Fast analysis of ink dyes using the Agilent 1290 Infinity LC System coupled to Agilent 6140 single quadrupole LC/MS System for forensic analysis of ink pens and markers

Application Note

Forensics

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Abstract

In forensics, the analysis of ink writings from documentation is required for authentication or crime analysis. Ten organic ink dye components typically found in ink pens were separated using the Agilent 1290 Infinity LC System and quantified using the Agilent 6140 single quadrupole LC/MS System. The dyes were separated in less than 3.5 min using a sub-2-µm, 30 mm column. Analysis of ink markings on paper from five black and five blue pens were matched to standard using retention time, mass-to-charge ratio and UV/Vis spectral matching. The results show that the pens can be distinguished from each other based on the percentage of ink dye content.

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Introduction

Determining the degradation and the source of ink play an important role during the forensic analysis of writings[1,2]. In this Application Note, ink source determination is demonstrated by comparing ink markings on paper from 10 pens against 10 external dye standards. The 10 external dye standards (Table 1) were separated on a sub-2-µm column and quantified using an Agilent 6140 single quadrupole LC/MS System. The recovery analysis of these dyes was performed from paper samples using an optimized extraction method. Ink markings on paper made by five black and five blue ink markers, ball point, and gel pens were matched with the standards using retention time (RT), mass-to-charge ratio (*m/z*) and UV/Vis spectra. Certain types of dyes were found to exist in different proportions in different pens. Therefore, the source of the ink can be linked to a specific type of pen in a relatively short amount of time using this method.

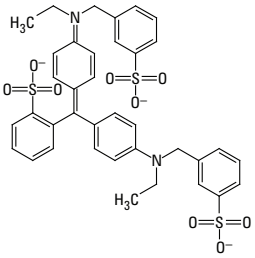
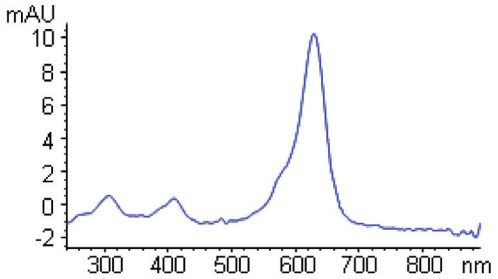
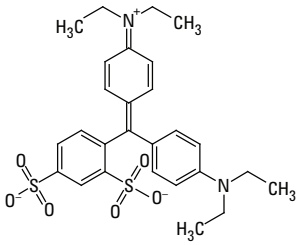
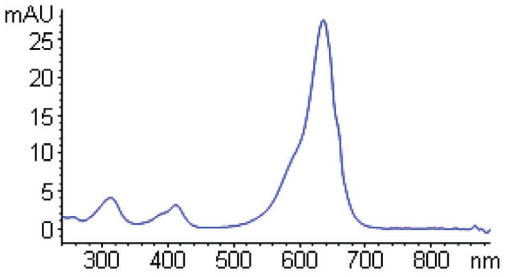
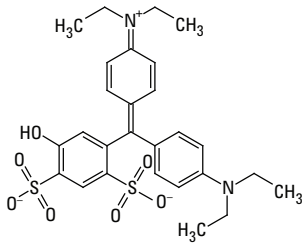
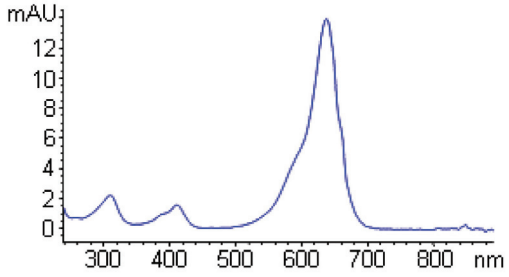
Ink Dye (abbreviation)	Structure	UV/Vis Spectra
Acid blue 9 (AB9)		 λ max: 630
Patent Blue VF (PBVF)		 λ max: 636
Patent Blue V (PBV)		 λ max: 636

Table 1
Structures of 10 ink dyes used in the experiment along with the UV spectra from 230 nm to 900 nm. (continued)

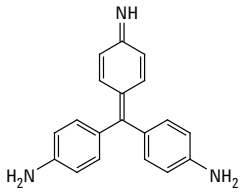
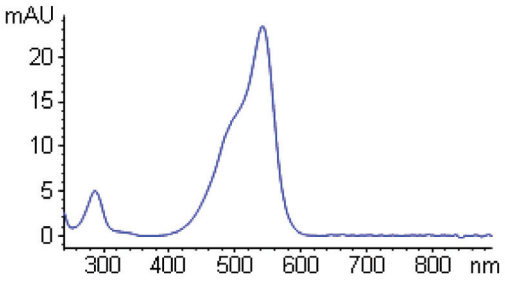
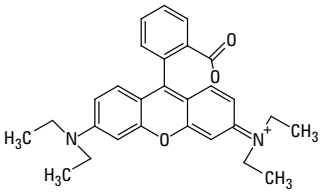
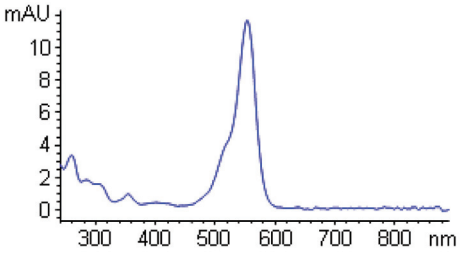
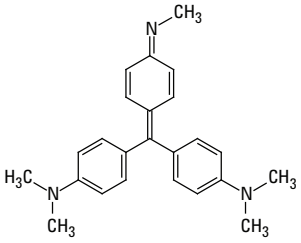
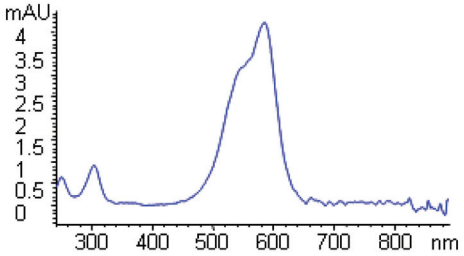
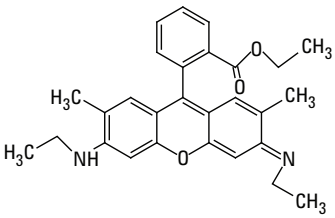
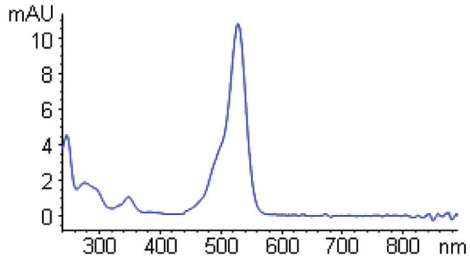
Ink Dye (abbreviation)	Structure	UV/Vis Spectra
Pararosaniline hydrochloride (PAH)		 λ max: 542
Rhodamine B (RB)		 λ max: 554
Methyl violet B base (MVB)		 λ max: 580
Rhodamine 6G (R6G)		 λ max: 528

Table 1
Structures of 10 ink dyes used in the experiment along with the UV spectra from 230 nm to 900 nm. (continued)

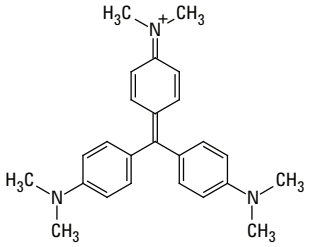
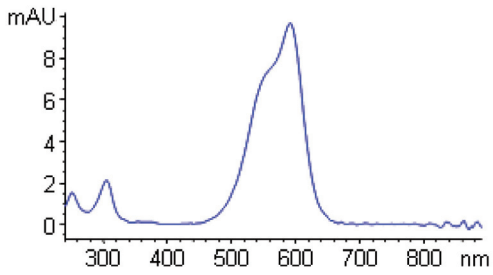
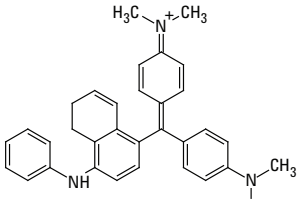
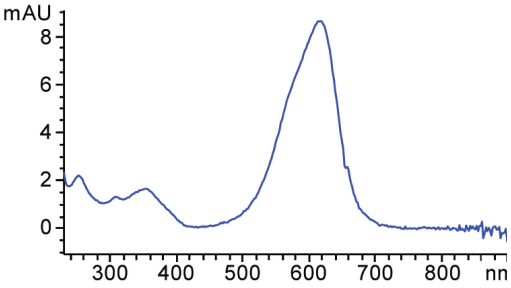
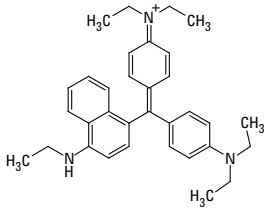
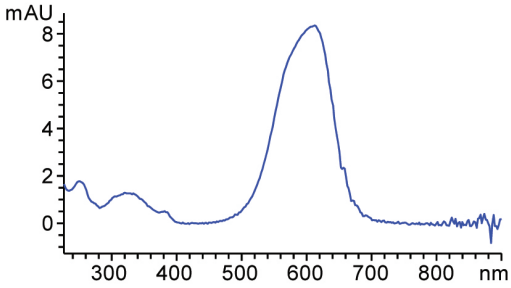
Ink Dye (abbreviation)	Structure	UV/Vis Spectra
Crystal Violet (CV)		 λ max: 592
Victoria blue b (VBB)		 λ max: 616
Victoria pure blue BO (VPBBO)		 λ max: 612

Table 1
Structures of 10 ink dyes used in the experiment along with the UV spectra from 230 nm to 900 nm.

Experimental

The 10 ink dye standards were purchased from Sigma Aldrich. Five black and five blue markers or ball point, gel pens were purchased from local stores for analysis. The mobile phase modifiers used were of LC-MS grade. Acetonitrile used was super gradient from Labscan.

Ten ink dye standard stock and linearity solutions: Standard stock solution was prepared in 100% methanol. Mixed linearity solution was prepared to the concentrations of 0.1 ppm, 0.5 ppm, 1 ppm, 2 ppm and 10 ppm in 50% mobile phase A and 50% mobile phase B. Six replicate experiments were performed using 0.5 ppm standard solution to obtain retention time and reproducibility values.

Recovery studies and extraction procedure: Dye mixture in the amount of 25 µL of 10 ppm (0.125 ppm) was added on 75 gram per square meter (gsm) paper and air dried. One milliliter of acetonitrile was added to the paper and vortexed for 30 sec followed by sonication for 10 sec. One milliliter of buffer A was then added followed by vortex for 30 sec and sonication for 1 min. The recovered amounts from the linearity results were compared against the expected amount of 0.125 ppm to determine the recovery percentage. The pens were used to fill a circle of 7-mm diameter on a paper. Samples were taken directly for analysis. Single ion monitoring (SIM) mode was used in the mass spectral acquisition.

Experimental Parameters	Details	
Column	Agilent ZORBAX SB-Aq 30 mm × 2.1 mm, 1.8 µm, p/n 824700-914; operated at 25 °C	
Mobile phase	Buffer A: Ammonium formate buffer pH 4.0 (190 µL of formic acid and 0.64 g of ammonium formate in 1L of water) Buffer B: 100% acetonitrile	
Step gradient run	Run time (min): 4.2 min 0 min – 20% B 0.01 min – 32% B 1.0 min – 34% B 1.1 min - 47% B 2.5 min – 50% B 2.6 min – 65% B 3.5 min – 75% B 3.6 min – 100% B 4.2 min – 100% B 4.3 min – 20% B 5.0 min – 20% B	
Flow	0.7 mL/min from 0 to 1 min 1.0 mL/min from 1 to 5 min	
Injection volume	1 µL, needle wash at flush port for 4 sec with 100% methanol	
Diode array detector (DAD) detection	Spectral acquisition at 2 nm step from 230 nm to 900 nm using Agilent 1200 Series DAD SL connected in series to an Agilent 1290 Infinity LC system	
Agilent 6140 single quadrupole LC/MS System	Drying gas	12.0 L/min
	Nebulizer pressure	40 psig
	Dry gas temperature	300 °C
	Capillary Voltage (+)	4000 V
	ESI Source: Positive mode	
	SIM mode, peak width 0.05 min	

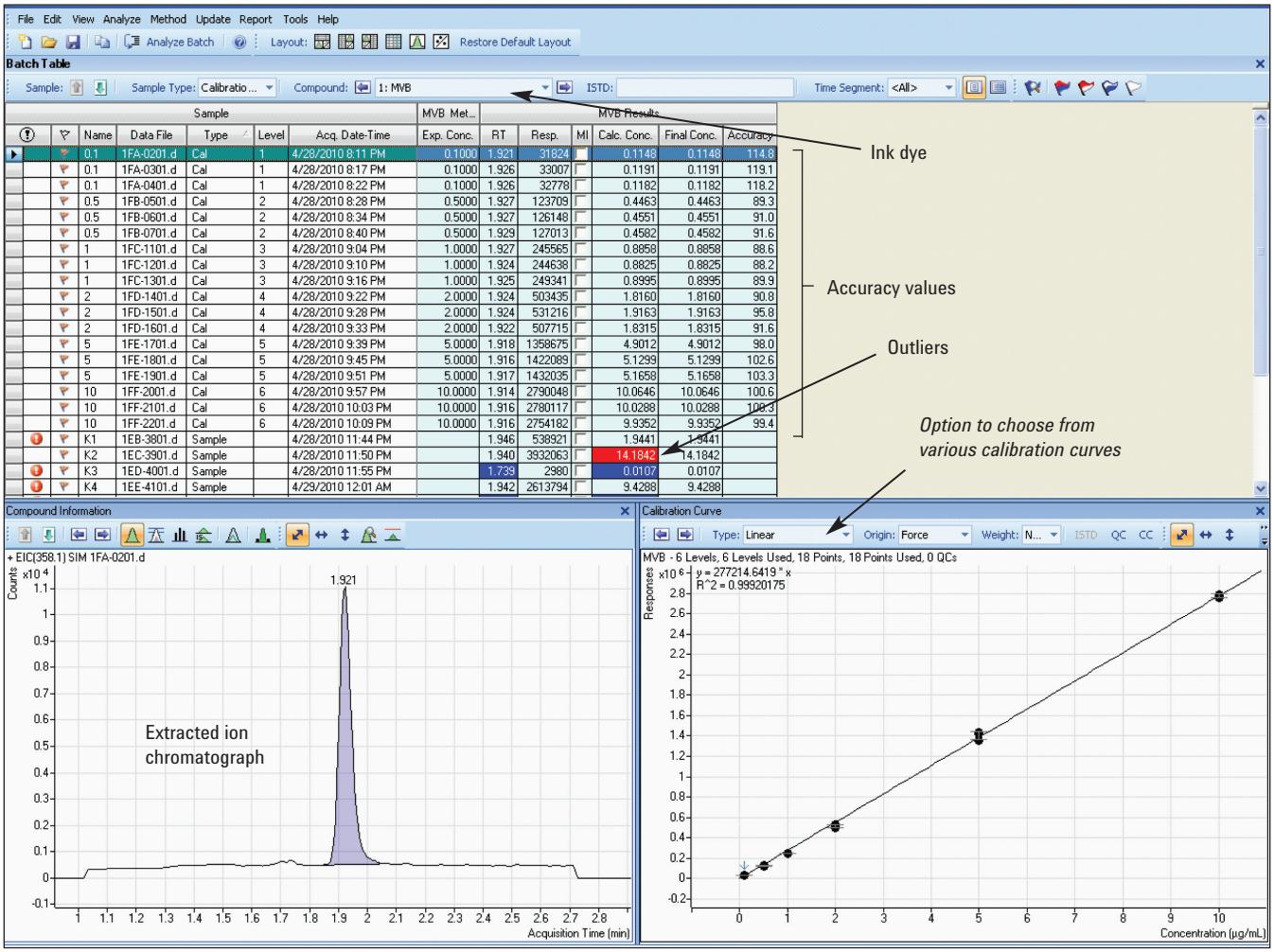


Figure 1
Snapshot of quantitative software for data analysis. Sample information, EIC and linearity curves are displayed in the same screen.

The data acquisition was performed using ChemStation B.04.02 software and the data files were converted online as a post acquisition step to MassHunter files using the MassHunter LC/SQ Integration Software (B.02.00). Data analysis was subsequently performed using MassHunter Quantitative Analysis software (B.03.01).

ChemStation data files were efficiently converted to MassHunter data files and all recovery and linearity data were processed using MassHunter Quantitative Analysis software (Figure 1).

Results and Discussion

A mixture of 10 ink dyes was analyzed using an Agilent 1290 Infinity LC system and an Agilent 6140 single quadrupole LC/MS System. All peaks were resolved well using a step gradient from 20% B to 100% B with a 30 mm Agilent ZORBAX SB-Aq, 1.8 µm column. The mobile phase (Buffer A) with pH 4.0 was found to be ideal to elute all ten ink dyes with good peak shape and resolution. The short gradient time ran from 20% B to 32% B and helped to separate PBV from PAH. The step gradient continued with partial isocratic steps of 32% B to 34% B and later of 47% B to 50% B. This helped to reduce the elution time of ink dyes from RB to CV, thereby reducing the overall run time. The specificity of the method was increased by operating the LC/MS in time programmed SIM mode. Here, three time segments were added in data acquisition: 0 – 1 min, 1–2.7 min, 2.7–5 min. This was done to contain specific molecular ions in each time segment (determined empirically) and to increase the dwell time (Table 2). Figure 2 shows the MS total ion chromatogram (SIM mode) for the 0.5 ppm standard mix of 10 ink dyes.

The Agilent 1290 Infinity DAD operates in the range of 190–640 nm while Agilent 1200 DAD SL has a specification range from 190–950 nm. Since some ink dyes have spectra that go beyond 640 nm, an Agilent 1200 Series DAD SL was connected to the Agilent 1290 Infinity LC System in series along with the Agilent 6140 single quadrupole LC/MS System. The advantages of MS-based detection are increased sensitivity and selectivity. These parameters along with UV-based detection and RT matching, provide accurate confirmation of dyes in pens.

The precision of the method (Table 2) using six replicates of 0.5 ppm solution show standard deviation (SD) for retention time to be less than 0.003 min and the RSD for area response to be less than 3.0. The linearity at six concentration levels shows the correlation coefficient (R^2) to be greater than 0.99. Recovery of the standard dyes from dried paper samples using the recovery procedure effectively extracted out all of the 10 ink dyes. The results from recovery experiments show a recovery range of 89% to 110% for all ten ink dyes from paper.

Ink dye analysis from pens

RT, *m/z* and UV/Vis spectral matches from standards were used to confirm the identity of the dyes from paper markings. Representative analysis results from two pens are shown in Figure 2. MVB, CV and VBB were identified in black pen 4 while VPBBO was present in blue pen 3. In the pen markings tested here, typically 5 out of 10 tested ink dye standards were found. These five dyes also were within the calibration range. The results in Table 3 show the specific ratios in which the 5 ink dyes occur in different pen markings. Analysis of the ratios of dyes present in the paper markings can possibly be traced to the origin of the pen. Nevertheless, there are some exemptions; for example, black pen 2 and 4 markings on paper show similar formulations of dyes while black pen 5 mark-

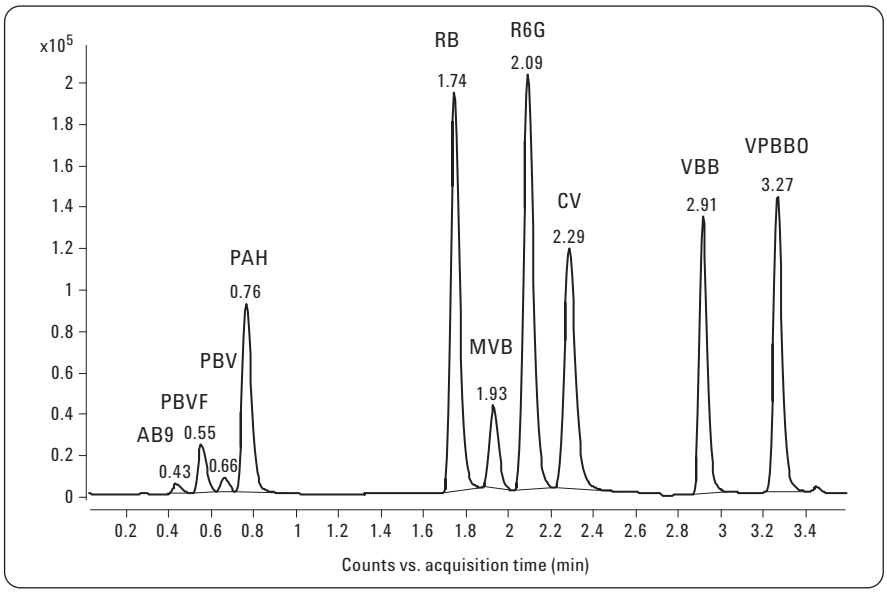


Figure 2
Total ion chromatogram (TIC) of the mixture of 10 ink dyes operated in time programmed SIM mode.

Abbreviated dye name	Molecular ion $(M+H)^+$	Fragmentor voltage (V)	Retention time (min)	SD of RT, n=6	RSD of Peak Area, n=6	Correlation Coefficient R^2	Average recovery % N=3
Time Segment: 0 – 1 min							
AB9	749.0	147	0.432	0.002	1.82	0.998	89
PBVF	545.0	123	0.552	0.002	1.63	0.991	106
PBV	561.0	96	0.660	0.002	1.24	0.992	110
PAH	288.1	120	0.762	0.002	2.08	0.998	106
Time Segment: 1 – 2.7 min							
RB	443.1	99	1.746	0.001	2.04	0.998	106
MVB	358.1	135	1.928	0.001	2.62	0.999	108
R6G	443.2	101	2.090	0.001	2.19	0.999	106
CV	372.2	156	2.284	0.001	1.78	0.998	104
Time segment: 2.7 – 5 min							
VBB	470.2	156	2.914	0.001	2.27	0.998	107
VPBBO	478.2	123	3.265	0.001	2.13	0.999	105

Table 2
Molecular ion, fragmentor voltage and retention time of 10 ink dyes acquired using SIM mode using time segments. The RT SD and area RSD were calculated from six replicate injections of 0.5 ppm standard solutions. The correlation coefficient represents the linearity samples at six concentration levels (0.1 – 10 ppm, three replicated each). Recovery of standards from paper ranged from 89% to 110%.

ings did not contain any of the ink dyes tested here. This suggests that additional dye standards are needed to make a comprehensive database for forensic analysis of documentation.

Conclusions

Ten ink dyes were separated in less than 3.5 min with excellent retention time reproducibility (SD < 0.003) while the area precision was less than RSD 3%. The recoveries of inks from paper ranged from 89 to 110%. Analyses of ink markings on paper from ten randomly selected pens mostly show five dyes in various combinations. This ratio of ink dyes helps to identify the origin of the pen. RT, *m/z* and UV/Vis spectral matching with external standards were used to confirm the identity of the compounds extracted from paper. The MassHunter LC/SQ Integration Software efficiently converted ChemStation files to MassHunter data files. The data processing was effectively performed on MassHunter Quantitative Analysis software. The combination of the Agilent 1290 Infinity LC System and Agilent 6140 single quadrupole LC/MS System is an efficient tool in forensic applications that include authentication, and crime analysis of documentations.

References

1.
H. Zou, Z. Wang, N. Ye, R. Xu, R. Zhu, B. Wang, G. Gu, "NACE discrimination of black ballpoint pen inks," *Chromatographia*, 67: 483-486, 2008.

2.
Y. Liu, J. Yu, M. Xie, Y. Chen, G. Jiang, Y. Gao, "Studies on the degradation of blue gel pen dyes by ion-pairing high performance liquid chromatography and electrospray tandem mass spectrometry," *J. Chromatography A*, 1125: 95-103, 2006.

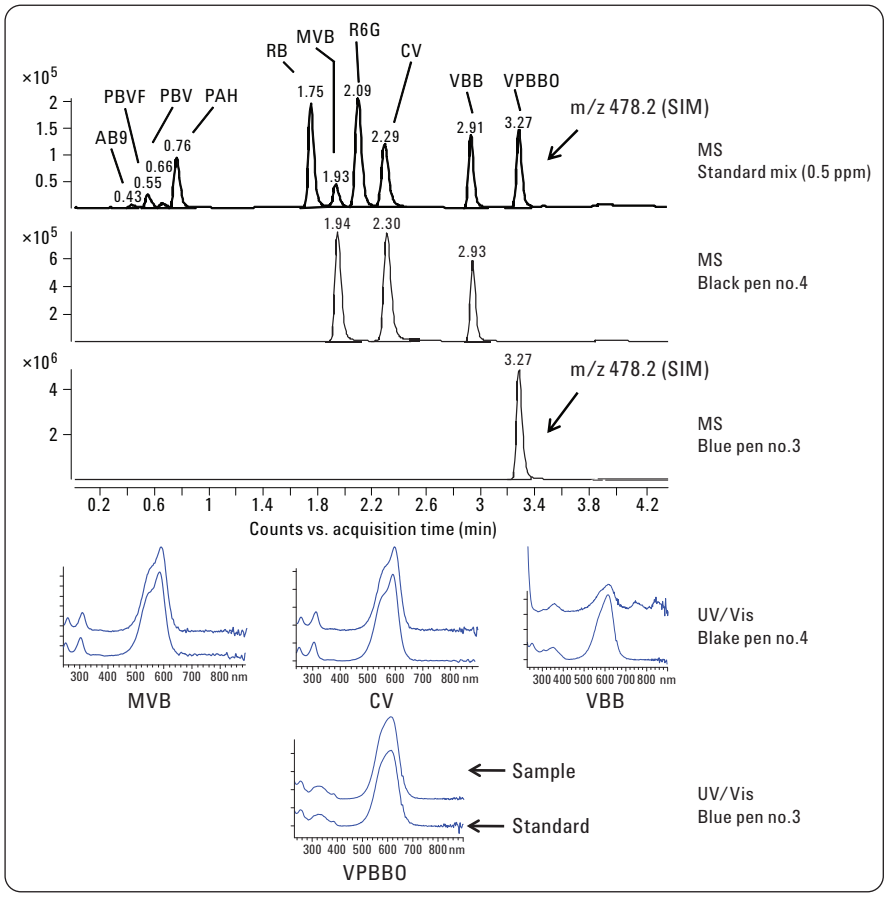


Figure 3
TIC showing the detection of MVB, CV and VBB dyes in black pen #4 and Blue pen #3 confirmed using *m/z* (SIM mode), RT and UV spectral matching. A small aberration at 656 nm seen in VBB and VPBBO spectra is the deuterium lamp peak.

Paper markings from pens	AB9	MVB	CV	VBB	VPBBO
Black pen 1	0	41	59	0	0
Black pen 2	0	63	23	14	0
Black pen 3	100	0	0	0	0
Black pen 4	0	62	22	16	0
Black pen 5	0	0	0	0	0
Blue pen 1	0	11	0	89	0
Blue pen 2	0	39	18	43	0
Blue pen 3	0	0	0	0	100
Blue pen 4	0	50	0	50	0
Blue pen 5	0	19	0	81	0

Table3
The ratio of five ink dyes that exists in paper markings from ten commercial black and blue pens.

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August 1, 2010
Publication Number 5990-6205EN

Agilent Technologies