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DEVELOPMENT OF A VALIDATED ZIC-HILIC METHOD COMBINED WITH ULTRAVIOLET DETECTION FOR THE DETERMINATION OF RUTIN CONTENTS IN SEVEN DIFFERENT TEA PLANTS

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Abstract

Tea is a drink widely consumed throughout the world and contains active compounds containing anti-parasite, cancer-resistant, and anti-oxidant substances. Such compounds include flavonoids which are essential for human health where one of the key flavonoid compounds found in many plants, notably tea, is rutin. In this paper, the objective of the ZIC-HILIC column is to explain the chromatographic features of the rutin retention behavior and determination in tea types. Hydrophilic interactions that establish a rutin mechanism separation. Several methods for extracting tea were used and during this study, these methods have been successful. The research technique was successfully used for extracting different teas. The results show that HILIC is efficient and sensitive and tea rutin quality can be determined. The validated procedure was used successfully for the extraction test in tea types. The findings showed that the HILIC mode was simple and efficient and that the rutin content of tea types can be determined. For one ZIC-HILIC column and linear range, the calibration curve was made. The standard curve was produced for ZIC-HILIC column, and linear range (0.05-2 μgmL^{-1}), RSD% (0.485 ± 0.135), LOD (0.030 μgmL^{-1}), LOQ (0.105 $\mu g\, mL^{-1}$).

Key words: Rutin, Tea, Flavonoids, Zwitterionic hydrophilic interaction, UV detection.

Introduction

Tea is considered to be a way of life and culture for many people. Tea is an excellent beverage featuring many bioactive compounds such as polyphenols, caffeine, theobromine, amino acids, inorganic salts (Sharma *et al.,* 2018). It is one of the affluent flavonoid natural sources that increased consumption of tea and flavonoids were related to reduced CVD risk (Serban *et al.,* 2015). Tea is a rich source of antioxidants because tea contains antioxidants useful for fighting free radicals and reducing cellular exposure to damage, thus reducing the potential of many chronic diseases, polyphenols are worth noting Polyphenols. It is known that tea fosters the protection of human intestines Studies have shown that in the intestine, bacteria can play an important role in reducing the risk of many diseases, such as inflammatory bowel diseases Obesity, cardiovascular disease, and type 2 diabetes, in addition to cancer, will increase the growth of beneficial bacteria with polyphenol compounds present in the tea and insert harmful ones into the growth that helps preserve intestinal health. Tea is also distinguished by the ability to reduce cancer risk where researchers believe that some antioxidant substances in tea, such as polyphenols and catechins, may reduce the risk of certain types of cancer. Tea reduces the risk that Parkinson's disease develops drinking tea has been found to help lower the stress hormone known as cortisol (Chung *et al.,* 2013, Cao *et al.,* 2016, Hursel *et al.,* 2009, Yang *et al.,* 2014, Qi and Li, 2014, Serban *et al.,* 2015, Wang *et al.,* 2019). Consequently, given the importance of tea for human health and the prevalence of its use by many people, different types of tea exist. Different types of tea that are common and used in many countries and are available in local markets have been used in this current study. Black tea, green tea, sage tea, olive leaf tea, ginger tea, fenugreek tea, pomegranate tea, hibiscus tea, malicia tea, chamomile tea, and moringa tea are among those styles. The flavonoids are phenolic compounds in most herbal

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products, wine, a large number of fruit juices, and tea. A typical tricyclic chemical composition (C6–C3–C6) is characteristic of such an active compound. flavonoids can be categorized into (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones, etc.) (Batra and Sharma, 2013, Budisan *et al.,* 2019) on account of the amount and arrangement of their carbon atoms. The rutin (Quercetin-3 rutinoside) shown in Fig. 1 is major flavonoid found in many plants, buckwheat, fruits, supplements, in wine, and Capers, spices, black olives, asparagus, blackberries, red berries, tomatoes, and plums are also found in many types of tea (Wang *et al.,* 2011). The compound includes many pharmacological activities, such as anti-cancer, anti-allergic and anti-inflammatory effects (Kruzlicova *et al.,* 2012). because of its biological effects, it has antioxidant and anti-inflammatory properties, it has been commonly used for disease management (López *et al.,* 2012). Flavonoids, and particularly rutin, are of great importance because of their numerous benefits to humans. Rutin can be used in drugs and is considered to be of cosmetic value (Aziz *et al.,* 2015, Gong *et al.,* 2010). There are several analytical methods for estimating rutin, including chromatographic analysis methods and, in particular, high-performance liquid chromatography technique (HPLC) with ultraviolet detection (UV) (Zou *et al.,* 2017, Jakovljeviæ *et al.,* 2020, Ji *et al.,* 2018, Huang *et al.,* 2019). A few studies to

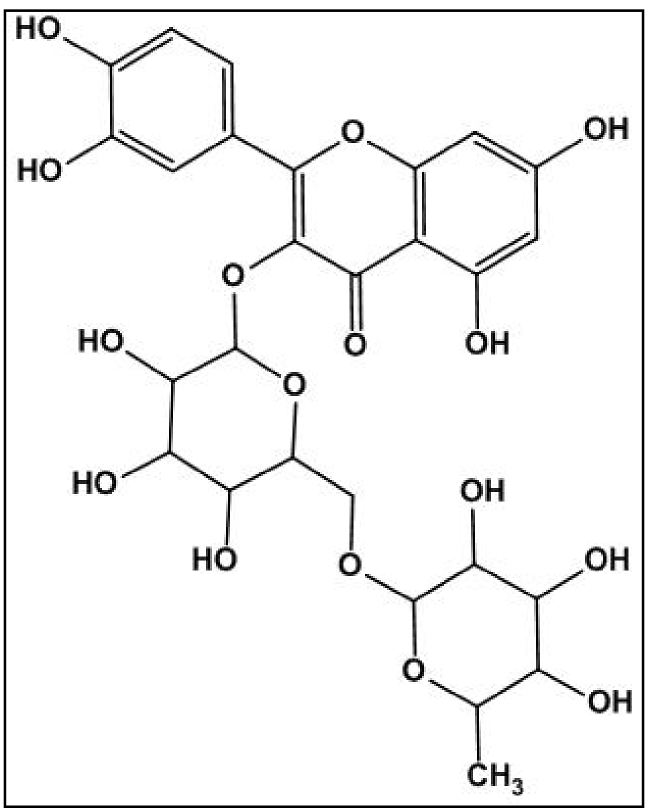

Fig. 1: Chemical structure of Rutin.

separate rutin using HILIC columns with MS detection (McCalley, 2017, Sentkowska *et al.,* 2016, Sentkowska *et al.,* 2013). It must be remembered that a major study by Rashid and his co-workers, who investigated the mechanisms of 2-deoxyuridine, amino acids and carboxylic acid separation and analysis using the columns of the ZIC HILIC, discussed the convergence of that research (Yaqout Abd Al-Hakeem Hamed and Rasheed, 2020, Ashraf Saad Rasheed and Rashid, 2020, Ashraf Saad Rasheed *et al.,* 2019, Abbas and Rasheed, 2018, Seubert and Saad Rasheed, 2017, Rasheed *et al.,* 2017, S Rasheed and Seubert, 2016, Al-Phalahy and Rasheed, 2016, Al-Phalahy *et al.,* 2016, Abbas and Rasheed, 2017a, Abbas and Rasheed, 2017b). Thus, during this study, we would aim to separate and analyze rutin using HILIC columns with UV detection. The hydrophilic liquid chromatography (HILIC) is a type of normal-phase liquid chromatography (NP-LC), but the mechanism for retention is more complex and is not fully comprehend (Bernal *et al.,* 2011). HILIC increased success is because HILIC is complementary to reverse phase liquid chromatography (RPLC) and is, in most situations, the best option for separating compounds that are not properly retained in RPLC conditions (McCalley, 2007). Several research projects suggested that the HILIC retention mechanism was very complicated, in which the analysts were partitioned between the mobile phase and the water layer and adsorbent interactions (hydrogen bonds or dipole-dipole) were possible (Cubbon *et al.,* 2007).

Materials and Methods

Millipore filters (0.45 μm) were used to purify the solution. As far as chemicals are concerned, Sigma-Aldrich obtained acetate, sodium acetate, Acetonitrile, and rutin. 0.1 μs / cm of Millipore Water conductivity has been used (System-US Millipores). A collection of teas such as green and black tea, ginger tea, hibiscus tea, moringian tea, and fenugreek tea. This set of teas was taken from Iraq's local markets.

Chromatographic devices and chromatographic condition

With a 45 μm membrane filter, the eluent was filtered and degassed to form an acetonitrile and acetate buffer. A flow of 0.5 mL/min was used. Rutin analyzes have been conducted by using the ultraviolet field Wavelength of 330 nm. The chromatography is 330 nm detected, the amount injected is 10 μL, the flow rate is 0.5 mL/min and 35^{o}C. Merck SeQuant (100 mm×4.6 mm I.D.) was used for the commercial column ZIC-HILIC. For Merck Hitachi HPLC, the L-6200 gradient pump and the UV-visible L-4200 is supplied with a 20μL injection loop. PH analyzes were performed on the pH 740 (WTW). My chromatography can be studied by using photographic software from the N2000 workstation.

Preparation of stock solution for Rutin

A Rutin solution was prepared to provide stock rutin solutions for stock (50 µgmL^{-1}), dissolving accurately a rutin amount (5 mg) in 50 ml of the eluent. In the mobile phase, the result was dissolved and filtered even more by a through 0.45 µm.

The general method for extracting and evaluating various seven tea types

Various tea kinds have been taken such as Black tea, green tea, olive leaf tea, ginger tea, fenugreek tea, pomegranate tea, and moringa tea. Tea leaves were taken for all the types used and well cleaned and then dried and crushed and turned into fine flour in preparation for the extraction process. One gram has been taken for each type of tea, where 30 ml of ethanol 70% has been added to it and has been given over a period of time. After stirring, it was placed in an ultrasound machine bath at 50^{0}C for 40 min and after that time, the product was filtered using 0.45 µm filter and then placed in the refrigerator until the time of use. The extraction process for all teas has been repeated three times.

Results and Discussion

Optimizing the separation of Rutin

As a Flavonoid model, Rutin has been selected to test the HILIC retention mechanism using industrial (ZIC-HILIC) ACN acetate buffers. In Fig. 2 the Rutin chromatogram appears. 90% ACN and 30 mM acetate buffers are subject to condition (pH 5.5). Components of the mobile phase, the pH effect and the eluent concentration effect as discussed below.

The effect of ACN content on rutin retention

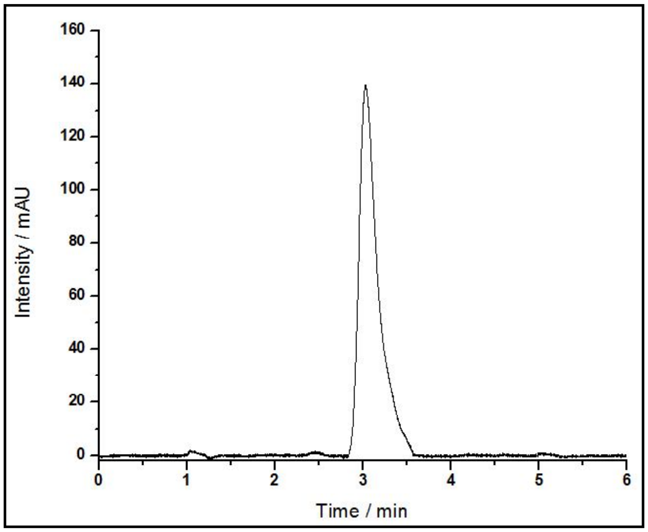

Fig. 2: Chromatogram of rutin using the ZIC-HILIC column.

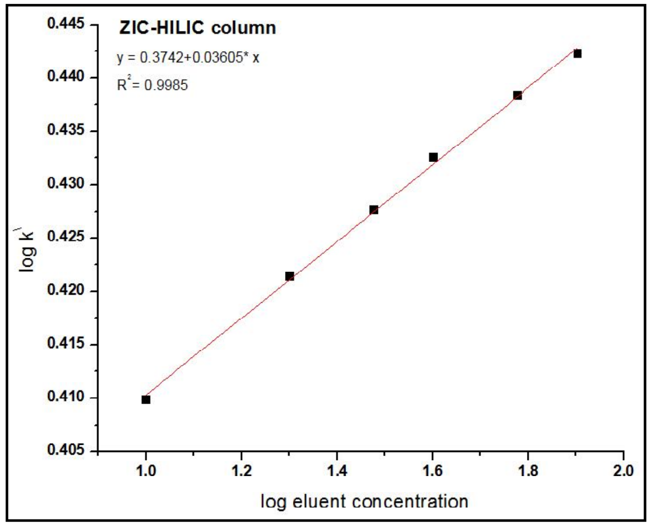

Fig. 4: Retention of rutin as a variation of buffer concentration.

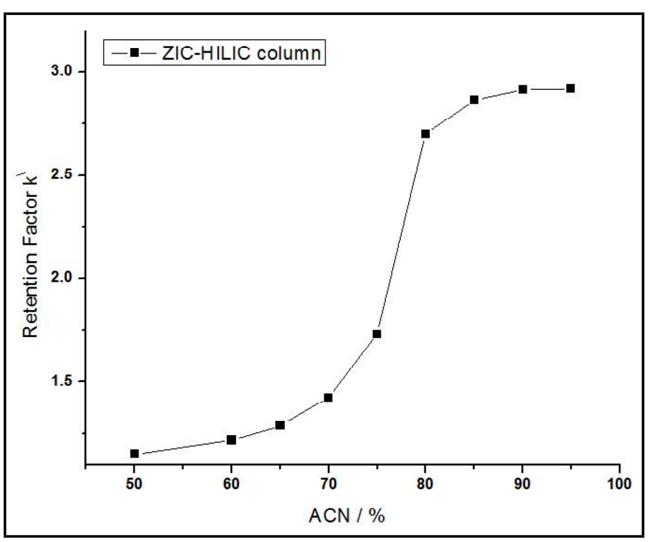

Fig. 3: Retention of rutin as an ACN content variation.

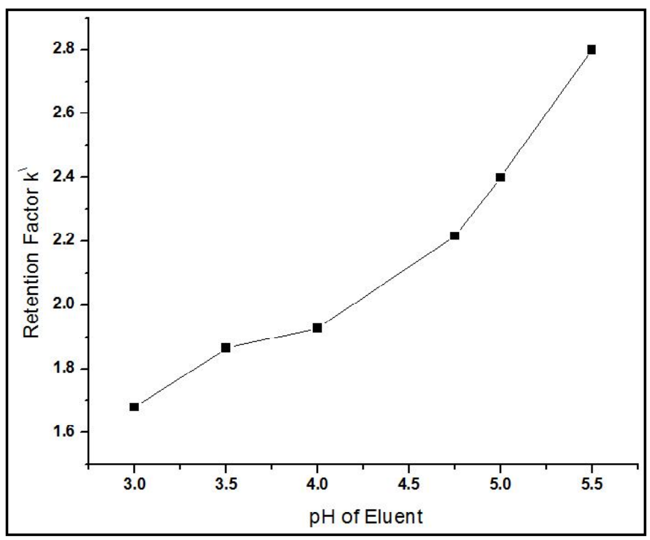

Fig. 5: Retention of rutin as a variation of eluent pH.

The effect of eluent ACN on rutin retention at 5.5 pH 35 mM NaOAc / HAc has been observed. The hydrophilic interaction components of Rutin (HILIC) tend to increase in the proportion of eluent ACN from 60% to 95%. The reason for this behavior is rutin; this column shows the HILIC rutin behavior Fig. 3 that was the roduct of the rutin log POW (-0.87).

The effect of the concentration of buffer on the retention of rutin

Salt is applied to the eluent to control electrostatic interactions between solutions and exchangers. The NaOAc / Hac buffer effect on eluent rutin retention is recorded in the eluent of 10-80 mM (pH 5.5) in 90% ACN. The results are in Fig. 4. Rising buffer concentrations in the NaOAc / HAc eluent increase the rutin retention factor in the column, which is closely linked to that of the stationary HILIC material step. To increase eluent concentrations, the retention of the substance to be analyzed in HILIC is increased due to fracture being the intramolecular ion pairs.

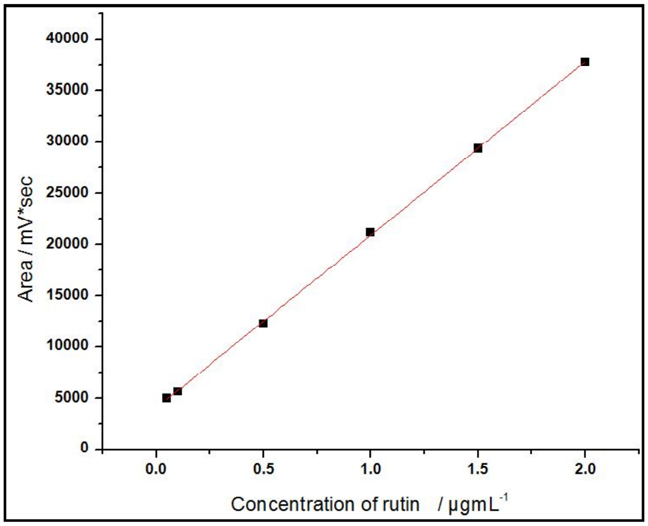

Fig. 6: Standard curve for rutin using the ZIC-HILIC column.

Table 1: The evidence of performance studies.

Parameter	ZIC-HILIC technique
Linearity[a] ($\mu g.ml^{-1}$)	0.05-2
Regressiona equationa	y=4063.55+16886.08*x
R^2	0.9996
LOD ($\mu g.mL^{-1}$)	0.030
LOQ ($\mu g.mL^{-1}$)	0.105

Table 2: Methodological performance of rutin on the same day as on different days.

Same-Day Analysisn=6					Day-to-Day Analysisn=6			
Rutin Taken ($\mu g.mL^{-1}$)	Rutin Found ($\mu g.mL^{-1}$)	% Rec.	% Erel.	% RSD	Rutin Found ($\mu g.mL^{-1}$)	% Rec.	% Erel.	% RSD
0.5	0.493	98.60	-1.40	0.62	0.495	99.00	-1.00	0.71
1.5	1.495	99.66	-1.34	0.35	1.493	99.53	-0.47	0.43

Table 3: The performance of rutin in seven tea forms was examined.

Name of tea	Rutinmg/mg (n=4)
Black tea	8.30±1.23
Green tea	9.44±1.65
Olive leaf tea	1.12±0.03
Ginger tea	0.22±0.03
Fenugreek tea	22.33±0.42
Pomegranate tea	4.36±0.23
Moringa tea	2.10±0.06

Eluent pH effect on the retention of rutin

The next enhanced eluent composition can be applied with a change in eluent pH. The eluent pH must be changed to complete rutin separation in HILIC mode. The pH increased from 3 to 5.5 at a steady buffer concentration of 35 mM and 90 % ACN. Rutin retention factor increases as seen in Fig. 5. It is because rutin is deprotonated in the hydroxyl group. That describes the predicted physicochemical rutin results. The value of pKa ranges from just under 6.38. When pH increases to 5.5 in the mobile process the analytes are detonated.

Calibration graph

The rutin of the standard curve is generated via the rutin concentration of the column ZIC-HILIC Fig. 7 and shows concentration (0.05-2 μgmL^{-1}).

Statistical analysis

The HILIC mode is used for the statistical results of the rutin calibration graph table 1. RSD % and % recovery in the stationary phase on the same day and different days is calculated for the accuracy and precision in table 2.

Rutin determination in tea samples

The ZIC-HILIC proposed method was used in the evaluation of rutin in seven tea types; the findings are illustrated in table 3.

Conclusions

This paper has established a HILIC method to isolate and estimate the amount of rutin in several teas. The stationary ZIC-HILIC shows HILIC interaction with rutin. This is due to the hydrophilicity behavior of rutin behaves with the ZIC-HILIC columns. As the process of extraction used for different teas has shown good rutin amounts for every tea form, as described in the above tables, this suggests the reliability and consistency of the procedure used in the rutin study.

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