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Highly sensitive solid forms discrimination on the whole tablet of the active ingredients in quercetin dietary supplements by NMR crystallography approaches

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ABSTRACT

Similarly to synthetic drugs, the exact crystalline form of active ingredients in solid formulations of dietary supplements may directly influence the dissolution rate, bioavailability, and stability of the final product, but this information is usually not provided by manufacturers. Working on the examples of two commercial quercetin dietary supplements a quick, reliable, and sensitive method is introduced for quercetin solid forms discrimination directly on the marketed products, without the need for prior sample preparation. It exploits the complementarity between solid-state Nuclear Magnetic Resonance (ss-NMR) and Powder X-Ray Diffraction (PXRD), which proved essential for performing a complete and accurate solid-state characterization of the two commercial products, and for obtaining new insights into the complex quercetin solid-forms landscape. The method can be readily generalized also to other dietary supplements based on bio-flavonoids/polyphenols.

1. Introduction

The global nutraceuticals product market reached $142 billion in 2011 and is expected to grow up to $205 billion by 2017, according to a recent market report [1] from Transparency Market Research, Albany, NY. An important class of dietary supplements sold worldwide is that of polyphenols supplements: among them, the bio-flavonoids based products are the most representative. Bio-flavonoids are naturally occurring polyphenolic compounds that are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine [2]. They have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and antioxidant activities. A *meta*-analysis of 9 cohort studies (comprising >220.000 men and women) showed that fruit and vegetable consumption was inversely associated with the risk of cardiovascular diseases [3].

Despite their documented health benefits, a significant percentage of the population is not consuming sufficient quantities of dietary polyphenols as a result of inadequate fruit and vegetable intakes caused by the modern lifestyle. Consequently, an alternative source of flavonoids that has become extremely popular in the recent past is dietary supplements. The biggest problem with the increase consumption of nutraceuticals is that they are not regulated to the degree that are medicines. This allows manufacturers to market supplements without fully testing them for efficacy or potential side effects [4]. As such, there are increasing demands for critically evaluating whether vascular health-promoting and other positive properties of flavonoid-rich diets can be replaced by purified flavonoids in dietary supplements. The solid formulations (tablets, capsules) of dietary supplements rise an additional concern. On the one hand side, the exact solid/crystalline form of the active ingredient is important to be known, because it may directly influence the dissolution rate and bioavailability of the final product, in a similar way that was found for synthetic drugs [5]. On the other hand side, however, this information is seldom provided by manufacturers.

The present study addresses exactly this issue. Benefiting from the remarkable progress that the emerging field of NMR crystallography [6] has witnessed in the last decade, it is shown here on the example of two representative commercial quercetin dietary supplements, that the combination of solid-state Nuclear Magnetic Resonance (ss-NMR) spectroscopy and Powder X-Ray Diffraction (PXRD) may offer a convenient tool for solid-form determination of active ingredients directly on the marketed product, without the need for prior sample preparation procedures. The power of NMR

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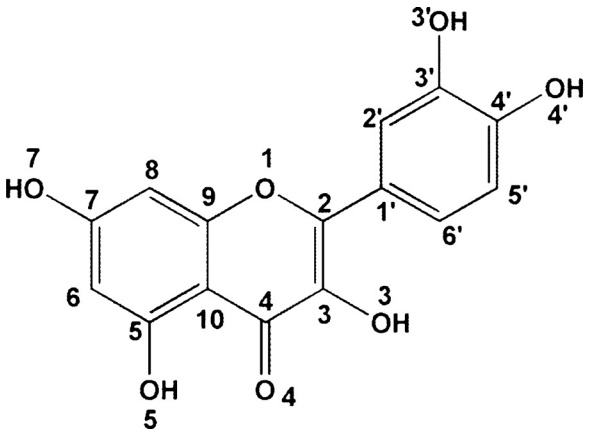

Fig. 1. Chemical structure of quercetin, with atom labelling.

crystallography in its traditional use – crystal structure determination from powders of solids that cannot be grown as single crystals – comes from the complementarity of the information provided by the two techniques [7]: local structural details are probed with the highest sensitivity by ss-NMR, whereas PXRD is very accurate in detecting long range ordering and crystal symmetries. Recent examples [8–10] of structural determination by NMR crystallography with accuracy close to that of single-crystal X-Ray diffraction perfectly illustrates this power.

The quercetin dietary supplements were chosen in our study for the following reasons: (i) quercetin (3,3′,4′,5,7-pentahydroxyflavone, Fig. 1) represents the most common flavonoid in the human diet [11] that has been widely investigated for its anti-inflammatory, antioxidant, antiviral, and anti-carcinogenic effects [12–15]; (ii) the known solid forms of quercetin: anhydrate [16], monohydrate [17] and dihydrate [18] have the crystal structures determined; (iii) the extraction process [19] does not necessarily provide quercetin in a pure solid form, but often in a mixture of different forms, as shown in a study on products from Brazilian sources [20].

The methods borrowed from NMR crystallography were used here with the main purpose of setting up a procedure for quick and reliable characterization of quercetin dietary supplements with respect to the solid-form purity of the incorporated quercetin extract—the challenge was to discriminate what particular form is present in the tablet/capsule, given the fact that this is a complex mixture containing also a number of excipients alongside with the active ingredient(s). The results obtained on the investigated quercetin products demonstrate that the complementarity between ss-NMR and PXRD is essential for fully addressing this issue. In particular, PXRD proved very useful for quick identification of the major crystalline components (both active ingredients, and excipients) mentioned by the manufacturers in the specifications of the two quercetin products. Though, the detection limit was found quite poor, which appears to be inherent to this technique when applied on complex mixtures of powdered components. By contrast, ^{13}C ss-NMR spectra were shown sensitive in distinguishing the quercetin solid forms in their commercial formulation, even in the presence of additional active ingredient(s) and excipients. Besides the major dihydrate quercetin component, small amounts of another solid form were also found in the both supplements. Most of the ^{13}C ss-NMR lines pointed towards the anhydrous quercetin, but small discrepancies observed for two other lines prompted us to investigate the possibility of having an unknown quercetin solid form. After extended studies, this new form was identified as being either the ethanol or methanol solvate (both showed almost identical PXRD patterns and ^{13}C ss-NMR spectra) of quercetin. The crystal structure could be determined by an NMR crystallography approach only for the quercetin-methanol solvate, because the ethanol solvate could not be obtained in a pure solid form. Subsequent studies have shown that the solvates are not fully stable: they suffer de-solvation to the anhydrous quercetin. Although demonstrated only on two quercetin dietary supplements, the employed approach can be readily generalized also to other quercetin/flavonoid commercial products.

Table 1
The PXRD characteristic peaks of the components for the two supplementary samples, *Quer_O* and *Quer_N*.

Quer_O		*Quer_N*	
2θ (°)	Components	2θ (°)	Components
6.26	Quer_dihy/Stearic acid	5.28	Magnesium stearate
10.82	Quer_dihy	6.25	Quer_dihy
11.61	Stearic acid	10.51	Ascorbic acid
12.43	Quer_dihy	10.81	Quer_dihy
13.63	Quer_dihy	11.67	Magnesium stearate
14.19	Quer_dihy	12.49	Quer_dihy
15.87	Quer_dihy	14.00	Ascorbic acid
16.20	Quer_dihy	15.76	Quer_dihy/Ascorbic acid
17.27	Quer_dihy	16.13	Quer_dihy/Ascorbic acid
17.98	Quer_dihy	17.35	Quer_dihy/Ascorbic acid
20.98	Stearic acid	19.68	Magnesium stearate
21.58	Stearic acid	19.88	Ascorbic acid
23.88	Quer_dihy	21.33	Ascorbic acid
24.46	Quer_dihy	23.51	Magnesium stearat
24.82	Quer_dihy	23.89	Quer_dihy
26.60	Quer_dihy	24.42	Quer_dihy
27.43	Quer_dihy	25.30	Ascorbic acid
29.35	Stearic acid	26.65	Quer_dihy
30.50	Stearic acid	27.21	Ascorbic acid
34.17	Stearic acid	27.42	Quer_dihy
34.45	Stearic acid	29.58	Ascorbic acid
36.85	Stearic acid	30.08	Ascorbic acid
37.16	Stearic acid	31.78	Silica
		32.00	Silica
		34.69	Ascorbic acid

2. Materials and methods

2.1. Samples

Two commercial quercetin dietary supplements originating from well-known manufacturers in the U.S. and Canada were purchased from trusted suppliers on the Romanian market: ***Quercetin*** from *Natrol Inc*, USA (*Quer_N*), and ***Quercetin*** from *Organika*, Canada (*Quer_O*). The samples considered for PXRD and ss-NMR analysis were used as received in the case of *Quer_N,* which is sold as capsules, or subjected only to moderate grinding (*Quer_O*, which is sold as tablets). Quercetin dihydrate (*Quer_dihy*, purity 97%) was purchased from Alfa Aesar and anhydrous quercetin (*Quer_anhy*, purity 97%), from Sigma Aldrich. Both samples were used as received without further recrystallization.

According to the dosage indicated by the manufacturers, a *Quer_N* capsule (weight: 1.016 g) contains quercetin (250 mg), vitamin C (500 mg), and a citrus bioflavonoid complex (50 mg) as active ingredients, and gelatin, magnesium stearate, silica, and water as excipients. For *Quer_O* (tablet weight: 1.165 g) the active ingredients are represented by a mixture of quercetin (400 mg) and bromelain derived from the stem of pineapples (100 mg), whereas the excipients by calcium phosphate dibasic dihydrate, magnesium stearate, microcrystalline cellulose, croscarmellose sodium, and stearic acid. For the both supplements, no indication about the amount of the excipients is given. Also, the quercetin crystalline form, namely dihydrate, is explicitly stated only in the case of *Quer_O*.