TECHNICAL NOTE

Ian R. Tebbett,[1] *Ph.D.; Inga A. Tulloch,*[1] *M.Sc.; and Per Knutsen,*[2] *M.S.*

The Analysis of Nail Varnishes by High Performance Liquid Chromatography

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ABSTRACT: A method using high performance liquid chromatography (HPLC) has been developed for the analysis and comparison of nail varnishes.

KEYWORDS: forensic science, criminalistics, nail varnishes, chromatographic analysis, high performance liquid chromatography

Attacks on females occasionally result in the victim breaking her fingernails in the ensuing struggle. It is not uncommon therefore for broken nails to be found either at the locus or on the accused's clothing, as a contact trace. Such a situation was recently encountered in Norway. In this particular case, it was necessary to compare nail varnish from fragments of nail found on the suspect's clothes with that worn by a murdered girl. High performance liquid chromatography (HPLC) of methanolic extracts of the nail varnish produced similar chromatographic patterns in each case. This paper reports the results of further work on the analysis of nail varnishes by HPLC. Previous published work in this area has concentrated on the analysis of dyes present in the varnish [*1-3*]. In contrast, we have sought to obtain a chromatographic pattern or "fingerprint" characteristic of individual nail varnishes.

The basic ingredients of nail varnishes are traditionally nitrocellulose combined with a plasticizer, a modifying resin, coloring agents, and a suitable solvent [*4*]. The nitrocellulose forms a tough film which is both waterproof and resistant to abrasion. Modifying resins which are generally of the aryl sulphonamide/formaldehyde type are added to give a good lustre to nail varnish films and to improve their hardness. The color of the varnish is produced by the incorporation of an insoluble lake usually combined with titanium dioxide to give a creamy appearance. These compounds are dissolved in a suitable solvent which tends

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[1]Lecturer and research assistant, respectively, Forensic Science Unit, University of Strathclyde, Glasgow, Scotland.

[2]Forensic scientist, Forensic Science Department, National Bureau of Crime Investigation, Oslo, Norway.

to be a mixture of alcohols, aromatic hydrocarbons, and aliphatic hydrocarbons. In addition, "pearly" nail varnishes also contain guanine (2-amino, 6-hydroxy purine), a crystalline substance which imparts the pearl appearance.

Experimental Procedure

The chromatographic system consisted of a Spectra Physics Sp800 XR extended range LC pump which was used to deliver solvent at 1 mL/min. The eluent was monitored at 220 nm with a Pye Unicam PU4020 variable wavelength ultraviolet detector. The column was a 25-cm by 4.5-mm inside diameter (ID) Spherisorb 5-μm ODS (Jones Chromatography, Glamorgan, UK) fitted with a Negretti and Zambra injection system incorporating a 20-μL loop. Separation was achieved with a gradient elution starting from 75% water, 25% acetonitrile changing to 100% acetonitrile linearly in 45 min. All solvents used were of HPLC grade (Rathburn Chemicals, Scotland, U.K.).

Both wet and dry samples of some 70 different nail varnishes were examined by this method. One drop of the nail varnish was diluted with methanol (0.5 mL) vortexed and then centrifuged at 2000 rpm for 5 min to remove any insoluble material. Twenty-microlitre samples of the supernanant were then injected on to the HPLC column. Varnishes which had been applied to fingernails and allowed to dry for 1 h were then removed using a cotton wool swab soaked in methanol. The swab was then placed in 0.5 mL of methanol and vortexed for 5 min. These extracts were also centrifuged before analysis of the supernanant by HPLC. Dried nail varnish was removed from nail clippings by placing the whole fragment into methanol and similarly vortexing and centrifuging.

The reproducibility of the chromatographic patterns obtained was determined by injecting the same sample five times. Also batch-to-batch variation of the nail varnishes was examined by injecting samples of the same varnish but with different manufacturers' batch numbers.

Results and Discussion

A chromatogram typical of that obtained for a wet sample of nail varnish is shown in Fig. 1. A chromatogram of the same sample after it had been applied to a fingernail and allowed to dry for 1 h, before removal with methanol, is represented in Fig. 2. The differences observed between the two chromatograms can be explained by the evaporation of volatile solvents. Each of the 70 varnishes tested produced different chromatograms under the conditions described (see Figs. 3 through 5). Samples which appeared to be of similar color but produced by different manufacturers, and also samples supplied by the same manufacturer but differing only in color, could all be readily differentiated on the basis of their chromatographic patterns.

The analysis of the same sample five times produced identical chromatographic traces, indicating that the procedure is reproducible. No difference was seen in the trace produced by two samples of the same nail varnish having different batch numbers. Also no problems were encountered in the analysis of small quantities of nail varnish as found on fragments of nail. The sensitivity of the technique was such that a detector range of 0.64 AUFS was adequate for the detection of very small amounts (2.3 mg of dried varnish in 0.5 mL of methanol). Chromatographic analysis of a methanol extract of a blank cotton wool swab showed no interfering peaks.

Conclusion

An HPLC system, using a gradient elution technique, has been developed for the analysis and comparison of nail varnishes. Some 70 different varnishes have been examined and each

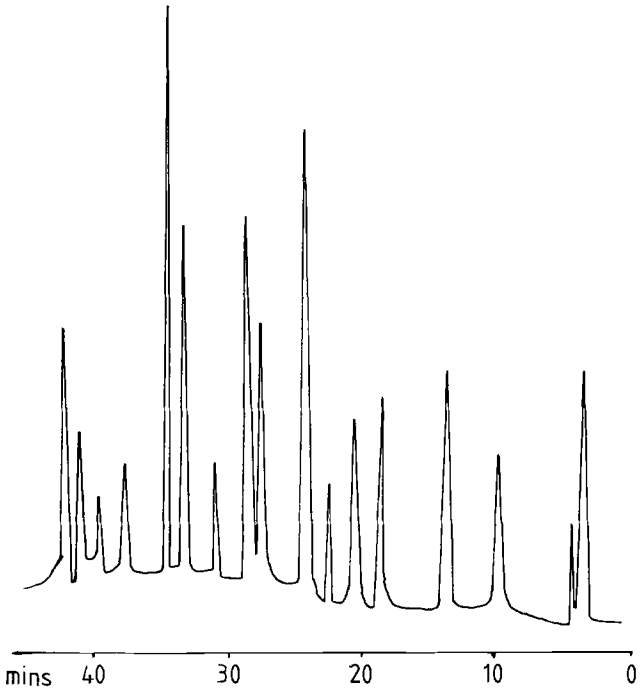

FIG. 1—*HPLC chromatogram of clear red (wet) nail varnish.*

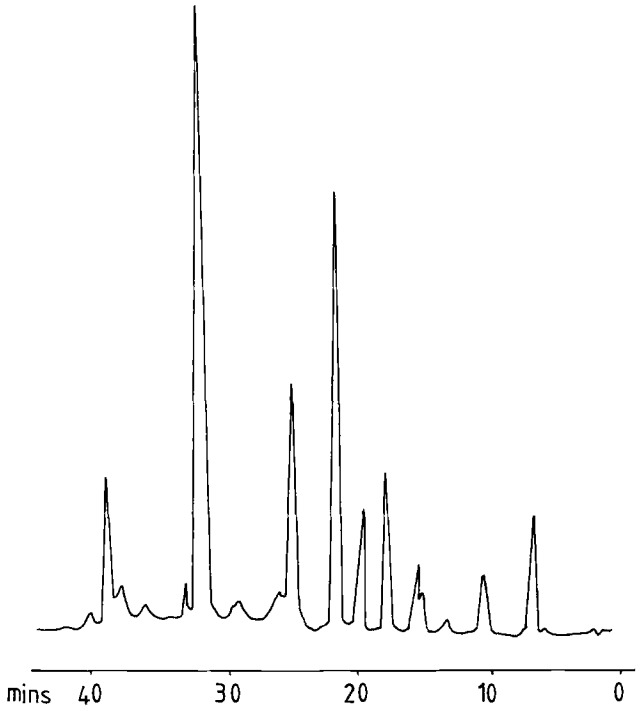

FIG. 2—*HPLC chromatogram of clear red (dry) nail varnish.*

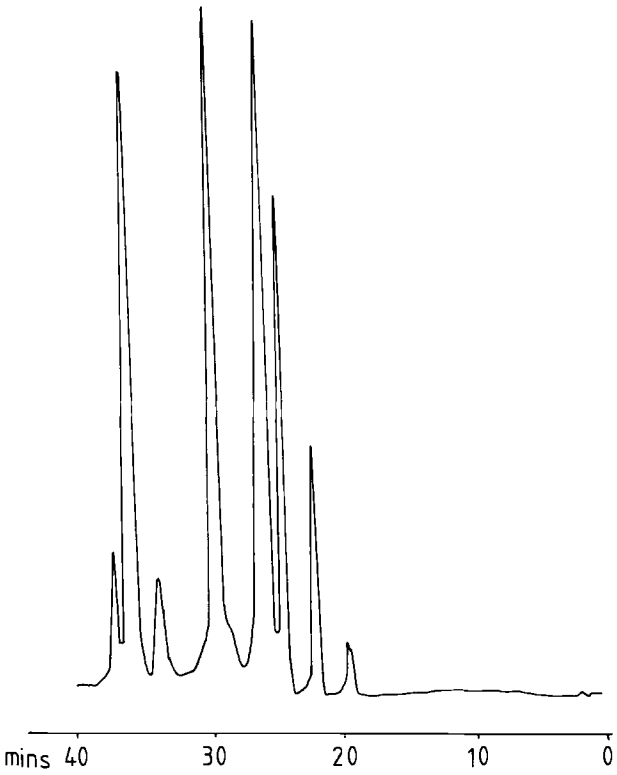

FIG. 3—*HPLC chromatogram of clear colorless nail varnish. Note the lack of peaks between 0 and 20 min.*

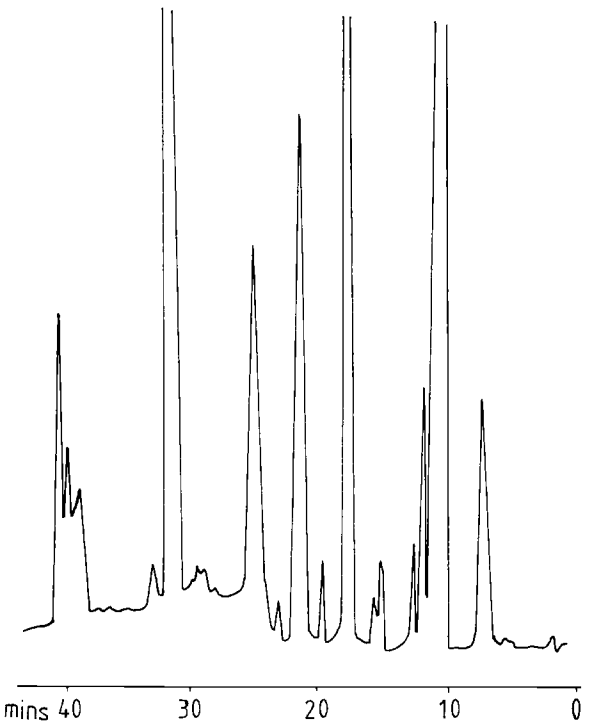

FIG. 4—*HPLC chromatogram of pearly nail varnish.*

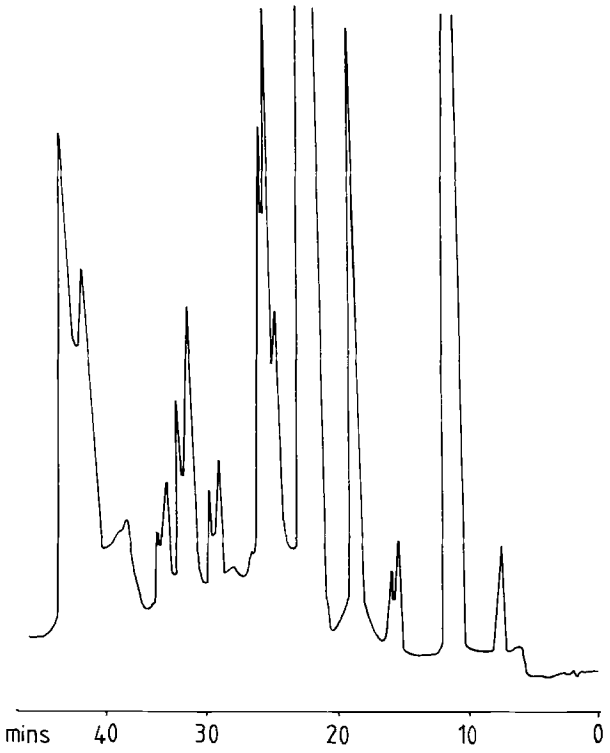

FIG. 5—*HPLC chromatogram of cream nail varnish.*

produced a different chromatographic pattern under the conditions described. This method may therefore be used for relating fragments of nail varnish found either as a contact trace on the accused, or at the scene of the crime, with that worn by the victim.

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Address requests for reprints or additional information to
Dr. Ian R. Tebbett
Forensic Science Unit
University of Strathclyde
204 George St.
Glasgow, G1 1XW Scotland