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Examinations of Cosmetic Smudges Including Transesterification and Gas Chromatographic/ Mass Spectrometric Analysis

REFERENCE: Keagy, R. L., **"Examinations of Cosmetic Smudges Including Transesterification and Gas Chromatographic/Mass Spectrometric Analysis,"** *Journal of Forensic Sciences*, JFSCA, Vol. 28, No. 3, July 1983, pp. 623-631.

ABSTRACT: Smeared traces of a cosmetic product may be transferred from a female victim to an assailant during crimes of violence. The procedure followed in the FBI Laboratory to characterize a cosmetic-like smudge is described, including extraction from a substrate, comparison with known specimens, and determination of the major inorganic and organic constituents used in the cosmetic formulation. A derivatization method used to transesterify oils and fatty acids commonly present in cosmetics is explained whereby the fatty substances are converted to methyl esters and subsequently identified by gas chromatography/mass spectrometry. This method is applicable to castor oil-based lipsticks as well as cosmetic powders, creams, and lotions that contain oils and fatty acids as main ingredients. Four examples are presented of forensic science evidence recently examined by the scheme of analysis set forth.

KEYWORDS: forensic science, cosmetics, chemical analysis, lipstick, castor oil, transesterification, gas chromatography/mass spectrometry

The FBI Laboratory receives hundreds of requests each year for microscopic examination of forensic science evidence in cases involving violent crimes such as assaults, robberies, rapes, and murders. Many of these cases involve direct contact between the assailant and a female victim. A transfer of some type of cosmetic product is possible and, consequently, the clothing or body of the suspect may bear smeared traces of a cosmetic. The analysis of these smudges could provide circumstantial evidence connecting a suspect and victim or placing a suspect at the crime scene. Other cases in which cosmetic smudges may be valuable physical evidence include bank robberies where cosmetics have been used to disguise the appearance of a suspect, hit-and-run accidents involving pedestrians or joggers, and burglaries where a trace of lipstick on a discarded cigarette butt may lead to a suspect.

Since the FBI Laboratory does not maintain a collection of all commercially available cosmetic products and only general information on proprietary chemical formulations can be readily obtained, no attempt is made to identify positively a cosmetic by trade name or manufacturer to the exclusion of all others. Trace components unique to a product usually cannot be extracted and identified from the limited amount of smudge typically encountered. Therefore, only the *major* organic and inorganic constituents are considered when attempting to classify a smear as a particular type of cosmetic product. Such circumstantial evidence can be combined with other "class"-type information gained from serological and hair and fiber

Received for publication 18 Aug. 1982; revised manuscript received 12 Oct. 1982; accepted for publication 13 Oct. 1982.

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examinations. If known specimens are submitted by investigators for comparison purposes, then instrumental analyses such as those described below are performed until a determination can be made that either the questioned smudge is different, or, that it could have originated from the source represented by the known specimen.

Method

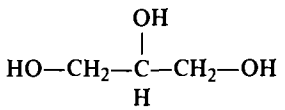
The first step in the investigation of a cosmetic-like smudge involves a microscopic examination of the evidence at approximately ×20, noting color, texture, viscosity, hardness, and the presence of any pearlescent pigment. Samples sufficient for trace elemental analysis are obtained by mechanically removing colored particles from the substrate. Data from the light emission spectrograph and the scanning electron microscope with an energy dispersive X-ray analyzer (SEM-EDXA) [*1*] can indicate synthetic pearlizing pigments like bismuth oxychloride or titanium-coated mica particles; barium and calcium "lake" colors; and "earth" pigments and fillers such as kaolin clay, iron oxide, and titanium dioxide [*1,2*].

The cosmetic smudge can often be substantially removed from cutout bits of fabric using methanol with an ultrasonic cleaner. Insolubles are separated by centrifuging, then subjected to X-ray diffraction analysis which complements the results of the elemental determination. The methanol extract normally contains some "fatty" materials used in cosmetics (an isopropyl myristate emollient, for example) and is analyzed by gas chromatography/mass spectrometry (GC/MS) after filtering and, subsequently, after the transesterification procedure described below. Organic analysis should also be performed after extracting another portion of the stained area with chloroform or, alternately, a trichloroethylene/acetone solution [*3*]. These stronger solvents will dissolve any waxes or oil soluble compounds present and can be used if the substrate is not affected.

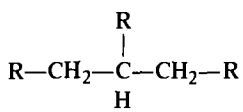
Lipstick is perhaps the most widely used cosmetic product [*2,4*] and therefore frequently encountered in forensic science evidence. After a lipstick smudge is extracted from the substrate using one of the above solvents, thin-layer chromatography (TLC) can be used for comparison with known lipstick specimens. An effective developing solvent used with V-Tech silica gel GF high performance (HP) TLC plates is chloroform/methanol, 3:1; or, better development and separation may sometimes be obtained with the Smalldon solvent system No. 3 used for ink comparison [*5*]: isoamyl alcohol/acetone/water/ammonia, 50:50:30:1. A two-dimensional TLC system reported by Andrasko [*1*] may give the best characterization of all color additives in the lipstick.

A smeared deposit of lipstick on a synthetic fabric may be heavy enough to allow transfer directly to a salt plate by merely wiping the stained area. Subsequent analysis by infrared spectroscopy should reveal a composite spectrum of primarily castor oil and wax [*6*]. Castor oil, the major organic component of many lipstick formulations, may account for greater than one half the total weight of the lipstick. It functions as the base material for a blend of waxes and emollients, as a dispersant for pigments and pearlizers, and as a solvent for the dyes [*2,4*].

Castor oil is a vegetable oil, or liquid fat, composed of fatty acid esters of glycerol. Most naturally occurring fatty acids exist in combined forms known as glycerides [*7*].



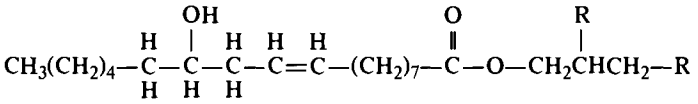
glycerol



a triglyceride

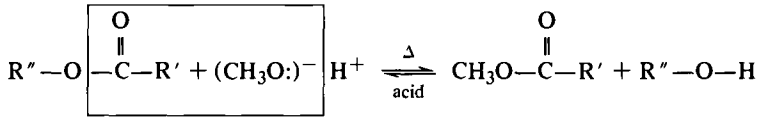
where R represents a fatty acid group. The fatty acid composition of the triglyceride molecules of castor oil [*8*] is approximately 87% ricinoleic, 7% oleic, 3% linoleic, 2% palmitic, and 1% stearic.

Castor oil is unique among natural fats and oils because its principal component is an unsaturated, hydroxy fatty acid, ricinoleic acid [*8,9*],



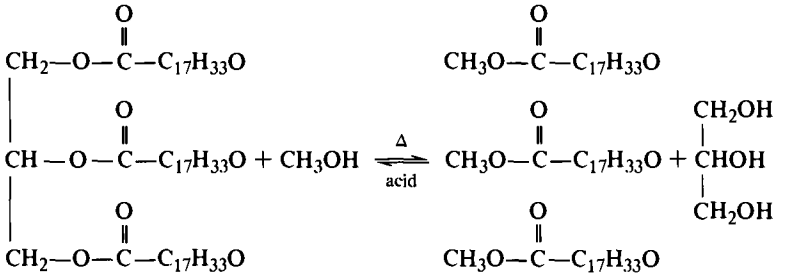
a castor oil triglyceride where R represents additional ricinoleic acid groups.

The GC/MS identification of castor oil in a lipstick smudge is preceded by a derivatization process called transesterification which is necessary to obtain optimum chromatographic performance as well as readily identifiable mass spectra. Transesterification is the procedure by which the triglyceride molecules of castor oil are broken apart and methyl esters of the parent fatty acids are formed in the presence of methanol and a sulfuric acid catalyst [*7,10*]. Chromatograms of castor oil before and after transesterification are shown in Figs. 1 and 2, respectively. In transesterification, or "alcoholysis," one alcohol is capable of displacing another from an ester [*10*].



an ester methanol a methyl ester an alcohol

If R′C(O)O in the above reaction represents a fatty acid group from ricinoleic acid combined with a glyceryl unit (R″) to form a triglyceride, then the transesterification reaction [*10*] proceeds as follows:



castor oil triglyceride based on ricinoleic acid 3 (methyl ricinoleate) glycerol

Procedure and Instrumentation

The filtered extract of a suspected cosmetic smudge was reduced to 2 mL by solvent evaporation, then combined with a 4-mL portion of the transesterification reagent consisting of 90 mL of methanol, 30 mL of benzene, and 1 mL of concentrated sulfuric acid [*3,11*]. After gently heating the mixture in a Blok heater for about 30 min with occasional vigorous agita-

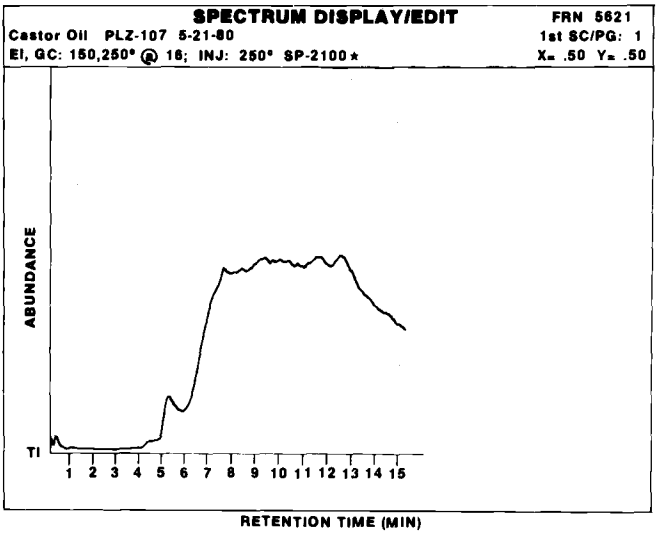


FIG. 1—*Total ion chromatogram of castor oil (Chem Service, Inc. Plasticizer Collection, PLZ-107). Note the unsatisfactory chromatography of the oil in the nonpolar column used.*

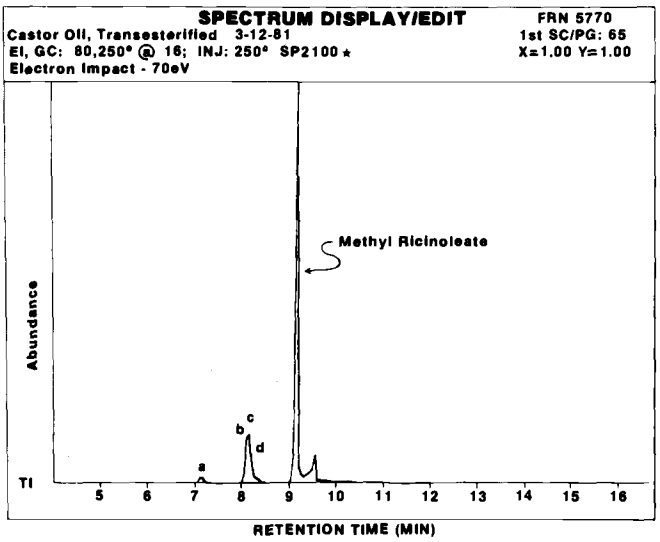


FIG. 2—*Total ion chromatogram of castor oil after transesterification yields methyl ricinoleate and other fatty acid methyl esters (retention times in parentheses).* a *is methyl palmitate (7.2).* b *is methyl linoleate (8.1),* c *is methyl oleate (8.2), and* d *is methyl stearate (8.4).*

tion using a test tube vibrator, the capped mixture was allowed to stand at room temperature for 24 h.

Ethyl ether (5 mL) was used to separate the methyl ricinoleate product in the transesterified extract of a lipstick stain [*3,11*]. Other fatty acid methyl esters are produced depending on the cosmetic involved (see Example Case 4). In order to remove excess methanol and acid, the ether solution was washed several times with water. Each time the mixture was shaken in a large test tube and the aqueous phase pipetted from the bottom and discarded. The ether

phase was evaporated under a stream of dry air leaving the extracted fatty acid ester(s) in a small volume of benzene.

The chromatographic column used with the Hewlett-Packard model 5982A GC/MS system for organic analysis of cosmetic smudges was a 74-cm, 2-mm inner diameter glass column (H-P configuration 5), packed with 2% SP 2100 on 100–120 mesh Supelcoport.

After separation of the methyl esters with ether, microlitre samples were injected (inlet temperature 250°C) onto the GC column which was heated from 80 to 250°C at 16°/min. The helium carrier gas flow was 30 cm^3/min. The compounds separated and eluted from the column were ionized by electron impact (EI) at 70 eV and analyzed by the quadrupole mass spectrometer. The internal temperatures of the ion source and mass filter were 150 and 110°C, respectively.

Results

The compound of primary interest derivatized from the castor oil in lipstick smudges, methyl ricinoleate, was identified in a transesterified extract by comparing the retention time with a known standard and by the EI mass spectrum. The methyl ricinoleate standard was obtained from Supelco Inc., Bellefonte, PA, and its retention time in the system described above was 9.1 min. As shown in Fig. 3, the principal product in the transesterified extract of a known Avon lipstick is methyl ricinoleate.

The electron impact mass spectra for known methyl ricinoleate, castor oil (transesterified) and a lipstick extract (transesterified) are graphically compared in Fig. 4. The base peak of each spectrum is at *m/e* 55, but the ions of greatest significance occur at *m/e* 166 (second most abundant ion), 198, 263, and 294 ($M\text{-}H_2O$).

The similarity of the mass spectra of methyl ricinoleate (molecular weight 312) and methyl 10-undecenoate (molecular weight 198) should be noted. During pyrolysis of castor oil or ricinoleic acid, bond cleavage and rearrangement yield 10-undecenoic acid [*9*]. In like manner, during ionization of the ricinoleic acid methyl ester in the mass spectrometer source, the resulting fragmentation pattern found experimentally is essentially the same as for the refer-

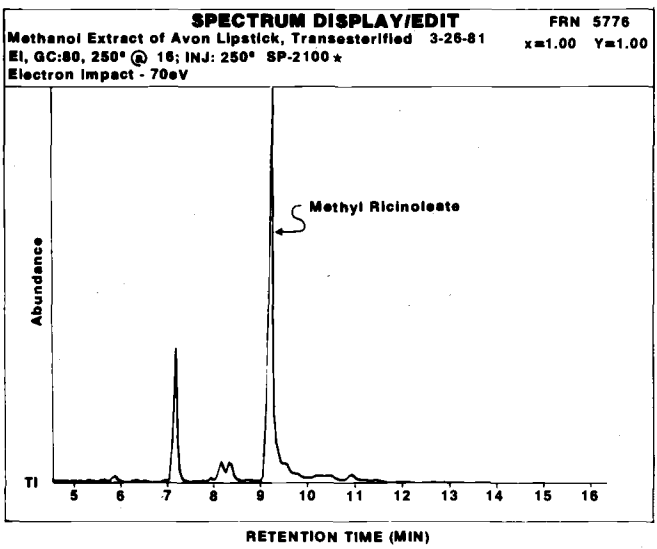


FIG. 3—*Total ion chromatogram of a transesterified methanol extract of an Avon lipstick showing the principal product, methyl ricinoleate, with a retention time of 9.1 min.*

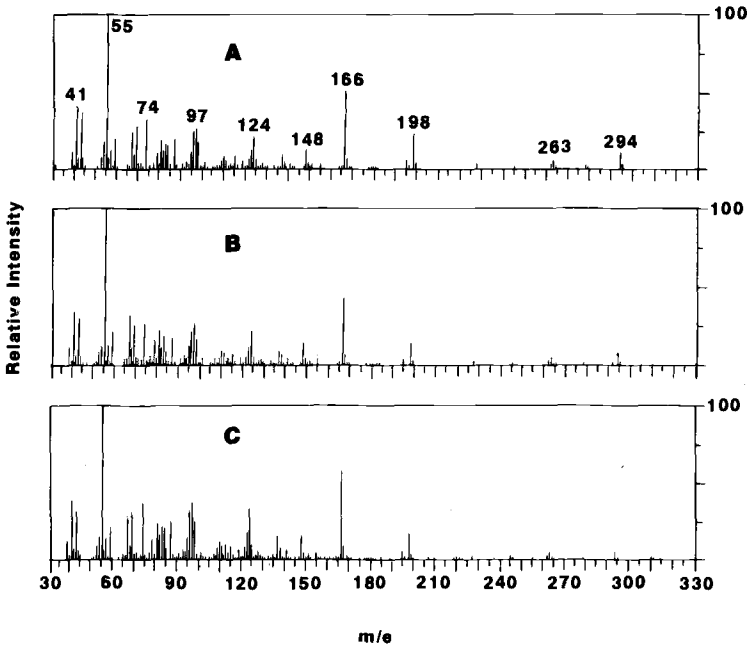


FIG. 4—*Electron impact mass spectra of total ion chromatogram peaks at a GC retention time of 9.1 min for* a, *methyl ricinoleate (Supelco, Inc. standard);* b, *castor oil (transesterified); and* c, *methanol extract of Avon lipstick (transesterified).*

ence spectrum of 10-undecenoic acid methyl ester [*12*]. Figure 5 shows the mass spectra of the two structurally related compounds.

Application

The transesterification procedure and GC/MS identification of methyl ricinoleate, the castor oil derivative, have yielded significant results in several recent cases.

Case 1

Soon after a rape was committed, a suspect was apprehended who had a smeared reddish substance on one of his hands, allegedly from silencing the victim's screams by holding his hand over her mouth. A stained tissue used by the investigator to wipe off the reddish substance was submitted to the FBI Laboratory. The transesterified extract of the tissue contained methyl ricinoleate, indicating castor oil, and additional comparisons made with the victim's own lipstick were carried out using the scanning electron microscope for trace elemental analysis and thin-layer chromatography. The lipstick determination was used in conjunction with hair and fiber and serological examinations.

Case 2

Examination of a pink-stained cigarette butt discovered at the crime scene after a burglary disclosed the presence of castor oil (Fig. 6) and a synthetic pearlizing pigment. A cosmetic analysis of cigarette butts may have lesser evidentiary value than a serology examination since blood type can sometimes be determined from saliva.

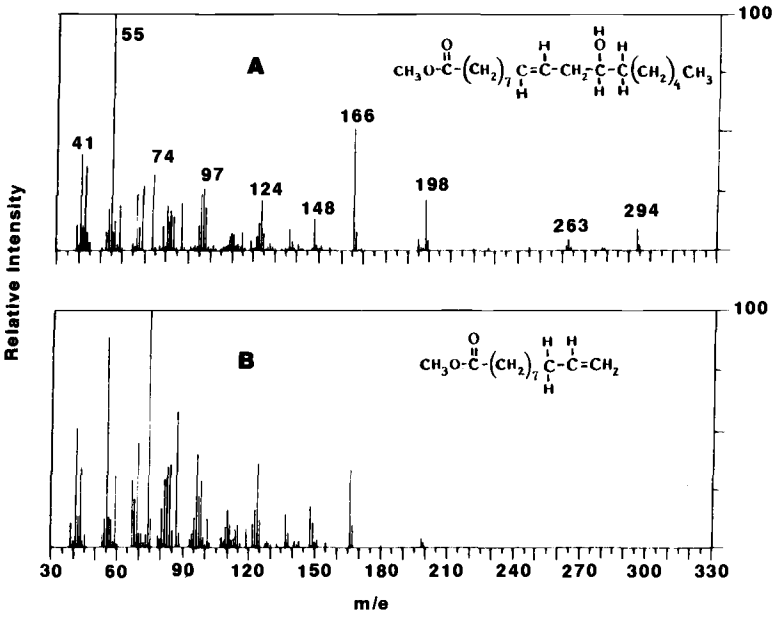


FIG. 5—*Electron impact mass spectra for* a, *methyl ricinoleate and* b, *methyl 10-undecenoate* [12].

Case 3

A murder victim was found nude with writing across her chest. Organic and inorganic analysis of residue scraped from the skin revealed that the suspect's crude message had been written with lipstick.

Case 4

Transesterification was also used in the identification of brownish smeared deposits on a towel allegedly used to clean the face of a bank robber who was shown by bank surveillance cameras to have been wearing heavy makeup during the robbery. Investigators submitted for comparison a known specimen of Max Factor pancake makeup. The manufacturer's label lists a fatty material, stearic acid, as the major organic ingredient. The commercial product of stearic acid consists mainly of a mixture of palmitic (45%) and stearic (55%) acids [*13*]. A portion of the brownish deposit was removed from the cotton towel using methanol and an ultrasonic cleaner. The insoluble fillers and pigments from the stained towel were analyzed by the emission spectrograph and X-ray diffractometer revealing talc, titanium dioxide, iron oxide, and kaolin. Another portion of the stained area was extracted with chloroform, filtered, and transesterified. The major compounds subsequently identified by GC/MS were the methyl esters formed by derivatizing the palmitic and stearic acids present in the smears of face powder. The areas under the chromatogram peaks in Fig. 7 for these two compounds represent the approximate ratio expected for the commercial stearic acid.

Conclusion

The general scheme of analysis for suspected cosmetic smudges, including extraction from a substrate, sample preparation, and instrumental determination of major organic and inor-

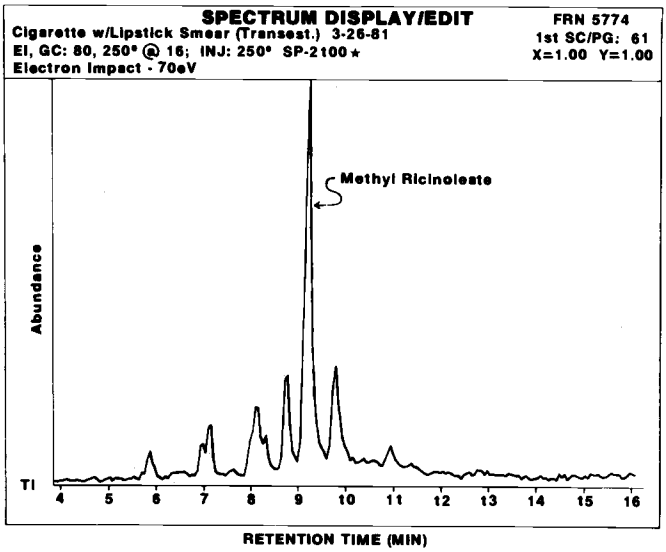


FIG. 6—*Total ion chromatogram showing the principal derivative of castor oil identified in the transesterified methanol extract of a cigarette butt bearing a lipstick stain.*

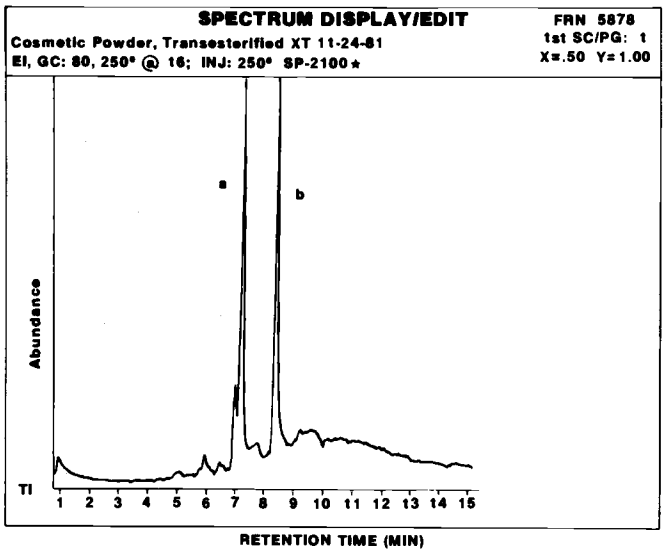


FIG. 7—*Total ion chromatogram of the transesterified chloroform extract of Max Factor pancake makeup. Methyl palmitate* (a) *and methyl stearate* (b) *were derivatized from the fatty acid ingredient.*

ganic constituents, may be followed regardless of the type of smudge, whether the source from which it originated was a cosmetic face powder, cream, lotion, rouge, eye makeup, or lipstick.

The transesterification procedure and identification of the resulting methyl ester derivatives may be used not only with castor oil-based lipsticks, but also with most cosmetics containing oils or fatty acids or both as bases, conditioners, texturizers, or emollients.

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