

Chromatography of Non-Steroidal Anti-Inflammatory Drugs: New Achievements

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Abstract

The newest results in the chromatographic analysis of non-steroidal anti-inflammatory drugs present in various organic and inorganic accompanying matrices are collected and critically evaluated. Examples for the application of pre-concentration and pre-purification methods for the enhancement of the selectivity and sensitivity of chromatographic technologies are presented. The advantages and disadvantages of the different chromatographic methods are discussed and the separation capacities of the techniques are compared. The application of various chromatographic procedures for the separation and quantitative determination of non-steroidal anti-inflammatory drug (ibuprofen, diclofenac, genfibrozil, ketoprofen, diflunisal, indoprofen, fenoprofen, meclofenamic acid, naproxen, mexiletin) is discussed in detail.

Keywords: Non-steroidal anti-inflammatory drugs; Gas chromatography; Liquid chromatographic technologies; Electrically driven separation methods

Abbreviations: BGE: Background Electrolyte; BSTFA: Bis(trimethylsilyl) Trifluoro acetamide; CZE: Capillary Zone Electrophoresis; DLLME: Dispersive Liquid-liquid Micro extraction; EME: Electro membrane Extraction; ESI-MS/MS: Electrospray Ionization Tandem Mass Spectrometry; FS: Full Scan; GC-MS: Gas Chromatography-Mass Spectrometry; HF-LPME: liquid-phase Micro extraction; HPLC: High Performance Liquid Chromatography; KET: Ketoprofen; KET-G: Ketoprofen Glucuronide; LC-MS: Liquid chromatography-Mass Spectrometry; LOD: Limit of Detection; LOQ: Limit of Quantitation; MBR: Membrane Bioreactor; MET: Metoprolol; MLR: Multivariate Regression Analysis; MS/MS: Tandem Mass Spectrometry; MTBSTFA: N-methyl-N-[tert-butyl-dimethyl-silyl]trifluoroacetamide; MSTFA: N-methyl-N-trimethylsilyl-trifluoroacetamide; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; ODS: Octadecylsilica; PAR: Paracetamol; PAR-G: Paracetamol Glucuronide; PAR-S: Paracetamol Sulfate; PRO: Propanolol; PRO-S: 4-Hydroxy Propanolol Sulfate; QqQ: Triple Quadrupole Sstem; Q-TOF: Quadrupole time of flight system; RP-HPLC: Reversed High Performance Liquid Chromatography; RSD: Relative Standard Deviation; SAL: Salicylic Acid; SOT: Sotalol; SPE: Solid-Phase Extraction; SIM: Selective Ion Monitoring; STP: Sewage Treatment Plant; TMCS: Trimethyl Chlorosilane; UHPLC: Ultra High Performance Liquid Chromatography

Introduction

Chromatographic technologies have been developed as powerful separation methods suitable for the separation and quantitative determination of analytes with very similar molecular structure [1]. Various chromatographic techniques such as gas chromatography (GC), liquid chromatographic procedures (thin-layer chromatography, high performance liquid chromatography, HPLC, ultra performance liquid chromatography), and electrically driven systems found application in the separation and quantitative determination of non-steroidal anti-inflammatory drugs (NSAIDs). Electrically driven separation systems include techniques employing simultaneously adsorption and electrical forces for the separation of similar compounds. These compounds are frequently used in human health care, can pollute food products and can cause environmental pollution.

The objectives of the present review are the compilation and concise evaluation on the newest results obtained in the chromatographic analysis of NSAIDs present in human and animal organisms and environmental samples. Moreover, the review deals with the brief enumeration of the techniques employed and the critical evaluation of the result.

Theoretical studies

A reversed-phase (RP)-HPLC system was employed for the assessment of the detector response of NSAIDs using fractional experimental design and multivariate regression analysis (MLR). The detector response was optimized and new retention parameters were computed. Isocratic measurements were performed on an octadecylsilica (ODS) column (250 mm × 3.0 mm i.d.) under thermostated conditions. The pH of the mobile phases was set by 20 mM phosphate and/or 20 mM citrate buffer. The flow rate was 1 mL/min in each eluent system. It was stated that the experimental data can be applied in structure-chromatographic response relationship studies and can be used to derive new retention parameters [2].

A novel hybrid type RP stationary phase: 1,3-alternate 25,27-bis-[cyanopropoxy]-26,28-bis-[3-propoxy]-calix[4]arene-bonded was synthesized and its characteristics were investigated in detail: surface coverage, hydrophobic selectivity, aromatic selectivity, shape selectivity, hydrogen bonding capacity, residue metal content, and silanol activity and were compared with similar stationary phases previously synthesized. The investigations indicated that polynuclear aromatic hydrocarbons, NSAIDs and sulfonamides can be easily separated in this new stationary phase [3].

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Human studies

GC-MS (gas chromatography-mass spectrometry) found application in the investigation of the influence of NSAIDs on the synthetic and endogenous androgenic anabolic steroids in urine. Ibuprofen, diclofenac, paracetamol, and propyphenazone were included in the experiments. Target compounds were derivatized with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA)/NH₄/Dithioerythritol (1000:2:4: v/v/w/w). The result indicated that the NSAIDs influence markedly the urinary concentration of endogenous androgen steroids [4].

The concentration of ceftriaxone was measured in the presence of NSAIDs by RP-HPLC in formulations and human serum. Analytes were separated on an RP-HPLC column (250 x 4.6 mm; particle size, 5 µm) at ambient temperature. Isocratic mobile phase consisted of methanol-water-acetonitrile (80:15:5 v/v/v). The pH of the mobile phase was set to 2.8 by ortho-phosphoric, the flow rate was 1.0 mL/min, analytes were detected at 270 nm. The inter- and intra-day RSDs were lower than 2.0%, the recovery was higher than 98.1%. Due to the accuracy, selectivity, sensitivity and reproducibility the method was proposed for the measurements of tiaprofenic acid, naproxen sodium, flurbiprofen, diclofenac acid and mefenamic acid [5].

RP-HPLC found application in the separation and quantitative determination of diltiazem and NSAIDs in human serum and pharmaceutical formulations. Measurements were carried out on a C18 column the mobile phase was methanol-water (80:20, v/v). The pH of the mobile phase was set to 3.1 with phosphoric acid (85%). The flow rate started at 0.5 mL/min and then increased to 1 mL/min. Analytes were detected at 240 nm. The results of validation process indicated that the method is reliable [6]. A similar RP-HPLC method was employed for the simultaneous determination of captopril in the presence of NSAIDs in pharmaceutical formulations and human serum. Measurements were performed on an ODS column (25 x 0.46 cm; particle size, 5 µm). Column was not thermostated. Analyses were carried out at ambient temperature. Mobile phase consisted of methanol-water (80:20, v/v). Flow rate was 1.0 mL/min; analytes were detected at 227 nm. LOD and LOQ for flurbiprofen, ibuprofen, diclofenac sodium, and mefenamic acid were 0.2, 1, 2, and 0.4 ng/mL while the corresponding LOQs were 0.9, 2.9, 8 and 1 ng/mL. It was established that the method is rapid, accurate (recovery >98.1%), selective and reproducible [7].

HPLC was employed for the study of the concentration change of rebamipide in the small bowel tissue of healthy subjects. It was established that the concentration of rebamipide in the jejunum is sufficient to protect for NSAID-induced gastrointestinal complications [8].

SPE followed by ultra high performance liquid chromatography (UHPLC) was employed for the simultaneous analysis of blockers (Sotalol SOT, metoprolol MET, propranolol PRO), NSAIDs (paracetamol PAR, ketoprofen KET, salicylic acid SAL), and their metabolites (paracetamol sulfate PAR-S, paracetamol glucuronide PAR-G, ketoprofen glucuronide KET-G, o-desmethylmetoprolol D-MET, -hydroxymetoprolol MET-H, 4-hydroxypropranolol sulfate PRO-S). Separation was performed on a C18 Fast Gradient monolithic column (50 mm x 2 mm). It was stated that the method can be applied for the analysis of the aforementioned analytes in human urine and water samples [9].

Capillary zone electrophoresis has also been employed for the determination of five NSAIDs in human serum and river water samples.

Target compounds were pre-concentrated by the electro kinetic supercharging focusing. The various parameters of the technique such as sample pH; concentration of leading stacker, BGE composition, electro kinetic injection time, composition and hydrodynamic injection of the solvent plug and of the terminating stacker was investigated in detail. It was established that the LOD of the method was 0.08 µg/L with a relative standard deviation of <1.03% [10].

Animal products

HPLC coupled with electro-spray ionization tandem mass spectrometry (ESI/MS/MS) was employed for the determination of NSAIDs in bovine plasma and milk. The samples were acidified and the target compounds were extracted with acetonitrile. The extracts were further purified by SPE. It was established that the accuracy of the method varied between 73 and 109% [11].

Enzymatic hydrolysis, extraction, SPE pre-concentration followed with RP-HPLC was applied for the determination of nine NSAIDs in swine, horse and chicken muscles. Muscle samples were vortex mixed, hydrolyzed with β-glucuronidase and extracted three times with acetonitrile. Extract was purified on an alumina N cartridge, concentrated and loaded on an ODS SPE cartridge. Analytes were eluted with 4 mL of n-hexane-ethyl acetate (1:1, v/v). The component of the gradient were acetonitrile (phase A) and 0.1% formic acid (phase B). Starting mobile phase composition was 10% A held 2 min; concentration of A increased to 60% in 15 min (2 min hold), the content of A increased to 80% from 17- to 22 min (final hold 3 min). The flow rate was 0.25 mL/min, the column temperature 30°C. It was stated that the method is suitable for the separation and quantitative determination of NSAIDs in animal muscles [12].

RP-HPLC with fluorescence detection was applied for the confirmatory analysis of some non-steroidal anti-inflammatory drugs in bovine milk. Flurbiprofen, carprofen, naproxen, vedaprofen, 5-hydroxy-flunixin, niflumic acid, mefenamic acid, meclofenamic acid and tolfenamic acid were included in the investigations. LOQs varied from 0.25-20.0 µg/kg [13].

Pharmaceutical formulations

An RP-HPLC method was developed for the measurement of the concentration of tenoxicam in raw material and pharmaceutical preparations. Stationary phase consisted of octadecyl silica. Separation was performed in isocratic mode employing buffer/acetonitrile (40:60, v/v) mobile phase. The flow rate was set to 1 mL/min. The linearity, accuracy, robustness, and intermediate precision of the method were validated statistically. The impact of the concentration of organic modifier in the mobile phase, pH, flow rate, and detection wavelength was also investigated. It was established that the flow rate exerts the highest impact on the robustness of the method. Due to its simplicity, accuracy, sensitivity, and precision the technique was proposed for routine quality control analyses [14].

Environmental matrices

Although the overwhelming majority of the chromatographic procedures applies various liquid chromatographic technologies for the analysis of NSAIDs GC-MS has also been employed. Thus, GC-MS technique was applied for the determination of ibuprofen, naproxen, diclofenac and ketoprofen in wastewater. SPE using octadecylsilica cartridges was used for the extraction of target compounds. The relative standard deviations (RSDs) of the repeatability and reproducibility of the SPE method was lower than 10%, the recovery values were >98%.

Analytes were derivatised with bis (trimethylsilyl) trifluoroacetamide (BSTFA) and 1% trimethyl chlorosilane (TMCS). LOD values were between 0.37 and 3.1 ng/L [15].

GC-MS has also application in the analysis of river water. The trimethylsilyl (TMS) oxime ester derivatives of NSAIDs were prepared and used for GC analysis. A new MS/MS data acquisition method was developed and compared with the traditional full scan (FS) and selective ion monitoring (SIM). The comparison included the RSD values. LOQ data for FS, SIM and MS/MS technologies markedly depended on the acquisition method: ibuprofen; 1.0, 0.43, 0.41; naproxen; 1.1, 1.0, 0.42; ketoprofen, 2.6, 1.0, 0.49; diclofenac; 1.4, 0.41, and 0.21. It was established that the novel MS/MS data acquisition method is superior to both FS and SIM procedures [16].

The application of trimethylsilyl diazomethane (TMSD) as derivatizing agent for the GC analysis of NSAIDs (ibuprofen, ketoprofen and naproxen) has also been reported. The results were compared with those obtained by methylation using boron trifluoride methanol solution, and silylation with a mixture of N, O-bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (99:1, v/v). It was stated that the method is simple, fast, efficient, and can be applied for the trace analysis of drugs in environmental matrices [17].

Both GC-MS and LC-MS were employed for the analysis of NSAIDs such as ibuprofen, naproxen, ketoprofen, diclofenac in tap water, river water and wastewater. Analytes were preconcentrated with SPE. Cartridges were conditioned with 3 mL of ethylacetate, methanol (3 mL) and rinsed with 3 mL of ultrapure water. Target compounds were eluted with 2×1 mL of ethylacetate. Analytes were derivatised before GC-MS measurements with N-methyl-N-[ter-butyl dimethylsilyl] trifluoroacetamide (MTBSTFA). Separations were carried out on a capillary column (30 m × 0.25 mm × 0.25 μm) Temperature gradient started at 65°C (2 min), increased to 180°C at 30°C/min, then ramped to 300°C at 5°C/min (final hold 12 min. Target ions applied for quantification were m/z 263 for ibuprofen, 287 for naproxen, 311 for ketoprofen and 352 and 354 for diclofenac. SPE technique used for the preconcentration of analytes before LC-MS analysis conditioned the cartridges with 5 mL of methanol followed by 5 mL of ultra-pure water. The cartridges were washed with 5 mL of water and then dried. Analytes were eluted with 2 × 4 mL of methanol. Separations were performed on an ODS column using isocratic separation mode: methanol with 5 mM NH₄ acetate and water with 5 mM NH₄ acetate. It was concluded from the results that GC-MS was superior for the determination of ibuprofen, ketoprofen and naproxen in complex matrices. It was further established that neither the filtration step nor the filter material influence significantly the reproducibility of the measurements [18].

An LC-MS technique was employed for the determination of pharmaceuticals in membrane bioreactors (MBR) applying submerged membranes. Analytes were pre-concentrated by SPE. After loading the cartridges were washed with ultra-pure water and were dried in nitrogen stream at 30°C. Pharmaceuticals were eluted by 6 × 1 mL of methanol. Sewage treatment plant (STP) effluents were dried in nitrogen stream and dissolved in methanol-water (1:1, v/v) before LC-MS measurement. Spectrometer in the positive mode was applied for the analysis of roxytromycin, sulfamethoxazole, trimethoprim, acetaminofen, and their metabolites. Ketoprofen, naproxen, and their metabolites were determined in the negative mode. LC measurements were carried out on a RP5 column (150 × 2.1 mm). Components of gradient elution were methanol: water (90:10, v/v, solvent A), and water: methanol (90:10, v/v) both containing 2 mM ammonium

acetate. Separation started with 20% A, the concentration of B increased linearly to 90% A within 12 min. The flow rate was 0.2 mL/min. It was found that the removal of NSAIDs were more efficacies than that of antibiotics. The decomposition rate increased with increasing contact time in MBR.

An LC-MS/MS method was developed for the determination of pharmaceuticals in sea water.

Analytes included were antibiotics, NAIDs, beta-blockers, lipid regulators and one psychiatric drug. The pH of the water samples was adjusted to 7±0.5 before SPE. Cartridges were preconditioned with 5 mL of methanol and 5 mL of water. After loading the cartridges were washed with water, and dried in vacuum. Analytes were eluted with 5 mL of acetone and 2 × 5 mL of methanol. The separation of the target compounds was performed on an ODY column (250 × 4 mm; particle size, 5 μm). The components of the mobile phase were 0.02 M formic acid in water (solvent A and acetonitrile (solvent B). Linear gradient (0.3 mL/min started with 60% A. Concentration of solvent A increased linearly to 100% in 20 min. The optimal ionization source working parameters were: sheath gas flow rate, 80 arbitrary unite (a.u.); auxiliary gas, 20 a.u.; capillary temperature 350°C; capillary voltage 14V; and tube lens offset, 20V.

Dispersive liquid-liquid micro extraction (DLLME) followed with liquid chromatography-tandem mass spectrometry was employed for the determination of ibuprofen, ketoprofen, naproxen and diclofenac in river and tap waters. Optimal extraction efficacy was achieved by using two-step extraction with sonification. Chloroform was used as extracting and acetone as dispersing agent. LOQs were between 0.5 and 10 ng/L. It was established that DLLME is a simple, rapid and sensitive method for the preconcentration of pharmaceuticals in environmental water samples [19].

Micro emulsion electrokinetic chromatography has also found application in this separation and quantitative determination of ten NSAIDs in water. It was found that the concentration and type of the organic modifier exert a significant influence on the efficacy of separation. It was further established that the application of field-amplified sample injection enhanced the detection sensitivity. LOD of NSAIDs was between 0.03 and 0.3 μg/L. It was stated that the method is suitable for the analysis of NSAIDs in water samples after solid-phase extraction (SPE) [20].

An electromembrane extraction (EME) method was developed and successfully applied for the simultaneous extraction of acidic NSAIDs and basic beta blockers. The efficacy of the procedure was controlled by gas chromatography-mass spectrometry (GC-MS) and the results were compared with those archived by SPE. Before extraction SPE cartridges were conditioned with 5 mL of ultrapure water, 5 mL of 1-hexane, 5 mL of ethyl acetate, 10 mL of methanol and 10 mL of ultrapure water. Extraction was carried out under vacuum; analytes were eluted with 5 mL of methanol. GC-MS measurements were performed in a fused silica capillary column (30 m × 0.25 mm i.d.; film thickness, 0.25 μm). The injector temperature was 300°C, the MS interface temperature was 280°C. The oven temperature program started at 70°C (2 min hold, ramped to 240°C at 10°C/min (8 min hold), increased to 300°C at 10°C/min (5 min hold). The procedure has been successfully employed for the analysis of drugs in wastewater samples [21].

Supports containing different alkyl chains and covered with magnetite were synthesized and employed for the preconcentration of four NSAIDs (acetaminofen, naproxen, diclofenac and ibuprofen).

The magnetic support was dispersed in the samples with the nonionic surfactants Triton X-100, then the support was magnetically isolated and the target compounds were eluted with methanol. Analytes were separated on an ODS column (150 mm × 4.6 mm i.d.) using isocratic separation mode. Mobile phase consisted of methanol-phosphate buffer (2:1, v/v), pH 2.5, 0.01 mol/L. The flow rate was 1.0 mL/min; separations were performed at 25°C. The highest recovery (>90%) was achieved with support containing octyl chain at pH 3. LOD varied between 1-2 µg/L, repeatability was lower than 5% in all cases. It was found that the method can be applied for the analysis of these NSAIDs in wastewater samples [22].

LC-MS, triple-quadrupole (QqQ) system and a quadrupole time-of-flight system (Q-TOF) were employed for the investigation of the decomposition rate of NSAIDs and their chlorination and bromination. It was established that salicylic acid, naproxen, diclofenac and indomethacin were degraded under strong chlorinated conditions (10 mg/L Cl₂, 24 h). The data indicated that the concentration of chlorine exerts the highest influence on the decomposition and the decomposition process started by the aromatic substitution of one or two hydrogens by chlorine and/or bromide. The method was successfully applied for the determination of target compounds in drinking water and wastewater samples [23].

LC combined with tandem mass-spectrometry was used for the measurement of acetaminophen and its metabolites acetaminophen glucuronide and acetaminophen sulfate in wastewater. The occurrence of these metabolites in waste water was established [24].

A novel method consisting of three phase hollow fiber liquid-phase micro-extraction (HF-LPME) followed by liquid chromatography-mass spectrometry was employed for the analysis of four NSAIDs (ketoprofen, naproxen, diclofenac, and ibuprofen) in sewage sludge. Enrichment factors were between 2761 and 3254 in pure water. The RSD values of repeatability and inter-day precision of sewage sludge samples varied from 10-18% and 7-15%, respectively [25].

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