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Review

Chromatographic, capillary electrophoretic and capillary electrochromatographic techniques in the analysis of flavonoids

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Abstract

An overview is presented of chromatographic methods currently in use to determine flavonoids, including free aglycones, their corresponding glycosides, one by one, and, in the presence of each other. As a basis of selection, the following approaches can be distinguished: critical evaluation of the preliminary steps (extraction/isolation and hydrolysis) as well as the separation, identification and quantitation of constituents both on the basic research level and/or subsequently to various work up procedures. Chromatographic techniques were discussed after extraction/isolation of various flavonoids from several natural matrices. Papers were classified and compared from analytical point of view, primarily on the chromatographic, secondly on the detection techniques applied.

Keywords: Reviews; Flavonoid's analysis; Chromatography; Aglycone; Flavonoid glycoside

Contents

Abbreviations: ACN, acetonitrile; APCI, atmospheric pressure chemical ionization; API, atmospheric pressure ionization; BHT, butylated hydroxytoluene; BSA, bis(trimethylsilyl)acetamide; BSTFA, bis(trimethylsilyl)trifluoroacetamide; CE, capillary electrophoresis; CEC, capillary electrochromatography; CID, collision induced dissociation; CL, chemiluminescence; CLND, chemiluminescence nitrogen detection; D, detection; DAD, photodiode array detection; DMF, dimethylformamide; EC, electron-capture detection; ED, electrochemical detection; ELSD, evaporative light scattering detection; ESI, electrospray ionization; FAB, fast atom bombardment; FID, flame ionization detection; FL, fluorescence; GC, gas chromatography; Glu, glucoside; Gly, glycoside; HMDS, hexamethyl disilazane; HPLC, high-performance liquid chromatography; IT, ion trap; ITD, ion trap detection; LC–MS, liquid chromatography–mass spectrometry; LOD, limit of detection; MECC, micellar electrokinetic capillary chromatography; Met, methanol; MS, mass spectrometry; MTBSTFA, *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide; NI, negative ionization; NMR, nuclear magnetic resonance; PB, particle beam; PI, positive ionization; PLE, pressurized liquid extraction; QqQ, triple quadrupole; Q-TOF-MS, quadrupole time of flight mass spectrometry; RDA, retro Diels-Alder; RI, refractive index; RP, reverse phase; R.S.D., relative standard deviation; S/N, signal to noice; SCF, supercritical fluid chromatography; SDDC, sodium diethyl dithiocarbamte; SFE, supercritical fluid extraction; SIM, selective ion monitoring; SPE, solid–phase extraction; TBHQ, *tert*-butylhydroxyquinone; TFA, trifluoroacetic acid; TIC, total ion current; TMCS, trimethylchlorosilane; TMS, trimethylsilyl; UV, Ultraviolet

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1. Introduction

In spite of the relevancy of flavonoids and the tremendous amount of papers dealing with the identification and quantitation of different flavonoid species in various matrices, according to author's knowledge, there is no recent review article associated with their chromatographic analysis. This fact is not a chance, it is thoroughly associated with the complexity of the task: with the very special chemical, physical and structural properties of flavonoids, with the huge number of papers containing several contradictions. All these characteristics deter analytical chemists from dealing with the topic.

The chemical structure of their main representatives, belonging to various groups of compounds, such as anthocyanidins, catechins, flavones, flavonols, flavanones and isoflavones, are depicted in Fig. 1. However, due to the intrinsic features of flavonoids:

(i) ready to transformation/oxidation/reduction processes, intra and intermolecular rearrangements,
(ii) being different in number and positions of their hydroxyl groups, and
(iii) being linked to several saccharides of various structures and degree of polymerization, the versions of a single flavonoid might be huge. Taking into account the diversities of the possible species, it turned out that theoretically more than 2×10^6 flavonoids can exist and for the time being more than 2×10^3 species have been already identified [1]. For instance, quercetin alone does have more than 179 glycosides [2,3].

The relevancy of these group of organics (being regarded as versatile, beneficial impact furnishing natural compounds) is associated with their invaluable physiological/biological and practical characteristics [4,5]. It has been confirmed that flavonoids of polyphenolic structure and of antioxidant characteristics (identified in almost all plants, vegetables and fruits, mainly in the form of their β-glycosides [5]), in living organism become absorbed very fast [4]. According to epidemiological studies, flavonoid containing vegetables and fruits demonstrate a protective effect against cancer, stroke and coronary heart disease related to their antioxidant properties [5]. Antimutagenetic activity of flavonoids (sourced from the heartwood of Rhus verniciflua) was proven on bromobenzene treated rats [6].

Thus, on the basis of the usefulness and importance of these types of natural compounds, it seemed to be a need to summarize recent chromatographic procedures.

No doubt about it, the complete analysis of a flavonoid-including its detailed structure, configuration of its ring positions, numbers of double bonds, free and substituted hydroxyl groups and additional substituents of the rings-needs a cooperation of analytical chemists, as well as the availability of advanced separation and identification techniques, at once [chromatography, nuclear magnetic resonance (NMR) and mass spectrometry (MS), etc.]. Certainly, the best solution of this task would be a HPLC–MS–NMR apparatus that can fulfill all these requirements, simultaneously [7]. Unfortunately, only a few laboratories are equipped with this relatively new system. However, simpler approaches, such as high-performance liquid chromatogra-