

A Simple And Validated Spectrophotometric Analysis Method For The Hydrophobic Drug Fenofibrate In Natural Oils

Hayder Jaafar Sadeq^{1*}, Mowafaq M. Ghareeb²,

^{1,2}Department of pharmaceuticals, college of pharmacy, Baghdad University, Iraq
Email: haydersadique@yahoo.com, Email: mopharmacy@gmail.com

*Corresponding Author: Hayder Jaafar Sadeq

*Department of pharmaceuticals, college of pharmacy, Baghdad University, Iraq, Email: haydersadique@yahoo.com,
Email: Haider.sadiq1101@copharm.uobaghdad.edu.iq, Mobile telephone number: 006947901741433
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Abstract

Objective: In this work, sodium lauryl sulfate (SLS) was used to present a simple spectrophotometry approach to more accurate quantification of fenofibrate (FENO) concentration in five natural oils (soybean oil, lemon oil, black seed oil, cardamom oil, and garlic oil). In the published work either organic solvent was used which may result in false results due to the dissolving of oil by organic solvent, or high-performance liquid chromatography (HPLC) was utilized, which is a sophisticated instrument that may not be available at every research facility.

Methods: construction of a calibration curve using a series of FENO concentrations added to 0.5% SLS phosphate buffer solution and measuring the absorbance by spectroscopy at 289 nm. The drug samples in different oils were diluted with 0.5% SLS phosphate buffer solution prior to direct reading by spectroscopy. According to the International Conference on Harmonization requirements, the method was validated regarding specificity, precision, accuracy, and determination range.

Results: FENO gave absorbance maxima at 289 nm in 0.5% SLS solution, the beer's law was obeyed in the range of 0.0125 to 0.05 mg/ml. High linearity (in the scope of 10 - 125 mg, $R^2=1$), highly specific, good accuracy (95- 104), precision (SD< 10%), and a wide range of detection, indicate validation of the procedure.

Conclusion: the proposed method for FENO analysis is simple, fast, and promising for easy determination of FENO in bulk and pharmaceuticals.

Keywords: UV-spectrophotometer, sodium lauryl sulfate, fenofibrate, natural oils

INTRODUCTION

Fenofibrate belongs to the group of fibrate, with chemical name 2-[4-(4-chlorobenzoyl) phenoxy] 2-methyl-propanoic acid, 1 methylethyl ester (fig. 1) which is a fibric acid derivative that can markedly reduce VLDL (very low-density lipoprotein) and hence triglyceride, with modest (approximately 10% reduction in LDL (low-density lipoprotein) [1, 2]. Fenofibrate is a class II drug with very poor water solubility; low melting point (78°C); the presence of fat food enhances its absorption. All of the above make this drug a good candidate for the lipid-based dosage form [3, 4].

Measuring the exact amount of drug in oil is a crucial task in the lipid-based dosage form. It is imperative to predict the solubility of the drug of interest in different oils, estimate the drug content in formulation, and calculate the percent released during in-vitro dissolution [5]. Many methods were followed to achieve the exact determination of the drug; on the top of them is using the HPLC instrument, which can provide precise measuring. Still, it is a complex device that may not be accessible at any time [6, 7]. The direct observation of the gradual addition of the drug to oils was used to predict the solubility of various drugs in oils. By weighing a portion of the drug, solubilizes it in oil until saturation; nonetheless, it is of no adoption for drug content measurement and drug release in various media [8]. On the other hand, using an organic solvent (acetone, ethanol, or ethyl acetate) can dissolve the drug and the oil, which will result in false results when introduced to a UV-visible spectrophotometer to quantify the drug at its absorbance of lambda max, making it unspecific method [9]. Even if the organic solvent is used to extract the active ingredient, such as methanol, incomplete extraction will result [10].

Here came the necessity for a simple, validated approach to measuring the amount of drug in oils; sodium lauryl sulfate (SLS) is an anionic surfactant (fig. 1) utilized as a solubilizing agent to enhance the dissolution of many drugs; emulsifying agent; modified-release agent; penetration enhancer; tablet and capsule lubricant [11].

When applied as a wetting agent, SLS could alleviate the surface tension between hydrophobic drugs and aqueous media to prevent the self-aggregation of drug particles, therefore improving the effective surface area of dissolution. Above the critical micelle concentration (CMC), SLS monomers aggregate to form micelles that can solubilize hydrophobic drugs [12].

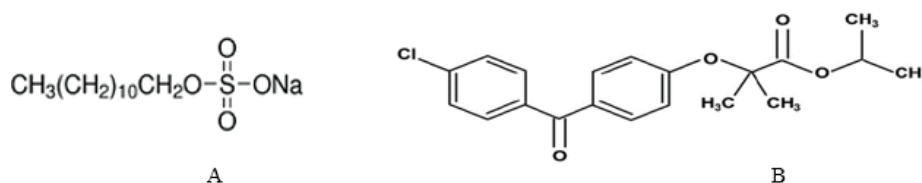


Fig. 1: sodium lauryl sulfate (A) and fenofibrate (B) chemical structure.

Nevertheless, SLS did not use before to predict the solubility of drugs in various natural oils. In this work, SLS solution in 0.5% will be used to measure fenofibrate concentration in oils using a UV- spectrophotometer. This method is simple, economical, and very accurate. It is the first time this technique has been used in natural oils to the researcher's knowledge.

MATERIAL AND METHODS:

Reagents and chemicals: Fenofibrate was purchased from Hyper Chem China. Sodium lauryl sulfate (SLS) was purchased from Thomas Baker India. Deionized water (AL- JOUD Company, Iraq) was used throughout the experiment.

Instruments: A Shimadzu UV-1601 PC double beam UV-visible spectrophotometer with a 1 cm quartz cell attached to an IBM computer was utilized. UVPC personal spectroscopy software was used (version 3.7). The wavelength-scanning speed was 2800 nm/min, and the spectral bandwidth was 2 nm. PLS-Toolbox 2.0 was used to analyze all of the data, which was conducted using MATLAB® 6.5.

Construction of calibration curve of fenofibrate

An accurately weighed quantity of FENO was transferred into a 100mL volumetric flask; then diluted up to the mark with an SLS buffer solution to get a 0.05 mg/mL standard stock solution. The stock solution was scanned using an ultraviolet (UV) and visible spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). Different aliquots of working stock solution were pipetted to prepare a series of concentrations from 0.0125, 0.025, 0.0375, 0.05 mg/ml. The calibration curve was constructed by plotting the concentration vs. absorbance of FENO from the above readings [13].

Solubility of fenofibrate in natural oils

An excess amount of FENO is added to 3 ml of natural oils (soybean oil, lemon oil, black seed oil, cardamom oil, and garlic oil) in test tubes. All five-tube were incubated in a shaking water bath (using BS-11 instrument) at 25 °C for 72 hours. At the end of the time scheduled for solubility study, test tubes are centrifuged at 5000 rpm for 30 minutes. The supernatant is filtered by a Millipore syringe 0.45 µm. 250 mg of that supernatant is diluted with 25 ml 0.5% SLS phosphate buffer solution; further dilution was performed until Beer-lambert law is obeyed [5,14].

Measuring drug content in the oil-based formula:

Accurately weighed amounts of lipid-based formulas prepared for another study; are diluted with 25 ml of 0.5% SLS phosphate buffer solution; further dilution is needed. FENO absorbance is estimated by UV spectroscopy at its lambda max [15].

Validation of the method:

Different weights of FENO (10, 50,100 & 125 mg) were dissolved separately in 5 ml of five natural oils. Fourteen milliliters of 0.5% SLS phosphate buffer solution were added to 1ml of oil containing the drug. One milliliter of the previous solution is taken and diluted with 39 ml SLS solution, filtered by 0.45 µm Millipore syringe, and measured at 289 nm by UV-spectrophotometer. Each experiment is triplicate [16]. Following the International Conference on Harmonization (ICH) recommendations, Validation was carried out.

Specificity:

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities, and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical process. The specificity of the method was determined by analyzing different oils tested to establish the lack of interference at lambda max of FENO [17]. In this work, five different natural oils were used to investigate the specificity of the procedure.

Precision and accuracy:

By definition, the **precision** of analytical procedure is the closeness of agreement between a series of measurements. 10, 50, 100, and 125 mg of FENO were used to examine the precision of the procedure.

Accuracy: By definition, it is how close a measurement is to the accepted value. 10, 50, 100, and 125 mg of FENO were accurately weighed and considered accepted value [18].

Linearity:

A linear relationship should be evaluated across the range of the analytical procedure by obtaining the regression value (R^2), which was calculated by plotting accepted values to the measured value [19].

Range:

The specified range is generally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical approach provides an acceptable degree of linearity, accuracy, and precision when applied to samples containing analysts within or at the extremes of the specified range of the analytical procedure [20].

RESULT AND DISCUSSION

Calibration curve:

The result of the construction of the calibration curve is shown in (fig. 2) the drug was found to obey Beer's Law in the concentration range of 0.0125- 0.5 mg/ml. The lambda max of FENO in 0.5% SLS solution was 289nm, as illustrated in (fig. 3) [13, 21].

Solubility of fenofibrate in natural oils:

The result of the solubility study of FENO in various natural oils is illustrated in (table 1) [22]

Table 1: solubility of fenofibrate in natural oils.

Oils	Fenofibrate dissolved (mg)
Soybean oil	87.5
Lemon oil	6.02
Black seed oil	22.5
Cardamom oil	50.87
Garlic oil	31.45

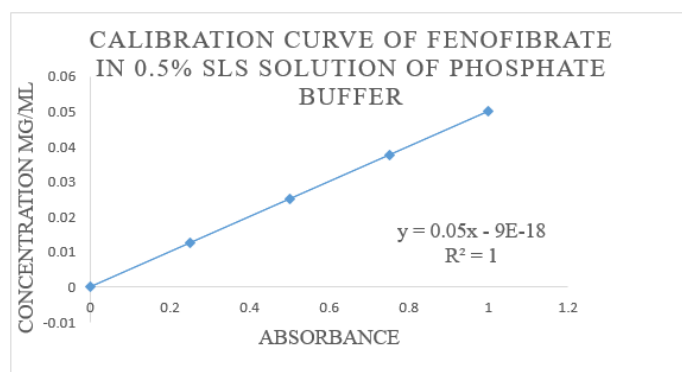


Fig. 2: calibration curve of fenofibrate in 0.5% SLS phosphate buffer solution.

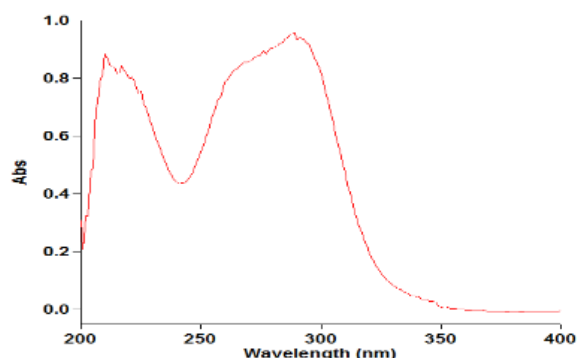


Fig. 3: fenofibrate scan in 0.5% SLS phosphate buffer solution

Drug content:

The drug content of the thirteen lipid-based formulas prepared for another study was between 98.03 % \pm 0.79 and 99.66 % \pm 0.32. The results were within an acceptable range, as stated in United States Pharmacopoeia requirement for drug content [15]. Statistically, a non-significant difference ($p > 0.05$) was found among tested formulas for their drug content percent [23].

VALIDATION OF THE METHOD:

Specificity:

The method used is shown to be specific for FENO in different oils at its lambda max of 289nm in SLS buffer solution due to the complete solubilization of drug by SLS from oils [24].

Precision & accuracy:

Precision was evaluated by determining the relative standard deviation (RSD) of three measurements, as illustrated in (table 2) for all five natural oil. Accuracy is accomplished since all results are close to the weighted values and lie within the range of 95 to 104. Precision is also confirmed because all results are close to each other, and SD% is less than 10% [25].

Table 2: Accuracy and precision of the method in different natural oils:

	Fenofibrate (mg)	mean	SD	SD%	Accuracy
Soybean oil	20	20.63	0.62	1.26	95.23-98.03
	50	51.16	0.62	1.21	96.15-98.03
	100	102	0.4	0.39	97.56-98.52
	125	126.5	0.4	0.31	98.42-99.2
Lemon oil	20	20.63	0.62	1.26	95.23-98.03
	50	51	1.22	2.39	95.23-98.03
	100	100.73	0.98	0.97	98.13-100.5
	125	125.5	0.88	0.7	98.89-100.5
Black seed oil	20	20.4	0.6377	3.125	95.69-102.5
	50	50.4	0.58	1.15	98.03-100.8
	100	100.4	0.8	0.79	98.81-100.7
	125	125.46	0.92	0.73	98.89-100.64
Cardamom oil	20	20.45	0.679	3.32	95.01-102.5
	50	50.46	1.04	1.03	97.65-101.41
	100	100.5	1.04	1.03	98.42-100.9
	125	125.3	0.9	0.71	98.97-100.7
Garlic oil	20	20.3	0.787	3.87	95.23-104.16
	50	50.4	0.86	1.7	97.65-101.62
	100	100.63	0.67	0.66	98.71-100.3
	125	125.3	0.65	0.51	99.12-100.4

Linearity:

The mean of the calculated value is plotted against the weighted value; a linear relationship is obtained with an R^2 value equal to 1. linearity means that the result is directly proportional to the measured value [26], As shown in (fig. 4-8) below.

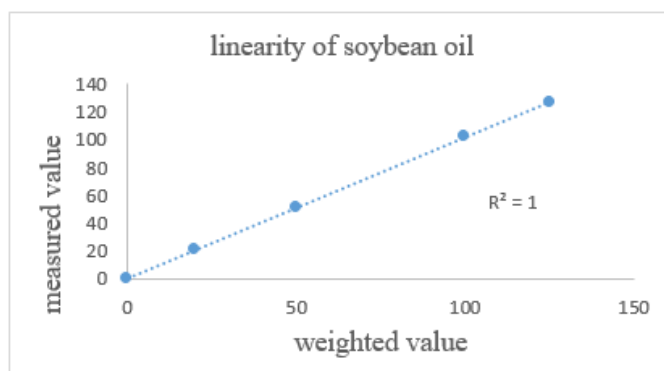


Fig. 4: the linearity of fenofibrate in soybean oil

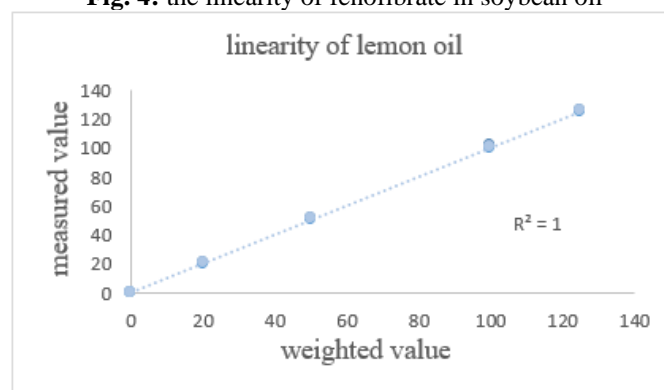


Fig. 5: The linearity of fenofibrate in lemon oil

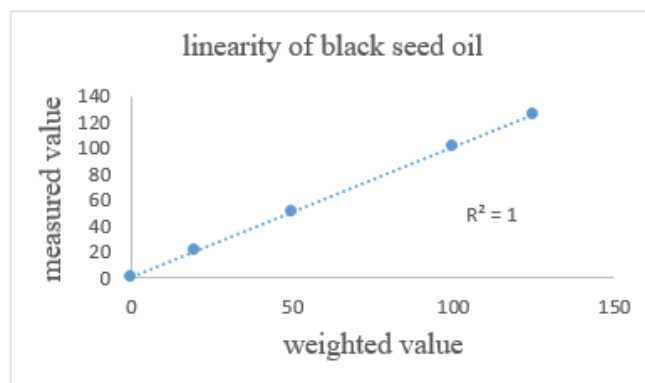


Fig. 6: The linearity of fenofibrate in black seed oil

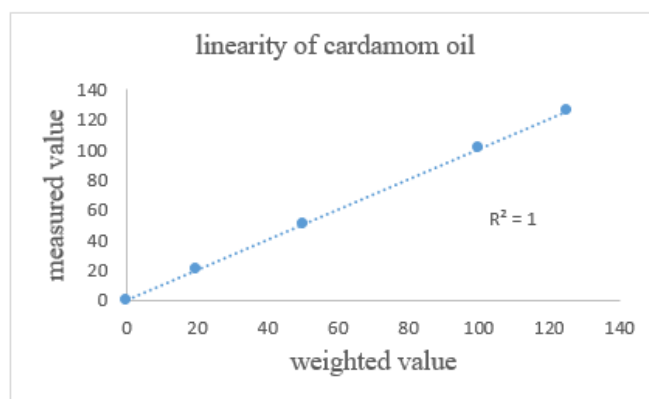


Fig. 7: The linearity of fenofibrate in cardamom oil

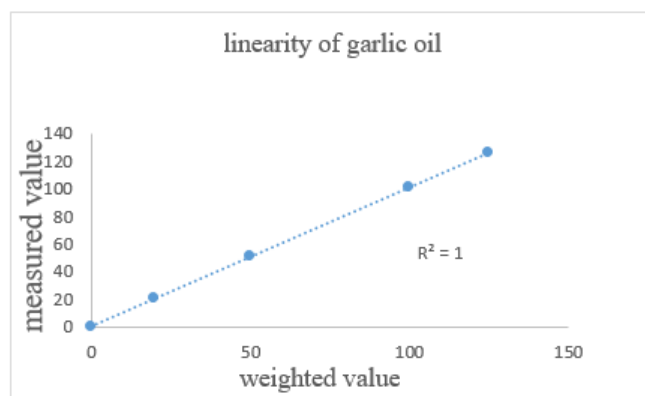


Fig. 8: The linearity of fenofibrate in garlic oil

Range:

Since linearity, accuracy & precision were established previously, the range can be confirmed between 20mg & 125mg in this experiment [27].

CONCLUSION:

We evaluated a rapid and straightforward method for determining the concentration of fenofibrate in natural oils using spectrophotometry. The technique is simple, specific, precise, and accurate. From the results of all validation parameters, it can be concluded that the present method will help determine fenofibrate in oils and different formulations with the desired precision and accuracy. Based on the outcome of the solubility study, soya bean oil was selected as an oil phase in the development of formulations for further research.

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