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Modern Chromatographic Methods for Determination Flavonoids

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Abstract

Flavonoids are a prominent group of plant phenolics—(polyphenol group) extensively present in the human diet and associated with health promotion. Flavonoids are essential in various pharmaceutical, nutraceutical, and cosmetic applications. To collect and categorize these compounds, we comprehensively assessed High-performance liquid chromatography (HPLC) techniques published to analyze flavonoids in various matrices. Most documented methods employed liquid chromatography with ultraviolet detection (HPLC-UV) and tandem mass spectrometry (LC-MS/MS) methods. Among chromatographic techniques, reversed-phase liquid chromatography remains the predominant and efficient method for separating various analytes. This review explores the primary chromatographic methods used to separate and quantify flavonoids in herbs, fruits, and biological samples. This article examines recent literature on applying liquid chromatography with different detection methods for analyzing flavonoids. To examine recent literature on applying liquid chromatography with different detection methods for analyzing flavonoids. It offers valuable insights to researchers as they prepare to develop quantification methods.

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1. Introduction

Flavonoids are Natural products that provide a rich source of bioactive compounds for various potential uses [1,2]. Today, natural medicines are meeting the primary healthcare needs of most people in countries and gaining greater attention as healthcare costs increase. They have been widely employed as traditional medicines for centuries and have been a subject of the scientific community in the last decades since increasing evidence connects them with health benefits and multiple disease prevention [3, 4]. Its value also grows because of the negative consequences for human and environmental safety of synthetic additives and their processing [5,6]. Generally, a natural product's functional and technical properties are correlated with its elements, concentration, and potential interactions. These bioactive components often have to be isolated and extracted from the raw material and then used as a sample, product, or ingredient. A secondary metabolism compound, (phytochemicals), is the source of most functional foods' bioactive properties. Phytochemicals are plant-producing chemicals that play a magnificent role in plant metabolism. Phytochemicals are not classified as essential nutrients but can be of significant biological importance. Many phytochemical classes are available, including Phenolic compounds (flavonoids, phenolic acids, tannins, stilbenes, coumarins, and lignans), carotenoids, phytosterols, alkaloids, terpenes [7-10]. The fundamental structural characteristic of phenolic compounds is an aromatic ring containing one or more group of hydroxyl groups [11,12]. Flavonoids are the most bountiful phenolic compounds commonly formed by two aromatic rings (termed A and B) connected by an oxygenated heterocyclic ring (the C ring) [13]. Many flavonoids share the same basic structural motif consisting of 15 carbon atoms arranged in a skeleton of C6-C3-C6, forming a phenylbenzopyrane

structure [14] (Figure 1). Flavonoids are widely found in fruits, food, vegetables, herbs, flowers, supplements, chocolate, tea, wine, and other plant sources [15].

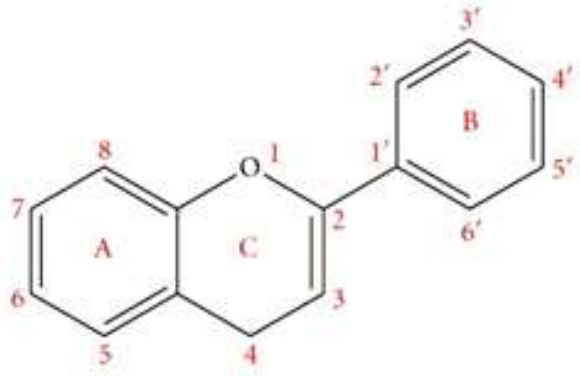

Figure 1. Show the Flavonoid structure [15].

2. Classification of flavonoid structures

The various types of flavonoids exhibit variations in the oxidation and substitution pattern on the C ring, leading to the classification of flavonoids into six distinct groups (see figure 2), flavones, flavonols, flavanones, isoflavonoids, flavanols, and anthocyanidins [16].

2.1. Flavones

Flavones are among the significant flavonoid subgroups; flavones are distinguished by including a keto group in C4 and, as in C2, a 3-double bond. The A-ring is derived from phloroglucinol, and the B-ring can be replaced in positions C3′, C4′, or C5′. Flavones, like apigenin, luteolin, wogonin, and baicalein, are structurally similar to flavonols but are not oxygenated at C-3 [17]. Flavones are not widely distributed, although celery, parsley, and some herbs have been found in substantial amounts. Citrus species contain polymethoxylated flavones such as nobiletin and tangeretin [18, 19].

2.2. Flavonols

Flavonols are flavons with a group of OH in C3. The most popular flavonols, kaempferol, quercetin, isorhamnetin, myricetin, and fisetin, are commonly found in the whole plant kingdom with the immunity of fungi and algae [20]. Glycosides are typically seen in the 5, 7, 3′4 ‹′, and 5′, respectively positions. Rich sources of flavonols include onions, kale, tomatoes, lettuce, oranges, grapes, and berries. Tea and red wine are also sources of flavonols besides fruits and vegetables [21]. Because of their various glycosylation behaviors, quercetin and rutin, for example, are probably the biggest and most popular subgroups of flavonoids in fruits and vegetables [22].

2.3. Flavanones

Flavanones are also a significant category, usually found in citrus fruits like oranges, lemons, and grapes. Sources of this class of Flavonos are hesperidin, naringenin, and eriodictyol [23]. Because of their free radical scavenging, flavonoids have various beneficial effects with several health benefits. Flavanones, also known as dihydroflavones, feature a saturated C ring; thus, unlike flavones, the double bond between positions 2 and 3 is saturated, the only structural distinction between the two flavonoid subgroups [24].

2.4. Isoflavonoids

Isoflavonoids are a broad and distinctive flavonoid subset. Isoflavones have a B-ring attached to C-3 instead of C-2. The distribution of isoflavonoids in the plant kingdom is limited mainly to soy and other leguminous plants [25]. Isoflavonoids have a tremendous potential to attack a variety of diseases. Isoflavones such as genistein and daidzein are often considered phyto-estrogenic in certain models due to their estrogenic activity [26].

2.5. Flavanols

Flavanols are the 3-hydroxy subaltern of flavanones. These are also flavan 3-ols, as the OH group is often connected to location 3 of the C ring [27]. There is no double bond between positions 2 and 3 as opposed to many flavonoids. Flavanols are often found in bananas, apples, blueberries, peaches, pears, and tea [28].

2.6. Anthocyanins

The anthocyanins belong to the phytochemicals flavonoid category, a group that is abundant in teas, honey, wines, vegetables, seeds, nuts, olive oil, cocoa, and cereals [29]. Anthocyanins are responsible for the red, blue, or purple color of many edible fruits, such as peaches, bananas, eggplants, and berries. The most common anthocyanidin glycosides are pelargonidine, cyanidine, delfinidine, pionidine, betonidine, and malvidine [30]. Anthocyanins play an essential role in preventing free radical damages, which lead to aging and the creation of many diseases; in addition to their ability to protect cells, tissues, and vital organs against free radicals/oxidant stress, anthocyanins

have many other effects. The anthocyanins positively affect the intestines' health when interacting with microflora, which can help reduce inflammatory markers, correlate with many chronic illnesses, and support hormone balance [31, 32].

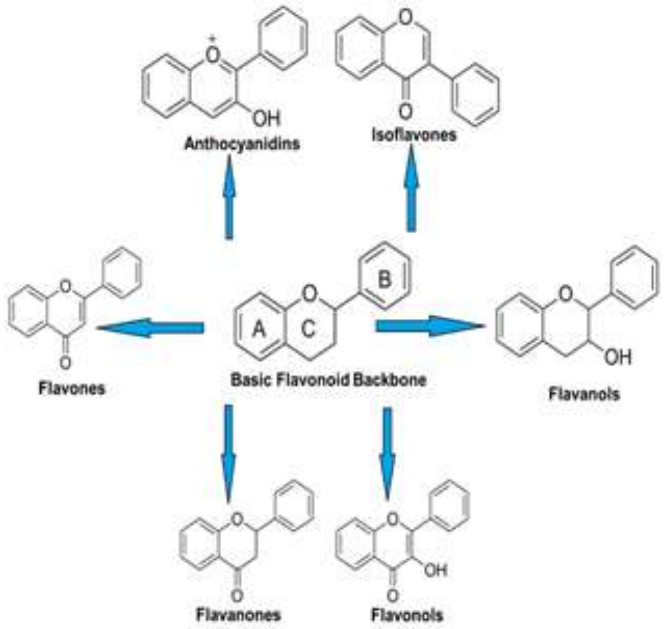

Figure 2. The families of flavonoids

3. Flavonoids ' Role in Life

Due to the importance of flavonoids and their role in life, their role in these points will be discussed.

3.1. A health perspective

For many human diseases (Figure 3), flavonoids play a significant therapeutic role, and these diseases are described below.

3.1.1. Prevention of cancer

Recent research from epidemiologic and lab experiments indicates that flavonoid intake lowers the risk of several types of cancer [33]. Eating fruits and vegetables protects the mouth, pharynx, larynx, esophagus, and stomach cancers. Different kinds of flavonoids have already been recognized as having antiproliferative effects in different cancers, including quercetin, rutin, genistein, naringenin, fisetin, daidzein, luteolin, kaempferol, and apigenin [34, 35]. Where it has been observed that these compounds have opposite effects on various cancers and cancer prevention, including rectum, colon, prostate, breast, thyroid, lung, and ovarian cancer [36-40].

3.1.2. Cardiovascular health

Cardiovascular disease (CVD) is originated by heart and blood vessel problems, including heart attack and vascular disease (stroke). It is well known that the leading causes of heart failure, high blood pressure, and artery damage are cigarette smoke, lethargy and immobility, and poor nutrition [41]. Some foods and drinks are derived from plants, including chocolate, much tea and its kind (black tea, green tea, and white tea), wine, and juices such as raspberry juice, grapefruit, orange, and lemon [42]. These foods and drinks contain many phenolic compounds, including flavonols, flavanols, and anthocyanins, which significantly affect treating heart problems, blood pressure, and blood vessels and their impact on promoting cardiovascular health [43-45].

3.1.3. Neurodegenerative prevention

One of the causes of these diseases is the slow and gradual loss of nerve cells in specific brain regions. Although studies have been conducted to understand the factors that cause nerve cell damage to various diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and other neurological diseases, no evidence has been provided. However, no treatments have benefited patients with these diseases, as most of these diseases are delayed in diagnosis and remain at an advanced stage without symptoms [43, 46, 47]. As a result, flavonoids played an important role in developing a novel generation of effective therapeutic agents for treating these diseases. There was a correlation between the regular intake of flavonoids and the reduced risk of neurodegenerative diseases. These compounds showed protective properties by interacting with cell signaling pathways leading to transcription and translation, in addition to their antioxidant properties [17, 48].

3.1.4. Asthma prevention

Asthma is one of the most chronic diseases characterized by bronchitis and reduced airflow. Genetic factors and environmental influences may be one of the causes of this disease. Although the precise causes of this disease are still unclear, dietary modification is assumed to be an environmental consequence. Many studies have shown that eating vegetables and fruit rich in flavonoids (quercetin, rutin, and fisetin) can give

adults and young adults enough against asthma or other allergies that affect humans [49-51].

3.1.5. Diabetes treatment

Diabetes is a chronic illness characterized by elevated blood glucose levels due to insulin defect or secretion [52]. One of the reasons for diabetes is a lack of physical activity and obesity, as well as a genetic factor [53]. Diabetes may cause damage such as kidney failure and body functions, such as heart, neuropathy, eyes, and blood vessels. As a result, the patient is subject to some symptoms, including frequent urination and thirst, loss of body weight, and other symptoms. Research has shown that flavonoids function to regenerate pancreatic cells and improve insulin secretion by taking anti-diabetic action [54, 55].

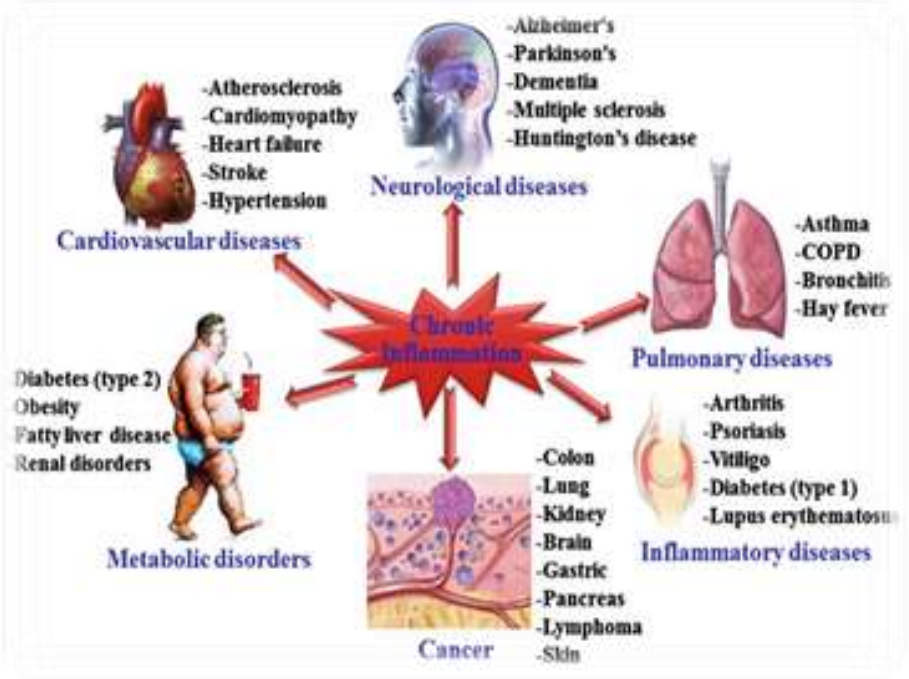

Figure 3: The most famous chronic diseases.

4. The role of Flavonoids as Antioxidants

The antioxidant properties of flavonoids are one of the most particular characteristics attributed to these molecules, which play a crucial role in preserving disease [56]. Antioxidants are essential because they shield our bodies from harmful toxic substances. Free radicals in our bodies, -atoms or molecules with a single unpaired electron,—these compounds are highly unstable trying to steal an electron from other particles or molecules [57]. When free radicals form they can make an uncountable number of these free radicals [58]. The loss of an electron is sometimes so damaging in our body, especially when a free radical produces another free radical, that the cell cannot function or start working abnormally anymore because free radicals are regarded as the major cause [59]. Nevertheless, flavonoids act as free radical scavenger antioxidants that interact with free radicals in the body and neutralize them before they can destroy or damage cells. Flavonoid oxidation effects can be caused by different processes, such as the movement of electrons from free radical species to flavonoids or indirectly by the metal chelation of the catalysts [60, 61].

5. Flavonoids ' Role as Enzyme Inhibitors

To control some diseases that affect humans, an enzyme inhibitor must be used, but there is concern resulting from synthetic enzyme inhibitors because of their serious side effects. Therefore, natural inhibitors of herbal origin, such as flavonoids, are currently preferable [62, 63]. Flavonoids are known to have the advantage of inhibiting a group of enzymes. It has inhibitory effects on various enzymes based on their structures. An example of such enzymes (α-glucosidase, α-amylase, Pancreatic lipase, Aldose reductase, Reverse transcriptase, Proteasome, and Cholinesterases) [64-66].

Therefore, we will test some compounds for the essential flavonoids.

6. Sources of Flavonoid Compounds

Flavonoid compounds (quercetin, rutin, genistein, and fisetin) are present in many Food (Figure 4) and beverages such as vegetables, fruits, herbs, tea, coffee, supplements, wine, beer, bee products, nuts, and seeds. Table 1 shows the presence of flavonoids in food and drink.

7. Analysis of Flavonoids

Due to many flavonoids, many different methods and techniques have been used to determine and measure them accurately. However, selective and sensitive analytical methods are needed to analyze flavonoids. With its various modifications, liquid chromatography (LC) has proved highly effective in the research of flavonoids. High-performance liquid chromatography (HPLC) is an advanced and suitable technique for liquid chromatography (LC) used in the separation and analysis of several compounds such as flavonoids since they are simple to use, quick, high precision, accurate, and not restricted to sample compound volatility or stability. The significant benefit of HPLC is that certain detectors such as Ultraviolet (UV), Diode Array Detector (DAD), Evaporative light scattering detector (ELSD), Tandem Mass spectrometry (MS/MS), Pulsed amperometric detection (PAD), and NMR may be correlated with this. The approaches for flavonoid analysis are listed in Table 2.

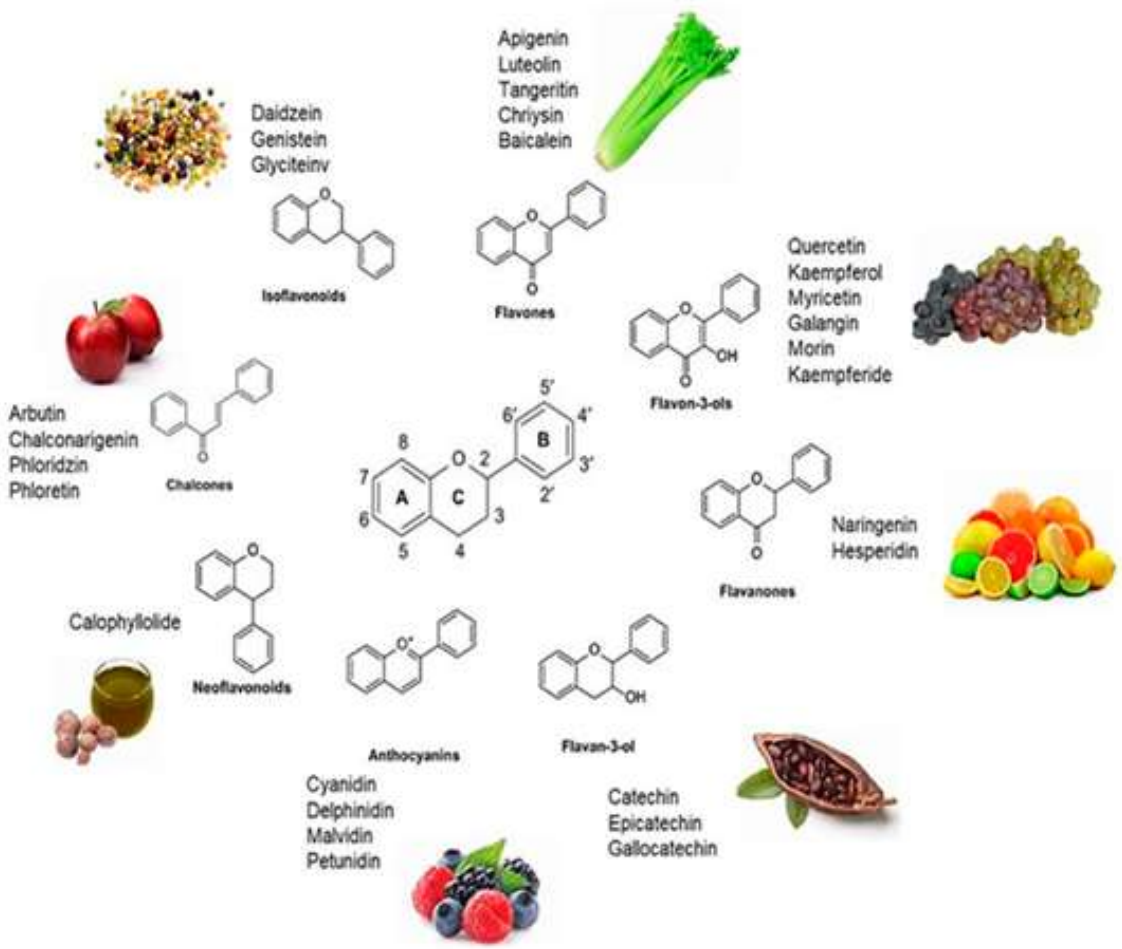

Figure 4. The presence of flavonoids in some foods and beverages [67].

Table 1. The table shows the essential compounds present in various foods and beverages.

Groups	Compounds	Food sources
Flavones	Sinensetin Disometin Nobiletin Tangeretin Tricetin Quercetogetin Apegenin Luteolin Chrysin Heptamethoxyflavone	Parsley, Celery, Capsicum Pepper, Citrus species, Onions, Apples, Grapes, Wines and Tea.
Flavonols	Quercetin Rutin Fisetin Kaempferol Myricetin Isorhamnetin	Yellow onion, Curly kale, Leek, Cherry, tomato, Broccoli, Apple, Green and black tea, Black grapes, kiwis, cucumbers and Blueberry.
Flavanones	Naringenin Hesperetin Dihydrobinetin Dihydrofisetin Dihydroquercetin	Orange juice, Grapefruit juice, and Lemon juice.
Isoflavonoids	Genistein Daidzein	Soybean, Soy flour, lupin and Tofu.
Flavanols	Catechin Taxifolin Silymarin Afzelechol Silibinin Fisetinidol Robinetinidol Gallocatechin	Cocoa, Cocoa beverages, Chocolates, bananas, apples, blueberries, peaches, pears, and tea.
Anthocyanins	Malvidin Cyanidin Peonidin Petunidin Delphinidin Pelargonidin	Blackcurrant, Cherry, Rhubarb, Plum, Strawberry, Red Wine, Red Cabbage.

Table 2. The overview of flavonoids determination using chromatographic methods.

Column	Mobile phase	Detection	Statistical data analysis	Application	Ref.
Targa C_{18}	ACN: H_3PO_4 buffer (30:70)	UV-vis 280 nm	100-600 μgmL^{-1} $R^2 = 0.9990$ % RSD = 0.13 LOD = 0.01μgmL^{-1} LOQ= 0.15μgmL^{-1}	Chocolates	[68]
C_{18}	MeOH: PO_4 buffer (40:60)	DAD 260 nm	0.5-10 μgmL^{-1} $R^2 = 0.9997$ % RSD = 1.58	Propolis	[69]

			% Rec = 95.71 LOD= 0.06 μgmL^{-1} LOQ= 0.22 μgmL^{-1}		
ZORBAX SB-C18	MeOH: HCOOH buffer (80:20)	UV_ESI-MS 330-367nm	2.0-20 μgmL^{-1} R^2 = 0.996 % RSD = 0.03 % Rec = 99.24 LO = 0.04 μgmL^{-1} LOQ= 0.05 μgmL^{-1}	citrus fruit	[70]
C_{18}	ACN: CH_3COOH: NH_4COO buffer (Gradient)	UV_MS 280-330 nm	12-11 μgmL^{-1} R^2 = 0.998 % Rec = 97.36% LOD = 25 µg mL^{-1} LOQ = 37 µg mL^{-1}	orange juice	[71]
Zorbax SB RP C-18	ACN:H_3PO_4 buffer (70:30 v/v)	PDA 254 nm	1-50 μgmL^{-1} R^2 = 0.995 % RSD =0.46 % Rec = 99.6 LOD=0.12 μgmL^{-1} LOQ =0.43 μgmL^{-1}	Juice	[72]
C_{18}	ACN: H_2O (29:71 v/v)	NMR	1.3 - 12 μgmL^{-1} R^2 = 0.9995 RSD% = 1.394 Rec% = 99.76 LOD = 10 μgmL^{-1} LOQ = 50 μgmL^{-1}	Fruit of Citrus	[73]
C_{18}	ACN: H_2O (60:40 v/v)	UV-vis 210_280 nm	2.5-100 μgmL^{-1} R^2 = 0.9990 % RSD = 2.9 % Rec = 92.2 LOD = 22 $ngmL^{-1}$ LOQ = 75 $ngmL^{-1}$	Citrus Juice	[74]
C_{18}	ACN: water (Gradient)	PAD 283-328nm	55-95 μgmL^{-1} R^2 = 0.9984 % RSD = 2.5% % Rec = 98.60 % LOD = 2.8 μgmL^{-1} LOQ = 8.5 μgmL^{-1}	Grapefruit juice	[75]
C_{18}	MeOH: H_2O in 10M CH_3COO buffer (40:60 v/v)	UV-Vis 356nm	1.4-14.3 μgmL^{-1} R^2 = 0.9990 % RSD =0.074 LOD =0.32 $ngmL^{-1}$	Apple juice	[76]
C_{18}	MeOH: HCOOH (Gradient)	DA280nm	10-60 μgmL^{-1} R^2 = 0.9941 % RSD =0.56-5.91	berry fruits	[77]

			LOD =0.90µgmL^{-1}		
XDB-C8	ACN: H2O: HCOOH (4:88:8v/v)	UV-Vis 515nm	0.1-1 mgmL^{-1} R^2 = 0.9995 % RSD =80 LOD =102µgmL^{-1} LOQ =105µgmL^{-1}	Grapefruit	[78]
C_{18}	ACN: CH_3COOH (Gradient)	DAD 280-320 nm	0.5-100 µgmL^{-1} R^2 = 0.9996 %Rec=99.5 LOD =0.15 µgmL^{-1} LOQ =0.42 µgmL^{-1}	Broccoli	[٧٩]
C_{18}	MeOH: H2O: H_3PO_4 (Gradient)	UV-Vis 190-600nm	1-80 mgmL^{-1} R^2 = 0.9990 %Rec=93.07 LOD=1.1 µgmL^{-1} LOQ=1.5 µgmL^{-1}	Berry	[٨٠]
C_{18}	ACN:CH_3COOH (90 :10v/v)	DAD	0.28-22.4 mgmL^{-1} R^2 = 0.9990 Rec=96.7 LOD=1.15 LOQ=0.25	Quinoa seeds	[81]
C_{18}	ACN: H_2O (75:25v/v)	PAD	5-25 µgmL^{-1} R^2 = 0.9990 Rec=116 LOD=0.04µgmL-1 LOQ=0.013µgmL^{-1} RSD=0.19	Soy	[82]
C_{18}	ACN: $(NH_4)_3PO_4$ (70 :30v/v)	UV-Vis 254nm	24-130 mgmL^{-1} R^2 = 0.9995 %Rec=91 LOD=1.31 µgmL^{-1} LOQ=1.17 µgmL^{-} %RSD=0.16	Soy	[83]
C_{18}	MeOH: CH_3COOH (Gradient)	ECD	4-52 µgmL^{-1} R^2 = 0.9990 %Rec=95 LOD=3 µgmL^{-1} LOQ=5.2 µgmL^{-1} %RSD=0.62	Dried flowers	[84]
C_{18}	MeOH:CH3COOH: H_2O (10:2:88 v/v)	UV-Vis 510nm	10-200 mgmL^{-1} R^2 = 0.9090 %Rec=90.28 LOD=1.2 µgmL^{-1}	Plant	[85]

Column	Mobile phase	Detection	Statistical data analysis	Application	Ref.
C_{18}	ACN: H_2O (60:40v/v)	Uv-Vis 370 nm	1-10 $mgmL^{-1}$ R^2 = 0.9995 %Rec=93 LOD=15 μgmL^{-1} LOQ=8 μgmL^{-1} %RSD=0.53	Moringa leaves	[86]
C_{18}	ACN: H_2O (50:50 v/v)	ECD	1-10 μgmL^{-1} R^2 = 0.9990 LOD=0.5 μgmL^{-1} %Rec=98 %RSD=9.2	Plasma	[87]
C_{18}	MeOH: CH_3COOH (Gradient)	PAD 254nm	7.8-250 $ngmL^{-1}$ R^2 = 0.9999 %Rec=99.3 LOD=1.73 μgmL^{-1} LOQ=4.01 μgmL^{-1} %RSD=0.1	Plant	[88]
C_{18}	(A) 30% MeOH in 0.05% CH_3COOH (B) 23.8 mM $C_6H_8O_7$, 67 mM EDTA (C) 100% ACN	DAD (290, 375 and 390) nm	50-500 $ngmL^{-1}$ R^2 = 0.9999 %Rec=97 LOQ=5 $ngmL^{-1}$ %RSD=3	Urine	[89]
C_{18}	MeOH: H_2O (50:50 v/v)	Uv-Vis 360nm	0.005-0.03 μgmL^{-1} R^2 = 0.9940 %Rec=96.1 LOD=3μgmL^{-1} LOQ=5.1 μgmL^{-1} %RSD=0.23	Plant	[90]
C_{18}	MeOH: HCOOH (gradient)	MS	1-1000 $ngmL^{-1}$ R^2 = 0.9981 %Rec=85-115 %RSD=1.9	Plasma	[91]
C_{18}	ACN: H_3PO_4 (Gradient)	Uv-Vis	0.2-2.5 μgmL^{-1} R^2 = 0.9990 %Rec=94.3 LOD=4.9μgmL^{-1} LOQ=0.4 μgmL^{-1} %RSD=1. 3	Serum	[92]
C_{18}	MeOH: HCOOH (80:20 v/v)	DAD 258nm	1-300 μgmL^{-1} R^2 = 0.9995 %Rec=98-100.2 LOD=0.39μgmL^{-1} LOQ=1.20 μgmL^{-1} %RSD=2. 18	Plant	[93]
C_{18}	MeOH: 0.1% H_3PO_4inH_2O (60:40 v/v)	Uv-Vis 258 nm	١٠-200 μgmL^{-1} R^2 =0.9995 %Rec=99.08 LOD=7μgmL^{-1} LOQ=10 μgmL^{-1} %RSD=0. 12	Herbs	[94]

Column	Mobile phase	Detection	Statistical data analysis	Application	Ref.
C_{18}	ACN: H_2O (50:50 v/v)	Uv-Vis 210nm	١٠-50 µgmL^{-1} R^2 = 0.9990 %RSD=0. 27 LOD=1.1µgmL^{-1}	Herbs	[95]
C_{18}	0.01 M H_2SO_4: ACN: MeOH:CH_3COOH (73:18:4:5 v/v)	Uv-Vis 353nm	3-15 µgmL^{-1} R^2 = 0.9997 %Rec=90.6 LOD=2.4µgmL^{-1} LOQ=1.3 µgmL^{-1} %RSD=4. 5	Plant	[96]
C_{18}	ACN: H_3PO_4 (15:85 v/v)	Uv-Vis 254nm	1.5-48 µgmL^{-1} R^2 = 0.9993 %Rec=99.9-100.45 LOD=1.7µgmL^{-1} %RSD=2. 39	Herbs	[97]
C_{18}	ACN: NH4OAc: EDTA:CH_3COOH (16.5:82.5:1v/v/v)	Uv-Vis 370 nm	3-1000 ngmL^{-1} R^2 = 0.9999 %Rec=98.2 LOD=0.75ngmL^{-1} LOQ=5 ngmL^{-1} %RSD=1. 3	Plasma	[98]
C_{18}	ACN: HCOOH (Gradient)	Uv-Vis 254 nm	2-20 µgmL^{-1} R^2 = 0.9974 %Rec=99.40 LOD=1.4µgmL^{-1}	Ginkgo biloba	[99]
C_{18}	ACN: HCOOH (Gradient)	MS-MS	25-2000 ngmL^{-1} R^2 = 0.9992 %Rec=68.9 LOD=2.4ngmL^{-1} %RSD=3. 8	Plasma	[100]
C_{18}	ACN: H2O: HCOOH (13:87:1 v/v/v)	ECD	1-15 ngmL^{-1} R^2 = 0.9990 %Rec=90.1 LOD=0.86ngmL^{-1} %RSD=0. 68	Buckwheat	[101]
C_{18}	ACN: H_3PO_4 (Gradient)	Uv-Vis 360nm	10-60 µgmL^{-1} R^2 = 0.9990 %Rec=90.3 LOD=1.73µgmL^{-1} LOQ=8.19 µgmL^{-1} %RSD=1. 3	Plant	[102]
C_{18}	ACN: HCOOH (Gradient)	DAD-EIS-MS	10-100 µgmL^{-1} R^2 = 0.9960 %Rec=86.5- 93.1 LOD=1. 3µgmL^{-1} %RSD=1. 08	Herb	[103]

C_{18}	ACN: HCOOH (Gradient)	QTOF-MS-MS	1-20 μgmL^{-1} R^2 = 0.9990 %Rec=98.0 LOD=1. 52μgmL^{-1} %RSD=2. 24	Juice	[104]
C_{18}	ACN: CH_3COOH (Gradient)	DAD 260,330 nm	20-200 $mgmL^{-1}$ R^2 = 0.9985 %Rec=94.0 LOD= μgmL^{-1} %RSD=0. 33	Herbs	[105]
C_{18}	MeOH: H_3PO_4 (Gradient)	Uv-Vis 350nm	2.4-14.3μgmL^{-1} R^2 = 0.9805-0.9995 %Rec=93.5-98.5 LOD=2. 3μgmL^{-1} %RSD=0. 4-7.2	Herbs	[106]
C_{18}	ACN: CH_3COOH (Gradient)	Uv-Vis 272,280 and310 nm	5-60 μgmL^{-1} R^2 = 0.9990 %Rec=96-103 LOD=0. 02μgmL^{-1} LOQ=0. 07μgmL^{-1} %RSD=0. 037	Herbs	[107]
C_{18}	MeOH: H2O: H_3PO_4 (48.5:51.5:0.25v/v/v)	Uv-Vis 360	0.5-25 μgmL^{-1} R^2 = 0.9990 %Rec=99.8 LOD=1. 62μgmL^{-1} %RSD=1. 23	Herbs	[108]
C_{18}	MeOH: H_3PO_4 (Gradient)	DAD	1.32-2.46 $mgmL^{-1}$ R^2 = 0.9999 %Rec=95.6 LOD=0. 16$mgmL^{-1}$ LOQ=1. 32$mgmL^{-1}$ %RSD=3. 01	Herbs	[109]
C_{18}	MeOH: H_2O (40: 60v/v)	Uv-Vis 370 nm	10-50 $mgmL^{-1}$ R^2 = 0.9999 %Rec=96.0 LOD=2. 44$mgmL^{-1}$ %RSD=2. 21	Herbs	[110]
C_{18}	ACN: HCOOH (Gradient)	Uv-Vis 210, 254, and 280 nm.	45-451μgmL^{-1} R^2 = 0.9992 %Rec=93.0 LOD=0. 22μgmL^{-1} LOQ=0. 72μgmL^{-1} %RSD=1.31	Herbs	[111]
C_{18}	THF: ACN: H_3PO_4 (20:3:77, v/v/v)	Uv-Vis 360	1.74-87μgmL^{-1} R^2 = 0.9980 %Rec=97.9 LOD=0. 3$ngmL^{-1}$ LOQ=1$ngmL^{-1}$ %RSD=0.02	Fruits	[112]

C_{18}	ACN: H_2O (Gradient)	MS_MS	40-100 $ngmL^{-1}$ R^2 = 0.9995 %Rec=97.2 LOQ=0.4$ngmL^{-1}$ %RSD=7.54	Herbs	[113]
C_{18}	H_2O: MeOH:ACN : 0.02% FAA (gradient)	MS	6-192 μgmL^{-1} R^2 = 0.9998 %Rec=95 LOD=1. 5μgmL^{-1} LOQ=3μgmL^{-1} %RSD=2.66	Herbs	[114]
C_{18}	MeOH: H_3PO_4 (45:55v/v)	DAD 259 nm	40-400 μgmL^{-1} R^2 = 0.9910 %Rec=87.46 LOD=3.3μgmL^{-1} %RSD=0.27	Herbs	[115]
C_{18}	ACN: CH_3COOH (Gradient)	UV-VIS 260 nm	1-100 μgmL^{-1} R^2 = 0.9195 %Rec=93 LOD=2.5μgmL^{-1} %RSD=0.22	Herbs	[116]
C_{18}	ACN:CH_3COOH (Gradient)	MS-MS	0.1-20 μgmL^{-1} R^2 = 0.9880 %Rec=96.4 LOD=1. 33μgmL^{-1} LOQ=4.47μgmL^{-1} %RSD=0.95	Fruits	[117]
C_{18}	ACN: HCOOH (Gradient)	MS-MS	0.5-200 $ngmL^{-1}$ R^2 = 0.9946 %Rec=98.2 LOQ=0.5$ngmL^{-1}$ %RSD=7.7	Plasma	[118]
C_{18}	ACN:CH_3COOH (Gradient)	DAD 370nm	2.5-100 μgmL^{-1} R^2 = 0.9996 %Rec=99.98 LOD=3μgmL^{-1} %RSD=1.2	Honey	[119]
C_{18}	ACN: CH3COOH:MeOH (Gradient)	FD 360nm	2-2000 $ngmL^{-1}$ R^2 = 0.9957 %Rec=86.9 LOD=1.5$ngmL^{-1}$ %RSD=1.3	Juices	[120]

C_{18}	MeOH: CH_3COOH (Gradient)	MS-MS	0.25-126.5 $ngmL^{-1}$ R^2 = 0.9915 %Rec=95.2 LOQ=2$ngmL^{-1}$ %RSD=0.15	Herbs	[121]
C_{18}	MeOH: HCOOH (Gradient)	MS-MS	0.64-300 $ngmL^{-1}$ R^2 = 0.9973 %Rec=98.0 LOQ=1.07$ngmL^{-1}$ %RSD=9.96	Plasma	[122]
C_{18}	MeOH: HCOOH (Gradient)	MS-MS	5-1000 $ngmL^{-1}$ R^2 = 0.9990 %Rec=95.2 LOQ=0.5$ngmL^{-1}$ %RSD=3.3	Plasma	[123]
C_{18}	ACN: H_3PO_4 (Gradient)	UV-VIS 255 nm	1-50 μgmL^{-1} R^2 = 0.9979 %Rec=95.0 LOD=2μgmL^{-1} %RSD=4	Herbs	[124]
C_{18}	ACN: H_3PO_4 (36:64, v/v)	UV-VIS 288nm	0.4-50 μgmL^{-1} R^2 = 0.9990 %Rec=99.0 LOD=0.02μgmL^{-1} LOQ=0.4μgmL^{-1} %RSD=2.2	Herbs	[125]
C_{18}	MeOH: NH_4OAc (Gradient)	UV-VIS 336 nm	25-400 μgmL^{-1} R^2 = 0.990 %Rec=94.6 LOQ=25$ngmL^{-1}$ %RSD=6.2	Plant	[126]
C_{18}	MeOH: $C_6H_8O_7$ (Gradient)	DAD 354 nm	0.14-177.2 μgmL^{-1} R^2 = 0.9990 %Rec=99.7 LOD=0.3μgmL^{-1} LOQ=1.2μgmL^{-1} %RSD=0.9	Herbs	[127]
C_{18}	MeOH:ACN:CH_3COOH:H_3PO_4:H_2O (40:20:0.05:0.05:40v/v/v/v/v)	UV-VIS 352 nm	0.25-5 μgmL^{-1} R^2 = 0.9995 %Rec=97.6 LOD=0.05μgmL^{-1} LOQ=0.15μgmL^{-1} %RSD=1.433	Plant	[128]

Column	Mobile phase	Detection	Statistical data analysis	Application	Ref.
C_{18}	ACN: TFA (34:66 v/v)	PAD 270nm	200-500 $ngmL^{-1}$ R^2 = 0.9996 %Rec=100.90 LOD=11.44μgmL^{-1} LOQ=38.15μgmL^{-1} %RSD=0.71	Plant	[129]
C_{18}	MeOH: NH_4OAc (Gradient)	MS-MS	25-1500 $ngmL^{-1}$ R^2 = 0.9960 %Rec=97.7 LOD=1.32$ngmL^{-1}$ LOQ=3.96$ngmL^{-1}$ %RSD=0.41	Plant	[130]
C_{18}	ACN: H_3PO_4 (47:53v/v)	UV-VIS 370nm	1-10 μgmL^{-1} R^2 = 0.9990 %Rec=98.5 LOD=2.1μgmL^{-1} LOQ=3.4μgmL^{-1} %RSD=1.22	Plant	[131]
C_{18}	ACN: $HClO_4$ (Gradient)	UV-VIS 330nm	1-1000 $ngmL^{-1}$ R^2 = 0.9999 %Rec=101.3 LOD=84$ngmL^{-1}$ LOQ=225$ngmL^{-1}$ %RSD=0.77	Plant	[132]
C_{18}	ACN: TFA (Gradient)	UV-VIS 260 nm	3.12-100 μgmL^{-1} R^2 = 0.9994 %Rec=96.2 LOD=0.44μgmL^{-1} LOQ=1.47μgmL^{-1} %RSD=0.154	Vegetables	[133]
C_{18}	ACN: HCOOH (Gradient)	PAD 254nm	18.1-63.4 μgmL^{-1} R^2 = 0.9998 %Rec=91.53 LOD=2.1μgmL^{-1} LOQ=3.6μgmL^{-1} %RSD=2	Herbs	[134]
C_{18}	ACN: MeOH (50:50 v/v)	UV-VIS 256nm	5-25 μgmL^{-1} R^2 = 0.9990 %Rec=92.3 LOD=0.6μgmL^{-1} %RSD=0.21	Plant	[135]
C_{18}	ACN: CH_3COOH (Gradient)	PAD	75-300 μgmL^{-1} R^2 = 0.9996 %Rec=96.5 LOD=1.2μgmL^{-1} %RSD=0.7	Plant	[136]

Column	Mobile phase	Detection	Statistical data analysis	Application	Ref.
C_{18}	ACN: FAA (Gradient)	MS	1.89-250 μgmL^{-1} R^2 = 0.9997 %Rec=99.1 LOD=0.8μgmL^{-1} %RSD=2	Plant	[137]
C_{18}	ACN:CH_3COOH (Gradient)	UV-VIS 330nm	0.25-10 μgmL^{-1} R^2 = 0.9999 %Rec=98.3 LOQ=0.312μgmL^{-1} %RSD=8.81	Plant	[138]
C_{18}	ACN: H_3PO_4 (Gradient)	DAD 285nm	1.04-104 μgmL^{-1} R^2 = 0.9990 %Rec=101 LOD=4μgmL^{-1} %RSD=0.2	Fruits	[139]
C_{18}	MeOH: ACN:H_2O: CH_3COOH (20:10:70:1v/v/v/v)	ED	0.03-13.49 $mgmL^{-1}$ R^2 = 0.9986 %Rec=101 LOD=0.06$mgmL^{-1}$ LOQ=0.02$mgmL^{-1}$ %RSD=0.05	Plant	[140]
C_{18}	ACN: H_3PO_4 (Gradient)	UV-VIS 517nm	0.03-13.49 $mgmL^{-1}$ R^2 = 0.9990 %Rec=91.4 LOD=3$mgmL^{-1}$ %RSD=0.38	Plant	[141]

8. Conclusions

Liquid chromatography is a very flexible analytical method that can be coupled with various detectors. HPLC methods combined with UV or mass spectroscopy are the primary analytical techniques for determining flavonoids in food and biological samples. Most of the methods reviewed in this study have employed HPLC systems coupled with UV detectors when a more straightforward and cost-effective approach is preferred for clinical monitoring. Over the past decade (the duration of the review), many methods have used MS detectors due to their enhanced selectivity and sensitivity. This review aimed to provide researchers with a comprehensive overview before developing a quantification method.

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