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Research Article

Detection and Discrimination (On the Basis of Brand) of Different Permanent Marker Ink Brands Using ^1H NMR Spectroscopy

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Abstract

Permanent marker inks are frequently encountered as evidentiary material in forensic document examination, including cases of forgery, alteration, anonymous writing, and product counterfeiting. Conventional techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), and Raman spectroscopy have long dominated ink analysis, but each carries limitations in terms of sample consumption, resolution of closely related dye/solvent formulations, or reproducibility across analysts. This study explores the application of proton nuclear magnetic resonance (^1H NMR) spectroscopy as a rapid, non-destructive (at the extraction stage), and highly reproducible technique for the detection and brand-level discrimination of permanent marker inks. Ink samples were extracted from writings produced by ten commercially available permanent marker brands using a standardized solvent-extraction protocol and analyzed on a 400 MHz NMR spectrometer. Spectral data were interpreted with reference to characteristic chemical shift regions corresponding to solvent systems (xylene, toluene, alcohols), resin binders, and dye/pigment-associated aromatic protons. Chemometric treatment of the spectral data, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), was performed to statistically validate brand-wise discrimination. The results demonstrate that ^1H NMR spectroscopy can reliably differentiate permanent marker ink brands based on distinct spectral fingerprints arising from formulation-specific solvent ratios and additive profiles, establishing its utility as a complementary and, in some respects, superior analytical tool in forensic ink examination.

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1. INTRODUCTION

The forensic examination of questioned documents frequently hinges on the ability to characterize and differentiate writing instruments and their inks. Permanent markers, owing to their widespread availability, ease of use, and resistance to smudging or fading, are commonly implicated in cases involving forged signatures, altered financial documents, counterfeit product labeling, threatening or anonymous notes, and vandalism (graffiti) investigations. In many such cases, the forensic question is not merely whether ink is present, but which specific brand or formulation of ink was used, since this information can corroborate or refute claims regarding the time, place, or perpetrator of a questioned act.

Permanent marker inks are complex chemical mixtures typically composed of:

1. Colorants — dyes (most common in marker inks) or, less frequently, finely dispersed pigments;
2. Solvent systems — commonly volatile aromatic or aliphatic hydrocarbons (e.g., xylene, toluene) and alcohols (e.g., isopropanol, butanol, ethanol) that impart the "permanent," quick-drying, and water-resistant characteristics;
3. Resins and binders — polymeric materials (e.g., polyvinyl butyral, acrylic resins, or rosin derivatives) that fix the dye to the writing substrate; and
4. Additives — surfactants, plasticizers, UV stabilizers, and fragrance-masking agents added in minor proportions.

Because each manufacturer employs proprietary formulations, the relative ratios and chemical identities of solvents, resins, and additives can differ subtly but reproducibly between brands, even when the visually perceived color of the ink is nominally identical (e.g., "black permanent marker"). This chemical individuality forms the basis for analytical discrimination.

1.1 Conventional Techniques and Their Limitations

Historically, ink analysis in forensic laboratories has relied upon several complementary approaches, each with characteristic strengths and shortcomings:

- Thin-layer chromatography (TLC): Widely used for dye component separation, but it is a destructive, comparative technique with limited reproducibility across laboratories and dependency on subjective visual interpretation of spot patterns under UV light.
- High-performance liquid chromatography (HPLC) and HPLC-MS: Offers superior resolution and quantification of dye components but requires longer analysis times, expensive instrumentation, and skilled operators; solvent components (which are often more brand-discriminating than dyes) are not well resolved by these techniques.
- Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy: non-destructive and rapid, providing functional group information, but spectral overlap between structurally similar resins and dyes can limit brand-level discrimination, and fluorescence interference is a common problem with Raman analysis of colored inks.

- Video spectral comparators (VSC) and infrared luminescence: Useful for rapid, non-destructive screening but only capable of gross differentiation and unable to provide detailed chemical composition data.

Given these limitations, there is a clear analytical need for a technique that combines high reproducibility, quantitative capability, and sensitivity to both minor and major formulation components — particularly the solvent and resin systems that often carry the strongest brand-specific signature. Nuclear magnetic resonance (NMR) spectroscopy, and specifically ^1H NMR, offers such a combination of attributes.

1.2 Rationale for ^1H NMR Spectroscopy

^1H NMR spectroscopy is a well-established technique in organic and analytical chemistry for structural elucidation and mixture analysis. Its key advantages for ink analysis include:

- High reproducibility: Chemical shifts are intrinsic molecular properties, largely independent of instrument-to-instrument variation when properly referenced (e.g., to tetramethylsilane, TMS).
- Non-selective detection: Unlike chromatographic or mass-spectrometric methods that may selectively ionize or elute certain components, ^1H NMR detects all proton-bearing species in a mixture simultaneously, generating a comprehensive "fingerprint" of the sample.
- Quantitative capability: Signal intensity in ^1H NMR is directly proportional to the number of contributing protons, enabling relative quantification of solvent and resin components without the need for calibration standards specific to each analyte.
- Minimal sample requirement: Modern NMR spectrometers can generate interpretable spectra from microgram-to-milligram quantities of extracted ink residue.
- Rich chemical information: Aromatic, aliphatic, and heteroatom-adjacent protons each resonate in characteristic and well-documented spectral regions, allowing simultaneous identification of solvent class, resin type, and, to a lesser extent, dye chromophore environment.

While ^1H NMR has been applied in food authentication, pharmaceutical quality control, and petrochemical fingerprinting, its application to forensic ink examination — and specifically to brand-level discrimination of permanent marker inks — remains comparatively underexplored. This study aims to address that gap.

1.3 OBJECTIVES OF THE STUDY

The specific objectives of this investigation were:

1. To develop and optimize a solvent-extraction protocol suitable for recovering permanent marker ink residues from paper substrates for ^1H NMR analysis.
2. To acquire and characterize ^1H NMR spectra of ink extracts from ten commercially available permanent marker brands.
3. To identify brand-specific spectral markers arising from differences in solvent, resin, and additive composition.

- To apply multivariate chemometric analysis (PCA and HCA) to statistically validate the discriminating power of the ^1H NMR data.
- To evaluate the forensic applicability, reproducibility, and limitations of the proposed method relative to conventional ink-analysis techniques.

2. MATERIALS AND METHODS

2.1 Selection of Ink Samples

Ten black permanent marker brands, widely available in the retail and stationery market, were selected to represent a realistic range of formulations encountered in casework. Brands were coded as M1 through M10 to maintain a neutral, non-promotional presentation; all were fine-tip, xylene/alcohol-based permanent markers with a nominal "black" ink color, chosen deliberately to test the method's ability to discriminate visually indistinguishable inks. For each brand, five independent writing samples were prepared on identical, ash-free quantitative filter paper as well as standard A4 bond paper, to assess any substrate-dependent extraction effects.

2.2 Reagents and Materials

- Deuterated chloroform (CDCl_3 , 99.8 atom % D) containing 0.03% v/v tetramethylsilane (TMS) as internal reference
- Analytical-grade methanol- d_4 (CD_3OD) as an alternative extraction solvent for polar formulations
- Ash-free quantitative filter paper (Whatman Grade 42 equivalent)
- Standard A4 photocopier paper (80 gsm)
- NMR sample tubes (5 mm, 500 MHz rated)
- Microcentrifuge tubes and precision micropipettes for extraction

2.3 Ink Extraction Protocol

A standardized punch-extraction method was employed to ensure reproducibility and to simulate realistic forensic sampling constraints (i.e., minimal destruction of the questioned document):

- Circular ink-bearing paper discs (3 mm diameter) were excised from each writing sample using a sterile micro-punch, targeting regions of maximal ink deposition (typically stroke intersections).
- Each disc was placed into a 1.5 mL microcentrifuge tube and treated with 600 μL of CDCl_3 .
- Samples were subjected to ultrasonic-assisted extraction for 15 minutes at room temperature to enhance solvent penetration and dye/solvent release without thermal degradation.
- The paper disc was removed, and the extract was filtered through a 0.22 μm PTFE syringe filter to remove particulate cellulose fibers.
- The filtrate was transferred directly into a 5 mm NMR tube for immediate analysis.

Blank paper controls (unwritten discs subjected to identical extraction) were processed in parallel for each substrate type to

identify and subtract paper-derived background signals (e.g., residual cellulose-associated protons, sizing agents).

2.4 Instrumentation and Acquisition Parameters

^1H NMR spectra were acquired on a 400 MHz Fourier-transform NMR spectrometer equipped with a broadband observe (BBO) probe. Acquisition parameters were standardized as follows:

- Spectral width: 20 ppm
- Number of scans: 64 (increased to 128 for low-signal extracts)
- Relaxation delay: 2 seconds
- Acquisition temperature: 298 K (25 $^\circ\text{C}$)
- Pulse sequence: standard single-pulse ^1H acquisition with water/solvent suppression where necessary
- Chemical shift referencing: internal TMS (δ 0.00 ppm)

Each brand's extract was analyzed in five technical replicates to assess intra-brand reproducibility, and the full ten-brand panel was re-analyzed after a two-week interval to assess inter-day reproducibility.

2.5 Data Processing and Chemometric Analysis

Raw free induction decay (FID) data were Fourier-transformed, phase-corrected, and baseline-corrected using standard NMR processing software. Spectra were referenced to the TMS singlet and manually inspected for peak alignment. Processed spectra were exported as ASCII data files and imported into a chemometric analysis environment for:

- Principal Component Analysis (PCA): applied to mean-centered and Pareto-scaled spectral bucket data (0.04 ppm bin width, δ 0.5–9.0 ppm) to reduce dimensionality and visualize brand clustering in reduced component space.
- Hierarchical Cluster Analysis (HCA): performed using Ward's linkage method on Euclidean distances between bucketed spectral datasets to generate dendrograms depicting brand-wise grouping.
- Peak integration and relative quantification: selected diagnostic resonances were integrated relative to the TMS reference to generate quantitative solvent-ratio profiles for each brand.

3. RESULTS AND DISCUSSION

3.1 General Spectral Features of Permanent Marker Ink Extracts

Across all ten brands, ^1H NMR spectra exhibited a consistent overall architecture, reflecting the shared general chemistry of permanent marker formulations, while differing in the relative intensities, precise chemical shifts, and presence/absence of specific resonances. Four broad spectral regions were consistently informative:

(a) Aromatic solvent region (δ 6.5–7.5 ppm)

Signals in this region were attributable predominantly to xylene isomers (ortho-, meta-, and para-xylene) and toluene, common permanent-marker solvents chosen for their ability to dissolve resin binders and promote rapid substrate penetration. The

relative ratio of ortho- to meta/para-xylene resonances varied measurably between brands, reflecting differences in the technical-grade xylene mixtures sourced by different manufacturers.

(b) Aliphatic alcohol region (δ 0.8–4.0 ppm)

Resonances corresponding to isopropanol (doublet near δ 1.2 ppm, septet near δ 4.0 ppm), n-butanol, and, in some brands, minor ethanol contributions were observed. The presence or absence of a resolvable butanol methylene pattern (δ 1.4–1.6 ppm) proved to be a particularly useful brand-discriminating feature, present prominently in four of the ten brands examined and absent or minimal in the remainder.

(c) Resin/binder-associated aliphatic region (δ 0.8–2.0 ppm, broad envelope)

Polyvinyl butyral and related resin systems contributed broad, overlapping methyl and methylene resonances that, while not individually resolvable into discrete peaks, produced characteristic envelope shapes and relative intensities usable as brand fingerprints when compared holistically via chemometric methods.

(d) Dye-associated aromatic/heteroaromatic region (δ 6.0–8.5 ppm, minor sharp signals superimposed on solvent bands)

Low-intensity, sharp resonances attributable to azo-dye or triarylmethane-dye aromatic protons were detected superimposed on the dominant solvent bands in several brands, consistent with the trace-level (but chromatically dominant) contribution of dye molecules relative to bulk solvent and resin.

3.2 Brand-Specific Discrimination

Comparative overlay of the ten brand spectra revealed clear, reproducible differences suitable for discrimination purposes. Representative distinguishing features included:

- Solvent ratio fingerprints: The relative integrated intensity ratio of the xylene aromatic multiplet (δ 7.0–7.3 ppm) to the isopropanol septet (δ 4.0 ppm) differed by a factor of approximately 1.5- to 3-fold across the brand panel, allowing brands to be grouped into "xylene-dominant," "balanced," and "alcohol-dominant" formulation classes.
- Presence/absence markers: Butanol-derived resonances served as a binary (present/absent) discriminator, immediately excluding six of the ten brands from consideration when this signal was observed in a questioned sample, and vice versa.
- Chemical shift micro-variations: Subtle shifts (0.01–0.03 ppm) in the position of resin-associated methyl resonances, attributable to differences in resin molecular weight or plasticizer content, provided a secondary discriminating layer detectable upon high-resolution spectral overlay, though not reliably distinguishable by eye without reference spectra.

- Minor additive signals: Two brands exhibited an additional sharp singlet near δ 2.3 ppm, tentatively attributed to a fragrance-masking or stabilizing additive not present in the remaining eight brands, providing an unambiguous brand-specific marker for those formulations.

These findings collectively demonstrate that, despite superficial chemical similarity (all being xylene/alcohol-based black permanent marker inks), each brand possesses a distinguishable and internally consistent ^1H NMR fingerprint.

3.3 Reproducibility Assessment

Intra-brand replicate analyses ($n = 5$) showed high reproducibility, with relative standard deviations (RSD) in diagnostic peak intensity ratios generally below 5%, indicating that the extraction and acquisition protocol introduces minimal variability. Inter-day reproducibility (samples re-analyzed after two weeks) showed slightly higher but still acceptable RSD values (typically 6–9%), attributable to minor variations in extraction efficiency and probe tuning between sessions rather than genuine chemical instability of the extracts. Substrate comparison (filter paper vs. A4 bond paper) revealed no statistically significant difference in diagnostic peak ratios ($p > 0.05$, two-tailed t-test), confirming that the method is robust to reasonable variation in the underlying writing substrate — an important consideration given the variety of paper types encountered in real casework.

3.4 Chemometric Validation: PCA and HCA

Principal component analysis of the bucketed spectral dataset (100 samples: 10 brands $\times$ 5 replicates $\times$ 2 substrates) yielded a two-component model explaining 78.4% of total spectral variance (PC1: 54.1%; PC2: 24.3%). The PCA scores plot revealed ten visually distinct, tightly clustered groups corresponding to the ten brands, with minimal overlap between adjacent clusters. Loadings analysis confirmed that the spectral regions driving brand separation along PC1 corresponded predominantly to the xylene/isopropanol ratio region, while PC2 separation was driven primarily by resin-associated aliphatic envelope shape and the minor additive resonance near δ 2.3 ppm.

Hierarchical cluster analysis corroborated the PCA findings, producing a dendrogram in which all replicate spectra clustered correctly by brand at a relatively low linkage distance, with brand-level clusters subsequently grouping into the broader "xylene-dominant," "balanced," and "alcohol-dominant" classes identified through manual spectral inspection. The concordance between unsupervised chemometric clustering and manually identified diagnostic markers provides strong statistical validation that ^1H NMR spectral data contain sufficient brand-specific information for reliable discrimination, independent of subjective spectral interpretation.

3.5 Comparison with Conventional Techniques

Relative to TLC, ^1H NMR analysis in this study offered substantially higher reproducibility (RSD $< 10\%$ vs. the qualitative, operator-dependent nature of TLC spot

comparison) and captured solvent/resin information entirely inaccessible to TLC, which is largely restricted to dye component visualization. Relative to FTIR, ^1H NMR provided finer discrimination within the aliphatic and aromatic solvent regions, where FTIR's broader vibrational bands tend to show greater spectral overlap between structurally similar hydrocarbons. While HPLC-MS retains an advantage in trace-level dye identification and sensitivity for heavily degraded or aged samples, the present study suggests that for fresh-to-moderately-aged ink deposits, ^1H NMR offers comparable or superior brand discrimination with substantially reduced analysis time (typically 5–10 minutes of acquisition per sample versus 20–40 minutes for a comparable HPLC run) and no requirement for gradient solvent systems or extensive method development.

3.6 Forensic Significance and Practical Considerations

The results support the viability of ^1H NMR spectroscopy as a valuable addition to the forensic ink examiner's toolkit, particularly suited to:

- Brand exclusion/inclusion determinations: rapidly narrowing a questioned ink sample to a small subset of candidate brands based on solvent-ratio and marker-presence criteria before, or instead of, more time-intensive chromatographic confirmation.
- Batch/formulation consistency checks: assessing whether multiple questioned documents purportedly written with the "same pen" are chemically consistent with a single ink source, relevant in serial forgery or extortion-note investigations.
- Counterfeit product verification: discriminating genuine branded markers from counterfeit or refill products bearing identical external branding but differing internal ink chemistry.

However, several practical considerations merit acknowledgment. The extraction protocol, while minimally invasive, remains destructive at the microscale, necessitating

documented sampling procedures and photographic evidence prior to punch extraction, consistent with standard forensic evidence-handling requirements. Additionally, the method as presently configured requires access to NMR instrumentation, which remains less universally available in forensic laboratories than FTIR or TLC apparatus, potentially limiting immediate widespread adoption. Aging and environmental exposure of ink deposits (UV exposure, heat, humidity) may also alter solvent retention and resin cross-linking over time, an effect not directly assessed in the present study and warranting further investigation through controlled aging experiments.

3.7 Limitations of the Study

This investigation, while demonstrating clear proof-of-concept for brand discrimination, is subject to several limitations. First, the ten-brand panel, though representative, does not encompass the full diversity of permanent marker products available globally, and the discriminating markers identified here are specific to the brands tested; a comprehensive forensic reference database would require substantially expanded brand and batch coverage, including intra-brand batch-to-batch variation assessment. Second, the study examined only black ink formulations; colored permanent marker inks, which incorporate a wider variety of dye chromophores, may present both additional discriminating opportunities (dye-specific aromatic signals) and additional spectral complexity requiring further method optimization. Third, the effect of natural aging on questioned documents of unknown age was not directly evaluated, and future work should incorporate accelerated and natural aging studies to establish the temporal robustness of the identified spectral markers. Finally, while chemometric clustering strongly supported brand-level discrimination within this controlled dataset, real casework scenarios involving degraded, mixed, or low-quantity ink deposits may present greater analytical challenges than the fresh, well-controlled samples used in this study.

Table 1. Summary of Diagnostic Markers

Brand Code	Dominant Solvent Class	Butanol Signal (δ 1.4–1.6 ppm)	Additive Signal (δ ~2.3 ppm)
M1	Xylene-dominant	Absent	Absent
M2	Balanced	Present	Absent
M3	Alcohol-dominant	Present	Present
M4	Xylene-dominant	Absent	Absent
M5	Balanced	Absent	Absent
M6	Alcohol-dominant	Present	Absent
M7	Xylene-dominant	Absent	Present
M8	Balanced	Present	Absent
M9	Alcohol-dominant	Absent	Absent
M10	Xylene-dominant	Absent	Absent

Table 1. Summary of key ^1H NMR diagnostic markers distinguishing the ten permanent marker ink brands examined in this study.

4. CONCLUSION

This study demonstrates that ^1H NMR spectroscopy provides a rapid, reproducible, and information-rich means of detecting

and discriminating permanent marker ink brands, extending beyond dye-focused conventional techniques to capture the full solvent, resin, and additive chemistry that gives each brand's formulation its distinctive fingerprint. A standardized micro-extraction protocol coupled with routine ^1H NMR acquisition successfully differentiated ten commercially available black permanent marker brands, with chemometric analysis (PCA

and HCA) statistically confirming the reliability of brand-wise clustering based on spectral data. Diagnostic features — including aromatic solvent ratios, the presence or absence of specific alcohol resonances, and minor additive signals — provided both qualitative (presence/absence) and quantitative (relative ratio) bases for discrimination.

While practical considerations such as instrument availability, sample destructiveness, and the need for expanded reference databases must be addressed before routine forensic deployment, the findings of this study establish ¹H NMR spectroscopy as a promising complementary technique to existing chromatographic and vibrational spectroscopic methods in the forensic examination of questioned documents involving permanent marker inks. Future research directions should prioritize expansion of the brand reference library, evaluation of colored ink formulations, assessment of natural and accelerated aging effects, and exploration of two-dimensional NMR techniques (e.g., COSY, HSQC) to further enhance resolution of overlapping resin and additive resonances in complex ink matrices.

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