Extraction of light and heavy filth from lipstick

David M. Malakauskas*[1] and Jason Nickelsen[1]

Southeast Food and Feed Laboratory, Office of Regulatory Affairs, U.S. Food and Drug Administration, 60 8th Street, Atlanta, GA 30309

*Contact information:
David.Malakauskas@fda.hhs.gov

Abstract

Lipstick is a commonly used cosmetic and is available in many different formulations. Lipstick tends to be a heterogeneous mixture of primarily waxes and oils. This base composition makes use of current filth extraction methods difficult, since most available methods are based on food matrices with dominant hydrophilic properties. In this study, we developed a method to extract light and heavy filth from lipsticks and performed an intralaboratory validation. We used hot mineral oil to dissolve and reduce the viscosity of the lipstick and then sieved and filtered the solution to recover filth elements. Five analysts participated in the validation. Recoveries of the five filth elements--plastic, foil, glass, elytral squares and mouse hairs—ranged from 86% to 96%. Recoveries of the different filth elements by all participating analysts were consistent. We propose using our method for future regulatory samples that require extraction of filth elements from lipstick.

Key Words: filth, lipstick, validation, method

Introduction

Lipstick is a common cosmetic used worldwide to enhance physical appearance. When formulating lipsticks, manufacturers must consider numerous aesthetic, physical and chemical properties, such as safety, texture and spreadability (1, 2). This necessitates use of complex formulations including oils, emollients, waxes and color additive lakes (3, 4). Moreover, reflective materials and skin conditioners are also frequently included in lipstick formulations (5).

Microanalytical filth analyses are routinely performed on food products. Most analytical food methods rely on water solubility or acid hydrolysis and heat to release filth elements from food matrices, with relatively small amounts of lipids (e.g., pigments in capsicums) being removed with alcohols (6), chloroform (7), or detergent (8). However, these methods are ineffective for lipsticks, which are made almost entirely of lipids. Moreover, these methods have been validated on foods rather than on cosmetics.

The Southeast Food and Feed Laboratory recently received a consumer complaint alleging adulterated lipsticks. At the time of the complaint, there was no official method of analysis available for this product, necessitating development of a novel method. Using a trial and error approach, we developed a method for solubilizing lipid-rich lipsticks and extracting filth elements contained therein. The present study reports a novel method for extraction of light and heavy filth from lipstick and presents the results of a single lab validation.

Experimental

Equipment List

Graduated cylinders, Class A (50 mL and 100 mL)
Glass beakers, 400 mL
Filter paper, #8 ruled (90 mm), AOAC 945.75B(i)
Top-loading balance (± 0.1 g minimum sensitivity recommended)
Plain-weave no. 230 mesh standard testing sieve with pan, AOAC 945.75B(r)
Vacuum filtration apparatus with Hirsch funnel, AOAC 945.75B(k)
Widefield stereo microscope, AOAC 945.75B(o)(2)
Magnetic stirring bar and stirrer hot plate, AOAC 945.75B(n) (hotplates with temperature displays are recommended).

Reagent List

Isopropanol, AOAC 945.75C(dd)
40% Isopropanol diluted with deionized water or other water free of extraneous materials, AOAC 945.75A

Mineral Oil, AOAC 945.75C(p): Paraffin oil, white, light, 125/135 Saybolt Universal viscosity, specific gravity 0.840 – 0.860
Igepal CO-630 (IUPAC name: octylphenoxypolyethoxyethanol), AOAC 945.75C(j)
Detergent Solution: 10% solution of Igepal CO-630 in deionized water or other water free of extraneous materials, AOAC 945.75A

Method

1. Obtain one 400-mL beaker per subsample.
2. Add 50 mL mineral oil to beaker and transfer the beaker to a balance. Tare the balance.
3. Add 5 g of lipstick to the beaker with mineral oil.
4. Add a magnetic stir bar to the beaker with the lipstick and mineral oil.
5. While stirring lipstick and mineral oil at low speed (ca. 100 rpm--do not allow a vortex to form while stirring), gently melt the lipstick on a hotplate to avoid charring. Do not proceed to the next step until the lipstick is fully dissolved, including any residue adhering to the sides of the beaker.
6. Add 20 mL of room-temperature Igepal CO-630 to beaker and stir until well mixed.
7. Slowly add 100 ml of 100% isopropanol to beaker. NOTE: Adding isopropanol to melted lipstick ≥160°C too quickly can cause vigorous spattering, which can create a hazard. Increase speed of stirring to high (ca. 400 rpm) until a deep vortex is formed. Bring contents to a light boil while stirring.
8. Once the beaker has reached a light boil, remove beaker from hotplate and pour its contents directly onto No. 230 sieve nested on pan to trap potential filth elements. The pan should not be angled because a flat sieve allows for more efficient drainage of the lipstick-reagent solution. Rinse stir bar over sieve using 10% Igepal CO-630 solution followed by hot water and examine to ensure no filth elements remain on the bar.
9. Discard filtrate in bottom pan into hazardous waste container.
10. Rinse the beaker well, alternating between room-temperature 10% Igepal CO-630 solution and hot tap water (hot water at SFFL is 54°C though a specific temperature is not required). Pour all rinse materials onto the sieve at a sink. Continue to wash the beaker with the Igepal solution and hot tap water until the lipstick residue is completely washed from the beaker. Failure to completely rinse lipstick from the beaker can cause filth elements to remain on the sides of the beaker. Some samples, such as those with with titanium dioxide, may require mechanical disruption of residue with a gloved hand or rubber policeman. In this case, wash glove or tool over sieve with detergent solution and hot water after use and inspect for filth elements prior to starting the next step.
11. Wash remaining residue on the sieve in a sink with a forceful stream of hot tap water, adding 10% Igepal CO-630 to disperse melted lipstick as needed—be sure to wash the sides of the sieve as well as the mesh. Wash all matrix residue through the sieve before proceeding to the next step. Lipstick with glitter-like ingredients may take longer to rinse.

12. While holding sieve at ca. 30˚ angle, rinse the filth elements to one side of the sieve using hot water and 10% Igepal CO-630. Rinse the sieve with 40% isopropanol to wash filth elements into a beaker. The sieve must be rinsed thoroughly multiple times to ensure all filth elements are transferred to the beaker (Reference AOAC 970.66B(a) Special Techniques–Wet Sieving Technique).
13. Transfer the beaker contents to ruled filter paper with aid of vacuum filtration apparatus. If lipstick sample has a large amount of residual glitter that could not be rinsed through the sieve, filter onto multiple filter papers.
14. Examine the paper for filth elements using microscopy.

Intralaboratory Validation

Mouse (*Mus musculus* L., 1758) hairs ranging in length from 2 to 5 mm, elytral squares of ca. 0.2 mm × 0.2 mm cut from *Sitophilus oryzae* (L., 1763), plastic pieces of ca. 0.5 mm × 2.0 mm cut from 2-oz. plastic, disposable soufflés cups, metal pieces of ca. 0.5 mm × 0.5 mm cut from a disposable aluminum baking dish and glass shards of ca. 0.5 mm × 2.0 mm from a broken test tube were used as spike materials. Mouse hairs and elytral squares were used for consistency with other filth validation methods. The metal, glass and plastic pieces represented alleged adulterants from the consumer complaint. Ten of each of the spike elements were placed on circular filter paper for each trial run. Spike materials were rinsed from filter papers with isopropanol into melted lipstick immediately following the addition of the Igepal CO-630 detergent (step 6 of method) to test recoveries.

Five analysts from the Southeast Food and Feed Laboratory participated in the validation study. Each analyst performed the method on a random sample and checked spike recoveries once, before collecting data, to familiarize themselves with the method. Following this practice run, each analyst completed trials of a sample blank and two random lipstick samples. Each of the three trials was performed in random order and at different times to reduce statistical sample dependence.

To determine if there was a difference in recoveries of spiked elements between analysts, a chi-squared test was performed. Runs were combined for individual analysts prior to the chi-squared analysis. A Spearman's Rank test was used to determine if amount of lipstick analyzed affected recoveries of filth elements. Descriptive statistics were calculated on the raw data from the 14 total spike recovery runs. Statistical analyses were performed with R version 3.6.1 (9).

Results

In total, analysts performed the extraction method on 9 brands of lipstick and 14 different colors. Lipstick quantities tested ranged from 1.9 g to 5.4 g (n = 14), with a mean mass of 3.5 g (SD = 1.2 g). Recoveries of filth elements from lipstick samples were consistent among analysts (df = 16, $\chi^2 = 4.891$, $p > 0.99$). Mean filth recoveries were at least 93% for all elements except for hair, which was recovered at a mean of 83%. Coefficients of variation of filth recovery between runs ranged from 6.6% to 13% (Table 1). Quantity of lipstick analyzed did not appear to influence recoveries of hairs (df = 12, $r_s = 0.42$, $p = 0.13$), glass pieces (df = 12, $r_s = 0.31$, $p = 0.28$), plastic pieces (df = 12, $r_s = -0.35$, $p = 0.22$), elytral squares (df = 12, $r_s = -0.17$, $p = 0.55$), or foil pieces (df = 12, $r_s = -0.14$, $p = 0.62$). Negative controls (blanks) showed no evidence of contamination.

Table 1. Filth elements recovered from spiked samples of lipstick.

Analyst*	Plastic	Foil	Glass	Elytra	Hairs
A_1	10	9	9	10	10
A_2	10	10	10	10	9
B_1	10	10	9	8	7
B_2	10	8	8	10	8
C_1	10	10	8	9	10
C_2	10	10	10	10	8
C_3	8	10	10	8	9
C_4	10	9	10	10	10
D_1	9	10	10	9	9
D_2	10	10	10	9	7
D_3	10	10	10	9	9
D_4	9	8	10	9	10
E_1	10	8	10	9	7
E_2	9	10	10	10	8
Avg. % Rec.	96	94	96	93	86
% Coeff. of Var.	6.6	9.0	7.9	7.8	13

*Subscripts denote independent runs performed by an analyst

Discussion

Our method for extracting filth from lipstick is simple and effective. Our intralaboratory validation showed consistent recovery of different filth elements between five different analysts, suggesting this method is robust, and will likely be effective in other regulatory laboratories.

Recoveries by analysts were generally over 90%; hair recoveries were the exception at 86%. While we found no previous studies on filth recovery from cosmetics in the literature, our recovery levels were similar to those in numerous food methods. For example, authors of a method to extract filth from corn- and rice-based cereals, reported rodent hair recoveries of 80% with a trap flask and 82% with a percolator. Insect fragments were recovered at 91% with a trap flask and 86% with a percolator (10). A cheese-based filth analysis recovered 91% of rodent hairs and 97% of insect fragments (11), and in an analysis of dried soup products, 79% of rodent hairs and 96% of insect fragments were recovered (12).

We used mouse hair and beetle elytral squares as spiked filth elements for consistency with other filth recovery validation methods and to facilitate comparison of recovery levels (13). In the present study, rodent hairs were recovered at a lower level than other filth elements, though this does not appear to be uncommon in filth analysis methods. For example, a validation study for a fish paste method recovered 82% of rodent hairs and 91% of insect fragments (14). Sixty-five percent of rodent hairs and 99% of insect fragments were recovered in a validation of filth isolation from pickled ginger (15). A filth extraction from chocolate showed a particulary stark contrast in recoveries with a rodent hair recovery of only 3% versus 69% of insect fragments (16).

The reason for lower recoveries of hairs in our method is not known, though hypotheses about lower hair recoveries have been made by authors of other studies. Improper stirring technique during an oil dispersion step has been proposed to be a factor in methods that use a trap flask (13). However, our method does not involve a trap flask. Entanglement with the matrix (e.g., plant trichomes) has also been proposed (17) though lipstick is largely liquid when melted, and seems to lack ingredients that could physically entangle hairs. We speculate lower recoveries of hairs in our method may be due to hairs becoming entangled in or passing through the sieve mesh during the washing step. Alternatively, the hair may be more difficult to rinse off of sieves and beakers due to the large surface area of hair relative to its volume and its ability to adhere to equipment when wet or coated with oil. Regardless, hair recoveries using our method were in line with hair recoveries from established methods for food matrices.

Our method takes advantage of the lipophilic nature of mineral oil to dissolve lipstick, as well as the decrease in viscosity of waxes and mineral oil with increasing temperatures. In earlier optimization trials of this method, we poured lipstick onto a sieve immediately after dissolution as opposed to heating the solution to boiling first. This resulted in markedly more lipstick residue in the sieve and required more rinsing with hot water and detergent. Heating the lipstick-mineral oil solution to a boil decreased manual labor by reducing the effort required to wash the product through a sieve.

We added filth elements to the lipstick before the addition of isopropanol to reduce potential loss of filth elements from handling. Filth elements for spikes were prepared on a piece of filter paper. We found, through trial and error, the best way to consistently transfer all filth elements from the paper to the beaker was to rinse them into the lipstick with isopropanol. The disadvantage of this method was that the filth elements were not present for the entire heating and boiling process. To address this shortcoming and to ensure the robustness of our method, we ran two subsamples with filth elements added manually to the mineral oil prior to the addition of lipstick. These subsamples were then heated to a vigorous boil on a hotplate set to 370°C. The elements were boiled for 15 minutes. Filth elements were recovered and examined micrsocopically for damage, following this procedure. All filth elements recovered were intact. This procedure also demonstrated that future analysts will not damage filth elements by using higher melting temperatures than we used during validation trials, showing our method is not temperature sensitive.

Earlier trials of our method neglected the addition of detergent to the lipstick-mineral oil solution. Lack of detergent appeared to increase adherence of filth elements to the sieve and beaker, requiring additional rinsing effort to maintain high recoveries. We speculate that the addition of detergent helps to free the filth elements of lipstick residue, making it easier to rinse the filth elements from the sieve and beaker. Addition of 100% isopropanol to the lipstick-mineral oil mixture further decreases the viscosity of the solution, solubilizes dyes, and facilitates rinsing through the sieve.

Use of a no. 140 sieve in earlier trials allowed for more viscous, cooler solutions to pass through the mesh of the sieve. However, recoveries of filth elements appeared to be lower, especially organic elements. Two possibilities for lower recoveries include: 1) filth elements were able to pass through the larger mesh size and 2) the tendency to use cooler, more viscous solutions in earlier trials left more lipstick residue on filth elements, trapping them in the sieve and beaker.

We presented a novel method for the extraction of light and heavy filth from lipstick in this study. Recoveries for all filth elements were at least 86% and consistent among analysts in our lab. We propose our method be used in future regulatory samples involving filth in lipstick samples.

Acknowledgments

We thank Christine Cash, Kantrell Smith and Anita Washington for help with method validation and feedback. We thank Cheryl Love for administrative support and the editor and reviewers for their time and suggestions to improve this manuscript.

References

1. Kasparavičienė, G., A. Savickas, Z. Kalvenienė, S. Velžienė, L. Kubilienė, and J. Bernatonienė. 2016. Evaluation of beeswax influence on physical properties of lipstick using instrumental and sensory methods. Evidence-Based Complementary and Alternative Medicine, Article ID 3816460, 8 pp. https://doi.org/10.1155/2016/3816460
2. Egan, R.R. and B.J. Hoffman. 1967. Fatty glycols and isostearyl alcohol as lipstick components. Journal of the American Oil Chemists' Society 45:726-728. https://doi.org/10.1007/BF02631942
3. Waskitho, D., E. Lukitaningsih, Sudjadi, and A. Rohman. 2016. Analysis of lard in lipstick formulation using FTIR spectroscopy and multivariate calibration: a comparison of three

extraction methods. Journal of Oleo Science 65:815-824. https://doi.org/10.5650/jos.ess15294

4. DeClermont-Gallerande, H., V. Chavardes, and L. Zastrow. 1999. Crystal clear transparent lipstick formulation based on solidified oils. International Journal of Cosmetic Science 21:413-424. https://doi.org/10.1046/j.1467-2494.1999.211909.x
5. Zanotti, F., S. Masiello, S. Bader, M. Guarneri, and D. Vojnović. 1998. Experimental design applied to the formulation of lipsticks with particular features. International Journal of Cosmetic Science 20:217-221. https://doi.org/10.1046/j.1467-2494.1998.176607.x
6. AOAC Official Method of Analysis 978.22. Light filth in capsicums (ground). In: Latimer, G.W., editor, Official Methods of Analysis of AOAC International, 21st ed., 2019. Online: http://www.eoma.aoac.org/
7. AOAC Official Method of Analysis 988.16. Filth in ground coffee and coffee substitutes. In: Latimer, G.W., editor, Official Methods of Analysis of AOAC International, 21st ed., 2019. Online: http://www.eoma.aoac.org/
8. AOAC Official Method of Analysis 970.70. Light filth in white breads and high-fat products. In: Latimer, G.W., editor, Official Methods of Analysis of AOAC International, 21st ed., 2019. Online: http://www.eoma.aoac.org/
9. R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
10. Dent, R.G., A.L. Roaf, and P.M.Jr., Brickey. 1970. Extraction of light filth from corn- and rice-based ready-to-eat breakfast cereals and corn-based snack foods. Journal of the Association of Official Analytical Chemists 53:558-560. https://doi.org/10.1093/jaoac/53.3.558
11. Nakashima, M.J. 1991. Extension of use of LIB 3485 to cheeses for light filth analysis. U.S. Food and Drug Administration. Laboratory Information Bulletin, No. 3618.
12. Roy, R.R. 1980. The extraction of light filth from dried soup products. U.S. Food and Drug Administration. Laboratory Information Bulletin, No. 2324.
13. Brickey, P.M, Jr., J.S. Gecan, J.J. Thrasher, and W.V. Eisenberg. 1968. Notes on microanalytical techniques in the analysis of foods for extraneous materials. Journal of Official Analytical Chemists 51:872-876. https://doi.org/10.1093/jaoac/51.4.872
14. Glaze, L.E. 1992. Extraction of light filth from fish paste and sauce (Bagoong) not containing spice: collaborative study. Journal of AOAC International 75:263-265. https://doi.org/10.1093/jaoac/75.2.263
15. Haynos, R. 1984. Extraction of filth from pickled ginger. U.S. Food and Drug Administration. Laboratory Information Bulletin, No. 2833.
16. Shostak, M. 1976. Improved method for determination of rodent hairs in chocolate products. U.S. Food and Drug Administration. Laboratory Information Bulletin, No. 2060.
17. Dimov, M.N., M.B. Atui, A.M. Silva, M.A.M. Marciano, V.P.A. Franco, L.B. Chasin, L.A. Silva, M.I.A. Fioravanti, and M.M.L. Oliveira. 2010. Aspects of the methodology validation for light filth in fruit pulp. 10th International Working Conference on Stored Product Protection, No. 425. https://doi.org/10.5073/jka.2010.425.167.059