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DEVELOPMENT AND VALIDATION OF NOVEL ANALYTICAL METHODS FOR ASSAY OF EFAVIRENZ IN PHARMACEUTICAL FORMULATIONS

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Abstract:

A simple and specific visible spectrophotometry method was developed and validated in order to assay of Efavirenz in pharmaceuticals .The analogy of the method was based on the reactivity of primary aromatic amine which is resulted due to the hydrolysis of Efavirenz and treated with 2 % w/v solution of p-dimethylamino benzaldehyde, a yellow colour chromogen is obtained as a Schiff's base which can be estimated at 406 nm using a spectrophotometer serves the basis for proposed method. The linearity for spectrophotometric methods was established it obey beer's law in the concentration range 100-500 µg/ml and the correlation coefficient was found as 0.9995. Limit of detection and limit of quantitation was found to be 0.125 and 0.375 for the developed method. The % RSD was found as 0.688 and 0.87 for intra-assay (repeatability) and intermediate precision respectively. The % recovery of the proposed method lies in the range 100.98-101.47. The method is simple and suitable for the determination of Efavirenz in formulation.

Keywords: *Efavirenz, spectrophotometry, p-dimethylamino benzaldehyde, validation.*

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INTRODUCTION:

Efavirenz can be chemically described as**(s)** -6-chloro – (cyclopropylethyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one (figure 1). The trade name of Efavirenz is Sustiva, Scrotin. It belongs to the class of Non-nucleoside reverse transcriptase inhibitors (NNRTI's) of category Antiretroviral. The molecular mass of Efavirenz 315.7gm/mol. It is freely soluble in methanol, dichloromethane, water. It is administered orally and the available dose is 600 mg daily in divided doses. It is a medicine approved by the U.S. Food and drug administration (FDA) for the treatment of HIV infection in adults and in children 3 months of age and older who weigh at least 7 pounds 12 ounces (3.5 kg). It is always used in combination with other HIV medicines. It is never used alone and is always used in combination with other drugs. Psychiatric symptoms are insomnia, nightmares, confusion, memory loss, anxiety are common. Efavirenz lowers blood levels of most protease inhibitors and also Garlic supplements may decrease Efavirenz blood levels. The Bioavailability of Efavirenz is 40-45% and Protein binding is about 99.5-99.75%.The Onset of actions 3-5 h and Biological half life is 40-55hrs and finally excreted through Urine and faeces.

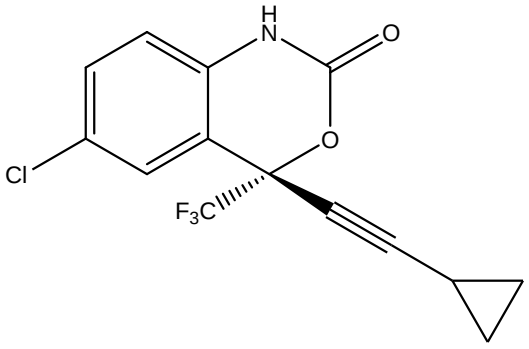


Fig. 1: **Chemical structure of Efavirenz**

A thorough literature search has been revealed that there were few analytical methods including LC-MS/MS for determination of efavirenz in dried breast milk spots [1], LC-tandem mass spectrometry method for estimation of the drug in human plasma [2] and in saliva [3]. Most of the reported methods were based on HPLC with UV detection [4-15] and with fluorescence detection [16] for the estimation of the drug in human plasma and quantitation of efavirenz alone and or with other antiviral agents using RP-HPLC [17-19]. An attempt was made to develop a specific and accurate method to quantitatively estimate efavirenz in API and dosage forms since there has been no visible spectrophotometric method developed by hydrolyzing the efavirenz and subsequent estimation by treatment with p-dimethylamino benzaldehyde.

MATERIALS AND METHODS:

Chemicals and reagents

Efavirenz (pure drug) was taken as a reference sample from hetero drugs Ltd., Hyderabad. PDAB (p-dimethylamino benzaldehyde) reagent from Qualigens fine chemicals Mumbai, while zinc dust and H_2SO_4 & HCl from S.D. fine chemicals limited, Mumbai. Ethanol from Changshu Yangyuan chemicals, china.

Instrumentation:

Double beam UV-Visible Spectrophotometry of model ELICO SL 210 is most frequently preferred in pharmaceutical analysis. It consists of radiation source ,monochromator, sample holder and detector, in addition electrical weight (model-DS-852 G**)** and sonicator (model -1.5L 50H) were also used in the study.

Acid hydrolysis of Efavirenz:

Accurately weighed 0.3 g of drug and dissolved it in 50 mL of water and 1g of zinc dust with 3-4 drops of concentrated sulphuric acid. The solution thus obtained was filtered through a cotton wool and volume was made with the diluent.

Base hydrolysis of Efavirenz:

Accurately weighed 0.3 g of drug and dissolved it in 50 mL of water and 1g of zinc dust with 1mL of 5 % potassium hydroxide solution. The solution thus obtained was filtered through a cotton wool and volume was made with the diluent.

Preparation of standard solution:

Accurately weighed 100 mg of Efavirenz and dissolve it in a 50 mL volumetric flask containing methanol. the volume was made up with methanol and filtered through a Whatman paper. Carefully transferred 5 mL of filtrate into an another volumetric flask and volume

was adjusted with methanol to get the working standard solutions.

Calibration curve for Efavirenz:
To a series of volumetric flasks, added standard drug solution of Efavirenz as aliquots, to this added 2ml of methanolic NaOH and 2 ml of 2 % w/v solution of PDAB reagent. The resulting solution was mixed homogeneously and heated at 80 ^{o}C on a sand bath for 10 min. The solution was cooled and diluted with methanol. The solutions were scanned for λ_{max} using a double beam UV-VIS spectrophotometer. Absorbance of all the solutions were recorded at 406 nm. A graph was plotted by taking concentration of standard Efavirenz on x-axis and absorbance on y-axis.

Assay of Efavirenz:
The randomly selected 20 tablets were powdered in a mortar from this, the powder equivalent to 100 mg of Efavirenz drug was taken and dissolved in 50 mL of methanol. To this added 1g of zinc dust with 3-4 drops of concentrated sulphuric acid. The contents were mixed and the solution thus obtained was filtered through a cotton wool and volume was made with the diluent.
2ml of methanolic NaOH and 2ml of PDAB was added. The solution was heated on a sand bath at 80^{o}c for 10min.The solution was cooled and diluted with ethanol. Finally, the amount of drug present in sample was computed from its calibration graph.

Validation of a proposed method:
The developed method of analysis was validated as per ICHQ2 (R1) for the parameters like specificity, linearity, accuracy, precision, limit of detection, limit of quantification.
a. Specificity
The specificity of the method was established by measuring the interference, if any, observed due to the 0.1N NaOH solvent at the wavelength maxima of Efavirenz. No significant absorbance due to 0.1N NaOH was observed at 406nm.
b. Linearity: Linearity of the method was established by determining by absorbance of different concentrations from 100-500 µg/ml of Efavirenz drug substance over a normal sample preparation. Each level was measured in triplicate. The calibration curve, as plot of absorbance Vs concentration in µg/ml of Efavirenz, was found to be rectilinear for 100, 200, 300, 400, 500 µg/ml concentrations.
c. Accuracy: The accuracy of the method was established by adding the Efavirenz test standard solutions of the pre analyzed tablet formulation. The analysis at each level was performs in triplicate and mean recovery was measured.
d. Precision: The assay of the same batch was performed in six replicates and the percentage relative standard deviation (% RSD) measured. The % RSD was found to be not more than 0.2 %.

RESULTS AND DISCUSSION:

Optimisation of method
An accurate and precise method has been developed to estimate the efavirenz in tablet formulations. Initially the drug was hydrolysed by dilute alkalies such as 0.1M sodium hydroxide and 0.1 M potassium hydroxide solutions to achieve primary aromatic amine. The method was optimised by controlling the experimental factors like nature of the aromatic aldehydes, concentration of the reagent and concentration of base in addition with nature of diluents used. The absorption spectrum of efavirenz in methanol was presented in figure.1. The hydrolysed efavirenz is treated with 2 % w/v solution of *p*-dimethylamino benzaldehyde, a yellow coloured chromogen (figure 2) is obtained due to the formation Schiff's base which can be estimated at 406 nm using a spectrophotometer serves the basis for current proposed method. The analogy of the proposed method based on the reactivity of primary aromatic amine with *p*-dimethylamino benzaldehyde was represented in figure 3.

Validation of developed method
The proposed method was validated according to guidelines given by ICH Q2(R1).Specificity of the method was evaluated and the method found satisfactory as there was no interference due to the excipients present in the formulation with the method. The linearity for spectrophotometric methods was established it obeys beer's law in the concentration range 100-500 µg/ml at 406nm. The correlation coefficient was found to be 0.995 which indicates that the proposed method is linear over the stated concentration range. Limit of detection and limit of quantitation was found to be 0.125 and 0.375 for the developed method. The regression analysis data was presented in table 2. The calibration curve of the method was given in figure 1. Precision was performed on the sample of six determinations and % RSD was determined. The % RSD was found as 0.688 and 0.87 for intra-assay (repeatability) and intermediate precision respectively. Low % RSD values indicating the method is highly precise. The results of precision were given in table 3 and 4. In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powders a

known quantity of standard efavirenz was added and contents were reanalysed by the proposed method. The % recovery of the proposed method lies in the range 100.98-101.47. High % recovery studies proven that the method has good accuracy. The results of accuracy were given in table 5. The proposed method has been successfully applied to commercially available formulations efavirenz and the results were presented in table 6.

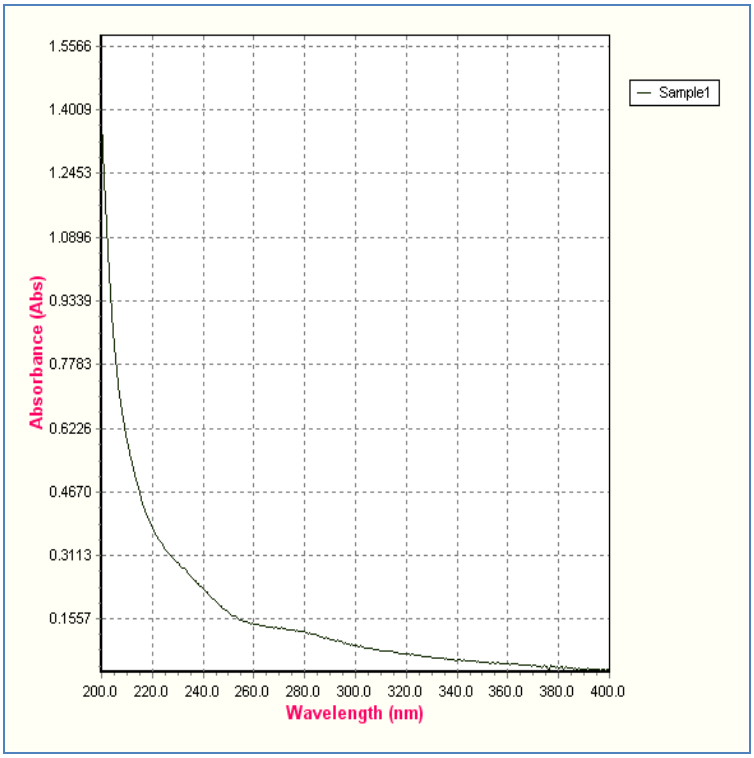


Fig. 2: UV spectrum of Efavirenz in methanol

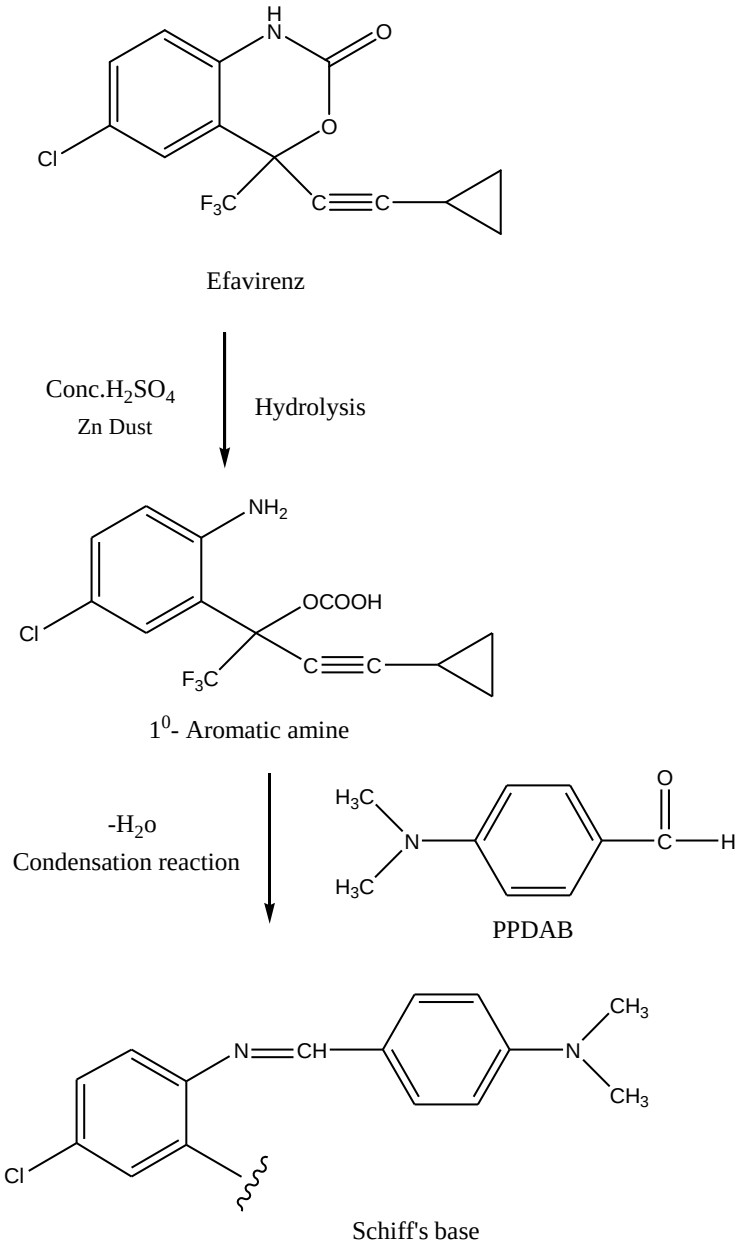
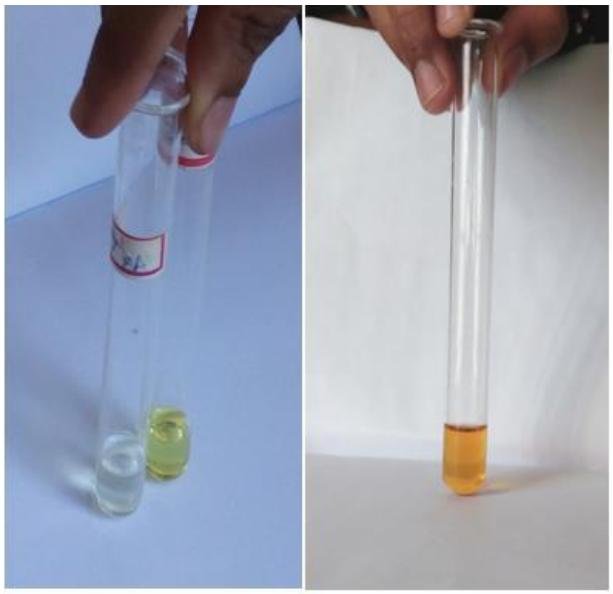


Fig. 3 : Proposed mechanism involved in production of chromogen



a b

C

Fig. 4: Optimization of method; a) Solution of base hydrolyzed Efavirenz. b) Solution of acid hydrolyzed Efavirenz. c) Calibration standards of Efavirenz

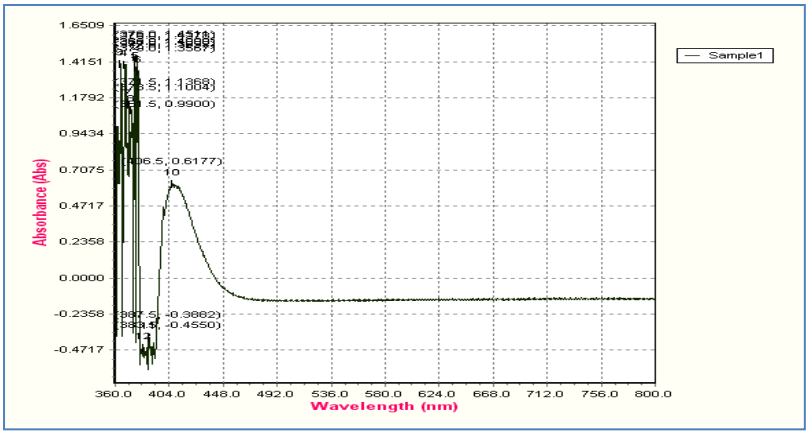


Fig. 5: Absorption spectrum of Efavirenz with PDAB reagent

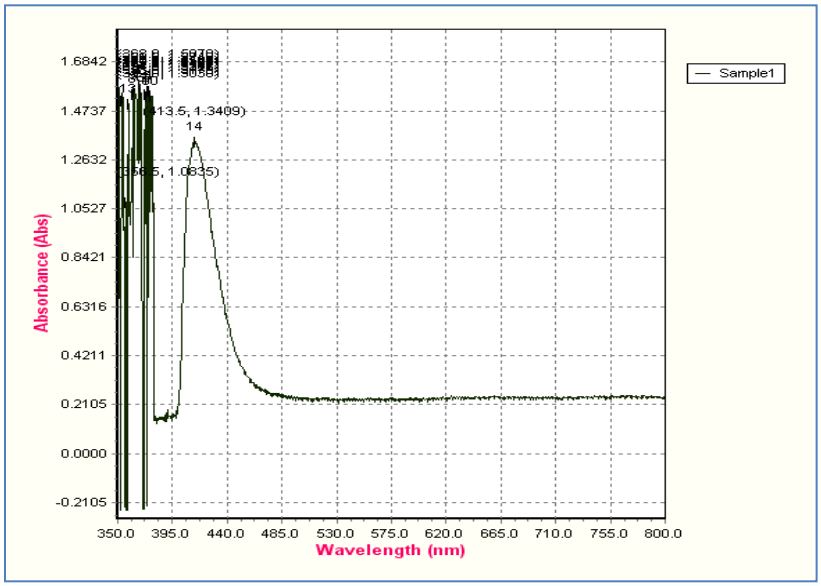


Fig. 6: Visible spectrum of coloured derivative of Efavirenz 2

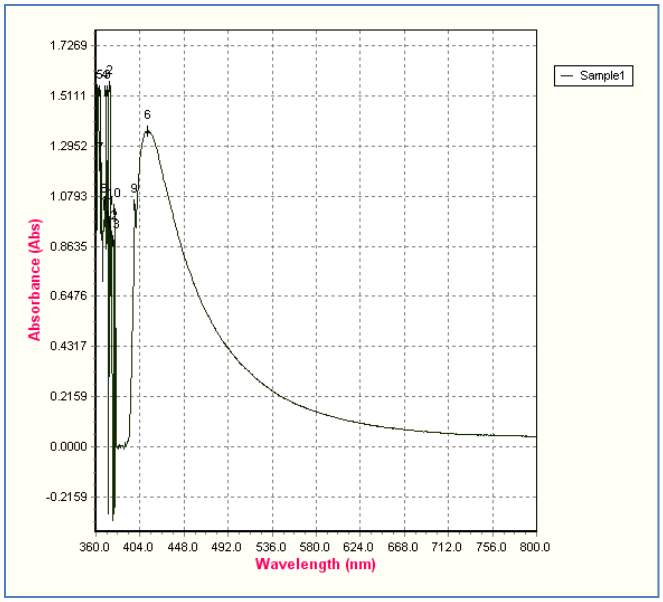


Fig. 7: Visible spectrum of coloured derivative of Efavirenz 3

Table 1: Linearity data of Efavirenz

S. No.	Concentration (mg/mL)	Absorbance at 406 nm
1	0.1	0.21
2	0.2	0.40
3	0.3	0.65
4	0.4	0.83
5	0.5	0.98

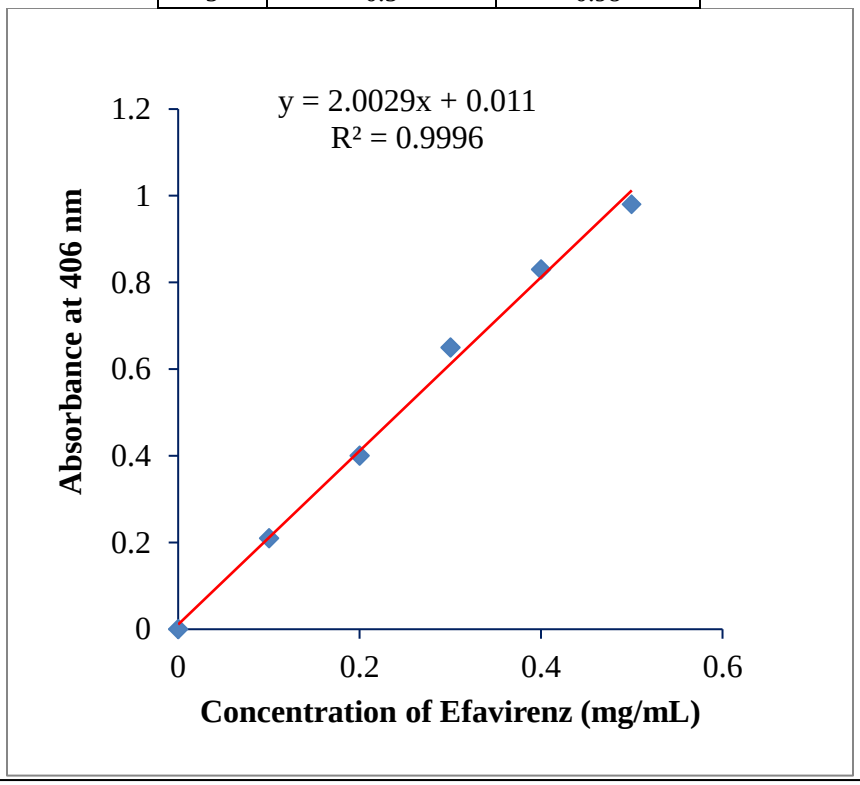


Fig. 8: Calibration curve for Efavirenz

Table 2: Linear regression data of the proposed visible method of Efavirenz

Parameters	**Method**
Detection wave length	By visible at 406 nm
Linearity range (mg/mL)	0.1-0.5 mg/mL
Regression equation	Y= 2.002x + 0.011
Slope (b)	2.002
Intercept (a)	0.011
Standard deviation of slope (s_b)	0.0012
Standard deviation of intercept (s_a)	0.0016
Standard error of estimation (Se)	0.0005
Correlation coefficient (r^2)	0.995
% Relative standard deviation i.e., coefficient of variation (CV)	0.85
% range of errors	x±0.00241
0.05 significance levels	0.000125
0.01 significance level	0.000112

Table 3: Results of Precision study (Intra-assay)

Name of Sample	**Absorbance**	**Amount found (mg) ± S.D***
EFAVIR 600	0.65	598.56 ± 0.0045
	0.64	599.13 ±0.0026
	0.65	597.89 ±0.0054
	0.66	596.87 ± 0.0035
	0.63	596.45 ± 0.0048
	0.66	599.23 ± 0.0042
% RSD#	0.726	0.688

**= mean of three determinations*

 # = six determinations

Table 4: Accuracy study (Recovery data) of Efavirenz

Level of study (%)	**% Recovery***	**SD***
75	101.48	0.056
100	100.98	0.041
125	101.75	0.064

**= mean of three*

Table 5: Assay results of Efavirenz

S. No.	**Name of the Formulation**	**Labeled amount (mg)**	**Amount found (mean ± SD*)**
1	EFAVIRENZ	600	599.23 ± 0.0042
2	SUSTIVA	600	599.13 ±0.0026

**= average of six determinations*

CONCLUSION:

The goal of project work is to develop novel, simple, reliable, accurate and precise analytical methods for the quantitative analysis of Efavirenz in pharmaceutical formulations and validation of developed methods using ICHQ2(R1) guidelines. A spectrophotometric method for quantifying EFA in tablet has been developed and validated. The method is selective, accurate, precise, and linear over the

concentration range studied. The method is simple and suitable for the determination of EFA in formulation.

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