Paper-based Colorimetric Assay: Rapid Detection of HVA in Urine Samples

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Research Article

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Abstract

Diagnosing and monitoring of Parkinson's disease, schizophrenia, and certain mood disorders involves detecting homovanillic acid an essential aspect excreted through urine. In this study, we explored novel approaches for detecting homovanillic acid (HVA) in urine samples, aiming for cost-effective and on-site analysis methods. Traditional techniques like spectrophotometric determination and HPLC, while sensitive and selective, are often impractical due to their expense and lack of portability. Instead, we investigated the utility of paper-based analytical devices (PADs), which have gained popularity for various analytical applications including chemical element identification and environmental contamination assessment. We utilized different types of Whatmann filter papers (No. 1, No. 2, No. 4) and chromatography paper as substrates for the PADs, and employed combination of potassium ferrocyanide and ferric chloride reagent for HVA detection. Our experiments revealed positive results, indicating the efficacy of this approach. Optimization experiments identified 30 minutes to 3 hours as the optimal immersion times, with a subsequent 30-minute drying period at room temperature. Notably, variations in performance were observed among the different filter papers, with Whatmann CF6 paper exhibiting significant differences compared to the others in terms of HVA detection efficiency.

1. INTRODUCTION

Homovanillic acid (HVA) monitoring is often done in clinical settings to assess dopamine metabolism. It's particularly useful in conditions where dopamine levels are implicated, such as Parkinson's disease, schizophrenia, and certain mood disorders [1]. In medical practice, measuring HVA levels can provide insights into the activity of dopamine-producing neurons in the brain. Abnormal levels of HVA may indicate dysfunction in dopamine regulation, which can help clinicians in diagnosing and managing various neurological and psychiatric conditions. Many HPLC methods, utilizing diverse sample extraction methods alongside analytical columns and either electrochemical or fluorimetric detection, have been developed. HPLC coupled with electrochemical detection is the preferred approach for detecting HVA due to its high sensitivity. However, this method is relatively nonspecific and can produce complex chromatograms if isolation steps are omitted. Therefore, most HPLC techniques using electrochemical detection to identify biogenic amines first separate interfering compounds using extraction columns. Though time-consuming, these procedures could provide significant advantages in routine clinical investigations.

Various analytical methods, such as spectrophotometric determination [2] and high-performance liquid chromatography (HPLC) [3], are commonly employed to detect homovanillic acid (HVA). However, despite their precision and sensitivity, these techniques necessitate expensive equipment and are impractical for on-site analysis. To address this limitation, there's a growing interest in developing indicator strips or optical sensor membranes for detecting chemical substances in herbal medicine. Some researchers opt for polymers as substrate materials for this purpose [4, 5]. Paper-based analytical devices (PADs) have recently emerged as a straightforward, cost-efficient, and user-friendly tool for environmental and biological analyses [6–8]. They have been successfully applied for on-site analysis of

organic molecules [9], metals [6], and pesticides[10]. A prevalent detection method in PADs involves a colorimetric approach, utilizing specific reagents to identify and detect analytes in samples [11–13].

In this study, we opted combination of Potassium Ferric cyanide + Ferric chloride as the colorimetric reagent for detecting HVA. Potassium Ferric cyanide + Ferric chloride was chosen as metal ions forms complex with HVA compound, making it a suitable colorimetric reagent. We screened the reagent and applied it to various types of Whatman filter papers, including Whatman No. 1, No. 4, and No. 6 [14]. The selectivity, sensitivity, and practicality of this paper-based analytical device (PAD) for HVA detection were also evaluated. The results indicate that this PAD is effective for detecting HVA in urine samples.

2. MATERIAL AND METHODS

All chemicals utilized were of analytical grade and were employed without additional purification. HVA was obtained from Sigma Aldrich Pvt. Ltd. Potassium ferro cyanide was purchased from Himedia whereas Ferric chloride was purchased from Lobachemie. Different grades of whatmann papers were purchased from whatmann grade 1 (whatmann), Whatmann CF4 (Cytiva), Whatmann CF6 (Cytiva) respectively. The absorbance was measured using a UV-Visible spectrophotometer model 1900 Shimadzu, utilizing a 1.0 cm quartz cell.

2.1 Reagent preparation

Weighed quantity of Potassium Ferric cyanide 24 mg + Ferric chloride 257 mg was dissolved in 10 ml of distilled water and stirred completely at room temperature.

2.2 Design and Optimization of Specific Reagent Tests on Paper

Different grade whatmann 1, CF4, CF6 filter paper (fig 18) were cut into 1cm*5cm size

⇩

Stock solution of Pot. Ferric cyanide 24 mg in 10 ml + Ferric chloride 257 mg into 10 ml of distilled water was prepared

⇩

Soak the cut whatmann paper for 30, 60, 120, 180 mins

⇩

Allow whatmann paper to dry at R.T for further use

2.3 Optimization and selection of dip strip base for qualitative detection of HVA

At different time interval (i.e 30, 60, 120, 180 mins) the different grade of Whatmann filter paper grade A: Whatmann No 1, B: Whatmann CF4, C: Whatmann CF6 strips were soaked in reagent reaction mixture and dried at R.T. The developed strip for HVA were dipped in 100 µg/ml of standard HVA sol. and time required for color development were recorded.

2.4 Qualitative detection by dip strip at different HVA concentrations

The optimized strip for HVA were treated with different concentration of HVA (25, 10, 1 mg/ml) and (1 µg/ml) for determining the minimum concentration to be detected by developed dip strip for HVA.

2.5 Real time analysis by dip strip in urine sample

Before analysis the urine samples were extracted 0.1 M perchloric acid and then dip strip was dipped into the sample then any color change on strip was observed for qualitative detection of HVA.

2.6 Prototype design of dip strip

Based on the earlier studies reported the material to be used was selected [15]. From point of care diagnosis approach the design of the strip was done as dip strip from point of ease of use, qualitative detection and economical use.

2.7 Concentration dependent color codes

From stock solution of standard HVA (10 mg in 10 ml) different dilutions of HVA were prepared from 10 µg/ml to 1 mg/ml and treated with reaction mixture to identify the detection limit of HVA concentration with developed strip.

2.8 Validation of dip strip

Stability testing of strip was performed at 1, 2, 3 months to assess the stability of dip strip to produce the color change when dipped in HVA solution.

Human ethical permission

- For human pilot study the proposal was submitted to Institutional Ethics committee at Dr. D. Y. Patil Homoeopathic Medical College and Research Center, Pimpri, Pune 411018.
- The proposal was sanctioned by IEC protocol no DYPHMCRC/E-04/2023, dated 30/4/2023.
- The enrolment of participants was done by taking their oral informed consent from patient by the neurologist and guiding them about the risks, benefits, awareness, and purpose of study experiment procedure.
- The study was performed in accordance with relevant guidelines and regulations as suggested by the ethics committee.

4. RESULT AND DISCUSSION

3.1 Design and Optimization of dip strip for qualitative detection of HVA in urine sample paper

In this study, Whatman filter papers No. 4 and No. 6, along with Whatman chromatography paper No. 1, were employed. Whatman No. 4 filter paper features pore sizes ranging from 20 to 25 μm and exhibits rapid capillary action. Whatman chromatography paper No. 1 is widely used for chromatography analysis, while Whatman chromatography paper No. 6, with its pore size of 3 μm, is specifically utilized for water analysis. Figure 1 illustrates the color change of Whatman filter paper in response to varying immersion times in the presence of HVA.

Whatmann filter paper grade A: Whatmann No 1, B: Whatmann CF4, C: Whatmann CF6

The outcome demonstrates that 30 mins is the ideal immersion time for strips reaction mixture pot. ferrocyanide + ferric chloride since the color intensity is higher during this period. Particμlarly the pore size, every Whatman fiłter paper is unique. All 3 Whatmann paper no 1, CF4, CF6 were able to absorb HVA on paper, absorb chemicals in essentially the same ways. The ideal drying period was completed at room temperature. Approximately one hour is required for the paper to dry completely at room temperature thus used as drying time for the paper utilized. Figure 2 depicts the paper-based analytical device's design. This design's material is simple and cost-effective. This pattern was created for every filter paper.

3.2 Performance of Paper-Based Analytical Device

To perform the sensitivity test for each paper strip prepared, we determined the minimum detectable concentration of HVA that the PAD can identify. The varying concentrations of HVA was weighed. In the PAD, (25, 10, 1 mg/ml, 1 μg/ml) of HVA was added. It was noted that the presence of HVA caused a color shift in the PAD. Whatmann CF6 paper changes color quickly when it is submerged in an HVA solution. Significant change in color was absorbed as per concentration. According to the sensitivity data, the lowest concentration that may be measured is 1mg/ml because at this concentration reagent still exhibit the proper color changes; at concentrations below this, the reaction mixture pot. ferrocyanide + ferric chloride does not produce any discoloration.

A selectivity test was carried out to ascertain the ability of the paper-based analytical device (PAD) to detect HVA. The test was conducted by dipping PAD into a HVA solution added in artificial urine. Figure 3 demonstrates that the reaction mixture pot. ferrocyanide + ferric chloride showed change in color when placed on a PAD that had been dipped in artificial urine. The reaction mixture pot. ferrocyanide + ferric chloride produces a green to blue color change on PAD dipped in HVA. This data shows that PAD is selective for detecting HVA because it reacts differently with other interfering chemicals, resμlting in a different color change.

3.3 Real time application of developed dipstrip for HVA in urine sample

The real time application of this established qualitative & quantitative method was performed by analysis in human urine. The fasting urine sample of human was collected for qualitative analysis by developed strip and quantitative analysis by UV spectroscopy was performed. The results obtained by UV method was found to be of significant difference in levels for biogenic amines & its metabolites concentration when normal group was compared with PD, AD, Depression patients, whereas non significant difference in results obtained by UV method & developed strip for HVA (Fig. 4,5).

3.4 Concentration based color codes

Qualitative detection of HVA was possible based on color intensity the concentration can be depicted in Table 1.

3.5 Stability of paper-based analytical device's (PAD)

The stability of paper strip was checked at 1, 2, 3 months for reproducibility of detection of HVA in urine sample. The detection of HVA by paper strip was possible upto 3 months (Fig. 6) as the pot. ferrocyanide + ferric chloride present on paper base was possible to change color from green to blue. After 3 months the pot. ferrocyanide + ferric chloride did not show any color change.

5. CONCLUSION

The combination of potassium ferrocyanide and ferric chloride was chosen for HVA detection and subsequently applied to the PAD. The PAD exhibited favorable sensitivity and selectivity towards 5-HIAA. Moreover, the developed PAD successfully detected HVA in urine samples, corroborating the UV data. This method offers simplicity, rapidity, instrument-free operation, and selectivity for on-site HVA analysis in urine samples.

Declarations

CONFLICT OF INTEREST

NIL.

FUNDING SOURCE

Self-funded.

Author Contribution

Vrushali Bhalchim: literature review, conceptualization, experimental work, manuscript draftingVaishali Undale: Conceptualization, Critical reviewsSunil Shewale: Manuscript drafting and analysis

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Data Availability

All data generated or analysed during this study are included in this published article. Yes, the data is available for supporting the research findings in the previously reported studies for determination of catecholamines and their metabolites in urine samples by HPLC (ref no 3). The concept of PAD for qualitative determination has been developed earlier for "Design and optimization of colorimetric paper-based analytical device for rapid detection of allopurinol in herbal medicine" (ref no 14). The present research work is novel as there are no reported studies available on development of PAD for determination of catecholamine and metabolites.

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Table

Table 1 is available in the Supplementary Files section.

Figures

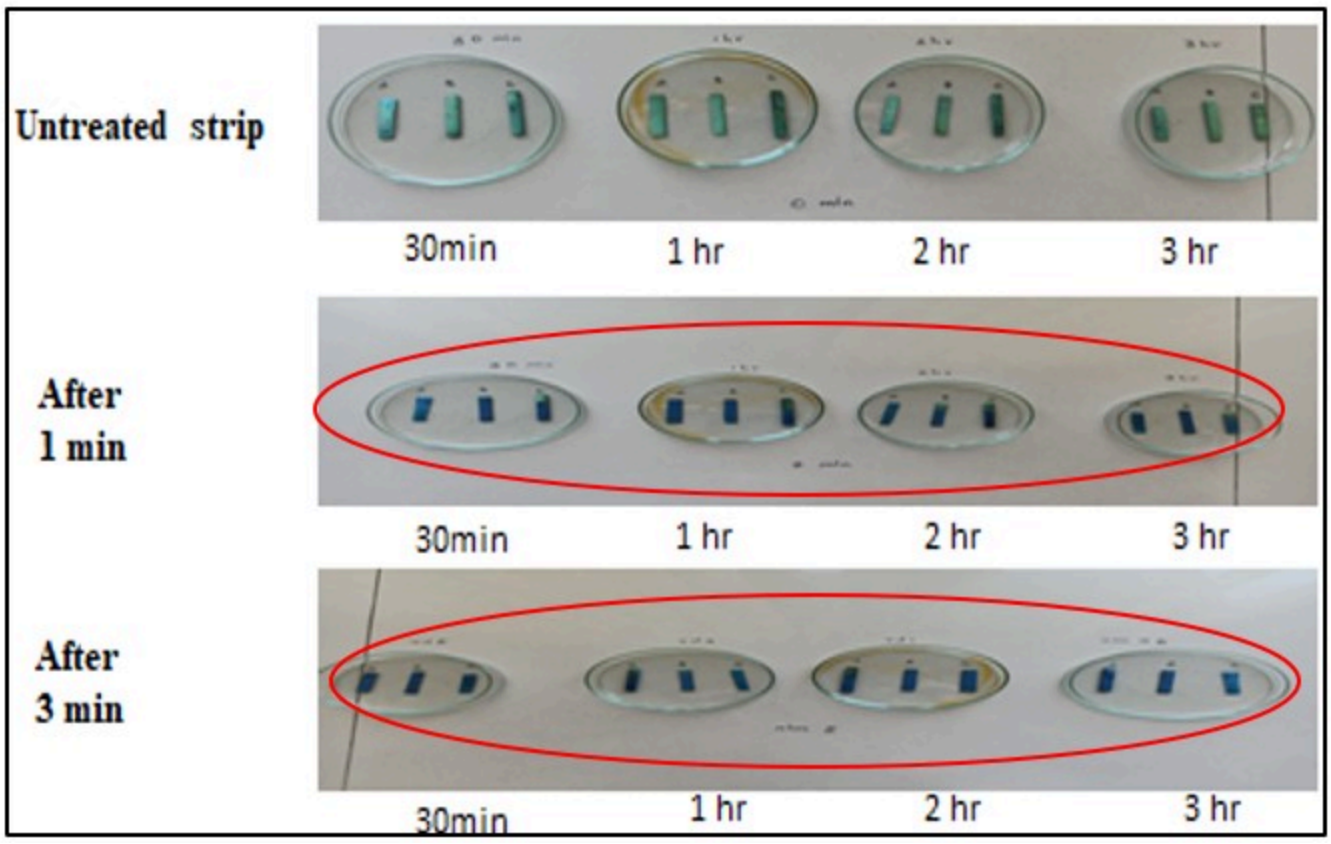

Figure 1

The whatmann paper pieces were soaked into reaction mixture for 30 min, 1hr, 2hr, 3hr better results were obtained after soaking paper for 30 mins in mixture and air dried.

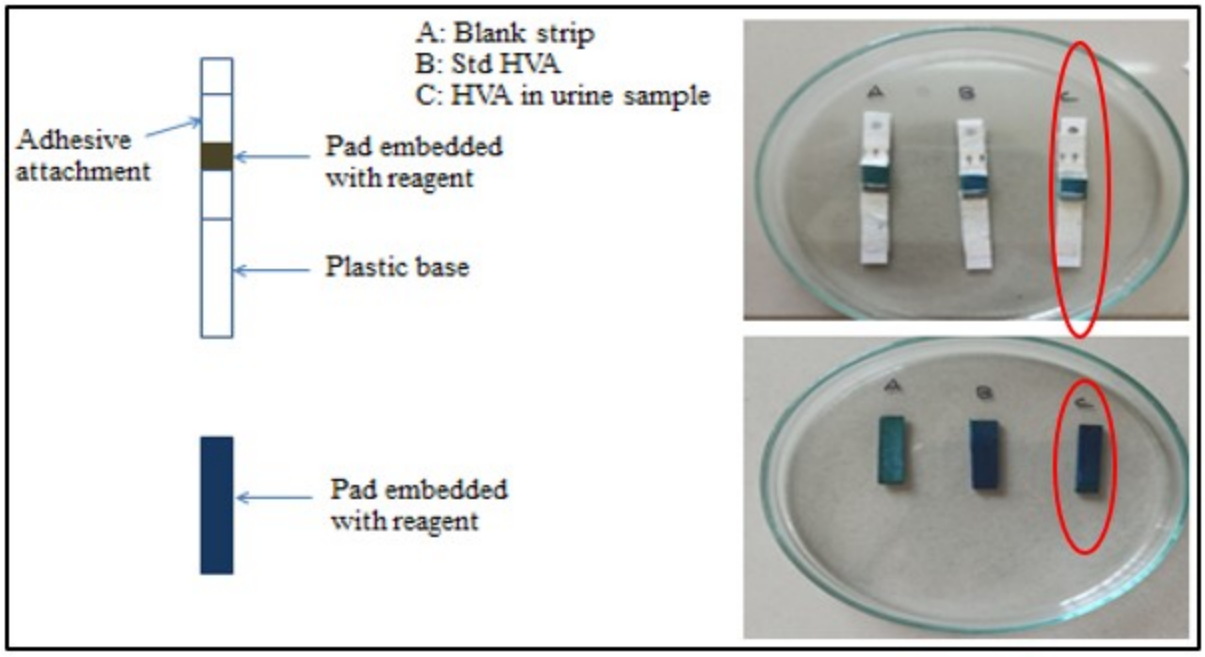

Figure 2

Design of paper-based analytical device

Figure 3

The developed dip strip were then dipped into urine samples time required to developed color was 1 min.

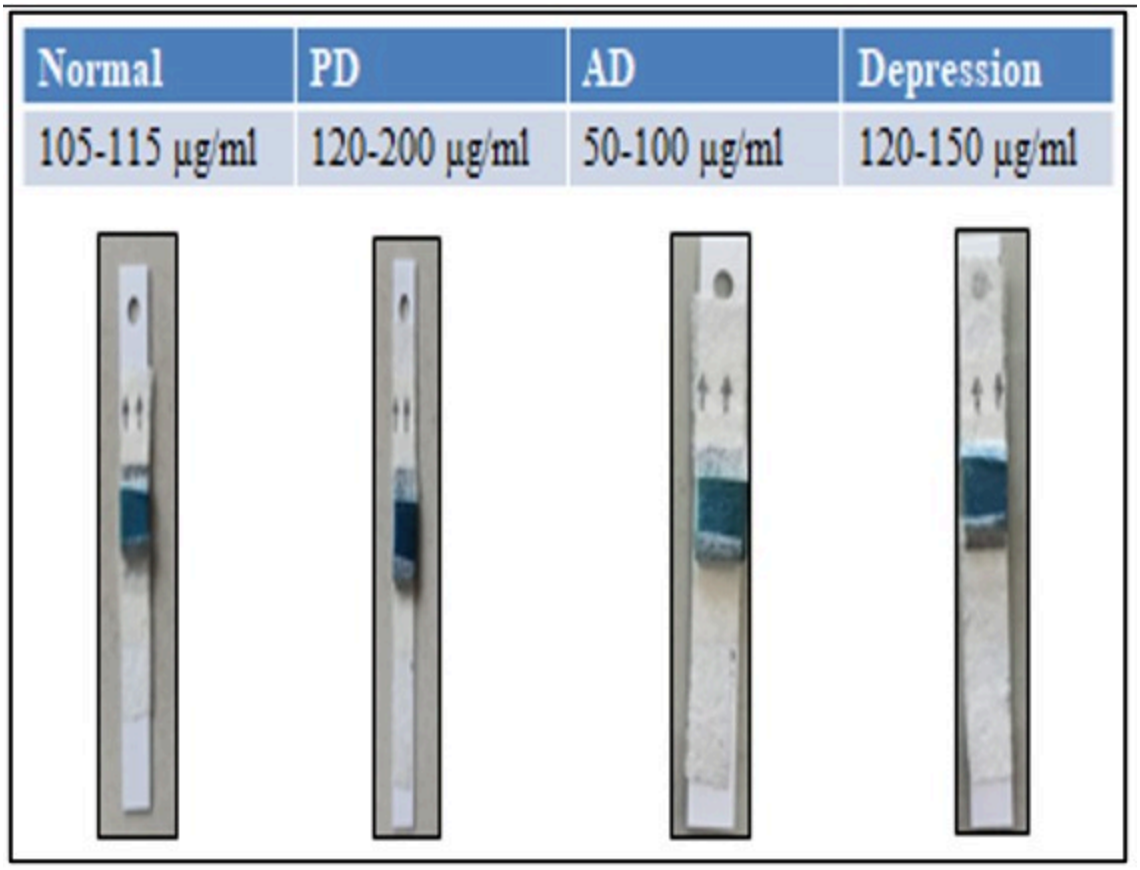

Figure 4

Qualitative detection of HVA level in human urine by dip strip

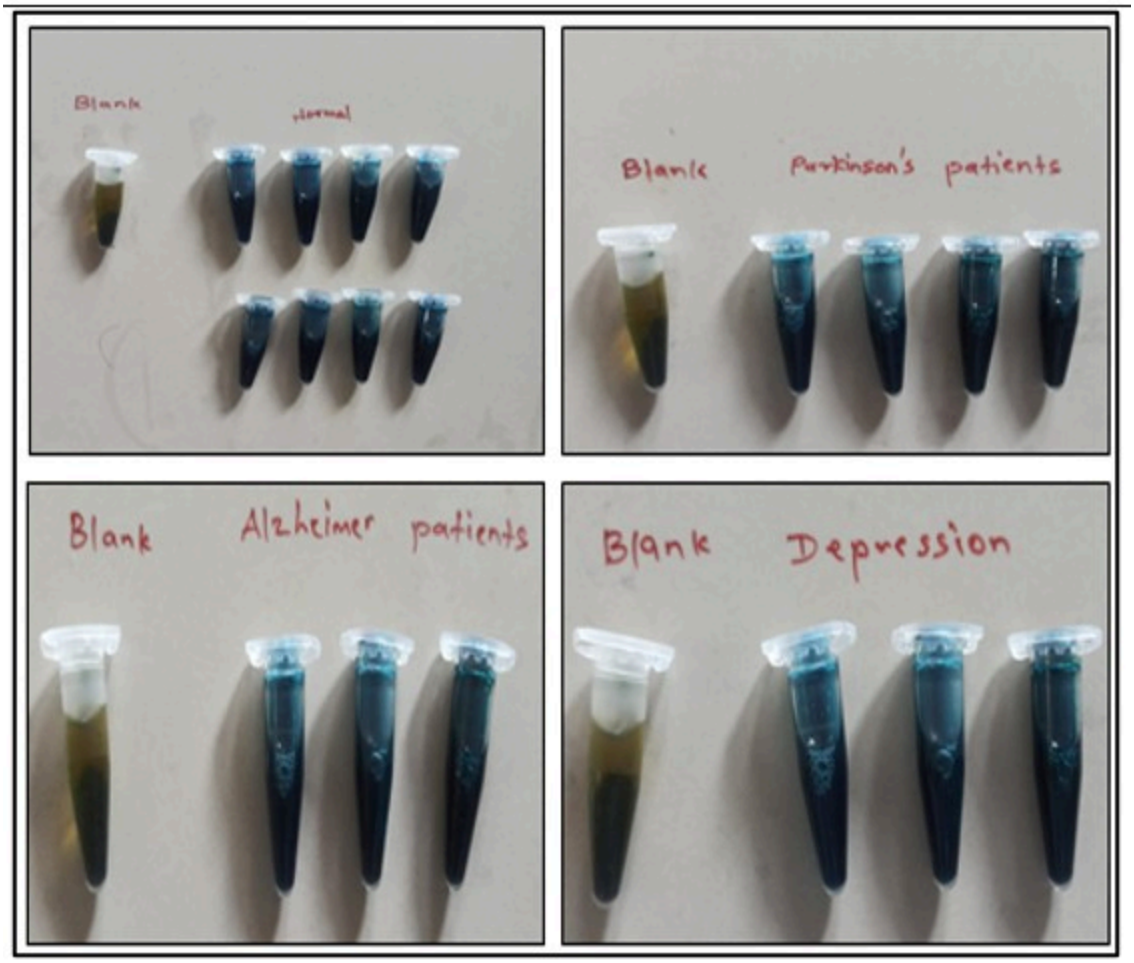

Figure 5

Qualitative detection of HVA level in human urine by UV method

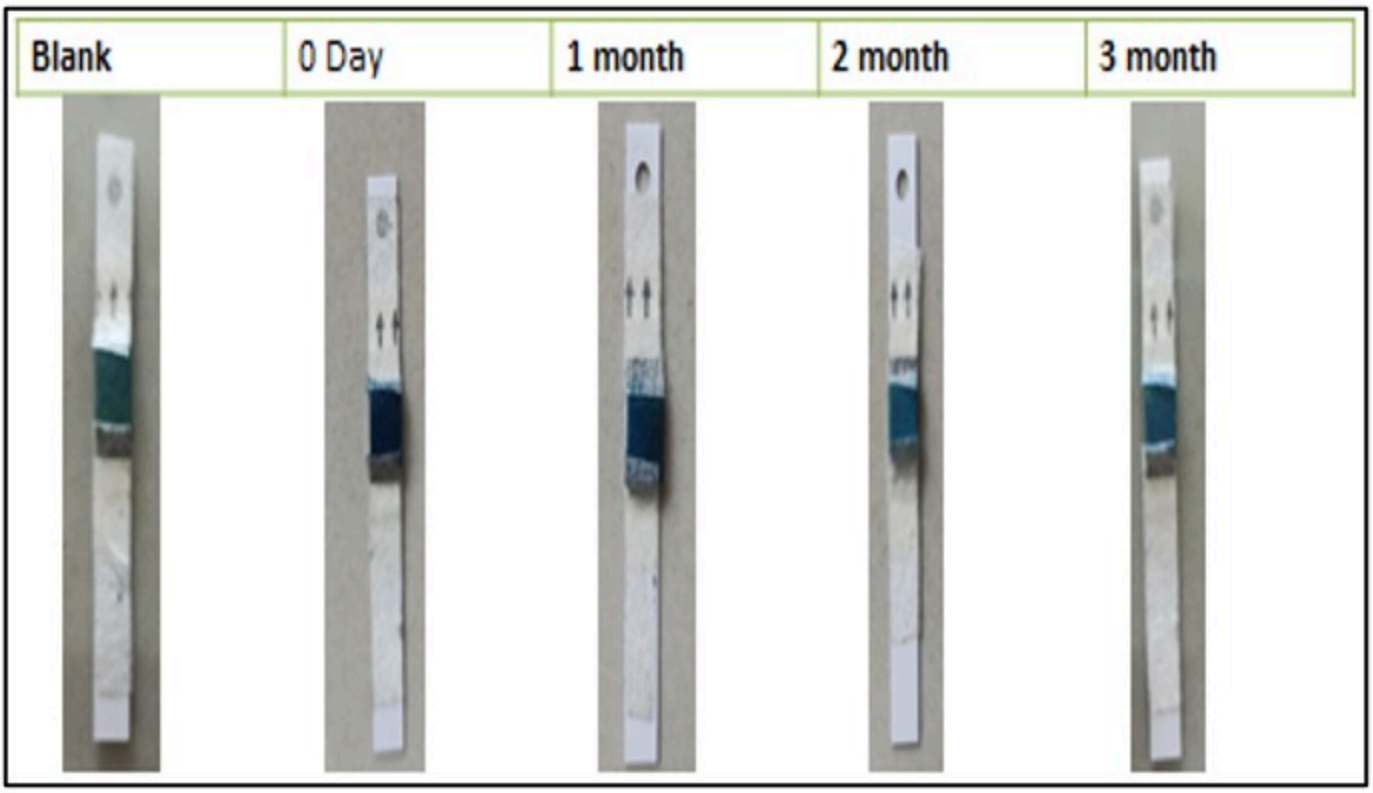

Figure 6

Stability testing of paper-based analytical device's (PAD)

Supplementary Files

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- table.docx