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Review article

# Nano-liquid chromatography applied to enantiomers separation

Salvatore Fanali *

*Institute of Chemical Methodologies, Italian National Research Council (C.N.R.), 00015, Monterotondo, Italy*

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ABSTRACT

This paper presents the state of the art concerning the separation of chiral compounds by means of nano-liquid chromatography (nano-LC). The enantiomers' separation and determination are a subject of fundamental importance in various application fields such as pharmaceutical industry, biomedicine, food, agrochemical etc. Nano-LC is a miniaturized chromatographic technique offering some advantages over conventional ones such as low consumption of mobile phase, sample volume and amount of chiral stationary phase, reduced costs etc. This is reported in the first part of the paper illustrating the features of the nano-LC. In addition, chiral resolution methods are briefly illustrated. Some chiral selectors, used in high-performance liquid chromatography have also been applied in nano-LC including cyclodextrins, glycopeptide antibiotics, modified polysaccharides etc. This is discussed in the second part of the review. Finally some examples of the applications available in literature are reported.



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## 1. Introduction

Nano-liquid chromatography (nano-LC) is a recent developed micro fluidic technique, mainly used for analytical purposes, offering some advantages over conventional high-performance liquid chromatography (HPLC). Because its features, this miniaturized technique has gained more and more interest in various application fields resulting either alternative and/or complementary to HPLC.

Analytes separation takes place into capillary columns containing selected stationary phases (SPs) under the effect of a mobile phase (MP) delivered at low flow rates (10–700 nL/min).

The SP can be either coated or bonded to i) the capillary wall (open tubular-LC, OTLC), ii) particles (packed) and iii) silica or polymeric (monoliths). The column (usually of fused silica material) has an I.D. in the range 10–100 μm. Because of the reduced flow rate, nano-LC results in a higher mass sensitivity when compared

* Correspondence to: Institute of Chemical Methodologies, Italian National Research Council (C.N.R.), Area della Ricerca di Roma I, Via Salaria km 29.300-00015, Monterotondo, Italy.
*E-mail address:* salvatore.fanali@cnr.it

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to HPLC. This feature is related to a lower chromatographic dilution [1]. In addition very small volumes of MP are consumed for experiments resulting in cheaper operation than HPLC. Finally the flow rate allows a perfect coupling with mass spectrometry (MS) that, on the other hand, is the best choice of detectors available.

After the pioneering work done by Karlsson and Novotny [2] who demonstrated the high efficiency and shorter analysis time employing columns with small inner diameters, several other authors reported studies dealing with nano-LC considering both theoretical, instrumental and methodological approaches [1,3–11].

Because of its potential advantages over other analytical techniques, nano-LC has been successfully applied to the analysis of a large number of compounds currently studied in different application fields such as proteomics, pharmaceutical, agrochemical, food chemistry, environmental, biomedicine, chiral etc.

The separation of enantiomers is a very important issue of great interest in the research field as these compounds are present in nature and are implicated in several biological processes, including those linked to human health. In the pharmaceutical field, many drugs possess chiral centres and therefore can exist as one or more couples of enantiomers with the possibility to interact differently in various biological processes. Often one of the two enantiomers shows greater pharmacological activity, however, in some cases can be even harmful. Therefore, very often, several drugs are marketed as a single enantiomer.

Since these compounds possess quite similar physical chemical properties, their structure differs for the spatial orientation of substituent groups at asymmetric centre. Thus their separation is a difficult task. However in presence of a chiral environment, two enantiomers can react in a different way on forming diastereoisomeric complexes exhibiting different properties and thus promoting their separation even in a non-chiral environment.

Considering the importance of chiral compounds, especially for human health, there is clearly a need to proceed to their separation, quantitation and characterization. Therefore analytical methods capable to determine accurately and reliably these compounds are required.

In addition to conventional techniques such as HPLC, supercritical fluid chromatography (SFC), GC, thin-layer chromatography (TLC), miniaturized techniques such as capillary electrophoresis (CE) and nano-LC are currently applied in this field.

Aim of this paper is to report about the use of nano-LC in the specific field of enantiomers separation. The main features of the technique will be briefly discussed. In addition, the currently used chiral stationary phases (CSPs) and the choice of experimental conditions will also be illustrated. Finally some selected applications will also be presented The paper is dedicated to the memory of Prof. Hanfa Zou (Dalian, PRC) who enormously contributed to develop nano-LC in various application fields [10,11].

## 2. Key features, instrumentation and usefulness of nano-LC

Nano-LC is a miniaturized liquid chromatographic technique where analytes separation take place into capillaries, usually of fused silica material, with very thin diameter (10–100 μm I.D.) being applied in different fields also including enantiomers separation. In this case, the capillary contains a chiral selector (CS) either bonded or adsorbed on the capillary wall (OTLC) or to packed particles or included/bonded to polymeric material (monolithic). Finally the CS can also be added to the MP.

The MP is delivered at low flow-rates (10–700 nL/min) and thus the technique offers a very high mass sensitivity. This feature is ascribed to the lower peaks chromatographic dilution. As can be observed in the following equation [1].

$$D = \frac{c_o}{c_{\max}} = \frac{\varepsilon \pi r^2 (1+k)\sqrt{2\pi LH}}{V_{inj}} \quad (1)$$

With $c_o$ and $c_{\max}$ are the sample concentrations at the injection and at the peak maximum, respectively, $r$ the column radius, $L$ the column length $H$ the plate height, $\epsilon$ the column porosity and $V_{inj}$ is the sample volume injected. It is clear that a decrease of the capillary radius causes a lower value of $D$. The low flow rates offer additional advantages over LC techniques, e.g., lower consumption of MP with consequent limited costs of organic solvents and waste; perfect coupling with mass spectrometry. The high sensitivity above mentioned needs some more clear information, especially for those that are not familiar with nano-LC. In fact since the injected sample volumes are very low in comparison with HPLC (only 10–60 nL), the sensitivity, especially when analyzing compounds in complex matrices, is lower and therefore it is necessary to consider some pre-concentration steps in the method development. This can be done off-line utilizing, e.g., liquid-liquid extraction (LL), solid-phase extraction (SPE), molecular imprinted polymers (MIP) etc. In addition, one simple method is realized injecting relatively high sample volumes into the capillary columns after selecting carefully the solvent utilized for analytes solution. The sample solvent must have lower elution strength than the MP obtaining a focusing effect. In this way analytes are pre-concentrated at the entrance of the column, as a sharp zone, increasing the sensitivity. In one of our previous work [12] volumes of some β-blocker enantiomers in the range 50–2100 nL were injected and analyzed with a capillary packed with vancomycin bonded to silica particles for the enantioresolution. 1500 nL was the optimum volume injected without affecting separation and efficiency and achieving good sensitivity. Other authors reported about focusing approach for other type of applications [8,13–17].

Although nano-LC possesses the above mentioned features it is note worth mentioning that dedicated instrumentation has to be used, e.g., pumping systems capable to deliver flows at levels of nL/min, detectors with reduced cell volumes and at high frequency, nano-injectors, tube connection of thin diameters. Last but not least, appropriate interfaces (nano-spray) for MS connection. These approaches are necessary to minimize extra column band broadening that could cause a decrease of chromatographic efficiency with consequent loose of resolution. Some commercial instrumentation dedicated to nano-LC are available, however it can also be laboratory assembled. Conventional HPLC pumps, equipped with a mechanical split in order to have the nano-flow, could be used to deliver the MP. Detection makes use of capillary electrophoresis (CE) UV-detector. Here the path length of the cell is the radius of the capillary. It has also been demonstrated that nano-LC can also be carried out utilizing CE commercial apparatus. This was done by our group for the separation of racemic loxiglumide employing a short column (7 cm packed) containing silica modified with a teicoplanin [18]. Usually with laboratory assembled instrumentation, isocratic elution mode is not a problem, nevertheless to use a gradient mode is more difficult. However the literature offers some examples where a gradient elution can be carried out. For example Cappiello et al. [19] proposed the use of a ten port valve with several loops (μL volume) containing MPs of different composition. The valve was moved and controlled by a computer The use of MS is a need to determine the analytes mass and to proceed with the characterization. Although different MS type have been coupled with the nano-LC, e.g., single quadrupole, ion-trap, time of flight (ToF), good results have been achieved with electrospray sources (ESI). Recently electron ionization (EI) has been