DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ANALYSIS OF COPPER PHTHALOCYANINE

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Abstract

A method was developed and validated for quantitative determination of copper phthalocyanine in blue ballpoint pen ink by high performance liquid chromatography (HPLC). Chromatographic separation was performed on Inertsil C8 (150 x 4.6 mm, 5 μm) column with 0.1% diluted phosphoric acid (H_3PO_4) and acetonitrile (80:20, v/v) as mobile phase and UV detector at 219 nm was used. Copper phthalocyanine in the ink sample was converted to phthalimide by oxidation with saturated potassium dichromate at 90°C for 30 min. The phthalimide was achieved at 7.1 min retention time by HPLC-UV. The linearity was established in the range of 0.07 to 2.5 μg/mL. The validation in terms of accuracy and precision was found to be 98.22% recovery and 1.88% relative standard deviation, respectively. Detection limit was found to be 0.6 ng/mL. The developed method was applied for various kinds of the blue ballpoint pen inks containing copper phthalocyanine and would be evident requiring in forensic science for questioned document examination.

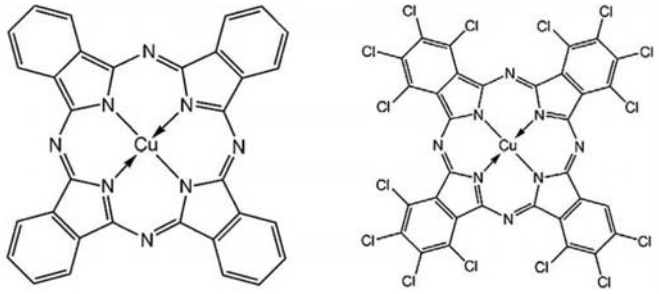
Keywords: forensic science, ballpoint pen, copper phthalocyanine, high performance liquid chromatography

Introduction

Document examination is important for forensic science investigating and can be identifying forgery and establishing the authenticity of documents in dispute. The most common type of examination involves handwriting or signatures because there are individual features that distinguish. In addition, chemical analysis of questioned documents is an important tool available to forensic examiner. It is often performed to determine the date of document or the time that ink could have been once placed on the paper (Ezcurra et al., 2010) which can be analyzed for types, colors or components in pen inks.

Ink is one of the hardest problems to solve in forensic science because it is the great variety of types and colors. Although it is difficult to determine an individual pen that was used to write a document, it is feasible to identify the brand of pen (Kher et al., 2006). The most type of ink in these investigations is a ballpoint pen ink. It dominates the writing instrument market (Andrasko, 2001). The colorants in writing inks are soluble and insoluble in the solvent which can be dyes and pigments, respectively. In the blue pen inks, the most dyes and pigments are used, Victory Blue (VB), the Methyl Violet group (Crystal Violet, CV; Methyl Violet, MV, and tetramethyl-pararosanilines, TPR), and the copper phthalocyanines (CuPc)

(Ezcurra et al., 2010). The phthalocyanines (Pc) are complex compounds. They have atom (M) in the center of the molecule that can be replaced by metal ions such as zinc, copper, cobalt, nickel, etc. (Brykina et al., 1998). The most compounds introduced for use as pigments, dyes or lacquers for artistic as shown in Figure 1 are copper phthalocyanine (CuPc) and copper perchlorophthalocyanine ($CuCl_{16}Pc$). The both have replaced by copper in the center atom but they are different in that the hydrogen atom (x). It can be replaced by hydrogen atoms or chlorine atoms, respectively. The properties of both pigments are deep colors, are insolubility in any solvent except concentrated sulfuric acid (H_2SO_4) and have a very high thermal stability (Fisher, 1992) and in all instances they are reprecipitated by dilution with water (Dahlen, 1939).



(a) (b)

Figure 1 Chemical structures of (a) copper phthalocyanine (CuPc) and (b) copper perchlorophthalocyanine ($CuCl_{16}Pc$)

Several techniques of chemical methods have been applied to the analysis of pen inks including spectrophotometric methods (microspectrophotometry, FTIR, and X-ray microanalysis) and separation methods (TLC, HPLC, GC-MS, and CE) (Williams et al. 2009). Thin layer chromatography (TLC) is the simplest of those methods and it is suitable for the separation of the dyes or pigments in a pen ink. However, copper phthalocyanine pigment is still mainly restricted to the observation of colors after treatment with concentrated sulfuric acid and because of their general insolubility, CuPc does not migrate during TLC development and substances remain at the starting position on the TLC plate. The chromatographic result is thus not obtained and measured. HPLC is one of applicable methods for analysis of phthalimide derived from copper phthalocyanines pigment (Fisher 1992). So, the objective of this research was to develop and validate the HPLC method for analysis of the copper phthalocyanines pigment in the blue pen inks. The results of which can be easily document examination.

Experimental

Materials

Copper phthalocyanines (CuPc) standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). Concentrated sulphuric acid and ortho-phosphoric acid were from Qrëc (New Zealand). Sodium hydroxide was from Rankem (New Delhi, India) and potassium dichromate was from VWR BDH Prolabo (Leuven, Belgium). Acetonitrile and methanol were of HPLC grade purchased from Fisher Scientific (Pittsburgh, PA, USA). Milli-Q Water from a Millipore filtration system (Bedford, MA, USA) was used.

Oxidation method

The analysis of copper phthalocyanine (CuPc) pigment is based its phthalimide by oxidation of the pigment (Figure 2). The CuPc standard was weighed and was dissolved in 10 mL concentrated sulphuric acid, mixed and heated to 80 °C for 10 min followed by sonication for 15 min, resulting in the dark green color of the solution. After that, 10 mL of a saturated aqueous potassium dichromate solution was added and heated to 90 °C for 30 min. Removal of excess dichromate and dilution of sulphuric acid was carried out by addition of 5 mL of 0.05 M sodium hydroxide in methanol-water (1:1, v/v). The volume of the solution was then brought up to 50 mL using Milli-Q Water.

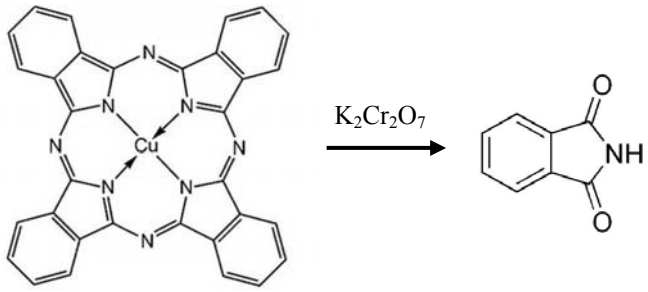


Figure 2 The oxidation of copper phthalocyanine by potassium dichromate

HPLC Method

LC-20A Shimadzu (Japan) was used for the method validation which developed by Fisher 1992 and the application for phthalic acid analysis. The system equipped with SPD-M20A Shimadzu PDA detector. Chromatographic separations were performed on C8 Inertsil column (4.6 mm ID × 15 cm), attached to a guard column connected and UV detection. The column temperature was maintained at 40 °C. The mobile phase contained 0.1% diluted aqueous phosphoric acid (H_3PO_4) and acetonitrile (80:20, v/v). The flow rate was 1 mL/min for 10 min of analysis time with injection volume of 20 µL. The detection wavelength 219 nm was selected because it gives a UV maximum and provides the sensitivity needed for quantification of the phthalimide (Figure 3).

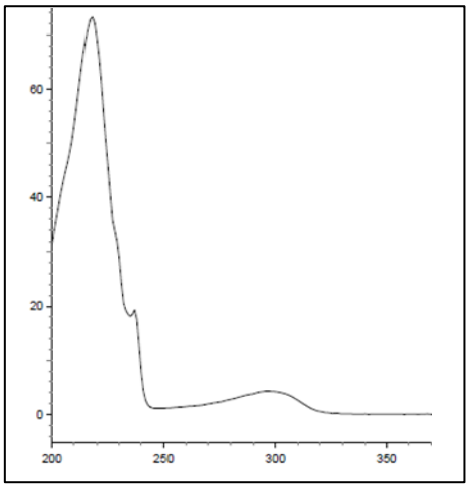


Figure 3 UV spectrum of phthalimide with the maximum wavelength at 219 nm

Validation of HPLC Method (Yavuz et al., 2007)

Linearity range

The range of an analytical procedure is the interval between upper and lower concentrations of analyte in the sample. The linearity of the analytical procedure is its ability to obtain test results that are directly proportional to the concentration of the analyte in the sample. If there is a linear relationship, the test results should be evaluated by calculation of a regression line. Six concentrations between 0.07-2.5 µg/mL of the phthalimide solutions were prepared to give the linearity of the method.

Precision and Accuracy

Precision is usually expressed as the variance, standard deviation or coefficient of variation (CV) of a series of measurement. Precision was evaluated by performing repeatability and intermediate precision. Accuracy should be reported as percent recovery by the assay of the known added amount of analyte in the sample. The assay concentrations of 0.312, 0.625 and 1.25 µg/mL were prepared for the accuracy of the method. Ten replicates were made for each concentration and the percent recoveries were calculated.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) may also be calculated based on the standard deviation (SD) of the response and the slope of the calibration curve (S) at levels approximating the LOD and LOQ according to the formula: LOD = 3.3(SD/S) and LOQ = 10(SD/S). The standard deviation of the response can be determined based on the standard deviation of y-intercept of regression line.

Results and Discussion

Validation of HPLC Method.

The method validation was done including linear range, precision, accuracy, LOD and LOQ. The copper phthalocyanine was converted to phthalimide. The standard solution of copper phthalocyanine (2 mg/mL) was subsequently diluted with water down to concentrations between 0.07 µg/mL and 2.5 µg/mL. This range covers the in vitro working range for phthalimide. The calibration curve of phthalimide is linear over the concentrations range of 0.07 - 2.5 µg/mL with r^2 of 0.9978. The regression line and equation are shown in Figure 4.

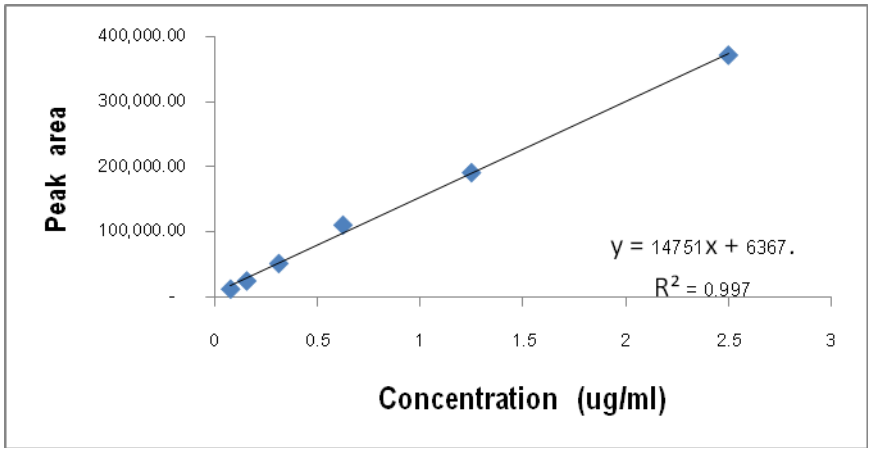


Figure 4 The regression line representing linearity of phthalimide solution

Ten samples were prepared at the same concentration (0.03 µg/mL) to evaluate the method precision, resulting in its CV(%) of 1.88%, which was considerably acceptable. For intermediate precision, this includes additional variability factors due to the standard, operator, equipment, reagents, etc., a similar dependency. Intermediate precision was evaluated to show the variation between the days. The samples (0.03 µg/mL) were prepared within 4 consecutive days and the CV(%) was also acceptable as shown in Table 1.

Table 1 The validated results expressed in terms of repeatability and intermediate precision

Sample (0.03 µg/mL)	Repeatability		Intermediate precision	
	Concentration (µg/ml)	Retention times	Concentration (µg/mL)	Retention times
1	0.630	7.12	0.598	7.09
2	0.619	7.12	0.584	7.10
3	0.616	7.11	0.581	7.11
4	0.613	7.11	0.588	7.08
5	0.615	7.12	0.584	7.09
6	0.652	7.13	0.611	7.12
7	0.620	7.12	0.593	7.10
8	0.617	7.12	0.588	7.08
9	0.608	7.11	0.576	7.09
10	0.617	7.12	0.584	7.11
Mean	**0.621**	**7.12**	**0.589**	**7.10**
CV(%)	**1.883**	**0.07**	**1.603**	**0.18**

The recovery of phthalimide was determined for each sample. The results are shown in Table 2, indicating CV of <2% for each concentration. Average percent recoveries were calculated for each concentration and the results were found to be 93.8% for 0.312 µg/mL, 99.3% for 0.625 µg/mL and 101.6% for 1.25 µg/mL. LOD and LOQ was found to be 0.6 and 2 ng/mL, respectively.

Table 2 The recovery study at three levels of spiked concentration

Sample	Recovery, %		
	0.312 µg/mL	0.625 µg/mL	1.25 µg/mL
1	91.82	100.73	99.76
2	93.02	98.96	101.42
3	94.18	98.52	102.02
4	94.97	98.04	100.63
5	94.40	98.44	101.79
6	92.12	104.29	100.87
7	94.79	99.14	102.53
8	94.53	98.68	102.13
9	93.80	97.23	101.44
10	94.65	98.79	102.97
Mean	**93.83**	**99.28**	**101.56**

Conclusion

The simple method for the analysis of pen inks is thin layer chromatography (TLC). It is suitable for the separation of dyes or pigments in a pen ink. However, copper phthalocyanines pigment is insoluble and CuPc do not migrate during TLC development and the substances remain at the starting position on the TLC plate. Removal of a small section of the ink followed by solvent extraction of the ink opens up more avenues of analysis. Thus, it was

aims to develop and validate the HPLC method for analysis the copper phthalocyanines pigment in pen inks and removal of a small section of the ink in the written paper. But the method required the oxidation of the copper phthalocyanine in the ink sample to be phthalimide prior to determination by HPLC-UV. The quantitation analysis was done after method validation. The HPLC method was found feasible for determining and quantifying the phthalimide in blue ballpoint pen. The developed method can be easily applied for document examination in forensic science.

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