

Evaluating 7.5 M Ammonium Acetate for the Extraction of DNA from Degraded Saliva Samples: A Comprehensive Forensic Evaluation

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Abstract

Recovering DNA from environmentally degraded biological samples remains one of the most persistent challenges in forensic science. Saliva, though highly valuable as a source of epithelial cells, is frequently exposed to detrimental environmental factors that accelerate chemical, enzymatic, and structural DNA degradation. This study thoroughly investigates the effectiveness of a 7.5 M ammonium acetate-based extraction protocol for the recovery of usable DNA from saliva subjected to controlled UV radiation, thermal degradation, and sodium hypochlorite exposure. DNA yield and purity were assessed using NanoDrop spectrophotometry, and STR profiling was performed using the VeriFiler™ Express PCR Amplification Kit. Results demonstrated that although degradation significantly impacted nucleic acid concentration and purity, amplifiable DNA was recovered, producing partial to near-complete STR profiles. These findings indicate that ammonium acetate precipitation is a cost-effective, non-toxic alternative for resource-limited laboratories, with important implications for forensic casework involving compromised biological evidence.

Keywords: Forensic DNA extraction, saliva evidence, degraded DNA, ammonium acetate precipitation, STR profiling

Introduction

Forensic samples often undergo significant degradation before being collected whether through environmental exposure, deliberate destruction, or natural biochemical decay. UV radiation induces pyrimidine dimers and conformational distortions. UV exposure can inhibit successful polymerase chain reaction (PCR) amplification, which is vital for DNA profiling [1]. Thermal exposure accelerates depurination and fragmentation, and can also affect cellular processes,

leading to the breakdown of protective proteins and metabolic components. The extent of damage depends on the specific environmental conditions and the stage of the cell cycle at the time of exposure [2]. Chemical agents such as detergents can affect DNA preservation, especially when biological stains are washed before collection. These actions introduce chemical variables that influence the preservation and recoverability of DNA as it can cause base modifications and changes in DNA structure [3].

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Among the various biological materials encountered in forensic casework, saliva represents a particularly common but often overlooked form of trace evidence. It is found on cigarette butts, bottles, envelopes, bite marks, clothing, and various food items [1, 4]. Despite its often invisible nature, saliva contains abundant epithelial cells capable of yielding nuclear DNA. Its non-invasive collection makes it favorable compared to blood or tissue samples [1, 5]. However, saliva residues on surfaces are readily exposed to heat, UV light, detergents, and moisture, making reliable DNA recovery comparatively difficult.

Despite its forensic value, the reliable recovery of DNA remains challenging, particularly when conventional extraction methods are applied to degraded samples. Organic extraction, although capable of producing high-quality and high-molecular-weight DNA, is labor-intensive, time-consuming, and requires the handling of hazardous reagents such as phenol and chloroform, which pose health and environmental risks [6, 7]. The Chelex method offers a rapid and cost-effective alternative, however, it produces single-strand DNA and does not effectively remove PCR inhibitors, such as haem and mucin, which can compromise downstream amplification and profiling. Consequently, its application is largely restricted to PCR-based analyses [6, 8].

Solid-phase extraction techniques, including silica column and magnetic bead-based systems, provide improved DNA purity and reproducibility. However, these methods rely on commercially available kits and specialized equipment, resulting in higher operational costs and limited accessibility in resource-constrained laboratories [1, 6].

In light of these challenges, ammonium acetate-based extraction method has gained attention as a promising alternative for forensic DNA isolation due to its ability to efficiently precipitate proteins and SDS while retaining nucleic acids in solution. Studies have demonstrated that 10 M ammonium acetate can yield high concentrations and purity of DNA from saliva samples, with reported 260/280 ratios between 1.8 and 2.0, indicating minimal protein contamination [9, 10]. Additionally, ammonium acetate forms ionic complexes with interfering metabolites, facilitating their removal and improving DNA quality [9]. While these findings highlight its effectiveness for fresh biological samples, the applicability of this method to degraded forensic evidence remains

limited. Therefore, further investigation is required to evaluate its reliability for compromised evidence, which is the primary focus of this study.

Materials and methods

This research was conducted under ethical approval granted by the University of Lincoln, United Kingdom (Ethics reference: 2025_12418).

Sample collection

Participants provided buccal saliva samples using sterile swabs. Samples were dried, labeled, and stored under controlled conditions prior to degradation.

Controlled degradation procedures

Three degradation conditions were designed to simulate realistic forensic environments (figure 1):

1. UV radiation for 0 (A), 30 (B), 60 (C), 120 (D) minutes in CL- 1000 Ultraviolet Crosslinker, set at 100 $\mu\text{J}/\text{cm}^2$. Simulating sunlight exposure which is common at outdoor crime scenes.
2. Thermal stress via an oven of Fisherbrand at 121°C under 15 p.s.i., for 0 (A), 30 (B), 60 (C), 120 (D) minutes. Simulating exposure to fire, high-temperature environments, or enclosed vehicles.
3. Chemical exposure with sodium hypochlorite (NaClO) with 0% (A), 25% (B), 50% (C), 100% (D) concentration for 3 hours. Simulating deliberate destruction or laundering of evidence.

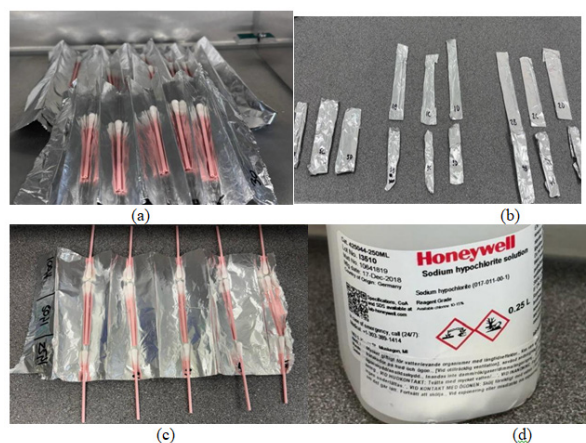


Figure 1: Samples ready for (a) UV degradation (b) thermal degradation (c) chemical degradation (d) Sodium hypochlorite administered

DNA extraction using ammonium acetate

During buffer preparation, all samples were stored in the refrigerator at 4°C to minimize further degradation. The extraction procedure was adapted from previous research in which the samples were treated with 10 M ammonium acetate^[10]. To minimize further DNA degradation during sample processing, lysis was performed in 5% SDS with 50 mM Tris-HCl solution. Strong SDS denatures protein, including nucleases, and enables effective lipid solubilization and disruption of plasma membrane without aggressive mechanical disruption (vortexing) and high temperatures. Each sample received 250 µL of prepared lysis buffer.

In addition, a blank control, containing only the lysis buffer and no biological material was included to monitor for contamination. Tubes were sealed with parafilm and foil, and were incubated at room temperature under gentle rotation (20 rpm) overnight (16 hours and 40 minutes). Post-lysis, samples were centrifuged at 13,300 rpm for 5 minutes.

To the lysate, 125 µL of 7.5 M ammonium acetate was added, yielding a final concentration of 2.5 M. The role of ammonium acetate is to precipitate the proteins and SDS while leaving DNA in the solution.

Samples were refrigerated at 4°C for 15 minutes, then centrifuged at 13,300 rpm for 15 minutes. Subsequently, 1.2 mL of 80% cold ethanol was added, and tubes were centrifuged at 3,000 rpm for 60 minutes at 4°C. A primary wash using 520 µL of 70% cold ethanol was performed, followed by centrifugation at 13,300 rpm for 10 minutes. Ethanol was discharged, and the tubes were air dried in an inverted position. The DNA pellet was then resuspended in nuclease-free deionized water and vortexed gently.

DNA quantification

NanoDrop spectrophotometry was used to assess DNA concentration and purity via A260/280 and A260/230 ratios. These indices provided insight into protein contamination, salt carryover, and overall extraction efficiency. The blank control was also analyzed using the NanoDrop to verify the absence of measurable DNA and assess background contamination.

STR profiling

For the DNA profiling process, a reduced volume PCR amplification protocol was adapted and validated based on the method outlined in previous research^[11]. Reduced-volume PCR was performed using VeriFiler™ Express with 5 µL total reaction volume for 26 PCR cycles, capillary electrophoresis via 3500xL Genetic Analyzer and allele calls were made using GeneMapper™ ID-X.

Accordingly, four samples from each degradation method were randomly selected, representing both higher and lower ends of DNA yield and purity to undergo DNA profiling to assess the extent of degradation and extraction efficiency. In addition to the experimental samples, Blank, PCR positive and PCR negative controls were included to monitor amplification success and detect potential contamination.

Statistical analysis

DNA concentration and purity (A260/280 and A260/230) were summarized using descriptive statistics. Differences in DNA purity across degradation methods (UV, thermal, and chemical) at three degradation intensities were evaluated using one-way ANOVA. Independent sample t-tests were then performed to compare each degraded group with its corresponding non-degraded control. To adjust for multiple comparisons within each degradation method, a Bonferroni correction was applied, resulting in an adjusted significance threshold of $p < 0.01$. All statistical tests were two-tailed. Statistical analyses were used to determine whether degradation significantly affected DNA purity following ammonium acetate extraction.

Results

DNA concentration

Referring to figure 2, UV exposure caused a clear, time-dependent decline in DNA concentration, with prolonged irradiation (120 minutes) producing severely reduced yields due to extensive UV-induced fragmentation. Thermal degradation also led to losses in DNA concentration, although the extent varied between samples. In contrast, chemical degradation using sodium hypochlorite produced highly inconsistent DNA concentration results. Some samples showed unexpectedly elevated apparent concentrations, likely due to the release of short, degraded DNA fragments that contributed to absorbance readings rather than intact DNA. Blank reading was 0.1 ng/µL.

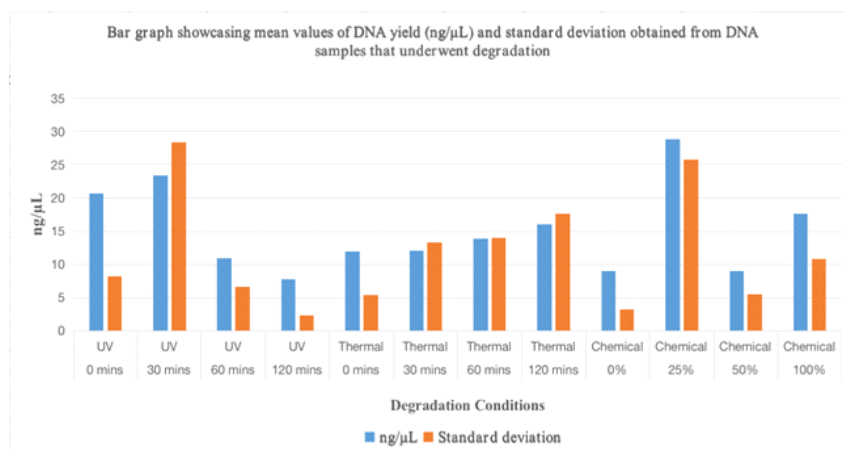


Figure 2: Bar graph showcasing mean values of DNA yield (ng/μL) and standard deviation obtained from DNA samples that underwent degradation

DNA Purity

Referring to Figure 3, UV-treated samples showed a gradual increase in A260/280 ratios with prolonged exposure, rising from approximately 1.4 at baseline to around 1.7 after 120 minutes, indicating a relative reduction in protein contamination despite ongoing DNA damage. Thermal degradation produced a more pronounced increase in A260/280 values over time, with ratios approaching or exceeding 1.8 after extended heating, suggesting possible RNA contamination or changes in nucleic acid composition

following heat-induced denaturation. In chemically degraded samples, A260/280 ratios also increased progressively with exposure, reaching values close to 1.8 at higher concentrations, indicating comparatively preserved purity under certain conditions. However, across all degradation models, A260/230 ratios consistently remained below 1.5, reflecting persistent salt and organic contaminant carryover, likely resulting from ammonium acetate residues and the use of a single ethanol wash step. Blank readings were - 0.44 (260/280) and 0.07 (260/230).

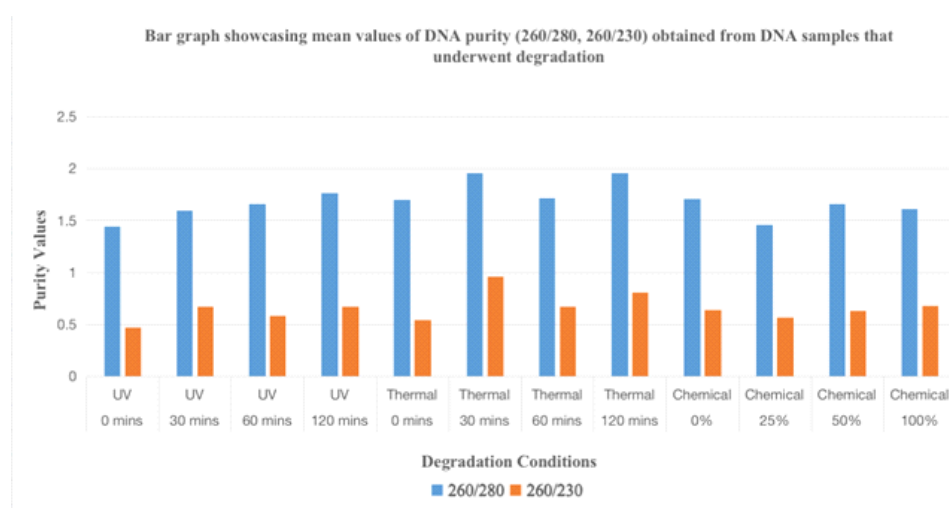


Figure 3: Bar graph showcasing mean values of DNA purity (260/280, 260/230) obtained from DNA samples that underwent degradation

STR Profiling Outcomes

Control samples (0 minute or 0% concentration) produced full profiles. Referring to table 1, as degradation intensity increased, allele dropouts became frequent, particularly after UV and thermal exposure. Chemical degradation preserved profile

quality slightly better, though degraded, samples still showed locus specific profiles. Some samples, like 3C-1, even after intense degradation, retained nearly complete profiles, highlighting individual sample variability and the robustness of the method. Negative controls yielded no peaks, confirming experimental validity.

Table 1: Comparison of STR locus recovery, allelic dropout, and profile completeness in saliva samples subjected to UV, thermal, and chemical degradation

Sample	Degradation Method	Condition (time/conc)	Total loci	Loci fully typed (n)	Allelic dropout (n)	No allele (n)	Overall profile status
1A-3	UV	0 min	22	≈20	D21S11, PENTA_D	0	Near-full profile, minor locus dropout
1D-2	UV	120 min	22	≈17-18	D21S11, D18S51, PENTA_E, D2S1338, PENTA_D	0	Partial profile, multiple dropouts
1D-3	UV	120 min	22	≈21	PENTA_D	0	Near full profile
1D-5	UV	120 min	22	≈20	TPOX, D21S11, PENTA_D	0	Near-full profile
2A-3	Thermal	0 min	22	≈20	D2S1338, D21S11, PENTA_D	0	Partial control profile, inherent weak loci
2A-4	Thermal	0 min	22	≈19-20	D16S539, TPOX, PENTA_E, D2S1338	0	Partial profile
2B-2	Thermal	30 min	22	≈20	D8S1179, D21S11, PENTA_E, D13S317, D6S1043	0	Moderate profile loss
2D-5	Thermal	120 min	22	≈15-16	TPOX (possible true homozygote), DS21S11, PENTA_E, D6S1043, D12S391	D18S51, D13S317, D7S820, PENTA_D	Severely degraded, partial profile
3A-2	Chemical	0 %	22	≈18-19	D8S1179, D21S11, PENTA_E, D13S317, D6S1043,	0	Near-full profile
3B-2	Chemical	25%	22	≈19-20	D21S11, D13S317, D6S1043	0	Near-full profile
3B-5	Chemical	25%	22	≈20	D21S11, PENTA_E	0	Near-full, mild dropout
3C-1	Chemical	100%	22	≈21	D13S317	0	Near-full profile despite severe degradation

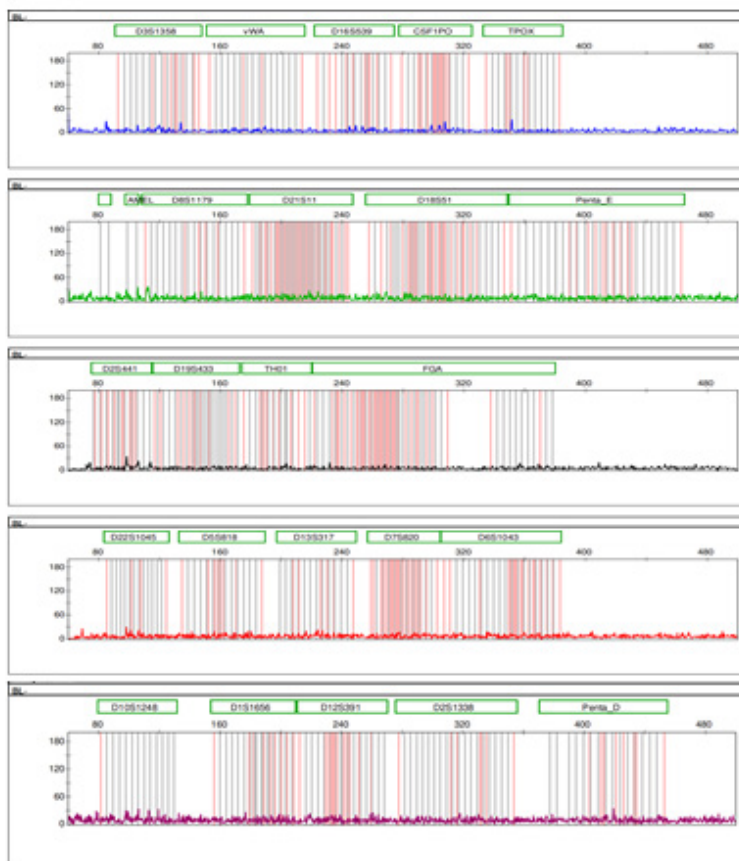
Statistical Results

Overall, the statistical findings indicate that mild early degradation can affect DNA purity, but severe UV, thermal, and chemical exposure produced

similar reductions across all conditions and did not surpass the corrected significance, the Bonferroni-adjusted threshold. (table 2).

Table 2: Summary of ANOVA and independent t-test results for DNA purity (A260/280) across degradation conditions

Analysis Type	Degradation Condition	Comparison	p-value	Significance after Bonferroni (p < 0.01)
ANOVA	Group 1 (30 min / 25%)	UV vs Thermal vs Chemical	0.000344	Yes
ANOVA	Group 2 (60 min / 50%)	UV vs Thermal vs Chemical	0.802	No
ANOVA	Group 3 (120 min / 100%)	UV vs Thermal vs Chemical	0.107	No
t-test	UV Degradation	Control vs 30 min	0.17	No
t-test	UV Degradation	Control vs 60 min	0.05	No
t-test	UV Degradation	Control vs 120 min	0.07	No
t-test	Thermal Degradation	Control vs 30 min	0.24	No
t-test	Thermal Degradation	Control vs 60 min	0.94	No
t-test	Thermal Degradation	Control vs 120 min	0.27	No
t-test	Chemical Degradation	Control vs 25%	0.02	No
t-test	Chemical Degradation	Control vs 50%	0.55	No
t-test	Chemical Degradation	Control vs 100%	0.30	No



(a)

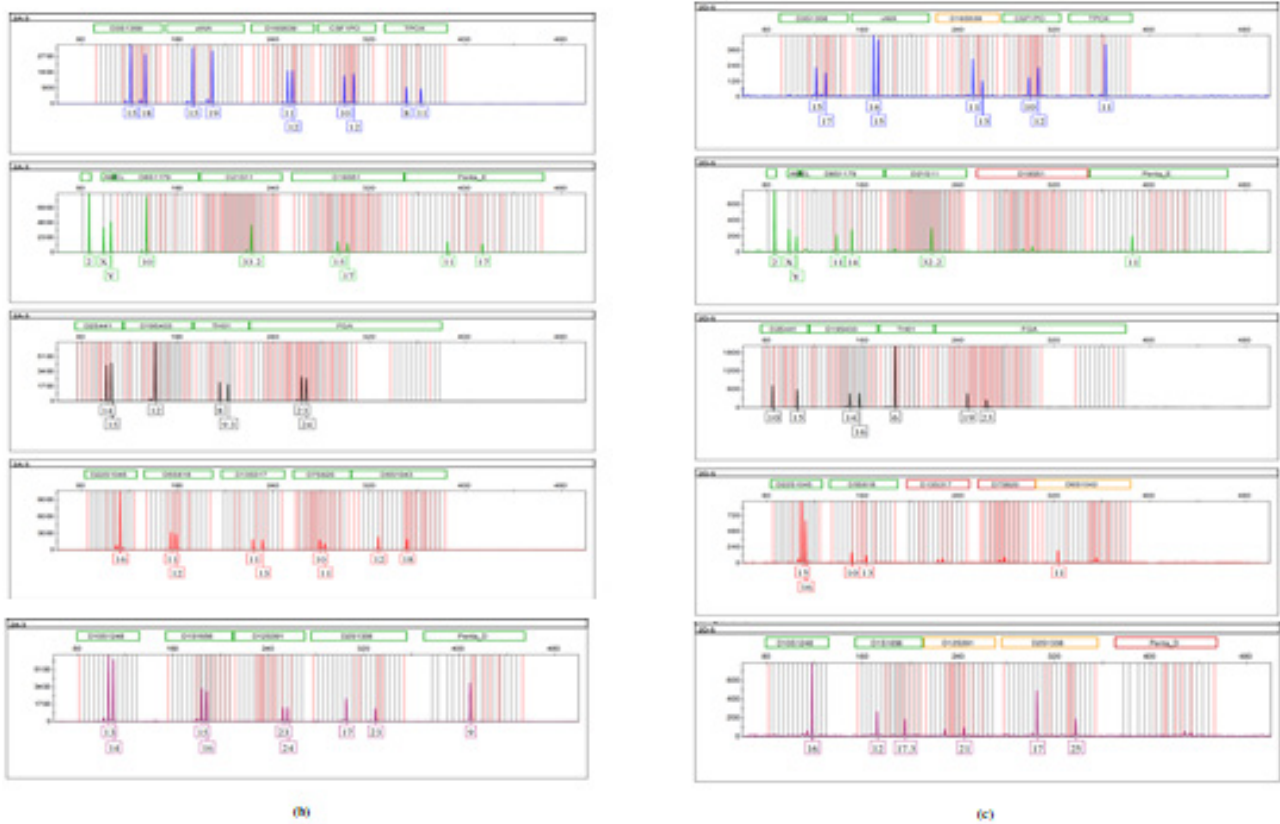


Figure 1: Comparative electropherograms illustrating STR amplification outcomes for (a) blank, confirming absence of contamination, (b) non-degraded saliva sample, demonstrating a balanced STR profile, and (c) thermally degraded saliva sample exposed for 120 minutes, showing pronounced allelic dropout and locus loss. These figures provides a clear visual comparison of profile quality against the impact of severe thermal degradation on STR performance.

Discussion

DNA degradation under simulated forensic conditions

The findings of this study confirm that ultraviolet radiation, heat, and chemical exposure adversely affect DNA integrity through mechanisms commonly encountered in forensic casework. UV exposure resulted in reduced DNA yield and partial STR profiles, reflecting both direct photochemical damage to pyrimidine bases and indirect oxidative damage mediated by reactive oxygen species. These effects are well documented in the literature and are known to interfere with polymerase activity during amplification, thereby limiting the recovery of complete genetic profiles from environmentally exposed samples [12, 13, 14]. Similarly, thermal exposure produced variable DNA yields and allelic dropout, particularly at larger loci, suggesting a combination of impaired DNA repair capacity

and the accumulation of unrepaired lesions. This is consistent with previous reports indicating that heat stress disrupts key DNA damage response pathways, even where the direct induction of strand breaks remains contested [2, 15, 16].

Chemical treatment, particularly with bleach-based agents, did not lead to complete DNA loss, supporting earlier observations that DNA retained within biological fluids can exhibit a degree of resistance to chemical degradation [17]. In this study, chemically exposed saliva samples yielded interpretable STR profiles following ammonium acetate extraction, indicating that visible cleaning or chemical treatment does not necessarily eliminate forensic value. Comparable findings have been reported in studies examining sodium hypochlorite exposure, where DNA persistence was influenced by concentration and exposure time rather than the mere presence of the chemical agent [3, 18].

Effectiveness of ammonium acetate for degraded DNA extraction

Previous studies have shown that ammonium acetate yields high-quality DNA with minimal protein contamination. Studies [10] reported average DNA concentrations of 1.48 mg/mL from human saliva using a 10 M ammonium acetate protocol, while demonstrated recovery of high-molecular-weight DNA with consistent A260/280 ratios between 1.8 and 2.0 [9]. The mechanism underlying this efficiency lies in ammonium acetate's ability to form ionic complexes with proteins, oxidized metabolites, and secondary cellular by-products, which migrate to the organic-aqueous interface and are removed during extraction, thereby enhancing DNA purity [9].

In the present study, a modified 7.5 M ammonium acetate protocol was applied to saliva samples subjected to UV, thermal, and chemical (bleach) degradation. This molarity was selected to evaluate that 7.5 M ammonium acetate can achieve DNA yields comparable to commercial kits while reducing salt carryover and solubility issues associated with higher concentrations, which is critical for downstream STR profiling [19]. Across all degradation conditions, DNA was successfully recovered, demonstrating the robustness of the method even under extreme environmental stress.

Despite variability in purity metrics, ammonium acetate consistently enabled DNA recovery from all degraded samples. Persistently low A260/230 ratios indicated residual salt contamination, a known limitation of ammonium acetate-based protocols, which can be mitigated through additional ethanol wash steps without compromising yield [20,21]. Importantly, ammonium acetate's volatility facilitates its removal during drying, reducing the risk of PCR inhibition compared to stronger salts and chaotropic agents [20].

These findings demonstrate that 7.5 M ammonium acetate is an effective extraction reagent for degraded forensic samples, capable of recovering usable DNA even under adverse environmental conditions. Its low cost, simplicity, and reduced inhibitor carryover highlights its forensic applicability, particularly in resource-limited settings and cases involving compromised biological evidence [9].

Interpreting DNA profiling and statistical outcomes

STR profiling demonstrated that the 7.5 M ammonium acetate extraction protocol reliably recovered amplifiable DNA from saliva samples subjected to UV, thermal, and chemical degradation, supporting its applicability to compromised forensic evidence. While partial to near-complete profiles were obtained across all conditions, degradation type and severity markedly influenced locus recovery. Larger loci, including D21S11 and PENTA_D, were particularly susceptible to allelic dropout and imbalance, consistent with the known vulnerability of long amplicons in fragmented DNA [22, 23]. UV exposure produced inconsistent outcomes at equivalent exposure times, reflecting the stochastic distribution of photochemical damage [24]. Thermal degradation caused more progressive and severe locus loss, likely due to fragmentation and depurination processes associated with prolonged heat exposure [15, 16]. In contrast, chemically degraded samples often retain interpretable profiles, supporting evidence that intracellular DNA may be partially shielded from hypochlorite-mediated damage [17].

Quantitative and statistical analyses highlighted the limited value of spectrophotometric measurements as predictors of STR success. NanoDrop derived concentration and purity ratios showed poor correlation with profiling performance, with low-quantity samples often yielding robust profiles and higher-yield samples exhibiting dropout, reflecting the inability of spectrophotometry to distinguish amplifiable DNA from degraded or contaminated nucleic acids [21,25]. One-way ANOVA revealed significant differences in A260/280 ratios between degradation methods only at early exposure stages, indicating that initial degradation mechanisms influence apparent purity, whereas prolonged exposure leads to convergent damage regardless of degradation method [12,13]. Notably, thermal degradation produced severe STR deterioration without corresponding changes in NanoDrop purity, likely due to interference from heat-altered proteins [2, 16].

Collectively, these results underscore that assessments of degraded forensic DNA must prioritize STR performance over quantitative purity metrics alone, integrating statistical trends with functional profiling outcomes to support forensic interpretation.

Comparison with traditional methods and previous studies

When compared with conventional forensic DNA extraction techniques, the 7.5 M ammonium acetate protocol demonstrated several practical and analytical advantages for degraded saliva samples. Phenol-chloroform extraction, although effective for recovering high-molecular-weight DNA, is labour intensive, hazardous, and involves multiple transfer steps that increase the risk of contamination and further degradation, particularly in compromised samples [6, 7]. In contrast, ammonium acetate offers a simpler and safer workflow while maintaining comparable DNA yields, as reported previously [9, 26]. Chelex-based methods, while rapid and inexpensive, retain PCR inhibitors and generate predominantly single-stranded DNA, limiting their suitability for chemically degraded samples. The successful recovery of near-complete STR profiles from bleach-treated samples in this study highlights the inhibitor removal capacity of ammonium acetate relative to Chelex, consistent with previous findings [9]. Silica-based and magnetic bead methods generally provide high purity and improved recovery of short fragments but are associated with higher costs, making them less accessible for routine or large-scale forensic analysis [27].

The findings of this study are consistent with, and extend, previous research on ammonium acetate extraction. Studies [9, 10] demonstrated high yields and purity from fresh saliva and buccal samples using higher molarities (10 M), whereas the present study shows that a reduced concentration (7.5 M) remains effective for environmentally degraded samples while minimizing salt carryover, with more than one ethanol wash step recommended [19]. As reported [28] ammonium acetate consistently produced the highest yields from fresh saliva. However, storage time significantly reduced recovery after 12 months, yield fell to 0.26 µg, reflecting the impact of prolonged cellular degradation. This aligns with the current findings that, ammonium acetate recovered usable DNA from all degradation conditions but with reduced yields in some severe cases such as in UV and thermal. Chemically degraded samples in this study exhibited comparatively higher DNA yields, consistent with the results reported [17].

Conclusion

This study demonstrates that a 7.5 M ammonium acetate-based extraction protocol can reliably recover amplifiable DNA from saliva samples exposed to UV, thermal, and chemical degradation, addressing a key gap in forensic research focused on compromised, degraded evidence. Although DNA yield and STR profile completeness declined with increasing degradation, interpretable profiles were obtained across all conditions, confirming the forensic applicability of this low-cost method. Degradation-specific trends were observed, including UV-induced photochemical damage, heat-related fragmentation and protein denaturation, and partial cellular protection against hypochlorite in chemically treated samples [17, 24]. In addition, spectrophotometric measurements were poor predictors of STR success, supporting previous findings that NanoDrop metrics do not reliably reflect functional DNA integrity [21, 25].

The protocol's simplicity, safety, and accessibility makes it particularly suitable for resource-limited laboratories. However, residual salt contamination and reliance on spectrophotometric quantification, limited analytical robustness [20, 24]. Future studies should focus on degradation-specific optimization, integration of qPCR-based quality assessment, and validation using ammonium acetate extraction with targeted clean-up or bead-based enrichment strategies and casework samples. With further refinement, ammonium acetate extraction has strong potential for routine forensic analysis of degraded biological evidence.

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Ethical Clearance/Statement of Ethics(*Instruction to authors: Explicitly state the name of the ethics committee clearing the study, along with the date and number*)

Ethics reference UoL2025_12418

Committee UG/PGT University of Lincoln

Date of Ethical Opinion 4 April 2025

Declaration of conflicts of interest statement if applicable: Not applicable

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