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UV spectroscopic method for determination of phenytoin in bulk and injection forms

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ABSTRACT

Recent study was conducted to develop a simple UV spectrophotometric method to determine Phenytoin in bulk and injection form according to official requirement and validate as per ICH guidelines. λ_{\max} of Phenytoin was found 202 nm. Linearity existed perceived in the concentration assortment 2-8 $\mu\text{g/ml}$ ($r^2 = 0.999$) for the method. The method was validated pertaining to linearity, precision and accuracy studies, LOD and LOQ consistent with ICH guidelines. The existent method was establish to be simple, linear, precise, accurate as well as sensitive and can be applied for routine quality control enquiry for the analysis of Phenytoin in bulk and injection form.

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Capsule Summary: A simple UV spectrophotometric method was developed to determine phenytoin in bulk and injection form according to official requirement and validate as per ICH guidelines.

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INTRODUCTION

Epilepsy is a highly shared disorder, regarded as seizures, which proceeds numerous forms and consequence from intermittent neuronal releases, the form of the seizure contingent on the chunk of the brain exaggerated (Madhavi et al., 2012). Phenytoin is extremely effective and broadly recommended anticonvulsant agent prescribed for the management of grand mal as well as psychomotor epilepsy. Phenytoin stayed prescribed to treat thought, mood and Behavior disorders, Neuro muscular disorders, cardiovascular disorders, Endocrine disorders and gastrointestinal disorders (Karthikeyan et al., 2009) It had better not to be given for management or prevention of eclamptic or else alcohol-related seizures. There is inadequate indication concerning its use in precluding febrile convulsions, treating or preventing seizures due to space

inhabiting abrasions or intracerebral haemorrhage and thrombosis (Gallop, 2010). It can be prescribed to control seizures arising through neurosurgery and to contrary digitalis-induced arrhythmia. Phenytoin is similarly prescribed in a 10% ointment preparation to endorse curing of ulcers in patients with diabetes. In the past, Phenytoin was prescribed for treatment of acute alcoholism, migraine, polyneuritis, pregnancy disorders, certain psychoses, in addition to trigeminal neuralgia. (Younes, N et al., 2006) The major effects of PS are to decrease excitatory neurotransmission and enhance GABA-mediated inhibition (Mulsa et al., 2012).

Phenytoin sodium (Fig. 1) is 5,5-diphenylimidazolidine-2, 4-dione sodium salt. Phenytoin sodium belongs to the category of drugs referred to as anticonvulsant and anti-epileptic. Phenytoin is one of the most commonly used antiepileptic medications in clinical practice for generalized seizures. It is prescribed to avoid as

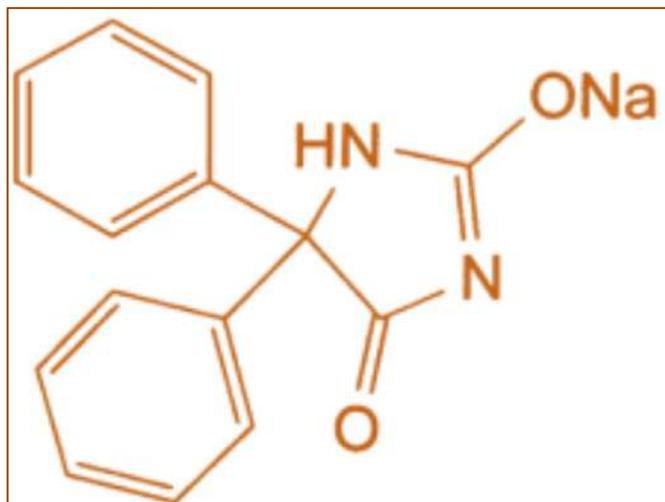


Fig. 1: Chemical Structure of Phenytoin

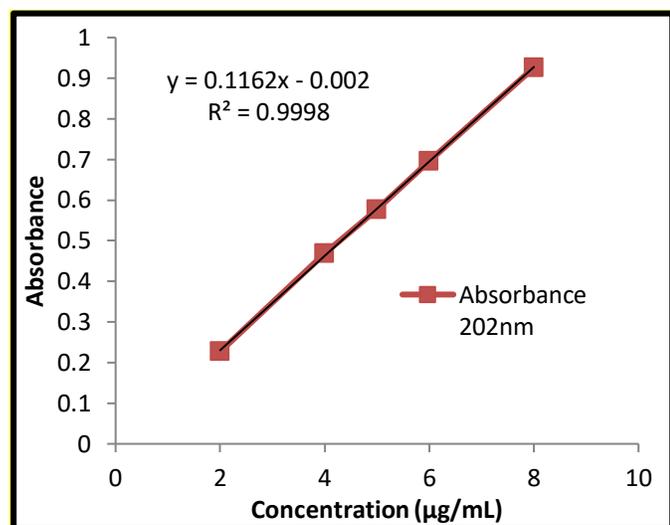


Fig. 2: Calibration curve for Phenytoin

well as control seizures. It acts by dropping the extent of seizure movement in the brain. Phenytoin works on sodium channels at neuronal cell membrane, regulating the extent of seizure commotion and plummeting seizure promulgation. Through endorsing sodium efflux commencing neurons, Phenytoin inclines to steady the threshold in contradiction of hyper impulsiveness instigated by extreme stimulation or ecological variations proficient of plummeting membrane sodium gradient. This embraces the lessening of post-tetanic potentiation on synapses, averts cortical seizure foci from exploding contiguous cortical areas (Shah et al., 2017). Phenytoin utilizes an advantageous consequence through reducing seizures simply for the duration of the first week subsequently severe head injury (Temkin et al., 1990). Magnesium sulfate is superior to Phenytoin for the preclusion of eclampsia in hypertensive pregnant women (Lucas et al., 1995). Steady-state serum-Phenytoin intensities

are dignified to regulate the dosage to attain the appropriate therapeutic concentration (Richens, and Dunlop, 1975).

Different Methods have been developed for HPLC and UV determination of Phenytoin sodium in bulk forms either alone or in combinations (Malsa et al., 2012; Shah et al., 2017; Bagade et al., 2014; Prasad et al., 1997; Lensmeyer et al., 1997; Lu-Steffes et al., 1982; Dykeman and Ecobichon, 1979; Bhatti et al., 1998; Sawchuk and Cartier, 1980; Abbaspour and Mirzajani, 2005; Mei and Williams, 1997; Dean et al., 1983; Kishore et al., 2003; Santagati et al., 2005; Vedsö et al., 1969; Castro et al., 1978; Löscher and Göbel, 1978; Cwik et al., 1997). But no UV method was available for the determination of Phenytoin sodium in Injection form. Our aim was to develop a simple, inexpensive and rapid method for the determination of Phenytoin sodium in both bulk and injection forms. The present research work describes the estimation of assay content of Phenytoin in active pharmaceutical ingredient (API) and ampoule dosage form using ultraviolet-Visible (UV-Vis) spectrophotometry technique. The work provides a sensitive, specific, as well as economical method for the determination of Phenytoin in very short time by the UV-Vis spectrophotometer. Distilled water is used as a diluent established on the drug solubility properties. Developed UV-Vis spectrophotometric method was validated with respect to Official guidelines.

MATERIAL AND METHODS

Chemical, reagents and instruments

Phenytoin, distilled water. Glass wares of pyrex material were incorporated. Weighing balance 'Shimadzu Japan' and Spectrophotometer (UV-1601), UV/Visible

Table 1: Calibration data of phenytoin for absorbance maxima

Concentration (µg/mL)	Absorbance
2	0.227
4	0.469
5	0.577
6	0.696
8	0.926

Table 2: Parameters from the calibration curve

Parameters	Observation at 202 nm
Calibration Curve	Linear
Expression	$y = mx + c$
Factor (y intercept)	0.002
Factor (slope)	0.1162
Coefficient (r2)	0.9998

spectrophotometer, Shimadzu Japan' were used.

Method development

Selection of wave length detection

Phenytoin was run on UV spectrophotometer at 200-400 nm and the λ_{\max} was found 202 nm.

Preparation of standard stock solution

Standard stock solution of phenytoin was organized by

suitable amount of solution was occupied to make auxiliary dilutions using distilled water to give 5 $\mu\text{g}/\text{ml}$. Absorbance was measured at 202 nm against standard solution in triplicate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limits of detection (LOD) can be defined as the lowest concentration of the analyte that the analytical method can reliably differentiate from the background. Limits of quantification (LOQ) can be defined as the lowest

Table 3: Accuracy and recovery of the developed method

S. No	Level	Amount recovered	% recovery
1	80	79.84	99.80
2	100	99.97	99.97
3	120	120.12	100.10

Table 4: Statistical evaluation of inter-day and intra-day precision studies

	Inter-day	Intra-day
Mean absorbance	0.5837	0.5857
SD	0.0006	0.0012
%RSD	0.0989	0.1972

thawing 10 mg of phenytoin in a 100 ml volumetric flask. Final volume was made up to 100 ml with distilled water to get working standard stock solution containing 100 $\mu\text{g}/\text{ml}$ of phenytoin and further dilutions were made by using distilled water.

Calibration curve for phenytoin

Serial dilutions were prepared with distilled water to obtain concentration in range 2-8 $\mu\text{g}/\text{ml}$. The spectrum was recorded, absorbance was measured at 202 nm and a calibration curve was plotted.

Validation parameters

According to ICH guidelines the aforementioned method was validated.

Linearity

The linearity of method was appraised by evaluating diverse concentrations of the standard solution of Phenytoin. Beer's law was obeyed in the concentration range 2-8 $\mu\text{g}/\text{ml}$.

Accuracy

A series of Samples were prepared for the range 80 to 120% for accuracy testing and the results were estimated by checking absorbance at 202 nm.

Precision

10mg of Phenytoin was deliberated precisely then dissolved in 100 ml of distilled water. From the standard stock solution

concentration that can be quantified with acceptable accuracy and precision.

Specificity

The specificity of the method was documented by preparing Placebo and sample. Both scanned at the wavelength of active.

Method application

Developed method was successfully applied to perform the assay of Phenytoin sodium injection available in the local market. The label claim of the injection was 250 mg per 5 milliliter. Suitable dilutions (5ppm) were made for both standards. A 20 mg of working standard was dissolved in distilled water and milliliter of this solution was diluted in 200 ml distilled water as a diluent. Solutions were prepared in duplicates for 2 milliliter of the injection was diluted in 200 ml volumetric flask using distilled water as a diluent and finally 2 ml of this solution was diluted in 200 ml flask using distilled water as a diluent. Absorbance of both solutions was measured at 202 nm on UV Spectrophotometer.

Table 5: LOD and LOQ of phenytoin

Parameters	Absorbance Maxima Method
LOD	0.0568 $\mu\text{g}/\text{mL}$
LOQ	0.1721 $\mu\text{g}/\text{mL}$

Table 6: Absorbance and percent assay of phenytoin

Phenytoin Injection	Absorbance At 202 nm	% Assay
Sample 01	0.614	100.41
Sample 02	0.616	100.74

RESULTS AND DISCUSSION

Optimization of UV-Vis spectrophotometric method conditions

The main purpose of the current method is to develop a simple, sensitive, and precise UV-Vis spectrophotometric method for the assessment of Phenytoin for the routine quantitative estimation of samples which will reduce dreary sample preparations, cost of resources and manpower obligatory to perform the analysis. The spectral analysis indicated that the λ_{max} of Phenytoin is 202 nm. Simple diluent was selected for the standard and sample solutions of Phenytoin drug substance. The developed method was applied for the assay of Phenytoin sodium injection available in local market of Karachi, Pakistan. Thus, the established UV-Vis spectroscopic method for the analysis of Phenytoin in its API and injection form enables analysis of several samples at the same time due to its simplicity in performing the analysis.

Method validation

According to ICH guidelines the aforementioned method was validated.

Linearity

The linearity graphs were plotted between the absorbance versus concentration to obtain the calibration curve. The data is recorded and shown in Table 1. The correlation coefficient was found to be 0.999. Linearity graph is shown in Figure 2. Results demonstrate that an excellent correlation between the absorbance and concentration of Phenytoin drug substance and shown in Table 2.

Accuracy

The percentage recovery results for Phenytoin were varied from 99.80% to 100.10% at three different concentration levels, and the results were shown in Table 3. Based on the % recovery data, it was concluded that the developed method is capable for the estimation of Phenytoin drug substance and is adequate for routine analysis.

Precision

The percent assay value difference was determined for solutions stored at room temperature for 24 hrs. Phenytoin solution found to be stable up to 24 hours at room temperature. Solution stability results at room temperature are shown in Table 4.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

LOD in addition to LOQ were appraised via the regression equation. Results are shown in Table 5.

Specificity

The developed method is specific for the aforementioned drug and no hindrance was observed in placebo and in sample. Assay of phenytoin sodium injection was performed on the developed method and found to be satisfactory. Results of the assay are shown in Table 6. So far, this simple UV spectrophotometric method can be used for the estimation of Phenytoin (Bansal et al., 2017; Gujjar et al., 2017; Jouyban-Gharamaleki et al., 2017; Kopsky and Keppel Hesselink, 2017; Margiotti et al., 2017; Selioutski et al., 2017; Serafini, 2017; Sridharan and S, 2017; Teo et al., 2017; Uribe-San-Martín et al., 2017), which is validated as per ICH guidelines.

CONCLUSIONS

UV-spectrophotometric method was established and was validated consistent with ICH guidelines for Phenytoin in bulk powder. Coefficient of correlation was found in the range of 0.999 for the drug as well as %RSD was < 2%. Therefore, it can be clinched that the developed method is accurate and precise and can be employed effectively for the assessment of Phenytoin in API and injection form.

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