Determination of the age of ballpoint pen ink by gas and densitometric thin-layer chromatography*

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Abstract

Two procedures for dating ballpoint inks are considered that use gas chromatography (a combination of the technique for determining the extent of extraction of ink volatile components and of the accelerated ageing technique) and densitometric thin-layer chromatography (separation of ink components and evaluation of the resulting chromatograms using a specially developed mass-independent technique that is also a very effective tool for the comparative TLC examination of similarly coloured inks, paints, fibres and other materials of forensic interest). The procedures have been used in many real case situations and the results of the examinations were accepted as conclusive evidence by courts of law.

1. Introduction

Gas chromatography (GC) and densitometric thin-layer chromatography (TLC) have been demonstrated to be useful tools for the solution of many problems frequently encountered in ink analysis, including ink dating problems [1–4]. Recently, five new procedures for dating ballpoint inks have been described [5,6]. Two of them, based on using chromatographic methods, are as follows.

(1) A GC method is used to determine the extent of extraction of ink volatile components, which decreases as ink ages on paper. The procedure considered in this paper combines the capabilities of this method and of the accelerated ageing technique. The procedure allows discrimi-

nation between "fresh" (age less than several months) and "old" ballpoint ink entries and it does not need dated reference entries written with ink having the same formula as that of the questioned ink.

(2) A TLC method is used for determining age changes in resins and other colourless non-volatile ballpoint ink components; these changes are detected by observing the resulting thin-layer chromatograms under UV illumination and evaluated by using scanning densitometry. The modified TLC procedure described in this paper includes a new, mass-independent approach to evaluating thin-layer chromatograms that allows one to obtain the values of an "ink ageing parameter" [7] directly proportional to the ratios of the masses of the separated ink components (dyes, resins, etc.). For this reason, the proposed procedure gives more reliable results for ink age determination than those obtainable with the

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widely used peak signal-to-peak signal ratio technique. The described approach is also a powerful tool for the comparative examination of similarly coloured inks, paints, fibres and other materials of forensic interest as its discriminating power is much greater than that usually produced by the peak ratioing technique [8–10].

2. Experimental

2.1. Materials

Up to 15-year-old entries written with Soyuz ballpoint inks of different colours having similar compositions of colourless components were analysed by GC. Entries of known ages (1 day, 1 month, 1, 2, 3 and 6 years old) written with a Parker blue ballpoint ink were analysed by TLC.

Camag N-11 polypropylene micro vials with cone-shaped interiors and a 10-μl Hamilton syringe were used.

2.2. Gas chromatography

A Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector and an HP split–splitless injection system was used. A SCOT column containing SP-1000 (polyethylene glycol 20M terminated with nitroterephthalic acid) (Supelco) (25 m × 0.5 mm I.D.) was used with nitrogen (4 p.s.i.) as the carrier gas at a flow-rate of 40 ml/min. The column oven temperature was programmed from 50°C (held for 0.5 min) at 10°C/min to 220°C (held for 6 min). The injection volume was 2 μl (splitless) at 250°C. A flame ionization detector was used at 250°C.

Each sample was obtained by cutting out a ca. 1-cm sliver of ink of approximately equal thickness from the paper using a safety razor and placed in a micro vial. A 10-μl volume of carbon tetrachloride as a "slowly extracting weak solvent", containing 10 μg/ml of benzyl alcohol as an internal stranded (if benzyl alcohol is detected in ink samples in significant amounts, another appropriate substance can be used as an internal

standard) was added and the vial was capped. After 30 min a ca. 2-μl aliquot of each sample was removed and analysed by GC.

The samples were removed from the extraction solutions, dried and placed into other micro vials. A second extraction was carried out for 1 min, stirring with a needle, with 10 μl of chloroform ("fast-extracting strong solvent") also containing benzyl alcohol in the same concentration. About 2 μl of each extract were removed and analysed by GC.

The masses of a vehicle component determined in each of the two extracts analysed (M_1 and M_2 for the first and second extractions, respectively) were calculated by means of the internal standard method. The percentage extraction [1,5], that is, the percentage of the mass of the ink vehicle component, %M, extracted in the "weak" solvent (relative to its total amount contained in the sample analysed), was calculated as follows:

$$\%M = [M_1/(M_1 + M_2)] \cdot 100$$

The values of %M obtained for all samples analysed were plotted against the age of the known ink entries (see Fig. 1).

2.3. Thin-layer chromatography

To obtain an "ageing curve", samples as two 1-cm slivers of ink of approximately equal thickness were taken from five entries of known ages (X_1–X_5). Three more samples were taken from a 2-year-old entry that was analysed as a questioned (Q) entry. Each sample was placed in a micro vial and extracted for 2 min with 15 μl of chloroform, stirring with a needle. A calibration standard solution was prepared by the treatment of eight 1-cm slivers taken from a 1-month-old entry with 60 μl of chloroform. Volumes of 10 μl of the obtained extracts and 5,8,11 and 15 μl of the calibration standard solution (calibration standards, S_1–S_4) were applied to a 20 × 10 cm precoated Merck HPTLC silica gel 60 F_{254} plate as 8-mm bands by means of a Camag Linomat-3 applicator. One-dimensional ascending development was performed with ethanol–acetone–hex-

ane (1:5:20, v/v/v). The development distance was 50 mm.

The resulting chromatograms contained zones of two ink components, A and B. For five samples, X_1–X_5, the relative proportion of these components was obviously linked with the age of the ink (see Fig. 2). The chromatograms were scanned densitometrically by reflectance in the absorbance mode for fluorescence quenching at 254 nm using a Camag TLC/HPTLC scanner (with a mercury lamp, monochromator bandwidth 30 nm, slit dimensions 0.3×5 mm and scanning speed 1 mm/s) connected to an SP4100 integrator (Spectra-Physics). The densitometric data obtained were evaluated with external standards in the following way.

For the calibration standards S_1–S_4, it was assumed that the contents of components A and B per zone (their real values are unknown, as follows from the procedure used for preparing the calibration standard solution) were equal to the corresponding values of the volumes of the calibration standard solution applied to the plate (see Table 1).

For each calibration standard component, A and B, a logarithmic (this function gave the best correlation coefficient in all non-linear calibrations that were tested in the given case) approximated calibration graph was constructed and then the contents C_A and C_B of components A and B per zone were determined for the chromatograms of the samples taken from the known and Q aged entries.

The ratio C_A/C_B was calculated for each sample. The values obtained were plotted against the actual age of the known ink entries and the age of the Q ink entry was determined (see Table 3 and Fig. 3).

3. Results and discussion

3.1. Gas chromatography

Figure 1 shows ageing curves obtained for Soyuz ballpoint inks of different colours having similar compositions of colourless components. The curves show that significant ageing taking place over a period from about 6 months to more than 2 years for different inks. After this period until the age of 15 years the extent of the extraction of the volatile component, phenoxyethanol, from the ink entries remained at about $20 \pm 10\%$.

An explanation of this result characterizing the mechanism of evaporation of volatile components (such as phenoxyethanol, phenoxyethoxyethanol and other high-boiling vehicles frequently used as the ingredients of ballpoint inks) from ageing inks has been given previously [5]. It was considered that the evaporation process includes a limiting stage of diffusion of a vehicle from the interior layers of the ink body

Table 1
Contents of components A and B in the chromatographic zones of the calibration standards

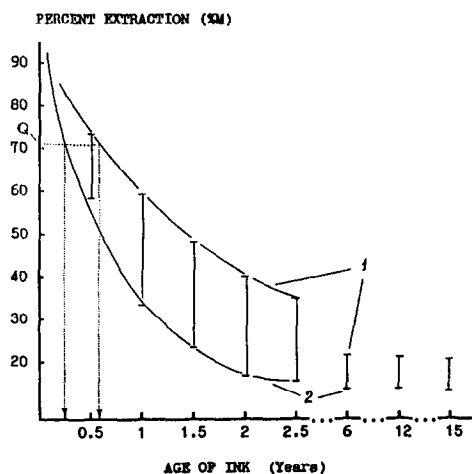| Standard[a]	Volume applied (μl)	Component content (mg per spot)	
		A	B
S_1	5	5	5
S_2	8	8	8
S_3	11	11	11
S_4	15	15	15

[a] Extract from the 1-month ink entry.

Fig. 1. Ageing curves obtained for violet, blue, green and black Soyuz ballpoint inks: 1 and 2 relate to the maximum and minimum values of %M obtained for the inks analysed.

to the surface of the film. For the same vehicle and thickness (depth) of the ink film, the efficiency of the diffusion process is mainly a function of the nature of ingredients of inks such as resins and polymers. Moreover, if the resin is capable of polymerizing, *i.e.*, of cross-linking, the diffusion process slows as an ink ages on paper, and at a certain stage of ageing it can virtually stop. For this reason the remaining ink volatile components can be detected in the ink line even after a long period of time; this is shown in Fig. 1 for up to 15-year-old entries written with Soyuz ballpoint inks.

In such situations, a "weak" solvent (with regard to hardened ink resins), being unable to penetrate inside an old ink line, extracts the ink volatile components only from its exterior layers. However, the newer the ink, the more exterior layers of the ink become available to the weak solvent, and hence a greater amount of the volatile components is extracted.

Fig. 1 is a good illustration of the above observation that the extraction efficiency of a "weak" solvent decreased from about 90% for fresh writings to about 20% for old writings.

It should be noted that the proposed method includes also an important stage that is carried out if the values of %M determined for the Q ink entry are larger than *ca.* 60%. In this event, another sample (1-cm sliver) is taken from the ink entry, heated moderately, *e.g.*, at 80°C for 5 min, and analysed as described under Experimental. The percentage extraction value, $\%M_t$, is calculated for the heated sample and compared with the value of %M that was determined for the unheated sample. If the difference between %M and $\%M_t$ is *ca.* 10% or larger, it can be concluded that the ink entry analysed is a fresh one. If the difference is less than 10%, it means that a more suitable "weak solvent" should be chosen for a given ink.

For example, as a result of studying the ageing process of many ballpoint inks of different formulae by using the proposed method, it has been established that if, for a given ink, the analytical results are $\%M > 70\%$ and $\%M - \%M_{t=80°C, 5\ min} > 10\%$, then the age of the ink analysed is less than *ca.* 6 months (depending on

the ink formula, this value may decrease to *ca.* 2 months).

As an example, Fig. 1 shows the results of the age determination obtained for the Q entry (in fact, it was a 3-month old entry written with a Soyuz blue ballpoint ink) using the proposed method.

The method demonstrated high efficiency in many actual case situations when it was necessary to determine whether the age of the Q entry was less than several months or not less than 1 year. Such cases are fairly typical when the investigator suspects that the given entry or signature was made after the time the investigation began. Some similar examples have been presented by Cantu [11].

3.2. Thin-layer chromatography

Fig. 2 demonstrates the view under UV illumination of the fragment of the thin-layer chromatogram (without the chromatographic zones of the paper's ingredients) and corresponding densitograms obtained for samples taken from entries written with Parker blue ballpoint ink. A and B represent separated colourless components of interest in the ink analysed.

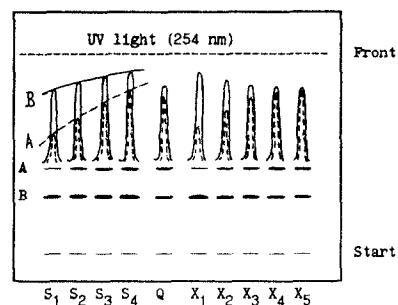It is clearly seen in Fig. 2 that there is an

Fig. 2. Fragment of the thin-layer chromatogram (UV detection at 254 nm) and corresponding densitograms obtained for Parker blue ballpoint ink entries of different ages. S_1–S_4 are calibration standards; Q relates to an entry of questionable age; X_1–X_5 relate to known ink entries: $X_1 = 1$ day, $X_2 = 1$ month, $X_3 = 1$ year, $X_4 = 3$ years, $X_5 = 6$ years old.

obvious link between the relative proportion of substances A and B and the age of the ink writings examined: the substance A/substance B ratio gradually increases as the ink ages (it is a minimum for a fresh, 1-day-old entry, the X_1 track, and maximum for a 6-year-old entry, the X_5 track).

As a rule, the relative proportions of the components separated by TLC are evaluated by obtaining related densitometric data and further by calculating the ratios of the components' peak signals [1–3,8,9]. However, this approach has been shown to produce erroneous results because, in densitometric TLC, when chromatograms are scanned by reflectance in the absorbance mode, the relationship between signal output (peak height or peak area) and the content of a separated zone is hardly ever a directly proportionality [10,12,13].

In this connection, a more reliable approach is offered here. It can be considered as a version of the external standard method for evaluating thin-layer chromatograms for cases typical in forensic analysis when information on the quantitative and even qualitative composition of samples to be analysed is not available and, therefore, calibration graphs cannot be obtained for the analytes. (Another way to avoid erroneous results produced by the signal-to-signal ratio technique includes the application of the approach based on the mass-independent version of the peak ratioing technique [10,13].)

The proposed method allows one to obtain the actual mathematical functions of signal *versus* content for any two components, A and B, of the materials analysed within a certain calibration range of the contents of these components, C_{min}–C_{max}. This calibration range is formed by applying at least four or five calibration standards on a TLC plate as follows.

If samples are sprayed on as narrow bands, different volumes of only one standard solution can be applied to form a calibration range, C_{min} – C_{max}. An important characteristic of the method is that the real values of C_{min} and C_{max} can be unknown to the examiner: only the values of C_{max}/C_{min} and C_i/C_{min} (where i relates to a calibration standard characterized by the content

of a component per zone that is less than C_{max} and larger than C_{min}) must be known, as was described under Experimental.

If samples are applied as spots, calibration standards should be prepared in different concentrations and spotted as a fixed constant volume: multiple spotting of a single standard solution to generate a calibration graph is not acceptable for accurate quantification as there is no simple correlation between signal response for a constant amount of substance and spot size in scanning densitometry [12]. In this case, the contents of components A and B per zone are assumed to be equal (or directly proportional, if only dilution factors, not real concentrations, are known for the calibration standard solutions) to the corresponding concentrations of the calibration standard solutions. Hence the value of the ratio of the contents of any component in the chromatographic zones corresponding to any two prepared calibration solutions will be equal to the value showing how many times one of these solutions is more (or less) concentrated than the other.

Further, for each component A and B, an appropriate approximation function is found and used as a calibration function for calculating the contents, C_A and C_B, of components A and B per spot of the samples taken from the entries of the known and questionable ages. Although these content values are not real, this is not sufficient for the considered method: the main point is that the ratios of these values, C_A/C_B, are independent of mass, in contrast to the peak ratioing technique that is based on using the mass-dependent values of $signal_A/signal_B$ (see Table 2).

Tables 2 and 3 show peak-height values calculated by an integrator for the separated components of the Parker ink analysed, PH_A (for component A) and PH_B (for component B), and the values characterizing the relative proportions of the components A and B calculated by using the peak rationing technique (fourth column) and the proposed "content" ratioing method (last column).

Fig. 3 shows ageing curves obtained for the Parker ink by plotting the values of the ratios

Table 2
Data obtained for calibration standards

Standard	Integrator reading[a]		Ratio of peaks, PH_A/PH_B	Ratio of contents[a], C_A/C_B
	PH_A	PH_b		
S_1	31 032	90 506	0.34	1.02
S_2	51 716	99 126	0.52	0.98
S_3	73 261	107 745	0.68	0.96
S_4	88 782	112 056	0.79	1.04
Mean			0.58	1.00
R.S.D.			0.34	0.04

[a] Peak heights, PH_A and PH_B, were plotted against the contents C_A and C_B (see Table 1). As a result, the following regression equations and correlation coefficients (r^2) of the logarithmic calibration graphs were obtained: $PH_A = -56\,496 + 53\,529 \log C_A$ ($r^2 = 0.9929$) and $PH_B = 57\,816 + 20\,258 \log C_B$ ($r^2 = 0.9894$). Using these equations, the values of C_A and C_B were recalculated for each standard, S_1–S_4, and used for calculating the content ratio values listed in the last column.

listed in the last two columns of Table 3 against the actual age of the known ink entries.

The results of determining the age of the Q entry (in fact, the age of this entry was 2 years) are also shown in Fig. 3 and presented in Table 3.

It is clearly follows from Fig. 3 and the data in Tables 2 and 3 that, in comparison with the peak ratioing technique, the proposed mass-independent content ratioing method gives a significant increase in the accuracy and precision of ink age determination.

It should also be noted that this method can be successfully applied to a comparative TLC examination of similarly coloured inks, paints, fibres and other materials of forensic interest, as

Table 3
Data obtained for ink entries

Ink entry	Integrator reading		Ratio of peaks, PH_A/PH_B	Ratio of contents, C_A/C_B
	PH_A	PH_B		
X_1	39 659	110 334	0.36	0.45
X_2	64 644	103 435	0.63	1.01
X_3	79 735	94 817	0.84	2.04
X_4	84 472	94 388	0.89	2.27
X_5	88 351	93 093	0.95	2.60
Q	81 887	93 529	0.88	2.26
	87 963	96 975	0.91	2.14
	80 168	93 394	0.85	2.20
Age determined for the Q entry (years)			1.3	1.4
			2.6	2.4
			4.1	3.0

MASS-INDEPENDENT

RATIO OF "CONTENTS" (a)

MASS-DEPENDENT

RATIO OF PEAK SIGNALS (b)
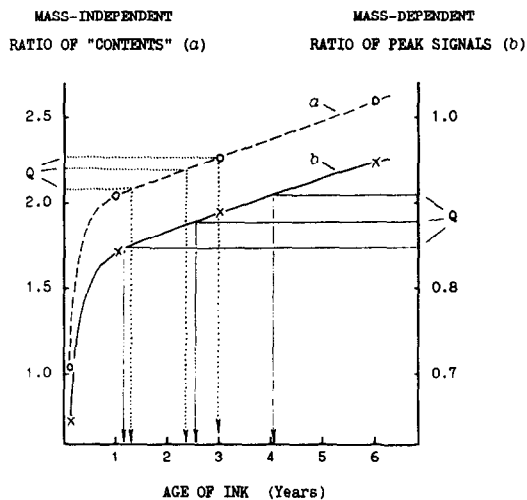

AGE OF INK (Years)

Fig. 3. Ageing curves obtained for the Parker ink using (a) the content ratioing method and (b) the peak ratioing technique.

its discriminating power is much greater than that usually produced by the widely used mass-dependent signal-to-signal ratio technique.

4. Conclusions

Two complementary methods for dating ball-point inks have been considered. The method using GC allows discrimination between fresh (age not greater than a few months) and old ballpoint inks, including inks with formulae unknown to the examiner. It is effective for analysing ballpoint inks that contain phenoxy-ethanol, phenoxyethoxyethanol or similar high-boiling vehicles.

The method using TLC allows the detection of age changes in resins and other non-volatile ink components. It includes a new procedure for evaluating thin-layer chromatograms of separated ink components. Being mass-independent, this procedure gives much more correct results for dating inks than those obtained with the aid of the widely used signal-to-signal ratio technique.

Both methods, together and separately, have been used in many actual case situations and the results of the examinations have been accepted as conclusive evidence by courts.

Further work is necessary to evaluate the limits of the applicability of the methods to numerous inks that are on the market.

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