
Forensic Analysis of Phenolphthalein in Trap Cases- A Critical Review

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ABSTRACT:

This paper is focused on the review of the scientific literature and succinctly recent progress related to the forensic examination of phenolphthalein in trap cases. Paper summarizes the current state of knowledge of the forensic examination of phenolphthalein. Attempt have been made to collect the suggested methods of forensic analysis of colored and faded/decolorized phenolphthalein solution (hand wash in trap cases). Mechanism of Colour change of the phenolphthalein and its break down products is also highlighted. This paper may create an understanding and can produce a coherent argument on this topic.

Key Words: *Forensic, Trap cases, phenolphthalein,*

INTRODUCTION:

Professor Edmond Locard's famous theory of exchange can be summed up as "every criminal leaves a trace". Locard said, in his 1934 publication "La police et les methods scientifiques": "Any action of an individual, and obviously, the violent actions of a crime, cannot occur without leaving a trace." In other words, there is always going to be physical evidence left behind at a crime scene by the criminal. The criminal will also take away physical evidence from the crime scene'¹. The vigilance and Anticorruption agencies employ 'Locards Principle of exchange' to trap the accused while accepting the bribe amount. Law enforcement agencies arrange a trap for illegal transaction (bribe) with the help of the complainant where in the accused is given currency notes on which chemical like phenolphthalein powder has been applied. If the accused touches the notes then the part of the chemical, which may be in traces, is transferred on his hands or fingers if it is kept in pocket, bag, briefcase or file etc the chemical may also be transferred on these objects and when these are washed with a colorless solution of sodium carbonate, which becomes

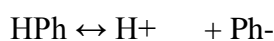
immediately pink confirming the touching of currency notes/ transferred of phenolphthalein to the object. Intensity of developed pink color depends on the quantity of phenolphthalein which is transferred. These washings are being collected and sent to the forensic laboratories along with other relevant articles to establish the presence of phenolphthalein. Establishing the presence of phenolphthalein in the solution is vital evidence in the court. Generally, the cases where currency note coated with phenolphthalein powder were collected from the possession of an accused. The offence are generally registered against the accused under Section 161 of Indian Penal Code² and Sections 7, 12, 13(1)(d) and 13(2) of the Prevention of Corruption Act, 1988². In order, to lucidly establish whether or not these currency notes were smeared with phenolphthalein powder and presence of phenolphthalein is confirmed by applying conventional chemical tests and demonstrated to explain the intrinsic value of the phenolphthalein. (See, *State vs K.Sundararajan*, CrI.A.No.74 of 1999, Madras High Court) However, in some cases it fails because of fading of pink colour or even conversion to colourless. Generally controversy arises in interpretation of section 161 and 165 of IPC (See, *State of Madras v. Vaidyanatha Iyer*, 1958 Mad W N (Notes) 31, *In Re: K.V. Ayyaswamy*, 1965 Cri. L. J 281(AP), *Vinayak V. Joshi vs The State*, AIR 1968 P H 120) specially where conducting the Phenolphthalein test yielded not showing positive result.

Phenolphthalein is known to give pink color in alkaline at a concentration as low as 0.005 x 10⁻³ % (5µg per 100ml). The pink color of this solution persists for some days to some months depending upon the quantity of phenolphthalein and the strength of alkali solution. • It gradually fades and turns in to colorless. Presence of phenolphthalein plays vital role to prove the guilt therefore to face this situation forensic scientist has to explain the technical reason. The colored phenolphthalein solution has a tendency to gradually fade away due to chemical changes with passage of time varying from days to months .its intensity may considerably decrease and the alkaline solution may become colorless. The fading away of the color of alkaline phenolphthalein solution can be due to two reasons i.e. either the PH of solution is out of limit /high or phenolphthalein has chemically broken down in to 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 air³. Decomposition of pink color (Phenolphthalein in alkaline solutions) has been studied to understand the manner of development of initial pink color and its subsequent fading away through irreversible

chemical changes. The basic data so acquired Can be utilized to interpret the chemical decomposition of phenolphthalein in alkaline solutions under prolong storage and with the use of strong alkaline solutions in the courts when ever needed.

MECHANISM OF COLOR CHANGE:

Color change of the phenolphthalein explained by the theory of indicator. According to Ostwald's theory” all indicators are either weak acids or weak bases in which the color of the ionized form is different from the color before dissociation”⁴. Kiruthiga⁵ described that color change is due to ionization of the acid-base indicator. The unionized form has different color than the ionized form and the ionization of the indicator is largely affected in acids and bases as it is either a weak acid or a weak base. In case, the indicator is a weak acid, its ionization is very much low in acids 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. Ostwald theory can be illustrated as follows: Phenolphthalein: It can be represented as HPh. It ionizes in solution to a small extent as: Phenolphthalein: It can be represented as HPh. It ionises in solution to a small extent as:



Colorless Pink

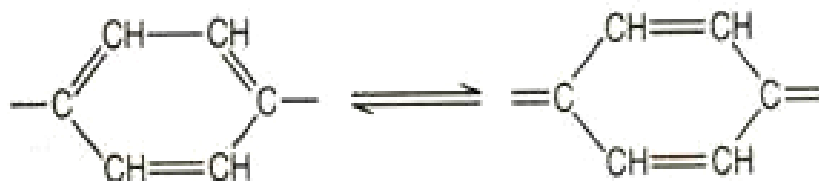
Applying law of mass action,

$$K = \frac{[\text{H}^+][\text{Ph}^-]}{[\text{HPh}]}$$

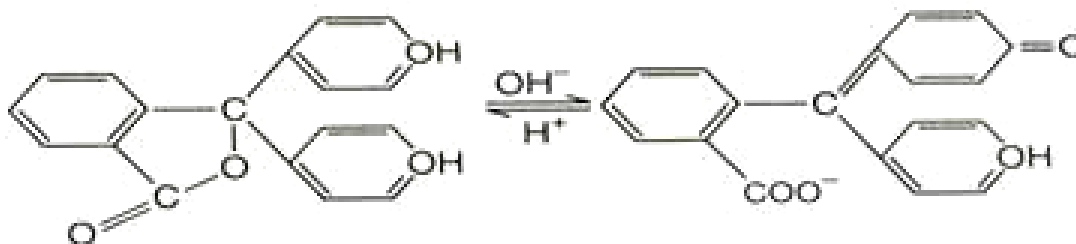
The undissociated molecules of phenolphthalein are colorless while Ph⁻ ions are pink in color. In presence of an acid the ionization of HPh is practically negligible as the equilibrium shifts to left hand side due to high concentration of H⁺ ions. Thus, the solution would remain colorless. On addition of alkali, hydrogen ions are removed by OH⁻ ions in the form of water molecules and the equilibrium shifts to right hand side. Thus, the concentration of Ph⁻ ions increases in solution and they impart pink color to the solution(see/Theory of indicators, www.srmuniv.ac.in/d)

QUINONOID THEORY:

According to this theory: (a) The acid-base indicators exist in two tautomeric forms having different structures. Two forms are in equilibrium. One form is termed benzenoid form and the other quinonoid form.

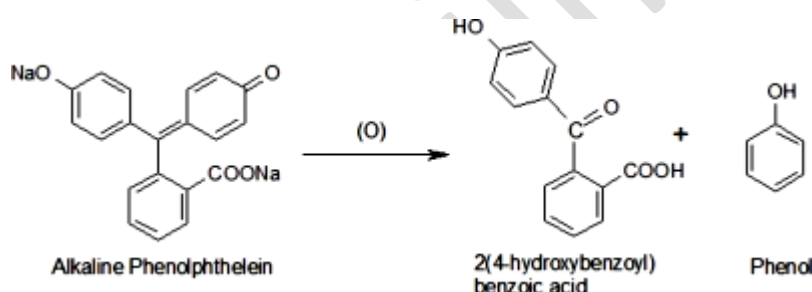


(b) The two forms have different colors. The color change is due to the interconversion of one tautomeric form into the other. (c) One form mainly exists in acidic medium and the other in alkaline medium⁵. Thus, the medium changes from acidic to alkaline or vice-versa. The change in pH converts one tautomeric form into the other and thus, the color change occurs. Phenolphthalein has the benzenoid form in acidic medium and thus, it is colorless while it has the quinonoid form in alkaline medium which has a pink color.



In forensic laboratories, the common presence of sodium carbonate and phenolphthalein is confirmed following the conventional methods of chemical analysis, detailed methodology has been described in the working procedure manual published by Directorate of Forensic Sciences, MHA, Government of India⁶ and various methods related to chemical analysis, chromatographic and spectroscopic methods have been also reported from time to time, Teotia⁷ has reviewed & summarized different methods of forensic analysis of phenolphthalein.

Phenolphthalein is a weak acid; its unionized molecules are colorless while on ionization give pink color. There are so many methods like color tests, thin layer chromatography, high performance liquid chromatography, high performance thin layer chromatography, Spectrophotometry have been used for the identification of phenolphthalein in the sodium carbonate washings. Examination of chemicals used in trap cases reviewed by Raghu Khimani⁸, author discussed analysis of Phenolphthalein by TLC and UV spectroscopy and concluded that the chemical break down of the alkaline phenolphthalein is caused by the oxygen present in the air and suggested that this can be prevented by the addition of hydroquinone which is strong antioxidant combines with the oxygen more rapidly than alkaline phenolphthalein does and thus retards the above reaction. It can be easily mixed with phenolphthalein powder before dusting the currency notes. The presence of hydroquinone will provide another parameter to prove the transfer of money especially in those cases when the plea is taken by the culprit that he uses phenolphthalein tablets as laxative. The presence of hydroquinone can be easily established on the basis of chemical and instrumental methods. Raghu Khimani⁸ has also highlighted the troubleshooting in trap cases and coated the fading of color solution and conversion of phenolphthalein in to 2[4-hydroxy benzoyl] benzoic acid and phenol by chemical reactions.

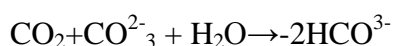


Examination of Phenolphthalein

Generally appropriate amount of hand wash (pink or colorless solutions) is acidified with dil. Hydrochloric acid drop wise with stirring till Ph of the test solution reaches up to about 4 to 5. Solvent ether is used for the extraction in two or three steps and extracts so obtained are combined and evaporated. The residue thus obtained is used for different tests e.g. color tests, thin layer chromatography and spectrophotometric analysis etc.

Tests for Phenolphthalein-

Sodium carbonate –Phenolphthalein test⁹-This test depends on the fact that phenolphthalein is turned pink by soluble carbonates and become colorless by soluble hydrogen carbonates hence if the carbon dioxide is liberated by dil acids from carbonates is allowed to come in to contact with Phenolphthalein solution colored pink by sodium carbonate solution It may be identified by the decolorization which takes place



The concentration of sodium carbonate solution must be such as not to be decolorized under the conditions of the experiment by the carbon dioxide in the atmosphere.

Acid alkali test:^{9,10} -Pink colored solution (hand wash of accused) is acidified with few drops of dil hydrochloric acid. The pink color disappears. Then few drops of dil sodium carbonate is added, the pink color reappears. The appearing and disappearing of pink color indicates the presence of phenolphthalein.

Folin Ciocalteu's test¹¹

About 2ml of the folin-ciocalteu reagent followed by 2 ml of 20% sodium carbonate is added to the test sample, Blue color indicates the positive test for the presence of phenolphthalein.

Baggi & Murthy¹² has also suggested a new color Test for detection of Phenolphthalein.

TLC method .

Thin layer chromatographic identification of phenolphthalein is reported by using Benzene :Dioxan: and Acetic acid(75:15:5)¹³ , Chloroform: Acetone(80:20)¹⁴ and Ethyl acetate : Methanol: Strong Ammonia(27 to 30%)¹⁴ as solvent systems and tlc plates coated with silica gel G/silica gel/GF 254. Visualization is recommended with dil sodium carbonate solution/acidified potassium permanganate solution¹⁴ (1% solution of permanganate in 0.25% sulphuric acid) and UV light.

A tlc method coupled with normal UV spectrophotometric analysis has been reported by Narayan Swamy et al³ . Dhole and Shinde¹⁵ reported a tlc method using a better spray reagent of diazotized ,p-nitroaniline followed by dil. NaOH giving a stable azo dye coupled with derivative UV absorption spectrophotometry before and after spraying the Diazo-coupling reagent. Geeta and Baggi¹⁶ developed and reported a simple, rapid, reverse phase liquid chromatographic method for the determination of phenolphthalein in commercial

formulations has been. Three mobile phases suggested, out of which the mobile phase consisting of only methanol has been found to be most satisfactory. The method is linear over the concentration range of 0-70 pml. A mean recovery of 99.8% was reported. The proposed high-performance liquid chromatographic method was verified for linearity, precision, accuracy, reproducibility, and applicability claimed by the authors.

HPLC Methods

Nandode and Dhole¹⁷ reported the forensic examination of phenolphthalein in anticorruption trap cases by HPLC. Diethyl ether extract obtained by acidifying carbonate solution and was concentrated, evaporated to dryness and the residue was dissolved in methanol. The methanolic extract was analyzed on HPLC system consisted of Hewlett packed HPLC unit with RP- C-18, ODS column(Lichrospher-100-RP-18,5µm,250mmLx4mmID) Variable wavelength absorption detector fitted with Datajet –CH-1-integrator. and isocratic mobile phase of water : methanol (7:5:2:5) at the flow rate of 1ml/min and UV absorption detection at 277nm used. A flow rate 1 ml/min and UV absorption detection at 277nm at ambient temperatures were maintained throughout the experiment. Integrator condition were kept at Attn – 1, chart speed- 1 cm/min, threshold = 2, peak width = 0.004. The characteristic peak of phenolphthalein was reported RT=2.5 min at 277nm of UV absorption detector of HPLC.

Chauhan, Chelian and Ramteke¹⁸ studied the HPLC instrumental method for the identification of traces of phenolphthalein in decolorized alkaline solution in trap cases and reported that it has been their observation that the trap solution referred for analysis, some of the cases which are even less than one month old, a few of the exhibits have been found decolorized /colorless in alkaline solution. The decolorized samples were processed with diethyl ether in acidic medium for extraction of phenolphthalein and the extracts were concentrated and analyzed by HPTLC. Phenolphthalein has been identified on the basis of its maximum absorbance at 220nm wavelength. Further the densitometric scanning for spectrum of each decolorized sample between 200nm to 300nm wavelength range reconfirmed the presence of phenolphthalein by using HPTLC instrument. The proposed method was claimed as rapid, simple efficient, sensitive and selective for the detection and identification of traces of phenolphthalein in decolorized alkaline solution, which can be used for routine analysis of such type of crime exhibits. HPLC grade water, chloroform, acetone & diethyl ether and HPTLC pre- coated silica gel 60F -254 (20X20CM) glass plates were used for the study. Solvent system of chloroform :acetone (9:1) was used for development of plate up to a

distance of 10cm. the HPTLC system (DESAGA) consisted of AS30 sample applicator equipped with a 100ml syringe, zero grade nitrogen gas cylinder and a TLC scanner (densitometer CD 60 with deuterium lamp) operated using software “ProQuant” loaded on a personal computer. The developed and dried plate was studied on HPTLC densitometer for maximum absorbance at a wavelength of 220 nm and densitometric scanning between 200 nm to 300 nm wavelengths for spectrum of each extract

Narayanswami et.al³ reported that phenolphthalein in saturated calcium hydroxide and dilute sodium carbonate solution after prolonged storage gave rise to certain break down products. These have been isolated and identified as 2(4hydroxybenzoyl) benzoic acid and phenols they applied this data in conforming presence of phenolphthalein in stored and decolorized alkaline solutions of phenolphthalein. Authors reported the ultraviolet absorption curve of phenolphthalein and its break down products they also performed the tlc analysis of ether extract of phenolphthalein using silica gel plates and solvent system 75:15:0.1 benzene: Dioxane: Acetic acid . Natural ferric chloride solution was used as spraying reagent

An interesting case was reported by Kasthuri et al¹⁹ wherein the hand washings were not visibly pink as some reddish yellow color was predominating. The normal pink color of phenolphthalein in sodium carbonate was masked by the yellow coloring matter from the turmeric coating observed on the hands of the accused woman officer authors examined & tried to explain the turmeric interference in identification of phenolphthalein ,they described that curcumin is the coloring pigment present in turmeric which imparts the yellow color. It is 1,7 bis(4-hydroxy –methoxy phenol)-1,6-heptodiene-3,5dione and also known as natural yellow3. It is a polyphenolic compound that gives brownish red color in alkaline medium. phenolphthalein is 3,3bis (p-hydroxy phenyl) phthalide. It gives pink-red color in alkaline solution. Authors argued that since both turmeric yellow from turmeric coating found on the hands and phenolphthalein were transferred, the sodium carbonate wash solution turned reddish yellow in color. Both turmeric yellow and phenolphthalein contain poly phenolic compound which are responsible for the major interference in the chemical analysis, separation by TLC posed greater problem ,both the compound gave the R_f values even three different solvent systems were tried however two dimensional TLC found to be much useful to overcome the interference .UV absorption of phenolphthalein and turmeric are all most the same because of the presence of polyphenols but visible absorption solved

the interference and the presence of phenolphthalein was characterized by visible spectroscopy they further confirm the identity of phenolphthalein by FTIR spectroscopy.

Pihlainen et al.²⁰ reported rapid identification and quantization of compounds of forensic interest using fast liquid chromatography-ion trap mass spectrometry and library searching. They described a fast liquid chromatography-electro-spray tandem mass spectrometric (LC-ESI-MS-MS) method by using a monolithic column, gradient elution and ion trap. mass spectrometer was developed for 14 forensically interesting and chemically different compounds. All compounds were eluted within 2.5 min and the total analysis time was 5 min including stabilization time required for the next injection. All the compounds, basics, neutrals and acids were efficiently ionized by positive ion ESI. A laboratory library including MS-MS spectra and retention times was developed and tested. Results with 476 standard samples and 50 authentic samples showed that the compounds studied can be unambiguously identified with the library. A quantitative method was developed for the compounds using external calibration. The evaluation process showed good linearity of the method and reasonable repeatability. Limits of detection ranged reported from 10.0 to 50.0 ng/ml

Sharma et al.²¹ reported the [detection and quantization of Trace Phenolphthalein by Liquid Chromatography-Tandem Mass Spectrometry](#), they mentioned that Phenolphthalein, an acid-base indicator and is an useful reagent in forensic science for apprehending erring officials accepting bribes in graft or trap cases. The pink-colored alkaline hand washes originating from the phenolphthalein-smear notes can easily be determined spectrophotometrically. But in many cases, colored solution turns colorless with time, which renders the genuineness of bribe cases doubtful to the judiciary. Claiming that no method is known till now for the detection and identification of phenolphthalein in colorless forensic exhibits with positive proof some authors used Liquid chromatography-tandem mass spectrometry which was found to be most sensitive, accurate method capable of detection and quantitation of trace phenolphthalein in colorless forensic exhibits with positive proof. The detection limit and calibration curve of phenolphthalein was found to be 1.66 pg/L or ng/mL, and $r^2 = 0.9974$ respectively.

CONCLUSION

The fading away of the color of alkaline phenolphthalein solution in trap cases can be due to two reasons i.e. either the PH of solution is out of limit /high or phenolphthalein has chemically broken down in to 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 . Various methods of analysis as reviewed and data so acquired can be utilized by the forensic scientists to interpret the chemical decomposition of phenolphthalein in alkaline solutions under prolong storage and with the use of strong alkaline solutions in the courts..

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