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Comprehensive evaluation of antioxidant activity: A chemometric approach using principal component analysis

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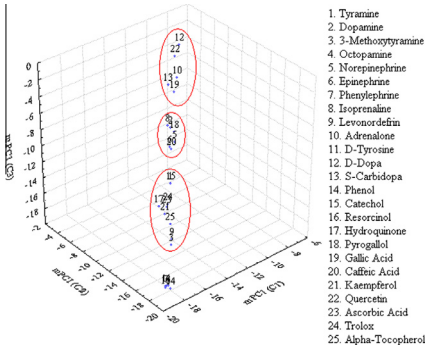
HIGHLIGHTS

- A novel DPPH· assay combined with a chemometric approach was proposed.
- Relevant wavelengths for the radical scavenging activity evaluation were selected.
- New potential antioxidant activity indices were proposed and evaluated.
- The radical scavenging profile of biogenic amines and related drugs was evaluated.
- The study offers the possibility of significant reduction of experimentally work.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel chemometric approach is described for evaluating the radical scavenging activity of biogenic amine related compounds by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) procedure and principal component analysis (PCA) tool. By a comprehensive chemometric investigation of variations in the radical scavenging profiles provided by the full-range UV–Vis spectra for different test duration and different relative concentrations (different molar ratio – [AH]/[DPPH·]) of the investigated compounds, new antioxidant activity evaluation parameters were proposed. The new proposed parameters (PC1, mPC1, maxPC1) are in good agreement with the reference DPPH· results (% RSA and IC_{50} derived from the reference DPPH· test), obtained for the investigated amines and reference antioxidants. Much more, the PCA profiles are better patterns for the comprehensive characterization of radical scavenging activity of compounds, allowing visualization of complex information by a simple graphical representation and underlying the (dis)similarity of compounds related both to the reaction kinetics and compounds concentration.

Introduction

The range of both naturally occurring and synthetic antioxidants applied in clinical settings has appreciably expanded since aging and degenerative diseases were related to the oxidation of biological components induced by reactive oxygen species (ROS). Also different experimental methods have been developed to evaluate the antioxidant activity effectiveness and a lot of data have been accumulated to describe kinetic process of radical-induced oxidation on biological experimental materials [1]. Besides research under real circumstances including *in vivo* studies, the *in vitro* convenient methods were also used to test the antioxidant effectiveness under relatively simple and controlled circumstances [2] and the chemical principles for determining antioxidant capacities were investigated [3].

In vitro antioxidant activity evaluation methods show extreme diversity, some of them involving a distinct oxidation step followed by measurement of the outcome but in other instances, there is no clear distinction between the various steps in the procedure [4]. Most of the reported methods are based on measurements using

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individual procedures as accelerated stability tests [5,6], peroxide value [7–9], diene conjugation [10,11], measurements of free radicals [12–16] or FRAP (ferric reducing antioxidant power) assay [17,18]. Tests including a large group of antiradical activity determinations still arouse scientist's interest as they are very useful even for preliminary analysis. In this area, much effort have been devoted to the development of chemical methods to evaluate antioxidant ability based on reaction with different radical species of biological significance (such as $O_2^{\cdot-}$, $OH^{\cdot}$, $NO^{\cdot}$ or lipid peroxyl radicals) [2] but tests using radical species such as 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot+}$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) and N,N-dimethyl-p-phenylendiamine (DMPD) are also quite popular [19,20]. These tests have been used for many decades to study the mechanism of hydrogen-atom donation from certain substances or the antioxidant activity of compounds with –SH, –OH and –NH groups [21].

Methods of expressing antioxidant activity results appear also to be as varied as the methods of measurement [22]. The large diversity of radical scavenging assays that differ from each other in terms of reaction conditions or in the form that results are expressed make difficult the comparison of results. As a consequence, efforts have been made during the last years to standardize analytical methods and provide valid guidelines that should be pursued by future researchers. In this order, new methodologies and parameters were proposed and different regression tools and multivariate mathematical approaches were applied to find insights into the antiradical process and generate a complete antioxidant profile of different classes of compounds [23–30]. Therefore new chemometric derived parameters for expressing results are trying to be used which more or less serve the same purpose. Most of them form the basis of the newer test methods such as the ABTS/TEAC (trolox equivalent antioxidant capacity) and different $DPPH^{\cdot}$ assays [31,32]. While the spectrophotometric technique was intensively used in the $DPPH^{\cdot}$ scavenging assays, however, to our knowledge, no research has been reported as yet to implement this technique based through the chemometric analysis of the full-range spectrum information (within the wavelength of 200–800 nm). Moreover, the short history of antioxidants employed in the treatment of central nervous system (CNS) disorders [33] still receives increasing attention. In this field efforts are likely to be directed at finding more powerful methods to investigate the free-radical-scavenging profiles and antioxidant properties of existing drugs in order to enhance their applicability as antioxidants [34] and improve their therapeutic applications [35]. According to recent studies, some biogenic amines (important compounds involved in usual metabolic processes and supporting the diagnosis of many diseases) and related drugs proved to have effective *in vitro* antioxidant and radical scavenging activity [36–38] but the complex profiles of these properties are still unclear.

In view of the above considerations, the present study aims to use the chemometric tools in order to find new potential antioxidant activity evaluation parameters based on the analysis of the information within the full-range UV–Vis spectra. The graphical approach to exploratory data analysis is also described and illustrated with data obtained on complex antiradical profiles of biogenic amines, related drugs and reference antioxidants.

Materials and methods

Reagents and solutions

All chemicals were of analytical reagent grade. Standard biogenic amines (tyramine, 3-methoxytyramine hydrochloride, dopamine hydrochloride, (±)-octopamine hydrochloride, (−)-norepinephrine, (−)-epinephrine, isoprenaline hydrochloride, (−)-3,4-dihydroxy-norephedrine (levonordefrin), 3′,4′-dihydroxy-2-(methylamino)acetophenone hydrochloride (adrenalone), 3,4-dihydroxy-D-phenyl-alanine (D-dopa), S-(−)-carbidopa and D-tyrosine), phenolic compounds (phenol, pyrocatechol (catechol), resorcinol, hydroquinone, pyrogallol, gallic acid and caffeic acid), flavonols (kaempferol and quercetin) and some reference antioxidants (ascorbic acid, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and (+)-α-tocopherol) were purchased from Sigma–Aldrich (Steinheim, Germany). 2,2-diphenyl-1-picrylhydrazyl free radical (95%) ($DPPH^{\cdot}$) was from Alfa Aesar (Karlsruhe, Germany). Analytical-grade methanol was purchased from Chemical Company (Iaşi, Romania).

For the $DPPH^{\cdot}$ assay a 0.15 mM solution was prepared by dissolving appropriate required amount of $DPPH^{\cdot}$ in methanol. This solution was prepared daily and protected from light throughout the analysis time in order to minimize the loss of free radical activity. Stock solutions of 1×10^{-1} M concentration were prepared for each of the investigated compounds by dissolving appropriate required amount of standard in 100 mL methanol in all cases. Working solutions of different concentrations (in range 2×10^{-5}–7×10^{-5} M) were prepared daily by rigorous dilution of the stock solution in all cases.

Reference $DPPH^{\cdot}$ method

To evaluate the new proposed methodology, the results obtained by the reference $DPPH^{\cdot}$ test [21] (with some minor modifications) were used. Also, for a comparative purpose, in the study, we used a dataset of antioxidant activity evaluation parameters (% RSA and IC_{50} values for a set of amines, related drugs and reference antioxidants), experimental determined by us and already published [38].

New $DPPH^{\cdot}$ method

The effect of biogenic amines, related drugs and reference antioxidants on the $DPPH^{\cdot}$ radical absorbance profiles was monitored by recording full-range UV–Vis spectra (in the range 200–800 nm) for different relative concentrations (the molar ratio $[AH]/[DPPH^{\cdot}] = 0.07$ (C1); 0.10 (C2); 0.13 (C3); 0.17 (C4) and 0.23 (C5) mole/mole) by adding 1 mL standard solution prepared in methanol to 2 mL of $DPPH^{\cdot}$ methanolic solution (0.15 mM) in all cases. To monitor the reaction kinetics, the spectra were also recorded in all cases at different time intervals (T1–T7) (1, 5, 10, 15, 20, 25 and 30 min after the reaction was started) until the reaction reached an equilibrium. Methanol was used as a blank solution and $DPPH^{\cdot}$ solution (2 mL) with additional methanol (1 mL) served as reference for the standard $DPPH^{\cdot}$ spectra. All measurements were performed in duplicate and the reactions were carried out at room temperature (22 °C).

Spectra acquisition

A Jasco V-550 UV–VIS spectrophotometer with double beam system with single monochromator (Tokyo, Japan), in absorbance mode, was used for spectra acquisition in the range of 200–800 nm. The acquired spectra were stored after the smoothing process. The Spectra Manager for Windows 95/NT version 1.53.04 (1995–2002, Jasco Corporation) software package was used for the spectra acquisition control, smoothing process, storage and spectral data digitization.

Spectra processing and PCA analysis

Spectra processing are commonly used to improve the performance of the spectral data. In our case the spectral processing