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Quantitative structure–retention relationships applied to liquid chromatography gradient elution method for the determination of carbonyl-2,4-dinitrophenylhydrazone compounds

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ABSTRACT

A usual method for the determination of aldehydes and ketones in different matrices consists of a derivatization with 2,4-dinitrophenylhydrazine (DNPH) followed by HPLC-UV analysis. In the present work, a HPLC-UV gradient elution method has been applied to the analysis of 13 aldehydes and ketones-DNPH in automotive emission samples. In addition to these 13 compounds-DNPH, several carbonyl-DNPH compounds (linear, ramified and cyclic, saturated and unsaturated compounds) have been analyzed by HPLC-UV. Quantitative structure–retention relationships (QSRR) methods have been applied to predict the logarithm of capacity factor ($\log k'$) of carbonyl-DNPH compounds. According to its physicochemical meaning, combinations of 2 and 3 molecular descriptors have been proposed in order to achieve higher correlation with $\log k'$. Using linear and non-linear QSRR methodologies, the resulting prediction models allowed the screening of the most probable carbonyl-DNPH derivative candidates that correspond to unknown compounds detected in automotive emission samples. This information has been useful for their identification by UPLC®–MS/MS. In addition, the chromatographic retention of different carbonyl-DNPH compound families was studied using two HPLC isocratic methods working with two orthogonal stationary phases (octadecylpolyethoxysilane and cyanopropyl). Differences between the retention indexes obtained for each column were used for classifying carbonyl-DNPH into compounds families.

1. Introduction

It is well known that the chemical properties of a compound depend on both its molecular structure and the conditions of its immediate environment. The theoretical description of chromatographic retention behavior using physicochemical properties derived from chemical structure of analytes and from the effect of both mobile and stationary phase, is known as quantitative structure–retention relationships (QSRR) methods [1].

Models obtained with QSRR can be applied to, for example, predict the retention times of unknown compounds, identify the most meaningful structural descriptors or gain insight into the separation mechanism operating in the chromatographic system [2].

QSRR methodologies have been developed in our group since 1975. This work was initiated by Gassiot, Carbó et al. with the establishment of linear relationships between gas chromatographic retention parameters and molecular properties (total energy and localized charge) for sterol acetates [3] and cyclohexane derivatives [4]. Indeed, the first empirical QSRR equation can be attributed to Gassiot et al. [5].

In 1980, Gassiot-Matas and Firpo-Pamies [6] found that gas chromatographic retention index could be explained using two descriptors: the first-order molecular connectivity index and the square of dipolar moment. The first-order molecular connectivity index was also found to be satisfactory for the elution order prediction of isoalkane hydrocarbons in gas chromatography [7]. This descriptor also correlates with HPLC retention index working in linear gradient elution mode for a set of 2-n-ketoalkanes-2,

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4-dinitrophenylhydrazones. Similar results were found using the number of carbon atoms as molecular descriptor [8].

With the inclusion of octanol/water partition coefficient logarithm in the QSRR study of 2-n-ketoalkanes-2,4-dinitrophenylhydrazones [9], the attempt to extend Ervin sz. Kováts methodology to HPLC was successfully achieved.

In the present work, QSRR methodologies were used for the study of carbonyl DNPH-derivatives analyzed by HPLC. The presence of carbonyl compounds plays an important role in many processes, including food chemistry (e.g., determination of beer flavor [10]) and environmental, health and safety sciences [11,12]. Carbonyl compounds in the atmosphere can act as atmospheric pollutants taking part in the photochemical formation of tropospheric ozone [13]. These compounds can reach the atmosphere from the exhaust of motor vehicles and other equipment using hydrocarbon and alcohol as fuels [11]. A usual method for the analysis of carbonyl compounds involves HPLC-UV analysis of previously obtained 2,4-dinitrophenylhydrazones (DNPH) derivatives [8,14].

In the case of vehicle emissions, samples are collected using silica cartridges impregnated with 2,4-dinitrophenylhydrazine [15]. Aldehydes and ketones contained in the gaseous emissions react with 2,4-dinitrophenylhydrazine and form stable hydrazone derivatives. The carbonyl-DNPH compounds are later eluted using acetonitrile and analyzed by reversed-phase liquid chromatography coupled with UV detector. Based on CEPA (California Environmental Protection Agency) standard operating process 104 [12], our group developed a HPLC-UV method in gradient elution mode for the analysis of 13 aldehydes and ketones-DNPH contained in automotive emission samples. In this method, the use of tetrahydrofuran (THF) in the mobile phase is essential to achieve good resolution between acetone-DNPH and prop-2-enal-DNPH, and between 2-butanone-DNPH and but-2-enal-DNPH, which coelute when acetonitrile/water solvent is used.

In order to characterize the chromatographic retention of different families of carbonyl-DNPH (linear, ramified and cyclic, saturated and unsaturated compounds), the data set of the initial 13 carbonyl-DNPH compounds was increased to 34 and was analyzed by the HPLC-UV gradient elution method developed. The use of gradient elution and the presence of THF in the mobile phase difficult the establishment of QSRR models for the chromatographic method used [2].

Not much information about QSRR application to carbonyl compounds analyzed by liquid chromatography was found [16,17]. Literature concerning QSRR methods applied to carbonyl compounds was mainly refereed to gas chromatography, in which the retention behavior of ketones and aldehydes can be predicted by QSRR models based on physical properties (mainly log P and molecular weight [18]) by a combination of solvent surface area, dipole moment, energy of HOMO, isotropic average polarizability and average hyperpolarizability at 0 eV electric field [19], or using different topological indexes [20–24]. Bibliographic applications of QSRR in HPLC were referred to reversed-phase HPLC in isocratic mode analysis of n-aldehydes, alk-2-ene aldehydes and alkan-2-ones-DNPH [25] (were carbon number was defined as the main descriptor).

The nature of molecular descriptors used in the establishment of the QSRR models becomes a key factor in ensuring its predictive capacity. In isocratic elution mode, calculated octanol/water partition coefficient logarithms have been extensively used [1,2]. Accurate correlations can be obtained (in both isocratic and gradient elution modes) through the integration of other descriptors related to the total dipole moment, the electron excess charge of the most negatively charged atom and the water-accessible molecular surface area [1,26]. In relation to HPLC gradient elution mode applications, good accuracy results have been obtained using linear solvent strength (LSS) model combined with Partial Least Squares (PLS) model [27–29]. However, it requires performing at least two linear gradient experimental measures (at different times) and it cannot be used for predictive purposes.

In the present work, PLS methodology was compared with Artificial Neural Networks (ANN) in the establishment of the QSRR prediction models. The logarithm of capacity factor ($\log k'$) was the selected chromatographic parameter used in the chromatographic retention characterization of the carbonyl-DNPH compounds. Established QSRR models were useful in unknown compounds identification of automotive emission samples. After that, the use of two stationary phases with orthogonal character (octadecylpolyethoxysilane and cyanopropyl) has allowed to classify carbonyl-DNPH compounds into different families.

2. Experimental

2.1. Equipment

Standards and samples analysis were carried out using a Waters 2690 HPLC liquid chromatograph (Waters Technologies Corporation, Milford, MA, USA). A 2489 UV/vis dual-wavelength absorbance detector at 360 nm (Waters Technologies Corporation, USA) was used for the detection of DNPH-compounds. The whole chromatographic system was connected to a computer with Empower 2 software. The two chromatographic columns used to determine carbonyl-DNPH retention times were an XBridge™ C_{18} column, 75 mm × 4.6 mm and 2.5 μm particle size (Waters Technologies Corporation, USA) and a LiChrosphere® column 100 CN, 125 mm × 4 mm and 5 μm particle size (Merck KGaA, Darmstadt, Germany).

Some standards and samples were also analyzed using a Waters 2690 HPLC coupled to a 996PDA Waters detector. Analyses were carried out using an XBridge™ C_{18} gradient elution method. The diode array detector was operating in the UV–vis range from 200 to 450 nm with a spectral resolution of 1.2 nm. The sampling rate was 1 spectrum/s.

A 6890 HRGC coupled to an electronic impact 5973 MS detector (Agilent Technologies, USA) was used for the identification and confirmation of the major component of the carbonyl-DNPH derivatives synthesized in our laboratory. The analysis was carried out using a HP-5MS column (J&W, Agilent Technologies, USA) of 30 m × 0.25 cm of internal diameter and 0.25 μm of film thickness.

The UPLC®–MS/MS analysis of standards and samples were carried out using an Acquity UPLC® H-Class chromatograph and a QqQ MS Waters XevoTQS detector (Waters Technologies Corporation, Milford, MA, USA). An Acquity UPLC® BEH C_{18} (Waters Technologies Corporation, USA) column of 50 mm × 2.1 mm and 1.7 μm particle size was used.

2.2. Chemicals

All the solvents used were of HPLC purity grade. Acetonitrile (ACN) was purchased from VWR International LLC (USA), tetrahydrofuran (THF) was purchased from Scharlab S.L. (Catalonia, Spain), methanol was purchased from Panreac Química S.A.U. (Catalonia, Spain) and water was obtained from a Milli-Q Water Purification System (Waters, Milford, MA, USA).

DNPH-coated sampling cartridges (Sep-Pak® DNPH-Silica) were purchased from Waters Technologies Corporation (Milford, MA, USA).

A standard mix solution containing 13 carbonyl-DNPH compounds (methanal-DNPH, ethanal-DNPH, 2-propanone-DNPH, prop-2-enal-DNPH, propanal-DNPH, but-2-enal-DNPH, butan-2-one-DNPH, 2-methylprop-2-enal-DNPH, butanal-DNPH,