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Artigo

FIGURES OF MERIT EVALUATION OF GC/MS METHOD FOR QUANTIFICATION OF 2-PHENOXYETHANOL FROM BALLPOINT PEN INK LINES AND DETERMINATION OF THE INFLUENCE OF SUPPORT PAPER ON SOLVENT EXTRACTION

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Brazilian Forensic Scientists are frequently asked about ballpoint pen manuscripts chronology in documents suspected to be forged. In the recent years, many methods like quantification of 2-PE (2-phenoxyethanol) have been developed on this subject. The validation of these methods only recently has been a concern between scientific forensic community. Researchers studied the behavior of ink on ageing, but few of them concerned about the influence of the paper on the extraction of 2-PE. This study performed the figures of merit evaluation of the quantification of 2-PE using GC/MS, testing the main parameters involved in the reliability of the method (linearity, repeatability, limits of detection and quantification, accuracy and robustness). After, based on a full factorial design with four factors and two repetitions, the authors tested kind of paper, grammage of paper, ink color and three ink ages, to verify the paper influence on the quantity of 2-PE from ink, trough GC/MS analysis. The merit parameters evaluation showed that the method is linear, precise, accurate and robust. The results for the effect of paper showed main effects of the factors and the existence of interactions effects between the kind of paper and paper grammage, and between the kind of paper and other factors.

Keywords: forensic documentoscopy; ballpoint pen; ink ageing; 2-phenoxyethanol; factorial design; support paper; figures of merit.

INTRODUCTION

Ink dating on questioned documents has been a great challenging on document examiners routine. Many official documents are forged using manuscripts with ballpoint pens and dated earlier than they really are. Ageing processes of an ink follow complex paths that are considerably influenced by several factors other than time, which may accelerate or slow down the aging, like: (i) initial composition of the ink; (ii) physical and chemical properties of the substrate; (iii) storage conditions (temperature, light, air flux, humidity etc.).[1] Many current methods developed try to establish a protocol of ink dating by dye or pigment degradation.[2-9] However, Aginsky (1998) already stated that is more correct to measure ink volatile components than dyes to date an ink, because dyes are easily extracted from surface layers of ink lines no matter the ink age.[10]

Volatile solvents in ballpoint inks are also used to assess the age of ink on paper. Solvent evaporation from ink on paper can be divided into an initial fast process, and after a few hours to days, the evaporation slows down and reaches a low, steady state. The aging curve shows significant aging taking place over a period of 3 months, and after this, until 15 years the extent of extraction of the volatile component has been kept at a level of about 20%.[11,12]

The solvent 2-phenoxyethanol (2-PE) was identified in 85% and 83% of the black and blue inks, respectively, from 633 ballpoint inks collection of the United States Secret Service.[13] Many authors proposed methods of ink dating, based on the behavior of 2-PE after the deposition of the ink on paper, like ageing curves of 2-PE concentration versus age or the manuscript,[12] artificial ageing for modelling natural aging process,[14] and others.

The validation of these methods only recently has been a concern between scientific forensic community. Published works presented interesting ideas and promising orientations, but measurement errors are rarely mentioned in the literature. The analytical dating methods require a considerable amount of time and resources and it is important not underestimate the task of ensuring their scientific validity before implementing them in practice[1]. Koenig *et al*.,[15,16] in previous publications, determined the limits of reliable measurements of 2-PE quantities and the repeatability of the liquid extraction method, and the repeatability and reproducibility of the Termal Desorption/Gas Chromatography-Mass Spectrometry method.[16]

Recently, Diaz-Santana *et al.*[17] attempt to develop a method integrating gas chromatography with mass spectrometry (GC-MS) with high-pressure liquid chromatography with photodiode array detection (HPLC-DAD) to study the relative variations between different compounds and the generation of degradation products such as phenol, from MontBlanc© pens, during 45 months. They used multiple regression model for each ink type to estimate the exposure time of the ink on paper with a maximum error of between 4 and 7 months.

In the same way, Koenig and Weyermann[18] very recently published a study evaluating the potential of seven ageing parameters for ink dating purposes: phenoxyethanol quantity, relative peak areas (RPA), three solvent loss ratios (R%, R%*, NR%) and two solvent loss parameters ($R_{NORM,}$ NR_{NORM}). These were calculated over approximately one year for 25 inks selected from a large database to represent different ageing behaviours.

It is essential to make certain that predicted differences provoked by aging are in fact higher than measurement errors. For ink solvents, the detection and the quantification limits (LoD and LoQ) play an important role in determining a threshold at which the method is not applicable anymore.[1]

Following these principles, the influence of different types of paper (substrate structure) on the drying process (evaporation of solvents) and extraction process of these solvents should not be underestimated. The paper porosity can differ quite widely as well as the paper chemistry (alkaline or acidic, fillers, detergents, additives).[1]

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Nowadays, the offices are using recycled paper besides white paper, once the recycled paper has an ecological appeal. However, the recycled paper do not use bleaching process and has a completely different porosity. In the casuistic of handwriting exams of Brazilian Federal Police, from 2010 to 2018, 1.86% of questioned manuscripts were done in recycled paper support documents. This percentage is not so expressive, but it exists and there is an increase tendency.[19]

The authors are not yet in accordance about the influence of paper type on dating of an ink: Weyermann *et al.* 2011[1] cited that Aginsky stated that it is a strong dependence on paper type for his method, while Bügler *et al.*[12] thought it to be negligible, but at the end of the article stated that there was a paper effect.

As a part of a sequence of studies from our research group,[20,21] this paper aimed to evaluate the main figures of merit of the method of 2-PE extraction and quantification, and to verify the influence of different types of paper on the extraction of 2-PE from ballpoint ink lines.

EXPERIMENTAL

Merit parameters evaluation

The important aspects of scientific reliability and validation for an analytical method were summarized early by Horwitz: reproducibility (between-laboratory precision), repeatability (within-laboratory precision), systematic error or bias (accuracy), selectivity and limits of reliable measurements.[15] The present study concerned to determine the linearity of the quantification curves of 2-PE; the repeatability (intra-assay precision); intermediate precision (different days and different operators); the reproducibility (using other GC/MS located in another laboratory); the detection and quantification limits of the method; the robustness (little variations in method parameters); and the detection of a possible matrix effect, that could alter the signal response. All the figures of merit evaluation process were based on guidelines and specialized publications.[16, 22-24]

Preparation of samples for the evaluation of merit parameters

The calibration curves were made dissolving a standard of 2-PE (Fluka) in methanol HPLC grade (Merck), at concentrations 3.53×10^{-6} μg mL^{-1}, 2.06×10^{-4} μg mL^{-1}, 4.13×10^{-4} μg mL^{-1}, 0.00275 μg mL^{-1}, 0.0275 μg mL^{-1} and 0.05 μg mL^{-1}, with o-cresol (Sigma-Aldrich) as internal standard, at a concentration of 0.01046 μg mL^{-1}. It were made three replicates of the curve, to evaluate the linearity of the curves, using Grubbs test to detect outlier values and the homoscedasticity of the data. The regression equation was calculated by the regression plot between 2-PE concentrations × relative peak area (area of 2-PE peak/area of Internal Standard peak).

Limits of Detection and Quantification

The regression curves obtained were used to calculate the limits of detection and quantification (LoD and LoQ), using the formulas (1) and (2):

$$LoQ = \frac{3\sqrt{MSR}}{slope\ of\ regressionline} \quad (1)$$

$$LoD = \frac{10\sqrt{MSR}}{slope\ of\ regressionline} \quad (2)$$

MSR = Mean Square Residual.

Repeatability

The repeatability was calculated from measurements carried out with the same instrument by the same operator in the same day (intra-assay) and in different days (inter-assay), and from measurements carried out with the same instrument with different operators in the same day. The measurements were made from samples with three different concentrations of 2-PE (0.33 ng mL^{-1}; 0.0044 μg mL^{-1}; 0.10 μg mL^{-1}), and internal standard o-cresol (0.01046 μg mL^{-1}) with six replicates for each concentration. The evaluation of acceptance of precision characteristics of the method was evaluated through Relative Standard Deviation (RSD) equation (3):

$$RSD = \frac{SD}{Mean} \quad (3)$$

RSD= relative standard deviation; SD= standard deviation; Mean = concentration media of replicates.

The RSD should be equal or inferior of 10% to be accepted.

Accuracy

The accuracy of the method was made determining the Error (E%) (Equation 4) between the 2-PE concentration obtained when compared to the expected concentration of the analite. It were performed three assays with 2-PE in different concentrations (0.00275 μg mL^{-1}, 0.0275 μg mL^{-1} and 0.05 μg mL^{-1}), with internal standard o-cresol (0.01046 μg mL^{-1}), performing three replicates for each concentration, and the accuracy was determined for each nominal concentration level of the analite. The values for the replicate contents at each level of concentration should show a maximum error of 10% when compared to the expected. The accuracy evaluation of this study was based on the Brazilian Institute of Metrology Guidelines.[25]

$$E\% = \frac{X_{lab} - X_v}{X_{lab}} \times 100 \quad (4)$$

X_{lab} = experimental result of 2-PE concentration; X_v = nominal value of 2-PE concentration.

Robustness

The robustness was evaluated performing the GC/MS method with small changes in the defined parameters, performing eight experiments designed trough *Youden* test, as exposed in Table 1: It was made five repetitions for each treatment, and it was injected a 0.1784 μg mL^{-1} 2-PE solution, with internal standard o-cresol (0.01046 μg mL^{-1}). To quantification of 2-PE for robustness, the calibration curve was the same already validated.

The evaluation of the results were made by evaluation of the relative difference between the concentration of 2-PE without changes in the method parameters and the concentration of 2-PE measured in each assay, and to be accepted, this relative difference should not be superior of 10%.

GC/MS analysis

The Gas Chromatograph used for the analysis was an Agilent model 6890N coupled with mass selective detector, model 5973 (at 70 eV) using a DB-WAX capillary column (30 m x 250 μm x 0.25 μm). The chromatographic conditions were as follows: injector at 180 °C, in splitless mode for 3 min; Oven at the initial temperature of 50 °C, with ramp with rate of 18 °C per minute to 204 °C, 5 °C per minute to 230 °C. The injection volume was 2 μL and the helium flow was 1.3 mL/min. For the identification and quantification of *o-cresol* and 2-PE it was used the Single Ion Monitoring (SIM) method, where the monitored ions were those with mass / charge ratio (*m/z*) of 108; 107; 91; 77 for o-cresol and 138; 94; 77 and 66 for 2-PE, with gain

Table 1. Youden test for robustness evaluation

Parameter	Validated	Assay							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Flow (mL min^{-1})	1.3	1.2	1.2	1.2	1.2	1.4	1.4	1.4	1.4
$T_{injector}$ (°C)	180	185	185	175	175	185	185	175	175
Ti_{oven} (°C)	50	55	45	55	45	55	45	55	45
Purge time	3	4	4	2	2	2	2	4	4
$V_{injection}$ (µL)	2	2.1	1.9	2.1	1.9	1.9	2.1	1.9	2.1

* Tinjector – injector temperature; Tioven- oven initial temperature; Vinjection – injection volume.

factor 2, MS Source 230 ºC, and MS Quadrupole 150 ºC. The ion used for quantification purposes were m/z 108 for o-cresol and m/z 94 for 2-phenxyethanol.

Influence of the paper on the extraction of 2-PE

To determine the influence of different types of paper in the efficiency of quantification of 2-PE, a full factorial design was elaborated using the software Minitab 17©, with four factors in the levels discriminated in Table 2, with two replicates.

The white paper used was a brand without chlorine and the recycled paper was a paper without bleaching process. The grammage of the paper was chosen based on the grammage available in the Brazilian book stores and specific for the different kinds of papers.

Samples of 1 cm straight lines of blue and black Bic Cristal ballpoint pen made on the two kinds and grammages of papers were cutted, inserted in a glass vial and extracted with 250 µL of a solution of 0.01 µg mL^{-1} of the internal standard *o*-cresol in Methanol HPLC grade. After homogenization in a vortex, the extraction solution from the pen ink samples were injected in the same GC column, with the same instrumental conditions used in the method for evaluation of merit parameters, previous described.

Table 2. Factors *versus* levels of the factorial design

Factor	Level
Kind of Paper	White
	Recycled
Paper Grammage	High (90 g/m^2)
	Low (75 g/m^2)
Ink Color	Blue
	Black
Ink Age	Recently applied
	One year
	Two years

Quantitative analyzes were made by comparing the ratio of 2-PE and internal standard obtained areas with the analytical curve obtained for 2-PE standards (with internal standard o-cresol constant concentration of 0,01 µg mL^{-1}) in concentrations of 0.05; 1.0; and 1.5 µg mL^{-1}. Statistical analyzes were performed using Minitab 17 Statistical Software© (Minitab, Pennsylvania, USA).

RESULTS AND DISCUSSION

Evaluation of the merit figures

Calibration curve and linearity

The analytical calibration curve constructed with the 2-PE standard area rated to the internal standard area obtained for each concentration of three analytical replicates, showed linearity with an R^2 of 0.999, and the regression equation obtained was $y = 6143.9x + 5.5234$.

The Analysis of Variance of the regression was significant, with an F of 22857.31 (95% of significance). The residuals were homoscedastic and there were not observed outliers.

These results are consistent with the conclusion of Koenig *et al.*,[26] where those authors, besides the determination of 2-PE concentration in the ink formula, they tested three different approaches of quantification of 2-PE: "solvent loss ratio R% (involves ratio of a sample and the same sample artificially aged with heat); "Relative Peak Areas between compounds (RPA)"; and "solvent loss ratio using RPA R%". The authors concluded that the calculation of RPA values proved to be the most promising ageing parameter, and the R% was the least reproducible. The RPA are the ratio between the peak area of 2-PE and the peak area of a stable compound of the pen ink formulation.

Very recently, Koenig and Weyermann[18] continued this study and concluded testing seven ageing parameters for ink dating purposes: the phenoxyethanol quantity, relative peak area (RPA), three solvent loss ratios (R%, R%*, NR%) and two solvent loss parameters ($R_{NORM,}$ NR_{NORM}) — that natural ageing parameters (NR% and NR_{NORM}) were not suitable ageing parameters for ink entries older than a few weeks. The R% ratio consist of the area of the 2-PE peak, decreased from the area of the 2-PE peak extracted from the same sample which would have been artificially aged, divided by the area of 2-PE and multiplied by 100. In the NR% parameter, instead of the 2-FE area of artificially aged sample, it would be used the peak area of 2-PE from a one month naturally aged sample.

The PE quantity, R% and R_{NORM} allowed to follow the ageing of the selected inks over the whole time frame and were identified as the most promising.

Although the ballpoint pen ink is a complex matrix, the electron impact source of the Mass Spectrometer is able to complete ionize the 2-PE molecule, and in the literature authors until now did not report ionization suppression in GC/MS analysis of 2-PE in pen ink matrix.[12,18,26,27] Because of this, it is reliable to use the curve after injection of standard solutions to determine 2-PE after extraction process.

Limits of detection and quantification

The limits of detection and quantification (LoD and LoQ) calculated with the analytical regression curve of the 2-PE standard solutions were 0.00194 µg mL^{-1} and 0.0064 µg mL^{-1}, respectively.

The LoQ obtained in our quantification curve was lower than the result obtained by Koenig *et al.*,[26] that was 0.010 µg mL^{-1} for LoQ. However, the detection limits obtained for the present study was higher than 0.003 µg mL^{-1} obtained for LoD in Koenig *et al.*[26] work. The limits of reliable measurements of 2-PE are rarely mentioned

in the literature, but an earlier paper from the same authors[27] also presented LoQ higher than the limits determined in this study.

Accuracy

The accuracy of the method was reliable and fulfill the acceptance criteria of up to 10% of Maximum Error (E%) between the nominal and the determined values of 2-PE concentration (Table 3).

The accuracy in 2-PE measurements has a special importance because in the majority of the cases, the ballpoint ink manuscripts have low quantities of 2-PE, in the order of nanograms. Generally in trace level, the acceptance limits for accuracy are larger than 10%, however, even with the concentration of 0,00275 µg mL-1 of 2-PE, the E% was 6,89%, showing that the method is sensible and reliable.

Repeatability

The repeatability was evaluated through the RSD (Relative Standard Deviation) for six replicates of three different levels of 2-PE concentration, for the same operator, in the same day and equipment (intra-assay); for six replicates of three different levels of 2-PE concentration for different operators, in the same day and instrument (inter-assay 1) and for six replicates of three different levels for the same operator and instrument, but in different days (inter-assay 2). The results reached the acceptance criteria for the repeatability (Table 4).

It can be seen that the RSD% obtained increases as the 2-PE concentration decreases, that is expected for too low concentrations. This results were compatible with those obtained by Koenig *et al.*[27] for repetitions of samples of ballpoint pen strokes and control solutions, analyzed using Thermal Desorption/GC/MS method, were the amounts of 2-PE detected were from 2.28 ng.cm-1 (~0.136 µg mL^{-1}) to 16.81ng.cm-1 (~1.0 µg mL^{-1}), much higher than the concentrations used in this study.

Robustness

The robustness was evaluated by the relative difference between the concentration of 2-PE without changes in the method parameters and the concentration of 2-PE measured in each assay. For all treatments performed, this relative difference was not superior of 10% .

In the literature, the authors study ballpoint pen ageing using different pens. Although they studied a good number of individuals (from 30 to up to 85), the type of pen is not always reported.[1]

Sometimes, forensic community consider as proof of validation only the acceptance of a method by the courts, however if the method is probably too delicate to be reproduced correctly by scientific colleagues, so this should be strongly questioned. All dating methods should follow complete validation following adequate criteria.[1]

Influence of the paper on the extraction of 2-PE

The concentrations of 2-PE were obtained after analyzing the assays organized by the Full Factorial Design, using the software Minitab 17. Figure 1a shows the chromatogram of one of the samples, and the Figure 1b is the same chromatogram showing the main extracted ions of 2-PE and the internal standard *o*-cresol, at the retention times of 8.96 and 8.05 respectively. The Single Ion Monitoring method proved to be sensitive and specific. In the sequence, it was performed the analysis of results, obtaining the "Main Effect Plot for Response Variable" and the "Interaction Plot for Response Variable", where it could be seen the main effects and the interactions effects between the target characteristics (Figure 2 and 3). In the same way that the validation assays, the calibration curve had R^2 = 0,998, with homocedastic residuals.

The Main Effects graph shows that the recycled paper has a positive effect, increasing the quantities of 2-PE extracted in relation to white paper. At the same time, the high grammage also has a positive effect on the extraction of 2-PE, possibly because a higher paper weight is related with a higher absorption of solvent.

Divergent from a previous work by Carvalho,[28] the black pens provided a lower quantity of 2-PE, having a negative effect, while the blue pens had a positive effect. However, this factorial study takes in account just one brand of pen (Bic Cristal), while the previous work analyzed several brands of pens, and the behavior had a positive bias in direction to more solvent in black pens.[28]

As expected, the recently strokes provided a greater quantity of 2-PE, and the quantities of strokes with one year and two years of age did not differ on the quantity of solvent extracted.

The plot of interactions "kind of paper versus paper grammage" shows that there is a positive interaction when the paper is recycled and the grammage is high. This could be understood because the recycled paper has porous surface and the pulp of the paper can hold for more time the solvent, especially when the paper has a higher wheight.

The plot of interactions "kind of paper versus ink color" shows that there is a positive interaction between the recycled paper and the color of the ink. The blue ink presents more quantity of 2-PE extracted than the black ink, when the paper is recycled; even if this occurs with the white paper, the difference is higher for recycled paper.

Table 3. Results for accuracy of 2-PE measurements

	Low Concentration (0.00275 µg mL^{-1})			Medium Concentration (0.0275 µg mL^{-1})			High Concentration (0.055 µg mL^{-1})		
	Measured Conc.*	Theoretical Conc.	E (%)	Measured Conc.*	Theoretical Conc.	E (%)	Measured Conc.	Theoretical Conc.*	RSD (%)
1	0.00256	0.00275	6.89	0.0289	0.0275	5.19	0.054326	0.055	1.22

*Mean of three replicates.

Table 4. Results for Repeatability of 2-PE measurements

RSD (%)								
Low Concentration (0.00033 µg mL^{-1})			Medium Concentration (0.0044 µg mL^{-1})			High Concentration (0.1 µg mL^{-1})		
Intra-assay	Inter-assay 1 (≠ operator)	Inter-assay 2 (≠ day)	Intra-assay	Inter-assay 1 (≠ operator)	Inter-assay 2 (≠ day)	Intra-assay	Inter-assay 1 (≠ operator)	Inter-assay 2 (≠ day)
6.85	9.55	10	3.51	2.62	4.7	0.637	0.81	2.12

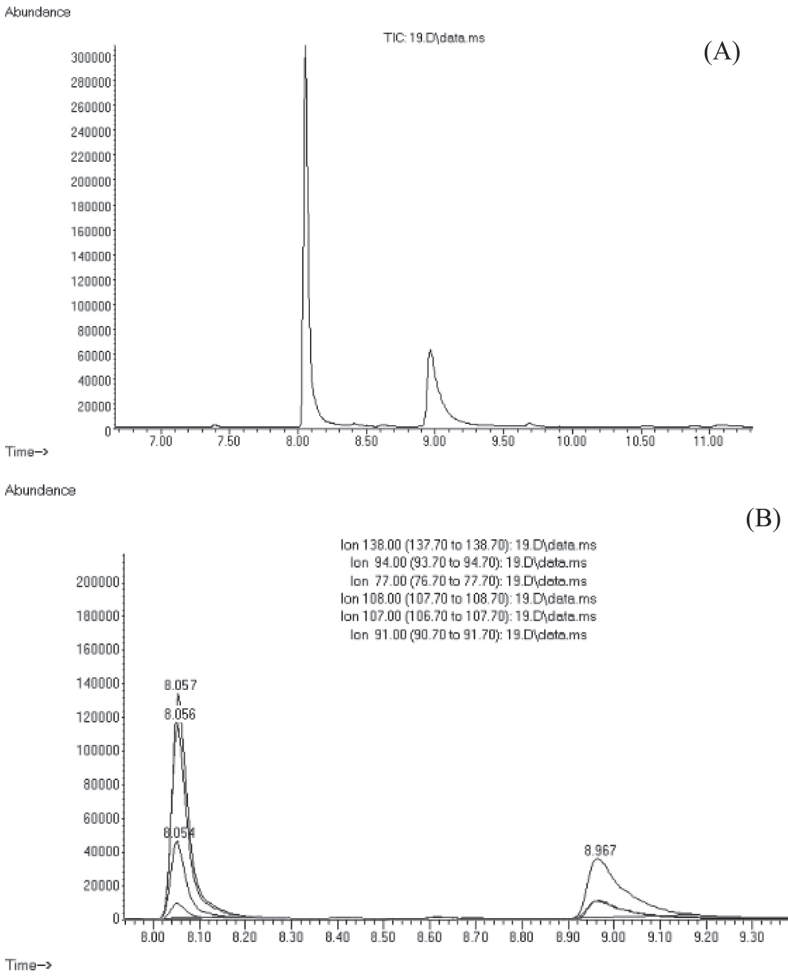

Figure 1. A. *Total Ion Chromatogram of an ink sample from the Factorial Project (assay 19), showing 2-PE peak at RT 8.96, and Internal Standard peak at RT 8.05.* ***B.*** *Extracted ion chromatogram of the same sample, showing the main ions of 2-PE and Internal Standard*

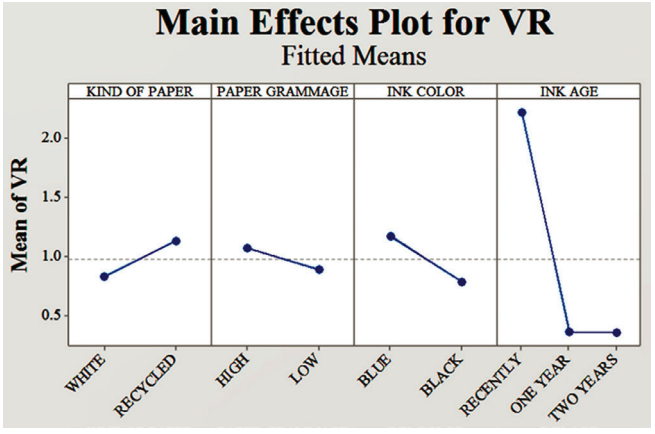

Figure 2. *Main Effects of the variables analyzed: kind of paper, paper grammage, ink color and ink age. *VR = Response Variable (2-phenoxyethanol concentration - μg mL^{-1})*

The high grammage of the paper also has a positive interaction effect with the color of ink, although this interaction is slightly positive, resulting in a difference of quantity of 2-PE slightly superior between blue and black ink for heavy paper than for paper with low grammage.

In the plots "Kind of Paper*Ink Age", and "Grammage of Paper*Ink Age", it can be seen that for one year and two year age there is no interaction effect of factors, because the lines are parallel.

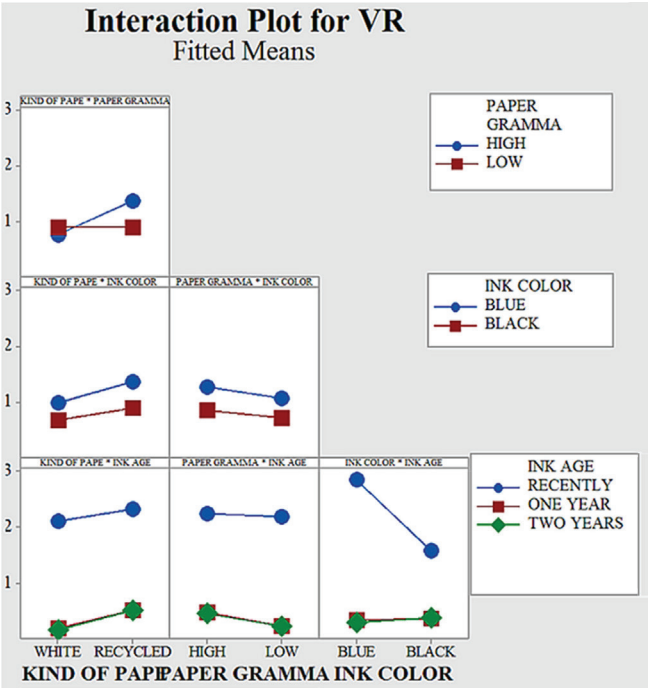

Figure 3. *Graphs of interactions between the variables studied, showing the combined effects. *VR = response variable (2-phenoxyethanol concentration - μg mL^{-1})*

However, comparing the results of fresh samples (recently applied) with aged samples, there is an interaction effect. The recycled paper had a more positive effect on quantity of 2-PE extracted from aged strokes, in comparison with white paper, than the fresh strokes. For the fresh strokes, the recycled paper was less influent in the extraction of 2-PE than in aged strokes, proving one more time that recycled paper retain longer the solvents, and the inks apposed in white paper showed a higher evaporation of the 2-PE. The same conclusion can be made analyzing the "Paper Grammage*Ink Age" plot, where the paper with higher weight provided more quantity of 2-PE extracted from old samples than for recently samples.

Looking closer to the plot "Ink color*Ink Age", there is interaction effect between the ink color and one year and two year age, although it is a slightly interaction. For blue samples, the amount of 2-PE detected at one year was higher than for two years. However, for the black pens, in the older samples there was no difference in the quantity of 2-PE, no matter the time. Between recently samples and aged samples, black pens showed less difference on the concentration of 2-PE than the blue pens, which even starting from a higher quantity of solvents the quantity of solvents detected after two years was below of the quantities detected for black ink.

What it can get from all these experiments is that the kind of paper exerts importance in the 2-PE quantification. Bügler *et al.*[12] proposed threshold values calculated from the rate of desorption of 2-PE in different temperatures, to document dating. These authors considered in the conclusion that the kind of paper had an influence in the method. Aginsky[10] also stated that two inks being compared must be written on paper of the same composition. According to this author, papers of different compositions sorbed significantly different amounts of a dye, and the reason for this is that the ability of paper to sorb ink dyes strongly depends on its composition – fiber composition: the larger the wood pulp content in the paper, the more the sorption of dye. However, Aginsky[10] concerned about the dyes, and not about the solvents. The present study confirmed statistically that papers with different compositions could alter the absorption and retention of a specific ink solvent.

Besides of that, as a document ages, both the ink and the paper age, and the effects of paper ageing on ink ageing may be small, but the document examiner should know how to delete this effect, doing relative comparisons.[29]

Recently, Koenig *et al.*[26] also concerned about the influence of paper in the ageing of ballpoint pens, looking on solvent evaporation. They studied the same ink in two different white papers with grammage of 80 g m^2, Xerox Business and Xerox Performer. Even being the same paper weight and color, this authors found a small effect on values as these related on the Xerox Business were slightly higher than for Xerox Performer.

The present study observed that recycled paper and high grammage paper could influence greatly the quantification of 2-PE, so the requirement to the paper be the same for two ink lines to be compared should not be neglected.

CONCLUSIONS

This study focused on the evaluation of the main merit parameters of the quantification method for 2-PE, a solvent used in ballpoint pens, that is the main substance studied for the determination of a manuscript age in forensic documents. The verification of the influence of a paper support of a document, in the quantification of 2-PE, is of remarkable importance in order to avoid mistaken conclusions.

The method of GC/MS proved to be reliable for 2-PE detection and quantification, once the main merit figures were successfully evaluated showing results under the limit values recommended in validation guidelines. In order to continue the evaluation of the 2-PE quantification method, blind testing on realistic samples could be performed to check the reliability of the method under real casework conditions.

Relating to the experiment testing the influence of kind of paper on 2-PE concentrations, it can be concluded that the kind of paper exerts importance in the 2-PE quantification. In the literature, we found that two different sheets of white paper with the same weight can influence the evaporation of 2-PE and in consequence the pen stroke dating. Until now, the literature[22,27,30,31] only used white paper with grammage varying from 75 up to 80 g m^2 in ballpoint ink dating studies. However, with growth of initiatives aimed at the recycling and preservation of natural resources, recycled paper will become a routine paper in offices, and the ageing behavior of pen inks in this kind of paper should be studied.

Based on these results, the forensic examiner have to be aware of the importance to compare manuscripts on the same document and to have much more concern when comparing different documents.

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