Use and abuse of retention indices in essential oils analysis

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Abstract

The identification of essential oil (EO) constituents is a challenging task. Automatic searching in mass spectra libraries is not sufficient, as compounds in EO, usually, present isomeric structural relationships. Even before the coupling of gas chromatographs to mass spectrometry (MS) lead to commercial equipment, identification of EO components was possible by co-injection of authentic standards, whenever available, and/or by the calculation of their retention indices (RI). The RI concept was developed by Kóvats in 1958 to be used in isothermal analyses, as was the common approach at that time. A few years later, van den Dool and Kratz published a RI modification for programmed-temperature analyses. RI do provide a useful tentative indication of the possible molecule(s), especially if combined with MS data. After more than 60 years the system proved its efficacy. However, a worrying trend is the increasing number of articles in which the original concepts or RI are misused, leading to misidentification and, what is worse, propagating wrong data which, in turn, serve as a (wrong) basis for new misidentification, perpetuating the propagation of error. Herein we discuss some causes of this misuse and propose a roadmap for the reliable use of retention indices.

1. Introduction

For those who are younger than 50, it is hard to imagine how essential oil (EO) constituents were isolated, quantified and identified. To have a good picture, see the book of Ernest Günther [1]. All changed when gas chromatography (GC) was developed in the 1950s. As all chromatography, GC is a separation technique, and identification requires additional techniques. Kováts indices [2] and van den Dool and Kratz linear retention indices [3], made identification possible when no internal standards were available, which is quite common in natural products chemistry. The use of retention indices successfully contributed to expand the applications of GC especially in the analysis of EO. A few years after the appearance of GC, its hyphenation to MS was developed. Now, the fast development of commercial equipment and, latterly the arrival of computers, libraries of spectra and automatic searching software have made GC–MS one of the most (if not *the* most) important and widespread tool for the analysis of volatiles in general, and essential oils in particular [4]. However, with automation, and less training and skilled operators required, reduced critical evaluation of generated data, leading to misuse, misidentification of compounds and, in some cases, abuse in the use of retention indices is apparent. Consequently, more publications containing identification errors appear. Some of these commonly found misidentifications are discussed and some simple procedures proposed to, at least, reduce their occurrence. As a case study, the confusion regarding the identification of cymene isomers is presented and discussed.

2. Material and Methods

Standards of *o*-cymene, *m*-cymene, *p*-cymene, *n*-decane, *n*-undecane, *n*-dodecane and *n*-tridecane were purchased from Sigma-Aldrich Co. (Milwaukee, WI). Dichloromethane "Absolv" grade was used as solvent and purchased from Tedia (Brazil). Two stock solutions were prepared: one with *n*-decane and *n*-undecane and another with *n*-dodecane and *n*-tridecane. Each cymene standard was added separately to two vials, one with the C_{10}-C_{11} and other with the C_{12}-C_{13} stock solutions, so that six test solutions were obtained. The GC–MS analyses were performed using an Agilent 7890A GC coupled to an Agilent 5975C mass detector in electron ionization mode, at 70 eV. A DB-5ms fused silica capillary column (5%-phenyl-95%-methylsilicone, 30 m × 0.25 mm × 0.25 μm, Agilent) and an HP-INNOWax (polyethylene glycol, 25 m × 0.25 mm × 0.20 μm, Agilent) were used. The injector was maintained at 250 ºC. A volume of 1.0 μL of each test sample was injected in split mode (1:50). The oven temperature was kept constant at 130 ºC for isothermal analyses and was varied from 60 to 240 ºC at a rate of 3 ºC/min for temperature programmed analyses. The carrier gas used was helium (1.0 mL/min). The transfer line

was kept at 260 ºC, the ionization source at 220 ºC and analyzer at 150 ºC. The mass scan range was from 40 to 350 u at a rate of 3.15 scans/s. All data were processed using ChemStation software (Agilent Technologies). Mass spectra were interpreted using commercial spectrum libraries, which also incorporate retention index values, from various sources.

3. Results

Since neither MS nor RI are adequate for isomer differentiation, identification is best based on injection of authentic standards, which is quite a reasonable requirement in this case, since all cymenes are available commercially. Under the experimental conditions and using capillary columns comprising 5%-phenyl-95%-methylsilicone (DB-5, HP-5 or similar), the first compound to elute is *m*-cymene, closely followed by *p*-cymene with *o*-cymene coming as a last, well resolved peak. Because the difference in the RI of *m*-cymene and *p*-cymene is only two or three units, these isomers can be reliably identified only by using standards, since noticeable deviations from the tabulated values of the RI can be observed when different non-polar phases are used (and even when using the same type of phase but from different manufacturers).

4. Discussion and Conclusions

Perhaps the easiest way to solve the identification problem for cymenes would be to change the stationary phase from a non-polar to a polar one. Unfortunately, the problem remains. In polyethylene glycol phases, *meta* and *para* isomers elute very close too. This also highlights that newer stationary phases, such as ionic liquids, simply do not have tabulated listings of RI data, so this excludes these phases from using RI databases to support identification, most likely delegating MS to be the only identification available with the attendant risks this implies. The cymene isomers case is illustrative, but several other types of error, invariably related to poor judgement, including poor peer-review processing, result in much wrong data published. The misuse of GC–MS data and, particularly, of retention indices in the analysis of EO is clearly related to a poor or lack of understanding on the concept of RI and its limitations leading to, not rarely, abuse on the conclusions authors could get from the analytical information. To minimize errors and use RI properly, simple procedures can be applied. A roadmap for using retention indices is proposed [5].

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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