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Mass spectrometric and quantum chemical treatments of molecular and ionic interactions of apigenine-O-glucoside – stochastic dynamics

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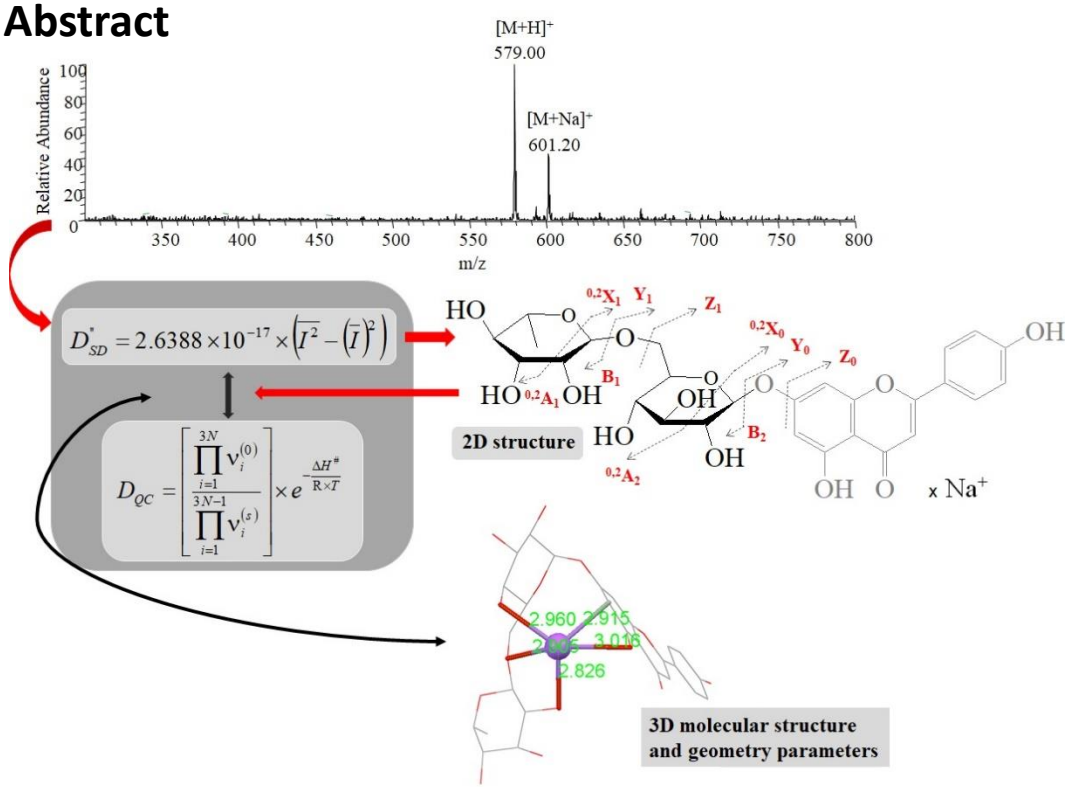
Graphical Abstract



Abstract:

The study answers to a question: How are 3D molecular and electronic structures; energetics; thermodynamics, kinetics and diffusion of mass spectrometric **(MS)** ions correlate quantitatively? Despite, irreplaceable application of the mass spectrometry to many interdisciplinary research fields as a robust analytical instrumentation, due to its superior method performances, there is a lacuna in this issue, which as yet is not been unambiguously resolved. Our innovative stochastic dynamic **(SD)** quantitative MS approach has emerged in recent few years and empirically justified in claiming that exact functions among measurable *variable intensity* of peaks of ions; their *3D molecular and electronic structures*, reaction rates, *free Gibbs energy* and *diffusion* exist, and the relations behave as our model formulas say. They treat MS variables highly accurately, precisely, selectively, sensitively, and exactly, respectively. It seems clearly that, we must have reliable empirical facts for such claims, because of many MS ionization phenomena are still far from well understood. However, the SD equations provide reasons to think that they might be warranted in moving toward an exact answer to the question. Herein, we provide new empirical facts and pro-arguments, examining ESI(+)-CID-MS^n data (n = 1–4) on apigenin-O-glucoside (1) via the SD formulas. The study argues for that the SD theory provides best quantitative explanation of MS observable subtle electronic effects of complex molecules from perspective of electronic structures, exhibiting a set of tautomers, proton and charge transfer effects, like analyte (1). *Ab initio* and semi-empirical static methods, molecular dynamics and chemometrics were carried out, as well.

Keywords: 3D molecular structure; flavonoids; mass spectrometry; quantum chemistry; stochastic dynamics

Introduction

If we take an understanding of an irreplaceability of the *mass spectrometric methods* as a primary concept, herein, then the introductory section must concentrate on arguments for convincing the reader of this statement. The first issue that we would like to consider aims at illustrating a significant importance of the contribution to the *analytical chemistry* looking at the object apigenin-O-glucoside. It belongs to chemical class of naturally occurring products **(NPs)** called *flavonoid-O-glycosides*, representing a diverse group of plant secondary metabolites, amongst others [1]. At about 2 % of total carbon photosynthesized by plants in the environment is converted into derivatives of flavonoids (**FLs**.) The largest part FLs are bonded chemically to carbohydrates ranging from monoglycosidic to pentaglycosidic derivatives. Their biological activity and beneficial effects to humans are another source of pro-arguments for significance of our study. These NPs exhibit a broad spectrum of biological functions, such as, an antioxidant activity; photosensitization or light screening; energy transfer; antimicrobial function; morphogenesis; feeding repellent activity; sex determination; regulation of plant growth hormones, *et cetera*. FLs beneficial pharmacological activity to human health includes an anti-inflammatory and antiallergic role; in addition to, an antiviral activity. Flavonoids are used to treat atherosclerosis, dementia, cardiovascular diseases, and more, as well. The most important biological function of FLs is their antioxidant activity. It is best documented function of almost all groups of such compounds. They affect on many systems generating free radicals in living cells, due to their lower redox potentials; thus, forming less reactive flavonoid-containing radicals comparing with initial highly oxidizing free radicals. The latter mechanism of biological activity has been assigned to beneficial function of flavonoids regarding process of lipid peroxidation leading to a damage of cellular membranes and to cell death. Flavonoids interact with nitric oxide, thus preventing its bonding to superoxide free radicals, in producing highly damaging peroxynitrite. Also, they are involved into Fenton reaction. Despite, research effort devoted to metabolism of dietary FLs are scarce and frequently contradictory, but very relevant to their beneficial effects on human health. Many molecular level mechanisms involving flavonoid-X-glucosides (X = O or C atoms) or corresponding aglycones are objects of current disputes.

Owing to a widespread distribution of flavonoid-X-glucosides in plants, including eligible ones and their implementation into human diet and health they are objects to a significant increasing attention looking at enormous research effort concentrated on their mass spectrometric qualitative, quantitative and structural analyses, respectively.

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The employment of MS methods is chiefly based on their superior instrumental features determine a great, irreplaceable and indispensable application to the analytical practice, generally. The complementary application of experimental MS databases, statistical approaches and chemometrics has yielded to a crucial impact of mass spectrometry on metabolomics, plant-omics, lipidomics, proteomics, peptidomics, macromolecular conjugates and biopharmaceuticals, microbial screening, genomics, food omics, metallomics and many more fields of the *analytical research*.

Also, MS represents a method of choice for studying noncovalent interactions **(NCI)**, including those among flavonoids, flavonoid-X-glycosides and proteins; or, in general, among small biologically active compounds and biologically active macromolecules. The NCI play a crucial role in understanding of cellular processes in living systems. Thus, MS methods become methods of choice for purposes of *structural biology*, as well.

We stress on soft ionization MS instrumentation, in particular, focusing the reader's attention on *electrospray ionization* **(ESI)** *mass spectrometry* as a soft- ionization method, among others. It is capable of transferring analytes from solution to a so-called *gas-phase* (or continuum,) without to change 3D molecular conformation of analytes ion in solution, even talking about complexes having millimolar dissociation constants. Therefore, ESI-MS is capable of examining precisely interacting molecular ensembles by an experimental design of conditions of measurements ensuring a mimic different physiological condition. The great applicability of ESI-MS method to determine K_d constants of flavonoids to cytochrome-c has been presented [2]. However, regarding, content of the current contribution and its major goal, we should emphasise, that there have been obtained high standard deviations (sd(yEr±) = 0.025–0.24) of K_d data, which might be a result from employment of I^{tot} values in determining K_d data, as the authors have highlighted, explicitly. Despite, MS becomes primary a tool of qualitative, quantitative and structural analyses of NPs, emphasizing as well as mechanisms of chemical reactions, which are closely connected with molecular/ionic covalent and NCI and chemical bonding, in a general context.

The issue of *MS method performances* is of significant importance from the perspective of quantitative treatment of chemical interactions not only within the framework of our SD theory, but also from perspective of available *kinetics, thermodynamics* and *diffusion theories* and model formulas for data-processing of measurable variables by another authors. *The known methods for data-processing of measurable variables predict intensity profiles by means of statistical analysis of a large set of spectrometric databases, thus deducing phenomenological relations of intensity of analyte ions* [3–9].

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So far, there are few attempts to predict relative intensity values of experimentally observable m/z peaks, which are limited to a comparative analysis of probability of retaining perturbation or change of fragment species under collision induced dissociation **(CID)** MS operation mode. Due to, *fluctuation* of experimental intensity values of MS peaks, however, such predictions are highly approximative. Furthermore, there is a drawback of statistical approaches, due to the fact that they approximate that major ions in theoretical MS spectrum have equal intensity values regardless of molecular and physical properties of analytes or experimental conditions of measurements. In other words, the statistical approaches discussed lastly not only do not account for experimental *fluctuations* of measurable variables, but also even do not consider them, essentially. Many statistical tools use data on m/z-values. The data on intensity is rarely accounted for available statistical algorithms. The major reason for this drawback is that currently available quantitative methods unable to predict and quantify data on MS intensity on chemical fragment reactions. The *fluctuations* of measurable variable *intensity* are unable to be neglected neither qualitatively, nor quantitatively, however. **The issue is of particular importance studying biological samples, where matrix effects are notably detected and shifting of baseline is encountered**. **The long-term reliability of analytical data can be altered during a long time of operation.** Despite, there have been developed methods of *external calibration*, *internal standard* or *standard addition*, respectively. Thus, the instrument drift can hardly be corrected by them. The method of external calibration could be used to solve the latter problem, but it is laborious. The *isotope labeling of internal standards* is another approach, among others, widely used to correct variation of measurable outcome, due to differences in composition of analyte and instrument drift. However, there is a lack of available isotope labeled compounds for all analytes. Despite, theoretically both the shift of m/z-values and intensity fluctuation can be reduced by means of calibration method, thus allowing a reliable analysis with significantly improved analytical method performances. However, in the context of this paragraph of the introductory section it becomes clear that employment of statistical approaches to determine quantitatively analytes in mixture using MS databases has rather deductive capability. This strategy does not provide unambiguous and exact determination neither qualitatively nor quantitatively or structurally about analytes.

Given that, there are reasons to think that methodological developments of quantitative MS methods capable of determining unambiguously and exactly analytes, in particular, highlighting methods having predictive capability contribute crucially not only to develop mass spectrometry as a robust and irreplaceable analytical method, but also to develop aforementioned interdisciplinary areas of research using broadly mass spectrometry to analyze qualitatively, quantitatively and molecular structurally analytes, in addition to determine experimentally molecular properties, thermodynamics, kinetics or diffusion parameters of their ion/molecular interactions and reactions, respectively.

The study concentrates on development of our innovative general *stochastic dynamic theory* and model **formulas (1)** and **(2)** analyzing exactly fluctuations of measurable variables, in particular looking at outcome *intensity of the peaks of analyte ions* from the point of view of chemometrics, thus revealing the actual capability of the MS methods as highly accurate, precise, selective and sensitive analytical instrumental methods for qualitative, quantitative and structural analysis, respectively. Also, it concentrates on application of our theory to 3D structural determination of very complex analytical object from the perspective of molecular conformation and subtle electronic effects (below.) Therefore, there lies a major question: What yields to exact possible formulas of laws, which the temporal behavior of mass spectrometric variable *intensity* obeys within the framework of our theory and model equations?

$$D_{SD}^{tot} = \sum_{i}^{n} D_{SD}^{i} = \sum_{i}^{n} \left(1.3194.10^{-17}.A^{i}.\frac{\overline{I_i^2} - \left(\overline{I_i}\right)^2}{\left(I_i - \overline{I_i}\right)^2} \right)$$

Eq.1

Equations (1) and **(2)** do tell us that measurable outcome *intensity* is treated as a stochastic variable with its average value *per* span of scan time, instead of over the whole time of a measurement as an average quantity.

$$D_{SD}^{''} = 2.6388 \times 10^{-17} \times \left(\overline{I^2} - \left(\overline{I}\right)^2 \right)$$

Eq.2

('A^i' denotes statistical parameter obtained as a result from SineSqr approximation of relation between experimental intensity data and scan time of measurements $(I-\langle I\rangle)^2 = f(t)$, while 'I' denotes absolute intensity value.)

Within the framework of this theory there are excellent to exact ($|r| = 1$) chemometrics correlating linearly between theory and experiment over a statistically representative set of organic, inorganic and metal-organic analytes, respectively. Moreover, there has been found that D_{SD} data correlate linearly with so-called *quantum chemical diffusion parameter* (D_{QC}) according to Arrhenius's theory (**equation (3)**) Therefore, **equation (1)** establishes a direct relation among MS outcome *intensity*, measurable parameters and factors, 3D molecular conformations and electronic structures of analytes, respectively [10–21].

$$D_{QC} = \underbrace{\left[\frac{\prod_{i=1}^{3N} \nu_i^{(0)}}{\prod_{i=1}^{3N-1} \nu_i^{(s)}} \right]}_{\Delta S^{\#}} \times e^{-\frac{\Delta H^{\#}}{R \times T}}$$

Eq.3

('R' denotes universal gas constant (R = 8.314463 $kg.m^2.K^{-1}.mol^{-1}.s^{-2}$); T means temperature; *enthalpy* ($\Delta H^{\#}$) and *entropy* ($\Delta S^{\#}$), $\nu_i^{(0)}$ and $\nu_i^{(s)}$ molecular vibrations at ground and transition states. The $\Delta S^{\#}$ is written in harmonic approximation.)

Formula (1) not only provides accurate, precise, selective and sensitive quantification of experimental variables, but also is applicable to exact MS based 3D structural analysis. This fact extends significantly the capability of mass spectrometry, far beyond its routine application to the qualitative and quantitative analytical chemistry. Chemometric analysis of mass spectrometric, single crystal X-ray diffraction and quantum chemical data on 3D structures of a set of model compounds has shown $|r| = 0.9557_5–0.9998_1$ examining D_{QC} and D_{SD} parameters [21]. **Equation (2)** is derived from **equation (1)** [18,19].

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Results and discussion

1. Mass spectrometric analysis of flavonoids

1.1. Figure of merits

We have chosen to begin presentation of new MS results from analysis of apigenin-O-glucoside (1) (see the chemical diagram depicted in the graphical abstract) with an expression of *figure-of-merits*. The focus of a reader's attention is on chemometrics of uncertainty of MS measurements, owing to the fact that the major goal of this study is to show validity, empirical testability and applicability of our new SD formulas to the analytical practice. It seems to us persuasively to illustrate its great advantages for quantifying MS variables, in particular, looking at absolute intensity data on observable peaks of analyte ions.

The results from measurements correspond to mean values, which should be presented with *uncertainty statements*, in order to do claim of precision, accuracy, selectively and sensitivity of any theory used to quantify MS variables. The *standard uncertainty* is expressed *via* the so-called *standard deviation* within the framework of observations under repeatability conditions [1]. The analysis involves estimation of two types of uncertainty (types A and B,) thus accounting for *systematic* — the total systematic error is called *bias* — and *random errors* of measurements. This manner of structuring of experimental and theoretical outcomes would seem to be most apparent in claims of applicability, advantages and limitations of our theory comparing with known MS methods for quantitative, semi-quantitative and structural analyses, respectively. It follows, generally, the guiding principle of analytical quantitative studies, that *the results are quantitative, only when there is assessed corresponding error contributions to them*. In other words, this sub-section attempts to answer the following question: How can we be sure that our results are *exact*? The answer to such question is simply that: *If the experimental variables show standard deviation sd(yEr±) = 0, then this means that they are absolute or exact*.

Figure 1 illustrates experimental ESI(+)-CID-MS spectra of analyte (1) in solution. **Figures 2–5** and tables — for instance, **Table 1** — depict statistical analysis and variables. A large number of statistical methods are applicable to normally distributed variables. The question which should be also addressed is: How can we determine the *normality* of measurable variables? We use Shapiro-Wilk test and W-statistics. It has the best power, capable of evaluating normality of random variables. Large W-value means a normal distribution of a set of variables. The results show that the outcome does not follow a normal distribution. Despite, Shapiro-Wilk test rejects hypothesis about a normal distribution. However, it cannot confirm it.

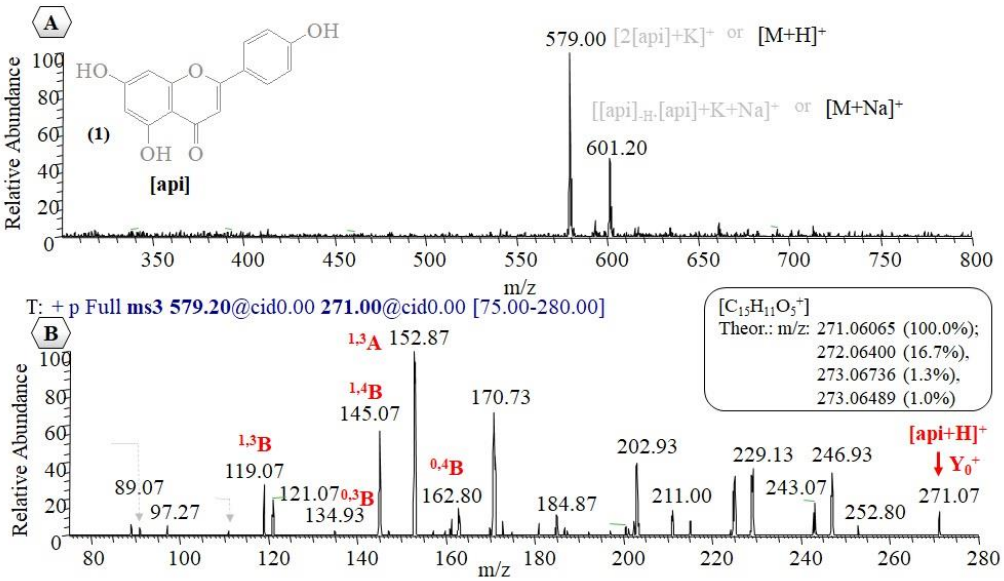


Figure 1. ESI(+)-CID-MS (A) and CID-MS^3 (B) spectra of analyte (1); chemical diagram of the flavonoid apigenine [api]; theoretical m/z data and isotope intensity ratios; assignment of MS peaks of analyte ions

The probability distribution of measurable variables and location of mean values of m/z-data are illustrated graphically as well as using probability-histograms (**Figure 4**.) There is used statistical t- and F-tests supposing a normal distribution of datasets looking at distributions of m/z-variables. As can be seen from tabulated data the decision rule states that there is a lack of significant difference among m/z-values. The chemometrics determine the justification used to this study, that experimental results from different sets of *m/z*-values *per* span of scan time belong to same fragment ions, despite, different measurements, concentration levels of analytes in solution and CID-MS^n operation modes, respectively. Thus, we argue plausibly that D_{SD} data according to **equations (1) and (2)** used *per* span of scan time and within different tandem MS operation modes reflect same molecular ions.

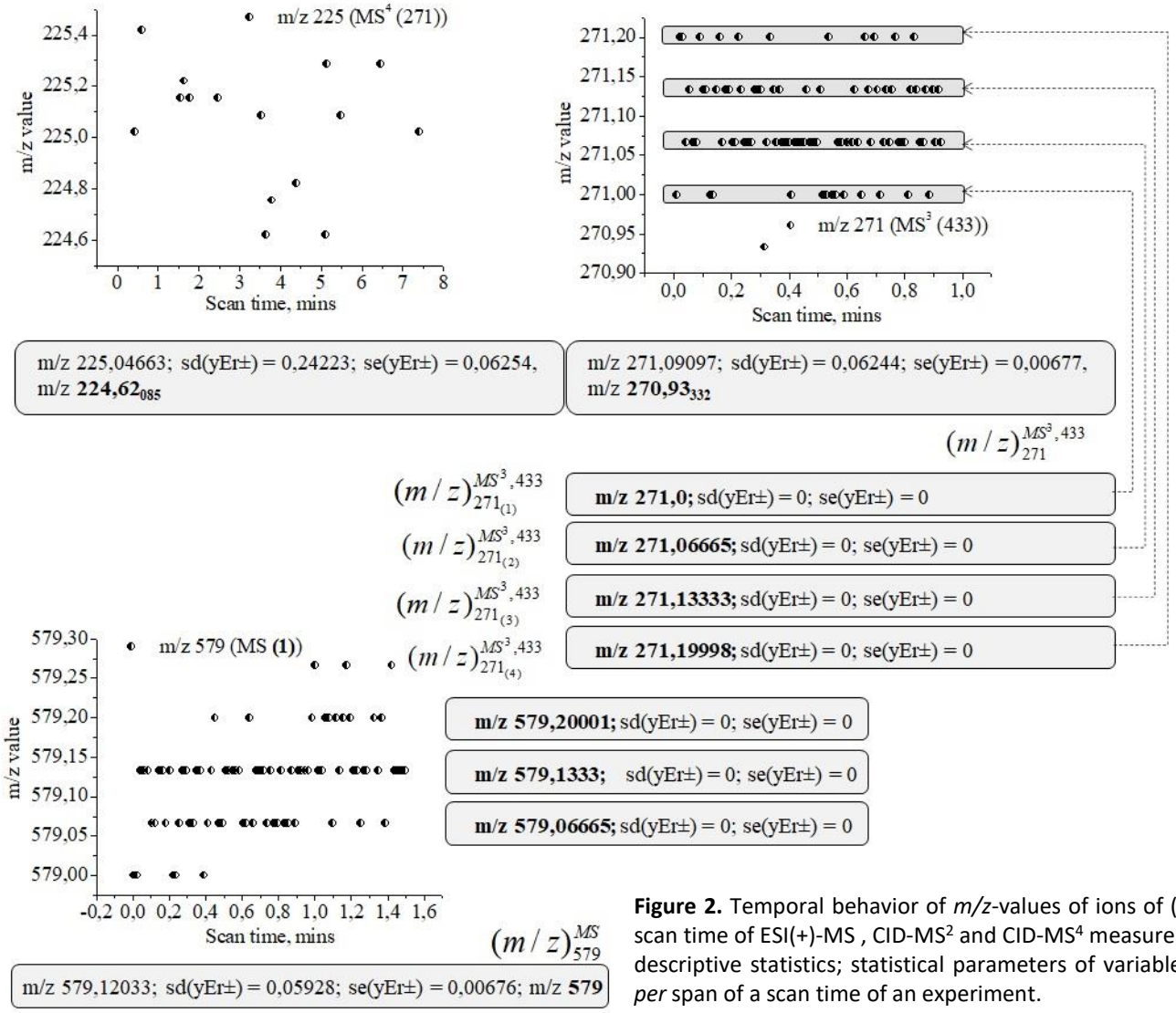


Figure 2. Temporal behavior of *m/z*-values of ions of (1) at *m/z* 271 and 579 with respect to scan time of ESI(+)-MS , CID-MS^2 and CID-MS^4 measurements of ions at m/z 579, 433 and 271; descriptive statistics; statistical parameters of variables over whole time of experiment and *per* span of a scan time of an experiment.

Coming to a broad and concrete application of our SD equations to the analytical practice, we should stress, that according to chemometrics (**Figure 5**), the two sets of m/z data on ions at m/z 91, 111, 153, 163, 171, 203, 225, 229, 243 and 247 (**Figure 1**) obtained as result from CID-MS^3 and CID-MS^4 measurements of ion of (1) at m/z 271 lack of differences ($|r| = 0.9999_9$.) Therefore, an obvious advantage of SD **equations (1) and (2)**, among other, is that the two of them give an exact quantification of the variable MS intensity.

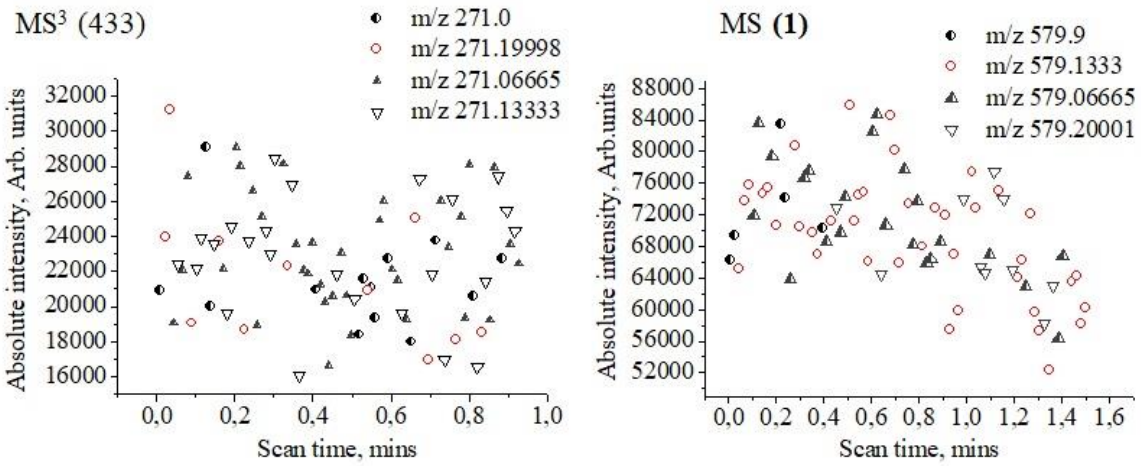


Figure 3. Temporal behavior of absolute values of MS intensity of analyte ions at *m/z* 579 and 271 observed in mass spectra under single and tandem MS^3 operation modes with respect to scan time of measurements; CID-MS^3 operation mode is applied to ion at m/z 433.

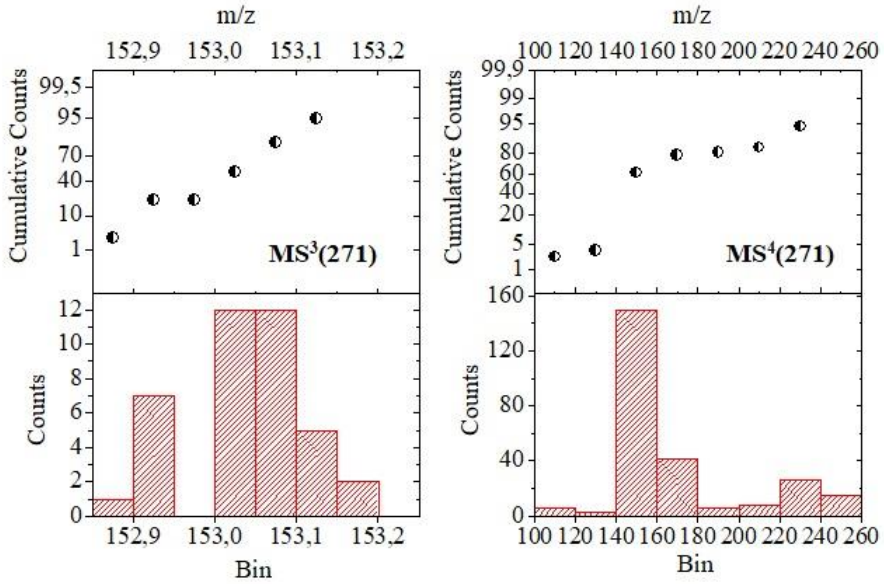
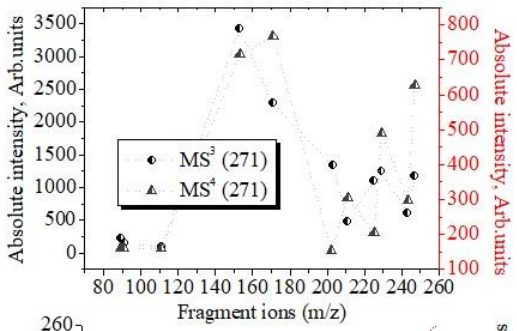
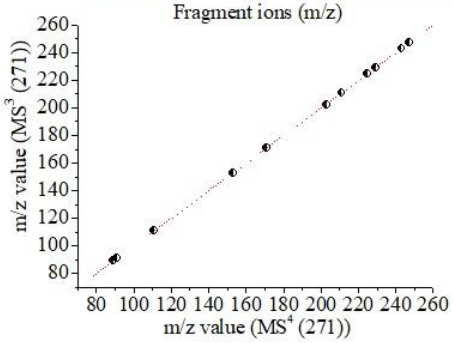
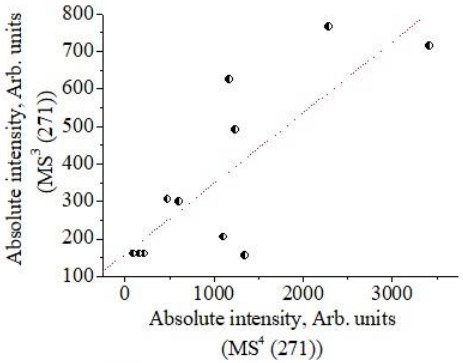


Figure 4. Histograms and probability distribution of *m/z*-values of MS ions of (1) with respect to different experimental CID-MS^n conditions (n = 3 and 4) applied to ions at m/z 271.







	Mean	Error	
A	0,05396	0,23679	
B	0,99957	0,00126	
r	SD	N	*p*
0,99999	0,23907	11	<0.0001

	Mean	Error	
A	159,44743	69,80831	
B	0,18869	0,04772	
r	SD	N	*p*
0,79667	152,29458	11	0,00333

Figure 5. Correlative analysis between absolute intensity values [arb.units] of analyte ions with respect to CID-MS^3 and CID-MS^4 experiments of ion at m/z 271; and between m/z-data of these species under the same experimental conditions; chemometrics.

Table 1. One way ANOVA chemometrics of MS variables of ions at *m/z* 271.0 and 271.19998 of (1) under CID MS^3 experiment of ion at m/z 433; power test

One way ANOVA test (*m/z* 309)					
Dataset	N	Mean	sd(yEr±)	se(yEr±)	
MS^3 (433)	11	271.19998_{1689}	0	0	
MS^3 (433)	13	271	0	0	
H_0: The means of all selected datasets are equal					
H_1: The means of one or more selected datasets are different					
Source	DoF	SS	MS$^{\#}$	F-value	P-value
Model	1	0.23828_{9695}	0.23828_{9695}	0	0
Error	22	0	0		
At the 0.001 level, the population means are significantly different.					
α		Sample size			Power
1.10^{-3}		24			$9.999.10^{-4}$

1.2. Mass spectrometric fragmentation paths of apigenin-O-glucoside under collision induced dissociation operation modes

Fragment processes under CID-MS^n conditions have been very successfully used to 2D structural analysis of flavonoids, including, their glycosides [2–9]. Frequently, a MS analysis involves at least MS^3 operation mode in order to identify aglycone residues. The first steps (MS and MS^2 modes) chiefly affect on labile glycosidic bonds, thus, producing a free aglycone ion. Despite, data on CID-MS^2 process show ion at m/z 271 (Y_0^+,) leading to a series of product species of API. The fragment paths of aglycone cation ($[M+H]^+$) provide very information about flavonoid identification in NPs, in particular, studying isomeric aglycones, which are often found in plant extracts [22–29]. For this reason, we concentrate on quantitative and 3D structural analyses of flavonoid residue of analyte (1). The protonated API corresponds to Y_0^+ peak. (The MS fragment ions of flavonoid-O-glycosides are assigned according to the nomenclature by Domon and Costello; thus, we use it, herein, as well.) The Y_0^+ ion is produced as a result from a cleavage of O–C bonds connecting between flavonoids and carbohydrate residues; and between monosaccharides and glycoside fragment leading to Y_0^+ and Y_1^+ ions as products, indicating presence of a carbohydrate connected with phenolic hydroxyl group of flavonoids. The MS peak at m/z 433 of (1) is assigned to Y_1^+.

The MS spectra of flavonoids often exhibit peaks of ionic adducts of alkali metal or ammonium cations under ESI(+) and ESI(-) operation modes. For this reason, our theoretical analysis includes energetics of species producing peaks at m/z 579 and 433 not only detailing on protonated flavonoid-O-glycoside molecular skeleton and its fragment ions, but also molecular/ion interacting adducts of alkali metal ions of dimers of API species (not shown, herein, but detailed on [1].)

To seek an adequate assignment of MS peaks of (1) there are needed employment of a set of experimental and theoretical methods, thus avoiding confusion between observable phenomena and their explanation, due to complexity of analytical object from perspective of 3D molecular conformation, tautomerism, inter- and intramolecular PT and CT-effects, as well as a great competition among proton accepting and coordinating centres toward alkali metal ions of the molecule of (1).

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Despite, the assignment of fragment ions of API in **Figures 6** and **7**, we should stress that very frequently it appears inadequate to fully and completely account for observable empirically MS phenomena of flavonoid-X-glycosides (X = O or C.) The assignment of species of (1) cannot be defined unambiguously by means of known statistical approaches to determine fragment paths, due to reasons discussed in preceding sub-sections. Therefore, an assignment of fragment ions according to these figures remains to a large extend probabilistic. Nevertheless, enormous effort devoted to MS analysis of *naturally occurring products* (NPs) containing flavonoids, might be said to be bundle into very important basic knowledge allowing for us to precise strictly fragment paths, 3D molecular and electronic structures of fragment species within the framework of our SD model formulas by means of a complementary application of MS and quantum chemical data. Since, common fragment paths of flavonoids under MS experimental conditions might not be so salient to the reader, chemical diagram of species of differently substituted flavonoids is presented, as well. Frequently, MS reactions of aglycone fragment of flavonoid-O-glycosides are characterized by neutral loss of CO and CO_2 molecules. There are reactions exhibiting a loss of $\bullet CH_3$ radical, as well. The comparative analysis between fragment paths of API under negative ESI(-)MS^n operation mode according to [30] and our data on positive ESI(+)-CID-MS^n operation mode of the same aglycone shows a set of common reactions. These are: m/z 269 ([api-H]$^-$), 241 ([api-H-CO]$^-$), 227 ([api-H-C_2H_4O]$^-$), 225 ([api-H-CO_2]$^-$), 201 ([api-H-C_3O_2]$^-$), 183 ([api-H-C_2H_2O-CO_2]$^-$), 181 ([api-H_{-2}.CO_2]$^-$), 159 ([api-H-C_3O_2-C_2H_2O]$^-$), 151 (^{1,3}A$^-$), 149 (^{1,4}B$^-$), 107 ([(^{1,3}A$^-$)-CO_2]$^-$) and 117 (^{1,3}B$^-$,) respectively. The assignment of the same type of fragment ions in our case are shown in **Figure 1**. **Figure 6** depicts differences in fragment reactions between ESI(-)-CID-MS^n and ESI-CID-(+)-MS^n operation modes of API, detailed, herein. Looking at retro Diels-Alder fragment reaction of ^{1,3}A$^-$ ion of API under ESI(-)-CID-MS^n conditions (m/z 151) which further produces a fragment species at m/z 107 of ([(^{1,3}A$^-$)-CO_2]$^-$), we might underline that under positive operation mode API exhibits a relative abundance MS peak at m/z 153 of ^{1,3}A$^+$ cation. To [(^{1,3}A$^+$)-CO_2]$^+$ cation there is assigned MS peak at m/z 109.06. However, as can be expected and as work [30] has underlined explicitly, the molecular structures of pairs of ^{1,3}A$^+$/^{1,3}A$^-$ and [(^{1,3}A$^-$)-CO_2]$^-$/[(^{1,3}A$^+$)-CO_2]$^+$ ions are different. The 3D molecular conformations and electronic structures as well as proton accepting capability of ^{1,3}A$^+$ and [(^{1,3}A$^+$)-CO_2]$^+$ cations are detailed in work [1].

[30] Fabre, N.; Rustan, I.; De Hoffmann, E.; Quetin-Leclercq, J. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 707–715.

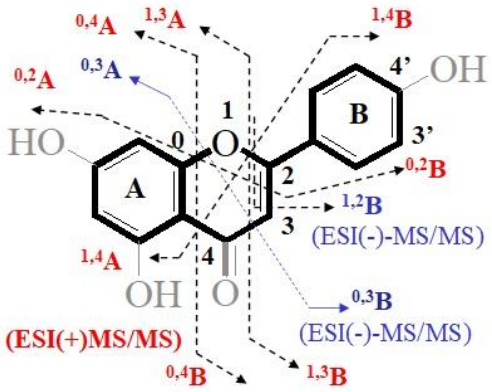


Figure 6. Chemical diagram of apigenin; fragment paths of API under ESI(+)- and ESI(-)-MS operation modes.

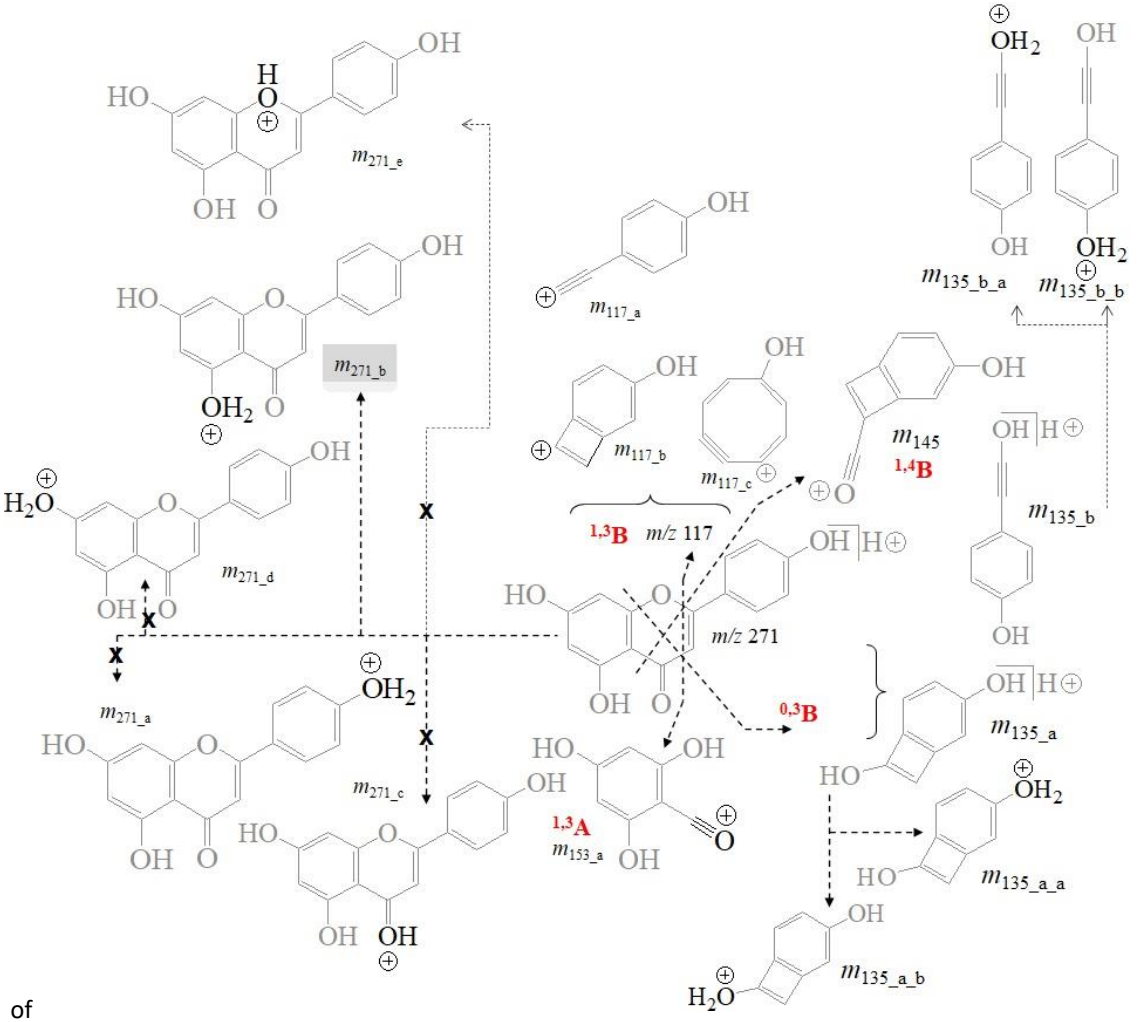


Figure 7. Chemical diagrams of fragment species of apigenin accounting for their different proton accepting capability; the most stable cations are obtained on the base on theoretical thermodynamics.

1.3. Mass spectrometric stochastic dynamic diffusion method and its application to study quantitatively apigenin

Within the framework of our *stochastic dynamic* theory [10–21] and model **equations (1)** and **(2)** there are examined quantitatively data on absolute intensity and TIC of fragment species of apigenin-O-glucoside (**Figure 8.)** In total experimental measurable variables of eighty-five fragment ions of analyte are quantified by means of chemometrics [1]. The absolute values of MS intensity with respect to different spans of scan time of measurements under single and tandem MS^n operation modes are tabulated [1]. The nonlinear approximation to experimental relationship $(I-\langle I\rangle)^2 = f(t)$ by means of SineSqr function in order to obtain values of statistical parameter A_i and D_{SD} data according to equation (1); as well as generation of random parameters in order to obtain lnP1 values according to the general SD concept behind formulas (1) and (2) [17] leads to lnP1 = 17.053 (**Table 2** and [1]), which appears same quantity despite analytes [10–21]. It has been stressed in many of our contributions devoted to develop SD theory that the temporal behavior of the variable *intensity* of a mass spectrometric experiment obeys a certain law and this law is namely equation (1). Thus, the results from the analysis of API presented for first time in the literature provides new pro-argument of this statement. The latter tables listed chemometric parameters according to the SD theory, in addition to, experimental diffusion D_{SD} and D_{SD}'' data according equations (1) and (2) examined in this chapter, as well. Statistical data on SineSqr fitting of relationship $(I-\langle I\rangle)^2 = f(t)$ depending on complexity of temporal behavior of experimental outcome accounting for absolute intensity values of analyte MS peaks and number of points *per* span of scan time are depicted in **Figure 9**. Due to complexity of MS isotope shape of experimental spectra of API there has been used as well as curve-fitting approach to MS ions at m/z 119, 121, 163 and 579, respectively [1].

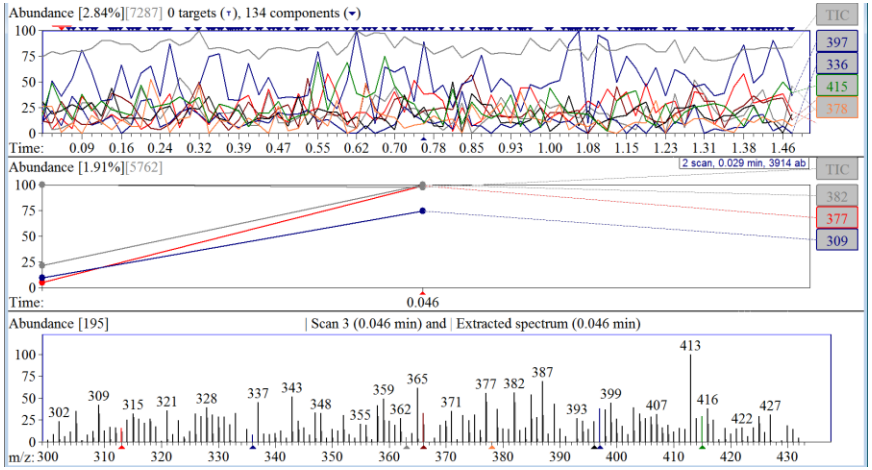
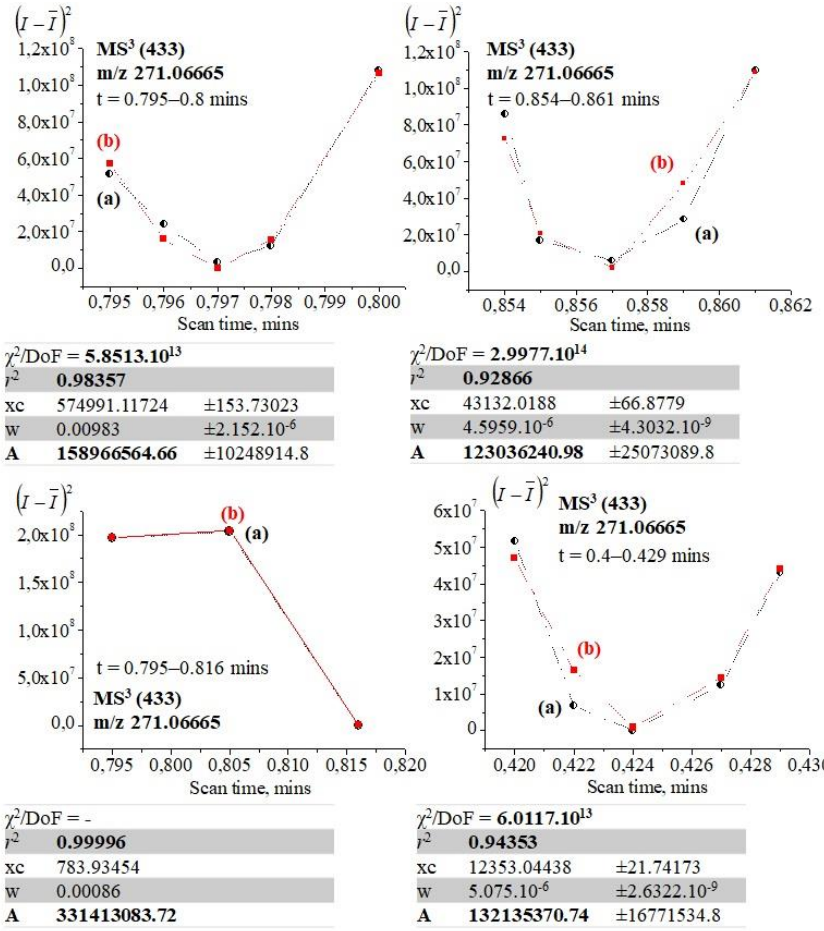


Figure 8. Absolute experimental intensity [arb. units] of analyte MS ions of (1) *versus* scan time [mins]; TIC *versus* time [mins] of MS and CID-MS^2 spectra of (1).

Table 2. Experimental CID–MS^3 parameters of MS peak at m/z 271.13333 of (1) at applied collision at ion at m/z 433; σ^2 and σ'^2 – variance parameters; P1 – parameters according to [10–21]; D_{SD} – diffusion parameter according to **equation (1)**; D''_{SD} – diffusion parameter according to **equation (2)**; scan time (t) [mins]

t [mins]	0.109–0.132
<I>	132816.33_3
$<(I)>^2$	17640178400.11_1
$<(I)^2>$	17640178591
σ^2	190.8889
$<(I-<(I)>)^2>$	190.8881_5
lnP1	17.05313_{98}
D_{SD}	$8.24403901083822.10^{-15}$
D''_{SD}	$5.0371760.10^{-15}$



χ^2/DoF = **5.8513.10^{13}**

r^2	**0.98357**	
xc	574991.11724	±153.73023
w	0.00983	$\pm 2.152.10^{-6}$
A	**158966564.66**	±10248914.8

χ^2/DoF = **2.9977.10^{14}**

r^2	**0.92866**	
xc	43132.0188	±66.8779
w	$4.5959.10^{-6}$	$\pm 4.3032.10^{-9}$
A	**123036240.98**	±25073089.8

χ^2/DoF = -

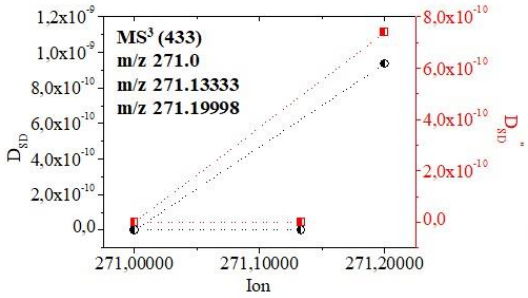
r^2	**0.99996**	
xc	783.93454	
w	0.00086	
A	**331413083.72**	

χ^2/DoF = **6.0117.10^{13}**

r^2	**0.94353**	
xc	12353.04438	±21.74173
w	$5.075.10^{-6}$	$\pm 2.6322.10^{-9}$
A	**132135370.74**	±16771534.8

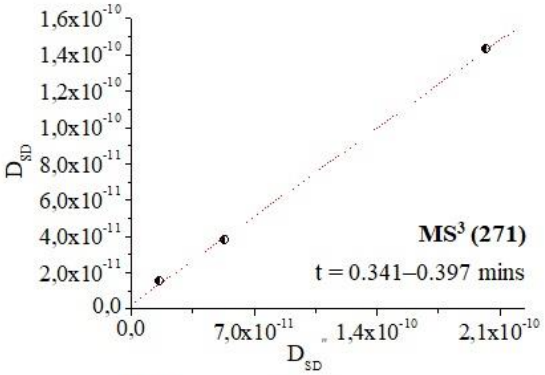
Figure 9. ESI-MS experimental $(I-<I>)^2 = f(t)$ relation (a) and approximation to SineSqr function (b) of MS intensity values *per* span of scan time $(I-<I>)^2$ *versus* scan time [mins] of ions of (1) at m/z 271.06665; chemometrics.

Figure 10 argues that linear correlation between D_{SD} and D_{SD}'' data according to equations (1) and (2) is warranted as far as there is |r|=1. There have been examined quantitatively diffusion results from ions at m/z 271, 271.1333 and 271.19998 obtained under CID-MS^3 experiment applied to Y_1^+-ion. The same is true looking at correlation between the discussed diffusion parameters, however, obtained within the framework of different CID-MS^3 and CID-MS^4 operation modes applied to ion at m/z 271 (**Figure 11**.) There are obtained |r|=0,9997–0.99929. As the latter figure also illustrates excellent-to-exact chemometric method performances are obtained over differents spans of scan time. Looking at data on MS ion of (1) at m/z 336, 366, 378 and 396 [1], it is further argued that claim about that D_{SD} and D_{SD}'' data from basic equation (1) and those obtained within the framework of simplistic model formulas (2) is warranted (|r|=99961.) It can give a probabilistic support of latter view, as far as, there are examined species having very love abundance (consider the intensity data on [1].) As can be seen, even employment of lowest intensity value I = 66 arb. units of ion at m/z 378 does not affect, in fact, on method performances, which are characterized by the lastly written excellent coefficient of linear correlation |r|. A particularly important result illustrates **Figure 12** showing a correlative analysis between D_{SD}'' data on ions at m/z 271 and 579. There is a linear correlation exhibiting |r|=99185.

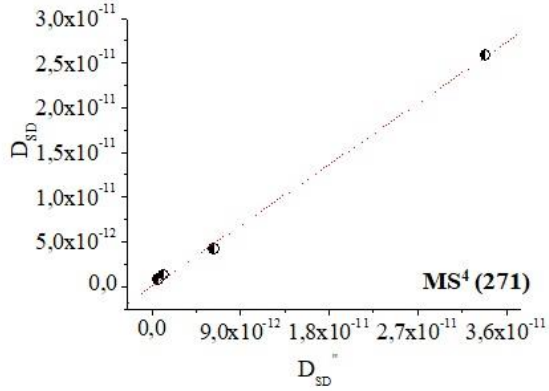


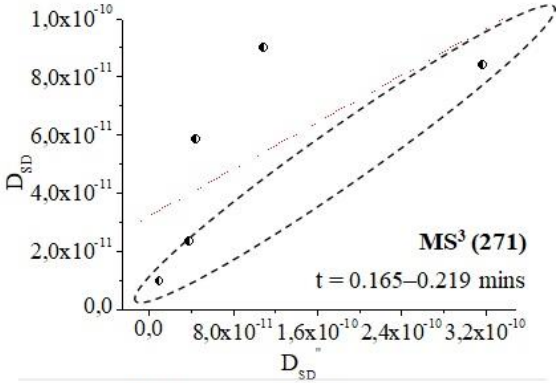
	Mean	Error	
A	$4{,}58202.10^{-14}$	$1{,}9377.10^{-15}$	
B	0,79047	$3{,}5878.10^{-6}$	
r	SD	N	*p*
1	$2.74.10^{-15}$	3	0,10449

Figure 10. Relation between D_{SD} and D_{SD}'' data on ions at m/z 271.0, 271.1333 and 271.19998 obtained under CID-MS^3 process of Y_1^+ ion; chemometrics.



	Mean	Error	
A	$2{,}8125.10^{-12}$	$2{,}0377.10^{-12}$	
B	0,69177	0,01681	
r	SD	N	*p*
0,9997	$2{,}3432.10^{-12}$	3	0,51158



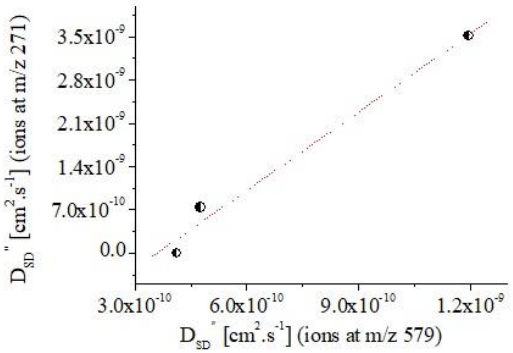


	Mean	Error	
A	$3{,}213.10^{-11}$	$1{,}8052.10^{-11}$	
B	0,20173	0,11857	
r	SD	N	*p*
0,70076	$2{,}954.10^{-11}$	5	0,6498

	Mean	Error	
A	$4{,}5842.10^{-12}$	$1{,}354.10^{-11}$	
B	0,80704	0,21781	
r	SD	N	*p*
0,93426	$1{,}58.10^{-11}$	4	0,6168

	Mean	Error	
A	$2{,}4.10^{-14}$	$3{,}4.10^{-13}$	
B	0,75791	0,02017	
r	SD	N	*p*
0,99929	$5{,}5.10^{-13}$	4	0,85503

Figure 11. Relations between D_{SD} and D_{SD}'' data on ions at m/z 119, 145, 153, 171 and 203 obtained under CID-MS^3 and CID-MS^4 experiments of ion at m/z 271; chemometrics.



	Value	Error	
A	$-1.515.10^{-9}$	$4.23.10^{-10}$	
B	4.22267	0.54255	
r	SD	N	*p*
0.99185	$3.34.10^{-10}$	3	0.08135

Figure 12. Relation between D_{SD} and D_{SD}'' data on ions at m/z 579.0, 579.1333 and 579.06665, respectively; chemometrics.

Figures 10–12 show that not only equation (2) as references [18,19] have proven, but also equation (1) are highly reliable formulas for exact analysis of analyte concentration in solution by ESI-MS^n method leading to plausible results.

It, herein, has been argued that this is namely so. At this point we should mention that report [31] has made attempt to correlate MS intensity values with respect to concentration of analytes in solution, however, achieving a significantly lower chemometric method performances comparing with superior data reported to reserpine and steroids according to our model **formulas (1)** and **(2)**. Work [31] shows coefficients of linear correlation |r| = 0.9944–0.9976. The data on quantification of analytes in solution *via* Taylor's dispersion approach to MS variables has been detailed on [32,33]. It treats diffusion process under ESI-MS experiments, as well as, describing convective and diffusive transport phenomena [33]. The approximation of the system to a laminar flow, leads to a complex mathematical solution and model equation (see detail on [1]) connecting between analyte concentration and diffusion parameter. Its application to ESI-MS data, however, has shown high error contribution to mean value; or great standard deviation or uncertainty. There is obtained diffusion value $11.9\pm1.0.10^{-10}$ $cm^2.s^{-1}$ [33]. Furthermore, there is a lack of linear relation between analyte concentration in solution and experimental measurable intensity data on MS ions.

[31] Yan, X.; Li,X.; Zhang, C.; Xu, Y.; Cooks, R. *J. Am. Soc. Mass Spectrom*. **2017**, *28*, 1175–1181.
[32] Taylor, G. *Royal Soc. London A*, **1953**, *219*, 186.
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2. Theoretical quantum chemical data

2.1. A brief overview

The experimental chemical science currently routinely employs the great capability of methods of computational quantum chemistry of providing highly accurate-to-exact information about reaction kinetics, thermodynamics or diffusion as well as 3D molecular conformation and electronic structures of molecules. This fact stems from the significant predictive capability of the computational quantum chemistry of molecular and electronic structures within the framework of *ab initio* methods where there is a lack of experimental and semi-empirical data used to compute highly accurately multidimensional structure and chemical reactivity of molecules. Since, the functional relationships among 3D molecular and electronic structures, chemical reactivity, thermodynamics stability, experimental factors and parameters of chemical reactions, in general, become far more complicated, due to complex physical interactions among nuclei and electrons of molecules and environmental parameters, the observable phenomena relating chemistry of matter are fully described when there is a complementary employment of experiment and theory in order to gain in-depth understanding of these relationships.

The computational quantum chemistry is broadly used to study MS phenomena, as well [34–39]. Despite, the most recent contributions to this field [39], however, no version of theory outside the theoretical framework of our SD concept, developed, so far, to predict and quantify accurately experimental variable '*intensity*' due to reasons sketched above (see, for instance, some of the most recent developments [39].) The same is true for all known theories that say that thermodynamics, kinetics and diffusion parameters can be obtained mass spectrometrically by means of I^{tot}-values. That is why our innovative formulas differ significantly from all known model equations used to quantify MS outcome '*intensity*'.

Throughout this work, in general; and this sub-section, in particular, we refer, namely, to great impact of the quantum chemistry on development of methods of mass spectrometry not only as robust analytical instrumentation for purposes of the quantitative chemical analysis, but also on its great capability of exact determining of 3D molecular and electronic structures of analytes; furthermore, accounting for subtle electronic effects of tautomers, proton and CT-effects, from a practical point of view.

[34] Grimme, S. *Angew. Chem. Int. Ed.* **2013**, *52*, 6306–6312.
[35] Bauer, C.; Grimme, S. *J. Phys. Chem. A* **2016**, *120*, 3755–3766.
[36] Cautereels, J.; Claeys, M.; Geldof, D.; Blockhuys, F. *J. Mass. Spectrom*. **2016**, *51*, 602–614.
[37] Bauer, C.; Grimme, S. *J. Phys. Chem. A* **2014**, *118*, 11479–1148.
[38] Song, K.; Spezia, R. *Theoretical mass spectrometry*, Walter de Gruyter GmbH, 2018, Berlin/Boston, pp. 1–229.
[39] Schreckenbach, S.; Anderson, J.; Koopman, J.; Grimme, S.; Simpson, M.; Jobst, K. *J. Am. Soc. Mass Spectrom.* **2021**, [https://doi.org/10.1021/jasms.1c00078].

2.2. Correlative analysis between theoretical quantum chemical diffusion data and experimental stochastic dynamic diffusion parameters

As we have observed, MS spectra of (1) exhibit a set of m/z values belonging to MS ions at m/z 579 and 271 (**Figures 1** and **2**.) These are : m/z 579.0; 579,1333, 579.06665, 579.20001, 271.0, 271.06665, 271.1333 and 271.19998, respectively. Yet, the results raise very important questions whether: (a) The MS peaks at m/z 579 and 271 correspond to only two analyte ions having unique 3D conformations and electronic structures; (b) the values belong to different analytes in context molecular structure; or (c) these quantities belong to different tautomers of two molecular ions, meaning that under certain experimental conditions the perturbation of electronic structures must exist; and its becomes measurable quantitatively by means of mass spectrometry? The latter question (c) suggests that soft-ionization MS methods provide not only a faithful measurement from perspective of chemometric, but also is capable of accounting for subtle electronic effects. Therefore, *via* our SD theory the MS capability is extended crucially to a highly precise quantification of measurable variables at five decimal signs as our chemometric results highlight, but also determination of subtle electronic effects and electronic structures within the framework of given molecular 3D conformation. Nonetheless, even if the answer to the later question (c) is positive, then there arises an additional question: (d) What exactly is the connection between pairs of species having identical m/z-values at the five-decimal sign? In other words, there lies a question: Do the pairs of species showing m/z 271/579, 271.1333/579.1333 and 271.06665/579.06665 are mutually connected with each other within the framework of identical ion/molecular interaction schemes or chemical reactions owing to the fact that there is excellent linear correlation between the D_{SD}''-data of these ions according to equation (2)? Thus, we must distinguish among different m/z data-sets belonging to characteristic MS ions at m/z 271 and 579 from perspective of molecular 3D conformation and electronic effects; if any. In doing so, we find ourselves completely able to address questions (a)–(d) employing high accuracy method of computational quantum chemistry, due to reasons mentioned before.

Thus, most stable geometry and energetics of a set of possible tautomers of the flavonoid fragment of (1) are examined, herein (**Figure 13**.).

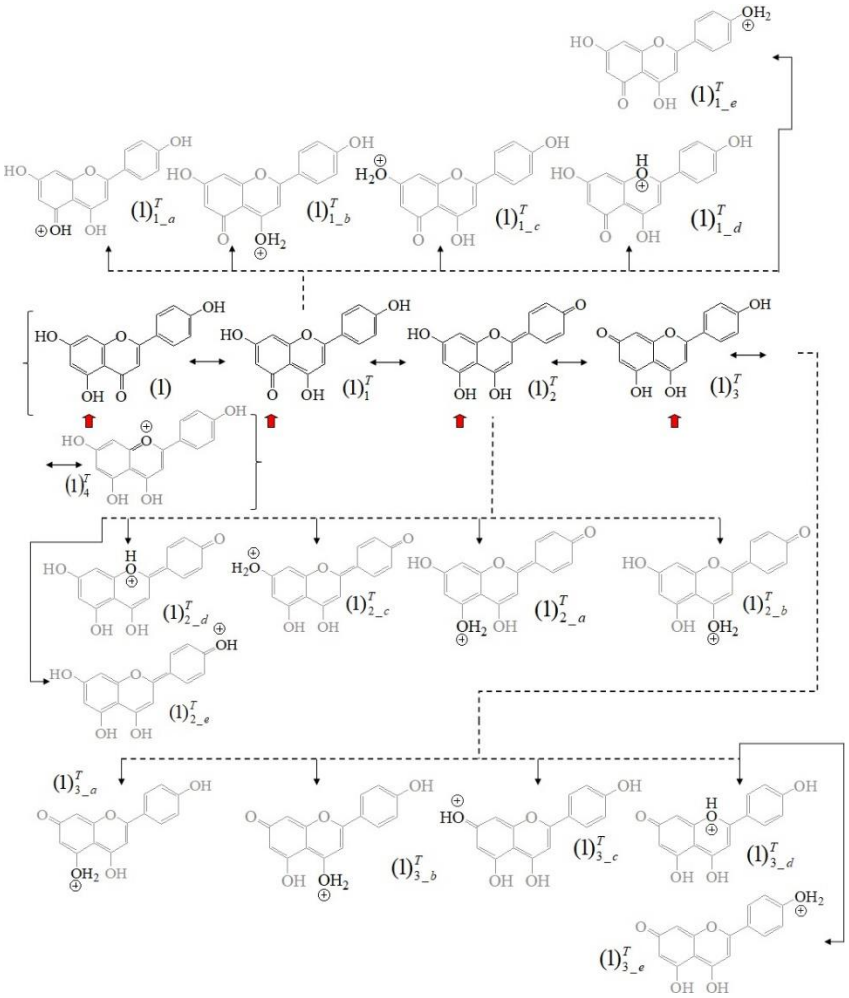
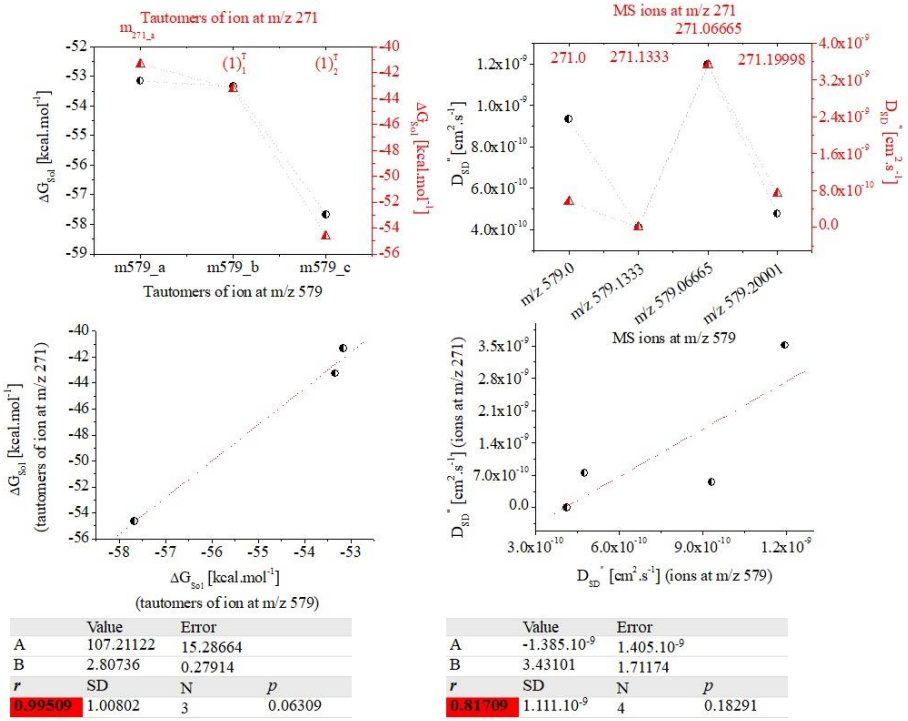


Figure 13. Tautomeric forms of flavonoide-fragment of (1) and their proton accepting capability.

Figure 14 correlates between theoretical thermodynamics in solution and experimental diffusion data on ions at m/z 579.0; 579, 1333, 579.06665, 579.20001, 271.0, 271.06665, 271.1333 and 271.19998, respectively. A |r| = 0.9951 is obtained looking at relationship $\Delta G_{Sol}(271_{\_i}) = f(\Delta G_{Sol}(579_{\_j}))$, where 'i' denotes different most stable tautomers of ions at m/z 271, while 'j' shows most stable forms of ions at m/z 579. The chemometrics of relationship among diffusion D_{SD}'' data on same species obtained according to **equation (2)** shown $|r| = 0.9918_5$–0.8170_9. The good-to-excellent chemometrics illustrate convincingly that mutual connection between pairs of ionic species 271.0/579.0, 271.1333/579.1333 and 271.06665/579.0665 could only be properly understood if there is used understanding that fragment CID reaction of each of molecular charged tautomers of analyte parent ion $[M+H]^+$ (m/z 579) produces corresponding Y_0^+ ion at m/z 271; or there are chemical reactions: m/z (579.0) → m/z (271.0), m/z (579.1333) → m/z (271.1333) and m/z (579.06665) → m/z (271.06665), respectively (**Figure 15**.) The deviation of ΔG_{Sol} data on ions at 579.20001 and 271.19998 from linear correlation assumes that there is a lack of m/z (579.20001) → m/z (271.19998) process.



	Value	Error	
A	107.21122	15.28664	
B	2.80736	0.27914	
r	SD	N	*p*
0.99509	1.00802	3	0.06309

	Value	Error	
A	$-1.385.10^{-9}$	$1.405.10^{-9}$	
B	3.43101	1.71174	
r	SD	N	*p*
0.81709	$1.111.10^{-9}$	4	0.18291

Figure 14. Relation between theoretical ΔG_{Sol} [kcal.mol^{-1}] data on pairs of ions at m/z 271.0/579.0, 271.1333/579.1333, 271.06665/579.0665 and 271.19998/579.20001; Relation between experimental mass spectrometric $D_{SD}^{''}$ [kcal.mol^{-1}] data on pairs of ions at m/z 271.0/579.0, 271.1333/579.1333, 271.06665/579.0665 and 271.19998/579.20001; chemometrics.

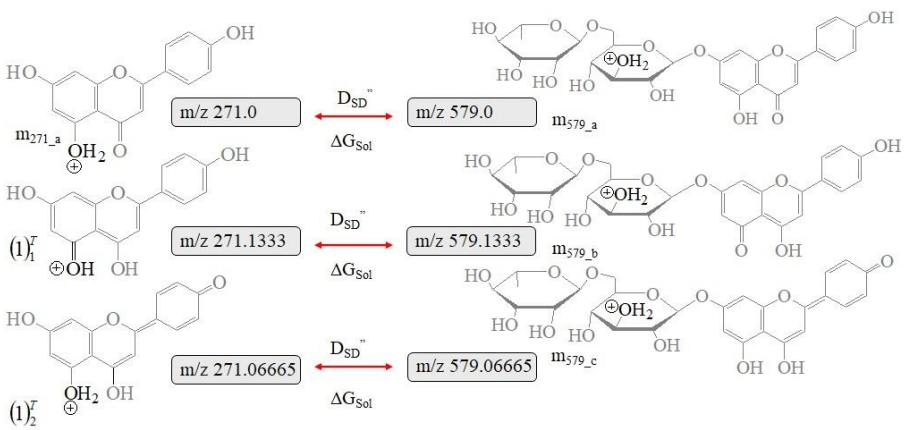


Figure 15. Chemical diagrams and connection by pairs of ions at m/z 271.0/579.0, 271.1333/579.1333 and 271.06665/579.06665 with respect to their different tautomeric forms.

The correlative analysis between D_{QC} and $D_{SD}^{''}$ data according to **equations (2)** and **(3)** is depicted in **Figure 16**. Remarkably, there is an excellent linear correlation between these parameters of tautomers 271_b, 271_(1)$^{T}_{1}$ and 271_(1)$^{T}_{2}$ showing |r| = 0.9578. The conclusion that emerges from the chemometrics is that $D_{SD}^{''}$ parameters according to our theory are not only capable of determining exact 3D molecular structures of analyte cations as has been show in review-article [21], but also is capable of accounting quantitatively for subtle electronic effects, thus distinguishing precisely among tautomers of analytes species; if any. This example can also be used to illustrate persuasively the great capability of experimental soft ionization mass spectrometry of absolute determination of multidimensional molecular conformations distinguishing among subtle electronic intra and intermolecular phenomena, when measurable variables are quantified within the framework of model equations **(1)** and **(2)** as well as when MS results are correlated quantitatively in chemometric terms with quantum chemical static and MD computations within the framework of Arrhenius's theory and **formula (3)**.

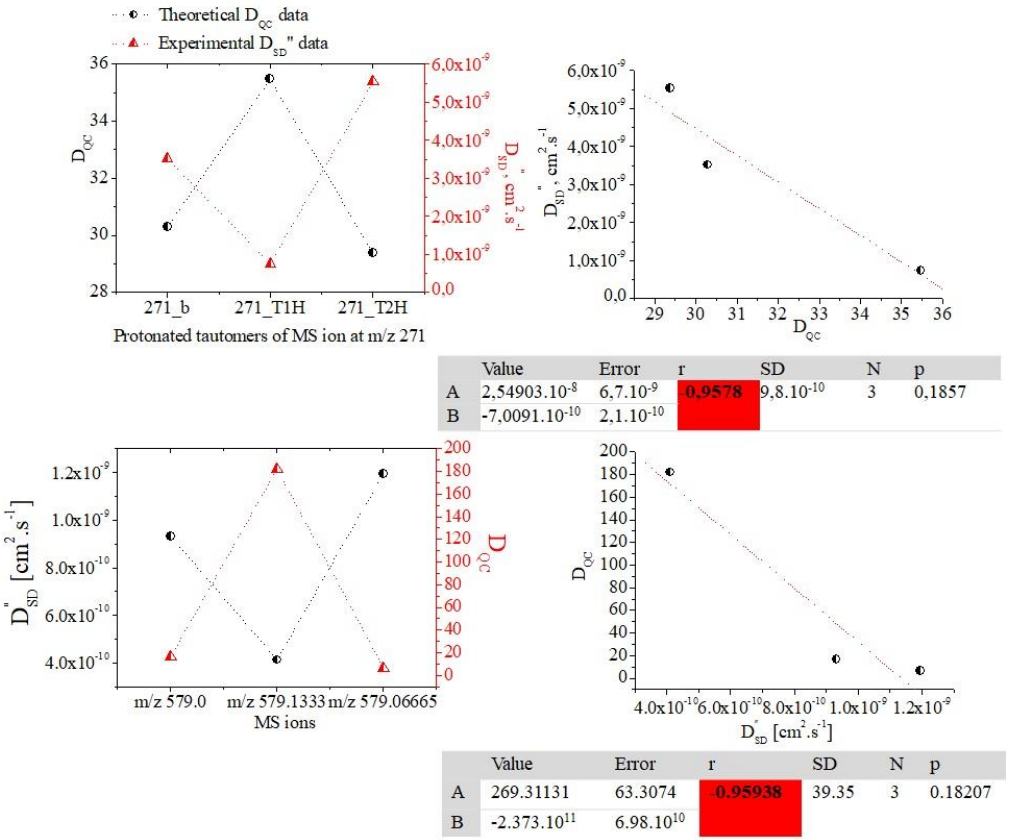


Figure 16. Relation between theoretical D_{QC} data and experimental $D_{SD}^{''}$ [$cm^2.s^{-1}$] data on ions at m/z 271.0, 579.0, 271.1333, 579.1333, 271.06665, 579.0665, 271.19998, 579.20001 with respect to their different tautomers; chemometrics

Conclusions

We draw particular conclusions, herein, which are related to important results from experimental and theoretical *stochastic dynamic mass spectrometric* data on our innovative theory and model **equations (1)** and **(2)** as well as theoretical quantum chemical results from gas-phase and solution thermochemistry, 3D molecular conformations and electronic effects of tautomers of apigenine-O-glucoside and its fragment species under ESI(+)-CID-MS^n experimental conditions, which correlated linearly from perspective of chemometrics and exhibit significant coefficient of linear correlation (|r|.) The latter statistical parameter is used to highly reliable quantitative criterion assessing mutual connection between experimental or theoretical datasets of values or both of these. There has been found that:

(A)The MS peak of analyte cation $[M+H]^+$ (m/z 579) and its Y_0^+ fragment cation (m/z 271) exhibit a set of common sub-peaks at m/z 271.0, 271.1333, 271.06665, 579.0, 579.1333 and 579.06665 corresponding to three different tautomers of the flavonoid fragment of the analyte. The D_{SD}'' data according to **equation (2)** calculated *per* span of experimental scan time, which treat statistically each data-set of measurable m/z variables correlate excellent with quantum chemical diffusion D_{QC} parameters of these different tautomers obtained within the framework of *Arrhenius's transition state theory* (**equation (3)**.) The functional relationship $D_{SD}'' = f(D_{QC})$ shows |r|=0.95938 and 0.9578;

(B)The fragment CID-MS^n processes of $[M+H]^+$ ion yielding to Y_0^+ cation is carried out without perturbation of electronic structures of tautomers. There are observed mass spectrometrically not only various tautomers of analyte species, but also these excellent distinguishable tautomeric forms are stable under collision induced reactions of cleavage of carbohydrate fragment of flavonoid-O-glycoside within the framework of processes: $579.0 \rightarrow 271.0$, $579.1333 \rightarrow 271.1333$ and $579.06665 \rightarrow 271.06665$, respectively. A |r| = 0.99187 value is obtained, examining mutual linear relation among D_{SD}'' data on the shown pairs of m/z-values;

(C)Excellent chemometric method performance ($|r|=0.99968_6$) has been obtained examining linear relation $D_{SD}'' = f(D_{QC})$ of characteristic MS ions of apigenin-O-glucoside at m/z 91, 111 and 153, as well.

(D)The linear relationship $D_{SD}'' = f(D_{SD})$ of diffusion parameters according to **equations (1)** and **(2)** within the framework of the discussed stochastic dynamic theory of tautomers of analyte ions shows |r| = 0.99929–1;

(E)The D_{SD}'' diffusion data correlate linearly with theoretical quantum chemical solvation *free Gibbs energy* parameters yielding to |r| = 0.54159–0.95432 depending on type of tautomer of analyte fragment ions.

Conclusions

Looking at points (A)–(E) we might underline that, as has been discussed above, there are different views resulting from various kinetics, thermodynamics and diffusion theories and model equations in analyzing quantitatively experimental measurable variables of soft-ionization mass spectrometric methods. However, the presented, herein, new results from analysis of a very complex from perspective of electronic structure and effects analyte object such as apigenin-O-glucoside exhibiting a set of tautometic forms, proton and charge transfer effects again show excellent-to-exact (|r| = 1 and sd(yEr±) = 0) chemometric method performances correlating between theory and experiment, like those reported previously of application of our SD theory to different classes of organics, metal-organics and inorganics [10–21]. The chemometrics show superior statistical parameters comparing with available data on quantitative analysis within the framework of various know theories and model formulas. The soundless of our arguments supporting a claim of an exact accuracy, precision, selectivity, sensitivity and reliability of our *mass spectrometric stochastic dynamic* data on **equations (1)** and **(2)** and their universal applicability to the analytical practice encompassing qualitative, quantitative and multidimensional structural *analytical chemistry* could easy be evaluated looking at the presented chemometrics.

Since, the chapter applies model SD equations of exact data processing of temporal behavior of measurable variable of analyte MS peaks, theoretical high accuracy quantum chemical data, experimental high accuracy MS results from (1) and chemometrics, the new pro-arguments toward validity and empirical testability of our model formulas be an interaction between theory and experiment. Perhaps, the most prominent result, amongst others, comes from quantification and exact determination from perspective of chemometrics of tautomeric forms of apigenin-O-glucoside, mass spectrometrically. Therefore, amongst many theories and model formulas, which have attempted to solve the problem of quantitative relations among molecular properties and 3D molecular structure of analytes, experimental mass spectrometric parameters and factors; analyte reaction kinetics, thermodynamics and diffusion of species only our model formulas, so far, argued persuasively and empirically for that the method of mass spectrometry are not only capable of providing exact parameters of the chemical reactions and ion/molecular interactions, but also of determining absolutely 3D molecular and electronic structures of analytes accounting for subtle electronic effects of tautomers and inter-, respectively, intramolecular proton transfer effects; if any.

Owing to the broad interdisciplinary application of the methods of *mass spectrometry* to a large scale of research fields, we hope that a broad universal audience are able to evaluate adequately the acceptability of the pro-argumentation of our innovative view and aforementioned formulas of *quantitative mass spectrometry*, thus pretending to reach a broad even universal application of our method not only to the fundamental research, but also to the analytical practice.

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