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Qualitative and Quantitative Analysis of Anti-hypertensive Drug Atenolol with Coffee

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GRAPHICAL ABSTRACT

100 mg of atenolol tablet (left)
Standard caffeine powder (right)

ABSTRACT

Normally, sufferers of high blood pressure and elderly also consumed coffee as beverages during taking of atenolol drugs. Coffee on the other hand is also a stimulant to the central nervous system which can keep them alert. In this study, UV-Vis and FTIR-ATR spectroscopy method were used for qualitative and quantitative analysis of caffeine towards atenolol consumption. From the UV-Vis spectrum, the relationship between the concentrations and the absorbance exhibited good linearity with correlation coefficient 0.9924 for atenolol and 0.9989 for caffeine at 273 nm wavelength. The calculated amount of lowest caffeine concentration was 2.15 ± 0.04 µg/mL and the percentage of error in caffeine was obtained at 1.7%. In atenolol sample, the concentration was obtained at 56.36 ± 0.11 µg/mL with 0.19% percentage error. At wavelength of 273 ± 1 nm, the 60 µg/mL atenolol solution mixed with 2, 4, 6, 8, 10 and 12 µg/mL standard caffeine after 15 minutes of stirring at room temperature displayed the increasing relationship. Further analysis by using FTIR-ATR showed 3 different peaks peaking at 1736 cm^{-1}, 1365 cm^{-1} and 1217 cm^{-1} consecutively in 4 and 6 µg/mL caffeine added. In comparison with 60 µg/mL atenolol solution, the 3 main bands were detected at 3325 cm^{-1}, 2109 cm^{-1} and 1636 cm^{-1}.

Keywords: Atenolol, high blood pressure, coffee, UV-Vis spectroscopy, FTIR-ATR spectroscopy

1. INTRODUCTION

Hypertension usually develops when increasing in age and it affects almost everyone after a period of time. Hypertension phenomenon is likely to happen when blood is pumped by heart through the narrower size of arteries. Arteries size become narrower when there is formation of plaque. Plaque is the mixture of bad cholesterol inside the arteries walls. Thus, this will result in an increase amount of resistance to blood flow. The unfavourable long-term hypertension may cause health issue for instance, heart disease due to high pressure of the blood flow against the artery walls. Without further treatment of hypertension, patient might be distressed with stroke, premature death, and myocardial infarction [1].

Hypertension is merely classified as non-communicable diseases (NCD) in which it is not passed from one to another. NCDs typically progressing slowly and several risk factors associated with NCDs are high intake of fat in food, lack in physical exercises, uncontrolled alcohol intake, and tobacco consumption. NCDs kill almost 41 million people each year, similar to 71% of all deaths reported globally [2]. There is about 35.1% which corresponds to 5.8 million of Malaysian adults who experience hypertension [3].

There are numerous types of medications used to treat patients with hypertension. Firstly, beta-blockers which include atenolol, acebutolol, timolol, and metoprolol. Secondly, is diuretics which helps to reduce blood pressure by flushing out imprudent water and sodium through urination as well as potassium content in the human body. ACE (Angiotensin-converting enzyme) also could be used for high blood pressure treatment. Other than atenolol, Felodipine (Plendil) is also another type of calcium channel blocker used to aid high blood pressure.

Once atenolol medications have been prescribed by the elderly, it is advisable not to stop the medicine unless being instructed by the doctors. Anti-hypertensive drugs should be taken separately with coffee or might need to have few hours of duration period in between to reduce any side effects. A 150 ml cup of coffee has 60-120 mg of caffeine [4]. During ingestion of caffeine into human body, it takes around 2.5-4.5 hours for the caffeine to reduce to half of its initial amount [5]. Small changes in the way these medications work will increase the risk of significant side effects that is observable drug interactions. The interactions caused fatigue, faint and dizziness to human.

This research will emphasize on the development of a simple method to study the interaction effect of caffeine towards atenolol consumption, to spike various concentrations of caffeine to atenolol sample and to chemical characterize qualitative and quantitative analysis of the samples using UV-Vis and FTIR-ATR spectroscopy.

2. EXPERIMENTAL

In this chapter, there are two major parts of the research which are the qualitative and quantitative analysis of atenolol, caffeine and mixed solution of fixed atenolol concentration with varied caffeine concentration using UV-Vis spectroscopy. Then, the research proceeds with a qualitative analysis of mixed solution of fixed atenolol concentration with varied caffeine concentration using FTIR-ATR spectroscopy.

Firstly, the analysis of atenolol and standard caffeine will be started by buying atenolol from pharmacy and standard caffeine from BDH Chemicals Ltd. Meanwhile, the atenolol tablet was ground into powder and dissolve in 0.1M HCl. Then, the caffeine powder is directly dissolve in 1L of distilled water to make up 1000 µg/mL stock solution.

Next, several series of concentration of atenolol were prepared which comprises of 20, 40, 60, 80, 100 and 120 µg/mL to analyse it using UV-Vis spectroscopy and calibration curve of atenolol was plotted. Several series of concentration of caffeine were also prepared which comprises of 2, 4, 6, 8, 10 and 12 µg/mL to analyse it using UV-Vis spectroscopy and calibration curve of caffeine was plotted. Several series of concentration of caffeine was prepared after mixed with atenolol in order to analyse it using UV-Vis spectroscopy.

One of the concentration of atenolol was taken for further analysis at fifteen-minute reaction time. The solution was analysed repeatedly for ten times in order to determine the percentage of error. The percentage of error can be calculated from the result obtained based on formula as below:

$$\% \, error = \frac{\frac{\Sigma|X - \bar{X}|}{10}}{\bar{X}} \times 100\% \tag{1}$$

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis of atenolol sample using UV-Vis spectroscopy

The UV-Vis spectrum of atenolol samples was recorded at 200 to 400 nm using Shimadzu Ultra Violet Visible (UV-Vis) spectroscopy. Figure 1 showed the UV-vis spectrum of atenolol at 200 to 400 nm. The atenolol samples were put inside a quartz cuvette during the analysis.

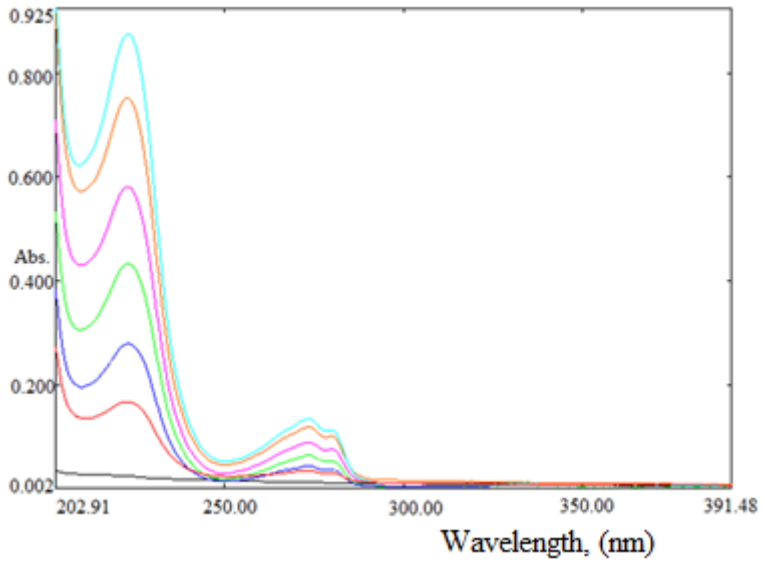

Figure 1. Atenolol UV-Vis spectrum

Figure 2 showed the calibration curve of absorbance against atenolol concentration. It shows the correlation value of atenolol concentration to the respective absorbance's. This calibration curve was very useful to determine quantitatively the concentration of atenolol upon spiking of caffeine based on the absorbance recorded.

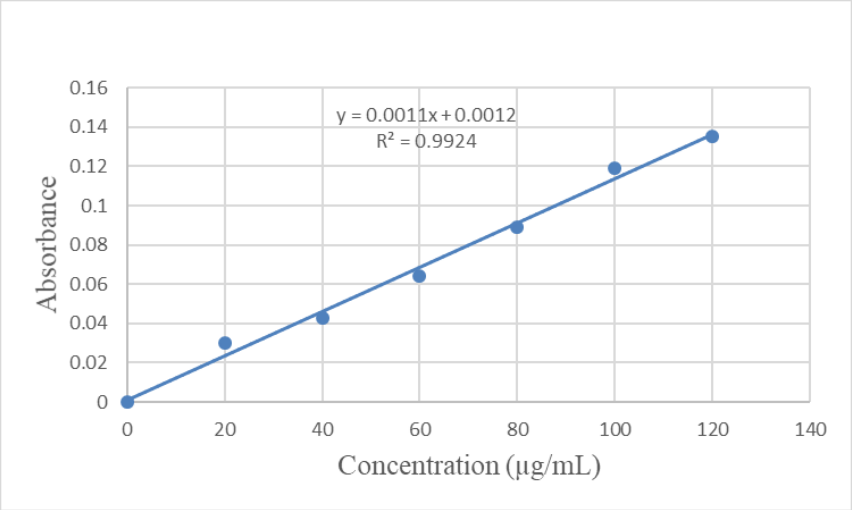

Figure 2. Calibration curve of atenolol against 0.1M HCl

In addition to the qualitative and quantitative analysis to the concentration of atenolol, some analytical calculations were done for a method validation. The percentage of error was evaluated by testing ten replicates of 60 µg/mL standard solution of atenolol within a day and the result as shown in Table 1 below. From the calculation, the percentage of error for atenolol was 0.19%.

Table 1. Results of ten replicates of 60 µg/mL standard solution of atenolol

Number of replicates	Absorbance	Concentration (µg/mL)
1	0.064	57.0909
2	0.065	58.0000
3	0.064	57.0909
4	0.062	55.2727
5	0.063	56.1818
6	0.063	56.1818
7	0.063	56.1818
8	0.059	52.5455
9	0.065	58.0000
10	0.064	57.0909

3.2. Quantitative Analysis of Caffeine Sample Using UV-Vis Spectroscopy

Analysis of caffeine in white powder form using UV-Vis spectroscopy gave the absorbance of caffeine at 273 ± 1 nm wavelength. The absorbances were different based on the concentration of caffeine dissolved in the distilled water. The UV-Vis spectrum of the caffeine solutions as shown in the Figure 3 were recorded at 200-400 nm using Shimadzu Ultraviolet-Visible (UV-Vis) spectroscopy using distilled water as blank.

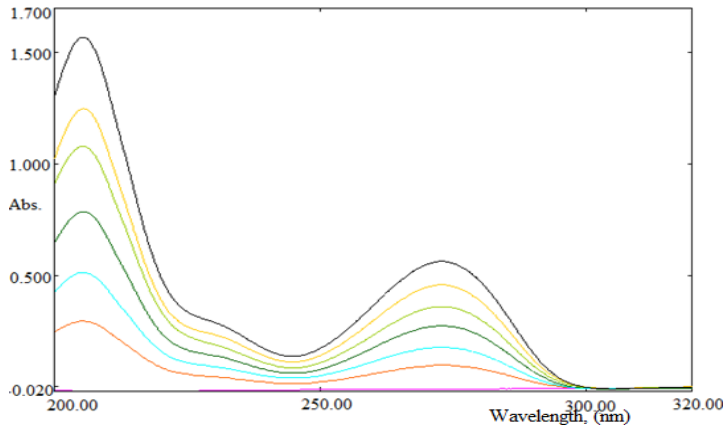

Figure 3. UV-vis spectrum of caffeine

Figure 4 showed the calibration curve of absorbance against caffeine concentration at wavelength of 273 nm. It shows the correlation value of caffeine concentration to the respective absorbance.

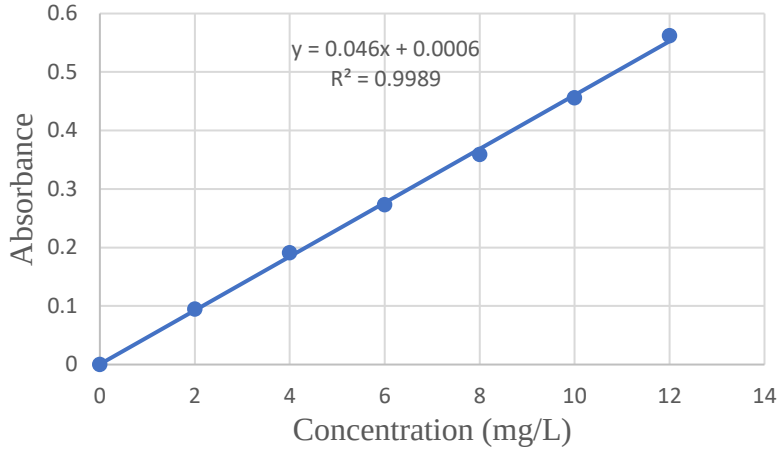

Figure 4. Calibration curve of standard caffeine at 273 ± 1 nm wavelength

In addition to the qualitative and quantitative analysis to the concentration of caffeine, some analytical calculations were done to validate the method. The percentage of error was evaluated by testing ten replicates of 2 µg/mL standard solution caffeine within the day. The result was presented by Table 2 as shown below. The percentage of error for caffeine was 1.7% obtained from calculation.

Table 2. Results of ten replicates of 2 µg/mL standard solution of caffeine

Number of replicates	Absorbance	Concentration (µg/mL)
1	0.098	2.1174
2	0.098	2.1174
3	0.095	2.0522
4	0.101	2.1826
5	0.099	2.1391
6	0.102	2.2043
7	0.102	2.2043
8	0.100	2.1609
9	0.101	2.1826
10	0.100	2.1609

3.3 Determination of Atenolol Concentration After Addition of Caffeine

To determine the amount of atenolol after addition of 2, 4, 6, 8, 10 and 12 µg/ml of caffeine, the samples were analysed using UV-Vis spectroscopy. From the spectrum obtained as shown in Figure 5, the absorbance of the sample was recorded at 273 nm wavelength which were recorded and presented as shown by Table 3. Based on the experimental observation, the absorbance increase when the caffeine concentration added to the atenolol increases.

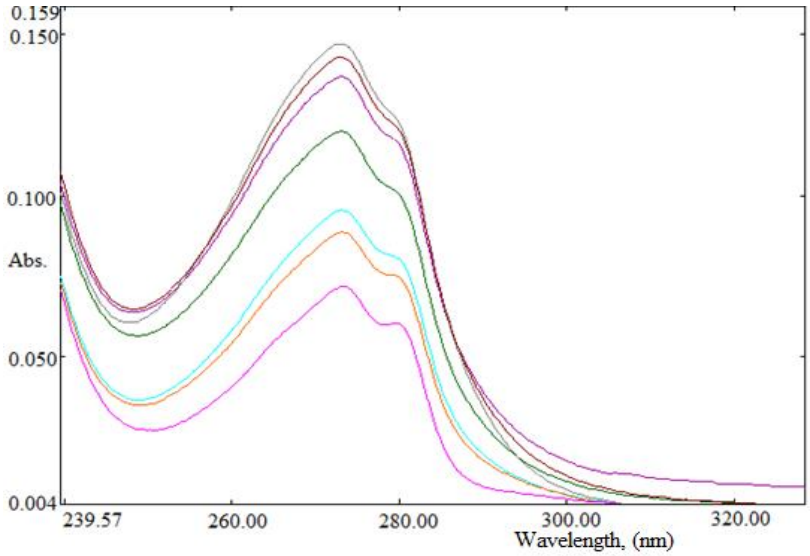

Figure 5. Effect of addition of caffeine into 60 µg/mL atenolol

Table 3 The absorbance value with respect to the concentration of caffeine added

Concentration of caffeine added (µg/mL)	Absorbance	Concentration of atenolol after addition of caffeine (µg/mL)
0	0.072	64.364
2	0.089	79.818
4	0.096	86.182
6	0.120	108.000
8	0.137	123.455
10	0.143	128.909

Quantification analysis of concentration of caffeine in the sample was done using calibration curve equation y = 0.0011x + 0.0012. Calculated amount of atenolol concentration were 79.82 ± 0.11 µg/mL, 86.18 ± 0.11 µg/mL and 108.00 ± 0.11 µg/mL after addition of caffeine. Before the addition of caffeine, the atenolol concentration was 64.36 ± 0.11 µg/mL.

3.4. Qualitative Analysis of Atenolol Spiked with Caffeine Using FTIR-ATR

Infrared spectroscopy deals with the infrared part of the electromagnetic spectrum. It measures the absorption of different IR frequencies by a sample positioned in the path of an IR beam. Currently, infrared spectroscopy is one of the most used techniques in industry. With the rapid development of infrared spectroscopic instrumentation software and hardware, the application of this technique has expanded into many areas of food research. Nowadays, it has become a powerful, non-destructive, and fast technology for food quality analysis and control. FTIR-ATR technique used to identify the functional groups present in the atenolol, caffeine and mixture of atenolol with caffeine respectively. The FTIR spectrum deduced the peaks of transmittance against wavenumber. The following Figure 6 showed the FTIR-ATR overlay spectrum of caffeine spiked to atenolol.

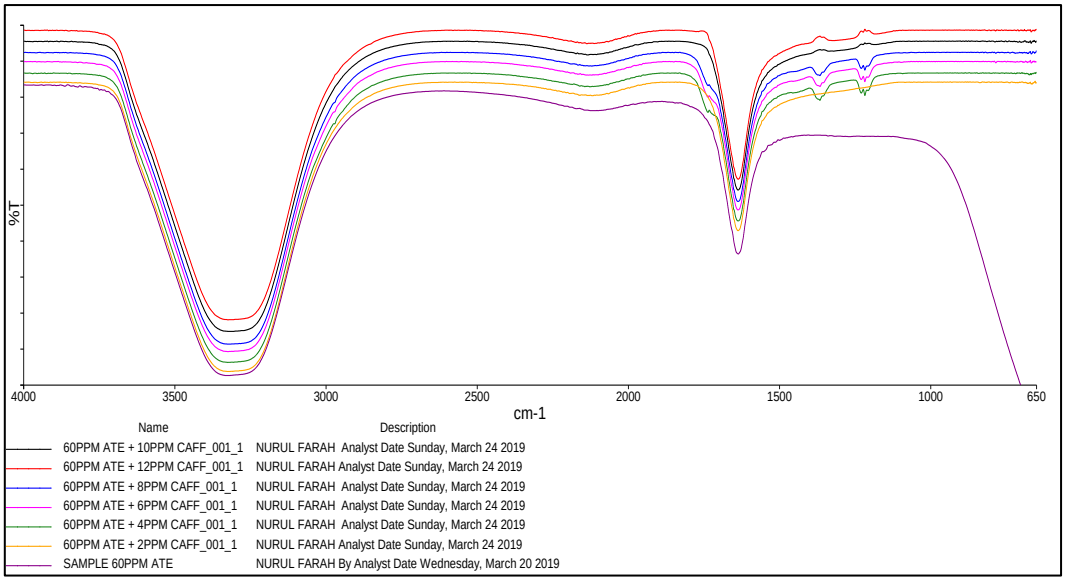

Figure 6. FTIR overlay spectrum of caffeine added to atenolol

From the spectrum of FTIR-ATR, we can see the difference between the atenolol sample and the atenolol with caffeine sample. As in the atenolol sample, the three absorption bands were at 3325 cm^{-1}, 2109 cm^{-1} and 1636 cm^{-1}. In the atenolol plus caffeine samples, there were three different shoulder peaks peaking at 1736 cm^{-1}, 1365 cm^{-1} and 1217 cm^{-1}. The broad and intense peak around 3325 cm^{-1} suspected of the presence of hydroxyl (OH^-). The (C=C) shows the moderate peak at 1636 cm^{-1}. The (C-O) group shows peak around 1217 cm^{-1}. Aromatic double bond (C=C) shows peak shifted at 1736 cm^{-1} and 1365 cm^{-1}.

4. CONCLUSION

Through this study, two methods were successfully identified the effect of caffeine added to atenolol. These two proposed methods are simple, and easy in handling for analysis of caffeine towards atenolol consumption. The next objective was successfully attained by preparing 2-12 µg/ml caffeine dissolved in distilled water with fixed concentration of atenolol. The calibration curve of atenolol and caffeine has obtained good linearity coefficient. Straight line equation was used to determine concentration of atenolol upon addition of caffeine. The last objective was also successfully achieved when the combination of these analytical method proved that it is able to do qualitative and quantitative analysis of atenolol with caffeine. Throughout the study, an increment in absorbance using UV-Vis spectroscopy and existence of absorption band using FTIR-ATR spectroscopy were observed. The development of the spectroscopy technique in screening, detection and confirmatory of atenolol and caffeine hopefully can help the elderly especially people who suffered from hypertension to be more aware of the possible effects that may occurred upon taking atenolol drug with drinking coffee as beverages.

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