



Assay of Sapropterin Dihydrochloride in Tablet Dosage Form Using Aromatic Aldehyde

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Abstract

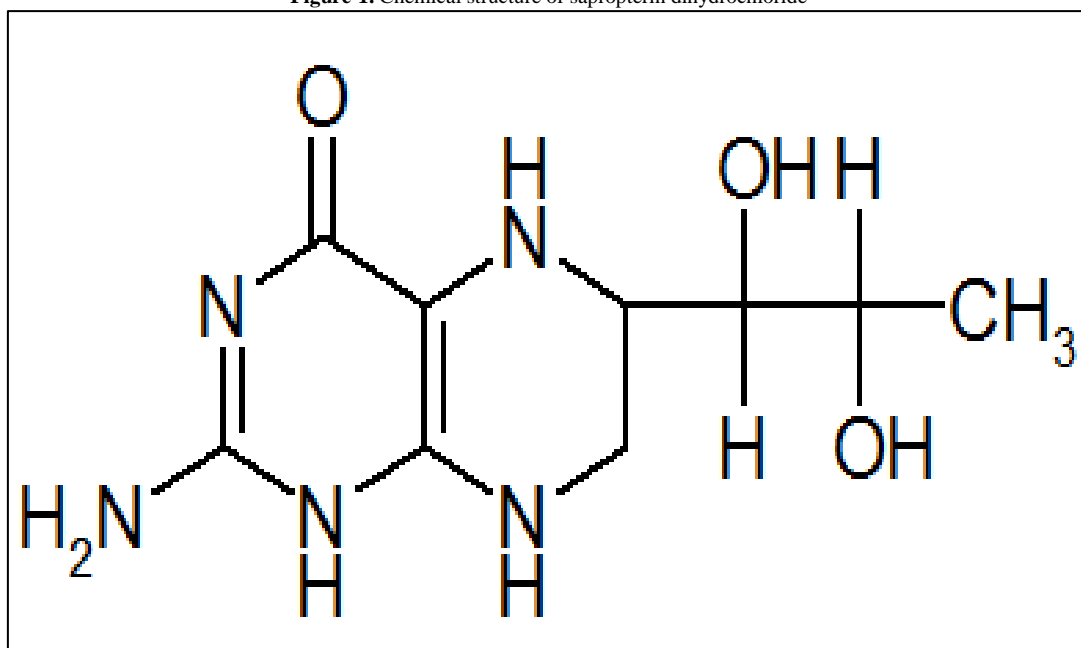
A simple and sensitive visible spectrophotometric method has been developed for the determination of sapropterin dihydrochloride in pure and tablet dosage forms. This method is based on the condensation reaction of drug with aromatic aldehyde such as Para dimethyl amino cinnamaldehyde (PDAC) in the presence of sulphuric acid in non aqueous medium and formed purple red colored Schiff base product with an absorption maximum of 534nm. The Beer's law obeyed in the concentration range of 10-30µg/ml. The proposed method is applied to commercial available Kuvan tablets and the results are statistically compared with those obtained by the reference UV method and validated by recovery studies.

Keywords: Estimation; PDAC; Regression analysis; Tablets; Visible spectrometry.

1. Introduction

Sapropterin (SAP) (Figure 1) is an enzyme cofactor and oral form of a synthetic preparation of the dihydrochloride salt of naturally occurring tetrahydrobiopterin(BH₄) [1-3]. It is chemically designated as (6R)-2-amino-6-[(1R,2S)-1,2-dihydroxypropyl]-6,7,8-tetrahydro-4(1H)-pteridinone dihydrochloride. Its empirical formula is C₉H₁₅N₅O₃·2HCl representing molecular weight of 314.17. It is an off white to slightly yellow crystalline powder that is soluble in water and methanol. BH₄ works with phenylalanine hydroxylase to metabolize phenylalanine (Phe). Sapropterin dihydrochloride tablets are indicated to reduce blood phenylalanine (Phe) levels in patients with hyperphenylalaninemia (HPA) due to tetrahydrobiopterin- (BH₄⁻) responsive Phenylketonuria (PKU) and to be used in conjunction with a Phe-restricted diet.

Figure-1. Chemical structure of sapropterin dihydrochloride



Literature survey reveals that no spectrophotometric method was reported. Only analytical methods such as HPLC [4-8], have been reported for the determination of SAP in biological fluids and formulations. For routine analysis, simple, rapid and cost effective visible spectrophotometric methods are required and preferred. The availability of the UV-Visible spectrophotometric methods with high sensitivity and selectivity will be very useful for quality control analysis and small scale pharmaceutical industries. The functional groups present in the drug have

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not been exploited properly in developing visible spectrophotometric methods. Nevertheless, there is a need for development of sensitive accurate and flexible visible spectrophotometric methods for the determination of SAP in pharmaceutical preparations. So the authors have made some attempts in this direction and succeeded in developing this method based on the condensation reaction of drug with PDAC as a reagent Gowri, *et al.* [9] under specified experimental conditions.

The proposed method for SAP determination has many advantages over other analytical methods due to its rapidity, normal cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and not costly. All the analytical reagents are inexpensive and available in any analytical laboratory. The method can be extended for the routine quality control analysis of pharmaceutical products containing SAP.

2. Materials and Methods (Experimental)

2.1. Apparatus and Chemicals

A Shimadzu double UV/Visible spectrophotometer model-1800 with 10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361 was used for pH measurements. All the chemicals used were of analytical grade. PDAC (E. Merck, 0.1% w/v $6.31 \times 10^{-3} \text{M}$) in methanol, Sulphuric acid (14M) was prepared.

2.2. Preparation of Standard Drug Stock Solution

The standard stock solution (1mg/ml) of SAP was prepared by dissolving 100mg of SAP in 100 ml methanol. This solution was further diluted stepwise with the same solvent to obtain working standard solution concentration of $100 \mu\text{g/ml}$. The prepared stock solution was stored at 4°C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

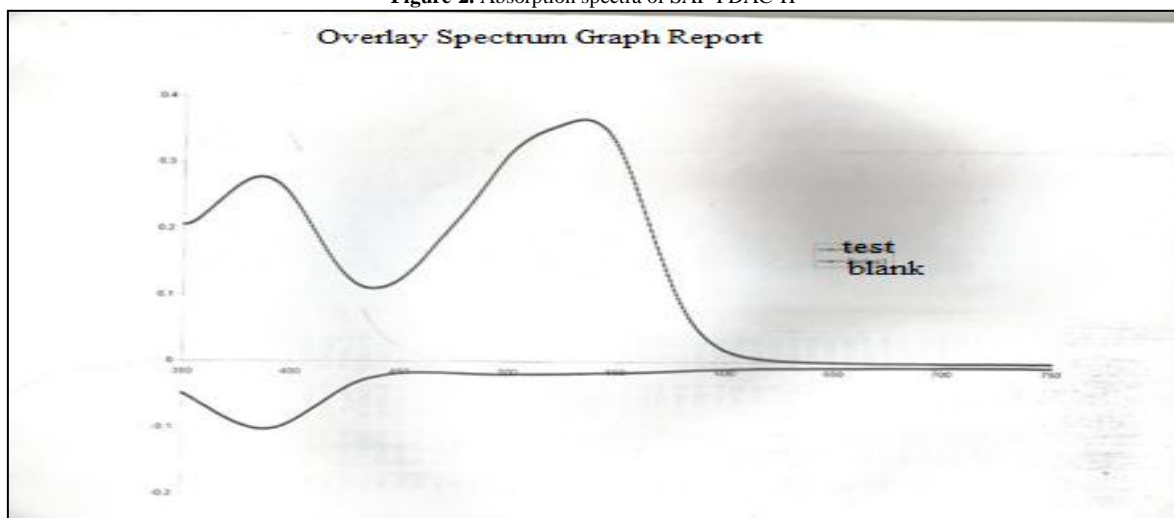
2.3. Preparation of Sample Solution

About 20 tablets were weighed to get average tablet weight and pulverized. The powder equivalent to 100mg of SAP was weighed and dispersed in 25ml of IPA, sonicated for 30 minutes and filtered through Whatman filter paper No 41. The filtrate was evaporated and the residue was dissolved in 100 ml of methanol (1mg/ml). It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

2.4. Determination of Wavelength Maximum (λ_{max})

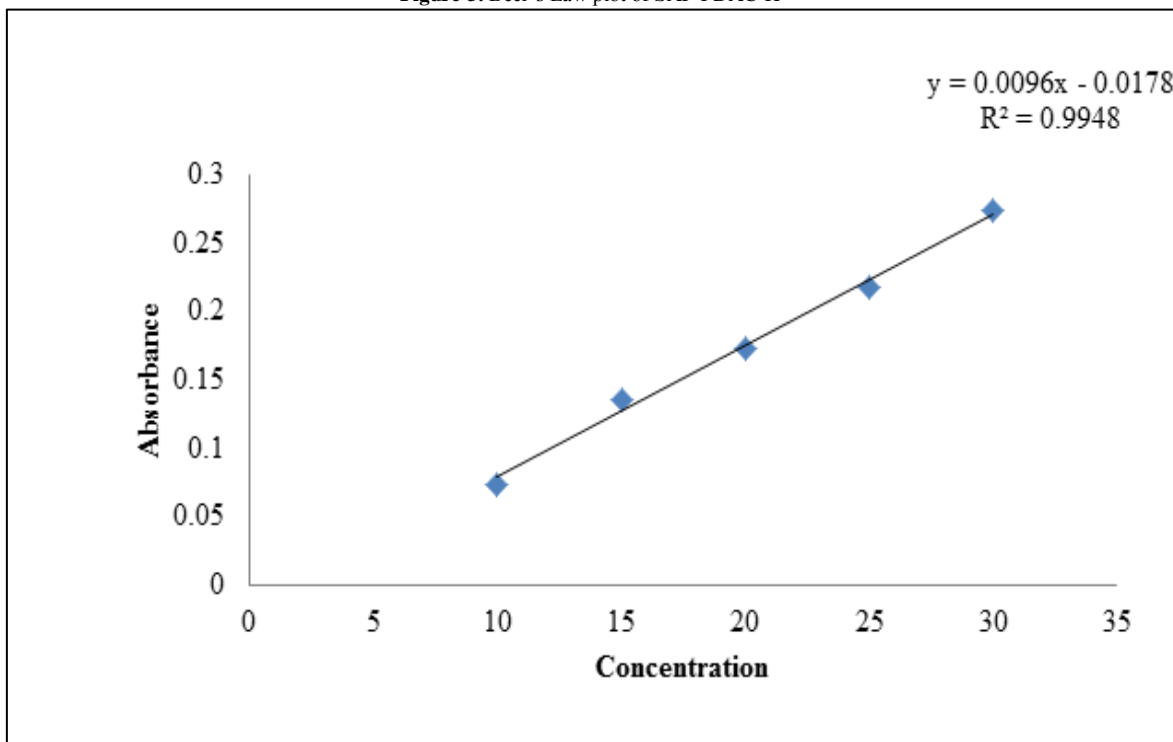
The 3.0 ml of working standard solution of SAP ($100 \mu\text{g/ml}$) in methanol was taken in 10ml calibrated tubes and volume of test tube adjusted to 3.0ml with methanol. To this 1.0 ml of PDAC ($6.31 \times 10^{-3} \text{M}$) and 1.0 ml of concentrated sulphuric acid (14M) were added, while cooling under a tap with constant shaking and kept in water bath at 60°C for 10min. cooled and diluted to the mark with methanol and sonicated for 1 min. to get a concentration of $30 \mu\text{g/ml}$. In order to investigate the wavelength maximum, the above standard stock solution was scanned in the range of 400-660nm by UV-Visible spectrophotometer. From the spectra (Figure 2), it was concluded that 534nm is the most appropriate wavelength for analyzing SAP with suitable sensitivity.

Figure-2. Absorption spectra of SAP-PDAC- H^+



2.5. Preparation of Calibration Curve

Aliquots of alcoholic standard drug SAP solution ($1.0 \text{ml} - 3.0 \text{ml}$, $100 \mu\text{gml}^{-1}$) was transferred into a series of 10 ml calibrated tubes and volume of each test tube adjusted to 3.0 ml with methanol. To each of these tubes 1.0 ml of ($6.31 \times 10^{-3} \text{M}$) PDAC and 1.0 ml Conc. H_2SO_4 (14M) were added while cooling under a tap with constant shaking and kept in water bath at 60°C for 10 minutes. Then the flasks were cooled and made up to the mark with methanol. The absorbance was measured within 10 minutes at 534 nm against a reagent blank prepared simultaneously omitting drug. The amount of drug present was calculated from its calibration graph (Figure 3)

Figure-3. Beer's Law plot of SAP-PDAC-H⁺

3. Results and Discussions

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits) were calculated and Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (Se), % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table 1.

Kuvan tablets containing SAP were successfully analyzed by the proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t- and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in Table 2

Table-1. Optical Characteristics, Precision and Accuracy of Proposed Method

Parameter	Values
λ_{\max} (nm)	534
Beer's law limit ($\mu\text{g/ml}$)	10-30
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ abs. unit)	0.011560694
Molar absorptivity (Litre/mole/cm)	27175.705
Correlation Coefficient	0.994
Regression equation (Y)*	
Intercept (a)	-0.017
Slope(b)	0.009
%RSD**	2.02
% Range of errors (95% Confidence limits)	
0.05 significance level	2.12
0.01 significance level	3.32

*Y = a + b x, where Y is the absorbance and x is the concentration of SAP in $\mu\text{g/ml}$

**calculated from six determinations

The interference studies in the determination of SAP in pharmaceutical formulation revealed that the normally existing excipients and additives like starch, talc, stearic acid, boric acid, gelatin, magnesium carbonate and sodium lauryl sulphate were found not to interfere even when present in excess (1-100 folds). However, preliminary clean up procedure with CHCl_3 is necessary prior to the estimation of SAP in formulations if lactose is present. The ingredients usually present in formulations of SAP did not interfere with the proposed analytical method. Among the four aromatic aldehydes (vanillin, PDAC, PDAB and anisaldehydes) tried, all of them responded. But PDAC was preferred as they were found to be better sensitivity in the assay of SAP. MS Excel Software-2007 used for

calculations and graphs. The proposed method is found to be simple, sensitive and accurate and can be used for the routine quality control analysis of SAP in bulk and dosage forms.

Table-2. Analysis of Sap in Pharmaceutical Formulations

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Method			Found by Reference Method \pm SD	#% Recovery by Proposed Method \pm SD
			**Amount found \pm SD	t	F		
SAP-PDAC-H ⁺	Tablet-1	100	98.47 \pm 1.04	1.36	1.1	98.04 \pm 1.07	98.471 \pm 1.04

* Tablet- 1: KUVAN tablets of Bio Marin Pharmaceuticals Inc USA

S**Average \pm Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits t =2.57 and F = 5.05.

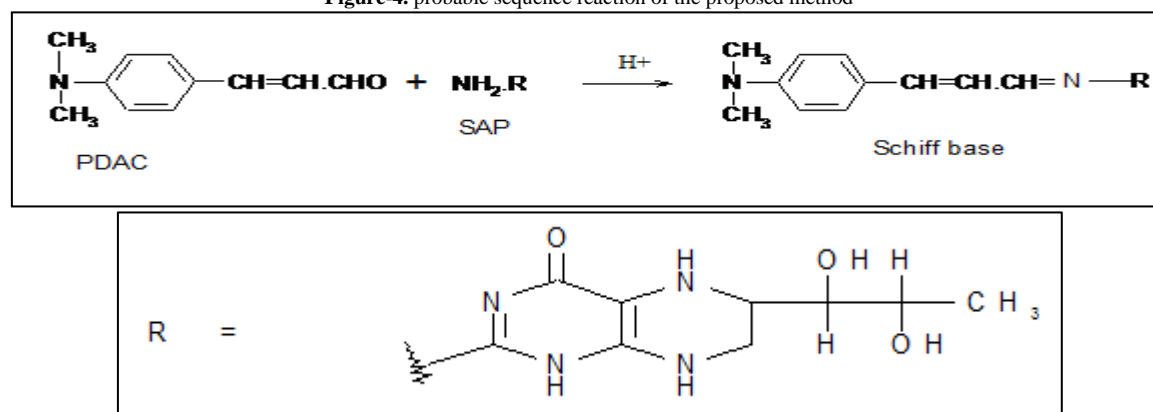
Recovery of 10mg added to the pre analyzed sample (average of three determinations).

Reference method (UV method) using methanol developed in our laboratory (λ_{\max} =224nm).

3.1. Chemistry of Colored Species

In the present investigation, it was observed that SAP (due to presence of Hetero cyclic amino moiety) furnish a colored condensation product with aromatic aldehyde (PDAC which are better in sensitivity than vanillin, PDAB and anisaldehyde) in the presence of H₂SO₄ in non aqueous medium. The formation of colored species with the reagent may be assigned through above analogy as shown in Figure 4.

Figure-4. probable sequence reaction of the proposed method



4. Conclusion

The reagents utilized in the proposed method are normal cost, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed method possesses reasonable precision, accuracy and is simple, sensitive and can be used as alternative method to the reported ones for the routine determination of SAP depending on the need and situation.

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References

- [1] Available: <http://www.drugbank.ca/drugs/DB00360>
- [2] Martindale The complete drug reference, 2383(2009). vol. 36,
- [3] The Merck Index, 2001. 13th ed., p. 1501.
- [4] Bhavani, V., Siva, R. T., and Jamelunnisa, B., 2014. "Stress degradation behavior of Sapropterin dihydrochloride and development of suitable stability indicating RP-HPLC method for the determination of sapropterin dihydrochloride in tablet dosage form." *Indo American Journal of Pharmaceutical Research*, vol. 4, pp. 3077-3092.
- [5] Janaki, P. P., Appala, R. N., and Parvathalu, G., 2012. "The estimation of Sapropterin dihydrochloride in tablet dosage form by RP-HPLC." *Asian J. Pharm. Ana.*, vol. 2, pp. 110-113.
- [6] Khan, A., Bhojani, M., Rajput, M., Revathi, P., Biswas, S., and Arifkhan, 2013. "Development and validation of a LC-tandem mass spectrophotometry method for direct determination of tetrahydrobiopterin-an enzyme cofactor in human plasma." *International Journal of Pharmaceutical and Chemical Sciences*, vol. 2, pp. 695-704.

- [7] Viplava, P. U., Syam, b. M., and Kalyana, R. B., 2017. "Sensitive visible spectrophotometric methods development for estimation of Sapropterin dihydrochloride in tablet dosage form." *Research Journal of Pharmaceutical Dosage Forms and Technology*, vol. 9, pp. 33-37.
- [8] Viplava, P. U., Syam, b. M., and Kalyana, R. B., 2017. "Determination of Sapropterin dihydrochloride in solid dosage forms by visible spectrophotometry." *Asian Journal of Pharmaceutical Analysis*, vol. 7, pp. 75-78.
- [9] Gowri, S. D., Sastry, C. S. P., and Narayana, R. M., 1991. "The determination of resorcinol amines with aromatic aldehydes." *Indian Drugs*, vol. 28, pp. 269-273.