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Precursors of oxidative hair dyes in hair colouring formulations

Analytical chemical control of chemical substances and chemical preparations

Arbejdsrapport fra DMU, nr. 175

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Data sheet

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Abstract

In the present study, 18 hair colouring formulations and 4 henna products were analysed for the content of 19 precursors of oxidative hair dyes to check their compliance with the Cosmetic Directive. The henna products were also analysed for the content of Lawsone. Toluene-2,5-diamine was present in 16/18 of hair colouring formulations, resorcinol in 15/18 products, 2-methylresorcinol in 6/18 products, 4-chlororesorcinol in 4/18 products, 2,7-naphthalenediol in 3/18 products, 4-aminophenol in 6/18 products, and 2-aminophenol was present in 1/18 of the products. Only one of the four henna products contained Lawsone. 1,4-phenylenediamine was not present in any of the investigated products. The contents of precursors of hair dyes in the investigated products were in compliance with the Cosmetic Directive.

Keywords:	EU Cosmetic Directive (kosmetik bekendtgørelse), hair colouring formulations, henna, oxidation hair dyes, phenylenediamines, toluenediamines, aminophenols, resorcinol, 4-chlororesorcinol, 2-methyl resorcinol, 1-naphthol, 2,7-naphthalenediol, hydroquinone, 2-amino-3-hydroxypyridine, 4-hydroxy-2-methylaniline, Lawsone
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Contents

Summary

The EU Cosmetic Directive regulates the contents of precursors of oxidative hair dyes and colorants in hair care formulations. In the present study, 18 hair colouring formulations and 4 henna products were analysed for the content of 19 precursors of hair dyes to check their compliance with the Cosmetic Directive. The henna products were also analysed for the content of Lawsone. Furthermore, the henna products and two single-component hair dyeing formulations were analysed for the contents of some permitted orange, red and yellow colorants.

Toluene-2,5-diamine was present in 16/18 of hair colouring formulations, resorcinol in 15/18 products, 2-methylresorcinol in 6/18 products, 4-chlororesorcinol in 4/18 products, 2,7-naphthalenediol in 3/18 products, 4-aminophenol in 6/18 products, and 2-aminophenol was present in 1/18 of the products. Three of the hair colouring formulations were found to contain 3-aminophenol and one of the products contained 2-amino-3-hydroxypyridine. However, the identification and determination of 3-aminophenol and 2-amino-3-hydroxypyridine in five of the products labelled for the contents of these substances could not be performed, because of the overlap of the chromatographic peaks of these two compounds, when analysed by the HPLC method used in the present study. All four henna products and the two single-component hair colouring formulations did not contain any of the target precursors of hair dyes or target colorants. Only one of the four henna products contained Lawsone. 1,4-phenylenediamine was not present in any of the investigated products.

The contents of precursors of hair dyes in the investigated products were in compliance with the Cosmetic Directive.

Present work has been performed as technical support to the Danish Environmental Protection Agency.

Resumé

Indholdet af præcursorer af oxidations hårfarver og farvestoffer i hårfarvnings produkter er reguleret af Miljø- og Energiministeriets bekendtgørelse om kosmetiske produkter. I nærværende undersøgelse er indholdet af 19 præcursorer af oxidations hårfarver bestemt i 18 hårfarvningsprodukter og fire henna produkter for at kontrollere om disse er i overensstemmelse med bekendtgørelse om kosmetik produkter. Henna produkterne er også undersøgt for indholdet af Lawsone. Herudover er indholdet af udvalgte røde, gule og orange farvestoffer er undersøgt i alle henna produkter samt i to enkelt komponent hårfarvnings produkter.

Der er fundet toluene-2,5-diamin i 16/18 hårfarvnings produkter, resorcinol i 15/18 produkter, 2-methylresorcinol i 6/18 produkter, 4-chlororesorcinol i 4/18 produkter, 2,7-naphthalenediol i 3/18 produkter, 4-aminophenol i 6/18 produkter, og 2-aminophenol er fundet i 1/18 produkter. Tre af de hårfarvningsprodukter indeholdt 3-aminophenol, og et af produkterne indeholdt 2-amino-3-hydroxypyridine. Identifikationen af 3-aminophenol og 2-amino-3-hydrodxypyridin var dog ikke muligt i fem produkter, der var mærket for at indeholde disse stoffer. Årsagen hertil er at de kromatografiske toppe af disse to stoffer overlapper ved den anvendte HPLC metode. Alle fire henna produkter og de to enkeltkomponent hårfarver indeholdt ingen af de undersøgte præcursorer eller farvestoffer. Kun en af de fire henna produkter indeholdt 0,24% Lawsone. Ingen af de undersøgte produkter indeholdt 1,4-phenylenediamin.

Indholdet af præcursorer af oxidations hårfarver i de undersøgte produkter er i overensstemmelse med kosmetik bekendtgørelsen.

Undersøgelsen er udført som faglig bistand til Miljøstyrelsen.

1 Introduction

Hair dyeing formulations belong to three categories, i.e. for temporary, for semi-permanent and for permanent colouring of hair. The permanent hair dyeing formulations, also called oxidative hair dyes, are generally marketed as two-component kits. One component contains the dye precursors (such as *p*-phenylene diamine, 2,5-diaminotoluene, N,N-bis(2-hydroxymethyl)-p-phenylene diamine, p-aminophenol etc.) and couplers (such as resorcinol, chlororesorcinol, methyl resorcinol, α-naphthol, m-aminophenol, m-phenylenediamine, etc.) in an alkaline soap or syndet base, and the other component is a stabilised solution of hydrogen peroxide (1). The two components are mixed immediately prior to use. The precursors and peroxide diffuse into the hair shaft, where colour formation takes place after a cascade of chemical reactions. The dye precursors are oxidised by hydrogen peroxide to p-benzoquinone imines / diimines, which are reactive intermediates in the colour formation. The couplers, which are relatively stable to hydrogen peroxide, undergo rapid reaction with the intermediates resulting in dinuclear, trinuclear or polynuclear colorant molecules (2). These molecules are too large to escape from the hair structure. Hydrogen peroxide in the oxidative hair dyeing formulations also serves as bleaching agent for the natural pigment of the hair. The colour formation (shades) is dependent on the precursors and direct dyes present in the dyeing solution, pH of the dyeing solution and the time of contact of the dyeing solution with the hair.

The contents of certain precursors of oxidative hair dyes in hair colouring formulations are restricted according to Annex III of the EU Cosmetic Directive / Danish Statutory Order (3, 4). Moreover, some substances are banned in these formulations according to Annex II of the Directive. To check the compliance of permanent hair dyeing formulations with the Cosmetic Directive, the contents of some selected precursors of oxidative hair dyes (Table 1) have been analysed in the commonly used hair dyeing products in the present investigation. Some commonly used henna products were included in the study to check whether these products contained target oxidative hair dye precursors or some colorants.

The present work has been performed as technical support to the Danish EPA.

Table 1: The investigated precursors of oxidative hair dyes.

Dye precursor	***CAS Nr.***	***Maximum authorised concentration***
1,4-phenylenediamine (PDA)	106-50-3	6% (total PDA including derivatives and salts)
1,3- phenylenediamine	108-45-2	not permitted
1,2- phenylenediamine	95-54-5	not permitted
Ttoluene-2,5-diamine (2,5-TDA)	95-70-5	10% (total TDA including derivatives and salts)
Toluene-2,6- diamine	823-40-5	not permitted
Toluene-2,4- diamine	95-80-7	not permitted
Toluene-3,4- diamine	496-72-0	10% (total TDA including derivatives and salts)
2-aminophenol	95-55-6	2%
3-aminophenol	591-77-5	2%
4-aminophenol	123-30-8	
Resorcinol	108-46-3	5%
2-methyl resorcinol	608-25-3	2%
4-chlororesorcinol	95-88-5	
1-naphthol	90-15-3	2%
2,7-naphthalenediol	582-17-2	1%
1,5-naphthalenediol	83-56-7	1%
Hydroquinone	123-31-9	0.3%
2-amino-3-hydroxy pyridine	16867-03-1	
2,3,5,6-tetraaminopyridine	5392-28-9	
2-hydroxy-1,4-naphthalenedione (Lawsone)*	83-72-7	

* Lawsone is a natural constituent of henna. It is not a precursor of oxidative hair dyes.

2 Products

DEPA collected 22 products for hair dyeing for non-professional use from Danish retail outlets in May 2002. Of these, 18 products were hair colouring formulations and four of the products were henna/modified henna. Among the hair colouring formulations, 16 products were 2-3 component systems, one of which contained precursors of oxidation hair dyes. The remaining two hair colouring formulations were single component products. The products analysed in the present investigation are described in Table 2.

Table 2: The investigated hair dyeing formulations

DMU Reg. No.	***MST No.***	***Product Identification***	***Production/ Import***
2-326	636	LIVE Toner, colour 62	Germany
2-327	637	LIVE Toner, colour 99	Germany
2-328	638	VITAL Colors, colour 86	Germany
2-329	639	Vital Colors, colour 30	Germany
2-330	640	POLY Brilliance, colour 878	Germany
2-331	641	POLY Brilliance, colour 890	Germany
2-332	642	Country Colors, colour 63	Germany
2-333	643	LIVE unlimited colors, colour 10	Germany
2-334	644	LIVE unlimited colors, colour 99	Germany
2-335	645	Casting Color Spa, colour 31	France
2-336	646	Casting Color Spa, colour 44E	France
2-337	647	Réctical Preference, colour 1	France
2-338	648	Féria Color, colour 3.66	France
2-339	649	Féria Color, colour 5.60	France
2-340	650	Natéa, colour 4.26	France
2-341	651	Natéa, colour 10.13	France
2-342	652	Open color, colour 7.4	France
2-343	653	Open color, colour 4.45	France
2-344	654	Sort Henna	Denmark
2-345	655	Henné Color, colour Aubum	France
2-346	656	Henné Color, colour black	France
2-347	657	Henna Dark Hair	Denmark

3 Analysis

The contents of hair dye precursors were analysed by high performance liquid chromatography (HPLC) employing diode array detection as described before (5, 6). The method was checked for sensitivity, calibration range, repeatability of determination and recovery from the relevant samples spiked with target substances.

All 24 samples were first analysed for the identification of the target precursors of oxidative hair dyes. This was followed by the determination of the identified substances in freshly prepared sample extracts. Calibration curves used for the determination were prepared by the analysis of freshly prepared calibration solutions, at three concentration levels, together with sample extracts. Two extracts of each sample were prepared for the analysis and each sample extract was analysed two times by HPLC.

The HPLC method for the analysis of oxidative hair dye precursors was also used for the analysis of Lawsone in the henna samples. The method was validated for the identification and determination of Lawsone: limit of detection, calibration curve, repeatability and recovery from a henna sample. The four henna samples were extracted in two ways: 1) ultrasonic treatment of 1 g sample in 10 ml HPLC mobile phase, and 2) the samples were cooked with water according to the prescriptions described on the respective packages, followed by extraction of the mixture with the HPLC mobile phase. Fresh sample extracts were analysed by HPLC for the identification and determination of Lawsone.

All four henna products and the two single component hair dyeing formulations (Sample No. 2-0326 and 2-0327) were analysed for the content of colorants: commonly used colorants in cosmetic products as described elsewhere (7). The method for the identification of colorants was exactly the same as described before (7). The validity of the method was checked by the analysis of reference colours CI 15850, CI 19140 and CI 45370. The identification of Lawsone in henna extracts was also confirmed by the analytical method used for colorants.

4 Results and Discussion

The previously described method for the analysis of precursors of hair dyes (5, 6) was checked for sensitivity, calibration range, repeatability of determination and recovery from the relevant samples spiked with target substances. The detection limits of various target substances were in the range 0.0005% - 0.0025%. The calibration curves of the target substances were linear (r^2 >0.998) in the investigated concentration range 0.001% - 0.05%. The repeatability of determination checked by ten repeated injections of 2,5-TDA, 1,4-PDA, 4-aminophenol and resorcinol at two concentration levels, 0.005% and 0.02%, were within 6%. The recovery of these four substances from two samples spiked to concentration levels 0.1% and 1% were 87-98%.

The target substances in the sample extracts were identified by matching both the retention time and the 220 nm - 400 nm UV-spectrum of the peaks in the HPLC chromatogram with those of standard target substances, analysed under the identical conditions. The HPLC chromatogram and the spectrum match of identified precursors of oxidative hair dyes in two of the products are shown in Figures 1 and 2.

The concentration of the identified substances was determined using the calibration curves prepared by the analysis of the calibration solutions in the same *sample set* in which sample extracts were also analysed. The content of precursors of oxidative hair dyes, among the target substances, in the investigated products is described in Table 2. All four henna products and two of the hair colour formulations (single-component formulations) did not contain any of the target precursors of oxidative hair dyes. Toluene-2,5-diamine was present in 16/18 of hair colour formulation at trace level (not determined due to interference) to maximum 3.63%. Resorcinol (0.002 - 1.13%) was present in 15/18 of the hair colour formulations, 2-methylresorcinol (0.11 - 1.55%) in 6/18 products, 4-chlororesorcinol (0.01 - 0.26%) in 4/18 products, 2,7-naphthalenediol (0.03 - 0.83%) in 3/18 products, 4-aminophenol (0.31- 1.12%) in 6/18 products, and 2-aminophenol (0.08%) was present in 1/18 of the products. Three of the hair colour formulations were found to contain 0.01- 0.94% 3-aminophenol and one of the products contained 2.06% 2-amino-3-hydroxypyridine. However, the identification and determination of 3-aminophenol and 2-amino-3-hydroxypyridine in five of the products labelled for the contents of these substances could not be performed, because the chromatographic peaks of these two compounds overlap in the HPLC analysis by the present method.

The results of the present investigation indicated that toluene-2,5-diamine was the commonly used precursor of hair dyes in the investigated products: all 2-and 3-component formulations were composed of a component that contained 2,5-TDA. Another well-known precursor of hair dyes, i.e. 1, 4-phenylenediamine (PPD) was not detected in any of the investigated products. Among the couplers, resorcinol appears to be commonly used as precursor of hair dyes, fol-

lowed by m-aminophenol, 2-methyl resorcinol and 4-chloro-resorcinol.

Natural henna is known to contain 1-2% Lawsone, which is responsible for colouring skin orange-red. The identification of Lawsone in the henna extracts was performed by two HPLC methods: by the method used for the precursors of oxidative hair dyes as well as by the method used for the analysis of colorants. Only one of the henna products was found to contain Lawsone (0,24%). Thus, it is obvious that some products containing Lawsone-free henna are marketed as henna. It was, therefore, interesting to investigate the content of synthetic colorants present in the Lawsone-free henna products. A limited investigation revealed that none of the commonly used red, orange and yellow colorants in cosmetic product (7) were present in the four henna products. The investigation of content of colorants in the two single-component hair colour formulations revealed that these products also did not contain any of the target red yellow and orange colorants.

The contents of precursors of oxidative hair dyes in the investigated products were in compliance with the Cosmetic Directive.

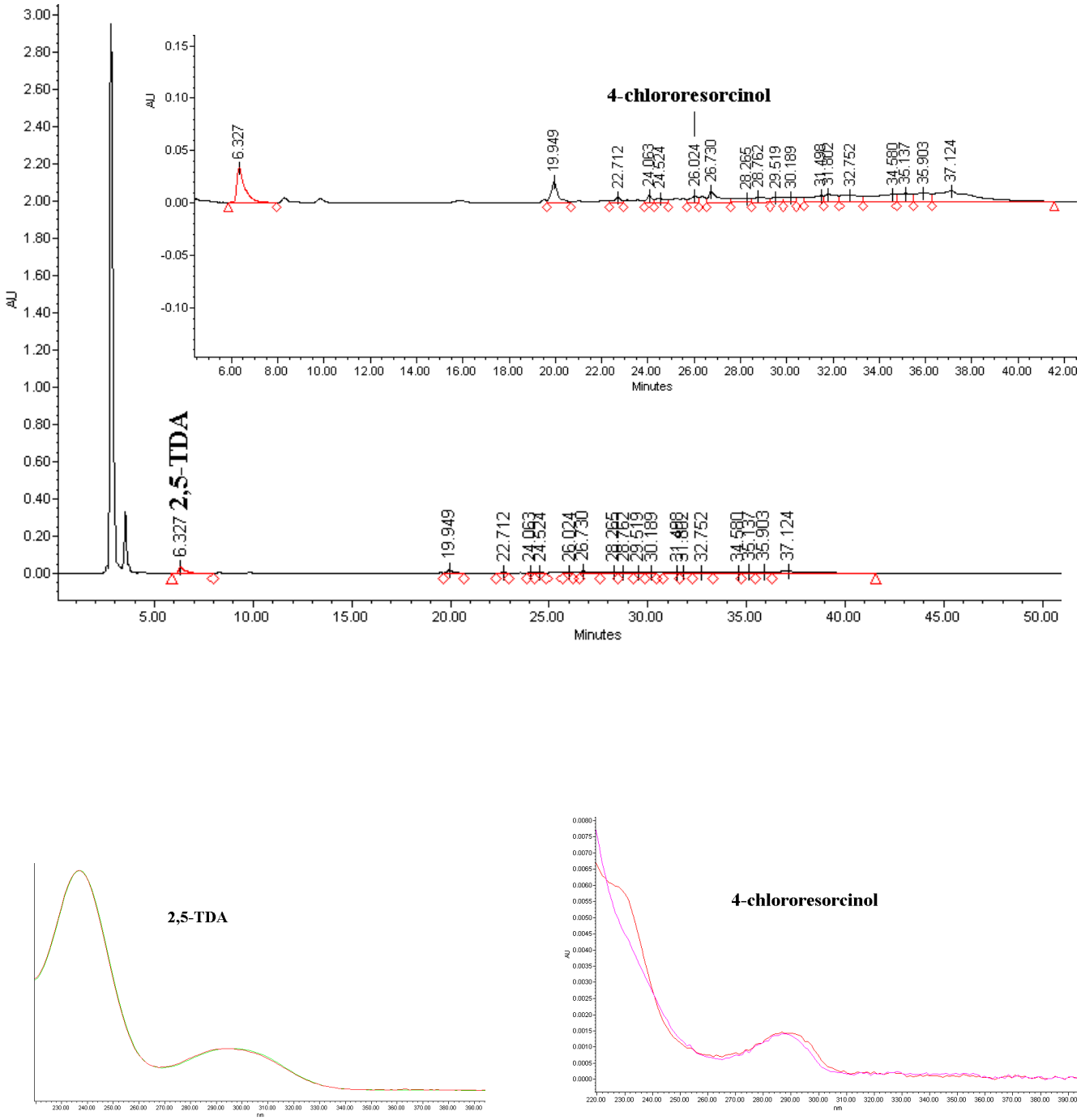

Figure 1: HPLC chromatogram of sample 2-0330 and UV spectrum match of the identified substances.

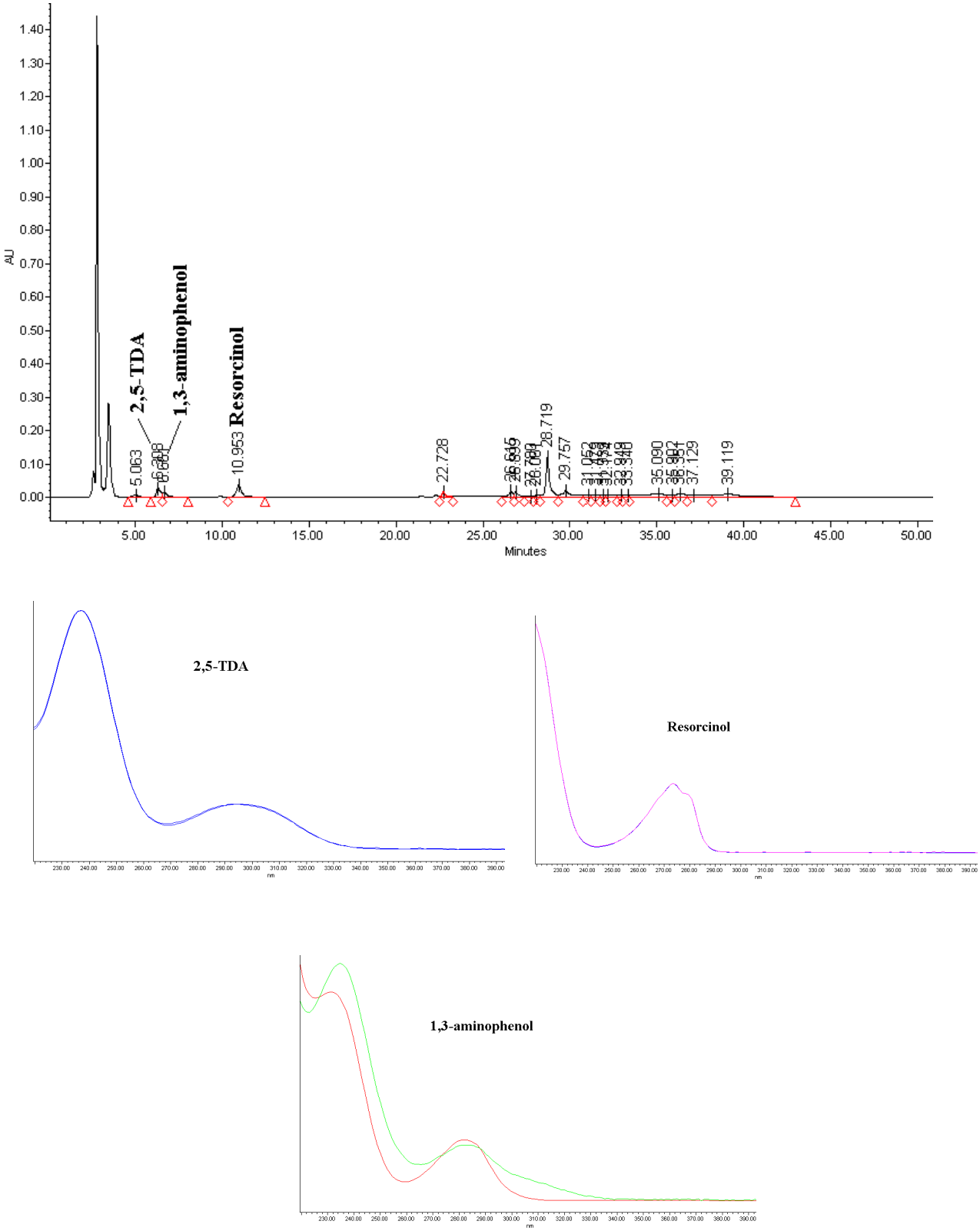

Figure 2: HPLC chromatogram of sample 2-0341 and UV spectrum match of the identified substances.

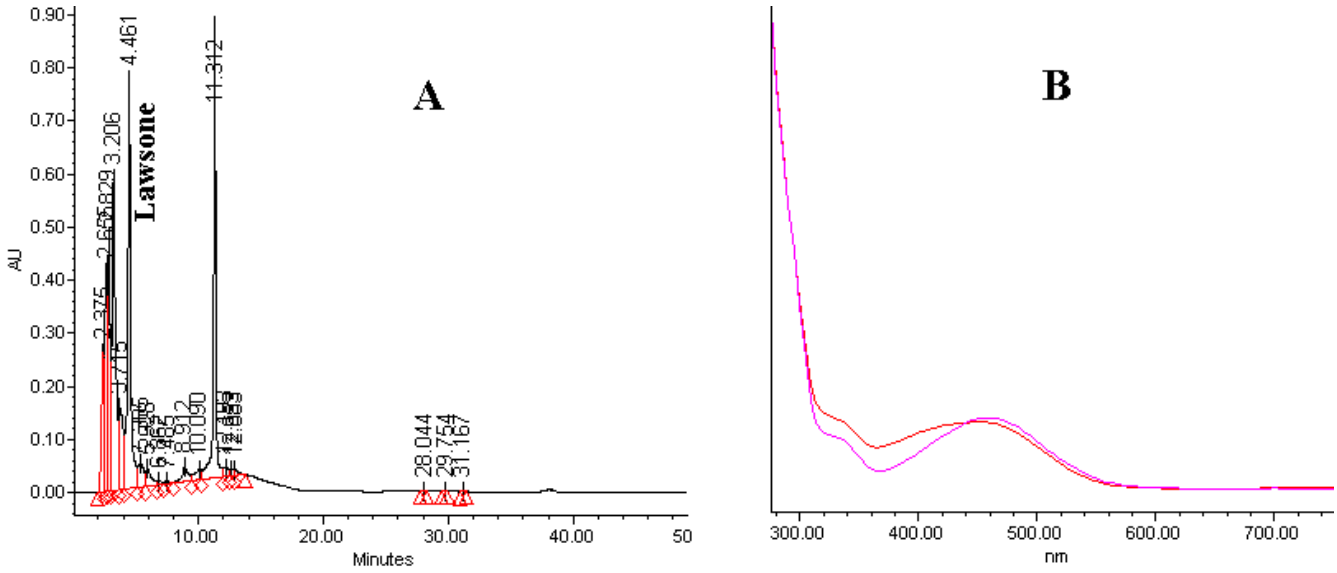

Figure 3: Identification of Lawson in sample 2-0345. HPLC chromatogram (A) and spectrum match (B)

Table 2: The contents of target precursors of oxidative hair dyes in the investigated products

DMU No.	***MST No.***	***Labelling***	***Identified***	***Concentration %(m/m)***	***Remarks***
2-326	636	None of the target substances	None of the target substances	-	
2-327	637	None of the target substances	None of the target substances	-	
2-328	638	Toluene-2,5-diamine Resorcinol 2-methylresorcinol 2,7-naphthalenediol 3-aminophenol* 2-amino-3-hydroxypyridine*	Toluene-2,5-diamine Resorcinol 2-methylresorcinol 2,7-naphthalenediol	1,1742 0,1433 0,4330 0,0249	*
2-329	639	May contain Toluene-2,5-diamine Resorcinol 4-chlororesorcinol 2- naphthalenediol 2-methylresorcinol 3-aminophenol* 2-amino-3-hydroxypyridine*	 Toluene-2,5-diamine Resorcinol 4-chlororesorcinol	 0,1286 0,0216 0,2241	*
2-330	640	Toluene-2,5-diamine Resorcinol 2-methylresorcinol 2-7-naphthaenediol	Toluene-2,5-diamine Resorcinol 2-methylresorcinol 2,7-naphthaenediol	Trace 0,2420 0,6923 0,833	2,5-TDA could not be determined due to interference
2-331	641	Toluene-2,5-diamine Resorcinol 2-methylresorcinol 2,7-naphthalenediol	Toluene-2,5-diamine Resorcinol 2-methylresorcinol 2,7-naphthalenediol	2,0166 0,4625 0,1063 0,4645	

*****HPLC peaks of and 3-aminophenol and 2-amino-3-hydroxypyridine overlap. Therefore, these substances can not be identified when both of these are present in a product

Table 2: continued.

DMU No.	***MST No.***	***Labelling***	***Identified***	***Concentration %(m/m)***	***Remarks***
2-332	642	May contain Toluene-2,5-diamine Resorcinol 2-methylresorcinol 4-chlororesorcinol 2,7-naphthalenediol 3-aminophenol* 2-amino-3-hydroxypyridine*	 Toluene-2,5-diamine Resorcinol 2-methylresorcinol 4-chlororesorcinol	 0,0678 0,2952 1,5493 0,0472	*
2-333	643	May contain Toluene-2,5-diamine Resorcinol 2-methylresorcinol 4-chlororesorcinol 2,7-naphthalenediol 1-naphthol 3-aminophenol* 2-amino-3-hydroxypyridine*	 Toluene-2,5-diamine 4-chlororesorcinol	 0,0783 0,0107	*
2-334	644	Toluene-2,5-diamine Resorcinol 4-chlororesorcinol	Toluene-2,5-diamine Resorcinol 4-chlororesorcinol	3,6282 1,1278 0,2617	
2-335	645	Toluene-2,5-diamine Resorcinol 2-aminophenol 4-aminophenol 2-methylresorcinol	Toluene-2,5-diamine Resorcinol 2-aminophenol 4-aminophenol 2-methylresorcinol	1,1045 0,1828 0,0756 1,196 0,3466	
2-336	646	Toluene-2,5-diamine Resorcinol 3-aminophenol 4-aminophenol	Toluene-2,5-diamine Resorcinol 3-aminophenol 4-aminophenol	0,0918 0,0708 0,0099 0,3067	

*****HPLC peaks of and 3-aminophenol and 2-amino-3-hydroxypyridine overlap. Therefore, these substances can not be identified when both of these are present in a product

Table 2: Continued.

DMU No.	***MST No.***	***Labelling***	***Identified***	***Concentration %(m/m)***	***Remarks***
2-337	647	Toluene-2,5-diamine Resorcinol 3-aminophenol	Toluene-2,5-diamine Resorcinol 3-aminophenol	3,9881 0,3904 0.9355	
2-338	648	Toluene-2,5-diamine Resorcinol 4-aminophenol 2-amino-3-hydroxypyridine	Toluene-2,5-diamine Resorcinol 4-Aminophnol 2-amino-3-hydroxypyridine	1,9308 0,0023 0,4438 2,0642	Interference between 2,5 TDA and 2-amino-3-hydroxypyridine
2-339	649	Toluene-2,5-diamine Resorcinol 4-aminophenol	Toluene-2,5-diamine Resorcinol 4-aminophenol	1,1132 0,8833 0,3791	
2-340	650	2,5-TDA Resorcinol	Toluene-2,5-diamine Resorcinol	1,2393 0,0065	
2-341	651	Toluene-2,5-diamine Resorcinol 3-aminophenol	Toluene-2,5-diamine Resorcinol 3-aminophenol	0,0697 0,0682 0,0137	
2-342	652	Toluene-2,5-diamine Resorcinol 4-aminophenol 2-methylresorcinol	Toluene-2,5-diamine Resorcinol 4-aminophenol 2-methylresorcinol	0,1967 0,0375 0,4578 0,1113	
2-343	653	Toluene-2,5-diamine Resorcinol 4-aminophenol 3-aminophenol* 2-amino-3-hydroxypyridine*	Toluene-2,5-diamine Resorcinol 4-aminophenol	1,2759 0,1743 1,1739	*
2-344	654	Pulveriseret blad fra henna busk og intet andet	None of the target substances	-	
2-345	655	Henne´ natural 98%, Lawsonia inermis	Lawsone	0,2478%	
2-346	656	Indigofera augentea, henna 5%	None of the target substances	-	
2-347	657	Indigofera tinctoria, Henne´dark	None of the target substances	-	

*****HPLC peaks of and 3-aminophenol and 2-amino-3-hydroxypyridine overlap. Therefore, these substances can not be identified, when both of these are present in a product

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