Research Article

Indian Internet Journal of Forensic Medicine and Toxicology
Year 2026, Vol.24 (1)

Fabrication and Optimization of Paper-based Analytical Devices (Pads) for Preliminary Analysis of Sedative-hypnotics

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Submission: 7 May 2026 **Revision:** 17 June 2026

DOI: https://doi.org/10.48165/iijfmt.2026.24.01.04

ABSTRACT

Sedative-hypnotics are frequently encountered in drug-facilitated crimes and overdosage cases due to their rapid pharmacological action, amnesic effects, and ease of administration in beverages or food matrices. Conventional forensic screening techniques such as colour tests and thin-layer chromatography (TLC) remain widely used but comes with limitation including subjectivity, limited sensitivity, and infrastructure dependency. Paper-based analytical devices (PADs) have emerged as promising alternatives offering low-cost, portable, and field-deployable analytical platforms. In the present study, five PAD configurations were fabricated using wax imprinting to generate hydrophobic barriers and hydrophilic microchannels. The devices were systematically optimised by evaluating sample retention efficiency, solvent migration behaviour, reagent-sample interaction, and colour development uniformity. Comparative analysis revealed that channel geometry significantly influenced capillary flow dynamics and analytical performance. Among the tested designs, the PAD incorporating a central rectangular interaction chamber demonstrated superior solvent retention, enhanced colour stability, and improved reproducibility. These devices can be effectively used to detect barbiturates, benzodiazepines, z-drugs, etc. The reaction output can be further interpreted for screening and presumptive examination both in field as well as laboratory. The optimised PAD configuration provides a reliable platform for rapid presumptive screening of sedative-hypnotics and supports the integration of portable colorimetric analysis in forensic field investigations.

Keywords: Paper-based Analytical Devices (PADs); Forensic analysis; Barbiturates; Benzodiazepines; Optimization of PADs

INTRODUCTION

Paper as a Medium for Colorimetric Reaction

The application of paper in colorimetric reactions can be traced back in literature back to the work reported by Yagoda.[(1)] He reported the use of paper impregnated with suitable reagent as one of the earliest developments in the art of analytical chemistry. It can further be linked to as early as 23 to 79 AD, to the work reported by Pliny for detection of ferrous sulphate. The author had initiated the confinement of certain areas or zones to carry out colour tests, with reagents impregnated on paper. In 1949, Muller & Clegg developed a method for rapid and automatic examination of paper chromatograms. The idea of confined zones or channels was utilized to improve the diffusion process and work with smaller quantities. Cellulose-based paper acts as a porous microfluidic substrate enabling capillary-driven flow. [(2)]

Paper matrix offers various advantages. It supports reagent immobilisation and uniform colorimetric response. Provides high surface area and wicking properties for rapid analytical reactions. It enables low-cost, disposable, and field-deployable analytical platforms. The selection of paper substrate however should incorporate scientific reasoning and approach. [(3)]

Fabrication Method of PADs

Several techniques are being employed for fabrication of PADs. It includes inkjet printing, wax printing,

photolithography, laser treatment, and stamping. [4-7] They are chosen as per the requirement of the analyst or producer, depending on resolution, cost-effectiveness, and speed. Wax printing technique is generally used for mass production. However, there is a need for an approach which offers low-cost, scalable, and easy device production particularly focusing on forensic analysis context and low-scale production. The device should encompass the capacity to facilitate detection and further analysis specifically to commonly encountered analytes such as sedative-hypnotics, narcotic drugs and psychoactive substances, explosive residues, heavy metals,insecticides,etc.[8-9]

Optimisation of PAD Designs

Current studies specifically focus on detection of various analytes, but the emphasis upon the choice of paper, channel geometry, solvent migration, migration time, sample retention, and sample retention is very limited. The current work aims to optimise and fabricate PADs keeping the above-mentioned factors under key consideration. Different PAD geometries should be checked and optimized for attaining efficient analytical performance. Structural variations in hydrophilic channels and hydrophobic barriers can be fabricated and the designs can be evaluated based on selected parameters.

Forensic Applicability

Paper-based analytical devices have strong potential in forensic analysis, particularly for preliminary screening. It can be used both in field as well as laboratory set-up. With the mandatory visit and thorough scientific investigation of crimes under the new criminal laws in India, these types of analytical field-friendly platforms are quite suitable.

The current research aims at fabrication and optimisation of paper-based analytical devices. It involved selection of an appropriate cellulose substrate followed by wax imprinting to generate hydrophobic barriers and hydrophilic channels. Post-fabrication treatment for wax fixation and channel stabilization. Multiple PAD geometries were fabricated to investigate fluid transport behaviour and reaction efficiency. Each design was evaluated for sample retention, solvent migration time, and colour development uniformity following reagent interaction. Comparative optimisation was used for the identification of the most effective design, demonstrating balanced capillary flow and enhanced analytical performance suitable for forensic screening applications.

MATERIALS AND METHODS

The following materials were used in this study- Whatman filter paper #1 and #41, Wax sticks, Hot air oven, Methanol, Chloroform, Stationary items, etc.

Selection of Paper Substrate

Different cellulose-based paper substrates were preliminarily screened to identify a suitable medium for fabrication of paper-based analytical devices (PADs). Paper types were evaluated for their solvent wicking consistency, fibre integrity, and thermal stability. Heat resistance testing was conducted to assess the ability of the substrate to withstand thermal treatment required for hydrophobic barrier fixation. Paper samples were exposed to controlled heating conditions, and structural deformation, discoloration, and wax penetration behaviour were monitored. Flow behaviour of each paper type was examined through solvent wicking tests. A fixed volume of solvent was applied to the paper surface, and migration behaviour was visually observed and comparatively assessed for uniformity and reproducibility. Microscopic examination was performed to evaluate fibre distribution, pore uniformity, and surface morphology, which influence capillary-driven solvent transport. The selected substrate demonstrated consistent fibre distribution, stable pore architecture, reproducible solvent flow behaviour, and reliable hydrophilic channel formation, and was therefore chosen for device fabrication.

Fabrication of Paper-Based Analytical Devices (PADs)

PADs were fabricated using a wax imprinting technique to generate hydrophobic barriers and define hydrophilic channels. Wax patterns were painted onto the paper substrate using pre-designed templates to create structured analytical zones. Following wax deposition, thermal treatment was carried out using a hot air oven to facilitate wax penetration through the paper thickness. This process created well-defined hydrophobic barriers, enabling controlled solvent migration through designated hydrophilic channels. The fabrication method was designed to be cost-effective, reproducible, and scalable. Device preparation required minimal instrumentation and allowed rapid batch production of PADs suitable for analytical screening applications.

Optimisation of PAD Designs

Five distinct PAD geometries were designed and fabricated to optimise analytical performance. Structural variations were introduced in channel architecture, sample loading zones, and test zone configurations to evaluate solvent flow dynamics and reagent-sample interaction efficiency. Each PAD design was systematically evaluated based on flow efficiency, reaction uniformity, solvent migration rate, and sample retention capacity.

EVALUATION OF PAD DESIGNS

Sample Extract Retention

Sample loading zones of each PAD design were assessed

for extract retention capacity and leakage prevention. Controlled retention was considered essential for ensuring reproducible solvent migration and analytical consistency.

Sample Flow Towards Test Zone

Directional solvent transport from the sample zone to the test zone was evaluated by observing solvent migration behaviour across hydrophilic channels. Channel geometry and channel width were examined for their influence on solvent distribution uniformity.

Reagent–Sample Interaction

Reaction efficiency was assessed through observation of colour development within the test zones. The intensity and uniformity of colour formation were used as indicators of effective reagent-sample interaction. The influence of channel design and reagent positioning on reaction kinetics was comparatively studied.

Solvent Migration Time

Solvent migration time was measured by recording the duration required for solvent to travel from the sample loading zone to the test zones. Migration time was used as an indicator of analytical response speed and operational practicality.

Selection of Optimised PAD Design

The optimal PAD configuration was selected based on combined evaluation of solvent flow efficiency, sample retention performance, reaction uniformity, and colour reproducibility. The selected design demonstrated balanced fluid transport, stable reaction zones, and operational simplicity suitable for forensic analytical screening.

RESULTS

Paper Substrate Selection

Among the evaluated paper substrates, significant variation was observed in fibre morphology, solvent wicking behaviour, and heat tolerance. Certain substrates demonstrated irregular solvent migration due to heterogeneous pore distribution, while others exhibited structural deformation during thermal treatment. The selected paper substrate i.e. Whatman filter paper #41, exhibited uniform fibre distribution and consistent pore architecture, facilitating stable capillary-driven solvent transport. Heat resistance testing confirmed that the substrate maintained structural integrity during thermal barrier fixation. The chosen paper substrate also offered better retention, separation, and final reaction colour. These characteristics enabled reproducible hydrophilic channel formation and reliable analytical zone development, making it suitable for PAD fabrication.

Fabrication Performance of PADs

Wax imprinting followed by thermal treatment successfully generated hydrophobic barriers across all fabricated devices. Wax penetration through the paper matrix produced clearly defined hydrophilic channels without lateral leakage. The fabrication approach demonstrated high reproducibility and operational simplicity. The method required minimal equipment and allowed rapid device preparation, supporting its applicability in resource-limited forensic environments.

Optimisation of PAD Geometries

Five PAD geometries were comparatively evaluated to identify an optimised design capable of ensuring efficient fluid transport and uniform analytical response. Structural differences significantly influenced solvent migration dynamics and reaction behaviour. Comparative Evaluation of PAD Designs is given in Table: 1.

Table 1: Comparative depiction of optimization parameters across PAD designs

PAD Design	Channel Geometry	Multiplex Capability	Sample Retention	Flow Behaviour	Reaction Interaction	Migration Time	Overall Performance
PAD #1	Symmetrical Y-branch	High	Moderate	Uniform branching	Good	Moderate	Reliable multiplex distribution
PAD #2	Compact Y-branch	High	Moderate	Faster directional flow	Good	Moderate-Low	Improved flow efficiency
PAD #3	Parallel dumbbell dual channel	Moderate	Moderate	Independent channel flow	Moderate	Moderate	Suitable for replicate testing
PAD #4	Central interaction chamber dumbbell	Low	High	Stable solvent retention	Very Good	Moderate	Enhanced colour development
PAD #5	Short dumbbell	Low	Low-Moderate	Rapid migration	Moderate	Low	Fast screening capability

Selection of Optimised PAD Design

Among the evaluated geometries, PAD #4 demonstrated superior analytical performance. The central compartment structure provided effective sample retention, while the rectangular channel configuration enabled uniform solvent distribution towards the test zones. It exhibited consistent reagent–sample interaction, uniform colour development, and reproducible solvent migration behaviour. The design also demonstrated balanced analytical response time and operational stability, making it the most suitable configuration for forensic screening applications. The optimised PAD configuration enhances analytical reliability while maintaining simplicity and cost-effectiveness, supporting its potential application in rapid field-based forensic drug detection. It also demonstrates structural characteristics that directly support forensic field screening requirements. The incorporation of a central hydrophilic interaction chamber facilitates controlled fluid dispersion and extended analyte–reagent contact time, enabling efficient colour development even under low analyte concentration conditions. Such structural modulation enhances reaction stability and reduces signal

variability commonly observed in rapid flow channel designs.

DISCUSSION

Comparative evaluation of the five fabricated PAD configurations demonstrated that channel geometry significantly influenced solvent migration behaviour, sample retention, and reagent-sample interaction efficiency. Multiplex branching designs (PAD #1 and PAD #2) enabled simultaneous sample distribution to multiple test zones but exhibited minor variability in solvent flow due to channel resistance differences. Parallel dual-channel configuration (PAD #3) allowed replicate testing but showed moderate flow inconsistency between channels. Linear dumbbell design (PAD #5) facilitated rapid solvent transport; however, reduced interaction time occasionally resulted in lower colour development intensity. In contrast, PAD #4, incorporating a central rectangular interaction chamber, demonstrated optimal analytical performance by providing controlled solvent migration and enhanced analyte–reagent contact time. The expanded interaction zone improved solvent retention, stabilized colour formation, and minimized inter-trial variability. The results collectively indicate that fluidic modulation through strategic channel expansion can significantly enhance analytical reliability in paper-based microfluidic platforms. Based on these findings, PAD #4 was selected as the optimised configuration for subsequent forensic screening applications. Despite demonstrating improved analytical performance, the optimised PAD configuration exhibits certain limitations that require further investigation. The current optimisation was primarily based on qualitative visual assessment of colour intensity and solvent migration behaviour. Although replicate experiments indicated reproducible performance, quantitative statistical validation using digital image analysis or densitometric measurement was not incorporated in the present study. Additionally, the fabricated PADs were evaluated under controlled laboratory conditions using standard solvent systems. Environmental parameters such as temperature, humidity, and variable sample matrix composition may influence capillary flow behaviour and reaction kinetics in field applications. Variations in manual sample deposition may also introduce minor inconsistencies in solvent migration and colour development. Furthermore, long-term storage stability of wax-imprinted hydrophobic barriers and pre-loaded reagents was not evaluated. The durability of the fabricated devices under prolonged storage and transportation conditions remains an area requiring systematic study.

CONCLUSION

The development of optimized paper-based analytical devices represents a significant advancement in rapid forensic drug screening methodologies. The incorporation of a central interaction chamber in PAD #4 enhances analytical reliability by improving reagent-analyte contact efficiency and stabilizing colour formation, thereby reducing subjectivity associated with conventional presumptive tests. The simplified fabrication strategy and minimal reagent requirement enable cost-effective device production, making the platform particularly suitable for resource-limited forensic laboratories. From a practical standpoint, the optimized PAD design supports rapid on-site screening of suspected sedative-hypnotics in complex matrices such as beverages, biological fluids, and seized drug samples. The portability and ease of operation reduce dependence on sophisticated instrumentation during preliminary investigations and facilitate timely decision-making in forensic casework. Furthermore, the device architecture is compatible with digital image colorimetric analysis, enabling future integration with smartphone-assisted quantification and chemometric evaluation. Collectively, the developed PAD platform demonstrates strong potential as a reliable presumptive screening tool that can enhance efficiency, accessibility, and evidentiary value in forensic drug analysis. Future research may focus on integrating digital image colorimetry and smartphone-assisted analysis for quantitative evaluation of colour intensity, thereby enhancing analytical precision and objectivity. Validation studies involving diverse forensic matrices such as beverages, biological fluids, and seized drug extracts would further establish practical applicability of the optimised PAD design. Advanced fabrication approaches including automated wax printing or screen printing may improve reproducibility and enable large-scale production. Incorporation of multiplex reagent zones and integration with chemometric analysis could expand the analytical capability of PADs for multi-drug screening. Additionally, systematic evaluation of device stability, reagent shelf life, and environmental tolerance would support real-world forensic deployment. The development of standardized operating protocols and validation according to forensic analytical guidelines would further strengthen the evidentiary reliability of PAD-based screening platforms.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Execution, Data collection, Data interpretation, Manuscript writing – Rahul Das

Data interpretation, Reviewing, Supervision – Dr. Suhas

Ballal

SOURCE OF FUNDING

None.

ETHICAL CLEARANCE

Not applicable

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How to Cite this Article: Das R., Ballal S.. (2026). Fabrication and Optimization of Paper-based Analytical Devices (Pads) for Preliminary Analysis of Sedative-hypnotics. *IIJFMT*, ***24***(1), 16-20. https://doi.org/10.48165/iijfmt.2026.24.01.04