DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETER METHOD FOR THE QUICK ESTIMATION OF OLIVE LEAF EXTRACT (OLE) IN PHARMACEUTICAL FORMULATION

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ABSTRACT

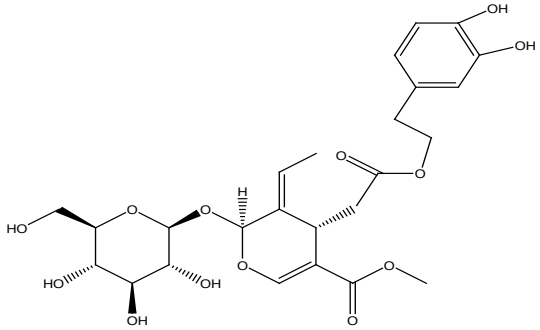
Olive (Olea europaea), a rich source of natural antioxidant is native to the Mediterranean region. The main active constituents of olive include oleic acid, phenolic constituents like hydroxytyrosol and oleuropein and squalene are abundantly found in its leaf. Therefore, the aim of our research is to develop and validate simple, sensitive and specific spectrophotometric method for olive leaf extract (OLE). The developed method was validated with respect to linearity, accuracy, precision and specificity. The adequate drug solubility and maximum sensitivity were found in ethanol. The λ_{max} or the absorption maxima of the drug was found to be 279 nm. The samples were prepared in ethanol and method obeyed Beers law in concentration ranges 5- 50 µg/ml. The content of OLE in herbal pharmaceutical formulation was determined. The results of analysis were validated statistically and confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of olive leaf extract in herbal pharmaceutical formulation.

KEY WORDS: UV spectroscopy, Olive leaf extract, Validation.

INTRODUCTION

Olive tree is native to Asia Minor and Syria, but is cultivated in all Mediterranean countries as well as in Chile, Peru, South Australia and the USA[(1,2)]. Olive trees have been cultivated for thousands of years, but the immune and circulatory benefits of olive leaf have only recently become fully realized. Olive leaf was first used medicinally in Ancient Egypt and was a symbol of heavenly power. More recent knowledge of the olive leaf's medicinal properties dates back to the early 1800s when pulverised leaves were used in a drink to lower fevers. A few decades later, green olive leaves were used in tea as a treatment for malaria[(3)]. Numerous scientific studies have been added to investigate the extract's beneficial properties, and olive leaf extracts are suggested as its hypotensive effects[(4)], antioxidant properties[(5)], hypoglycemic[(6-8)], antimicrobial[(9)], radioprotective[(10)], antiatherogenic[(11,12)], anti-inflammatory[(13,14)], and hepatoprotective[(15)]. Olive leaves contain around 60~90 mg per gram (dry weight) oleuropein, additionaly significant levels of a glucosidic esterof elenolic acid and hydroxytyrosol (3,4-dihydrophenylethanol) (figure 1).

Literature survey reveals that several high pressure liquid chromatography (HPLC) methods are developed and validated for oleuropein[(16-19)] but there is no reported UV-visible method for quick estimation of olive leaf extract. Hence, a simple UV spectrophotometer method was developed and validated as per International Conference on Harmonization (ICH) Q2A. As the formulations are available without combinations of any drugs, there is a need for coming up with analytical method which is simple, sensitive, rapid and accurate for estimation of OLE in pharmaceutical preparations. Therefore, the aim of the present work is to develop and validate a method for the analysis by UV-Visible spectrophotometer which is easily adaptable as a routine in quality testing laboratories. This has enabled us to reduce total time of analysis besides taking care of the error caused due to in complete extraction and use of internal standard [(20)].



(Figure 1) Structure of oleuropein

MATERIALS AND CHEMICALS

Chloroform, acetone and ethanol were purchased from Sigma Aldrich, Germany. Herbal capsules containing olive leaf extract were purchased from Oliver PhytoFlore Les laboratories, Arrazi. Agilent Carry 60, UV- Vis spectrophotometer was used for the analysis. All chemicals and reagents used were of analytical grade.

Preparation of olive leaf extract

The olive leaves were collected from olive orchid of Misurata, Libya. The collected leaves were cleaned and dried under shade (at ambient temperature). The

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dried olive leaves were weighed (2.5 kg) and stored in desiccator. The extraction was done by simple maceration process. The dried leaves were mixed in ethanol, placed for 15 days under room temperature with occasional shaking. The mixture was filtered with muslin cloth, simple filter paper and then finally with Whatmann filter paper to obtain clear liquid extracts. The clear liquid extract was dried in oven at 40 ˚C to obtain green colored crude extract, which was stored in desiccator until further use.

METHOD OPTIMIZATION

Selection and optimization of solvent

It is well known that the solvent does exert a profound influence on the quality and shape of the peak[13]. The choices of solvents for UV method development are: chloroform, acetone, ethanol etc. Different solvents were tried. Out of which ethanol satisfied all the conditions related to peak quality and non-interference at the specified wavelength.

Preparation of standard stock solution of OLE

The stock solution (100 µg/ml) of OLE was prepared by dissolving accurately about 10 mg of drug in sufficient quantity of ethanol and then volume was adjusted to 100 ml with ethanol. Further, a series of dilution was made with ethanol.

Calibration curve of olive leaf extract

A series of calibrated 10 ml volumetric flask was taken and appropriate aliquots of the working stock solution of OLE were withdrawn and diluted up to 10 ml with ethanol. The absorbance was measured at absorption maxima 279 nm against the blank without the OLE. Absorption maxima and Beer's law limits were recorded and data, that prove the linearity and obeys Beer's law limits, were noted. The linear correlation between these concentrations (x-axis) and absorbance (y-axis) were graphically presented. The slope (m), intercept (c) and correlation coefficient (R^2) were calculated for the linear equation ($y=mx\pm c$) by regression.

METHOD VALIDATION

The method was validated for linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ) and system suitability by the following procedures:

Linearity

Ten different concentrations of OLE were analyzed and their calibration curve was constructed in the specified concentration range (5-50 µg/ml). The calibration plots were generated by replicate analysis (n = 3) at all concentration levels and the linear relationship was evaluated.

Precision

The precision was examined by performing the intraday and interday assays of three replicate injections of the mixture of standard solutions at three concentration levels (15, 35 and 50µg/ml). The intraday assay precision was performed with the interval of 4 h in 1 day, while the inter-day assay precision was performed over 3 days.

Accuracy by recovery

The accuracy of the method was determined by calculating the recoveries of OLE by the method of standard addition. A known amount of standard (50%, 100% and 150%) was added to pre-analyzed sample solution, and the amount of the standard was estimated by measuring the absorbance and by fitting these values to the straight-line equation of calibration curve.

Limit of detection and limit of quantification

The LOD and LOQ of OLE were determined by using standard deviation of the response and slope approach as defined in ICH guidelines. LOD and LOQ values were calculated using the relation:

$LOD=3.3\delta /S$

$LOQ=10\ \delta /S$

Where, δ= standard deviation of residuals from the curve; S= slope of the curve

Ruggedness

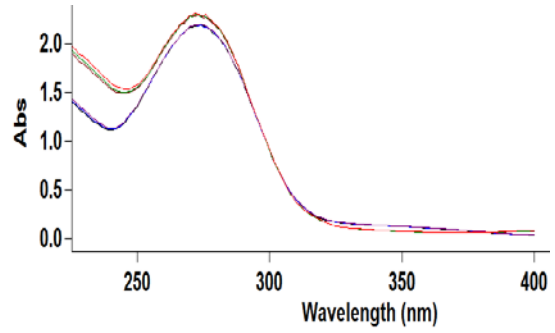
Ruggedness of this method was determined by analyzing the OLE with different equipment on different days and by different analysts.

Determination of OLE in pharmaceutical formulation

A pharmaceutical formulation (soft gelatin capsule) claiming 25 mg of OLE was taken and the drug content was taken out by cutting the shell. The drug content was transferred into 100 ml volumetric flask containing 30 ml of ethanol and made up to the mark using ethanol. An appropriate volume, 0.1 ml of this solution was transferred to 10 ml volumetric flask and the volume was made up to the mark using ethanol to give concentration of 2.5 µg/ml. The resulting solution was scanned on spectrophotometer in the UV range 200 - 400 nm. The concentrations of the drug were calculated using linear regression equations.

RESULT AND DISCUSSION

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of OLE will reduce unnecessary tedious sample preparations, the cost of materials and labor. OLE has a UV-absorbing molecule with specific chromospheres in the structure that absorbs at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The absorption spectrum of OLE in ethanol was shown in (figure 2).



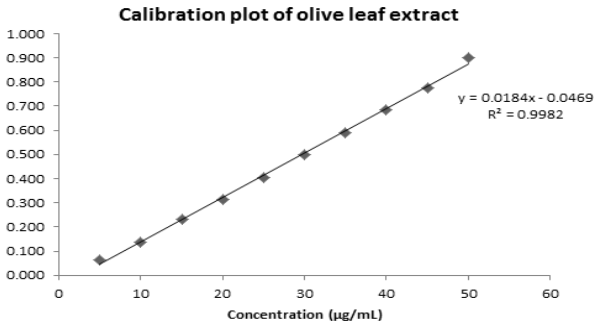
(Figure 2) Absorption spectra of olive leaf extract

A calibration curve was constructed in the range of the concentrations of 5-50 µg/ml, which also obeyed Beer's law as shown in (table 1).

(Table 1) Calibration plot of olive leaf extract

S. No.	Concentration (µg/ml)	Absorbance ± S.D (n=3)	%RSD	S. E	95% confidence interval	
					Lower value	Upper value
1	5	0.062±0.0010	1.613	0.0006	0.060	0.064
2	10	0.137±0.0020	1.460	0.0012	0.132	0.142
3	15	0.231±0.0024	1.088	0.0015	0.225	0.238
4	20	0.315±0.0031	0.971	0.0018	0.307	0.322
5	25	0.406±0.0025	0.620	0.0015	0.399	0.412
6	30	0.501±0.0028	0.503	0.0015	0.494	0.507
7	35	0.589±0.0035	0.597	0.0020	0.580	0.597
8	40	0.686±0.0042	0.607	0.0024	0.676	0.697
9	45	0.777±0.0026	0.341	0.0015	0.770	0.784
10	50	0.901± 0.0055	0.611	0.0032	0.887	0.914

The regression equation was found to be y = 0.0184x - 0.0469. The correlation coefficient (r^2) of the standard curve was found to be greater than 0.998 (figure 3). The OLE was scanned for the entire UV region for λ_{max} and it was found to be 279 nm.



(Figure 3) Calibration plot of olive leaf extract

Precision

The precision of the method was expressed in terms of % relative standard deviation (%RSD). The %RSD values found to be less than 2 for intra-day and interday precision, which indicated that the proposed method is precise for analysis. The results are expressed in (table 2).

(Table 2) Intraday and Interday Precision

S. No.	Concentration (µg/mL)	Interday precision		Intraday precision	
		Absorbance ± SD (n=3)	%RSD	Absorbance ± SD (n=3)	%RSD
1	15	0.231±0.0035	1.522	0.232±0.0040	1.745
2	35	0.588±0.0085	1.446	0.590±0.0080	1.356
3	50	0.895±0.0098	1.100	0.897±0.0134	1.498

Accuracy by recovery

Accuracy of the method was confirmed by recovery study from marketed formulation at three levels of standard addition. % Recovery for OLE was found to be 99.38-102.63%. The recovery studies are reported in (table 3).

(Table 3) Accuracy by recovery

S. No.	Initial Concentration (µg/ml) [A]	Addition of known quantity (µg/ml) [B]	A+B	Absorbance	Concentration recovered (µg)	% Recovery	Average recovery n=3
1	20	10	30	0.506	30.05	100.16	99.38%
2				0.498	29.61	98.71	
3				0.501	29.78	99.26	
4	20	20	40	0.686	39.83	99.58	100.21%
5				0.691	40.10	100.26	
6				0.695	40.32	100.80	
7	20	30	50	0.892	51.03	102.05	102.63%
8				0.898	51.35	102.71	
9				0.902	51.57	103.14	

Limit of detection and limit of quantification

The LOD and LOQ for OLE were found to be 0.988µg/ml and 2.993 µg/ml respectively.

Ruggedness

Absorbance was measured for same concentration solutions, six times. The results are given in (table 4).

Specificity

The specificity was the ability of an analytical procedure to measure accurately an analyte in presence of components that may be expected to present in sample matrix. Standard and sample solution were prepared separately and standard solution was spiked with sample solution and the absorbance was found at 279 nm which showed that the method was

specific and was not affected in presence of any other component in the sample (figure 2).

(Table 4) Ruggedness

S. No.	Concentration (µg/ml)	Analyst I Amount found % ± S. D	Analyst II Amount found % ± S. D
1	30	99.08±0.21	101.55±0.38

Analysis of OLE in pharmaceutical formulation

The concentrations of the drug were calculated from linear regression equations. The % assay was found between 98.34% - 100.77% as shown in (table5).

(Table5) Analysis of olive extract in marketed preparation

Sample	Concentration (µg/ml)	Absorbance n=3	% Assay
Test (marketed preparation)	50	0.893	98.34% 100.77% 99%
		0.906	
		0.895	
Standard (Plant extract)	50	0.908	
		0.899	
		0.904	

CONCLUSION

UV spectrophotometric method has been developed and validated for quick estimation of olive leaf extract in herbal formulations. The proposed methods are accurate and precise as indicated by good recoveries of the drugs and low % RSD values. All the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. The proposed methods could be applied for routine analysis and in quality control laboratories for quantitative determination of the olive leaf extract both in the pure and dosage forms.

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