3	15.8	3	5.8
SL	0.024	3	3.7
LH	0.029	3	1.7

n = number of chromatographic measurements; SD, standard derivation; CV, coefficient of variation; NB1, NB2, NB3, SL, and LH: investigated samples.

adequate and that quality control was deficient. The results reinforced the importance of overseeing cosmetic products, mainly those of unknown origin. Then, many people were exposure to high levels of formaldehyde, with all its health damages. In this context, the importance of the chemical analysis resolved a legal scenario and was essential to justice sphere.

This analytical method is fast; sample preparation is simple and does not require many steps, whereas the chromatographic run lasts less than 10 min. The product obtained as the intermediate DNPH intermediate is colorful, which indicates its actual formation in the reaction.

The developed methodology afforded reliable results for the determination of formaldehyde, a low molecular weight and volatile compound. In the forensic context, this is a valuable finding—this analysis covers a large analytical range and gives incontestable results within a short period.

Conclusions

The method proposed in this work is potentially applicable in routine Forensic Analysis for both confirmatory purpose and quantification of formaldehyde in suspect products. Indeed, the method is simple, cost-effective, and accurate for derivatization of formaldehyde with DNPH. It is reliable in a large working range. Sample preparation is fast and simple. The application of the developed method to the analysis of some commercial products revealed a large amount of formaldehyde in their composition, which ought to involve health hazards to the consumer and legal complications.

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