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Pharmaceutical Drug Analysis In Biological And Environmental Matrices By Nano Liquid Chromatography: A Step Toward Lab On Chip

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Abstract: The exposure and occurrence of the residues of drugs and pharmaceuticals has become very common in different matrices such as biological samples (e.g., blood, plasma, urine) or in environmental samples, such as soil, sediment, and water. These residues may accumulate in the human body and may create detrimental effects after long-term exposure. The detection of the presence of these toxic components even up to the nano level or lower level may be carried out by the application of advanced hyphenated analytical instrumentation. In this direction, various chromatographic analytical methods are available, but recently, nanoliquid chromatography (NLC) has become more popular among researchers. Nanoanalysis of pharmaceutical drug residues by nano-liquid chromatography has been a state-of-the-art in the area of research and innovation. Its application has become very important in recent years. NLC is a novel method and is applicable for drug analysis, especially for the separation and detection of chiral compounds. In industrial sectors such as pharma, agrochemical and biomedicine or in research laboratories, enantiomeric separation and determination in samples have received much attention from authorities and researchers. Nano-LC has benefits over other conventional chiral separation have been explained. The basic science of normal separation and chiral separation along with the application of NLC in drug analysis has been well described and reviewed. Moreover, chiral recognition mechanisms are important and are presented herein. Various modalities of Nano-LC have been described in the article as a step toward Lab on Chip with a green chemistry approach.

Keywords: pharmaceutical compounds • chiral drug • analysis • nanoliquid chromatography • HPLC

Introduction

The science of separation is an essential part of any analytical work in the area of different scientific studies of chemistry, biology, biotechnology, pharmaceutical science, environmental science or agricultural sciences. During the 1960s, Horvath and coworkers (1967) applied columns having small inner diameters for the liquid chromatographic (LC) analytical studies. After that, Karlsson and Novotny (1988) reported the idea of nano liquid chromatography (nano-LC) during the year of 1988. The researchers found that the separation effectiveness of slurry-packed LC micro columns with small inner diameters was very high. Conventional chromatographic analysis by using high performance liquid chromatograph (HPLC) has been completed using columns of

3.5 to 4.6 mm inner diameters with flow rates of 1.0 ml per min, whereas columns with smaller internal diameters ranging from 20 to 100 µm using flow rates of nanoliters per minute are known as nanocolumns and are used in nanoliquid chromatography (Cutillas 2005, Satio and Greibrokk 2004). Many research and development related tasks have been performed by scientists in the area of separation science and technology, especially in liquid chromatographic modalities.

Simply, nanochromatography may be defined as "a chromatographic technique having samples volume in nanoliter, flow rate of mobile phase in nano per minute with the detection limit of nanogram per level" (Ali et al 2009*)*. Truly complete nanochromatography is called lab-on-chip chromatography and is normally termed nanochromatography from a broader perspective.

Nano-Liquid Chromatography (NANO-LC):

Chervet et al (1996) described the separations using different columns, such as the columns having i.d. size from 0.50–1.0 mm have been explained as micro-LC; columns having i.d. size from 100 to 500 mm are described as capillary-LC and the separations performed using the columns of 10–100 mm i.d. are expressed as nano-LC which covers the separations in microchips because nano-HPLC columns on chips have an i.d. of 20 to 100 mm. Finally, the whole nanochromatography is just achievable on a chip, which is termed lab-on-chip chromatography or nanochromatography (NC) from a broader perspective. When we discuss about the liquid chromatography, it is known as nanoliquid chromatography (Ali et al 2009).

The basic principle of NLC is similar to that of conventional methods. The main terms and related formulas or equations of chromatographic analysis, such as retention factor (k), separation factor (α), and resolution factors (Rs), Number of a theoretical plate (N), Chromatographic Dilution (D), the flow rate (F) in a column etc. are important to understand and can be calculated using the following standard equations (Meyer 1993).

Retention Factor (k):

$$k = \frac{t_r - t_0}{t_0}$$

where:

t_r = the retention time of the separated analyte in minutes/seconds

t_0 = dead time i.e. solvent front in minutes/seconds

Separation factor (α):

$$\alpha = \frac{k_1}{k_2}$$

Where:

k_1 and k_2 are retention factors.

Resolution factor (Rs):

$$R_s = 2\Delta t_r / (w_1 + w_2)$$

where:

Δt_r = time difference of the retention times of two peaks of the separated analyte

w_1 = width of the base of peak 1,

w_2 = width of the base of peak 2

When the individual values of Separation factor and Resolution factor are 1 or more than 1, then the chromatographic separation is described as complete separation whereas if the individual values of these factors are less than 1, then the separation is called as partial or incomplete separation.

Number of a theoretical plate (N):

The number of theoretical plate describes the quality of any column or chip. The value of number of theoretical plate can be achieved from the given equation:

$$N = 16(t_r / w)^2$$

where:

t_r = retention time of the peak

w = width of the peak at the base

A larger value of N means a complicated mixture of a sample can be separated through the column.

Chromatographic Dilution (D):

The dilution process of injected analyte in a column may change the separation efficiency. Such dilution is known as chromatographic dilution (D) and is expressed by the following equation:

$$D = \frac{C_0}{C_{max}} = \frac{\pi d_c^2 \, \varepsilon \, (1+k)\sqrt{2LH\pi}}{4V_{inj}}$$

where:

C_0 = Initial concentration of the analyte

C_{max} = Final concentration of the analyte

d_c = Internal diameter of the column

ε = Complete porosity of the column

L = Column length

H = plate height

V_{inj} = Volume of injected sample

k = retention factor

Flow rate (F) in a column:

$$F = \frac{u\pi d_c^2 \epsilon}{4}$$

Where:

u = mobile phase's linear velocity

d_c = Internal diameter of the column

ε = Total porosity of the column

Nano – High Performance Liquid Chromatography (HPLC): Currently, nano-HPLC, as a modern separation science for the analysis of biological and environmental matrices, has become a very important tool. The addition of a precolumn in nano-HPLC instrumentation makes this technique more popular and useful because of the small size of the separation channel. Silica based RP microcolumns, have been applied in nano-HPLC by researchers (Ishihama 2005). Better separation of several molecules in nano-HPLC analysis has been achieved by using a monolithic silica column (polymer-based with molecular imprinting) (Ishihama 2005; Ericson 2000). Under Lab on Chip-based scientific studies, microfluidic nano-HPLC chips are in practice in pharmaceutical drug analysis in biological and environmental matrices.

Application of Nano-liquid Chromatography in Drug Analysis: During the 1980s, attempts to miniaturize the HPLC were started in the direction of the progress of packed micro-columns by applying fused silica capillaries

having 20–250 µm i.d. size and flow rate of 0.02 to10 µL/min for better separation efficiency (Takeuchi and Ishii 1980, Yang 1982).

Zhu et al (2012) studied the bio-activity profiling of small volume samples by nanoliquid chromatography attached to microarray bioassaying through high-resolution fractionation with a Dionex Ultimate 3000 nano LC system. The researchers used a column with 0.3 mm i.d. and 5 mm length that was packed with PepMap 100 $C_{18}$with a 5 µm pore size using acetonitrile–water (2:98; v/v) as the solvent (with 0.1% TFA) with a flow rate of 10 µL per minute. After 6 min, the trapping column was switched in line with another analytical C_{18} column with 75 µm i.d., 150 mm length and a 2 µm pore size.This whole analytical study was completed using a gradient elution with a mobile phase comprised of solvent A, i.e., water : acetonitrile : formic acid in a ratio 98:2:0.1 (v/v/v) respectively, and solvent B, i.e., acetonitrile : water : formic acid with the ratio of 98:2:0.1 (v/v/v) respectively.

Zhu et al (2012) worked on microfluidic chip-based nano liquid chromatography coupled to tandem mass spectrometry using a 25 mm, 500 nL enrichment column packed with ZORBAX 80 SB-C_{18} with a 5 µm particle size, a 150 mm×75 µm separation column packed with ZORBAX 80 SB-C_{18} with a 5 µm particle size, in the determination of drugs and metabolites such as morphine, codeine, cocaine, benzoylecgonine, cocaethylene, norcocaine, 6-acetylmorphine, phencyclidine, amphetamine, methamphetamine, MDMA, MDA, MDEA, and methadone in the hair samples of drug abusers at the Shenzhen Detoxification Institute (Shenzhen, China) and compared them with conventional HPLC–MS/MS analysis.

Langford and Lurie (2022) reviewed the use of micro, capillary, and nanoliquid chromatography in forensic studies and described the benefits, disadvantages, and usefulness of micro flow and nano liquid chromatography. Jornet-Martínez et al (2021) used a portable nano liquid chromatography in which the LOD was 100 µg/mL in scopolamine analysis in beverages with a 100 mm × 150 µm i.d. column packed with an ODS stationary phase.

Auditore and his team (2013) performed a study of enantiomeric separation of a powerful vasodilator drug, amlodipine, used to cure hypertension and its two chiral impurities by nano LC using a novel polysaccharide-based chiral stationary phase based on cellulose tris (4-chloro-3-methylphenylcarbamate). In this method, capillary columns (100 µm packed with the chiral stationary phase) and mobile phase of a mixture of Acetonitrile : water (90:10, v/v) using 5 mM ammonium borate buffer at pH 9.0 obtained the complete separation of the three pairs of enantiomers within 30 min in nano-LC analysis.

Duan et al (2012) employed a nano-LC/nanospray-MS process to attain a lesser limit of quantification in a study of high-throughput method development for exact quantification of therapeutic monoclonal antibodies.

André and Guillaume (2022) developed a cetylcholinesterase nano LC capillary column (75 µm i.d. × 50 mm) as a nano Bio LC column in the study of acetylcholinesterase molecular targets for the rapid screening of inhibitor candidates and to evaluate the action mechanism. Xu and coworkers (2018) developed an approach for the fabrication of a vancomycin-functionalized polymer-based monolith as a chiral stationary phase for nano LC and obtained success in partial enantio-separation for drugs, specially thalidomide, colchicine and some β-blockers.

Liu et al (2012) found that the nano-LC system which was coupled with tandem mass spectrometer in the identification of metabolites from in vitro and in vivo samples was approximately 1000-fold more useful than

HPLC/MS. Fanali et al (2004) successfully used a hepta-Tyr antibiotic-modified silica packed stationary phase in enantiomeric resolution of D, L-loxiglumide in a nano liquid chromatographic study.

Demir and Aydoğan (2022) successfully studied chloramphenicol and chloramphenicol glucuronide in milk and honey samples using a Monolith column (graphene oxide-fumed silica nanoparticle-based column) of size 50 µm x 100 mm with a flow rate of 500 nl/min using a UV detector. Jornet-Martínez et al (2021) performed a portable nano-LC comparative study of scopolamine in beverage samples using an ODS stationary phase of 150 µm x100 mm at 200 nL/min with a UV–VIS detector. Chmela and coworkers (2002) designed and tested a miniaturized hydrodynamic chromatography chip with a size of 1µm x 1000 µm and integrated it with a 300 pL injector in the separation work of nanospheres, macromolecules, synthetic polymers, biopolymers, etc., as a rapid, capable and low solvent utilization technology.

Famiglini et al (2005) analysed the 29 endocrine disrupting compounds, like polyaromatic hydrocarbons and phenol pesticides, using nano-HPLC–MS (C_{18} column with 75 µm i.d. in marine water) method. The research group described a limit of detection (LOD) between 0.4 and 118.7 ng/L. Some researchers have used SVD, ECD and LIF detectors and PDMS, quartz, and glass-based chip in separation and identification of dopamine compound (Yamamoto et al 2002, Cellar and Kennedy 2006, Lacher 2004)

Chiral Separation Mechanisms: The chiral recognition mechanisms in nano-liquid chromatography and enantiomer distinction have an impact on various analytical fields that deal with bio-active compounds, especially in drug discovery, development, chiral pollutants, etc. In NLC, the chiral recognition mechanisms are similar as in conventional liquid chromatography. However, specific molecules, such as polysaccharides, cyclodextrins (cyclic oligosaccharides), crown ethers, proteins, macrocyclic glycopeptide antibiotics, ligand exchangers and Pirkle-type molecules (brush-type), are usually used as chiral selectors by researchers due to the presence of some special characters like chiral cavities, grooves, baskets, and loops in these molecules. Researchers have reported that after injection of a sample in NLC, the enantiomers of the racemic drug make temporary diastereoisomeric complexes with the addition of a chiral mobile phase in a chiral column. The stability of these complexes depends on specific interactions, such as hydrogen bonding, van der waals forces, dipole interactions, anionic and cationic bonding, and steric forces in column (Ali et al 2009, Rocco et al 2013, Kallenborn et al 2021, Enein and Ali 2009, 2003, Ali et al 2009, Ali et al 2010, Sharma 2014, Chander et al 2021)

In addition, the aromatic moieties may be involved in ∏-∏ donor-acceptor interactions, which depend on the other factors of the binding process (Kasat 2008, Yamamoto et al 2002). The binding mechanism in polysaccharide CSPs (cellulose or amylose based) has been supported by different NMR, IR and computational studies (Yamamoto et al 2002, Ye et al 2007, Lämmerhofer et al 2010, D'Orazio 2020, Aydoğan, 2002).

Conclusion

Currently, state-of-the-art nano-liquid chromatographic separation methods have been developed as crucial tools with higher sensitivity and resolving power. The separation as well as identification of pharmaceutical drugs in biological and environmental samples upto the nano level using chip-based nano-liquid chromatography has received increasing attention from scientists. Nano-LC has potential

advantages over conventional separation techniques in the analysis of drugs and pharmaceuticals in biological and environmental samples using new generation columns such as fewer samples, less consumption of solvents in the mobile phase, easy coupling with mass spectrometry (MS), less waste generation, and reproducibility of results. Various miniaturized analytical devices, such as glass chips, quartz chips, silica chips, polymer chips, and plastic chips, have been designed and developed. However, more development with specific modifications is challenging, but researchers and technologists are still working on it and dedicated to lower-level detection of drug residues in various matrices with modified and fabricated chips in lab-on-chip-based NLC methods.

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