ADVANCES IN DISCRIMINATION OF DYED TEXTILE FIBERS USING CAPILLARY ELECTROPHORESIS/MASS SPECTROMETRY

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Abstract

The premise of this research is that improved forensic discrimination for forensic fiber examinations may be achievable by extraction of the dye from the fiber, followed by trace analysis by a high resolution separation technique. Protocols have been developed for extraction of unknown dyes from textile fibers based on three CE methods for dyes from six textile dye classes. Although capillary electrophoresis (CE)/diode array detection (DAD) is useful for longer fiber lengths, a more sensitive and selective technique such as CE/mass spectrometry (MS) is needed to analyze the small amount of dye (2-200 ng) present on forensically relevant fiber samples. Although this approach is destructive to the sample, only an extremely small sample is required (~2 mm of a single 15 μ diameter fiber). Automated micro-extractions and CE offer reproducible sensitive analyses. CE/MS can separate extracted dye components and provide semi-quantitative estimates of dye amounts as well as qualitative information to identify dyes.

Introduction

Fibers have achieved growing forensic significance because of their ubiquitous presence in commercial products and at crime scenes. However, discovery of a fiber and its identification as a particular fiber type may not, by itself, provide support for a forensic investigation. The history of fiber examinations is characterized by a search for increased discrimination to render such

trace evidence more specific and discriminmating.[1] The analysis of dye components following extraction from a fiber has the potential of increasing discrimination of fibers by comparison of formulations of the dye(s) employed, relative amounts of dye present, and spectral fingerprints of individual extracted dyes. Because recovered fibers range from 2-10 mm in length and contain 2-200 ng of dye,[2] methods for analysis of extracted fiber dyes need to be sensitive.

Thin layer chromatography (TLC),[3-14] high performance liquid chromatography (HPLC),[15,16] and capillary electrophoresis (CE)[17,18] have been used for separation of extracted dyes. TLC requires a relatively large amount of extracted dye for identification of all components, and TLC reproducibility can be lacking. Concerns that dilution of injected dye concentrations in HPLC columns (4-5 mm ID, 10-25 cm in length) may impact limits of detection have also been voiced.[16,19] More efficient columns (narrow internal diameter, small particles, low dead-volume connectors, high pressure) can potentially ease these problems, but gradient elution may be required to separate complex dye mixtures. Although detection limits of 200 pg/dye using single or multi-wavelength detection have been reported, extracts of short fiber lengths of light dyes shades sometimes yielded insufficient dye.[15]

CE is an excellent tool for dye analysis because many dyes are ionized, depending on pH. CE has other advantages: high efficiency, high selectivity, short analysis times/high sample throughput, simplicity and ease of automation, low organic solvent consumption/waste, low sample requirement (< 50 nL injected), and relatively low running costs. CE capillaries are reusable, inexpensive, and can be used at a higher pH than most silica-based HPLC columns.[20] Basic, reactive, and acid dyes have been separated with high resolution using CE.[12,17,21-33]

The primary objective of the research presented here was to devise micro-scale extraction protocols for dyes from four fiber types (acrylic, cotton, nylon, polyester) and to develop CE

protocols compatible with detection by diode array and mass spectrometry. MS detection offers the dual capabilities of low detection limits and qualitative identification of dyes by molecular weight and mass fragmentation. It is assumed here that the polymer type (acrylic, cotton, nylon, polyester) for any unknown fiber can be determined using polarized light microscopy[34] or infrared spectroscopy prior to extraction and analysis. A further objective was the demonstration of CE-DAD-MS analysis of dye extract from 2 mm length single fibers.

Methods and Materials

Solvents were added to single fibers in a 96-well plate on a BioMek 2000 automated extraction workstation (Beckman-Coulter, Fullerton, CA) programmed extraction of specific fiber-dye combinations. A plastic lid clamped over the plate minimized solvent evaporation during extraction. Chemicals were analytical reagent grade. Dye standards, referenced by Colour Index names, and fabric samples from manufacturers were used as provided.

Nylon fibers were treated with equal parts of aqueous ammonia, pyridine, and water (66 μL each), heated at 100 °C for 60 min.[35] Complete extraction indicated presence of an acid dye.

Cotton fibers may be dyed with direct, reactive or vat dyes. The first stage of cotton fiber extraction used 60:40 pyridine:water using (120 μL pyridine, 80 μL water) at 100 °C for 60 min; complete extraction indicated a *direct dye* is present. If incomplete extraction occurred, 200 μL of 1.5% NaOH solution was added, and the plate heated at 100 °C for 60 min. A complete extraction signified that a reactive dye was present. If extraction was still incomplete, a vat dye may be present. A 200 μL volume of reducing agent solution (0.8 g sodium dithionite, 0.5 g of NaOH, 5.0 mL H_2O, 33.0 mL of 1,2-dimethoxy ethane, and 66.0 mL of H_2O) was added to the remaining fiber sample in a 96-well plate, and heated at 100 °C for 30 min. When the plate was

placed in a fume hood without the cover, air oxidized the extracted vat dye to water insoluble pigment with a corresponding color change.[36]

Acrylic fibers were treated with 50:50 formic acid:water (total volume 200 µL) at 100 °C for 60 min. Complete extraction after this step indicated that a basic dye is present.[37]

Polyester fibers were treated with 200 µL of chlorobenzene at 100 °C for 60 min. Complete extraction indicated presence of a disperse dye.[38]

All extracts were dried at 60 °C to remove extraction solvents and reconstituted for capillary electrophoresis by adding 190 µL water. CE separations were performed with a PACE-MDQ CE system (Beckman-Coulter) equipped with a diode array detector, using 50 mm capillaries (Polymicro Technologies, Phoenix, AZ). The CE methods employed group into three protocols with conditions selected to provide efficient separation of multiple dye components and to be sufficiently volatile for electrospray ionization (ESI)-MS detection.

Separation of acid, direct, and reactive dyes, from cotton and nylon fibers, was done with an anionic buffer system of 15 mM ammonium acetate in acetonitrile-water (40:60, v/v) at pH 9.3.[39] Vat dyes from cotton fibers can be separated after the addition of a reducing agent, sodium dithionite, to this same buffer.[36,39] Extracts from acrylic fibers containing basic cationic dyes were analyzed with a cationic buffer system of 45 mM ammonium acetate buffer in acetonitrile-water (60:40, v/v) at pH 4.7.[39] Separation of hydrophobic disperse dyes from polyester was performed by non-aqueous CE using 80 mM ammonium acetate in acetonitrile-methanol (75:25, v/v) at pH 9. For CE-DAD analysis, hydrodynamic injections were made at 0.2 psi for 2 s.[38,39]

Separations were performed at 25 °C with an applied voltage of 30 kV for the analysis of direct, reactive, acid, and vat dyes and with 20 kV for the analysis of cationic dyes. For CE-DAD-MS analyses, an external light source was modified to position the DAD window close to

the MS source. Light from a 75 W xenon lamp (Craic Technologies, Altadena, CA) was focused using a 2.5 cm diameter lens onto a 1 mm diameter optical fiber, through a standard 0.8 mm aperture located at a capillary window, and absorbance from 190 to 600 nm was monitored. A Micromass Q-Tof Micro (Waters Corporation, Milford, MA) with a sheath flow interface[13] to an ESI source was employed for MS. For positive CE-MS, the sheath liquid was 50/50 methanol/water with 1% formic acid at 1.7 μL/min. The nebulization gas was set at 8 psi, and the ESI voltage and cone voltage were 3.7 kV and 17 V, respectively. In negative mode, the sheath liquid was 50/50 isopropyl alcohol/water with 1% triethylamine at 10 μL/min. The nebulization gas was set at 15 psi, and the ESI voltage and cone voltage were 4.8 kV and 44 V, respectively. Hydrodynamic injections were performed at 1 psi for 5 s with the ESI voltage off.

Results and Discussion

CE analysis of anionic acid, direct, reactive, or vat dyes extracted from nylon and cotton requires a high pH buffer to ensure that solutes were negatively charged.[39] Anionic dyes are soluble in water and were separated using a buffer consisting of 15 mM ammonium acetate in 40:60 acetonitrile:water at pH 9.3. Figure 1 shows the separation achieved for five acid, four direct, and five reactive dyes within 15 min. Vat dyes are insoluble pigments that are applied to cotton in the reduced water-soluble form and oxidized back to the water insoluble form on the fiber. Vat dyes were extracted from cotton using sodium dithionite to reduce vat dyes to their water-soluble *leuco* form. After oxidation during drying, dyes were reconstituted in acetonitrile:water (40:60, v/v). By adding sodium dithionite to the anionic CE buffer, vat dye extracts can be analyzed using the anionic CE protocol as for acid, direct, and reactive dyes.[36,39] Figure 2 displays the separation of the major and minor components of three vat dye standards. Small peaks represent minor components present in the three dyes and not in the blank.

Cationic basic dyes from acrylic require a low pH buffer for CE analysis. The CE separation shown in Figure 3 was performed using a 45 mM ammonium acetate buffer in 60:40 acetonitrile:water at pH 4.7.[39]

Disperse dyes are poorly soluble in water and require non-aqueous CE conditions, such as a conductive electrolyte dissolved in organic solvent(s). Separations of disperse dyes (not shown) were achieved using an electrophoretic medium consisting of 80 mM ammonium acetate and 75% acetonitrile in methanol at pH 9.[38,39]

CE/DAD enables comparison of fiber dye extracts based on peak migration times and their UV/visible spectra. Figure 4 shows separation of a mixture of three blue acid dyes extracted from a 10 cm fiber. The spectra are quite similar and could be difficult to distinguish; micro-spectrophotometry conducted directly on the fiber produces a mixture spectrum that hides the presence of three dyes. Elution order is consistent with the molecular structures shown: larger anions and anions of lower charge elute first. However, UV/visible detection may not be sensitive enough for use with extracts from the short fiber lengths generally encountered in forensic cases.[40] For extracts from fibers lengths close to 1 cm, baseline noise increases relative to the dye peak height. As the signal-to-noise ratio decreases, usefulness of the UV/visible spectrum for component identification is diminished. CE/MS detection reduces the length of fiber required to detect dye components extracted from single fibers. Figure 5 shows reconstructed ion electropherograms of an extract from a single 2-mm length of acrylic fiber that was dyed with three cationic basic dyes. The dye peaks are easily detected and mass spectra from the extract match well spectra from dye standards.

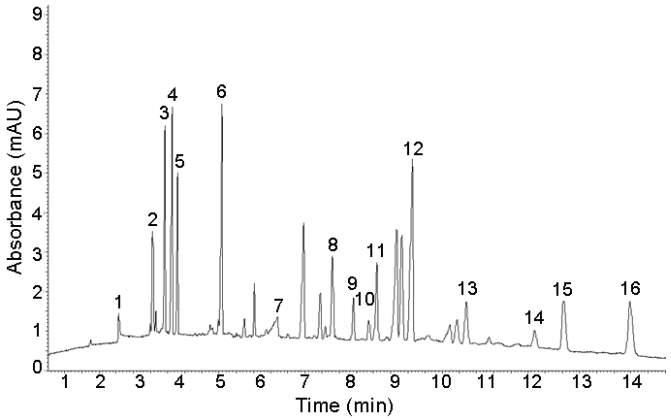

FIG. 1−Electropherogram at 214 nm for fourteen acid, direct, and reactive dyes (1 mg/mL).[39] Peak identification: (1) neutrals; (2) acid blue 239; (3) acid yellow 156; (4) acid blue 324; (5) acid red 337; (6) acid dye, no C.I.; (7) direct red 84; (8) direct orange 39; (9) direct yellow 58, (10) direct blue 71, first peak; (11) direct blue 71, second peak; (12) reactive blue 250; (13) reactive red 198; (14) reactive blue 220; (15) reactive red 180; (16) reactive red 239.

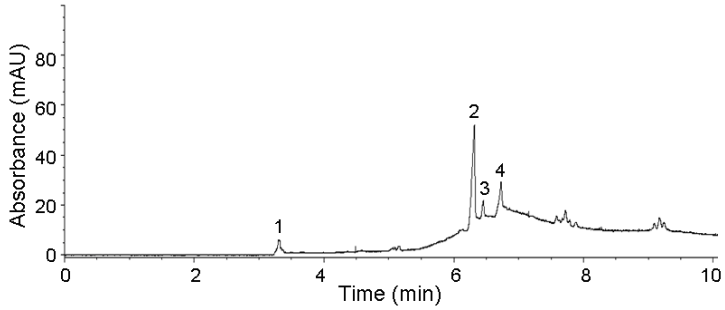

FIG. 2−Electropherogram at 214 nm for three vat dyes (1 mg/mL).[39] Peak identification: (1) neutrals; (2) vat yellow 2; (3) vat orange 2; and (4) vat black 16.

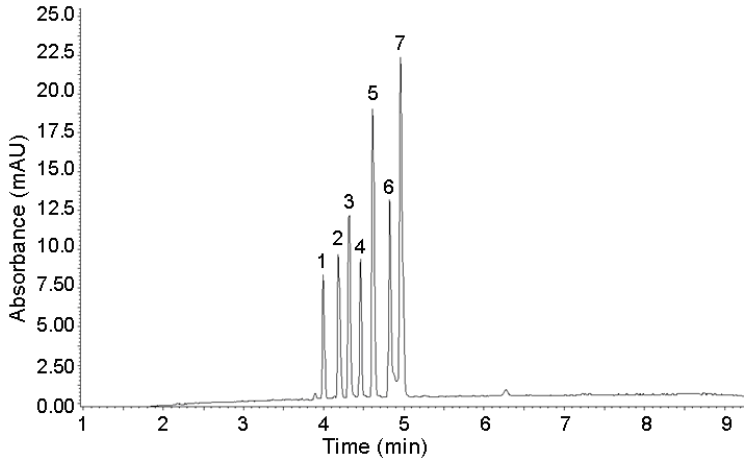

FIG. 3−Electropherogram at 214 nm for seven cationic dyes (1 mg/mL).[39] Peak identification: (1) basic red 22; (2) basic yellow 21; (3) basic blue 159; (4) basic red 14; (5) basic blue 41; (6) basic blue 45; (7) basic red 18.

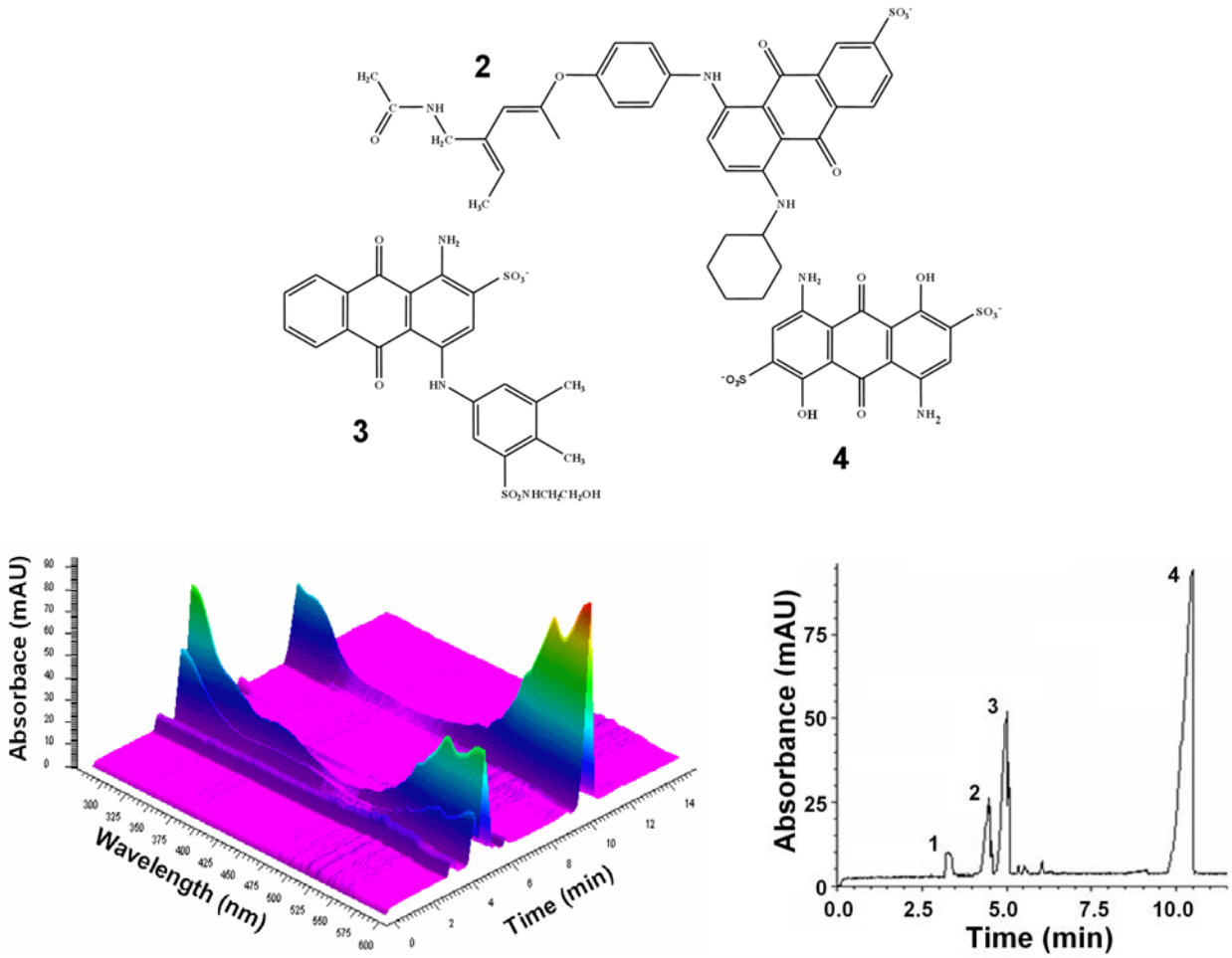

FIG. 4– Separation of three acid dyes extracted from a 10 cm nylon fiber. Peak identification: (1) neutrals; (2) acid blue 239; (3) acid blue 277; and (4) acid blue 45. Diode array electropherogram shown at left, electropherogram taken at 600 nm shown at right, and structures shown at top.

Conclusions

Microextraction and capillary electrophoresis methods have been developed to span the analysis of nylon, cotton, polyester, and acrylic fibers dyed with six different classes of textile dyes, *i.e.*, acid, direct, reactive, vat, cationic, and disperse dyes. The analysis of dye extracts from single textile fibers by CE/DAD has been demonstrated down to 1 cm fiber lengths and by CE/MS down to 2 mm fiber lengths. Further research should further characterize detection limits for extraction of dyes from various fiber types and validate these protocols on case work samples.

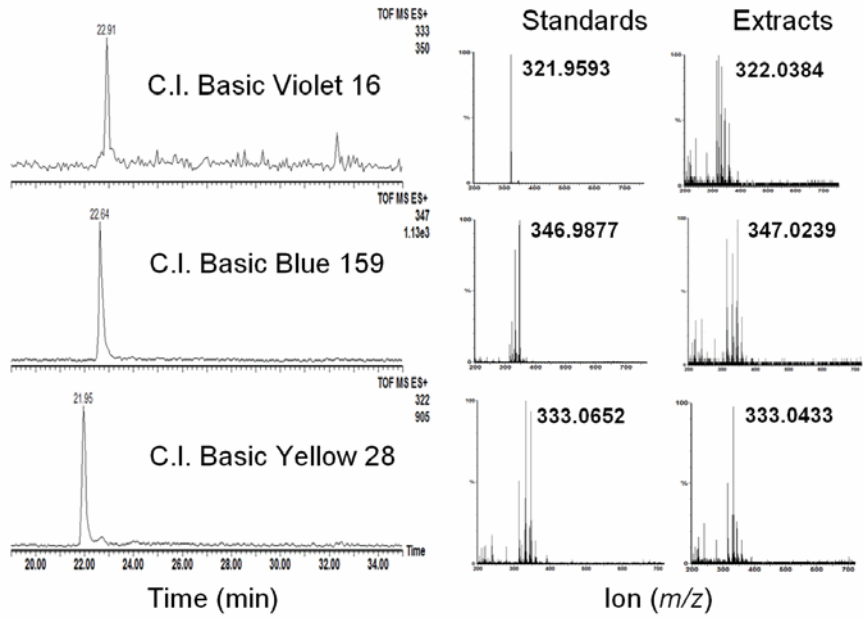

FIG. 5− (left) Reconstructed ion electropherograms for three basic dyes extracted from a single 2 cm acrylic fiber. (right) Comparisons of mass spectra for dye standards and extracted dye peaks (ion masses shown).

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References

1. Robertson J, Grieve M. Eds., Forensic Examination of Fibres 2nd edition. London: Taylor & Francis: 1999.
2. Macrae R, Smalldon KW. J Forensic Sci 1979; 24: 109-116.
3. Macrae R, Dudley RJ, Smalldon KW. J Forensic Sci 1979, 24: 117-129.
4. Resua R. J Forensic Sci 1980, 25: 168-173.
5. Shaw IC. Analyst 1980, 105: 729-730.
6. Home JM, Dudley RJ. Forensic Sci Int 1981, 17: 71-78.
7. Beattie B, Roberts H, Dudley RJ. J Forensic Sci 1981, 21: 233-237.
8. Hartshorne AW, Laing DK. Forensic Sci Int 1984, 25: 133-141.
9. Wiggins KG, Crabtree SR, March BM. J Forensic Sci 1996, 41: 1042-1045.

10. Laing DK, Boughey L, Hartshorne AW. J Forensic Sci 1990, 30: 299-307.
11. Laing DK, Hartshorne AW, Bennett DC. J Forensic Sci 1990, 30: 309-315.
12. Rendle DF, Wiggins KG. Review of Progress in Coloration and Related Topics, 1995, 25: 29-34.
13. Rendle DF, Crabtree SR, Wiggins KG, Salter MT. J Soc Dye Colour 1994, 110: 338-341.
14. Crabtree SR, Rendle DF, Wiggins KG, Salter MT. J. Soc. Dye. Colour 1995, 111: 100-102.
15. Wheals BB, White PC, Paterson MD. J. Chromatogr. 1985; 350: 205-215.
16. Laing DK, Gill R, Blacklaws C, Bickley HM. J Chromatogr 1988; 442: 187-208.
17. Sirén H, Sulkava R. J Chromatogr A 1995; 717: 149-155.
18. Xu X, Leijenhorst H, Van den Hoven P, De Koeijer JA, Logtenberg H. Sci Justice 2001; 41: 93-105.
19. Wiggins KG, Cook R, Turner, YJ. J Forensic Sci 1988; 33: 998-1007.
20. Frazier RA, Ames JM, Nursten HE. Capillary Electrophoresis for Food Analysis-Method Development. Royal Society of Chemistry: Cambridge, 2000.
21. Croft SN, Lewis DM. Dyes and Pigments 1992, 18: 309-317.
22. Croft SN, Hinks D. Textile Chemist and Colorist 1993, 25: 47-51.
23. Burkinshaw, SM, Hinks D, Lewis DM. J Chromatogr 1993, 640: 413-417.
24. Burkinshaw SM, Hinks D, Lewis DM. In: Special Publication-Royal Society of Chemistry, 1993, Vol. 122: 93-100.
25. Croft SN, Hinks D. J Soc Dye Colour 1992, 108: 546-551.
26. Takeda S, Tanaka Y, Nishimura Y, Yamane M, Siroma Z, Wakida S. J Chromatogr A 1999, 853: 503-509.
27. Borros S, Barbera G, Biada J, Agullo N. Dyes and Pigments 1999, 43: 189-196.
28. Burkinshaw, S.M.; Graham, C. Dyes and Pigments 1997, *34*, 307-319.
29. Burkinshaw SM, Son YA, Bide MJ. Dyes and Pigments 2001, 48: 245-251.
30. Riu J, Schonsee I, Barcelo D. J Mass Spectrom 1998, 33: 653-663.
31. Riu J, Eichhorn P, Guerrero JA, Knepper TP, Barcelo D. J Chromatogr A 2000, 889: 221-229.
32. Riu J, Barcelo D. Analyst 2001, 126: 825-828.
33. Robertson J, Wells RJ, Pailthorpe MT, David S, Aumatell A, Clark R. Advances in Forensic Sciences, Proceedings of the Meeting of the International Association of Forensic Sciences, 13th, Duesseldorf: Aug. 22-28, 1993.
34. Stoeffler SF. J Forensic Sci 1996; 41: 297-299.
35. Stefan AR, Dockery CR, Nieuwland AA, Roberson SN, Hendrix JE, Morgan SL. Submitted to J Forensic Sci 2007.
36. Dockery CR, Stefan AR, Nieuwland AA, Roberson SN, Baguley BM, Hendrix JE, Morgan SL. Submitted to J Forensic Sci 2007.
37. Stefan AR, Dockery CR, Nieuwland AA, Hendrix JE, Morgan SL. Submitted to J Forensic Sci 2007.
38. Baguley BM, Stefan AR, Dockery CR, Hendrix JE, Morgan SL. Submitted to J Forensic Sci 2007.
39. Morgan SL, Vann, BC, Baguley BM, Stefan AR. Submitted to J Forensic Sci 2007.
40. Tuinman AA, Lewis LA, Samuel A, Lewis S. Anal Chem 2003, 75: 2753-2760.