

Dissociation of phenolphthalein helps in solving Bribe Trap Case: Forensic approach

RAHUL VERMA¹ AND SUNANDA PRAMANIK²

¹Department of Forensic Science, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, 221005, India

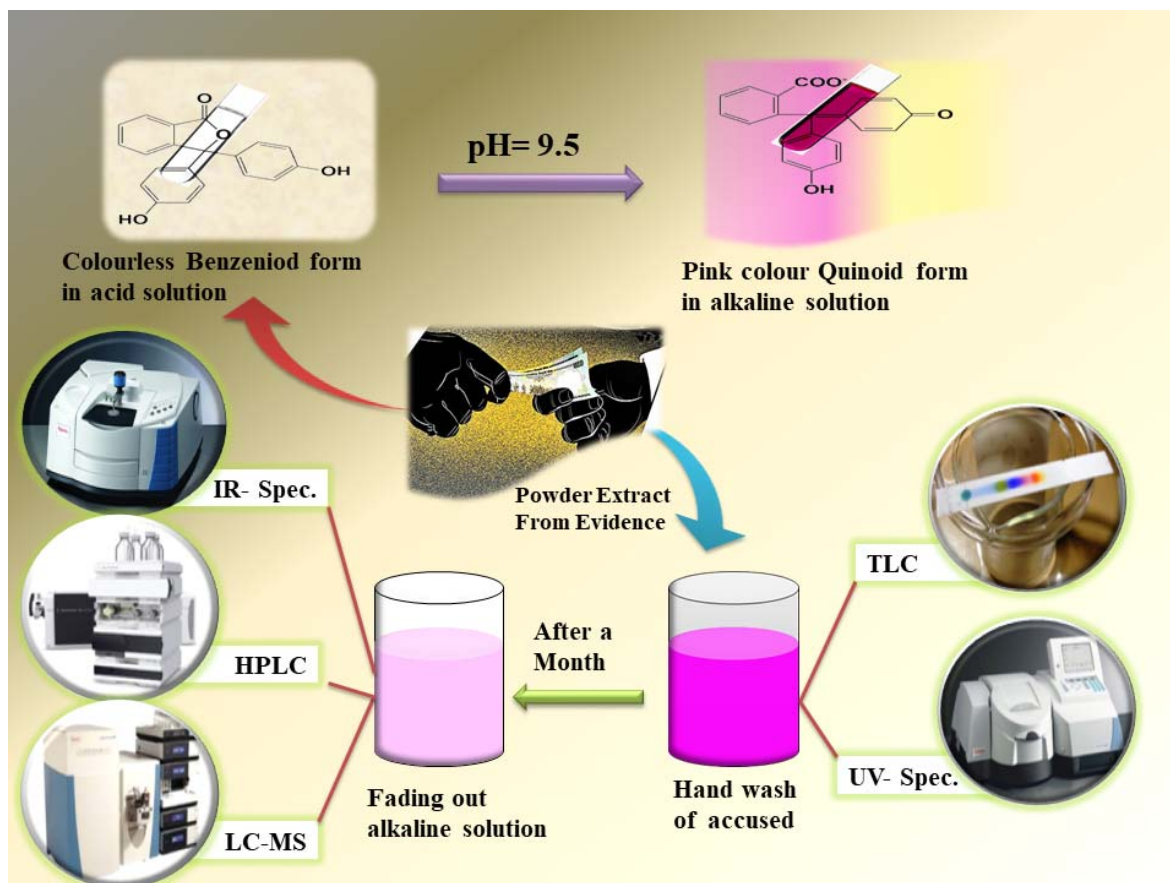
²Faculty of Science, Department of Chemistry, University of Allahabad, Katra road, Prayagraj, Uttar Pradesh, 211002, India

Correspondence Author: Rahulbhuchem@gmail.com
+917753073265

Abstract: Phenolphthalein renders pink color visualization on dissociation. It is an important constituent often used in solving bribe trap cases. An alkaline hand wash of phenolphthalein powder, smeared on a currency note, confidential paper, and other official documents was originating pink color. Its presence was determined by various analytical methods. But in many cases, the visualization of pink color becomes faded with time. Further, this problem was minimized by adding a small amount of hydroquinone. Na₂CO₃ or KOH bases were used to collect smeared phenolphthalein powder by hand wash of suspected person. The shreds of transferred evidence were collected from the accused body, hand and clothes are submitted to further examination in Forensic Science Laboratories (FSLs). In this review article, TLC, IR, UV-Visible Spectrophotometer, HPLC, and LC-MS/MS analytical method was used for analysis. Our aim and concern are concise to test the presence of phenolphthalein, using various analytical techniques. That can help establish scientific evidence in criminal bribery trap cases.

KEYWORD: Bribe trap case; Bribery; Phenolphthalein; Currency notes, Infra-red technique, Uv-spectrophotometer; HPLC; Liquid chromatography-tandem mass spectrometry.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The digital and advanced analytical method applied to study bribe crime is a genuine area of forensic identification. The methodology will increase the interest in forensic chemical science and advanced digital method of crime. Hence, the method used to introduce the idea and principle practice of trapping methods by the chemicals Law enforcement agency. Phenolphthalein powder is smooth, white in color generally smeared in small quantity on the confidential document, currency note and other objects are likely to come in contact with hands, cloths^[1]. Trace objects were washed with an alkaline solution such as sodium carbonate or potassium hydroxide. The colorless solution gives pink color visualization confirming the touching of the confidential document^[2]. An alkaline solution was collected and sent to the forensic laboratories for analysis. The instrumental analysis confirmative assessment it can be considered as important evidence in court trials. But, the collected alkaline solution was starting to fading (equilibrium shift to backward) after a few days or months. It was depending on the strength of phenolphthalein quantity in alkaline solution. The equilibrium shifted to backward reaction due to two reasons, i.e. either the concentration of H^+ is higher or breaking down of phenolphthalein into other colorless products, i.e. 2[4-hydroxy-benzoyl]-benzoic acid and phenol which may be caused by the action of alkali, heat, light and the oxygen present in the air. Phenolphthalein gives pink color in alkaline at a low concentration. This gives positive proves of the suspect with contact with currency notes^[3]. Bribe cases involve the use of various tracing materials such as staining material, fluorescent powder, chemical detector, radioactive indicator. According to, "Ostwald's theory" all indicators exist as either weak acids or weak bases. The color change is due to dissociation of the acid-base indicator. The color of the dissociated form is different from the color of the undissociated form of phenolphthalein. The ionization of weak acid indicator is very low in acid due to common H^+ ions, while it is fairly ionized in alkalies. Similarly, if the indicator is a weak base, its ionization is large in acids and low in alkalies due to common OH^- ions^[4].

Chemistry of Phenolphthalein: Undissociated state of phenolphthalein showed colorless visualization, while dissociation gives pink color. Equilibrium shifts to the left-hand side in the acidic medium due to more concentration of H^+ ions. Therefore, the solution would remain colorless. The excess of H^+ ions was neutralized by the addition of alkaline to form water molecule and equilibrium shift right-hand side. Yadav and Goutam reported in his article, the phenolphthalein exists in three forms shown in figure 1 (a) both tautomeric structure of phenolphthalein indicator having a different structure. i.e., quinonoid form (pink color) and benzenoid form (colorless). (b) The color of both forms would be different due to the inter-conversion of tautomeric form into each other. (c) In both form one form exist in acidic medium and other was exist in alkaline medium. The change in the tautomeric structure would be responsible for the change in color by changing the pH of the medium^[5-6]. The demonstration of sodium carbonate and phenolphthalein present in the collected exhibit was done by various chemical sampling methods shown in table 1. If a positive result comes through these chemical tests it was proved the sodium carbonate and phenolphthalein present in forensic exhibit^[7-12].

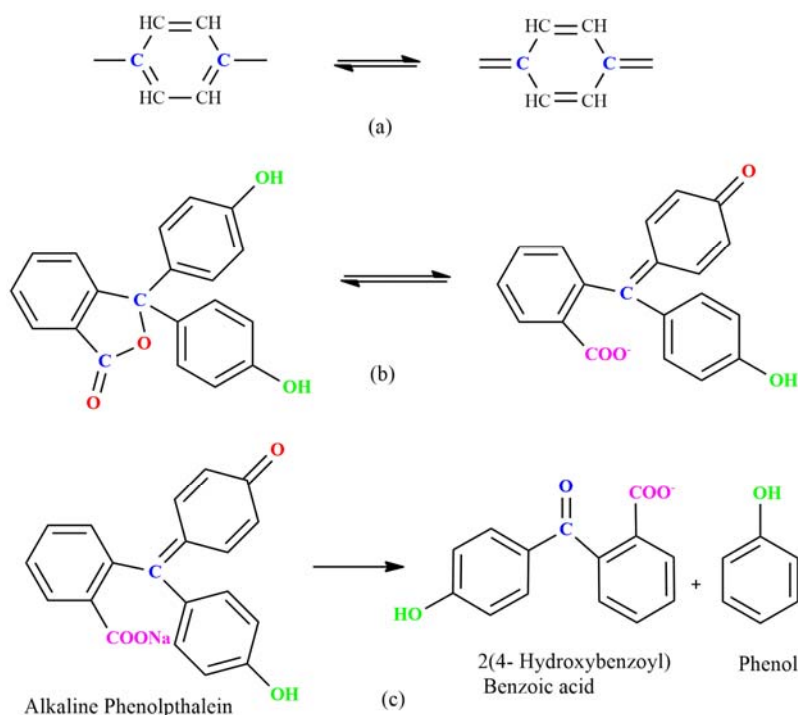


Figure 1: Quinonoid theory showed (a) tautomeric structure; (b) inter-conversion of tautomeric structure; (c) existence of phenolphthalein in both acidic and basic medium.

In the forensic science laboratories commonly, the presence of sodium carbonate and phenolphthalein is confirmed by the conventional methods of chemical analysis. The detailed methodology has been described in the working procedure manual published by the Directorate of Forensic sciences. The offense is generally registered against the accused under Section 161 of Indian Penal Code and Sections 7, 12, 13(1) (d) and 13(2) of the Prevention of Corruption Act, 1988^[13]. The expression establishes either these confidential documents or notes were smeared with phenolphthalein powder or not. Some analytical tests of sodium carbonate and phenolphthalein shown in table 1 demonstrated to explain the spontaneous value. (*See*, Harish Chand Khurana vs State Thr. CrI No. 953/2004, Cbi/Acb New Delhi on 30 May 2012). Section 171 B of the Indian Penal Code defines bribery and section 171 E deals with the punishment of bribery. Each acceptance of illegal content whether preceded by demand would cover by prevention of corruption Act 1988 with punishment is 6 months to 7 years with the fine^[14]. Advanced spectroscopic methods have been also reported and summarized different methods of Forensic analysis of phenolphthalein. Forensic analysis of phenolphthalein in solutions collected in trap cases has already been reported by various workers. In the present review, our aim to cover the various analytical method of analysis of trap cases such as TLC, UV spectrophotometer, infrared spectrophotometry, HPLC, LC-MS/MS has been used to detect the presence of phenolphthalein in sodium carbonate powder.

2. MATERIAL AND METHODS

Chemical solvent such as acetonitrile, distilled water, acetic acid, sodium hydroxide, acetone, chloroform, ethyl acetate, diethyl ether, potassium hydroxide, sodium carbonate, hexane, staining chemicals (Phenolphthalein), fluorescent powder, chemoluminescence, radioactive material and some chemicals used to identify the trace evidence of trap case called tracing chemicals. The various analytical method used for the examination of phenolphthalein exhibit in bribe cases briefly discuss in table 2.

2.1. Spotting Method Using TLC

Numbers of polar and non-polar solvent systems were used as a mobile phase. Silica gel used as a stationary phase in column packing. The spotting method very often used in analytical chemistry using silica gel, TLC plate. For the identification of phenolphthalein, the TLC plate was developed in an appropriate solvent system and spread with suitable spraying solvent systems to develop color. The Pink color spot was shown in acid-base system^[15]. Visualization is recommended with dilute sodium carbonate solution/acidified (1% solution of permanganate in 0.25% sulphuric acid) potassium permanganate solution and UV light. A TLC method coupled with normal UV spectrophotometric analysis has been reported by Pandey A^[16]. The chemical used as spraying reagent, which helps to develop the color visualization in TLC plates was shown in table 3. Dhole and Shinde^[17] reported a TLC method using a better spray reagent of diazotized, p-nitroaniline followed by alkaline solution giving a stable azo-dye coupled with derivative UV absorption spectrophotometry before and after spraying the Diazo-coupling reagent. Geeta and Baggi^[18] reported and developed a new chromatographic method for the determination of phenolphthalein; it was a simple, rapid and reverse-phase chromatographic method.

2.2. UV-Spectroscopy

Phenolphthalein gives maximum absorbance of 220-280nm spectra wavelength in free aldehyde^[19]. Normal UV spectra of phenolphthalein exhibit after extraction in methanol give λ_{max} of 277nm. Pithava and Pandey reported, absorbance and λ_{max} of phenolphthalein in two conditions, i.e., firstly sample kept in constant normality and secondly sample kept in constant weight. It was observed that, as the time increases, absorbance slightly decreases which may be due to the color intensity variations observed in phenolphthalein solution.

2.3. FT-IR Spectroscopy analysis

The collected sample was successfully analyzed by FTIR. The main use of FTIR is to determine the functional group in the molecule. FTIR Method is non-destructive and thus suitable for the analysis of forensic exhibits. Yadav and Goutam were reported the comparative analysis of a pure and contaminated sample of phenolphthalein with sodium carbonate. In both cases, the presence of phenolphthalein was characterized by their spectral data. The presence of phenolphthalein was confirmed with the help of FTIR using sodium carbonate at the initial stage of the solution.

2.4. High-performance liquid chromatography (HPLC)

The collected sample was extracted with acetone or chloroform in extracting funnel. The organic layer was separated and evaporated up to dryness. The evaporated sample was then reconstituted in methanol or acetonitrile. These prepared samples were further injected (5-10 μ ml of volume) in the HPLC injector port. Isocratic mode delivery system was used for the mobile phase solvent in which the mobile phase used was acetonitrile: distilled water containing 0.1M of acetic acid. A flow rate of 1 ml/min and 277 nm of UV absorption detection at ambient temperatures were maintained throughout the experiment^[20]. Exhibit sample was acidified by sodium carbonate solution and extracted in diethyl ether. This sample evaporated to dryness and the residue dissolved in methanol or acetonitrile was reported by Nandode and Dhole^[21] in the examination of phenolphthalein in anticorruption trap cases by HPLC. Chauhan, Chelian and Ramteke^[22] discuss the

identification of traces of phenolphthalein in trap case was reported in some cases which are even less than one month old, these older exhibits were found to be colorless in alkaline medium. The colorless samples were processed with diethyl ether in an acidic medium for extraction of phenolphthalein and the extracts were concentrated and analyzed by HPTLC. Confirmation of phenolphthalein present in the exhibit was done based on its maximum absorbance wavelength at 210-220nm in spectra. Further, the densitometric scanning for the spectrum of each decolorized sample between 210nm to 280nm wavelength range reconfirmed the presence of phenolphthalein by using the HPTLC instrument.

Table 1: Sodium carbonate and phenolphthalein was determined by various chemical analysis methods

Compound/ Ions	Name of Test	Reference
Test for Sodium ions	Uranyl Zinc Acetate Test	8
Test for Carbonate ions	Barium Chloride test	10
	Magnesium Sulphate test	10
	Silver Nitrate test	10
Test for phenolphthalein	Acid –Alkali Test	10,11
	Folin-Ciocalteu's reagent test	12

Table 2: List of spotting methods was used to determine the presence of phenolphthalein.

Mobile Phase	Visualizing reagent	References
Benzene: Dioxane: Acetic acid (75: 15: 10)	UV light	11
Chloroform: Acetone (4:10)	Exposed to ammonia vapor	12
Ethyl acetate: Methanol: Ammonia (80:10:5)	Spray with a neutral ferric chloride solution	12
Stationary phase: Silica Gel G	Iodine fumes (With 1 % solution of potassium permanganate in 0.25 M sulphuric acid.)	12

Table 3: List of the analytical methods was used to determine the presence of phenolphthalein exhibit in bribe cases.

Analytical Method	Mobile Phase/ Methodology	Extraction Process	Spraying Reagent/ Detector	Result/ Conclusion	Ref.
Infra-Red Spectroscopy	IR Affinity-1S (Simadzu, Japan), λ range (4000-400 cm ⁻¹), sampling (Sample + KBr in dispersion cell)	Sodium Carbonate washing	DLATGS detector equipped with temperature control mechanism	Absorption: 3859.56 (vO-H), 3435.22, 3294.43, and 3016.67 (vO-H, intermolecular bond), 2492.03 (v O=C=O), 1778.37 (v C=O), 1404.37, 1294.24, 763.81, 644.22, and 578.64 cm ⁻¹ .	[4]
UV- Vis Spectroscopy	Shimadzu UV 2550, λ range (190- 800nm)	Hand wash in Na ₂ CO ₃ , Extracted in diethyl ether	Photo Multiplier Tube	λ_{max} = 279nm (acidic), 553.8nm (alkaline) LOD= 0.27 μ g/ml Linear range 1.0-40 \times 10 ⁻⁹	[4]
TLC	TLC plate of Silica gel G: Mobile phase: Chloroform: acetone 4: 1	Sodium Carbonate washing extracted in ether	ammonia vapor or iodine fumes for visual development	Pink color spot of phenolphthalein was developed on the TLC plate.	[15]
TLC	TLC plate of Silica gel G: a) Chloroform: Acetone (30: 20), b) Ethyl acetate: Diethyl ether (40: 10)	Sodium Carbonate washing	a) NaOH/ Dil. KOH b) Dil. KOH	a) Pink color, RF 0.9298 b) Pink color, RF 0.9633	[16]

UV- Vis Spectroscopy	T9DCS spectrometer, λ range (400-800 nm)	Washing method by NaOH	Photo Multiplier Tube	$\lambda_{\text{max}} = 553\text{-}532 \text{ nm}$	[16]
HPLC	Perkin Elmer, Column (150 x 4.5 μ m), ACN: Water with few drops of 0.1M acetic acid, Isocratic method, I.V. 1 μ l, F.R. 1.5 ml/min.	Solvent extraction in ether	Detector: UV-Vis (230nm) and Run time: 30 min.	LOD=1.66 ng/mL, and linearity (r = 0.9974).	[16]
HPLC	Reverse Phase C-18, column (Lichrospher-100-RP-18,5 μ m,250mmLx4mm ID)	Acidifying carbonate solution extract obtained by Diethyl ether	UV Detector	RT=2.5 min at 277nm of wavelength	[20]
LC-MS	3200 QTRAP LC-MS/MS, C ₁₈ column, methanol: ammonium acetate (80:20),	Hand wash in Na ₂ CO ₃ , Extracted in diethyl ether	ESI	LOD= 1.66ng/ml Linear range= 2.0-160 $\times 10^{-9}$	[21]

3. CONCLUSION

Unfortunately, the pink color solution of phenolphthalein was decline to fading with time due to the equilibrium shift to a backward reaction. The intensity of the exhibit sample was decreased after a few months or days. Finally, when physical evidence was offered in the court, it takes several months to years for final decision. Furthermore, the visualization of exhibit samples was almost colorless in alkaline solution. This created a problematic presence of phenolphthalein in the bribe case at the time court support on the visual appearance of pink color. In addition to a small quantity of hydroquinone in an alkaline solution, this pink color was retained. Nowadays, the forensic expert team was collected sodium carbonate hand washed samples and determine the presence of phenolphthalein using a sophisticated analytical method (such as UV, FTIR HPLC, and GC-MS). The thin layer chromatographic method is one of the oldest methods in forensic laboratories. In which exhibit sample was spotted and run in the suitable solvent and both NaOH and KOH solvent was used as spraying reagent. Better result of developed TLC was disposed of by ethyl acetate: diethyl ether (80:20%), chloroform: acetone solvent system used as a mobile phase. Iodine fumes, neutral ferric chloride spraying solutions were used as developing reagents exposed to ammonia vapor under UV light. Teotia studies similar methods to conducted extraction methods in ether solution and further studied by Thin Layer Chromatography (TLC) using silica gel plates. Sudhir Kumar Shukla was used to derivative a method in UV-spectrophotometry for determination of phenolphthalein in the collected hand washed sample from cloth currency notes and other confidential documents in bribe trap cases^[24]. A similar study was reported by Pandey in her article that the amount and concentration of phenolphthalein in two conditions. It was observed that, as the time increases, absorbance slightly decreases which may be due to the color intensity variations observed in phenolphthalein solution. From the FTIR method, the analysis of phenolphthalein with sodium carbonate can only be done in powdered form. Yadav reported in his article the analyzed mixture of phenolphthalein and sodium carbonate by FTIR method. The principle absorption bands were observed at 3859.56 (b, ν O-H), 3435.22, 3294.43, and 3016.67 (ν O-H, intermolecular bond), 2492 .03 (ν O=C=O), 1778.37 (ν C=O), 1404.37, 1294.24, 1068.56, 1022.27, 900.76, 802.39, 763.81, 763.81, 644.22, and 578.64 cm^{-1} . From the HPLC method, the analysis of phenolphthalein was observed that the solvent system used in the method found to be suitable for the analysis it may be methanol, acetone, acetonitrile with Mili-Q water or buffer. A flow rate of 1 ml/min and 277 nm of UV absorption detection at ambient temperatures were maintained throughout the experiment. Pihlainen using liquid chromatography-ion trap mass spectrometry in his article. They briefly elaborate liquid chromatography-electrospray ionization method coupled with tandem mass spectrometric (LC-MS/MS) using a monolithic column, gradient method. Ion trap-mass spectrometer was developed for rapid examination of phenolphthalein exhibit and their different intermediate chemical compounds. The elution time was very rapid for all compounds and less time required for total analysis. ESI positive mode of ionization was efficiently used for all acidic, neutral, alkaline medium of the compound. Resultant spectra were developed a distinguishable RT and MS/MS peak area. These developed methods show good linearity and reasonable reproducibility. LOD range was reported from 10.0 to 50.0ng/ml. Sharma^[25] reported phenolphthalein is self-indicator and useful reagent in

forensic science for the examination of a bribe in graft and trap cases in his article the detection and quantization of trace phenolphthalein by LC-MS/MS Spectrometry. The author uses LC-MS to give positive proof of colorless phenolphthalein present in the forensic exhibit^[26]. No other method claiming the colorless detection of phenolphthalein. The collected hand was of smeared phenolphthalein power on currency notes was easily examined by liquid chromatography-tandem mass spectrometry. The calibration curve and LOD of phenolphthalein were found to be $r^2=0.9974$ and 1.66ng/ml respectively. The introduced method was rapid, simply efficient, sensitive and selective for the examination and identification of traces of phenolphthalein in decolorized alkaline solution. This can be used for routine analysis of such type of crime exhibits.

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AUTHOR CONTRIBUTION STATEMENT

RAHUL VERMA¹

The author collects the data of the forensic bribe trap case and its analytical methods. The main focus area in analytical as well the forensic point of view such as law and punishment related to bribing trap case. Both authors discuss and briefly analyze different analytical methods from literature and references to solve bribe trap case.

SUNANDA PRAMANIK²

The author focus on chemical change and the nature of phenolphthalein reaction occurs with base and their problems such as the feeding of color. The chemistry part of this review article was done by Sunanda Pramanik.

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