A New Chromogenic Reagent for the Determination of Phenobarbitone-Na

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Summary: Phenobarbitone-Na reacts with amino acetic acid in alkaline media after heating for 3 min at 100°C to give an orange yellow color having maximum absorbance at 330 nm. The reaction is selective for phenobarbitone-Na with 0.01 mg as visual limit of identification and provides a basis for a new spectrophotometric determination. The color reaction obeys Beer's Law from 0.01 mg to 3 mg/ml of Phenobarbital-Na and the relative standard deviation is 0.85%. Other drugs like atropine, phenytoin-Na benzodiazepine, chlorpromazine, etc were also studied for the interferences in the developed procedure.

Introduction

Phenobarbital-Na is a derivative of barbituric acid. It is a sedative, hypnotic and an epileptic drug. It is used for neuroses, related tension states and mild prolonged sedation in hypertension, coronary artery disease, functional gastrointestinal disorders and preoperative apprehensions. However like other CNS-depressant drugs, Phenobarbital-Na is abused and continuous use cause addiction. Other adverse effects are interstitial nephritis, hepatic dysfunction, and impairment of memory concentration, restlessness and rapid eye movement. [1, 2]

Many analytical techniques have been employed for the determination of Phenobarbital-Na. In the chromatographic method [3] the separation and determination of Phenobarbital-Na was carried out with methanol modified supercritical fluid and carbamazepine was used as an internal standard. The analytes were resolved on a Shendon-phenyl 5µm coloumn with 11.8% methanol in (1.5ml/min) and detected at 230 nm. Barbital, phenylbarbital and isoamylbarbital were determined by oscillopolarographic titration [4] using sodium diethyldithiocarbamate. In the HPLC [5], HPLC diod-assay [6] and caplliry GC [7] procedures Phenobarbital was determined by GLC C18 coloumn, methanolic 0.005 mol/l ml sodium hexanesulfonate solution (pH 3.0 was adjusted by 68% potassium dihydrogen phosphate buffer) with triethylamine (30:70) as the mobile phase, and detection at the wavelength of 220 nm [5], while optimal chromatographic conditions with extensive studies of the stability of the system had to be established [6]. In the spectrophotometric procedures [8-10], different colored charge transfer complexes depending on their solvent polarity were developed for the determination of Phenobarbital-Na, thiopental and phenytoin. Due to the fast development of the color complexes, thin layer chromatography was employed for the complex formation and detection [8], while elaborate extraction and filtration procedures were involved prior to the UV determination [9] and there is an error of about 5% in the simultaneous quantification of analytes [10] in the capillary electrophoresis [11, 12] liquid-liquid extraction or solid-phase extraction was carried out prior to the electrophoresis separation which was performed in the absence of the electro somatic flow [11], whereas non crossed lined acrylamide-N-isopropyleamide copolymers were used as a buffer additives in the capillary electrophoretic separation of structurally similar solutes after totally eliminating the contribution of electro somatic flow [12]. In the gas chromatography-atomic emission detection [13] the relative standard deviation was 10.21%. Stringent control of conditions is required in fluorescence polarization immunoassay [14] voltametry [15] and GC-MS [16].

During a systematic study of drug of abuse it was found that Phenobarbital-Na reacts with amino acetic acid in the presence of sodium hydroxide to give an orange-yellow color having maximum absorbance at 330 nm. The reaction obeys Beer's Law from 0.01 mg to 3 mg/ml and has 0.1mg/ml as visual limit of identification. The color reaction has yet not been reported in literature. The present method is simple accurate, precise and sensitive. The amount of other drugs like atropine, benzodiazepine, hlorpromazine, methohexitaland phenytoin-Na which does not interfere in the determination of pheno-

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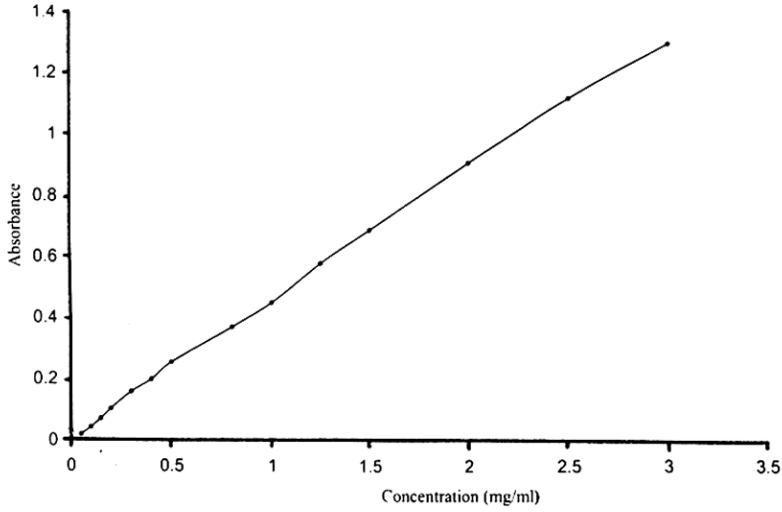

Fig. 1: Calibration curve of phenobarbitone-Na

barbitone-Na by the developed procedure was also studied.

Results and Discussion

Absorption Spectrum of the Colored Complex

Phenobarbital-Na reacts with amino acetic acid at pH 12.50 when heated for 3 min at 100°C to give an orange yellow color, the absorption maxima of which, under optimum conditions lies at 330 nm (Fig .2)

Effect of Color Producing Reagent and pH

Amino acetic acid was used as a color-producing reagent. It was found that 30mg/ml of amino acetic acid gave maximum color (Fig. 3) above and below this concentration the color intensity diminish and color become unstable. When Phenobarbital-Na is mixed with amino acetic acid without the addition of sodium hydroxide the pH of the contents was 3.35 and there was no color production, however on the addition of 1.5 ml of 1 N

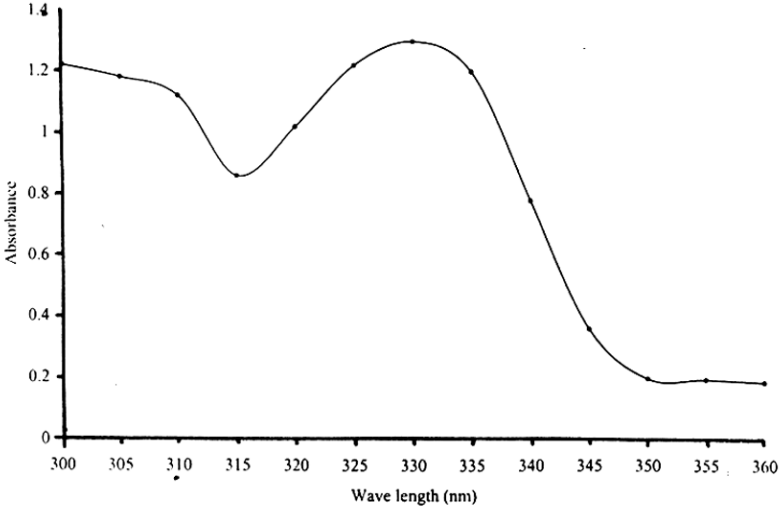

Fig. 2: Absorption spectra of phenobarbitone-Na

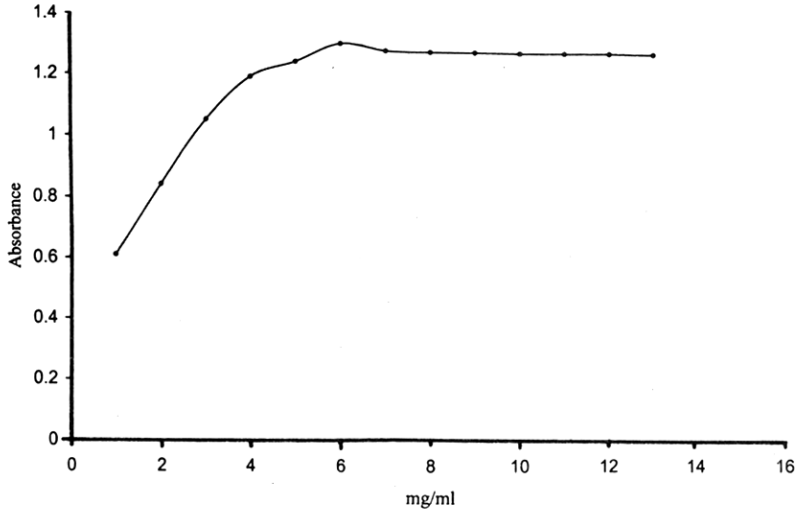

Fig. 3: Effect of color producing reagent

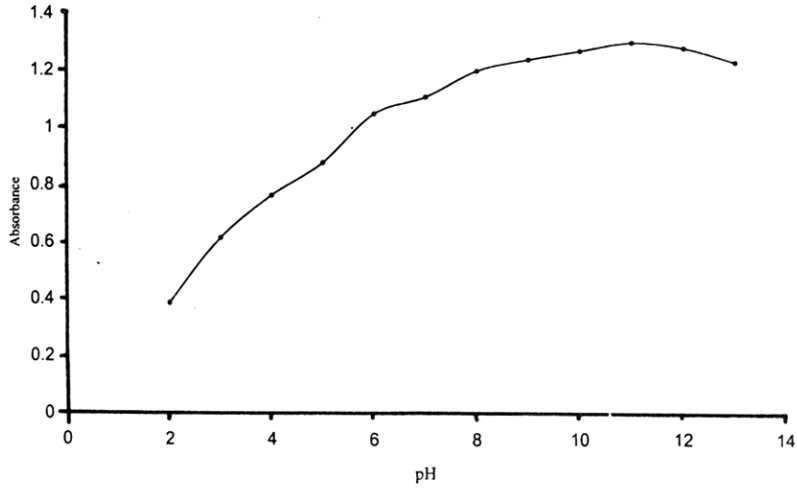

Fig. 4: Effect of pH

sodium hydroxide pH of the solution rises to 12.5 and maximum color intensity was achieved (Fig. 4). The probable mechanism of the color reaction is that Phenobarbital-Na having a weak acidic group reacts with sodium hydroxide to give a nitrogen containing anion, which acts as a strong nucleophile [17]. This nucleophile then attacks the electrophilic carbon of amino acetic acid giving an unstable di-hydroxy adduct which loses a water molecule to give an orange-yellow colored acid derivative showing maximum absorbance at 330 nm. The over all reaction is the replacement of OH group of amino acetic acid by a nucleophile.

Effect of Temperature and Time

The effect of temperature is shown in Fig. 5. The color does not developed at room temperature. With the rise of temperature the color intensity increased and was stable at 100°C.

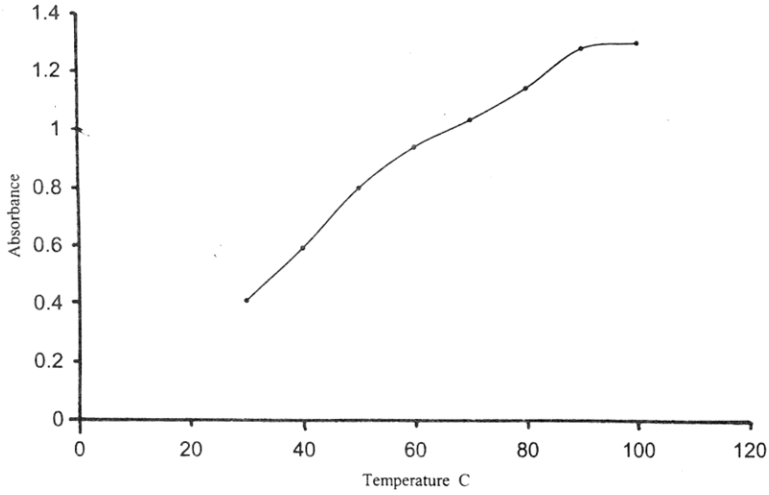

Fig. 5: Effect of temperature

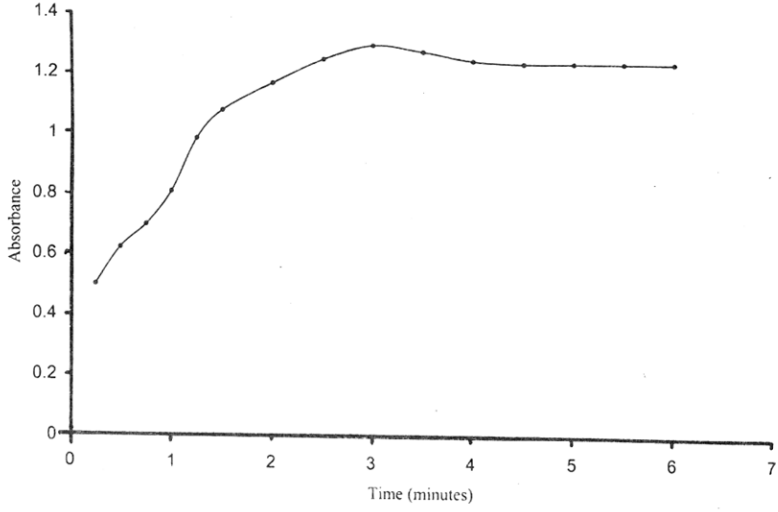

Fig. 6: Effect of heating time

The effect of heating time is shown in Fig. 6. It was found that heating for 3 min at 100°C gave maximum color, above and below these times and temperatures the color intensity decreased.

Effect of organic solvents

Different organic solvents such as chloroform, hexane, carbon tetrachloride, dichloromethane, benzene, xylene and amylalcohol were tested for color extraction and for stability. Since none were effective therefore no organic solvent was employed.

Sensitivity of the developed procedure

The results for the determination of Phenobarbital-Na are shown in Tables 1 and 2, which show the sensitivity, validity and reproducibility of the method. It is also reasonably precise and accurate, as the amount taken from identical samples is known and the amount found does not exceed the relative standard deviation of 0.85% (c.f Table 1). The optimization has been done at lower analyte concentration. The calibration graph is linear in the range of 0.01mg to 3 mg/ml. The apparent molar

Table 1:Determination of Phenobarbital-Na from pure solution

Phenobarbitone-Na Taken (mg)	Phenobarbitone-Na found* (mg)	Relative standandard deviation (%)
0.10	0.098	0.85
0.15	0.148	0.61
0.20	0.199	0.78
0.25	0.251	0.83
0.50	0.508	0.37
1.00	1.02	0.15
1.50	1.409	0.31
2.00	2.05	0.40
2.50	2.47	0.67
3.00	3.07	0.25

*Every reading is an average of three independent measurements

Table 2: Optical characteristics, precision and accuracy of the proposed method

Parameters	Values
λ_{max} (nm)	330
Beer's Law limit (mg/ml,C)	0.01-3.0
Molar Absorbitivity ($mol^{-1}mol^{-1}$)	0.1085×10^4
Limit of detection (mg/ml)	0.01
Regression equation (Y)*	
Slope (b)	0.466
Intercept (a)	0.033
Correlation coefficient (r)	0.999
Relative standard deviation (RSD)**	0.85
% age range of error (confidence limit) at (95%) confidence level	100.08±0.947

*Y=a+Cb Where C is the concentration of analyte (mg/ml) and Y is the absorbance unit

**Calculated from five independent measurements.

Table-3: Quantitative assessment of tolerable amount of other drugs.

Drugs	Maximum Amount not Interfering (%)
Acetyl salicylic acid	247
Atropine	109
Barbituric acid	150
Benzodiazepine	219
Brucin	267
Buscopan	183
Chloral hydrate	195
Chlorpromazine	291
Haloperidol	175
Phenytoin-Na	130
Propanolol-HCl	210

*The value is the percentage of the drug with respect to the 1mg/ml of phenobarbitol-Na that causes ±0.01 change in absorbance

absorbitivity is 1.085×10^3 $mol^{-1}cm^{-1}$ and the regression equation [18] was calculated by the method of least square from fourteen points, each of which is the average of five determinations. The correlation between absorbance and concentration is 0.999 in terms of correlation coefficient.

Interferences of other drugs

The quantitative assessment of tolerable amount of different drugs (w/v) under the experimental condition is given in Table 3. Various amounts of diverse interfering compounds like atropine, benzodiazepine, chlorpromazine, phenytoin-Na were added to a fixed amount of Phenobarbital-Na (1mg/ml) and the recommended procedure for the spectrophotometric determination was followed.

Applications of the developed procedure

The proposed method is successfully applied for the quality control of pure Phenobarbital-Na and in pharmaceutical dosage form as shown in Table 4. The excepients in the formulations did not interfere with the analysis.

Experimental

Apparatus

Hitachi U 1100 spectrophotometer with 1 cm silica cells was used to measure the absorbance. Beckman Zero metric pH meter and graduated pipettes were employed.

Reagents

Analytical grade chemicals and doubly distilled water were used.

Phenobarbital-Na (Unexo Labs. Pvt. Ltd.) Standard solution (w/v) (1mg/ml) was prepared by dissolving 100 mg in distilled water to give a stock solution, which was further diluted as required. 1% (w/v) amino acetic acid (Light and Co. Winbrook) was prepared by dissolving 1 gm of amino acetic acid

Table-4: Determination of Phenobarbital-Na from pharmaceutical preparations

Drug	Trade name	Pharmaceutical preprations	Amount Present	Amount found* (mg)	Recovery (%)
Phenobarbital-Na	Phenobarbital (Unexo Labs. (Pvt.) Ltd.)	Tablets	30.0	29.89	99.60
Phenobarbital-Na	Phenotab (Wilson's Pharma)	Tablets	30.0	30.05	100.60
Phenobarbital-Na	Phenobarbitone(Ethical Laboratories Pvt Ltd)	Tablets	60.0	58.99	98.13
Phenobarbital-Na	Phenobarbitone (Remington Pharma)	Tablets	30.0	29.18	97.26
Phenobarbital-Na	Phenobarbital (Dosaco Labs.)	Tablets	30.0	30.12	100.40

in distilled water. 1 M sodium hydroxide was prepared in distilled water.

General Procedure

To an aliquot of Phenobarbital-Na containing 0.01mg to 3 mg/ml was added 3 ml of 1% amino acetic acid and the pH of the solution was adjusted to 12.50 by the addition of 1.5 ml of 1 M sodium hydroxide. The contents were then heated for 3 min in a water bath at 100°C. The volume was made up to 10 ml with distilled water. The resulting absorbance of the orange- yellow color was measured at 330 nm, employing all reagents except Phenobarbital-Na as a blank. The experiment was repeated with different volumes of standard Phenobarbital-Na solution and a calibration curve was prepared (Fig. 1). The color reaction obeys Beer's Law from 0.01 mg to 3 mg/ml of Phenobarbital-Na

Procedure for Studying Interfering Compounds

To an aliquot containing 1mg/ml of Phenobarbital-Na, different amounts of various interfering organic compounds like atropine, benzodiazepine, chlorpromazine, phenytoin-Na (1 mg/ml) (w/v) were added individually until the solution shows the same (±0.01) absorbance as that of pure Phenobarbital-Na with out the addition of the organic compounds, under experimental conditions as described in general procedure. The amount of other drugs that did not interfere was calculated in percentage with respect to the amount of Phenobarbital-Na.

Procedure for Determination of Phenobarbital-Na in Pharmaceutical Preparations

Tablets of phenobarbitone-Na: Tablets containing 30 mg, 250 mg or 500 mg phenobarbitone-Na were powdered weighed, dissolved in distilled water and filtered. The filtrate was diluted further to get a 1 mg/ml solution of Phenobarbital-Na. An aliquot containing 0.01mg to 3mg/ ml was taken, the procedure was followed as described above and the absorbance was measured at 330 nm. The quantity per tablet was calculated from standard calibration graph.

Injections of phenobarbitone-Na: The appropriate volume of injection was taken and diluted with distilled water to get 1 mg/ml solution of phenobarbitone-Na. The above procedure was followed using an aliquot containing 0.01mg to 3 mg/ml and the absorbance was measured at 330 nm. The quantity per injection was calculated from the standard calibration graph

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