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A Novel Method for Forensic Examination of Bribery Cases Using Alpha-Naphtholphthalein

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ABSTRACT

Background: Corruption is one of the major issues of India and other third world countries. Anti-corruption Bureau, detective agencies and various Forensic Science Laboratories make use of indicator dyes/detective dyes, such as, Phenolphthalein and Anthracene for catching a person involved in taking bribe, red-handed. This study aims to solve the issue of fading out of colour and fluorescence due to extraneous substances, often encountered in case of traditional dyes. To overcome the limitations faced in case of traditional dyes, a new and innovative approach by using α-naphtholphthalein [3,3-Bis(4-hydroxynaphthalen-1-yl)-2-benzofuran-1(3H)-one] has been developed. **Results**: The sensitivity of the method was evaluated and limit of detection (LOD) and limit of quantification (LOQ) were found to be 243.2005 µg/mL or ppm and 736.9712 µg/mL or ppm respectively, with a regression coefficient of 0.987. **Conclusion:** Two pathways have been suggested in this study – Aerosol spray method and the traditional wash-solution method. The resulting wash solution and the turquoise blue coloured solution have been analysed using Acid-Alkali test, pH test, Thin Layer Chromatography (TLC), Colorimetry and UV Spectrophotometry.

Keywords: Trap case, Detective dye, Corruption.

1. Introduction

Corruption has plagued mankind and civilization throughout history. It can be found in various forms and at multiple levels in the society. Corruption encompasses personal gain at the cost of a breach in ethics and integrity (Jain 2001; Bahoo et al. 2020; Shapovalov 2022). Furthermore, the trust towards governing bodies gets hampered to a certain extent. International initiatives and conventions have been established as a result of efforts to eliminate corruption, which have attracted global attention. A foundation for international cooperation in preventing and

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combating corruption is provided by the United Nations Convention against Corruption (UNCAC). Leading anti-corruption organisation Transparency International evaluates and ranks nations based on perceived levels of corruption, offering insightful comparisons (Sharshova 2021; Teremetskyi et al. 2021; Dewantara et al. 2021).

India has implemented institutional reforms and legislative measures to combat corruption in recent years. The Lokpal and other anti-corruption organisations were created with the intention of looking into and punishing corrupt behaviour. The Right to Information Act's implementation has improved the openness and accountability of government operations (Topchii et al. 2021; Hock 2021; Schoultz et al. 2021).

Forensic Chemistry acts as a tool in the scientific examination of these cases. The application of chemical techniques and principles to aid in legal arena has certainly aided in the examination of corruption cases. Trap cases, sometimes known as anti-corruption cases, are the deliberate employment of undercover operations to identify and capture people involved in corrupt activities. In these situations, law enforcement or anti-corruption organisations send out undercover agents who pretend to be eager participants in corrupt operations, like giving bribes or carrying out fraudulent transactions. Trap cases are used to gather information, elicit admissions, and finally prosecute corrupt individuals by exposing secret networks, discouraging future criminals, and bolstering the rule of law, such operations are essential in the battle against corruption.

The conventional methods used to administer a trap case encompasses the use of Phenolphthalein and Anthracene. They act as colour changing dye and has eminent importance. However, there are a set of short-comings faced with these dyes (Ekici et al. 2021; McGee et al. 2023; Verma & Pramanik 2019). As a result, many trap cases has failed to convict the suspected bribe-taker due to a number of procedural flaws and legal difficulties. One of the issues associated with the use of this ancient dye is the decolorization of pink coloured solution from hand washings of the accused during trapping (Yadav & Goutam 2014; Supreme Court of India 2010; Hindustan 2015). The current study was carried out to address the limitations. In this study, we discuss a novel and effective technique for its application in the examination of trap cases under the realm of Forensic Chemistry.

2. Methods

The chemicals used in this study consisted of: α-naphtholphthalein (Loba Chemie Pvt Ltd), Sodium Carbonate Anhydrous (Nice Chemicals Pvt Ltd), Distilled water, Aerosol sprayer, Feather brush, TLC Silica gel 60 F254 plates from Merck KGaA Germany, Absolute Ethanol (Changshu Hongsheng Fine Chemical Co. Ltd.), Colorimeter (Electronics India - 312) and UV-Vis Spectrophotometer (Electronics India - 2373).The preliminary tests in this research study were performed on cellulose-based paper of 2.5 inch × 2.5 inch and on bare hand of one of the authors. Currency note was not used in this study to prevent any form of damage to the currency note and follow Section 489 (Devadas 2016) and Section 3 (Jayakumar 2011) of the Indian Penal Code and the Prevention of Damage to Public Property Act, 1984 respectively.

To maintain reproducibility, the study was performed by the authors by simulating a trap case. Two alternative pathways are suggested to detect the dye – Aerosol spray method and wash-solution method.

In this study, the human subject was one of the authors, and the study was conducted maintaining the ethical standards. The dye was transferred on the palm region and was immediately washed off, after noting down the result. The study was conducted effectively from December 2022 till July 2023.

2.1 Aerosol spray method

α-naphtholphthalein, was smeared over a piece of paper using a fine feather brush (9" Plume and 20" Overall). An alkaline solution of 1% Na_2CO_3 was then sprayed over the paper, maintaining a distance of 15 centimetres. Similar steps were followed for the examination on bare hand.

The paper was further collected and kept at ideal room temperature. As per Locard's Principle of Exchange (Mistek et al. 2018), the powder got transferred to the hand of the subject. The alkaline solution was then sprayed evenly over the hand, maintaining a distance of about 15 centimetres.

2.2 Wash solution method

A paper, portraying a currency note was smeared with α-naphtholphthalein. The paper was collected with bare hand by one of the authors and kept in his custody. Distilled water was poured on the hand and the wash-solution was collected in a beaker. About 3-4 drops of 1% Na_2CO_3 solution was then added into the beaker.
To preserve and present the evidences in the court of law, documentation in the form of Photography and Videography of the exhibits are usually carried out. In this case, documentation of the Turquoise blue colour of the paper, hand and wash-solution were carried out.

2.3 Analysis

The wash solution was further analysed to establish the presence of α-naphtholphthalein in the sample. The detection of the analyte was carried out through Acid-alkali test, pH test, Thin Layer Chromatography, Colorimetry and UV-Vis Spectroscopy. The analyte, α-naphtholphthalein was extracted from the wash solution using solvent extraction method. To the wash solution, 5 ml of Ethanol was added and was left undisturbed for 6 hours. The ethanolic extract was used for further analysis. The choice of solvent was made based on the solubility of α-naphtholphthalein.
The acid-alkali test was performed using alkaline 1% Na_2CO_3 solution and HCl. To about 2-3 drops of the extract, a drop of the alkaline solution was added, which was followed by the addition of 2 drops of HCl. To the same solution, a drop of the alkaline solution was added over again. To check repeatability, the test was conducted in triplicate. The pH of the wash solution and the turquoise blue alkaline solution was measured using pH paper and pH meter.

The extract obtained after solvent extraction was subjected to Thin Layer Chromatography using readymade silica gel 60 F254 plate of 10 cm × 2 cm size as the stationary phase and three different solvent systems were selected based upon Stahl's Triangle as the mobile phase. Table 1 depicts the different types of solvent systems used. The chambers were allowed to saturate with the vapours from the solvent system and the samples were run for about 8 minutes. The developed plates were then visualised using direct visualisation method and the Rf values were calculated.

Table 1: Solvent systems used for TLC

Sl. No.	Solvent system	Ratio
1	Ethyl Acetate: Acetone	70:30
2	Chloroform: Acetone	70:30
3	Ethyl Acetate: Water	80:20

A simulated case approach was opted as a reference for performing colorimetry. A minimum amount of the powder (0.01 g) was taken in 10 ml of distilled water, followed by the addition of ethanol for extraction of the analyte. To the extract, about 1 ml of an alkaline solution of 1% Na_2CO_3 was added, which resulted to the formation of an oxidized form of α-naphtholphthalein, which appears turquoise blue in colour. One part of the blue coloured solution and three parts of Ethanol were mixed in a cuvette to dilute the dark coloured liquid with very high intensity. Measurement of absorbance values under different wavelengths in the visible range was further carried out. Graphical representations of the same were obtained using MS Office suite.

A standard stock solution of α-naphtholphthalein of 2000 µg/mL or 2000 ppm was prepared by dissolving 50 mg of α-naphtholphthalein in 25 mL of Ethanol (EtOH). From the standard stock solution, a reference stock solution was prepared by mixing 5 mL of stock solution and diluting it up to 10 mL with EtOH, to obtain 1000 µg/mL or 1000 ppm reference stock solution. The reference solution of α-naphtholphthalein (1000 µg/mL) was scanned in the wavelength region of 400 - 600 nm at an interval of 1 second. Five working solutions of varying concentrations – 200 µg/mL or 200 ppm; 400 µg/mL or 400 ppm; 600 µg/mL or 600 ppm; 800 µg/mL or 800 ppm; and 1000 µg/mL or 1000 ppm, were further prepared by using dilution method. These solutions were subjected to linearity test as per ICH Q2 (R1) guidelines. The absorbance values of these solutions were measured using EtOH as blank and the obtained data was used for plotting the calibration curve to determine the linear range.

LOD and LOQ for the assay were calculated by using the following formula (Uhrovčík, J. 2014).
LOD = 3.3 × (standard deviation of the y-intercept of the regression line/slope of the calibration curve)
LOQ = 10 × (standard deviation of the y-intercept of the regression line/slope of the calibration curve)

The data of absorbance obtained at different concentrations (Absorbance vs Concentration) were plotted using MS Office Suite and the standard equation and Regression Coefficient (R^2 value) were determined.

3. Results

3.1 Wash solution and Aerosol spray study

The results showed the formation of a turquoise blue colour on the hand as well as paper. This change in colour is due to the oxidation of α-naphtholphthalein in alkaline medium. The resultant blue solution was kept for over 8 months in room temperature without any fading out of colour, as is seen in case of phenolphthalein.

3.2 Acid-alkali test and pH study

The blue colour changes into colourless on addition of acid i.e. HCl and attains back the blue colour on further addition of the alkaline solution, as shown in Figure 1. The pH was found to be 7 for the wash solution and 10 for the turquoise blue solution.

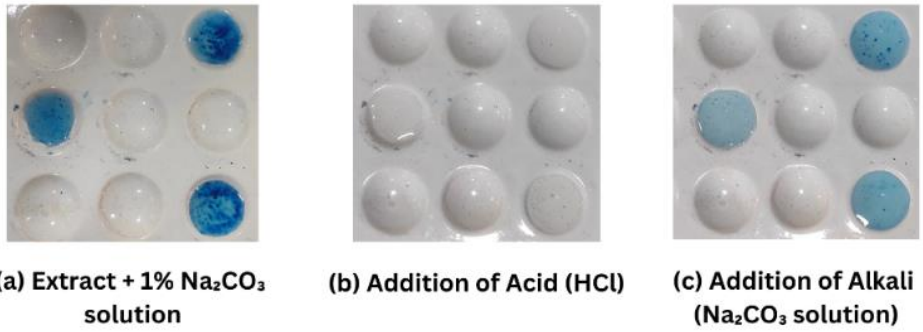

Figure 1: Acid – alkali test

3.3 Thin Layer Chromatography

Crimson-red spots were formed on the plate, which were visualised using direct visualisation. The Rf values for all the three systems were found to be 0.89, 0.86 and 0.90 respectively, as given in Table 2. These values can be used for identification of the analyte from the wash solution in trap case examination.

Table 2: Result obtained after TLC

Sl. No.	Solvent System	Rf
1	Ethyl Acetate: Acetone	0.89
2	Chloroform: Acetone	0.86
3	Ethyl Acetate: Water	0.90

3.4 Colorimetry

The H-atoms from -OH functional groups of α-naphtholphthalein, reacts with the available OH^- ions from the alkali added, and forms C=O and COOH groups. The conjugated and unsaturated structure containing carbonyl and carboxylic acid functional groups which acts as chromophores, produces a turquoise-blue coloured solution which is used for colorimetric analysis. Readings of the Absorbance values for multiple wavelengths obtained, are listed in Table 3. It shows that the maximum absorbance took place at 520 nm under green light. Figure 2 and 3 depicts the graphical representation of Absorbance values and wavelengths in the visible range.

Table 3: Table showing the results obtained after Colorimetry

Sl. No.	Wavelength (nm)	Colour	Absorbance
1	400	Violet	1.27
2	450	Blue	1.16
3	490	Greenish blue	1.69
4	520	Green	1.76
5	540	Yellowish green	1.15
6	570	Yellow	1.16
7	620	Orange	1.01
8	680	Red	0.96

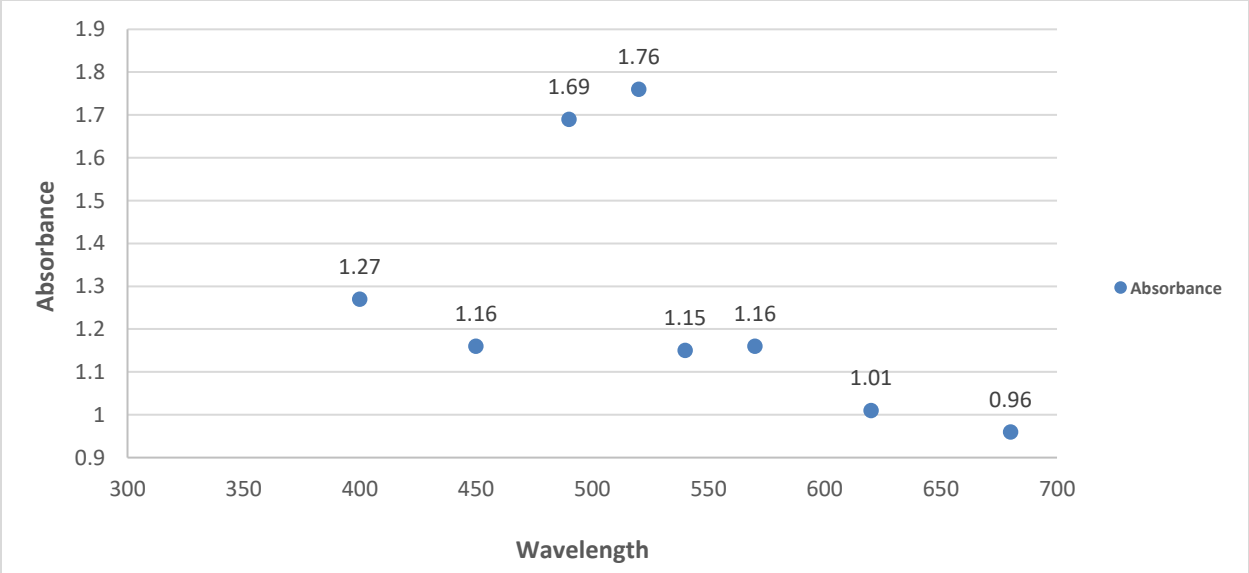

Figure 2: Scatter plot of Wavelength vs Absorbance

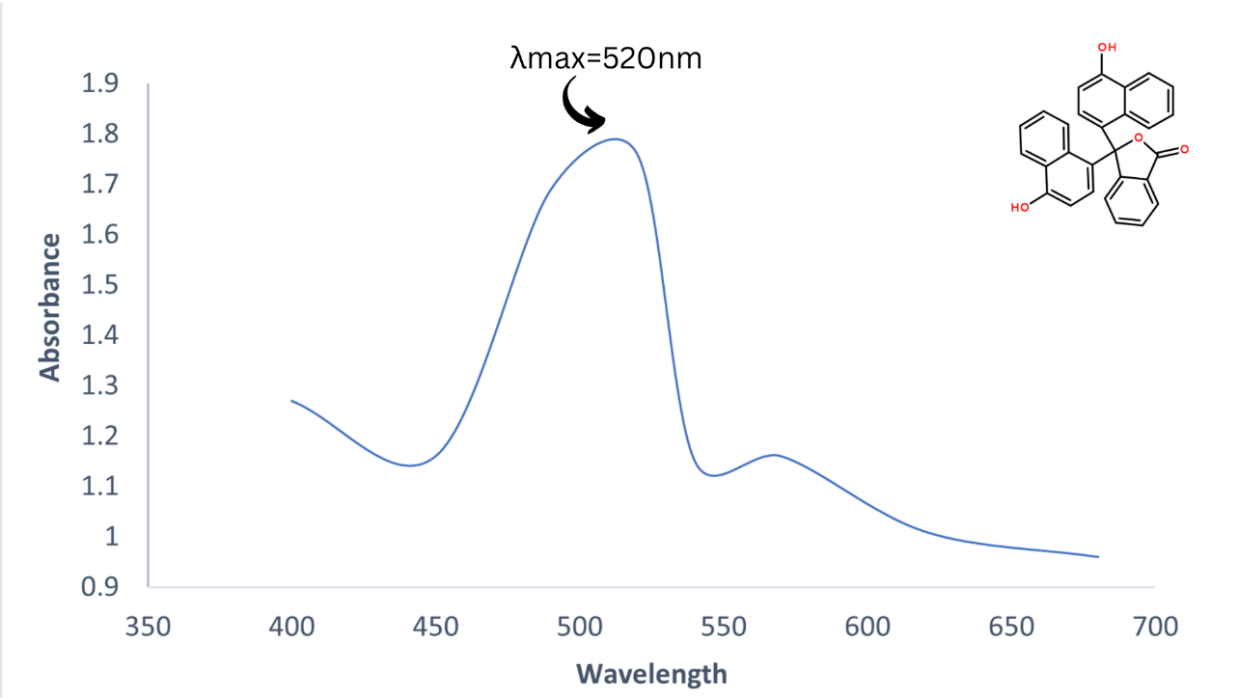

Figure 3: Spectrum generated via dataset from Colorimetry of α-naphtholphthalein

3.5 UV-Vis Spectrophotometry

Determination of wavelength of maximum absorption (λmax):

The wavelength of maximum absorbance (λmax) was found to be 531 nm, which was determined using light source in the wavelength range of 400 – 600 nm and at an interval of 1 second. Table 4 gives the results given by the spectrometric analysis.

Linearity and Range:

The absorbance of the prepared standard solutions (200 – 1000 ppm) were determined at 531 nm. The mean absorbance was found to be 0.13844. The plot of absorbance versus concentration (Fig. 5) obeyed Beer-Lambert's law in the concentration range mentioned above. However, the linear range where the trendline plot showed the best result was 200 – 600 µg/mL or ppm.

LOD and LOQ:

The LOD and LOQ were found to be 243.2005 µg/mL or ppm and 736.9712 µg/mL or ppm respectively. This indicates that the proposed method is sensitive.

Calibration curve and Regression Coefficient:

The calibration curve, plotted between absorbance and concentration showed a linear trendline with regression coefficient of 0.987. The standard equation obtained was y = 0.0002x - 0.0008, wherein the slope and intercept were found to be 0.0002 and - 0.0008 respectively. This equation

can be utilized for determining concentration of the analyte under study for unknown concentrations. The graph has been given in Figure 4.

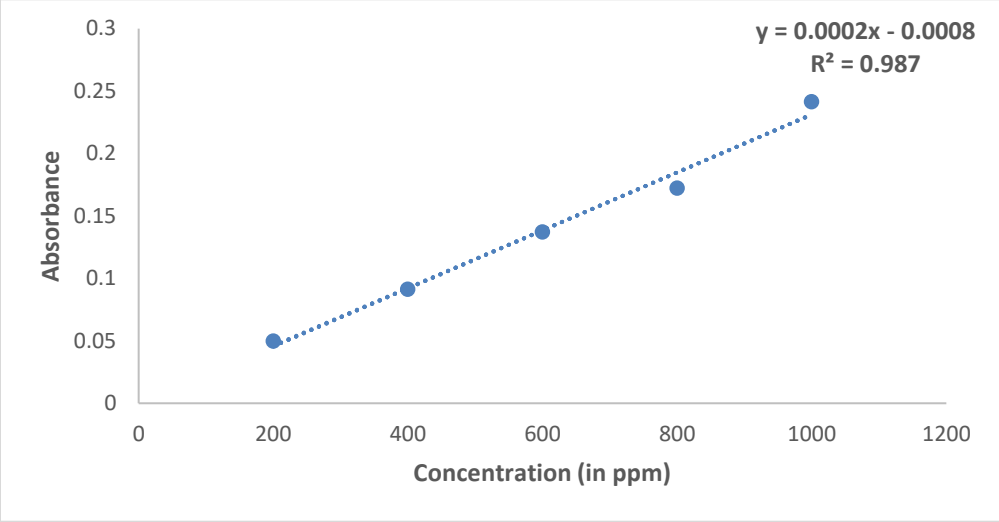

Figure 4: Calibration curve obtained from UV-Vis Spectroscopy dataset

Table 4: Table showing the results obtained from Spectroscopic analysis at 531 nm

Sl. No.	Concentration (µg/mL or ppm)	Absorbance
1	200	0.0499
2	400	0.0914
3	600	0.1371
4	800	0.1723
5	1000	0.2415

4. Discussion

The present study contributes towards the examination of evidences associated with trap cases. α-naphtholphthalein showed a number of improvements over previously used chemicals, such as phenolphthalein and anthracene, in the context of bribery trap cases. Due to its ability to change colour when exposed to alkaline substances, phenolphthalein has long been preferred in forensic applications. This characteristic makes it a valuable tool for marking bribe money. The efficacy of long-term evidence retention can be diminished by the hue of phenolphthalein fading with time The admissibility of the wash solution becomes challenging in such cases (Yadav & Goutam 2014). As our study has shown, α-naphtholphthalein maintains its colour for longer period of time, which is a clear benefit in situations when evidence may not be evaluated right away. Because of this characteristic, α-naphtholphthalein is a reliable indication, upholding forensic integrity in actual circumstances where delays in the evaluation of evidence are frequent.

The stable coloured product formation in this case, is due to a fused naphthalene based structure.

This contributes towards an extended π-Conjugation, increased resonance stability, lesser ring strain and better thermodynamic stability than that of phenolphthalein, which is a benzenoid based structure (Rabari et al. 2024; Durana et al. 2024; Yusuf 2024). The use of phenolphthalein in pharmaceutical formulations, however, poses a risk of cross-reactivity or false positives in forensic analyses. On the other hand, our research indicates that α-naphtholphthalein is not a component of medicinal items, which greatly improves the specificity of detection in forensic settings.

Another marker that was utilized in the past, anthracene, relies on a blue fluorescence for detection, which can pose challenges. Although fluorescence-based techniques can be quite sensitive, other compounds that glow in identical settings can interact with them. Results may become unclear, especially in areas with high concentrations of artificial or naturally occurring fluorescent compounds. Furthermore, in environments with limited resources, it may not always be possible to obtain the specialist equipment needed for fluorescence detection (Singh 2022; Basu 2021). However, α-naphtholphthalein is a more practical choice for simple identification since it produces a visually vivid colour that is easier to recognize without relying on fluorescence. α-naphtholphthalein does not exhibit any colour upon washing with tap water. Hence, the suspect will not be aware of the presence of the detective dye on his or her hands, if the person tries to wash the hands with normal tap water. The detective team can easily nab the person by spraying the alkaline solution on the suspect's hands and associated articles. The currently used dye is not a part of any medication (Working Procedure Manual: Chemistry 2021; National Institute of Criminology and Forensic Science 2021).

As per Material Safety Data Sheet (MSDS), no acute toxicity and carcinogenic effects has been reported (Regulatory affairs-Thermo Fisher Scientific 2021). To sum up, α-naphtholphthalein has a number of advantages over phenolphthalein and anthracene, such as increased colour stability, improved specificity, and avoidance of fluorescence-dependent detection.

5. Conclusions

A new method of examination of trap cases has been developed. The use of α-naphtholphthalein, as a detective dye has been suggested by the authors. The data from this study can be used for examination of trap cases and analysis of the chemical powder used for execution of the trap. The results of Thin Layer Chromatography, Colorimetry and UV Spectrophotometry can be utilised for its application in real time scenario. The use of colorimetry combined with UV-Vis Spectroscopy has been reported in this study. The dataset from the colorimetric analysis and characteristic wavelength from spectroscopic analysis shall be useful in the detection and identification of the detective dye. The linear range of the method, LOD and LOQ have also been reported. Rf values were calculated post Thin Layer Chromatography of the sample. The Rf values for Ethyl Acetate: Acetone, Chloroform: Acetone, and Ethyl Acetate: Water systems were found to be 0.89, 0.86, and 0.90 respectively. The wavelength of maximum absorbance (λmax) from UV-Vis Spectrometric analysis was determined to be at 531 nm. The sensitivity of the method was evaluated and LOD and LOQ were found to be 243.2005 μg/mL and 736.9712 μg/mL respectively. A regression coefficient of 0.987 was determined. The current innovation finds its application in

legal sector, particularly for catching a person involved in bribe-taking. Anti-Corruption Bureau, Forensic Science Laboratories (FSLs) and other detective agencies can make use of this method. This study also provides a scope for further research in this less explored area.

Competing Interests

The authors declare that there are no conflict of interests.

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