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Determination of Preservatives in Finger Paints with HPLC

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Introduction

Finger paints allow children to draw without having to use a paint brush. Besides water as solvent, ingredients such as binding agents, extenders, humectants, surfactants, colourants, embittering agents and preservatives are used in commercial products. Water based finger paints must have preservatives in order to avoid microbiological spoilage. Some of these are known allergens and therefore legal restrictions, similar to those applied for cosmetics, are under way to regulate their use (1). As far as we know, there is only one paper discussing the determination of preservatives, namely formaldehyde, in finger paints (2). We therefore present our two HPLC methods which together allow for the determination of 42 preservatives. The first method is used for the determination of 40 uv-absorbing, mostly aromatic compounds such as parabenes, aromatic alcohols and halogenated aromatics. The second method is used especially for the determination of two polar isothiazolinone compounds, which are applied in far lower concentrations.

Methods

Materials and instruments

Table-top centrifuge, Hereaus Primo (BGB, Anwil); sonicator, Branson 3510 (Merck, Zürich); 0.45 µm nylon filter for HPLC, Titan (Schmidlin, Neuheim); vortex (Bender & Hobein, Basel); quaternary gradient HPLC system consisting of: low pressure mixing quaternary gradient pump (P4000, narrow bore configuration), autosampler (AS 3000), photo diode array detector (UV 6000LP fitted with 2 µl 10 mm flowcell) and data station (Chromquest) all from Thermo Finnigan, Allschwil; column for method 1: Phenomenex Luna, phenyl-hexyl, 3 µm, 100 × 4.6 mm; guard column: Phenomenex phenyl-propyl; column for isothiazolinones:

Phenomenex Prodigy ODS-3, 3 μ m, 100 \times 4.0 mm; guard column: Phenomenex C18, all from Brechbühler, Schlieren.

Reagents

Formic acid, 98–100%, e.g. Merck 100264; methanol for HPLC gradient grade, e.g. SDS 09337G16; acetonitrile for HPLC gradient grade, e.g. Lichrosolv, Merck 00030; tetrahydrofuran for HPLC., e.g. Merck (08101); demin. water for HPLC.

Reference materials for method 1

Reference materials for method 1 are subdivided into *A*: those preservatives which are allowed in finger paints (1), *B*: preservatives, which are used in cosmetics but are not on the positive list for finger paints (3) and *C*: preservatives, which are either explicitly forbidden in cosmetics (4) or are not used in cosmetics and are not on the positive list (3).

A

1. benzoic acid, 99.9% (Merck 135) CAS-Nr. 65-85-0,
2. methyl benzoate, $\geq 99\%$ (Merck 106059) CAS-Nr. 93-58-3,
3. ethyl benzoate, $\geq 99\%$ (Fluka 12360) CAS-Nr. 93-89-0,
4. propyl benzoate, 99% (Aldrich 30, 700-9) CAS-Nr. 2315-68-6,
5. butyl benzoate, $\geq 98\%$ (Fluka 12410) CAS-Nr. 136-60-7,
6. sorbic acid, $\geq 99.0\%$ (Merck 100662) CAS-Nr. 110-44-1,
7. 2-phenylphenol, $> 98\%$ (Fluka 54890) CAS-Nr. 90-43-7,
8. 4-hydroxybenzoic acid, 99% (Fluka 54630) CAS-Nr. 99-96-7,
9. 4-hydroxy-methyl benzoate, $\geq 98\%$ (Fluka 54750) CAS-Nr. 99-76-3,
10. 4-hydroxy-ethyl benzoate, $\geq 99\%$ (Fluka 54660) CAS-Nr. 120-47-8,
11. 4-hydroxy-n-propyl benzoate, $\geq 99\%$ (Fluka 54790) CAS-Nr. 94-13-3,
12. 4-hydroxy-n-butyl benzoate, $\geq 99\%$ (Fluka 54680) CAS-Nr. 94-26-8,
13. 4-hydroxy-isopropyl benzoate, $\geq 98\%$ (Avocado 13930) CAS-Nr. 4191-73-5
14. 4-hydroxy-isobutyl benzoate, (K&K 202548) CAS-Nr. 4247-02-3,
15. 4-hydroxy-benzyl benzoate, $\geq 98\%$ (Fluka 54670) CAS-Nr. 94-18-8,
16. dehydroacetic acid, 98% (Aldrich D290-0) CAS-Nr. 520-45-6,
17. 2,4-dichlorobenzyl alcohol, 99% (Aldrich 14,666-8) CAS-Nr. 1777-82-8,
18. triclocarban, 99% (Aldrich 10,593-7) CAS-Nr. 101-20-2,
19. 4-chloro-3,5-xyleneol, 99.5% (Sigma C-4394) CAS-Nr. 88-04-0,
20. 2-phenoxyethanol, $\geq 98\%$ (Fluka 77699) CAS-Nr. 122-99-6,
21. climbazole, 99.7% (Riedel-de Haën 45401) CAS-Nr. 38083-17-9,
22. benzyl alcohol, $> 97\%$ (Merck 981) CAS-Nr. 100-51-6,
23. bromochlorophene, 98% (Merck 3281) CAS-15435-29-7,
24. 3-methyl-4-isopropyl phenol, 99% (Aldrich 31,643) CAS-Nr. 3228-02-2,
25. chlorophene, 98% (Chem Service PS-170) CAS-Nr. 120-32-1
26. chlorophenesine, 99% (Avocado 17386) CAS-Nr. 104-29-0

B

27. 1-phenoxy-2-propanol, $\geq 93\%$ (Aldrich 48,442-3) CAS-Nr. 770-35-4
28. 4-chloro-m-cresol, $\geq 98\%$ (Fluka 24940) CAS-Nr. 35421-08-0
29. triclosan, $\geq 99\%$ (Ciba SC) CAS-Nr. 3380-34-5
30. phenol, 99.5% (Merck 206) CAS-Nr. 108-95-2
31. salicylic acid, $\geq 99\%$ (Fluka 84210) CAS-Nr. 69-72-7
32. phenyl salicylate $\geq 98.0\%$ (Fluka 84340) CAS-Nr. 118-55-8
33. dichlorophene, $\geq 98\%$ (Riedel-de Haën 35992) CAS-Nr. 97-23-4
34. chloramine-T tri hydrate, $\geq 99\%$ (Merck 102426) CAS-Nr. 127-65-1

C

35. hexachlorophene, 99% (Aldrich 23,458-3) CAS-Nr. 70-30-4
36. 2-n-octyl-4-isothiazolin-3-one, 45–48% (Kathon 893F, Christ Chemie) CAS-Nr. 26530-20-1
37. 4,5-dichloro-2-octyl-3(2H)-isothiazolone, 99% (Kathon 287T, Christ Chemie) CAS-Nr. 64359-81-5
38. 1,2-benzisothiazolone, 100% (Acima) CAS-Nr. 2634-33-5
39. 4-Chloro-2-isopropyl-5-methylphenol, 99% (Lancaster) CAS-Nr. 89-68-9
40. Methyl salicylate, $\geq 99\%$ (Fluka) CAS 119-36-8

Reference materials for method 2 (Isothiazolinones):

41. 2-Methyl-4-isothiazolin-3-one (MI), $> 98\%$ (Sigma M-6045) CAS-Nr. 26172-54-3
42. 5-chloro-2-methyl-4-isothiazolin-3-one (MCI), 1.1% (Fluka 00344 or Kathon CG, Christ Chemie) CAS-Nr. 26172-55-4 (note: MCI is only available as a mixture with MI)

Procedures

Extractant for method-1 preservatives: 1% methanolic formic acid

Fill 500 ml methanol in a 1000 ml flask. Add 10 ml of formic acid, shake and dilute to 1000 ml with methanol.

Extractant for MI and MCI: 1% aqueous formic acid

Dilute 10 ml of formic acid to 1000 ml with water.

Calibration solutions for HPLC

Stock solutions for method 1 preservatives

Note: For routine analysis it is convenient to calibrate with the most often found preservatives only. Prepare 20 ml solutions of 100 mg of compounds 1, 6,

8–15, 26 and 29 and 200 mg of compounds 20 and 22 in methanol. Solutions can be stored in a refrigerator for at least one month.

Stock solution for MI/MCI

Weigh 600 mg of a MI/MCI mixture with a certified content in a flask and dilute to 10 ml with methanol (600 mg of mixture contains 0.375% MI and 1.15% MCI).

Calibration solutions for method 1 preservatives

Pipette 1 ml of each stock solution into the same flask and dilute to 20 ml with 0.1% methanolic formic acid (calibration solution (CS) 1: 2.5 µg/10 µl). Dilute 5 ml of CS 1 to 10 ml (CS 2: 1.25 µg/10 µl), 5 ml to 20 ml (CS 3: 0.625 µg/10 µl), 1 ml CS 1 to 10 ml (CS 4: 0.25 µg/10 µl) and 1 ml to 20 ml (CS 5: 0.125 µg/10 µl) with methanolic formic acid. Solutions can be stored at 4°C for at least one week.

Calibration solution for MI/MCI

Pipette 1 ml of stock solution into a flask and dilute to 20 ml with 0.1% aqueous formic acid (dilution A). Pipette 1 ml of dilution 1 into a flask and dilute as described above (dilution B). Pipette 1 ml of dilution B into a flask and dilute to 10 ml (dilution C). Calibrate with 5 µl dilution C (0.2 ng MI, 0.7 ng MCI), 10 µl dilution C (0.5 ng MI, 1.5 ng MCI), 20 µl dilution C (0.9 ng MI, 3 ng MCI), 5 µl dilution B (2 ng MI, 7 ng MCI), 10 µl dilution B (4 ng MI, 14 ng MCI), 20 µl dilution B (8 ng MI, 28 ng MCI) and 50 µl dilution B (20 ng MI, 70 ng MCI). (Note: exact amounts depend on weighed-in quantity.)

HPLC parameters and eluants

Method 1 preservatives

Temperature: 40°C, flow rate: 1.3 ml/min, ternary gradient: see gradient time table (table 1), run time: 38 min, sample injection volume: 10 µl, detection wave-

Table 1
Gradient time table for method 1 preservatives

Min	MeCN	0.1% HCOOH	MeOH/THF*
0	10%	85%	5%
7	10%	85%	5%
15	20%	70%	10%
23.3	35%	55%	10%
28	67%	23%	10%
31	85%	5%	10%
34.1	85%	5%	10%
34.2	10%	85%	5%
38	10%	85%	5%

* Mixture of 1 volume part methanol with one volume part THF

length: 230, 260 and 300 nm, rate: 10 Hz, bandwidth: 11 nm, DAD: wavelength range: 220–360 nm, rate: 1 Hz, resolution 1 nm, bandwidth 1 nm, measuring time: 35 min. Note: wash column with approximately 30 ml acetonitrile after each series of samples.

MCI, MI

Temperature: 35°C, flow rate: 1.0 ml/min, run time: 14 min, sample injection volume: 20 µl, detection wavelength: 280 nm, rate: 10 Hz, bandwidth: 11 nm, rise time: 0.2 sec. DAD: wavelength range: 220–360 nm, resolution: 1 nm, bandwidth: 1 nm, rate: 1 Hz, binary gradient system 0.2% aqueous formic acid, methanol (table 2).

Table 2
Gradient time table for MCI/MI

Min	Methanol	0.2% HCOOH
0	5%	95%
9	55%	45%
9.1	90%	10%
11.6	90%	10%
11.7	5%	95%
17	5%	95%

Sample preparation

Method 1 preservatives

Weigh 1 g of sample in a 50 ml stoppered Erlenmeyer flask. Add 20 ml 1% methanolic formic acid. Put stopper on flask and shake thoroughly on a vortex. Place for 15 min in an ultra-sonic bath. Centrifuge suspension for 5 min at 4000 rpm. Filter a 2 ml aliquot of the supernatant through a 0.45 µm filter disc. This solution is ready for injection.

MCI/MI

Weigh 1 g of sample in a 50 ml stoppered Erlenmeyer flask. Add 20 ml 1% aqueous formic acid. Put stopper on flask and shake thoroughly on a vortex. Place for 15 min in an ultra-sonic bath. Centrifuge suspension for 5 min at 4000 rpm. Filter a 2 ml aliquot of the supernatant through a 0.45 µm filter disc. This solution is ready for injection.

Evaluation of chromatograms

Calculate peak areas at 230 nm (compounds 1–5, 17, 19, 21, 23–26, 28, 33, 35 and 39), 260 nm (6, 8–15, 18, 20, 22, 27, 29–30 and 36–37) and 300 nm (7, 16, 31–32, 38 and 40) for method 1 and at 280 nm for method 2. Peak height calculation gives

more accurate results if an interference by a close eluting component occurs. Detection wavelength can be optimized if interferences occur. Peaks are assigned according to retention time and UV spectrum.

Quality control

In case of positive samples, identification should be verified and recovery rates determined by repeating the procedure with a sample aliquot spiked with the presumed preservative. Individual calibration curves or calibration factors must be established for every compound found.

Results and Discussion

We originally developed similar methods for the determination of preservatives in cosmetics with the aim of being capable of separating about 40 compounds of interest in one HPLC run. This allowed for a very effective supervision of the legal limits of preservatives and their correct declaration and meant a great progress compared to our older method (5), which was rather time consuming and lacked the resolution power necessary for this task. As separation problems are rather smaller in fingerpaints and only slight modifications were necessary, we could rely heavily on our experience with cosmetic samples.

Method 1

Preliminary testing of stationary phases showed that phenyl-hexyl columns had the highest selectivity with acceptable peak shapes, so method 1 development was further pursued on this phase.

Work with hundreds of cosmetic samples showed us that of over 40 possible uv-active preservatives, only about a dozen are actually used. We therefore concentrated our effort mainly on the separation and quantitation of these substances, the difficult part being the separation of the often found polar preservatives 1, 6, 8, 9, 16, 20, 22, 27. Further difficulties with the compounds 30, 31 and 38 which we never found in cosmetics also had to be met.

With the exception of long chained parabenes, apolar compounds were only occasionally found. Achieving a high peak resolution at high retention times therefore wasn't that important for a reliable identification.

For a method 1 separation on a routine basis, a run time of 40 minutes proved to be sufficient (fig. 1). Even though not all peaks are baseline resolved, samples containing a problematic combination of preservatives are only rarely encountered. A serious coelution problem only exists for 4-chloro-3,5-xyleneol and 3-methyl-4-isopropyl phenol. Both which we never found in our samples. We therefore think it highly improbable that this combination would be encountered in the commodities mentioned. Selectivity wasn't a problem for fingerpaint samples.

With all the advantages a ternary (pseudo quaternary) gradient has concerning resolution, method transfer from one HPLC instrument to another sometimes has

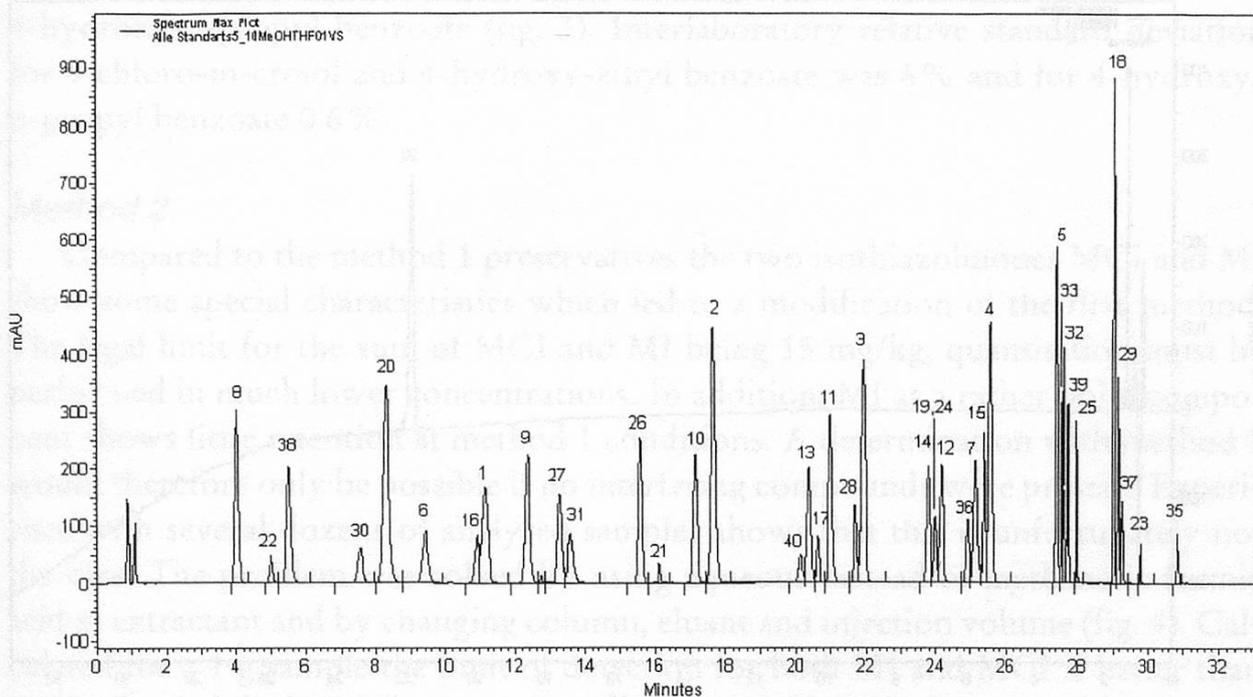


Figure 1 Method 1: Chromatogram at spectrum maximum of a reference solution containing all preservatives except chloramin-T (34). Coelution of 4-chloro-3,5-xyleneol (19) and 3-methyl-4-isopropyl-phenol (24)

to be done with a slight adaptation of the gradient. This procedure is necessary when variations in eluant mixing, resulting in small differences on the composition of the mobile phase, influence the retention times of polar compounds.

With the first method, aromatic preservatives can be detected even in the lower mg/kg range. The highest limit of detection (LOD) was observed for benzylic alcohol with about 40 mg/kg. Almost all the other preservatives had considerably lower LODs lying in the range of 0.5 to 5 mg/kg. As we know from our analyses of cosmetics, application concentrations of most of the preservatives are in the upper mg/kg and g/kg range. The limit of quantitation is therefore established by the lowest concentration used for calibration. Calculated for a 1 g sample, range of quantitation is between 0.025 % (rsp. 0.05 % for compounds 20 and 22) and 0.5 % (rsp. 1 %). According to prEN 71-7 (1), the lowest legal limits for method 1 preservatives in fingerprints are 0.1 % (Bromochlorophene and 3-methyl-4-isopropyl phenol) and 1 % (benzylic alcohol and phenoxyethanol).

This demonstrates that the method is on one hand suitable for supervising limits and on the other hand sensitive enough to detect illegal respectively unallowed preservatives such as hexachlorophene, benzisothiazolone, 2-n-octyl-4-isothiazolin-3-one or 4,5-dichloro-2-octyl-3(2H)-isothiazolone.

The method was used in an interlaboratory test by four laboratories on two fingerprint samples. Sample one contained about 0.3 % 4-chloro-m-cresol (fig. 2),

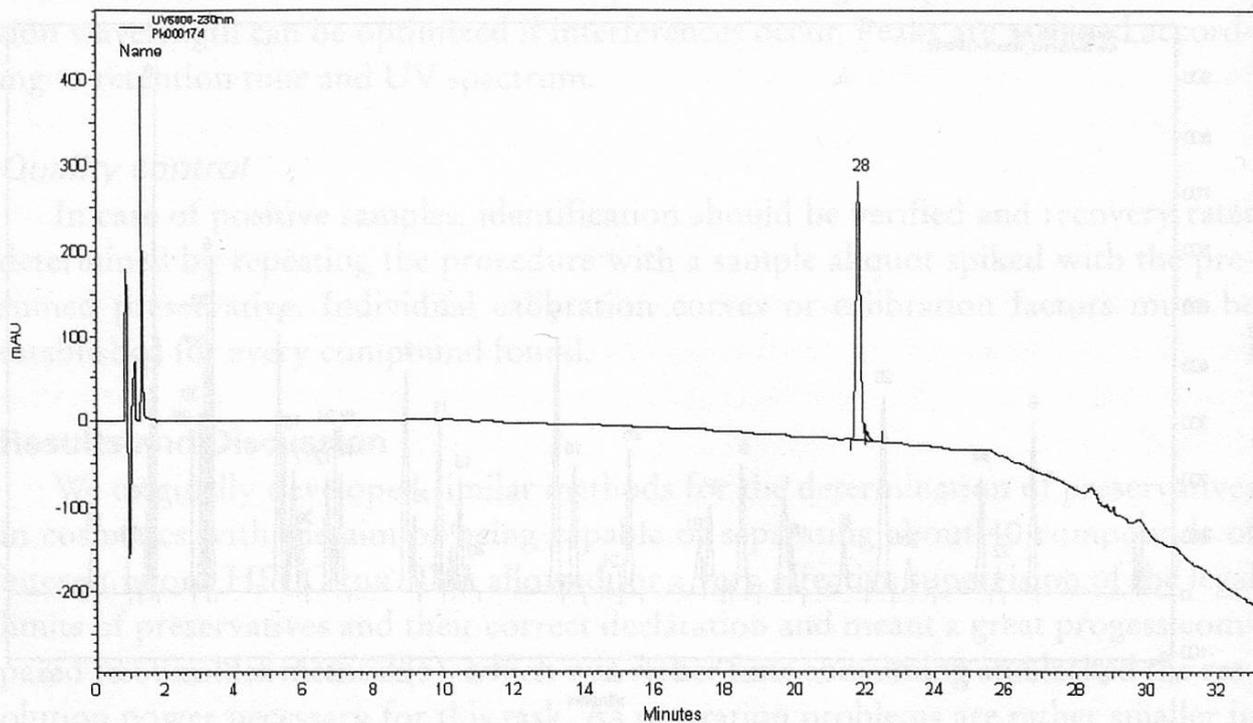


Figure 2 Method 1: Chromatogram at 260 nm of a finger paint sample containing 0.26% 4-chloro-m-cresol

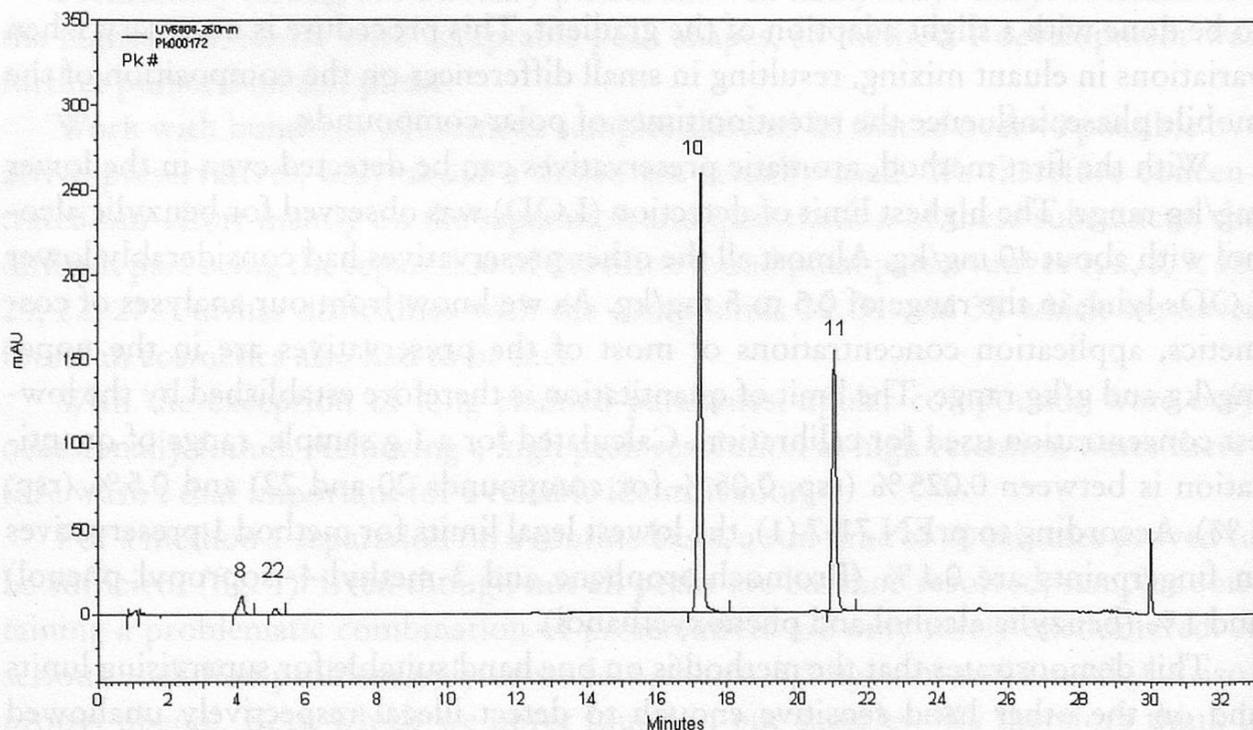


Figure 3 Method 1: Chromatogram at 260 nm of a finger paint sample containing 0.2% 4-hydroxy-n-propyl-benzoate and 0.1% 4-hydroxy-ethyl-benzoate. Traces of 4-hydroxy-benzoic acid and benzylic alcohol are visible

sample two contained about 0.2% 4-hydroxy-ethyl benzoate and about 0.1% 4-hydroxy-n-propyl benzoate (fig. 3). Interlaboratory relative standard deviation for 4-chloro-m-cresol and 4-hydroxy-ethyl benzoate was 6% and for 4-hydroxy-n-propyl benzoate 0.6%.

Method 2

Compared to the method 1 preservatives the two isothiazolinones MCI and MI show some special characteristics which led to a modification of the first method: The legal limit for the sum of MCI and MI being 15 mg/kg, quantitation must be performed in much lower concentrations. In addition, MI as a rather polar component shows little retention at method 1 conditions. A determination with method 1 would therefore only be possible if no interfering compounds were present. Experience with several dozens of analysed samples shows that this is unfortunately not the case. The problem was solved by using aqueous instead of methanolic formic acid as extractant and by changing column, eluant and injection volume (fig. 4). Calculated for a 1 g sample the limit of detection for both MI and MCI is better than 0.1 mg/kg and the limit of quantitation is 0.3 mg/kg for MI and 0.8 mg/kg for MCI. The method is therefore sensitive enough for supervising the legal limit of 15 mg/kg for the sum of MI and MCI. As with method 1, we didn't encounter selectivity problems with the 29 samples of the market survey. The identity of MCI and MI can easily be established by comparison of retention times, uv spectra and by cochro-

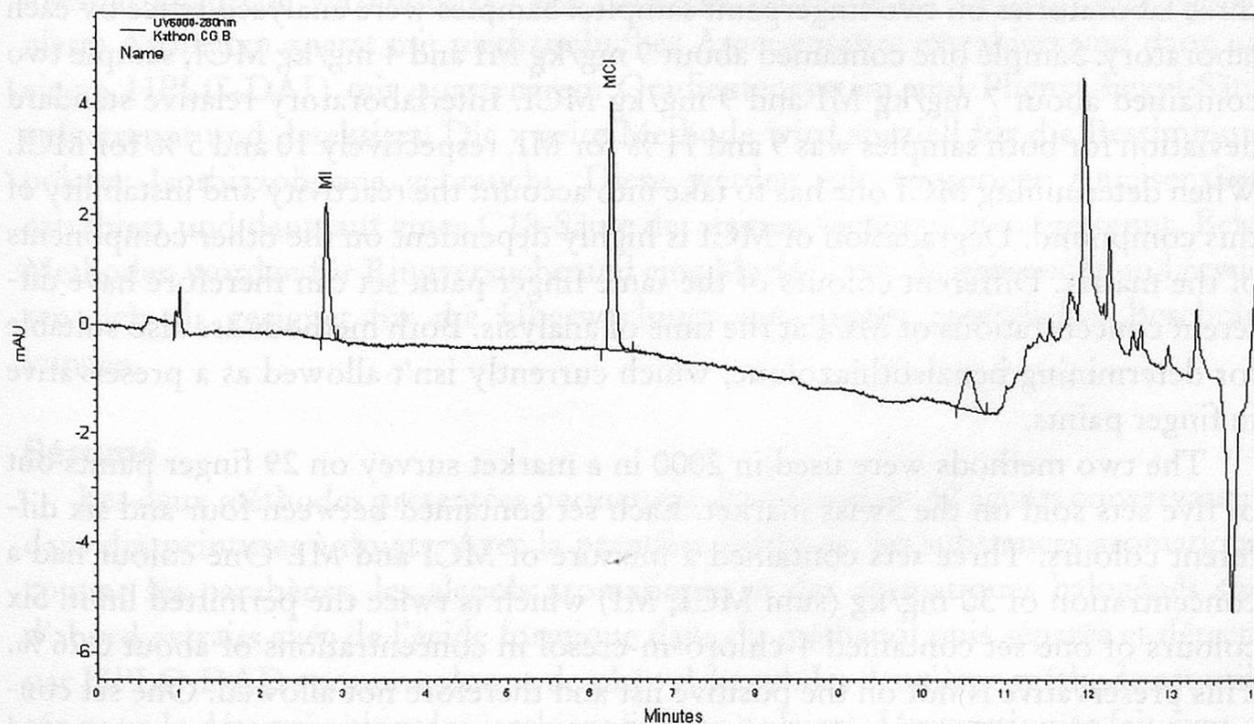


Figure 4 Method 2: Reference solution containing 2 ng MI and 7 ng MCI

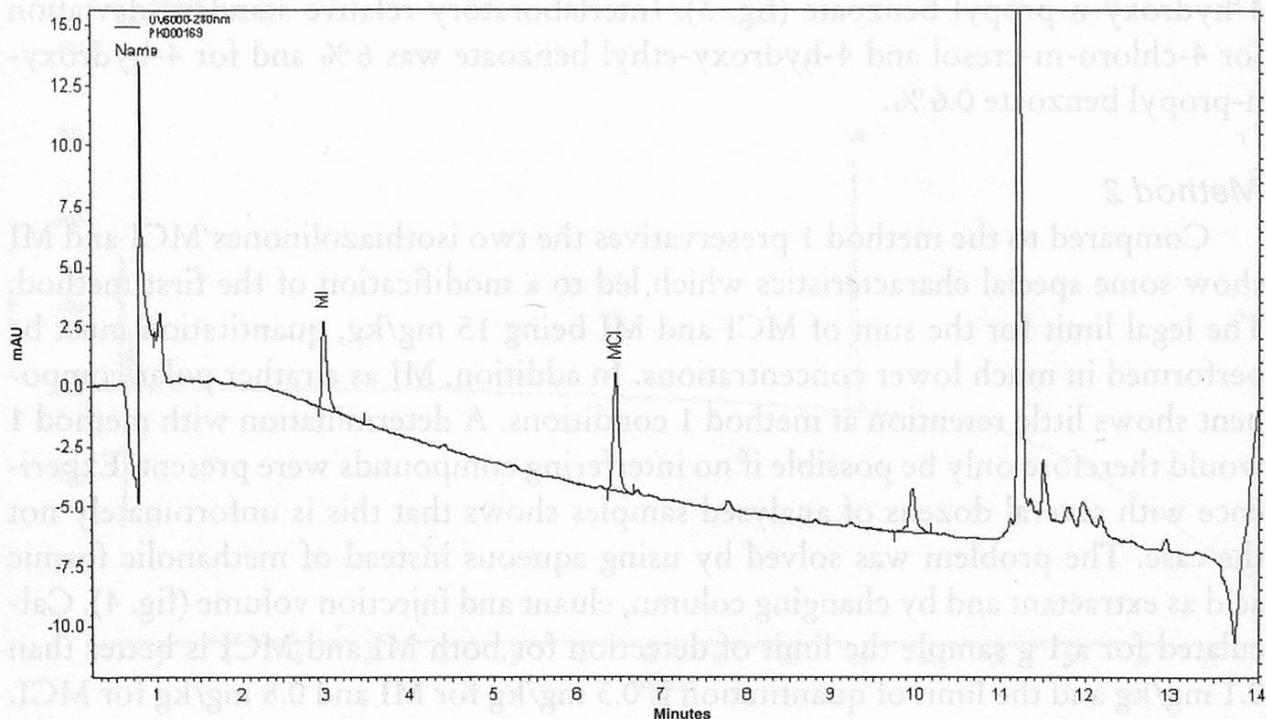


Figure 5 Method 2: Chromatogram of a finger paint sample containing 6 mg/kg MI and 8 mg/kg MCI

matograms of spiked samples. The method was used in an interlaboratory test by three laboratories on two fingerpaint samples. Samples were analysed twice by each laboratory. Sample one contained about 9 mg/kg MI and 4 mg/kg MCI, sample two contained about 7 mg/kg MI and 9 mg/kg MCI. Interlaboratory relative standard deviation for both samples was 9 and 11 % for MI, respectively 10 and 5 % for MCI. When determining MCI one has to take into account the reactivity and instability of this compound. Degradation of MCI is highly dependent on the other components of the matrix. Different colours of the same finger paint set can therefore have different concentrations of MCI at the time of analysis. Both methods are also suitable for determining benzisothiazolone, which currently isn't allowed as a preservative in finger paints.

The two methods were used in 2000 in a market survey on 29 finger paints out of five sets sold on the Swiss market. Each set contained between four and six different colours. Three sets contained a mixture of MCI and MI. One colour had a concentration of 30 mg/kg (sum MCI, MI) which is twice the permitted limit. Six colours of one set contained 4-chloro-m-cresol in concentrations of about 0.26 %. This preservative is not on the positive list and therefore not allowed. One set contained a mixture of 0.2 % 4-hydroxy-ethyl benzoate and 0.1 % 4-hydroxy-n-propyl benzoate. Using another HPLC method with an electro-chemical detector, we also found bronopol in one set. In interpreting the results of the survey one has to take

into account, that prEN 71-7 was (and still is) a provisional document. As our results for MCI/MI and 4-chloro-m-cresol show, the definitive norm will certainly have an impact on the use of preservatives in finger paints.

Acknowledgements

We would like to thank the following laboratories for participating in the round robin tests: Clariant (Schweiz) AG, Qualitätskontrolle/Chromatographie, Muttenz; Kantonales Laboratorium Zürich; Laboratoire cantonal vaudois, Epalinges.

Summary

Two HPLC methods for the determination of 42 preservatives in finger paints are presented. With the first method mostly aromatic compounds such as parabenes, aromatic alcohols and halogenated aromatics are first extracted with methanolic formic acid and then separated and detected on a quaternary gradient HPLC-DAD-system using a phenyl-hexyl column. The second method is used for the determination of polar isothiazolinones which are extracted with aqueous formic acid and separated on a third generation C18 column. Both methods were used in interlaboratory tests and in a market survey and proved to be suitable for supervising future legal restrictions.

Zusammenfassung

Es werden zwei HPLC-Methoden für die Bestimmung von insgesamt 42 Konservierungsmitteln in Fingerfarben vorgestellt. Mit der ersten Methode werden meist aromatische Verbindungen wie Parabene, aromatische Alkohole und halogenierte Aromaten zuerst mit methanolischer Ameisensäure extrahiert und dann auf einem HPLC-DAD mit quaternärem Gradientensystem und Phenyl-hexyl-Säule aufgetrennt und detektiert. Die zweite Methode wird speziell für die Bestimmung polarer Isothiazolinone gebraucht. Diese werden mit wässriger Ameisensäure extrahiert und dann auf einer C18-Säule der dritten Generation aufgetrennt. Beide Methoden wurden für Ringversuche und eine Marktübersicht gebraucht und erwiesen sich als geeignet für die Überwachung zukünftiger gesetzlicher Beschränkungen.

Résumé

Les deux méthodes présentées permettent de déterminer 42 agents conservateurs dans des peintures à doigts. Avec la première méthode, les substances aromatiques comme les parabènes, les alcools aromatiques et des aromatiques halogénés sont d'abord extraits avec de l'acide formique dans du méthanol puis séparés et détectés par HPLC-DAD sur une colonne de phényl-hexyl. La deuxième méthode est utilisée pour la détermination des isothiazolinones polaires. L'extraction se fait avec de l'acide formique dilué. La séparation est exécutée sur une colonne C18 de la troisième génération. Les deux méthodes ont été contrôlées par des tests interlabora-

toires et ont été appliquées pour un contrôle du marché. Elles se sont avérées être des méthodes utilisables pour vérifier le respect des exigences légales.

Key words

Preservatives, Finger paints, Toys, HPLC

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