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Identification And Determination Of Fisetin In Black And Green Teas By ZIC-HILIC Column Coupled With UV Detection

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Abstract

ABSTRACT

This study aims to introduce a novel method that included zwitterion chromatography-hydrophilic interaction chromatography with a UV detection system for analysis and identification of the fisetin in black and green teas. Flavonoids like fisetin have been shown to have multiple beneficial effects on human health that are available in many fruits, beverages, herbs, vegetables, and nutritional supplements herbs are the main source of flavonoids. Hydrophobic and hydrophilic interactions forming a fisetin mechanism to distinguish mixed mode. The validated process has been successfully used for the extraction test in herbs. Fisetin has been separated in the ZIC-HILIC column using acetonitrile and sodium acetate buffer as the mobile phase. The results showed that the HILIC mode was simple and effective, and could be used to identify the fisetin content in samples of black tea leaves and green tea leaves. The calibration curve was produced in commercial ZIC-HILIC column and linear range (0.01-7 µgmL-1), RSD% (1.32), LOD (0.008 µgmL-1), LOQ (0.024 µgmL-1).

Keywords: Fisetin, Black tea, Green tea, hydrophilic interaction chromatography, flavonoids

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INTRODUCTION

During these decades researchers have focused on the identification of biologically active compounds in herbal preparations, their mechanisms of action, and their effectiveness [1]. As many biologically active chemical components of these herbal extracts exist, Flavonoids are the most important of them. Flavonoids play an important role in protecting against chronic disease cases including diabetes, cardiac disease, blood vessels, asthma, nervous system disorders, prostate, and cancer [2]. Numerous studies have already shown that diets high in these flavonoids are a close association with a lower prevalence of chronic diseases, such as cardiovascular diseases, type two diabetes, neurodegenerative diseases, and even cancers [3-5]. This is due to their antioxidant, anti-inflammatory, anti-tumor activities and antiviral activities [6]. The classification of flavonoids is based on their chemical structures, which vary in the number of double bonds, the number of oxygen-containing alternatives, and their relative location. There are six known flavonoid varieties: flavanones, flavonoids, flavones, isoflavones, 3-uls flavans, and anthocyanidins. Flavonoids are present in many foods consumption by a human, including onions, broccoli, various fruits, and vegetables such as parsley and celery [7]. Flavonoids are also present in tea, coffee, bananas, grapes, red wine, berries, and soybeans. Fisetin (Figure 1) is considered to be one of the most important flavonoids found in many fruits, teas, cucumbers, and onions. Because fisetin has anti-oxidant and anti-inflammatory effects, its anti-cancer potential has been explored, and this has made it a powerful factor in the prevention and treatment of various types of cancer, in particular prostate, colon, and breast cancer [8]. There are a lot of studies dealing with the study of separating and estimating many flavonoids, the most important of which are chromatographic analytical methods in which columns of C_{18} are used. Here, researchers face many problems when using these columns, including the retention time for separating polar compounds is very little, or that these columns do not separate such materials. On this basis, the problems of the C18 columns have now been resolved using the HILIC columns. Consequently, the HILIC is suggested as another new solution for highly polar and hydrophilic compounds [9]. In HILIC mode, in most cases, a mixture of water or buffer and organic acetonitrile is used in the stationary polar phase. The structural differences in the HILIC type stationary phases are larger than those observed in the reverse phase and this has given the HILIC columns an important benefit in dealing with problems in the classical columns and making the HILIC columns the favorite of many researchers in this field [10]. Currently, there are studies in which HILIC columns have been used for various applications e.g. nucleosides, carboxylic acids, amino acids, and pharmaceutical [11-23]. For flavonoids, few studies have been used to separate and estimate flavonoids and to use HILIC columns with MS detector [24-27]. However, there is a recent single analysis to separation and estimate using HILIC columns with UV detection carried out by Rasheed and Co-worker [28]. It gave us the motivation to investigate in separation and estimation Fisetin using HILIC columns with UV detection. As a result, a new method has been introduced to estimate Fisetin in some important herbs. Since decades many people around the world have used herbs to treat human diseases [29, 30]. These herbs are of great interest, because they have natural benefits and high efficiency. Black tea leaf and green tea leaf as both contain many flavonoids, These plant leaves have become increasingly common because of their active function in promoting health activities such as antioxidants, anti-cancer, anti-inflammatory, immune, and hypoglycemic [31]. On this basis in this paper, the components of these leaves have been studied.

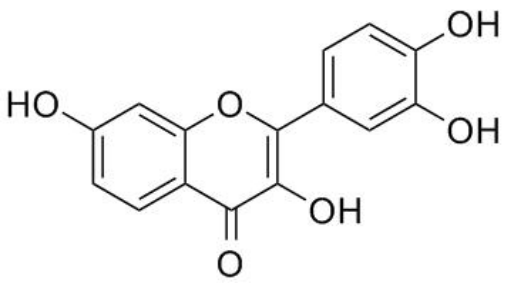

Figure 1: Chemical structure Fisetin.

MATERIALS AND TECHNIQUES

Chemical reagents and materials

As for chemicals, acetic acid, sodium acetate, acetonitrile, fisetin were purchased from Sigma-Aldrich. 0.1 µs / cm of conductivity from Millipore Water System (USA). Millipore filters (0.45 µm) filtered the solution. Black and green tea leaves have been brought from the local market in Iraq.

Instrumentation and chromatographic requirements

Merck Hitachi HPLC system equipped with L-6200 Pump and UV detector Visible type L-4200. The mobile phase used composed of acetonitrile and acetate buffer at a flow rate of 0.5 mL/min. Quantitative measurement of fisetin was performed with a 350 nm. The volume of injection was 10 µL. The column used in the separation process was kept at a temperature of 35 ^{0}C. The data was analyzed using the N2000 workstation software. All separations were performed on the ZIC-HILIC commercial column from Merck SeQuant (100 mm x 4.6 mm ID).

Preparation of the stock solution of Fisetin

A stock solution of fisetin was prepared by dissolving 10 mg of fisetin in 100 ml of acetonitrile in a volumetric flask. The result was then dissolved and filtered through a 0.45 µm filter.

Samples preparation

Black and green tea leaves were brought from the local market in Iraq and the herbs were of Chinese origin as they were imported from China in 2019. The leaves of these plants were taken and well cleaned, then dried, crushed, and turned into fine flour in preparation for the extraction procedure. Two grams were taken for each type of herb as 20 ml of 80 % ethanol was added to the herb and given over a period of time. After stirring, the product was placed in the ultrasound machine bath at 60 ° C for 30 minutes and then filtered with a 0.45 µm filter and then placed in the refrigerator for a period of time. The extraction procedure for these herbs has been repeated three times.

THE RESULTS AND DISCUSSIONS

Study separation mechanism

For the evaluation of the HILIC retention mechanism in the commercial column (ZIC-HILIC) with acetonitrile and acetate buffers. Fisetin was selected as a flavonoid model. It is important to investigate the effect of changing the ingredient of the mobile phase, pH buffer, and the buffer concentration.

The effect of changing acetonitrile content on fisetin retention

The effect of changing acetonitrile content on the fisetin retention interaction at constant pH 5 and 30 mM acetate buffer was observed. Fisetin behavior is in reversed-phase (RP), with the level of ACN eluent continuing to increase from 60% to 95%. This behavior is caused by fisetin hydrophobicity; in this column, the reverse phase (RP) fisetin interaction was seen (Figure 2), which was attributed to fisetin log P_{ow} (1.81).

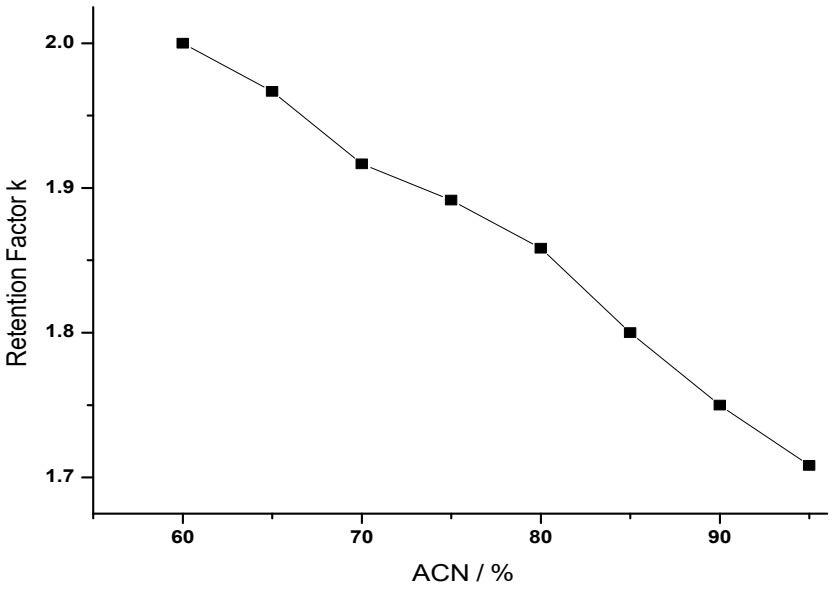

Figure 2: Effect of changing acetonitrile content on fisetin interaction.

The effect of changing of acetate buffer concentration on the retention of fisetin

Solute behavior has increased in hydrophilic mode with an increased buffer concentration, leading to intramolecular ion pairs deactivation. In this way, the linearization of the functional column groups is improved, even though acetonitrile exists [19]. Remarkably, fisetin shows increased retention factors when the holding acetonitrile at 85% and pH at 5 whilst acetate buffer from 20 to 80 mM has been increased (Figure 3). However, it can at least be shown that the hydrophilicity of the analytes and electrostatic influences known from the ZIC play a role. Besides, the quality of the phase separation between the mobile and pseudo-stationary phase also seems to have an impact on the chromatographic separations. The separation of fisetin is considered to be mainly based on the formation of a pseudo-stationary water layer on the column stationary phase between which fast partitioning occurs with the rich organic solvent mobile phase [32, 33].

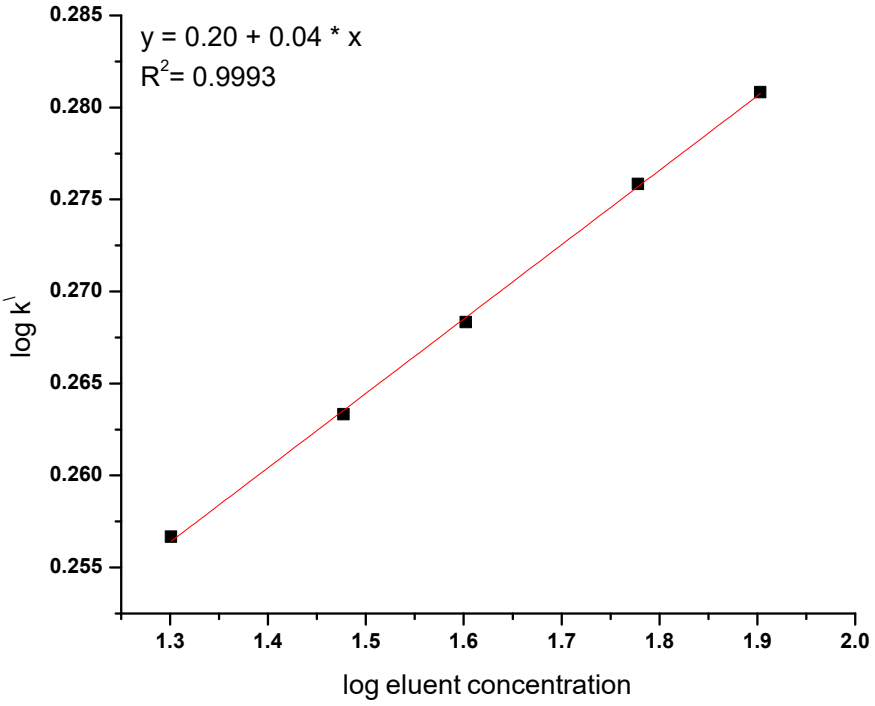

Figure 3: Effect of changing acetate buffer concentration on retention fisetin interaction

The effect of changing of acetate buffer pH on the retention of fisetin

This significant effect was studied to remove or reduce the strong electrostatic attraction of charged between analyses and stationery HILIC materials. The eluent pH must be changed to complete the fisetin separation in HILIC mode. At steady buffer concentrations of 30 mM and 85% of acetonitrile, the pH improved from 3 to 5.5. As in Figure 4, fisetin raises the retention factor this is because the group of hydroxyls is separated in fisetin. This depends on the physical and chemical data of the fisetin predicted. pKa value range from just under 6.32 and the analyzes are certainly detonated if the pH in the mobile phase rises to 5.5.

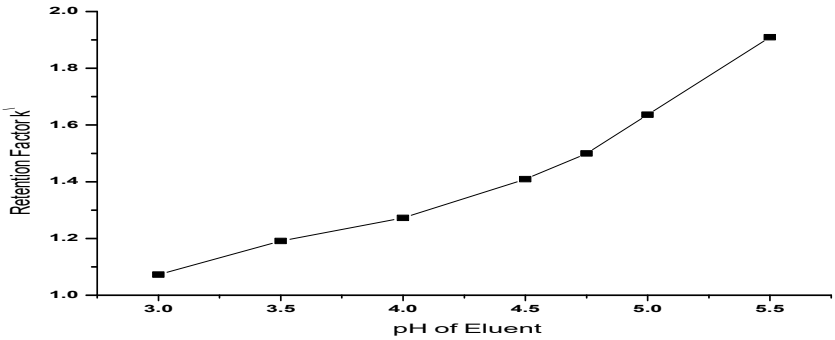

Figure 4: Effect of changing acetate buffer pH on retention fisetin interaction

Optimizing the separation of fisetin

Once the separation mechanism of fisetin has been examined, the effect on the contents of acetonitrile, the concentration of the acetate buffer, and the eluent buffer pH has been calculated. The best condition was 85% acetonitrile and acetate buffer 30 mM-pH 5 for separation of fisetin. The separation fisetin chromatogram as shown in Figure 5.

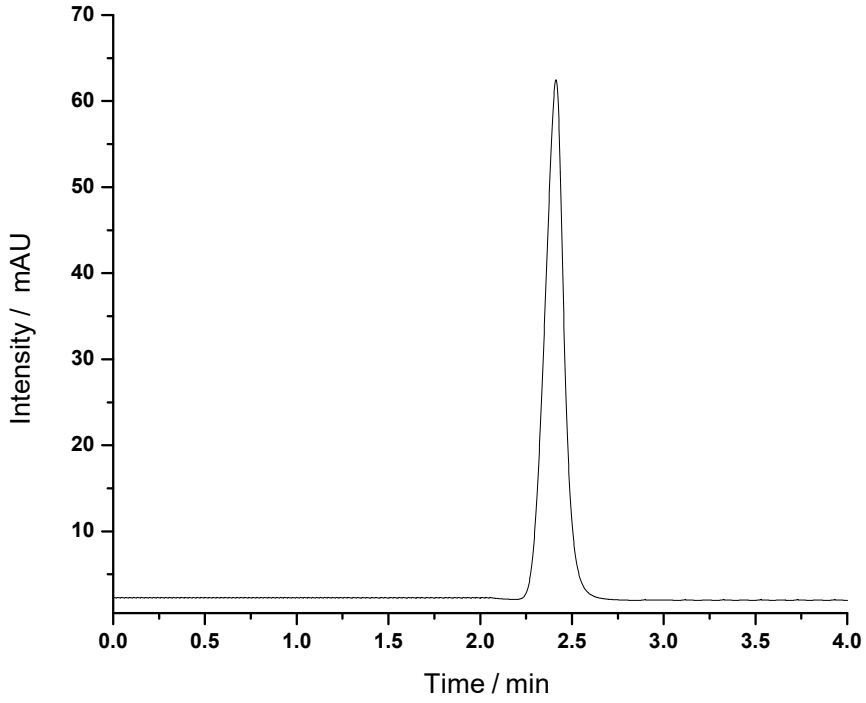

Figure 5: Chromatogram for the separations Fisetin in ZIC-HILIC column

Linearity and range

In this study, the method suggested was linearity checked by plotting peak area against fisetin concentration. In a concentration range of 0.01 to 7 µg mL^{-1}, the plot of the peak area against respective fisetin concentrations was found to be linear (Figure 6). Table 1, which displayed the findings of linearity, regression equation, and fisetin calibration statistics. The findings indicate a strong association of the peak area with the concentration of fisetin.

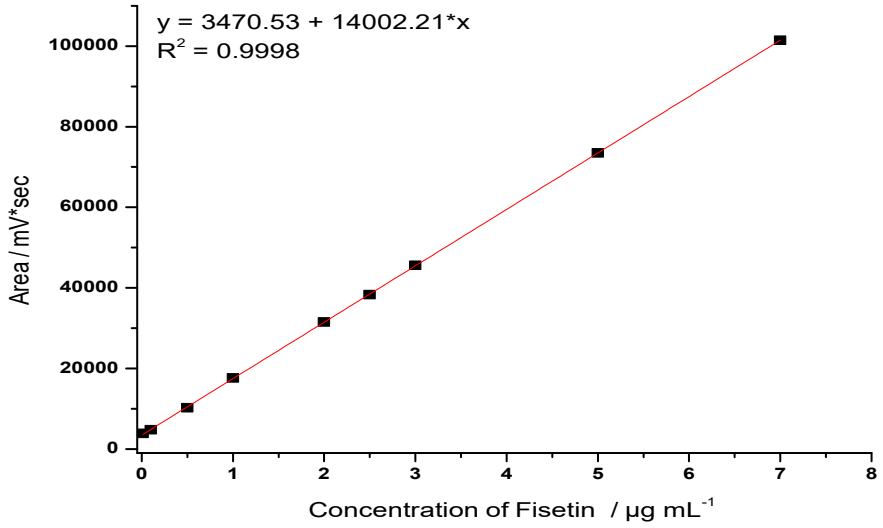

Figure 6: Linearity curve of fisetin.

Table 1: The results obtained from this study.

Parameter	Proposed method

Linearity (µg.ml^{-1})	0.01-7
Regression equation	y = 3470.53 + 14002.21*x
R^2	0.9998
LOD (µg.ml^{-1})	0.008
LOQ (µg.ml^{-1})	0.024

Statistical analysis

On the same day and on different days, where accuracy and accuracy were measured, as well as RSD% and Rec. Calculated percentage. This relatively small assumptions and high recovery values explain the proposed method that works in (Table 2).

Table 2: Statistical reliability and accuracy of fisetin on the same day and various days.

	Same-Day Analysis n=5				Day-to-Day Analysis n=5			
Taken (µg.mL^{-1})	**Found (µg.mL^{-1})**	**% Rec.**	**% Erel.**	**%RSD**	**Found (µg.mL^{-1})**	**% Rec.**	**% Erel.**	**%RSD**
1	0.98	98.00	- 2.00	1.32	0.985	98.50	- 1.50	1.45
3	2.96	98.66	-1.34	1.20	2.98	99.33	- 0.67	1.34

Fisetin determination in some tea samples

The proposed method by using the ZIC-HILIC column was utilized for the determinations of fisetin in black and green tea leave samples have been successfully and that is through the results of the study are listed in Table 3.

Table 3: The fisetin content in tea samples

flavonoid	Black tea leaves mg/g*	Green tea leaves mg/g
Fisetin	0.0750 ± 0.004	0.2695 ± 0.066

***** Contents (mg/g) as mean + SD, are expresses (n = 5).

CONCLUSIONS

This newly developed method was successfully applied to determine the amount of fisetin in these plant's leaves. The study also discussed the technique of extracting and analyzing the ingredients in these herbs. The analytical procedure demonstrated the effectiveness, practicability, and viability of this new methodology with high precision, sensitivity, and repeatability. This is the result of the use of such columns, which are the ZIC-HILIC columns, which dealt with problems that obstruct the separation process when using the C18 columns. This analysis showed that fisetin was used in black tea leaves and green tea leaves. The newly established approach used samples are left according to the effects of this. The evolution of the fisetin content in these leaves samples has been first studied, based on the method developed.

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