



# On the chemical identification and determination of flavonoids in solid-state

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## ABSTRACT

The paper presented with qualitative and quantitative analysis of alkaloids in solid-state, using the excitations within the THz-spectroscopic region of  $300\text{--}30\text{ cm}^{-1}$  ( $9.00\text{--}0.9\text{ THz}$ ). Series of nine plant flavonoids (FLs) and their mixtures were analyzed both qualitatively and quantitatively. For first time in the literature was reported the quantitative analysis of alkaloids and their mixtures within the THz-region using the solid-state Raman spectroscopy, and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS). The calibration and validation, concentration limit of detection (*LOD*), limit of quantification (*LOQ*), and linearity limit (*LL*) were obtained. The chemometric nonlinear and linear approaches for analysis and interpretation of the quantities were applied. The results obtained were compared with a parallel *QA*, using the calibrated and validated HPLC electrospray ionization (ESI) mass spectrometric method, electronic absorption (EAs) and CD spectroscopy. The metrology, including accuracy, measurement repeatability (intra-serial precision condition of measurement), measurement precision, trueness of the measurement, and reproducibility of the measurement, measurement bias and errors of the measurements were discussed.

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## 1. Introduction

The recent developments on the qualitative and quantitative analysis (*QA*) of small molecules by THz-spectroscopy [1d,e] for the needs of the forensic science have been shown successful results on the identification of many explosives, chemical and biological agents [1,2]. The *QA* of the solid-binary mixtures has been shown the concentration  $LOD \pm 5\%$  of the analyzing of the macro component [1e]. Recent analytical studies of the excitation phenomena of pharmaceuticals within the same region, by the solid-state Raman spectroscopy [3] have been provided data for concentration *LOD* of 1.34% of the analyte, using the nonlinear chemometric approaches [3i,j]. It has been shown as well that the complementary application of the vibrational spectroscopy and MALDI method expand the capability for analysis of multicomponent mixtures within concentration range  $10^{-5}$  to  $10^{-7}$  mol/l [3j]. The individual spectroscopic profiles in the vibrational spectra of molecules in solid-state, observed within the THz-region and the high intensity of the observed excitations, expand the applicability of the method for *QA* of the properties of the evidences that could be attributed to a common source with an extremely high degree of certainty [2e–h]. In this respect, the development of the method for the needs the forensic chemistry, lie in the systematic investigations of the different classes of natural products (NPs),

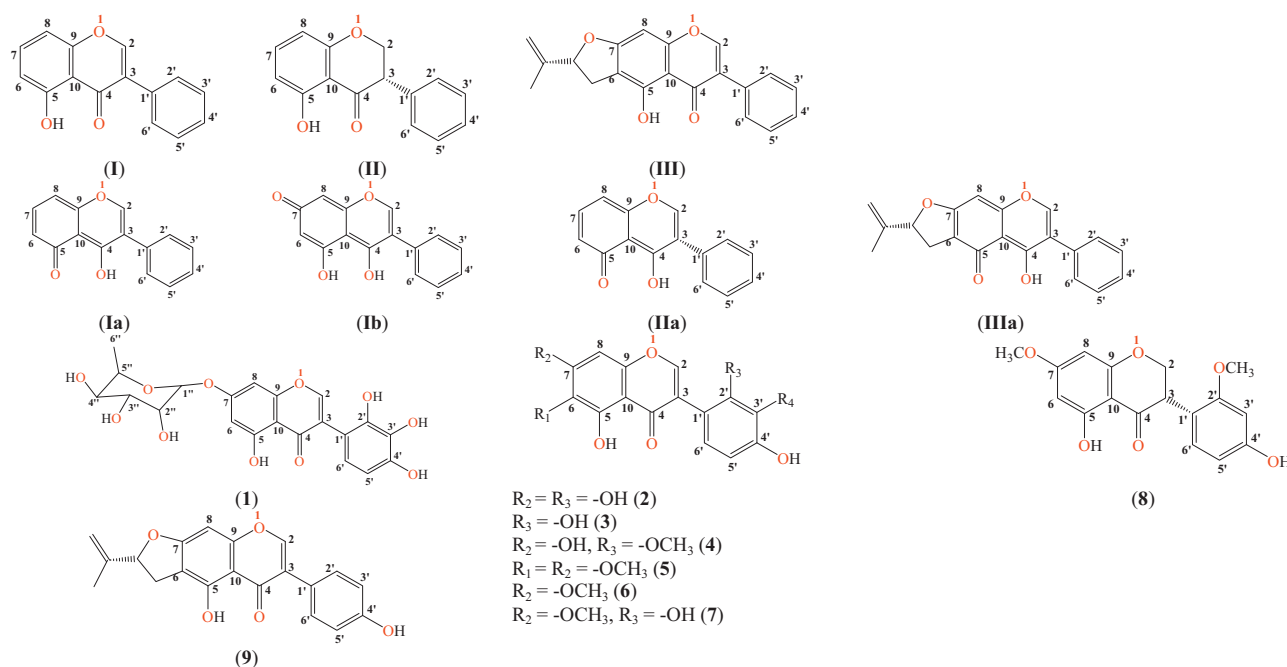
semi- and synthetic analogous, updating the databases, and optimizing the software allowing quickly, conveniently and accurately usage of the method for obtaining of the pertinent investigative information [2]. Thus, the paper reported a complementary analytical application of solid-state Raman spectroscopic and mass spectrometric study of nine flavonoids (Scheme 1) as well as their mixtures. The validation of the methods, metrology including, accuracy, precision, measurement repeatability, trueness of the measurements, reproducibility of the measurement, measurement bias and errors of the measurements, concentration *LOD* and *LOQs*, as well as *LL* were discussed.

## 2. Experimental

### 2.1. Materials

The FLs were obtained according [3]. The synthesis of bis(R-(+)-1-phenylethylammonium) squarate **Sd-(1)**, was obtained by mixing of squaric acid (Sigma) and R-(+)-1-phenylethylamine at molar ratio 2:1 in 20 ml solvent mixture methanol:water 1:1 under stirring for 30 min at  $150^\circ\text{C}$ . The resulting colorless crystals, were filtered off, washed with  $\text{CH}_3\text{OH}$  and dried on  $\text{P}_2\text{O}_5$  at 298 K. The data from the performed elemental analysis were: found: C, 67.40; H, 6.76; N, 7.88; calcd. for  $\text{C}_{20}\text{H}_{24}\text{O}_4\text{N}_2$ : C, 67.38; H, 6.79; N, 7.86%, yield 91%, respectively.

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**Scheme 1.** Chemical diagrams of FLs (1)–(9); common molecular skeleta (I–III), the resonance (Ia,b, IIa, IIIa) forms, depending of the pH.

## 2.2. Physical methods

The X-ray diffraction intensities were measured on a Bruker Smart X2S (Bruker AXS, Karlsruhe, Germany) diffractometer, using micro source Mo- $K_{\alpha}$  radiation and employing the  $\omega$  scan mode. The data were corrected for Lorentz and polarization effects. An absorption correction procedure, based on multiple scanned reflections was applied. The Sd-(1) structure was solved by direct methods using SHELXS-97 [4] and was refined by full-matrix least-squares refinement against  $F^2$  [4]. Anisotropic displacement parameters were introduced for all non-hydrogen atoms. The hydrogen atoms attached to carbon were placed at calculated positions and refined allowing them to ride on the parent carbon atom. The hydrogen atoms bound to nitrogen and the oxygen were constrained to the positions which were confirmed from the difference map and refined with the appropriate riding model. The experimental data were summarized in Table S1. The spectra were recorded at ambient conditions ( $T = 298$  K,  $P = 1$  atm). The powder X-ray diffraction measurements were carried out on polycrystalline samples, proving the crystallographically obtained space systems. The XRD patterns were obtained using a Rigaku MiniFlex powder diffraction system (Rigaku Corp., Osaka, Japan), equipped with a horizontal goniometer in the  $\theta/2\theta$  mode. The X-ray source was nickel-filtered K- $\alpha$  emission of copper (1.54056 Å). Samples were packed into an aluminum holder using a back-fill procedure and will be scanned over the range of  $50$ – $6^\circ 2\theta$ , at a scan rate of  $0.5^\circ 2\theta/\text{min}$ . Using a data acquisition rate of 1 point per second, the scanning parameters equate to a step size of  $0.0084^\circ 2\theta$ . Calibration of each powder pattern will be effected using the characteristic scattering peaks of aluminum at  $44.738$  and  $38.472^\circ 2\theta$ . The method was applied for elucidation of the crystallographic space system of the matrixes during the same preparation technique for MALDI measurements.

HPLC–MS/MS measurements were made using TSQ 7000 instrument (Thermo Fisher Inc., Rockville, MD, USA). Two mobile phase compositions were used: (A) 0.1% (v/v) aqueous HCOOH and (B) 0.1% (v/v) HCOOH in  $\text{CH}_3\text{CN}$ . Electrospray ionization mass spectrometry. A triple quadrupole mass spectrometer (TSQ 7000 Thermo Electron, Dreieich, Germany) equipped with an ESI 2 source was used and operated at the following conditions: capillary

temperature  $180^\circ\text{C}$ ; sheath gas 60 psi, corona  $4.5\ \mu\text{A}$  and spray voltage 4.5 kV. Sample was dissolved in acetonitrile ( $1\ \text{mg ml}^{-1}$ ) and was injected in the ion source by an autosampler (Surveyor) with a flow of pure acetonitrile ( $0.2\ \text{ml/min}$ ). Data processing was performed by Excalibur 1.4 software. A standard LTQ Orbitrap XL (Thermo Fisher Inc.) instrument was used for the MALDI-MS measurements, using the UV laser source at 337 nm. An overall mass range of  $m/z$  100–1000 was scanned simultaneously in the Orbitrap analyzer. The ImageQuest 1.0.1 program package was used. The laser energy values were within 12.5–17.2  $\mu\text{J}$ . The numbers of averaged laser shots lies within 16–108, the MALDI flow rate values were within 24.8–27.2; the acquisition time was within 30.2–137.2 min, the corresponding elapsed scan time range lies within 20.2–3.77 s, respectively.

Raman spectra in solid-state were recorded on: (i) Nicolet NXR 9610 FT-Raman spectrometer (both instruments were products of Thermo Electron Corporation, Baltimore, MD, USA), equipped with the semiconductor laser operating source at 976 nm. The resolution of  $0.09\ \text{cm}^{-1}$  was over the spectral range 100–3705  $\text{cm}^{-1}$  and (ii) Horiba Jobin–Yvon Inc. (Edison, NJ, USA) 60000 triple monochromator spectrometer equipped with a Spectra-Physics Inc. (Mountain View, CA, USA) model 164 argon ion laser operated on the 514.5 nm line. The resolution of  $1.0\ \text{cm}^{-1}$  was over the spectral range 20–2500  $\text{cm}^{-1}$ . A triple integrated laser system was used for the variation of the excitation energy at 532, 633 and 785 nm, respectively. The laser power used was 100 mW, with a spectral band-space of 3  $\text{cm}^{-1}$ . The spectra were recorded at ambient conditions ( $T = 298$  K,  $P = 1$  atm). The Raman spectra of the solid crystal powders were measured in a glass capillary.

Chromatographic confirmation about the purity of the studied compounds was performed with a Gynkotek (Germering, Germany) HPLC instrument, equipped with a preparative Kromasil 100 C18 column ( $250\ \text{mm} \times 20\ \text{mm}$ ,  $7\ \mu\text{m}$ ; Eka Chemicals, Bohus, Sweden) and a UV detector set at 250 nm. The mobile phase was acetonitrile:water (90:10, v/v) at a flow rate of 4 ml/min. The analytical HPLC was performed on a Phenomenex (Torrance, CA, USA) RP-18 column (Jupiter 300,  $150\ \text{mm} \times 2\ \text{mm}$ ,  $3\ \mu\text{m}$ ) under the same chromatographic conditions as above. The QA was performed on a Shimadzu UFLC XR (Kyoto, Japan) instrument, equipped with

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