INTERNATIONAL JOURNAL
OF
ADVANCED PHARMACEUTICAL SCIENCES
Volume 1, Issue 01, 82-99. ISSN:2456-8147

ANALYTICAL METHOD DEVELOPMENT OF ANANDAMIDE DRUG BY USING UV SPECTROSCOPY

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Article Received on

05.10.17

Revised on

13.10.17

Accepted on

30.10.17

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ABSTRACT

Analytical chemistry is essentially involved with the determination. Analytical chemistry is essentially involved with the determination. of the chemical composition of matter but, identification of substance, the elucidation of its structure and quantitative analysis of its composition square measure the aspects lined by modern analytical techniques. In this study, an effort was created to develop an analytical technique for the estimation of Anandamide in tablet dose type. The tablet dose sort of Anandamide was subjected for the estimation by exploitation numerous analytical techniques like uv spectroscopic analysis. The uv studies were carried out using methanol as medium .Developed technique was found to conform the Beer's law and therefore the assay and recovery studies that was carried out shows that the tactic is straightforward.

Keyword: Anandamide , Absorbance, LOD,LOQ

Introduction

Ultraviolet spectroscopic analysis cares with the study of ultraviolet radiation, that ranges from 200 nm to four hundred nm. Compounds, that are colored, together absorb the radiation in visible region (400-800 nm). In every ultraviolet radiation and visual spectrographic analysis, exclusively the electrons absorb the energy, thereby undergoing transition from ground state to excited state. Usually this can be often characteristic to the character of the electrons gift inside the compound. The intensity of absorption depends on the concentration of the compound and thus the path-length as given by Beer's law.[1,2,4]

General Methodology for UV Method Development:

Absorption spectrophotometry is that the activity of the selective absorption of Absorption spectrophotometry is that the activity of the selective absorption of magnetic force radiations having a particular and slender wavelength reading, by atoms molecules or ions. Ultra-violet and visual absorption bands ar as a results of magnetic and electric force transitions inside the region of 200-780 nm.

One of the key applications of ultraviolet radiation and visual spectrographic analysis is that the quantitative determination of drugs. An unknown amount of a known compound in the solution, if it obeys Beer's law, is determined by victimisation the following equationLog (I_o /I) = € CL

Where,

I_o = Intensity of the incident energy

I = Intensity of the emergent energy

C = Concentration

L = Thickness of the absorber (normally 10 mm or 1cm)

€ = Molar absorptivity for concentration in mol/lit.

Here the principle concerned is "Any molecule has either n, or a mixture of those electrons. These bonding and non-bonding (n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks, the natures of electrons gift and thus the molecular structure will be elucidated" [3,5-11]

Validation Of Analytical Parameters In Pharmaceutical Analysis By Uv Spectrophotometry[12-15]

1. Sensitivity :

The knowledge of the sensitivity of areaction is important and generally three methods are commonly employed for expressing sensitivity.

Sandell's sensitivity :

Here the number of micrograms of drugs is converted into the colored product is determined; which in a column solution of cm^2/cross-section shows an absorbance of 0.002 or 0.005 .It is expressed as µg of drug / cm^2

Molar Extinction Co-efficient:

It may be calculated from the equation

Molar Extiction Co-efficient= A / IC

Where

A = absorbance

C = Concentration of colored species (Mole/I)

I= light path length (cm) (Expressed as 1 $mole^{-1}$ cm^{-1}

2. Correlation coefficient (r^2):

When the changes in one variable are associated or followed by changes in the different, it's referred to as correlation. The numerical live of correlation is termed the coefficient of correlation and is outlined by the relation.

$$r = \frac{C - \sum A \sum C/N]}{\sqrt{[[\sum C^2 - \sum (\sum C)^2]/N\ [\sum A^2 - (\sum A)^2/N]]}}$$

3. Precision :

The exactness (or reproducibility) of the projected technique was determined by analyzing an equivalent concentration (3 / 4th of the higher Beer's law limit) of the drug in freshly ready take a look at resolution, eight times. The set of absorbance values geted were then accustomed obtain the quality deviation, that is expressed in absorbance units or as a proportion of mean absorbance.

$$S = \sqrt{\sum(X - x)^2 / N - 1}$$

Where

x = observed values

X = Arithmetic mean = $\sum x / N$

N = Number of deviations

For sensible interpretation it's a lot of convenient to specific 'S' in terms of % of the approximate average of the vary of study is employed within the calculation of 'S'. this can be known as co-efficient of variation or % relative variance (% RSD).

C.V OR %RSD = 100* S/X

It is customary to use probability limits 0.05 level (95 % of the readings will be within the calculated limits x ± a, where a = t.s / √n, t value is 2.365 from Students table) and 0.01 level (99 % of the readings will be within the limits x ± b, where b = t.s / √n, t value is 3.499 from students table for eight determinations).

% range of error at p = 0.05 level = ± 100 a / x

% range of error at p = 0.01 level = ± 100 a / x

4. Accuracy :

The accuracy of the recommended procedure is evaluated by comparing the values obtained in the proposed and reported methods.

a)Percent recovery studies :

Recovery studies by adding known quantities of drug to previously analyzed pharmaceutical preparations are followed using proposed procedure. To study percent recovery, fixed amount of the sample is taken in a series of volumetric flasks and three different levels of standard solutions are

added. Each level of the added drug is repeated six times. The total amount of the drug is then determined by the proposed method.

The percent recovery is calculated by using the equation:

$$\% \text{ Recovery} = \frac{N.\sum xy - (\sum x)(\sum y)}{N\sum x^2 - (\sum x)^2} \times 100$$

Where x = amount of drug added in mg/g of sample

y = amount of drug found in mg/g of sample

N = total number of observations

6. Interference studies :

The effects of a wide range of excipients and other additives usually present in formulations on the determinations under optimum conditions were investigated.

In the initial interference studies, a fixed concentration of the drug was determined several times by the recommended procedure in the presence of a suitable (1 - 100 fold) molar excess of the foreign compound under investigation and its effect on the absorbance of solution was noted. The foreign compound was considered to be not interfering at these concentrations if it consistently produces an error less than 3 % in the absorbance produced in pure solution.

Determination of pharmaceutical compound by UV spectroscopy can be done in two ways.

1) Qualitative analysis
2) Quantitative analysis

The basic criteria for determination of a compound by UV spectroscopy in the compound must have a conjugated double bond in its structure. So that the electronic excitation occurs when it absorbs in UV light at the region of 200 - 400 nm.

Any of the following electronic excitation takes place by the compounds when absorbs UV light. Possible electronic transitions of л, σ and *n* electrons are;

- ✓ σ - σ * Transitions
- ✓ n - σ* Transitions
- ✓ n - л*and л - л* Transitions

The absorption maxima of the unknown compounds can be calculated mathematically by

The given compound solution should obey Beer-lamberts law. Method development of the given pharmaceutical compound by UV spectroscopy can be done by

1. Qualitative analysis

a. Determining the effect of conjugation, geometric isomerism, alkyl substitution and number of rings in structural analysis of organic compounds.
b. Detection of impurities.
c. Structure elucidation of organic compounds.

2. Quantitative analysis

1) Assay of substance in single component samples

a) Single standard (or) direct comparison method :

In this method the absorbance of a standard solution of known concentration and a sample solution is measured. The concentration of unknown can be calculated by using the formula,

$$C2 = C1 \times \frac{A_2}{A1}$$

Where,

A_1, A_2 = Absorbance of standard and sample

C_1, C_2 = Concentration of standard and sample

b) Calibration curve method or multiple standard method :

A calibration curve is plotted using concentration vs absorbance value of five (or) more standard solutions. A straight line is drawn either through maximum number of point or in such a way that there is equal magnitude of positive and negative errors that is line of best fit. From the

absorbance of the sample solution and using the calibration curve, the concentration of drug, amount and the percentage purity can be calculated.

c) Standard absorptivity value method :

In this method, the use of standard A 1 %, 1 o ε alues a e used i o de to dete i e its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance.

2) Assay of substances in multicomponent samples

In multicomponent samples spectral interference can arise which is known as irrelevant non-specific absorption, it arises from absorption by other materials and impurities that may be present. spectral selectivity and detection sensitivity can be enhanced significantly by a number of chemical or instrumental techniques, which include difference, higher derivative and dual-wavelength spectrophotometry. Such methods and certain graphic techniques such as the Morton-Stubbs method, can contribute in different ways to reduce the general problem of spectral interference in quantitative spectroscopy. when interference arises specifically from the spectral overlap of two or more well defined components, a number of methods can be applied to measure the individual concentrations.

They are

- ✓ Simultaneous equation method.
- ✓ Derivative spectroscopy method.
- ✓ Absorbance ratio method.
- ✓ Chemical derivatization method.
- ✓ Area under curve method.
- ✓ Two wave length method.

Simultaneous equation method

When a sample contains two absorbing drugs x &, at the λ_{max} of the other. It is possible to determine both drugs at the same time without separating them from each other

The simultaneous equation of the two drugs in combined dosage form. once the equation parameters are set out, the it is just found out from a simultaneous equation at λ_{max} points of the two drugs. Then this measured absorbance are substituted in the equation and solved out for the determination of the two drugs.[15]

Data required for the construction of simultaneous equation the steps should be followed is

A) The λ_{max} of the two drugs should be found out by using the reference standards of the two drugs.

B) The calibration curve should be plotted for each drug and the linearity range should be found out.

C) The wavelength λ_{max} points of two drugs should be measured and their absorptivity values should be calculated.

D) The absorbance value of tablet formulation at the two wavelengths should be measured and recorded.

A set of two simultaneous equations were framed using the absorptivity values as given below: $A_1 = ax_1\ Cx + ay_1\ Cy$

$A_2 = ax_2 C_x + Ay_2 Cy_2$

Criteria to obtaining maximum precision

1. The ratios

A_2 / A_1 ay_2 / ay_1

ax_2 / ax_1 A_2 / A_1

Should be outside the range of 0.1 to 2.0 for the precise determination of the two drugs x & y

2. The λ_{max} of x & y should be dissimilar
3. The two components should not react chemically
4. The total absorbance of sample = absorbance of x + absorbance of y.

Advantages

a. Simple and it can be employed for routine analysis of a combination of drugs.

b. Very less time is required for analysis when the absorptivity values are determined.

c. Only two selected wavelengths are enough for the determination of absorbance of samples.

Derivative spectroscopy method

For the purpose of spectral analysis in order to relate chemical structure to electronic transitions for analytical situations in which mixtures contribute interfering absorption, a method of manipulating the spectral data called derivative spectroscopy. In this technique spectra are obtained by plotting the first or higher order derivative of absorbance or transmittance with respect to wavelength versus the wavelength. Often these plots reveal spectral details, which is lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference can sometimes be made easily or more accurately.

Chemical derivatisation method

Indirect Spectrophotometric assays are based on conversion of the analyte by a chemical reagent to a derivative that has different spectral properties. This method is employed if the analyte absorbs weekly in the UV region, a more sensitive method of assay is obtained by converting the substance to a derivative with a more intensely absorbing chromophore.

AREA UNDER CURVE METHOD

In this method, the absorptivity values (μ_1 and μ_2) of each of the two drugs were determined at the selected wavelength range. Total area under curve of a mixture wavelength range. This method is applicable when the μ_{max} of the two components is reasonably dissimilar, the two components do not interact chemically and both the component must be soluble in same solvent.

The methods deviated when overlapping of UV spectra of two drugs significantly and large difference in labeled strength. The accuracy of the method depends upon the nature of solvent, pH of solution, temperature, high electrolyte concentration and the presence of interfering substances.[16-18]

MATERIALS AND METHOD

Make : Shimadzu

Model : UV – 1700 series

Wavelength range : 90 ~ 1100 nm

Spectral band-width (Resolution) : 1 nm or less (190 - 900 nm)

Wavelength display : 0.1 nm Units

Wavelength setting : 0.1 nm units

Wavelength accuracy : ± 0.3nm on-board automatic wavelength

Wavelength repeatability : ± 0.1 nm

Light source switching : Automatic switching with wavelength Range can be set anywhere in range from

295 to 364 nm. (340.8 nm is recommended)

Stray light : 0.04% or less (220 nm: NaI 10 g / L)

0.04% or less (340 nm: $NaNo_2$ 50 g / L)

Photometric system : double beam optics

Photometric range : Absorbance - 0.5 ~ 3.0 Abs

(When uncorrected baseline curve isWithin 0.5 Abs)

Transmittance - 0~300 %

Recording range : Absorbance - 3.99~3.99 Abs

Transmittance – 399 ~ 399 %

Photometric accuracy : ± 0.004 Abs (at 1.0 Abs)

± 0.002 Abs (at 0.5 Abs)

Photometric repeatability : ± 0.002 Abs (at 1.0 Abs)

± 0.001 Abs (at 0.5 Abs

Auto zero function : Auto zero key enables one-touch setting

Base line correction : Automatic correction using computer memory

Deuterium lamp (Socket Type) - Light source position automatic adjustment mechanism. Built-in lamp lighting time displays function.

Reagents :

- ✓ Anandamide (pure sample)
- ✓ Formulation-1
- ✓ Formulation-2
- ✓ Methanol

Preparation of standard stock :

Standard stock solution of anandamide was prepared by dissolving 20 mg of drug in 50 ml of methanol in 50 ml of volumetric flask to get a concentration of 0.4 mg / ml. (400 µg / ml.).

Preparation of working standard solution :

The prepared stock solution was further diluted with methanol to get working standard solutions of 100 µg / The standard solutions of anandamide were transferred separately into a series of 10 ml volumetric flasks and diluted to 10 ml with methanol (1 - 10 µg / ml).

Preparation of sample solution:

The powder equivalent to 10 mg of anandamide was dissolved in 10 ml of methanol, sonicated for 30 mins, and filtered. 0.1 ml and 0.2 ml from the filtrate were taken and further diluted with methanol up to 10 ml. The absorbance of the prepared solutions were measured at 250 nm against methanol as blank and the drug content in each tablet was estimated by using the standard graph.

Evaluation of uv parameters

- ✓ Absorption maximum.
- ✓ Beers law concentration, showing linearity and range.
- ✓ Assay of the tablet formulation.
- ✓ Recovery studies.

Absorption maxima:

Stock solution was suitably diluted with methanol so as to contain 10 µg / ml of anandamide. This solution was subjected to scanning in the U.V region between 200 - 400 nm and found that anandamide maximum absorbance at 250 nm.

Beer's law concentration range

Linearity and calibration:

The stock solution of anandamide was suitably diluted with solvent solution to provide varying concentrations of 1 - 10 µg / ml. which were scanned at 250 nm and the absorbances were plotted against concentration.

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80 %, 100 %, and 120 %) of bulk samples of anandamide within the linearity range were taken and added to the pre-analyzed formulation of concentration 5 □g / ml. From that percentage recovery values were calculated.

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ of Anandamide were determined by using standard deviation of the response and slope approach as defined in International conference on harmonization (ICH) guidelines

Repeatability studies:

Repeatability is given by inter-day and intra-day precision. Intra-day precision was determined by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was determined by analyzing the three different concentration of the drug for three days. The precision of the assay was determined and % RSD was found.

Results And Discussion

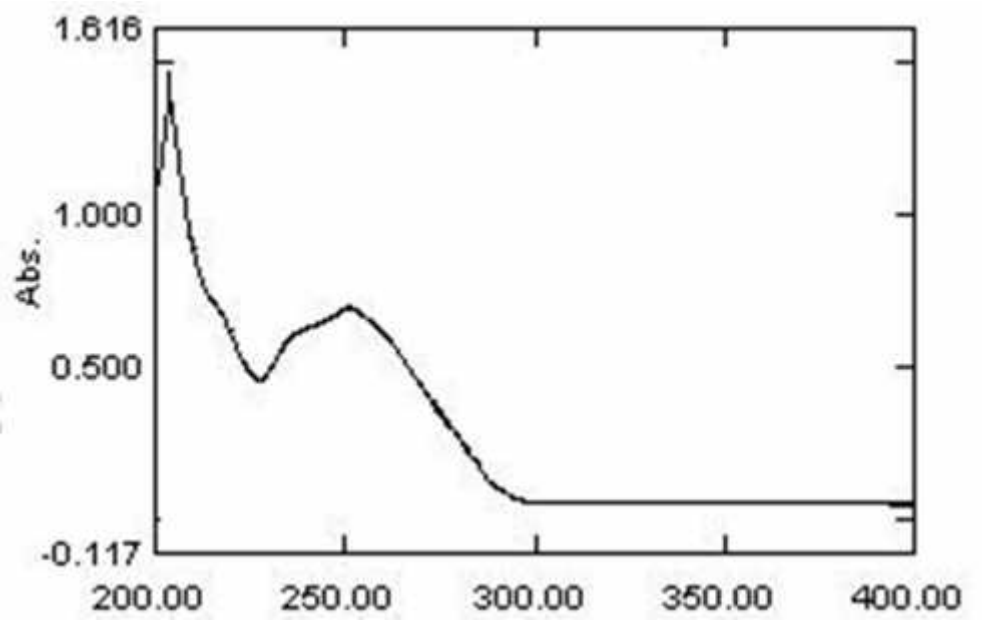
Uv Spectroscopic Method

UV spectroscopic method for the estimation of anandamide with

1. Absorption Maxima:

The absorption maxima of the proposed drug were determined by scanning in the entire UV region between 200 - 400 nm. Absorption maxima were found to be 250 nm (Fig1.1).

Fig 1. Identification spectrum of anandamide by UV spectroscopy.



2. Beer's law concentration range Linearity:

The method was validated by linearity studies which were performed by plotting different concentrations of standard solution against their respective absorbances and calibration curve was drawn at 1 - 10 µg / ml. The values were given in Table-1.1 and calibration curve was in (Figure 3) . A correlation co-coefficient value was found to be 0.998329 which was shown in their respective figure. The optical characteristics of were shown in Table-1.5.

Figure 2 - Overlain spectra of anandamide by UV-Spectroscopy

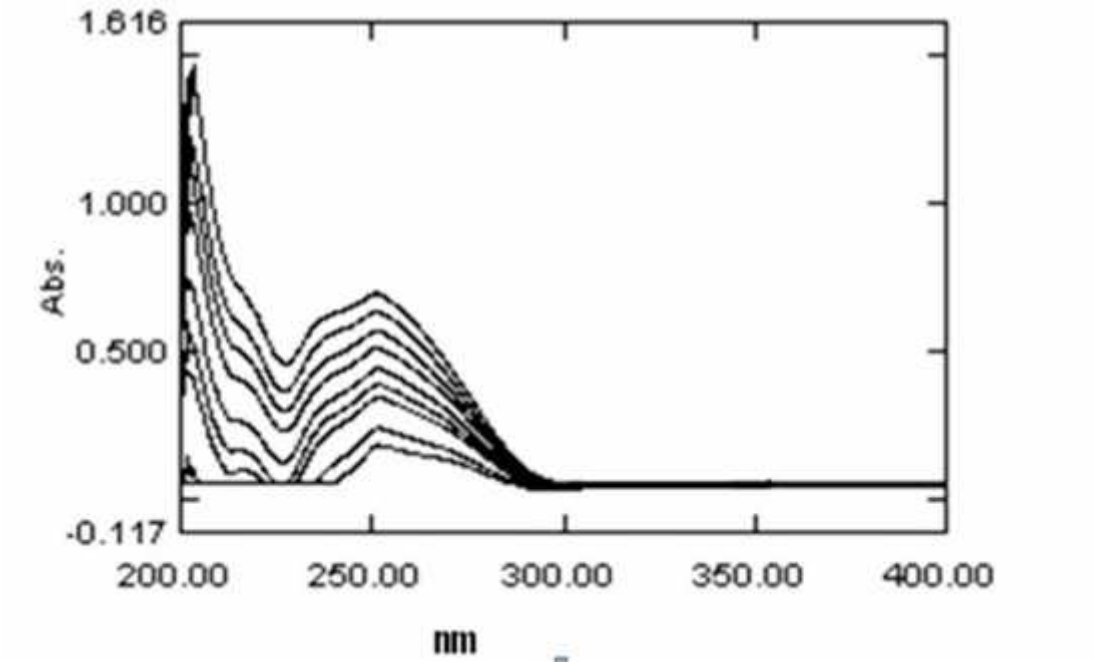


Fig 3- Linearity of anandamide by UV spectroscopy

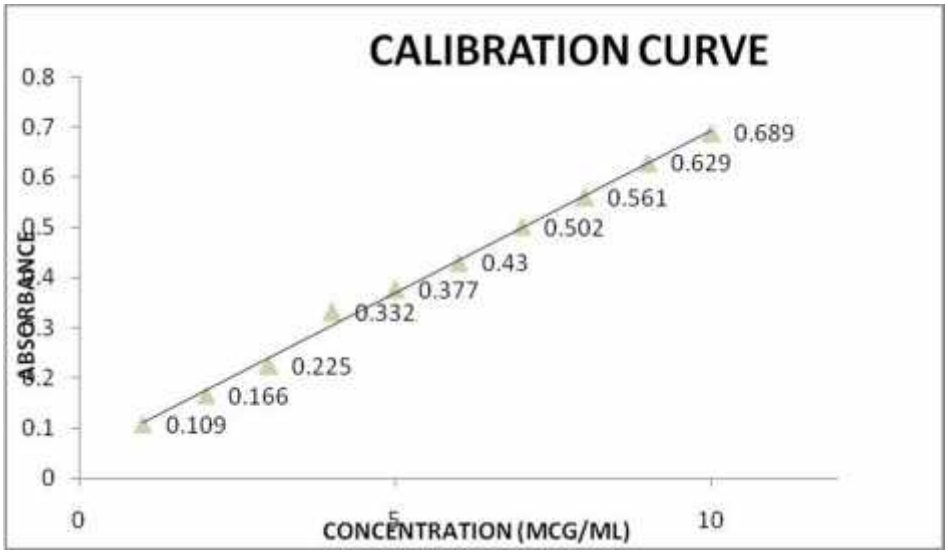


Table 1- Linearity studies of anandamide by UV spectroscopy

Concentration(µg/ml)	Absorbance
1	0.19
2	0.166
3	0.225
4	0.332
5	0.377
6	0.43
7	0.502
8	0.561
9	0.629

Assay :

The quantitative estimation was carried out on marketed tablets formulation-1and formulation-2 by taking concentration of 10 µg / ml of each formulation was scanned over the range of 200 - 400 nm and the absorbances at 250 nm..

Percentage relative standard deviation was found to be 0.296 and 0.290 respectively.

Percentage purity values were obtained as 99.51 % for Formulation-1 and 99.36 % for

Formulation-2

Table.2- Assay of anandamide by UV spectroscopy

Formulation	Labeled amount*(mg)	Observed amount*(mg)	% amount found (mg)	% RSD
Formulation-1	20	19.903 - 0.0589	99.51	0.296
Formulation-2	20	19.872 - 0.0577	99.36	0.29

* Each value is average of three determinations ± standard deviation.

Accuracy:

The proposed method was validated by recovery analysis performed by adding known concentrations of standard drugs to preanalysed sample solution at three different levels. Percentage recovery range was found to be within 98 – 102 %. (Table-3)

Table 3- Accuracy data of anandamide by UV spectroscopy

Sample ID	Concentration (µg / ml)		% Recovery of Pure Drug	Statistical Analysis	
	Pure drug	Formulation.			
S1 : 80 %	8	10	97.5	Mean	97.3
S2 : 80 %	8	10	97.2	SD	0.07
S3 : 80 %	8	10	97.2	% RSD	0.072
S4 : 100 %	10	10	98.7	Mean	98.43
S5 : 100 %	10	10	98.4	SD	0.1909
S6 : 100 %	10	10	98.2	% RSD	0.193
S7 : 120 %	12	10	98.6	Mean	98.43
S8 : 120 %	12	10	98.4	SD	0.1202
S9 : 120 %	12	10	98.3	% RSD	0.1219

Repeatability Studies:

Repeatability is given by inter-day and intra-day precision. Intra-day precision was determined by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was determined by analyzing the three different concentration of the drug for three days in a week. The precision of the assay was determined and % RSD was found (Table -4). Intra-day and Inter-day readings (n = 6):

Table 4 - Precision data of anandamide by UV spectroscopy

Concentrations	Absorbance	Statistical analysis
2	0.0194	S.D. - 0.00018
		% R.S.D.- 0.93
5	0.0292	S.D. - 0.00013
		% R.S.D.-0.45
10	0.0431	S.D. - 0.00031
		% R.S.D.- 0.73

Summary and Conclusion

I conclude that the developed methods are simple, accurate, sensitive and precise and found to be with in prescribed limits. And these methods can be used to quantify the pure and formulations containing anandamide.

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