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A NEW METHOD IN SOME GERMAN GRAPE WINES USING ZIC-HILIC TECHNOLOGY WITH UV DETECTION TO SEPARATE AND IDENTIFY QUERCETIN.

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Abstract

Quercetin has a variety of positive pharmacological effects and potential future treatment. Cancer cell growth has been reported *in vitro* and cancer growth in laboratory animals has been reduced. Besides induction and progression prevention of human cancer. Quercetin has anti-tumor, antioxidant, antibacterial, anti-inflammatory and analgesic therapeutic roles. The goal is to describe the chromatographic properties of the ZIC-HILIC column for the study of the quercetin retention and quantitative in wine samples. Hydrophobic and hydrophilic interactions that establish a quercetin mechanism for mixed-mode separation. The validated method for the extraction test in wine was successfully used. The results indicated that the HILIC mode was clear and efficient and could be used for the identification of the quercetin content in wine samples. The calibration curve was produced for one exchanger and linear range (0.01-3 μgmL^{-1}), RSD% (1.26 ± 0.06), LOD (0.012 μgmL^{-1}), LOQ (0.042 $\mu g\, mL^{-1}$).

Key words: Quercetin, wine, ZIC-HILIC, Hydrophobic and hydrophilic, grape.

Introduction

Wine is one of the most consumed drinks in the world. Consumption has increased significantly as a result of knowledge of the health-promoting characteristics of wines that are conveyed by various polyphenolic compounds such as flavonoids, anthocyanins, tannins (Moreno-Arribas and Suáldea, 2016). It's one of the earliest, if not the oldest, alcoholic beverages known to man, that's been around since the dawn of civilization some 8,000 years ago. Polyphenolic compounds have been very important in the last decade because their role in evaluating the quality of the wine (color and taste) and the importance of these compounds in the medical field (antioxidant, antitubicide and coronary heart disease (CHD) was determined. Flavonoids are commonly the most abundant phenolic compounds (Panche *et al*., 2016; Fernandes *et al*., 2017; Palma-Duran *et al*., 2017). The different types of flavonoids vary in the oxidation and substitution pattern of the C ring, which results in six groups of flavonoids Flavones, flavonols, flavanones, isoflavonoids, flavanols and anthocyanidins (Rauter *et al*., 2018). Previous studies have shown that flavonoids, like quercetin, have an impact on many illnesses, such as cardiovascular disease, obesity and other chronic diseases. Quercetin is one of the ubiquitous flavonols found in vegetables, fruit, wine and tea, as well as nutritional supplements such as glycosides (Lesjak *et al*., 2018). Quercetin has the advantage of antioxidant, anti-inflammatory, anti-viral, anti-allergic and enzymatic inhibitory activity. Quercetin has an anticancer function, like breast, leukemia and colon and ovarian cancer. And its role in the reduction of cardiovascular disease (David *et al*., 2016). Studies show that quercetin has been analyzed using HPLC technology and that the columns used are RPLC. However, few studies have been done to separate quercetin (Fig. 1) using a technique Hydrophilic interaction liquid chromatography (HILIC). Newly, research and studies on Hydrophilic interaction liquid chromatography (HILIC), along with several stationary phases produced for HILIC, have increased significantly. HILIC is a type of normal-phase liquid chromatography and has enticed the attention of

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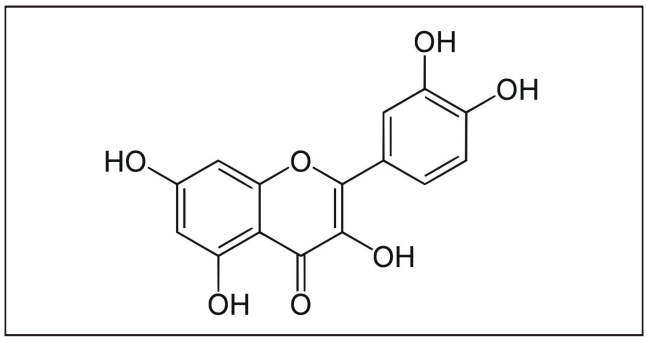

Fig. 1: Chemical structure of Quercetin.

researchers in various fields of science who are investigating the separation of polar compounds. In HILIC mode, a mixture of water and an organic solvent that is often acetonitrile (ACN) is most commonly used With Polar Stationary Phase. HILIC is a good alternative to the reverse phase (RPLC) in chromatographic separation (Buszewski and Noga, 2012). Many HILIC applications have been observed in previous periods to separate and analyze several compounds, such as nucleotides, nucleosides, carbohydrates, amino acids, proteins and peptides (Urban and Jandera, 2013; Van Nuijs *et al*., 2011; Wuhrer *et al*., 2009; Ashraf Saad Rasheed and Rashid, 2020; Periat *et al*., 2015; Boersema *et al*., 2008; Spagou *et al*., 2010; Yaqout Abd Al-Hakeem Hamed and Rasheed, 2020). Typically, the HILIC separation silica gel is used as stationary phases with chemically bonded amino, amido, cyano, diol, carbamate or polymer matrices (Ciminiello *et al*., 2005). The retention mechanism is a mixed-mode, which may be divided into adsorption, ion exchange, hydrogen bond formation, dipole-dipole and other interactions, depending on the conditions in which they occur (Euerby *et al*., 2015). Depending on the column sort, the polarity and ionization of the sample as well as of the mobile phase content, various interactions are involved in the total HILIC retention. Hydrophilic Liquid Chromatography-HILIC using the Zwitterionic Ion Column is the next separation technique with its importance accelerating very quickly because it is suitable for separating water-loving and ionic compounds. It should be noted that the aqueous phase is used as a mobile phase in ZIC and aqueous-organic as a mobile phase in ZIC-HILIC. ZIC-HILIC technology has become the first preferred technique for field researchers. Chromatography, because it combines reversible and normal phase chromatography with ion exchange. Zwitterionic stationary phases, mostly sulfobetaine varieties have been found to be adequate retention in comparison with diolicine and bare silica columns for a wide range of application to check polyphenols (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*., 2016a; Abbas and Rasheed, 2017a; Abbas and Rasheed, 2017b). It is worth mentioning that reference is made to the convergence of this study from an important study carried out by Rasheed and Co-worker, which studied the separation and analysis of amino acid and carboxylic acid by using ZIC HILIC column. So the quercetin will be analyzed using a column (ZIC–HILIC) with UV detection (Al-Phalahy *et al*., 2016b; Rasheed *et al*., 2017). Here, a new method was introduced for the analysis and extraction of quercetin in wines.

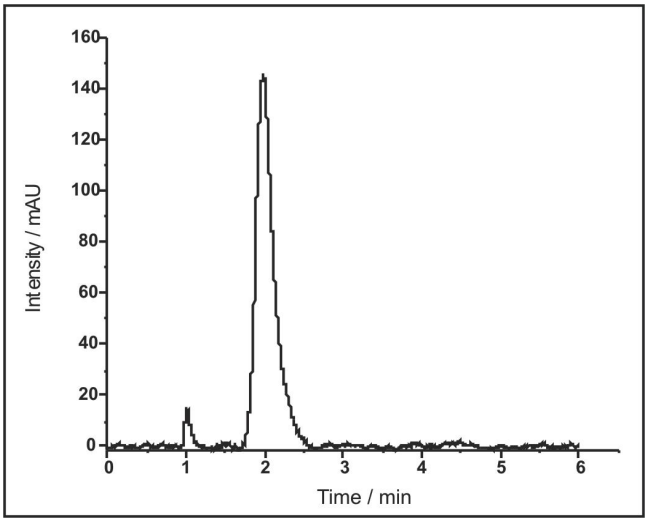

Fig. 2: A typical chromatogram of quercetin.

Materials and Methods

Chemical reagents and materials

The solution was purified by Millipore filters (0.45 μm). As for chemicals, for example, acetic acid (HAc), Sodium Acetate (NaOAc), Acetonitrile (ACN), Quercetin, were purchased from Sigma-Aldrich and 0.1 μs / cm conductivity from Millipore Water (System-US

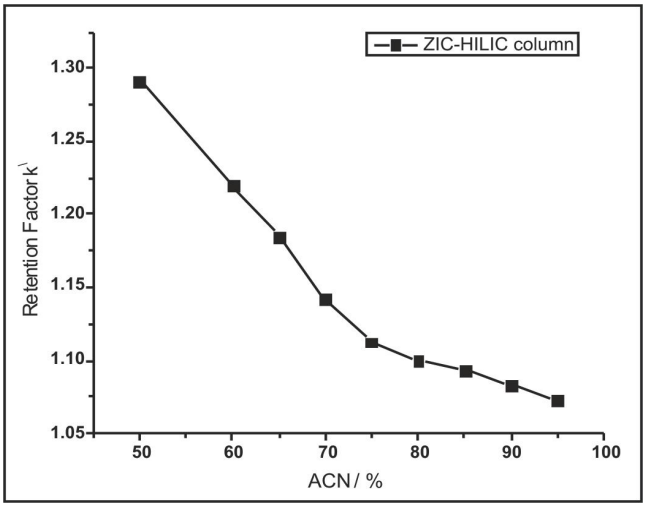

Fig. 3: Retention behavior of quercetin as a function of ACN content.

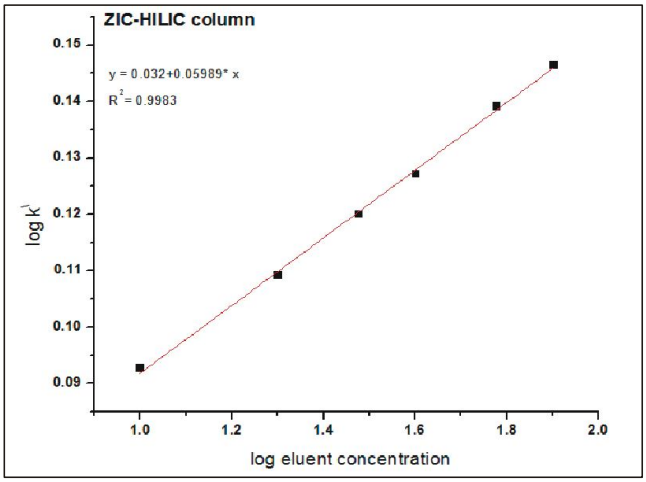

Fig. 4: Retention behavior of quercetin as a function of buffer concentration.

Millipores). Two examples of wine will be taken the first type is (Anselmann pinot noir) as for the second type is (Hensel Und Gretel), both of which are German-produced and have also been brought from Germany.

Instrumentation

A 20 µL injection loop is used for the Merck Hitachi HPLC system, including the L-6200 gradient pump and the UV-visible L-4200. On the pH 740 (WTW), pH analysis was conducted. The N2000 workstation photographic software is used to track and analyze my chromatography.

Chromatographic conditions

The eluent formed a buffer of acetonitrile and acetate by using a 45 µm membrane filter, the eluent has been filtered and degassed. The flow rate was used at 0.5 mL/min. Flavonoid analysis was carried out using the Wavelength 330 nm ultraviolet area. The chromatographic condition is 330 nm detection, injection volume of 10 µL,

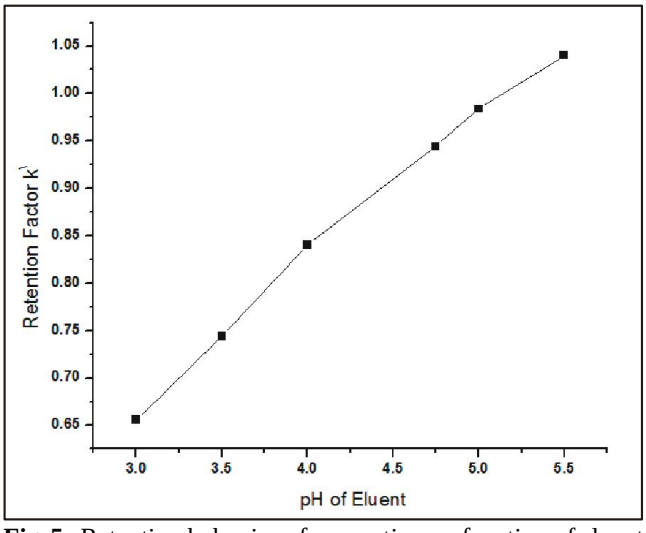

Fig. 5: Retention behavior of quercetin as a function of eluent pH.

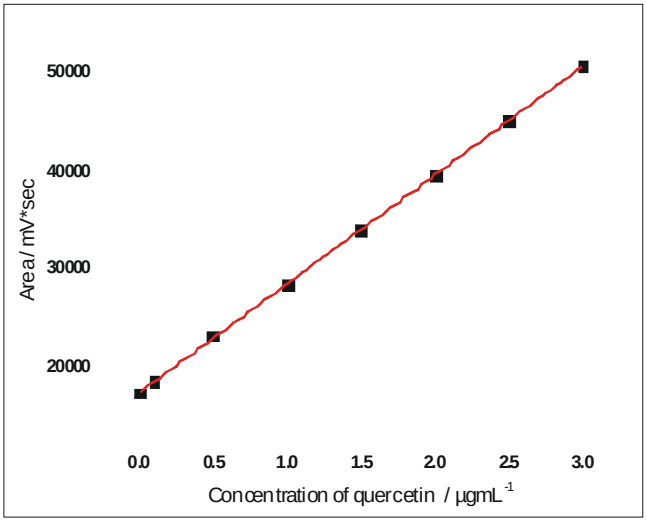

Fig. 6: Linearity graph for quercetin using the ZIC-HILIC column.

the flow rate of 0.5 mL/min, Temperature is 35°C. The commercial column ZIC-HILIC has been collected respectively from Merck SeQuant and Advanced Materials Technology (100 mm × 4.6 mm I.D.).

Preparation of the quercetin stock solution

A quercetin solution was prepared to provide stock quercetin solutions for stock (100 µg mL^{-1}), dissolving accurately a quercetin amount (10 mg) in 100 ml of an eluent. In the mobile phase, the result was dissolved and filtered even more by a through 0.45 µm.

Samples preparation

Commercially available grape wine samples (Anselmann pinot noir) and (Hensel Und Gretel) were purchased and analyzed directly, both of which are German-based and were also brought from Germany. Both samples have been filtered by 0.45 µm filter before injection. The samples have been diluted ten times with the respective mobile phases mentioned above for chromatographic analyzes.

Results and Discussion

Separation and identification of quercetin

Quercetin was selected as a Flavonoid model for assessing the HILIC retention mechanism with ACN acetate buffers in the commercial column (ZIC-HILIC). The quercetin chromatogram shows in (Fig. 2). Under

Table 1: The evidence of the study of results.

Parameter	ZIC-HILIC method
Linearity (µg.ml^{-1})	0.01-3
Regression equation	y= 17583.24+11021.99*x
R^2	0.9998
LOD (µg.mL^{-1})	0.012
LOQ (µg.mL^{-1})	0.042

Table 2: Quercetin methodological exactness and precision both on the same day and on different days.

Taken ($\mu g.mL^{-1}$)	Same-Day Analysis n = 5				Day-to-Day Analysis n = 5			
	Found ($\mu g.mL^{-1}$)	% Rec.	% Erel.	% RSD	Found ($\mu g.mL^{-1}$)	% 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

condition 90% ACN and 30 mM acetate buffers (pH 5.5). To find out about quercetin behavior in this column ZIC- HILIC, it is important to study the effect of changing the mobile phase's components, the effect of pH and the effect of eluent concentration as discussed below.

ACN impacts quercetin retention

The impact of the eluent ACN content on the retention behavior of the quercetin was studied at 5.5 pH 35 mM NaOAc / HAc. Quercetin has reversed-phase (RP) behavior, with the percentage of ACN eluent continuing to rise from 60% to 95%. Quercetin hydrophobicity is the reason for this behavior; in this column, the reversed-phase (RP) activity of quercetin is shown (Fig. 3), which was due to the log POW of quercetin (2.16) [27].

Eluent concentration impacts in quercetin retention

Salt is usually added to the eluent to monitor solute/ exchanger electrostatic interactions. The effect of the NaOAc / Hac buffer on the eluent retention behavior of quercetin has been reported in the 10-80 mM (pH 5.5) at 90% ACN in the eluent. The results are shown in (Fig. 3). Increasing buffer concentrations in the eluent of NaOAc / HAc increase the quercetin retention factor in the column. This demeanor is closely related to that of the HILIC content stationary phase. To increase eluent concentrations, the retention of analytes in HILIC is increased because the intramolecular ion pairs are fracture.

Eluent pH impacts in quercetin retention

The next improved composition of the eluent can be applied with a change in eluent pH. To complete quercetin separation in HILIC mode, the eluent pH must be changed. The pH improved from 3 to 5.5 at a steady buffer concentration of 35 mM and 90% ACN. As shown in fig. 5 quercetin retention factor increases. This is because the hydroxyl group is deprotonated in quercetin. This represents the physicochemical data of quercetin that are predicted. The pKa values range from just fewer than 6.38. When pH in the mobile process is increased to 5.5, the analytes are certainly detonated.

Table 3: The quality of quercetin examined two wine samples.

Name of wine	Year of vintage	Quercetin µg/mL (n=3)
Anselmann pinot noir-Germany	2012	1.65 ± 0.47
Hensel Und Gretel-Germany	2015	0.55 ± 0.12

Calibration graph

The graph quercetin is generated by plotting the quercetin concentration against the peak area and showing concentration (0.01-3 μgmL^{-1}) of the ZIC-HILIC column (Fig. 6).

Statistical data information

The corresponding calibration curve used a thorough assessment of quercetin under HILIC circumstances and record statistics in table 1. Accuracy and precision were measured on the same day and different days and RSD percent and Rec. percent were determined. The relatively small defaults and high recuperation values indicate that the proposed method is successful (Table 2).

Quercetin determination in wine samples

In the evaluation of quercetin in two wine samples, the ZIC-HILIC proposed method was successfully used; the results are described in table 3.

Conclusions

In this study, a new method of evaluating quercetin without sample pretreatment was developed using the ZIC-HILIC method. The findings demonstrated the usability, practicability and feasibility of this new method with high precision, sensitivity and repeatability and also a strong resolution of the quercetin in wine samples. Quercetin was the dominant flavonol in wine samples, according to the findings of the wine samples. Based on the method developed, the evolution of quercetin content in wine samples was studied for the first time, to our knowledge.

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